# Inventory of microbiological species living in spent nuclear fuel pools : towards the identification of radioresistant species

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# THÈSE

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dans **l'École Doctorale Chimie et Sciences du Vivant**

## Analyse des espèces biologiques vivant dans les piscines des installations nucléaires : vers l'identification d'espèces radiorésistantes

## Inventory of microbiological species living in spent nuclear fuel pools: Towards the identification of radioresistant species

Thèse soutenue publiquement le **30 octobre 2018**,
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# Abbreviations

**BHI:** brain heart infusion
**bp:** base pair
**kb:** kilobase
**Bq:** Becquerel
**cfu:** colony forming unit
**CNV:** copy number variation
**$D_{10}$:** ionizing radiation dose needed to eliminate 90% of a population
**Da:** dalton
**ddNTP:** dideoxyrubonucleoside triphosphate
**dNTP:** deoxyrubonucleoside triphosphate
**DNA:** desoxyribonucleic acid
**dsDNA:** double-stranded DNA
**rDNA:** ribosomal DNA
**EPS:** exopolysaccharide
**ESDSA:** extended synthesis-dependent strand annealing
**eV:** electron volt
**FA-VL:** faible activité vie longue (low activity and long-life wastes)
**FMA-VC:** faible à moyenne activité vie courte (low and medium activity with short-life wastes)
**GSB:** glycerol strain buffer
**GSH:** reduced glutathione
**GSSG:** oxidized glutathione
**Gy:** Gray
**HA-VL:** haute activité vie longue (high activity and long-life wastes)
**HGP:** human genome project
**HPP:** human proteome project
**HR:** homologous recombination
**ISS:** international space station
**ITS:** internal transcribed spacer
**LB:** Luria-Bertani (or lysogeny broth)
**$LD_{50}$:** lethal dose 50, the dose for which 50% of a population dies
**MA-VL:** moyenne activité vie longue (medium activity and long-life wastes)
**MIC:** microbial influenced corrosion
**MIT:** Mascot identity threshold
**MnSOD:** manganese superoxide dismutase
**MS:** mass spectrometer
**NA:** nutrient agar
**nanoLC:** nanoscale liquid chromatography
**NB:** nutrient broth
**NGS:** next generation sequencing
**NHEJ:** non-homologous end-joining

**NPP:** nuclear power plant
**OTU:** operational taxonomic unit
**PCR:** polymerase chain reaction
**PSM:** peptide-spectrum match
**RNA:** ribonucleic acid
**rRNA:** ribosomal RNA
**rDNA:** ribosomal DNA
**ROS:** reactive oxygen species
**RT:** room temperature
**SBS:** sequencing by synthesis
**SDSA:** synthesis-dependent strand annealing
**SMRT:** single-molecule real-time
**SNFP:** spent nuclear fuel pool
**SNP:** single nucleotide polymorphism
**SODs:** superoxide dismutases
**SSA:** single-strand annealing
**ssDNA:** single-stranded DNA
**Sv:** Sievert
**TFA:** très faible activté (low activity wastes)
**TOF:** time of flight
**TSA:** tryptic soy agar
**TSB:** tryptic soy broth
**TSM:** taxon-spectrum match
**UV:** ultraviolet
**VBNC:** viable but non-culturable bacteria
**VTC:** vie très courte (very short-life wastes)

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## *Bibliographical introduction*

# 1. Bibliographical introduction

## 1.1 Origin of life and diversity of microorganisms

Living organisms can be divided into three domains: Bacteria, Archaea and Eucarya (all Eukaryotes). This classification was first introduced in 1977 by Woese and Fox after the phylogenetic analysis of ribosomal RNA sequences of a large number of organisms [1]. Bacteria and Archaea are prokaryotes, meaning that they do not possess a nucleus, unlike Eukaryotes, which possess a nucleus. All animals and plants, including fungi, yeasts and micro-algae, belong to the Eucarya domain. The simplified tree of life obtained with the current available genomics data is presented in Figure 1.1.

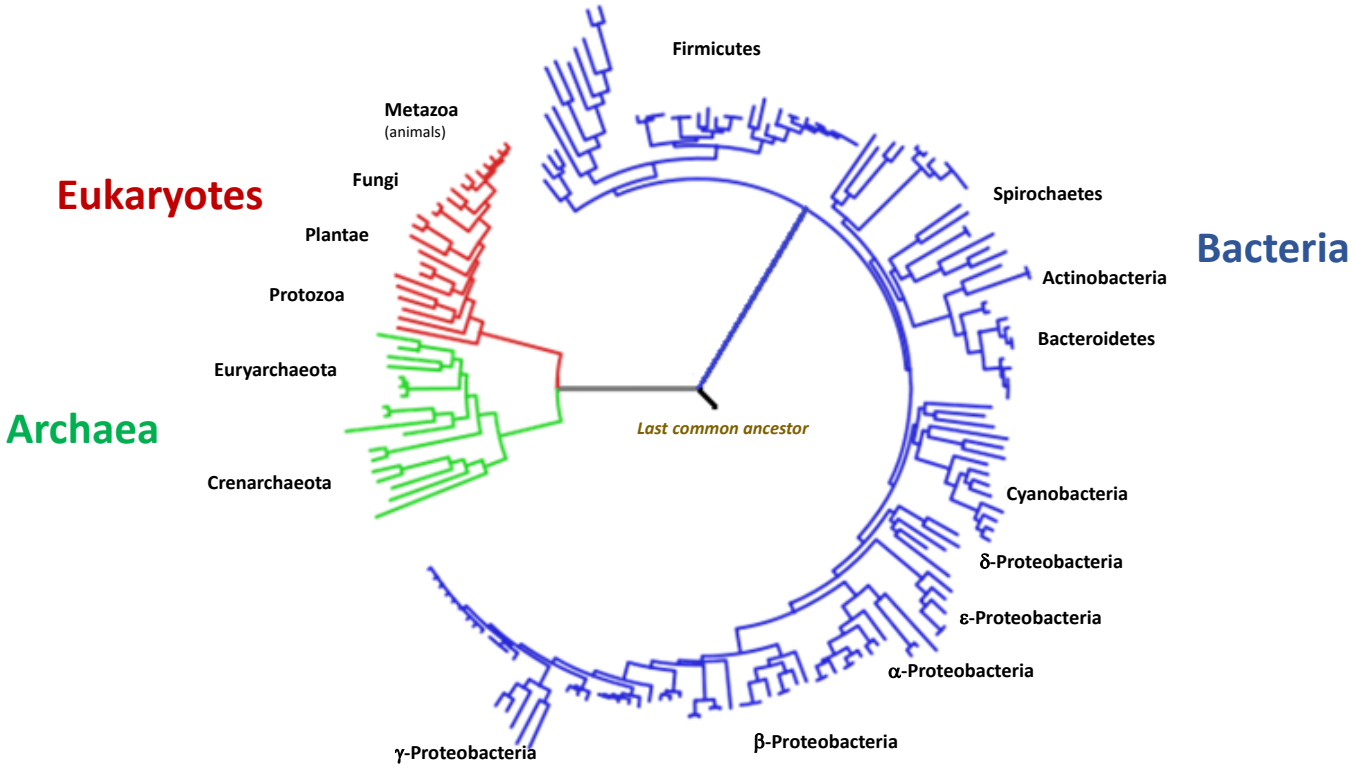



*Figure 1.1: Tree of life (non-exhaustive) (adapted from [2])*

In 1998, Whitman et *al*. estimated the population of prokaryotes on Earth as being around 4 to $6.10^{30}$ cells [3], the Prokaryotes kingdom representing most of life on Earth.

The Earth was formed around 4.5 billion years. The first confirmed evidence of life has been evaluated to be 3.5 billion years old thanks to a microfossil, during the Archean eon [4, 5]. However some indirect evidence could place life earlier on Earth, between 3.8 and 4.1 billion years ago [4-6].

When life first appeared on Earth, the conditions were extremes, when compared to the current ones. Indeed, the first organisms were anaerobic since no free oxygen was present in the atmosphere. Moreover, the ozone layer was inexistent due to the absence of free oxygen, hence the UV rays received from the young Sun were stronger than today and not filtered by the ozone layer. The Earth's surface temperatures may also have been as high as 70°C in the Archean eon [7]. However, liquid water was likely found beneath the surface in hydrothermal systems [8]. Finally the natural radioactivity present on Earth was higher than today due to radioactive decay (cf. 1.4.2). Free oxygen in the atmosphere appeared millions of years after

the first cell. If life could have arisen in such harsh conditions, it is not a surprise to find microorganisms in a multitude of metabolic niches.

Microorganisms can colonize almost every location on Earth, from animal's gut to the bottom of the ocean and the atmosphere. Microorganisms can also be found in unexpected environment and exhibit resistance properties to what humans consider as extreme conditions. Those microorganisms can be extremophiles.

## 1.2 Extremophiles

According to Rothschild and Mancinelli, "*an organism that thrives in an extreme environment is an extremophile; in more than one extreme it is a polyextremophile*"[9]. An extremophile can thus survive physical and biochemical conditions where humans, most animals, and plants cannot survive.

Some microorganisms can subsist and even flourish in extreme conditions for which multicellular organisms cannot survive. Extremophile organisms such as bacteria and archaea have indeed been found in hydrothermal vent, acidic or alkaline media, extremely low or high temperature, high pressure, etc. Some of them cannot grow without those extreme conditions [10]. Because they have the ability to occupy every potential environment microorganisms are the most diverse and abundant cellular life forms on Earth [10-13].

Knowing that life can be found in almost every place and conditions, it is not a surprise to find radioresistant microorganisms. One bacterium, identified in 1956 by Arthur W. Anderson, has been proven capable to resist high radiation level, up to 20 kGy (cf. 1.4.1). This unusual bacterium is *Deinococcus radiodurans* (former *Micrococcus radiodurans*) (Fig. 1.2) [14]. The $LD_{50}$ within 30 days for human is around 4 Gy and a dose around 40 or 50 Gy compulsorily leads to the death of the person, according to the IRSN (Institut de Radioprotection et de Sûreté Nucléaire – French Radioprotection and Nuclear Safety Institute).

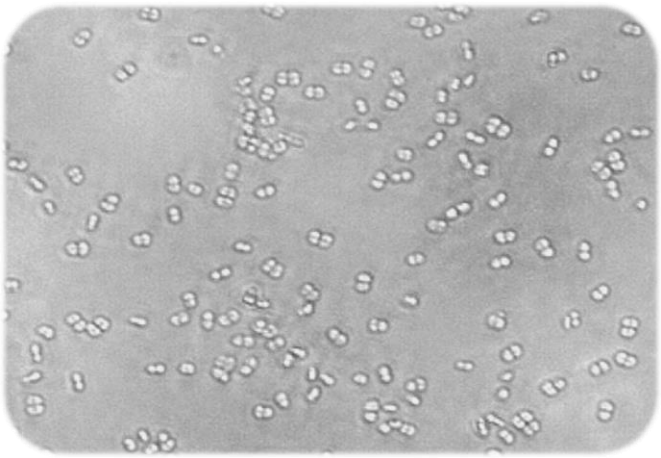


***Figure 1.2: Deinococcus radiodurans observed using a photon microscopy (x40)***

## 1.3 Common environmental stress and microorganism responses

Throughout evolution, extremophiles have also developed mechanisms to be able to live within the constraints of their environment. For example, microorganisms can be found in the hot springs of Yellowstone. These springs are not only extremely hot (up to 93°C) but also acidic (pH between 2 and 3). As an example, *Thermus aquaticus* was discovered in one of Yellowstone's hot spring in 1978 by Brock [15].

This bacterium had a great influence on biotechnology since its DNA polymerase (called Taq polymerase) was later used for PCRs (polymerase chain reaction) to amplify DNA fragments.

To survive the different environmental conditions, whether favorable or unfavorable, microorganisms have evolved and developed different metabolic ways to survive. Some of them can be the activation of a certain living form, such as biofilms or spores. Others can be the activation of pathways in order to counter the negative effects of the stresses on the cell and its components, such as oxidation stress for aerobic organisms and the different cell defenses that exist to block its negative impact. The most common environmental stresses can be a deficiency of nutrients, desiccation, presence of antibiotics, predator and food competitor, etc.

### 1.3.1 Biofilms

In unfavorable environments, microorganisms, as a mean to survive, can form biofilms, either composed by the same species or by a consortia of different species, genus or even kingdom, *e.g.* bacteria with other bacteria, fungi, micro-algae… This morphological state protects them, since they behave as a complex multicellular organism and communicate with each bacterium, fungal or algal cell through quorum sensing mechanisms. This phenomenon not only preserves them and gives them higher chances of survival but also allows them to concentrate nutrients taken from the medium [16]. Stewart and Costerton reviewed in 2001 the ability of biofilms to protect the microorganisms against antibiotics. Some bacteria that did not present any antibiotic resistance genes could survive antibiotics when in biofilms [17]. It is now widely accepted that biofilms are predominant in aquatic ecosystems [18-21].

The formation of biofilms in an aqueous environment relies on several steps. First of all bacterial cells come into contact with a solid surface and can attached in a reversible way. They can then grow and proliferate, leading to an irreversible attachment due to the secretion of exopolysaccharide (EPS). This leads to the formation of a thin layer of biofilm, which will then mature and give a complex structure [22]. The biofilm formation is eased by the presence of EPS. Once a mature biofilm is formed, it can further enhance bacterial deposition. This explains why, once a biofilm has been observed, it is extremely difficult to eliminate: if only one cell is present, the biofilm can grow again. It is thus necessary to either eliminate the entire biofilm with drastic methods or to regularly clean the surface on which the biofilm has grown [22].

The need to eliminate biofilms can be explained by several reasons. It can contaminate drinkable water pipes and provoke diseases like in 1998 and 1999 where several cases of legionnaires' disease, due to *Legionella* species, were reported in Paris. Moreover, the presence of a biofilm can damage the material it is attached to and cause corrosion.

In nuclear installations, the main danger the biofilms could provoke is their ability to induce corrosion. The degradation process of the biofilm's support is presented in Figure 1.3. At first, a thick biofilm is formed and an anaerobic zone is created next to the colonized surface. The microorganisms then develop colonies and complex microbiota, catching ions and creating localized chemical and physical gradients at the metal surface. The metal can then dissolves, leading to corrosion, or, as it called when caused by microorganisms, Microbial Influenced Corrosion (MIC) [23].

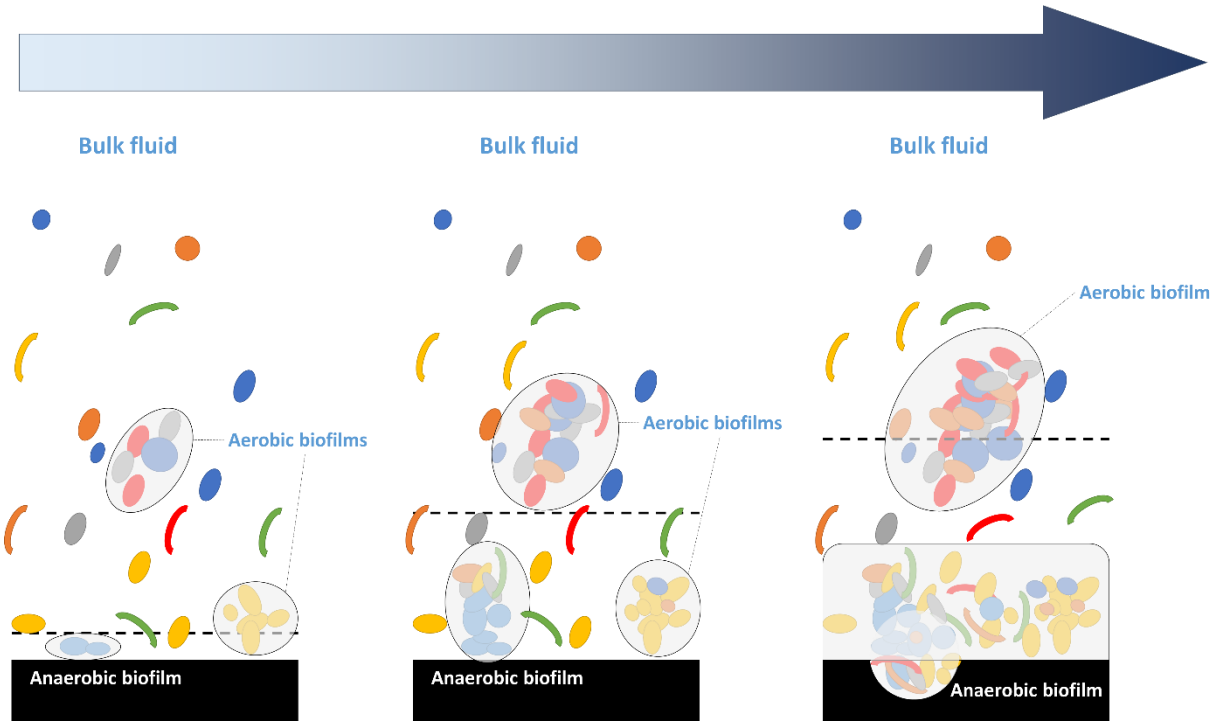



***Figure 1.3: Biofilm leading to biocorrosion (adapted from [23]).*** *The cells first attached to the surface and a thin anaerobic biofilm is formed. This biofilm grows and matures until it changes the physical and chemical conditions of the surface. These changes can lead to the corrosion of the surface.*

The formation of biofilms can thus protect the microorganisms but can also have negative influences on their environment, such as propagating diseases or provoke corrosion in a human made environment.

However the response to an environmental stress can not only be the growth under a biofilm form but also the ability to either form spores (Gram positive bacteria) or enter a viable but non-culturable state.

### 1.3.2 Sporulation and Viable but Non-Culturable Bacteria (VBNC)

Besides biofilm formation, microorganisms have developed other ways to resist environmental stresses. Indeed most bacteria can slow their metabolism down. This state, called the viable but non-culturable state, possesses the metabolic activity needed to survive but is extremely low [24-26]. Some gram positive bacteria can sporulate, leading to spore(s) formation. It is well known that spores are more resistant to stresses than the vegetative form [27-29].

#### *1.3.2.1 Sporulation*

Only Gram positive bacteria can sporulate. Usually bacteria sporulate when the environmental conditions are unfavorable to growth or life. Indeed bacteria can form spore in nutriment starvation or to prevent desiccation. But spores are also a resistant microbial form to ionizing radiation or antibiotics. Moreover, it has been proved that bacterial spores can survive in space, amongst the cosmic radiation and the vacuum [30, 31]. Usually, one bacterial cell produces one spore. However depending on the conditions some bacteria can produce two or more spores [32]. The main different steps of spore formation in bacteria are schematized in Figure 1.4. During its maturation in the cell, a spore is called “forespore”.

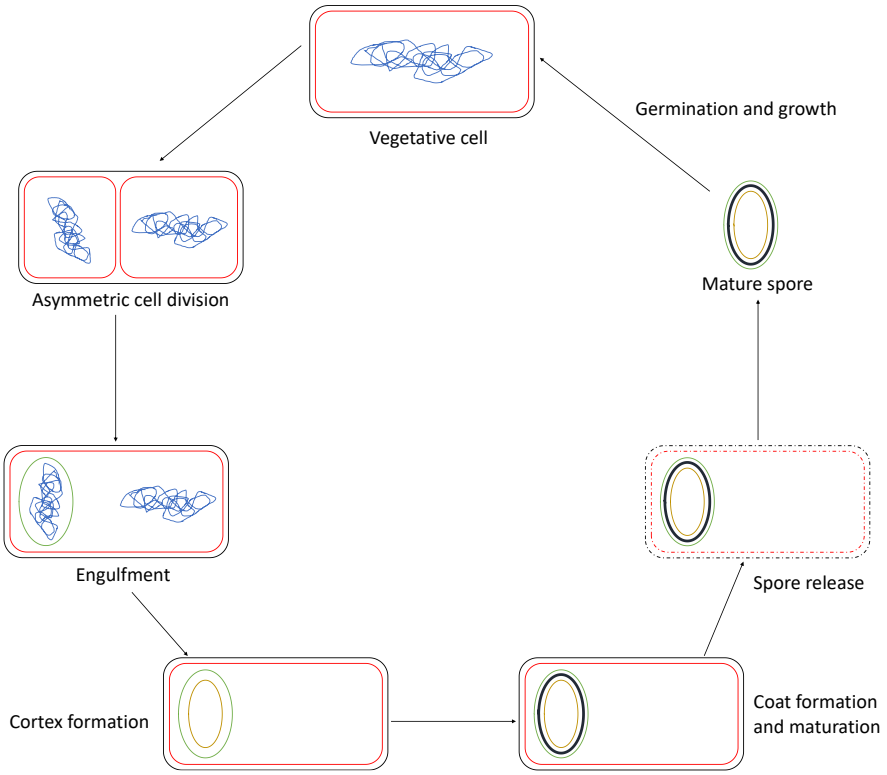



***Figure 1.4: Bacterial sporulation steps (adapted from [33]).*** *A vegetative cell will divide asymmetrically. One cell will lead to the formation of the spore while the mother cell will go under a programmed cell death to release the mature spore.*

When the conditions become unfavorable to the bacterium, regulation factors and different genes involved in sporulation are activated. This activation leads to an asymmetric cell division as shown in Figure 1.4. The smaller part becomes the forespore while the bigger one will be considered as the mother cell. Once the spore is mature enough, the mother cell experiences a programmed cell death and lysis. This cell death leads to the release of the mature spore [32]. Once the conditions are proper enough, the spore can germinate and transform into a new vegetative cell. However, while sporulation can be an effective way to survive environmental stresses, not all bacteria have the ability to form spore.

### *1.3.2.2 Viable but Non-Culturable Bacteria (VBNC)*

The VBNC state is empirically defined as a state in which cells are viable but not able to divide on non-selective growth medium, leading thus to a lack of visible growth. The first published description of such a state was in 1982 by Xu et al. in which they observed the ability of *Escherichia coli* and *Vibrio cholerae* to undergo a dormant-like state in response to starvation [34]. The VBNC response differs from other bacterial responses to stress. Moreover cells can remain VBNC for a long period of time, up to several years, meaning that this state is not a temporary response. These characteristics explain why the VBNC state is thought to be a long-term survival mechanism initiated in response to environmental stresses. Those stresses can include starvation but also UV light, extreme temperatures, toxic molecules, radiation, etc. [35]. One inconvenient of the VBNC state is that, in order to be able to grow the bacteria, the optimum conditions must be found. It has been estimated that, in water, such as the oceans, lakes, and rivers, bacteria are mainly present under a VBNC state and represent between 90 to 99.9% of the bacterial cells [36]. This means that only 0.1 to 10% of aquatic and environmental bacteria are culturable with the classic laboratory

methods. Using different direct analysis methods, such as metagenomics (cf. 1.6.2.2), it is possible to identify new microbial species, genera, families or phyla even for unculturable bacteria. Tara Expedition is currently analyzing the oceans around the globe to highlight some of the diversity present in the oceans.

During this PhD thesis, the isolated microorganisms were subjected to three main environmental stresses such as starvation, ionizing radiation and radionuclides. The survivability of isolated bacteria were tested in presence of ionizing radiation and selected radionuclides.

## 1.4 Unusual environmental stresses: ionizing stress, radiation and radionuclides

### 1.4.1 Ionizing stress and radiation

Ionizing radiation is a radiation that carries enough energy to damage the matter and can liberate electrons from atoms or molecules, thereby ionizing them. These radiations thus impact the living organisms. Figure 1.5 represents the different radiations that exist in the environment.

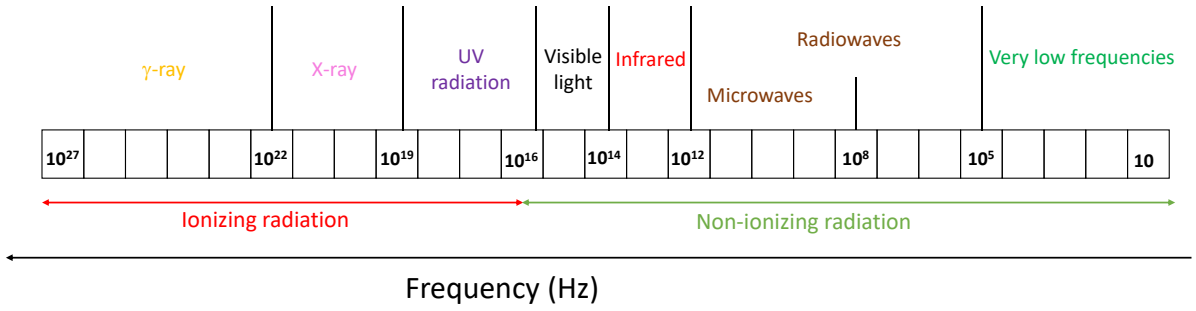



***Figure 1.5: Ionizing and Non-ionizing radiations.*** *Radiations are electromagnetic waves, consisting of oscillating electric and magnetic fields traveling through space. Depending on the frequency (in Hz) of the wave, the radiation can be visible (visible light) or invisible to the human eye and ionizing or not.*

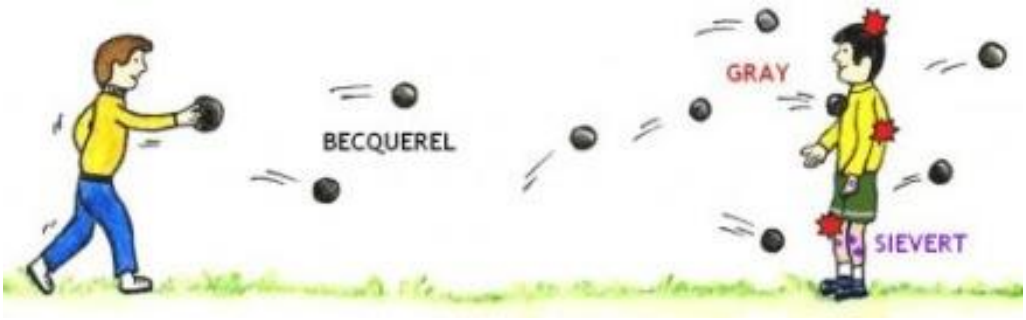
Gamma ray, X-ray, and the higher ultraviolet part of the electromagnetic spectrum are ionizing radiations. The lower ultraviolet part of the electromagnetic spectrum, and also the lower part of the spectrum below UV, including visible light (along with nearly all types of laser light), infrared, microwaves, and radio waves are all considered non-ionizing radiations. The boundary between ionizing and non-ionizing electromagnetic radiation that occurs in the ultraviolet is not sharply defined, since different molecules and atoms ionize at different energies. Conventional definition places the boundary at a photon energy between 10 eV and 33 eV in the ultraviolet. The electronvolt (eV) represents the energy carried by an electron or a photon for example.

Part of radioactivity belongs to the higher frequencies of ionizing radiation. When an atom is unstable, it disintegrates to reach a more stable state (this state can be achieved after one or several disintegrations). During this transition, energy and ionizing radiation are emitted. This process is called “radioactive decay”.

Some ionizing radiation occurs from natural sources, due to cosmic and terrestrial radiation. For example, the Sun emits UV rays that reach the Earth and are not entirely stopped by the ozone layer. Each year, γ-rays are received on Earth from cosmic rays.

Several units can be used to evaluate radioactivity and are explained in Scheme 1.1:

- Becquerel (Bq): it measures the number of disintegrations of atoms during one second. It thus represents the energy released by the atoms;
- Gray (Gy): it assesses the energy received and thus absorbed by a material (organic and inorganic). It can be seen as how much energy you received from the energy that was send;
- Sievert (Sv): it estimates the effect of the radiation on the organism.

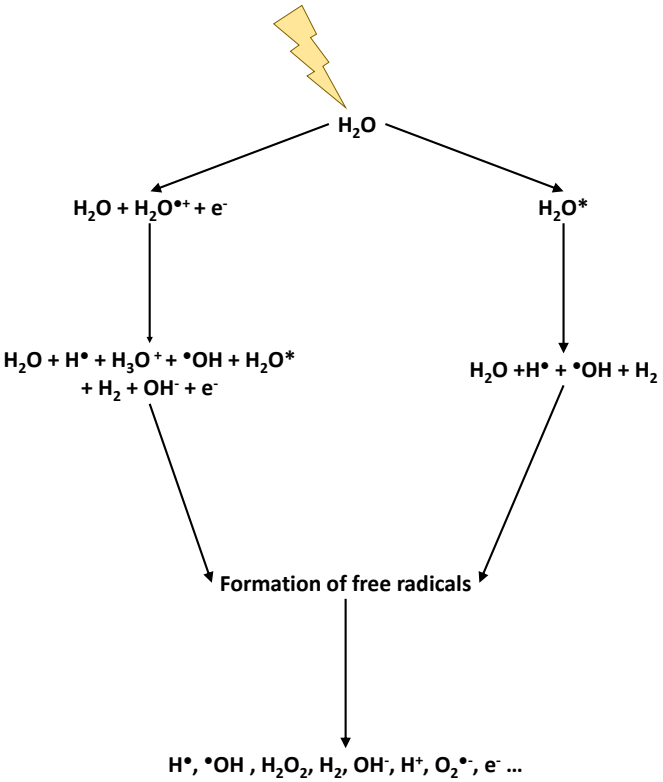



*Scheme 1.1: Schematic view of the different units used in radioactivity (IRSN)*

#### *1.4.1.1 Effect of ionizing radiation on living cells*

Ionizing radiation can provoke the formation of Reactive Oxygen Species (ROS), which alters molecules and thus cells.

Ionizing radiations have two effects on DNA. It can either create direct double-strand breaks (around 20% of the damages in eukaryotes) or lead to the formation of ROS due to water radiolysis (around 80% of the damages in eukaryotes) [37-39]. Water radiolysis leads to the creation of several molecules such as free radicals (Scheme 1.2) that can interact with cell molecules and damage proteins, lipids and DNA.




***Scheme 1.2: Water radiolysis and molecules formed (adapted from [40]).*** *Ionizing radiation can interact with a water molecule. This water molecule will then be modified and lead to the formation of superoxides, free radicals and molecules highly reactive to oxygen.*

Once ROS are formed, they induce negative effects on molecules, and on the structure and functionality of living organisms. ROS can indeed impact proteins and lipids, which themselves can damage DNA due to the by-products of lipid peroxidation [41] or after exposure of histones proteins to free radicals [42-44]. Moreover when enzymes used to repair DNA are damaged they cannot properly function.

### *1.4.1.2 Reactive oxygen species (ROS)*

#### 1.4.1.2.1 Nature and origins

Reactive oxygen species (ROS) are chemically reactive chemical species containing oxygen. ROS are molecules that are more reactive than $O_2$. These molecules are naturally produced by the aerobic metabolism [45]. However, external stresses can also be responsible of production of ROS in cells, such as high temperature, desiccation, UV, irradiation, etc. They include superoxides, oxygen radicals but also non-radical derivatives of $O_2$, such as $H_2O_2$, as detailed in Table 1.1.

*Table 1.1: Different forms of ROS [46]*

| Free radicals | Non-radicals |
|---|---|
| Superoxide, $O_2^{\bullet-}$ | Hydrogen peroxide, $H_2O_2$ |
| Hydroxyl, $OH^{\bullet}$ | Hypobromous acid, HOBr |
| Hydroperoxyl, $HO_2^{\bullet}$ | Hypochlorous acid, HOCl |
| Carbonate, $CO_3^{\bullet-}$ | Singlet oxygen, $O_2\,^1\Delta g$ |
| Peroxyl, $RO_2^{\bullet}$ | Organic peroxides, ROOH |
| Alkoxyl, $RO^{\bullet}$ | Peroxynitrite, $ONOO^-$ |
| Carbon dioxide, $CO_2^{\bullet-}$ | Peroxynitrate, $O_2NOO^-$ |
| Singlet, $O_2\,^1\Sigma g^+$ | Peroxynitrous acid, ONOOH |
| | Peroxynitric acid, $O_2NOOH$ |
| | Nitrosoperoxycarbonate, $ONOOCO_2^-$ |
| | Peroxymonocarbonate, $HOOCO_2^-$ |

The ROS are mainly due to the presence of free oxygen in the atmosphere. During Earth early age, free oxygen was present in less than 0.1% in the atmosphere: the first cells were confronted from low to inexistent levels of oxidative stress. Then, the anaerobic microorganisms present had to evolve to cope with the oxidation induced by the rising levels of oxygen on Earth. Microorganisms had three possibilities to cope with the presence of high quantity of free oxygen: i) death, ii) life in anaerobic environments or iii) implementation of antioxidant defenses and aerobic metabolism. Most of living organisms have been able to survive by the use of pathways to repair the damages caused by aerobic respiration and environmental oxidative stress.

#### 1.4.1.2.2 Consequences on the cell

A significant part of the initial damage done to the cells by ionizing radiation is due to $OH^{\bullet}$ which leads to ROS. ROS formation can damage three main biological molecules: the proteins, the lipids and the nucleic acids. ROS induce several damages, including lipid peroxidation, protein oxidation, oxidative alterations to DNA, and inactivate enzymes. Lipids are the main targets of ROS in the cell. Lipid peroxidation starts a chain reaction and form several radicals. For example, if only one lipid becomes a lipid peroxide, it can create as many peroxides as it possesses carbon chains. Lipid peroxidation can not only decrease the membrane fluidity but can also enhance the release of internal $H_2O$ into the external medium as well as injuring membrane proteins. Moreover in some bacteria, the DNA is close to or attached to the membrane which means that it can be damaged during lipid peroxidation. Other deleterious effects of ionizing radiation and $OH^{\bullet}$ are their interactions with proteins. $OH^{\bullet}$ can indeed oxidize proteins and decrease their activities or even inactivate them. Proteins are essential to cell metabolism and damages repair. When proteins are inactivated or when their activity decreases they can damage the cells by malfunctioning and thus also not be able to repair damages. Furthermore, $OH^{\bullet}$ can also interact directly with DNA by inducing single- and double-strand breaks, modifying nucleotide bases, and damaging the deoxyribose. The deleterious effects of ionizing radiation are enhanced by the presence of $O_2$ [46].

#### 1.4.1.2.3 Defenses against ROS

To be able to survive despite the presence of ROS, cells have developed several ways to fight the ROS and their negative effects. Cells have active mechanisms such as DNA repair but can also use passive

mechanisms. The high condensation of the genome, as well as the histone-like protein HU associated with bacterial nucleoid, are involved in cell survival after γ-radiation [47, 48]. It has also been noticed that a high [Fe]/[Mn] ratio is often involved with radioresistance [49]. Moreover some cells can possess antioxidant molecules. Active and passive mechanisms have been identified in radiation resistance.

#### 1.4.1.2.3.1 Antioxidants

Antioxidant molecules are known to prevent the oxidative damages of molecules on the cells. Halliwell and Gutteridge have defined in 2015 an antioxidant molecule as "*any substance that delays, prevents, or removes oxidative damage to a target molecule*" [46]. The presence of ROS and oxidative stress activates genes coding for molecules involved in ROS scavenging and proteins involved in cell protection.

Some families of antioxidants have been shown to help microorganisms to grow in ionizing radiating environments, such as in space or in the facility of the reactor of Chernobyl [50-52]. Such class of molecules is present in all aerobic organisms to counter the negative effects of oxidation during the aerobic metabolism. Some of these molecules are pigments. One well-known antioxidant pigment is melanin. It can be observed on different domains of life: Eukarya (in almost every animals and fungi) and in some bacteria. It is admitted that melanin can protect against UV, solar or γ-radiation as well as metal toxicity due to, for instance, its ability to scavenge free radicals [53-55]. Melanised fungi can be found in extreme environments, including Arctic and Antarctic [56, 57], and, more surprisingly, the walls of the damaged reactor of Chernobyl, where they are exposed to a high constant radiation field [54]. Melanised fungi can also be found in another astonishing place where radiation levels are higher than in classical environment: the International Space Station (ISS) [58, 59]. Melanins are highly effective scavengers of free radicals [60] and have electron transfer properties [61]. It has also been shown and reviewed that the degree of radio-resistance of an organism is associated with the quantity of produced melanin [59].

Another family of pigments involved in the response to oxidative stress are the carotenoids. They represent organic pigments that are produced by plants, algae, as well as several bacteria and fungi. Carotenoids are lipophilic and have the ability to protect some bacteria to UV radiation and ionizing radiation [62-64]. For example*, Deinococcus radiodurans*, the most known radioresistant organism, contains high levels of carotenoids. Several carotenoids, such as sarcinaxanthin have been proved to have an anti-oxidative activity [65]. It has been shown that, amongst the carotenoids, deinoxanthin, found in *D*. *radiodurans*, exhibited the highest scavenging activity, followed by lycopene, lutein, zeaxanthin and, finally, β-carotene [63] [66, 67]. The three pigments described above are not the only antioxidant pigments present in microorganisms. Other pigments, belonging to different families of molecule, such as mycosporine or scytonemin, as well as several other molecules, play a role in the defense against oxidative stress [68, 69].

Other molecules such as glutathione are also involved in the response of oxidative stress. Glutathione is present in high concentration in several living cells, including microorganisms. It has however not yet been identified in strictly anaerobic microorganisms. It exists under different form: oxidized (GSSG) or reduced (GSH). In cells, most of the glutathione is under the reduced form [46, 70]. Reduced glutathione has been proved to be a radioprotective molecule [71] by scavenging $OH^{\bullet}$ radical and by being involved in the following reaction:

$$RO_2^{\bullet} + GSH \rightarrow RO_2H + GS^{\bullet}$$, where $GS^{\bullet}$ is an oxidized molecule [46]

Glutathione can be also involved in DNA repair after single and double strand breaks due to ionizing radiation [72]. It was indeed shown that GSH was involved in the non-homologous end-joining DNA repair pathway when joining both ends [73]. Moreover, GSH and glutathione S-transferase were shown to be involved in metal stress defenses, such as uranium, copper or cadmium response mechanisms, and even some radionuclides counteraction by chelating the metals [46, 70, 74-76].

One known class of enzymes involved in ROS scavenging is the superoxide dismutase (SOD). This enzyme can react and remove the different ROS present, such as $H_2O_2$, $O_2^{\bullet -}$, $OH^{\bullet}$, etc [46]. SODs are present in eukaryotes as well as prokaryotes and archaea [77]. In animal cells, it has been shown that the overexpression of Manganese SODs (MnSODs) leads to a higher radiation resistance [78]. Moreover, MnSOD is present in *D. radiodurans* and has been proved to be involved in the radioresistance mechanism implemented by the bacterium [79, 80]. Some anaerobic microorganisms possess SODs to help them survive temporary $O_2$ exposure [46].

#### 1.4.1.2.3.2 The role of Manganese

Antioxidant enzymes protect biomolecules from ROS-mediated damages. This represent the first line of protection against oxidative stress. The second line is manganese. Indeed, a high [Mn]/[Fe] ratio can be observed in resistant organisms. For example, a ratio of 0.24 was observed in *D. radiodurans* while it dropped to 0.0072 in *E. coli* [81]. A high [Mn]/[Fe] ratio increases the capacity to prevent the formation of iron-dependent ROS through the Fenton reaction (Scheme 1.3). It is also thought that $Mn^{2+}$ ions do not catalyze the formation of $OH^{\bullet}$ from $H_2O_2$, thus preventing the formation of this ROS [46, 82].Furthermore, intracellular manganese can also be protective by scavenging ROS. This high ratio does not directly prevent DNA double-strand breaks but it can mimic the activities of antioxidant enzymes such as superoxide dismutases and catalases [83].

| | | |
|---|---|---|
| **$Mn^{2+}$**: dismutation (SOD-like) | $2\ O_2^{\bullet -} + 2\ H^+$ | $\rightarrow\ O_2 + H_2O_2$ |
| dismutation (catalase-like) | $2\ H_2O_2$ | $\rightarrow\ O_2 + 2\ H_2O$ |
| **Haber-Weiss reaction** | $O_2^{\bullet -} + H_2O_2$ | $\rightarrow\ O_2 + OH^- + HO^{\bullet}$ |
| **$Fe^{3+}$**: | $Fe^{3+} + O_2^{\bullet -}$ | $\rightarrow\ Fe^{2+} + O_2$ |
| **$Fe^{2+}$**: Fenton reaction | $H_2O_2 + Fe^{2+}$ | $\rightarrow\ Fe^{3+} + OH^- + HO^{\bullet}$ |

*Scheme 1.3: Reaction of $H_2O_2$ with $Mn^{2+}$ and $Fe^{2+}$ [49]*

Finally, some enzymes involved in ROS scavenging contain manganese or are manganese-dependent. For example, one known SODs in bacteria is MnSOD, which is involved in ROS scavenging. It has been shown that *D. radiodurans* uses the manganese properties to be more tolerant to ionizing radiation [81, 84].

#### *1.4.1.3 DNA protection and repair*

##### 1.4.1.3.1 Physical protection of DNA

One of the different characteristics of *D. radiodurans* that allows its survival to high radiation levels is its highly compacted DNA [47, 48] which could limit the dispersion of radiation-induced DNA fragments. It was also suggested that *D. radiodurans* and D. *deserti*, two highly resistant bacteria, contained a low diversity of nucleoid associated proteins, with the histone-like HU protein and DNA-gyrase being the main proteins involved in their nucleoids [48]. The HU protein is involved in supercoiling of DNA. The shape of genomic DNA from *D. radiodurans* was determined in absence of ionizing stress and was observed as a ring-like (toroidal) shape in the stationary-state while it was dispersed in some cells during the growing state, allowing access for the replicative enzymes [85]. Furthermore, *D. radiodurans* grows as compartmentalized cells, each compartment having its own genomic DNA in multicopy. After an irradiation at 15 kGy, this peculiar DNA shape was maintained for 1 hour after exposure in culture. However, few hours after irradiation, the DNA toroidal shape changed and the DNA presented an open S-like structure. Then the DNA started spreading between the compartments through a hole in the membrane. Three hours after exposure, the spread DNA started coalescing between each other [85]. It was suggested that this feature is involved in the radioresistance of *D. radiodurans*. It was also determined that the DNA from *D. radiodurans* contains high amounts of $Mn^{2+}$ [86]. The nucleoid morphologies of different bacterial species, either radioresistant or radiosensitive, has been determined. It was shown that the radioresistant species possessed a highly condensed genome while the DNA from the radiosensitive species was uniformly distributed in the cells [87]. The authors of this study suggested that no obvious relationship between the ring-like shape observed by Lebowitz et *al.* and the radioresistance property of bacteria exists. The condensation of the DNA, rather than its shape, may play a role in radioresistance.

Finally, it was also suggested that *D. radiodurans* DNA could be attached to the membrane. This peculiar structure could act as a support to ensure the correct restitution of the genome. Indeed, DNA from un-irradiated *D. radiodurans* cells was found to be associated with the membrane complex, while, after X-ray irradiation, the damaged DNA was dissociated from the membranes. After incubation in proper conditions, the DNA was re-associated to the membrane and reformed the DNA-membrane complex [88].

The shape of DNA only helps preventing DSBs while DNA systems have been implemented by the cell to repair the damaged DNA.

##### 1.4.1.3.2 Enzymatic pathways: DNA repair

Depending on the kingdom living organisms have developed different pathways to repair damaged DNA, *i.e.* eukaryotes or prokaryotes. Prokaryotes can possess the same chromosome in several copies in their cells, presenting the advantage of providing an intact copy for DSBs repair.

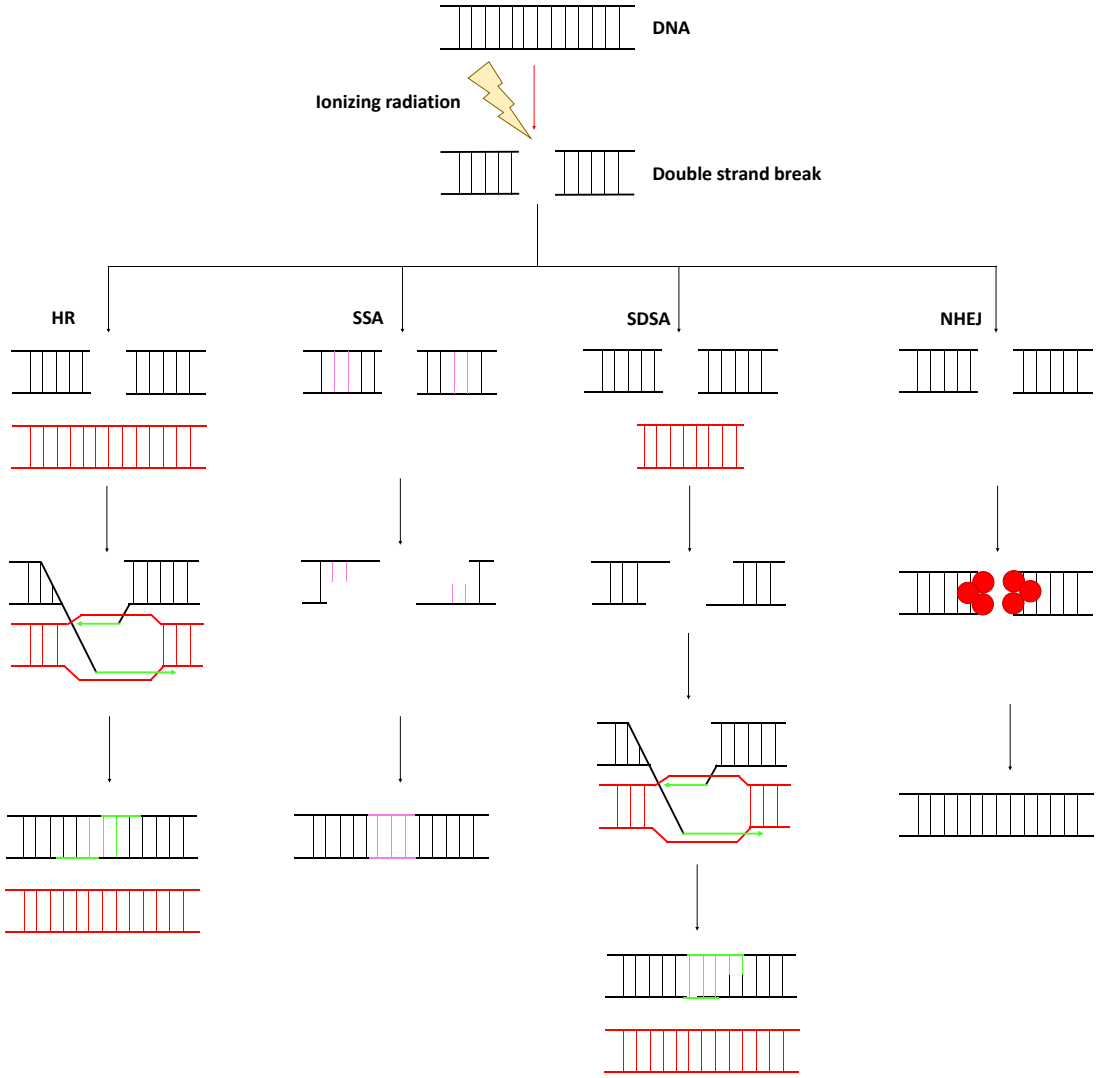



***Figure 1.6: The four main pathways used to repair double strand breaks in DNA (adapted from[83]).*** *Homologous Recombination (HR) is the main pathway in DNA double-strand break repair in bacteria. It uses an intact homologous DNA to restore the correct DNA sequence at damaged sites. Single-Strand Annealing (SSA) and Synthesis-Dependent Strand Annealing (SDSA) are the main DNA repair pathways found in D. radiodurans: the ends of the DSB are resected to produce a long 3'-ended single stranded DNA (ssDNA). Non-Homologous End-Joining (NHEJ) is the main pathway in DNA double-strand break repair in eukaryotes. This pathway is based on the binding of double-strand DNA carrying strand breaks nucleoids. Proteins ligates the end of the break together.*

As presented in Figure 1.6 four known pathways involved to repair DSBs in DNA are particularly common and used. These four pathways are the homologous recombination (HR), the single-strand annealing (SSA), the synthesis-dependent strand annealing (SDSA), and the non-homologous end-joining (NHEJ).

**Homologous recombination**

The main pathway in DNA double-strand break repair in bacteria and *Saccharomyces cerevisiae* is the homologous recombination (HR). It uses an intact homologous DNA to restore the correct DNA sequence at damaged sites (Fig.1.6). Two proteins are involved in this pathway: the RecA protein and the RecBCD enzyme [89]. The RecA protein is a recombinase and binds strongly to ssDNA in long clusters in order to form a nucleoprotein filament. It can hold a single strand and a double strand together, joining the DSB to the intact homologous DNA fragment [90, 91]. The basal level of RecA in *D. radiodurans* has been estimated to be around 11000 proteins per cell [92]. After a 15 kGy irradiation, the gene expression of *recA* is 8 fold

induced [93]. In *Deinococcus deserti* the levels of RecA were 4.5 fold induced 22 hours after a 3 kGy irradiation [94].

**Single-strand annealing and synthesis-dependent strand annealing**

These two DNA repair pathways are present in *D. radiodurans* [95]. Single-strand annealing (SSA) can occur when a DSB is made between two repeated sequences oriented in the same direction in the genome. The DNA repair through this pathway is schematized in Figure 1.6. In this pathway, the ends of the DSB are resected to produce a long 3'-ended single stranded DNA (ssDNA). The complementary strands can thus directly rejoin, through the annealing of complementary single-stranded [96]. The DNA overhangs are degraded so the ligation can occur. This process does not preserve the whole genome since the flanking regions of the break are excised.

Synthesis-dependent strand annealing (SDSA) is a variant of SSA. As in SSA, the ends of the DSB are resected to create a ssDNA. A protein then binds to the DNA and form a nucleoprotein filament. A heteroduplex DNA with a homologous DNA is formed, creating a D-loop. This overlap is followed by DNA synthesis beyond the original break site using the homologous DNA sequence as a template, which restores the missing sequence information at the break point unlike SSA [97].

**Non-homologous end-joining**

The non-homologous end-joining (NHEJ) is the main pathway in DNA double-strand break repair in eukaryotes. It has recently been identified and characterized in bacteria. This pathway is based on the binding of double-strand DNA carrying strand breaks nucleoids, as exposed in Figure 1.6 [83]. Indeed, after a DSB, several proteins can be involved in order to ligate the two ends of the break. Nucleases excise the ends to make them compatible. A ligase then joins both ends. This pathway can lead to alterations of the sequence at the break location since it does not need a homologous DNA template.

**Base excision repair and Nucleotide excision repair**

The base excision repair is a mechanism used to repair deaminated, alkylated and oxidized bases. It is highly conserved in all three kingdoms and is initiated by DNA glycolases. The base excision repair pathway can be used by organisms when DNA was damaged by oxidation, deamination and alkylation. The first step of this pathway is the removal of the damaged base with a DNA glycolase. The remaining sugar is then excised with an AP-endonuclease and a 5'-phosphodiesterase. A new nucleotide is then added to repair the damaged site with a DNA polymerase and the enzymatic reaction is over after a DNA ligase has ligated the new nucleotide to the DNA [98]. It has been found that *D. radiodurans* possess more DNA glycolases than most of the bacteria [99]. Some pathways, such as Nucleotide Excision Repair (NER) has been shown to have no incidence in radioresistance in *D. radiodurans* [100]. In the NER pathway the damaged nucleotide is detected by the UvrA protein which binds the UvrABC complex to the DNA. The damaged nucleotide and several surrounding nucleotides are uncoiled by the UvrAB complex before being incised by the UvrBC complex [101]. The incision complex is then removed by a DNA helicase. The gap created is filled by the action of DNA polymerase. In the final step a DNA ligase seals the newly completed repair DNA [102].

**Other pathways**

Other not often used DNA repair pathways exist in microorganisms. Some of them were recently discovered and are not very well described, such as micro-homology-mediated recombination. Other DNA repair pathway may exist but have not yet been discovered.

### 1.4.2 Radionuclides

Apart from ionizing radiation and aerobic respiration, other uncommon environments can lead to oxidative stresses such as metallic stress.

#### *1.4.2.1 Radionuclides description*

A radionuclide is an atom with an excess of nuclear energy due to its atomic mass number. This excess of energy makes it unstable: the atom can emit gamma radiation to release the energy, transfer it to one of its electron to release it as a conversion electron. The atom can create and emit a new particle, alpha or beta, from the nucleus. These processes are called radioactive decay and are considered as ionizing radiation. This decay can produce either a stable atom or another unstable atom. If an unstable atom is created, the radioactive decay continues until it reaches a stable nuclide. The duration of the decay is estimated through the half-life, *i.e.* the time it takes to decompose half of the atoms. The shorter the half-time is, the strongest the radioactivity. For example, $^{24}$Na has a half-time of 15 hours and is highly radioactive and dangerous, while $^{238}$U has an estimated half-life of 4.5 billion years. This uranium isotope is thus less dangerous in terms of radioactivity than this sodium isotope. If naturally only time can stabilize radionuclides, a new method is being developed to faster stabilize radionuclides. This method is called transmutation. Its principle relies on the transformation of long half-life radionuclides (up to hundreds of thousand years) in stable elements or with shorter half-life. To do so a neutron must be absorbed by the radionuclide's nucleus. According to SCK●CEN and CEA transmutation is however still in development and cannot nowadays be used industrially. Finally, not all radionuclides are targeted with this technic [103].

##### 1.4.2.1.1 Natural radionuclides

Radionuclides naturally occur on Earth and can be classified in three categories. Natural radionuclides can have extraterrestrial origins as well as an Earth's crust origin. The first category, the primordial radionuclides, are nuclides that existed in their current form at least since the Earth's formation, meaning that their half-lives are long enough to still be present today. To be considered as a primordial radionuclide, the half-life must be greater than 100 million year. $^{235}$U and $^{238}$U are primordial radionuclides [104].

The second category, called secondary radionuclides, are isotopes derived from the decay of primordial radionuclides. For example, $^{222}$Rn and $^{210}$Po are decay products: the radon 222 is naturally formed during $^{238}$U decay chain while the polonium 210 is formed during $^{222}$Rn decay chain. $^{210}$Po decays in stable lead ($^{206}$Pb). The last category contains the cosmogenic isotopes, such as $^{14}$C or $^{24}$Na for example. They are created when a high-energy cosmic ray interacts with the nucleus of an atom. Cosmogenic radionuclides are formed on Earth with the interaction of the cosmic ray and with an atom present in the atmosphere or on the ground, such as soil or rocks. They can also be produced in extraterrestrial materials, such as asteroids. Some locations on Earth are more radioactive than others due to the presence of natural radionuclides in the soils, such as the states of Minas Gerais and Goias as well as the state Espírito Santo in Brazil near the Atlantic Ocean [105]. In the Kerala state, India, high levels of $^{238}$U, $^{232}$Th and $^{40}$K can naturally be found [106].

#### 1.4.2.1.2 Man-made radionuclides

Currently humans use nuclear fission to produce energy and more specifically electricity. During this process, radionuclides are formed and energy is liberated. These radionuclides can be by-products of nuclear fission. For research purpose or medicine some radionuclides are produced to be commercialized.

Several diagnostic methods depend on radioactivity. The combination of radioactive tracers with imaging devices allows the doctors to study flows taking place in the body. A molecule, chosen to target the observed organ, is tagged with a short-life radionuclide (mainly $^{99m}$Tc). Aside from diagnosis and imaging radionuclides can also be used in radiotherapy. This treatment is used for some cancers, such as bone cancer or thyroid cancer, $^{131}$I being used for the latter. Several radionuclides linked to molecules, such as antibodies, are currently under clinical trial to treat cancers [107]. For brain tumors a knife containing $^{60}$Co can be used as an external radiation source on the cancerous cells to destroy them [108].

In a nuclear reactor, the fuels (most commonly $^{235}$U and $^{239}$Pu) are activated by a neutron to start a nuclear chain reaction. For uranium the neutron collides with an atom of $^{235}$U which will then be activated and will decompose itself in neutrons and fission products. The fission products can also be unstable while the neutrons will activate other atoms of uranium. In a nuclear reactor, the chain reaction is controlled, meaning that neutrons absorbers are installed to absorb the majority of the neutrons. It will thus not activate too much uranium atoms at the same time and exponentially increase the released energy (Fig. 1.7). To be able to protect the workers of nuclear installations, several measures exist. First of all, the fuels are stocked in a pool during a working cycle, the water acting as a shield against the radiation, and the heat emitted by the chain reaction and the decay of radionuclides. Once the fuel is too old to produce enough energy, at the end of a cycle, it is removed and placed in a spent nuclear fuel pool (SNFP) to allow the decay of radionuclides so that no ionizing radiation are emitted. When a sodium-potassium alloy is used as a coolant in nuclear reactors, $^{24}$Na is created, meaning that the coolant is itself radioactive. However, since its half-life is around 15 hours, when the reactor is in shut down, no radioactivity in the coolant can be detected after a few days. Cobalt 60 is naturally produced in nuclear facilities and can be found in nuclear effluents. It is a neutronic activation product from materials from nuclear reactors.

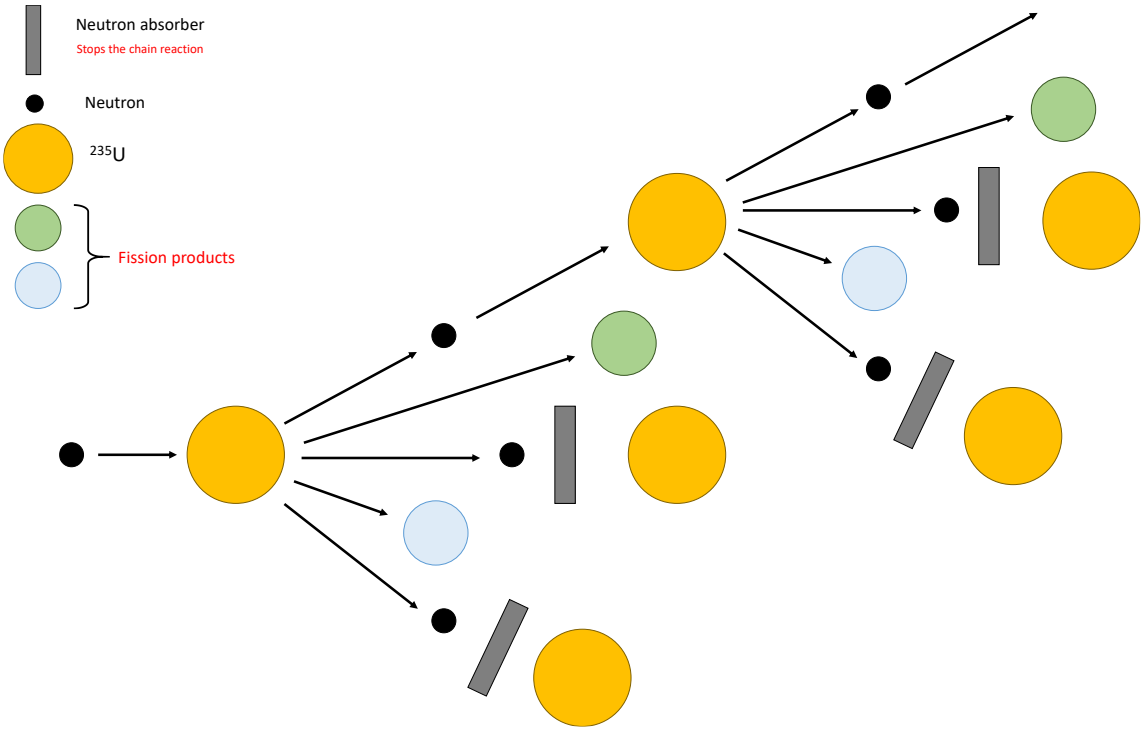



*Figure 1.7: Nuclear chain reaction occurring in a nuclear reactor*

In France, there are currently 58 nuclear reactors, distributed in 19 facilities, to produce electricity. Moreover 8 nuclear reactors used for research are also present in France, 7 of them are present on a CEA center. All these installations produce radioactive wastes that are treated accordingly to their nature. In France, the radioactive wastes can be classified and treated accordingly to their half-life duration and their activity [109-111]. Short-life wastes contain all radionuclides with a half-life smaller than 31 years while those present in long-life wastes have a half-life greater than 31 years (cf. Appendix 1).

Several new treatments are being studied, such as chemical and physical treatments (*e.g.* the separation-transmutation process) or the use of biotechnology to concentrate radioactivity (*e.g.* the use of *Coccomyxa actinabiotis* [112], or the identification of organisms able to decontaminate radionuclides [113-115]). It is interesting to notice that, in France, electricity production generates 62% of the wastes, while the 28% left are produced by research, medicine or the army.

#### 1.4.2.1.3 Radionuclides release in nature

Human activities may lead to environmental release of radionuclides, either because of nuclear waste treatment, mining or because of the use of fertilizers. Indeed uranium has a high affinity with phosphate, added in the soils as fertilizers. From 1972 to 2013 the addition of phosphate as fertilizers went from 31 to 8 kg/ha of agricultural soils. Naturally, there is only 0.1 to 3.7 kg/ha of phosphate in agricultural soils (Ministère de la Transition Ecologique et Solidaire). The uranium concentration in commonly used Brazilian fertilizers is between 5.17 and 54.3 mg of U/kg [116]. This value can reach 700 mg of U/kg of fertilizer for some fertilizers (personal manufacturer's information). In Europe, the mean value of uranium in soil is around 2 mg of U/kg of soil (IRSN) [117]. Mining activities can also be a source of radionuclide

contamination. Depending on the mining process some uranium can leaked in the environment. For example acid mine drainage is formed by oxidation of metal sulfides present in the ore by acidophiles, such as *Leptospirillum* [12, 118]. Acidic mine water may contain heavy metals and radionuclides, such as uranium [119]. Finally, another source of massive release of radionuclides is nuclear accidents and nuclear weapons assays. After the Chernobyl and Fukushima accidents, the area around the facilities were forbidden because of the large amount of radioactivity and radionuclides present in the soils and waters [120]. For Chernobyl this area measures 30 km radius from the power plant while the one implemented in Fukushima measures 20 km. The radionuclide pollution occurring after a nuclear accident can travel through hundreds to thousands of kilometers (Fig. 1.8). After the Chernobyl accident, radionuclides, mainly $^{137}Cs$, arrived in France and are still present 30 years later in some area, such as in Vercors, in the French Alps [121].

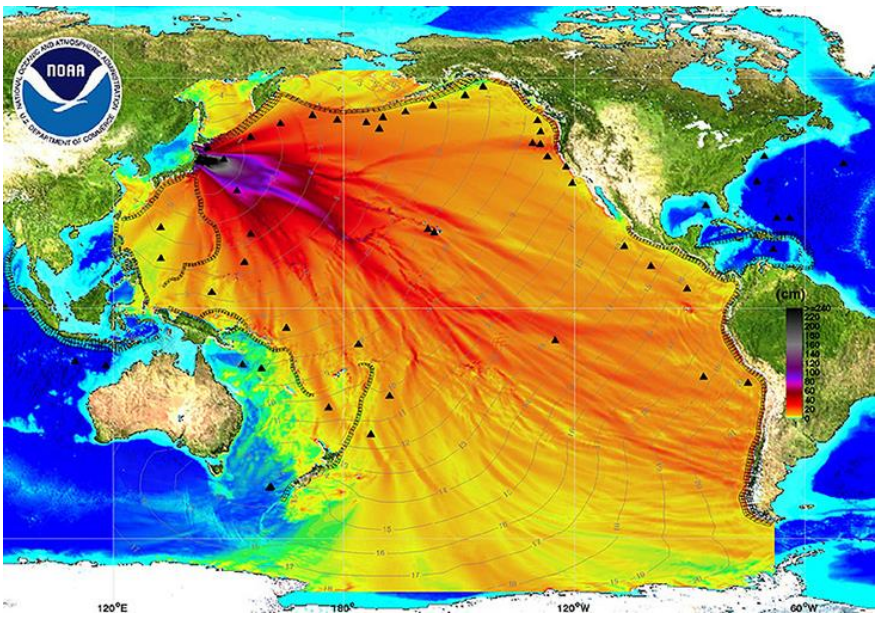



*Figure 1.8: Nuclear fallout after the Fukushima nuclear accident (NOAA)*

### *1.4.2.2 Radionuclide resistance in microorganisms*

All these radionuclide contaminations can affect life and be lethal. However, some organisms are more resistant to radionuclides than others. Even though several organisms can resist and accumulate radionuclides, such as plants [122-125], this paragraph will only treat the resistance of microorganisms.

Radionuclides, beside the negative effects due to radiation emission, can also be toxic to the cell because of their chemical form as most of them are metal isotopes. At high concentration metals are toxic to organisms. Some metals are essential for organisms and can be enzymatic co-factors, such as cobalt, copper or zinc. However at levels higher than needed by the cells these metals can be toxic for the organism. To regulate the levels of the essential metals, membrane transports are used [126]. It was shown that metal resistance is often associated with antibiotic resistance [127, 128], and metal resistance is often carried by a plasmid [129]. The resistance to essential metal is usually carried by the chromosome while the resistance to non-essential metal is often present on plasmids [130]. Nonessential metals have a greater affinity to thiol-containing groups and oxygen sites than essential metals. The resulting alterations in the conformational structure of nucleic acids and proteins are toxic for the cells. The osmotic balance is also changed, leading to potential lethal effects [126].

Microorganisms which can resist radionuclides have developed several ways of survival. One of them is linked to the oxidative stress produced by the radionuclides, for which several pathways exist to counter ROS lethal effects, mainly the antioxidant mechanisms such as glutathione or SODs (cf.1.4.1.1). It has also been noticed that the interactions between bacteria and radionuclides are similar to the ones with heavy metals [131].

Some microorganisms can be resistant to radionuclides by preventing them to enter the intracellular environment. The permeability of the cell membrane can decrease due to a gene mutation preventing the production of a porin in *Eschericha coli* for example [132, 133]. Some bacteria can naturally produce an exopolysaccharide coating which can possess attachment sites for metal cations but also bioabsorb metal ions and prevent them from interacting with the cell [126, 134, 135]. Some *Klebsiella aerogenes* strains exhibited a resistance twice greater to cadmium when the exopolysaccharide was present [135]. Radionuclides can also be sorbed to the cell membrane component. The cell does not need to be alive to bioadsorb but there is a quick saturation as well as desorption due to cation present in the medium and environment [131].

Some bacteria can bioaccumulate radionuclides. The active accumulation requires energy and can be dependent or independent from the metabolism. These accumulations can occur because of the presence of a transporter or if the radionuclide has an effect on the permeability of the membrane [131]. Metals that are non-essential for the cell usually borrow normal nutrient transport systems to penetrate in the cell but are rapidly exported [126]. Once the metal has entered the cell several mechanisms can be implemented. First of all, the cell can use transporters to export the nuclide back to the extracellular environment. For example, in the *Microbacterium* A9 strain uranium was found to be adsorbed by carboxyl and phosphoryl groups present on the cell membrane [136]. In the same strain, the presence of a metabolism-dependent transporter has also been described but was not identified. It is however known that ATPase dependent transporters can be used by bacteria to export metallic anions and cations into the extracellular environment [137, 138]. In the last two mechanisms, the cell accumulates the nuclide before exporting the metal back into the extracellular environment. Bacteria exporting the nuclide back to the extracellular environment cannot be used for a bioaccumulation process. Secondly, the cell can also perform an intracellular sequestration of the atom through protein bindings (*e.g.* metallothionein, GSH, cysteine-rich protein) to prevent the exposure of essential cellular components once the metal has entered the cell. Some bacteria also possess enzymes to partially detoxify the nuclide by modifying its chemical form. Some enzymes can indeed reduce the nuclide [126].The reduction of uranium or cobalt is implemented by different enzymes. As an example, the cytochrome $c_3$ in *Desulfovibrio vulgaris* was proved to reduce uranium [139]. It has been shown that some iron-reducing bacteria can reduce cobalt, such as *Shewanella alga* or *Geobacter sulfurreducens*. Some bacteria, such as *Shewanella oneidensis*, are able to preserve energy for anaerobic growth via the reduction of uranium [140]. The number of proteins involved in the phosphate metabolism of the *Microbacterium* A9 strain was increased in the presence of uranium [141].

#### *1.4.2.3 Decontamination of radionuclides*

Today's treatments to decontaminate radionuclides from the environment are expensive and tedious. To decontaminate soils, different methods exist and can be categorized in three classes: outside of the site, on the site and *in situ*. For the first two classes the soil needs to be excavated before treatment. The soil can be treated differently but all methods are either expensive or harmful for the environment [142].

Phytoremediation can also be used. However, while it is less expensive and less damaging to the environment than excavation and soil washing, its efficiency was shown to be much lower (a maximum of 24% for phytoremediation in low contaminated soils when soil excavation presents a 100% efficiency) [143]. Moreover phytoremediation for a contaminated site has been estimated to take 5 to 20 years meaning that the land will be unusable during this period [144]. More recently the use of metal resistant bacteria able to produce biosurfactants have been studied to increase phytoremediation efficiency [145]. Biosurfactants can form complexes with metals and increase their bioavailability for plants.

Several microorganisms have been studied for their ability to bioreduce, bioaccumulate and/or biosorb radionuclides. Among them, bacteria, algae and fungi have been identified [113, 146-152]. The advantages of radionuclides accumulating microorganisms can be numerous. They can in fact concentrate the pollution inside their cells and thus reduce the volume of wastes to store. Microorganisms able to change the toxicity and solubility of radionuclides can be used in the environment, either to immobilize radionuclides or to accelerate their removal [115]. Several studies have been performed *in situ* with the natural microbiota present in contaminated soils. It was noticed that the addition of a surfactant was necessary to maintain uranium concentration at low levels in groundwater (under 0.1 µM) [153-155]. It was also noticed that the main genus found in the tested environments was *Geobacter* [156]. Moreover the implementation of a biodecontamination process for contaminated environments, such as nuclear effluents, is possible and has already been studied at laboratory scale and plant scale for the micro-algae *Coccomyxa actinabiotis* [112]. Such process should be highly efficient on large volume of waste with low concentration of radionuclides. It should also be cheaper than the existing processes and more "eco-friendly" since it does not need any chemicals. Furthermore, the use of a green technology for the nuclear industry could be well accepted by the public opinion. Microorganisms that have shown to possess the greater accumulation of a specific radionuclide can be used in biotechnologies to decontaminate nuclear effluent and be used in bioremediation. Even though several microorganisms have been identified as potential candidate for bioremediation process, large-scale experiments for industrial use have yet to be implemented.

## 1.5 Radioresistant organisms

The ability of life to counter any threat, whether it is biological or environmental, has led to organisms resistant to different stresses. Since 1956, it is known that organisms can be highly radioresistant with the discovery of *Deinococcus radiodurans* by Anderson et *al*. [14]. Several radioresistant organisms have been discovered in natural environments and in man-made radioactive environments, such as nuclear facilities.

### 1.5.1 Radioresistant organisms found in natural environments

The ability of some bacteria to be radioresistant has been well investigated using *D. radiodurans* as a biological model. It has been observed that the capacity to withstand high levels of radiation is not part of an evolutionary mechanism due to the presence of high radiation but is the result of a secondary consequence associated to desiccation and oxidation resistance [157, 158]. There is indeed naturally no such high level of radioactivity in the world as the one found in nuclear facilities (50 mGy/year nonetheless can be observed in the south-west of India and in a region of Brazil, while radioactivity found in the rest of the world is around 2.4 and 3.6 mGy/year). There are however regions in the world with very low bioavailable water level and high UV radiation which can cause oxidation, such as the Sahara and Atacama

deserts, Antarctica, Arctic and other locations. It is in those natural environments that radioresistant microorganisms have mainly been found.

In order to survive these harsh conditions microorganisms have developed ways to counter the lethal effects of desiccation and oxidation. Some microorganisms present in dry and bright environments can yet resist to stresses different from desiccation and oxidation. They can in fact exhibit radioresistance properties thanks to a complex metabolism developed in order to survive the oxidative stress [157, 159]. Radioresistance characteristics have been found in all three kingdoms, including multicellular organisms. However, most radioresistant organisms are microorganisms, mainly bacteria but also some fungi, archaea, and algae. Some radioresistant organisms from different kingdoms are described below.

#### *1.5.1.1 Tardigrade*

*Figure 1.9: Tardigrade (SEM)*

One exceptional family of animals that belongs to the Tardigrada phylum, based on their morphology and genome, can resist several extreme conditions. Tardigrades (Fig. 1.9) were discovered in 1773 and can be found in very diverse environments all over the world. They are small invertebrates (up to 2.1 mm in length). Currently, circa 1200 species are known. These animals are unusual for several reasons. They can be found in various environments, from the bottom of the oceans to the top of a mountain, in freshwater, marine and terrestrial environments. They can resist extreme temperatures (from -272.8 to 150°C) and pressures (from 200 hPa to 7500 MPa; the atmospheric pressure being around 1023 hPa). The terrestrial species need a thin film of water around them to stay active. They can enter into a cryoptobiotic state which allows them to resist anaerobic environments, cold temperature, excess salinity and desiccation. During the cryoptobiotic state, their metabolism decreases drastically to very low level and can occur several times in every stage of their life, from youth to adult form. Their ability to resist such harsh conditions makes them interesting model to study the effect of space on the organisms [160]. Tardigrades exhibits resistance to high doses of radiation. Depending on the species, this resistance can change. The survival of *Milnesium tardigradum* after $\gamma$-irradiation was studied, at short- and long-term. The $LD_{50}$ 2 hours after irradiation was around 5.5 kGy and dropped to 5 kGy 48 hours after exposition. This species could only laid eggs under a 2 kGy irradiation, while *Richtersius coronifer* could laid eggs after a 5 kGy irradiation. However, the eggs produced after a 2k Gy irradiation did not hatch [161]. The genome of two tardigrade species, *Hypsibius dujardini* and *Ramazzottius varieornatus*, have recently been sequenced [162]. The authors observed the loss of genes involved the ROS producing pathways. Furthermore, they found several genes involved in ROS defenses, such as genes coding for SODs, genes coding for proteins involved in desiccation tolerance, or trehalose, a sugar known to protect cells against desiccation. Trehalose was only found in *R. varieornatus*. In the genome of *R. varieornatus*, genes encoding proteins considered to protect DNA have been identified, such as the Damage suppressor protein (Dsup) which is believed to prevent DSBs [163, 164]. A gene coding for an homologous protein was not identified in *H. dujardini* genome [162].

Tardigrades are thus animals able to resist extreme conditions. However, amongst prokaryotes, those abilities are not so uncommon.

### 1.5.1.2 *Deinococcus* species

All discovered bacteria belonging to the *Deinococcus* genus are radioresistant [165]. The most famous one, *Deinococcus radiodurans* (Fig. 1.10) was the first radioresistant organism discovered so its ability to survive radiation has been extensively studied and several reviews have been published [39, 47, 99, 157, 159, 166, 167]. Most of the mechanisms found to be involved in radioresistance were identified using *D. radiodurans*, such as high levels of $Mn^{2+}$ or several *recA* gene copies for example. The ability to survive radiation can be measured with the $D_{10}$, indicating the dose needed to eliminate 90% of the living population.

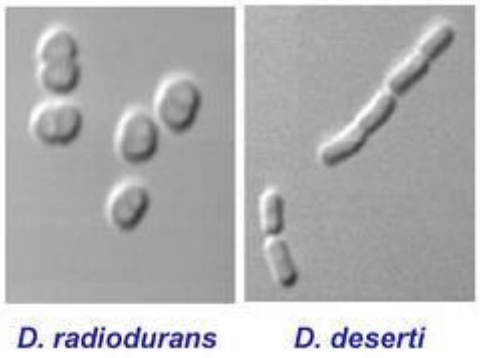



*Figure 1.10: Deinococcus radiodurans and Deinococcus deserti under a microscope (CEA - Genoscope)*

Another bacterium, belonging to the *Deinococcus* genus, has been recently discovered. This bacterium, *Deinococcus deserti* (Fig. 1.10), was isolated from Sahara sand samples by CEA scientists [168]. It was isolated after the sand sample irradiation at 15 kGy with γ-ray and deeply analyzed by genomics, transcriptomics and shotgun proteomics. It has been shown that *D. deserti* is able to survive desiccation for 40 days with a living rate of 50%. Its ability to survive desiccation is related to the DNA repair found in *D. radiodurans*. It was determined that *D. deserti* possesses three different *recA* genes, highlighting the need for various versions of the RecA protein involved in DNA repair. The genome of *D. deserti* also exhibited a large variety of genes involved in DNA repair [169]. In *Deinococcus deserti* the levels of RecA were 4.5 fold induced 22 hours after a 3 kGy irradiation while the levels of DNA gyrase were induced 2.9 fold 22hours after the irradiation. Other proteins involved in SSA and ESDSA DNA repair pathways were also up-regulated after the 3 kGy irradiation of *D. deserti*, such as the DdrB protein induced 11.2 fold 6 hours after the irradiation [94].

Nine *Deinococcus* species were isolated after the irradiation of soil samples from the Sonoran Desert (0 to 30 kGy) and were proved to resist a 10 kGy irradiation. *D. hohokamensis*, *D. pimensis*, and *D. yavapaiensis* were isolated after a 30 kGy irradiation of the sample [170].

### 1.5.1.3 *Chroococcidiopsis* species

Another genus, belonging to the *Cyanobacteria* phylum, possesses radioresistant species. Several species from this genus have been isolated from the Negev desert in Israel. The *Chroococcidiopsis* genus (Fig. 1.11) was studied for its ability to survive oxidative stress and its potential ability to survive in space and under Martian conditions [171, 172].

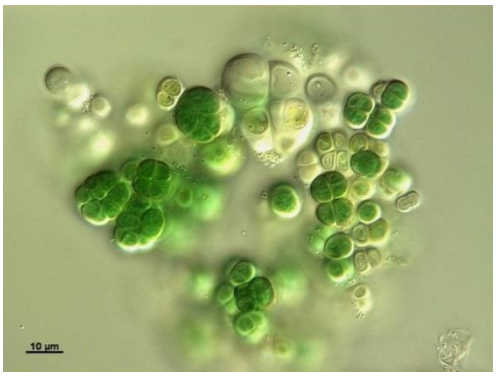



*Figure 1.11: Chroococcidiopsis sp. under a microscope (Universität Rostock)*

Several members of the *Chroococcidiopsis* genus were irradiated at several doses. Depending on the strains, the survival to X-ray at an exposure of 2.5 kGy was between 35 to 80%. All the tested strains survived after a 15 kGy exposure while no survival has been detected at 20 kGy [173].

#### 1.5.1.4 Thermococcus gammatolerans

An archaeon, *Thermococcus gammatolerans*, was isolated from samples of an active chimney recovered from a hydrothermal site in Guaymas Basin after a 30 kGy irradiation [13]. This strain presented an unaffected growth after a 3 kGy irradiation. *T. gammatolerans* exhibited an enhanced number of oxidized nucleosides after a 5 kGy irradiation. However the number of oxidized nucleosides decreased one hour after the irradiation, indicating the presence of an efficient DNA repair system. Two putative enzymes involved in BER were identified by next-generation proteomics and transcriptomics. These two enzymes were present before the irradiation and their transcription was up-regulated after a 5 kGy irradiation (2 to 3 fold)[174]. The ability of *T. gammatolerans* to resist radiation was proved to be growth phase-independent, unlike *D. radiodurans* [175].

A brief comparison of the gamma-radiation resistance of some microorganisms found in nature and in spent nuclear fuel pools can be found in Table 1.2. *Escherichia coli* is present as a radiosensitive strain.

***Table 1.2: $D_{10}$ or $LD_{50}$ in kGy of different microorganisms.*** *E. coli is not considered as radioresistant. $D_{10}$: indicates the dose needed to eliminate 90% of the population ([47, 159, 176-179])*

| **Microorganisms** | **Radioresistance ($D_{10}$) (kGy)** |
|---|---|
| *Escherichia coli* | 0.1 to 0.2 |
| *Aspergillus fumigatus* | 0.63 |
| *Methylobacterium radiotolerans* | 1 |
| *Micrococcus luteus* | 1 |
| *Chroococcidiopsis* spp. | 4 |
| *Deinococcus deserti* | >7.5 |
| *Deinococcus radiodurans* | 10 |
| *Coccomyxa actinabiotis* | $LD_{50}$ : 10 |
| *Thermococcus gammatolerans* | 30 |

### 1.5.2 Radioresistant microorganisms found in man-made environments

Several organisms exhibit radioresistance properties due to their ability to survive extreme conditions, such as desiccation and high light intensity. However some organisms have been isolated from environments presenting high radiation levels that are man-made, presenting thus radiation levels never found elsewhere on Earth. The organisms living there have been selected for their ability to survive radiation and evolved accordingly.

To avoid overheating and protect staff, nuclear fuels are submerged in water. The water present in this pool is a deionized water. Due to the presence of high levels of radiation, high quantity of dissolved radionuclides and the lack of nutrients, people have thought for a long time that no life could be found in such environment. Different microorganisms were found living in the cooling water of nuclear reactors. Their presence was made possible by their ability to form biofilms and their resistance to ionizing stress. Different microorganisms, such as bacteria, fungi and a micro-alga, have already been found in several Nuclear Power Plants (NPPs) and Spent Nuclear Fuel Pools (SNFPs) around the world [176, 180, 181]. The first report of life present in these environments was in 1997, at the Savannah River Site, a nuclear facility in the USA [182].

Furthermore, Microbial Influenced Corrosion (MIC) has been previously described from a water sample of a SNFP. The water was sampled aseptically and enriched with liquid medium in the presence of metal coupons. After several months of incubation, the coupons were recovered and the biofilm removed. Corrosion was observed on the metal due to MIC. Figure 1.12 highlights the presence of corrosion with gaps measuring less than 2 µm with a depth around 100 nm. This experiment highlighted the presence of microorganisms in the water of this nuclear pool and their ability to do MIC [183].

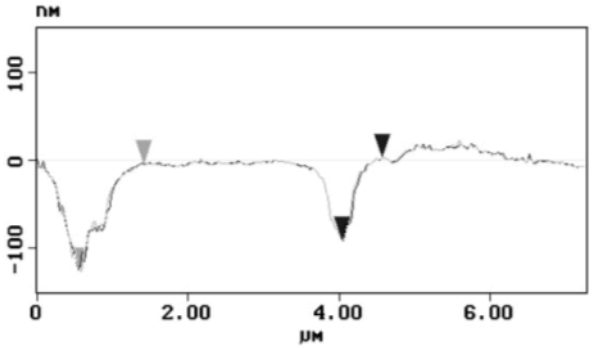



***Figure 1.12: Section analysis of a steel coupon after removal of a biofilm (adapted from [183]).*** *The curve represents the surface of a metal coupon after removal of a biofilm and observation via an Atomic Force Microscope. Corrosion are indicated with arrows. Two gaps can be observed, measuring less than 2 µm with a depth around 100 nm.*

In different countries, including Hungary and in particular in Spain, studies of the microorganisms present in the water of nuclear facilities have been performed and several bacteria have been isolated through biofilm formation. In the SNFP of Cofrentes in Spain, 56 different bacteria species have been reported to be present. Researchers recovered biofilms from the pool and cultivated them. Biofilms can absorb radionuclides and hence become highly radioactive: it should thus be handle with caution [180, 184]. Among the bacteria isolated from the SNFP of Cofrentes, a majority of *Proteobacteria* were identified (cf. Appendix 2). A fungi identified as *Aspergillus fumigatus* was also identified in this pool [181]. Bacteria identified as *Kocuria palustris*, *Micrococcus luteus*, and an *Ochrobactrum* sp., isolated from an interim spent fuel storage pool, were able to accumulate cesium and cobalt [152]. The microorganisms that have already

been found in SNFPs and NPPs are bacteria, a few fungi and one micro-algae living in biofilms. They are listed in Appendix 2. The isolated microorganisms from SNFPs were mainly Gram negative bacteria belonging to the *Proteobacteria* phylum. Among the few Gram positive bacteria identified, the majority belonged to the *Bacillus* genus (*Firmicutes* phylum). Some bacteria were found to belong to the *Actinobacteria* phylum. In these studies there were no indication whether the Gram positive bacteria were present under a vegetative or a spore form in the water [152, 177, 181, 184-192]. More recently, the use of a direct identification approach consisting on sequencing the amplicon of the 16S rDNA of the microbiota was used (cf. 1.6). Different bacterial phyla were identified in a nuclear storage basin, including *Acidobacteria*, *Actinobacteria*, *Planctomycetes*, *Proteobacteria*, *Nitrospirae* and *Deinoccous-Thermus* [193].

Aside from bacteria, several eukaryotes have been found in SNFPs. For example, a micro-algae has been found in a spent nuclear fuel in France. This organism, called *Coccomyxa actinabiotis* (Fig. 1.13), has been proved to withstand radiation of 20 kGy [176], capacity only found hitherto in prokaryotes, in particular *D. radiodurans*. It was also observed that it could accumulate silver, carbon-14, cobalt, cesium, and uranium [112, 176, 194, 195]. A process using this algae was developed at a laboratory scale and displayed encouraging results regarding the use of biotechnology to decontaminate nuclear effluent [112]. *C. actinabiotis* indeed exhibited radioactive silver decontamination abilities similar to the ion-exchange resins currently used in nuclear facilities [176].

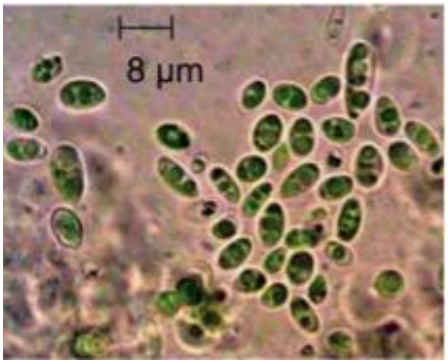



*Figure 1.13: Coccomyxa actinabiotis observed using a microscope (x40) (C. Rivasseau – CEA)*

Several fungi have been isolated near the damaged reactor of Chernobyl [52] and presented what the authors called “radiotropism” [196]. The irradiated fungi grew towards the direction of the radioactive sources and it was the first and only time such phenomenon was observed. The fungi *Cladosporium sphaerospermum* and *Penicillium hirsutum* were the main fungi identified in the walls of the damaged nuclear reactor of Chernobyl [52]. It was found that the irradiation of fungal spores led to a slower growth and a decrease metabolism [197].

In order to achieve a complete picture of the different microorganisms present in those highly unfavorable environments, direct identification methods should be used, such as metagenomics, already used for Tara expedition for example [198] and metaproteomics.

## 1.6 Microorganisms analysis in samples

### 1.6.1 After a cultivation step – morphological and biochemical identification

Before the apparition of sequencing, microbiologists used different tests to identify and classify the microorganisms. These methods needed the cultivation and isolation of organisms to obtain pure culture. First of all, the morphology of the cell and the colony was determined, as well as a gram coloration was also performed. Secondly, the biochemical profile of the bacterium was determined using several tests. These tests are still being used to describe a new microbial species.

### 1.6.2 Genetics rises and metagenomics

When organisms are closely related, they share most of their DNA sequence. It is largely accepted that a similarity of 98.65% or more in the 16S rDNA sequence indicates that the organisms belong to the same species [199, 200]. This number drops to an identity between 70 (in 1987) to 95-96% for the entire genome depending on the threshold used [199, 201]. However, the gray area around the limits of the taxonomic DNA identity caused some issues concerning some species. Indeed, a same species could have had, or still have, different names and be categorized between two different species, particularly when sequencing started to be used and the error rate was quite high [202, 203].

#### *1.6.2.1 Ribosomal DNA sequencing*

The sequencing of the 16S rDNA (prokaryotes) and 18S rDNA or Internal Transcribed Spacer (ITS), a gene located between the small- and the large-subunits of the 18S rDNA (eukaryotes), can be performed to identify microorganisms. This method allows the identification of an organism at a deep taxonomical level.

16S and 18S rRNA and ITS genes are used to determine the taxonomy of an organism [204, 205]. This DNA region is similarly organized between eukaryotes and prokaryotes, as seen in Figure 1.14.

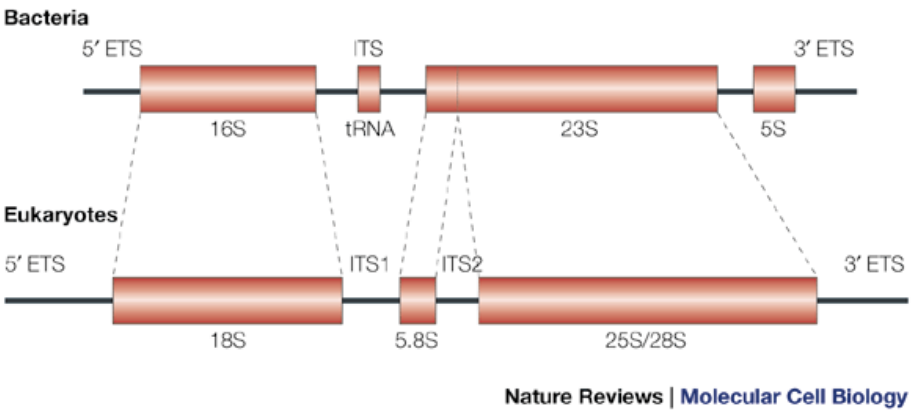



*Figure 1.14: Comparison between the ribosomal genes in bacteria and eukaryotes [206]*

The advantages of these genes are their conserved and hypervariable regions. The conserved regions allow the design of primer used to amplify and sequence this gene. The hypervariable regions, though conserved throughout the evolution between closely related members, allow the identification of the organism. Figure 1.15 presents the rRNA 16S gene of *Escherichia coli* with the nine variable regions (in orange) and the

conserved regions (in white). For the 16S rDNA, a very used universal primer pair is the 27F/1492R (5'-AGAGTTTGATCMTGGCTCAG-3' and 5'-TACGGYTACCTTGTTACGACTT-3' respectively) providing a fragment of 1465 pb [207, 208]. This pair amplifies all nine variable regions of the 16S rDNA.

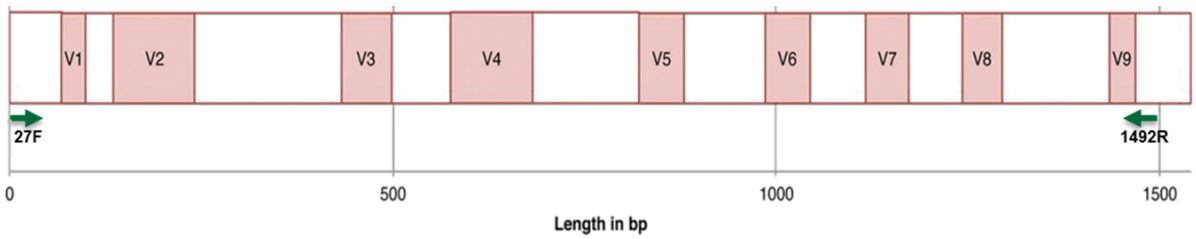



*Figure 1.15: 16S rRNA gene of Escherichia coli showing the nine variable regions and the position of the 27F/1492R primers (adapted from [209])*

The organization of this gene is similar between all bacteria and its architecture varies slightly between bacteria and archaea. However even though the sequence is smaller in archaea than in bacteria it still possesses nine variable regions that permit the identification of the microorganism (Fig. 1.16).

The variable regions can also be seen when the 16S rDNA sequences of several bacteria and archaea are compared between each other and plot into one figure (Fig. 1.16). It indeed highlights the variable regions (in red) and the conserved ones (in blue). The score of each nucleotide position was determined and, according to the obtained similarity of the nucleotide present in one specific position for all the tested sequences, a color was attributed. The redder the more different the nucleotide present in the specific position between sequences is. According to Figure 1.16, and for the bacteria tested, some regions are more different within another than the rest. Indeed V1, V2, V3 and V6 appear highly different, followed by V4, allowing thus to have a deeper identification of the organism.

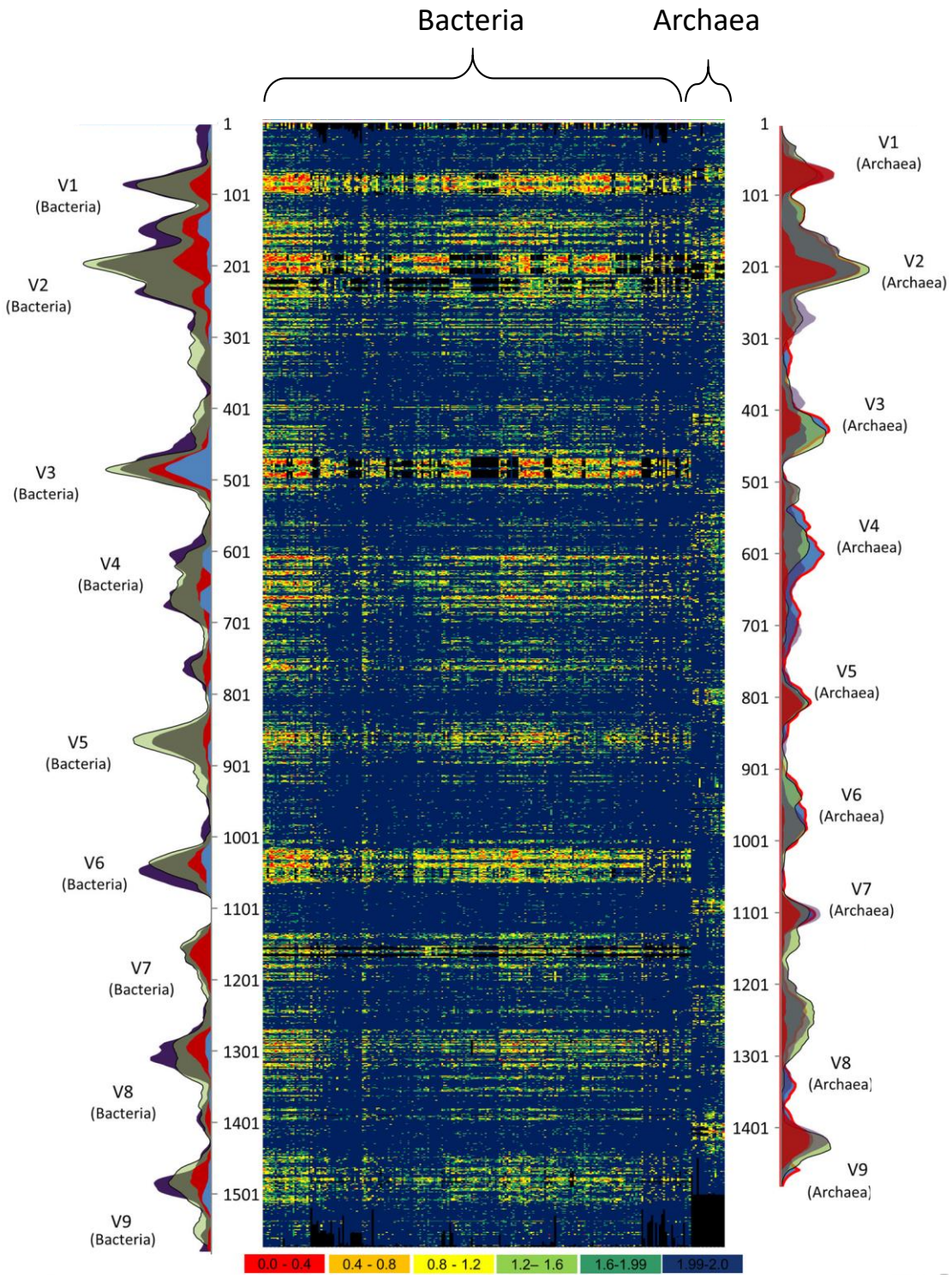



*Figure 1.16: Comparison of the 16S rDNA of different bacteria and archaea (red: different nucleotides; blue: same nucleotide) (adapted from [210])*

The difference of variability between the regions can be explained by the organization of the 16S rRNA gene (Fig. 1.17). The variable regions are in fact more accessible to mutations or transcription errors because the heap in these areas are less important than in the conserved regions.

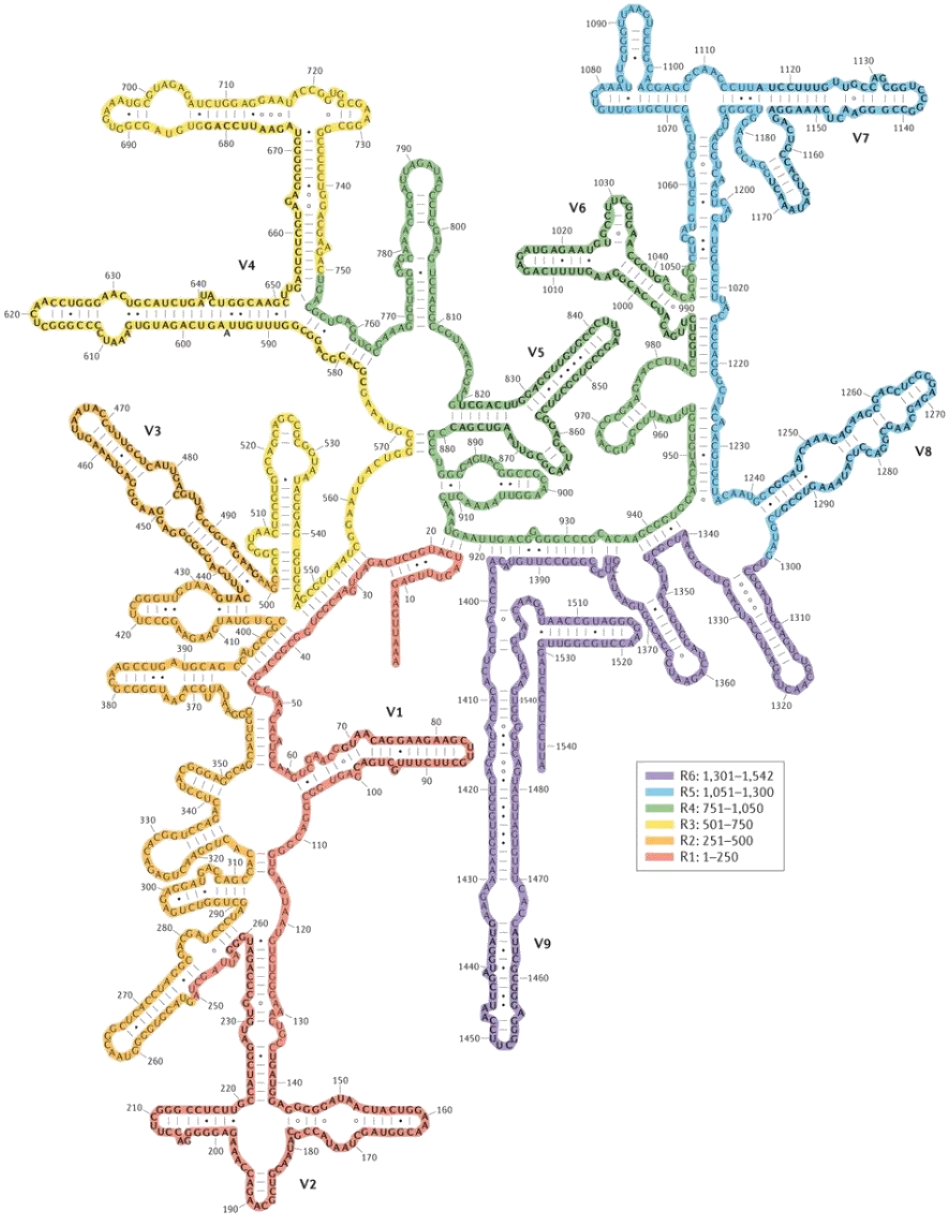



***Figure 1.17: Organization of the bacterial 16S rDNA [200].*** *The variable regions present a smaller steric hindrance, making them more sensitive to mutations.*

Because most of the microorganisms cannot be observed after culture, scientists have only recently become aware of their presence through shotgun sequencing of conserved marker genes like 16S or 18S rRNA for prokaryotes and eukaryotes respectively. The use of sequencing, particularly the metagenomic sequencing of one specific region of DNA, enables the discovery of new organisms. The metagenomic analysis is therefore a powerful tool to reference and inventory all bacterial species in water and other environments.

In the last 10 years the most commonly used next generation sequencing platform for amplicon sequencing has been the Roche 454. Many bioinformatics pipelines and denoising algorithms have been developed for Roche 454 but also for other sequencing technologies, making metagenomics a robust approach to investigate diversity.

### *1.6.2.2 Metagenetics*

#### 1.6.2.2.1 Metagenetics principle

To analyze the population of a microbiota and identify the present microorganisms, the sequencing of the amplicons of a metagenome from chosen regions of the 16S rDNA can be performed. While no specific term exists for this method [211], the term “metagenetics” will be used all along this thesis to talk about 16S or 18S rDNA amplicon sequencing. Metagenomics and metagenetics differ slightly. Metagenomics is the shot-gun sequencing of a metagenome while metagenetics is the sequencing of a specific gene (*e.g.* 16S rDNA) in a metagenome.

Different methods can be used to sequence the 16S rRNA gene. One of the most commonly used is the MiSeq sequencing platform from Illumina. However, this method is better suited for small fragments, *i.e.* sequencing between the regions V1 and V3 or V3 and V4 or either V4 alone. This method is not recommended if the sequence of the whole genome needs to be known. Illumina has also developed HiSeq sequencing, which can sequence smaller fragments than the MiSeq technology [212]. The evolution of sequencing methods and apparition of new technologies provided several generations of sequencing platforms.

To be able to sequence and analyze metagenetics results, several steps are needed. Once the microbial sample has been recovered and the DNA extracted, the targeted region is being amplified by PCR. The resulting amplicons are then sequenced according to the used technology. The results obtained after sequencing need to be analyzed using bioinformatics (Fig. 1.18). Softwares are used to eliminate any PCR and sequencing errors as well as chimeras but also enable the identification of a specific sequence. To do so, an algorithm gathers similar sequences in Operational Taxonomic Units (OTUs). The determined OTUs are then compared to genetic databases, such as NCBI. Different softwares exist to treat the results obtained by metagenetics, such as mothur, OCToPUS, 16S Classifier, or MG-RAST. Mothur was developed by Schloss et al. in 2009 [213]. This software uses algorithms previously developed to identify microorganisms. To treat the raw data obtained after the metagenetic sequencing using mothur, the elimination of chimeras and false sequences must be done. These steps can be performed on mothur but the control command must be written. To do so several algorithms were developed and mothur uses them (*e.g.* Uchime, CATCh). The sequences are then clustered into OTUs depending on their identity with a 0.10 distance cut-off. To identify the clustered OTUs a database (*e.g.* SILVA, Ribosomal Database Project) must be downloaded. OCToPUS presents the advantage of cleaning the sequences (chimeras, singleton, etc.) without entering the control command. It does automatically the same steps than mothur. A cut-off of 0.03 was chosen to cluster the OTUs [210]. The use of 16S Classifier does not need informatic skills and is freely available. It uses an internet interface in which the raw metagenetics results are loaded. It presented a better performance on the identification of 16S rDNA region compared to the use of RDP Classifier [214]. MG-RAST is an open-submission data portal for processing and analyzing metagenomic datasets. It can be used for a metagenetic purpose (identification of microorganisms) but also for metagenomics analysis on several genes present in a microbiota. It is mainly use for meta-data analysis [215]. Among the three presented software analysis, the easier to use is 16S Classifier.

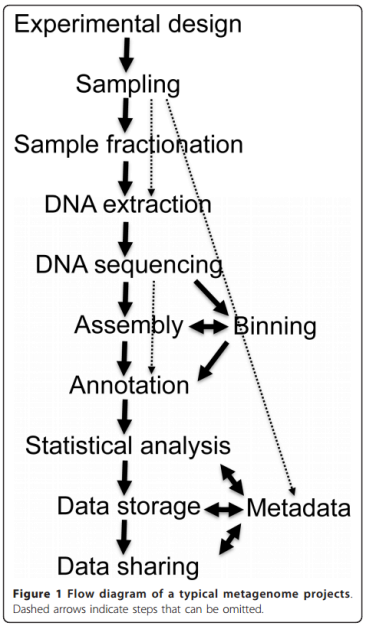



*Figure 1.18:Experimental design of metagenomics [216]*

The advantage of metagenetics on direct samples relies on the fact that VBNC can be identify from environmental samples after a direct DNA extraction and sequencing. It is also an extremely sensitive method since the material needed for this method is in the order of nanograms thanks to the sensitivity of the existing technologies. Thanks to metagenetics, several species and genera but also phyla have been discovered [217-219]. Until the unknown organisms can be isolated and studied in more details, their name begins with the term "*Candidatus*", *e.g.* "*Candidatus* Burkholderia verschuerenii" or "Candidate phylum WS1". For example, the "Candidate Division OP10" was separated into two candidate divisions (WS1 and FBP) and the *Armatimonadates* phylum after the isolation and identification of the type strain *Armatimonas rosea* YO-36$^T$ [220, 221]. Some of these microorganisms can be well described with an almost complete genome thanks to metagenomics.

Two characteristics can define third-generation sequencing: first of all, no PCR is needed before sequencing which leads to shorter DNA preparation time for sequencing and secondly, the signal is captured in real time [222]. Several sequencing technologies exist and the main ones are described in Table 1.3 which sums up their main advantages and disadvantages. This comparison can provide information on which method should be used according to the exigence on the analysis and the granted budget. The Illumina MiSeq platform features the biggest output for lowest reagent cost, the SOLiD system presents the highest accuracy, and the PacBio system has the longest read lengths. MinIon seems the most promising system because of its price, speed and its ability to sequence extremely long fragments. Since this technique is still in development it will be considered as truly promising if its accuracy increases [223].

*Table 1.3: Comparison of the main sequencing technologies [222-227]*

| Platform | | Sequencing chemistry | Read length | Accuracy | Running time | Reagent Cost / Mb | Advantages | Disadvantages |
|---|---|---|---|---|---|---|---|---|
| **Sanger method** | | Dideoxy chain terminator | ≈ 750 bp | 99.999% | NA | NA | Low error rate<br>Read lenght (750 bp)<br>High quality | Expensive<br>Slow<br>Cloning needed |
| **PacBio** | | Real-time | ≈ 1000 bp | < 90% | 15 min - 4 hours | 7 - 38 $ | Long reads (up to 30+ kb with a mean size of 4,2 to 8,5 kb)<br>Near random distribition of errors (increase the coverage to correct the errors)<br>No PCR bias<br>Capability of directly detecting modified nucleotides<br>Fast | High error rate for the Standard Long reads<br>Still in development<br>Expensive |
| **Roche 454** | *GS Junior Titanium* | Pyrosequencing | ≈ 400 bp | 99% | 10 hours | 22 $ | Multiplexing (multiple samples at the same time) | Expensive |
| | *FLX Titanium* | | 400 - 600 bp | 99.99% | 10 hours | 12 $ | Long read length | High error rate |
| | *FLX+* | | 1000 bp | 99.99% | 23 hours | 7 $ | Very long read lenght | Reagent and upgrade issues |
| **Illumina** | *MiSeq* | Reversible terminator | 150 x 2 bp | > 99.9% | 4 - 39 hours | 1 $ | Low error rate | Short read length (but the best from Illumina) |
| | *HiSeq 2000* | Sequencing by synthesis | 50 - 100 bp | 98% | 3 - 10 days | 0.02 $ | Cheap<br>Fast<br>Large volume<br>High throughput | Slow<br>Few reads |
| **Ion Torrent** | *PGM* | Ion semiconductor | ≈ 200 bp | ≈ 99% | 2 hours | 0.01 - 0.09 $ | Fast<br>Low error rates<br>Cheap<br>Easy to use<br>No optics used | Higher error rates than Illumina |
| **ABI SOLiD** | *3730 xl* | Dideoxy chain terminator | 400 - 900 bp | 99.999% | 20 min - 3 hours | 2308 $ | Low cost for very small studies<br>Accuracy | Expensive for large amount of data<br>Short read length |
| | *5500 xl* | Sequencing by Oligo | 60 x 2 bp | NA | 6 days | < 0.07 $ | Cheap | Slow |
| | *SOLiD v4* | Ligation Detection | 50 x 2 bp | 99.94% | 7 - 14 days | 0.13 $ | Accuracy | |
| **Oxford Nanopore Technologies** | *MinIon* | Real-time | up to or > 50 kb | 97% for the 2nd generation | ≈ 9 kb/hour<br>150 Mb/hour<br>no time limitation | 0.01 - 0.04 $ | Fast and possibility to stop and restart the sequencing whenever needed<br>Long read<br>Cheap | High error rate (frequently above 10% but improving with the new generations) |

The increasing throughput of sequencing technology was followed by the development of different bioinformatics tools to treat and analyze the large set of genome data. Thanks to metagenomics, scientific knowledge on microbiota from different samples (environmental, human…) has drastically increased over the last decade.

#### 1.6.2.2.2 Example of microbiota analysis using metagenetics

Metagenomics has been made well-known thanks to the Sorcerer Expedition [228, 229], and then the Tara Expedition. In the later project, the microbiota present in 68 oceanic locations at three different depths (0 to 1 km depth) was established [230]. They highlighted the fact that more circa 50% of the bacterial diversity in the oceans were composed of *Proteobacteria* at all three depths, mainly $\alpha$- and $\gamma$-*Proteobacteria*. Temperature and dissolved oxygen were shown to influence the microbial diversity and evenness [198]. MOCAT and MetaGeneMark were used to assemble the sequences and determine gene predictions. In 2016 the impact of uranium contamination in river was assessed [231]. While the diversity at the phylum taxonomic level was not impacted by the uranium pollution, the evenness was greater in the unpolluted sample. In the uranium polluted sample *Proteobacteria* indeed dominated the diversity (47.5%) while in the unpolluted sample *Bacteroidetes* was the main phylum (27.7%). At the species level the unpolluted sample presented a higher diversity. This study pointed out the change occurring in the microbial community in presence of uranium as well as the presence in higher quantity of rare species in the polluted sample. The microbiota present in this uranium polluted river contained mainly Gram negative bacteria compared to the unpolluted sample which contained more Gram positive bacteria. These results also displayed the power of metagenetics to easily compare samples [231].

In 2018 the first metagenetic inventory of a spent nuclear fuel pool (at the Savanah Rive Site) was published [193]. This study was meant to highlight the need to follow the microbiota present in SNFP to anticipate any changes the water storage condition. White precipitant samples from 13 different locations and depths were recovered though filtration. These samples were sequenced and analyzed. The mothur pipeline was used to analyze the sequencing data. After analysis, the samples were mainly composed of *Proteobacteria*. The main identified families were *Burkholderiaceae*, *Hyphomicrobiaceae* and *Comamonadaceae*. At the genus identification level, the main identified genera were *Nitrospira* and *Pedomicrobium*. In this study the use of V4V5 regions lessened the number of sequences belonging to the *Nitrospirae* and *Actinobacteria* phyla compared to the V1V2 sequencing (12.8% to 0.5% and 2.5% to 0.3% respectively). This study confirmed the empirical tendency of the presence in SNFPs of a majority of non-spore forming bacteria [185]. The SNFP from the Savanah River Site did not seem to contain any *Firmicutes* unlike the SNFP of Cofrentes [181, 184-187].

### 1.6.3 Characterization using metaproteomics

#### *1.6.3.1 Principle*

Metaproteomics is the study of all proteins contained in samples recovered directly from environmental sources. This recent methodology can highlight the microbial mechanisms involved in an ecosystem but also may help to identify organisms. For this, all the proteins are extracted and peptides are generated by proteolysis by trypsin. Then, the peptides are resolved by reverse phase chromatography and analyzed by tandem mass spectrometry. Thanks to bioinformatics and new tandem mass spectrometry technologies, it

is now possible to determine the origins of described peptides from a sample composed of a consortium of organisms [232]. The use of tandem mass spectrometers (MS/MS) revolutionized proteomics, allowing to obtain more precise and faster results than the 2D-PAGE gel previously used [233]. Current technology can identify proteins of populations that comprise at least 1% of the community and for which closely related genomic sequences are available [234].

To analyze a sample via metaproteomics, the cellular pellet is lysed and the proteins are recovered. Once the proteins are extracted from the sample, they are hydrolyzed into peptides which are then analyzed through a nanoLC MS/MS. The MS/MS spectra are then interpreted against a protein sequence database representative of the sample. The organisms from which the peptides and thus the proteins came from can be identified on this basis.

The advantages of metaproteomics not only include the identification of organisms but also highlights the mechanisms implemented in the analyzed sample and community. Furthermore, metaproteomics can be a superior approach to metagenetics for reasons such as the proportion of a specific species, which cannot be assessed using a genetic approach. The number of 16S rRNA gene copies depends indeed of the species [235] but also of the incubation conditions and growth rate which can influence the proportion of identified bacteria. The biases produced by the DNA extraction method, the PCR and the sequencing can also influence the final proportion of one specific gene [236]. These factors can thus influence the overall proportion of a given OTU in the sample. At the present stage, metaproteomics is not considered as a quantitative method. Indeed, the exhaustiveness of the databases used for the interpretation influences the results obtained as well as the number of identified specific peptides of a peculiar taxonomic level. Bias can also occur during protein extraction. Metaproteomics, metagenomics and metagenetics are thus highly complementary, but difficult to perform on the same sample.

#### *1.6.3.2 Example of microbiota identification using metaproteomics*

Several protein extraction protocols can exist and should be adapted to the type of the sample to be analyzed. However protein extraction and peptide purification protocols are relatively similar: they use a cell lysis method such as bead-beating or sonication and then, proteins are hydrolyzed into peptides with trypsin [237]. Nanoscale liquid chromatography coupled online to a tandem mass spectrometry (nanoLC-MS/MS) is the current technique used to analyze complex peptide mixtures. The chromatography separates the molecules along their hydrophobicity on a reverse phase column. After the analysis of their molecular weight, the peptides are fragmented into small pieces and the molecular weight of these fragments is established by mass spectrometry. For correct fragmentation, the goal is to isolate the intended precursor peptide ion from the others, a step generally done by a quadrupole mass filter. Once the MS/MS spectrum acquired and then interpreted into a Peptide-to-Spectrum Match (PSM), the amino-acid sequence identified is used to decipher the nature of the original protein it came from. To do so, the MASCOT software is a possible tool which principle is the comparison of the experimental MS/MS spectrum with the most probable MS/MS spectra theoretically drawn from all the possible peptide sequences from the protein database [238-241]. The lack of information concerning environmental organisms in the public protein databases and the lack of proper annotation [242] can be a major obstacle for using metaproteomics in environmental samples.

The combined use of metagenomics and metaproteomics can be useful to identify microorganisms present in a community as well as their interactions within each other and the environment. For example in an acid mine drainage in Richmond, the dominant biofilm bacteria belonged to the *Leptospirillum* group II (metagenetics results). Using metaproteomics and a dedicated protein sequence database 48% of the predicted proteins from *Leptospirillum* goup II were identified. Many proteins involved in protein refolding and response to oxidative stress were identified [243, 244]. The biofilms present in the acid mine drainage from the Richmond mine were highly studied in terms of metagenomics and metaproteomics due to their low diversity and abundant biomass [245]. No cultivation step were thus needed and the interactions observed were the ones actually happening *in situ*.

The biological diversity of six different aquatic environments has been studied using metaproteomics. The authors confirmed their results with the ones obtained using metagenetics. The identification between both methods were comparable, indicating that metaproteomics could be used as an alternative to metagenetics [246]. The microbial assessment of the Chesapeake Bay was also performed without any cultivation step [246]. However, in both studies, the water volume sample to be analyze was comprised between 10 and 20 L.

## 1.7 PhD project

### 1.7.1 Framework and objectives of the PhD project

This PhD was carried out in the framework of the INFERNUS project leaded by Corinne RIVASSEAU and funded by the Programme Transversal de Toxicologie from the CEA. The goal of this project was to analyze the microorganisms present in French spent nuclear fuel pools. Their ability to resist ionizing radiation and to concentrate specific radionuclides were determined after isolation and identification. This project was divided in two parts: first the inventory of the microbial genera present in French SNFPs using direct molecular approaches, then the identification of the main mechanisms involved in radioresistance and radionuclides resistance and accumulation.

This project was of great interest for the CEA for several reasons. With the knowledge of the microbial community in SNFPs, microbiological controls can be performed to prevent the apparition of corrosion and any chemical changes in the water due to microbiological activity. This will ensure the safety and integrity of nuclear storage and installation. A second reason concerned a more fundamental area in radiobiology and radionuclides toxicity in microorganisms and thus highlighting the mechanisms and genes implemented to resist these stresses. The last reason was the potential use of some microorganisms in biotechnology process for decontaminating metal pollution, in the same spirit as it was demonstrated using *Coccomyxa actinabiotis*.

The INFERNUS project involved 5 teams. Team 1 is Team Plant, Stress and Metals from the Cell & Plant Physiology Laboratory in CEA Grenoble. This team studies on heavy metals and their impact on vegetal cells and organisms. They have discovered the *Coccomyxa actinabiotis* microalgae and developed biotechnological process with this radiotolerant organism. Team 2 is the Technological Innovations for Detection and Diagnosis Laboratory (Li2D) from the CEA Marcoule. This team is expert in proteomics, bioinformatics and high-resolution mass spectrometry techniques, and has already studied several radiotolerant microorganisms (*D. deserti, T. gammatolerans* among others). Team 3 is the Bioenergetics and Proteins Engineering Laboratory in Marseille (CNRS). They work on eukaryotes (mainly algae) and can

reconstruct an intact genome from shotgun metagenomics results. Team 4 comprises the two studied nuclear installations in CEA Saclay and brought expertise for sampling. The last team, Team 5, is the Microbiology group from the SCK●CEN (Belgium). They have developed an expertise in metagenomics analysis of environmental sample as well as several analysis pipelines. Team 1 and Team 2 have been working together since 2012 for the characterization of *Coccomyxa actinabiotis* and the mechanisms it implemented after an irradiation and the addition of radionuclides.

In the framework of this ambitious project, my PhD thesis had for objectives to find radioresistant microorganisms that can also accumulate radionuclides. The only easily accessible environments where radiation and radionuclides can be found in high quantity, for CEA personal, were spent nuclear fuel pools and the cooling pools of nuclear reactors. The organisms living there may have developed radioresistance mechanisms and a tolerance for radionuclides. By doing a complete inventory of the microbiota present in those environments using direct methods, microorganisms can be detected and latter isolated and studied. The sampling and analysis condition were first determined and optimized (cf. Chapter II). Several samplings were carried out in two different pools. One pool is used to store radioactive cobalt sources while the other is the cooling pool of a uranium reactor core. In the second pool one sampling was performed during the reactor last working cycle and one sampling was carried out a year after its definitive shutdown. Water samples and biofilms were collected in both pools and analyzed using i) metagenetics and the OCToPUS software (developed by the SCK●CEN) and ii) metaproteomics, more specifically phylopeptidomics (a very innovative method patented by the Li2D and currently still under development) (cf. Chapter III). The identification of microorganisms through metaproteomics is currently based on the fact that some peptides are specific to a distinct species or strain and, thus, by analyzing the peptides from a consortium of microorganisms, it is possible to determine which organisms were present in a sample. However, since metaproteomics is still developing, several factors are limiting this method [242]. Because metaproteomics may use genomics and metagenomics data, it is important to be careful to the presence of possible contaminants in the metagenomics data before interpreting the proteomics results [242]. To identify microorganisms, informatic softwares have been developed and are currently used to avoid false-positive (Fig. 1.19). This method was developed and patented by the Li2D. Another advantage is that it can identify and analyze prokaryotes as well as eukaryotes on the same run, using the same databases at the same price. According to LGC Genomics, a sequencing company, wanting to identify both prokaryotes and eukaryotes is possible. In that aim one pair of primer that can amplify regions of the 16S rDNA plus a pair to amplify regions of the 18S rDNA must be used as well as a pair to amplify the ITS (for fungi [247]). This is not only experimentally heavier but also more expansive, and unfortunately a method not quantitative. In comparison, phylopeptidomics has been proved to be a relatively good quantitative method.

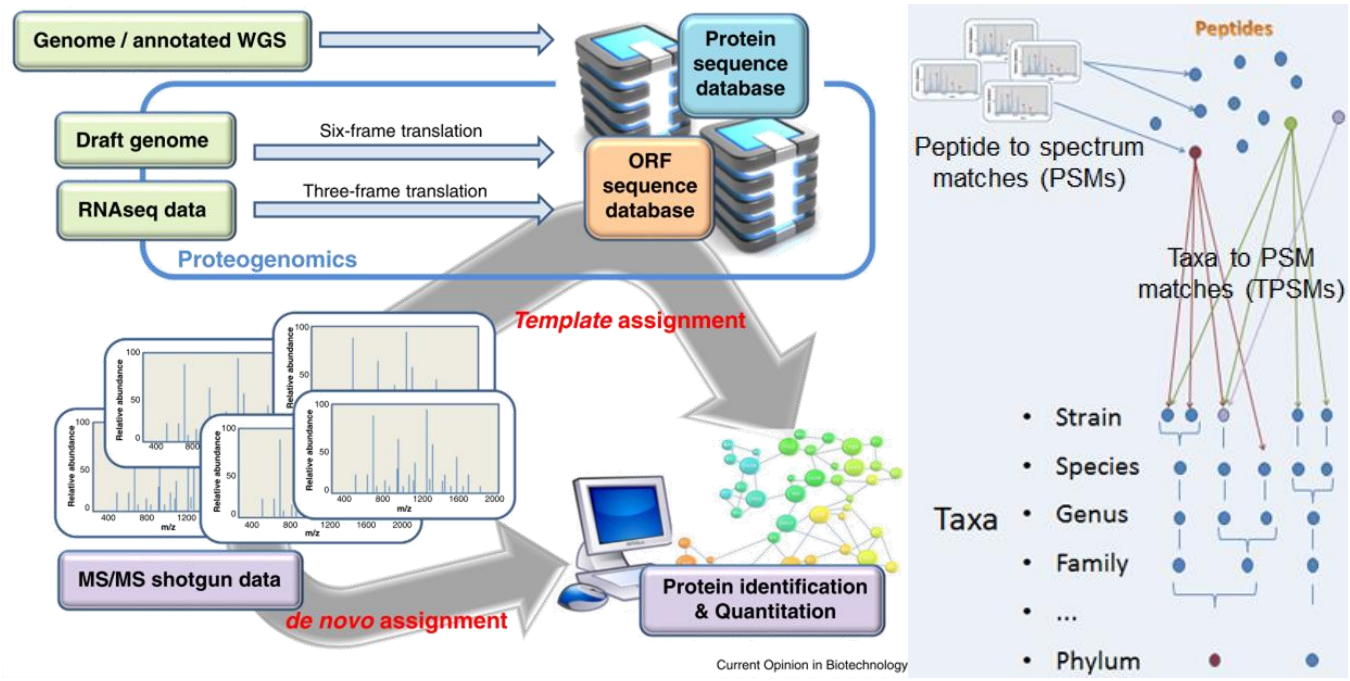



***Figure 1.19: Scheme of the metaproteomics identification method [248].*** *Interpretation of MS/MS spectra, against protein sequence database or de novo. Protein sequence databases are derived from genomics and/or RNAseq or draft genome data. In this latter case, unannotated nucleic acid molecules are translated into open reading frames (ORFs), either along the six or three possible frames. Further proteogenomics approaches may be used to improve the protein sequence database. Tandem mass spectrometry data, acquired on peptides from a given organism or microbiome, are assigned against the most appropriate protein sequence database, giving peptide-to-spectrum matches. These are then interpreted in order to infer which proteins are present and to quantify these items.*

The goal was to be able to isolate those microorganisms after identifying the diversity present in these waters. Several microorganisms were isolated using different culture methods and media. The isolated bacteria, fungi and one micro-alga were identified after the sequencing of the 16S rDNA, the 18S rDNA and the ITS for the bacteria, alga, and fungi respectively. The characterization of the isolated bacteria in terms of radioresistance and radionuclides accumulation had also been performed. First their ability to resist γ-radiation was tested. Radionuclides emit radiation in addition to the metallic stress induced by the atom. The accumulation of uranium and cobalt (not radioactive) was also assessed (cf. Chapter IV). Uranium accumulation is of great interest in terms of environmental pollution while cobalt accumulation is interesting to decontaminate nuclear effluents. In the second pool dissolved radioactive cobalt emitted circa $1.3x10^5$ Bq/m$^3$ and cobalt particles were found in recovered biofilms. The biofilms with the cobalt particles emitted up to 80 Bq per biofilm. Cobalt is thus a potential water contaminant of nuclear installations. Three cobalt accumulating bacterial strains were isolated from a Slovak SNFP [152] while several uranium accumulating bacteria were found in various environments. After the Fukushima accident uranium was released in the environment and was shown to be associated with cesium in microparticles at a 4 km distance from the melted reactor. The use of uranium and cesium accumulating bacteria could be used in this situation [249].

The bacteria that exhibited great radionuclides accumulation performances could be studied to highlight the mechanisms implemented to resist radiations and accumulate radionuclides. In the longer term these microorganisms may be used to decontaminate nuclear effluents and for bioremediation.

## 1.7.2 Description of the pools

### *1.7.2.1 Pool 1*

Pool 1 is located at CEA – Saclay, near Paris. It is an irradiating pool, used to store radioactive cobalt sources. The sources are used to perform irradiation experiments on different materials. This pool does not contain any radionuclides, making thus an excellent choice to develop and implement protocols of analyzing the microorganisms present in its water. The water used to fill the pool is de-mineralized.

In 2015, the purification system filtered the water every day for circa 10 minutes. The characteristics and composition of the water of this pool on July 2015 are presented in Table 1.4. Several ions ($Cl^-$, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$) can be used as nutrients by microbial cells.

*Table 1.4: Characteristics and composition of the water of Pool 1 in July 2015*

| | pH | σ at 25°c (µS/cm) | COD (mg/L) | MIS (mg/L) | $F^-$ (mg/L) | $Cl^-$ (mg/L) | $Br^-$ (mg/L) | $NO_3^-$ (mg/L) | $PO_4^{3-}$ (mg/L) | $SO_4^{2-}$ (mg/L) | $Na^+$ (mg/L) | $K^+$ (mg/L) | $Ca^{2+}$ (mg/L) | $Mg^{2+}$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **LOQ** | - | 2,00E+01 | 4,00E+00 | 2,00E+00 | 1,00E-01 | 5,00E-01 | 9,40E-02 | 5,00E+00 | 2,00E-01 | 1,00E+02 | 5,00E+00 | 1,80E-01 | 5,00E+00 | 4,30E-01 |
| **Pool 1** | 8 | 1,27E+02 | <4,0E+00 | <2,0E+00 | - | 2,70E+00 | - | - | - | - | 5,80E+00 | 1,60E+00 | 4,10E+01 | 1,70E+00 |
| **Inc (%)** | - | 3 | 10 | - | - | 21 | - | - | - | - | 13 | 29 | 15 | 25 |
| **Measuring T°C** | - | 21,6 | 21,9 | - | - | - | - | - | - | - | - | - | - | - |

The dose received by the nearest spot on the wall (less than 20 cm) while radioactive sources are present is around 100 Gy/h. In the middle of the sources, the dose is around 5000 Gy/h. Figures 1.20 and 1.21 illustrate the pool and the position of the sources when present. Natural light is present on this pool due to the presence of windows and the northern wall can be highlighted several hours of a day.

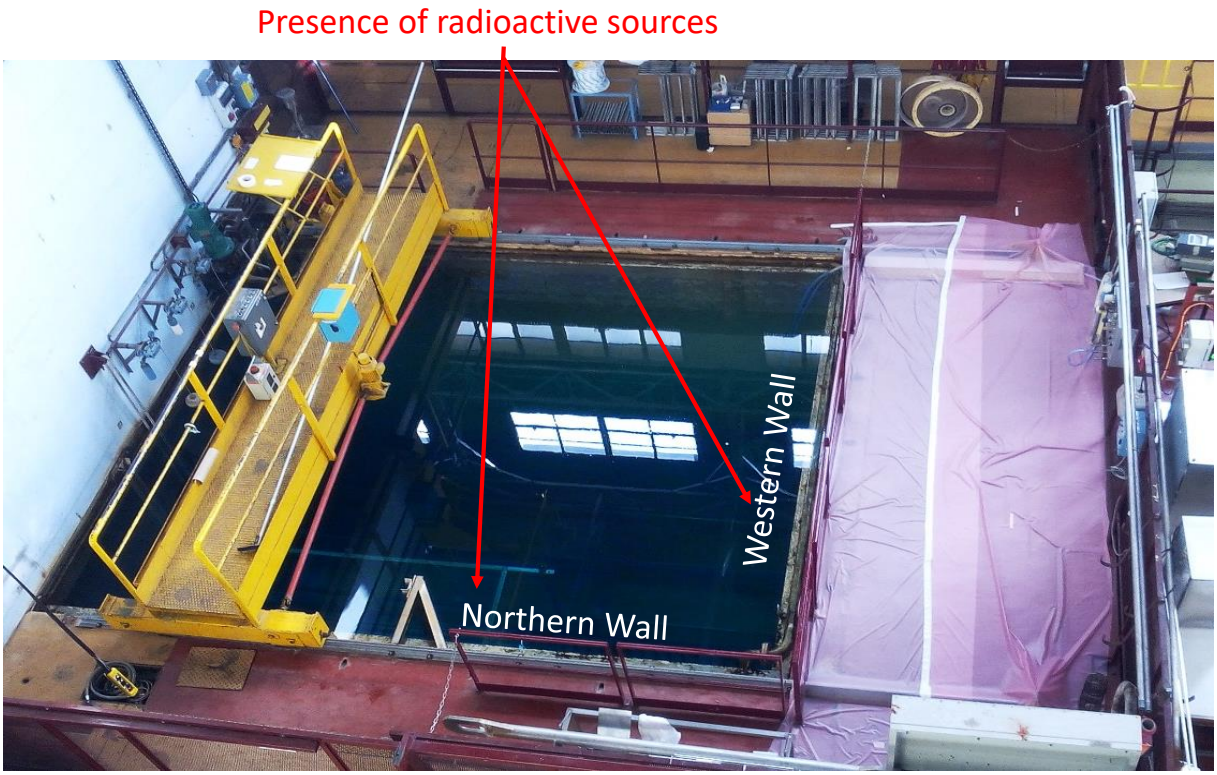



*Figure 1.20: Overall view of Pool 1*

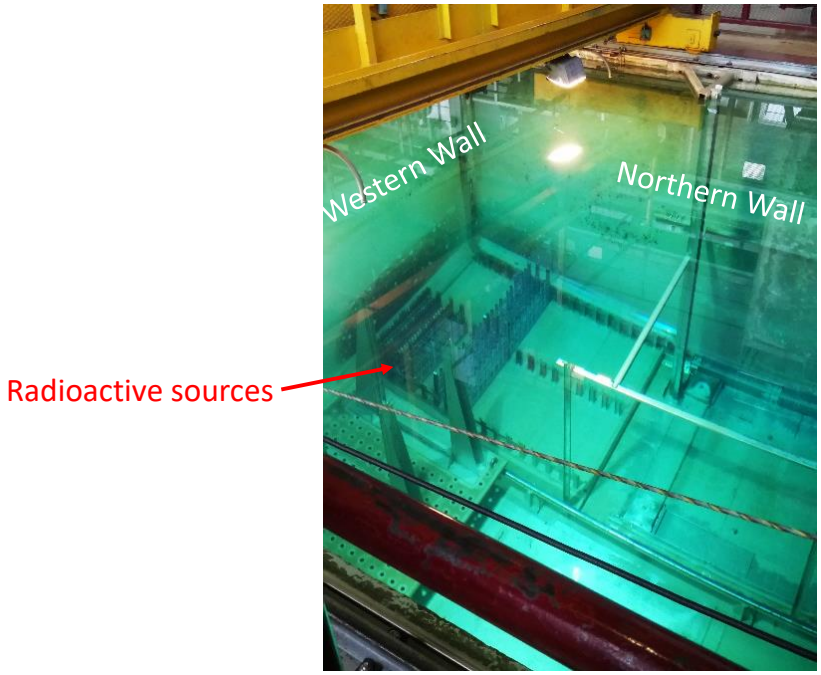



*Figure 1.21: Pool 1 containing the $^{60}Co$ radioactive sources, between the western and the northern wall*

In July 2016, the purification system of the pool has been changed, leading to a decrease in the concentration of ions. The new system also increased the filtration flow. The physico-chemistry parameters of the pool were changed, leading to a probable change in the composition of the microbiota present in the water. The water composition, after the implementation of the new filtration system, is presented in Table 1.5. A decrease of ions concentration was observed between 2015 and July 2016. The $Cl^-$ concentration was similar between 2015 and July 2016 (2.7 and 2.2 mg/L respectively) while the other detected ions in 2015 were not detected in 2016. $NO_3^-$ was present in 2016 (5.5 mg/L) when none was detected in 2015.

*Table 1.5: Characteristics and composition of the water of Pool 1 in July 2016 after the purification system change*

| | pH | σ at 25°c (µS/cm) | COD (mg/L) | MIS (mg/L) | $F^-$ (mg/L) | $Cl^-$ (mg/L) | $Br^-$ (mg/L) | $NO_3^-$ (mg/L) | $PO_4^{3-}$ (mg/L) | $SO_4^{2-}$ (mg/L) | $Na^+$ (mg/L) | $K^+$ (mg/L) | $Ca^{2+}$ (mg/L) | $Mg^{2+}$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOQ | - | 2,00E+01 | 4,00E+00 | 2,00E+00 | 1,00E-01 | 5,00E-01 | 9,40E-02 | 5,00E+00 | 2,00E-01 | 1,00E+01 | 5,00E+00 | 1,80E-01 | 5,00E+00 | 4,30E-01 |
| Pool 1 | 7,9 | 1,40E+02 | <4,0E+00 | <2,0E+00 | <1,0E-01 | 2,20E+00 | <9,40E-02 | 5,50E+00 | - | <1,00E+01 | - | - | - | - |
| Inc (%) | 3 | 9 | - | - | - | 23 | - | 16 | - | - | - | - | - | - |
| Measuring T°C | 23,2 | 23,2 | - | - | - | - | - | - | - | - | - | - | - | - |

The pool is deeply cleaned annually in December. Several sampling campaigns were performed during this study. The first one, in November 2015, occurred before the cleaning of the pool. The second one, in June 2016, occurred just before the change of the purification system. Only 6 months had happened between the previous cleaning and the sampling. The last sampling, finally, occurred in March 2017, after the purification system had been changed. The different samplings were performed on several walls and in the water at different depths and are detailed in III.4.

### *1.7.2.2 Pool 2*

This pool, located at CEA – Saclay, used to be a nuclear reactor until its last run in December 2015. Its main purpose was to produce radionuclides for medical goals. The power created by this nuclear reactor was around 70 MWth [250]. The water use to cool the core was thus highly radioactive and still contains

radionuclides. Due to radioactivity the recovered water has to be treated on the site. Small volume of water was cultivating in several liquid media and agar plate. DNA was extracted on site to eliminate radionuclides. A chemistry laboratory, located on the site, was used to treat the samples, complemented with an electric Bunsen burner to have a sterility environment around the samples. To concentrate microorganisms, a centrifuge and a vacuum pump, with sterile 0.20 µm filters were also used. The samples, once treated, and controlled to allow an exit from the restricted area, were treated at the LPCV in Grenoble (DNA and protein extractions) or Li2D in Marcoule (liquid kinetic).

The tritium activity is $2.49 \times 10^8$ Bq/m$^3$ (both during operation and at shutdown) while the $\gamma$-emissions were $2.6 \times 10^9$ Bq/m$^3$ when operating and $3.25 \times 10^6$ Bq/m$^3$ when shutdown. The composition of the water, both when operating and stationed is presented in Table 1.6. $^{24}$Na represents more than 99% of the activity of the water during operation. However its period is quite short: in around 15 hours, half of the isotope has been disintegrated. The radioactivity contained in this pool consists of $\beta$- and $\gamma$-emissions.

*Table 1.6: Radionuclides composition of the water in Pool 2*

**Operating**

| Isotope | Period | Gamma% | Activity (Bq/m$^3$) |
|---|---|---|---|
| $^{7}$Be | 53,20 days | 10,430 | 3,965E+06 |
| $^{24}$Na | 14,96 hours | 99,994 | 2,899E+09 |
| $^{41}$Ar | 1,83 hours | 99,160 | 1,659E+06 |
| $^{51}$Cr | 27,70 days | 9,850 | 2,063E+05 |
| $^{54}$Mn | 312,20 days | | |
| $^{56}$Mn | 2,58 hours | 98,870 | 1,142E+06 |
| $^{85}$Kr | 10,71 years | 0,437 | 3,532E+06 |
| $^{99}$Mo | 2,75 days | | |
| $^{99m}$Tc | 6,01 hours | 89,000 | 2,896E+07 |
| $^{122}$Sb | 2,70 days | | |
| $^{137}$Cs | 30,15 years | | |
| $^{187}$W | 23,90 hours | | |
| $^{186}$Re | 3,77 days | | |
| $^{188}$Re | 16,98 hours | | |
| $^{197m}$Hg | 23,80 hours | | |

**Shutdown**

| Isotope | Period | Gamma% | Activity (Bq/m$^3$) |
|---|---|---|---|
| $^{51}$Cr | 27,70 days | 12,2 | 1,59E+06 |
| $^{54}$Mn | 312,20 days | 20,1 | 3,60E+04 |
| $^{58}$Co | 70,86 days | 8,2 | 2,50E+05 |
| $^{60}$Co | 5,2714 years | 12,2 | 1,28E+05 |
| $^{99}$Mo | 2,75 days | 40,0 | 1,66E+05 |
| $^{99m}$Tc | 6,01 hours | 40,0 | 2,50E+05 |
| $^{122}$Sb | 2,70 days | 10,2 | 1,73E+05 |
| $^{124}$Sb | 60,2 days | 8,2 | 2,81E+05 |
| $^{186}$Re | 3,77 days | 22,1 | 3,71E+05 |

This pool is 11 meters depth and the nuclear reactor is located at 9.5 meters depth. A schematic view of the reactor is presented in Figure 1.22. There are also a footbridge, some fuel bundles, and other objects that had to stay in the water until they are safe to be placed into the nearby canals until their radioactivity decayed enough to be safely removed.

Artificial light is present all day on this pool. During a working cycle, the pool is at 38°C under the heavy water layer and at 30±1°C at the bottom. The chimney possess its own water system which circulates less (both during operation and at shutdown) than in any other pool's location. In operation (2015) pH was 7.0 while pH was 5.4 in 2017. Whatever the operating mode, the pool water is continuously filtered at a 500 m$^3$/h flow rate, and is purified on ion exchange resins at 30 m$^3$/h, meaning a turnover of the water of this 536 m$^3$ pool in 18 h.

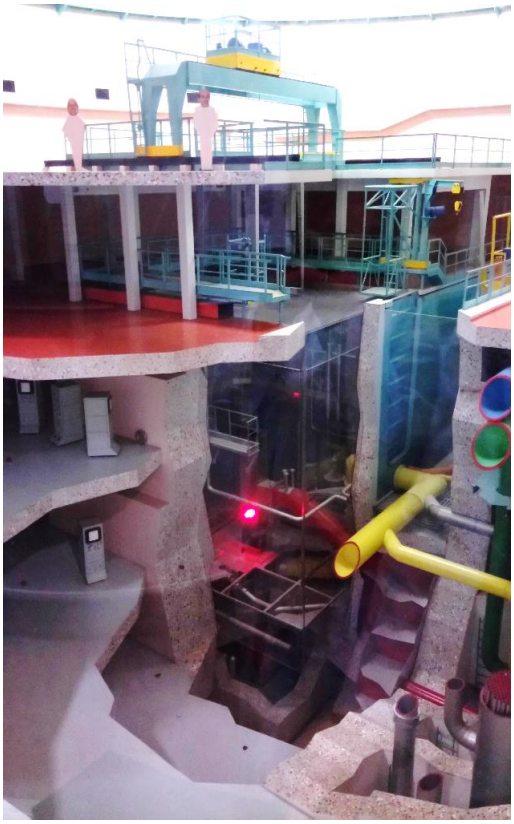


*Figure 1.22: Schematic view of Pool 2 reactor (red light: position of the reactor)*

In this pool, the selection pressure is quite high. There are indeed very few nutrients, the presence of radionuclides in the water and high levels of radiation.

Two campaigns were performed in this pool. One in 2015 during the last working cycle and one in 2017, more than a year after its definitive shutdown.

# Chapter II:

## *Methods development and optimization*

## 2. Methods development and optimization

In this chapter, several methods used for this thesis were developed and optimized. As seen in the introduction (sections 1.6.2 and 1.6.3), different methods exist to analyze a microbial community. The most recent ones, direct metagenetics and direct metaproteomics, have been used during this thesis.

First of all, metagenetics results should be properly analyzed and trusted. In several metagenetics studies several phyla were present in very few quantity, smaller than 0.01% or even less [231, 251, 252]. In one study, to be sure to eliminate false-positive, only taxa with an abundance greater than 2% were presented [253]. However when setting a cut-off as high as 2%, the risk of removing true positives, *i.e.* interesting sequences obtained from the sample, is important. The determination of a cut-off through the sequencing and analysis of a standard is highly recommended to eliminate false-positive. However the risk of eliminating organisms actually present can be high. The cut-off should be low enough to select no false-positive but keep sequences from organisms present in low concentration. In the last decade, progresses have been made in metagenetics, not only in terms of technology's improvement but also in terms of algorithms and softwares used to analyze the results. To determine the sequencing an analysis errors a DNA standard was used. A V4 and V4V5 regions sequencing were performed on this standard.

Metaproteomics is an approach used at first to highlight the interactions existing in a microbial community after the identification of the community members with metagenetics [234, 243-245, 254-256]. Nowadays it can be used to identify the organisms present in a sample, a concept named proteotyping [246, 257-260]. Here, for the first time we have applied a new approach dubbed phylopeptidomics to identify the community members of biofilms and liquid cultures. In this chapter, we present in details this approach and a case study.

Last, we investigated whether cultivation in liquid media modified the composition of the sampled microflora, taking into account that long-term culture usually resulted in poorer diversity. For this, we monitored by metaproteomics the microbial composition of a series of liquid cultures.

During this PhD, two pools were studied, one being a pool used to stock $^{60}$Co radioactive sources to do irradiation experiments on materials (Pool 1) while the other pool is the one used to cool $^{235}$U radioactive sources (Pool 2). The latter (Pool 2) used to produce radionuclides for medicine (cf. Chapter III.1 for detailed description). Briefly, three campaigns were performed in Pool 1 (in 2015, 2016, and 2017) and two in Pool 2 (in 2015 during the last working cycle and in 2017, more than a year after its definitive shutdown).

## 2.1 Direct analysis: method development and optimization

### 2.1.1 Comparison of two 16S rDNA regions metagenetics sequencing to identify microbial diversity in an aquatic environment complemented with microbial harvesting and DNA extraction optimization (*article in preparation*)


**Abstract**

The 16S rDNA sequencing been widely used in the identification of microbial community. Thanks to this technology uncultured organisms can be identified. However during the different steps prior to sequencing and during sequencing different biases can occur. To be able to trust a metagenetics result a cut-off taking into account false-positive and false-negative should be used. In this study a DNA sequencing of the V4 and V4V5 regions of 16S rDNA from a ZymoBIOMICS™ Microbial Community DNA Standard was performed. After Illumina MiSeq sequencing and OCToPUS analysis of the results, a 0.016% cut-off was identified after a V4V5 sequencing. A 0.025% cut-off was identified after a V4 sequencing. This cut-off allowed us, using the same sequencing and analysis methods, to eliminate false-positive while maintaining as much taxonomical information present (*i.e.* false-negative). The V4V5 sequencing, while presenting a lower quality, allowed the identification at a deeper taxonomic level than the V4 sequencing.


**Introduction**

Since the birth of metagenetics and the rise of NGS (next generation sequencing), the analysis of microbial communities took a new start and highlighted the presence of new taxa. It has been estimated that between 0.1 to 10% of microorganisms present in water cannot be culturable [36]. The use of a direct analysis approach should allow the identification of unknown organisms. Thanks to metagenetics a whole new microbial world was discovered in environmental samples [219, 228, 229, 261, 262]. Metagenetics sequencing can use one or several hypervariable regions present on the 16S rDNA. There is however no consensus on which region(s) should be sued to identify bacteria [263-265]. The choice of the region(s) can depend on several factors: the homology between different species on a peculiar region, the ability to amplify a chosen region, as well as the length of the fragment to be amplified and sequenced. In a study the sequencing of the V4 region gave the highest diversity compared to the other tested regions (V2, V3, V6, V6V7, V8, and V9) [266]. A previous study analyzed the differences between a V4 or a V4V5 from a mock community [267]. However these mocks were chosen to assess the accuracy of OCToPUS a novel pipeline. Despite NGS advantages the read length of sequencing is not long enough to sequence the 1500 pb constituting the whole 16S rDNA gene. New long read NGS methods are being developed such as PacBio and MinIon but their price and their error rate are not optimal enough today to be used for a metagenetic approach [268, 269]. To sequence region of the 16S rDNA, a first step of PCR is necessary. During this amplification step, biases can occur, especially if the region is GC rich. It has been observed that there were large discrepancies in the proportion of gram negative bacteria established using next generation sequencing when compared to the results of direct microscopy, and culture-based methods. The proportion of gram negative bacteria was underestimated using NGS [270]. Moreover, even though metagenetics can indeed identify the microorganisms present in a population the DNA extraction should be optimized to recover DNA from every cells [271]. Biases cannot only occur during DNA extraction and PCR steps, but also

sequencing. These biases can lead to a change in proportion of each OTU [236] or the absence of some microorganisms for which DNA was not completely extracted and not properly amplified [236]. Finally the use of the DNA extraction protocol can influence the obtained results. In 2016, a study showed the results of 21 different DNA extraction protocol. They found out that the use of a phenol-chloroform-isoamyl alcohol and a protocol using the Qiagen-based lysis extraction presented the most reliable results. Furthermore these two extraction protocols were reproducible between different laboratories. However this study was performed on human feces. No harvesting of the microorganisms was thus necessary [272]. Human feces have a high bacterial density (about $0.9x10^{11}$ bacterial/g fresh feces [273]) compared to water (about $3x10^4$ bacteria/mL in seawater [274], $5.8x10^6$ bacteria/mL in lakes [275] or about $10^6$ cells/L in spent nuclear fuel pool water [cf. Chapter III]). The bacterial number is thus higher in feces and less biological material is needed. Several liters of water would be necessary to obtain as much bacteria as 1 gram of feces.

The aim of this study was to assess the impact of i) which 16S rDNA region primers (V4 or V4V5) performed best, using a microbial community DNA standard as control, ii) how cells were harvested from the water samples (either by filtration or by centrifugation), and iii) how DNA was extracted.

## Material and Methods

### *Sampling*

At three different locations (Loc. A, B, and C) in a shutdown nuclear reactor pool, 8 liters of water were recovered and stored into sterile plastic container. Among these 8 liters, only 3 were used to extract DNA. One liter was centrifuged at 16000 *g* for 20 min at 10°C to harvest the microbial community. The two other liters were filtered on two different 0.22 µm hydrophilic polyethersulfone filters (MicroFunnelTM Supor®, Pall Corporation, USA). The filters were introduced into sterile 50 mL Falcon tubes for transportation. The initial microbial cell density in water sampled was assessed with a Malassez cell at $2.7x10^6$ cell/L, $1.4x10^6$ cell/L, and below $0.75x10^6$ cell/L at Loc. A, Loc. B, and Loc. C, respectively, using light microscopy after microorganism concentration by centrifugation. The filters and the pellet were preserved at + 4°C prior to DNA extraction. The radiations dose at sampling location A (bottom of the pool) was below detection limits (< 0.1 µGy/h), about 15 µGy/h at sampling location B (above the chimney), and about 25 Gy/h at sampling location C (inside the chimney).

### *DNA extraction*

The DNA was extracted with two different methods. The DNA from the microbial communities of half the filters was extracted with DNeasy® PowerWater® kit from Qiagen (Germany) according to the manufacturer's instructions. The centrifuged water and the other half of the filters were extracted using a phenol-chloroform method, which was adapted from Vilchez-Vargas et al. [276]. The liquid sample was centrifuged 20 minutes at 20000 *g* at room temperature and the supernatant discarded. One milliliter of lysis buffer (100 mM Tris pH 8; 100 mM EDTA pH 8; 100 mM NaCl; 1% polyvinylpyrrolidone; 2% SDS) was added and the pellet resuspend. The mixture was then transferred to a glass bead (0.1 mm) tube from Mobio. The tube was disrupted three times at 30 Hz for 60 seconds with 30 seconds break between each run with a disruptor MM 300 from Retsch/Qiagen. The tube was then centrifuged 5 minutes at 20000 *g* at room temperature and the supernatant removed and transferred into a new tube. One milliliter of phenol-chloroform-isoamyl alcohol (25:24:1) was added. The sample was then vortexed and vigorously mixed by

inverting the tube. The supernatant was recovered and transferred into a new tube after a centrifugation step of 1 minute at 17000 *g* at 4°C. Seven hundred microliters of chloroform were added to the sample. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered after a centrifugation step of 1 minute at 17000 *g* at 4°C. The supernatant was divided into two tubes (around 450 µl in each) and 45 µl of sodium acetate 3 M and 500 µL of isopropyl alcohol were added into each tube. The samples were then mixed by inverting the tubes and put overnight at -20°C. The tubes were centrifuged 30 minutes at 17000 *g* at 4°C and the supernatant removed. Two steps of washing were performed by adding 750 µL of 75% ethanol and a centrifugation step of 5 minutes at 17000 *g* at 4°C. The supernatant was removed and the pellet dried with a heating block at 55°C. Sixty microliters of sterile milliQ water were added to dissolve the pellet. To have a more pure DNA and remove any trace of alcohol, the sample was put on a 0.05 µm filter disposed on sterile water for 15 minutes before being transferred into a new tube. The different methods were called method 1, method 2, and method 3 for centrifugation followed by phenol-chloroform-isoamyl alcohol DNA extraction, filtration followed by phenol-chloroform-isoamyl alcohol DNA extraction, and filtration followed by the DNeasy® PowerWater® kit respectively.

The association of centrifugation and phenol-chloroform-isoamyl alcohol extraction was called method 1. Methods 2 and 3 correspond to filtration followed by a phenol-chloroform-isoamyl alcohol extraction or the PowerWater® extraction respectively. To test the quality of the sequencing and determine the number of false-positive, 10 µL of a ZymoBIOMICS™ Microbial Community DNA Standard (10 ng/µL) was sent for sequencing (100 ng sent to be sequenced).

***Sequencing***

The sequencing of genomic DNA on 16S rDNA was performed by LGC Genomics, UK. The sequencing was conducted as followed. The PCRs included about 1-10 ng of DNA extract (total volume 1 µL), 15 pmol of each forward primer and reverse primer (in 20 µL volume of 1 x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µL of BioStabII PCR Enhancer (Sigma) and additionally 0.2 µl of DNase (Artic Zymes) for the 16S PCR. For each sample, the forward and reverse primers had the same 10-nt barcode sequence. Each PCRs were carried out for 20 cycles, dependent on the primer pair, using the following parameters: 1 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s and primers without inline barcodes have been used (341F/1061R). For the second round 1 µL PCR product from the first round was used and the PCR conditions were the same as before. For this reaction barcoded primers have been added to amplify the V4V5 regions (515YF-926R: 5'-GTGYCAGCMGCCGCGGTAA-3' and 5'-CCGYCAATTYMTTTRAGTTT-3' respectively). The primers used to amplify V4 region were 515F/806R (5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-GGACTACHVGGGTWTCTAAT-3' respectively).

DNA concentration of amplicons of interest was determined by gel electrophoresis. About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying different barcodes. The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification on MiniElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

***Data analysis***

The raw data obtained after sequencing were analyzed using the OCToPUS software [210]. This software eliminated any singleton sequences, chimeras, clustered according to a threshold similar sequences in OTUs, and assigned each OTU to a taxonomical level using the RDP database (Ribosomal Database Project).

## Results

***Determination of 16S rDNA regions sequencing***

*Determination of a threshold with V4 and V4V5 amplification using ZymoBIOMICS™ Microbial Community DNA Standard*

The DNA sequenced by LGC Genomics presented little differences with its theoretical composition after a sequencing of the V4 and V4V5 regions of the 16S rDNA in its composition and proportion. Moreover some sequences, belonging to absent bacteria in the mock were identified after sequencing (Table 2.1, Fig. 2.1 & 2.2). The theoretical proportion of each OTU sequences in the ZymoBIOMICS$^{TM}$ DNA standard was similar to the one found after the sequencing of the V4V5 regions of the 16S rDNA (Fig. 2.1, Table 2.1). The presence after sequencing of absent sequences in the sample was also noticed. They were however present in low quantity, representing less than 0.03% of the sequences (Fig. 2.1). Fourteen bacterial sequences could not be clustered with a known OTU. Among these sequences several OTUs were identified as "Bacteria_unclassified". All "Bacteria_unclassified" OTUs were regrouped under the name of "Bacteria_unclassified" in Table 2.1. Among the false-positive sequences identified after sequencing and analysis using OCToPUS, the maximum of sequences clustered in a single OTU was 4 (data not shown). The number of "Bacteria_unclassified" represented around 0.015% of the sample. This mock highlighted the detection of false-positives. With the number of unclassified bacterial sequences a 0.016% threshold could be chosen to analyze data from microbial samples obtained after a V4V5 Illumina MiSeq sequencing and an OCToPUS analysis (the number of the false-positive OTU presenting the higher number of sequences with a 0.001% margin).

*Table 2.1: Theoretical proportion of each bacterium in the ZymoBIOMICS™ DNA standard and the proportions obtained after a V4 and a V4V5 sequencing with Illumina MiSeq*

| | ZymoBIOMICS™ DNA standard theoretical composition | V4V5 sequencing | | V4 sequencing | |
|---|---|---|---|---|---|
| | Proportion (%) | Number of sequences | Proportion (%) | Number of sequences | Proportion (%) |
| **Bacillus** | **15,7** | 17027 | 18,6 | 28673 | 21,5 |
| **Lactobacillus** | **18,8** | 16283 | 17,8 | 9895 | 7,4 |
| **Staphylococcus** | **13,3** | 13483 | 14,7 | 25039 | 18,8 |
| **Listeria** | **15,9** | 12338 | 13,5 | 20457 | 15,3 |
| **Salmonella** | **11,3** | 11141 | 12,1 | 14919 | 11,2 |
| **Escherichia/Shigella** | **10** | 9043 | 9,9 | 16100 | 12,1 |
| **Enterococcus** | **10,4** | 7311 | 8,0 | 11936 | 9,0 |
| **Pseudomonas** | **4,6** | 5055 | 5,5 | 6173 | 4,6 |
| **Bacteria_unclassified** | | 14 | 0,0 | | |
| **Porphyrobacter** | | 4 | 0,0 | | |
| **Limnobacter** | | 2 | 0,0 | 8 | 0,0 |
| **Methylobacterium** | | 2 | 0,0 | 3 | 0,0 |
| **Lactobacillales_unclassified** | | 2 | 0,0 | | |
| **Enterobacteriaceae_unclassified** | | | | 33 | 0,0 |
| **Bacilli_unclassified** | | | | 58 | 0,0 |
| **Bacillales_unclassified** | | | | 21 | 0,0 |
| **Clostridiaceae_1_unclassified** | | | | 2 | 0,0 |
| **Acinetobacter** | | | | 2 | 0,0 |

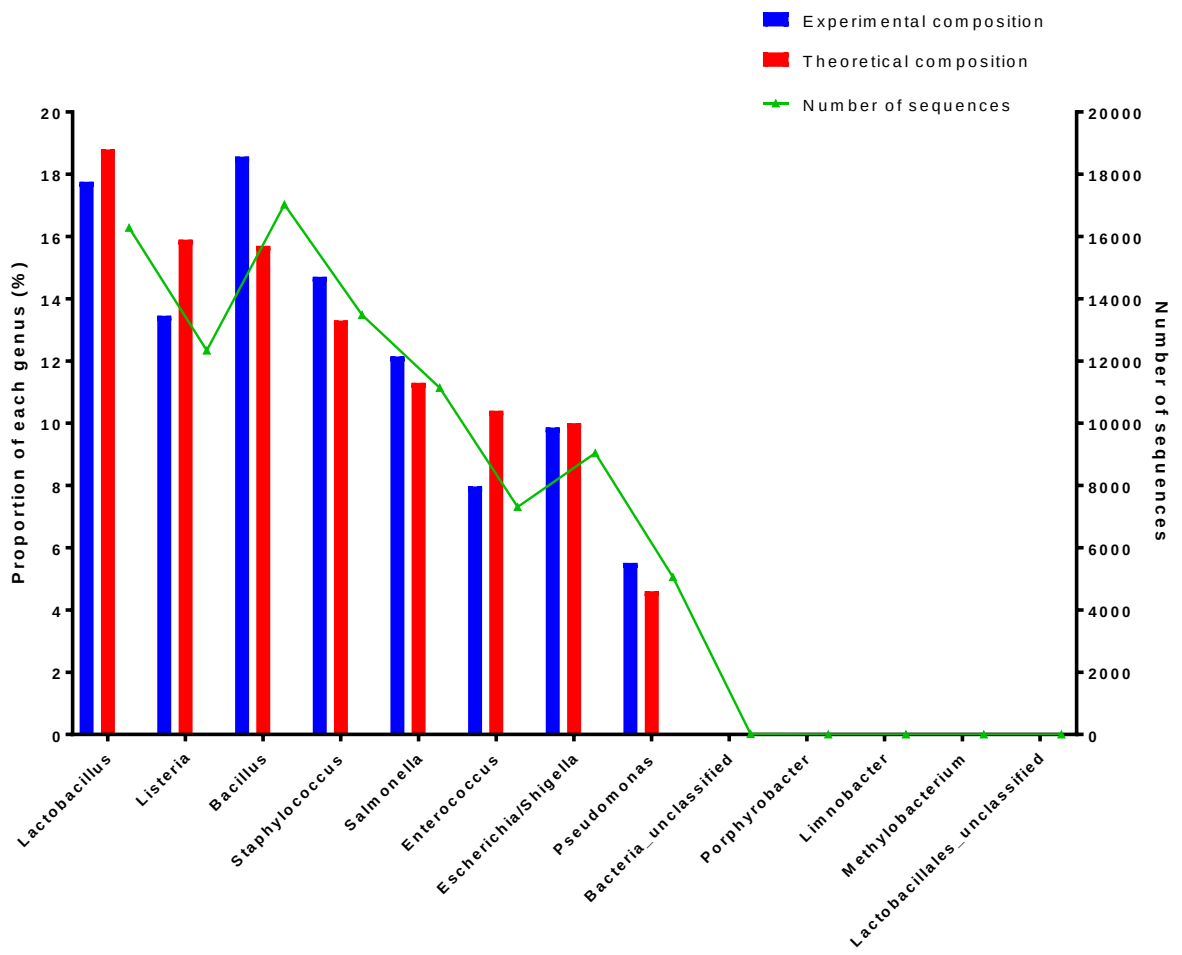



***Figure 2.1: Mock analysis after a V4V5 metagenetics sequencing.*** *Comparison between the theoretical composition of the mock sample (red) and the experimentally established composition (blue) identified after a V4V5 metagenomics sequencing and OCToPUS analysis. Green curve: number of sequences identified for each OTUs*

The identification quality after a V4 16S rDNA sequencing was assessed. Differences could be observed concerning the proportion of each bacteria in the mock (Fig. 2.2). Moreover, the detection of false-positive was also noticed and in a bigger proportion than after a V4V5 regions sequencing.

After the V4 sequencing and analysis, the identification of *Lactobacillus* was lower than expected (7.4%) while the proportion of *Bacillus* was greater (21.5%), as *Staphylococcus* (18.8%) (Fig. 2.2). Theoretically the proportion of *Lactobacillus* was 18.8% and the one of *Bacillus* was 15.7% while the proportion of *Staphylococcus* was 13.3%. It appeared that the excess of sequences identified as *Bacillus* and *Staphylococcus* could correspond in reality to the missing sequences observed for *Lactobacillus*. These three genera all belong to the *Bacillales* order and are thus phylogenetically close. A 5.8% and 5.5% differences (for *Bacillus* and *Staphylococcus* respectively) can indeed be observed between a V4 sequencing and the theoretical composition of the DNA standard. The sum of both differences is 11.3%, which is similar to the 11.4% missing sequences in *Lactobacillus*. Different OTUs, not present in the sample, were also identified. However, they represent less than 0.1% of the sample and sequences (Table 2.1). The number of sequences of the first OTU not present in the sample was 33, out of 133319 sequences (0.025%). This OTU was identified as “Enterobacteriaceae_unclassified” (Table 2.1). The proportion of false-positive represented 0.025% of the sample after a V4 sequencing analysis. The cut-off, with a margin, would be settle at 0.026%.

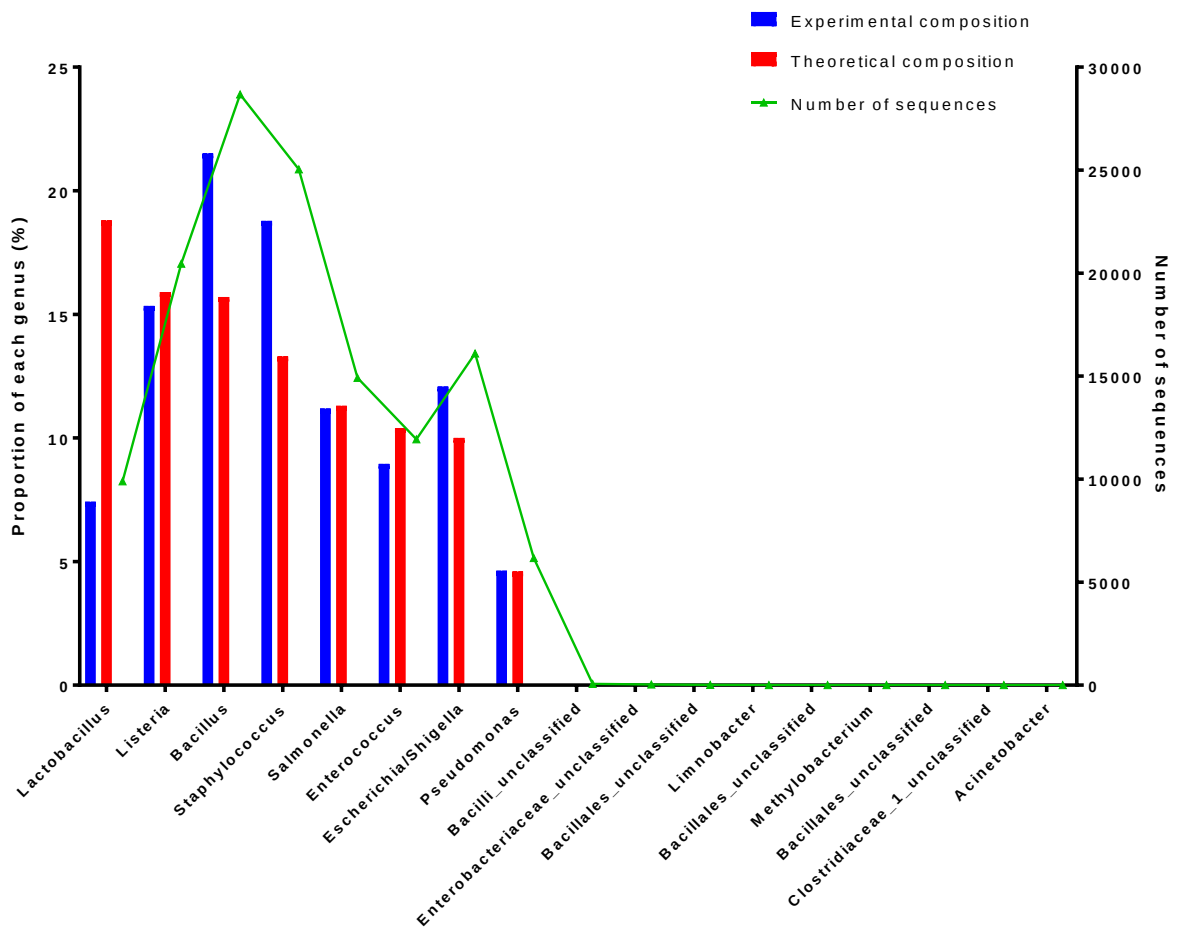



***Figure 2.2: Mock analysis after a V4 metagenetics sequencing.*** *Comparison between the theoretical composition of the mock sample (red) and the experimentally established composition (blue) identified after a V4 metagenomics sequencing and OCToPUS analysis. Green curve: number of sequences identified for each OTUs*

*Determination of taxonomical level identification and sequencing quality for both V4 and V4V5 sequencing*

Differences could be observed between a V4 and a V4V5 sequencing with the theoretical composition of the DNA standard. These differences were greater with a V4 sequencing than a V4V5 sequencing (Table 2.2). The under-identification of *Lactobacillus* after a V4 sequencing can be highlighted by the observed standard deviation (more than 8). Similarly the over-identification of *Bacillus* and *Staphylococcus* were also observed (4.1 and 3.9 respectively). The standard deviation with the theoretical DNA standard was closer to 0 with the V4V5 sequencing compared to the V4 sequencing. A V4V5 sequencing appeared thus to be more precise in OTU attribution than a V4 sequencing.

*Table 2.2: Standard deviation of each OTU after a V4 and V4V5 sequencing compared to the theoretical mock*

| | Standard deviation with the theoretical composition | |
|---|---|---|
| | V4V5 sequencing | V4 sequencing |
| **Bacillus** | 2,03 | 4,11 |
| **Lactobacillus** | 0,74 | 8,05 |
| **Staphylococcus** | 0,99 | 3,88 |
| **Listeria** | 1,73 | 0,39 |
| **Salmonella** | 0,60 | 0,08 |
| **Escherichia/Shigella** | 0,10 | 1,47 |
| **Enterococcus** | 1,72 | 1,02 |
| **Pseudomonas** | 0,65 | 0,02 |

As expected, the taxonomical identification with OCToPUS was deeper after a V4V5 sequencing than after a V4 sequencing. The information loss between the family and genus taxonomical levels was indeed smaller for V4V5 (9.9%) than for V4 (12.1%). Some loss was also observed between the class and the order level after the analysis and identification of the V4 sequencing (Table 2.3).

*Table 2.3: Number of sequences identified at a specific taxonomic level for the V4V5 and the V4 sequencing and percentage of loss between each taxonomical level*

| | V4V5 | | V4 | |
|---|---|---|---|---|
| | *Number of sequences* | *Loss (%)* | *Number of sequences* | *Loss (%)* |
| **Kingdom level** | 91705 | | 133319 | |
| **Phylum level** | 91691 | 0,015 | 133319 | 0 |
| **Class level** | 91691 | 0 | 133319 | 0 |
| **Order level** | 91691 | 0 | 133261 | 0,044 |
| **Family level** | 91689 | 0,002 | 133240 | 0,016 |
| **Genus level** | 82646 | 9,861 | 117105 | 12,103 |

The quality of sequencing and analysis were also compared. Using only a V4 metagenetic sequencing, the number of errors and unattributed sequences was lower than using a V4V5 sequencing (Fig. 2.3). If 66% of the reads were kept and analyzed after a V4 sequencing, only 52% were kept after a V4V5 sequencing.

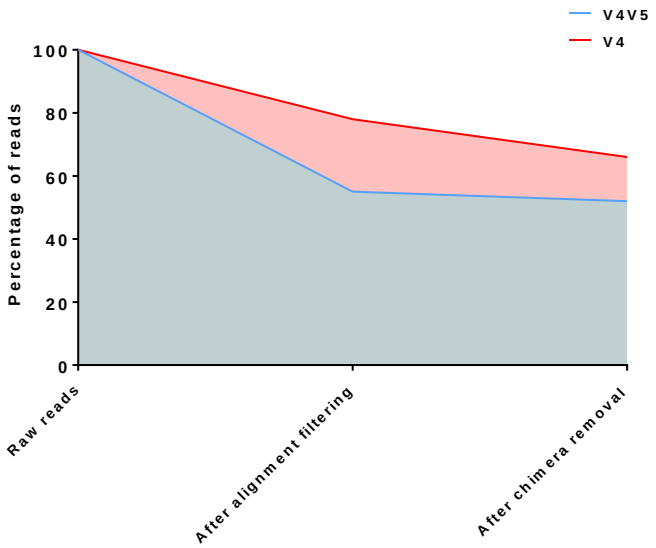



***Figure 2.3: Reads removal on the mock sample after a V4 or V4V5 metagenetic sequencing.*** *In blue: % of V4V5 reads obtained after each treatment, in red: % of V4 reads obtained after each treatment.*

Furthermore the sequencing error rate determined by OCToPUS was lower for the V4 sequencing (0.01%), even though both error rates were extremely low (Table 2.4), confirming the sequencing quality performed by LGC Genomics and the analysis of OCToPUS.

*Table 2.4: Sequencing error rates determined after OCToPUS treatment on the ZymoBIOMICS™ mock sample after a V4 and V4V5 sequencing*

| | V4 | V4V5 |
|---|---|---|
| Error rate (%) | 0.01 | 0.02 |

The sequencing quality was thus greater after a V4 sequencing, which can be explained by the size of the amplicon (291 bp for V4 *vs.* 411 bp for V4V5). With 291 bp it is possible to have overlapping sequences after sequencing, allowing a better obtained sequence quality. With 411 bp only the end of the sequences can overlap. The sequences' end obtained after sequencing is of lower quality than the beginning [277].

Despite better sequencing quality, V4 sequencing does not offer a satisfying taxonomical identification level compared to V4V5 sequencing. It also presented a greater threshold (0.025%) than V4V5 sequencing (0.016%) concerning the identification of sequences that were not present in the initial sample.

***Comparison of different methods for microbial harvesting and DNA extraction***

Different samplings were performed at three different locations (A: bottom of the pool, B: above the chimney, and C: inside the chimney) in the pool. Three different methods were tested: centrifugation of the water followed by DNA extraction with phenol-chloroform-isoamyl alcohol (method 1), filtration of the water followed by DNA extraction with phenol-chloroform- isoamyl alcohol (method 2), and filtration of the water followed by DNA extraction with DNeasy® PowerWater® kit (method 3). The objective was to test different harvesting and extraction methods to obtain as much diversity present in water as possible.

After sequencing and OCToPUS analysis, 100000 ± 5000 sequences were obtained using method 3. Only 3696 sequences were obtained with method 1 at location C after analysis. Nonetheless 84000 ± 15000 sequences were obtained with method 1 at locations A and B. About 64500 ± 355000 sequences were obtained using method 2 after OCToPUS analysis (Table 2.5).

*Table 2.5: Number of sequences obtained after a V4V5 Illumina MiSeq sequencing and after the OCToPUS analysis*

| | **Number of sequences** |
|---|---|
| **Loc. A - meth. 1** | 71157 |
| **Loc. A - meth. 2** | 99970 |
| **Loc. A - meth. 3** | 127024 |
| **Loc. B - meth. 1** | 100394 |
| **Loc. B - meth. 2** | 45166 |
| **Loc. B - meth. 3** | 105307 |
| **Loc. C - meth. 1** | 3696 |
| **Loc. C - meth. 2** | 28977 |
| **Loc. C - meth. 3** | 95759 |

For the OTUs common to the different methods, the proportion was determined using the mean of the values obtained for each method. Some differences could be observed between the three methods. However the main OTUs were all common. A few OTUs were present at only one location and in a very weak proportion (Fig. 2.4). It can be observed that few differences were observed between method 3 and methods 1&2 at locations A and B (only 2 and 7 genera only detected with method 3, respectively). The opposite was observed for the sample collected at location C. 20 genera were only detected using method 3 and only 3 were common to all three methods at location C. When comparing methods 1 and 3 or 2 and 3, no genera were common to both methods (except for the 3 genera common to all three methods). This observation could be confirmed by the ANOVA analysis (Table 2.6). It can be observed that at both locations A and B, methods 1 and 2 were different within each other. Methods 2 and 3 were not significantly different. However at location C, methods 1 and 2 were similar while method 3 was significantly different to methods 1 and 2 (Table 2.6). Table 2.6 should however be considered carefully since no replicates were performed.

***Table 2.6: ANOVA analysis of the three different methods.*** *Mann-Whitney pairwise with raw p values and uncorrected significance (obtained from Past software [278]). In bold: significant difference obtained when p-value<0.05.*

**Location A**

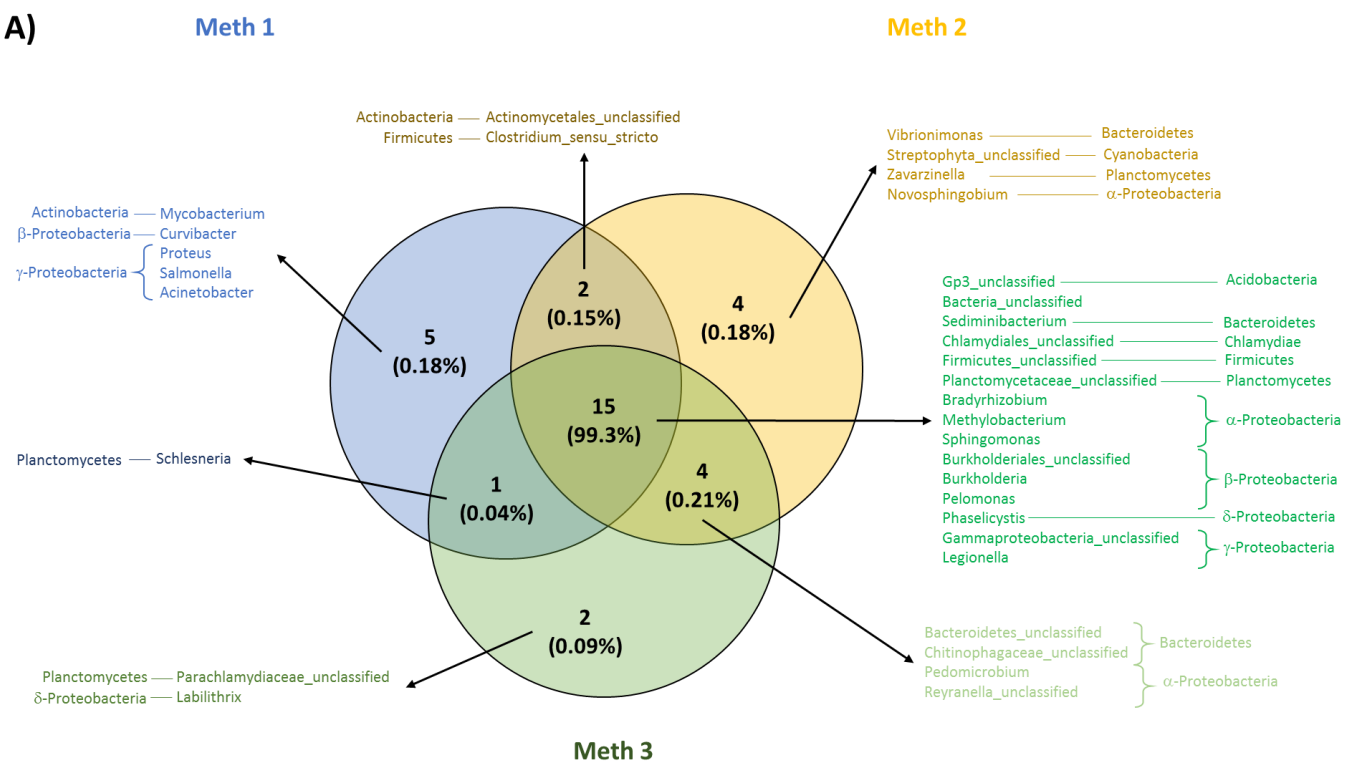
| | Meth. 1 | Meth. 2 | Meth. 3 |
|---|---|---|---|
| **Meth. 1** | | **0,000** | **0,008** |
| **Meth. 2** | **0,000** | | 0,221 |
| **Meth. 3** | **0,008** | 0,221 | |

**Location B**

| | Meth. 1 | Meth. 2 | Meth. 3 |
|---|---|---|---|
| **Meth. 1** | | **0,024** | 0,889 |
| **Meth. 2** | **0,024** | | 0,075 |
| **Meth. 3** | 0,889 | 0,075 | |

**Location C**

| | Meth. 1 | Meth. 2 | Meth. 3 |
|---|---|---|---|
| **Meth. 1** | | 0,244 | **0,000** |
| **Meth. 2** | 0,244 | | **0,000** |
| **Meth. 3** | **0,000** | **0,000** | |



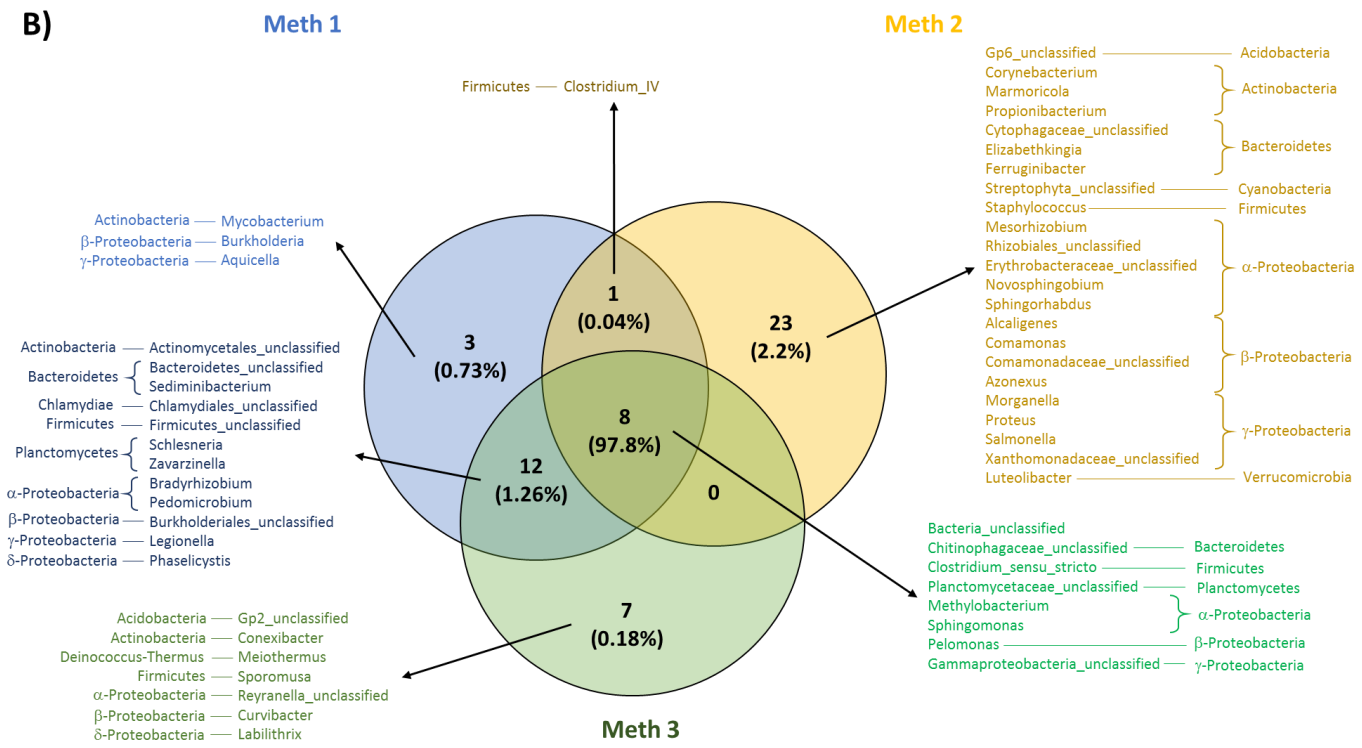



***Figure 2.4: Venn diagram after a V4V5 metagenetics sequencing at A) Loc. A, B) Loc. B, C) Loc. C.*** *Only the OTUs above the 0.016% threshold were selected. In brackets: proportion of the OTUs in the sample.*

## Discussion

The sequencing error rate observed on the mock sample for the V4 metagenetic sequencing was lower than the error rate on the V4V5 sequencing (0.01% *vs*. 0.02% respectively). However both can be considered as good and similar sequencing error rates. The high number of eliminated reads (Fig. 2.3) can be explained by a poor DNA quality and/or quantity sent to be sequenced (cf. Appendix 3). The quality of the V4 sequencing was sensitively higher due to the length of the region. V4 is indeed smaller than V4V5, allowing a bigger read depth for one DNA amplicon.

The identification of the OTUs after a V4 sequencing was not as precise as after a V4V5 sequencing. The attribution of some closely related organisms can indeed have been mixed. For example, the excess of sequences identified as *Bacillus* and *Staphylococcus* could correspond in reality to the missing sequences observed for *Lactobacillus*. These three genera all belong to the *Bacillales* order and are thus phylogenetically close. It has been previously shown that closely related bacteria cannot be solely identified using V4 sequencing, such as the *Enterobacteriaceae* and *Peptostreptococcaceae* family [279]. Moreover the number of false-positive was higher in the V4 sequencing of the mock sample compared to the V4V5 sequencing. Considering the higher number of false-positive and the lack of depth for the taxonomical identification after a V4 sequencing, this study confirms that V4V5 regions of the 16S rDNA sequencing is recommended over the sequencing of only V4 region.

The methods of microbial harvesting and filtration as well as which DNA extraction protocol was used are complementary. However when looking at the reads number, method 1 presented less sequences, perhaps due to a lack of microorganisms: during the centrifugation, the cell density was so low that not all microorganisms could have been pelleted. It was shown that at two different locations, methods 1 and 2 were significantly different while method 3 was similar to method 2. An important discrepancy between centrifugation (method 1) and filtration (methods 2 and 3) could be observed at location C (between 67000 and 97000 sequences differences with methods 2 and 3 respectively). In this location method 3 and was significantly different to methods 1 and 2, but methods 1 and 2 were similar. The differences observed between the three methods depending on their sampling's location could be explained by the low number of sequences (3696) obtained with method 1 at location C. The second lowest sequence number was 28977 (obtained using method 2 at location C) while the greater sequence number was 127027 (obtained using method 3 at location A) (Table 2.5). This low number of analyzed sequences could have influenced the observed diversity which would have caused the lack of differences between methods 1 and 2 at location C while being extremely different to method 3 at the same location (95759 obtained sequences).

## Conclusion

Metagenetics has been widely used [198, 229-231, 266, 272, 280-282]. There are however biases introduced during the several steps of DNA extraction and sequencing. To overcome these biases the use of a threshold should be used to eliminate any false-positive in the analysis. Eliminating false-positives appears to be critical to analyze microbiota present in extreme environments. In a nuclear reactor cooling pool the identification of different microbial genera can be of great interest and results should be trusted. By identifying a sequence artefact in such environment could lead researches in the wrong direction. The attribution at the genus level could also be biased by the length of the sequence and the close phylogenetic relationship between several genera. In this study the sequencing of the V4V5 regions was recommended

over the sequencing of only the V4 region. Even though V4 presented a better quality of read it also lacked of taxonomical identification. The taxonomical identification level presented a better depth after a V4V5 sequencing. Moreover the number of false-positive was higher after a V4 sequencing compared to the one obtained after a V4V5 sequencing. As a result to this study a threshold of 0.016% is suggested when using an Illumina MiSeq metagenetics sequencing of the V4V5 regions of the 16S rDNA followed with an OCToPUS analysis.

Both centrifugation and filtration can be used to harvest the microorganisms. However filtration provided more sequence number than centrifugation. Filtration was also faster and easier to implement than centrifugation, especially for large volumes. The authors recommend using both methods to treat liquid samples. It is however suggested to favor filtration if only one method is to be used. Even though more diversity was detected using method 3, it provided a lot of false-positive sequences and the quantity of DNA obtained after DNA extraction was lower than after a phenol-chloroform-isoamyl alcohol (Appendix 3). The phenol-chloroform-isoamyl alcohol DNA extraction has to be performed in a secure environment and under a fumehood. For this reasons this protocol, even if the DNA is extracted in higher quantity, is not to be favored in constrained environment. Depending on the sampling and DNA extraction condition the above recommendation could be optimized to recover as much organisms and DNA as possible.

### 2.1.2 Taxonomical composition of a biofilm sampled from a nuclear fuel pool assessed by metaproteomics (*article in preparation*)


## Abstract

Sampling small amounts of biofilm samples from difficult environments such as biofilm present on walls from a pool used to store nuclear elements does not give many analytical options if taxonomical characterization and estimation of the different biomass contributions are the objectives. While DNA extraction and 16S/18S rRNA amplification and sequencing is the most widely applied methodology, its reliability has been challenged as yields can be species-dependent. In this work, we propose a new proteotyping approach consisting in metaproteomics data acquisition and interpretation against a generalist database. Peptide sequence information obtained by high resolution tandem mass spectrometry is transformed into useful taxonomical information that allows to obtain the different biomass contributions at different taxonomical ranks. This novel methodology is applied for the first time to analyse the composition of biofilms harvested from a pool used to store radioactive sources in a nuclear facility from minute amounts of material. We report the identification of three genera: *Sphingomonas*, *Caulobacter*, and *Acidovorax*.


## Introduction

Some microorganisms can subsist and even flourish in extreme conditions in which most multicellular organisms, like animals or humans, cannot survive. Bacteria, fungi, archaea, and micro-algae have indeed been found in acidic or alkaline media, in environments with extremely low or high temperature, high pressure, or high salt concentration [12, 13, 52, 168, 176, 283]. Some of these organisms are known to withstand high radiation doses. *Deinococcus radiodurans* was the first radioresistant organism described in 1956 [14]. It is able to withstand ionizing radiation doses as high as 20 kGy with a $LD_{50}$ of 6 to 10 kGy [81], that is 1000 to 2000 times the human $LD_{50}$. Since then, many radioresistant microorganisms have been reported, such as *Deinococcus deserti* isolated from the Sahara desert, the archaea *Thermococcus gammatolerans* isolated from a deep-sea hydrothermal vent, the green micro-algae *Coccomyxa actinabiotis* isolated from a pool used to store spent nuclear fuels and several fungi isolated particularly from the Chernobyl damaged nuclear reactor [13, 52, 168, 176]. While numerous studies have been focussed on deciphering the molecular mechanisms of these model organisms explaining their remarkable tolerance properties, *in situ* environmental studies are relatively scarce. A variety of bacteria belonging to the genera *Bacillus, Burkholderia, Micrococcus, Pseudomonas, Ralstonia* or *Staphylococcus* have been identified in a French storage pool and in the cooling pool of a Spanish nuclear power plant [184, 185, 189].

In nature, many microorganisms can form biofilms as a mean to colonize a specific environment and facilitate their long-term fate. For this, different microorganisms stick generally onto a surface, embedded within a self-produced matrix of extracellular polymeric substances, and may further colonize their surroundings. This specific growth pattern is indeed more efficient than the planktonic form, because protective against external conditions and their associated changes. Inside a biofilm, the pH can be appropriately modified, and nutrients and organic material may be more easily recycled, creating more favourable conditions for microorganism survival and growth [16, 284]. The advantages obtained by

growing as biofilms can explain the fact that in aquatic environment less than 0.1% of the bacteria are under planktonic form [16]. As a consequence, microbial biofilms have been detected in very inhospitable places such as used nuclear fuel pools [176, 181].

Assessing the microbial diversity of aquatic environments is usually done by extraction of the DNA content from the sample, followed by 16 or 18S rRNA gene amplification and sequencing [211, 285]. This approach has been challenged because the number of copies of the 16S rRNA gene per chromosome and the number of copies of the chromosomes in bacteria may be different depending on the strains and their physiological state [235]. Moreover, the DNA extraction may be not rigorously identical depending on the microorganisms present and the type of biofilms to analyse. Last, the amplification of 16S rRNA gene from archaea or bacteria, and the amplification of 18S rRNA gene from eukaryotes are done with different sets of primers. Moreover, within the same taxonomical group the neighbouring sequences of the 16/18S rRNA may differ influencing the amplification rate. Thus, for all these reasons the amplification may not be scrupulously identical depending on the strains and phyla present in the sample. Alternatives for taxonomy purpose may rely on the cataloguing of genomic sequences (metagenomics) or protein sequences (proteotyping). While metagenomics gives a glimpse on the potential of the microorganisms present in a sample, metaproteomics is devoted to the global study of proteins present in a complex sample comprising numerous different organisms for better understanding the pathways with key roles and the physiology of the main players, and study the interactions of a microbial community with its environment [286].

Metaproteomics has been used to get new insights into biofilms [14-16]. Several studies have been performed on acid mine drainage biofilms in the USA where metaproteomics coupled with metagenomics showed the power of combining both methods to study closely related microorganisms within their environment. The genotype of the main phyla and the location of the sample were shown to influence the proportion or the presence of a bacteria belonging to the *Leptospirillum* genus, the dominant organisms in the biofilm [244]. Proteomics combined with genomics provided direct insights into the functioning of the organisms constituting such biofilms in their natural environment, where they have to challenge extreme acidity and metal toxicity. These investigations also provided insights into the functioning of these organisms as consortia, highlighting how essential functions such as nitrogen fixation and biofilm polymer production are partitioned among members of the community. Last, one highly abundant protein of initially unknown function was found to be an iron-oxidizing cytochrome, a key enzyme for energy production in these biofilms [244]. Comparative proteogenomics of these communities revealed how genomic variation in closely related bacteria could contribute to their ecological divergence [287]. Metaproteomics highlighted diverse post-translational modifications specific to the biofilm growth stage [288]. These microbial communities were further grown in bioreactors and metaproteomics revealed metabolic characteristics such as species-specific hydrogen isotope fractionation in proteins [289], nitrogen flows through the consortia [290], and change in the proteome of individual organisms within the biofilm resulting from an increase in temperature [291]. Other studies on environmental biofilm communities have addressed marine biofilms [239, 292] and wastewater treatment biofilms [293, 294]. Taxonomical classification and functional potential of microbial communities harvested from ship hulls have been obtained [292], and the activities of key microorganisms responsible for organic pollutant degradation in anaerobic wastewater treatment biofilms have been investigated [294]. The most common strategy for conducting a metaproteomics study is first to collect metagenomics data on the same sample in order to obtain a protein sequence database specific of the sample. For this, either a systematic six frame translation of the reads or contigs may be performed or their tentative annotation, leading to an inflated

proteogenomics protein sequence database. Then, MS/MS spectra recorded from the peptides generated by proteolysis of the extracted proteins are assigned based on this metagenomics information [292, 294]. However, for precious samples obtained in low amount, or for a quicker analysis, obtaining this metagenomics information may be far too complex.

In this work, we propose a new proteotyping approach consisting in metaproteomics data acquisition and interpretation against a generalist database. Then, the assigned peptides are analysed as a whole dataset in terms of taxonomical information. This method is applied for the first time to decipher the composition of biofilms harvested from a pool used to store radioactive sources in a nuclear facility. A procedure for recovering few milligrams of biological material from this hostile environment and compatible to proteotyping of mixtures of microorganisms is also proposed.

## Materials and Methods

***Installation studied***

The biofilm samples were recovered in a 5.5 m deep pool used to store radioactive sources and perform irradiation experiments in CEA – Saclay, France. The pool contained reverse osmosis-purified water supplemented with tap water. Its conductivity, pH and temperature were 127 µS/cm, pH 7.8, and 26°C, respectively. The analysis of the major anions and cations revealed the presence of chloride, sodium, potassium, calcium, and magnesium at respective mean concentrations of 2.2 mg/L, 5.9 mg/L, 5.8 mg/L, 1.6 mg/L, 41 mg/L, and 1.7 mg/L. Nitrates, phosphates, and sulphates were below the quantification limit of 5 mg/L, 0.2 mg/L, and 10 mg/L, respectively. No radionuclides were detected. The water was filtered daily on resins for 1 hr. When irradiation sources were present in the pool, the dose rate on the wall on which the biofilms were collected reached 100 Gy/h.

***Biofilm collection***

Biofilms were sampled using sterile Spontex® sponges (Spontex-Mappa). For each sampling, a sponge was fixed with duct tape on a metal pole and was then used to scrub the wall under water. After scrubbing a surface of about 25 $cm^2$, the pole was slowly pulled up, the duct tape was removed, and the sponge was introduced into a sterile hermetic bag for transport. Once in the laboratory, each sponge containing the biofilm was then introduced inside a 1 L sterile flask with 200 mL of sterile water. To obtain the biofilm, the sponge was stirred with a sterile spatula and the flask was shaken. The water was recovered and centrifuged for 20 min at 15,000 *g* at room temperature. The entire supernatant but 1 mL was discarded and the pellet was dissolved in this mL volume. For the metaproteomics analysis, a volume of 750 µL of the suspension was stored at -80°C prior to metaproteomics analysis. The rest was preserved in Glycerol Strain Buffer (65% glycerol v/v, 0.1 M $MgSO_4$, 0.025 M Tris-HCl pH 8), flash-frozen in liquid nitrogen, and stored at -80°C.

***Protein extraction and trypsin proteolysis***

After thawing the sample tube on ice, cells and cellular debris were precipitated by adding a volume of trichloroacetic acid solution prepared at 50% in water to obtain a final concentration in acid of 10%. The sample was then centrifuged for 10 min at 16,000 g. The resulting pellet was dissolved in a volume of 250 µL of LDS 1X Laemmli buffer (Invitrogen) consisting in 106 mM Tris/HCl, 141 mM Tris base, 2% lithium

dodecyl sulfate, 10% glycerol, 0.51 mM EDTA, 0.22 mM SERVA Blue G250, 0.175 mM phenol red, buffered to pH 8.5 and supplemented with 2.5% beta-mercaptoethanol. The sample was heated at 99°C for 5 min followed by a treatment for 5 min in an ultrasonic bath. The sample was introduced into a 2 mL Precellys tube (Bertin Technologies) containing 200 mg of glass beads. The samples were subjected to 3 cycles of grinding at 6,500 rpm for 20 sec followed by 30 sec rest by means of a Precellys grinder (Bertin technologies). After grinding, cellular debris were centrifuged for 1 min at 16,000g. The supernatant was heated for 10 min at 99°C. Then, the sample was loaded onto a 4–12% gradient 12-well NuPAGE gel (Invitrogen). The proteins were subjected to electrophoresis for 5 min. After Coomassie Blue Safe staining (Invitrogen), the polyacrylamide band corresponding to the whole proteome was sliced. The polyacrylamide band was then destained with ultra-pure water, reduced with dithiothreitol, and treated with iodoacetamide, before proteolysis with Trypsin Gold Mass Spectrometry Grade (Promega) in the presence of 0.01% ProteaseMAX surfactant (Promega), as previously described by Hartmann et al. [295].

***NanoLC-MS/MS analysis***

The extracted peptides (10 µL) were analyzed with an ESI-Q Exactive HF mass spectrometer (Thermo) equipped with an ultra-high field Orbitrap analyser and coupled to an Ultimate 3000 176 RSL Nano LC System (ThermoFisher). This analysis was performed in data-dependent mode*, i.e.* fragmentation and MS/MS spectra acquisition was done on specific peptide ions identified by their molecular masses divided by their electric charge. Peptides were injected onto a reverse phase Acclaim PepMap 100 C18 column (3 µm, 100 Å, 75 µm id x 500 mm) and resolved at a flow rate of 0.2 µL/min with a 60 min gradient of $CH_3CN$ in presence of 0.1% formic acid. The gradient during MS/MS acquisition consisted in 50 min from 2 to 20% $CH_3CN$, followed by 10 min from 20 to 32% $CH_3CN$. A quick wash of the column was done with an additional gradient from 32 to 72% $CH_3CN$ in 1 min, before column equilibration with 2% $CH_3CN$. The Q-Exactive HF instrument was operated with specific parameters with a Top20 strategy: full scan mass spectra were acquired from m/z 350 to 1,500 *m/z* with an Automatic Gain Control Target and a resolution set at $3x10^6$ ions and 60,000, respectively, for the MS scan, and $1x10^5$ ions and 15,000, respectively, for the MS/MS scan. MS/MS scan acquisition was initiated when the threshold intensity of 170,000 was reached. The maximum IT was 60 msec and the isolation window for the quadrupole was 1.6 *m/z*. Only ions with potential charge states of 2+ and 3+ were selected for MS/MS with a dynamic exclusion of 10 sec.

***Proteotyping MS/MS data interpretation***

For the proteotyping interpretation of MS/MS spectra, the database used was the National Center for Biotechnology Information non-redundant fasta file (NCBI-nr) which was downloaded the 9th of November, 2015 as ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nr.gz. This version comprises 76,068,736 protein sequence entries totalling 27,658,295,194 amino acids. Corresponding taxonomy files gi_taxid_prot.dmp, names.dmp and nodes.dmp were downloaded the same day as ftp://ftp.ncbi.nlm.nih.gov/pub/taxonomy/taxdmp.zip. The Mascot search engine (Matrix science) was used for peptide inference. Peptide-to-MS/MS spectrum assignation with Mascot was done with the following parameters: full trypsin specificity, maximum of one missed cleavage, mass tolerances of 5 ppm on the parent ion and 0.02 Da on the MS/MS, static modification of carboxyamidomethylated cysteine (+57.0215), and oxidized methionine (+15.9949) as dynamic modification. Post-processing of Mascot dat files was done as follows with Python version 2.6.6 procedures. Mascot DAT files were parsed using the Python version of Matrix Science msparser version 2.5.1 with function ms_peptidesummary

(http://www.matrixscience.com/msparser.html). Peptide-Spectrum Matches (PSMs) were validated with a Mascot expectation value below 0.05 using Mascot identity threshold (MIT), and allowing multiple PSMs per MS/MS spectrum. Peptides associated to spectra were assigned to taxa using NCBI databases. In the 2015 version of NCBI data files, each header of the nr.fasta file was the concatenation of the complete set of protein identifiers matching the sequence, with a gi identifier per entry. The taxonomy file gi_taxid_prot.dmp was used to perform gi to taxid matching. For "WP_" Refseq accessions, file ftp.ncbi.nlm.nih.gov/refseq/release/release-catalog/release73.AutonomousProtein2Genomic dated 12/14/2015 was used to complement gi to taxa mapping. PSMs were directly mapped to taxa using both peptide to gi from Mascot, and gi to taxa mappings. In addition to the raw number of PSMs per taxon obtained (further called TSMs for Taxon-Spectrum Matches), a collation of information was performed following the NCBI taxonomical tree from direct assignation to "canonical" taxonomical levels: species, genus, family, order, class, phylum, superkingdom. For each taxon at each level, an identification of matching peptide sequences and TSMs was performed, as well as a count of specific or unique peptide sequences and corresponding specific PSMs.

## Results

***Selection of the nuclear facility and biofilm sampling***

We proposed a direct sampling of biofilm from the walls of a pool used to store radioactive sources. In such peculiar environment, it appears difficult to introduce experimental support and let biofilms grow for several weeks or months. The pool chosen for this case study did not contain any radionuclides, letting a direct manipulation of collected biofilms for full characterization with omics tools. The facility selected is used to carry out irradiation experiments with cobalt irradiation sources either positioned in a concrete blockhouse or in the pool next to the bottom. During storage, sources are maintained under water. In the pool, they are positioned at specific locations near the bottom northern wall. The pool is supplied with reverse osmosis-purified water supplemented with tap water, and its water is oligotrophic and slightly alkaline. It contains very few nutrients, the main macronutrients such as nitrate, phosphate, and sulphate being undetectable. The water surface is in contact with the air. Figure 2.5 shows the pool surface and the strategy for protein extraction, peptide analysis by tandem mass spectrometry and metaproteomics analysis of the data. A first biofilm (Biofilm A) was collected at 4 m below the surface water on a wall intermittently irradiated by gamma radiations at a dose rate of 100 Gy/h. Another biofilm (Biofilm B) was collected near the surface water of this wall (1 m deep). Two other biofilms were collected at 4 m below the surface (Biofilm C) and near the surface (Biofilm D) of the western wall.

Most investigations performed on biofilms in aquatic environment were carried out either by direct sampling at the air/water interface or after removal of the biofilm support from the water and scraping the biofilm with a spatula [239, 296, 297]. In this study however, the support could not be taken out of the water, nor could a diver be sent to collect the biofilms. Here, we proposed to detach directly underwater the biofilm from the wall with a sponge rather than with a spatula in order to recover more easily the sample and avoid the sample loss when brought back to the surface. We choose to use a Spontex abrasive sponge, a cellulose sponge equipped with a scour abrasive pad made of synthetic fibers chemically bonded together with a resin. Sampling at 4 m below the surface water necessitated to use a sponge fixed onto a pole with duct tape. This specific sponge enabled an easy manipulation several meters under water as the sponge fibers imprisonned part of the biofilm, allowing its recovery. After scrubbing the wall, the sponge was

recovered and some biofilm material could be directly observed by means of a slight yellowish color. After tape removal, the sponge was stored inside a sterile hermetic plastic bag. Once in the analytical laboratory, the sponge was subjected to vigorous shaking with sterile water in order to detach the biological material from the sponge fibers. After sponge removal, the resulting liquid was centrifuged for pelleting the biological material. The supernatant was removed and the biological pellet was stored at -80°C until metaproteomics analysis.

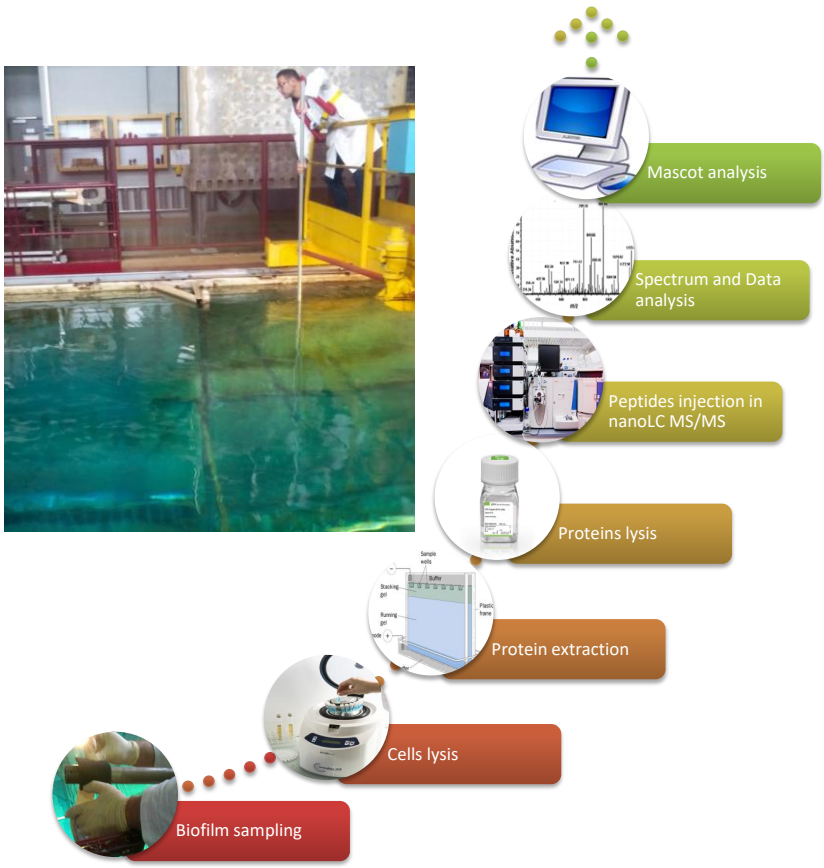



*Figure 2.5: Strategy implemented to collect the biofilms and for their direct analysis using metaproteomics*

***Proteotyping the microbial composition of biofilm by phylopeptidomics***

Microbial cells were lyzed by bead beating in presence of Laemmli buffer. The resulting proteins were loaded for a short migration onto a SDS-PAGE gel. As shown in Figure 2.5, the denatured polypeptides were fragmented into peptides with trypsin and the resulting peptides were characterized by high resolution tandem mass spectrometry. Due to scarce biological material recovered from the sponge, only one analytical run could be performed per sample. For proteotyping the microbial composition of biofilm, we applied the phylopeptidomics methodology which allows accurate quantification of the relative ratios of mixtures of microorganisms (Pible, Armengaud, et *al.*, submitted). Briefly, the MS/MS spectra were interpreted against NCBInr, a generalist database comprising annotated proteomes from several tens of thousands of reference organisms. After this metaproteomics interpretation of mass spectrometry data, a MS/MS spectrum may correspond to a peptide sequence found in the database. If this peptide sequence is

shared with various organisms or not, this specific taxonomical information is kept at the different possible taxon ranks, resulting in taxon-to-spectrum matches (TSMs). This information is complementary to the number of peptide sequences specific of one taxonomical branch, which should logically ascertain the presence of this taxonomical branch in the sample, *i.e.* taxon-specific peptide sequence. The proteotyping capability of the phylopeptidomics method applied here (version 1.0) is based on the validation of taxa, from the superkingdom taxonomical level down to the subspecies level, using both corresponding specific peptides and additional TSMs added to the pool of TSMs attributed to taxa already validated.

The taxonomical ranks considered in this study are: superkingdom, phylum, class, order, family, genus, species, and subspecies, forming lineages starting at the top Bacteria-Eukarya-Virus-Archaea (BEVA) taxon and going down the list of ranks. At each taxonomical level, children taxa of already validated parent taxa (BEVA is pre-validated) were assessed using the following thresholds: 7 specific peptides, or max(#totalTSMs/100, 30) added TSMs (ThTSMs). At each taxonomical rank, an ordering of pertaining taxa was performed per reverse number of specific peptides. The top taxon per parent was compared to the specific peptides threshold, cycling through parent taxa. TSMs corresponding to validated taxa were added to a pool of TSMs at the taxonomical rank (ValRankTSMs). This was reiterated for the next taxa in order, one per parent, until no more taxa with enough specific peptides could be found. Subtaxa were then reordered by the number of corresponding TSMs excluding ValRankTSMs, and compared to ThTSMs, cycling though parent taxa. The process was ended when the number of ValRankTSMs (rank) was above 0.992 × ValRankTSMs (parent) or when no additional taxon could meet validation criteria. The conjunction of a Mascot MIT p-value of 0.05 which is very stringent for large databases such as NCBInr, and of high thresholds (7 specific peptides to validate a taxon on a large database and reduced MS/MS data sets is very drastic) concur to the selection of high confidence taxa, at the expense of sensitivity.

Table 2.7 shows the number of MS/MS spectra recorded per sample. It is comprised between 4,657 (Biofilm B) and 25,777 (Biofilm C). This number is directly correlated to the quantity of protein material recovered per sample. Roughly, it corresponds to the equivalent of 0.6 mg of biological material as other experiment done with pure microbial culture in the same analytical conditions yielded 50 thousands MS/MS spectra for 1.9 mg of wet biological material. Therefore, here Biofilm B was the less rich in terms of extracted proteins. However, this dataset acquired within 1 h of tandem mass spectrometry is still relatively loaded in information as a total of 594 peptide sequences could be assigned in MIT mode. The percentage of MS/MS spectra assigned per sample varies between 10% (Biofilm C) to 16% (Biofilm B). This number illustrates the conjunction of two factors: an expected lack of comprehensiveness of the generalist database on the one hand, and a possible decrease of the quality of some MS/MS spectra due to low ion signal on the other hand. However, these percentages compared favourably with other proteomics search results on non-model organisms [298].

*Table 2.7: MS/MS spectra acquired per LC-MS/MS run and their interpretation.*

| | Biofilm A | Biofilm B | Biofilm C | Biofilm D |
|---|---|---|---|---|
| # MS/MS spectra | 14677 | 4657 | 25777 | 16052 |
| # TSMs All taxa | 2095 | 728 | 2645 | 1744 |
| # peptide sequences | 1387 | 594 | 1931 | 1325 |

**Identifying the microorganisms present in Biofilm A as case study**

Table 2.8 reports the numbers of TSMs and specific peptides for Biofilm A at the different possible taxonomical ranks. A total of 1,168 TSMs and 724 unique taxon-specific peptide sequences were exclusive of the *Bacteria* superkindgdom. Among these, the very large majority are exclusive of only one phylum: *Proteobacteria* with 1,136 TSMs and 580 unique taxon-specific peptide sequences. We noted that when focusing our attention from the superkingdom to the phylum taxonomical rank the decrease in terms of unique taxon-specific peptide sequences (-20%) is sharper than the decrease in terms of TSMs (-3%). This discrepancy arises from the peptide sequences belonging to highly conserved parts of housekeeping proteins systematically found in bacteria. While these peptides will not be considered as specific of the *Proteobacteria* phylum, they contribute to the bacterial signal. Because no other phylum is represented in the dataset (*i.e.* validated with enough specific peptides), the whole specific bacterial signal can be then assigned to the Proteobacteria. The same reasoning can be applied at a lower taxonomical rank. At the Class rank, two children taxa could be validated: *Alphaproteobacteria* and *Betaproteobacteria* with 493 and 55 unique taxon-specific peptides, respectively. The corresponding quantities of the two classes could be roughly assessed through their respective TSMs: 1011 and 171, respectively. At the Order rank, the *Betaproteobacteria* taxon was explained by the presence of a unique order: *Burkholderiales* with 47 unique peptides and 160 TSMs. The *Alphaproteobacteria* taxon signal is subdivided into two children orders: *Sphingomonadales* and *Caulobacterales*. More taxonomical precision could be obtained at the Family and Genus levels. A unique genus and family could be delineated per order by phylopeptidomics. Finally, the *Sphingomonas* (*Sphingomonadaceae*), *Caulobacter* (*Caulobacteraceae*), and *Acidovorax* (*Comamonadaceae*) genera were validated with 340, 33, and 16 unique taxon-specific peptides, respectively. Their respective quantities may be roughly assessed by their phylopeptidome signal (TSMs): 80% (898), 11% (122) and 9% (96). The proportion of each genus would be significantly different if based on the number of taxon-specific specific peptides: 87% (340), 8% (33), and 4% (16). Indeed, the number of taxon-specific specific peptides may differ significantly due to the phylogenetic relationships between the organisms present in the sample. Therefore, it is not a reliable parameter for quantifying the ratio of organisms.

While the decrease of the number of specific peptides is rather sharp along the taxonomical ranks considered (from 724 at the Superkingdom taxonomical rank till 389 at the Genus taxonomical rank), the TSMs parameter is rather stable (from 1,168 till 1,111, respectively). Thus, no other main contributor to the biofilm could explain the proteomic signal such as an unsequenced branch of life of bacteria. The identification of organisms at a lower taxonomical rank than the genus rank would require more starting material and a more comprehensive database for the three identified genera, as the biofilm may comprised several closely related species that have been not yet genome sequence and reported in the NCBInr database. However, the main most-closely related species identified by means of their specific peptides were: *Sphingomonas sp. Leaf257* (TaxID 1736309), *Sphingomonas parapaucimobilis* (TaxID 28213), *Caulobacter vibrioides* (TaxID 155892) and *Acidovorax sp. CF316* (TaxID 1144317). Because the number of genome sequenced bacteria with clear species taxonomy in these important environmental groups of bacteria is still rather low (*i.e.* 45 for *Sphingomonas*, 5 for *Caulobacter*, and 17 for *Acidovorax*), we cannot ascertain at the present stage the accuracy of the species taxonomical rank. Here, the results illustrate that at least certain species heterogeneity exist for the *Sphingomonas* genus in Biofilm A.

*Table 2.8: Number of taxa-to-spectrum matching (TSMs) and taxon-specific peptides (spePEPs) detected at the different taxonomical ranks in the four biofilms.*

| Taxonomy | | Biofilm A | | Biofilm B | | Biofilm C | | Biofilm D | |
|---|---|---|---|---|---|---|---|---|---|
| **Rank** | **Taxa** | **# TSMs** | **# spePEPs** | **# TSMs** | **# spePEPs** | **# TSMs** | **# spePEPs** | **# TSMs** | **# spePEPs** |
| Superkingdom | Bacteria | 1168 | 724 | 577 | 427 | 1313 | 913 | 676 | 484 |
| Phylum | Proteobacteria | 1136 | 580 | 572 | 326 | 1264 | 743 | 634 | 367 |
| Class | Alphaproteobacteria | 1011 | 493 | 547 | 303 | 1205 | 703 | 551 | 316 |
| | Betaproteobacteria | 171 | 55 | 42 | 9 | - | - | 99 | 24 |
| Order | Sphingomonadales | 903 | 393 | 523 | 264 | 1142 | 589 | 484 | 1134 |
| | Caulobacterales | 148 | 39 | 51 | 14 | 87 | 10 | 70 | 1464 |
| | Burkholderiales | 160 | 47 | 33 | 9 | - | - | 90 | 1178 |
| Family | Sphingomonadaceae | 903 | 383 | 523 | 259 | 1140 | 568 | 484 | 255 |
| | Caulobacteraceae | 148 | 39 | 51 | 14 | 87 | 10 | 70 | 22 |
| | Comamonadaceae | 119 | 29 | 20 | 3 | - | - | 52 | 13 |
| Genus | Sphingomonas | 893 | 340 | 521 | 226 | 1131 | 497 | 478 | 214 |
| | Caulobacter | 122 | 33 | 35 | 9 | 52 | 7 | 53 | 18 |
| | Acidovorax | 96 | 16 | 16 | 3 | - | - | 40 | 8 |

**Biofilm C stands out from the others**

Table 2.8 presents also the phylopeptidomics data for the three other biofilms. For the Biofilm B biofilm, a low quantity of material was noted from the number of MS/MS spectra recorded (4,657 compared to 14,677 for Biofilm A). Although this decrease is important (a third of Biofilm A), the phylopeptidics signal could be assigned to the genus level as previously described for Biofilm A. Here, the presence of three orders could be validated: *Sphingomonadales, Caulobacterales*, and *Burkholderiales*, with 393, 39 and 47 unique specific peptides, respectively. At the Family taxonomical rank, enough signal could certified for the presence of *Sphingomonadaceae* (259 taxon-specific peptides) and *Caulobacteraceae* (14 taxon-specific peptides), but not for *Comamonadaceae* (only 3 taxon-specific peptides). Logically, the same occurs at the Genus taxonomical rank, with 226, 9 and 3 taxon-specific peptides for the presence of *Sphingomonas*, *Caulobacter*, and *Acidovorax*, respectively. Regarding Biofilm D, a large quantity of MS/MS spectra was recorded. In this case, a rather low level of TSMs was assigned to the superkingdom Bacteria (676) compared to Biofilm A (1168). Anyway, the three genera present in this sample are unambiguously identified by phylopeptidomics: *Sphingomonas*, *Caulobacter*, and *Acidovorax*, certified by 214, 18 and 8 taxon-specific peptides, respectively. The latest biofilm sample, Biofilm C, was the largest MS/MS spectra dataset*, i.e.* corresponding to the most material rich sample. In this case, only one class is identified (*Alphaproteobacteria*) and with a large number of taxon-specific peptides (703). Two orders, *Sphingomonadales* and *Caulobacterales*, were contributing to this signal. The genera identified were: *Sphingomonas* and *Caulobacter*, with 497 and 7 taxon-specific peptides, respectively. Therefore, Biofilm C stands out from the others as no trace of the presence of *Acidovorax* could be detected in this biofilm while large amount of material was available.

Figure 2.6 shows the ratio of microorganisms established for the four sampled biofilms based on the TSMs criterion at two taxonomical ranks, Genus and Order. In the four biofilms, *Sphingomonas* was the main genus contributor. *Sphingomonas* and *Caulobacter* were systematically found. Remarkably, *Acidovorax* was another genus detectable in Biofilms A and D, as well as faintly perceived in Biofilm B (only formerly identified by three taxon-specific peptides) but explaining a *Burkholderiales* MS/MS-certified signal (nine taxon-specific peptides). The Biofilm C signal was differing from the others due to the absence of *Betaproteobacteria* phylopeptidomics signal. Some slight discrepancies in terms of relative ratio of each genus are noted between Genus and Order taxonomical ranks (Fig. 2.6). However, because the Order taxonomical rank benefit of more genome sequence microorganisms in the database and therefore protein sequences, its reliability in terms of quantitation should be better than the Genus taxonomical rank.

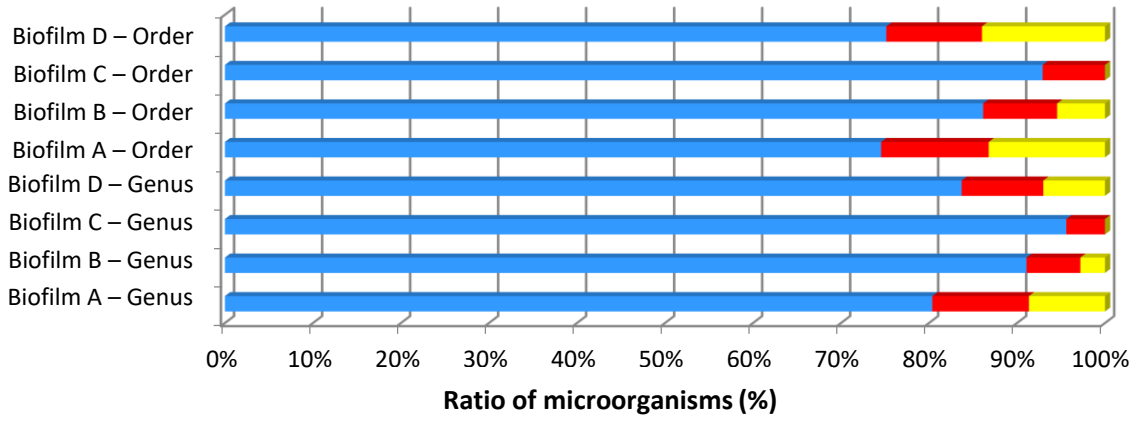



***Figure 2.6: Proportion of each genus in the four biofilms after a direct phylopeptidomics analysis.*** *The biofilms were recovered from four locations (Biofilms D, C, B, and A). The ratios of microorganisms were established either by Order-specific TSMs or Genus-specific TSMs.* Sphingomonas, Caulobacter, *and* Acidovorax *are shown in Blue, Red, and Yellow, respectively.*

## Discussion

Sampling of minute amounts of precious biofilm samples from difficult environments such as a pool used to store nuclear elements does not give many analytical options if taxonomical characterization and estimation of the different biomass contributions are the objectives. Extracting the DNA present and analysing the taxonomy either by 16S/18S rRNA amplification and sequencing, or metagenomics, is a first option. The reliability of this approach has been challenged as bulk DNA extraction and rRNA PCR amplification is a problematic methodology because their yields can be species-dependent [242, 299]. Furthermore, the use of only rRNA information tends to be insufficient for current taxonomy purpose as a relatively high percentage (at least five per cent) of 16S RNA sequence records were estimated to contain substantial anomalies [300]. Here, we explored another option relying on peptide extraction and identification by high resolution tandem mass spectrometry. The wealth of information obtained in terms of taxonomy appears large because peptide sequence encoded with 20 possible different amino acids is highly condensed (while nucleic acids are encoded with 4 nucleotides), and thus discriminative. Here, a total of 724 bacteria-specific peptides were identified for Biofilm A with the equivalent of 0.6 mg of starting biological material. With an average length of 10 amino acids per peptide, these peptides are totalling 7,240 amino acid positions. Because two amino acids (leucine and isoleucine) are indistinguishable by tandem mass spectrometry, the theoretical combinations of all amino acids lead to an impressive number of combinations ($19^{7240}$), which can be theoretically obtained by sequencing the equivalent of a nucleic acid sequence totalling 42 trillions of nucleotide positions. However, because of Life history starting from a common ancestor, the molecular relationships between organisms and the constraints of protein folding for a stable structure and function, the sequences possibilities are not as huge. Consequently, the differences between nucleic acid sequencing and peptide sequencing may be not as tremendous.

Here, we have shown that phylopeptidomics can help to establish the composition of microorganisms present in biofilms and estimate their biomass contributions directly through their peptide signal. Metaproteomics is more accurate for biomass estimates than sequencing methods as recently established with specific standards [301]. Here our methodology differs significantly from the metaproteomics strategy

developed by Kleiner and others [301-303], as the peptidome signal is assigned to taxonomical information while metaproteomics relies on protein inference and then assignation to taxonomical information of the detected proteins. By bypassing the protein inference problem, phylopeptidomics gives a more direct and unbiaised view of the taxonomical units present in the sample, and thus a better estimation of their biomass contributions.

The limits of the phylopeptidomics approach may be discussed here. First, the current lack of the NCBInr database to comprehensively represent the biodiversity on Earth is trivial. The method may not be applicable for identifying new microorganisms from branches of Life not yet genome sequenced, or for accurately give the most exact taxonomical rank (species, subspecies, or strains ranks). Currently, the generous efforts done by the scientific community in terms of full genome sequencing of pathogens, emergent pathogens, and environmental samples help to fill this gap. Consequently, the phylopeptidomics method should be more powerful and efficient with further database updates. A second caveat is the current limit of detection of tandem mass spectrometers and the impossibility to amplify the peptide signal like with nucleic acid PCR. However, we have shown here that an amount of approximately 0.18 mg of starting biological material was sufficient to give the three main microbial components of Biofilm B with a Q-Exactive HF tandem mass spectrometer, and their respective biomass contributions. For sure, this current threshold may be lowered with novel tandem mass spectrometers as suppliers tend to increase regularly the sensitivity and speed acquisition of their instruments. These limits have to be balanced with the analytical speed and cost achieved by the phylopeptidomics methodology as only 60 min of tandem mass measurements were needed per sample in the present study.

For sampling directly biofilms from aquatic environments without introducing any specific support, we proposed the use of a Spontex® sponge that, as shown here, is able to scrub the biofilm and imprison part of the biofilm, allowing its recovery. The low amount of material obtained even at 4 m depth in the pool was sufficient for its analysis by means of phylopeptidomics.

The three genera observed in these samples, *Sphingomonas*, *Caulobacter*, and *Acidovorax*, are all environmental bacteria. The genus *Sphingomonas* is known as largely ubiquitous. It has been found in different environments, such as marine and fresh water environments, soils, and deep subsurfaces [304, 305]. Noteworthy, bacteria belonging to the *Sphingomonas* genus have already been identified in spent nuclear fuel pools from the power plant of Cofrentes, Valencia (Spain) [185] and the Idaho Nuclear Technology and Engineering Center, USA [188]. Some *Sphingomonas* are known to be multi-resistant but are not considered as extremophiles *per se*. The strain AntH11 [306], isolated from the Antarctic Dry Valleys, encompasses in its genome numerous stress response genes (116 in total), an enzymatic arsenal for facing the extreme environmental conditions encountered in Antarctica, *i.e.* hyper aridity, UV radiation and cold temperatures [306]. Another *Sphingomonas* strain exhibited a high resistance to UV radiation with a 10% survival at a 1500 $J.m^{-2}$ UV dose [307]. The genus *Caulobacter* is also ubiquitous and can be found in aquatic environments, freshwater sediment and soils [308, 309] and even associated with plants [310]. One species of *Caulobacter* has been proven to grow with 200 µM of uranium at pH 7 [311]. Bacteria belonging to the *Acidovorax* genus have been isolated from lawn soil, water, commensal flora, the water of an activated sludge plant, and has also been described as a phytopathogenic agent [312-314]. Some *Acidovorax* strains can use the oxidation of $H_2$ as an energy source [315]. $H_2$ can be formed by water radiolysis. To our knowledge, this is the first time the genus *Acidovorax* has been described in an environment bathed with ionizing radiation.

## Conclusions

In this work, we have shown that the microbial composition of biofilms could be directly analysed using metaproteomics. An original methodology has been developed for the remote sampling of biofilms, as well as for the data treatment of the MS/MS spectra obtained after protein extraction, a procedure that we named phylopeptidomics. The strategy developed here can be used to identify microorganisms present in any kind of samples and decipher their respective biomass contributions.

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## 2.2 Proteotyping the fraction of microbiomal community from a nuclear pool able to grow in liquid cultures

**Abstract**

To study the diversity present in an aquatic environment and observe its evolution in liquid medium throughout time, a kinetic can be performed in diluted rich liquid media. By increasing the number of media and dilution some diversity can be observed, however the use of two or three media and two different dilutions could be enough to identify the main genera present in the water of constraint environments. In this study the water of two nuclear pools have been studied over a liquid kinetic. This study allowed the optimization of kinetic's conditions. The kinetics highlighted the presence of a majority of *Proteobacteria* in both pools after culture. This study present the first description after culture of a water sample from a working nuclear reactor. This study highlighted the presence of a great diversity present in nuclear pools although the samples that could be used were in low amounts. The study of two different pools, including one pool with a change in the living condition, brought to light the differences in microbiota.

### 2.2.1 Introduction

Microbial biotechnology relies heavily on isolation of novel microorganisms with specific characteristics and their characterization for the most appropriate use as biocatalysts [316]. Many biotechnology-relevant microorganisms have been isolated from extreme environments. However, it is known that only a relatively low number (0.1 to 10%) of the microorganisms present in complex environmental samples can be culturable in normal laboratory conditions [36]. To increase the chances to obtain more isolates from complex environmental samples, new strategies relying on the screening of numerous media and growth conditions have been recently proposed [270, 317]. Remarkably, culturomics was shown to be more efficient than metagenomics to comprehensively understand the gut microbiota of a patient [318].

Another question regarding isolation of microorganisms is how to propagate the initial sample. Today, the sample is directly used for culturomics screening after its dilution. However, a complementary strategy is to propagate the sample to obtain a larger quantity of the consortium of microorganisms, either before screening and isolation, or their characterization as a consortium in terms of biotechnology application. Maintaining the diversity of such a consortium is far to be trivial but will be of interest to understand the interactions between the key players of the consortium. The molecular tools to analyze the diversity of a consortium are metagenetics (16S/18S rRNA amplification and sequencing), metagenomics, and proteotyping by mass spectrometry. Whole-Cell MALDI-TOF mass spectrometry has revolutionized the clinical microbiology laboratory as in some minutes a microbial isolate can be identified in terms of species [319]. However, this approach is still not really well adapted to environmental microbiology because of the lack of comprehensiveness of the MALDI-TOF databases for environmental species. Proteogenomics definition of biomarkers was shown to be of interest for screening new isolates by whole-cell MALDI-TOF [320, 321] but its usage still remains scarce. Recently, proteotyping by tandem mass spectrometry has shown to be efficient to analyze more complex samples (Pible, Armengaud, et *al*., submitted). We have developed this latter approach and recently proposed phylopeptidomics as a powerful tool to identify the microorganisms present in a complex sample and assess their respective biomass contributions (Pible, Armengaud, et *al*., submitted). Phylopeptidomics can give within a few hours an extensive picture of the microbial consortium and thus can be implemented in novel strategies for microbial isolation.

Here, we proposed to investigate by means of phylopeptidomics whether cultivation in rich but diluted liquid media of microbial communities sampled from two pools of nuclear facilities affect their diversity. For this, we sampled different microbial communities and cultivate them in various conditions. We followed their evolution along time and found that a rather stable community can be maintained for various days and months.

## 2.2.2 Material and Methods

### *2.2.2.1 Description of pools and sampling*

The water from two pools located in the CEA – Saclay center was sampled in this study. Pool 1 is used to stored $^{60}$Co radioactive sources to perform irradiation experiments on materials. This pool does not contain any radionuclides.

In 2015, the purification system filtered the water every day for circa 10 minutes. The characteristics and composition of the water of this pool on July 2015 are presented in Table 2.9. Several ions ($Cl^-$, $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$) can be used as nutrients by microbial cells.

***Table 2.9: Characteristics and composition of the water of Pool 1 in July 2015***

| | pH | σ at 25°c (µS/cm) | COD (mg/L) | MIS (mg/L) | $F^-$ (mg/L) | $Cl^-$ (mg/L) | $Br^-$ (mg/L) | $NO_3^-$ (mg/L) | $PO_4^{3-}$ (mg/L) | $SO_4^{2-}$ (mg/L) | $Na^+$ (mg/L) | $K^+$ (mg/L) | $Ca^{2+}$ (mg/L) | $Mg^{2+}$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **LOQ** | - | 2,00E+01 | 4,00E+00 | 2,00E+00 | 1,00E-01 | 5,00E-01 | 9,40E-02 | 5,00E+00 | 2,00E-01 | 1,00E+02 | 5,00E+00 | 1,80E-01 | 5,00E+00 | 4,30E-01 |
| **Pool 1** | 8 | 1,27E+02 | <4,0E+00 | <2,0E+00 | - | 2,70E+00 | - | - | - | - | 5,80E+00 | 1,60E+00 | 4,10E+01 | 1,70E+00 |
| **Inc (%)** | - | 3 | 10 | - | - | 21 | - | - | - | - | 13 | 29 | 15 | 25 |
| **Measuring T°C** | - | 21,6 | 21,9 | - | - | - | - | - | - | - | - | - | - | - |

Pool 2 is used to cool $^{235}$U radioactive sources. This reactor used to produce radionuclides for medicine before its definitive shutdown in December 2015. While in operation, the pH 7.0 pool's demineralized water (conductivity 0.5 µS/cm), maintained at 35°C, contained the β-emitter $^3$H (2.49x10$^8$ Bq/m$^3$) and the γ-emitter radionuclides $^{24}$Na (2.2x10$^9$ Bq/m$^3$), $^7$Be (4.0x10$^7$ Bq/m$^3$), $^{27}$Mg, $^{41}$Ar, $^{56}$Mn, $^{99}$mTc, $^{122}$Sb, $^{187}$W, $^{188}$Re, $^{197}$mHg yielding a total γ activity of 2.6 10$^9$ Bq/m$^3$. During the reactor shutdown pH 5.4 pool's demineralized water (conductivity 0.5 µS/cm) contained the β-emitter $^3$H (2.49x10$^8$ Bq/m$^3$) and the γ-emitters $^{51}$Cr (1.6x10$^6$ Bq/m$^3$), $^{186}$Re (3.7x10$^5$ Bq/m$^3$), $^{124}$Sb (2.8x10$^5$ Bq/m$^3$), $^{58}$Co (2.5x10$^5$ Bq/m$^3$), $^{99m}$Tc (2.5x10$^5$ Bq/m$^3$), $^{122}$Sb (1.7x10$^5$ Bq/m$^3$), $^{99}$Mo (1.7x10$^5$ Bq/m$^3$), $^{60}$Co (1.3x10$^5$ Bq/m$^3$), and $^{54}$Mn (3.6x10$^4$ Bq/m$^3$), yielding a total γ activity of 3.25x10$^6$ Bq/m$^3$.

To sample water from Pool 1, a 1.5 L sampling bottle (Wildco, USA) was used to sample 6 L of water. The recovered water was transferred in sterile bottles (PETG Media Bottle Sterile, VWR). For Pool 2, a 2 L water volume was collected using a permanently installed pipe extensively purged before sampling. The water was centrifuged at 15,000 *g* for 20 min at room temperature for both pools. The supernatant was discarded and the microbial pellet resuspended in 1 mL of supernatant. From this milliliter, 50 µL were used as starters for liquid and solid cultures. The water from Pool 1 was sampled in November 2015 in five different locations, one in the middle of the pool, and four near the walls after rubbing the walls to resuspend any possible biofilm present. Sample A was collected near the northern wall at a 4 m depth while sample B was recovered at the near the same wall but at a 1 m depth. Sample C was collected near the bottom of the western wall (at a 4 m depth) and sample D was recovered near the same wall at a 1 m depth. Finally sample E was collected in the middle of the pool at a 1 m depth (Fig. 2.7).

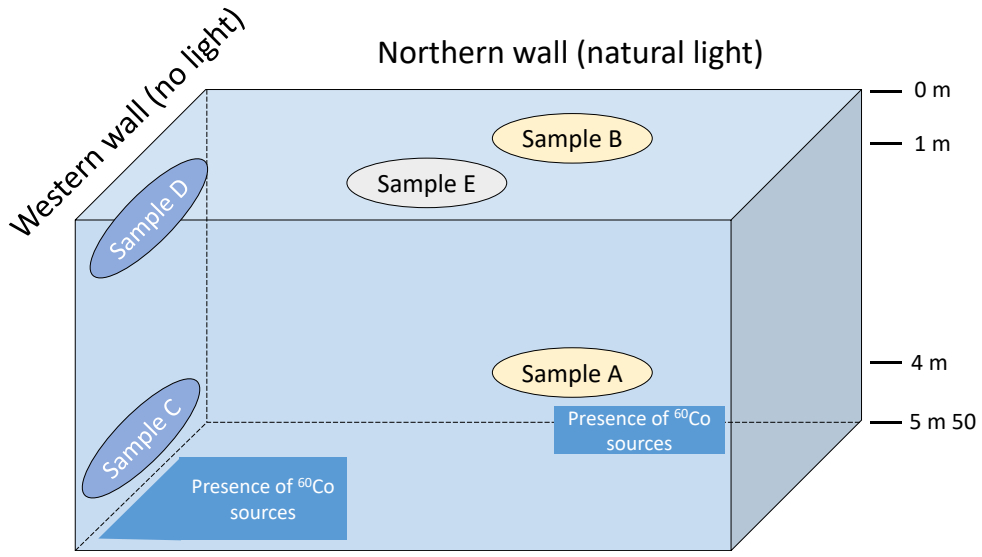



***Figure 2.7: Schematic view of Pool 1.*** *Samples A, B, and E can be highlighted by natural light during day time while samples C and D are always in the shadow. The location of the cobalt sources are indicated when they are present. The sources near the western wall are present more often than the ones near the northern wall.*

The water from Pool 2 was sampled first in 2015 in location A (Loc. Asample F), 2 m away from a working nuclear reactor at a depth of 9 m. Loc. A. In 2017, three samples were recovered from location A (sample G), location B (sample H) and location C (sample I) as shown in Fig. 2.8. The radiation dose rate at each location in 2017 was <1 µGy/h, 15 µGy/h and 25 Gy/h for Location A, Locaction B and Location C, respectively. The latter samples were harvested a year after the reactor's definitive shutdown.

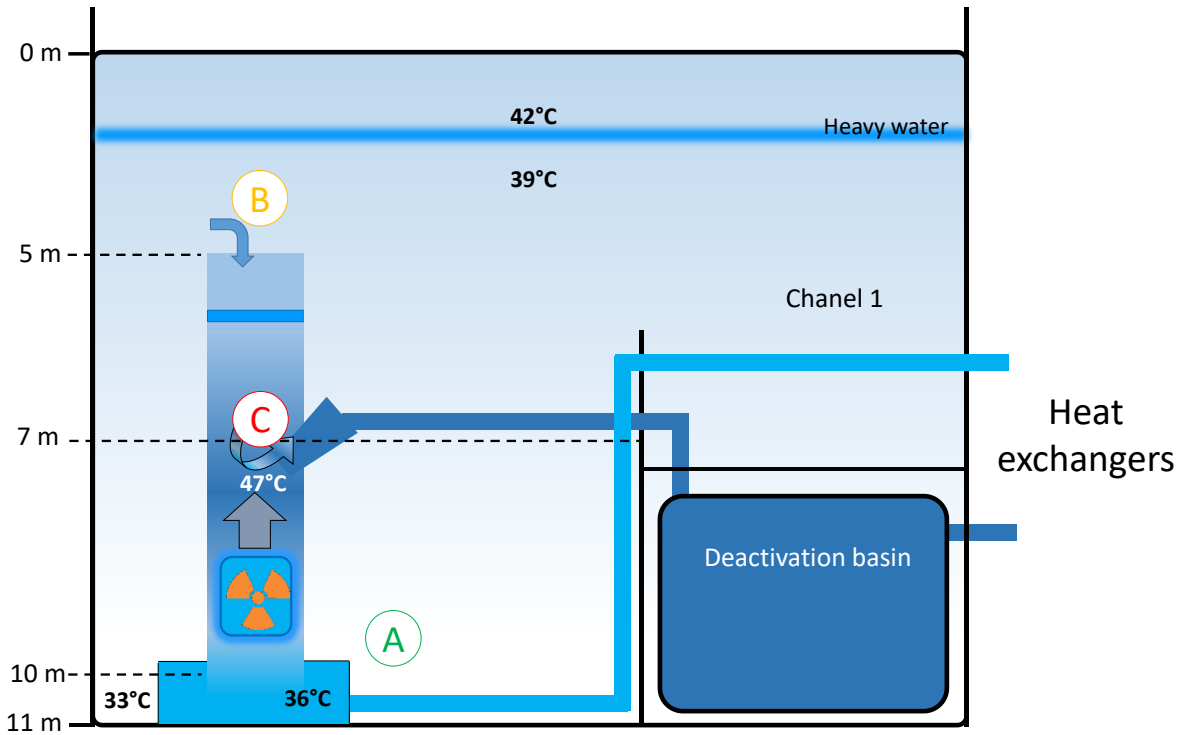



***Figure 2.8: Sampling locations of the different samples in Pool 2 (simplified and adapted from [250])***

Samples from 2015 (samples A to E and sample F) were used for a preliminary experiment to settle the analytical parameters.

#### *2.2.2.2 Cultivation and sampling for proteotyping*

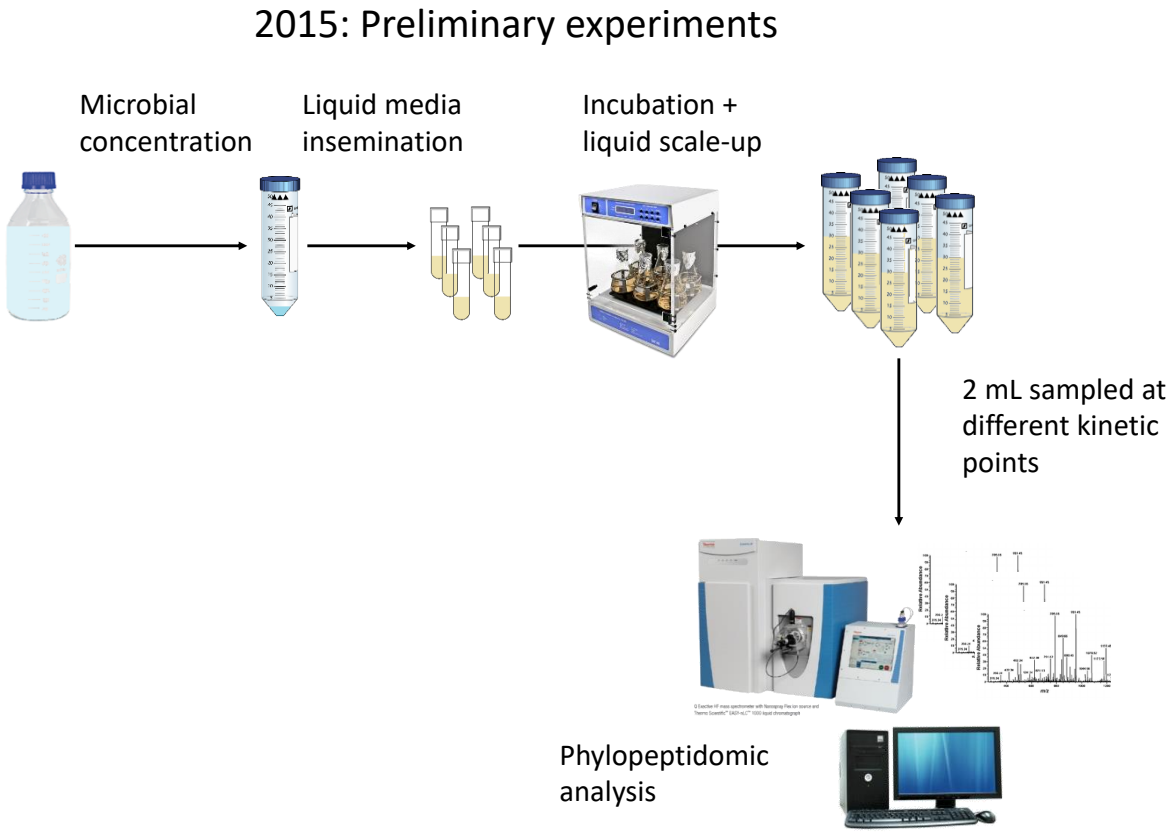
##### 2.2.2.2.1 Preliminary experiments in Pools 1 and 2

The 1.0 mL concentrate obtained after centrifuging 6L of water was estimated to contain at least $12x10^6$ cells per mL. Among the mL recovered only 50 µL were meant to be cultivated in liquid media. The rest was used for metagenetics sequencing, solid cultures and -80°C preservation in Glycerol Strain Buffer (GSB) (65% glycerol v/v, 0.1 M $MgSO_4$, 0.025 M Tris-HCl pH 8) (cf. Chapter III.4). The 50 µL of liquid sample were divided between different liquid media (4 µL per liquid culture for Pool 1 and 6 µL for Pool 2). For Pool 1, the sampling in 2015 was cultivated in Luria Bertani broth (LB) diluted six times (1/6) and in LB diluted twelve times (1/12). For Pool 2, in 2015, the water was cultivated in Brain Heart Infusion (BHI) diluted 10 times (Sigma, Germany), LB diluted twice (Roth, Germany), Tryptic Soy Broth (TSB) diluted twice (Sigma), and Nutrient Broth (NB) diluted twice (Sigma). Dilutions were performed with sterile milliQ water.

Liquid cultures of 6 mL were initiated with samples from Pool 1 or Pool 2. The cultures were incubated at 26°C for Pool 1 under vigorous shaking (200 rpm). After one week of cultivation, the 6 mL cultures were transferred into a 30 mL culture from the corresponding medium. These cultures were then sampled after 45 days, 137 days, 173 days, and 227 days. For the cultures initiated with the first sampling in Pool 2 in 2015, the temperature of incubation was 28°C and the kinetic points were: 82 days, 153 days, and 207 days. These kinetic points were chosen to have enough material to be analyzed using phylopeptidomics. Samples from Pool 1 presented a faster growth than Pool 2. After the second point of kinetic from Pool 1 (day 137), it had been realized that microbial material needed for phylopeptidomics analysis could be reduced. Starting from day 173 in Pool 1 and day 153 in Pool 2 the kinetic points were closer (sampled every months and a half for Pool 1 and two months for Pool 2). Each sample was preserved at -80°C in GSB.

For each kinetic point, 2 mL of culture were sampled and centrifuged at 20000 *g* for 5 minutes at room temperature. The supernatant was removed and the resulting wet pellet weighed. The tubes were then flash-frozen in liquid nitrogen and stored at -80°C before being analyzed by metaproteomics. The protocol used is presented in Scheme 2.1.

These preliminary allowed us to select liquid media and isolate interesting organisms from Pool 2 after sample A direct metagenetics analysis (cf. Chapter III.4).




*Scheme 2.1: Preliminary experiments protocol schematized*

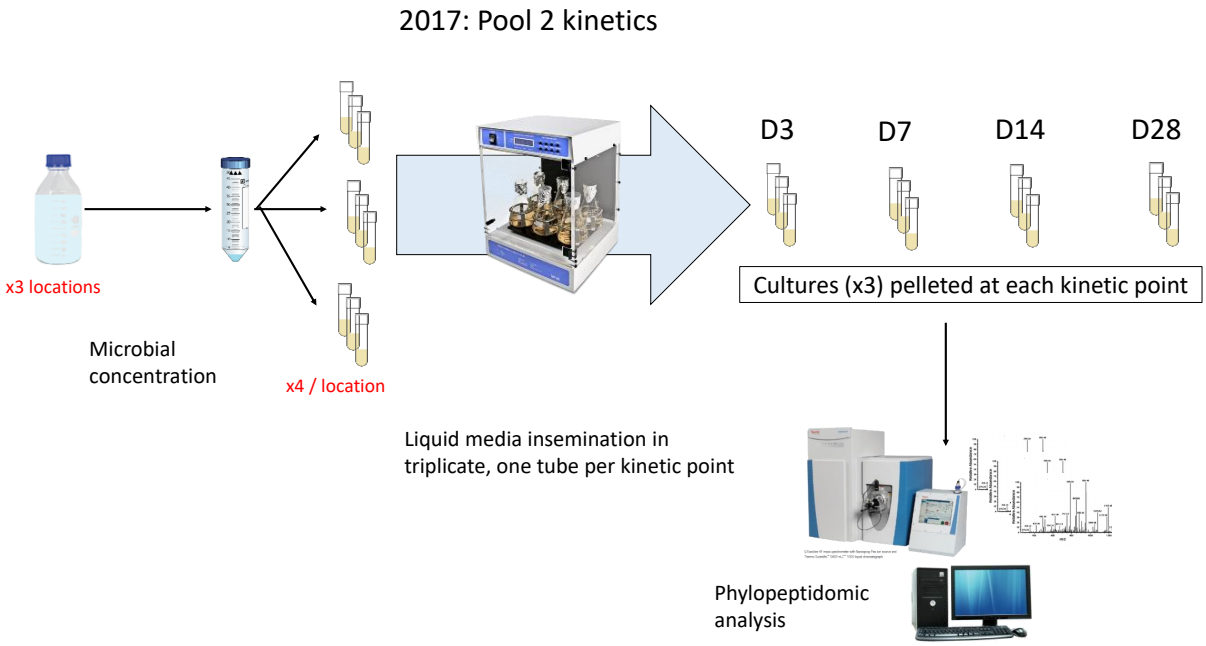
#### 2.2.2.2.2 Water cultivation from Pool 2 in 2017

In 2017, 600 mL of samplings were centrifuged for 20 min at 16000 *g* at 10°C. Microbial pellet was resuspended in about 3-4 mL of supernatant. 16 to 150 µL of the mix were cultivated in each culture (depending on the number of cultures and volume of recovered mix).The water was cultivated in 5 mL of LB diluted twelve times and Reasoner´s 2A (R2A) diluted three times (HiMedia Laboratories Pvt. Ltd, USA). Dilutions were performed with sterile milliQ water. These media were chosen according to the preliminary results and in order to isolate *Variovorax* present in more than 95% in the sample (cf. Chapter III.4). Each kinetic point was individual to avoid any contamination.

For the second sampling in Pool 2, performed in 2017, the kinetic points were: 3 days, 7 days, 14 days, and 21 days. Each culture was performed in triplicate. For each kinetic point per liquid medium, three cultures were analyzed by metaproteomics, and one culture was preserved at -80°C in four aliquots.

For each kinetic point, 5 mL of culture were sampled and centrifuged at 20000 *g* for 5 minutes at room temperature. The supernatant was removed and the resulting wet pellet weighed. The tubes were then flash-frozen in liquid nitrogen and stored at -80°C before being analyzed by metaproteomics.

The protocol used is presented in Scheme 2.2.




*Scheme 2.2: Pool 2 2017 samplings' treatment protocol schematized*

### 2.2.2.3 Metaproteomics analysis

The metaproteomics analysis was done as previously described (cf. Taxonomical composition of a biofilm sampled from a nuclear fuel pool assessed by metaproteomics). Briefly, samples were thawed on ice, and a volume of 59 µL of Laemmli buffer LDS1X (Invitrogen) was added per mg of wet bacterial pellet as described by Hartmann et *al* [295]. After denaturation for 5 min at 99°C, the protein sample was loaded onto a 4–12% gradient 10-well NuPAGE gel (Invitrogen). The proteins were subjected to electrophoresis for 5 min and then stained with Coomassie Blue Safe staining (Invitrogen) as recommended by the supplier. The polyacrylamide band corresponding to the whole proteome was sliced, then unstained with ultra-pure water, reduced with dithiothreitol, and treated with iodoacetamide. It was then subjected to proteolysis with Trypsin Gold Mass Spectrometry Grade (Promega) in the presence of 0.01% ProteaseMAX surfactant (Promega), as previously described [322]. The resulting peptides were analyzed with an ESI-Q Exactive HF mass spectrometer (Thermo) equipped with an ultra-high field Orbitrap analyser and coupled to an Ultimate 3000 176 RSL Nano LC System (ThermoFisher). A volume of 10 µL of peptides was injected onto a reverse phase Acclaim PepMap 100 C18 column (3 µm, 100 Å, 75 µm id x 500 mm) and resolved at a flow rate of 0.2 µL/min with a 60 min gradient of CH3CN in presence of 0.1% formic acid. The Q-Exactive HF instrument was operated with a Top20 strategy with only ions with potential charge states of 2+ and 3+ selected for MS/MS and with a dynamic exclusion of 10 sec. The proteotyping interpretation of MS/MS spectra was done with the National Center for Biotechnology Information non-redundant fasta file (NCBI-nr) database with the Mascot search engine (Matrix science). Peptide -to-MS/MS spectrum assignation was done with full trypsin specificity, maximum of one missed cleavage, mass tolerances of 5 ppm on the parent ion and 0.02 Da on the MS/MS, static modification of carboxyamidomethylated cysteine (+57.0215), and oxidized methionine (+15.9949) as dynamic modification. Post-processing of Mascot dat files was done as previously indicated (reference public biofilm).

## 2.2.3 Results

### *2.2.3.1 Preliminary results*

#### 2.2.3.1.1 Phylopeptidomics analysis of the water from Pool 1 campaign

Pool 1 is used to store $^{60}$Co radioactive sources to perform irradiation experiments on materials. We first sampled water from the middle of the pool (sample E), inoculated LB diluted media (1/6 and 1/12), and monitor the diversity of organisms by phylopeptidomics. As shown in Table 2.10, this sample contained $2x10^6$ cells per liter as estimated by microscopy with a Malassez cell. Therefore, the quantity of microbial cells in the 50 µL aliquot of sample that was used for inoculating the medium is really low: 100 cells.

***Table 2.10: Cell count at the five different water sampling location using a Malassez cell.***

| **Location** | **A** | **B** | **C** | **D** | **E** |
|---|---|---|---|---|---|
| **Total cell count ($10^6$ cells/L)** | 2.0 ± 1.2 | 2.0 ± 1.6 | 4.5 ± 0.5 | 2.0 ± 0.9 | 2.0 ± 0.8 |

After 45 days of culture, an aliquot of each culture was sampled and the proteins present were extracted, proteolyzed by trypsin, and the resulting peptides were analyzed by high resolution tandem mass spectrometry.

Additional samples were taken from the two cultures after 137, 173 and 227 days without renewing the media. Phylopeptidomics analysis was performed in the same conditions on all these samples. As reported in Table 2.13, the number of families detected for the LB 1/6 and LB 1/12 cultures is 20 and 16, and the number of genus detected is 27 and 20, respectively. Even after a long incubation period, no drastic loss of biodiversity could be observed, nor the preponderance of one genus over the others.

Mainly genera belonging to the *Proteobacteria* phylum (and more specifically *α-Proteobacteria*) were identified in both kinetics. The main genus present in Sample E during both kinetics was *Caulobacter* (between 17 to 70% in LB 1/6 and 13 to 52% in LB 1/12). 2 to 9% of the samples were composed by *Bacillus*. The *Xanthobacter* genus was also present in both kinetics and represented up to 13% of the samples. One eukaryotic phylum was identified in LB 1/12 with the presence of the *Fusarium* fungus at day 173 (Tables 2.11 & 2.12).

*Table 2.11: Proportion of each genus during the 2015 kinetic of Sample E in LB 1/6*

| | | | | | Sample E 1/6 | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | D 45 | D 137 | D 173 | D 227 |
| Phylum | Class | Order | Family | Genus | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Nocardiaceae | Rhodococcus | | 0,80 | | 0,88 |
| | | | Streptomycetaceae | Streptomyces | | | 4,21 | |
| bacterium YEK0313_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus | | 3,29 | 3,22 | |
| Cyanobacteria | Hormogoneae | Nostocales | Aphanizomenonaceae | Nodularia | | | 0,27 | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 3,02 | 4,59 | 4,25 | 8,71 |
| Proteobacteria | Alphapreoteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | 70,24 | 28,12 | 17,29 | 21,09 |
| | | Rhizobiales | Bradyrhizobiaceae | Bosea | | 7,35 | 6,97 | 7,76 |
| | | | | Bradyrhizobium | 5,77 | 5,56 | 5,39 | 6,02 |
| | | | Hyphomicrobiaceae | Blastochloris | | 3,05 | 2,85 | |
| | | | | Hyphomicrobium | | | | 3,32 |
| | | | Methylobacteriaceae | Methylobacterium | | | 5,41 | |
| | | | | Microvirga | 7,73 | | | |
| | | | Methylocystaceae | Methylocystis | | | | 3,91 |
| | | | | Pleomorphomonas | | | 2,53 | |
| | | | Phyllobacteriaceae | Mesorhizobium | | | 4,49 | 5,03 |
| | | | Rhizobiaceae | Rhizobium | | 5,02 | 4,72 | 5,23 |
| | | | Rhodobiaceae | Parvibaculum | | | 1,97 | |
| | | | Xanthobacteraceae | Azorhizobium | | 7,94 | 7,95 | 8,71 |
| | | | | Xanthobacter | | 11,37 | 11,90 | 12,65 |
| | | Rhodobacterales | Rhodobacteraceae | Paracoccus | | 2,80 | 2,32 | 2,60 |
| | | Rhodospirillales | Rhodospirillaceae | Azospirillum | | 3,49 | 3,17 | 3,34 |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | | 5,19 | 3,79 | 4,64 |
| | | | | Sphingopyxis | 5,73 | 2,40 | | |
| | Betaproteobacteria | Burkholderiales | Burkholderiaceae | Burkholderia | | 2,56 | 2,09 | |
| | | | Comamonadaceae | Acidovorax | | 1,91 | 1,32 | 1,57 |
| | | | | Pelomonas | 1,33 | | | |
| | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 6,18 | 4,57 | 3,87 | 4,53 |

*Table 2.12: Proportion of each genus during the 2015 kinetic of Sample E in LB 1/12*

| | | | | | Sample E 1/12 | | |
|---|---|---|---|---|---|---|---|
| | | | | | D 45 | D 137 | D 173 |
| Phylum | Class | Order | Family | Genus | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Nocardiaceae | Nocardia | | | 0,97 |
| | | | Streptomycetaceae | Streptomyces | 5,58 | 5,30 | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 8,84 | 2,88 | 2,39 |
| Proteobacteria | Alphapreoteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | 51,68 | 29,08 | 12,92 |
| | | Rhizobiales | Bradyrhizobiaceae | Bosea | 6,73 | 3,39 | 4,76 |
| | | | Hyphomicrobiaceae | Blastochloris | | | 2,11 |
| | | | Methylobacteriaceae | Microvirga | | 3,10 | |
| | | | Xanthobacteraceae | Azorhizobium | | | 4,44 |
| | | | | Xanthobacter | 4,36 | 3,09 | 6,39 |
| | | Rhodobacterales | Rhodobacteraceae | Paracoccus | | 2,16 | 2,09 |
| | | Rhodospirillales | Rhodospirillaceae | Skermanella | | | 1,93 |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter | | | 2,79 |
| | | | Sphingomonadaceae | Sphingobium | | | 4,61 |
| | | | | Sphingomonas | 9,54 | 4,83 | 9,16 |
| | | | | Sphingopyxis | 8,43 | 2,33 | 3,86 |
| | Betaproteobacteria | Burkholderiales | Burkholderiaceae | Burkholderia | | | 3,97 |
| | | | Comamonadaceae | Acidovorax | | 31,50 | 14,76 |
| | | | | Hylemonella | | 7,16 | |
| | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 4,83 | 5,17 | 4,28 |
| Ascomycota | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | | | 18,57 |

*Table 2.13: Number of identified families and genera during the kinetic for each sample*

| | Northern wall | | | | Western wall | | | | Middle of the pool | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample A LB 1/6 | Sample A LB 1/12 | Sample B LB 1/6 | Sample B LB 1/12 | Sample C LB 1/6 | Sample C LB 1/12 | Sample D 1/LB 6 | Sample D LB 1/12 | Sample E LB 1/6 | Sample E LB 1/12 |
| Number of families | 23 | 20 | 25 | 23 | 22 | 33 | 34 | 33 | 20 | 16 |
| Number of genera | 33 | 32 | 34 | 32 | 33 | 49 | 49 | 50 | 27 | 20 |

#### 2.2.3.1.2 Phylopeptidomics analysis of samples from Pool 1 after wall scrubbing

We sampled additional water samples after scrubbing the walls to detach biofilms. Four samples could be harvested (Samples A to D). In this case, the water contained small biofilm particles that were collected by centrifugation. As shown in Table 2.10, the total cell counts were relatively similar for all the samples ($2.0x10^6$ cells per liter), except the Sample C which was shown to contain $4.5x10^6$ cells per liter. Cultures in LB 1/6 and LB 1/12 were inoculated with these four water samples. The samples harvested near the western wall presented the most diversity when in culture. Between 49 and 50 genera were identified from the top location sample while between 33 and 49 genera were identified at the bottom location. The northern wall presented a smaller diversity with only 32 to 34 identified genera (Table 2.13). The phylopeptidomics analysis of the resulting kinetics is shown in Tables 2.14 to 2.17.

In Sample A, *Caulobacter*, *Sphingomonas*, and *Sphingopyxis* were the main identified genera (Table 2.14). They were present in all kinetic points at both dilutions. *Caulobacter* represented between 7 to 43% of the samples. 6 to 19% of the biomass belonged to *Sphingomonas* while 3 to 10% belonged to *Sphingopyxis*. The genera *Pseudomonas* and *Bacillus* were also present during these kinetics (0 to 6% and 0 to 3% respectively). The genus *Fusarium* appeared at D 137 in LB 1/12 represented almost 7% of the identified peptides in LB 1/12 at D 173 (Table 2.14).

In Sample B one of the main identified genera was *Streptomyces* (4 to 5% for both dilutions) (Table 2.15). This genus was present in all kinetic point at a 1/12 dilution and only present at the first two kinetic points (D 45 and 137) at a 1/6 dilution. The genus *Caulobacter* was present in greater proportion in LB 1/6 than LB 1/12 (14 to 27% and 3 to 22% respectively). This change in proportion observed between both dilutions could imply an important nutrient need of *Caulobacter* cells. *Sphingomonas* represented between 6 to 29% of the samples and were present in similar proportion in both dilutions (6 to 23% in LB 1/6 and 11 to 29% in LB 1/12). The *Pseudomonas* genus was present in similar proportion in both kinetics, and represented about 4% of the total biomass. *Acidovorax* was mainly detected in LB 1/12 (3 to 29%). In LB 1/6 this genus represented 0 to 4% of the sample (detection in LB 1/6 started at D 173). *Acidovorax* appeared to have a smaller growth rate than other bacteria and is not competitive enough in a rich medium. At greater dilution this bacterium seemed to thrive in a poor medium. The genus *Fusarium* represented almost 7% of the identified peptides in LB 1/12 at D 227 (Table 2.15).

In Sample C, *Sphingomonas* was the main genus (2 to 28%) (Table 2.16). In this location (bottom western wall) the *Caulobacter* genus present at only one kinetic point per dilution and represented 2% (LB 1/6) and 1% (LB 1/12) of the identified genera. *Hydrogenophaga* (β-Proteobacteria) was present in all kinetic points from both dilutions (between 1 to 8% in LB 1/6 and 5 to 6% in LB 1/12). *Pseudomonas* and *Bacillus* represented about 3 and 2% of the samples respectively (at both dilutions) (Table 2.16).

In Sample D, the main genus was *Caulobacter* in both kinetics (8 to 32%) (Table 2.17). Sphingomonas was also present in important proportion at all kinetic points in both dilutions (6 to 15%). Both *Rhizobium* and *Azorhizobium* genera were present in all kinetic points in LB 1/6 and appeared at D 137 in LB 1/12. They

represented between 2 to 5% (*Rhizobium*) and 2 to 7% (*Azorhizobium*) of the identified bacteria. At D 173 in LB 1/6, genus *Fusarium* represented 10% of the sample (Table 2.17).

*Table 2.14: Proportion of each genus during the 2015 kinetic of Sample A in LB at both dilutions*

| | | | | | Sample A 1/6 | | | | Sample A 1/12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | D 45 | D 137 | D 173 | D 227 | D 45 | D 137 | D 173 | D 227 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Mycobacteriaceae | Mycobacterium | | | | | | 1,44 | | |
| | | | Nocardiaceae | Rhodococcus | | | 0,84 | 0,75 | | | | 0,78 |
| | | | Streptomycetaceae | Streptomyces | | 4,07 | | | | 4,95 | 4,17 | |
| bacterium YEK0313_class_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus | | | | 5,90 | 4,02 | | 2,86 | 3,06 |
| Bacteroidetes | Flavobacteria | Flavobacteriales | Flavobacteriaceae | Chryseobacterium or Bergeyella | | | | 0,35 | | | | |
| Cyanobacteria | Cyanophyceae | Synechococcales | Synechococcaceae | Synechococcus | | | 1,07 | | | | | |
| | Hormogoneae | Nostocales | Nostocaceae | Anabaena | | 0,30 | | | | | | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | | 2,80 | 3,02 | 2,15 | 3,41 | 2,54 | 1,86 | 1,66 |
| Proteobacteria | Alphapreoteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | 43,26 | 11,40 | 13,76 | 11,14 | 30,71 | 6,53 | 12,23 | 12,70 |
| | | Rhizobiales | Beijerinckiaceae | Chelatococcus | | | | 3,32 | | | | |
| | | | Bradyrhizobiaceae | Bosea | | 6,09 | 7,37 | 6,64 | 4,93 | | 4,42 | 4,90 |
| | | | | Bradyrhizobium | 4,34 | 4,35 | 5,64 | 5,01 | | | 3,80 | 4,44 |
| | | | Hyphomicrobiaceae | Blastochloris | | | 3,05 | | | | | |
| | | | | Devosia | | | | 3,07 | | | | |
| | | | | Hyphomicrobium | | 2,61 | | | | | 2,21 | 2,32 |
| | | | Methylocystaceae | Methylocystis | | | | 3,43 | | | | |
| | | | Phyllobacteriaceae | Mesorhizobium | | | 4,78 | 4,18 | | | | |
| | | | Rhizobiaceae | Rhizobium | | 4,39 | 5,38 | 4,53 | | | | 3,66 |
| | | | Xanthobacteraceae | Azorhizobium | | 6,58 | 8,37 | 4,94 | | | 4,00 | 4,82 |
| | | | | Xanthobacter | | 9,85 | 12,61 | 6,48 | | 2,80 | 5,89 | 6,66 |
| | | Rhodobacterales | Rhodobacteraceae | Paracoccus | | 2,38 | 2,46 | | | | | |
| | | | | Rhodovulum | 9,63 | | | | | | 2,54 | 2,79 |
| | | Rhodospirillales | Rhodospirillaceae | Azospirillum | | 3,20 | | 3,26 | | | | 2,53 |
| | | | | Magnetospira | | | | | | | 1,02 | |
| | | | | Oceanibaculum | | | 1,84 | | | | | |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter | | | | | | 5,15 | 2,09 | |
| | | | Sphingomonadaceae | Novosphingobium | | 5,59 | | | | | | |
| | | | | Sphingobium | | 6,32 | 4,13 | | | 9,19 | 3,14 | |
| | | | | Sphingomonas | 10,99 | 13,13 | 7,73 | 6,29 | 17,61 | 18,86 | 6,49 | 5,94 |
| | | | | Sphingopyxis | 10,00 | 5,00 | 3,32 | 2,98 | 4,80 | 7,18 | 2,94 | 2,50 |
| | Betaproteobacteria | Burkholderiales | Burkholderiaceae | Burkholderia | | 2,04 | 2,79 | 3,68 | | | 3,55 | 3,77 |
| | | | Comamonadaceae | Acidovorax | 6,44 | 3,98 | | 11,74 | 10,18 | 30,64 | 8,60 | 6,83 |
| | | | | Hydrogenophaga | 9,86 | 1,08 | | 3,98 | | | 4,33 | 4,31 |
| | | | | Hylemonella | | | | | 6,96 | | 3,80 | 4,20 |
| | | | | Limnohabitans | | | | | | | 4,52 | 4,48 |
| | | | | Pelomonas | | | | | 15,18 | | 2,93 | 2,89 |
| | | | | Polaromonas | | | | | | | | 4,61 |
| | | | | Rhodoferax | | | | | | | | 4,38 |
| | | | Oxalobacteraceae | Massilia | | | | | | | 2,22 | |
| | | Nitrosomonadales | Sterolibacteriaceae | Methyloversatilis | | 1,22 | 3,58 | 1,90 | | 2,29 | | |
| | Gammaproteobacteria | Pseudomonales | Pseudomonaceae | Pseudomonas | 5,47 | 3,62 | 4,39 | 4,27 | | 4,92 | 3,77 | 3,96 |
| | | Xanthomonadales | Xanthomonadaceae | Pseudoxanthomonas | | | | | 2,22 | | | |
| Ascomycota | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | | | 3,87 | | | 3,52 | 6,64 | 1,81 |

*Table 2.15: Proportion of each genus during the 2015 kinetic of Sample B in LB at both dilutions*

| | | | | | Sample B 1/6 | | | | Sample B 1/12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | D 45 | D 137 | D 173 | D 227 | D 45 | D 137 | D 173 | D 227 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Nocardiaceae | Nocardia | | | | | | | | 1,02 |
| | | | | Rhodococcus | | | 0,75 | 0,84 | | | | |
| | | | Streptomycetaceae | Streptomyces | 4,87 | 5,02 | | | 5,04 | 4,37 | 3,80 | 5,08 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Prevotellaceae | Prevotella | | | | | | | | 0,85 |
| | Sphingobacteria | Sphingobacteriales | Cytophagaceae | Dyadobacter | | | | | | | 0,33 | |
| bacterium YEK0313_class_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus | | 3,32 | 6,28 | | | 4,29 | 1,49 | 2,61 |
| Cyanobacteria | Hormogoneae | Nostocales | Hapalosiphonaceae | Mastigocladus | | | | | | | | 0,63 |
| | | | Nostocaceae | Anabaena | | | | 0,40 | | | | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 5,48 | 2,65 | 2,61 | 2,37 | 2,91 | 1,83 | | 2,32 |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | | | | | 1,35 | |
| Proteobacteria | Alphapreoteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | 25,26 | 27,23 | 14,93 | 14,01 | 7,19 | 22,43 | 3,44 | 4,38 |
| | | Rhizobiales | Beijerinckiaceae | Chelatococcus | | | 3,22 | | | | | |
| | | | Bradyrhizobiaceae | Bosea | | 5,81 | 7,13 | 8,45 | | 5,64 | | |
| | | | | Bradyrhizobium | 3,61 | | 5,26 | 6,27 | | 5,00 | 2,16 | 3,78 |
| | | | Hyphomicrobiaceae | Blastochloris | | | | 3,63 | | | | |
| | | | | Devosia | | | 3,13 | | | | | |
| | | | | Hyphomicrobium | | | | | | 2,82 | | |
| | | | Methylobacteriaceae | Methylobacterium | | | 6,33 | 6,53 | | 5,21 | | |
| | | | Methylocystaceae | Methylocystis | | | 3,45 | | | | | |
| | | | | Pleomorphomonas | | | | 3,17 | | | | |
| | | | Phyllobacteriaceae | Mesorhizobium | | | | 5,17 | | | | |
| | | | Rhizobiaceae | Rhizobium | | 4,94 | 4,51 | 5,78 | 3,91 | 4,48 | | |
| | | | Rhodobiaceae | Parvibaculum | | | | 2,54 | | | | |
| | | | Xanthobacteraceae | Azorhizobium | | | 4,82 | 9,82 | | 4,90 | | |
| | | | | Xanthobacter | 3,43 | 4,55 | 6,27 | 14,59 | | 6,95 | 1,90 | 3,58 |
| | | Rhodobacterales | Rhodobacteraceae | Paracoccus | | 3,37 | | 2,81 | | 2,41 | 1,32 | |
| | | | | Rhodovulum | | | 3,47 | | | | | |
| | | Rhodospirillales | Rhodospirillaceae | Azospirillum | | | 3,23 | 3,68 | | 3,28 | | 2,93 |
| | | | | Rhodovibrio | 1,59 | | | | | | | |
| | | | | Thalassobaculum | | | | | | | 1,19 | |
| | | Sphingomonadales | Erythrobacteraceae | Erythrobacter | 6,75 | | | | 8,91 | | 3,92 | |
| | | | | Porphyrobacter | | | | | | | | 3,60 |
| | | | Sphingomonadaceae | Sphingobium | 9,78 | | | | 13,72 | | 5,89 | 6,80 |
| | | | | Sphingomonas | 20,88 | 23,42 | 7,98 | 6,17 | 28,57 | 11,14 | 11,83 | 13,75 |
| | | | | Sphingopyxis | 7,98 | 11,18 | 3,78 | | 10,54 | 3,74 | 4,77 | 5,68 |
| | Betaproteobacteria | Burkholderiales | Burkholderiaceae | Burkholderia | | | 2,61 | | | 2,87 | 4,54 | |
| | | | Comamonadaceae | Acidovorax | | | 4,01 | 1,19 | 14,05 | 2,59 | 27,97 | 29,44 |
| | | | | Hydrogenophaga | 3,35 | 3,78 | 2,14 | | | | | |
| | | | | Pelomonas | | | | | | 1,86 | | |
| | | | | Variovorax | | | | | | | 8,87 | |
| | | | | Verminephrobacter | | | | | | | 8,89 | |
| | | | Methylibium_family | Methylibium | 2,43 | | | | | | | |
| | | Nitrosomonadales | Sterolibacteriaceae | Methyloversatilis | | | | 1,52 | | | | 2,02 |
| | Gammaproteobacteria | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 4,59 | 4,72 | 4,10 | | 4,48 | 4,20 | 3,55 | 4,75 |
| | | Xanthomonadales | Xanthomonadaceae | Pseudoxanthomonas | | | | 1,05 | | | | |
| Ascomycota | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | | | | | 0,68 | | 2,80 | 6,79 |

*Table 2.16: Proportion of each genus during the 2015 kinetic of Sample C in LB at both dilutions*

| | | | | | Sample C 1/6 | | | | Sample C 1/12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | D 45 | D 137 | D 173 | D 227 | D 45 | D 137 | D 173 | D 227 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Mycobacteriaceae | Mycobacterium | | | | | | 1,07 | | |
| | | | Nocardiaceae | Nocardia | | 0,79 | 0,83 | 0,93 | | | | |
| | | | Streptomycetaceae | Streptomyces | 4,61 | 3,71 | | | 3,14 | 3,85 | 3,45 | 3,86 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | | 0,57 | 0,61 | | |
| | | | Porphyromonadaceae | Porphyromonas | | | | | | | | 0,29 |
| Cyanobacteria | Cyanophyceae | Oscillatoriales | Cyanothecaceae | Cyanothece | | | 0,28 | | | | | |
| | Hormogoneae | Nostocales | Nostocaceae | Cylindrospermum | | | | 0,24 | | | | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 2,58 | 1,53 | 1,73 | 2,13 | 1,70 | | 1,76 | |
| | | | Paenibacillaceae | Paenibacillus | | | | | | | | 1,39 |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | | | | 1,32 | | 1,37 |
| Proteobacteria | Alphapreoteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | | | 1,87 | | | | | |
| | | | | Caulobacter | | 2,41 | | | | | 1,01 | |
| | | | Bradyrhizobiaceae | Bosea | | 5,38 | 4,91 | 5,39 | | 4,07 | 2,87 | 3,68 |
| | | | | Bradyrhizobium | | 3,92 | 3,74 | 4,33 | | 3,53 | 2,67 | |
| | | | Hyphomicrobiaceae | Blastochloris | | 2,27 | | 2,30 | | | | |
| | | | | Hyphomicrobium | | | 2,20 | | | 1,77 | | |
| | | | Methylobacteriaceae | Methylobacterium | | 4,48 | 4,03 | | | | 2,47 | |
| | | | | Microvirga | | | | | 0,88 | 2,47 | | |
| | | | Phyllobacteriaceae | Mesorhizobium | | 3,37 | 3,48 | 3,65 | | 2,58 | | |
| | | | Rhizobiaceae | Rhizobium | | 3,91 | 3,69 | 4,12 | | 2,86 | | 3,17 |
| | | | Xanthobacteraceae | Azorhizobium | | 5,67 | 5,63 | 6,27 | | 4,16 | 3,01 | 3,40 |
| | | | | Xanthobacter | 3,23 | 8,65 | 8,64 | 9,23 | | 6,28 | 4,47 | 4,99 |
| | | Rhodobacterales | Rhodobacteraceae | Paracoccus | | | 1,82 | | | 1,50 | | |
| | | | | Rhodovulum | | 2,76 | | 2,86 | | | | |
| | | Rhodospirillales | Rhodospirillaceae | Azospirillum | | 2,86 | 2,60 | 2,89 | | | 1,61 | 2,37 |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter | | | | | | | | 2,67 |
| | | | | Erythrobacter | 8,31 | | | | | | | |
| | | | | Porphyrobacter | | 3,74 | | | | | | |
| | | | Sphingomonadaceae | Novosphingobium | 10,58 | 6,18 | 4,29 | | | | | |
| | | | | Sphingobium | 12,64 | 7,28 | 5,02 | 4,36 | | | | 4,46 |
| | | | | Sphingomonas | 27,73 | 16,41 | 10,29 | 8,55 | 1,57 | 3,10 | 2,01 | 8,50 |
| | | | | Sphingopyxis | 9,76 | 5,58 | 3,92 | 3,48 | 0,86 | | | 3,64 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | | | | | 3,36 | 7,85 | | |
| | | | Aquabacterium_family | Aquabacterium | | | | | 5,03 | | | |
| | | | Burkholderiaceae | Burkholderia | | 2,01 | 3,77 | 4,59 | 4,60 | 4,39 | 4,65 | 4,56 |
| | | | Burkholderiales bacterium JOSHI_001_family | Burkholderiales bacterium JOSHI_001_genus | | | | | 5,31 | | | |
| | | | Comamonadaceae | Acidovorax | 5,05 | | 10,66 | 18,70 | 6,98 | | 12,88 | 18,98 |
| | | | | Hydrogenophaga | 5,93 | 1,25 | 7,12 | 7,54 | 5,24 | 5,61 | 6,47 | 5,88 |
| | | | | Hylemonella | | | | | | 5,72 | 5,97 | 4,95 |
| | | | | Limnohabitans | | | | | | 5,79 | 7,01 | 6,60 |
| | | | | Pelomonas | | | | | 11,07 | 4,99 | 6,52 | 4,84 |
| | | | | Polaromonas | 5,20 | 1,36 | 3,83 | 4,23 | 5,59 | | 7,05 | |
| | | | | Ramlibacter | | | | | | 5,34 | 6,25 | |
| | | | | Thiomonas | | | | | 3,56 | | | |
| | | | | Variovorax | | | | | | 5,45 | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ideonella_family | Ideonella | | | | | 7,62 | | | |
| | | | Methylibium_family | Methylibium | | | 4,58 | | 8,88 | 5,53 | 6,82 | 5,81 |
| | | | Oxalobacteraceae | Massilia | | 1,15 | | | 3,78 | 3,13 | 3,48 | 3,36 |
| | | | Rhizobacter_family | Rhizobacter | | | | | 7,75 | | | |
| | | | Rubrivivax_family | Rubrivivax | | | | | 5,59 | | | |
| | | Neisseriales | Neisseriaceae | Chromobacterium | | | | | 2,07 | | | |
| | | | | Pseudogulbenkiania | | | | | | | 1,78 | |
| | | Rhodocyclales | Zoogloeaceae | Azoarcus | | | | | 2,20 | | | |
| | | | | Thauera | | | | | | 2,28 | 2,21 | |
| | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Enterobacter | | | 1,06 | | | | | 1,23 |
| | | | | Klebsiella | | | | | | 1,09 | | |
| | | Pseudomonales | Pseudomonaceae | Pseudomonas | 4,38 | 3,33 | | 4,20 | 2,64 | 3,67 | 3,38 | |
| Ascomycota | Dothideomycetes | Dothideales | Saccotheciaceae | Aureobasidium | | | | | | | 0,21 | |

*Table 2.17: Proportion of each genus during the 2015 kinetic of Sample D in LB at both dilutions*

| | | | | | Sample D 1/6 | | | | Sample D 1/12 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | D 45 | D 137 | D 173 | D 227 | D 45 | D 137 | D 173 | D 227 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Intrasporangiaceae | Janibacter | | 0,11 | | | | | | |
| | | | Micrococcaceae | Arthrobacter | | | | | | | 0,36 | 0,38 |
| | | | Nocardiaceae | Nocardia | | | | 0,52 | | | | |
| | | | | Rhodococcus | | | | | | 0,42 | | |
| | | | Streptomycetaceae | Streptomyces | 5,86 | 2,06 | 4,83 | 2,29 | 4,48 | 2,35 | 4,68 | 2,41 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | | 0,38 | | | |
| | | | Prevotellaceae | Prevotella | 0,54 | 0,29 | | | | | | |
| | Cytophagia | Cytophagales | Microscillaceae | Microscilla | | | | | | | 0,15 | |
| bacterium YEK0313_class_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus | 1,50 | 1,50 | 2,07 | 3,72 | | 4,32 | 5,03 | 4,73 |
| Cyanobacteria | Cyanophyceae | Synechococcales | Synechococcaceae | Synechococcus | | 0,36 | | | 3,18 | 2,65 | 2,75 | 2,33 |
| | Hormogoneae | Nostocales | Nostocaceae | Nostoc | 0,20 | | | | | | | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 5,55 | 3,57 | 5,91 | 5,77 | | | | |
| | | | Paenibacillaceae | Paenibacillus | | | 1,24 | 2,11 | | | | |
| | | | Staphylococcaceae | Staphylococcus | | | | | 3,19 | 2,17 | 1,71 | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | | | | | 0,79 | |
| | | | Lachnospiraceae | Roseburia | | | 0,31 | | | | | |
| Proteobacteria | Alphapreoteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | | 4,15 | | | | 3,25 | | |
| | | | | Caulobacter | 16,82 | 11,62 | 12,82 | 8,58 | 31,65 | 21,71 | 14,85 | 8,22 |
| | | Rhizobiales | Beijerinckiaceae | Chelatococcus | | | | 1,57 | | | | |
| | | | Bradyrhizobiaceae | Bosea | 7,42 | 2,57 | 3,73 | 6,96 | 0,56 | 6,22 | 5,66 | 5,56 |
| | | | | Bradyrhizobium | 3,63 | 3,88 | 2,73 | 5,25 | | 4,62 | 2,57 | 4,68 |
| | | | Hyphomicrobiaceae | Blastochloris | | | 1,43 | | | 1,21 | | 1,20 |
| | | | | Devosia | | | | 1,65 | | 1,45 | 1,72 | 1,42 |
| | | | | Hyphomicrobium | 2,17 | 1,08 | | 1,35 | | | 1,20 | |
| | | | Methylobacteriaceae | Methylobacterium | | 2,21 | | | 2,46 | 2,99 | | 2,60 |
| | | | | Microvirga | | | 2,34 | 2,09 | | 2,42 | | |
| | | | Methylocystaceae | Methylocystis | | | 1,74 | | | | | 1,42 |
| | | | | Methylopila | | | | 1,62 | | | | |
| | | | Phyllobacteriaceae | Mesorhizobium | | 1,75 | 2,25 | 4,46 | | | 2,26 | |
| | | | Rhizobiaceae | Rhizobium | 3,21 | 1,96 | 2,40 | 4,87 | | 4,53 | 4,34 | 2,15 |
| | | | Xanthobacteraceae | Azorhizobium | 5,55 | 2,69 | 3,72 | 7,47 | | 2,62 | 2,29 | 4,66 |
| | | | | Xanthobacter | 8,29 | 5,75 | 1,99 | 10,70 | | 5,13 | 6,04 | 6,34 |
| | | Rhodobacterales | Rhodobacteraceae | Paracoccus | 1,46 | 2,72 | | 2,36 | | 2,45 | | |
| | | | | Rhodovulum | | 1,28 | 1,73 | | | | 3,29 | 3,14 |
| | | Rhodospirillales | Rhodospirillaceae | Azospirillum | | 1,32 | 1,59 | 3,21 | | 1,59 | 3,29 | 3,18 |
| | | | | Magnetospira | | | | | | 0,62 | | |
| | | | | Thalassobaculum | | 0,90 | | | | | | |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter | | | 1,91 | | | | | |
| | | | | Erythrobacter | 4,03 | 3,44 | | | | | | 2,17 |
| | | | | Porphyrobacter | | | | | | | 1,97 | |
| | | | Sphingomonadaceae | Novosphingobium | | | | | | | 3,01 | |
| | | | | Sphingobium | 5,88 | 6,92 | 2,88 | | | | 3,63 | 3,16 |
| | | | | Sphingomonas | 15,01 | 14,87 | 8,42 | 6,17 | 5,92 | 7,90 | 7,39 | 8,52 |
| | | | | Sphingopyxis | 4,61 | 3,94 | 2,49 | 1,80 | | 1,47 | 4,02 | 3,59 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Azohydromonas | | | | | 3,47 | | | |
| | | | Burkholderiaceae | Burkholderia | | 1,58 | 3,97 | | 4,24 | 3,62 | 3,27 | 2,12 |
| | | | Comamonadaceae | Acidovorax | | 2,05 | 12,71 | 11,79 | 3,98 | 2,57 | 7,80 | 18,68 |
| | | | | Hydrogenophaga | | 0,83 | 1,71 | 2,21 | | 5,89 | 1,64 | 2,51 |
| | | | | Hylemonella | | | | | | 1,36 | | |
| | | | | Pelomonas | 3,66 | 2,80 | | | 6,56 | | 1,72 | 2,13 |
| | | | Ideonella_family | Ideonella | | 2,15 | | | 4,52 | | | |
| | | | Methylibium_family | Methylibium | | 2,36 | | | 5,32 | | | |
| | | | Oxalobacteraceae | Janthinobacterium | | | | | 2,29 | | | |
| | | | | Massilia | | 1,17 | | | | | 1,04 | 1,45 |
| | | | Rhizobacter_family | Rhizobacter | | 2,09 | | | 4,62 | | | |
| | | | Rubrivivax_family | Rubrivivax | | 1,53 | | | 3,52 | | | |
| | | Neisseriales | Chromobacteriaceae | Leeia | | | | | 0,98 | | | |
| | | Rhodocyclales | Zoogloeaceae | Thauera | | | | | 1,40 | | | |
| | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Enterobacter | | | | | | | | 0,66 |
| | | | | Escherichia | | | | | | | 1,57 | 0,60 |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 5,24 | 3,87 | 2,15 | | 3,94 | 4,46 | | |
| | | Xanthomonadales | Xanthomonadaceae | Lysobacter | | | 0,84 | 0,59 | | | | |
| | | | | Pseudoxanthomonas | | | | 0,65 | | | | |
| | Deltaproteobacteria | Myxococcales | Kofleriaceae | Haliangium | | | | 0,23 | | | | |
| Ascomycota | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | | | 10,09 | | | | | |

In the kinetic cultures, the main genus for Samples A, B, and D was *Caulobacter,* which represents 3%-70% in terms of biomass contribution. In Sample C this genus was only present at D 137 (2%) and D 173 (1%) in LB 1/6 and LB 1/12 respectively. The genus *Xanthobacter* was also present in high proportion in all samples of the kinetic, mainly starting after 4 months of cultivation. The third main genus found in all samples belonged to the *Sphingomonadaceae* family. The main genera found in all samples in average proportion were *Sphingomonas* and *Sphingopyxis*. Two other genera, *Sphingobium* and *Novosphingobium* were also present, but in smaller proportion and not in all samples.

#### 2.2.3.1.3 Phylopeptidomics analysis of the water from Pool 2 sampled during the 2015 campaign

For the Pool 2 samples, different media were used for the cultures: BHI and BHI diluted 10 times, LB and LB diluted twice, TSB and TSB diluted twice, and NB and NB diluted twice in water. The eight resulting kinetics were analyzed at days 82, 153 and 207.

Table 2.18 presents the phylopeptidomics results for the eight cultures. The main genera identified during the 2015 kinetic were *Leifsonia* (about 4%), *Bosea* (about 3%), *Bradyrhizobium* (22 to 36%), *Rhodopseudomonas* (3 to 4%), *Prosthecomicrobium* (about 4%), *Methylobacterium* (about 3%), *Mesorhizobium* (about 4%), *Rhizobium* (about 4%), and the *Trichoderma* fungi (4 to 7%). A majority of *Proteobacteria* were identified and more specifically *$\alpha$-Proteobacteria* during the 2015 kinetic.

A stability of the community in liquid culture over time could be observed in all media, starting from two and a half months of cultivation.

BHI medium (at both dilutions) presented a smaller diversity compared to the other media used. 26 genera were common to all media. These genera were among the main genera identified.

***Table 2.18: Proportion of each genus during the 2015 kinetic of Sample F.*** *Results from all different media were mixed. The mean of each genus in each medium is presented in this table.*

| | | | | | D 82 | D 153 | D 207 |
|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | Genus | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Nocardiaceae | Nocardia | 0,38 | 0,35 | 0,38 |
| | | Micrococcales | Cellulomonadaceae | Cellulomonas | | | 0,04 |
| | | | Microbacteriaceae | Agromyces | 1,13 | 1,09 | 1,04 |
| | | | | Arthrobacter | 0,68 | 0,67 | 0,82 |
| | | | | Cryocola | 2,00 | 1,97 | 1,81 |
| | | | | Leifsonia | 4,02 | 4,11 | 3,42 |
| | | | | Microbacterium | 1,46 | 1,40 | 1,36 |
| | | | Micrococcaceae | Micrococcus | 0,33 | 0,32 | 0,32 |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 3,21 | 2,57 | 4,00 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | 0,08 | |
| | | | Porphyromonadaceae | Porphyromonas | 0,05 | | |
| | | | Prevotellaceae | Prevotella | 0,08 | | |
| | | | Rikenellaceae | Rikenella | | | 0,02 |
| | Cytophagia | Cytophagales | Cytophagaceae | Runella | 0,18 | 0,19 | 0,16 |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | 0,00 | 0,00 | 0,11 |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 0,62 | 0,76 | 0,20 |
| | | | Paenibacillaceae | Paenibacillus | | 0,15 | 0,18 |
| | Clostridia | Clostridiales | Clostridiceae | Clostridium | 0,83 | 0,36 | 0,91 |
| | | | Lachnospiraceae | Butyrivibrio | 0,00 | 0,05 | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | 1,38 | 1,28 | 0,99 |
| | | Rhizobiales | Aurantimonadaceae | Aurantimonas | 0,47 | 0,72 | |
| | | | | Aureimonas | 1,67 | 1,46 | 1,55 |
| | | | Bartonellaceae | Bartonella | 1,98 | 1,73 | 1,31 |
| | | | Beijerinckiaceae | Beijerinckia | 0,83 | 1,23 | |
| | | | | Methylocapsa | | | 0,48 |
| | | | Bradyrhizobiaceae | Bosea | 3,28 | 3,34 | 3,41 |
| | | | | Bradyrhizobium | 22,42 | 23,85 | 34,54 |
| | | | | Rhodopseudomonas | 2,85 | 4,34 | 3,80 |
| | | | Brucellaceae | Ochrobactrum | 0,66 | 0,49 | 0,55 |
| | | | | Pseudochrobactrum | 0,38 | | |
| | | | Chelatococcaceae | Chelatococcus | 1,03 | | |
| | | | Hyphomicrobiaceae | Devosia | 2,37 | 1,59 | 0,60 |
| | | | | Hyphomicrobium | 1,99 | 1,85 | 1,79 |
| | | | | Prosthecomicrobium | 3,90 | 3,59 | 3,25 |
| | | | Methylobacteriaceae | Methylobacterium | 3,37 | 3,49 | 3,35 |
| | | | | Microvirga | 1,44 | 1,81 | 0,68 |
| | | | Methylocystaceae | Methylocystis | 0,54 | | |
| | | | | Pleomorphomonas | 1,70 | 2,11 | 1,96 |
| | | | Phyllobacteriaceae | Aminobacter | 1,86 | 1,01 | 0,59 |
| | | | | Mesorhizobium | 4,17 | 3,73 | 3,97 |
| | | | Rhizobiaceae | Rhizobium | 4,35 | 4,55 | 4,20 |
| | | | Rhodobiaceae | Afifella | 0,38 | | |
| | | | | Amorphus | 0,28 | 0,36 | 0,40 |
| | | | Xanthobacteraceae | Azorhizobium | 0,96 | 0,00 | 0,43 |
| | | | | Starkeya | | 0,44 | 0,00 |
| | | | | Xanthobacter | 1,64 | 1,54 | 1,62 |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas | 0,57 | 0,49 | 0,29 |
| | | | | Labrenzia | 0,75 | 1,89 | 1,66 |
| | | | Rhodobacteraceae | Pannonibacter | 2,06 | 1,29 | 0,99 |
| | | Rhodospirillales | Acetobacteraceae | Acetobacter | | 0,26 | |
| | | | | Roseomonas | 0,59 | | |
| | | | Rhodobacteraceae | Komagataeibacter | 0,00 | 0,56 | |
| | | | Rhodospirillaceae | Azospirillum | 1,77 | 1,83 | 1,86 |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | 2,41 | 2,15 | 1,58 |
| | Betaproteobacteria | Burkholderiales | Methylibium_family | Methylibium | 0,17 | 0,00 | 0,00 |
| | | | Burkholderiaceae | Burkholderia | 1,16 | 1,43 | 1,53 |
| | | | Comamonadaceae | Acidovorax | 0,15 | | 0,15 |
| | Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio | | 0,14 | |
| | | Desulfuromonadales | Geobacteraceae | Geobacter | 0,15 | | |
| | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Enterobacter | 0,00 | | 0,10 |
| | | | | Escherichia | 0,11 | | |
| | | | | Serratia | 0,12 | | |
| | | | Erwiniaceae | Pantoea | 0,05 | | 0,09 |
| | | Pseudomonadales | Moraxellaceae | Acinetobacter | | 0,08 | |
| | | | Pseudomonadaceae | Pseudomonas | 1,77 | 2,40 | 3,78 |
| Ascomycota | Eurotiomycetes | Eurotiales | Trichocomaceae | Talaromyces | | 0,12 | 0,07 |
| | Saccharomycetes | Saccharomycetales | Saccharomycetaceae | Zygosaccharomyces | 0,02 | | |
| | Sordariomycetes | Hypocreales | Clavicipitaceae | Metarhizium | 0,03 | | 0,06 |
| | | | Cordycipitaceae | Torrubiella | | 0,02 | |
| | | | Hypocreaceae | Trichoderma | 7,14 | 6,69 | 3,60 |
| | | | Nectriaceae | Fusarium | | 2,10 | |
| | | | | Neonectria | 0,03 | | |
| | | | Ophiocordycipitaceae | Ophiocordyceps | 0,04 | | |

To analyze microbial community in Pool 2 water in 2017 only two media were chosen: one general such as LB, and R2A, optimized for water samples [323].

### *2.2.3.2 Phylopeptidomics analysis of the water from Pool 2 sampled during the 2017 campaign*

In another campaign, we had the opportunity to sample once again Pool2 but at three different locations and initiated cultures in two liquid media: LB 1/12 and R2A 1/3. The latest medium was chosen on the basis that *Variovorax* was the main bacteria identified using metagenetics in Pool 2 during the 2015 campaign (cf. Chapter III.2). Based on the previous results, we choose to follow the culture in a short-term mode and sampled the cultures at 3, 7, 14, and 21 days. Only Sample H was analyzed at 3, 7, 14, and 21 days. Samples G and I were only analyzed at days 7 and 28.

#### 2.2.3.2.1 Kinetic from Sample G: microbial composition in the bottom of the pool in 2017

In the 2017 LB kinetic from Sample G, the main identified genera were *Sphingomonas* (2 to 65%), followed by *Sphingobium* (0 to 10%), *Pelomonas* (1 to 11%), *Rhizobium* (0 to 6.5%), and *Pseudomonas* (2 to 4%). *Streptomyces* genus was present in important proportion (about 32%) for only 1 triplicate at D7 and D28. In the other duplicates this genus represented between 2 and 4% of the sample. From this kinetic it can be observed that, if the triplicates were not similar within each other, a certain consistency can be seen between the 2 kinetic points. The first triplicate in D7 presented an important proportion of *Sphingomonas* (65%) when the second triplicate in D28 contained 49% of the same genus. Similarly *Pelomonas* represented about 4.7% of the sample at D7 and 3% at D28. The third triplicate at D7 and D28 also presented some similarity in microbial composition and proportion. *Streptomyces* represented at both kinetic points about 32% of the sample, while *Ralstonia* and *Burkholderia* were present at about 44% and 10% respectively (Fig. 2.9; Appendix 4, Table 6.4).

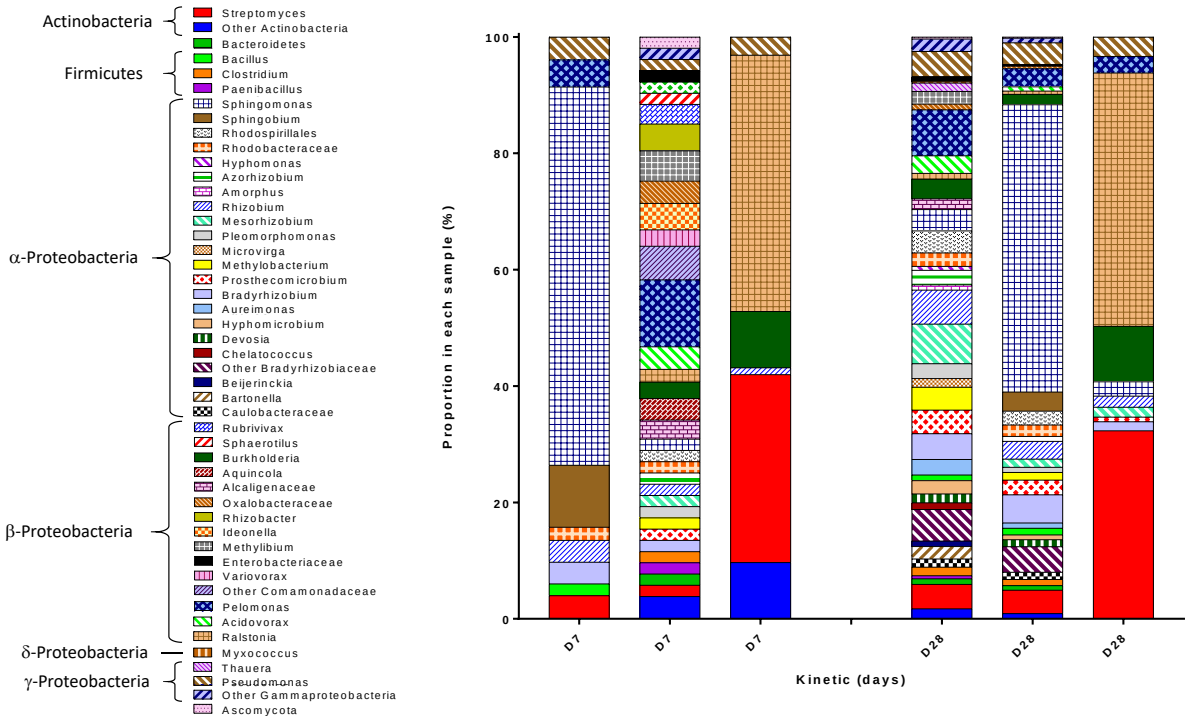



*Figure 2.9: Kinetic of the liquid culture of Sample G from Pool 2 in 2017 at the genus level in LB*

In the R2A kinetic, the genus *Pelomonas* was also present in important quantity (1 to 15%). The genera *Bradyrhizobium* and *Rhizobium* were also present in the kinetic at a proportion from 1.5 to 4.5% and 0 to 6% respectively (Fig. 2.10; Appendix 4, Table 6.5). In this kinetic the evenness of each genus was greater than in the other kinetics. Indeed the greater observed proportion in Sample G R2A kinetic was 15% for *Pelomonas* while in the other kinetics, *Sphingomonas* could represent up to 65% of the sample, and *Ralstonia* up to 40%.

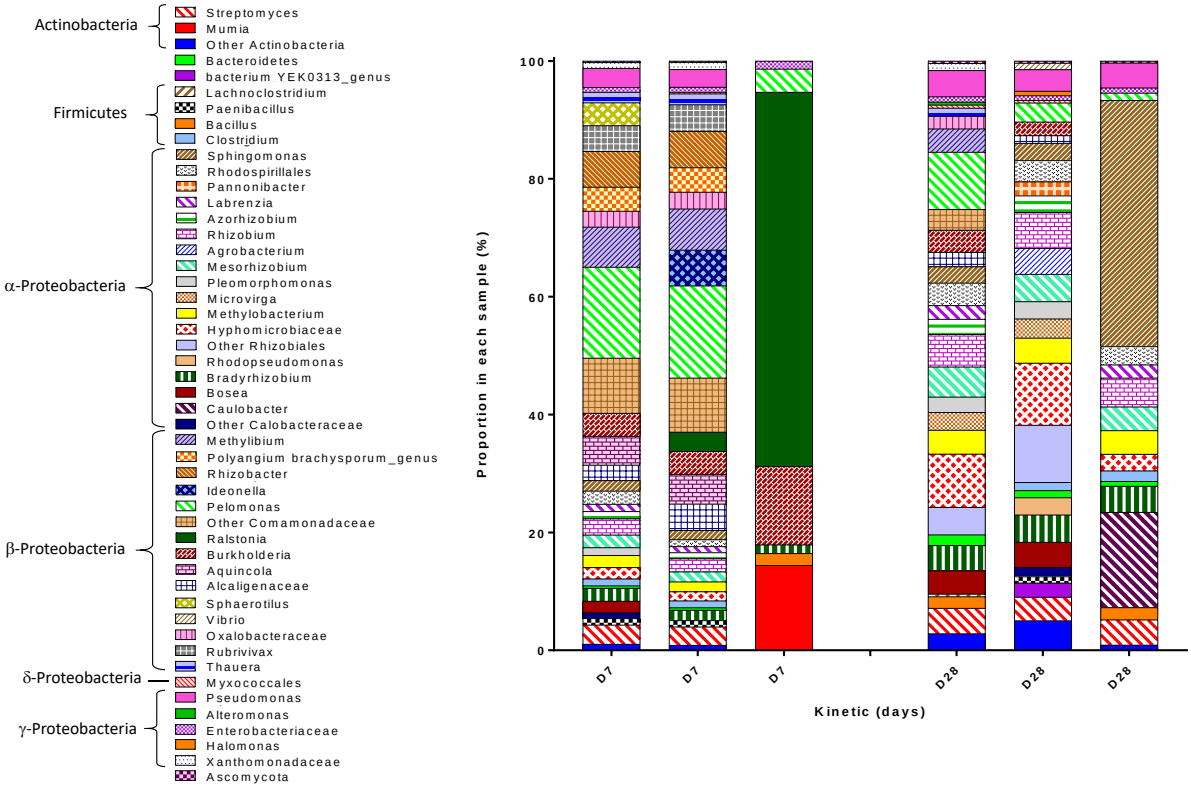



*Figure 2.10: Kinetic of the liquid culture of Sample G from Pool 2 in 2017 at the genus level in R2A*

Diversity was thus present at both kinetic points in both media (Fig. 2.9 & 2.10). A stability in the diversity could also be observed despite the visible growth of the microbial community (Fig. 2.11).

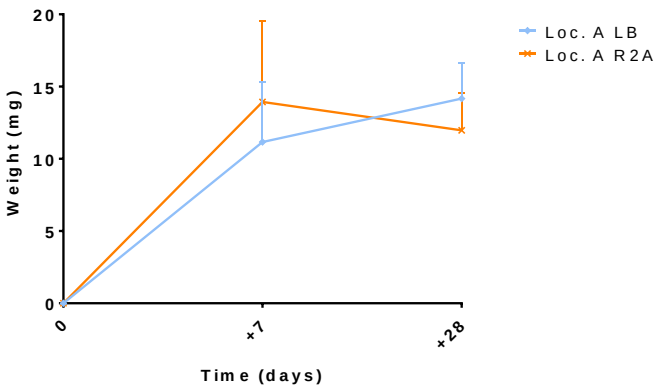



*Figure 2.11: Weight of Sample G pellet at each 2017 kinetic point*

#### 2.2.3.2.2 Kinetic from Sample H: microbial composition above the chimney in 2017

During the 2017 kinetic, the main genera found in Sample H in LB and R2A liquid cultures were the same: *Streptomyces*, *Bradyrhizobium*, *Rhizobium*, *Burkholderia*, *Acidovorax*, *Pelomonas*, *Methylibium*, *Rhizobacter*, *Rubrivivax*, and *Pseudomonas* (Appendix 4, Tables 6.6 & 6.7). As in the 2015 kinetic, some diversity could be observed in the cultures, starting from day 7 (Fig. 2.12 & 2.13; Appendix 4, Tables 6.6 & 6.7). The kinetic from Sample H were not especially even between the genera repartition.

In the LB kinetic, at D14, the *Sphingomonas* genus represented 72% of the identified and attributed peptides. Despite an observable similarity between the triplicates at D7, the triplicate appeared different at D14. For example triplicate 3 was composed of about 73% of *Sphingomonas* while the others contained 3 to 4% of this genus. Similarly triplicate 2 presented 24% of *Caulobacter* cells while it was only present in one other triplicate with a 1% proportion. At D28 the first two triplicates were similar both in composition and proportion. Triplicate 3 possessed more *Rhizobiales* genera than the other two. The proportion of phyla others than *Proteobacteria* was similar between each triplicate at D28 (Fig. 2.12; Appendix 4, Table 6.6).

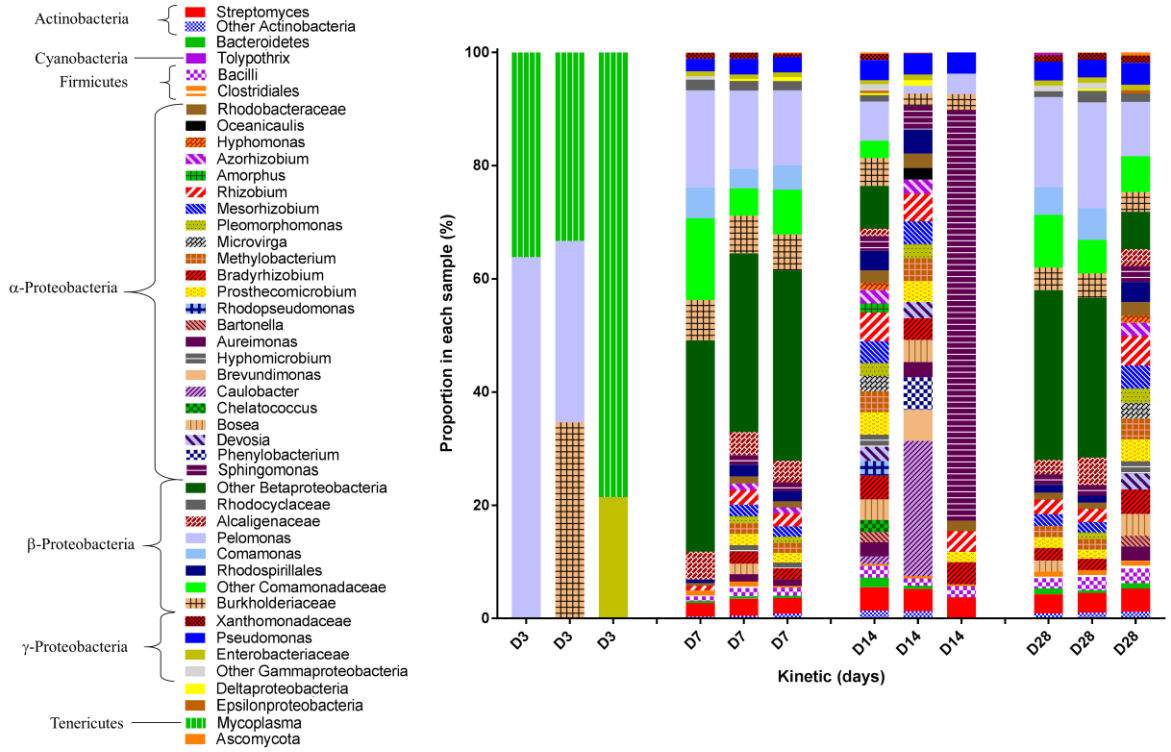



*Figure 2.12: Kinetic of the liquid culture of Sample H from Pool 2 in 2017 at the genus level in LB*

In R2A, *Pelomonas* was among the main genera and represented up to 22% of the sample (D28). At D7 the triplicates appeared similar within each other in terms of composition and proportion. The main genera present at D7 were *Streptomyces* (about 3%), *Bradyrhizobium*(about 2%), *Rhizobium* (about 2%), *Burkholderia* (about 3-4%), *Acidovorax* (5-6%), *Pelomonas* (about 15-18%), *Methylibium* (about 6-8%), *Rhizobacter* (about 5.5-7%), *Rubrivivax* (about 4-5%), and *Pseudomonas* (about 3%). At D14 and D28 the triplicates presented some differences in terms of composition and proportion. At D14 the *Bradyrhizobiaceae* family represented about 8% of triplicates 1 and 2 while it was only present in a 4% proportion in triplicate 3. Similarly, *Methylobacteriaceae* family represented about 7% of triplicates 2 and 2 but only 1% of triplicate 3. *Pelomonas* was however present in greater proportion in triplicate 3 (about

16%) compared to triplicates 1 and 2 (6 and 10% respectively) and *Variovorax* (about 4%), *Rhizobacter* (about 6%), and *Rubrivivax* (about 4%) were only present in triplicate 3. At D28 a major difference can be observed in triplicate 1: about 40% of the sample was composed by *Actinobacteria*, while the other triplicates were composed of about 5% of this phylum (Appendix 4, Table 6.7).

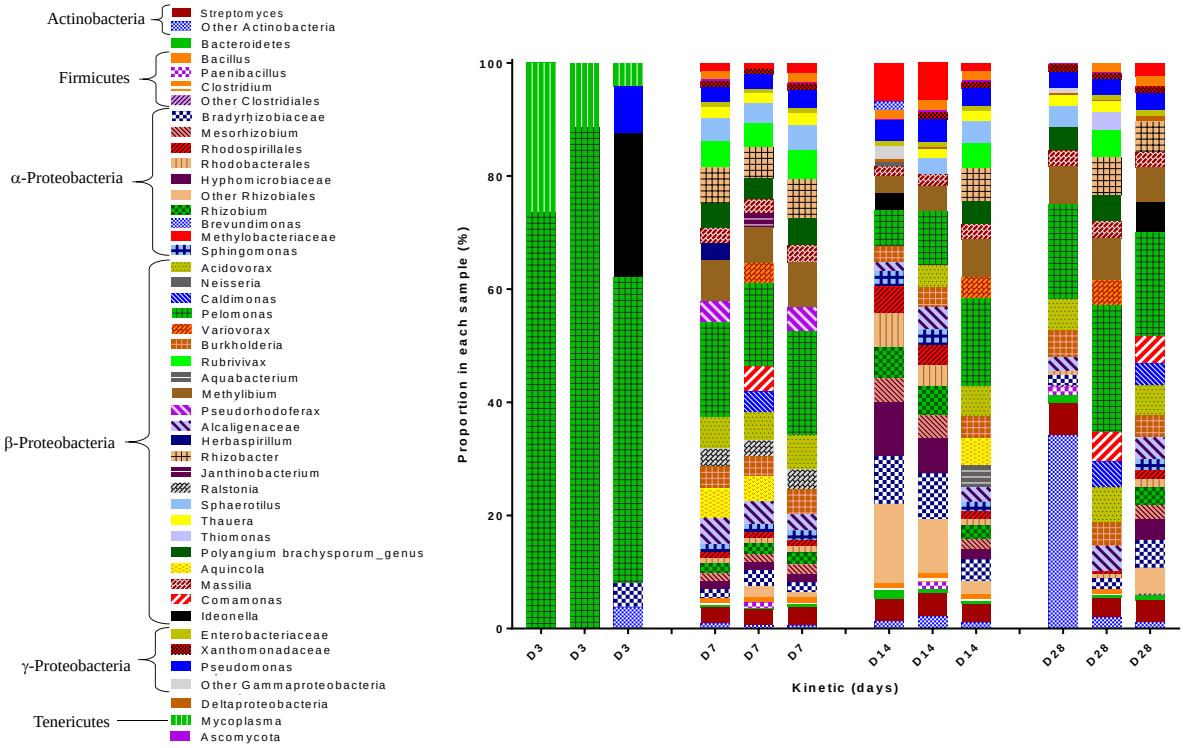



*Figure 2.13: Kinetic of the liquid culture of Sample H from Pool 2 in 2017 at the genus level in R2A*

A microbial diversity was identified in Sample H in both kinetics. A stability in the diversity could also be observed despite the visible growth of the microbial community (Fig. 2.14). Without addition of liquid media microorganisms were able to develop and grow even 3 months after insemination in a 6 mL nutritive media.

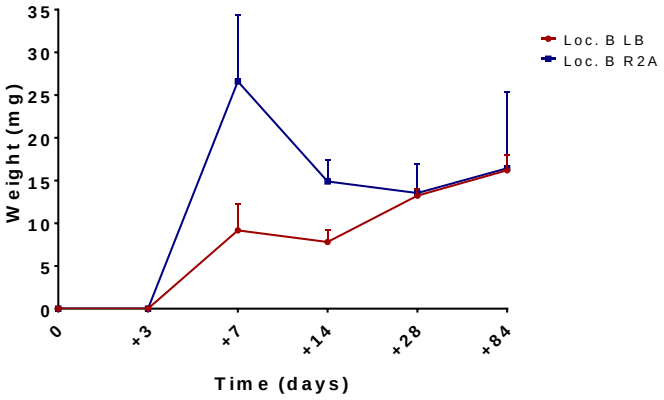



*Figure 2.14: Weight of Sample H pellet at each 2017 kinetic point*

### 2.2.3.2.3 Kinetic from Sample I: microbial composition inside the chimney in 2017

The stability could also be observed in Sample I between 7 and 28 days of liquid culture (Fig. 2.15 & 2.16). During the LB kinetic triplicates were different a D7. Triplicates 1 and 3 were similar while triplicate 2 was

different. In triplicates 1 and 3, *Streptomyces* was present at 20 to 30% in the sample when it was only present at about 2% in triplicate 2. Similarly, *Ralstonia* was detected in both triplicate 1 and 3 (40 and 26% respectively) while it was completely absent from triplicate 2. At D28 the triplicates appeared to be highly similar. In all triplicates, Streptomyces represented about 3% of the sample, *Bradyrhizobium* and *Methylobacterium* were present at about 2-3%, *Novosphingobium* represented between 10-12%, *Sphingomonas* represented between 10-11%, *Burkholderia* represented about 2% of the sample, and *Pseudomonas* represented about 3% (Fig. 2.15; Appendix 4, Table 6.8).

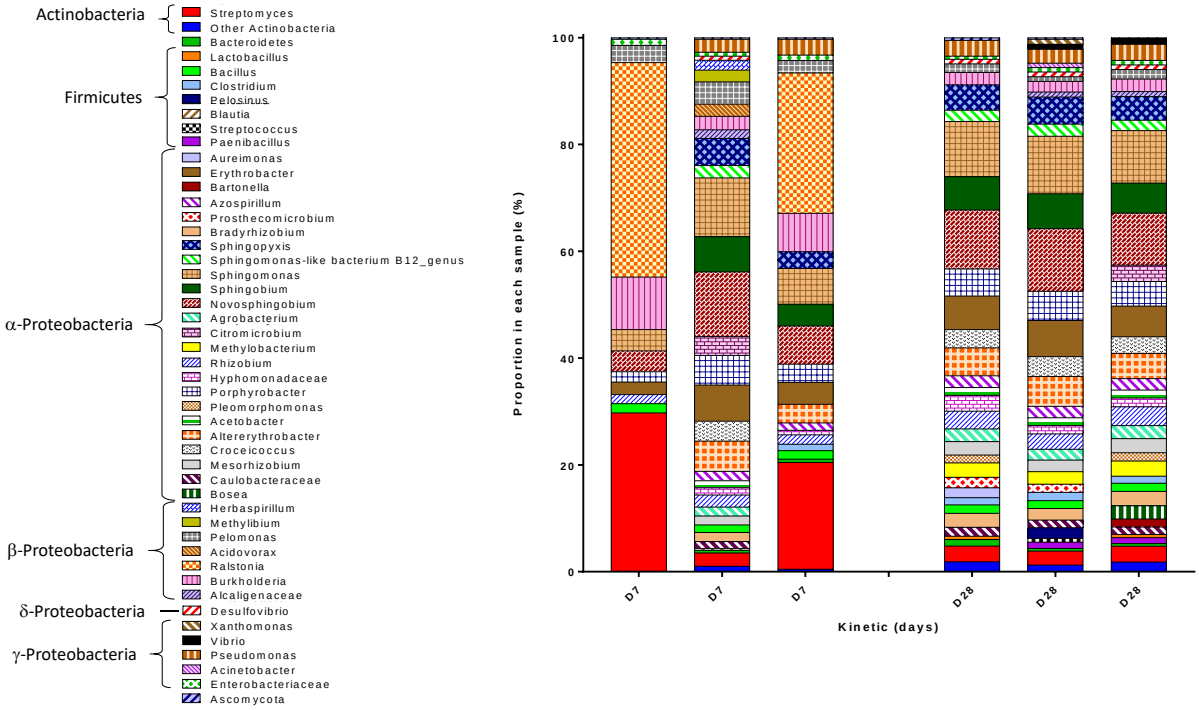



*Figure 2.15: Kinetic of the liquid culture of Sample I from Pool 2 in 2017 at the genus level in LB*

During the R2A kinetic the triplicate from D7 were similar. *Streptomyces* and *Bradyrhizobium* both represented about 2% of the samples, *Altererythrobacter* was present at about 6%, *Erythrobacter* was present at about 7% of the sample, *and Sphingomonas* represented about 12% of the sample for example. This similarity observed at D7 was not detected at D28 between the triplicates. If triplicates 1 and 2 were similar, triplicate 3 was different. *Steptomyces* represented about 18% of triplicate 3 when it was only present at about 3% in triplicates 1 and 2. Moreover *Ralstonia* was absent from triplicates 1 and 2 but represented 23% of triplicate 3. Several Rhizobiales, present in similar proportion in triplicates 1 and 2 were absent from triplicate 3 (*e.g. Bradyrhizobium* and *Mesorhizobium* at about 2% each, *Methylobacterium* at about 2%,…) (Fig. 2.16; Appendix 4, Table 6.9).

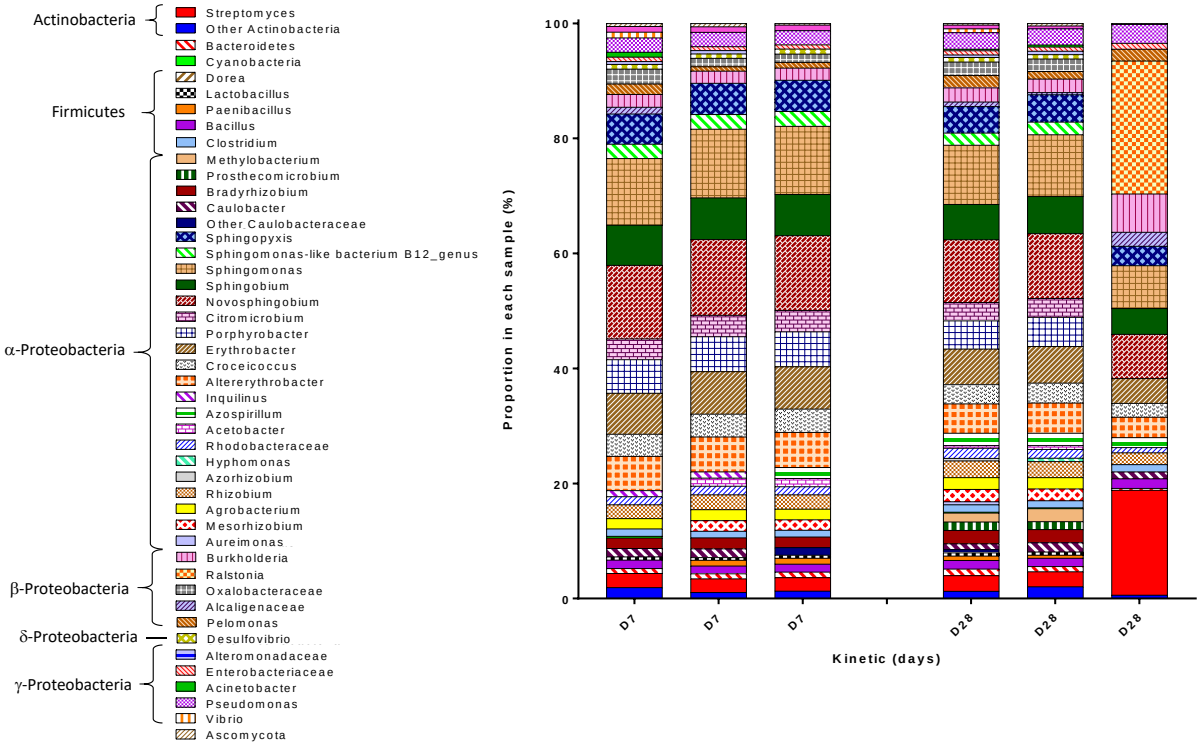



*Figure 2.16: Kinetic of the liquid culture of Sample I from Pool 2 in 2017 at the genus level in R2A*

A stability in the diversity could also be observed despite the visible growth of the microbial community (Fig. 2.17).

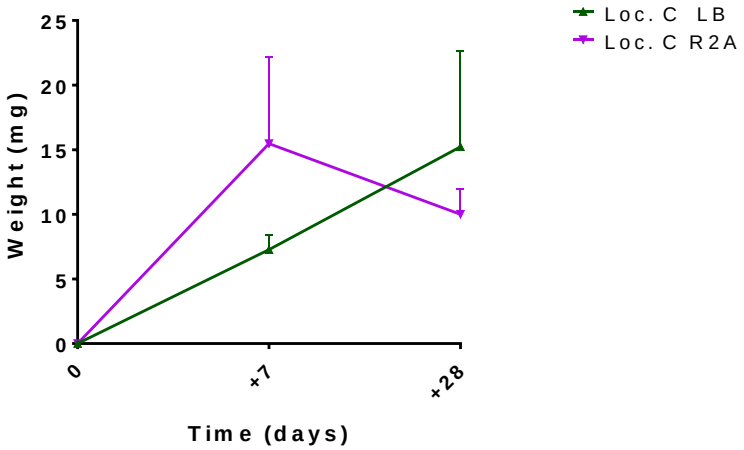



*Figure 2.17: Weight of Sample I pellet at each 2017 kinetic point*

During the different kinetics, the genus *Streptomyces* was present in all analysis at about 4% with the exception of some triplicate in Sample G in LB at D7, and Sample I in LB at D7 and R2A at D7 and D28 where *Streptomyces* represented between 20 and 30% of the identified peptides. The *Sphingomonadales* order was highly represented in the LB and R2A kinetics of Sample I, with the presence of *Sphingomonas*,

*Novosphingobium* and *Erythrobacter* being the most represented (Appendix 4, Tables 6.8 & 6.9). In the LB kinetic Sample I the genus *Ralstonia* was present in high quantity in two replicates at D7 (26 and 40%) but completely disappeared at D28. *Burkholderia* represented between 2 and 9% of the identified peptides during the LB kinetic from Sample I.

In all kinetics (location and media), the genus *Methylobacterium* was absent or present in very low quantity. The phylum *Ascomycota* (fungi) was also present in all kinetics from Pool 2, from the 2015 and the 2017 kinetics. However the fungi were identified in small proportion, less than 1% except for D7 in LB in Sample G (2%). In the 2015 kinetic the fungi represented between 3.5 to 9% of the identified peptides. In the latter the genus *Trichoderma* was highly represented (3.5 to 7%).

Figure 2.18 presents the microbial diversity comparison between all samples. Among the identified genera 46 were common to all three sampling's locations. Samples G and H were more similar within each other (30 genera in common) than with Sample I (5 common with Sample G, and 11 common with Sample H). Sample H presented the highest diversity. This diversity can be explained by the number of kinetic points (4 for Sample H *vs.* 2 for samples G and I).

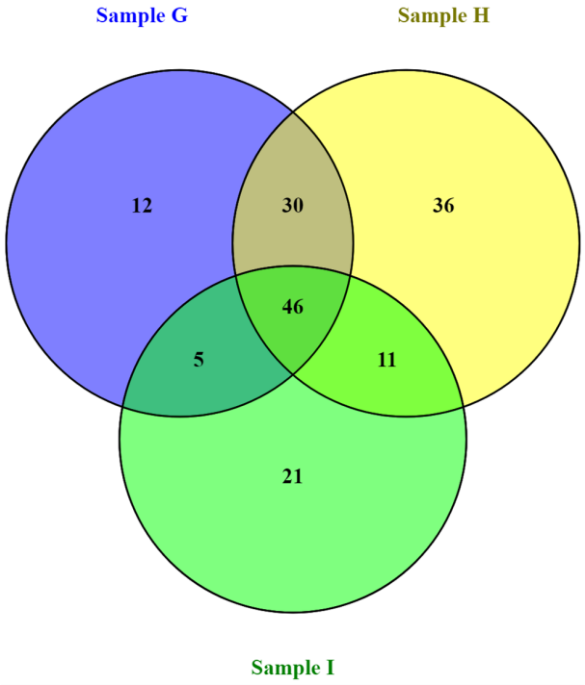



*Figure 2.18: Venn diagram of the kinetics from Samples G, H, and I represented the number of genera common to all samples, two samples or only one sample.*

### 2.2.4 Discussion

In the present study, we have shown that inoculating a culture with a low volume of water sampled from a pool allows in some days to obtain enough biological material to identify the main microbial players in the culture. We have observed that the microbial diversity of the culture is assessable when enough biological material is obtained and a certain stability of this microbial diversity settled over time in several diluted rich liquid media. The taxonomical identification at the genera taxonomical rank using phylopeptidomics, a novel metaproteomic approach, allows visualizing the presence of different Life kingdoms as prokaryotes and eukaryotes are distinguishable in the same analysis, and their respective biomass contributions.

For Pool 1, water diversity in the middle of the pool was lower (20 to 27 genera) than the other locations (32 to 50 genera). Samples A to D were collected after rubbing the walls to resuspend biofilms. It is known that biofilms are predominant in aquatic environments [18-21]. The resuspension of microorganisms from biofilms enriched the water sampling and could thus lead to a greater diversity. The western wall (39 to 55 genera) presented the most diversity after cultivation than the northern wall (32 to 34 genera). This diversity could be explained by the configuration of the pool. Cobalt sources, when present at the bottom of the northern wall, are a few centimeters away of the wall and a dose rate of circa 100 Gy/h can be measured. Near the western wall, sources are more often present but at a greater distance from the wall.

Pool 2 is used to cool the $^{235}U$ radioactive nuclear reactor and produce radionuclides for medicine. From this very extreme environment, water samples have been used to inoculate cultures in rich media diluted in water. These cultures exhibited some extended microbial diversity after one week of cultivation, and were not populate only with a few major players. Thus, this culture approach can help maintaining and amplifying microbial consortia by specific culture conditions. The main genera identified during this short-term kinetic experiment were present in all tested media during the 2017 kinetic of Pool 2. Some genera present in small proportion were only identified in one liquid medium, thus highlighting the interest of culturomics. Twenty-eight and 35 genera were identified only in LB or R2A respectively, while 98 genera were common to both media (Appendix 4, Table 6.10). The R2A medium thus exhibits a slightly higher propensity for maintaining diversity compared to the LB medium. Between the 2015 and 2017 kinetics from Pool 2, 20 genera were only found in 2015 while 108 genera were solely found in 2017. Among the 53 genera common to both kinetics, 5 genera belong to the Actinobacteria (*Nocardia*, *Cellulomonas*, *Arthrobacter*, *Microbacterium*, and *Streptomyces*), 4 genera belong to the *Bacteroidetes* (*Bacteroides*, *Porphyromonas*, *Prevotella*, and *Flavobacterium*), 4 genera belong to the *Firmicutes* (*Bacillus*, *Paenibacillus*, *Clostridium*, and *Butyrivibrio*), 4 genera belong to the *Ascomycota* (*Zygosaccharomyces*, *Metarhizium*, *Trichoderma*, and *Fusarium*). Finally 36 genera belonging to the Proteobacteria were common (27 $\alpha$-*Proteobacteria*, 3 $\beta$-*Proteobacteria*, 5 $\gamma$-*Proteobacteria*, and one $\delta$-*Proteobacteria*). The sample used for the 2015 kinetics originated from the cooling pool of a working nuclear reactor, and the microbiota was analyzed after at least 2.5 months of culture. The samples used for the 2017 kinetics originated from the same pool, but a year after the definitive shutdown of the pool. Moreover the microbiota was after 3, 7, 14 and 28 days of culture. A deep comparison between the results from the 2015 and 2017 kinetics is thus not applicable here.

Some strains present in these pools belonged to genera that had never been described in such environments before. Among the genera mainly present in the kinetics, *Xanthobacter*, *Caulobacter*, *Erythrobacter*, *Acidovorax*, *Methylibium*, *Streptomyces* and *Trichoderma* have never been described in SNFP. Some of these genera can however exhibit resistance to ionizing radiations. The isolation of these strains could help understanding the mechanisms implemented by the cell to resist the harsh condition prevailing in both pools by studying them in presence of the different stresses prevailing in both pools.

A majority of *Xanthobacter* strains can produce slime. It was proved that slime producer strains grow better than non-producer at a high oxygen concentration [315]. The fact that this peculiar genus was present in this pool could be explained by its ability to produce slime and its potential protective effect against radiation. Members of the *Erythrobacter* genus isolated in the Mediterranean Sea and the Pacific Ocean were found to be UV-B resistant [324]. The *Methylibium* genus was identified in 2006 [325]. In one study,

the frozen permafrost of Arctic was irradiated at 100 kGy and one strain of *Methylibium* survived this irradiation [326]. The genus *Streptomyces*, belonging to the *Actinobacteria* was also identified. This genus can form spores and is abundantly found in soils and can be found in aquatic environments. *Streptomyces rimosus* was found to resist gamma-radiation with a $D_{10}$ of 1 kGy [179]. A strain of *S. canarius*, isolated in Egypt, was shown to resist up to 25 kGy $\gamma$-irradiation [327]. Most of the isolated *Streptomyces* from this study exhibited a resistance to $\gamma$-radiation and only a few species did not survive doses higher than 5 kGy [327].

One genus of the *Ascomycota* phylum was particularly present in the kinetic from Pool 1: the *Trichoderma* fungus. It is commonly found in forest and in soil. In a study, spores from *T. viride* exhibited a $D_{10}$ between 1 and 1.5 kGy [328].

*Bosea*, *Bradyrhizobium*, *Rhizobium*, *Sphingomonas*, *Methylobacterium*, *Ralstonia*, *Brukholderia*, *Pelomonas*, *Pseudomonas*, *Leifsonia*, and *Bacillus* genera, identified during the different kinetics, have already been identified in the water of SNFPs [152, 177, 181, 183-189, 329].

A few differences in the microbial diversity could be observed between the kinetics obtained in Pool when compared to the ones obtained in Pool 2 in 2015 and in 2017 (Appendix 5, Fig. 6.2). Interestingly only 2 genera were common to both pools in 2015. The common genera were *Xanthobacter* and *Methylocystis*, belonging to the *$\alpha$-Proteobacteria*. The physical and chemical conditions of the pools were highly different during the two 2015 samplings. The main stresses in Pool 1 were nutritive stress and the presence of radiation near the radioactive sources. In Pool 2 the stresses were not only the lack of nutrients but also the presence of highly active radionuclides, such as $^{24}Na$ or $^{60}Co$, but also the presence of high radiation levels. As explained above some *Xanthobacter* species can produce slime which could protect them from radiation. The *Methylocystis* genus is methanotrophic and can resist desiccation, thus could have all the enzymatic arsenal to resist to high radiations that are known to cause the same DNA damages [315]. The kinetic from Pool 2 in 2017 presented the most unique genera with a total of 79 genera compared to the other two kinetics (34 and 18 genera for Pool 1 and Pool 2 2015, respectively). It can be explained by the number of phylopeptidomics analysis performed on these samples, higher than the other two kinetics. One point worth noticing was the absence of *Cyanobacteria* during the 2015 kinetic from Pool 2. All *Cyanobacteria* genera were indeed identified in Pool 1 in 2015 or in Pool 2 from the 2017 kinetic.

### 2.2.5 Conclusion

To study the diversity present in an aquatic environment and observe its evolution in liquid medium throughout time, a kinetic can be performed in diluted rich liquid media. By increasing the number of media and dilution some diversity can be observed, however the use of two or three media and two different dilutions could be enough to identify the main genera present in the water of constraint environments. As seen above, enough biological material could be obtained after day 7 in order to perform phylopeptidomics and assess the microbial diversity which persisted over time, up to several months. The use of a kinetic in diluted but rich media with phylopeptidomics monitoring allows exploring new perspectives for microbiology experiments. For example, it can be helpful to amplify the biological material and orient further agar-plate isolation of the most interesting microorganisms. Indeed several genera identified in both pools had never been described in similar environments. The main genera, such as *Sphingomonas*, *Ralstonia* or even *Methylobacterium* and *Bacillus* were also found in Spent Nuclear Fuel Pools all over the globe.

Moreover the main genera found in both Pool 1 and Pool 2 were common even though the pools are different.

A majority of *Proteobacteria* were identified in both pools after culture. This result is in accordance with a previous description of similar environment (SNFPs) where, surprisingly, mainly Gram negative bacteria had been isolated [185]. The presence large presence of Gram negative bacteria, unable to sporulate, can highlight the potential radioresistance of these organisms under a metabolically active form. Another explanation could be that these bacteria entered a viable but not culturable state in the pools and the liquid culture allowed them to become metabolically active again. However the selected media were favorable to Gram negative growth. In terms of population, Pools 1 and 2 from the 2017 kinetic were more similar than Pool 2 during the 2015 kinetic. The conditions in Pool 2 in 2017 were less stringent than in 2015. The 2015 sampling was performed during a working nuclear cycle while the sampling in 2017 was performed more than a year after the complete shutdown of the reactor. The radioactivity was thus lower and less radionuclides were present in the water in 2017.

This study highlighted the presence of a great diversity present in pools from nuclear facilities although the samples that could be used were in low amounts due to low cell density in the water and the inability to sample and treat more than 30 L. The study of two different pools, including one pool with a change in the living condition, brought to light the differences in microbiota. Each pool thus owned its own condition-dependent microbiota.

## 2.3 Chapter conclusion

In this chapter, it has been shown that the sequencing of the V4V5 regions allowed a better taxonomic identification when compared to the V4 sequencing. Moreover a 0.016% cut-off should be used when analyzing metagenetics data obtained after an Illumina MiSeq sequencing and OCToPUS analysis. Different methods can be used to harvest microorganisms and extract DNA. The different methods all have their strengths and weaknesses. However two harvesting methods should be used: filtration and centrifugation. Only one DNA extraction protocol can be used, especially if a phenol-chloroform-isoamyl alcohol based method is used.

It was also proved that using a new metaproteomic approach (phylopeptidomics) could enhance the knowledge of the microbial community present in extreme environments. The use of Spontex sponge to recover biofilms from a radioactive storage pool was proved to be adapted to the sampling conditions. Furthermore, even though the sensitivity of metaproteomics was lower than metagenetics, several genera were identified in the recovered biofilm. With more material, as demonstrated in 2.2 after a cultivation step, a numerous number of different genera from several phyla were identified using metaproteomics.

The taxonomical identification with metagenetics and metaproteomics could only be certain until the genus taxonomic level and not the species one. Using meta-analysis, particularly metagenetics, the depth of the analysis and the size of the sequenced region of the 16S rDNA were indeed too low and small to enable identification at the genus level. Moreover some genera can be phylogenetically extremely close and the sequencing of only a fragment of 16S rDNA cannot discriminate at the genus level. For example, after the V4V5 sequencing of the ZymoBIOMICS DNA standard the OCToPUS software could not discriminate whether one OTU belonged to the *Escherichia* genus or the *Shigella* one. The combination of both metagenetics and metaproteomics can not only provide more information about the diversity present in water from nuclear fuel pools but could present complementary results in microbiota analysis. Finally the use of a liquid culture in several diluted rich media enabled the identification of a large diversity of genera although the water sample was really low (50 µL). This method also presented the advantage that the great diversity is stable over time.

# Chapter III:

## *Deciphering the microbial composition of the cooling pools of nuclear facilities*

## 3. Deciphering the microbial composition of the cooling pools of nuclear facilities

It is now known that pools from nuclear facilities can harbor life. Few studies to study microbial diversity have been performed in nuclear pools. Different genera have been isolated in the spent nuclear fuel pools (SNFPs) of different facilities, in Spain, Hungary or in the USA [152, 177, 181, 183-188]. The microorganisms discovered in these pools were identified after a cultivation step. Thus they only represented a small part of the microbial diversity of microorganisms present in those environments. One recent study identified the bacterial population present in a spent nuclear fuel pool [193]. Using a metagenomic approach consisting in sequencing the amplicon of the 16S rRNA gene of the microbiota found in this storage basin, the authors found different phyla, including *Acidobacteria*, *Actinobacteria*, *Planctomycetes*, *Proteobacteria*, *Nitrospirae* and *Deinococcus-Thermus* [193]. In this peculiar SNFP, the majority of the sequences corresponded to the families *Burkholderiaceae* (23%), *Nitrospiraceae* (23%), *Hyphomicrobiaceae* (17%), and *Comamonadaceae* (6%). The evenness of the different families was quite important, due to the presence of some selection pressures (nutritive stress, presence of radiations and radionuclides). Depending on the pool's conditions the microbiota can be different when compared to another SNFP. Indeed, in the SNFP of Cofrentes, a majority of *Proteobacteria* and *Firmicutes*, as well as some *Actinobacteria* were identified while in a SNFP in Sweden, the main genus was *Meiothermus* [186, 190]. The physico-chemical composition of each pool may influence the microbiota composition as well as the hydrodynamics and maintenance procedures.

Till now no investigation have been performed on the pool containing a working nuclear reactor. Knowing the nature of the microbiota present in nuclear facilities might be important for several reasons, the first one being the maintenance of the reactor structure integrity. Some organisms can indeed modified the water composition, form biofilms and lead to corrosion. Secondly the radioactive risk is well controlled in nuclear facilities but the biological one is absent of all control. The safety of the staff present in nuclear facilities could not only depend on the radiation but also should take into consideration any possible biological contamination. Studying these organisms will open interesting industrial perspectives as the isolated strains could be implemented in a new nuclear effluents decontamination process and fundamental perspectives as their resistance and radionuclides accumulation pathways could be deciphered. In this chapter, the microbiota in two different pools was assessed at different locations and time points. It was thus possible to follow the microbiota stability or not depending on the sampling location and the pools' condition. The microbiota present in these pools were analyzed using meta-analysis.

Meta-analysis based on 16S rRNA amplicon sequencing has established itself as a powerful tool in various fields including medical and environmental applications to get insight into microbial community composition [198, 230, 285]. Despite some bias occurring from DNA extraction, PCR, the choice of the 16S rRNA region, the size of the fragment to sequence, and the quantity of microorganisms and DNA [236, 330-332], it offers unsurpassed insight into the identity and the diversity of the taxa present in a given sample. However, owing to the above-mentioned bias in DNA extraction, the variable number of copies of the 16S rRNA gene, and the variable number of copies of the chromosomes depending on the microorganism and on its physiological state, it is far from being quantitative [235]. Over the last 10 years, metaproteomics has been used to assess functional aspects of communities inside their environment. This method also allows a more quantitative approach compared to metagenetics [301]. In the first presented study, we explored for the first time the microbial communities present in the water of the cooling pool of an operating nuclear reactor's core. Microbial communities present in this pool were also investigated during reactor's

shutdown. In contrast to most previous studies conducted on spent nuclear fuel storage pools, we performed here non-culture selective high throughput meta-multi-omics approaches combining i) analysis of 16SrDNA gene amplification and sequencing, and ii) direct metaproteomics analysis.

In nature, many microorganisms can form biofilms as a mean to colonize a specific environment and facilitate their long-term fate. For this, different microorganisms stick generally onto a surface, are embedded within a self-produced matrix of extracellular polymeric substances and may further colonize their surroundings. This specific growth pattern is indeed more protective than the planktonic form (cf. Chapter I.3.1). It has been estimated that biofilms are predominant in aquatic ecosystems and represent more than 99.9% of the total microbial community [18]. Inside a biofilm, the pH can be appropriately modified, and nutrients and organic material may be more easily recycled, creating more favourable conditions for microorganism survival and growth [16, 284]. As a consequence, microbial biofilms have been detected in very inhospitable places such as used nuclear fuel pools [176, 181] or acid mine drainage [244, 256]. Biofilms can lead to corrosion and thus pose a threat to the integrity of the pool's walls. It has indeed been proved that Microbial Influenced Corrosion (MIC) can occur in these environments due to the presence of microorganisms [183]. Knowing the biofilm composition is important to get clues on how to prevent their development. In previous studies, microbial colonization of stainless steel and titanium coupons placed in a bioreactor connected with the water of a Spanish SNFP have been studied [184, 187]. Over a year the bioreactor stayed in the pool and pumped the water with an "on-off" cycle (pump was on for 2 h and off for 1 h). The metals were then recovered and the developed biofilms were analyzed after cultivation on solid media at 30°C [184, 187]. This biofilm concentrates some radionuclides: $^{60}$Co (about 80%), $^{59}$Fe (about 25%), $^{95}$Nb (about 13%), $^{65}$Zn (2-5%), and $^{54}$Mn (5-10%) after 174 days of immersion. Mainly Gram negative bacteria (more than 60% of *Proteobacteria* identified in all studies) were identified in SNFPs in all previous studies performed [152, 177, 181, 184-188, 191-193]. The goal of the second study was to study the diversity present in the biofilms collected in the cooling pool of a nuclear reactor. To do so eight different biofilms were recovered from 6 different locations in the pool and analyzed using a metagenetic analysis.

Finally, the study of the organisms living in a pool used to store radioactive $^{60}$Co sources has never been performed to our knowledge. Pools used to store radioactive cobalt sources do not contain radionuclides in their water. The only stresses present in these pool are ionizing radiation and nutritive stress. Studying the microbiota present in such environments, with only a radiative and nutritive stress can be of great interest to identify radioresistant organisms. Moreover these stresses are less stringent than the ones present in SNFPs, with lower dose rates and the use of tap water instead of demineralized water. A higher microbial diversity should thus be expected. In the third presented work the microbiota of a $^{60}$Co sources storage pool located in Saclay was studied. The presence of natural light in this infrastructure, placed in a hall with windows, could favor the development of photosynthetic organisms. Indeed, the presence of algae in presence of ionizing radiation has already been reported since a green micro-algae belonging to the *Coccomyxa* genus has been identified in a SNFP and can resist $\gamma$-radiation up to 20 kGy [176]. The filtration system of this pool had been modified in July 2016. The nature of the organisms present in this pool was studied at three different time points and at 6 different locations. Both water and biofilms were recovered for analysis. Moreover each previously studied pool seemed to possess its own microbiota despite the presence of common genera. Here, the aim of this study was to identify microorganisms present in a $^{60}$Co sources storage pool, establishing a space and time comprehensive microbial inventory.

## 3.1 Microbial composition of a nuclear reactor cooling pool during operation and at shutdown deciphered using multi meta-analysis (*article in preparation*)

**Abstract**

Water pools present inside nuclear facilities such as the ones used to store spent nuclear fuel constitute harsh environments where radiative, nutritive, and metallic stress prevail. Very few studies have investigated the microbial composition of such pools owing to the constraining handling conditions. This work presents the first complete inventory of microorganisms present in the core's cooling pool of a nuclear reactor. The microbial composition of the pool's water was investigated both during operation and at shut-down. To get the most exhaustive view of the microbiota composition, two direct and complementary meta-omics approaches were used, namely metagenetics and metaproteomics. They were complemented by the cultivation of the water samples in diluted media which has proven to be a valuable approach to identify the main microbial species when initially present in low concentration (article in preparation). Despite highly stringent conditions (presence of radiations and radionuclides at high concentration, lack of nutrients), 27 different OTUs were identified in the pool during operation, and 96 OTUs were identified at shutdown. A change in the microbiota composition occurred between the samples collected during operation and the ones collected after the shutdown. The main genus during operation was *Variovorax*, a genus which might use dihydrogen produced by water radiolysis as energy source. It completely disappeared at shutdown to the profit of *Methylobacterium*.

### 3.1.1 Materials and Methods

#### *3.1.1.1 Water sampling in the cooling pool of the nuclear reactor's core and characterization*

The basin studied is the cooling pool containing light water in direct contact with the 58 MW core of a nuclear reactor. It is 11 m deep, 7.5 m long, and 6.5 m wide. Water samples were collected at three different points of interest of the basin, called location A (Loc. A), location B (Loc. B), and location C (Loc. C). Loc. A corresponds to the bottom of the pool 2 m from the core at a depth of 9 m, Loc. B to the top of the chimney containing the nuclear core, and Loc. C to the inside of the chimney.

While the reactor was in operation, a 2 L water volume was collected at Loc. A on December 2015 using a permanently installed pipe extensively purged before sampling. When the reactor was shut down, a total volume of 8 L was collected on March 2017 at Loc. A using the same device, and at Loc. B and Loc. C using a sterile sampling vial attached to the lower end of a pole and opened at the sampling point (Wildco®).

While in operation, the pH 7.0 pool's demineralized water (conductivity 0.5 µS/cm), maintained at 35°C, contained the β-emitter $^{3}H$ (2.94x$10^{8}$ Bq/$m^{3}$) and the γ-emitter radionuclides $^{24}Na$ (2.9x$10^{9}$ Bq/$m^{3}$), $^{7}Be$ (4.0x$10^{7}$ Bq/$m^{3}$), $^{27}Mg$, $^{41}Ar$, $^{56}Mn$, $^{99}mTc$, $^{122}Sb$, $^{187}W$, $^{188}Re$, $^{197}mHg$ yielding a total γ activity of 3.1 $10^{9}$ Bq/$m^{3}$. During the reactor shutdown, the dose rates were below the threshold of 0.1 µGy/h, 15 µGy/h, and 25 Gy/h, at Loc. A, Loc. B, and Loc. C, respectively. The pH 5.4 pool's demineralized water (conductivity 0.5 µS/cm) contained the β-emitter $^{3}H$ (2.49x$10^{8}$ Bq/$m^{3}$) and the γ-emitters $^{51}Cr$ (1.6x$10^{6}$ Bq/$m^{3}$), $^{186}Re$ (3.7x$10^{5}$ Bq/$m^{3}$), $^{124}Sb$ (2.8x$10^{5}$ Bq/$m^{3}$), $^{58}Co$ (2.5x$10^{5}$ Bq/$m^{3}$), $^{99m}Tc$ (2.5x$10^{5}$ Bq/$m^{3}$), $^{122}Sb$ (1.7x$10^{5}$ Bq/$m^{3}$), $^{99}Mo$ (1.7x$10^{5}$ Bq/$m^{3}$), $^{60}Co$ (1.3x$10^{5}$ Bq/$m^{3}$), and $^{54}Mn$ (3.6x$10^{4}$ Bq/$m^{3}$), yielding a total γ activity of 3.25x$10^{6}$ Bq/$m^{3}$.

Whatever the operating mode, the pool water is continuously filtered at a 500 $m^3$/h flow rate, and is purified on ion exchange resins at 30 $m^3$/h, meaning a turnover of the water of this 536 $m^3$ pool in 18 h.

The initial microbial cell density in water sampled during the reactor shutdown was assessed using a Malassez cell and light microscopy after microorganisms' concentration by centrifugation of 50 mL of water.

#### *3.1.1.2 Water sample treatment and microbial communities harvesting*

Samples harvested during reactor shutdown were processed for microbial community assessment immediately after collection. Each sample was divided into 3 x 1 L for direct meta-analysis by amplicon sequencing, and 4.5 L for direct metaproteomic analysis. Microorganisms were concentrated and harvested either by centrifugation at 16,000 *g* for 20 min at 10°C (1 L) or by filtration on 0.22 µm hydrophilic polyethersulfone filters (MicroFunnelTM Supor®, Pall Corporation, USA) (2 x 1 L) (see article *Comparison of two 16S rDNA regions metagenetics sequencing to identify microbial diversity in an aquatic environment complemented with microbial harvesting and DNA extraction optimization* in preparation for details: cf. Chapter II.1.2). After centrifugation Samples collected during reactor operation were stored for 3 days at 4°C before handling to reduce their activity by radioactive decay of $^{24}$Na (half-life 15 hours) to 24 µS/h in contact with water, enabling its manipulation in controlled conditions. They were centrifuged and filtered as described above prior to DNA extraction for metagenetic analysis.

#### *3.1.1.3 DNA extraction, Illumina sequencing, and sequence data analysis for meta-amplicomic analyses*

Three methods were used for the treatment of samples harvested prior to amplicon sequencing, namely, centrifugation of 1 L of water followed by phenol-chloroform-isoamyl alcohol DNA extraction, filtration of 1 L of water followed by phenol-chloroform-isoamyl alcohol extraction, and filtration of 1 L of water followed by the DNeasy® PowerWater® kit (Qiagen, Germany) kit extraction.

DNA from the microbial communities of half the filters was extracted with DNeasy® PowerWater® kit from Qiagen (Germany) according to the manufacturer's instructions. The centrifuged water and the other half of the filters were extracted using a phenol-chloroform method, which was adapted from Vilchez-Vargas et al. [276]. The liquid sample was centrifuged 20 minutes at 20,000 *g* at room temperature and the supernatant discarded. One milliliter of lysis buffer (100 mM Tris pH 8; 100 mM EDTA pH 8; 100 mM NaCl; 1% polyvinylpyrrolidone; 2% SDS) was added and the pellet resuspend. The mixture was then transferred to a glass bead (0.1 mm) tube from Mobio. The tube was disrupted three times at 30 Hz for 60 seconds with 30 seconds break between each run with a disruptor MM 300 from Retsch/Qiagen. The tube was then centrifuged 5 minutes at 20,000 *g* at room temperature and the supernatant removed and transferred into a new tube. One milliliter of phenol-chloroform-isoamyl alcohol (25:24:1) was added. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered and transferred into a new tube after a centrifugation step of 1 minute at 17,000 *g* at 4°C. Seven hundred microliters of chloroform were added to the sample. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered after a centrifugation step of 1 minute at 17,000 *g* at 4°C. The supernatant was divided into two tubes (around 450 µl in each) and 45 µl of sodium acetate 3 M and 500 µL of isopropyl alcohol were added into each tube. The samples were then mixed by inverting the tubes and put overnight at -20°C. The tubes were centrifuged 30 minutes at 17,000 *g* at 4°C and the supernatant

removed. Two steps of washing were performed by adding 750 µL of 75% ethanol and a centrifugation step of 5 minutes at 17,000 *g* at 4°C. The supernatant was removed and the pellet dried with a heating block at 55°C. Sixty microliters of sterile milliQ water were added to dissolve the pellet. To have a more pure DNA and remove any trace of alcohol, the sample was put on a 0.05 µm filter disposed on sterile water for 15 minutes before being transferred into a new tube. The different methods were called method 1, method 2, and method 3 for centrifugation followed by phenol-chloroform-isoamyl alcohol DNA extraction, filtration followed by phenol-chloroform-isoamyl alcohol DNA extraction, and filtration followed by the DNeasy® PowerWater® kit respectively.

The sequencing of genomic DNA on 16S rDNA was performed by LGC Genomics, UK. The sequencing was conducted as followed. The PCRs included about 1-10 ng of DNA extract (total volume 1 µL), 15 pmol of each forward primer and reverse primer (in 20 µL volume of 1 x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µL of BioStabII PCR Enhancer (Sigma) and additionally 0.2 µl of DNase (Artic Zymes) for the 16S PCR. For each sample, the forward and reverse primers had the same 10-nt barcode sequence. Each PCRs were carried out for 20 cycles, dependent on the primer pair, using the following parameters: 1 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s and primers without inline barcodes have been used (341F/1061R). For the second round 1 µL PCR product from the first round was used and the PCR conditions were the same as before. For this reaction barcoded primers have been added to amplify the V4V5 regions (515YF-926R: 5'-GTGYCAGCMGCCGCGGTAA-3' and 5'-CCGYCAATTYMTTTRAGTTT-3' respectively). The primers used to amplify V4 region were 515F/806R (5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-GGACTACHVGGGTWTCTAAT-3' respectively).

DNA concentration of amplicons of interest was determined by gel electrophoresis. About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying different barcodes. The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification on MiniElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

Sequencing data were analyzed through the OCToPUS pipeline [210] for contig assembly, quality filtering, de-noising, chimera removal, and OTU clustering. The OTUs were then taxonomically assigned at 97% identity against the SILVA database [333-335] using the mothur software [213]. Based on the analysis of a mock sample, the cut-off threshold was set at 0.016%. The results of the different methods were compared in (article in preparation, cf. Chapter II.1.2). Sequencing results at each location were pooled and are presented here, using subsampling at 28977 reads. The main OTUs unidentified at the genus level were manually blasted against Nucleotide BLAST from NCBI.

#### *3.1.1.4 Protein extraction, peptide analysis using nanoLC/MS/MS, and taxonomic analysis by metaproteomics*

Water samples (4.5 L per sampling location) dedicated to metaproteomic analysis were filtered and stored at 4°C prior to their pretreatment which was carried out in a controlled laboratory. Filters were shook into 15 mL of sterile MilliQ water to resuspend the microorganisms. The water was centrifuged for 20 minutes at 20,000 *g* at 10°C. The supernatant was removed and the pellet resuspended in 720 µL of supernatant.

The metaproteomics analysis was done as previously described (cf. Taxonomical composition of a biofilm sampled from a nuclear fuel pool assessed by metaproteomics). Briefly, samples were thawed on ice, and a volume of 50 µL of Laemmli buffer LDS1X (Invitrogen) was added as described by Hartmann et *al* [295]. After denaturation for 5 min at 99°C, the protein sample was loaded onto a 4–12% gradient 10-well NuPAGE gel (Invitrogen). The proteins were subjected to electrophoresis for 5 min and then stained with Coomassie Blue Safe staining (Invitrogen) as recommended by the supplier. The polyacrylamide band corresponding to the whole proteome was sliced, then unstained with ultra-pure water, reduced with dithiothreitol, and treated with iodoacetamide. It was then subjected to proteolysis with Trypsin Gold Mass Spectrometry Grade (Promega) in the presence of 0.01% ProteaseMAX surfactant (Promega), as previously described [322]. The resulting peptides were analyzed with an ESI-Q Exactive HF mass spectrometer (Thermo) equipped with an ultra-high field Orbitrap analyser and coupled to an Ultimate 3000 176 RSL Nano LC System (ThermoFisher). A volume of 10 µL of peptides was injected onto a reverse phase Acclaim PepMap 100 C18 column (3 µm, 100 Å, 75 µm id x 500 mm) and resolved at a flow rate of 0.2 µL/min with a 60 min gradient of CH3CN in presence of 0.1% formic acid. The Q-Exactive HF instrument was operated with a Top20 strategy with only ions with potential charge states of 2+ and 3+ selected for MS/MS and with a dynamic exclusion of 10 sec. The proteotyping interpretation of MS/MS spectra was done with the National Center for Biotechnology Information non-redundant fasta file (NCBI-nr) database with the Mascot search engine (Matrix science). Peptide -to-MS/MS spectrum assignation was done with full trypsin specificity, maximum of one missed cleavage, mass tolerances of 5 ppm on the parent ion and 0.02 Da on the MS/MS, static modification of carboxyamidomethylated cysteine (+57.0215), and oxidized methionine (+15.9949) as dynamic modification. Post-processing of Mascot dat files was done as previously indicated (cf. Chapter II.1.2).

## 3.1.2 Results

### *3.1.2.1 Configuration of the pool*

The pool contains the uranium sources which consists of a stack of four casings used to position the fuel elements and to channel the cooling water. It includes the base with notably the water inlet, the core vessel with the fuel elements, the water outlet casing, and the chimney which limits the mixture between water leaving the core and the water in the reactor pool as described in Figure 3.1. In this configuration, water could only be sampled at Location A owing to the huge dose rate prevailing in the core unit. Pool water is highly radioactive, with a tritium activity of $2.4\pm0.2\text{x}10^{8}$ Bq/m$^3$ whatever the functioning mode, and a gamma activity reaching $2.6\text{x}10^{9}$ Bq/m$^3$ during operation and $1\text{x}10^{5}$ to $3\text{x}10^{6}$ Bq/m$^3$ during shutdown. The microbiota present in this pool were identified and compared in operation and during shutdown and at different collection sites. During shutdown, three sites displaying very different ionizing radiation dose rates were investigated. The dose rate at Location A, situated at the bottom of the pool at a distance of 2 m from the core unit, was below the detection threshold of 0.1 µGy/h. It was 15 µGy/h at Loc. B situated at the top of the chimney, and raised up to 25 Gy/h at Loc. C inside the chimney due to the activation of the structure elements whose radioactivity subsists once the reactor shut down.

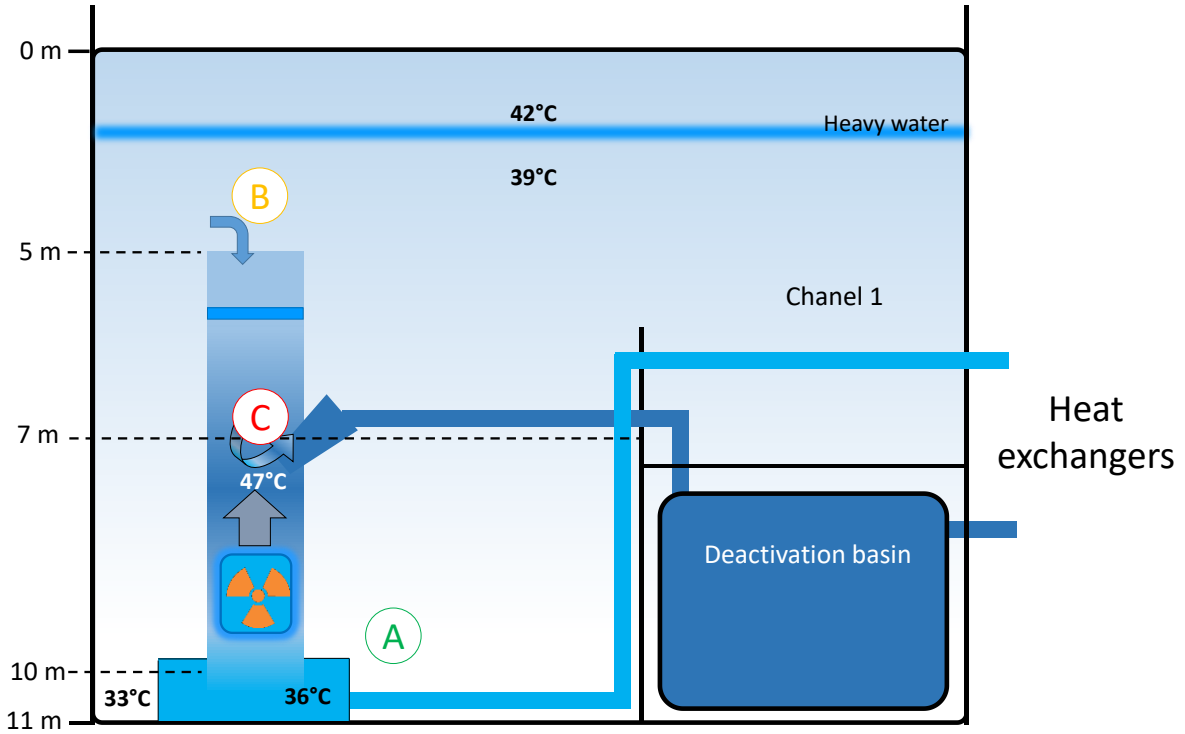



*Figure 3.1: Sampling locations of the different samples in Pool 2 (simplified and adapted from [250])*

The initial microbial cell density in water sampled during the reactor shutdown was assessed at 2.7±0.6x$10^3$ cells/mL, 1.4±0.2 x$10^3$ cells/mL, and 0.5±0.25 x$10^3$ cells/mL at Loc. A, Loc. B, and Loc. C, respectively. These values are in the range of the densities reported for microbial density in spent nuclear fuel pools [152, 184, 189, 193]. Unlike some pools where the water can be considered as stagnant, the continuous filtering and cleaning at a high flow rate of the water in the facility studied here enabled the renewal of the pool volume in 18 h and maintained microbial density at a low level.

### *3.1.2.2 Direct meta-analysis of communities present in the reactor core's cooling pool water*

**Microbiota present during reactor's operation (2015 samplings)**

The 2015 water sample had to be stored for three days to reduce its radioactivity at a level enabling its handling in controlled conditions via the deactivation of the short-lived radionuclide $^{24}$Na, whose activity decreases by half in 15 hours.

The evenness of the samples was below 1 (0.96 with method 1 and 0.68 with method 2) (Table 3.1). This can indicate the presence of one OTU dominating the sample.

***Table 3.1: 16S rRNA gene amplicon sequencing analysis from the 2015 samplings.*** *The read number represents the reads attributed to OTUs, and the OTUs number represents the number of OTUs above the 0.016% cut-off.*

| Sample | Raw reads | Read number | OTUs number | Shannon index |
|---|---|---|---|---|
| **Loc A - meth 1** | 50908 | 36387 | 20 | 0.96 |
| **Loc A - meth 2** | 53243 | 37293 | 17 | 0.68 |

A total of 26 genera belonging to five phyla were identified in the pool during a working cycle (Fig. 3.2). While this proportion can give some indications about the main genera present, it does not represent the proportion of each genus in the water sampled at this location. A majority of *Variovorax* (about 75%) was noticed in this pool, followed by the genus *Sphingomonas* (about 18%). The identified genera belonged mainly to the *Proteobacteria* phylum (16 genera). The other genera were divided between the *Actinobacteria* (4 genera representing 0.4% of the analyzed sequences), *Bacteroidetes* (2 genera representing 4.1% of the sequences), the *Cyanobacteria*, with the presence of "Streptophyta_unclassified" (one genus representing 0.2% of the sequences), and the *Firmicutes* (3 genera representing 0.3% of the sequences).

The description "Streptophyta_unclassified" could have two different meaning in this sample: it can i) highlight the presence of a micro-algae or ii) highlight the presence of *Cyanobacteria*. It has been hypothesized that chloroplast were *Cyanobacteria* after endocytosis [336, 337]. The Ribosomal Database Project associates this "Streptophyta" classification to the *Cyanobacteria* phylum (one *Cyanobacteria*-Chloroplast phylum) [335]. The presence of a micro-algae can be possible since a green micro-algae, *Coccomyxa actinabiotis*, has been isolated from a French SNFP [123, 338].

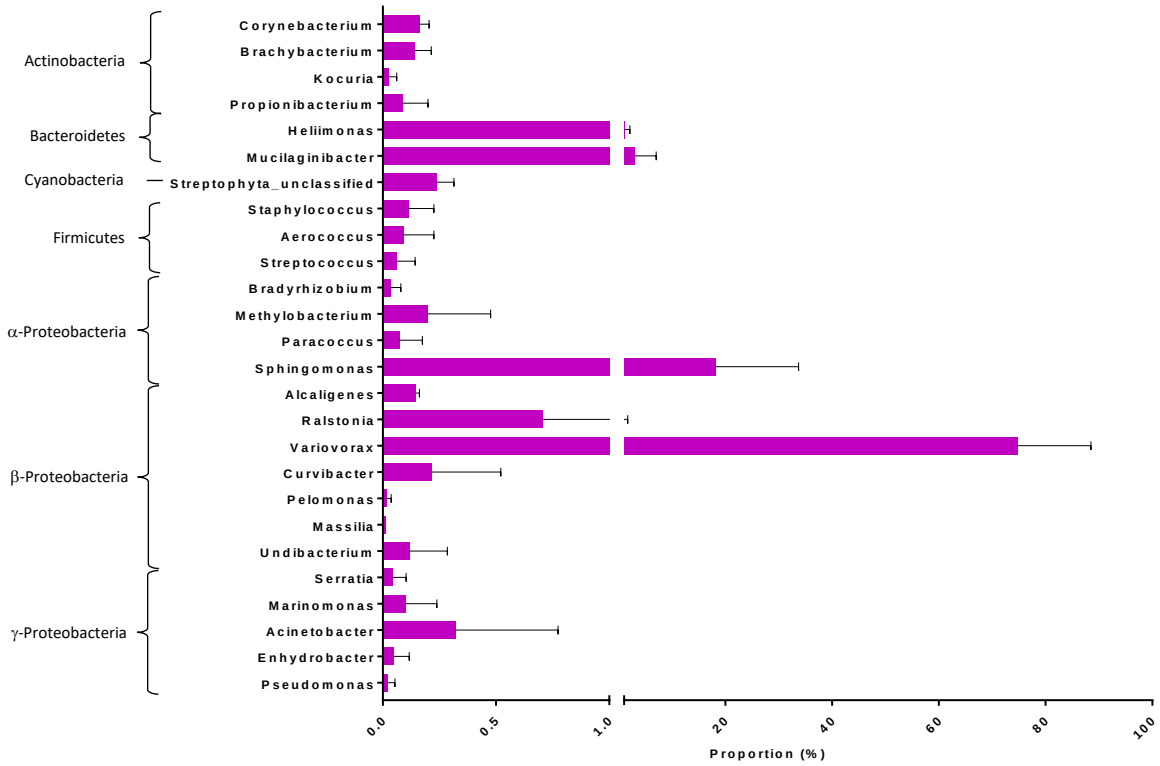



***Figure 3.2: Microbial diversity at the genus level present at location A (bottom of the pool) from the 2015 samplings.*** *Metagenomics results after a V4V5 sequencing and a 0.016% cut-off of all methods. The phyla (or class for the Proteobacteria) is indicated for each genus. A majority of Variovorax (about 75%) can be observed at this location. The second main genus was Sphingomonas (about 18%).*

In this pool a majority of *Proteobacteria* (about 95%), and more generally Gram negative bacteria (about 99%), have been identified, which was also observed by Bagwell et *al*. in the metagenetic analysis of the Savanah River Site SNFP [193].

**Microbiota present during reactor's shutdown (2017 samplings)**

*16S rRNA amplicon sequencing*

The evenness of the samples was quantified using the Shannon index. This index combines species richness and abundance into a single value of evenness [339]. When this index is close to 0 a single species dominates the sample. A greater evenness could be observed at Loc. C (Shannon index of 0.8±0.5) (Table 3.2) which was submitted to the highest dose rate of 25 Gy/h than at Loc. B (Shannon index of 0.5±0.4) where the dose rate was nearly $10^6$ lower, as well as at Loc. A in 2017 (Shannon index of 0.2±0.04) where the radiation dose rate was below the detection limit. A maximum of 38 OTUs was identified in these samples.

***Table 3.2: 16S rRNA gene amplicon sequencing analysis from the 2017 samplings.*** *The read number represents the reads attributed to OTUs, and the OTUs number represents the number of OTUs above the 0.016% cut-off.*

| Sample | Raw reads | Read number | OTUs number | Shannon index |
|---|---|---|---|---|
| **Loc A - meth 1** | 134752 | 71157 | 30 | 0.20 |
| **Loc A - meth 2** | 184589 | 99970 | 33 | 0.28 |
| **Loc A - meth 3** | 274423 | 127024 | 31 | 0.25 |
| **Loc B - meth 1** | 205579 | 100394 | 32 | 0.25 |
| **Loc B - meth 2** | 84569 | 45166 | 38 | 0.87 |
| **Loc B - meth 3** | 193922 | 105307 | 35 | 0.24 |
| **Loc C - meth 1** | 7167 | 3696 | 12 | 1.21 |
| **Loc C - meth 2** | 55638 | 28977 | 16 | 0.89 |
| **Loc C - meth 3** | 181945 | 95759 | 30 | 0.26 |

A year after the reactor definitive shutdown the microbiota present at Location A has been analyzed (Fig. 3.3). A total of 36 genera, belonging to 9 phyla were identified. In this sampling a majority of *Methylobacterium* was detected, representing about 97% of the sequences. No other genera represented more than 1% of the sample. Among the other genera, one genus (*Burkholderia*) represented more 0.45% of the sequences, while OTUs belonging to the *Chlamydiales* order represented about 0.5% of the analyzed sequences. OTUs not identified at the phylum level represented about 0.8% of the sequences.

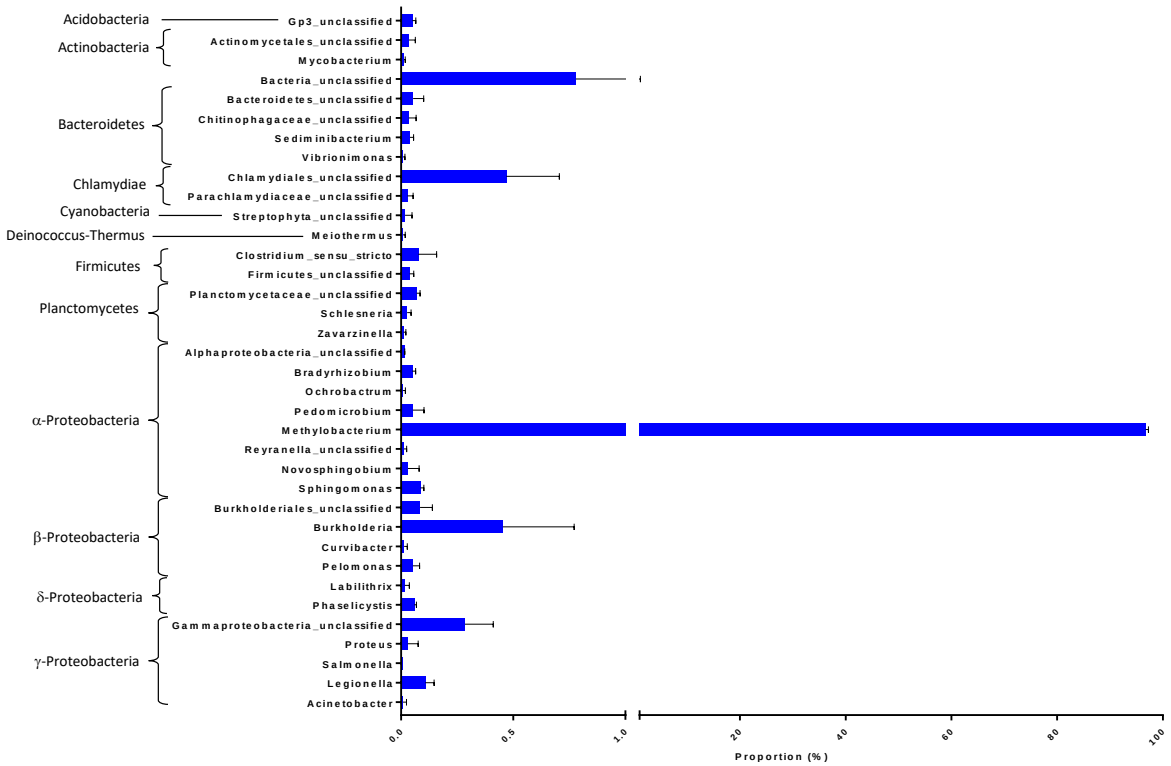



***Figure 3.3: Microbial diversity at the genus level present at location A (bottom of the pool) from the 2017 samplings.*** *Metagenomics results after a V4V5 sequencing and a 0.016% cut-off of all methods. The phyla (or class for the Proteobacteria) is indicated for each genus. A majority of Methylobacterium (about 97%) can be observed at this location.*

Similarly to the 2015 sampling, a majority of *Proteobacteria* (98%) was identified in this sample, and more generally Gram negative bacteria (99%). In this sample one genus (and more specifically, one OTU) dominated the identified sequences.

Comparison between the 2015 and 2017 samplings at Location A

A comparison between the two sampling dates at Location A was performed (Fig. 3.4). It can be observed that, while a majority of *Variovorax* was identified during the reactor's operation, no *Variovorax* was detected after the definitive shutdown of the reactor. Another genus, *Methylobacterium*, took over *Variovorax* as the main genus present in the water. The genus *Methylobacterium* was already present during the reactor's operation but at about 0.2%. A change in the microbiota can thus be observed. Moreover the number of sequences attributed to *Sphingomonas* went from 18% during operation to less than 0.1% after the reactor's shutdown. Finally, genera belonging to the *Actinobacteria* (*Corynebacterium*, *Brachybacterium*, *Kocuria*, and *Nocardioides*), the *Bacteroidetes* (*Heliimonas* and *Mucilaginibacter*) and the *γ-Proteobacteria* (*Serratia*, *Marinomonas*, *Acinetobacter*, *Enhydrobacter*, and *Pseudomonas*) were not detected during the reactor's shutdown while they were present in small quantities during operation (about 3% and 1% for *Mucilaginibacter* and *Heliimonas* respectively, below 1% for the others). Similarly some *Planctomycetes*, belonging to *Schlesneria* and *Zavarzinella* genera were identified in 2017 (during shutdown) at 0.03 and 0.01% respectively.

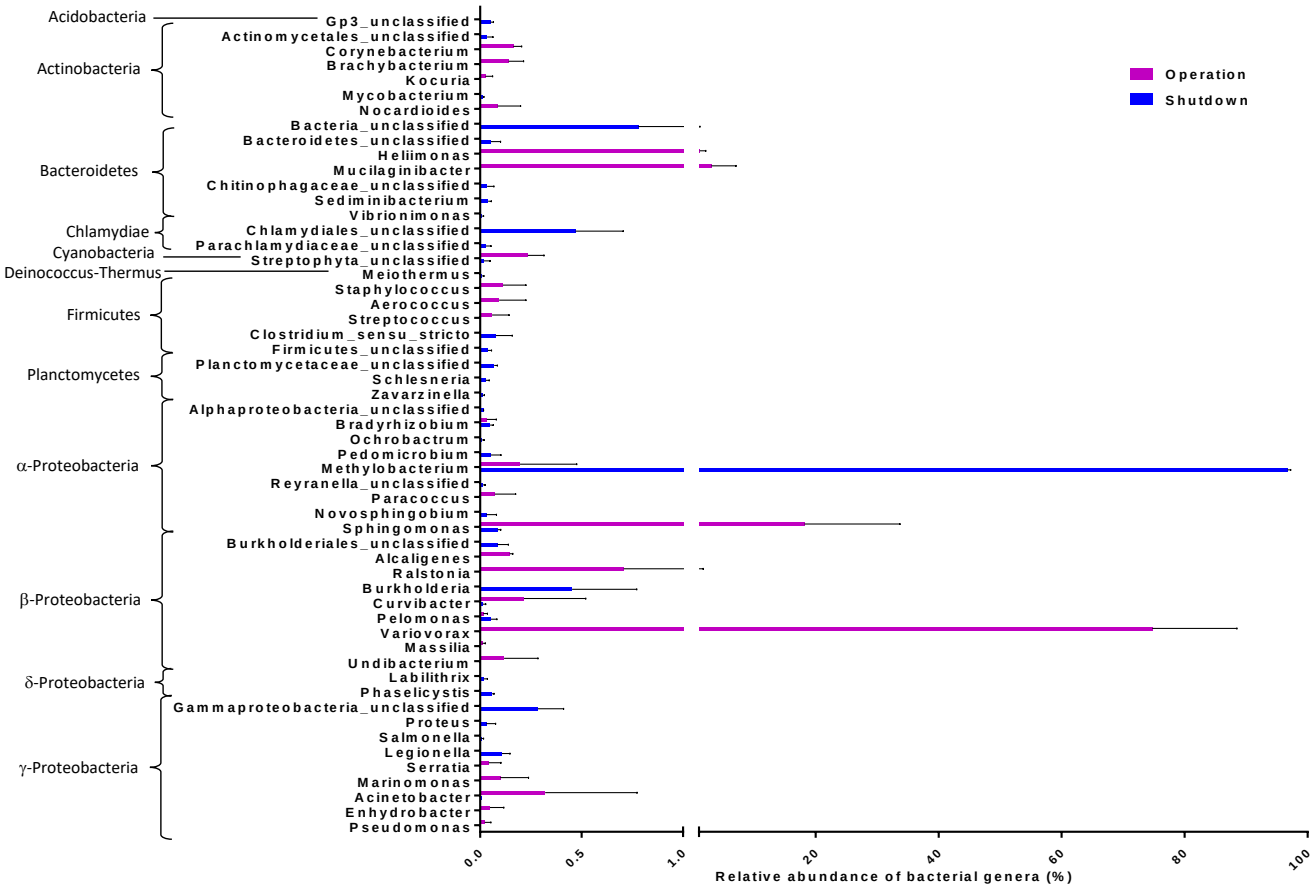



***Figure 3.4: Microbial communities present in the cooling pool of the reactor's core assessed during the reactor operation (2015) and at shutdown (2017). Meta-amplicomics 16S rRNA analysis at the family taxonomic level. Analysis from Loc. A samplings.*** *A change in proportion can be observed between the two samplings. Indeed, the genus Methylobacterium represented less than 1% of the sample harvested in operation, while the genus Variovorax represented about 75%. At shutdown, Methylobacterium contributed to around 95% of the sample while while Variovorax has not been identified in the in the sampling performed at shutdown.*

Diversity at Locations B and C

A total of 57 genera were identified in Location B (Fig. 3.5). As in Figure 3.5, a majority of *Methylobacterium* can be detected in the samples (about 87% of the sample). Another genera was present in proportions higher than 1%. *Sphingomonas* genus was detected in the samples and represented about 9% of the sequences. Among the detected genera, only 5 were above 0.1%, including *Methylobacterium* and *Sphingomonas*. *Clostridium sensu stricto*, *Burkholderia*, and *Pelomonas* each represented about 0.2% of the sequences. Several OTUs were only identified at the order level and two were above 0.1%: "Chlamydiales_unclassified" (0.4%) and "Rhizobiales_unclassified" (0.2%). The genus *Meiothermus* was detected in the samples but represented about 0.01% of the sequences. Nine phyla were detected at low abundance, including *Acidobacteria* (0.06%), *Actinobacteria* (0.1%), *Bacteroidetes* (0.3%), *Chlamydiae* (0.5%), *Cyanobacteria*-Chloroplast, with the presence of "Streptophyta_unclassified" (0.04%), *Deinococcus-Thermus* (0.01%), *Firmicutes* (0.3%), *Planctomycetes* (0.1%), and *Verrucomicrobia* (0.01%).

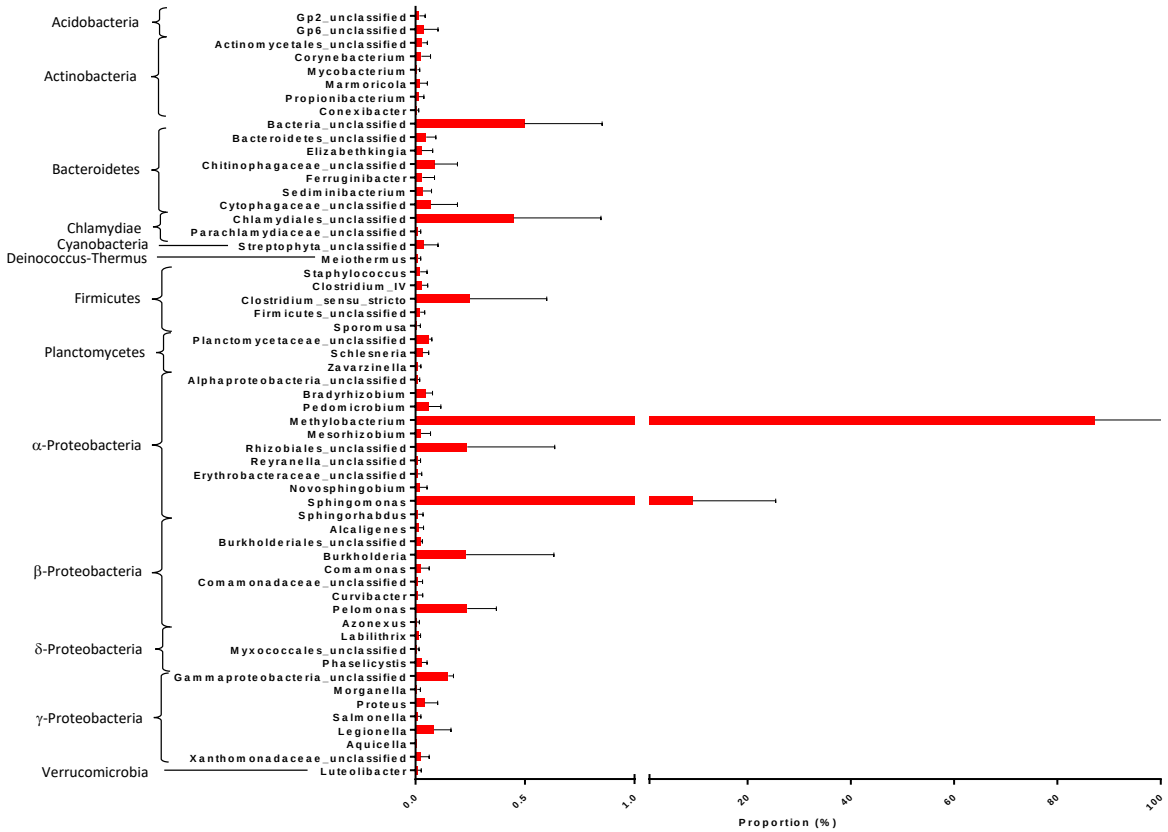



***Figure 3.5: Microbial diversity at the genus level present at location B (above the chimney) from the 2017 samplings.*** *Metagenomics results after a V4V5 sequencing and a 0.016% cut-off of all methods. The phyla (or class for the Proteobacteria) is indicated for each genus. A majority of Methylobacterium (about 87%) can be observed at this location. The second main genus was Sphingomonas (about 9%).*

Similarly to the 2015 and 2017 samplings at Location A, a majority of *Proteobacteria* (98%) was identified in this sample, and more generally Gram negative bacteria (99%). In this sample too one genus (and more specifically, one OTU) dominated the identified sequences.

A total of 38 genera belonging to 8 phyla were identified at location C in 2017. Figure 3.6 represents the proportion of each identified genus from the analyzed sequences. While this proportion can give some indications about the main genera present, it does not represent the proportion of each genus in the water sampled at location C. A majority of *Mehtylobacterium* (about 80%) was identified in this sample, followed by *Sphingomonas* (about 8%) and *Clostridium sensu stricto* (about 7%). Apart from these three genera, 35 genera in proportion lower than 1% were also identified. The genus *Meiothermus* was detected in very small quantity (less than 0.02%). At low abundance and low diversity level, bacteria belonging to the *Acidobacteria* (0.04%), *Actinobacteria* (0.8%), *Bacteroidetes* (0.1%), *Chlamydiae* (0.2%), *Deinococcus-Thermus* (0.01%) and *Planctomycetes* (0.03%) phyla have been identified.

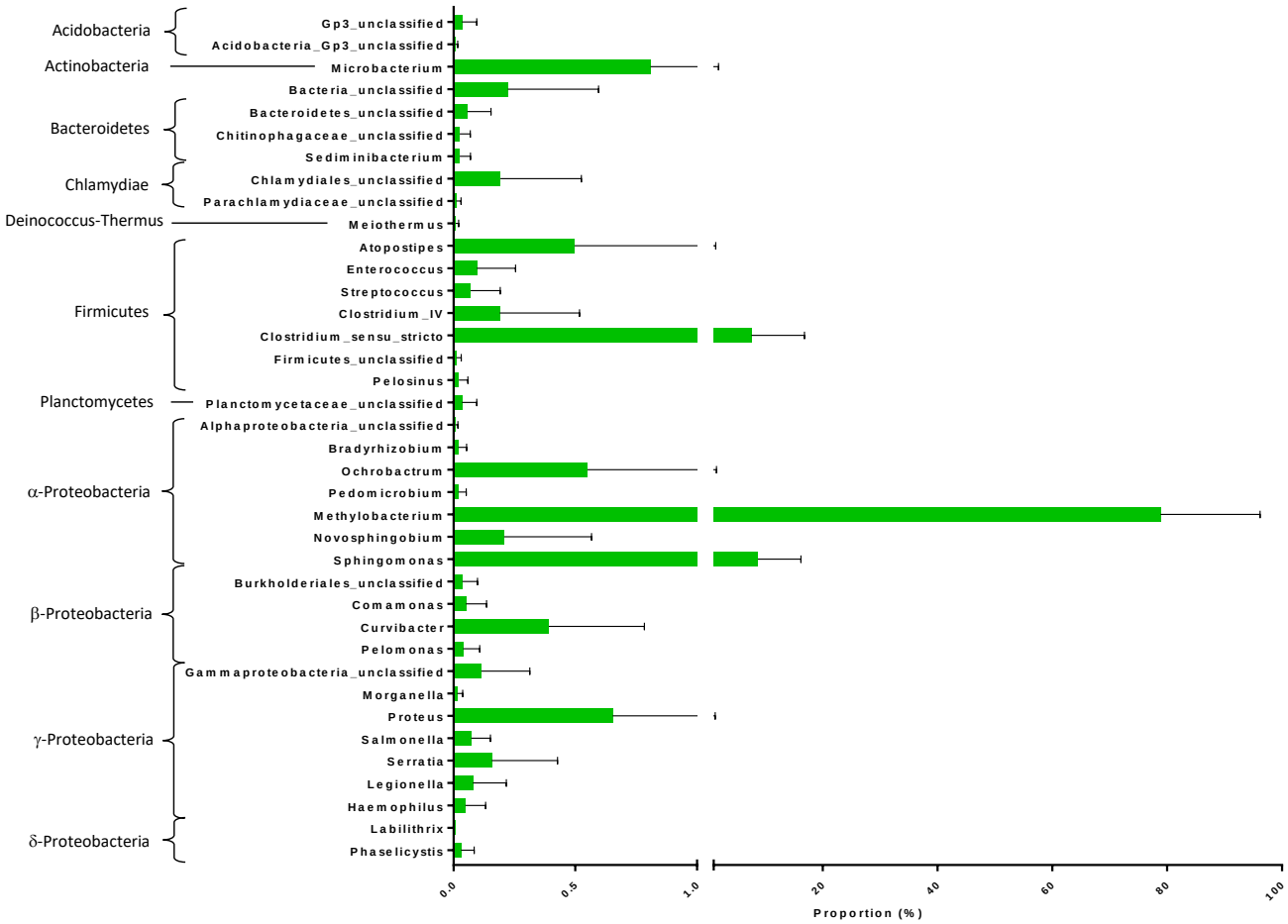



***Figure 3.6: Microbial diversity at the genus level present at location C (inside the chimney) from the 2017 samplings.*** *Metagenomics results after a V4V5 sequencing and a 0.016% cut-off of all methods. The phyla (or class for the Proteobacteria) is indicated for each genus. A majority of Methylobacterium (about 80%) can be observed at this location. The second main genus was Sphingomonas (about 8%), followed by Clostridium (about 7%).*

Unlike the two other 2017 samplings, *Proteobacteria* phylum represented 90% of the sequences while *Firmicutes* represented about 8%. Gram negative bacteria represented about 91% of the sample.

One OTU dominated all samples from the 2017 samplings and belonged to the *Methylobacterium* genus.

For each sampling, it could be noticed that only one OTU dominated the sample. In 2015, this OTU (OTU n°2) belonged to *Variovorax* genus and in 2017, this OTU (OTU n°1) belonged to *Methylobacterium* genus.

*Direct proteotyping with metaproteomics*

A direct metaproteomic approach was attempted after filtration of 4.5 L of water on the 2017 samplings. While the number of microorganisms present is relatively low for this approach ($12.2\pm2.7 \times 10^6$ cells/L, $6.3\pm0.9 \times 10^6$ cells/L, and $2.3\pm1.1 \times 10^6$ cells/L for locations A, B, and C respectively), enough signal could be recorded highlighting the presence of four bacterial phyla.

*Table 3.3: Proportion of each genus present in the nuclear pool during shutdown analyzed by metaproteomics*

| | | | | | Water | | |
|---|---|---|---|---|---|---|---|
| | | | | | Loc. A | Loc. B | Loc. C |
| Phylum | Class | Order | Family | Genus | % | % | % |
| Actinobacteria | Actinobacteria | Micrococcales | Microbacteriaceae | Microbacterium | 0,97 | | 0,48 |
| | | Micromonosporales | Micromonosporaceae | Asanoa | | | 27,32 |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 37,11 | 40,67 | 26,27 |
| Firmicutes | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | 1,00 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | Bradyrhizobium | 5,44 | | 3,34 |
| | | | Methylobacteriaceae | Methylobacterium | 47,15 | 51,77 | 34,05 |
| | | | Rhizobiaceae | Rhizobium | | | 3,13 |
| | Betaproteobacteria | Burkholderiales | Comamonadaceae | Comamonas | | | 0,65 |
| | | | | Pelomonas | 3,32 | 1,20 | |
| | Gammaproteobacteria | Enterobacteriales | Enterobacteriaceae | Escherichia | 1,08 | 1,20 | 0,61 |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 4,12 | 4,21 | 2,68 |
| Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | Mycoplasma | 0,82 | 0,96 | 0,47 |

The *Proteobacteria* phylum was present in all samples and represented 64%, 60%, and 48% of the peptide signal at locations A, B, and C respectively. The second main detected phylum was *Actinobacteria*, with 35%, 39% and 51% of the peptide signal at locations A, B, and C respectively (Table 3.3 and Fig. 3.7). In the Proteobacteria phylum, *Methylobacterium* was the main genus identified with 47%, 52%, and 34% of the total peptide signal in the sample at locations A, B, and C respectively. Similarly, *Streptomyces* was the second main genus of the samples at about 37%, 41%, and 26% of the samples at locations A, B, and C respectively. A third main genus in proportion, *Asanoa*, was only detected at Location C and represented about 37% of the sample. Highest diversity was observed at Loc. C. *Firmicutes*, with the genus *Clostridium*, was only detected in the sample collected at Location C and represented about 1% of the peptide signal. One phylum, *Tenericutes* with the presence of *Mycoplasma*, accounted only for 0.5% (Loc. C) to 1% (Loc. A&B) of the identified peptide signal in each sample.

Despite low signal, eleven different families were detected via this approach, among which six were common to the three locations. Minor identified genera included *Pseudomonas* (about 4% at locations A and B, about 3% at Loc. C), *Bradyrhizobium* (absent from Loc. B, about 5% at Loc. A and 3% at Loc. C), *Rhizobium* (about 3% at Loc. C), *Escherischia* (about 1% in all samples), *Microbacterium* (about 1% at Loc. A and 0.5% at Loc. C), and *Comamonas* (about 0.7% at Loc. C).

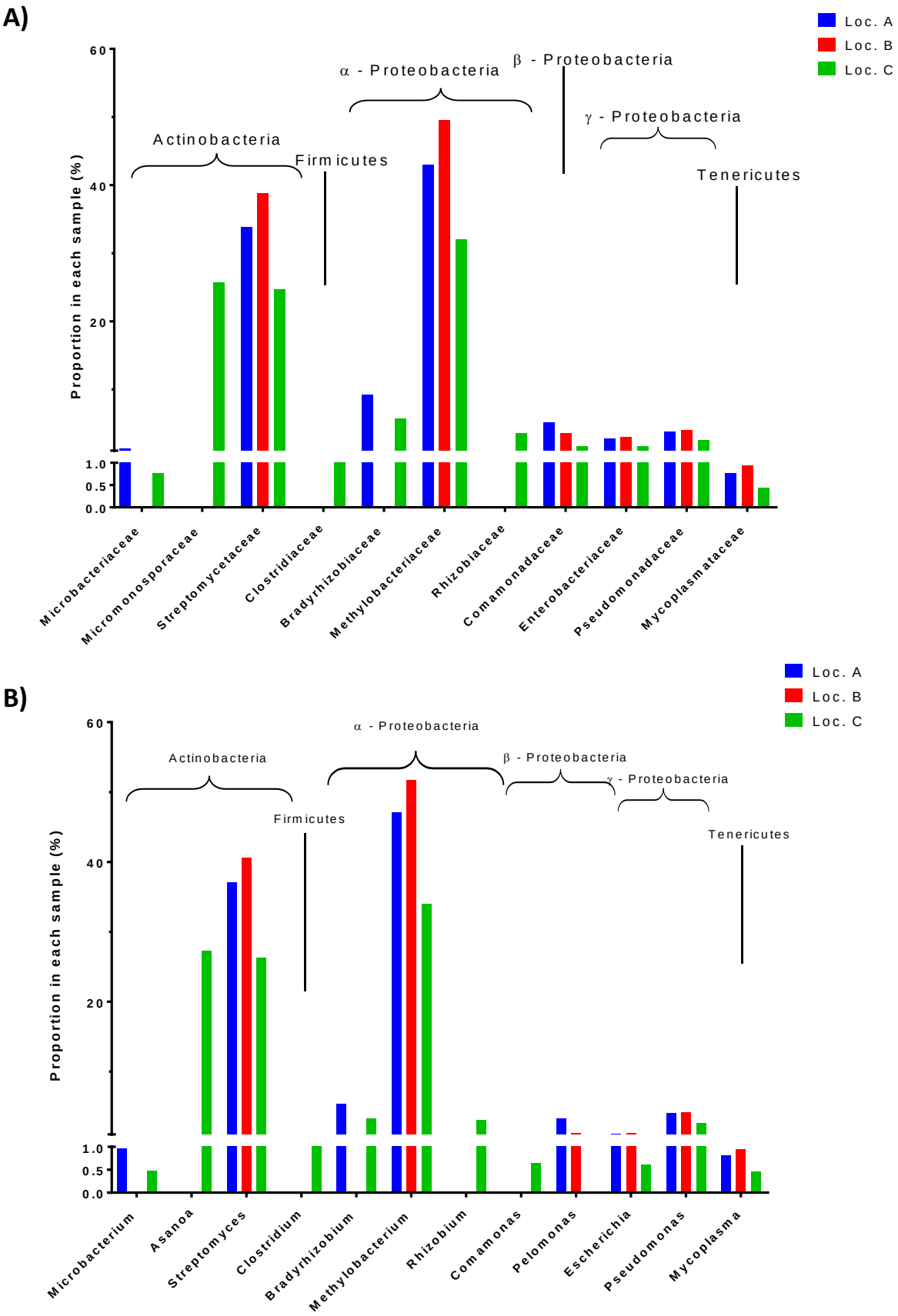



*Figure 3.7: Microbial communities present in the cooling pool of the reactor's core assessed during the reactor shutdown, analyzed by metaproteomics at A) the family taxonomic level, B) the genus taxonomic level.*

When comparing the three different sampling locations from the 2017 campaign, it can be noticed that most of the genera were common to all locations using both analysis methods. Indeed, 15 genera, representing 97.5% of the sequences were common to the three locations, including *Methylobacterium* and *Sphingomonas*, the two main genera identified in the samples. While no genus was solely identified in Loc. A, 11 genera (0.6% of the sequences) were only identified in Loc. B. Finally, 10 genera (1.8% of the sequences) were only identified in Loc. C (Appendix 6, Fig. 6.3).

Using metaproteomics, 5 genera were common to all locations, representing 84.4% of the peptide signal identified, including *Streptomyces*, *Methylobacterium*, and *Pelomonas*. No genus was solely found in locations A and B, but 4 genera were only identified in Loc. C, representing 32% of the peptide signal of this sample (Appendix 6, Fig. 6.4).

The comparison of the 16S rRNA amplicon sequencing and metaproteomic approaches to investigate the microorganisms present in the water of the core's cooling pool revealed that the most abundant genera were detected by both method (Appendix 6, Fig. 6.5). 55.9% of the peptide signal and 96.8% of the sequences in the water from Loc. A belonged to the same genera, while about 53% of the peptide signal and 87.5% of the sequences belonged to the same genera at Loc. B. At Loc. C, the common genera identified using metaproteomics and metagenetics represented about 38.5% of the peptide signal and 79.7% of the sequences. *Methylobacterium* was detected by both methods. However, while *Streptomyces* represented between 26 and 41% of the identified peptide signal, it was not detected using metagenetics.

### 3.1.3 Discussion

This work presented the first analysis of the microbiota present in the cooling pool of a nuclear reactor, both during operation and at shutdown. The environment in this pool was hostile due to the presence of high ionizing radiation, toxic radioactive metals and few nutrients. Despite these different stresses this pool hosted some bacterial diversity (27 OTUs during operation, 96 OTUs during shutdown). A majority of *Proteobacteria* were detected using metagenetics (all samples from all locations) and metaproteomics (from the 2017 samplings of locations A and B). *Firmicutes* were also brought to the fore by amplicon sequencing, and *Actinobacteria* by metaproteomics.

Despite the conditions prevailing in the pool during operation (presence of radiations, radionuclides and lack of nutrients), different bacteria have been identified using metagenetics. A total of 27 different OTUs were detected in the samples after the OCToPUS analysis, which is much lower than the 4,000 OTUs identified by Bagwell et *al*. in the Savanah River Site SNFP [193]. One genus can possess several OTUs, which can give an indication about the species number contained per genus. The evenness of the samples collected during reactor shutdown was quite low, with a Shannon index in the 0.2 to 1.2 range compared to other environmental samples [193]. In the results represented by Bagwell et *al*., the Shannon indexes of the samples collected in the SNFP were at about 5 [193].

Bacteria isolated from SNFPs in Spain, Argentina, and Slovakia after a cultivation step mainly belonged to the *Proteobacteria*, the *Firmicutes*, and the *Actinobacteria* [152, 181, 184-187, 192]. A recent study inventorying the population of a SNFP in the USA using amplicon sequencing also highlighted a majority of *Proteobacteria* (83%). However, *Methylobacterium* represented only 2% of the reads [193]. The main phyla found in this pool were similar to the ones detected in our study, including *Acidobacteria*, *Planctomycetes*,

*Actinobacteria*, *Bacteroidetes*, and *Deinococcus-Thermus*. All are environmental bacteria, which may have contaminated the pool owing to the traffic in and out of the reactor.

During the core's operation some microbial diversity was detected despite the presence of high radiation and radionuclides concentration in the water. To be able to analyze the water the decrease of radioactivity was mandatory before manipulation. The presence of one species dominating the sample, belonging to the *Variovorax* genus, could be a consequence of the harsh conditions present in the pool. This species was more suited to resist the prevailing conditions. At shutdown the dominant species was different meaning that *Variovorax* was no longer the best fitted organism. One *Methylobacterium* species was able to occupy the pool once the water radiolysis was over. At shutdown, it was possible to screen the population from different spots presenting an irradiation dose rate varying of a $10^8$ factor (from the 0.1 µGy/h at Loc. A to the 25 Gy/h at Loc. C). The high levels of radiation present at Loc. C could explain the lower cell density. Moreover the water present in Loc. C was more stagnant due to the pool's configuration.

Metaproteomics highlighted the presence of genera such as *Streptomyces*, *Asanoa*, *Bradyrhizobium*, and *Mycoplasma*, which were not detected by amplicon sequencing and represented about 36% of the relative protein abundance. The 16S rDNA gene amplification approach actually suffers from non-quantitative representativeness particularly for Gram-positive bacteria. It has been shown that most of the classical DNA extraction protocols considerably underestimate the Gram-positive bacteria fraction, probably owing to the higher mechanical resistance of their cell walls [272]. Several DNA extraction protocols advise the use of 0.1 mm beads associated with a mechanical lysis via the use of a Precellys® for example [272, 276, 340, 341], while another study proposes a 2h incubation of the sample in presence of lysis buffer at 65°C [342]. Metaproteomics actually made it possible to get away from metagenomics biases and to highlight the presence of two families of Gam-positive bacteria of the *Actinobacteria* phylum which accounted for 30% of the consortia composition, based on proteins. Even though biases also exist due to protein extraction, to the size of the cells, and to the less extensive databases, metaproteomics probably assesses more accurately the quantitative composition of the consortia [301].

All the genera detected in the core's cooling pool are environmental bacteria, which may have contaminated the pool owing to the traffic in and out of the reactor. They might either have been selected and/or have adapted to the specific conditions of the pool. Among the main genera present in this pool, members of the *Methylobacterium* genus are ubiquitous in nature, being found in freshwater and aquatic sediments [343]. They constitute common airborne organisms which are found in industrial environments. A *Methylobacterium* strain has recently been detected using metagenetics in a spent nuclear fuel storage basin at the Savannah River Site in the USA [193]. Some strains have been shown to exhibit resistance to gamma irradiation 10–40 times higher than that withstood by many other Gram-negative bacteria [315, 344, 345]. *M. extorquens* and *M. radiotolerans* presented a $D_{10}$ of 4.5 and 3.5 kGy, respectively [346], and one species (*Methylobacterium* sp. strain rad) was even isolated after irradiation of 20 kGy [346].

*Streptomyces* bacteria are aerobic, spore-forming, and environmental organisms [347]. *Streptomyces* can produce eumelanin, which is an antioxidant pigment, helping prevent the oxidative damages from ionizing radiation and ROS formation [68]. This genus was also identified in a contaminated soil sample from Chernobyl [348]. *Streptomyces radiopugnans* was isolated from a radiation-polluted soil in China and presented great survival after a 5 kGy irradiation and could resist up to 15 kGy [349]. *S. rimosus* was found to resist gamma-radiation with a $D_{10}$ of 1 kGy [179]. A strain of *S. canarius*, isolated in Egypt, was shown to

resist up to 25 kGy γ-irradiation [327]. Most of the isolated *Streptomyces* from this study exhibited a resistance to γ-radiation and only a few species did not survive doses higher than 5 kGy [327]. To our knowledge, this is the first time the genus *Streptomyces* was identified in a nuclear pool.

The genus *Sphingomonas* is also widely distributed in aquatic environments [315]. One strain isolated from the Antarctic Dry Valleys combined the ability to withstand UV radiations, hyper aridity, and low temperatures encountered in Antarctica. This strain contained in its genome numerous stress response genes (116 in total), including oxidative stress resistance genes [306]. Another strain (*Sphingomonas* sp. strain RB2256) exhibited a high resistance to UV radiation, with a $D_{10}$ of about 175. $J.m^{-2}$ [307]. Members of this genus were also identified in the Cofrentes SNFP in Spain and in a SNFP of the Idaho Nuclear Technology and Engineering Center in the USA, after a cultivation step [177, 185].

*Variovorax*, predominant during the reactor operation, is an environmental genus [315]. One *Variovorax paradoxus* strain was also identified in the SNFP of Cofrentes after a cultivation step [184]. The ability of some *Variovorax* strains to make use of hydrogen oxidation as an energy source [315, 350] might explains the prevalence of this genus during operation. $H_2$ is indeed formed through the radiolysis of water, which occurs when a reactor is operating. When the reactor is shut down, the level of $H_2$ drops. The advantage conferring by using $H_2$ as an energy source could explain *Variovorax* prevalence during the reactor's operation on other genera enable to use this energy. Other genera, not competitive enough during reactor functioning, might have been better fitted to the new conditions present in the pool during shutdown. This could explain the completely loss of *Variovorax* in 2017 to the profit of *Methylobacterium*.

The change of temperature and pH between operation (temperature of 35°C and pH 7.0) and shutdown (pH 5.4) could also partly explain the change in the microbiota, such as the presence of *Acidobacteria* during shutdown. However since no indication was obtained concerning the species taxonomical level of the bacteria, these explanations can only be hypotheses. No *Variovorax* nor *Methylobacterium* have been isolated from both campaigns, preventing the determination of the main physical or chemical factor responsible for this change.

The description "Streptophyta_unclassified" could have two different meaning in this sample: it can i) highlight the presence of a micro-algae or ii) highlight the presence of *Cyanobacteria*. It has been hypothesized that chloroplast were *Cyanobacteria* after endocytosis [336, 337]. The Ribosomal Database Project associates this "Streptophyta" classification to the *Cyanobacteria* phylum (one *Cyanobacteria*-Chloroplast phylum) [335]. The presence of a micro-algae can be possible since a green micro-algae, *Coccomyxa actinabiotis*, has been isolated from a French SNFP [123, 338].

The microbial diversity in the cooling pool of a nuclear reactor is thus important, with a domination of the genera *Variovorax* during operation and *Methylobacterium* during shutdown. This diversity was even greater than the one described in this work. It was indeed shown in Chapter II.2 that a microbial diversity was observed during the liquid culture kinetics. A total of 181 different genera were indeed identified during the kinetics.

The identification of the genus *Variovorax* in the pool dominating the microbiota present during a working cycle is an interesting discovery. The given hypothesis linking the high abundance of this genus and the metabolism of hydrogen produced by the reactor should however be confirmed with specific experiments. Studying the microorganisms present in the cooling pool of a working nuclear reactor will be needed to

confirm *Variovorax* predominance or other hydrogen-metabolism active microorganisms. Finally, a deeper metaproteomics analysis of water samples could be done in order to identify the possible metabolism pathways.

### 3.1.4 Acknowledgements

The authors gratefully thank M. Delage and C. Dubois (CEA, DEN/DDCC/UADS/SEROS, Saclay) for providing access to the facility, F. Coulon and E. Latil (CEA, DRF/PSAC/USPS/SPRE/SRP, Saclay) for completing the gamma spectrometry measurements, the radiological controls, and the transportation documents, S. Echivard (CEA, DEN/DDCC/UADS/SEROS) for the water sampling, P. Servan (CEA, Fontenay-aux Roses), and J. Timmins (IBS, Grenoble) for providing the D. radiodurans strain and discussion, and J. Martin-Laffon (DRF/BIG/PCV) for her help in bibliographic research. The authors gratefully acknowledge the Nuclear Toxicology Program of the CEA for financial support (INFERNUS project grant).

## 3.2 Unravelling the microbial community of biofilms present in a nuclear reactor pool (*article in preparation*)


**Abstract**

It is known that life can be found in spent nuclear fuel pools and nuclear reactor cooling pools. The aim of this study was to analyze microbial diversity present in biofilms collected from the cooling pool of a nuclear reactor The use of metagenetics revealed that the main dominant genus in all biofilms but one was *Clostridium*, as well as a high bacterial diversity. The identification of anaerobic bacteria in these samples raises microbial influenced corrosion concerns. Microbial monitoring of nuclear cooling pools and spent nuclear fuel pools is thus highly recommended.


### 3.2.1 Material and Methods

#### *3.2.1.1 Samplings*

Eight different scrubs were performed in a nuclear reactor cooling pool to recover biofilms. These samplings were harvested in 2017, one year after the definitive shutdown of the reactor. The location of the scrubs in relation to the reactor position in this pool is schematized in Figure 3.8. The scrub number 5 was the furthest to the reactor while Scrub 2 was the closest. Scrub 1 was used to recover the biofilm present on the footbridge under a spotlight at a depth of 4.5 meters (Fig. 3.9-1). The radioactivity level was below the detection limits (< 1 µGy/h) in this location (Table 3.4). Scrub 2 was made on an extinguished spot at a depth of 8 meters. Scrubs 3 and 7 were performed on the cofferdam, on the pool side at a depth of 1.5 meters while scrubs 4 and 8 were sampled on the same cofferdam but at a depth of 4 meters (Fig. 3.9-2). The biofilm recovered by scrub 5 was located on the opposite wall from the nuclear fuels in the pool at a 1 m depth. Due to the presence of visible deposit on the lid of a spent nuclear fuel at a 4 m depth, a scrub was performed (Scrub 6, Fig. 3.9-3). The recovered biofilms were highly radioactive (Table 3.4) and contained $^{137}Cs$ and $^{60}Co$.

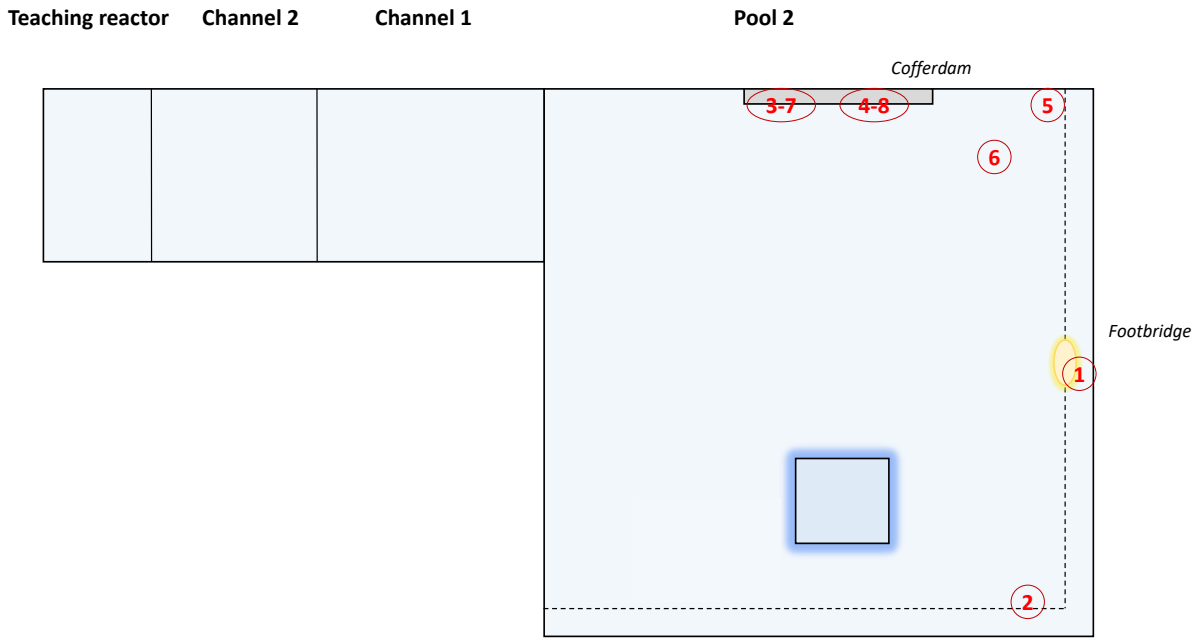



*Figure 3.8: Scheme of the scrubs location compared to the position of the reactor (reactor: box with the blue light)*

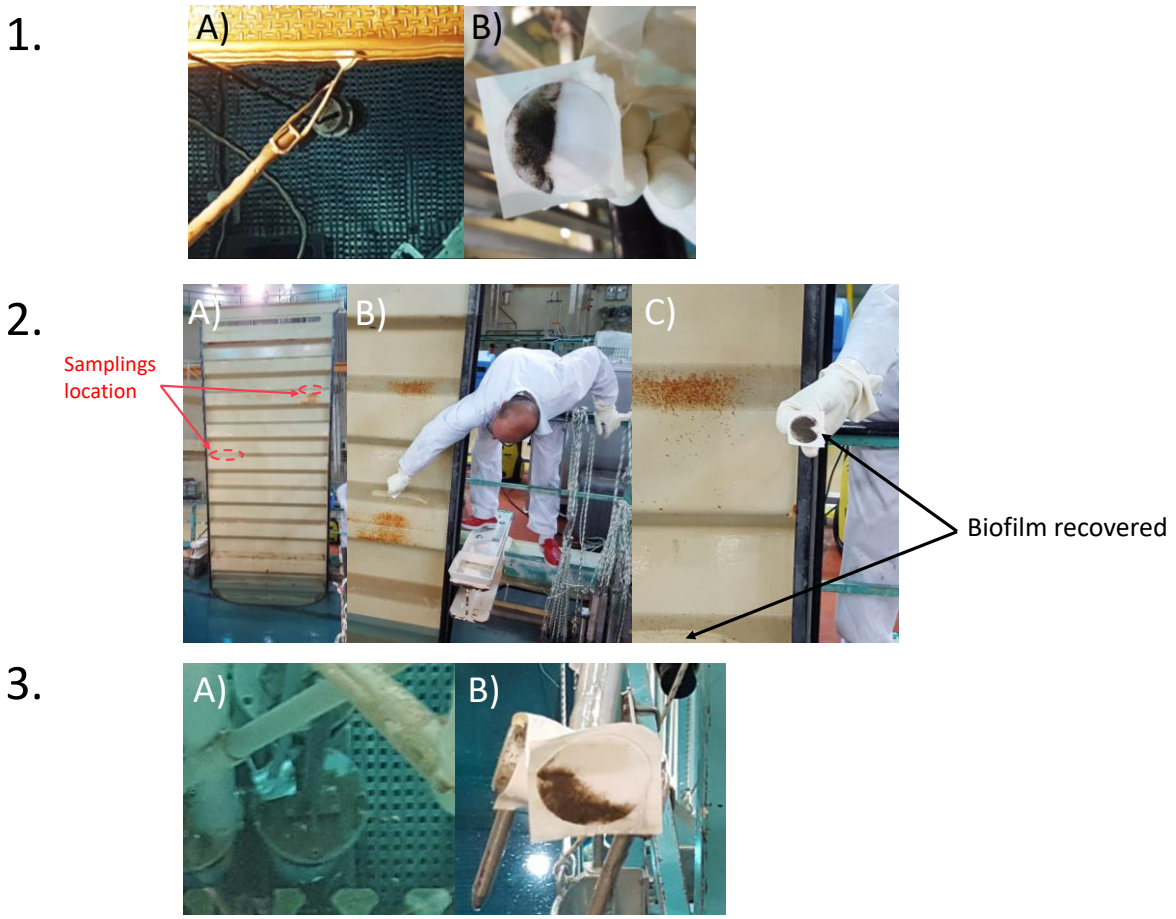



***Figure 3.9: Scrubs location and aspect.*** *(1) Scrub 1 location (A) and aspect after rubbing (B). The black part (B) represents the recovered sample. (2) Location (A and B) and aspect (C) of the scrubs 3 - 4 - 7 -8. Scrubs 3 and 7 were performed on the top of the cofferdam while 4 and 8 were located on the bottom of the cofferdam (A). (3) Scrub 6 location (A) and aspect after rubbing (B). The black part (B) represents the recovered sample.*

The scrubs were composed of cellulose and are used by the staff of nuclear facilities to determine the radiation level present on an object. The scrubs were either attached to a metal pole for an underwater sampling or directly used by the handler when the support could be brought out of the water (Fig. 3.9). The recovered scrubs were immediately placed inside sterile hermetic plastic bags to avoid contact with ambient air. In a sterile environment the scrubs were introduced into 50 mL sterile Falcon tubes with 15 mL of sterile milliQ water and shook to resuspend the recovered organisms. After the resuspension step, the presence of visible particles containing $^{60}Co$ and the emission of more than 800 counts per second measured with the radiagem 2000 (CANBERRA, USA) forced us to let the Falcons decant overnight at RT. The 5 top mL were recovered after a 1 min centrifugation at 100 *g*. 800 µL were used to extract DNA and 500 µL were cryopreserved in Glycerol Strain Buffer at -80°C preservation (65% glycerol v/v, 0.1 M $MgSO_4$, 0.025 M Tris-HCl pH 8). The gamma dose rate of each sample was determined using a γ-spectrometry (composed by a germanium detector EGC-10-180R and an ORION analyzer, the results were treated with the InterWinner software).

Due to the activity of the samples, the DNA extraction was performed in the radioactive room of the PCV laboratory in CEA – Grenoble. While this environment is not sterile, the fume hood was thoroughly cleaned prior manipulation.

#### *3.2.1.2 DNA extraction*

The DNA was extracted with a phenol-chloroform method, adapted from Vilchez-Vargas et al. [276]. The samples were centrifuged 20 minutes at 20,000 *g* at room temperature and the supernatant discarded. One milliliter of lysis buffer (100 mM Tris pH 8; 100 mM EDTA pH 8; 100 mM NaCl; 1% polyvinylpyrrolidone; 2% SDS) was added and the pellet resuspend. The mixture was then transferred into a glass bead (0.1 mm) tube from Mobio. The tube was disrupted three times at 30 Hz for 60 seconds with 30 seconds break between each run with a disruptor MM 300 from Retsch/Qiagen. The tube was then centrifuged 5 minutes at 20,000 *g* at room temperature and the supernatant removed and transferred into a new tube. One milliliter of phenol-chloroform-isoamyl alcohol (25:24:1) was added. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered and transferred into a new tube after a centrifugation step of 1 minute at 17,000 *g* at 4°C. Seven hundred microliters of chloroform were added to the sample. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered after a centrifugation step for 1 minute at 17000 *g* at 4°C. The supernatant was divided into two tubes (around 450 µL in each) and 45 µL of sodium acetate 3M and 500 µL of isopropyl alcohol were added into each tube. The samples were then mixed by inverting the tubes and put overnight at -20°C. The tubes were centrifuged 30 minutes at 17,000 *g* at 4°C and the supernatant removed. Two steps of washing were performed by adding 750 µL of 75% ethanol and a centrifugation step of 5 minutes at 17,000 *g* at 4°C. The supernatant was removed and the pellet dried with a heating block at 55°C. Sixty microliters of sterile milliQ water were added to dissolve the pellet. To have a more pure DNA and remove any trace of alcohol, the sample was put on a 0.05 µm filter disposed on sterile water for 15 minutes before being transferred into a new tube.

#### *3.2.1.3 Sequencing and data analysis*

The sequencing of the genomic DNA on 16S rDNA was performed by LGC Genomics, UK. The sequencing was conducted as followed.

The PCRs included about 1-10 ng of DNA extract (total volume 1 µL), 15 pmol of each forward primer and reverse primer (in 20 µL volume of 1 x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µL of BioStabII PCR Enhancer (Sigma) and additionally 0.2 µl of DNase (Artic Zymes) for the 16S PCR. For each sample, the forward and reverse primers had the same 10-nt barcode sequence. Each PCRs were carried out for 20 cycles, dependent on the primer pair, using the following parameters: 1 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s and primers without inline barcodes have been used (341F/1061R). For the second round 1 µL PCR product from the first round was used and the PCR conditions were the same as before. For this reaction barcoded primers have been added to amplify the V4V5 regions (515YF-926R: 5'-GTGYCAGCMGCCGCGGTAA-3' and 5'-CCGYCAATTYMTTTRAGTTT-3' respectively).

DNA concentration of amplicons of interest was determined by gel electrophoresis. About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying different barcodes. The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimer and other small

mispriming products, followed by an additional purification on MiniElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

Sequencing data were analyzed through the OCToPUS pipeline [210] for contig assembly, quality filtering, de-noising, chimera removal, and OTU clustering. The OTUs were then taxonomically assigned at 97% identity against the SILVA database [333-335] using the mothur software [213].

## 3.2.2 Results

### *3.2.2.1 Radioactivity of the samples*

The radioactivity of the biofilms was measured (Table 3.4). Scrubs 3 and 4, collected on the cofferdam, presented the highest gamma dose of 70 and 80 Bq respectively. Scrubs 1, 2 and 5 presented a gamma dose below 10 Bq. Scrub 5, presenting the lowest gamma dose of the samples (below 4 Bq) was collected at the opposite wall from the nuclear fuels.

***Table 3.4: Gamma dose of each sample measured using a γ-spectrometry (germanium detector EGC-10-180R and an ORION analyzer, the results were treated with the InterWinner software).***

| Scrub | Gamma dose (Bq) |
|---|---|
| **1** | 8 |
| **2** | <7 |
| **3** | 70 |
| **4** | 80 |
| **5** | <4 |
| **6** | 30 |
| **7** | 30 |
| **8** | 30 |

### *3.2.2.2 Metagenetics analysis of the samples*

In this analysis the rarefaction curves presented a reached plateau for each scrub, meaning that a comprehensive analysis was obtained with these data with only 5000 reads (Fig. 3.10). Diversity in scrubs 1, 2, 4, 5, 6, 7, and 8 was achieved at about 10-17 OTUs while it was achieved at about 45 OTUs in Scrub 3. Scrub 3 was thus more diverse in terms of microbial diversity than the other scrubs. However the diversity in these samples is far to be huge compared to most soil samples, air, or marine water [193, 231].

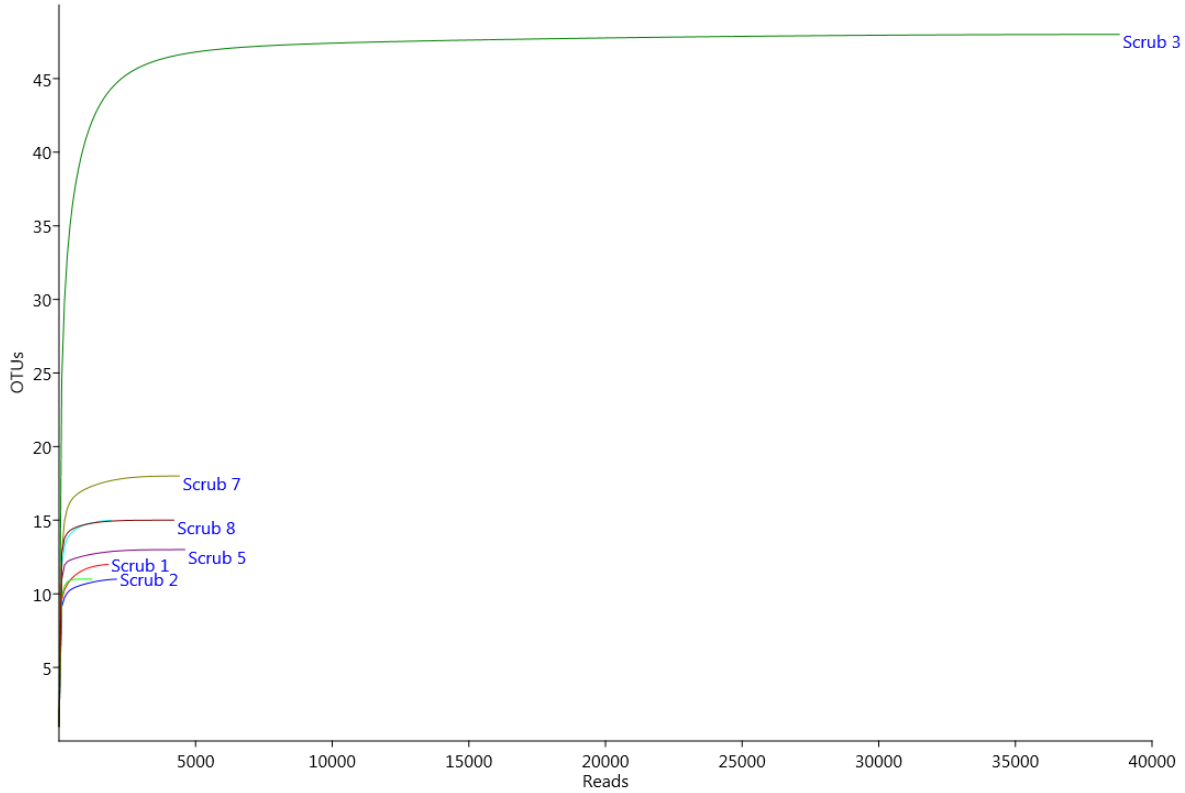



***Figure 3.10: Rarefaction curve of the scrubs (obtained from Past software [278]).*** *The diversity was reached quickly for all scrubs, except for scrub 3, which exhibited a greater diversity and reached a plateau above 5000 reads and at about 45 OTUs. The other scrubs reached a plateau faster, below 2000 reads and at about 10-17 OTUs.*

The evenness of the samples was quantified using the Shannon index. The evenness indicates whether the diversity is well divided between the different OTUs or if one OTU dominates the sample. This index combines species richness and abundance into a single value of evenness [339]. When this index is close to 0 a single species dominates the sample. The evenness of the different scrubs was important for each sample. The Shannon indexes were between 1.56 and 3.07 (Table 3.5). Using this index no sample was dominated by one genus, except for scrubs 5 and 6 since they possessed the lowest Shannon indexes (1.56 and 1.59 respectively).

***Table 3.5: Shannon index of the scrubs.*** *When this index is close to 0, one species dominates the sample.*

| Sample | Shannon index |
|---|---|
| **Scrub 1** | 1.76 |
| **Scrub 2** | 1.77 |
| **Scrub 3** | 3.07 |
| **Scrub 4** | 2.06 |
| **Scrub 5** | 1.56 |
| **Scrub 6** | 1.59 |
| **Scrub 7** | 1.85 |
| **Scrub 8** | 1.95 |

Seven phyla were identified in the different scrubs. The majority of the identified genera and families belonged to the *Proteobacteria* phylum (34 OTUs identified as *Proteobacteria*, and 17 OTUs identified as *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, and *Planctomycetes*). However the *Clostridiaceae* family (*Firmicutes*) was the main family present in these scrubs (more than 50% of the sequences were identified as *Clostridiceae*) (Fig. 3.11). After a metagenetic analysis, the majority of the scrubs were composed of 11 to 18 OTUs, except for Scrub 3 which consisted of 48 OTUs (including 14 OTUs identified as "Bacteria_unclassified" representing a total of 33% of the analyzed sequences from Scrub 3) (Appendix 7, Table 6.11). Two OTUs (OTU 8 and OTU 56) were identified by OCToPUS as "Enterobacteriaceae_unclassified". After comparison of the sequence of these two OTUs it appears that only a few nucleotides were different (Appendix 7, Fig. 6.6). When blasting the sequences on the nucleotide BLAST tool from NCBI, OTU 56 presented a great score with *Serratia* while the OTU 8 was identified as *Salmonella*. Both sequences presented a 100% coverage and identity with the corresponding organism.

Three phyla were identified in Scrub 1: *Actinobacteria* (5%), *Firmicutes* (57%), and *Proteobacteria* (38%). The sample was dominated by the genus *Clostridium sensu stricto* with about 51% of the identified sequences (*Firmicutes*), followed by *Proteus* with 15% of the sequences (*γ-Proteobacteria*), and *Ochrobactrum* with about 10% of the sequences (*α-Proteobacteria*). The genus *Mycetocola* (*Actinobacteria*) represented about 5% of the sequences, the genus *Pelomonas* (*α-Proteobacteria*) represented about 3% of the sequences, and the genus *Melissococcus* (*Firmicutes*) represented about 3% of the sequences. About 9% of the sequences were attributed to OTU 8 (corresponding to *Salmonella*) (Fig. 3.11; Appendix 7, Table 6.11).

Four phyla were identified in Scrub 2: *Actinobacteria* (6%), *Bacteroidetes* (0.1%), *Firmicutes* (56%), and *Proteobacteria* (39%). In this scrub too, a majority of *Clostridium sensu stricto* (45%) was identified. The second main genus was *Sphingomonas*, with 14% of the sequences. *Melissococcus* (10%), *Ochrobactrum* (9%), *Mycetocola* (6%), *Pelomonas* (5%), *Proteus* (5%), and OTU 8 (*Salmonella*) (5%) constituted the main genera identified (Fig. 3.11; Appendix 7, Table 6.11).

Scrub 3 represented the greater diversity and evenness among the different scrubs (Fig. 3.10, Table 3.8). This can be confirmed by the diversity present in the sample. Bacteria belonging to seven phyla were identified in this sample: *Actinobacteria* (1%), *Bacteroidetes* (3%), *Chloroflexi* (4%), *Deinococcus-Thermus* (3%), *Firmicutes* (11%), *Planctomycetes* (1%), and *Proteobacteria* (44%). In this sample, the genus *Clostridium sensu stricto* represented only 4% of the sequences. The main identified genera were *Sphingomonas* (7%), *Pelomonas* (7%), and *Methylobacterium* (7%), all belonging to the *Proteobacteria*. The genus *Sediminibacterium* represented 3% of the sequences and belongs to the *Bacteroidetes* phylum. Several OTUs, not identified at the genus level, were also present in important proportion. OTU 11, belonging to the *Comamonadaceae* family, represented 10% of the analyzed sequences. OTUs 12, 13, and 16, not identified at the phylum level, represented 9%, 9%, and 7% of the sample respectively. OTU 14, belonging to the *Clostridia* class, represented about 7% of the sequences. Four OTUs belonging to the *γ-Proteobacteria* class represented about 8% of the sequences, with one OTU representing more than 6% of the sequences. Finally, 3% of the sequences were attributed to OTU 22, which has been associated to the *Chloroflexi* phylum. *Chloroflexi* and *Deinococcus-Thermus* phyla were only identified in Scrub 3 (Fig. 3.11; Appendix 7, Table 6.11).

Five phyla were identified in Scrub 4: *Actinobacteria* (3%), *Bacteroidetes* (5%), *Firmicutes* (34%), *Planctomycetes* (0.3%), and *Proteobacteria* (58%). *Planctomycetes* phylum was only identified in scrubs 3

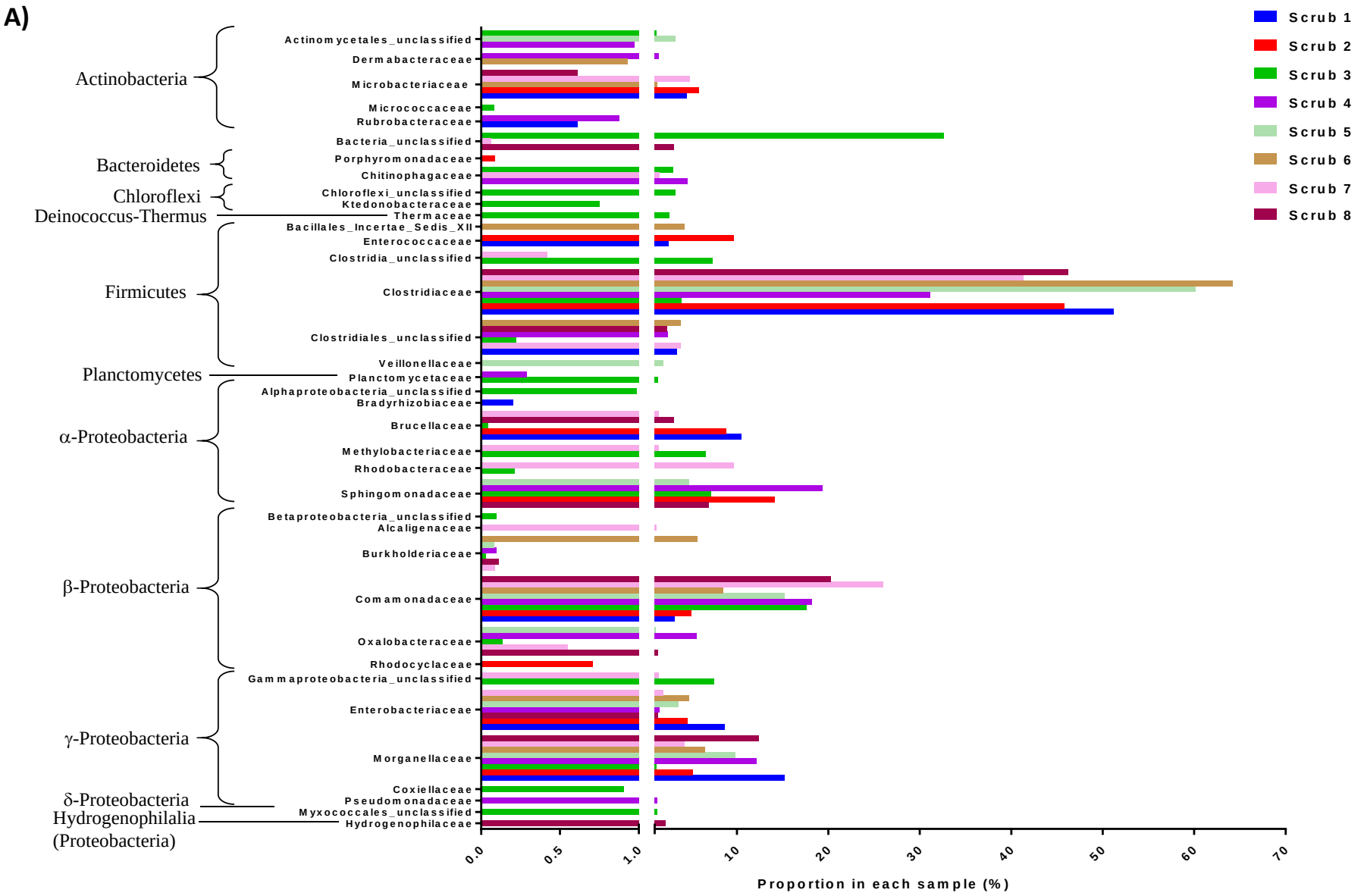
and 4. In this sample, *Clostridium sensu stricto* was the main genus identified (31%), followed by *Sphingomonas* (19%) and *Proteus* (12%). The genera *Curvibacter* (9%), *Diaphrobacter* (9%), *Herminiimonas* (6%), and *Sediminibacterium* (3%) were among the main genera identified (Fig. 3.11; Appendix 7, Table 6.11).

Bacteria belonging to three different phyla were identified in Scrub 5: *Actinobacteria* (3%), *Firmicutes* (62%), and *Proteobacteria* (35%). In this scrub, *Clostridium sensu stricto* represented 60% of the analyzed sequences, followed by *Curvibacter* (13%). The other main genera included *Proteus* (8%), OTU 8 (*Salmonella*) (4%), *Sphingomonas* (2%) (*Sphingomonadaceae* family represented 5% of the sample), *Pelomonas* (2%), and OTU 43, belonging to the *Actinomycetales* order (3%) (Fig. 3.11; Appendix 7, Table 6.11).

The same three phyla were also identified in Scrub 6: *Actinobacteria* (2%), *Firmicutes* (72%), and *Proteobacteria* (25%). In this sample too *Clostridium sensu stricto* was the main genus (64%), followed by *Diaphrobacter* (9%), *Proteus* (7%), *Wautersia* (6%), OTU 8 (*Salmonella*) (5%), and *Exiguobacterium* (4%). The genus *Mycetocola* represented about 1% of the sequences (Fig. 3.11; Appendix 7, Table 6.11).

Four phyla were identified in Scrub 7: *Actinobacteria* (5%), *Bacteroidetes* (2%), *Firmicutes* (46%), and *Proteobacteria* (48%). The main genus identified in this sample was *Clostridium sensu stricto* (41%), followed by *Curvibacter* (26%). The other main genera identified were *Mycetocola* (5%), *Proteus* (4%), *Sediminibacterium* (2%), *Methylobacterium* (1%), *Alcaligenes* (1%), OTU 56 (*Serratia*) (1%), or belonged to the *Rhodobacteraceae* family (OTU 36 - 8% and OTU 71 – 2%) (Fig. 3.11; Appendix 7, Table 6.11).

Finally, Scrub 8 was composed of bacteria belonging to three phyla: *Actinobacteria* (1%), *Firmicutes* (49%), and *Proteobacteria* (48%). Similarly to all scrubs but Scrub 3, *Clostridium sensu stricto* was the main genus identified (44%). *Curvibacter* (16%), Sphingomonas (7%), *Morganella* (7%), *Proteus* (6%), *Pelomonas* (4%), *Hydrogenophilus* (2%), and *Herminiimonas* (1%) have also been identified (Fig. 3.11; Appendix 7, Table 6.11).


A)
Actinobacteria
Bacteroidetes
Chloroflexi
Deinococcus-Thermus
Firmicutes
Planctomycetes
α-Proteobacteria
β-Proteobacteria
γ-Proteobacteria
δ-Proteobacteria
Hydrogenophilalia
(Proteobacteria)
Actinomycetales_unclassified
Dermabacteraceae
Microbacteriaceae
Micrococcaceae
Rubrobacteraceae
Bacteria_unclassified
Porphyromonadaceae
Chitinophagaceae
Chloroflexi_unclassified
Ktedonobacteraceae
Thermaceae
Bacillales_Incertae_Sedis_XII
Enterococcaceae
Clostridia_unclassified
Clostridiaceae
Clostridiales_unclassified
Veillonellaceae
Planctomycetaceae
Alphaproteobacteria_unclassified
Bradyrhizobiaceae
Brucellaceae
Methylobacteriaceae
Rhodobacteraceae
Sphingomonadaceae
Betaproteobacteria_unclassified
Alcaligenaceae
Burkholderiaceae
Comamonadaceae
Oxalobacteraceae
Rhodocyclaceae
Gammaproteobacteria_unclassified
Enterobacteriaceae
Morganellaceae
Coxiellaceae
Pseudomonadaceae
Myxococcales_unclassified
Hydrogenophilaceae
Scrub 1
Scrub 2
Scrub 3
Scrub 4
Scrub 5
Scrub 6
Scrub 7
Scrub 8
0.0
0.5
1.0
10
20
30
40
50
60
70
Proportion in each sample (%)

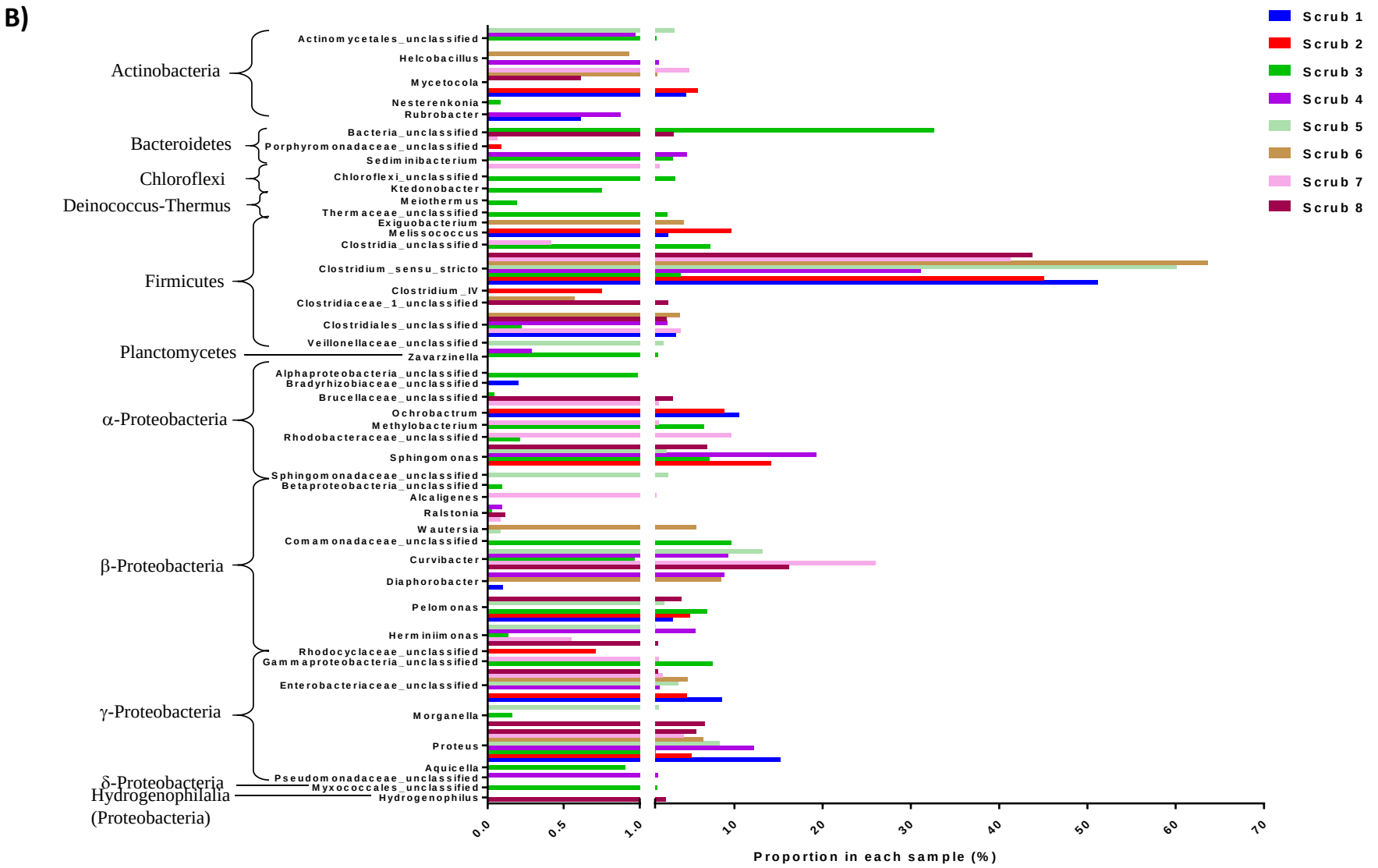



***Figure 3.11: Proportion of each OTU in the scrubs samples at the taxonomical level (A) family, and (B) genus.*** *A 0.016% cut-off was used.*

Several "Bacteria_unclassified" have been identified in Scrub 3. When manually aligning these OTUs' sequences with the nucleotide BLAST tool from NCBI, they were aligned with undescribed bacteria or with bacteria belonging to two different phyla. For example, OTU 16 presented a 100% coverage and 98-99% identity (with an E-value of 0.0) with one bacterium belonging to the *Acidobacteria* phylum and one bacterium belonging to the *Firmicutes* phylum (Fig. 3.12).

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|---|---|---|---|---|---|
| Uncultured Acidobacteria bacterium partial 16S rRNA gene, DGGE band 55 | 680 | 680 | 100% | 0.0 | 99% | HF546523.1 |
| Uncultured Firmicutes bacterium clone GASP-MB2W1_D08 16S ribosomal RNA gene, partial sequence | 658 | 658 | 100% | 0.0 | 99% | EF665320.1 |
| 16S rRNA amplicon fragment from a soil sample (ferralsol, Madagascar) resulting from a 16 days laboratory incubation experiment in the presence of 13C-er | 652 | 652 | 100% | 0.0 | 98% | FQ768488.1 |
| Uncultured Acidobacteria bacterium clone SEW_08_299 16S ribosomal RNA gene, partial sequence | 652 | 652 | 100% | 0.0 | 98% | HQ599002.1 |
| Uncultured bacterium clone Frankliniella occidentalis3 16S ribosomal RNA gene, partial sequence | 652 | 652 | 100% | 0.0 | 98% | JN793851.1 |
| Uncultured bacterium clone ADB-46 16S ribosomal RNA gene, partial sequence | 652 | 652 | 100% | 0.0 | 98% | HM366506.1 |
| Uncultured Firmicutes bacterium clone GASP-KC2S2_A06 16S ribosomal RNA gene, partial sequence | 652 | 652 | 100% | 0.0 | 98% | EU299693.1 |
| Uncultured bacterium clone OTU_3957 16S ribosomal RNA gene, partial sequence | 647 | 647 | 100% | 0.0 | 98% | MH315616.1 |
| 16S rRNA amplicon fragment from a soil sample (ferralsol, Madagascar) resulting from a 16 days laboratory incubation experiment in the presence of 13C-er | 647 | 647 | 100% | 0.0 | 98% | FQ767935.1 |
| Uncultured Acidobacteria bacterium clone A6YD14RM small subunit ribosomal RNA gene, partial sequence | 647 | 647 | 100% | 0.0 | 98% | FJ570274.1 |
| Uncultured Firmicutes bacterium clone GASP-KC2S2_E05 16S ribosomal RNA gene, partial sequence | 647 | 647 | 100% | 0.0 | 98% | EU299737.1 |

***Figure 3.12: Results of the BLAST query of OTU 16.*** *Nucleotide BLAST tool from NCBI was used for this alignment.*

### 3.2.3 Discussion

In all scrubs but Scrub 3, the genus *Clostridium sensu stricto* was the main genus identified (31 to 64%). Among the second main genera, *Sphingomonas*, *Curvibacter*, *Proteus*, and *Mycetocola* were identified. Some *Methylobacterium* were also identified in Scrub 3

The *Mycetocola* genus belongs to the *Actinobacteria* phylum. It is usually found on food (fruit, vegetables, cheese) [347]. To our knowledge it has never been described in similar environment. The *Sphingomonas* genus was identified in several SNFPs in Spain and in the USA after a cultivation step [185, 188]. One strain, isolated in the Antarctic Dry Valleys, exhibited in its genome the presence of oxidative stress resistance genes [306]. Another strain exhibited a high resistance to UV radiation with a 10% survival at a 1500 $J.m^{-2}$ UV dose [307]. The *Methylobacterium* genus is ubiquitous and can be found in aquatic environments [315]. Some strains have been shown to exhibit resistance to gamma irradiation 10–40 times higher than that exhibited by many other Gram-negative bacteria [315, 344, 345]. Several species can be radioresistant, such as *M. extorquens* and *M. radiotolerans* with a $D_{10}$ of 4.5 kGy and 3.5 kGy respectively [346]. This genus was found in SNFPs, in the water and the biofilms [184-186]. *Pelomonas* genus was identified via metagenetics in an American SNFP after a metagenetics analysis [193]. The genus *Curvibacter* was first identified in 2004 from well water [351]. A strain closely related to the *Curvibacter* genus was isolated from a SNFP in England after cultivation of the water in agar medium complemented with $CoCl_2$ and could resist cobalt toxicity up to 3 mM [352], while *E. coli* presented almost no growth at doses higher than 100 µM [353, 354]. This genus was found in scrubs 3 to 5 and scrubs 7 and 8. To our knowledge this is the first time this genus was found in a highly radioactive environment. The biofilms activity was in range of 4 to 80 Bq (Table 3.4) and was mainly due to the presence of $^{60}Co$. This bacterium can not only tolerate cobalt but can also survive high radiation levels. *Proteus* bacteria are facultative anaerobic. They can also produce an extracellular polysaccharide matrix. It was shown that the mean lethal dose (37% survival) of ionizing radiation was circa 100 Gy but some growth could be observed after a 2.4 kGy irradiation [355]. A *Proteus* strain could also resist the presence of $^{32}P$ in its culture up to a 8 µCi dose ($2.96x10^5$ Bq) [356]. The ability of *P. vulgaris* to adsorb cobalt ions on its membrane was pointed out in 1952 [357]. *Clostridium* are anaerobic *Firmicutes*

but some species can tolerate oxygen [358]. *C. subterminale* and *C. celerecrescens* had been found in a SNFP in the USA [188]. The other studies from other SNFPs were performed aerobically, explaining the absence of this genus in their studies. *Clostridium* spores were proved to resist a dose up to 8.5 kGy, and for some strain, up to 14 kGy [359, 360]. *Clostridium* cells can grow under a biofilm shape [361]. The radioresistance of spores may indicate that *Clostridium* cells were under spore shape in the biofilm. However *Clostridum* cells may have been under vegetative and spore shape in the biofilm, with spores being present in higher quantity than the vegetative cells. The high number of spores could explain the inability to detect *Clostridium* (and *Firmicutes*) using metaproteomics. It was shown that some *Clostridium* strains were able to reduce uranium [362].

Growth under a biofilm form is known to be advantageous to microorganisms. The extracellular matrix could have protected the cells from radiation and radionuclides. Several identified bacteria might have been able to survive the hostile conditions of the pool because of the presence of biofilms.

The presence of biofilm composed by anaerobic microbial genera can pose some concerns concerning MIC in nuclear installations [23, 363, 364]. Corrosion on spent fuel bundles can lead to a leak of the cooling element and thus change the characteristics of the water. Moreover corrosion on the pool' walls might lead at long term to a leak if untreated and unnoticed. The detection of biofilms and anaerobic bacteria is thus an important information concerning the monitoring of the pool's integrity and controlled conditions maintaining.

The composition of biofilms was highly different from the water composition observed in this pool in both 2015 and 2017 (cf. Microbial composition of a nuclear reactor cooling pool during operation and at shutdown deciphered using multi meta-analysis). The water was mainly composed by *Methylobacterium* genus at the time of the biofilms' sampling, while scrubs were mainly composed by *Clostridium* genus and genera belonging to the *Proteobacteria* phylum. This study highlighted the microbiota differences observed in the pool depending on their living form (vegetative or spores and biofilms). However the low number of reads from the biofilms' samples (between 1959 and 4747 reads for scrubs 1, 2, 4, 5, 6, and 7, and 38984 reads for Scrub 3) compared to the number of reads in the water samples (3696 for one sample and 28977 to 127024 reads for the others) could explain the observed difference.

### 3.2.4 Conclusion

This work completes the previous description of biofilms present in SNFPs all over the world. Most of the previous studies were performed after culture [152, 177, 181, 183-188, 192] and only one used a metagenetic approach [193]. However it is the first time that biofilms from the cooling pool of a nuclear reactor were sampled and analyzed. Despite the stresses present in such environment (lack of nutrient, presence of radionuclides and radioactivity) microorganisms were able to grow. The presence of radioactive cobalt particles in the biofilm did not prevent microbial growth. One main difference between these results and the previous studies was the abundant presence of *Clostridium* (*Firmicutes*). The *Firmicutes* phylum was present from 11% to 72% in the biofilms (metagenetics). This phylum did not represent as much in the previous studies, whether they were after cultivation [152, 181, 183-187] or using a metagenetic approach [193]. It was only in Scrub 4 that *Proteobacteria* was present for more than half of the sample (metagenetics results).

The presence of biofilms in this pool highlighted the presence of microbial diversity present in the cooling water of a nuclear reactor. The microorganisms found in these biofilms were similar between each other with the notable exception of Scrub 3. All scrubs were radioactive due to the presence of cobalt particles. Despite this radioactivity life was able to thrive in this pool under biofilms shape. Regardless of the potentiality of radioresistance research on these strains, the presence of biofilms and anaerobic bacteria could be a major concern for the pool's integrity and the physico-chemical changes possible. A deep cleaning of the pool or a monitoring of the cell density should thus be regularly performed in every nuclear pool.

### 3.2.5 Acknowledgements

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## 3.3 Direct meta-analyses reveal microbial diversity over space and time in a nuclear pool (*article in preparation*)

**Abstract**

Several studies exist about life found in spent nuclear fuel pools. However no data concerning the microbial diversity of the cooling pool used to store radioactive cobalt sources have been yet obtained. This work presents the first description of microbial diversity present in such environment. Using complementary methods (metagenetics and direct phylopeptidomics) several microorganisms have been identified in Pool 1, including bacteria, fungi, and micro-algae. The study over time of this pool highlighted a major microbial change when the pool's filtration system had been modified. A majority of *Cyanobacteria* was previously identified, replaced afterwards by *Limnobacter*. Different depths as well as different sampling locations have been studied for obtaining a comprehensive view of this specific infrastructure.

### 3.3.1 Material and Methods

#### *3.3.1.1 Sampling*

The pool is located in CEA-Saclay and is used to store $^{60}$Co sources. It is annually cleaned in December. A sampling bottle (Wildco®) was used to sample 1 L or 6 L of water (in November 2015 and March 2017 respectively). The recovered water was transferred in sterile bottles from VWR (PETG Media Bottle Sterile). The water was centrifuged at 15,000 *g* for 20 minutes at room temperature (RT). The supernatant was discarded and the microbial pellet resuspended in 1 mL of supernatant (in 2017). In 2015 800 mL were filtered through a 0.22 µm sterile hydrophilic polyethersulfone filters (MicroFunnelTM Supor®, Pall Corporation, USA) for DNA extraction. In 2017, 5 L were used for the metaproteomic analysis and 1 L for DNA extraction. In 2017, 5 L were centrifuged to be directly analyzed *via* metaproteomics and the liter left was centrifuged for DNA extraction. In June 2016, 2 L were sampled in the middle of the pool, near the surface (at a 1 m depth) while 2 L were recovered for the samples collected near the northern and western walls, near the surface (at a 1 m depth) and near the bottom of the pool (at a 4 m depth). The 2 L were centrifuged and directly analyzed using metaproteomics.

In 2015 and 2016, the water was sampled in five different locations, four near the walls after rubbing the walls with a metallic broom to resuspend any possible biofilm present, and one at the middle of the pool: the bottom and top of the northern wall (samples A and B respectively) and the bottom and the top of the western wall (samples C and D respectively) and water collected from the middle of the pool near the surface (sample E). The top samplings were performed at a 1 meter depth while the bottom samplings were performed at a 5 m 50 depth. The sample from the middle of the pool was performed at a depth of 1 meter. The mix water plus particles was recovered and analyzed using the same procedure than water samplings. Scrubs of the walls (A, B, C, and D) were also performed using sterile Spontex® sponges in 2016.

In 2017 the water was sampled in 6 different locations (A, B, C, D, E, and F corresponding to the sampling performed at the bottom of the middle of the pool) without rubbing of the walls. Scrubs on location A, B, C, and D, and the top and bottom corner between the western and the northern wall were performed in duplicate with sterile Spontex® sponges.

#### *3.3.1.2 DNA extraction*

The DNA was extracted with a phenol-chloroform method, adapted from Vilchez-Vargas et al. [276]. The samples were centrifuged 20 minutes at 20,000 *g* at room temperature and the supernatant discarded. One milliliter of lysis buffer (100 mM Tris pH 8; 100 mM EDTA pH 8; 100 mM NaCl; 1% polyvinylpyrrolidone; 2% SDS) was added and the pellet resuspend. The mixture was then transferred to a glass bead (0.1 mm) tube from Mobio (USA). The tube was disrupted three times at 30 Hz for 60 seconds with 30 seconds break between each run with a disruptor MM 300 from Retsch/Qiagen. The tube was then centrifuged 5 minutes at 20,000 *g* at room temperature and the supernatant removed and transferred into a new tube. One milliliter of phenol-chloroform-isoamyl alcohol (25:24:1) was added. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered and transferred into a new tube after a centrifugation step of 1 minute at 17,000 *g* at 4°C. Seven hundred microliters of chloroform were added to the sample. The sample was then vortexed and vigorously mixed by inverting the tube. The supernatant was recovered after a centrifugation step of 1 minute at 17,000 *g* at 4°C. The supernatant was divided into two tubes (around 450 µL in each) and 45 µL of sodium acetate 3M and 500 µL of isopropyl alcohol were added into each tube. The samples were then mixed by inverting the tubes and put overnight at -20°C. The tubes were centrifuged 30 minutes at 17,000 *g* at 4°C and the supernatant removed. Two steps of washing were performed by adding 750 µL of 75% ethanol and a centrifugation step of 5 minutes at 17,000 *g* at 4°C. The supernatant was removed and the pellet dried with a heating block at 55°C. Sixty microliters of sterile milliQ water were added to dissolve the pellet. To have a more pure DNA and remove any trace of alcohol, the sample was put on a 0.05 µm filter disposed on sterile water for 15 minutes before being transferred into a new tube.

#### *3.3.1.3 Sequencing and data analysis*

The sequencing of the genomic DNA on 16S rDNA was performed by LGC Genomics. The sequencing was conducted as followed. The PCRs included about 1-10 ng of DNA extract (total volume 1 µL), 15 pmol of each forward primer and reverse primer (in 20 µL volume of 1 x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 µL of BioStabII PCR Enhancer (Sigma) and additionally 0.2 µl of DNase (Artic Zymes) for the 16S PCR. For each sample, the forward and reverse primers had the same 10-nt barcode sequence. Each PCRs were carried out for 20 cycles, dependent on the primer pair, using the following parameters: 1 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s and primers without inline barcodes have been used (341F/1061R). For the second round 1 µL PCR product from the first round was used and the PCR conditions were the same as before. For this reaction barcoded primers have been added to amplify the V4V5 regions (515YF-926R: 5'-GTGYCAGCMGCCGCGGTAA-3' and 5'-CCGYCAATTYMTTTRAGTTT-3' respectively). DNA concentration of amplicons of interest was determined by gel electrophoresis. About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying different barcodes. The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification on MiniElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina). The raw data obtained after sequencing were analyzed using the OCToPUS software [210]. This software eliminated any singleton sequences, chimeras, clustered according to a threshold

similar sequences in OTUs, and assigned each OTU to a taxonomical level using the RDP database (Ribosomal Database Project).

#### *3.3.1.4 Direct metaproteomics: sample preparation and analysis*

The metaproteomics analysis was done as previously described (cf. Taxonomical composition of a biofilm sampled from a nuclear fuel pool assessed by metaproteomics). Briefly, samples were thawed on ice, and a volume of 57 µL of Laemmli buffer LDS1X (Invitrogen) was added per mg of wet bacterial pellet as described by Hartmann et *al* [295]. After denaturation for 5 min at 99°C, the protein sample was loaded onto a 4–12% gradient 10-well NuPAGE gel (Invitrogen). The proteins were subjected to electrophoresis for 5 min and then stained with Coomassie Blue Safe staining (Invitrogen) as recommended by the supplier. The polyacrylamide band corresponding to the whole proteome was sliced, then unstained with ultra-pure water, reduced with dithiothreitol, and treated with iodoacetamide. It was then subjected to proteolysis with Trypsin Gold Mass Spectrometry Grade (Promega) in the presence of 0.01% ProteaseMAX surfactant (Promega), as previously described [322]. The resulting peptides were analyzed with an ESI-Q Exactive HF mass spectrometer (Thermo) equipped with an ultra-high field Orbitrap analyser and coupled to an Ultimate 3000 176 RSL Nano LC System (ThermoFisher). A volume of 10 µL of peptides was injected onto a reverse phase Acclaim PepMap 100 C18 column (3 µm, 100 Å, 75 µm id x 500 mm) and resolved at a flow rate of 0.2 µL/min with a 60 min gradient of CH3CN in presence of 0.1% formic acid. The Q-Exactive HF instrument was operated with a Top20 strategy with only ions with potential charge states of 2+ and 3+ selected for MS/MS and with a dynamic exclusion of 10 sec. The proteotyping interpretation of MS/MS spectra was done with the National Center for Biotechnology Information non-redundant fasta file (NCBI-nr) database with the Mascot search engine (Matrix science). Peptide -to-MS/MS spectrum assignation was done with full trypsin specificity, maximum of one missed cleavage, mass tolerances of 5 ppm on the parent ion and 0.02 Da on the MS/MS, static modification of carboxyamidomethylated cysteine (+57.0215), and oxidized methionine (+15.9949) as dynamic modification. Post-processing of Mascot dat files was done as previously indicated (cf. Taxonomical composition of a biofilm sampled from a nuclear fuel pool assessed by metaproteomics).

### 3.3.2 Results

#### *3.3.2.1 Metagenetic analysis of water and biofilm samples during the 2015 sampling campaign*

The 2015 samplings were performed on Location E and on locations A, B, C, and D after rubbing the walls. The analyzed samples thus contained the microbiota present in the water and the one present on the walls, except for Location E.

The evenness of the samples was quantified using the Shannon index. The evenness indicates whether the diversity is well divided between the different OTUs or if one OTU dominates the sample. This index combines species richness and abundance into a single value of evenness [339]. When this index is close to 0 a single species dominates the sample. The samples presenting the greater evenness between the different genera were sample E and sample D filter (2.58 and 2.67 respectively). The samples collected at the bottom of the western wall as well as the scrub from the bottom northern wall presented the smaller evenness (0.47 to 0.63) (Table 3.5). A total of 12 phyla were identified in the water and the scrubs in 2015.

***Table 3.6: Shannon index of the 2015 samplings.*** *When this index is close to 0, one species dominates the sample.*

| Sample | Shannon index |
|---|---|
| **Sample E filter** | 2.58 |
| **Sample A scrub** | 0.63 |
| **Sample B filter** | 2.25 |
| **Sample B scrub 1** | 1.67 |
| **Sample B scrub 2** | 1.47 |
| **Sample C filter** | 0.57 |
| **Sample C scrub** | 0.47 |
| **Sample D filter** | 2.67 |
| **Sample D scrub 1** | 0.89 |
| **Sample D scrub 2** | 2.45 |

21 OTUs were identified in the scrub from Sample A. 125, 36, and 18 OTUs were identified in samples B filter, scrub 1 and scrub 2 respectively. Only 19 and 6 OTUs were identified in sample C filter and scrub respectively, while 106, 16, and 99 OTUs were identified in samples D filter, scrub 1 and scrub 2 respectively. Finally, 43 OTUs were present in sample E filter.

The diversity was reached quickly for all samples, except for sample B filter, which exhibited a greater diversity and reached a plateau above 250000 reads and at about 350 OTUs. The other samples reached a plateau faster, below 50000 reads. For sample D filter and sample D scrub 2 the plateau was reached at about 100 OTUs while the other samples reached the plateau below 50 OTUs (cf. Appendix 10, Fig. 6.14). This indicates that a comprehensive analysis was obtained with these data.

In the sample collected from the middle of the pool some bacterial diversity can be observed (Fig. 3.13). A majority of *Cyanobacteria* belonging mostly to the *Bacillariophyta* and also to *Streptophyta* families was identified (49.4% of the identified sequences; Appendix 8, Table 6.13). The evenness between the families and genera was greater than the one observed in 3.2- Microbial composition of a nuclear reactor cooling pool during operation and at shutdown deciphered using multi meta-analysis. Bacteria belonging to 7 phyla were identified (*Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Nitrospirae*, *Planctomycetes*, and *Proteobacteria*). In this sample the *Proteobacteria* phylum does not represent the majority of the microbiota.

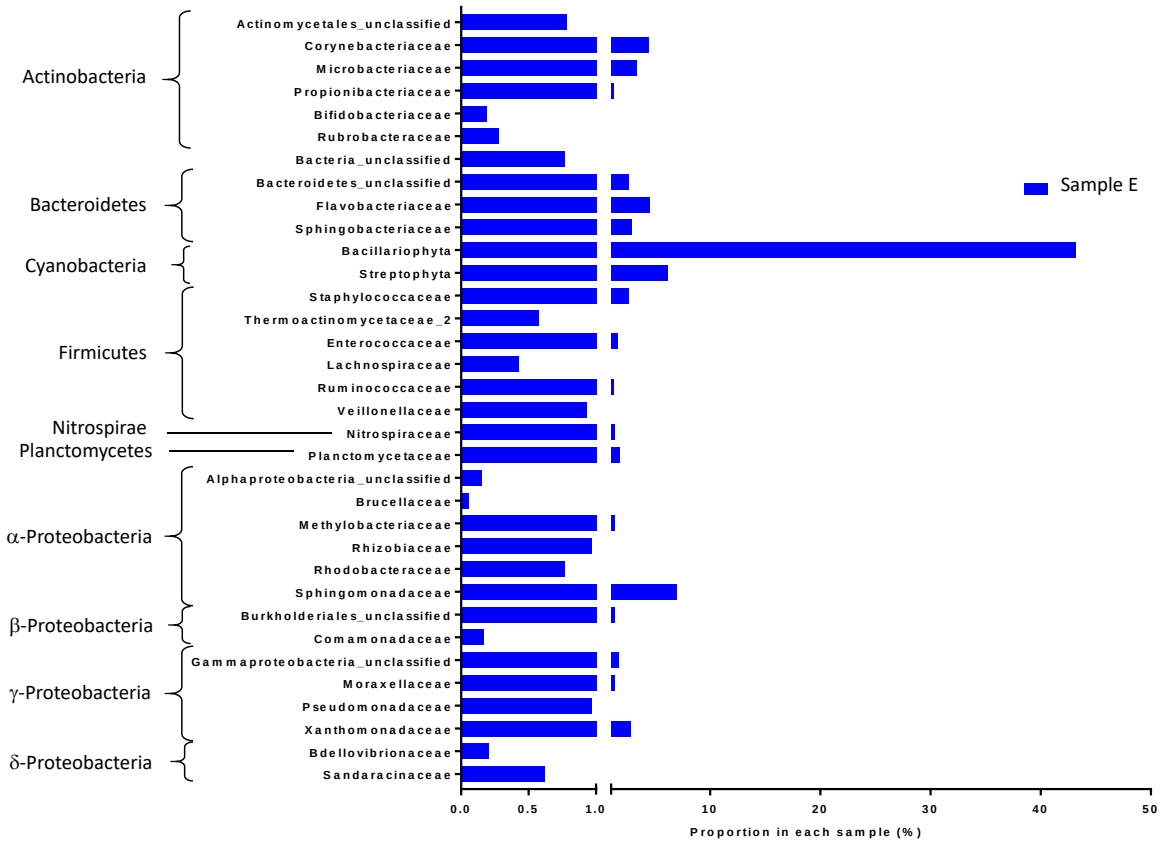



***Figure 3.13: Diversity analyzed at the family level from the water sample E after filtration using metagenetics.*** *A 0.016% cut-off was used.*

The sequencing of the nucleic acid extracted from water filtered after rubbing the Location A did not work and no sequences were obtained. No data are thus available for this sample. A majority of *Sphingomonadaceae* were observed in the scrub from Location A (87.8%). Only 1.6% of the analyzed sequences belonged to the *Streptophyta* family. Five phyla were identified in this sample with the *Proteobacteria* being the most represented. The four other phyla were the *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Firmicutes*. The evenness in this sample was lower than in the water sampled in the middle of the pool. The *Microbacteriaceae* (with the genus *Microbacterium*) was identified at a 4.6% proportion in the sample and *Sphingobacteriaceae* (with the genus *Mucilaginibacter*) was present at 2.4% (Fig. 3.14; Appendix 8, Table 6.13 and Fig. 6.8).

The water recovered after rubbing Location B was more diverse than the scrubs performed at the same location (47 families and 57 genera for the water *vs.* 14-21 families and 16-24 genera for the scrubs; Appendix 8, Table 6.13). In the water 59.4% of the analyzed sequences belonged to the *Bacillariophyta* family while only 2.9 and 3.4% were attributed to this family in scrub 1 and 2 respectively. The evenness in the water was smaller than in the scrubs from the same location. The other main families and genera in the water were *Sphingomonadaceae* (6% with 2.5% of *Blastomonas*), *Saprospiraceae* (4.7%), *Nitrospiraceae* (with the genus *Nitrospira*, 3.5%), *Rhodobacteraceae* (3.5% with 3.2% of *Rhodobacter*) and *Planctomycetaceae* (2.2%) (Fig. 3.14; Appendix 8, Table 3.13 and Fig. 6.8).

The biofilms recovered through scrubs at Location B were not similar in composition. In Scrub 1 a majority of *Sphingomonadaceae* (49.9% with 47.8% of *Sphingomonas*) was observed while only 12.6% of this family

(and genus) were identified in Scrub 2. Likewise 49.1% of bacteria belonging to the *Caulobacteraceae* (all identified as *Brevundimonas*) were found in Scrub 2 when only 0.02% of this family (and genus) were detected in Scrub 1. The *Microbacteriaceae* family represented 21.7 and 17.9% of scrubs 1 and 2 respectively. However while this family only includes the *Microbacterium* genus in Scrub 2, 16.6% could not be identified at the genus level (after nucleotide BLAST, several genera were possible for this OTU) in Scrub 1. The 5% left were associated with the *Microbacterium* genus. The *Methylobacteriaceae* family, with the *Methylobacterium* genus, represented 18.8 and 14.9% of scrubs 1 and 2 respectively. Finally the family *Bradyrhizobiaceae* was identified at 7.3 and 1.2% with the genus *Afipia* constituting 7.3 and 1.1% of scrubs 1 and 2 respectively (Fig. 3.14; Appendix 8, Table 6.13 and Fig. 6.8).

A total of nine phyla were identified at the northern wall, all depths and sampling method taken together.

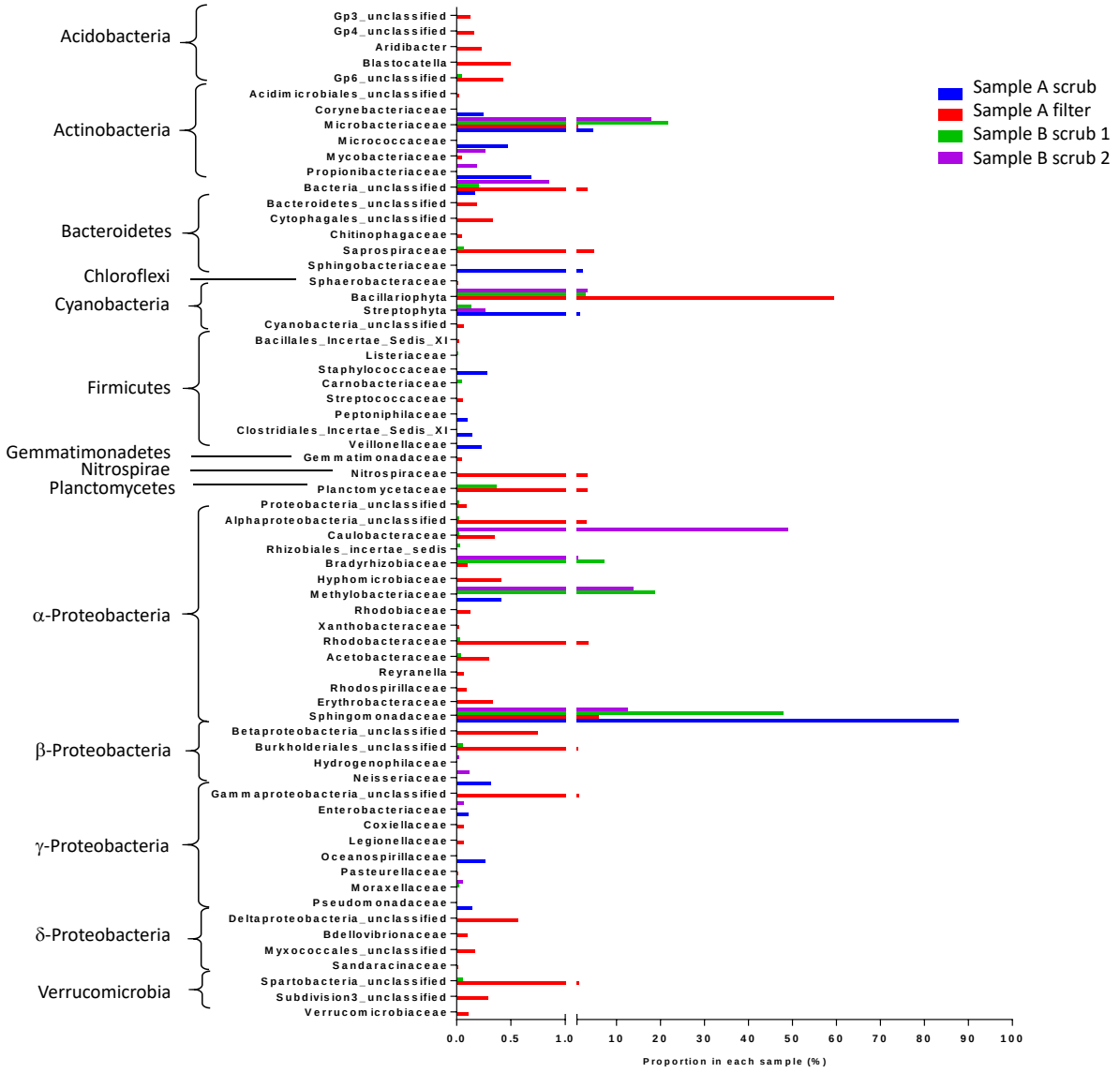



***Figure 3.14: Diversity analyzed at the family level from the samples collected at the northern wall using metagenetics.*** *A 0.016% cut-off was used.*

At the bottom of the western wall, the diversity was smaller compared to the other location of the pool. Only 28 genera were identified at Location C. The diversity of the scrub was lower than the scrub from the bottom northern wall (6 genera for Sample C biofilm *vs.* 18 genera for Sample A biofilm) (Appendix 8, Table 6.13). On both samples from the bottom western wall the evenness was low, with the *Sphingomonadaceae* family representing 1.7% of the filtered water and 85.5% of the scrub. In the filtered water a majority of *Commamonadaceae* was identified (87.6%) while no sequence belonging to this family was identified in the scrub (Fig. 3.15; Appendix 8, Table 6.13). Interestingly, the genus from the *Commamonadaceae* family was not identified but after alignment it appeared to be exactly the same sequence as OTU 2 from Pool 2 in 2015 (Fig. 3.16). In the water 7.6% of *Caulobacteraceae* (namely *Brevundimonas*), 2% of *Methylobacteriaceae* (*Methylobacterium*), and 1.7% *Sphingomonadaceae* (*Blastomonas*) were identified. In the scrub, the genus identified belonging to the *Sphingomonadaceae* was not *Blastomonas* but *Sphingomonas* (Appendix 8, Table 6.13 and Fig. 6.9). The other main genus from the scrub was *Stenotrophomonas* (belonging to the *Xanthomonadaceae*) and represented up to 13.4% of the scrub.

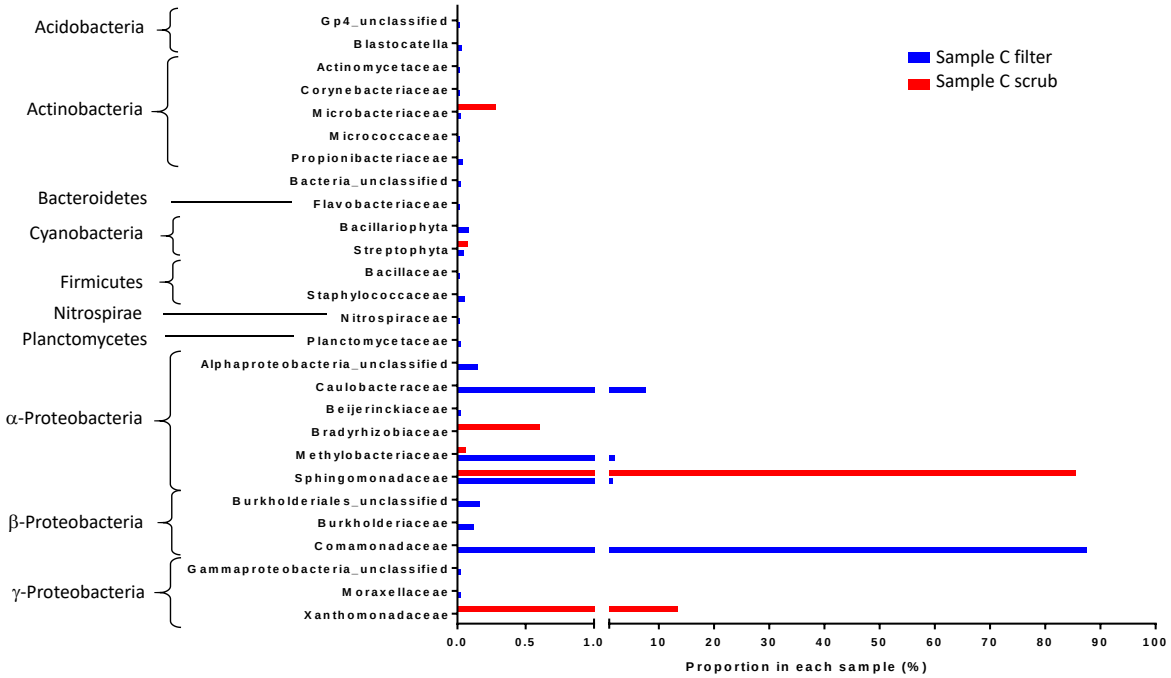



***Figure 3.15: Diversity analyzed at the family level from the samples collected at the bottom western wall using metagenetics.*** *A 0.016% cut-off was used.*

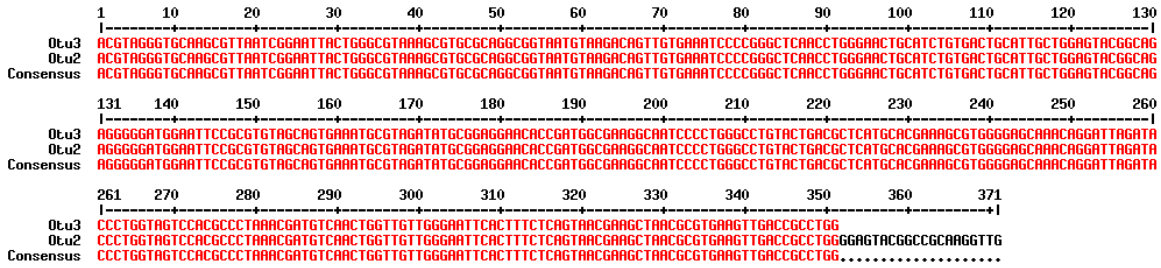



***Figure 3.16: Sequence homology between OTU 3 from Pool 1 in 2015 and OTU 2 from Pool 2 in 2015.*** *Alignment realized using MultAlin, INRA.*

The last location analyzed was the top western wall. In this analysis an important diversity was observed in the scrubs and in the filtered water (15 genera in Scrub 1, 51 genera in Scrub 2, and 64 genera in the water) (Appendix 8, Table 6.13). A total of 8 bacterial phyla were identified at this location.

In the water the phylum *Cyanobacteria* represented 30.8% of the analyzed sequences. 30.6% of the analyzed sequences were attributed to the *Bacillariophyta* family. The 0.2% left were attributed to the *Streptophyta* family (Fig. 3.17; Appendix 8, Table 6.13). The genera belonging to these two families were not identified (Appendix 8, Table 6.13, Fig. 6.10). In the water sample 22.6% of the sequences were identified as belonging to the *Burkholderiales* order but neither the family nor the genus were identified (Fig. 3.17; Appendix 8, Table 6.13). More than 4% of the sequences identified from the sample D filtered water were associated with the *Blastocatella* family (*Acidobacteria* phylum). In this sample the *Rhodobacteraceae* family represented almost 7% of the sequences. In this family, 3.1% belonged to the *Rhodobacter* and 3.9% were not identified at the genus level but belonged to the same OTU (Fig. 3.17; Appendix 8, Table 6.13, Fig. 6.10). The genera *Blastomonas* and *Sphingomonas* were also present at a proportion of 2.6 and 4.4% respectively.

The scrubs 1 and 2 were, as scrubs 1 and 2 from the sample B location, not similar in composition. In Scrub 1, 48.5% of the analyzed sequences were attributed to the *Methylobacteriaceae* family and more specifically to *Methylobacterium*, while this genus represented 18.2% of Scrub 2. The other main family of Scrub 1 was *Sphingomonadaceae* and more specifically *Sphingomonas* (49.2%) when this family (and genus) represented only 6.7% of Scrub 2. More diversity was observed in Scrub 2. The family *Bacillariophyta* displayed 36.5% of the sequences. The family *Caulobacteraceae* (composed by the genus *Brevundimonas*) represented 11.1% of the sample. In this scrub 8.7% of the OTU were attributed to the *Sphingomonadaceae* family, composed at 6.7% by the genus *Sphingomonas*. Finally, *Microbacterium* represented 3.6% of the sequences in this scrub (Fig. 3.17; Appendix 8, Table 6.13, Fig. 6.10).

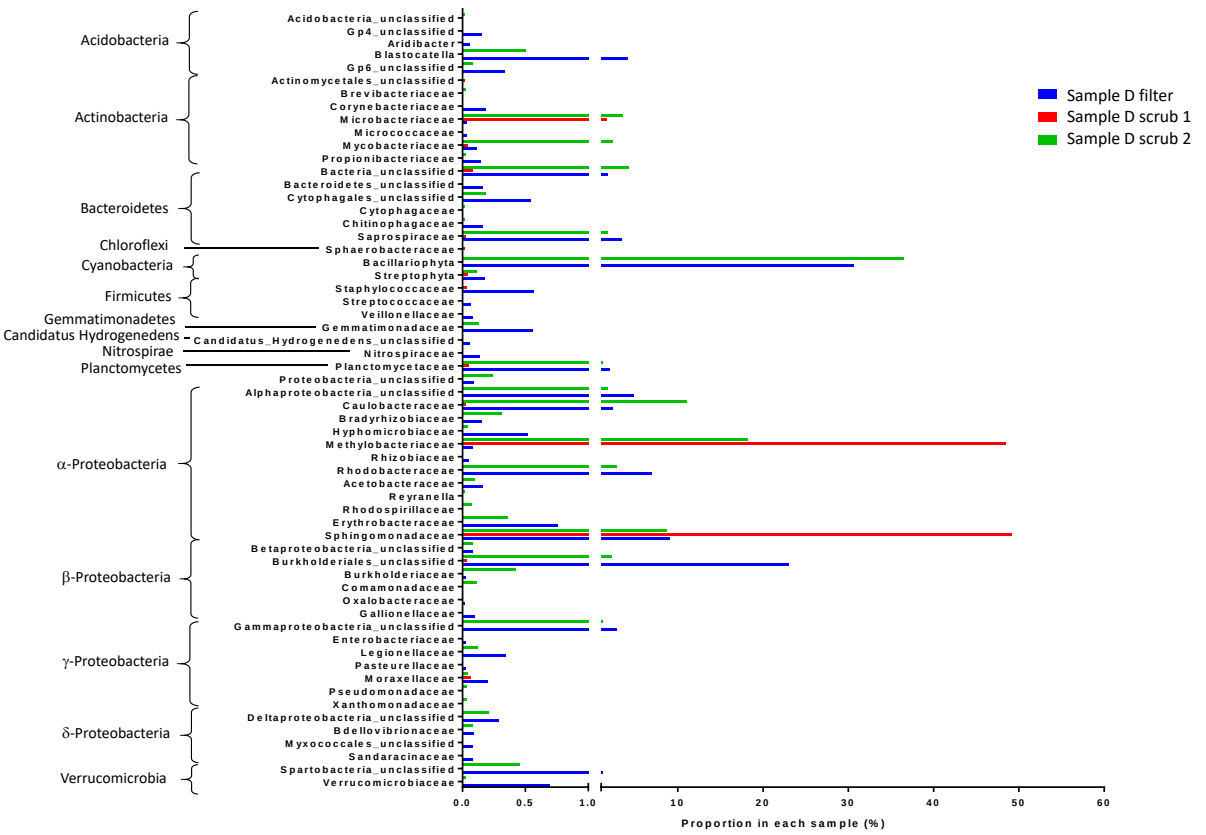



***Figure 3.17: Diversity observed at the family level from the samples collected at the top western wall using metagenetics.*** *A 0.016% cut-off was used.*

Despite differences observed between the water composition in the middle of the pool, near the walls after rubbing of the walls and the scrubs, several families and genera were found in almost all samples, such as *Microbacterium* (only absent from sample E), *Sphingomonas* (only present at 0.003% in the sample D filter and beyond the cut-off), and the phylum *Cyanobacteria* with the *Bacillariophyta* and *Streptophyta* families.

### *3.3.2.2 Metagenetic analysis of water and biofilm samples during the 2017 sampling campaign*

A total of 55 OTUs were identified in 2017 in sample A. In Sample B, 20 OTUs were identified while 58, 43, 15, and 13 OTUs were present in samples C, D, E, and F respectively. The evenness in the 2017 water samples was important for all samples but one. Sample F had indeed a Shannon index of 0.69 (Table 3.7). This sample, containing 13 OTUs (cf. Appendix 9), was thus dominated by one or two species.

***Table 3.7: Shannon index of the 2017 samplings.*** *When this index is close to 0, one species dominates the sample.*

| Sample | Shannon index |
|---|---|
| **Sample A** | 1.75 |
| **Sample B** | 1.25 |
| **Sample C** | 1.60 |
| **Sample D** | 1.58 |
| **Sample E** | 1.5 |
| **Sample F** | 0.69 |

A comparison of the diversity in the samples was performed using rarefaction curves (cf. Appendix 10). The maximum diversity plateau was reached quickly for all samples, except for sample B filter 2015, which exhibited a greater diversity and reached a plateau above 250000 reads and at about 350 OTUs. The other samples reached a plateau faster, below 50000 reads. Both samples E (filtration from 2015 and centrifugation from 2017) started to reach a plateau below 2000 reads and between 15 to 40 OTUs.

In 2017, after a change in the water filtration and conditions, six samplings were performed. Some differences between the top and the bottom samplings can be observed for all locations in the pool (Fig. 3.18 to 3.20; Appendix 9, Table 6.14, Fig. 6.11 to 3.13).

In the water collected from the middle of the pool, 20 genera were identified, belonging to 3 different phyla (*Actinobacteria*, *Firmicutes* and *Proteobacteria*). At both depths, the major family was *Burkholdericeae* at 76.5 and 60.2% for samples F and E respectively. The genus *Limnobacter* was the only genus from this family present in these samples. From Sample F the other main family present was *Sinobacteraceae* (and the genus *Nevskia*) at 20.5%. The last genus present at more than 1% in this sample was *Porphyrobacter*, from the *Erythrobacteraceae* family (1.5%). The evenness from sample E was greater (1.38 *vs.* 0.67 for sample F; Appendix 9 Table 6.14). The genus *Limnobacter* was present at 60.2% while bacteria belonging to *Clostridiaceae* represented 19.3% of the microbiota. Only the genus *Clostridium* was identified in this family. Two other *Firmicutes* were identified: *Staphylococcus* and *Enterococcus* (2.4 and 4.4% respectively). Finally two *γ-Proteobacteria* were also identified, belonging to the *Enterobacteriaceae* (*Proteus* genus) and *Pseudomonadaceae* (*Pseudomonas* genus) families (4 and 2.3% respectively). The water collected in the middle of the pool presented a low diversity (20 genera) (Fig. 3.18; Appendix 9, Table 6.14, Fig. 6.11).

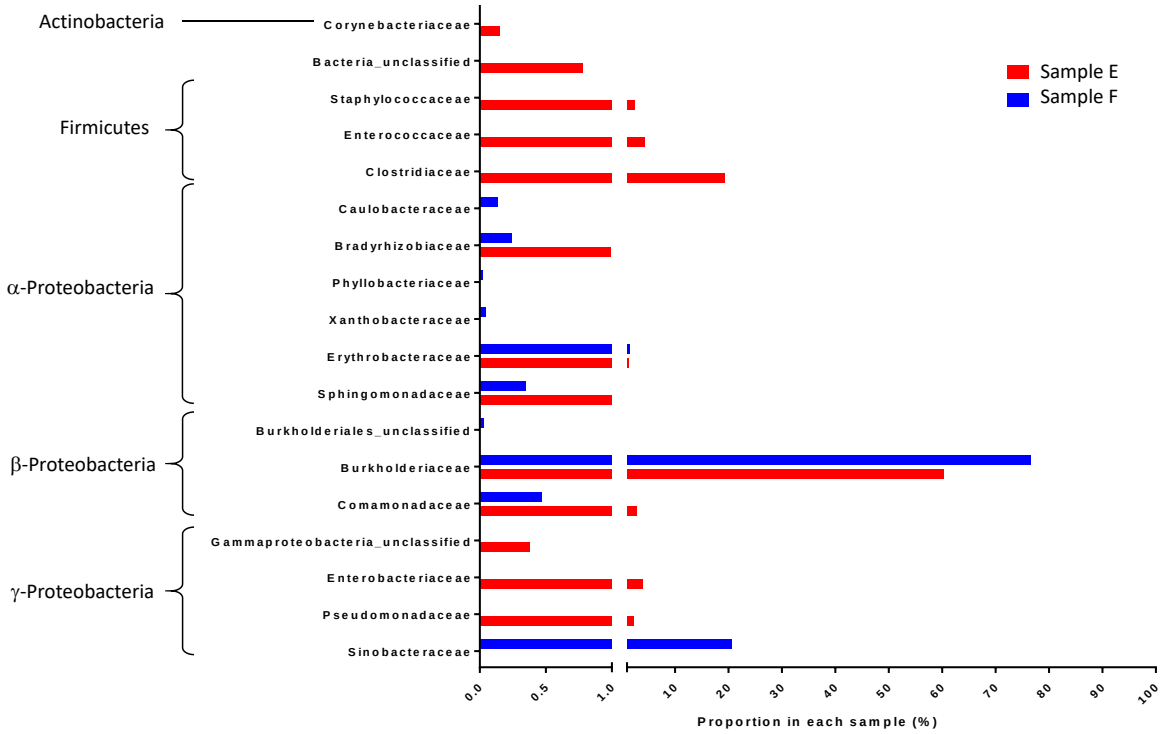



***Figure 3.18: Diversity observed at the family level from the samples collected at the middle of the pool using metagenetics in 2017.*** *A 0.016% cut-off was used.*

The water collected near the northern wall presented a higher diversity than the water from the middle of the pool (38 genera). A majority of *Burkholderiaceae* was identified in the sample B (76.5% of *Limnobacter*) while this family (and genus) represented only 21.3% of sample A. The families *Caulobacteraceae* (10.9 and 5.3% for samples A and B respectively) and *Sinobacteraceae* (5.7 and 2.4% for samples A and B respectively) were also represented in both depths with the genera *Brevundimonas* (10.9 and 5.3% for samples A and B respectively) and *Nevskia* (2.1 and 2.4% for samples A and B respectively). At the bottom northern wall the family *Erythrobacteraceae* (with the genus *Porphyrobacter*) represented 22.1% of the sample while this genus was present at only 1.3% in sample B. At the sample B location the family *Bacillariophyta* (2%) was identified (Fig. 3.19; Appendix 9, Table 6.14, Fig. 6.12).

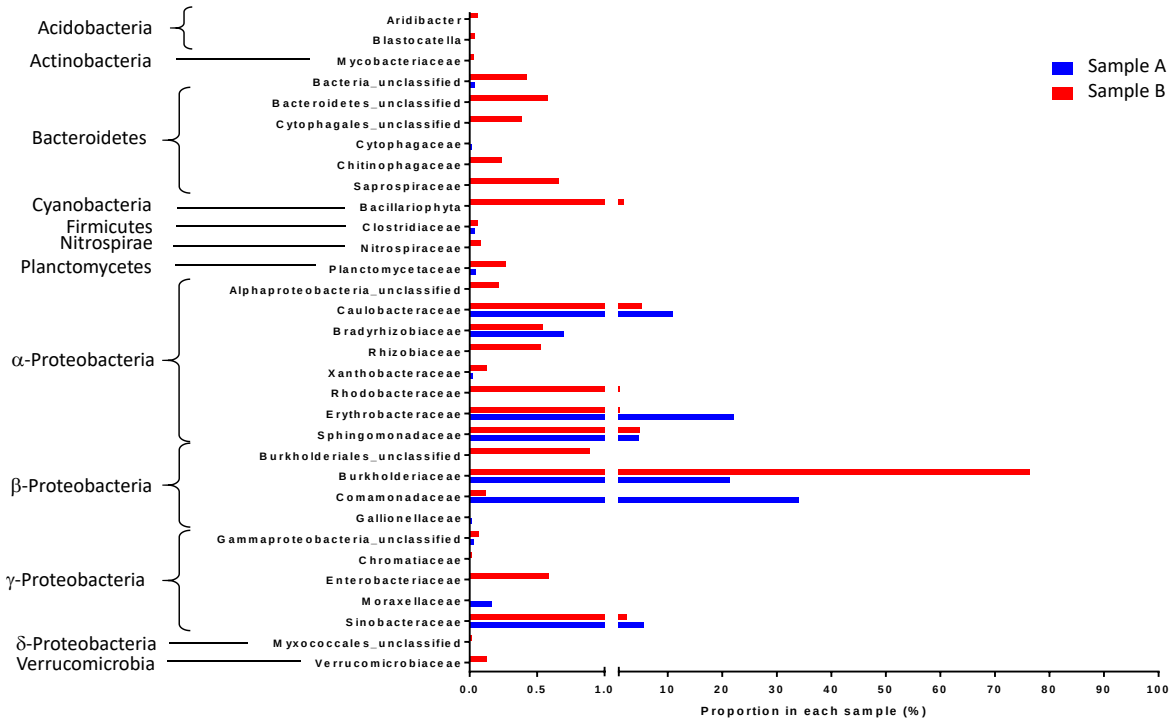



***Figure 3.19: Diversity observed at the family level from the samples collected at the northern wall using metagenetics in 2017.*** *A 0.016% cut-off was used.*

The diversity present near the western wall was the greater from all locations analyzed in 2017 (60 genera identified in the samples C and D). The main family at both depths was *Burkholderiaceae* (65.1 and 67.8% for samples C and D respectively). The genus *Limnobacter* was the only one present in this family in samples C while *Ralstonia* was also present (0.02%) in samples D along with *Limnobacter* (67.8%). The family *Erythrobacteraceae* was also identified at both depths (7% and 6.6% for samples C and D respectively), in which the genus *Porphyrobacter* represented 7 and 6.6%. The genus *Brevundimonas* was present at 3.7% in samples C *vs.* 1.9% in sample D. *Clostridium* represented 3.4% of sample D while was present at only 0.05% in sample C. In these samples the *Bacillariophyta* family was also present (0.03 and 0.4% in samples C and D respectively) (Fig. 3.20; Appendix 9, Table 6.14, Fig. 6.13). Fewer differences between the two depths at the western location were observed compared with the other two locations studied (northern wall and middle of the pool).

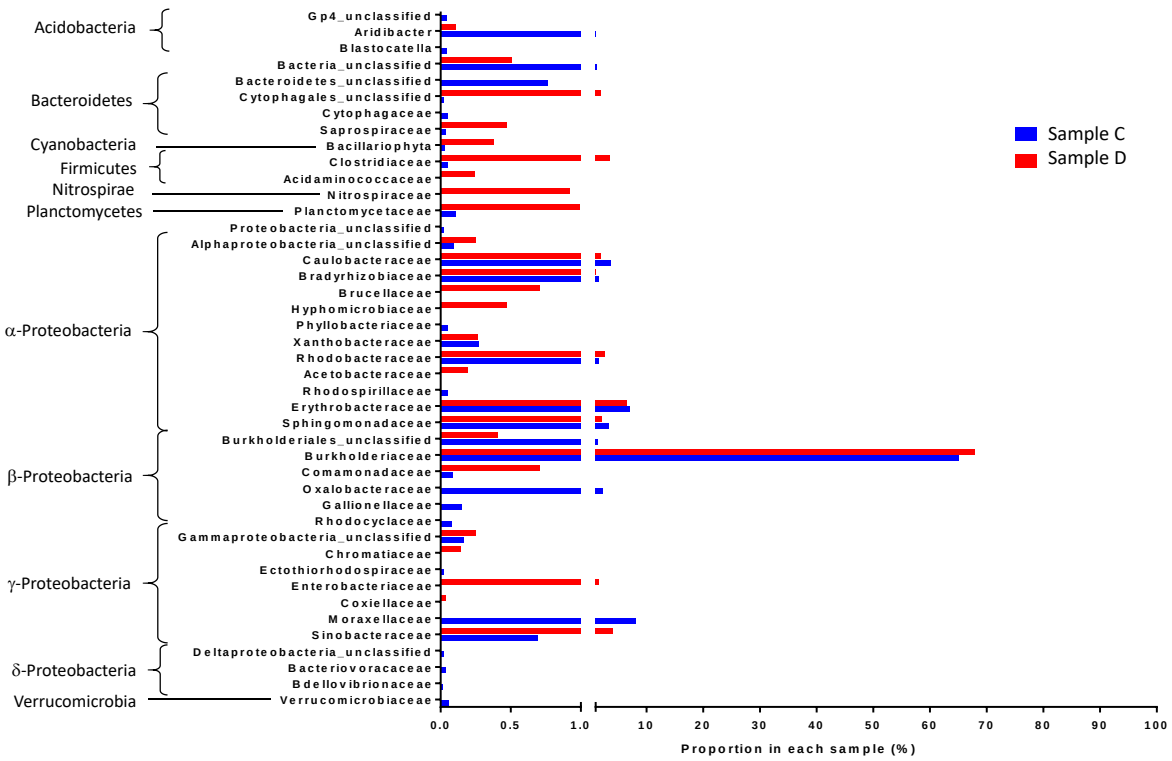



***Figure 3.20: Diversity observed at the family level from the samples collected at the western wall using metagenetics in 2017.*** *A 0.016% cut-off was used.*

A total of 9 phyla were identified in the water of this pool in the 2017 samplings. The *Cyanobacteria* phylum was not identified in the middle of the pool (both depth) and the bottom of the western wall.

The comparison of the two sampling dates after a metagenetics analysis and a subsampling at 21570 sequences is presented in Appendix 11. A total of 47 genera were common between the 2015 and 2017 samplings, all locations taken together. Three phyla were not common to both sampling date: *Chloroflexi* (only present in 2015), *Hydrogenedentes* (only present in 2015), and "*Candidatus* division WPS-1" (only present in 2017). The 2015 sampling exhibited more diversity than the 2017 sampling (106 genera *vs.* 65 genera respectively), due to the fact that more samples were recovered and analyzed in 2015 as well as the fact that the pool was cleaned 3 months before the 2017 sampling (for the 2015 sampling, the pool was cleaned a year before).

#### *3.3.2.3 Direct metaproteomics analysis of water and biofilms during the 2016 campaign*

After the 2016 water sampling, the cell density was assessed for the five location samplings. The water recovered near the northern and western walls at both depths was recovered after rubbing the walls to resuspend potential biofilms. The location with the more cell density was at the bottom of the western wall (Table 3.8).

*Table 3.8: Cell count at the five different water sampling location from the 2016 sampling*

| Location | A | B | C | D | E |
|---|---|---|---|---|---|
| **Total cell count ($10^6$ cells/L)** | 2.0 ± 1.2 | 2.0 ± 1.6 | 4.5 ± 0.5 | 2.0 ± 0.9 | 2.0 ± 0.8 |

Despite low material quantity (about 4 to 9 million cells in the 2 L samplings), a signal was detectable using direct metaproteomics in the water. As observed in Figure 3.21, the presence of some detectable families and genera highlighted the potential of proteotyping by direct metaproteomics. In these samples, no microorganisms were detectable at Location A. The family *Moraxellaceae* (with *Acinetobacter*) was the only microbial species detected at the top northern wall. The other three locations exhibited a higher diversity, with the presence of not only bacteria (*Proteobacteria*, *Firmicutes* and *Cyanobacteria*) but also 2 algae closely related to *Phaeodactylum* and *Thalassiosira*. With this analysis the proportion of algae and bacteria cannot be determined precisely in terms of cfu since algae cells are much bigger than bacterial cells and possess thus more proteins. However the algae related to *Phaedoactylum* was present in the water plus particle mix from the western wall at both depths as well as in the water collected at near the surface of the middle of the pool. The algae related to *Thalassiosira* was detected only at the top western wall sample. As shown in Figure 3.21 the biomass contribution assessed by metaproteomics is precise and indicates that for samples C, D, and E, 50% or more of the collected biomass is due to green algae. Another photosynthetic organism was also spotted in the water of this pool. This organism, named *Scytonema*, belongs to the *Cyanobacteria* phylum and was detected at both sampling depths near the western wall. The genus *Acidovorax* was detected in three locations (C, D, and E). No fungi were detected using direct metaproteomics in 2016 (Fig. 3.21). The diversity near the top western wall seemed to be higher compared to the other locations and depths (8 genera detected at samples D location), including 2 eukaryotes genera and one *Cyanobacteria* genus. Six genera were detected at the samples C location with three common genera with samples D location (*Acidovorax*, *Scytonema*, and *Phaeodatylum*). It was only at the sample C location that *Actinobacteria* were detected with the presence of *Rhodococcus* (Appendix 12, Table 6.15).

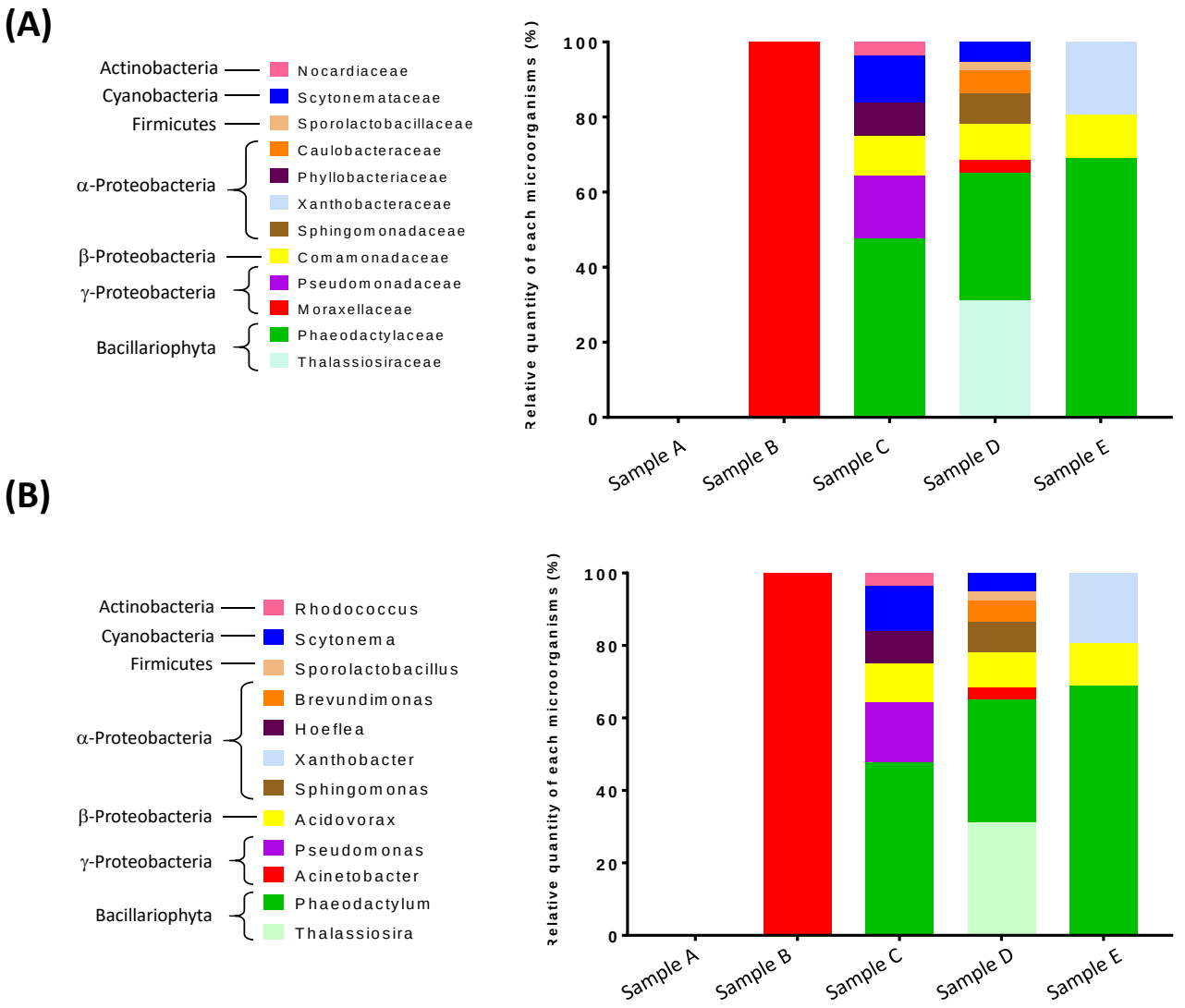



***Figure 3.21: Proportion of each genus from the water collected in 2016 before the change of filtration analyzed without cultivation step by metaproteomics****. For sample A, B, C, and D the water was recovered after rubbing the wall to resuspend potential biofilms. (A) Family level; (B) Genus level.*

Scrubs of the walls were also performed and analyzed using direct metaproteomics. The composition of the recovered biofilms was similar between all locations and depths. The *Sphingomonadaceae* and *Caulobacteraceae* families were found in each scrub. The *Sphingomonadaceae* was the main family detected for each sample (80.4 to 95.6%). The other family present in all samples was *Caulobacteraceae* (4.4 to 11%). The *Comamondaceae* family was not detected at the bottom of the western wall but was present in every other location (2.8 to 8.6%) (Fig. 3.22; Appendix 12, Table 6.16). Strangely, while the *Comamonadaceae* family (*Acidovorax* genus) was detected in the samples A, B, and D scrubs, it was only detected in the water plus particles mix collected near the western wall at both depths and was not identified near the northern wall using direct metaproteomics.

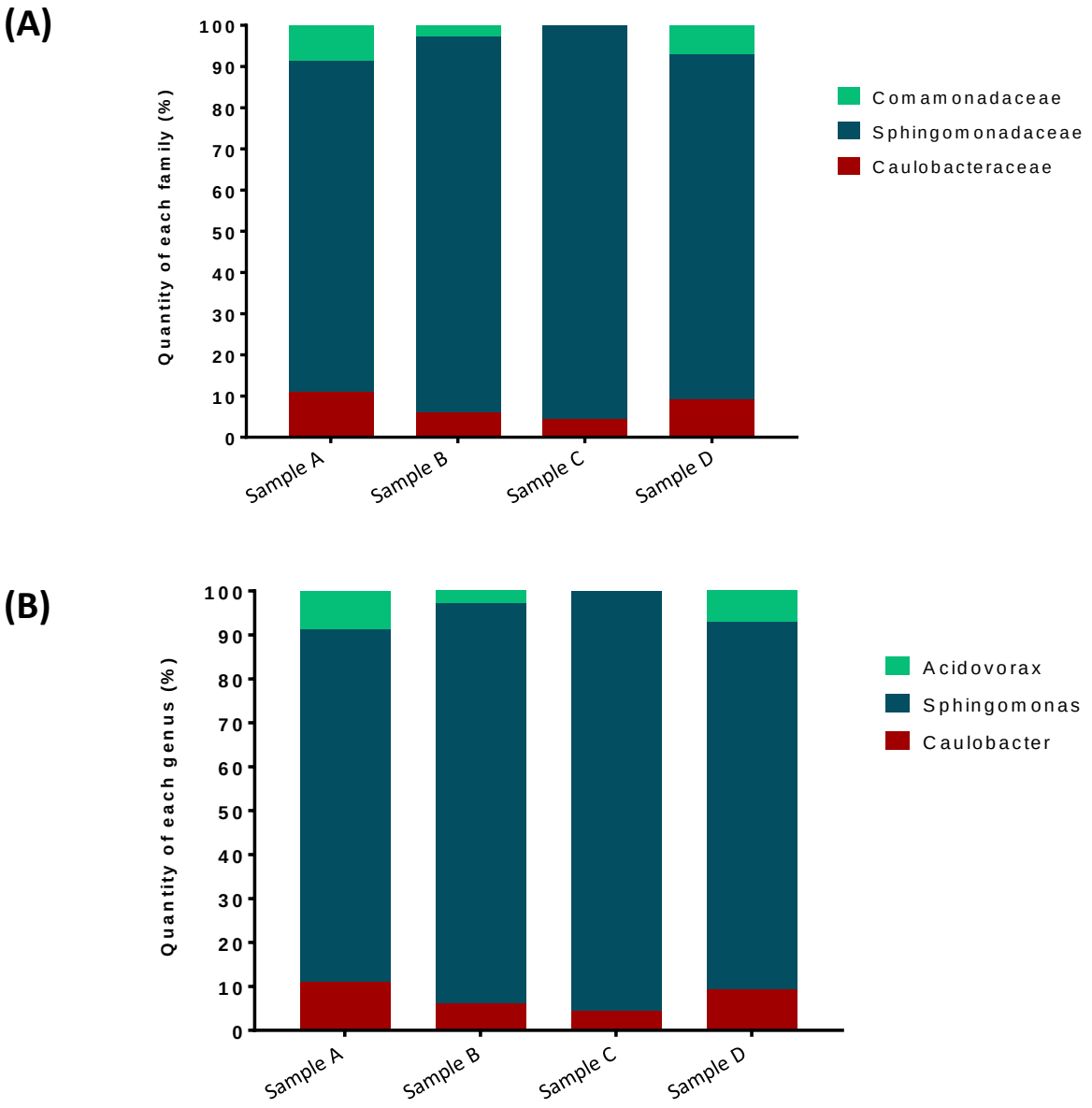



***Figure 3.22: Proportion of each genus from the scrubs collected in 2016 before the change of filtration analyzed without cultivation step by metaproteomics.*** *(A) Family level; (B) Genus level.*

### *3.3.2.4 Direct metaproteomics analysis of water and biofilms during the 2017 campaign*

During the 2017 analysis only the water was recovered, unlike 2016 when water and particles obtained after rubbing the walls were recovered. Low cell density was observed at locations A, B, C, D, and E (less than 2 million cells per L) (Table 3.9). Between 2.5 to 20 million cells were analyzed via metaproteomics (5 L for the analysis).

***Table 3.9: Cell count at the six different water sampling location from the 2017 sampling***

| Location | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| **Total cell count ($10^6$ cells/L)** | 0.5 ± 0.2 | 1.6 ± 5.0 | 1.5 ± 0.5 | 0.5 ± 0.2 | 1.0 ± 4.0 | 4.0 ± 1.1 |

The use of direct metaproteomics to analyze the water highlighted the diversity present in the water.

Some differences in the microbiota composition depending on the depth and location can be observed in the water after a direct metaproteomic analysis. The *Pseudomonadaceae* family (and genus *Pseudomonas*) were present in all samples, with a higher proportion in water sample F and water sample C (100%) when it was present at 20.2% in water sample D, 9.8 and 9.7% for water samples E and A respectively. The microbial composition between the water samples E and A seemed similar at the family level but not at the genus level. Several genera were present in sample E but not in sample A. The *Cytophagaceae* family (with *Hymenobacter*) represented 0.6% of the identified proteins in sample E while *Salmonella* was also present in the *Enterobacteraceae* family along with *Escherichia*. *Paenibacillus* was present in sample E (4.4%) and *Streptomyces* was present in sample A (6.6%). Those two samples appeared to be the more diverse after a metaproteomic analysis. The genera *Achromobacter* was also present in sample D (79.8%) and *Bacillus* was present in sample B (30.4%). A total of 4 different phyla was identified in these samples (Fig. 3.23; Appendix 12, Table 6.17). Unlike the 2016 samples, no *Cyanobacteria* nor algae were detected in 2017.

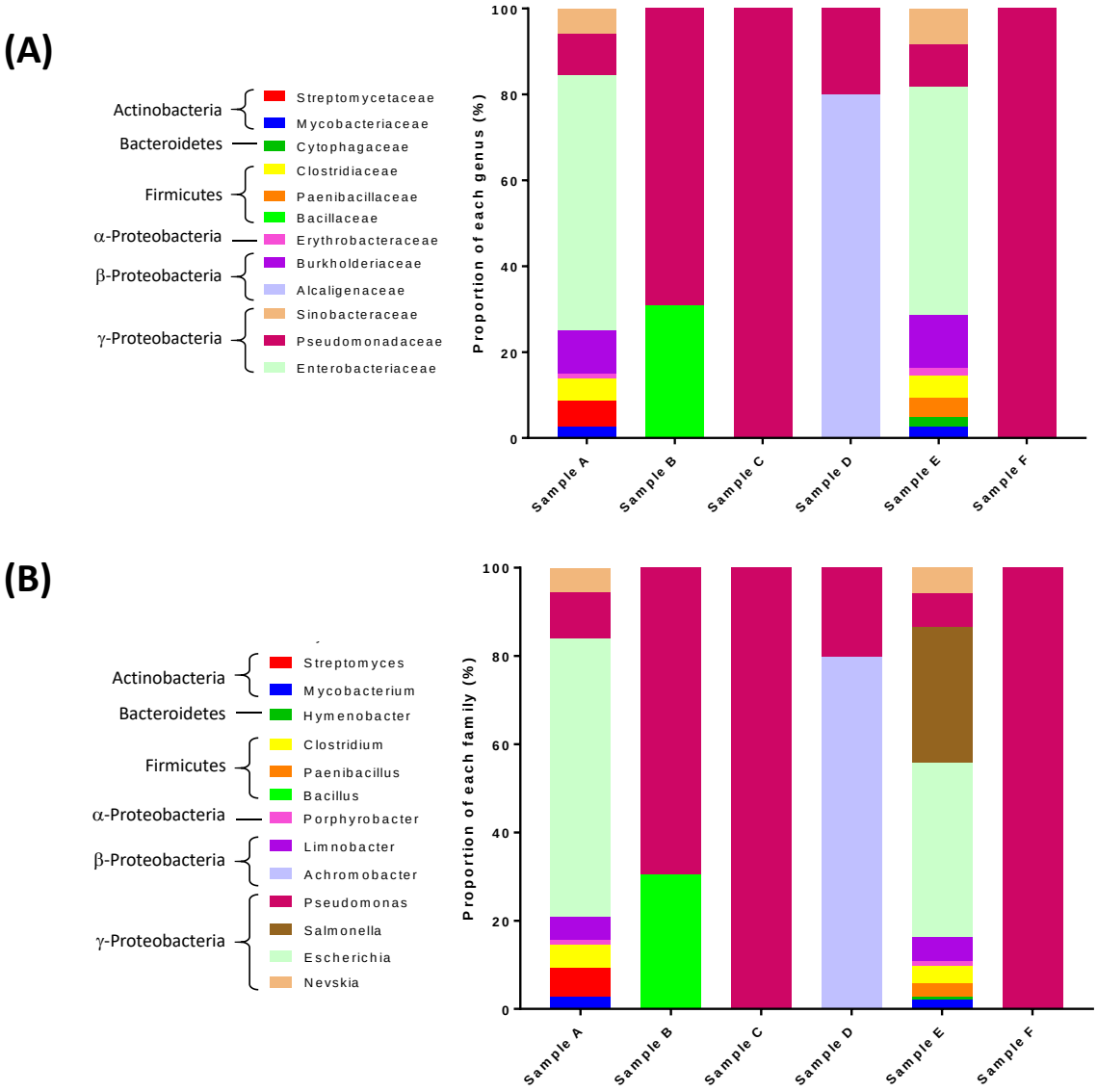



***Figure 3.23: Proportion of each genus from the water collected in 2017 after the change of filtration analyzed without cultivation step by metaproteomics****. (A) Family level; (B) Genus level.*

The biofilms recovery was performed in duplicates and analyzed using direct metaproteomics. As observed in Figure 3.24, the duplicates from all locations do not correspond between each other. This absence of similarity between the duplicates might be because of the poor cell density and maybe sign heterogeneity on each part of the same wall due to too early microbial colonization. The Spontex® sponges were indeed rubbed against the walls to recover biofilm. A small quantity of biofilm might have been recovered in this case. Moreover the cleaning of the pool, 3 months before, consisted in rubbing the walls to eliminate limescale, biofilms, etc. The new biofilms might have not grown sufficiently to be analyzed using direct metaproteomics. The diversity of these samples was limited after the metaproteomic analysis. Between one and three families and genera were observed per location. The presence of *Chroococcidiopsis* (*Cyanobacteria*) is worth noticing at sample C scrub and in the high corner of the walls. The 2 other genera found in more than one sample were *Pseudomonas* (sample D scrub 1 and sample B scrub 1) and *Rhizobium* (sample D scrub 2 and sample B scrub 2). The location at the bottom of the corner between the western and northern walls was the more diverse with the presence of *Bradyrhizobium*, *Selenomonas*, *Fusobacterium*, and *Neisseria*. A total of 4 different phyla were identified in these samples (Fig. 3.24; Appendix 12, Table 6.18).

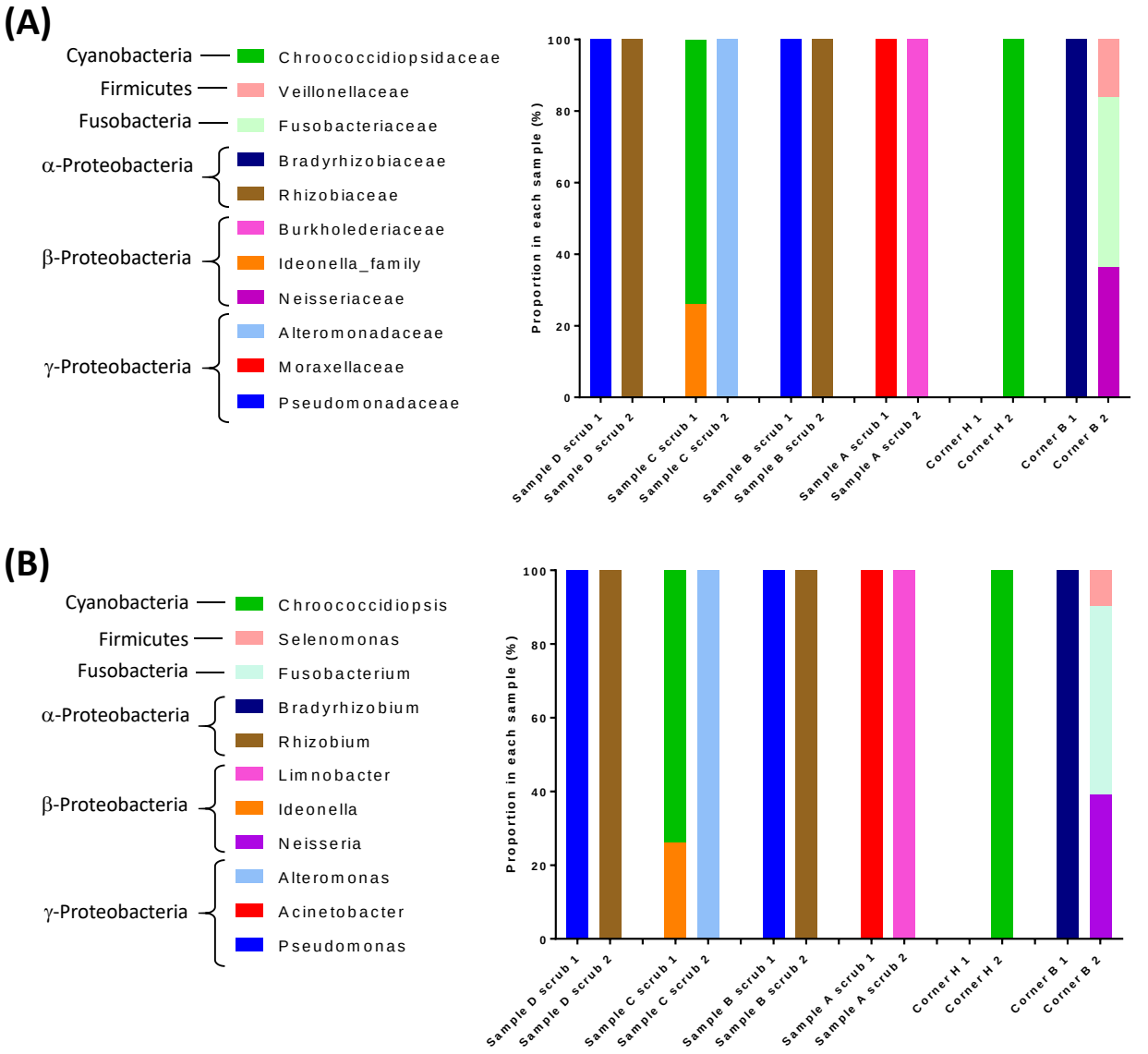



***Figure3.24: Proportion of each genus from the scrubs collected in 2017 after the change of filtration analyzed without cultivation step by metaproteomics**. (A) Family level; (B) Genus level.*

### 3.3.3 Discussion

Differences can be observed between the 2015 and 2017 samplings. In 2015, 239 OTUs were identified in both water and biofilms samples (205 OTUs were found in the water samples) while 106 OTUs were identified in the water in 2017. In 2015, 12 different phyla were identified in the water samples and in the biofilms using metagenetics: *Acidobacter*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Hydrogenedentes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia*. In 2017, 9 phyla were identified in the water samples: *Acidobacter*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia*.

In 2015 a high proportion of *Cyanobacteria* and more specifically the *Bacillariophyta* family was observed in several samples, up to 59.4%. In 2017 this family was present in only 3 samples at 2% maximum. Similarly the *Chloroflexi* phylum was only detected in 2015. This change in microbiota can also be observed when

doing a comparison between the 2015 and 2017 metagenetics results (cf. Appendix 11). In 2015 a majority of *Cyanobacteria* was observed while *Limnobacter* was the dominant genus in 2017. The detection of algae in the 2016 sampling may indicate the presence of a rich and diverse microbiota in this pool compared to Pool 2. Moreover the biofilm composition was different in 2016 (before the filtration system change) and in 2017 (after the change). In 2016, *Acidovorax*, *Sphingomonas* and *Caulobacter* were present in the biofilms but in 2017 none of these genera were detected using the same method (direct metaproteomics). The diversity in the biofilms was lower in 2017 than in 2016 (between 0 to 3 genera detected in one sample from the 6 sampling locations in duplicate in 2017 while 2 to 3 genera were found in 2016 in all 4 samples). Another interesting point is the fact that in 2016 the biofilms were similar within each other but the composition was quite different in 2017, except for the top samples from the western and northern walls.

It is unclear whether the *Bacillariophyta* family belongs to the *Cyanobacteria* phylum or is associated with the 16S rRNA gene present in algae chloroplast. Nonetheless this family was found in Chinese intertidal sediment [365]. The two OTUs present in this pool in 2015 and associated with *Bacillariophyta* were closely related with the chloroplastic DNA of different algae, including *Phaeodactylum* and *Thalassiosira* species (Fig. 3.25). These two algae genera were found after the metaproteomic analysis of the water and the water plus particles mix.

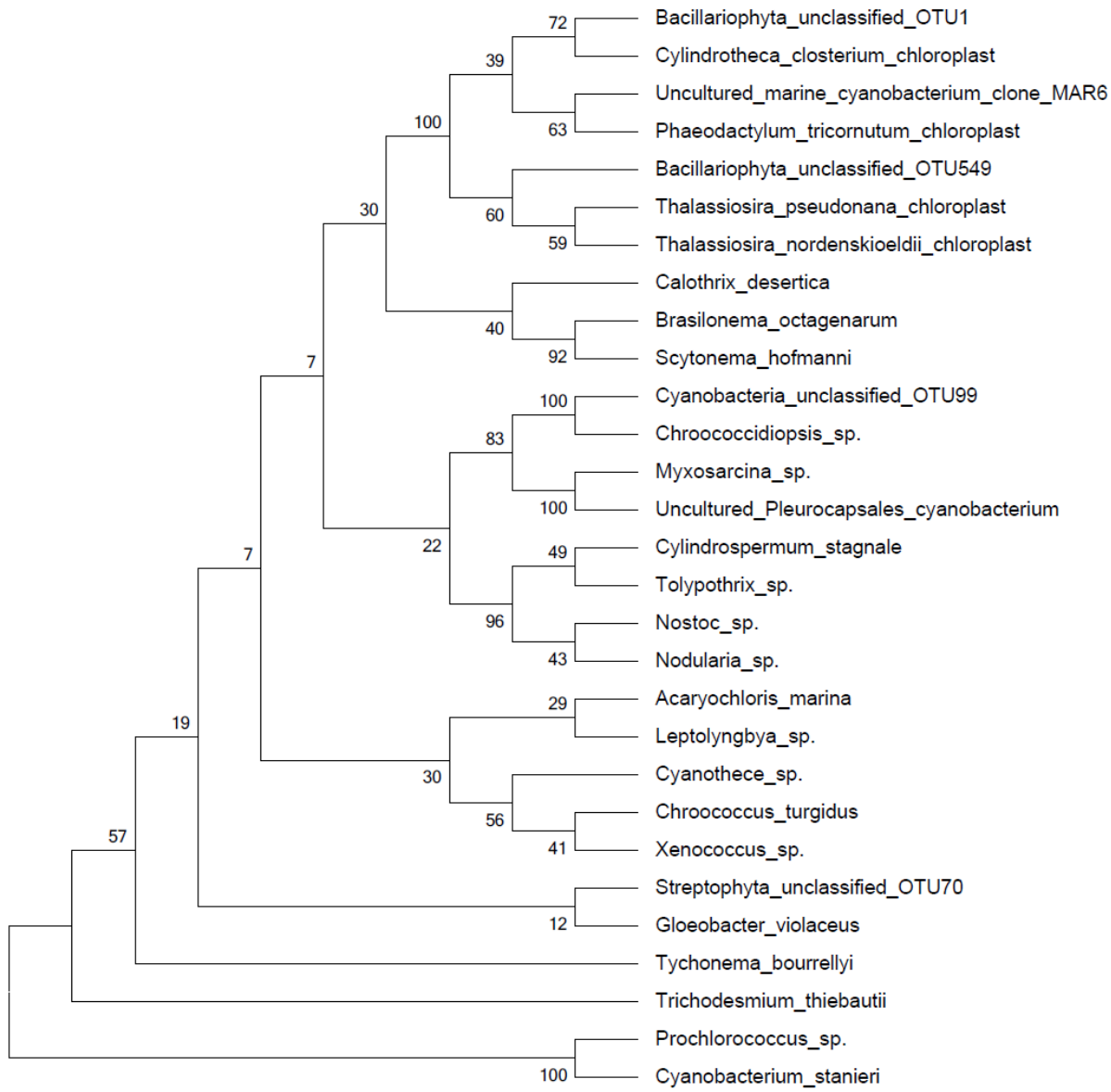



***Figure 3.25: Molecular Phylogenetic analysis of OTUs 1, 70, 99, and 549 (Cyanobacteria phylum) by Maximum Likelihood method.*** *The 16S rDNA sequences were obtained from NCBI. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [366]. The bootstrap consensus tree inferred from 500 replicates [2] is taken to represent the evolutionary history of the taxa analyzed [2]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [367]. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7011)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 12.2640% sites). The analysis involved 29 nucleotide sequences. All positions with less than 0% site coverage were eliminated. That is, fewer than 100% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 349 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [368].*

Amongst the diverse microorganisms found here, some can be related to extreme environment from previous works. For example, strains from the genus *Scytonema*, from the *Cyanobacteria* phylum, have been proved to resist radiation with a $D_{10}$ between 4 and 12 kGy and an $LD_{50}$ circa 8 kGy [369]. *Scytonema* bacteria produce scytonemin, an antioxidant pigment, allowing them to survive high levels of radiation [69, 370]. The cyanobacteria *Chroococcidiopsis* was studied for its ability to survive oxidative stress and its

potential ability to survive in space and under Martian conditions [171, 172]. Several members of the *Chroococcidiopsis* genus were irradiated at several doses. Depending on the strains, the survival to X-ray at an exposure of 2.5 kGy was between 35 to 80%. All the tested strains survived after a 15 kGy exposure while no survival has been detected at 20 kGy [173].

We also detected the presence of *Blastomonas*, which can be found in the environment and possess carotenoids [315]. However no data were available about potential radioresistance. This genus was not found in the different SNFPs analysis over the world. The genus *Limnobacter* was highly abundant in the 2017 sampling. This genus was not present in the different SNFPs analyzed. Strains closely related to the *Limnobacter* genus were identified in an acid mine drainage contaminated with several heavy metals [371]. The *Microbacterium* belong to the *Actinobacteria*. Several strains of this genus are UV resistant [372, 373]. Moreover, this genus was already found in the Cofrentes SNFP [184, 185], and one strain has been isolated from Chernobyl and shown by proteomics to overproduced specific proteins in response to uranium contamination [141]. Nitrite oxidation is the main energy source of *Nitrospira* bacteria. This bacterial genus can be found in aquatic environment, such as freshwater and activated sludge [165]. It is the dominant nitrite oxidizing bacteria genus in several aquatic habitats, as well as wastewater treatment systems and bioreactors [374]. Bacteria closely related to the *Nitrospira* genus were identified in a radon hot spring in Australia (radon levels in the water between 2000 and 5000 Bq/L) [375]. The *Brevundimonas* genus (*α-Proteobacteria*) can be found in water and soil [315]. One bacterial strain identified as a *Brevundimonas* was isolated from a hot spring containing high levels of radon. This strain was also proved to biosorb and desorb cadmium [376]. Another strain, isolated in the Antarctic Cry Valley, exhibited a $D_{10}$ of 1.5 kGy after a gamma-irradiation at -79°C and some viability was observed after a 7 kGy irradiation [377]. The *Sphingomonas* genus was identified in several SNFPs in Spain and in the USA after a cultivation step [185, 188]. One strain, isolated in the Antarctic Dry Valleys, exhibited in its genome the presence of oxidative stress resistance genes [306]. Some strains of the *Acidovorax* genus can use the oxidation of $H_2$ as an energy source [315]. This genus can be found in soil, water, and activated sludge for example. A species of *Acidovorax*, *A. facilis*, can accumulate uranium and is able to survive a concentration of 500 µM [378]. This genus has also been found in the SNFP of Savanah River Site [193]. Bacteria identified as *Stenotrophomonas* sp. have been isolated from Andean lakes and exhibited a great resistance to UV radiation [379, 380]. *Nevskia* bacteria can be found on the surface of freshwater environments. It can form a pellicle on water surface and create slime [381]. *N. ramosa* has an effective photorepair mechanism when exposing cells to light after a UV irradiation [382]. It has however never been described in SNFP or radioactive environment. *Blastocatella fastidiosa* was the first described species of *Acidobacteria* subdivision 4 and was isolated from a semiarid savanna soil [383]. *Blastocatella* genus was found in a heavy metal contaminated land in China [384]. This genus was isolated recently and little is known about it. Considering the environments these genera can be found, it appears that they possess performant defenses against oxidative stress induced by ionizing radiation, metallic stress, and desiccation.

### 3.3.4 Conclusion

In this pool a great diversity was observed. Prokaryotes and eukaryotes have been found living in this constraint environment thanks to a novel proteotyping methodology based on high-resolution tandem mass spectrometry. In some samples, the biomass contributions of prokaryotes and photosynthetic eukaryotes were found almost similar, highlighting the importance of microbial equilibrium as recently

observed [385]. Indeed, photosynthetic eukaryotes required for long-term stability that nutrient recycling by diverse heterotrophic bacteria takes place. The heterotrophes remineralize the inevitably leaked organic matter from photosynthetic organisms, making nutrients circulate in a mutualistic system. The combined use of metaproteomics and metagenetics allowed a deep analysis of the microbial diversity present in this pool. Some differences could be observed between the location and depth of samplings. However the main differences were noticed between the two different pool conditions. After the cleaning and change of filtration system the microbiota was indeed extremely different. The drastic decrease of *Bacillariophyta* and the increase of *Limnobacter* were the main changes observed.

The presence of algae in this pool could be interesting for several reasons. First of all, if we assume that the *Bacillariophyta* family indicated, in fact, the presence of algae (due to a chloroplastic DNA sequencing), this pool would have been composed of photosynthetic eukaryotes in high proportion in 2015. The isolation of such organisms could help discover new resistance mechanisms. Secondly it could indicate the presence of a complex microbiota in a constrained environment.

The biofilm composition also changed between 2015 and 2017. The presence of *Sphingomonas* in biofilms analyzed in 2015 (metagenetics) and 2016 (metaproteomics) in large proportion with its severe decrease in the 2017 biofilms could indicate an influence of the filtration and condition changes in the pool in opposite with the annual cleaning process.

While the use of metaproteomics was less sensitive than metagenetics it can be useful for quick analysis and monitoring of the pool's microbiota. A monitoring of the microbiota of SNFPs or radioactive sources storage pools should be performed annually to help prevent microbial influenced corrosion.

The microbial composition of this pool was different from the microbiota of SNFPs. The presence of *Bacillariophyta* was unique and never described in similar environment. Moreover no *Cyanobacteria* were found in the studied SNFPs over the world. However this pool does not contain radionuclides unlike the SNFPs studied over the world. These results indicates a dependence of the microbiota on the conditions prevailing in the pool. Each pool has its unique microbiota and a pool can present a change in the microbiota when one of the numerous parameters has been changed.

The protocols developed from this pool were used to analyze the microbiota present in the water and on biofilms in the cooling pool of a nuclear reactor. These methods could be used to unveil the microbial diversity present in constrained environments.

### 3.3.5 Acknowledgments

The authors thank J.-B. Baugé, D. Do Couto, D. Duval, D. Le Tutour, and M. Delage (CEA, Saclay). This work was founded by the Transverse Toxicology Program of the CEA.

## 3.4 Chapter conclusions

In this chapter it has been observed that an unexpected diversity could be seen in the water of nuclear storage pools. This diversity was assessed using direct analysis methods (metagenetics and direct metaproteomics) and liquid cultures (kinetic in different media). The combine use of all three methods allowed a deep inventory of the microbial diversity present in these pools. The microbiota present in both pools (analyzed with and without culture) were not similar to the already described microorganisms in similar environments.

Both pools presented different physico-chemical conditions between in each but also within each other. A change in the microbiota has been observed in both pools when the conditions were changed. In Pool 1 the main change was the filtration system while in Pool 2 the definitive shutdown of the reactor deeply modified the pool's conditions. Differences were observed in Pool 2 in the microbiota composition as expressed by the non-metric multidimensional scaling (NMDS) presented in Figure 3.26. Three groups could be observed: one group containing the analysis from the 2015 water sampling, one group with the 2017 water analysis and the final one containing the scrubs from 2017. Only one sample (Scrub 3) was apart from the other scrubs, probably because of its richness and evenness. The survival mechanisms implemented by the organisms in Pool 2 were most likely different between the bacteria identified in the water and the ones identified in the biofilms. Using direct metaproteomics could help identify these mechanisms.

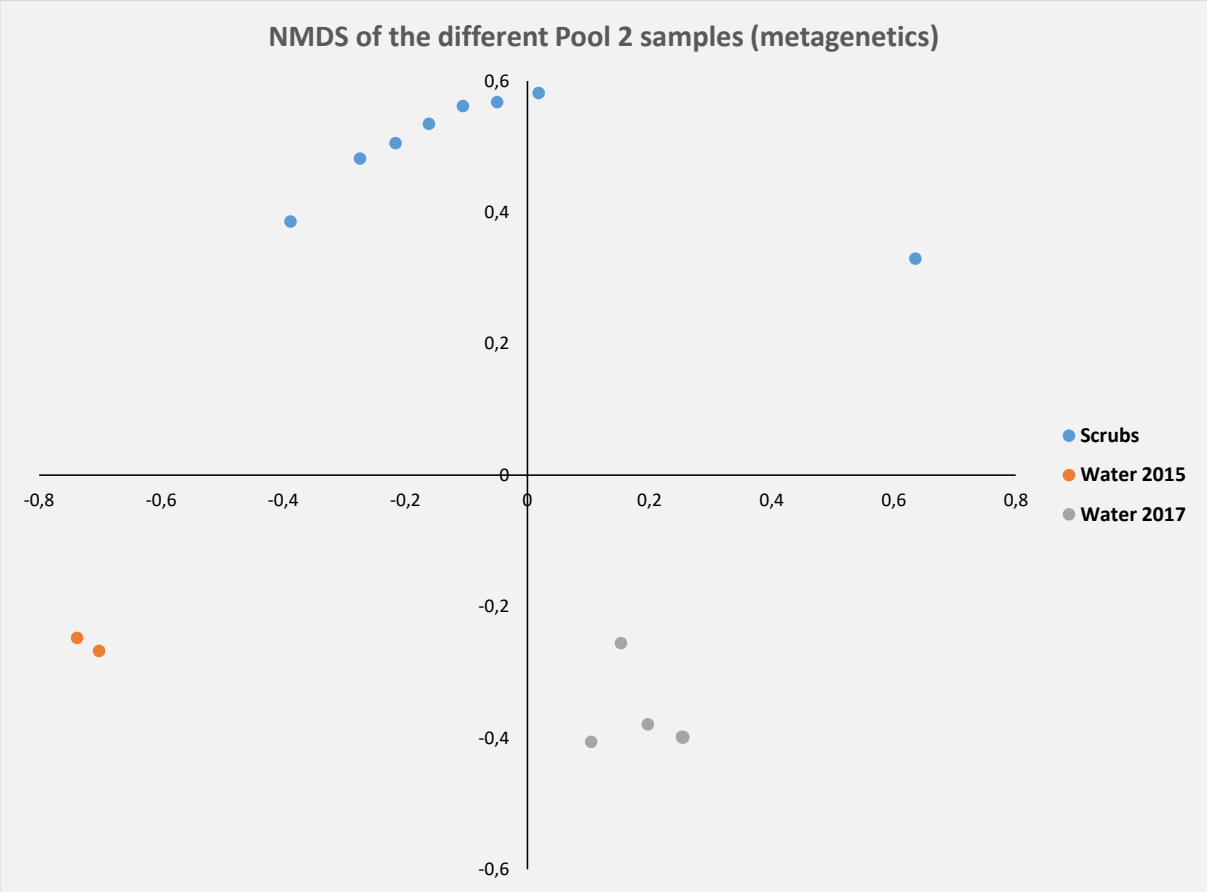



***Figure 3.26: Non-metric multidimensional scaling of the different samplings from Pool 2.*** *The results from the scrubs (blue) and the water analysis from 2015 (orange) and 2017 (grey) were obtained using the metagenetics results.*

As specified above the microbial composition of both pools was different despite the presence of common genera mainly belonging to the *Proteobacteria* phylum after metagenetics analysis (cf. Appendix 11). During the kinetic the same tendency could be observed, with 60 genera common to both pools (out of 215 genera) (cf. Chapter II, Appendix 5). This difference can be easily explained by the different conditions of the pools. Moreover Pool 1 is less stringent than Pool 2 in terms of nutrients and ionizing radiation. Furthermore no radionuclides were found in the water of Pool 1. Pool 1 was thus useful to develop methods to sample and analyze the microbial diversity present in nuclear environments.

The diversity observed in Pool 1 was greater than the one found in Pool 2, whether the conditions changed or not. Photosynthetic organisms were found in Pool 1 (*Cyanobacteria* and algae) in high proportion while less than 0.3% of *Cyanobacteria* were found in Pool 2 (during operation and at shutdown). The evenness in the microbial diversity was greater in Pool 1 than in the water of Pool 2. The biofilms collected in Pool 2 presented an evenness similar to the one found in Pool 1 (water and scrubs).

Several microorganisms including several radioresistant bacteria have been isolated from the water and biofilms sampled in Pool 2. No unknown bacteria at the genus level were isolated in this pool. Several bacteria were similar to unnamed already identified species (*i.e.* 100% hit with a bacteria identified at the genus level but not species one: for example, a hit with a *Bacillus* sp.). The ability to accumulate and survive different radionuclides was studied for the radioresistant bacteria isolated from Pool 2.

# Chapter IV:

*Radioresistance and bioaccumulation of uranium and cobalt by bacteria isolated from a nuclear reactor cooling pool*

# 4. Radioresistance and bioaccumulation of uranium and cobalt by bacteria isolated from a nuclear reactor cooling pool

## 4.1 Introduction

In France, there are currently 58 nuclear reactors, distributed in 19 facilities, to produce electricity. Moreover 8 nuclear reactors are used for research in France. All these installations produce radioactive wastes that are treated accordingly to their nature (cf. Chapter I, Appendix 1). Once treated the wastes are stored in deposits. Radioactive wastes management is an issue concerning the environment and public health and is taken into account when building a nuclear power plant. Nuclear energy is the third source of electric production in the world. In France, 77% of electricity is produced using nuclear fission (EDF). The volume of radioactive wastes will increase due to nuclear exploitation. The actual solution is waste underground burial but it is of limited space. One solution to reduce wastes volume could be changing the effluents decontamination methods. Nuclear effluents are currently mainly decontaminated via resins and chemical products. The use of biotechnology to reduce the volume of wastes produced by nuclear effluents is currently under consideration [112, 152, 386, 387]. Large volume of wastes with low radionuclides concentration could indeed be reduced using biotechnologies.

The advantages of radionuclides accumulating microorganisms can be numerous. They can in fact concentrate radionuclides inside their cells and thus reduce the volume of wastes to store. Microorganisms able to change the toxicity and solubility of radionuclides can be used in the environment, either to immobilize radionuclides or to accelerate their removal by changing their chemical form (*e.g.* reduction) [115]. Several studies have been performed *in situ* with the natural microbiota present in contaminated soils [153-155]. Among the microorganisms able to accumulate toxic metals and radionuclides, several fungi, bacteria and algae exhibited a great accumulation ability [113, 134, 135, 146, 147, 149, 150, 152, 195, 388-393]. A laboratory-scale process has been developed using the micro-algae *Coccomyxa actinabiotis* to depollute nuclear effluent [112]. Throughout time one evidence has come to light: organisms can be used to decontaminate low concentration of large volume of wastes [143, 176]. Microorganisms able to accumulate or reduce radionuclides are more likely to be found in an environment presenting this stress. To accumulate radionuclides and survive this accumulation microorganisms need to resist ionizing radiations. Nuclear pools provide an environment containing ionizing radiations and radionuclides. In these environments microorganisms able to accumulate radionuclide should be more easily discovered than in any other environments (with the exception of Chernobyl and Fukushima surroundings).

Since two decades we now know that life can be found in Spent Nuclear Fuel Pools (SNFP) [152, 181-187, 191-193]. The Spanish SNFP Cofrentes was deeply studied [181, 184-187]. Several microorganisms, mainly bacteria, have been isolated from these pools. To be able to survive the hostile conditions present in such environment (ionizing radiation, presence of radionuclides, lack of nutrients) the organisms must have developed mechanisms to resist the different stresses. They include biofilms' formation, sporulation or Viable But Non-Culturable state (VBNC) for example. Other mechanisms, independent from physiological shape, can be implemented to resist radiations and metallic stress. Microorganisms can resist metallic stress using different mechanisms. They can either i) accumulate the atom and detoxify it to potentially eliminate it or ii) prevent its penetration in the cell.

In this study the radioresistance of bacteria isolated from the cooling pool of a nuclear reactor (during operation and at shutdown) has been assessed. Their ability to accumulate cobalt and uranium was also

tested. The radionuclides $^{238}U$ and $^{60}Co$ were chosen since they are the most interesting in terms of bioremediation and bio-decontamination in the case of a nuclear incident or just to decontaminate nuclear liquid effluents.

Uranium is used as a fuel and uranium can be released in nature during nuclear accidents. Agriculture is however the main uranium contamination in soils (cf. Chapter I.4.2.1.3). Cobalt 60 can be produced in nuclear reactor when particles from the pool's structure and elements are activated by the reactor's neutronic flow. It usually represents about 47% of total gamma activity in liquid effluents in EDF power plants (IRSN website: https://www.irsn.fr/FR/Pages/Home.aspx). During treatment cobalt is dissolved into liquid effluents. In 1999 the $^{60}Co$ activity was estimated at about $3.21x10^{11}$ Bq in the SNFP of La Hague. In England, at the Sellafield nuclear fuel reprocessing and nuclear decommissioning site, the activity of cobalt in effluents was estimated at $1.5x10^{12}$ Bq in 1997 (IRSN website). Cobalt can be toxic for organisms and the environment [394-398]. It is thus necessary to depollute any cobalt contamination.

To decontaminate these radionuclides, several methods exist (cf. I.4.2.3 for uranium biodecontamination). Cobalt can be removed from waste using chemical precipitation, ion exchange, solvent extraction and evaporation. Chemical precipitation can be performed using a polyurethane foam or EDTA and iron. This method allowed cobalt extraction but was not the best method since about 50% of cobalt was recovered [399]. It can be improved using amine-functionalized silica nanoparticles [400]. This addition allowed a stable foam on a longer time. Bioengineering could allow a great radionuclides accumulation by microbial strains. An *Escherichia coli* strain as well as *Deinococcus radiodurans* R1 were transformed with Ni/Co transporter (gene from *Rhodopseudomonas palustris* CGA009 and *Novosphingobium aromaticivorans* F-199) to bioremove cobalt from spent decontamination solutions of nuclear power reactors. The modified *E. coli* was able to remove up to 85% of dissolved Co (of a 8 nM solution) in two cycles while modified *D. radiodurans* could remove 90% (of a 8.5 nM solution). These experiments revealed the ability to bioengineer bacterial strains to accumulate Co. However *D. radiodurans* exhibited a better accumulation than *E. coli*. Finally to remove Co present in the studied solutions, only 2 kg of modified bacteria were needed, which was less than the 50 kg estimated using a wild Co accumulating strain or the 8000 kg of ion-exchange resin existing [401, 402].

## 4.2 Material and Methods

### 4.2.1 Microbial strains isolation

The basin studied is the cooling pool containing water in direct contact with the core of a nuclear reactor located on the CEA-Saclay center. It is 11 m deep, 7.5 m long, and 6.5 m wide. While the reactor was in operation, a 2 L water volume was collected on December 2015 using a permanently installed pipe extensively purged before sampling. When the reactor was shut down, a total volume of 8 L was collected on March 2017 using the permanently installed pipe, as well as a sterile sampling vial (Wildco, USA) attached to the lower end of a pole and opened at the samplings points.

During reactor shutdown 0.5 L of the collected water were used for cultivation and cryopreservation. Microorganisms were concentrated and harvested by centrifugation at 16,000 *g* for 20 min at 10°C. The pellet was resuspended in 1 mL of supernatant. This milliliter was dispatched and cultivated in presence of 6 ml of liquid media over a kinetic culture. Two liquid media were used: Luria-Bertani (LB) diluted 1/12 (Roth) and Reasoner's 2A (R2A) diluted 1/3 (HiMedia Laboratories Pvt. Ltd.). Four kinetic points were

analyzed: 3 days, 7 days, 14 days and 21 days. The cultures were incubated at 28°C under shaking. To isolate strains, 100 µL of each kinetic point were plated at different dilution on the corresponding agar medium, namely LB diluted ten times and R2A diluted twice. Plates were incubated at room temperature until microbial growth was detected and then kept at 4°C. The microorganisms were isolated from these plates. For anaerobic cultures, 100 µL of the liquid cultures from day 28 of the kinetic from each location in LB medium were diluted in 1.9 mL of sterile water. One milliliter from this solution was introduced in BSR or BTR liquid media kit from Labège-CFG Services (Orléans, France). The cultures were incubated 2 months at room temperature without agitation. The isolation of microorganisms was performed on R2A diluted 1/2 agar plate introduced in anaerobic jar (GENbox anaer, Biomérieux, France). The bacteria isolated from the kinetic possessed the code-name "SKH".

Biofilms present in this pool in 2017 were recovered via cellulosic scrubs. After sampling the scrubs were placed on sterile hermetic plastic bags to be treated in a sterile environment. In a sterile environment the scrubs were introduced into 50 mL sterile Falcon tubes with 15 mL of sterile milliQ water to resuspend the recovered organisms. After the resuspension step, the presence of visible cobalt particles forced us to let the Falcons decant overnight at RT to eliminate as much radioactivity as possible to transport the samples outside of the radioactive zone. The 5 top milliliters were recovered the next day. Ten microliters were plated in different media for microbial isolation. The media used were LB (Roth, Germany), Tryptic Soy Agar (TSA), TSA diluted ½, Nutrient Agar (NA), NA diluted ½, (5 g tryptone, 2.5 g yeast extract, 1 g glucose, and 15 g agar per L), TCA diluted ½, Brain Heart Infusion (BHI), and BHI diluted 1/10 (Sigma, Germany).

Samples collected during reactor operation were stored for 3 days at 4°C before handling to reduce their activity by radioactive decay of $^{24}$Na (half-life of 15 hours), enabling its manipulation in controlled conditions. To isolate microbial strains harvested during reactor operation, 0.5 L water sample was centrifuged at 15,000 *g* for 20 min. The supernatant was discarded except for the last 4.5 mL which were used to resuspend the microbial pellet. Aliquots of 500 µL were plated on different agar media, namely LB (Roth, Germany), TSA, TSA diluted ½, NA, NA diluted ½, TCA, TCA diluted ½, BHI, and BHI diluted 1/10 (Sigma, Germany). Another 0.5 L water sample were filtered. Filters were stamped onto agar plates containing the same media. Plates were incubated at 28°C until microbial growth was detected and microorganisms were isolated by successive plating.

### 4.2.2 Strain identification

#### *4.2.2.1 16S rDNA and ITS sequencing*

Once the microorganisms were isolated, a colony PCR was performed. Amplification of 16S rDNA was performed to identify bacteria and ITS amplification was performed to identify fungi [57, 247, 403]. Once isolated, microorganisms were identified by 16S rDNA sequencing after PCR performed on a colony. Amplification of 16S rDNA was carried out using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') at an annealing temperature of 61°C. Amplification of ITS was carried out using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') at an annealing temperature of 55°C. The elongation for both amplifications was performed by the DNA Phusion polymerase (Thermo Fisher, USA) at 72°C for 45 s and 60 s for the 16S rDNA and the ITS, respectively. PCR products obtained after 35 cycles were gel purified (NucleoSpin® Gel and PCR Clean-up, Macherey-Nagel, Germany). The DNA was sequenced by GATC (Germany) with the

Suprem Run method. The obtained sequences were aligned against NCBI GenBank database using nucleotide BLAST.

#### *4.2.2.2 Phylopeptidomics*

Each bacteria isolated from the 2017 kinetic were identified using phylopeptidomics. The metaproteomics analysis was done as previously described (cf. Taxonomical composition of a biofilm sampled from a nuclear fuel pool assessed by metaproteomics).

Briefly, microbial colonies were recovered and a volume of X µL of Laemmli buffer LDS1X (Invitrogen) was added per mg of wet bacterial pellet as described by Hartmann et *al* [295]. After denaturation for 5 min at 99°C, the protein sample was loaded onto a 4–12% gradient 10-well NuPAGE gel (Invitrogen). The proteins were subjected to electrophoresis for 5 min and then stained with Coomassie Blue Safe staining (Invitrogen) as recommended by the supplier. The polyacrylamide band corresponding to the whole proteome was sliced, then unstained with ultra-pure water, reduced with dithiothreitol, and treated with iodoacetamide. It was then subjected to proteolysis with Trypsin Gold Mass Spectrometry Grade (Promega) in the presence of 0.01% ProteaseMAX surfactant (Promega), as previously described [322]. The resulting peptides were analyzed with an ESI-Q Exactive HF mass spectrometer (Thermo) equipped with an ultra-high field Orbitrap analyser and coupled to an Ultimate 3000 176 RSL Nano LC System (ThermoFisher). A volume of 10 µL of peptides was injected onto a reverse phase Acclaim PepMap 100 C18 column (3 µm, 100 Å, 75 µm id x 500 mm) and resolved at a flow rate of 0.2 µL/min with a 60 min gradient of CH3CN in presence of 0.1% formic acid. The Q-Exactive HF instrument was operated with a Top20 strategy with only ions with potential charge states of 2+ and 3+ selected for MS/MS and with a dynamic exclusion of 10 sec. The proteotyping interpretation of MS/MS spectra was done with the National Center for Biotechnology Information non-redundant fasta file (NCBI-nr) database with the Mascot search engine (Matrix science). Peptide -to-MS/MS spectrum assignation was done with full trypsin specificity, maximum of one missed cleavage, mass tolerances of 5 ppm on the parent ion and 0.02 Da on the MS/MS, static modification of carboxyamidomethylated cysteine (+57.0215), and oxidized methionine (+15.9949) as dynamic modification. Post-processing of Mascot dat files was done as previously indicated (reference public biofilm).

### 4.2.3 Characterization of radioresistance

Each isolated microorganisms was cultivated in liquid LB medium and subcultured twice. When the stationary phase was reached, 2 mL of each culture were centrifuged at 20,000 *g* for 5 min at room temperature. The supernatant was discarded. The pellet was washed twice with sterile NaCl 0.9% and resuspended in 1 mL of sterile NaCl 0.9%. Bacteria were then counted using a Malassez cell. $10^6$ cells were introduced into 200 µL of sterile liquid LB medium, in one single row of a 96-well plate. Bacteria were prepped the day before irradiation and the sealed plates were stored overnight at +4°C. They were irradiated at a dose rate of 2,200 Gy/hr using $^{60}$Co sources at room temperature (ARC-Nucléart, CEA - Grenoble), integrating total doses of 200, 500, 1000, and 1500 Gy. A control plate (0 Gy) was handled in the same conditions. After irradiation, the bacteria were incubated at 28°C on LB agar plate at two different dilutions corresponding to 100 and 1000 cells per plate. Experiments were performed in duplicate. When bacterial growth appeared on the control plate, after at least one week, the colonies were counted and

compared to the control to assess the percentage of survival. *Escherichia coli* strain DH5α and *Deinococcus radiodurans* were also tested in the same conditions as non radioresistant and highly radioresistant control species, respectively.

The bacterial irradiation was also performed in NaCl 0.9% instead of LB. The procedure was the same as the one detailed above.

### 4.2.4 Radionuclides accumulation studies

#### *4.2.4.1 Determining the screening conditions*

To determine the incubation time, the metal concentrations to use and to assess the impact of uranium on cell viability, a preliminary experiment was performed on six bacteria among the most radioresistant. The accumulation was assessed after 1 and 24 hours of contact with uranium. Direct viability was also performed at both time points. Briefly, 800 µL were centrifuged 15 min at 20,000 *g* at RT. The supernatant was recovered and the pellet was washed once with 1 mL of sterile NaCl 0.9% and a second time with $Na_2CO_3$ 10 mM to eliminate most uranium adsorption on the cell walls. The two different washes were pooled into one same tube. Direct cell viability was performed on the 200 µL culture left. The tubes were centrifuged 15 min at 20,000 *g* at RT and the supernatant discarded. The pellet was resuspended in 99.5 µL of NaCl 0.9%. 0.2 µL of propidium iodide (1 mg/mL) and 0.3 µL of fluorescein diacetate (2.5 mg/mL) were added. After 5 min incubation in the dark the tubes were centrifuged 5 min at 20,000 *g* at RT and the supernatant discarded. The pellet was washed twice with 100 µL of NaCl 0.9% and resuspended in 100 µL of NaCl 0.9%. Direct cell viability was observed using a microscope (Zeiss Axio Scope A1) and an epifluorescent lamp (HXP 120C). GFP filter (excitation: 457-487 nm; emission: 502-538 nm) was used to highlight living cells while FITC filter (excitation: 467-498 nm; emission: 513-556 nm) was used to highlight dead cells. Cell viability was analyzed using ImageJ (v. 1.46r) by assessing the surface of living and dead cells.

#### *4.2.4.2 Screening experiment*

Each isolated microorganisms (11 strains) was cultivated in liquid LB medium and subcultured twice. When the stationary phase was reached, 2 mL of each culture were centrifuged at 20,000 *g* for 5 min at room temperature. The supernatant was discarded. The pellet was washed twice with sterile NaCl 0.9% and resuspended in 1 mL of sterile NaCl 0.9%. Bacteria were then counted using a Malassez cell. $1.40x10^8$ cells were introduced in sterile NaCl 0.9% pH 5.5. Different metals were tested: $UO_2(NO_3)_2$ $6H_2O$ and $CoCl_2$ at three different concentration in duplicate: 5 – 50 and 500 µM. The final volume of the experiment was 1 mL of NaCl 0.9%. The tubes were incubated at 28°C with agitation (200 rpm). Control tubes with 5-50-500 µM of uranium or cobalt were done to measure the metal concentration really introduced. After 24h of incubation the tubes were centrifuged 15 min at 20,238 *g* at RT. 475 µL of supernatant were recovered and the rest of supernatant was discarded. The pellet was washed once (5 µM) or twice with 1 mL of sterile NaCl 0.9%. After centrifugation 475 µL of supernatant were recovered and the rest of supernatant was discarded. The two different washes were pooled into one same tube. Nitric acid ($HNO_3$) was added to the different collected fractions to a final concentration of 0.5%. The pellet was preserved at -20°C without addition of acid prior to mineralization.

The radionuclides $^{238}U$ and $^{60}Co$ were chosen since they are the most interesting in terms of bioremediation and bio-decontamination in the case of a nuclear incident or just to decontaminate nuclear liquid effluents.

Normal saline and a pH at 5.5 were chosen to avoid any possible speciation between the liquid media and the radionuclides.

#### 4.2.4.3 Metal analysis

Five hundred microliters of pure $HNO_3$ were added to the bacterial pellet. The mixture was placed into 15 mL mineralization tubes (Courtage Analyses Services, France). The tubes were placed in a HOT BLOCK Environmental Express for 2h30 at 90°C. At the end of the mineralization 4.5 mL of milliQ water were added to the mixture.

The three recovered fraction (pellet, supernatant, wash) were diluted in $HNO_3$ 0.5% as presented in Table 4.1. The concentration of metal was measured using an ICP-MS (Inductively Coupled Plasma Mass Spectrometry) iCAP RQ (Thermo Scientific, USA). The results were recovered and analyzed using the Qtegra software (Thermo Scientific, USA).

*Table 4.1: Dilution factors used to analyze metal concentration via the ICP-MS*

| | U | | | Co | | |
|---|---|---|---|---|---|---|
| | 5 µM | 50 µM | 500 µM | 5 µM | 50 µM | 500 µM |
| **Pellet** | 10 | 100 | 1000 | 10 | 10 | 10 |
| **Supernatant** | 100 | 10000 | 50000 | 10 | 1000 | 1000 |
| **Washes** | 100 | 100 | 1000 | 10 | 10 | 100 |

## 4.3 Results and discussions

### 4.3.1 Microbial identification

Microorganisms have been isolated from both campaigns. These microorganisms originated either directly from the samples or from the culture kinetics of the communities. Five fungi were identified in 2015 as well as 22 bacteria. In 2015 the identification was conducted using 16S rDNA and ITS sequencing for bacteria and fungi respectively. No fungi was isolated in 2017 but 24 bacteria were identified. Among these bacteria, the ones isolated during the kinetics were identified via phylopeptidomics and the ones isolated from biofilms were identified via 16S rDNA sequencing. Isolated strains belonged to different phyla and genera (Table 4.2). Only *Actinobacteria*, *Firmicutes* and *Proteobacteria* were isolated among the bacteria. A majority of *Firmicutes* and *Actinobacteria* were isolated during reactor's operation while a majority of *Proteobacteria* was isolated during shutdown.

Bacteria and fungi isolated from the 2015 sampling and the biofilms recovered in 2017 were coded "CEA-NOB-XXX" and "CEA-NOF-XXX" respectively. Bacteria isolated from the 2017 kinetic were coded "SKH XXX".

Among the isolated bacteria, only *Pantoea* sp. and *Rothia* sp. had never been described in SNFPs. The genus *Leifsonia* was found in an Argentinian SNFP [192], *Nocardia* genus and *Gordonia bronchialis* were found in a Spanish SNFP [181, 184, 185]. The species *Ralstonia pickettii* was found in several SNFPs in France and Spain [184, 186, 189], while this genus was also found in the Cofrentes SNFP [181, 185]. Many *Bacillus* species have been isolated in Spain and Argentina [184, 186, 192]. *Staphylococcus epidermidis* was found in Cofrentes [184, 186] and several *Staphylococcus* species were isolated from French and Spanish SNFPs [181, 185, 189]. *S. epidermidis* was also isolated from feathers of birds living in the Chernobyl exclusion area where the received dose was 0.1 µGy/h [404]. One *Streptococcus* species was found in Cofrentes [185]. *Micrococcus luteus* was found in different SNFPs over the world [152, 188] while several *Micrococcus*

species were found in France, Argentina and USA [177, 189, 192]. The genera *Mycobacterium*, *Cellulomonas*, *Bradyrhizobium*, and *Afipia* were all found in Cofrentes [186] [184, 185]. *Sphingomonas* genus was isolated from SNFPs in Spain and USA [185, 188]. *Acinetobacter* genus was found in French and Argentinian SNFPs [189, 192]. *Kocuria* genus was identified in a Slovak SNFP [152]. *Pelomonas* and *Mycobacterium* genera were identified using metagenetics in an American SNFP [193]. Concerning the fungi, only *Aspergillus* genus was isolated from the Cofrentes SNFP [181] (Table 4.2).

Several bacteria were identified using phylopeptidomics. For some of the tested colonies, several organisms were detected and identified. This phenomenon proved the corresponding bacterial culture was not pure. Interestingly the inability to identify some bacteria to the species level and the attribution of several taxon ID may indicate that these bacteria might be unknown. However the lack of exhaustiveness of protein databases might explain this phenomenon. Many isolated bacteria were matched to the same strain (*e.g. Ralstonia pickettii* with the *R. pickettii* strain ID 329) (Table 4.3). Among these bacteria, the genera *Ideonella*, *Aquincola*, *Acidovorax*, and *Methylibium* had never been described in SNFPs after cultivation. The analysis method might explain the inability to detect these genera in the previously described SNFPs. These bacteria were identified in impure cultures. Phylopeptidomics allows indeed the identification of several genera when simple 16S rDNA sequencing requires pure bacterial cultures. The identification of *Ideonella* with metagenetics in an American SNFP [193] could confirm this hypothesis.

Even though several bacteria were isolated from this pool in 2015 and 2017, an important proportion of the strains were similar and belonged to the same species or genera (e.g. *Bacillus* sp., *Ralstonia pickettii*, *Sphingomonas* sp., *Pelomonas* sp.). The chosen media might have not selected original strains that could need more exotic medium. A greater diversity could be discovered use the use of several different and specific media.

***Table 4.2: Identification of microorganisms isolated in the pool from the 2015 (operating reactor) and 2017 (shutdown reactor) samplings.*** *In 2015, all strains were identified via sequencing. In 2017 the "SKH XXX" bacteria were isolated from the kinetic and identified using phylopeptidomics while the "CEA-NOB-XXX" were isolated from scrubs and identified using 16S rDNA sequencing.*

| Phylum / Class | Species | Strain | Sampling date |
|---|---|---|---|
| Actinobacteria | *Micrococcus luteus* | CEA-NOB-001 | 2015 |
| | *Micrococcus luteus* | CEA-NOB-002 | |
| | *Rhodococcus corynebacterioides* | CEA-NOB-004 | |
| | *Kocuria koreensis* | CEA-NOB-006 | |
| | *Leifsonia* sp. | CEA-NOB-010 | |
| | *Streptomyces caeruleatus* | CEA-NOB-011 | |
| | *Gordonia bronchialis* | CEA-NOB-012 | |
| | *Leifsonia shinshuensis* | CEA-NOB-013 | |
| | *Leifsonia* sp. | CEA-NOB-014 | |
| | *Rothia dentocariosa* | CEA-NOB-015 | |
| | *Rothia mucilaginosa* | CEA-NOB-017 | |
| | *Gordonia bronchialis* | CEA-NOB-032 | |
| Firmicutes | *Bacillus* sp. | CEA-NOB-003 | |
| | *Staphylococcus epidermidis* | CEA-NOB-005 | |
| | *Bacillus pumilus* | CEA-NOB-008 | |
| | *Streptococcus sanguinis* | CEA-NOB-016 | |
| | *Bacillus thuringiensis* | CEA-NOB-031 | |
| | *Brevibacillus agri* | CEA-NOB-032 | |
| | *Bacillus* sp. | CEA-NOB-033 | |
| | *Bacillus thuringiensis* | CEA-NOB-034 | |
| α-Proteobacteria | *Rhizobiales* sp. | CEA-NOB-007 | |
| γ-Proteobacteria | *Acinetobacter johnsonii* | CEA-NOB-009 | |
| Ascomycota | *Penicillium chrysogenum* | CEA-NOF-001 | |
| | *Penicillium chrysogenum* | CEA-NOF-002 | |
| | *Aspergillus nidulans* | CEA-NOF-003 | |
| | *Aspergillus nidulans* | CEA-NOF-004 | |
| | *Chaetomium globosum* | CEA-NOF-005 | |
| Actinobacteria | *Nocardia niigatensis* | CEA-NOB-020 | 2017 |
| | *Mycobacterium aubagnense* | CEA-NOB-021 | |
| | *Mycobacterium* sp. | CEA-NOB-022 | |
| | *Nocardia niigatensis* | CEA-NOB-030 | |
| | *Cellulomonas* sp. | SKH434 | |
| | *Cellulomonas* sp. | SKH444 | |
| Firmicutes | *Bacillus* sp. | SKH448 | |
| α-Proteobacteria | *Bradyrhizobium* sp. | CEA-NOB-023 | |
| | *Bradyrhizobium* sp. | CEA-NOB-025 | |
| | *Afipia* sp. | CEA-NOB-026 | |
| | *Sphingomonas echinoides* | CEA-NOB-027 | |
| | *Sphingomonas echinoides* | CEA-NOB-028 | |
| | *Sphingomonas* sp. | SKH154 | |
| | *Sphingomonas* sp. | SKH86 | |
| β-Proteobacteria | *Pelomonas* sp. | CEA-NOB-018 | |
| | *Pelomonas puraquae* | CEA-NOB-019 | |
| | *Pelomonas puraquae* | CEA-NOB-024 | |
| | *Pelomonas* sp. | SKH111 | |
| | *Pelomonas* sp. | SKH104 | |
| | *Ralstonia pickettii* | CEA-NOB-029 | |
| | *Ralstonia pickettii* | SKH163 | |
| γ-Proteobacteria | *Pantoea vagans* | SKH445 | |
| | *Pantoea* sp. | SKH497 | |
| | *Pantoea* sp. | SKH446 | |

***Table 4.3: Identification of microorganisms isolated in the pool from the 2017 (shutdown reactor) samplings.*** *This identification was performed with phylopeptidomics. Taxon ID represent the name of the closest identified strain in NCBI. The secondary organisms indicate the presence of several strains in the tested colony.*

| Code | Growing temperature | Medium | Kinetic point isolation | Main oraganism | Taxon ID | Secondary organisms | Taxon ID | Secondary organisms | Taxon ID | Secondary organisms | Taxon ID |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SKH448 | RT | R2A 1/2 | 3 months | *Bacillus* sp. | 1581712 or 1579346 | | | | | | |
| SKH451 | 37°C | R2A 1/2 | 3 months | *Bacillus* sp. | 1581712 or 1579346 | *Pantoea* sp. | 1347370 or 470934 | | | | |
| SKH444 | RT | BTR | 28 days | *Cellulomonas* sp. | / | | | | | | |
| SKH434 | RT | BSR | 28 days | *Cellulomonas* sp. | 1346710 | | | | | | |
| SKH497 | 37°C | LB 1/10 | 3 months | *Pantoea* sp. | 1526743 | | | | | | |
| SKH446 | 37°C | LB 1/10 | 3 months | *Pantoea* sp. | 470934 | *Pantoea* sp. | 1526743 | | | | |
| SKH450 | 37°C | R2A 1/2 | 3 months | *Pantoea vagans* | 470934 | | | | | | |
| SKH85 | RT | LB 1/10 | 3 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736434 | *Ideonella* sp. | 1547922 | | |
| SKH113 | RT | LB 1/10 | 14 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736430 | *Methylibium* sp. | 1437443 or 1430884 | | |
| SKH112 | RT | LB 1/10 | 14 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736430 | *Sphingomonas* sp. | 59803 | | |
| SKH111 | RT | LB 1/10 | 14 days | *Pelomonas* sp. | 1736464 | | | | | | |
| SKH107 | RT | LB 1/10 | 14 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736430 | *Acidovorax* sp. | 1144317 | | |
| SKH104 | RT | LB 1/10 | 14 days | *Pelomonas* sp. | 1736464 | | | | | | |
| SKH118 | RT | R2A 1/2 | 14 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736434 | *Ideonella* sp. | 1547922 | | |
| SKH158 | RT | R2A 1/2 | 28 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736434 | *Ideonella* sp. | 1547922 | *Ralstonia pickettii* | 329 |
| SKH153 | RT | LB 1/10 | 28 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736430 | *Ideonella* sp. | 1547922 | | |
| SKH152 | RT | LB 1/10 | 28 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736434 | *Ideonella* sp. | 1547922 | | |
| SKH151 | RT | LB 1/10 | 28 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736434 | *Ideonella* sp. | 1547922 | | |
| SKH150 | RT | LB 1/10 | 28 days | *Pelomonas* sp. | 1736464 | *Pelomonas* sp. | 1736430 | *Ideonella* sp. | 1547922 | | |
| SKH91 | 37°C | R2A 1/2 | 7 days | *Ralstonia pickettii* | 329 | | | | | | |
| SKH110 | RT | R2A 1/2 | 14 days | *Ralstonia pickettii* | 329 | | | | | | |
| SKH109 | RT | R2A 1/2 | 14 days | *Ralstonia pickettii* | 329 | *Pelomonas* sp. | 1736464 | *Ideonella* sp. | 1547922 | | |
| SKH117 | RT | R2A 1/2 | 14 days | *Ralstonia pickettii* | 329 | *Pelomonas* sp. | 1736430 | *Aquincola tertiaricarbonis* | 391953 | | |
| SKH116 | RT | R2A 1/2 | 14 days | *Ralstonia pickettii* | 329 | *Pelomonas* sp. | 1736430 | | | | |
| SKH115 | RT | R2A 1/2 | 14 days | *Ralstonia pickettii* | 329 | *Pelomonas* sp. | 1736430 | | | | |
| SKH174 | RT | R2A 1/2 | 28 days | *Ralstonia pickettii* | 329 | | | | | | |
| SKH171 | RT | R2A 1/2 | 28 days | *Ralstonia pickettii* | 329 | *Pelomonas* sp. | 1736464 | | | | |
| SKH167 | RT | R2A 1/2 | 28 days | *Ralstonia pickettii* | 329 | *Pelomonas* sp. | 1736430 | | | | |
| SKH163 | RT | R2A 1/2 | 28 days | *Ralstonia pickettii* | 329 | | | | | | |
| SKH160 | RT | R2A 1/2 | 28 days | *Ralstonia pickettii* | 329 | | | | | | |
| SKH87 | RT | LB 1/10 | 3 days | *Sphingomonas* sp. | 59803 | | | | | | |
| SKH106 | RT | LB 1/10 | 14 days | *Sphingomonas echinoides* | 59803 | *Sphingomonas* sp. | 1564113 | *Pelomonas* sp. | 1736529 or 1736580 | | |
| SKH105 | RT | LB 1/10 | 14 days | *Sphingomonas echinoides* | 59803 | *Sphingomonas* sp. | 1564113 | *Pelomonas* sp. | 1736529 or 1736580 | | |
| SKH156 | RT | LB 1/10 | 28 days | *Sphingomonas elodea* | 179878 | *Sphingomonas* sp. | 194867 | *Sphingomonas* sp. | 194870 | | |
| SKH155 | RT | LB 1/10 | 28 days | *Sphingomonas elodea* | 179878 | *Sphingomonas* sp. | 194867 | *Sphingomonas* sp. | 194870 | | |
| SKH154 | RT | LB 1/10 | 28 days | *Sphingomonas* sp. | 179878 | | | | | | |

### 4.3.2 Radioresistance screening

The ability to survive radiations has been assessed on the isolated bacteria (from both campaigns) which were able to grow in liquid media (27 bacteria tested). Two controls were used to assess the experimental procedure: one radiosensitive (*E. coli*) and the other highly radioresistant (*D. radiodurans*). Bacteria radioresistance was assessed using two different conditions: an irradiation in NaCl 0.9% to prevent important metabolic activity and an irradiation in liquid LB to allow a metabolic activity. Only bacteria presenting a growth on control plates (0 Gy) were analyzed. Some of the isolated bacteria did not present any growth during the liquid culture and some irradiated bacteria did not present any growth on the agar plate, even the non-irradiated ones (7 bacteria). This explained the number of tested bacteria, lower than the number of isolated bacteria. Only 20 bacteria were thus studied.

Some bacteria which did not exhibit growth on agar control plate (0 Gy) after irradiation in NaCl exhibited bacterial growth when irradiated in liquid medium. The opposite was also true.

#### *4.3.2.1 Irradiation in NaCl*

After an irradiation in NaCl 0.9% *Deinococcus radiodurans* (positive control) presented low lethality after a 1 kGy irradiation. *Escherichia coli* (negative control) presented no survival after 500 Gy irradiation (Fig. 4.1). Both controls thus confirmed the chosen irradiation conditions. Four bacterial groups could be observed after the irradiation in NaCl. The first group, containing two *Bacillus* sp. and *Micrococcus luteus*, could well resist a 500 Gy irradiation (more than 40% survival) and presented a great viability after 1 kGy (between 10 to 27.1%). The second group presented a great viability after a 200 Gy irradiation but presented a survivability between 7.9 and 16.1% at 500 Gy. After a 1 kGy irradiation the survivability of the strains was between 0.14 and 1.8%. Bacteria in this group were *Pantoea vagans*, *Brevibacillus agri*, two *Leifsonia* sp., and *Gordonia bronchialis*. The third group was composed of the two *Nocardia niigatensis*, *Kocuria koreensis* and *Pantoea* sp. This group presented between 24.2 to 34.3% survival after a 200 Gy irradiation but exhibited a viability smaller than 10% at 500 Gy (4.5 to 9.9% viability). At 1 kGy less than 2% of the cells were alive. The last group was composed of two *B. thuringiensis*, *Sphingomonas* sp., and *Ralstonia pickettii*. These bacteria presented no survival after a 500 Gy irradiation. The last group presented a similar survivability profile than *E. coli* and should thus not be considered as radioresistant (Fig. 4.1; Appendix 13, Table 6.21).

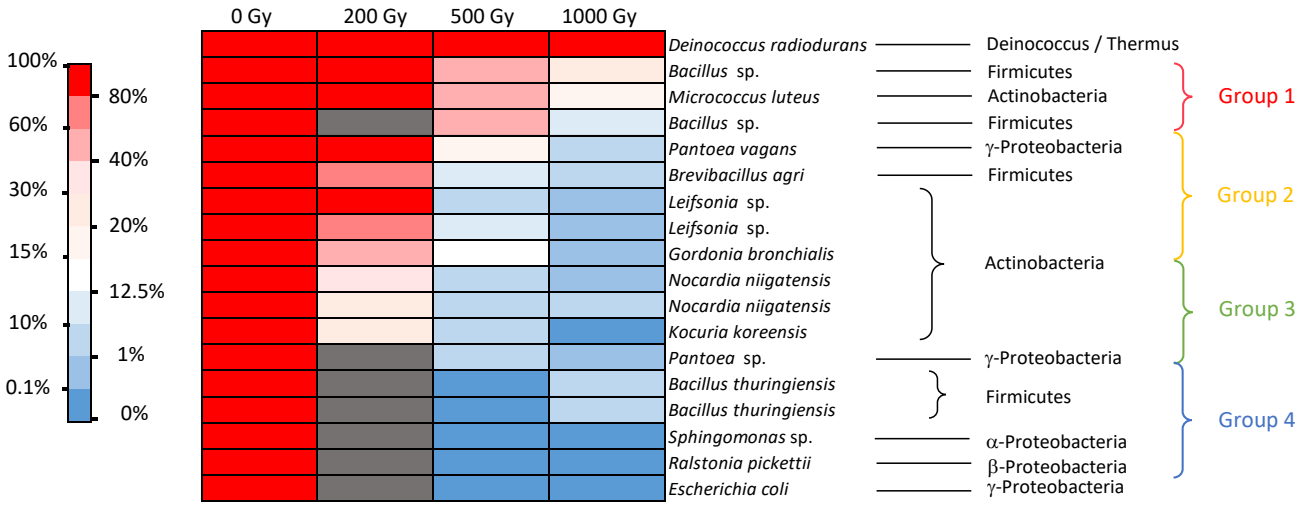



***Figure 4.1: Heat-map of the radioresistance of the isolated species in NaCl.*** *% of survival of the strains when compared to the control plate. In dark grey: no data available.*

### *4.3.2.2 Irradiation in LB medium*

Several bacteria could withstand a dose of 200 Gy while a smaller number could resist a 1 kGy irradiation (Fig. 4.2). From our results, four groups could be seen. The first one, a highly resistant one, which contained *Micrococcus luteus*, *Kocuria koreensis* and *Pantoea vagans* could well survive a 500 Gy irradiation (49.5 to 92.7%) and presented survival after a 1 kGy irradiation (3.7 to 37.2%) and beyond for *M. luteus*. The second group contained bacteria that could withstand a 500 Gy irradiation (2.1 to 14.4%) but presented no survival beyond this dose. The concerning bacteria were *Leifsonia* sp., *Kocuria koreensis*, two *Nocardia niigatensis*, *Brevibacillus agri*, and *Pantoea* sp. (two different strains). The third group survived well the 200 Gy irradiation (32.0 to 53.7%) but presented no growth after a 500 Gy irradiation and contained *Leifsonia* sp., *Godornia bronchialis*, *Bacillus pumilus*, and *Bacillus* sp. (two strains). Finally the last group contained *Pelomonas* sp., which exhibited a similar survival than *E. coli* and can thus be considered as radiosensitive (Fig. 4.2; Appendix 13, Table 6.22).

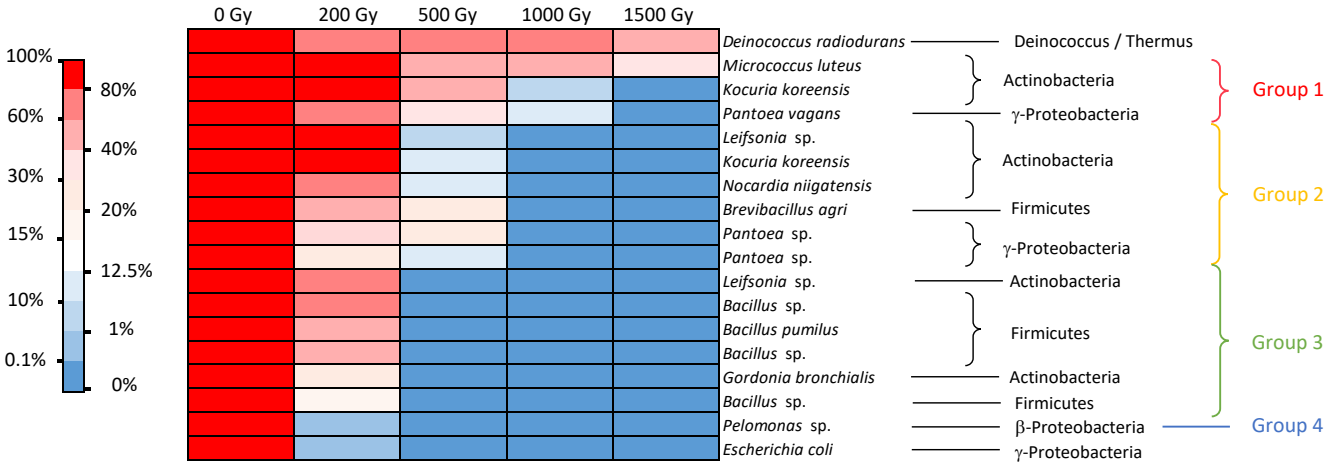



***Figure 4.2: Heat-map of the radioresistance of the isolated species in LB.*** *% of survival of the strains when compared to the control plate.*

It should be noted that, in the tested condition, while *E. coli* did not resist a dose higher than 200 Gy and presents high mortality at 200 Gy (less than 1% survival), *D. radiodurans* did not present a complete survival started from 200 Gy. This bacterium is known to survive up to 5 kGy irradiation without any mutation nor lethal effect when put in the optimum conditions [47]. However it was the bacterium that could best withstand the 1.5 kGy irradiation. These results showed that in these screening conditions the chosen protocol was adapted for a screening, but did not present the optimum conditions of survival for the strains. It is possible that, in the optimum conditions, the ability to resist radiations could be higher. Moreover this experiment only showed the survival after an acute irradiation. For a chronic irradiation it is possible that the dose received could be higher than 1.5 kGy and cells could survive this dose.

The irradiation in LB or NaCl did not change the survivability of *E. coli*. The irradiation of *D. radiodurans* was more lethal when it occurred in LB media than in NaCl. Several bacteria were influenced by the irradiation medium (liquid LB or NaCl). Seven strains presented a different survivability depending on the condition. *M. luteus* and *K. koreensis* had their resistance increased when the irradiation occurred in LB. The five other strains (*Leifsonia* sp., *G. bronchialis*, *B. agri*, and two *Bacillus* sp.) all presented a higher radioresistance after a NaCl irradiation (Appendix 13). It is possible that an irradiation in liquid media allowed the immediate induction of DNA repair and ROS scavenging mechanisms for *M. luteus* and *K. koreensis*. Having an active metabolism for these strains during irradiation might thus be an advantage. Concerning the five other strains the irradiation in LB was more lethal. One possible explanation could be the presence of an active metabolism due to the presence of nutritive environments, potentially enhancing the damages. In NaCl an almost inactive metabolism could have prevented damages, making them more repairable after cultivation. This phenomenon was observed by Hu et *al*. in 2005. *Vibrio parahaemolyticus* was irradiated in phosphate-buffered saline (PBS) or in oyster, where it can naturally be found. Its $LD_{50}$ (dose needed to eliminate 50% of a population) was slightly lower in oyster (0.57 kGy) than in PBS (0.60 kGy) [405]. This result is in contradiction with the study performed by Farag et al. in 1990. A *Listeria monocytogenes* strain was irradiated in TSB and phosphate buffer. $D_{10}$ for this strain was about 0.18 kGy in phosphate buffer and 0.21 kGy in TSB [406]. Both studies could explain the differences observed between the irradiation of our strains in NaCl or LB. They could also prove that each strain can react differently depending on the irradiation conditions. One strain could exhibit better survivability in NaCl or PBS when another strain will prefer irradiation in liquid broth.

No data concerning ionizing radiations of the species *Brevibacillus agri* and the genus *Brevibacillus* were previously found. In the literature, *M. luteus* was shown to survive a 1 kGy irradiation [177], *Kocuria koreensis* could resist an UV irradiation of 1 kGy [407], the genus *Leifsonia* was already found in similar environment (spent nuclear fuel pool) [192], while *Bacillus* possesses species that can be UV resistant [379]. The two tested *Bacillus* exhibited no cell viability a 500 Gy but presented some bacterial growth after a 1 kGy irradiation in NaCl. It might have been caused by the presence of resistant bacterial spores during irradiation [408-410]. *Pantoea* genus also possesses species (*P. agglomerans*) able to resist to a gamma-radiations [411], while the *Nocardia* genus contains species found in spent nuclear fuel pool [181, 184, 185]. This genus can also survive up to a 3 kGy irradiation, maybe due to its ability to sporulate [170]. The species *Gordonia bronchialis* was also found in a spent nuclear fuel pool in Spain [181, 184].

### 4.3.3 Bioaccumulation screening

Bacteria exhibiting radioresistance greater than *E. coli* (radiosentive control) were tested for radionuclides accumulation. Among them, highly radioresistant strains as well as strains never described for U or Co accumulation were selected.

#### *4.3.3.1 Uranium accumulation*

##### 4.3.3.1.1 Conditions screening

The uranium accumulation of the different strains was higher for all tested strains after 24 hours than 1 hour (Table 4.4). Some of the tested bacteria appeared to present a great accumulation capacity (*e.g.* *Leifsonia* sp. with a maximum accumulation at 457±50 nmol/$10^9$ cells, *Bacillus* sp. 448 at 247±55 nmol/$10^9$ cells, and *Kocuria koreensis* at 211±20 nmol/$10^9$ cells). Some duplicate presented a huge discrepancy between each other. For example, after 1 hour in presence of 200 µM of uranium, *M. luteus* presented a 10 fold difference between the two duplicates. For *K. koreensis* at 200 µM one duplicate accumulated 81.0 nmol for $10^9$ cells while the other one accumulated 20.7 nmol for $10^9$ cells. When looking at the uranium recovered in each fraction it can be noticed that some samples presented a dosed uranium higher than the uranium introduced while others exhibited some uranium loss compared to the quantity of uranium introduced and measured by ICP-MS (Appendix 14, Tables 6.24 & 6.25). Due to technical ICP-MS issues not all data could have been re-analyzed. The samples to re-analyze are highlighted in green in Appendices 14 and 15. At 20 and 200 µM cellular death was observed when compared with negative control and 5 µM (Appendix 14, Table 6.23).

***Table 4.4: Uranium accumulation for $10^9$ cells in nmol.*** *Preliminary results on the six tested bacteria.*

| | | ***Micrococcus luteus* 002** | ***Kocuria koreensis* 006** | ***Leifsonia* sp. 010** | ***Bacillus* sp. 033** | ***Bacillus* sp. 448** | ***Pantoea vagans* 450** |
|---|---|---|---|---|---|---|---|
| 1h00 | **5 µM - 1** | 8,64 | 14,52 | 25,76 | 10,56 | 1,82 | 4,31 |
| | **5 µM - 2** | 9,41 | 18,50 | 38,62 | 11,87 | 4,94 | 4,56 |
| | **20 µM - 1** | 33,90 | 18,32 | 38,11 | 12,55 | 49,88 | 18,06 |
| | **20 µM - 2** | 26,16 | 23,88 | 94,74 | 36,50 | 43,21 | 20,34 |
| | **200 µM - 1** | 38,13 | 80,98 | 72,60 | 55,66 | 81,93 | 96,33 |
| | **200 µM - 2** | 3,65 | 20,67 | 84,39 | 60,16 | 84,27 | 25,89 |
| 24h | **5 µM - 1** | 17,61 | 25,88 | 15,33 | 21,09 | 25,15 | 12,85 |
| | **5 µM - 2** | 13,72 | 29,91 | 12,93 | 30,73 | 19,44 | 12,72 |
| | **20 µM - 1** | 4,80 | 252,60 | 375,55 | 82,87 | 85,14 | 73,90 |
| | **20 µM - 2** | 5,08 | 116,26 | 301,89 | 83,60 | 78,65 | 77,94 |
| | **200 µM - 1** | 16,08 | 191,80 | 506,88 | 147,55 | 191,83 | 113,18 |
| | **200 µM - 2** | 50,21 | 231,87 | 407,76 | 133,17 | 303,11 | 93,98 |

In light of the results obtained with this screening conditions, a 24 hours accumulation was selected along with the 5 – 50 and 500 µM uranium concentrations. Direct cell viability was not tested.

##### 4.3.3.1.2 Uranium accumulation

Considering results obtained with the screening experiment, the incubation time with uranium was set at 24 hours.

After 24 hours of exposure to three different concentrations of uranium, its quantity in each fraction (pellet, supernatant, washes) was assessed (Appendix 14, Table 6.27). Almost no uranium was found in the supernatant and in the washes for the lower concentrations for some bacteria. *Kocuria koreensis* presented almost no uranium in the supernatant nor washes for the 5 – 10 and 50 µM concentrations. SKH 448 (*Bacillus* sp.) presented this pattern too. At the 5 µM concentration six bacteria almost entirely accumulated the uranium present in the medium (*Micrococcus luteus* 002, *Kocuria koreensis* 006, *Brevibacillus agri* 032, *Bacillus* sp. 033, *Bacillus* sp. 448 and *Pantoea* sp. 497) (Appendix 14, Table 6.27).

Concerning the accumulation of uranium, not all bacteria presented the same profile. Some bacteria presented indeed a high accumulation profile while the others accumulated less. For most bacteria the accumulation was higher at 500 µM. *N. niigatensis* 030, the two *Pantoea* strains and *Gordonia bronchialis* did not present a higher accumulation at 500 µM. *Bacillus* sp. 448 and *Brevibacillus agri* 032 both accumulated more than 650 nmol for $10^9$ cells at 500 µM. These two bacteria were the most accumulating bacteria at 500 µM. In presence of 1000 µM of uranium *B*. *agri* 032 was the only strain able to accumulate more than 200 nmol of U for $10^9$ cells. All bacteria presented a decrease in uranium accumulation at 1000 µM, suggesting this dose was too high and killed most of the cells (Fig. 4.3). The two *Bacillus* strains did not accumulate uranium with a same profile. *Bacillus* sp. 448 was the bacteria which accumulated the most uranium while *Bacillus* sp. 033 accumulated similarly than other strains such as *Micrococcus luteus* 002 or *Kocuria koreensis* 006. *Pantoea vagans* 450 exhibited an accumulation plateau below 100 nmol for $10^9$ cells at 500 and 1000 µM. This strain did thus not well survive a uranium concentration higher than 50 µM (Fig. 4.3). In the preliminary results accumulation of circa 100 nmol for $10^9$ cells was detected at 200 µM for this strain. At this dose *Pantoea vagans* 450 seemed to have lost cell viability since the accumulation was close to the ones found at 500 and 1000 µM. This observation was confirmed with direct cell viability for which only 23% of the bacteria were alive at 200 µM (Table 4.4; Appendix 14, Table 6.23).

The less accumulating strains were *Pantoea* sp. 497, *Gordonia bronchialis* 012, *Leifsonia* sp. 010, and both *Nocardia niigatensis*. Despite the low accumulation profile of the latter strains they can be considered as uranium accumulating strains. All strains, except for *N. niigatensis* 030, were indeed able to accumulate more than 100 nmol of uranium for $10^9$ cells (Fig. 4.3; Appendix 14, Table 6.27).

Beyond 500 µM of uranium the accumulation of *K. koreensis* 006 seemed to reach a plateau since more uranium were recovered after the washes (Appendix 14, Table 6.27).

A decrease in uranium accumulation can be observed at 1000 µM. This decrease can be explained by a cell mortality. It was indeed observed that a decrease in cell viability occurred at 20 and 200 µM during the preliminary screening (Appendix 14, Table 6.23).

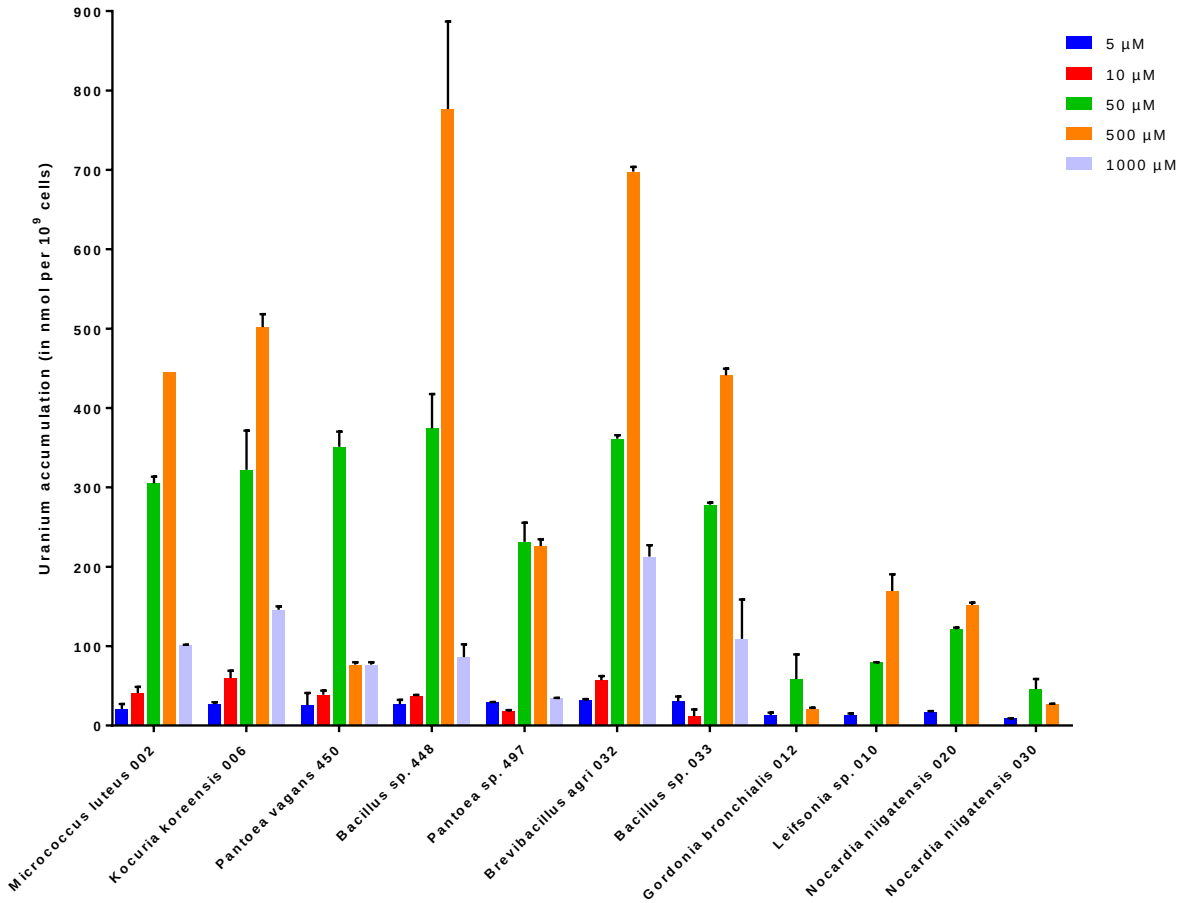



*Figure 4.3: Uranium accumulation at different concentration of 11 bacterial strains isolated from a nuclear reactor pool.*

No data is available in the literature concerning the interactions between *Gordonia* genus and uranium. Genus *Gordonia* is widely distributed in aquatic and terrestrial habitats [347]. *G. bronchialis* can be an opportunistic pathogen for human [412].

The genus *Brevibacillus* was found in a uranium contaminated site in the USA and was shown to reduce uranium [413]. No other information are available concerning this genus with uranium.

No data concerning the accumulation of uranium by *Kocuria koreensis* was found in the literature. This species was isolated in 2010 which could explain the absence of study. However the species *Kocuria erythromyxa* could adsorb 68% of a 0.1 µM $^{235}U$ solution. This strain was also able to adsorb plutonium [414, 415]. For these studies, no washes were performed. No data concerning uranium accumulation of *Nocardia niigatensis* is available in the literature. However *N. erythopolis* was able to adsorb 1.56 mmol/g dry weight [416].

The species *M. luteus* was proven to adsorb uranium. It was shown that it could adsorb up to 2.60 mmol/g dry weight [416]. In another study about 163 µmol/g of cells were adsorbed by *M. luteus*. It was also shown that a saturation occurred after 30 min at about 150 µmol/g of cells [389]. In all studies concerning the uranium accumulation by *M. luteus*, no washes were performed. Moreover the authors described the adsorption of uranium and not its absorption or accumulation. It is then highly probable that uranium is

adsorb by the cell wall. This hypothesis can be confirmed by the presence of significant uranium in the washes of our *M. luteus* 002 strain (Appendix 14, Table 6.27).

Different species and strains of *Bacillus* sp. were able to adsorb high amounts of uranium [417, 418]. Both vegetative and spore forms were able to adsorb uranium (up to 200-225 mg/g dry cells for vegetative form and 275-300 mg/g dry cells for spores). At least half of the uranium was recovered when washing the cells with EDTA/TRIS 0.01 M. The uranium was thus mainly present on the cell walls [418]. *B. subtilis* was able to adsorb up to 90% of uranium in a 1000 ppm (1 g/L or about 4 mM) in a pH 4.9 solution [419]. Moreover three *Bacillus* species (*B. licheniformis*, *B. megaterium*, and *B. subtilis*) could adsorb uranium from 159 to 220 µmol/g of cells [389]. In our study the two *Bacillus* strains were able to highly accumulate uranium, or, at least, strongly bind uranium on the cell's walls. This hypothesis is consistent with the analysis of uranium complexes formed at the surface of two different *Bacillus* species performed by Hening et *al*. (2011). The bacteria were washed three times with NaCl 0.9% and their uranium complexes were studied with an extended X-ray absorption fine structure spectroscopy. The authors discovered that uranium was strongly bound to phosphoryl residues on the cell surface [420]. *B. vallismortis* was able to biosorb (no wash performed) up to 100% of a 20 µM uranium solution [421].

Members of the *Pantoea* genus were able to accumulate uranium. *P. agglomerans* was proved to biosorb 81% of a 0.1 µM $^{235}U$ solution as well as other radionuclides [415]. However no wash was performed in this study and adsorption on the cell surface was probable. An important adsorption could be observed in our study where uranium was recovered in the washes (Appendix 14, Table 6.27). In a study, after 24 hours of contact, about 80 mg/g of cells was accumulated by *Pantoea* sp. TW18. This accumulation was obtained after three washes of the cells with distilled water [422].

One strain of *Leifsonia* was shown to remove about 68% of a solution of uranium at different concentration (8.4 to 126.05 µM). This strain was coated with biochar to enhance the adsorption of uranium with this strain. With this coating the bacterium was able to remove 99.82% of a 42 µM uranium solution [423].

#### *4.3.3.2 Cobalt accumulation*

After 24 hours of exposure to three different concentrations of cobalt, its quantity in each fraction (pellet, supernatant, washes) was assessed (Appendix 15, Table 6.29). Unlike uranium, the tested strains did not accumulate cobalt to large extents. Three strains did not accumulate any cobalt at any dose. These strains were *Bacillus* sp. 448 and 033, and *Brevibacillus agri*. *Kocuria koreensis* was the most accumulating strain with about 11 nmol of cobalt accumulated by $10^9$ cells. These strains could accumulate between 450 and 750 nmol of uranium for $10^9$ cells. The strains *Gordonia bronchialis*, *Nocardia niigatensis* 020, *Pantoea vagans*, and *Micrococcus luteus* accumulated cobalt in smaller proportion than *K. koreensis*. The latter strains accumulated cobalt at all tested concentrations. The strain *Pantoea* sp. 497 accumulated small quantities of cobalt at 30 and 300 µM (less than 0.5 nmol for $10^9$ cells). The strain *Leifsonia* sp. 010 presented no accumulation at 3 and 30 µM while about 0.5 nmol of cobalt were accumulated by $10^9$ cells at 300 µM. The strain *N. niigatensis* 030 presented an accumulation of about 0.8 nmol for $10^9$ cells only at 300 µM (Fig. 4.5; Appendix 15, Table 6.29).

The fraction containing the higher quantity of cobalt was the supernatant. A few was recovered in the washes, mainly at 300 µM (Appendix 15, Table 4.29). This indicated that not only the cobalt was not accumulated by the bacteria but it was not adsorbed or weakly fixed on the cells' surface. Cobalt is involved

in metabolism as an enzyme cofactor [394, 424]. It is thus naturally present in small quantity in bacterial cells and regulated. Due to cobalt toxicity at high levels transports to eliminate cobalt exists in order to maintain cobalt homeostasis [394, 425, 426]. The cobalt role in cell metabolism could explain the absence of cobalt accumulation in the tested strains. Furthermore bacteria low metabolism induced by NaCl could have prevent cobalt to enter the cell.

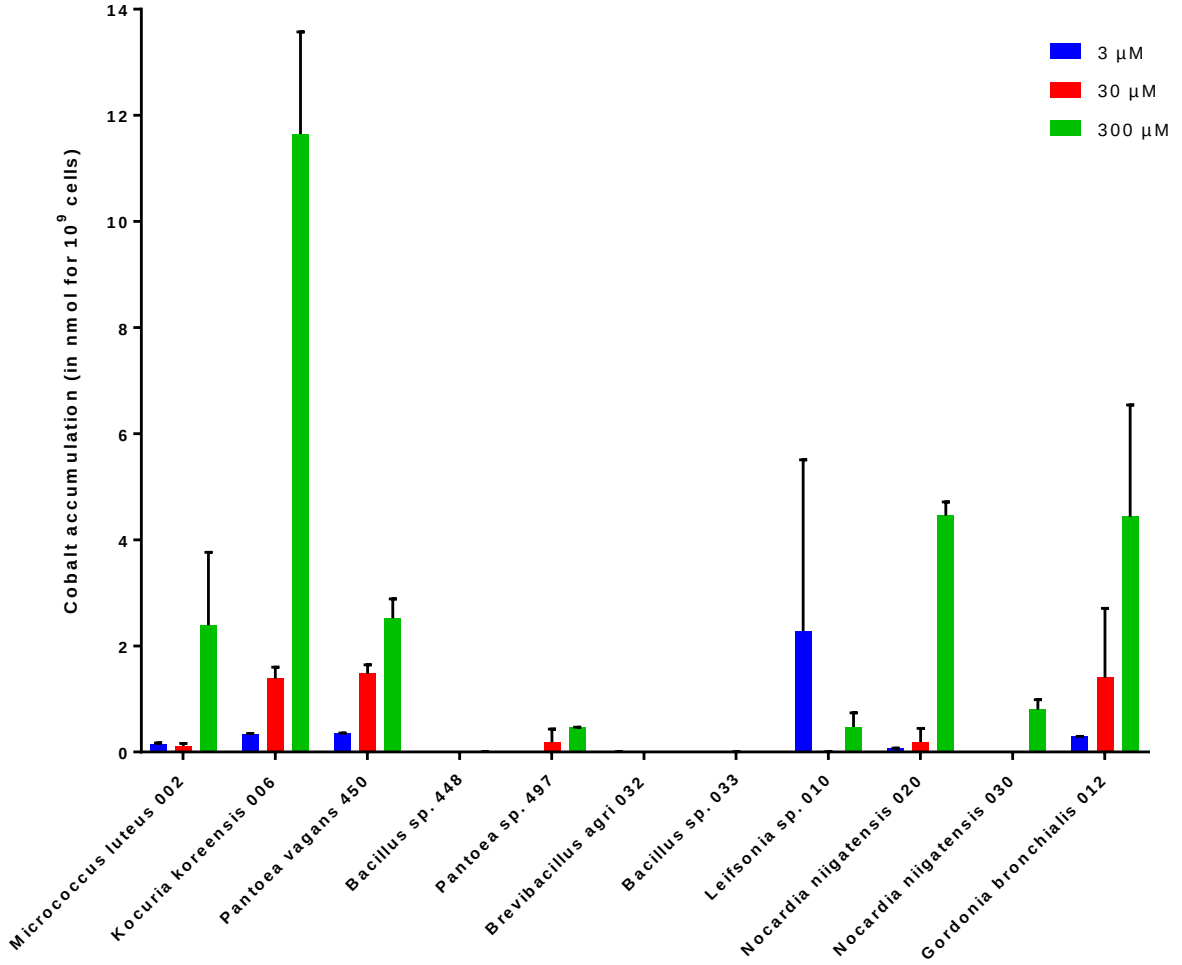



*Figure 4.4: Cobalt accumulation at different concentration of 11 bacterial strains isolated from a nuclear reactor pool.*

No data is available in literature concerning the interactions between the genera *Pantoea*, *Brevibacillus*, *Nocardia*, *Leifsonia*, and *Gordonia* with cobalt. The only found information concerning cobalt accumulation of the genus *Kocuria* concerned *K. palustris*. This species was able to accumulate cobalt at about 17 µmol/g dry weight [152].

A *M. luteus* strain isolated in a Slovak SNFP was able to accumulate cobalt at about 90 µmol/g dry weight [152]. Another *M. luteus* strain, isolated from an Algerian wastewater, could accumulate 12.8% (pH 5) to 79.4% (pH 8.5) of a 1.69 µM cobalt solution. At pH 5.5 this strain could accumulate 18.22% of the same solution [427]. Another *Micrococcus* species, *M. lylae*, was able to accumulate or adsorb up to 23 mg/g dry weight [388].

*Bacillus pumilus* was proved to be able to accumulate or adsorb cobalt up to 40 mg/g dry weight while *B. cereus*, in the same conditions, could only accumulate 6.5 mg/g dry weight [388]. Several strains of *Bacillus*

isolated from drain waters of a German uranium waste pile were able to partially accumulate cobalt. The tested vegetative cells were able to sorb up to 40% of a 0.7 µM cobalt solution when spores of the same strains were able to sorb up to 20% of the same solution. An extraction with 0.01 M EDTA-Tris was performed to assess the accumulation and adsorption proportion. In the vegetative cells, more than 50% of the sorbed cobalt was extracted while none was extracted in the spores [428]. These *Bacillus* strains were thus able to slightly accumulate cobalt but could adsorb it on their surface. Several *Bacillus* strains isolated from Indian uranium mine wastes were able to accumulate cobalt up to 150 µmol/g dry weight. However some of the *Bacillus* tested strains did not present any accumulation [429]. *B. lichenformis* was able to adsorb cobalt up to 20% of a 0.5 mM cobalt solution. Electron microscopic showed that cobalt was accumulated at the surface wall [430]. Our two *Bacillus* strains did not present any cobalt accumulation.

## 4.4 Conclusion

Many bacteria and some fungi were isolated from Pool 2 at shutdown and during operation. Most of the tested bacteria presented a radioresistance ability at 200 Gy. Some of them could even resist 1 kGy and beyond. The bacteria isolated during the reactor operation were among the most radioresistant ones. The different irradiation conditions slightly changed the bacterial ability to resist radiations during the screening. It should thus be remembered that these screenings did not represent the optimal irradiation conditions but allowed to quickly select resistant strains and eliminate sensitive strains. In LB irradiation, *M. luteus* 002 $D_{10}$ (dose needed to eliminate 90% of a population) was above 1.5 kGy. SKH 450 (*Pantoea vagans*) presented a $D_{10}$ between 1 and 1.5 kGy when *Pantoea* sp. 497 was between 500 and 1000 Gy. Both *Nocardia niigatensis* strains had a $D_{10}$ close to 500 Gy. *K. koreensis* had a $D_{10}$ between 500 and 1000 Gy. The other strains $D_{10}$ was between 200 and 500 Gy, except for *Pelomonas* sp. where the $D_{10}$ was below 200 Gy.

These bacteria were not only able to resist radiations but could also highly accumulate uranium. Some of them were able to almost entirely remove uranium for the solution at 5 µM (*Micrococcus luteus* 002, *Kocuria koreensis* 006, *Brevibacillus agri* 032, *Bacillus* sp. 033, *Bacillus* sp. 448 and *Pantoea* sp. 497) while only *K. koreensis* and *Bacillus* sp. 448 present presented almost no uranium in supernatant and washes at 50 µM. However at higher concentrations the ability to remove uranium from the medium decreased. *Bacillus* sp. 448 was the strain presenting the higher uranium accumulation.

Some bacteria isolated from this pool are described for the first time in such environment, however several isolated bacteria from this pool had already been described in several SNFPs around the world [152, 181, 184-186, 188, 189, 192].

A deeper study of *Micrococcus luteus* 002, *Kocuria koreensis* 006, *Brevibacillus agri* 032, *Bacillus* sp. 033, *Bacillus* sp. 448 and *Pantoea* sp. 497 could be considered to highlight accumulation mechanisms. Concerning cobalt accumulation, those strains were not adapted to solely decontaminate SNFPs effluent. They could be used as an additional process but other microorganisms were proved to be more efficient.

Several genera studied in this work had never been described concerning their ability to resist radiations or accumulate radionuclides. This work was the first description of uranium and cobalt accumulation for one and five genera respectively.

# Chapter V:

## *General Discussion, Conclusions, and Perspectives*

# 5. General discussion, conclusions, and perspectives

## 5.1 General discussion

### 5.1.1 The use of different analysis methods to highlight microbial diversity

To be able to identify microorganisms from a water sample, metagenetics and direct metaproteomics were used. In Chapter II, it was shown that the use of direct phylopeptidomics was possible to identify the main microbial genera present in a sample. The analysis of a biofilm recovered using sterile Spontex® enabled the identification of three main genera, all bacterial. With the identification of peptides related to one micro-algae (*Phaeodactylum*) along with peptides associated to two bacterial genera (*Acidovorax* and *Xanthobacter*) in a water sample, it was possible to identity in the same run prokaryotes and eukaryotes (Chapter III). This technique presented thus an advantage compared to metagenetics for which it is indeed possible to identify both prokaryotes and eukaryotes (with 16S rDNA, and 18S rDNA plus ITS sequencing) during the same run, but it is complicated and results were not guaranteed (personal information from LGC Genomics). However phylopeptidomics exhibits some disadvantages: first of all the sensitivity of mass spectrometers are lower than sequencing technologies; secondly the protein databases are less exhaustive than genetic databases. This innovative approach is thus promising to identify microorganisms present in a sample. When exploring biodiversity present in extreme environment using metagenetics, the information obtained via sequencing should be of high confidence. Unreliable sequences should thus be removed. It is known that biases can occur during DNA extraction, PCR and sequencing [236]. To be able to trust the results, *i.e.* lowering the false-positive rate, a cut-off should be proposed. This cut-off should be the perfect equilibrium between removing all sequences absent from the original sample (false-positives) but not removing sequences present in small quantities (false negatives). Despite extensive metagenetics published works, most of them present the metagenetics results without any cut-off after bioinformatics analysis using mothur or MG-RAST for example [193, 231, 296]. In one study, to be sure to eliminate false-positive, only taxa with an abundance greater than 2% were retained and presented [253]. However when setting a cut-off as high as 2%, the risk of removing true positives, *i.e.* interesting sequences obtained from the sample, is important. Mohd Shaufi et *al*. decided to remove rare OTUs with less than five sequences in all tested samples [431]. The issue of a cut-off to be able to trust results obtained from gut microbiota of clinical samples was assessed by White et *al*. [432]. To do so, they developed a statistical method to compare clinical metagenomic samples from two treatment populations on the basis of count data obtained via sequencing. However this method relies on p-values and does not give a cut-off if only one sample was sequenced [432]. In Chapter II a 0.016% cut-off was determined after an Illumina MiSeq sequencing of the regions V4V5 of the 16S rDNA from a DNA standard and an OCToPUS analysis. This cut-off was low enough to keep all genera present in the DNA standard but eliminated sequences belonging to bacteria not present in the DNA standard. With this cut-off we can be confident about the results presented in this work: no bacteria that were not present in the water samples could have been identified. However, another standard should be used for such analysis but does not yet exist. This standard should cover a large extent of dynamic range in terms of concentrations of microorganisms. Here, the standard used is a commercial standard that does not encompass sufficient diversity and lacks an important dynamic range. For example, no bacteria are present in very low concentrations near the established cut-off. Thus our results deserve to be further complemented with additional experiments.

We have also checked whether microbial cultures in liquid diluted media could be used for enrichment of some microorganisms for further isolation. We have monitored the biodiversity over a liquid kinetic and

have shown that the diversity is relatively large and even, while a microbial stability settled over time. While only the fraction of cultivable microorganisms can then be assessed, the data are worth to consider as biotechnological relevant microorganisms should be cultivable for being used.

All three methods highlighted the presence of unique genera that are in small quantities (*e.g*. *Ideonella*, *Mehtylibium* or *Rhizobacter* genera for the consortia cultivation; the *Acidobacteria* and *Verrucomicrobia* phyla, *Nitrospira*, *Vampirovibrio* genera for metagenetics; *Sporolactobacillus* and *Scytonema* genera for direct phylopeptidomics). These three methods were thus complementary for the microbial water analysis.

### 5.1.2 Microbial diversity in nuclear cooling pools

Two different pools were studied over time during this PhD. Pool 1 is used to store radioactive cobalt sources while Pool 2 is the cooling pool of a nuclear reactor. Several samplings were realized in these pools. The prevailing conditions in the pools had changed between two samplings. For Pool 1, the filtration system changed and the pool was cleaned a few months before the third sampling. For Pool 2 the first sampling was performed during the last operating cycle while the second sampling was realized a year after the reactor's definitive shutdown. Several observations were possible from these results.

From the different campaigns, between 0.5 to 4.0 million cells were used for DNA extraction to analyze water samples. No cell count could have been performed on the biofilms due to the small amount of biomass collected and the radioactivity in the biofilms collected in Pool 2. However, when looking at the saturation curves of all samples, it can be observed that a plateau was reached for each sample, indicating that the sequencing allowed a good inventory of the bacterial species present in the water.

- **Identification of a large extent of microbial diversity**

Using all three methods previously described and multiplying the samples of biofilms and waters, some diversity was observed in both pools. While a majority of bacteria was identified, several fungi and algae were also found. A total of 379 genera was identified in both pools. Compared to previous studies in which microorganisms have been isolated after a cultivation step (up to 56 isolated bacteria in Cofrentes) [152, 177, 181, 183-188, 192], this number can be judged as really important. However in a recent study performed in an American SNFP (Savanah River Site) using direct metagenetics, about 4000 OTUs were identified. This difference can be explained by the cell density in the water. Between $1.8x10^6$ to $2.8x10^8$ cells per liter had been identified in the USA SNFP [193], while 2 to $4.5x10^6$ cells per liter had been identified in Pool 1, and 0.75 to $2.7x10^6$ cells per liter had been identified in Pool 2. In our studies a maximum of 4 L were used to perform the metagenetics analysis. At the Savanah River Site SNFP no indication was given concerning the volume sampled. It was however specified that a peristaltic pump had been used. It is possible that more than 4 L had been sampled for metagenetics analysis.

A majority of *Proteobacteria* (205 genera), and more generally a majority of Gram negative bacteria were identified (about 283 genera) in both pools (136 and 163 genera belonging to the *Proteobacteria* for Pool 1 and Pool 2 respectively; 169 and 189 Gram negative bacteria in Pool 1 and Pool 2 respectively). This result is in accordance with a previous description of similar environment (SNFPs) where, mainly Gram negative bacteria had been isolated [185]. Similarly about 83% of *Proteobacteria* and 10% of *Acidobacteria* (Gram negative) were identified at the Savanah River Site SNFP [193].

Only 19 genera were always identified in both pools at both time point (2015 and 2017). This could imply that microorganisms can be metabolically active under these conditions and these pools are not just a place where airborne bacteria fall. Among these genera a majority of *Proteobacteria* was identified (14 genera), while 3 and 2 genera belonged to *Firmicutes* and *Actinobacteria* phyla respectively (Appendix 16). If both pools were to be compared, 88 genera would only be found in Pool 1 (no matter the sampling date) and 169 would only be found in Pool 2. 123 genera were common to both pools. The majority of the identified *Cyanobacteria* were found in Pool 1. At least two algae were also present in this pool, identified using direct metaproteomics. The "Bacillariophyta_unclassified" designation obtained using metagenetics could highlight the presence of algae, detected through their chloroplastic DNA [433]. It has indeed been hypothesized that chloroplast were *Cyanobacteria* after endocytosis [336, 337]. The preservation of 16S rDNA in photosynthetic eukaryotes could explain the presence of the *Bacillariophyta* family in Pool 1, dominating almost all samples in the 2015 sampling. Moreover proteins closely related to two microalgae (*Phaeodactylum* and *Thalassiosira*) were identified in this pool in the 2016 sampling before the filtration system change whereas from the 2015 water sampling one green microalgae, *Eustigmatos vischeri*, was isolated. *Phaeodactylum triconatum* exhibited a great resistance to a 0.16 $W.m^{-2}$ UV-B irradiation at the long term (after 7 days) and an enhanced UV-absorbing compounds and carotenoid production was observed (2.5 fold and 1.5 fold for UV-absorbing compounds and carotenoid respectively) [434]. *Thalassiosira pseudonana* presented some antioxidant response in presence of UVB with an increase of superoxide dismutase activity, involved in reactive oxygen species scavenging [435]. It could thus be possible that algal strains belonging to *Phaeodactylum* or *Thalassiosira* genera were present in Pool 1, despite the presence of ionizing radiations.

The majority of identified fungi were present during Pool 2 kinetics of 2015 and 2017. Fungi are able to form spores and can thus survive in hostile environment until better conditions are found. All *Chlamydiae* bacteria were identified in Pool 2 in 2017. *Planctomycetes* and *Chloroflexi* phyla were identified in both pools. However only the *Planctomycetes* phylum had already been described in a SNFP [193]. The conditions in Pool 2 in 2017 were less stringent than in 2015. The 2015 sampling was performed during a working nuclear cycle while the sampling in 2017 was performed more than a year after the complete shutdown of the reactor. The radioactivity was thus lower and less radionuclides were present in the water in 2017.

Despite the presence of great diversity, direct analysis highlighted the lack of evenness mainly in Pool 2 water. One OTU indeed represented at least 90% of the sample both in 2015 (*Variovorax*) and 2017 (*Methylobacterium*). In Pool 2 scrubs, the evenness was greater despite a lower diversity. For the 2017 sampling in Pool 1 the water was dominated by *Limnobacter* (21.1 to 76.6%). The stringent conditions prevailing in both pools might explain this observed domination.

All three genera belong to the *Proteobacteria* phylum. *Methylobacterium* is an $\alpha$-*Proteobacteria* while *Variovorax* and *Limnobacter* belong to the $\beta$-*Proteobacteria*, and more specifically to the *Burkholderial*es order.

The *Methylobacterium* genus is ubiquitous in nature, and can be found in freshwater and aquatic sediments [343]. This genus constitutes common airborne organisms which are found in industrial environments. *Methylobacterium* species are aerobic, chemoorganotrophic, and mesophilic bacteria [315]. A *Methylobacterium* strain has recently been detected in a spent nuclear fuel storage basin at the Savannah River Site in the USA [193]. The ability of this genus to resist a certain degree of desiccation, leading to oxidative stress, contributes to their survival in hostile environments [315]. Several mechanisms involved

in desiccation and gamma radiations resistance are indeed common. Some strains have been shown to exhibit resistance to gamma irradiation 10–40 times higher than that withstood by many other Gram-negative bacteria [315, 344, 345]. One species (*Methylobacterium* sp. strain rad) was even isolated after irradiation of 20 kGy [346], and *M. extorquens* and *M. radiotolerans* presented a $D_{10}$ of 4.5 and 3.5 kGy, respectively [346]. *M. extorquens* produces carotenoids [436], which are involved in oxidative stress defense [62-64]. The domination of this genus (between 60 and 97% of the samples) in Pool 2 in 2017 is thus not a surprise considering its ability to resist radiations and its presence in an American SNFP.

The genus *Variovorax* is aerobic and can be found in soils and water. It can contain carotenoid pigments, which are known to be antioxidant molecules (cf. Chapter I.4.1.2.3). This genus was predominant during the reactor operation (65 to 85% of the samples). It was also identified in the SNFP of Cofrentes after a cultivation step [184], but no *Variovorax* was identified in the Savanah River Site SNFP [193]. The genus *Variovorax* has also been identified in a uranium contaminated soil in China [437]. The ability of some *Variovorax* strains (biotype I) to make use of hydrogen oxidation as an energy source [315, 350] might explains the prevalence of this genus during operation. High amounts of $H_2$ are indeed formed due to water radiolysis when a nuclear reactor is operating.

The genus *Limnobacter* was first described in 2001 by Spring et *al*. with the identification of *L. thiooxidans*, a thiosulfate oxidizing bacteria [438]. *Limnobacter* species have been isolated from lake sediments, a volcanic deposit, Baltic Sea and soils [438-441]. Up to this day, only aerobic species have been described in this genus. This genus has never been described in the different SNFPs analyzed, whether it was with cultivation step or using metagenetics. Strains closely related to the *Limnobacter* genus were identified in an acid mine drainage contaminated with several heavy metals [371]. After a genome sequencing of *Limnobacter* sp. MED105, a putative protein involved in the bacterial oxidative stress defense system was potentially identified (protein A6GUX4) (https://www.uniprot.org/uniprot/A6GUX4). The ability to oxidize thiosulfate associated with the presence of a putative antioxidant molecule, and the identification of this genus in an acid mine drainage contaminated with heavy metals could be indications that the *Limnobacter* genus possesses performant mechanisms involved in resistance to oxidative stress.

The microbial composition of Pool 1 was different from the microbiota of other SNFPs and from Pool 2. The presence of *Bacillariophyta* (up to 60%) was unique and never described in similar environment. Moreover no *Cyanobacteria* were found in pools from nuclear facilities over the world. However this pool does not contain radionuclides unlike the SNFPs studied over the world. These results indicated a dependence of the microbiota on the conditions prevailing in the pool. Each pool has its unique microbiota and a pool can present a change in the microbiota when one parameter is changed.

Several OTUs identified with metagenetics were not described to the genus taxonomic level (142 OTUs and 71 OTUs for pools 1 and 2 respectively with a 0.016% cut-off) and several “Bacteria_unclassified” were present in the pools (25 OTUs and 7 OTUs for pools 1 and 2 respectively with a 0.016% cut-off). The diversity could thus be even greater.

- **Differences observed in a same pool**

Despite differences observed between Pool 1 and Pool 2, differences in the microbiota were observed between two sampling date. In Pool 1, between the 2015 and 2016 samplings and the 2017 sampling, the pool was deeply cleaned and a change in the filtration condition occurred. Before the change, several

*Cyanobacteria* were present and represented most of the samples. After the change a majority of *Proteobacteria* was observed and *Cyanobacteria* represented less than 2% of the samples. Few differences were observed between the sampling depths and locations.

Among the main bacteria identified in Pool 2 biofilms, *Clostridium* bacteria are able to form biofilms [442, 443]. It is known that microorganisms present in biofilms can benefit from each other, leading to a cell community in which each bacteria can help another one [16]. The interactions and advantages of bacteria to grow as biofilms is thus known and have been described in Chapter I.3.1. The advantage of life in a biofilm can be numerous compared to planktonic life: both aerobic and anaerobic bacteria can be found in biofilms, it can concentrate nutrients and locally change the pH [20]. The extracellular matrix can also provide a protection against physical and chemical stresses [16, 17, 21, 444, 445].

It is known that some *Variovorax* strains are able to use $H_2$ oxidation as an energy source [315, 350]. Water radiolysis leads to the formation, among others, of $H_2$. When the reactor was operating, $H_2$ was abundantly present in water due to radiolysis. In 2015 the dominance of *Variovorax* might have been possible thanks to $H_2$ and this bacterium ability to use $H_2$ as an energy source. When the reactor was definitively shutdown, the radiation diminished and thus the level of $H_2$ decreased too. Other genera, not competitive enough during reactor functioning, might have been better suited to the new conditions present in the pool during shutdown. This could explain the completely loss of *Variovorax* in 2017 to the profit of *Methylobacterium*.

This change in the microbiota was not only noticed via direct analysis (metagenetics) but also via the liquid culture kinetics of the water. Indeed, while $\beta$-*Proteobacteria* was the main observed class during the 2017 culture kinetic, $\alpha$-*Proteobacteria* dominated the 2015 kinetic. This could indicate that not only the main genera were different but also the minor genera, present in quantities too low to be detected by metagenetics. A deep change in the microbiota was thus influenced by the shutdown of the nuclear reactor. However the presence of the same *Firmicutes* and *Actinobacteria* genera (*Bacillus*, *Clostridium*, *Streptomyces*, *Nocardia*, etc.) may indicate the survivability of these organisms in a radiative environment, maybe present in the water in spore shape.

In Pool 2 the microbiota was completely different if the sample originated from a biofilm or a water sampling. Differences were also observed between the two sampling dates (2015: operating reactor, or 2017: shutdown of the reactor for more than a year). These differences can be easily observed in Figure 5.1, representing the non-metric multidimensional scaling (NMDS) of the different samplings from Pool 2. Three groups can be observed in this NMDS: the biofilms (in blue), the 2015 water sampling (in orange) and the 2017 water sampling (in grey). The biofilms were mainly composed of *Clostridium* bacteria. In 2015 water samples were dominated by *Variovorax* while in 2017 water was dominated by *Methylobacterium*.

*Clostridium* are anaerobic *Firmicutes* but some species can tolerate oxygen [358]. *C. subterminale* and *C. celerecrescens* had been found in a SNFP in the USA [188]. *Clostridium* spores were proved to resist a dose up to 8.5 kGy, and for some strain, up to 14 kGy [359, 360]. It was shown also that some *Clostridium* strains were able to reduce uranium [362]. The genus *Clostridium* is usually chemoorganotrophic, but some species can be chemoautotrophic [358]. For example, it was shown that a strain of *C. difficile* was able to grow with $CO_2$ and $H_2$ as energy source [446]. *C. perfringens* was proved to possess an adaptive response to oxidative stress (induced by $H_2O_2$), both in aerobic and anaerobic conditions [447]. *Clostridium* genus can thus resist oxidative stress, induced by $H_2O_2$ or by ionizing radiations. Moreover strains able to use $CO_2$ and $H_2$ as energy source can confer this genus an advantage in nuclear pool.

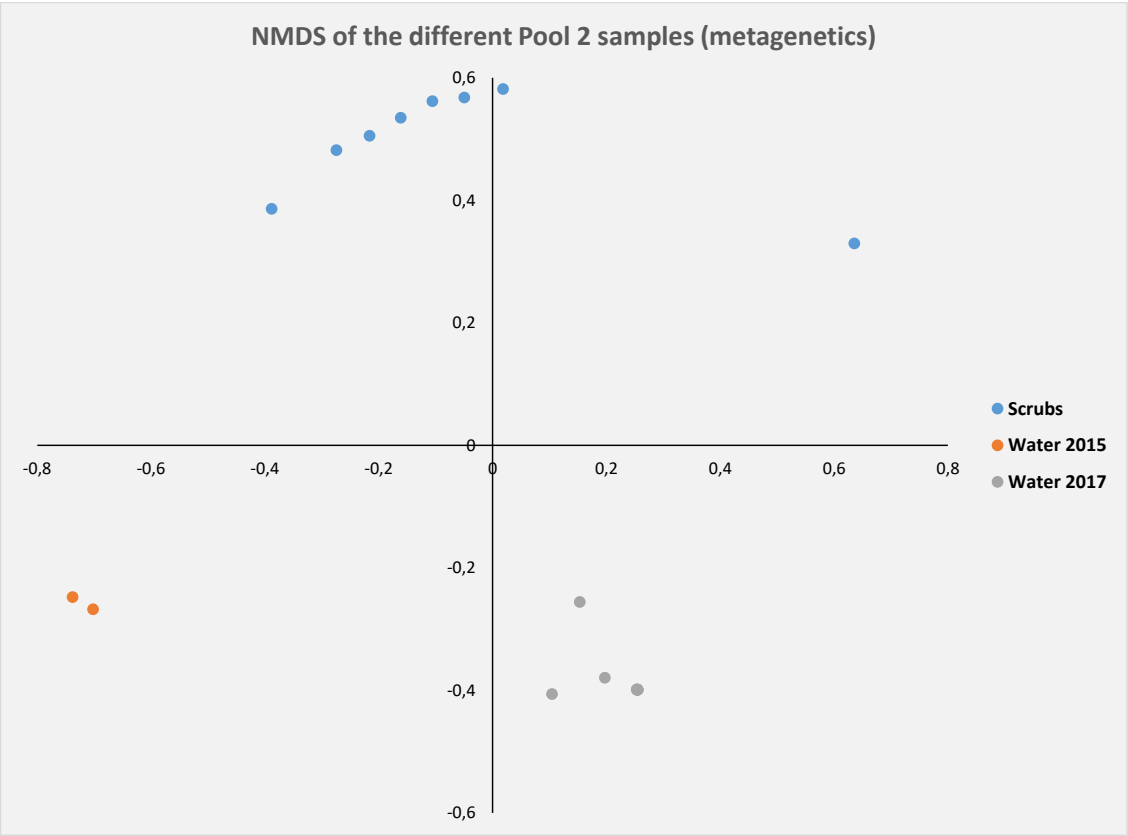



***Figure 5.1 : Non-metric multidimensional scaling of the different samplings from Pool 2.*** *The results from the scrubs (blue) and the water analysis from 2015 (orange) and 2017 (grey) were obtained using the metagenetics results.*

Oxygen diffusion is limited in a biofilm and is rapidly used by aerobic species, which can lead to an increase of facultative and obligate anaerobic bacteria [448]. The presence of *Clostridium* [449, 450], and more generally anaerobic and sulfate-reducing bacteria (SRB) [448], in the biofilms could lead to Microbial Induced Corrosion (MIC) [23, 363, 364]. Sulfate-reducing bacteria (*e.g. Desulfovibrio* genus) can produce sulfide, a corrosive compound. Acid-producing bacteria (*e.g. Thiobacillus* spp. can also provoke corrosion. Acids produced by microorganisms able to produce an exopolysaccharide matrix are concentrated at the metal surface, thus leading to corrosion of the metal [363]. It was also shown that sulfate-reducing bacteria can use acids produced by acid-producing bacteria as nutrients. SRB can thus proliferate at sites of corrosion due to the acid production of acid-producing bacteria [451]. Among the bacteria isolated from a corroding metal, *Sphingomonas capsulate* and *Methylobacterium* sp. have been identified [452]. In the scrubs recovered from Pool 2, two OTUs belonging to *Sphingomonas* genus and two OTUs belonging to *Methylobacterium* genus have been identified after metagenetics analysis in scrubs 2 to 5 and 8 for *Sphingomonas*, and scrubs 3 and 7 for *Methylobacterium*. The identification of genera described in MIC should be a concern since it can corrupt the walls' integrity and change the pool's conditions if an old fuel bundle is pierced, freeing radionuclides such as uranium or transuranium elements. This study highlighted the need of nuclear installation operators to regularly monitor the microbial community from their installations and regularly clean the pools. MIC has also been evaluated in a French nuclear waste underground disposal [453]. In this study, it was shown that high corrosion rates could be caused by

biooxidation of pyrites under aerobic conditions. Under anaerobic conditions, bacteria may lead to a localized corrosion at a longer term [453].

- **Several microorganisms never described in similar environment**

The use of three methods to identify microorganisms present in nuclear cooling pools enabled the identification of hundreds of genera, several of them never described in similar environments (*e.g. Xanthobacter*, *Caulobacter*, *Erythrobacter, Methylibium, Acidovorax, Blastomonas*, *Mycetocola* and *Streptomyces* genera). The identification of *Chloroflexi* in both pools might be the first description of this phylum in a radiative environment.

The isolation of these strains should be interesting and could help understanding the mechanisms implemented by the cell to resist the harsh conditions prevailing in both pools. Among the isolated microorganisms, no unknown genera were identified. All bacteria were identified at the genus or species level. Several organisms, after a 16S rDNA nucleotide identification using the BLAST tool from NCBI, were matched to undescribed but referenced bacteria.

### 5.1.3 Radioresistance and radionuclides accumulation of bacteria isolated from Pool 2

To identify bacteria able to accumulate radionuclides and thus resist radiations, only bacteria isolated in Pool 2 were studied. Pool 2 is indeed a nuclear reactor cooling pool (ionizing radiations and radionuclides) while Pool 1 is used to store radioactive cobalt sources (ionizing radiations near the sources and no radionuclides). The probability of finding bacteria able to accumulate radionuclides is thus higher in Pool 2 than in Pool 1.

Many bacteria and some fungi were isolated from Pool 2 at shutdown and during operation. Most of the tested bacteria presented a radioresistance ability at 200 Gy. Some of them could even resist 1 kGy and beyond (*e.g*. *Micrococcus luteus* CEA-NOB-002, *Kocuria koreensis* CEA-NOB-006, *Leifsonia* sp. CEA-NOB-010, *Pantoea vagans* SKH 497). The bacteria isolated during the reactor operation were among the most radioresistant ones. The different irradiation conditions slightly changed the bacterial ability to resist radiations during the screening. It should thus be remembered that these screenings did not represent the optimal irradiation conditions but allowed to quickly select resistant strains and eliminate sensitive strains. In LB irradiation, *M. luteus* 002 $D_{10}$ was above 1.5 kGy. SKH 450 (*Pantoea vagans*) presented a $D_{10}$ between 1 and 1.5 kGy when *Pantoea* sp. 497 was between 500 and 1000 Gy. Both *Nocardia niigatensis* strains had a $D_{10}$ close to 500 Gy. *K. koreensis* had a $D_{10}$ between 500 and 1000 Gy. The other strains $D_{10}$ was between 200 and 500 Gy, except for *Pelomonas* sp. where the $D_{10}$ was below 200 Gy.

Different radioresistant bacteria have been isolated in the SNFP of the Idaho National Engineering and Environmental Laboratory. All isolated bacteria resisted a 50 Gy irradiation dose while only *Corynebacterium aquaticum*, *Pseudomonas putida*, *Comamonas acidovorans*, *Gluconobacter cerinus*, *Micrococcus diversus*, *Rhodococcus rhodochrous* could resist a 600 Gy irradiation [177]. Radioresistant bacteria have also been isolated from the Cofrentes SNFP (Spain). Strains belonging to the *Stenotrophomonas*, *Mycobacterium*, *Nocardia*, *Burkholderia*, *Ralstonia*, and *Staphylococcus* genera presented $D_{10}$ values of about 1.4 kGy [184].

Bacteria isolated from Pool 2 were not only able to resist radiations but could also highly accumulate uranium. Some of them were able to almost entirely remove uranium for the solution at 5 µM (*Micrococcus luteus* 002, *Kocuria koreensis* 006, *Brevibacillus agri* 032, *Bacillus* sp. 033, *Bacillus* sp. 448 and *Pantoea* sp. 497) while only *K. koreensis* and *Bacillus* sp. 448 presented almost no uranium in supernatant and washes at 10 and 50 µM. However at higher concentrations the ability to remove uranium from the medium decreased. *Bacillus* sp. 448 was the strain presenting the higher uranium accumulation. The seven strains able to remove almost all uranium from a 5 µM solution and the two strains able to remove uranium from a 50 µM solution could be used in biotechnologies. Their ability is indeed of great interest to decontaminate diluted uranium from a large volume of effluent.

These strains did not accumulate cobalt. The cobalt concentrations might have been too important and killed the strains. However the chosen concentrations were used for other bacterial strains which exhibited accumulation and thus survival [152, 388, 430, 454]. Another possibility could be changing the incubation time. To be able to determine whether these strains could accumulate cobalt or not a kinetic with several time points (*e.g.* 1 hour, 12 hours, 24 hours) should be tested.

Radionuclides accumulating bacteria have previously been described in a Slovak SNFP: *Kocuria palustris*, *Micrococcus luteus*, and *Ochrobactrum* sp. were proved to accumulate Cs and Co after 48 and 24h of incubation respectively [152]. The Slovak isolated *M. luteus* strain was able to accumulate cobalt (up to 12.2 $\mu mol.g^{-1}$ dry weight) while the *M. luteus* strain isolated from Pool 2 did not present cobalt accumulation. This implies that the cobalt accumulation is not a characteristic of all *M. luteus* strains, unlike radioresistance. The latter characteristic was indeed identified in a *M. luteus* strain isolated in an American SNFP by Bruhn et *al*. and presented a $D_{10}$ above 1 kGy [177] which is comparable with the $D_{10}$ of the *M. luteus* isolated from Pool 2 (about 1.5 kGy).

In another study by Sarro et *al*., radionuclides accumulation properties were tested. It was proved that most of $^{60}Co$ was biosorbed and not bioaccumulate by the biofilms [184]. To develop a biotechnological process in order to decontaminate nuclear effluent, the sorption of radionuclides on biofilm matrix should be considered and could be used.

Our isolated strains allowed a great uranium decontamination at low uranium concentration after 24 hours. A deeper study of *Micrococcus luteus* 002, *Kocuria koreensis* 006, *Brevibacillus agri* 032, *Bacillus* sp. 033, *Bacillus* sp. 448 and *Pantoea* sp. 497 could be considered to highlight accumulation mechanisms. Concerning cobalt accumulation those strains were not adapted to solely decontaminate SNFPs effluent. They could be used as an additional process but other microorganisms were proved to be more efficient.

Several genera studied in this work had never been described concerning their ability to resist radiations or accumulate radionuclides. This work was the first description of uranium and cobalt accumulation for one (*Gordonia*) and five genera respectively (*Pantoea*, *Brevibacillus*, *Nocardia*, *Leifsonia*, and *Gordonia*).

## 5.2 General conclusions

Great diversity can be found in SNPFs and the cooling pool of nuclear reactors, both at shutdown and in operation. This PhD highlighted the presence of microbial diversity in aquatic nuclear environments. Several microorganisms identified in both pools (Pool 1 and Pool 2) were not identified in previous studies concerning microorganisms present in different SNFPs around the world. It was shown that each pool possesses its own microbiota depending on the physico-chemical conditions. However some microbial

families and genera were common to both pools and every SNFPs, such as *Bacillus* or *Sphingomonas*. The use of direct methods (phylopeptidomics and metagenetics) emphasized the presence of different phyla undescribed in such environments before. As previously noticed [152, 177, 181-188, 191-193] a majority of *Proteobacteria* and more generally a majority of Gram negative bacteria were identified in the studied pool. The conditions prevailing in Pool 1 (no radionuclides, natural light) enabled the growth of photosynthetic organisms. This diversity was detected using several methods. The combine use of three different complementing analysis methods allowed a complete overview of microbial diversity present in an environmental sample. Direct use of phylopeptidomics enabled eukaryotes and prokaryotes identification in a same run. Despite a lower sensitivity compared to metagenetics, phylopeptidomics was able to identify organisms (*e.g. Streptomyces*) which were not detected using metagenetics. We were also able to isolate and identify different bacteria and fungi. The isolation of radioresistant bacteria as well as uranium accumulating bacteria in Pool 2 proved that the implemented protocol can be used to identify and isolate radioresistant and radionuclides accumulating organisms, confirmed with the previous study of Tišáková et *al*. in which bacteria isolated from a SNFP were able to accumulate cobalt and cesium [152].

## 5.3 Perspectives

Despite the identification of different radioresistant bacteria able to accumulate uranium, none of them exhibited great cobalt accumulation abilities. Several methods could be employed to isolate more radioresistant and cobalt accumulating strains. First of all a radiative selection pressure could be used to isolate more radioresistant organisms. Using this approach some unknown organisms could be discovered. Secondly a cobalt selection pressure (*e.g*. cultivation in medium containing bioavailable cobalt) could be applied to water and biofilm samples. This method could be used to isolate resistant microorganisms to several radionuclides of industrial interest.

With the identification of hyperaccumulating strains it would be possible to develop a nuclear effluent treatment process to biodecontaminate radionuclides. A deep study of the optimum conditions to accumulate radionuclides as well as acquire knowledge about the cells behavior. If no strain exhibit the wanted properties (different radionuclides accumulation), a co-culture with different strains able to abundantly accumulate one or two different radionuclides could be implemented.

Finally, the identification of radioresistant and accumulating strains could be useful to highlight new resistance mechanisms implemented by strains. A complete genome sequencing and annotation should allow us to identify proteins and pathways involved in radioresistance and radionuclides accumulation. As seen in I.4.1.2 different pathways exist to counter the negative effect of ROS production due to ionizing radiations. Hence, by identifying genes involved in radioresistance and/or radionuclides accumulation, some strains could be bioengineered to develop an optimized biodecontamination process. To do so a deeper strain study should be performed to identify the optimum cultures conditions. Molecular tools should exist or be implemented to bioengineer these strains.

The main discovery of this PhD thesis is related to the identification of *Variovorax* in the pool of a functioning nuclear reactor. Our hypothesis linking the high abundance of this genus and the metabolism of hydrogen produced by the reactor should be explored and confirmed with specific experiments. Moreover, a deeper metaproteomics analysis of these samples could be done in order to explore the possible metabolism pathways. This discovery calls for novel investigation of microorganisms in functioning

nuclear facilities and confirmation of the predominance of *Variovorax* or other hydrogen-metabolism active microorganisms in such facilities. Finally, interesting analysis of the metabolic interactions between microorganisms could be done to identify the corresponding molecular mechanisms and understand the development of consortia and biofilm formation in such extreme environments.

# References


1. Woese, C.R. and G.E. Fox, *Phylogenetic structure of the prokaryotic domain: The primary kingdoms.* Proc. Natl. Acad. Sci. USA, 1977. **74**(11): p. 5088-5090.
2. Ciccarelli, F.D., et al., *Toward Automatic Reconstruction of a Highly Resolved Tree of Life.* Science, 2006. **311**: p. 1283-1287.
3. Whitman, W.B., D.C. Coleman, and W.J. Wiebe, *Prokaryotes: The unseen majority.* Proc. Natl. Acad. Sci. USA, 1998. **95**: p. 6578-6583.
4. Dodd, M.S., et al., *Evidence for early life in Earth's oldest hydrothermal vent precipitates.* Nature, 2017. **543**(7643): p. 60-64.
5. Bell, E.A., et al., *Potentially biogenic carbon preserved in a 4.1 billion-year-old zircon.* PNAS, 2015. **112**(47): p. 14518–14521.
6. Mojzsis, S.J., et al., *Evidence for life on Earth before 3,800 million years ago.* Nature, 1996. **384**: p. 55-59.
7. Knauth, L.P., *Origin and diagenesis of cherts: an isotopic perspective.* Isotopic signatures and sedimentary records, 1992. **43**: p. 123–152.
8. Lunine, J.I., *Physical conditions on the early Earth.* Philos Trans R Soc Lond B Biol Sci, 2006. **361**(1474): p. 1721-31.
9. Rothschild, L.J. and R.L. Mancinelli, *Life in extreme environments.* Nature, 2001. **409**: p. 1092-1101.
10. Pikuta, E.V., R.B. Hoover, and J. Tang, *Microbial extremophiles at the limits of life.* Crit Rev Microbiol, 2007. **33**(3): p. 183-209.
11. Rinke, C., et al., *Insights into the phylogeny and coding potential of microbial dark matter.* Nature, 2013. **499**(7459): p. 431-7.
12. Kay, C.M., A. Haanela, and D.B. Johnson, *Microorganisms in subterranean acidic waters within Europe's deepest metal mine.* Res Microbiol, 2014. **165**(9): p. 705-12.
13. Jolivet, E., et al., *Thermococcus gammatolerans sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation.* Int J Syst Evol Microbiol, 2003. **53**(Pt 3): p. 847-51.
14. Anderson, A.W., et al., *Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation.* Food Technology, 1956. **10**(1): p. 575-577.
15. Brock, T.D., *Thermophilic Microorganisms and Life at High Temperatures*. 1978, New-York: Springer-Verlag.
16. Costerton, J.W., et al., *Microbial Biofilms.* Annual Review of Microbiology, 1995. **49**: p. 711-745.
17. Stewart, P.S. and J. William Costerton, *Antibiotic resistance of bacteria in biofilms.* The Lancet, 2001. **358**(9276): p. 135-138.
18. Leclerc, H., *Relationships between common water bacteria and pathogens in drinking-water.* Heterotrophic Plate Counts and Drinking-water Safety. London: IWA Publishing, 2003: p. 80-118.
19. Geesey, G.G., et al., *Sessile bacteria: An important component of the microbial population in small mountain streams.* Limnology and Oceanography, 1978. **23**(6): p. 1214-1223.
20. Costerton, J.W., et al., *Biofilms, the Customized Microniche.* Journal of Bacteriology, 1994. **176**(8): p. 2137-2142.
21. Costerton, J.W., et al., *Bacterial biofilms in nature and disease.* Annu. Rev. Microbiol., 1987. **41**: p. 435-464.
22. Bozorg, A., I.D. Gates, and A. Sen, *Impact of biofilm on bacterial transport and deposition in porous media.* J Contam Hydrol, 2015. **183**: p. 109-20.
23. Borenstein, S., *Microbiologically Influenced Corrosion Handbook*, W.P. Ltd., Editor. 1994: Abington, Cambridge.
24. Oliver, J.D., *The viable but nonculturable state and cellular resuscitation.* Microbial Biosystems: New Frontiers, 2000: p. 723-730.
25. Oliver, J.D., *The Viable but Nonculturable State in Bacteria.* The Microbiological Society of Korea, 2005. **43**: p. 93-100.
26. Oliver, J.D., *Recent findings on the viable but nonculturable state in pathogenic bacteria.* FEMS Microbiology Reviews, 2010. **34**(4): p. 415-425.
27. Murrell, W.G. and W.J. Scott, *The Heat Resistance of Bacterial Spores at Various Water Activities* J. gen. Microbiol., 1966. **43**: p. 411-425.
28. Gould, G.W. and G.J. Dring, *Heat resistance of bacterial endospores and concept of an expanded osmoregulatory cortex.* Nature, 1975. **258**: p. 402-405.

29. Gould, G.W., *Recent Advances in the Understanding of Resistance and Dormancy in Bacterial Spores.* Journal of Applied Bacteriology, 1977. **42**: p. 297-309.
30. Horneck, G., H. Biicker, and G. Reitz, *Long-Term Survival of Bacterial Spores in Space.* Adv. Space Res. , 1994. **14**(10): p. 1041-1045.
31. Horneck, G., et al., *Resistance of bacterial endospores to outer space for planetary protection purposes--experiment PROTECT of the EXPOSE-E mission.* Astrobiology, 2012. **12**(5): p. 445-56.
32. Hutchison, E.A., D.A. Miller, and E.R. Angert, *Sporulation in Bacteria: Beyond the Standard Model.* Microbiol Spectr, 2014. **2**(5).
33. Leggett, M.J., et al., *Bacterial spore structures and their protective role in biocide resistance.* J Appl Microbiol, 2012. **113**(3): p. 485-98.
34. Xu, H.-S., et al., *Survival and viability of nonculturable Escherichia coli and Vibrio cholerae in the estuarine and marine environment.* Microbial Ecology, 1982. **8**(4).
35. Trevors, J.T., et al., *Bacterial gene expression at low temperatures.* Extremophiles, 2012. **16**(2): p. 167-76.
36. Stokell, J.R. and T.R. Steck, *Viable but Nonculturable Bacteria.* Encyclopedia of Life Sciences, 2012.
37. Sutherland, B.M., et al., *Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation.* PNAS, 2000. **97**(1): p. 103-108.
38. Gulston, M., et al., *Clustered DNA damage induced by γ radiation in human fibroblasts (HF19), hamster (V79-4) cells and plasmid DNA is revealed as Fpg and Nth sensitive sites.* Nucleic Acids Research, 2002. **30**(15): p. 3464-3472.
39. Ghosal, D., et al., *How radiation kills cells: survival of Deinococcus radiodurans and Shewanella oneidensis under oxidative stress.* FEMS Microbiol Rev, 2005. **29**(2): p. 361-75.
40. Azzam, E.I., J.P. Jay-Gerin, and D. Pain, *Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury.* Cancer Lett, 2012. **327**(1-2): p. 48-60.
41. Marnett, L.J., *Lipid peroxidation—DNA damage by malondialdehyde.* Mutation Research, 1999. **424**: p. 83-95.
42. Luxford, C., et al., *Histone H1- and other protein- and amino acid-hydroperoxides can give rise to free radicals which oxidize DNA.* Biochem. J. , 1999. **344**: p. 124-134.
43. Luxford, C., R.T. Dean, and M.J. Davies, *Radicals Derived from Histone Hydroperoxides Damage Nucleobases in RNA and DNA.* Chemical Research in Toxicology, 2000. **13**(7): p. 665-672.
44. Davies, M.J., *Protein oxidation and peroxidation.* Biochemical Journal, 2016. **473**(7): p. 805-825.
45. Decker, H. and K.E. van Holde, *Oxygen and the Evolution of Life*, in *Oxygen and the Evolution of Life*, Springer-Verlag, Editor. 2011, Springer-Verlag: Berlin. p. 43-59.
46. Halliwell, B. and J.M.C. Gutteridge, *Free Radicals in Biology & Medicine*. Fifth ed, ed. O.U. Press. 2015, New-York, USA: Oxford University Press.
47. Slade, D. and M. Radman, *Oxidative stress resistance in Deinococcus radiodurans.* Microbiol Mol Biol Rev, 2011. **75**(1): p. 133-91.
48. Toueille, M., et al., *A comparative proteomic approach to better define Deinococcus nucleoid specificities.* J Proteomics, 2012. **75**(9): p. 2588-600.
49. Latour, J.-M., *Manganese, the stress reliever.* Metallomics, 2015. **7**: p. 25-28.
50. Gomoiu, I., et al., *Fungal Spores Viability on the International Space Station.* Orig Life Evol Biosph, 2016. **46**(4): p. 403-418.
51. Aguileta, G., et al., *Lower prevalence but similar fitness in a parasitic fungus at higher radiation levels near Chernobyl.* Mol Ecol, 2016. **25**(14): p. 3370-83.
52. Zhdanova, N.N., et al., *Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor.* Mycological Research, 2000. **104**(12): p. 1421-1426.
53. Pacelli, C., et al., *Melanin is effective in protecting fast and slow growing fungi from various types of ionizing radiation.* Environmental Microbiology, 2017. **19**(4): p. 1612-1624.
54. Dadachova, E. and A. Casadevall, *Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin.* Curr Opin Microbiol, 2008. **11**(6): p. 525-31.
55. Butler, M.J. and A.W. Day, *Fungal melanins: a review.* Can. J. Microbiol. , 1998. **44**: p. 1115-1136.
56. Prabagaran, S.R., et al., *Marinomonas ushuaiensis sp. nov., isolated from coastal sea water in Ushuaia, Argentina, sub-Antarctica.* Int J Syst Evol Microbiol, 2005. **55**(Pt 1): p. 309-13.
57. Selbmann, L., et al., *Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert.* STUDIES IN MYCOLOGY, 2005(51): p. 1-32.
58. Alekhova, T.A., et al., *Monitoring of Microbial Degraders in Manned Space Station.* Applied Biochemistry and Microbiology, 2005. **41**(4): p. 382-389.

59. Nosanchuk, J.D. and A. Casadevall, *The contribution of melanin to microbial pathogenesis.* Cellular Microbiology, 2003. **5**(4): p. 203-223.
60. Sichel, G., et al., *In vitro scavenger activity of some flavonoids and melanins against $O_2^{-\bullet}$* Free Radical Biology and Medicine, 1991. **1**(11): p. 1-8.
61. Gan, E.V., H.F. Haberman, and I. Aravindakshan Menon, *Electron transfer properties of melanin.* 1976. **173**(2): p. 666-672.
62. Ordenes-Aenishanslins, N., et al., *Pigments from UV-resistant Antarctic bacteria as photosensitizers in Dye Sensitized Solar Cells.* J Photochem Photobiol B, 2016. **162**: p. 707-14.
63. Tian, B., et al., *Evaluation of the antioxidant effects of carotenoids from Deinococcus radiodurans through targeted mutagenesis, chemiluminescence, and DNA damage analyses.* Biochim Biophys Acta, 2007. **1770**(6): p. 902-11.
64. Tian, B., et al., *Effects of carotenoids from Deinococcus radiodurans on protein oxidation.* Lett Appl Microbiol, 2009. **49**(6): p. 689-94.
65. Osawa, A., et al., *Characterization and Antioxidative Activities of Rare $C_{50}$ Carotenoids-Sarcinaxanthin, Sarcinaxanthin Monoglucoside, and Sarcinaxanthin Diglucoside-Obtained from Micrococcus yunnanensis.* Journal of Oleo Science, 2010. **59**(12): p. 653-659.
66. Ligor, M., T. Trziszka, and B. Buszewski, *Study of Antioxidant Activity of Biologically Active Compounds Isolated from Green Vegetables by Coupled Analytical Techniques.* Food Analytical Methods, 2012. **6**(2): p. 630-636.
67. Fernandez-Sevilla, J.M., F.G. Acien Fernandez, and E. Molina Grima, *Biotechnological production of lutein and its applications.* Appl Microbiol Biotechnol, 2010. **86**(1): p. 27-40.
68. Gao, Q. and F. Garcia-Pichel, *Microbial ultraviolet sunscreens.* Nat Rev Microbiol, 2011. **9**(11): p. 791-802.
69. Rastogi, R.P., R.R. Sonani, and D. Madamwar, *Cyanobacterial Sunscreen Scytonemin: Role in Photoprotection and Biomedical Research.* Appl Biochem Biotechnol, 2015. **176**(6): p. 1551-63.
70. Penninckx, M.J. and M.T. Elskens, *Metabolism and Functions of Glutathione in Micro-organisms.* Advances in Microbial Physiology, 1993. **34**: p. 239-301.
71. Morse, M.L. and R.H. Dahl, *Cellular glutathione is a key to the oxygen effect in radiation damage.* Nature, 1978. **271**: p. 660-662.
72. Chatterjee, A., *Reduced glutathione: a radioprotector or a modulator of DNA-repair activity?* Nutrients, 2013. **5**(2): p. 525-42.
73. Dutta, A., et al., *Interaction of radiation- and bleomycin-induced lesions and influence of glutathione level on the interaction.* Mutagenesis, 2005. **20**(5): p. 329-335.
74. Hu, P., et al., *Whole-genome transcriptional analysis of heavy metal stresses in Caulobacter crescentus.* J Bacteriol, 2005. **187**(24): p. 8437-49.
75. Obeid, M.H., et al., *Mechanism of Attenuation of Uranyl Toxicity by Glutathione in Lactococcus lactis.* Appl Environ Microbiol, 2016. **82**(12): p. 3563-3571.
76. Preveral, S., et al., *Metal(loid)s and radionuclides cytotoxicity in Saccharomyces cerevisiae. Role of YCF1, glutathione and effect of buthionine sulfoximine.* Biochimie, 2006. **88**(11): p. 1651-63.
77. Miller, A.F., *Superoxide dismutases: ancient enzymes and new insights.* FEBS Lett, 2012. **586**(5): p. 585-95.
78. Maier, P., M.R. Veldwijk, and F. Wenz, *Radioprotective gene therapy.* Expert Opinion on Biological Therapy, 2011. **11**(9): p. 1135-1151.
79. Markillie, L.M., et al., *Targeted Mutagenesis by Duplication Insertion in the Radioresistant Bacterium Deinococcus radiodurans: Radiation Sensitivities of Catalase (katA) and Superoxide Dismutase (sodA) Mutants.* Journal of Bacteriology, 1999. **181**(2): p. 666-669.
80. Abreu, I.A., et al., *The kinetic mechanism of manganese-containing superoxide dismutase from Deinococcus radiodurans: a specialized enzyme for the elimination of high superoxide concentrations.* Biochemistry, 2008. **47**(8): p. 2350-2356.
81. Daly, M.J., et al., *Accumulation of Mn(II) in Deinococcus radiodurans Facilitates Gamma-Radiation Resistance.* Science, 2004. **306**: p. 1025-1028.
82. Sharma, A., et al., *Responses of Mn2+ speciation in Deinococcus radiodurans and Escherichia coli to gamma-radiation by advanced paramagnetic resonance methods.* Proc Natl Acad Sci U S A, 2013. **110**(15): p. 5945-50.
83. Confalonieri, F. and S. Sommer, *Bacterial and archaeal resistance to ionizing radiation.* Journal of Physics: Conference Series, 2011. **261**: p. 012005.

84. Daly, M.J., et al., *Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance.* PLoS BIOLOGY, 2007. **5**(4): p. 769-779.
85. Levin-Zaidman, S., et al., *Ringlike structure of the Deinococcus radiodurans genome: a key to radioresistance?* Science, 2003. **299**(5604): p. 254-256.
86. Lebowitz, P.J., L.S. Schwartzberg, and A.K. Bruce, *The In Vivo Association of Manganese with the Chromosome of Micrococcus Radiodurans.* Photochemistry and Photobiology, 1976(23): p. 45-50.
87. Zimmerman, J.M. and J.R. Battista, *A ring-like nucleoid is not necessary for radioresistance in the Deinococcaceae.* BMC Microbiol, 2005. **5**: p. 17.
88. Burrell, A.D., P. Feldschreiber, and C.J. Dean, *DNA-membrane association and the repair of double breaks in X-irradiated Micrococcus radiodurans.* Biochimica et Biophysica Acta, 1971. **247**(1): p. 38-53.
89. Cox, M.M., *Problems and Paradigms: Relating biochemistry to biology: How the recombinational repair function of RecA protein is manifested in its molecular properties.* BioEssays, 1993. **15**(9): p. 617-623.
90. Lesterlin, C., et al., *RecA bundles mediate homology pairing between distant sisters during DNA break repair.* Nature, 2014. **506**(7487): p. 249-53.
91. Amundsen, S.K., et al., *Intersubunit signaling in RecBCD enzyme, a complex protein machine regulated by Chi hot spots.* Genes Dev, 2007. **21**(24): p. 3296-307.
92. Bonacossa de Almeida, C., et al., *Quantification of RecA protein in Deinococcus radiodurans reveals involvement of RecA, but not LexA, in its regulation.* Mol Genet Genomics, 2002. **268**(1): p. 28-41.
93. Liu, Y., et al., *Transcriptome dynamics of Deinococcus radiodurans recovering from ionizing radiation.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 4191-6.
94. Dedieu, A., et al., *Major soluble proteome changes in Deinococcus deserti over the earliest stages following gamma-ray irradiation.* Proteome Science, 2013. **11**(3).
95. Zahradka, K., et al., *Reassembly of shattered chromosomes in Deinococcus radiodurans.* Nature, 2006. **443**(7111): p. 569-73.
96. Haber, J.E., *Partners and pathways: repairing a double-strand break.* Trends in Genetics, 2000. **16**(6): p. 259-264.
97. Helleday, T., et al., *DNA double-strand break repair: from mechanistic understanding to cancer treatment.* DNA Repair (Amst), 2007. **6**(7): p. 923-35.
98. Seeberg, E., L. Eide, and M. Bjørås, *The base excision repair pathway.* Trends in Biochemical Sciences, 1995. **20**(10): p. 391-397.
99. Timmins, J. and E. Moe, *A Decade of Biochemical and Structural Studies of the DNA Repair Machinery of Deinococcus radiodurans: Major Findings, Functional and Mechanistic Insight and Challenges.* Comput Struct Biotechnol J, 2016. **14**: p. 168-176.
100. Minton, K.W., *Repair of ionizing-radiation damage in the radiation resistant bacterium Deinococcus radiodurans.* Mutation Research, 1996. **363**: p. 1-7.
101. Truglio, J.J., et al., *Prokaryotic Nucleotide Excision Repair: The UvrABC System.* Chem. Rev., 2006. **106**(2): p. 233-252.
102. Van Houten, B., *Nucleotide excision repair in Escherichia coli.* Microbiol. Mol. Biol. Rev., 1990. **54**(1): p. 18-51.
103. CEA, *Avancées des recherches sur la séparation-transmutation et le multi-recyclage du plutonium dans les réacteurs à flux de neutrons rapides*. 2015, CEA-Saclay/DEN: Gif-sur-Yvette.
104. Eisenbud, M. and T.F. Gesell, *Environmental Radioactivity: From Natural, Industrial, and Military Sources*, ed. E. Academic Press. 1997, USA.
105. Malanca, A., et al., *Natural radioactivity in building materials from the Brazilian state of Espírito Santo.* Applied Radiation and Isotopes, 1995. **46**(12): p. 1387-1392.
106. Derin, M.T., et al., *Radionuclides and radiation indices of high background radiation area in Chavara-Neendakara placer deposits (Kerala, India).* PLoS One, 2012. **7**(11): p. e50468.
107. Devoogdt, N., et al., *Radio-labelled antibody fragments for use in the prevention and/or treatment of cancer*, U.S. Patent, Editor. 2018, Vrije Universiteit Brussel (VUB) USA. p. 69.
108. WNA. *Radioisotopes in Medicine*. 2018 June 2018 [cited 2018; Available from: http://www.world-nuclear.org/information-library/non-power-nuclear-applications/radioisotopes-research/radioisotopes-in-medicine.aspx.
109. ASN. *La gestion des déchets radioactifs*. 2016 [cited 2018; Available from: https://www.asn.fr/Informer/Dossiers-pedagogiques/La-gestion-des-dechets-radioactifs.
110. SFEN. *Le Traitement Des Déchets*. 2015 [cited 2018; Available from: http://www.sfen.org/energie-nucleaire/filiere-responsable/traitement-dechets.
111. IRSN, *La gestion des déchets radioactifs*. 2013, Institut de Radioprotection et de Sûreté Nucléaire.

112. de Gouvion Saint Cyr, D., et al., *Feasibility study of microfiltration for algae separation in an innovative nuclear effluents decontamination process.* Separation and Purification Technology, 2014. **125**: p. 126-135.
113. Gadd, G.M., *Heavy metal accumulation by bacteria and other microorganisms.* Experientia, 1990. **46**(8): p. 834-840.
114. Wang, C., et al., *Enhancement of radionuclide bio-decontamination by screening highly efficient microalgae for Sr biomineralization.* Lab on a Chip, 2018.
115. Roh, C., C. Kang, and J.R. Lloyd, *Microbial bioremediation processes for radioactive waste.* Korean Journal of Chemical Engineering, 2015. **32**(9): p. 1720-1726.
116. Yamazaki, I.M. and L.P. Geraldo, *Uranium content in phosphate fertilizers commercially produced in Brazil.* Applied Radiation and Isotopes, 2003. **59**(2-3): p. 133-136.
117. De Vos, W., et al. *Geochemical Atlas of Europe. Part 2 - Interpretation of Geochemical Maps, Additional Tables, Figures, Maps, and Related Publications.* 2006 [cited 2018; Available from: http://weppi.gtk.fi/publ/foregsatlas/article.php?id=15.
118. Campos, M.B., et al., *Environmental assessment of water from a uranium mine (Caldas, Minas Gerais State, Brazil) in a decommissioning operation.* Environmental Earth Sciences, 2011. **62**(4): p. 857-863.
119. Committee on Uranium Mining in Virginia, Committee on Earth Resources, and N.R. Council, *Uranium Mining in Virginia: Scientific, Technical, Environmental, Human Health and Safety, and Regulatory Aspects of Uranium Mining and Processing in Virginia* 2011: Washington DC. p. 370.
120. Mimura, T., et al., *Radioactive pollution and accumulation of radionuclides in wild plants in Fukushima.* J Plant Res, 2014. **127**(1): p. 5-10.
121. IRSN. *1986-2016 : Tchernobyl, 30 ans après - Impacts de l'accident de Tchernobyl en France et en Europe*. 2016 [cited 2018.
122. Laurette, J., et al., *Influence of uranium speciation on its accumulation and translocation in three plant species: Oilseed rape, sunflower and wheat.* Environmental and Experimental Botany, 2012. **77**: p. 96-107.
123. Doustaly, F., et al., *Uranium perturbs signaling and iron uptake response in Arabidopsis thaliana roots.* Metallomics, 2014. **6**(4): p. 809-21.
124. Laurette, J., et al., *Speciation of uranium in plants upon root accumulation and root-to-shoot translocation: A XAS and TEM study.* Environmental and Experimental Botany, 2012. **77**: p. 87-95.
125. Berthet, S., et al., *Arabidopsis thaliana plants challenged with uranium reveal new insights into iron and phosphate homeostasis.* New Phytologist, 2018. **217**(2): p. 657-670.
126. Bruins, M.R., S. Kapil, and F.W. Oehme, *Microbial resistance to metals in the environment.* Ecotoxicol Environ Saf, 2000. **45**(3): p. 198-207.
127. Nakahara, H., et al., *Linkage of Mercury, Cadmium, and Arsenate and Drug Resistance in Clinical Isolates of Pseudomonas aeruginosa*. Vol. 33. 1977. 975-6.
128. Sevim, A. and E. Sevim, *Plasmid Mediated Antibiotic and Heavy Metal Resistance in Bacillus Strains Isolated from Soils in Rize, Turkey.* Journal of Natural and Applied Science, 2015. **19**(2): p. 133-141.
129. Ji, G. and S. Silver, *Bacterial resistance mechanisms for heavy metals of environmental concern.* Journal of Industrial Microbiology, 1995. **14**(2): p. 61-75.
130. Silver, S. and M. Walderhaug, *Gene Regulation of Plasmid- and Chromosome-Determined Inorganic Ion Transport in Bacteria.* Microbiological Reviews, 1992. **56**(1): p. 195-228.
131. Shukla, A., P. Parmar, and M. Saraf, *Radiation, radionuclides and bacteria: An in-perspective review.* J Environ Radioact, 2017. **180**: p. 27-35.
132. Lutkenhaus, J.F., *Role of a major outer membrane protein in Escherichia coli.* Journal of Bacteriology, 1977. **131**(2): p. 631-637.
133. Rouch, D.A., B.T.O. Lee, and A.P. Morby, *Understanding cellular responses to toxic agents: a model for mechanism-choice in bacterial metal resistance.* Journal of Industrial Microbiology, 1995. **14**(2): p. 132-141.
134. Scott, J.A. and S.J. Palmer, *Cadmium bio-sorption by bacterial exopolysaccharide.* Biotechnology Letters, 1988. **10**(1): p. 21-24.
135. Scott, J.A. and S.J. Palmer, *Sites of cadmiun uptake in bacteria used for biosorption.* Applied Microbiology and Biotechnology, 1990. **33**(2): p. 221-225.
136. Theodorakopoulos, N., et al., *Use of combined microscopic and spectroscopic techniques to reveal interactions between uranium and Microbacterium sp. A9, a strain isolated from the Chernobyl exclusion zone.* J Hazard Mater, 2015. **285**: p. 285-93.

137. Nies, D.H. and S. Silver, *Ion efflux systems involved in bacterial metal resistances.* Journal of Industrial Microbiology, 1995. **14**(2): p. 186-199.
138. Silver, S., et al., *Bacterial resistance ATPases: primary pumps for exporting toxic cations and anions.* Trends in Biochemical Sciences, 1989. **14**(2): p. 76-80.
139. Payne, R.B., et al., *Uranium Reduction by Desulfovibrio desulfuricans Strain G20 and a Cytochrome c3 Mutant.* Applied and Environmental Microbiology, 2002. **68**(6): p. 3129-3132.
140. Lloyd, J.R., *Microbial reduction of metals and radionuclides.* FEMS Microbiology Reviews, 2003. **27**(2-3): p. 411-425.
141. Gallois, N., et al., *Proteogenomic insights into uranium tolerance of a Chernobyl's Microbacterium bacterial isolate.* Journal of Proteomics, 2018. **177**: p. 148-157.
142. Cazala, C., et al., *Gestion des sites potentiellement pollués par des substances radioactives*, G. méthodologique, Editor. 2011, IRSN.
143. Chen, W. and H. Li, *Cost-Effectiveness Analysis for Soil Heavy Metal Contamination Treatments.* Water, Air, & Soil Pollution, 2018. **229**(4).
144. Entry, J.A., et al., *Phytoremediation and reclamation of soils contaminated with radionuclides* ACS Symposium Series, 1997: p. 299-306.
145. Pacwa-Płociniczak, M., et al., *Environmental Applications of Biosurfactants: Recent Advances.* International Journal of Molecular Sciences, 2011. **12**(1): p. 633-654.
146. Avery, S.V., G.A. Codd, and G.M. Gadd, *Salt-stimulation of caesium accumulation in the euryhaline green microalga Chlorella salina: potential relevance to the development of a biological Cs-removal process.* Microbiology, 1993. **139**(9): p. 2239-2244.
147. Tykva, R., et al., *Bioaccumulation of uranium from waste water using different strains of Saccharomyces cerevisiae.* Nukleonika, 2009. **54**(2): p. 143-148.
148. Faison, B.D., et al., *Binding of Dissolved Strontium by Micrococcus luteus.* Applied And Environmental Microbiology, 1990. **56**(12): p. 3649-3656.
149. Watson, J.S., C.D. Scott, and B.D. Faison, *Adsorption of Sr by Immobilized Microorganisms.* Applied Biochemistry and Biotechnology, 1989. **20/21**: p. 699-709.
150. Tsuruta, T., *Bioaccumulation of uranium and thorium from the solution containing both elements using various microorganisms.* Journal of Alloys and Compounds, 2006. **408-412**: p. 1312-1315.
151. Adam, C. and J. Garnier-Laplace, *Bioaccumulation of silver-110m, cobalt-60, cesium-137, and manganese-54 by the freshwater algae Scenedesmus obliquus and Cyclotella meneghiana and by suspended matter collected during a summer bloom event.* Limnol. Oceanogr. , 2003. **48**(6): p. 2303–2313.
152. Tišáková, L., et al., *Bioaccumulation of $^{137}$Cs and $^{60}$Co by bacteria isolated from spent nuclear fuel pools.* Journal of Radioanalytical and Nuclear Chemistry, 2013. **295**(1): p. 737-748.
153. Anderson, R.T., et al., *Stimulating the In Situ Activity of Geobacter Species To Remove Uranium from the Groundwater of a Uranium-Contaminated Aquifer.* Applied and Environmental Microbiology, 2003. **69**(10): p. 5884-5891.
154. Istok, J.D., et al., *In Situ Bioreduction of Technetium and Uranium in a Nitrate-Contaminated Aquifer.* Environmental Science & Technology, 2004. **38**(2): p. 468-475.
155. Williams, K.H., et al., *Acetate Availability and its Influence on Sustainable Bioremediation of Uranium-Contaminated Groundwater.* Geomicrobiology Journal, 2011. **28**(5-6): p. 519-539.
156. Newsome, L., K. Morris, and J.R. Lloyd, *The biogeochemistry and bioremediation of uranium and other priority radionuclides.* Chemical Geology, 2014. **363**: p. 164-184.
157. Mattimore, V. and J.R. Battista, *Radioresistance of Deinococcus radiodurans: Function necessary to survive ionizing radiation are also necessary to survive prolonged desiccation.* Journal of Bacteriology, 1996. **178**(3): p. 633-637.
158. Musilova, M., et al., *Isolation of Radiation-Resistant Bacteria from Mars Analog Antarctic Dry Valleys by Preselection, and the Correlation between Radiation and Desiccation Resistance.* Astrobiology, 2015. **15**(12): p. 1076-90.
159. Cox, M.C. and J.R. Battista, *Deinococcus radiodurans – The consummate survivor.* Nature Reviews Microbiology, 2005. **3**: p. 882-892.
160. Weronika, E. and K. Lukasz, *Tardigrades in Space Research - Past and Future.* Orig Life Evol Biosph, 2017. **47**(4): p. 545-553.
161. Horikawa, D.D., et al., *Radiation tolerance in the tardigrade Milnesium tardigradum.* Int J Radiat Biol, 2006. **82**(12): p. 843-8.

162. Yoshida, Y., et al., *Comparative genomics of the tardigrades Hypsibius dujardini and Ramazzottius varieornatus.* PLoS Biol, 2017. **15**(7): p. e2002266.
163. Hashimoto, T., et al., *Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein.* Nat Commun, 2016. **7**: p. 12808.
164. Hashimoto, T. and T. Kunieda, *DNA Protection Protein, a Novel Mechanism of Radiation Tolerance: Lessons from Tardigrades.* Life (Basel), 2017. **7**(2).
165. Boone, D.R., R.W. Castenholtz, and G.M. Garrity, *Bergey's Manual of Systematic Bacteriology - The Archaea and the Deeply Branching and Phototrophic Bacteria*, ed. Springer. Vol. 1. 2001. 721.
166. Daly, M.J., *A new perspective on radiation resistance based on Deinococcus radiodurans.* Nature Reviews Microbiology, 2009. **7**: p. 237-245.
167. Battista, J.R., *Against all odds: The survival strategies of Deinococcus radiodurans.* Annual Review of Microbiology, 1997. **51**: p. 203-224.
168. de Groot, A., et al., *Deinococcus deserti sp. nov., a gamma-radiation-tolerant bacterium isolated from the Sahara Desert.* Int J Syst Evol Microbiol, 2005. **55**(Pt 6): p. 2441-6.
169. de Groot, A., et al., *Alliance of Proteomics and Genomics to Unravel the Specificities of Sahara Bacterium Deinococcus deserti.* PLoS Genetics 2009. **5**(3): p. 1-12.
170. Rainey, F.A., et al., *Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus Deinococcus obtained from a single soil sample.* Appl Environ Microbiol, 2005. **71**(9): p. 5225-35.
171. Baqué, M., et al., *The BOSS and BIOMEX space experiments on the EXPOSE-R2 mission: Endurance of the desert cyanobacterium Chroococcidiopsis under simulated space vacuum, Martian atmosphere, UVC radiation and temperature extremes.* Acta Astronautica, 2013. **91**: p. 180-186.
172. Baqué, M., et al., *Biofilm and planktonic lifestyles differently support the resistance of the desert cyanobacterium Chroococcidiopsis under space and Martian simulations.* Orig Life Evol Biosph, 2013. **43**(4-5): p. 377-89.
173. Billi, D., et al., *Ionizing-Radiation Resistance in the Desiccation-Tolerant Cyanobacterium Chroococcidiopsis.* Applied And Environmental Microbiology, 2000. **66**(4): p. 1489–1492.
174. Barbier, E., et al., *Oxidative DNA Damage and Repair in the Radioresistant Archaeon Thermococcus gammatolerans.* Chemical Research in Toxicology, 2016. **29**(11): p. 1796-1809.
175. Tapias, A., C. Leplat, and F. Confalonieri, *Recovery of ionizing-radiation damage after high doses of gamma ray in the hyperthermophilic archaeon Thermococcus gammatolerans.* Extremophiles, 2009. **13**(2): p. 333-343.
176. Rivasseau, C., et al., *An extremely radioresistant green eukaryote for radionuclide bio-decontamination in the nuclear industry.* Energy & Environmental Science, 2013. **6**(4): p. 1230.
177. Bruhn, D.F., et al., *Irradiation of microbes from spent nuclear fuel storage pool environments*, in *Global 99, ANS*. 1999: Jackson Hole, Wyoming.
178. Moreira, R.G., et al., *Factors Affecting Radiation D-Values (D10) of an Escherichia Coli Cocktail and Salmonella Typhimurium LT2 Inoculated in Fresh Produce.* Journal of Food Science, 2012. **77**(4): p. E104-E111.
179. Aziz, N.H., et al., *Effect of gamma radiation on the survival of fungal and actinomycetal florae contaminating medicinal plants.* Applied Radiation and Isotopes, 1997. **48**(1): p. 71-76.
180. Chicote, E., et al., *Isolation and identification of bacteria from spent nuclear fuel pools.* J. Ind. Microbiol. Biotechnol., 2005. **32**: p. 155-162.
181. Chicote, E., et al., *Biofouling on the walls of a spent nuclear fuel pool with radioactive ultrapure water.* Biofouling, 2004. **20**(1): p. 35-42.
182. Berry, C.J., J. Santo Domingo, and C.B. Fliermans, *Microbial Condition of Water Samples from Foreign Fuel Storage Facilities*. 1997.
183. Diósi, G., et al., *Corrosion influenced by biofilms during wet nuclear waste storage.* International Biodeterioration & Biodegradation, 2003. **51**(2): p. 151-156.
184. Sarro, M.I., A.M. Garcia, and D.A. Moreno, *Biofilm formation in spent nuclear fuel pools and bioremediation of radioactive water.* International Microbiology, 2005. **8**: p. 223-230.
185. Chicote, E., et al., *Isolation and identification of bacteria from spent nuclear fuel pools.* J Ind Microbiol Biotechnol, 2005. **32**(4): p. 155-62.
186. Sarro, M.I., et al., *Biofouling on austenitic stainless steels in spent nuclear fuel pools.* Materials and Corrosion, 2003. **54**: p. 535–540.
187. Sarro, M.I., et al., *Development and characterization of biofilms on stainless steel and titanium in spent nuclear fuel pools.* J Ind Microbiol Biotechnol, 2007. **34**(6): p. 433-41.

188. Bruhn, D.F., et al., *Microbial biofilm growth on irradiated, spent nuclear fuel cladding.* Journal of Nuclear Materials, 2009. **384**(2): p. 140-145.
189. Gales, G., et al., *Molecular hydrogen from water radiolysis as an energy source for bacterial growth in a basin containing irradiating waste.* FEMS Microbiol Lett, 2004. **240**(2): p. 155-62.
190. Masurat, P., E.C. Fru, and K. Pedersen, *Identification of Meiothermus as the dominant genus in a storage system for spent nuclear fuel.* Journal of Applied Microbiology, 2005. **98**(3): p. 727-40.
191. Forte Giacobone, A.F., et al., *Biocorrosion at Embalse Nuclear Power Plant. Analysis of the Effect of a Biocide Product.* Procedia Materials Science, 2015. **8**: p. 101-107.
192. Forte Giacobone, A.F., et al., *Microbiological induced corrosion of AA 6061 nuclear alloy in highly diluted media by Bacillus cereus RE 10.* International Biodeterioration & Biodegradation, 2011. **65**(8): p. 1161-1168.
193. Bagwell, C.E., et al., *Amplicon Sequencing Reveals Microbiological Signatures in Spent Nuclear Fuel Storage Basins.* Front Microbiol, 2018. **9**: p. 377.
194. Leonardo, T., et al., *Determination of elemental distribution in green micro-algae using synchrotron radiation nano X-ray fluorescence (SR-nXRF) and electron microscopy techniques--subcellular localization and quantitative imaging of silver and cobalt uptake by Coccomyxa actinabiotis.* Metallomics, 2014. **6**(2): p. 316-29.
195. Leonardo, T., et al., *Silver Accumulation in the Green Microalga Coccomyxa actinabiotis: Toxicity, in Situ Speciation, and Localization Investigated Using Synchrotron XAS, XRD, and TEM.* Environ Sci Technol, 2016. **50**(1): p. 359-67.
196. Zhdanova, N.N., et al., *Ionizing radiation attracts soil fungi.* Mycological Research, 2004. **108**(9): p. 1089-1096.
197. Mesquita, N., et al., *Flow cytometry as a tool to assess the effects of gamma radiation on the viability, growth and metabolic activity of fungal spores.* International Biodeterioration & Biodegradation, 2013. **84**: p. 250-257.
198. Sunagawa, S., et al., *Structure and function of the global ocean microbiome.* SCIENCE, 2015. **348**(6237).
199. Kim, M., et al., *Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes.* Int J Syst Evol Microbiol, 2014. **64**(Pt 2): p. 346-51.
200. Yarza, P., et al., *Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences.* Nat Rev Microbiol, 2014. **12**(9): p. 635-45.
201. Wayne, L.G., et al., *Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics* International Journal of Systematic and Evolutionary Microbiology, 1987. **37**: p. 463-464.
202. Achtman, M., et al., *Yersinia pestis, the cause of plague, is a recently emerged clone of Yersinia pseudotuberculosis.* PNAS, 1999. **96**(24): p. 14043-14048.
203. Teng, J.L., et al., *Phylogenomic Analyses and Reclassification of Species within the Genus Tsukamurella: Insights to Species Definition in the Post-genomic Era.* Front Microbiol, 2016. **7**: p. 1137.
204. Fredriksson, N.J., M. Hermansson, and B.M. Wilen, *The choice of PCR primers has great impact on assessments of bacterial community diversity and dynamics in a wastewater treatment plant.* PLoS One, 2013. **8**(10): p. e76431.
205. Olsen, G.J., et al., *Microbial Ecology and Evolution: a Ribosomal RNA Approach.* Ann. Rev. Microbiol., 1986. **40**: p. 337-365.
206. Lafontaine, D.L.J. and D. Tollervey, *The Function and Synthesis of Ribosomes.* Nature Reviews Molecular Cell Biology, 2001. **2**: p. 514-520
207. Frank, J.A., et al., *Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes.* Appl Environ Microbiol, 2008. **74**(8): p. 2461-70.
208. Mao, D.-P., et al., *Coverage evaluation of universal bacterial primers using the metagenomic datasets.* BMC Microbiology, 2012. **12**(66): p. 1-8.
209. Cox, M.J., W.O. Cookson, and M.F. Moffatt, *Sequencing the human microbiome in health and disease.* Hum Mol Genet, 2013. **22**(R1): p. R88-94.
210. Mysara, M., et al., *Reconciliation between operational taxonomic units and species boundaries.* FEMS Microbiol Ecol, 2017. **93**(4).
211. Esposito, A. and M. Kirschberg, *How many 16S-based studies should be included in a metagenomic conference? It may be a matter of etymology.* FEMS Microbiol Lett, 2014. **351**(2): p. 145-6.
212. Soergel, D.A., et al., *Selection of primers for optimal taxonomic classification of environmental 16S rRNA gene sequences.* ISME J, 2012. **6**(7): p. 1440-4.

213. Schloss, P.D., et al., *Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities.* Applied and Environmental Microbiology, 2009. **75**(23): p. 7537-7541.
214. Chaudhary, N., et al., *16S classifier: a tool for fast and accurate taxonomic classification of 16S rRNA hypervariable regions in metagenomic datasets.* PLoS One, 2015. **10**(2): p. e0116106.
215. Wilke, A., et al., *The MG-RAST metagenomics database and portal in 2015.* Nucleic Acids Research, 2016. **44**(D1): p. D590-D594.
216. Thomas, T., J. Gilbert, and F. Meyer, *Metagenomics - a guide from sampling to data analysis.* Microb Inform Exp, 2012. **2**(1): p. 3.
217. Youssef, N.H., et al., *Assessing the global phylum level diversity within the bacterial domain: A review.* Journal of Advanced Research, 2015. **6**(3): p. 269-282.
218. Hongoh, Y., et al., *Phylogenetic Diversity, Localization, and Cell Morphologies of Members of the Candidate Phylum TG3 and a Subphylum in the Phylum Fibrobacteres, Recently Discovered Bacterial Groups Dominant in Termite Guts.* Applied and Environmental Microbiology, 2006. **72**(10): p. 6780-6788.
219. Danczak, R.E., et al., *Members of the Candidate Phyla Radiation are functionally differentiated by carbon- and nitrogen-cycling capabilities.* Microbiome, 2017. **5**(1).
220. Tamaki, H., et al., *Armatimonas rosea gen. nov., sp. nov., of a novel bacterial phylum, Armatimonadetes phyl. nov., formally called the candidate phylum OP10.* International Journal of Systematic and Evolutionary Microbiology, 2011. **61**(6): p. 1442-1447.
221. Lee, K.C.Y., et al., *Phylogenetic Delineation of the Novel Phylum Armatimonadetes (Former Candidate Division OP10) and Definition of Two Novel Candidate Divisions.* Applied and Environmental Microbiology, 2013. **79**(7): p. 2484-2487.
222. Liu, L., et al., *Comparison of next-generation sequencing systems.* J Biomed Biotechnol, 2012. **2012**: p. 251364.
223. Leong, I., J. Skinner, and D. Love, *Application of Massively Parallel Sequencing in the Clinical Diagnostic Testing of Inherited Cardiac Conditions.* Medical Sciences, 2014. **2**(2): p. 98-126.
224. Chua, E.W. and P.Y. Ng, *MinION: A Novel Tool for Predicting Drug Hypersensitivity?* Front Pharmacol, 2016. **7**: p. 156.
225. Rhoads, A. and K.F. Au, *PacBio Sequencing and Its Applications.* Genomics Proteomics Bioinformatics, 2015. **13**(5): p. 278-89.
226. Quail, M.A., et al., *A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers.* BMC Genomics, 2012. **13**: p. 341-354.
227. Corporation, R.D., *454 Sequencing*. 1996, PixelMEDIA, Inc.
228. Venter, J.C., et al., *Environmental Genome Shotgun Sequencing of the Sargasso Sea.* Science, 2004. **304**(5667): p. 66-74.
229. Yooseph, S., et al., *The Sorcerer II Global Ocean Sampling expedition: expanding the universe of protein families.* PLoS Biol, 2007. **5**(3): p. e16.
230. Pesant, S., et al., *Open science resources for the discovery and analysis of Tara Oceans data.* Scientific Data, 2015. **2**: p. 150023.
231. Suriya, J., et al., *Assessment of bacterial community composition in response to uranium levels in sediment samples of sacred Cauvery River.* Appl Microbiol Biotechnol, 2017. **101**(2): p. 831-841.
232. Armengaud, J. and O. Pible, *Proteomics tracking the pathogenic bacteria.* Biofutur, 2015. **363**: p. 43-45.
233. Armengaud, J., *Microbiology and proteomics, getting the best of both worlds!* Environ Microbiol, 2013. **15**(1): p. 12-23.
234. VerBerkmoes, N.C., et al., *Systems biology: Functional analysis of natural microbial consortia using community proteomics.* Nat Rev Microbiol, 2009. **7**(3): p. 196-205.
235. Tourova, T.P., *Copy Number of Ribosomal Operons in Prokaryotes and Its Effect on Phylogenetic Analyses.* Microbiology, 2003. **72**(4): p. 389-402.
236. Brooks, J.P., et al., *The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies.* BMC Microbiol, 2015. **15**: p. 66.
237. Thelen, J.J. and J.A. Miernyk, *The proteomic future: where mass spectrometry should be taking us.* Biochem J, 2012. **444**(2): p. 169-81.
238. Perkins, D.N., et al., *Probability-based protein identification by searching sequence databases using mass spectrometry data.* Electrophoresis, 1999. **20**(18): p. 3551-3567.
239. Leary, D.H., et al., *Method development for metaproteomic analyses of marine biofilms.* Anal Chem, 2012. **84**(9): p. 4006-13.

240. Bouyssié, D., et al., *Mascot File Parsing and Quantification (MFPaQ), a New Software to Parse, Validate, and Quantify Proteomics Data Generated by ICAT and SILAC Mass Spectrometric Analyses.* Mol Cell Proteomics, 2007. **6**(9): p. 1221-1237.
241. Lacerda, C.M., et al., *Analysis of iTRAQ data using Mascot and Peaks quantification algorithms.* Brief Funct Genomic Proteomic, 2008. **7**(2): p. 119-26.
242. Pible, O. and J. Armengaud, *Improving the quality of genome, protein sequence, and taxonomy databases: a prerequisite for microbiome meta-omics 2.0.* Proteomics, 2015. **15**(20): p. 3418-23.
243. Ram, R.J., et al., *Community Proteomics of a Natural Microbial Biofilm.* Science, 2005. **308**: p. 1915-1920.
244. Goltsman, D.S., et al., *Community genomic and proteomic analyses of chemoautotrophic iron-oxidizing "Leptospirillum rubarum" (Group II) and "Leptospirillum ferrodiazotrophum" (Group III) bacteria in acid mine drainage biofilms.* Appl Environ Microbiol, 2009. **75**(13): p. 4599-615.
245. Lo, I., et al., *Strain-resolved community proteomics reveals recombining genomes of acidophilic bacteria.* Nature, 2007. **446**(7135): p. 537-41.
246. Kan, J., et al., *Metaproteomic analysis of Chesapeake Bay microbial communities.* Saline Systems, 2005. **1**: p. 7.
247. Luo, G. and T.G. Mitchell, *Rapid Identification of Pathogenic Fungi Directly from Cultures by Using Multiplex PCR.* Journal of Clinical Microbiology, 2002. **40**(8): p. 2860-2865.
248. Armengaud, J., *Next-generation proteomics faces new challenges in environmental biotechnology.* Curr Opin Biotechnol, 2016. **38**: p. 174-182.
249. Ochiai, A., et al., *Uranium Dioxides and Debris Fragments Released to the Environment with Cesium-Rich Microparticles from the Fukushima Daiichi Nuclear Power Plant.* Environmental Science & Technology, 2018. **52**(5): p. 2586-2594.
250. CEA/DEN/DANS/11-42, *INB 40 - Réacteurs OSIRIS et ISIS*

*Evalutation complémentaire de la sureté au regard de l'accident survenu à la centrale nucléaire de Fukushima Daiichi*. 2011, CEA.

251. Xiong, T., et al., *Implication of graphene oxide in Cd-contaminated soil: A case study of bacterial communities.* J Environ Manage, 2018. **205**: p. 99-106.
252. Molina, V., et al., *Bacterial Active Community Cycling in Response to Solar Radiation and Their Influence on Nutrient Changes in a High-Altitude Wetland.* Front Microbiol, 2016. **7**: p. 1823.
253. Henriques, A.C., R.M. Azevedo, and P. De Marco, *Metagenomic survey of methanesulfonic acid (MSA) catabolic genes in an Atlantic Ocean surface water sample and in a partial enrichment.* PeerJ, 2016. **4**: p. e2498.
254. Wilmes, P. and P.L. Bond, *Metaproteomics: studying functional gene expression in microbial ecosystems.* Trends Microbiol, 2006. **14**(2): p. 92-7.
255. Delmotte, N., et al., *Community proteogenomics reveals insights into the physiology of phyllosphere bacteria.* Proc Natl Acad Sci U S A, 2009. **106**(38): p. 16428-33.
256. Denef, V.J., et al., *Proteomics-inferred genome typing (PIGT) demonstrates inter-population recombination as a strategy for environmental adaptation.* Environ Microbiol, 2009. **11**(2): p. 313-25.
257. Tanca, A., et al., *Enrichment or depletion? The impact of stool pretreatment on metaproteomic characterization of the human gut microbiota.* Proteomics, 2015. **15**(20): p. 3474-85.
258. Hanson, B.T., I. Hewson, and E.L. Madsen, *Metaproteomic survey of six aquatic habitats: discovering the identities of microbial populations active in biogeochemical cycling.* Microb Ecol, 2014. **67**(3): p. 520-39.
259. Xiao, J., et al., *Metagenomic Taxonomy-Guided Database-Searching Strategy for Improving Metaproteomic Analysis.* J Proteome Res, 2018. **17**(4): p. 1596-1605.
260. Karlsson, R., et al., *Proteotyping: Proteomic characterization, classification and identification of microorganisms – A prospectus.* Systematic and Applied Microbiology, 2015. **38**(4): p. 246-257.
261. Kantor, R.S., et al., *Small genomes and sparse metabolisms of sediment-associated bacteria from four candidate phyla.* MBio, 2013. **4**(5): p. e00708-13.
262. Hedlund, B.P., et al., *Impact of single-cell genomics and metagenomics on the emerging view of extremophile "microbial dark matter".* Extremophiles, 2014. **18**(5): p. 865-75.
263. Armougom, F. and D. Raoult, *Exploring Microbial Diversity Using 16S rRNA High-Throughput Methods.* Journal of Computer Science and Systems Biology, 2009. **02**(01).
264. Kim, M., M. Morrison, and Z. Yu, *Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes.* Journal of Microbiological Methods, 2011. **84**(1): p. 81-87.
265. Wang, Y. and P.-Y. Qian, *Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies.* PLoS One, 2009. **4**(10): p. 1-9.

266. Sperling, J.L., et al., *Comparison of bacterial 16S rRNA variable regions for microbiome surveys of ticks.* Ticks Tick Borne Dis, 2017. **8**(4): p. 453-461.
267. Mysara, M., et al., *From reads to operational taxonomic units: an ensemble processing pipeline for MiSeq amplicon sequencing data.* Gigascience, 2017. **6**(2): p. 1-10.
268. Mardis, E.R., *Next-generation sequencing platforms.* Annu Rev Anal Chem (Palo Alto Calif), 2013. **6**: p. 287-303.
269. Brown, B.L., et al., *MinION™ nanopore sequencing of environmental metagenomes: a synthetic approach.* GigaScience, 2017. **6**(3).
270. Lagier, J.C., et al., *Human gut microbiota: repertoire and variations.* Front Cell Infect Microbiol, 2012. **2**: p. 136.
271. Schloss, P.D. and J. Handelsman, *Metagenomics for studying unculturable microorganisms: cutting the Gordian knot.* Genome Biol, 2005. **6**(8): p. 229.
272. Costea, P.I., et al., *Towards standards for human fecal sample processing in metagenomic studies.* Nat Biotechnol, 2017. **35**(11): p. 1069-1076.
273. Sender, R., S. Fuchs, and R. Milo, *Revised Estimates for the Number of Human and Bacteria Cells in the Body.* PLoS Biology, 2016. **14**(8): p. e1002533.
274. Jannasch, H.W. and G. Jones, *Bacterial Populations in Sea Water as Determined by Different Methods of Enumeration[1].* Limnology and Oceanography, 1959. **4**(2): p. 128-139.
275. Maranger, R. and D.F. Bird, *Viral abundance in aquatic systems: a comparison between marine and fresh waters* Marine Ecology Series, 1995. **121**: p. 217-226.
276. Vilchez-Vargas, R., et al., *Analysis of the microbial gene landscape and transcriptome for aromatic pollutants and alkane degradation using a novel internally calibrated microarray system.* Environ Microbiol, 2013. **15**(4): p. 1016-39.
277. Fuller, C.W., et al., *The challenges of sequencing by synthesis.* Nat Biotechnol, 2009. **27**(11): p. 1013-23.
278. Hammer, Ø., D.A.T. Harper, and P.D. Ryan, *2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis.* Palaeontologia Electronica, 2001. **4**(1): p. 1-9.
279. Jovel, J., et al., *Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics.* Front Microbiol, 2016. **7**: p. 459.
280. Tang, J., et al., *Metagenomic analysis of bacterial community composition and antibiotic resistance genes in a wastewater treatment plant and its receiving surface water.* Ecotoxicol Environ Saf, 2016. **132**: p. 260-9.
281. Mukherjee, N., et al., *Microbial Diversity of Source and Point-of-Use Water in Rural Haiti - A Pyrosequencing-Based Metagenomic Survey.* PLoS One, 2016. **11**(12): p. e0167353.
282. Oldham, A.L., et al., *Molecular methods resolve the bacterial composition of natural marine biofilms on galvanically coupled stainless steel cathodes.* J Ind Microbiol Biotechnol, 2017. **44**(2): p. 167-180.
283. Berestovskaya, Y.Y., et al., *A psychrotolerant Caulobacter sp. from Russian polar tundra soil.* Microbiology, 2006. **75**(3): p. 317-322.
284. Jefferson, K.K., *What drives bacteria to produce a biofilm?* FEMS Microbiol Lett, 2004. **236**(2): p. 163-73.
285. Audebert, C., et al., *Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota.* Sci Rep, 2016. **6**: p. 25255.
286. Jagtap, P., T. Griffin, and J. Armengaud, *Microbiomes – Embracing complexity.* Proteomics, 2015. **15**(20): p. 3405-3406.
287. Denef, V.J., et al., *Proteogenomic basis for ecological divergence of closely related bacteria in natural acidophilic microbial communities.* Proc Natl Acad Sci U S A, 2010. **107**(6): p. 2383-90.
288. Li, Z., et al., *Diverse and divergent protein post-translational modifications in two growth stages of a natural microbial community.* Nat Commun, 2014. **5**: p. 4405.
289. Fischer, C.R., et al., *Stable-isotope probing reveals that hydrogen isotope fractionation in proteins and lipids in a microbial community are different and species-specific.* ACS Chem Biol, 2013. **8**(8): p. 1755-63.
290. Justice, N.B., et al., *(15)N- and (2)H proteomic stable isotope probing links nitrogen flow to archaeal heterotrophic activity.* Environ Microbiol, 2014. **16**(10): p. 3224-37.
291. Mosier, A.C., et al., *Elevated temperature alters proteomic responses of individual organisms within a biofilm community.* ISME J, 2015. **9**(1): p. 180-94.
292. Leary, D.H., et al., *Integrated metagenomic and metaproteomic analyses of marine biofilm communities.* Biofouling, 2014. **30**(10): p. 1211-23.

293. Abram, F., E. Gunnigle, and V. O'Flaherty, *Optimisation of protein extraction and 2-DE for metaproteomics of microbial communities from anaerobic wastewater treatment biofilms.* Electrophoresis, 2009. **30**(23): p. 4149-51.
294. Wu, J.H., et al., *Community and proteomic analysis of methanogenic consortia degrading terephthalate.* Appl Environ Microbiol, 2013. **79**(1): p. 105-12.
295. Hartmann, E.M., et al., *Taking the Shortcut for High-Throughput Shotgun Proteomic Analysis of Bacteria*, in *Host-Bacteria Interactions: Methods and Protocols*, A.C. Vergunst and D. O'Callaghan, Editors. 2014, Springer New York: New York, NY. p. 275-285.
296. Gomez-Alvarez, V., R.P. Revetta, and J.W. Santo Domingo, *Metagenome analyses of corroded concrete wastewater pipe biofilms reveal a complex microbial system.* BMC Microbiology, 2012. **12**.
297. Sorokovikova, E.G., et al., *Diversity of cyanobacterial species and phylotypes in biofilms from the littoral zone of Lake Baikal.* J Microbiol, 2013. **51**(6): p. 757-65.
298. Trapp, J., et al., *Proteogenomic insights into the core-proteome of female reproductive tissues from crustacean amphipods.* J Proteomics, 2016. **135**: p. 51-61.
299. Klein, D.A., *Bulk Extraction-Based Microbial Ecology: Three Critical Questions.* Microbe 2011. **6**(9): p. 377.
300. Ashelford, K.E., et al., *At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies.* Appl Environ Microbiol, 2005. **71**(12): p. 7724-36.
301. Kleiner, M., et al., *Assessing species biomass contributions in microbial communities via metaproteomics.* Nat Commun, 2017. **8**(1): p. 1558.
302. Heyer, R., et al., *Metaproteomics of complex microbial communities in biogas plants.* Microb Biotechnol, 2015. **8**(5): p. 749-63.
303. Muth, T., et al., *MPA Portable: A Stand-Alone Software Package for Analyzing Metaproteome Samples on the Go.* Anal Chem, 2018. **90**(1): p. 685-689.
304. Ryan, M.P. and C.C. Adley, *Sphingomonas paucimobilis: a persistent Gram-negative nosocomial infectious organism.* J Hosp Infect, 2010. **75**(3): p. 153-7.
305. Cavicchioli, R., et al., *Sphingomonads from marine environments.* Journal of Industrial Microbiology & Biotechnology, 1999. **23**: p. 268-272.
306. Gunnigle, E., et al., *Draft genomic DNA sequence of the multi-resistant Sphingomonas sp. strain AntH11 isolated from an Antarctic hypolith.* FEMS Microbiol Lett, 2015. **362**(8): p. fnv037.
307. Joux, F., et al., *Marine Bacterial Isolates Display Diverse Responses to UV-B Radiation.* Applied And Environmental Microbiology, 1999. **65**(9): p. 3820–3827.
308. Jin, L., et al., *Caulobacter profunda sp. nov., isolated from deep freshwater sediment.* Int J Syst Evol Microbiol, 2014. **64**(Pt 3): p. 762-7.
309. Ariskina, E.V., et al., *Evaluation of the taxonomic diversity of prosthecate bacteria belonging to the genera Brevundimonas and Caulobacter isolated from various eurasian ecosystems by analysis of the 16S rRNA genes.* Microbiology, 2011. **80**(3): p. 403-410.
310. Edulamudi, P., et al., *Novel root nodule bacteria belonging to the genus Caulobacter.* Lett Appl Microbiol, 2011. **53**(6): p. 587-91.
311. Brzoska, R.M. and A. Bollmann, *The long-term effect of uranium and pH on the community composition of an artificial consortium.* FEMS Microbiol Ecol, 2016. **92**(1).
312. Willems, A., et al., *Acidovorax, a New Genus for Pseudomonas facilis, Pseudomonas delafieldii, E. Falsen (EF) Group 13, EF Group 16, and Several Clinical Isolates, with the Species Acidovorax facilis comb. nov. , Acidovorax delafieldii comb. nov., and Acidovorax temperans sp. nov.* International Journal of Systematic Bacteriology, 1990. **40**(4): p. 384-398.
313. Gardan, L., et al., *Acidovorax anthurii sp. nov., a new phytopathogenic bacterium which causes bacterial leaf-spot of anthurium.* International Journal of Systematic and Evolutionary Microbiology, 2000. **50**: p. 235-246.
314. Schulze, R., et al., *Genotypic Diversity of Acidovorax Strains Isolated from Activated Sludge and Description of Acidovorax defluvii sp. nov.* Systematic and Applied Microbiology, 1999. **22**(2): p. 205-214.
315. Brenner, D.J., N.R. Krieg, and J.T. Staley, *Bergey's Manual of Systematic Bacteriology - The Alpha-, Beta-, Delta and Epsilonproteobacteri, Part C* ed. Springer. Vol. 2. 2005. 1388.
316. Hartmann, E.M., et al., *Proteomics meets blue biotechnology: a wealth of novelties and opportunities.* Mar Genomics, 2014. **17**: p. 35-42.
317. Lagier, J.C., et al., *Culturing the human microbiota and culturomics.* Nat Rev Microbiol, 2018.
318. Dubourg, G., et al., *The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics.* Eur J Clin Microbiol Infect Dis, 2013. **32**(5): p. 637-45.

319. Lavigne, J.P., et al., *Mass spectrometry: a revolution in clinical microbiology?* Clin Chem Lab Med, 2013. **51**(2): p. 257-70.
320. Christie-Oleza, J.A., G. Miotello, and J. Armengaud, *Proteogenomic definition of biomarkers for the large Roseobacter clade and application for a quick screening of new environmental isolates.* J Proteome Res, 2013. **12**(11): p. 5331-9.
321. Durighello, E., et al., *Proteogenomic biomarkers for identification of Francisella species and subspecies by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry.* Anal Chem, 2014. **86**(19): p. 9394-8.
322. Clair, G., J. Armengaud, and C. Duport, *Restricting Fermentative Potential by Proteome Remodeling.* AN ADAPTIVE STRATEGY EVIDENCED IN BACILLUS CEREUS, 2012. **11**(6).
323. Reasoner, D.J. and E.E. Geldreich, *A new medium for the enumeration and subculture of bacteria from potable water.* Applied and Environmental Microbiology, 1985. **49**(1): p. 1-7.
324. Matallana-Surget, S., et al., *Response to UVB radiation and oxidative stress of marine bacteria isolated from South Pacific Ocean and Mediterranean Sea.* J Photochem Photobiol B, 2012. **117**: p. 254-61.
325. Nakatsu, C.H., et al., *Methylibium petroleiphilum gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the Betaproteobacteria.* Int J Syst Evol Microbiol, 2006. **56**(Pt 5): p. 983-9.
326. Cheptsov, V.S., et al., *100 kGy gamma-affected microbial communities within the ancient Arctic permafrost under simulated Martian conditions.* Extremophiles, 2017. **21**(6): p. 1057-1067.
327. Sakr, A.A., M.F. Ghaly, and M.F. Ali, *The Use of Gamma Irradiation in the Sterilization of Streptomyces Colonizing the Tempra Paintings in Ancient Egyptian Tombs* International Journal of Conservation Science, 2013. **4**(3): p. 283-294.
328. Maity, J.P., et al., *Effects of gamma radiation on fungi infected rice (in vitro).* Int J Radiat Biol, 2011. **87**(11): p. 1097-102.
329. Anellis, A., *Radioresistance of a Pseudomonas Species Isolated from the Omega West Reactor.* Radiation Research, 1961. **15**(6): p. 720-723.
330. Keisam, S., et al., *Quantifying the biases in metagenome mining for realistic assessment of microbial ecology of naturally fermented foods.* Sci Rep, 2016. **6**: p. 34155.
331. Gohl, D.M., et al., *Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies.* Nat Biotechnol, 2016. **34**(9): p. 942-9.
332. Jonsson, V., et al., *Statistical evaluation of methods for identification of differentially abundant genes in comparative metagenomics.* BMC Genomics, 2016. **17**: p. 78.
333. Pruesse, E., et al., *SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB.* Nucleic Acids Res, 2007. **35**(21): p. 7188-96.
334. Quast, C., et al., *The SILVA ribosomal RNA gene database project: improved data processing and web-based tools.* Nucleic Acids Res, 2013. **41**(Database issue): p. D590-6.
335. Yilmaz, P., et al., *The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks.* Nucleic Acids Res, 2014. **42**(Database issue): p. D643-8.
336. Gray, M.W., *The Endosymbiont Hypothesis Revisited*, in *International Review of Cytology*, D.R. Wolstenholme and K.W. Jeon, Editors. 1992, Academic Press. p. 233-357.
337. McFadden, G.I., et al., *Evidence that an amoeba acquired a chloroplast by retaining part of an engulfed eukaryotic alga.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(9): p. 3690-3694.
338. Rivasseau, C., et al., *Coccomyxa actinabiotis sp. nov. (Trebouxiophyceae, Chlorophyta), a new green microalga living in the spent fuel cooling pool of a nuclear reactor.* J Phycol, 2016. **52**(5): p. 689-703.
339. Shannon, C.E., *A Mathematical Theory of Communication.* Bell System Technical Journal, 1948. **27**(4): p. 623-656.
340. de Boer, R., et al., *Improved detection of microbial DNA after bead-beating before DNA isolation.* J Microbiol Methods, 2010. **80**(2): p. 209-11.
341. Ferrand, J., et al., *Comparison of seven methods for extraction of bacterial DNA from fecal and cecal samples of mice.* J Microbiol Methods, 2014. **105**: p. 180-5.
342. Atashpaz, S., et al., *A robust universal method for extraction of genomic DNA from bacterial species.* Microbiology, 2010. **79**(4): p. 538-542.
343. Gallego, V., M.T. Garcia, and A. Ventosa, *Methylobacterium variabile sp. nov., a methylotrophic bacterium isolated from an aquatic environment.* Int J Syst Evol Microbiol, 2005. **55**(Pt 4): p. 1429-33.
344. Ito, H. and H. Iizuka, *Taxonomic Studies on a Radio-resistant Pseudomonas.* Agricultural and Biological Chemistry, 1971. **35**(10): p. 1566-1571.

345. Green, P.N. and I.J. Bousfield, *Emendation of Methylobacterium Patt, Cole, and Hanson 1976; Methylobacterium rhodinum (Heumann 1962) comb. nov. corrig. ; Methylobacterium radiotolerans (Ito and Iizuka 1971) comb. nov. corrig. ; and Methylobacterium mesophilicum (Austin and Goodfellow 1979) comb. nov. .* International Journal of Systematic Bacteriology, 1983. **33**(4): p. 875-877.
346. Nogueira, F., M.L. Botelho, and R. Tenreiro, *Radioresistance studies in Methylobacterium spp.* Radiation Physics and Chemistry, 1998. **52**: p. 3.
347. Goodfellow, M., et al., *Bergey's Manual of Systematic Bacteriology - The Actinobacteria, Part A*, ed. Springer. Vol. 5. 2012. 2083.
348. Chapon, V., et al., *Microbial diversity in contaminated soils along the T22 trench of the Chernobyl experimental platform.* Applied Geochemistry, 2012. **27**(7): p. 1375-1383.
349. Mao, J., et al., *Streptomyces radiopugnans sp. nov., a radiation-resistant actinomycete isolated from radiation-polluted soil in China.* Int J Syst Evol Microbiol, 2007. **57**(Pt 11): p. 2578-82.
350. Davis, D.H., et al., *Proposal to reject the genus Hydrogenomonas: Taxonomic implications.* International Journal of Systematic and Evolutionary Microbiology, 1969. **19**(4): p. 375-390.
351. Ding, L. and A. Yokota, *Proposals of Curvibacter gracilis gen. nov., sp. nov. and Herbaspirillum putei sp. nov. for bacterial strains isolated from well water and reclassification of [Pseudomonas] huttiensis, [Pseudomonas] lanceolata, [Aquaspirillum] delicatum and [Aquaspirillum] autotrophicum as Herbaspirillum huttiense comb. nov., Curvibacter lanceolatus comb. nov., Curvibacter delicatus comb. nov. and Herbaspirillum autotrophicum comb. nov.* Int J Syst Evol Microbiol, 2004. **54**(Pt 6): p. 2223-30.
352. Dekker, L., T.H. Osborne, and J.M. Santini, *Isolation and identification of cobalt- and caesium-resistant bacteria from a nuclear fuel storage pond.* FEMS Microbiol Lett, 2014. **359**(1): p. 81-4.
353. Wu, L.F., et al., *Antagonistic effect of nickel on the fermentative growth of Escherichia coli K-12 and comparison of nickel and cobalt toxicity on the aerobic and anaerobic growth.* Environmental Health Perspectives, 1994. **102**(Suppl 3): p. 297-300.
354. Ranquet, C., et al., *Cobalt Stress in Escherichia coli: the Effect on the Iron-Sulfur Proteins.* Journal of Biological Chemistry, 2007. **282**(42): p. 30442-30451.
355. Nermut, M.V. and F. Herčík, *Effect of ionizing radiation on large bodies of Proteus vulgaris.* Folia Microbiologica, 1961. **6**(5): p. 306-310.
356. Mandel, P., M. Sensenbrenner, and G. Vincendon, *Action of Beta Radiation on the Proteus P18 Bacillus.* Nature, 1958. **182**: p. 674.
357. Neyland, M., P. Dunkel, and A.L. Schade, *The Uptake of Cobalt by Proteus vulgaris.* Microbiology, 1952. **7**(3-4): p. 409-416.
358. De Vos, P., et al., *Bergey's Manual of Systematic Bacteriology - The Firmicutes*, ed. Springer. Vol. 3. 2009. 1422.
359. Wheaton, E., G.B. Pratt, and J.M. Jackson, *Radioresistance of Five Strains of Clostridium Botulinum in Selected Food Product.* Food Science, 1961. **26**(4): p. 345-350.
360. Anellis, A. and R.B. Koch, *Comparative resistance of strains of Clostridium botulinum to gamma rays.* Appl Microbiol., 1962. **10**(4): p. 326-330.
361. Thapa, T., et al., *Multiple Factors Modulate Biofilm Formation by the Anaerobic Pathogen Clostridium difficile.* Journal of Bacteriology, 2012. **195**(3): p. 545-555.
362. Gao, W. and A.J. Francis, *Reduction of Uranium(VI) to Uranium(IV) by Clostridia.* Applied and Environmental Microbiology, 2008. **74**(14): p. 4580-4584.
363. Beech, I.B. and C.C. Gaylarde, *Recent advances in the study of biocorrosion: an overview.* Revista de Microbiologia, 1999. **30**: p. 117-190.
364. Little, B.J., et al., *Microbiologically Influenced Corrosion.* 2007.
365. Wang, Y., et al., *Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags.* Appl Environ Microbiol, 2012. **78**(23): p. 8264-71.
366. Tamura, K. and M. Nei, *Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees.* Molecular Biology and Evolution, 1993. **10**: p. 512-526.
367. Felsenstein, J., *Confidence limits on phylogenies: An approach using the bootstrap.* Evolution, 1985. **39**: p. 783-791.
368. Tamura, K., et al., *MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods.* Molecular Biology and Evolution, 2011. **28**(2731-2739).
369. Kraus, M.P., *Resistance of Blue-Green Algae to $^{60}$Co Gamma Radiation.* Radiation Botany, 1969. **9**: p. 481-489.

370. Rastogi, R.P., R.R. Sonani, and D. Madamwar, *The high-energy radiation protectant extracellular sheath pigment scytonemin and its reduced counterpart in the cyanobacterium Scytonema sp. R77DM.* Bioresour Technol, 2014. **171**: p. 396-400.
371. Fan, M., et al., *Microbial communities in riparian soils of a settling pond for mine drainage treatment.* Water Res, 2016. **96**: p. 198-207.
372. Williams, P.D., et al., *Effects of Ultraviolet Radiation on the Gram-positive marine bacterium Microbacterium maritypicum.* Curr Microbiol, 2007. **55**(1): p. 1-7.
373. Zhang, W., et al., *Microbacterium radiodurans sp. nov., a UV radiation-resistant bacterium isolated from soil.* Int J Syst Evol Microbiol, 2010. **60**(Pt 11): p. 2665-70.
374. Hovanec, T.A., et al., *Nitrospira-Like Bacteria Associated with Nitrite Oxidation in Freshwater Aquaria*. Applied and Environmental Microbiology, 1998. **64**(1): p. 258-264.
375. Anitori, R.P., et al., *A Culture-Independent Survey of the Bacterial Community in a Radon Hot Spring.* Astrobiology, 2002. **2**(3): p. 255-270.
376. Masoudzadeh, N., et al., *Biosorption of cadmium by Brevundimonas sp. ZF12 strain, a novel biosorbent isolated from hot-spring waters in high background radiation areas.* J Hazard Mater, 2011. **197**: p. 190-8.
377. Dartnell, L.R., et al., *Low-temperature ionizing radiation resistance of Deinococcus radiodurans and Antarctic Dry Valley bacteria.* Astrobiology, 2010. **10**(7): p. 717-32.
378. Gerber, U., et al., *Combined use of flow cytometry and microscopy to study the interactions between the gram-negative betaproteobacterium Acidovorax facilis and uranium(VI).* J Hazard Mater, 2016. **317**: p. 127-34.
379. Ordonez, O.F., et al., *Extremophile culture collection from Andean lakes: extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation.* Microb Ecol, 2009. **58**(3): p. 461-73.
380. Flores, M.R., et al., *Isolation of UV-B resistant bacteria from two high altitude Andean lakes (4,400 m) with saline and non saline conditions.* J. Gen. Appl. Microbiol., 2009. **55**: p. 447-458.
381. Brenner, D.J., N.R. Krieg, and J.T. Staley, *Bergey's Manual of Systematic Bacteriology - The Gammaproteobacteria, Part B*, ed. Springer. Vol. 2. 2005. 1106.
382. Stürmeyer, H., et al., *Ecophysiological and Phylogenetic Studies of Nevskia ramosa in Pure Culture.* Applied and Environmental Microbiology, 1998. **64**(5): p. 1890-1894.
383. Foesel, B.U., M. Rohde, and J. Overmann, *Blastocatella fastidiosa gen. nov., sp. nov., isolated from semiarid savanna soil - the first described species of Acidobacteria subdivision 4.* Syst Appl Microbiol, 2013. **36**(2): p. 82-9.
384. Guo, H., et al., *Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing.* Ecotoxicol Environ Saf, 2017. **144**: p. 300-306.
385. Christie-Oleza, J.A., et al., *Nutrient recycling facilitates long-term stability of marine microbial phototroph–heterotroph interactions.* Nature Microbiology, 2017. **2**: p. 17100.
386. Macaskie, L.E., *The Application of Biotechnology to the Treatment of Wastes Produced from the Nuclear Fuel Cycle: Biodegradation and Bioaccumulation as a Means of Treating Radionuclide-Containing Streams.* Critical Reviews in Biotechnology, 1991. **11**(1): p. 41-112.
387. Lloyd, J.R. and J.C. Renshaw, *Bioremediation of radioactive waste: radionuclide-microbe interactions in laboratory and field-scale studies.* Curr Opin Biotechnol, 2005. **16**(3): p. 254-60.
388. Hafez, M.B., et al., *Biosorption of some ions on different bacterial species from aqueous and radioactive waste solutions.* Journal of Radioanalytical and Nuclear Chemistry, 2002. **252**(1): p. 179-185.
389. Nakajima, A. and T. Tsuruta, *Competitive biosorption of thorium and uranium by Micrococcus luteus.* Journal of Radioanalytical and Nuclear Chemistry, 2004. **260**(1): p. 13-18.
390. Puyen, Z.M., et al., *Biosorption of lead and copper by heavy-metal tolerant Micrococcus luteus DE2008.* Bioresour Technol, 2012. **126**: p. 233-7.
391. Druteikienė, R., et al., *Interaction of biomass of aerobic bacteria and fungi with Pu(IV) at low pH.* Journal of Radioanalytical and Nuclear Chemistry, 2010. **286**(2): p. 387-391.
392. Ivshina, I.B., T.A. Peshkur, and V.P. Korobov, *Efficient Uptake of Cesium Ions by Rhodococcus Cells.* Microbiology, 2002. **71**(3): p. 357-361.
393. Ruggiero, C.E., et al., *Actinide and metal toxicity to prospective bioremediation bacteria.* Environ Microbiol, 2005. **7**(1): p. 88-97.
394. Barras, F. and M. Fontecave, *Cobalt stress in Escherichia coli and Salmonella enterica: molecular bases for toxicity and resistance.* Metallomics, 2011. **3**(11): p. 1130-4.

395. Lock, K., et al., *Ecotoxicity of cobalt to the springtail Folsomia candida.* Comp Biochem Physiol C Toxicol Pharmacol, 2004. **139**(4): p. 195-9.
396. Gault, N., et al., *Cobalt toxicity: chemical and radiological combined effects on HaCaT keratinocyte cell line.* Toxicol In Vitro, 2010. **24**(1): p. 92-8.
397. Nagajyoti, P.C., K.D. Lee, and T.V.M. Sreekanth, *Heavy metals, occurrence and toxicity for plants: a review.* Environmental Chemistry Letters, 2010. **8**(3): p. 199-216.
398. Gikas, P., *Single and combined effects of nickel (Ni(II)) and cobalt (Co(II)) ions on activated sludge and on other aerobic microorganisms: a review.* J Hazard Mater, 2008. **159**(2-3): p. 187-203.
399. Rao, S.V.S., et al., *Separation of Cobalt from Synthetic Intermediate and Decontamination Radioactive Wastes Using Polyurethane Foam.* Separation Science and Technology, 1997. **32**(18): p. 3007-3026.
400. Kim, C., et al., *Sorption of cobalt by amine-functionalized silica nanoparticles for foam decontamination of nuclear facilities.* Journal of Radioanalytical and Nuclear Chemistry, 2016. **310**(2): p. 841-847.
401. Gogada, R., et al., *Engineered Deinococcus radiodurans R1 with NiCoT genes for bioremoval of trace cobalt from spent decontamination solutions of nuclear power reactors.* Applied Microbiology and Biotechnology, 2015. **99**(21): p. 9203-9213.
402. Raghu, G., et al., *Bioremediation of trace cobalt from simulated spent decontamination solutions of nuclear power reactors using E. coli expressing NiCoT genes.* Applied Microbiology and Biotechnology, 2008. **81**(3): p. 571-578.
403. White, T.J., et al., *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*, in *PCR protocols: a guide to methods and applications*, A.P. Inc., Editor. 1990. p. 315-322.
404. Ruiz-Gonzalez, M.X., et al., *Resistance of Feather-Associated Bacteria to Intermediate Levels of Ionizing Radiation near Chernobyl.* Sci Rep, 2016. **6**: p. 22969.
405. Hu, X., et al., *Comparison of kinetic models to describe high pressure and gamma irradiation used to inactivate Vibrio vulnificus and Vibrio parahaemolyticus prepared in buffer solution and whole oysters.* 2005. **68**: p. 292-5.
406. Farag, M.D.E.-D.H., K. Shamsuzzaman, and J. Borsa, *Radiation Sensitivity of Listeria monocytogenes in Phosphate Buffer, Trypticase Soy Broth, and Poultry Feed.* Journal of Food Protection, 1990. **53**(8): p. 648-651.
407. Shukla, M., et al., *Multiple-stress tolerance of ionizing radiation-resistant bacterial isolates obtained from various habitats: correlation between stresses.* Curr Microbiol, 2007. **54**(2): p. 142-8.
408. Briggs, A., *The Resistances of Spores of the Genus Bacillus to Phenol, Heat and Radiation.* Journal of Applied Bacteriology, 1966. **29**(3): p. 490-504.
409. Moeller, R., et al., *Roles of the major, small, acid-soluble spore proteins and spore-specific and universal DNA repair mechanisms in resistance of Bacillus subtilis spores to ionizing radiation from X rays and high-energy charged-particle bombardment.* J Bacteriol, 2008. **190**(3): p. 1134-40.
410. Setlow, P., *Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals.* J Appl Microbiol, 2006. **101**(3): p. 514-25.
411. Dussault, D., et al., *Carotenoids' influence on radiotolerance of Pantoea agglomerans, a plant pathogen.* Lett Appl Microbiol, 2008. **47**(3): p. 208-13.
412. Tsukamura, M., *Proposal of a New Genus, Gordona, for Slightly Acid-fast Organisms Occurring in Sputa of Patients With Pulmonary Disease and in Soil.* Microbiology, 1971. **68**(1): p. 15-26.
413. Petrie, L., et al., *Enumeration and Characterization of Iron(III)-Reducing Microbial Communities from Acidic Subsurface Sediments Contaminated with Uranium(VI).* Applied and Environmental Microbiology, 2003. **69**(12): p. 7467-7479.
414. Nazina, T.N., et al., *Distribution and activity of microorganisms in the deep repository for liquid radioactive waste at the Siberian Chemical Combine.* Microbiology, 2006. **75**(6): p. 727-738.
415. Nazina, T.N., et al., *Microorganisms in a Disposal Site for Liquid Radioactive Wastes and Their Influence on Radionuclides.* Geomicrobiology Journal, 2010. **27**(5): p. 473-486.
416. Tsuruta, T., *Removal and recovery of uranyl ion using various microorganisms.* Journal of Bioscience and Bioengineering, 2002. **94**(1): p. 23-28.
417. Li, X., et al., *Biosorption of uranium on Bacillus sp. dwc-2: preliminary investigation on mechanism.* J Environ Radioact, 2014. **135**: p. 6-12.
418. Panak, P., et al., *Complex formation of U(VI) with Bacillus-isolates from a uranium mining waste pile.* Radiochimica Acta, 2000. **88**(2).
419. Fowle, D.A., J.B. Fein, and A.M. Martin, *Experimental Study of Uranyl Adsorption onto Bacillus subtilis.* Environ. Sci. Technol., 2000. **34**(17): p. 3737-3741.

420. Hennig, C., et al., *EXAFS investigation of uranium(VI) complexes formed at Bacillus cereus and Bacillus sphaericus surfaces*, in *Radiochimica Acta*. 2001. p. 625.
421. Ozdemir, S., et al., *Resistance, bioaccumulation and solid phase extraction of uranium (VI) by Bacillus vallismortis and its UV-vis spectrophotometric determination.* J Environ Radioact, 2017. **171**: p. 217-225.
422. Zhang, Z., et al., *Accumulation of U(VI) on the Pantoea sp. TW18 isolated from radionuclide-contaminated soils.* J Environ Radioact, 2018. **192**: p. 219-226.
423. Ding, L., et al., *Uranium adsorption and subsequent re-oxidation under aerobic conditions by Leifsonia sp. - Coated biochar as green trapping agent.* Environ Pollut, 2018. **242**(Pt A): p. 778-787.
424. Gottschalk, G., *Bacterial metabolism*. 2012: Springer Science & Business Media.
425. Ma, Z., F.E. Jacobsen, and D.P. Giedroc, *Coordination chemistry of bacterial metal transport and sensing.* Chem Rev, 2009. **109**(10): p. 4644-81.
426. Arguello, J.M., D. Raimunda, and M. Gonzalez-Guerrero, *Metal transport across biomembranes: emerging models for a distinct chemistry.* J Biol Chem, 2012. **287**(17): p. 13510-7.
427. Benmalek, Y. and M.L. Fardeau, *Isolation and characterization of metal-resistant bacterial strain from wastewater and evaluation of its capacity in metal-ions removal using living and dry bacterial cells.* International Journal of Environmental Science and Technology, 2016. **13**(9): p. 2153-2162.
428. Selenska-Pobell, S., et al., *Selective accumulation of heavy metals by three indigenous Bacillus strains, B. cereus, B. megaterium and B. sphaericus, from drain waters of a uranium waste pile.* FEMS Microbiology Ecology, 1999. **29**(1): p. 59-67.
429. Choudhary, S., et al., *Uranium and other heavy metal resistance and accumulation in bacteria isolated from uranium mine wastes.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2012. **47**(4): p. 622-37.
430. Hafez, M.B., A. Fouad, and W. El-Desouky, *Accumulation of some metal ions on Bacillus licheniformis.* Journal of Radioanalytical and Nuclear Chemistry, 2002. **251**(2): p. 249-252.
431. Mohd Shaufi, M.A., et al., *Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses.* Gut Pathog, 2015. **7**: p. 4.
432. White, J.R., N. Nagarajan, and M. Pop, *Statistical Methods for Detecting Differentially Abundant Features in Clinical Metagenomic Samples.* PLOS Computational Biology, 2009. **5**(4): p. e1000352.
433. Bengtsson, J., et al., *Metaxa: a software tool for automated detection and discrimination among ribosomal small subunit (12S/16S/18S) sequences of archaea, bacteria, eukaryotes, mitochondria, and chloroplasts in metagenomes and environmental sequencing datasets.* Antonie Van Leeuwenhoek, 2011. **100**(3): p. 471-5.
434. Shi, K., et al., *Impacts of enhanced UVB radiation on photosynthetic characteristics of the marine diatom Phaeodactylum tricornutum (Bacillariophyceae, Heterokontophyta).* Journal of Applied Phycology, 2017. **29**(3): p. 1287-1296.
435. Rijstenbil, J.W., *Assessment of oxidative stress in the planktonic diatom Thalassiosira pseudonana in response to UVA and UVB radiation.* Journal of Plankton Research, 2002. **24**(12): p. 1277-1288.
436. Van Dien, S.J., et al., *Genetic Characterization of the Carotenoid Biosynthetic Pathway in Methylobacterium extorquens AM1 and Isolation of a Colorless Mutant.* Applied and Environmental Microbiology, 2003. **69**(12): p. 7563-7566.
437. Yan, X., X. Luo, and M. Zhao, *Metagenomic analysis of microbial community in uranium-contaminated soil.* Appl Microbiol Biotechnol, 2016. **100**(1): p. 299-310.
438. Spring, S., P. Kampfer, and K.H. Schleifer, *Limnobacter thiooxidans gen. nov., sp. nov., a novel thiosulfate-oxidizing bacterium isolated from freshwater lake sediment.* International Journal of Systematic and Evolutionary Microbiology 2001. **51**: p. 1463–1470.
439. Lu, H., et al., *Limnobacter litoralis sp. nov., a thiosulfate-oxidizing, heterotrophic bacterium isolated from a volcanic deposit, and emended description of the genus Limnobacter.* Int J Syst Evol Microbiol, 2011. **61**(Pt 2): p. 404-7.
440. Vedler, E., et al., *Limnobacter spp. as newly detected phenol-degraders among Baltic Sea surface water bacteria characterised by comparative analysis of catabolic genes.* Syst Appl Microbiol, 2013. **36**(8): p. 525-32.
441. Nguyen, T.M. and J. Kim, *Limnobacter humi sp. nov., a thiosulfate-oxidizing, heterotrophic bacterium isolated from humus soil, and emended description of the genus Limnobacter Spring et al. 2001.* J Microbiol, 2017. **55**(7): p. 508-513.
442. Pantaleon, V., et al., *Biofilms of Clostridium species.* Anaerobe, 2014. **30**: p. 193-8.
443. Zablotni, A., et al., *Changes in the lipopolysaccharide of Proteus mirabilis 9B-m (O11a) clinical strain in response to planktonic or biofilm type of growth.* Med Microbiol Immunol, 2018. **207**(2): p. 129-139.

444. Costerton, J.W., *Introduction to biofilm.* International Journal of Antimicrobial Agents, 1999(11): p. 217-221.
445. Costerton, J.W., *Discussion - Introduction to biofilm.* International Journal of Antimicrobial Agents, 1999. **11**: p. 217-221.
446. Köpke, M., M. Straub, and P. Dürre, *Clostridium difficile Is an Autotrophic Bacterial Pathogen.* PLoS ONE, 2013. **8**(4): p. e62157.
447. Briolat, V. and G. Reysset, *Identification of the Clostridium perfringens Genes Involved in the Adaptive Response to Oxidative Stress.* Journal of Bacteriology, 2002. **184**(9): p. 2333-2343.
448. Hamilton, W.A., *Sulphate-Reducing Bacteria and Anaerobic Corrosion.* Annual Review of Microbiology, 1985. **39**(1): p. 195-217.
449. Machuca, L.L., R. Jeffrey, and R.E. Melchers, *Microorganisms associated with corrosion of structural steel in diverse atmospheres.* International Biodeterioration & Biodegradation, 2016. **114**: p. 234-243.
450. Monroy, O.A.R., et al., *Corrosion of API XL 52 steel in presence of Clostridium celerecrescens.* Materials and Corrosion, 2011. **62**(9): p. 878-883.
451. Soracco, R.J., et al., *Microbiologically influenced corrosion investigations in electric power generating stations*. 1988.
452. Webster, B.J., D.B. Wells, and P.J. Bremer, *The Influence of Potable Water Biofilms on Copper Corrosion*, in *CORROSION 96*. 1996, NACE International: Denver, Colorado. p. 14.
453. Féron, D. and D. Crusset, *Microbial induced corrosion in French concept of nuclear waste underground disposal.* Corrosion Engineering, Science and Technology, 2014. **49**(6): p. 540-547.
454. Staniland, S., et al., *Cobalt Uptake and Resistance to Trace Metals in Comamonas testosteroni Isolated From a Heavy-Metal Contaminated Site in the Zambian Copperbelt.* Geomicrobiology Journal, 2010. **27**(8): p. 656-668.

# Appendices

# Appendix 1: Nuclear waste treatments

**Very short-life wastes (VTC):** all radioactive elements with a half-life shorter than 100 days. Most of these wastes originate from medicine and research. They are stocked on the facility they are used until the radioactive decay is over.
*Treatment:* the VTC are then eliminated through the usual waste management ways according to their physical, chemical and biological characteristics.

**Low activity wastes (TFA):** wastes from the nuclear industry and are solid wastes (concrete, rubble…). It represents less than 30% of the radioactive wastes (Fig. 6.1).
*Treatment:* the TFA are stocked in "big bags" or in metal boxes in the department of Aude. An extremely low proportion of wastes are recycled in the nuclear industry. Lead can for example be melted to create protection against radiation in a nuclear installation.

**Low and medium activity with short-life wastes (FMA-VC):** mainly short-life radionuclides, such as $^{60}Co$ or $^{137}Cs$. They represents around 60% of the total waste volume (Fig. 6.1).
*Treatment:* the FMA-VC are stocked in the department of Aube. Before being stocked, the wastes are compacted or solidified and placed into a metal container or in concrete. Some of the waste can be incinerated. A "package" of FMA-VC waste is composed of 15-20% of radioactive waste and 80-85% of coating.

**Low activity and long-life wastes (FA-VL):** mainly graphite wastes from old reactors. This category represents around 7% of the total waste volume (Fig. 6.1).
*Treatment:* there is currently no existing treatment for those wastes. The producers of the wastes hoar them in the production site or specific installations until a solution is found.

**Medium activity and long-life wastes (MA-VL):** solid or liquid wastes from nuclear reactors. The radionuclides present have half-lives greater than 31 years. This category represent 3% if the total waste volume (Fig. 6.1).
*Treatment:* the MA-VL are compacted or placed in concrete and stocked by the producers or by Areva in La Hague. There is currently a project to burry those wastes at a Cigéo center (Centre industriel de stockage géologique) in Bure, near the surface.

**High activity and long-life wastes (HA-VL):** this category represents less than 0.2% of the total waste volume (Fig. 6.1) but produce more than 96% of the radioactivity produced by the wastes. They come from the treatment of the fuels used in a nuclear facility.
*Treatment:* these wastes are vitrified, so the radioactive radiation are blocked by the glass. The vitrified wastes are then introduced inside stainless steel containers and stocked by their producers, in the CEA Marcoule or by Areva in its site of La Hague and in the Gard. They are planned to be stored deeply underground by Cigéo in Bure.

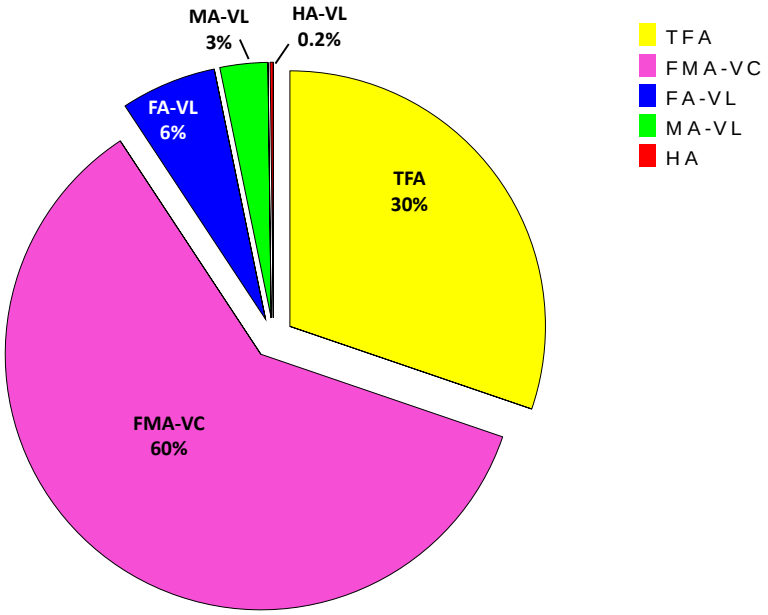



*Figure 6.1: Repartition of the French nuclear waste at the end of 2013*

# Appendix 2: Microorganisms found in nuclear facilities

***Table 6.1: Microorganisms identified in spent nuclear fuel pools after cultivation***

| Location | Sample | Microorganisms | Identification method | Reference |
|---|---|---|---|---|
| Spent nuclear fuel pool of the Cofrentes nuclear reactor, Valencia, Spain | Pool water | *Afipia sp.*<br>*Bradyrhizobium* sp.<br>*Burkholderia cepacia*<br>*Chryseobacterium meningosepticum*<br>*Methylobacterium* sp.<br>*Microbacterium* sp.<br>*Nocardia* sp.<br>*Pseudomonas saccharophila*<br>*Pseudomonas putida*<br>*Ralstonia mannitolilytica*<br>*Ralstonia* sp.<br>*Rhizobium* sp.<br>*Staphylococcus haemolyticus*<br>*Strenotrophomonas maltophilia*<br>*Streptococcus salivarius*<br>*Sphingomonas* sp.<br>Unidentified *alpha proteobacterium* | Culture and sequencing of the 16S rDNA amplicons | Chicote *et al*., 2005 |
| Idem | Biofilm formed on metallic coupons immersed for 34 months in the SNFP | *Bacillus cereus*<br>*Bacillus pumilus*<br>*Bacillus* sp.<br>*Brachybacterium* sp.<br>*Bradyrhizobium* sp.<br>*Burkholderia* sp.<br>*Mesorhizobium* sp.<br>*Methylobacterium* sp.<br>*Microbacterium paraoxidans*<br>*Mycobacterium* sp.<br>*Nocardia* sp.<br>*Pseudomonas saccharophila*<br>*Ralstonia detusculanense*<br>*Ralstonia mannitolilytica*<br>*Ralstonia picketii*<br>*Ralstonia* sp.<br>*Strenotrophomonas maltophilia*<br>*Staphylococcus succinus*<br>*Staphylococcus epidermidis*<br>*Staphylococcus* sp.<br>*Unidentified beta proteobacterium*<br>*Variovorax paradoxus*<br>*Xylophilus ampelinus* | Culture and sequencing of the 16S rDNA amplicons | Sarro *et al*., 2005 |
| Idem | Biofilm formed on metallic coupons immersed for 315 days in the SNFP | *Bacillus* sp.<br>*Cellulomonas* sp.<br>*Methylobacterium* sp.<br>*Nocardia pseudosporangifera*<br>*Pseudomonas saccharophila*<br>*Ralstonia picketii*<br>*Staphylococcus epidermidis*<br>*Staphylococcus haemolyticus*<br>*Strenotrophomonas maltophilia*<br>*Xylophilus ampelinus* | Culture and sequencing of the 16S rDNA amplicons | Sarro *et al*., 2003 |
| Cofrentes nuclear reactor, Valencia, Spain | Biofilm formed on the walls of the upper transference pool | *Aspergillus fumigatus*<br>*Bacillus licheniformis*<br>*Gordonia terrae*<br>*Nocardia seriolae*<br>*Ralstonia* sp.<br>*Staphylococcus saccharolyticus* | Culture and sequencing of the 16S rDNA amplicons | Chicote *et al*., 2004 |
| Spent fuel storage pool, Idaho Nuclear Technology and Engineering center, Idaho National Laboratory, USA | Pool water | *Corynebacterium aquaticum*<br>*Pseudomonas putida*<br>*Comamonas acidovorans*<br>*Gluconobacter cerinus*<br>*Micrococcus diversus*<br>*Rhodococcus rhodochrous*<br>Two strains of sulfate-reducing bacteria (SRB) | Culture and sequencing of the 16S rDNA amplicons | Bruhn *et al*., 1999 |

| | | | | |
|---|---|---|---|---|
| Idem | Pool water | *Aureobacterium testaceum* (formerly Microbacterium testaceum)<br>*Pseudomonas monteilii*<br>*Rhodococcus* sp.<br>*Pseudonocardia saturnea* (formerly *Amycolata saturnea* )<br>Sp. close to *Taxeobacter gelupurpurascens*<br>*Sphingomonas* sp.<br>*Micrococcus luteus* | | Bruhn *et al*., 2009 |
| Idem | INL simulated sludge | Sp. related to *Agromyces fucosus*<br>*Sphingomonas*<br>*Desulfotomaculum guttoideum* (SRB)<br>*Clostridium subterminale* (anaerobic)<br>*Clostridium celerecrescens* (anaerobic) | | Bruhn *et al*., 2009 |
| France | Water from a basin containing irradiating waste | *Acinetobacter*<br>*Bacillus*<br>*Burkholderia*<br>*Burkholderia cepacia*<br>*Delftia*<br>*Micrococcus*<br>*Pseudomonas*<br>*Ralstonia*<br>*Ralstonia pickettii*<br>*Staphylococcus* | Species identification: Culture and sequencing of the 16S rDNA amplicons | Galès *et al*., 2004 |
| Pool for interim storage of spent fuel, Atomic Energy Research Institute, Budapest, Hungary | Pool water | Not identified, aerobic and anaerobic isolates, six different bacteria isolated | | Diosi *et al*., 2003 |
| Spent nuclear fuel storage pool, Savannah River, SC, USA | Water from the receiving basin for off-site fuel | Sulfate Reducing bacteria, Acid Producing Bacteria, Viable Aerobic bacteria, Viable Anaerobic Bacteria (not identified) | | Berry *et al*., 1997 |
| Spent nuclear fuel storage basins, Savannah River Site, USA | Idem | Sulfate Reducing bacteria, Acid Producing Bacteria, non-identified aerobic and anaerobic bacteria | | Santo Domingo *et al*., 1998 |
| Nuclear fuel storage facilities in Chile, Swiss, Germany, Colombia, Canada, Japan, England, Italy | Water | Sulfate Reducing bacteria, Acid Producing Bacteria, Viable Aerobic bacteria, Viable Anaerobic Bacteria (not identified) | | Berry *et al*., 1997 |
| Interim spent fuel storage for nuclear power plants, Slovak Republic | Water | *Kocuria palustris*<br>*Micrococcus luteus*<br>*Ochrobactrum sp.*<br>*Pseudomonas aeruginosa* | Culture and sequencing of the 16S rDNA amplicon | Tisakova *et al*., 2012 |
| CLAB interim spent nuclear fuel storage pool, Sweden | Water | *Meiothermus* sp. | Culture and sequencing of the 16S rDNA amplicon | Masurat *et al*., 2005 |
| Spent Nuclear Fuel Pool, Argentina | Water | *Acinetobacter* sp.<br>*Aminobacter* sp.<br>*Arthrobacter* sp.<br>*Bacillus cereus*<br>*Bacillus firmus*<br>*Bosea* sp.<br>*Leifsonia* sp.<br>*Mesorhizobium* sp.<br>*Micrococcus* sp.<br>*Pseudomonas* sp.<br>*Pseudomonas lurida*<br>*Rhizobium* sp.<br>*Rhodococcus* sp.<br>*Sinorhizobium* sp. | Culture and sequencing of the 16S rDNA amplicon | Giacobone *et al*., 2011 |
| Spent nuclear fuel pool of a research reactor, France | Biofilm formed on a spotlight | *Coccomyxa actinabiotis* | Culture and sequencing of the 18S rDNA amplicon | Rivasseau *et al*., 2013 |

# Appendix 3: DNA concentration obtained after extraction

*Table 6.2: DNA concentration and quality obtained after the different DNA extraction methods, measured with a ThermoScientific Nanodrop 2000 Spectrophotometer.*

| | [DNA] (ng/µL) | A 260nm/280nm | A 260nm/230nm |
|---|---|---|---|
| **Loc. A - meth. 1** | 6,1 | 1,75 | 0,13 |
| **Loc. A - meth. 2** | 3,0 | 1,99 | 0,17 |
| **Loc. A - meth. 3** | 4,8 | 1,59 | 0,27 |
| **Loc. B - meth. 1** | 5,7 | 2,61 | 0,06 |
| **Loc. B - meth. 2** | 3,9 | 1,60 | 0,19 |
| **Loc. B - meth. 3** | 3,0 | 1,79 | 0,09 |
| **Loc. C - meth. 1** | 13,2 | 1,43 | 0,41 |
| **Loc. C - meth. 2** | 7,4 | 1,57 | 0,24 |
| **Loc. C - meth. 3** | 3,3 | 1,81 | 0,59 |

*Table 6.3: Cell concentration at each location*

| Location | Loc. A | Loc. B | Loc. C |
|---|---|---|---|
| **Total cells count ($10^6$ cells/L)** | 2,7 | 1,4 | < 0,75 |

The [DNA] was extremely low and can be explained by the low cell quantity. The DNA quality was also extremely poor and can be explained by the fact that the nanodrop's sensitivity for DNA is 50 ng/µL as well as the low cell quantity. Other DNA quantity method was impossible due to the lack of DNA and sample.

# Appendix 4: Kinetic cultures of Pool 2

*Table 6.4: Proportion of each genus during the 2017 kinetic in LB (sample G)*

| | | | | | D 7 | | | D 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Mycobacteriaceae | Mycobacterium | 0,00 | | | | 0,42 | |
| | | | Nocardiaceae | Nocardia | | 1,93 | | | 0,31 | |
| | | | | Mumia | | | 9,69 | | | |
| | | | | Rhodococcus | | | | 0,73 | | |
| | | Micrococcales | Microbacteriaceae | Microbacterium | | 1,93 | | 0,31 | 0,18 | |
| | | | Micrococcaceae | Arthrobacter | | | | 0,35 | | |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis | | | | 0,38 | | |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 4,02 | 1,93 | 32,32 | 4,19 | 4,03 | 32,34 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | 0,30 | 0,32 | |
| | Cytophagia | Cytophagales | Cytophagaceae | Rhodonellum | | | | | 0,22 | |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | | 1,93 | | 0,65 | 0,25 | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 2,02 | | | 0,96 | 1,16 | |
| | | | Paenibacillaceae | Paenibacillus | | 1,93 | | 0,54 | | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | 1,93 | | 1,47 | 1,05 | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Asticcacaulis | | | | 0,70 | | |
| | | | | Caulobacter | | | | 0,69 | 1,23 | |
| | | Rhizobiales | Aurantimonadaceae | Aureimonas | | | | 2,69 | 0,91 | |
| | | | Bartonellaceae | Bartonella | | | | 2,14 | | |
| | | | Beijerinckiaceae | Beijerinckia | | | | 0,95 | | |
| | | | Bradyrhizobiaceae | Afipia | | | | | 1,82 | |
| | | | | Bosea | | | | 4,02 | 1,23 | |
| | | | | Bradyrhizobium | 3,72 | 1,93 | | 4,43 | 4,84 | 1,55 |
| | | | | Rhodopseudomonas | | | | 1,42 | 1,35 | |
| | | | Chelatococcaceae | Chelatococcus | | | | 1,14 | | |
| | | | Hyphomicrobiaceae | Devosia | | | | 1,52 | 1,23 | |
| | | | | Hyphomicrobium | | | | 2,28 | 0,79 | |
| | | | | Prosthecomicrobium | | 1,93 | | 4,03 | 2,51 | 0,78 |
| | | | Methylobacteriaceae | Methylobacterium | | 1,93 | | 3,95 | 1,33 | |
| | | | | Microvirga | | | | 1,48 | | |
| | | | Methylocystaceae | Pleomorphomonas | | 1,93 | | 2,55 | 0,86 | |
| | | | Phyllobacteriaceae | Mesorhizobium | | 1,93 | | 6,82 | 1,43 | 1,73 |
| | | | Rhizobiaceae | Rhizobium | 3,74 | 1,93 | 1,16 | 5,83 | 3,04 | 1,88 |
| | | | Rhodobiaceae | Amorphus | | | | 0,91 | | |
| | | | Xanthobacteraceae | Azorhizobium | | 1,93 | | 2,49 | 0,84 | |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas | | | | 0,68 | | |
| | | | Rhodobacteraceae | Labrenzia | | 1,93 | | 1,21 | 1,34 | |
| | | | | Pannonibacter | | | | 1,10 | | |
| | | | | Paracoccus | | | | | 0,62 | |
| | | | | Rhodovulum | 2,26 | | | | | |
| | | Rhodospirillales | Acetobacteraceae | Roseomonas | | | | 1,44 | 0,58 | |
| | | | Rhodospirillaceae | Azospirillum | | 1,93 | | 2,35 | 1,80 | |
| | | Sphingomonadales | Sphingomonadaceae | Sphingobium | 10,66 | | | | 3,29 | |
| | | | | Sphingomonas | 65,00 | 1,93 | | 3,67 | 49,37 | 2,49 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | | | | 1,07 | | |
| | | | | Azohydromonas | | 3,28 | | 0,77 | | |
| | | | Aquincola_family | Aquincola | | 3,70 | | | | |
| | | | Burkholderiaceae | Burkholderia | | 2,84 | 9,68 | 3,37 | 1,78 | 9,53 |
| | | | | Ralstonia | | 2,20 | 44,04 | 0,99 | 0,57 | 43,53 |
| | | | Comamonadaceae | Acidovorax | | 3,85 | | 3,03 | 0,73 | |
| | | | | Caldimonas | | 3,04 | | | | |
| | | | | Pelomonas | 4,65 | 11,51 | | 7,90 | 3,12 | 2,84 |
| | | | | Pseudorhodoferax | | 2,73 | | | | |
| | | | | Variovorax | | 2,82 | | | | |
| | | | Ideonella_family | Ideonella | | 4,55 | | | | |
| | | | Oxalobacteraceae | Collimonas | | 1,93 | | | | |
| | | | | Massilia | | 1,93 | | 1,00 | 0,43 | |
| | | | Methylibium_family | Methylibium | | 5,17 | | 2,16 | | |
| | | | Rhizobacter_family | Rhizobacter | | 4,63 | | | | |
| | | | Rubrivivax_family | Rubrivivax | | 3,35 | | | | |
| | | | Sphaerotilus_family | Sphaerotilus | | 1,93 | | | | |
| | | Nitrosomonadales | Sterolibacteriaceae | Sulfuritalea | | 1,93 | | | | |
| | | Rhodocyclales | Zoogloeaceae | Thauera | | | | 1,46 | | |
| | Deltaproteobacteria | Myxococcales | Myxococcaceae | Myxococcus | | | | 0,24 | | |
| | Gammaproteobacteria | Alteromonadales | Pseudoalteromonadaceae | Pseudoalteromonas | | | | 0,35 | | |
| | | Enterobacterales | Enterobacteriaceae | Escherichia | | | | 0,86 | 0,26 | |
| | | | | Serratia | | 1,93 | | | | |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 3,91 | 1,93 | 3,12 | 4,32 | 3,76 | 3,33 |
| | | Vibrionales | Vibrionaceae | Vibrio | | | | 0,62 | 0,41 | |
| | | Xanthomonadales | Xanthomonadaceae | Xanthomonas | | 1,93 | | 1,15 | 0,38 | |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | | | | 0,20 | | |
| | | | | Penicillium | | | | 0,14 | | |
| | | Chaetothyriales | Herpotrichiellaceae | Exophiala | | | | | 0,20 | |
| | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | | 1,93 | | | | |

## *Table 6.5: Proportion of each genus during the 2017 kinetic in R2A (sample G)*

| | | | | | D 7 | | | D 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Mycobacteriaceae | Mycobacterium | | | | 1,35 | 1,41 | |
| | | | Nocardiaceae | Nocardia | 0,57 | 0,50 | | 0,85 | | |
| | | | | Rhodococcus | | | | | | 0,90 |
| | | Micrococcales | Cellulomonadaceae | Cellulomonas | | | | | 1,43 | |
| | | | Microbacteriaceae | Microbacterium | | 0,38 | | 0,62 | 0,72 | |
| | | | Micrococcaceae | Arthrobacter | 0,48 | | | | | |
| | | Propionibacteriales | Nocardioidaceae | Mumia | | | 14,43 | | | |
| | | | | Nocardioides | | | | | 0,72 | |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis | | | | | 0,73 | |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 3,25 | 3,09 | | 4,34 | 4,01 | 4,28 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | | 0,65 | |
| | | | Prevotellaceae | Prevotella | | | | 0,61 | | |
| | Chitinophagia | Chitinophagales | Chitinophagaceae | Sediminibacterium | | | | 0,49 | | |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | 0,42 | 0,42 | | 0,71 | 0,54 | 0,80 |
| bacterium YEK0313_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus | | | | | 2,38 | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | | | 2,02 | 1,94 | | 2,07 |
| | | | Paenibacillaceae | Paenibacillus | 1,11 | 1,09 | | | 1,09 | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | 1,15 | 1,15 | | | 1,36 | 1,82 |
| | | | Lachnospiraceae | Lachnoclostridium | | | | 0,44 | | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Asticcacaulis | | | | | 1,58 | |
| | | | | Brevundimonas | 0,96 | | | | | |
| | | | | Caulobacter | | | | | | 16,18 |
| | | Rhizobiales | Aurantimonadaceae | Aureimonas | | | | 2,59 | 2,98 | |
| | | | Bartonellaceae | Bartonella | | | | 2,09 | | |
| | | | Bradyrhizobiaceae | Bosea | 1,96 | | | 3,98 | 4,27 | |
| | | | | Bradyrhizobium | 2,21 | 1,76 | 1,47 | 4,27 | 4,64 | 4,43 |
| | | | | Rhodopseudomonas | | | | | 2,94 | |
| | | | Chelatococcaceae | Chelatococcus | | | | | 2,56 | |
| | | | Hyphomicrobiaceae | Devosia | | | | 2,71 | 3,22 | |
| | | | | Hyphomicrobium | | | | 2,31 | 2,52 | |
| | | | | Prosthecomicrobium | 1,96 | 1,56 | | 4,02 | 4,78 | 2,81 |
| | | | Methylobacteriaceae | Methylobacterium | 2,04 | 1,69 | | 4,05 | 4,25 | 4,01 |
| | | | | Microvirga | | | | 3,03 | 3,25 | |
| | | | Methylocystaceae | Pleomorphomonas | 1,32 | | | 2,61 | 2,95 | |
| | | | Phyllobacteriaceae | Mesorhizobium | 2,14 | 1,68 | | 5,08 | 4,62 | 4,03 |
| | | | Rhizobiaceae | Agrobacterium | | | | | 4,41 | |
| | | | | Kaistia | | | | | 2,34 | |
| | | | | Rhizobium | 2,76 | 2,25 | | 5,53 | 6,11 | 4,90 |
| | | | Rhodobiaceae | Amorphus | | | | | 1,84 | |
| | | | Xanthobacteraceae | Azorhizobium | 1,22 | 1,01 | | 2,56 | 2,80 | |
| | | Rhodobacterales | Rhodobacteraceae | Labrenzia | 1,24 | 1,03 | | 2,33 | | 2,23 |
| | | | | Pannonibacter | | | | | 2,37 | |
| | | Rhodospirillales | Acetobacteraceae | Acetobacter | 0,92 | | | | | |
| | | | | Roseomonas | | | | 1,45 | | |
| | | | Rhodospirillaceae | Azospirillum | 1,31 | 1,16 | | 2,35 | 2,32 | 3,09 |
| | | | Rhodospirillales bacterium_family | Rhodospirillales bacterium URHD0088_genus | | | | | 1,30 | |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | 1,78 | 1,60 | | 2,78 | 2,93 | 41,77 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | 2,62 | | | | 1,30 | |
| | | | | Azohydromonas | | 4,45 | | 2,45 | | |
| | | | Aquincola_family | Aquincola | 4,90 | 5,04 | | | | |
| | | | Burkholderiaceae | Burkholderia | 3,91 | 3,89 | 13,29 | 3,75 | 2,31 | |
| | | | | Ralstonia | | 3,26 | 63,49 | | | |
| | | | Comamonadaceae | Acidovorax | 5,40 | 5,46 | | 3,52 | | |
| | | | | Pelomonas | 15,44 | 15,63 | 3,93 | 9,70 | 3,23 | 1,26 |
| | | | | Pseudorhodoferax | | 3,73 | | | | |
| | | | | Variovorax | 3,95 | | | | | |
| | | | Ideonella_family | Ideonella | | 6,06 | | | | |
| | | | Methylibium_family | Methylibium | 6,81 | 6,98 | | 3,96 | | |
| | | | Oxalobacteraceae | Herbaspirillum | | | | 2,15 | | |
| | | | | Janthinobacterium | | 2,87 | | | | |
| | | | | Massilia | 2,71 | | | | | |
| | | | Polyangium brachysporum_family | Polyangium brachysporum_genus | 4,08 | 4,14 | | | | |
| | | | Rhizobacter_family | Rhizobacter | 6,04 | 6,19 | | | | |
| | | | Rubrivivax_family | Rubrivivax | 4,39 | 4,51 | | | | |
| | | | Sphaerotilus_family | Sphaerotilus | 3,87 | | | | | |
| | | Rhodocyclales | Zoogloeaceae | Thauera | 1,79 | 1,85 | | 1,37 | | |
| | Deltaproteobacteria | Myxococcales | Archangiaceae | Hyalangium | | | | | 0,44 | |
| | | | | Stigmatella | | 0,26 | | | | |
| | | | Myxococcaceae | Myxococcus | | | | 0,53 | | |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Alteromonas | | | | 0,51 | | |
| | | Enterobacterales | Enterobacteriaceae | Escherichia | | 0,85 | | 0,91 | 0,77 | |
| | | | | Salmonella | | | | | | 0,86 |
| | | | | Serratia | 0,81 | | 1,37 | | | |
| | | Oceanospirillales | Halomonadaceae | Halomonas | | | | | 0,78 | |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 3,24 | 3,02 | | 4,45 | 3,66 | 4,24 |
| | | Vibrionales | Vibrionaceae | Vibrio | | | | | 1,10 | |
| | | Xanthomonadales | Xanthomonadaceae | Pseudoxanthomonas | 0,99 | | | | | |
| | | | | Xanthomonas | | 1,19 | | 1,20 | | |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | | | | 0,41 | 0,36 | |
| | | Chaetothyriales | Herpotrichiellaceae | Exophiala | | | | | | 0,32 |
| | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | 0,25 | 0,23 | | | | |

## *Table 6.6: Proportion of each genus during the 2017 kinetic in LB (sample H)*

| | | | | | D 3 | | | D 7 | | | D 14 | | | D 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Corynebacterium | | | | 0,35 | | | | | | | | |
| | | | Gordoniaceae | Gordonia | | | | | | | | | | 0,44 | | |
| | | | Nocardiaceae | Nocardia | | | | | 0,48 | 0,45 | 0,66 | | | | 0,56 | 0,77 |
| | | | | Rhodococcus | | | | | | | | 0,69 | | | | |
| | | Micrococcales | Cellulomonadaceae | Cellulomonas | | | | | | | | | | | | 0,42 |
| | | | Microbacteriaceae | Microbacterium | | | | | | 0,38 | | 0,58 | | 0,45 | 0,44 | |
| | | | Micrococcaceae | Arthrobacter | | | | | | | 0,73 | | | | | |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | | | | 2,38 | 3,01 | 2,81 | 4,09 | 3,94 | 3,74 | 3,39 | 3,50 | 4,10 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | | | | 0,71 | | | 0,58 | | |
| | Cytophagia | Cytophagales | Cytophagaceae | Rhodonellum | | | | | | | 0,37 | | | | | 0,30 |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | | | | 0,34 | 0,40 | 0,38 | 0,63 | 0,59 | | 0,46 | 0,46 | 0,63 |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | | | | | 1,48 | 1,40 | 1,62 | | 1,93 | 1,75 | 1,75 | 1,85 |
| | | | Lactobacillaceae | Lactobacillus | | | | | | | 0,45 | | | | | |
| | | | Paenibacillaceae | Paenibacillus | | | | 0,83 | | | | 1,24 | | | | |
| | | | Streptococcaceae | Streptococcus | | | | | | | | | | | 0,59 | 0,74 |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | | 1,00 | 1,16 | | | | | 1,19 | 1,26 | 1,39 |
| | | | Lachnospiraceae | Butyrivibrio | | | | | | 0,28 | 0,35 | | | | | |
| | | | | Oribacterium | | | | | | | | | 0,32 | | | |
| | | | Peptococcaceae | Desulfosporosinus | | | | | | | | 0,43 | | | | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | | | | | | | 1,35 | 23,90 | | | | |
| | | | | Brevundimonas | | | | | | | | 5,50 | | | | |
| | | | | Phenylobacterium | | | | | | | | 5,73 | | | | |
| | | Rhizobiales | Aurantimonadaceae | Aureimonas | | | | | 1,29 | 1,22 | 2,48 | 2,67 | | | | 2,53 |
| | | | Bartonellaceae | Bartonella | | | | | | | 1,82 | | | | | 1,90 |
| | | | Beijerinckiaceae | Chelatococcus | | | | | | | 2,11 | | | | | |
| | | | Bradyrhizobiaceae | Bosea | | | | | 1,88 | | 3,67 | 3,92 | | 1,93 | | 3,85 |
| | | | | Bradyrhizobium | | | | | 2,13 | 1,93 | 4,17 | 3,82 | 3,95 | 2,23 | 1,97 | 4,31 |
| | | | | Rhodopseudomonas | | | | | | | 2,58 | | | | | |
| | | | Hyphomicrobiaceae | Devosia | | | | | | | 2,55 | 2,88 | | | | 2,83 |
| | | | | Hyphomicrobium | | | | | 1,15 | 1,03 | 2,12 | | | | | 2,16 |
| | | | | Prosthecomicrobium | | | | | 1,91 | 1,71 | 3,96 | 3,66 | 1,82 | 1,84 | 1,61 | 3,82 |
| | | | Methylobacteriaceae | Methylobacterium | | | | | 1,96 | 1,77 | 3,70 | 4,08 | | 2,05 | 1,90 | 3,74 |
| | | | | Microvirga | | | | | | | 2,74 | | | | | 2,74 |
| | | | Methylocystaceae | Pleomorphomonas | | | | | 1,25 | 1,10 | 2,30 | 2,49 | | | 1,15 | 2,58 |
| | | | Phyllobacteriaceae | Mesorhizobium | | | | | 1,99 | 1,80 | 3,79 | 3,97 | | 2,06 | 1,80 | 4,02 |
| | | | Rhizobiaceae | Rhizobium | | | | 0,87 | 2,59 | 2,30 | 5,11 | 4,94 | 3,66 | 2,62 | 2,36 | 5,17 |
| | | | Rhodobiaceae | Amorphus | | | | | | | 1,59 | | | | | |
| | | | Xanthobacteraceae | Azorhizobium | | | | | 1,16 | 1,05 | 2,36 | 2,50 | | | | 2,37 |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas | | | | | | | 1,21 | | | | | 1,28 |
| | | | | Oceanicaulis | | | | | | | | 2,02 | | | | |
| | | | Rhodobacteraceae | Labrenzia | | | | | 1,24 | 1,10 | 2,25 | 2,56 | | 1,22 | 1,09 | 2,40 |
| | | | | Paracoccus | | | | 0,45 | | | | | | | | |
| | | | | Rhodobacter | | | | | | | | | 1,87 | | | |
| | | Rhodospirillales | Acetobacteraceae | Acetobacter | | | | | 0,79 | | | | | | | |
| | | | | Roseomonas | | | | | | 0,66 | 1,33 | 1,52 | | | | 1,37 |
| | | | Rhodospirillaceae | Azospirillum | | | | 0,64 | 1,28 | 1,16 | 2,13 | 2,74 | | 1,43 | 1,25 | 2,17 |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | | | | | 1,67 | 1,51 | 2,57 | 4,19 | 72,58 | 1,82 | 1,87 | 2,83 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | | | | | | | | | | 2,55 | | |
| | | | | Azohydromonas | | | | 4,91 | 4,11 | 3,82 | | | | | 4,86 | 2,96 |
| | | | | Bordetella | | | | | | | 1,37 | | | | | |
| | | | Aquincola_family | Aquincola | | | | 5,71 | 4,73 | 4,47 | | | | | 5,34 | |
| | | | Burkholderiaceae | Burkholderia | | 34,62 | | 3,92 | 3,76 | 3,58 | 3,02 | 2,13 | 2,74 | 4,00 | 4,31 | 3,54 |
| | | | | Ralstonia | | | | 3,20 | 2,91 | 2,80 | 1,96 | | | | | |
| | | | Comamonadaceae | Acidovorax | | | | 5,73 | 4,80 | 4,62 | 3,00 | | | 5,42 | 5,94 | 3,77 |
| | | | | Caldimonas | | | | 4,60 | | | | | | | | |
| | | | | Comamonas | | | | 5,42 | 3,43 | 4,31 | | | | 4,85 | 5,60 | |
| | | | | Pelomonas | 63,79 | 32,05 | | 17,18 | 13,87 | 13,26 | 6,92 | 1,37 | 3,62 | 15,96 | 18,70 | 9,58 |
| | | | | Pseudorhodoferax | | | | | | 3,26 | | | | | | 2,54 |
| | | | | Variovorax | | | | 4,12 | | | | | | 3,88 | | |
| | | | Ideonella_family | Ideonella | | | | | 5,59 | 5,32 | 2,80 | | | 5,68 | | |
| | | | Leptothrix_family | Leptothrix | | | | 4,92 | | | | | | | | |
| | | | Methylibium_family | Methylibium | | | | 7,57 | 6,26 | 5,94 | 3,10 | | | 6,56 | 7,61 | 4,40 |
| | | | Oxalobacteraceae | Herbaspirillum | | | | | 2,77 | 2,64 | | | | | | |
| | | | | Janthinobacterium | | | | | | | | | | 2,79 | | 2,17 |
| | | | | Massilia | | | | 2,94 | 2,47 | 2,37 | 1,61 | | | 2,73 | 3,05 | |
| | | | Polyangium brachysporum_family | Polyangium brachysporum_genus | | | | | | 3,62 | | | | 4,02 | | |
| | | | Rhizobacter_family | Rhizobacter | | | | 6,77 | 5,62 | 5,32 | | | | | 6,67 | |
| | | | Rubrivivax_family | Rubrivivax | | | | 4,98 | 4,13 | 3,92 | | | | 4,33 | | |
| | | | Sphaerotilus_family | Sphaerotilus | | | | 4,46 | | | | | | 3,87 | 4,45 | |
| | | Neisseriales | Chromobacteriaceae | Andreprevotia | | | | | | | | | | | 1,10 | |
| | | Rhodocyclales | Rhodocyclaceae | Azoarcus | | | | | | | | | | | 2,01 | |
| | | | | Uliginosibacterium | | | | | | | | | | 1,03 | | |
| | | | | Thauera | | | | 1,89 | 1,69 | 1,62 | 1,15 | | | | | 1,47 |
| | Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio | | | | | | 0,70 | | 1,01 | | | | |
| | | Myxococcales | Cystobacteraceae | Cystobacter | | | | | 0,34 | | | | | | | |
| | | | | Hyalangium | | | | | | | 0,37 | | | | | |
| | | | Myxococcaceae | Myxococcus | | | | | | | | | | | 0,39 | |
| | Epsilonproteobacteria | Campylobacterales | Campylobacteraceae | Campylobacter | | | | | | | 0,41 | | | | | |
| | | | Helicobacteraceae | Helicobacter | | | | | | | | | | | | 0,60 |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Marinobacter | | | | | | | | | | 0,99 | 1,05 | |
| | | | | Paraglaciecola | | | | 0,61 | | | | | | | | |
| | | Enterobacterales | Enterobacteriaceae | Candidatus Blochmannia | | | 21,43 | | | | | | | | | |
| | | | | Escherichia | | | | | 0,80 | | 0,72 | | | 0,91 | | |
| | | | | Enterobacter | | | | 0,84 | | 0,84 | | 0,98 | | | 0,97 | 0,98 |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | | | | 2,25 | 2,80 | 2,67 | 3,57 | 3,77 | 3,76 | 3,31 | 3,11 | 3,89 |
| | | Vibrionales | Vibrionaceae | Vibrio | | | | | | | 1,15 | | | | | |
| | | Xanthomonadales | Xanthomonadaceae | Rhodanobacter | | | | | | 0,68 | | | | | | |
| | | | | Xanthomonas | | | | 1,02 | 1,04 | | 1,05 | | | 1,20 | 1,20 | 1,27 |
| Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | Mycoplasma | 36,21 | 33,33 | 78,57 | | | | | | | | | |
| Cyanobacteria | | Nostocales | Microchaetaceae | Tolypothrix | | | | | | | | | | 0,34 | | |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Penicillium | | | | 0,10 | | | 0,28 | | | | | |
| | | Chaetothyriales | Clavicipitaceae | Cladophialophora | | | | | | | | | | | | 0,22 |
| | Sordariomycetes | Hypocreales | Hypocreaceae | Metarhizium | | | | | 0,08 | | | | | 0,11 | | |
| | | | | Trichoderma | | | | | | | | | | | 0,11 | |
| | | | Nectriaceae | Fusarium | | | | | | 0,20 | | | | | | 0,35 |
| | | Sordariales | Madurella_family | Madurella | | | | | | | | 0,16 | | | | |

## *Table 6.7: Proportion of each genus during the 2017 kinetic in R2A (sample H)*

| | | | | | D 3 | | | D 7 | | | D 14 | | | D 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Actinomycetaceae | Actinomyces | | | | | | | | | | 2,82 | | |
| | | Bifidobacteriales | Bifidobacteriaceae | Bifidobacterium | | | | | | | | | | 1,10 | | |
| | | Corynebacteriales | Mycobacteriaceae | Mycobacterium | | | 3,75 | | | | | | | 3,29 | 0,98 | |
| | | | Nocardiaceae | Nocardia | | | | 0,48 | | | | 0,76 | 0,62 | 2,39 | | 0,67 |
| | | | | Rhodococcus | | | | | 0,48 | | 0,63 | | | | | |
| | | Micrococcales | Cellulomonadaceae | Actinotalea | | | | | | | | | | 3,59 | | |
| | | | | Cellulomonas | | | | | | | | | | 9,06 | | |
| | | | Intrasporangiaceae | Janibacter | | | | | 0,15 | | | | | | | |
| | | | Microbacteriaceae | Microbacterium | | | | | | | | | 0,44 | 2,66 | 0,48 | 0,47 |
| | | | Micrococcaceae | Arthrobacter | | | | 0,44 | | 0,53 | 0,68 | 0,76 | | 2,98 | | |
| | | | Promicromonosporaceae | Promicromonosporaceae bacterium W15_genus | | | | | | | | | | 2,41 | | |
| | | Propionibacteriales | Nocardioidaceae | Nocardioides | | | | | | | | | | 2,09 | | |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis | | | | | | | | 0,62 | | 1,79 | 0,53 | |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | | | | 2,87 | 2,68 | 3,26 | 3,90 | 4,19 | 3,32 | 5,55 | 3,38 | 3,81 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | | | | 0,67 | | | 0,85 | | |
| | Cytophagia | Cytophagales | Cytophagaceae | Rhodonellum | | | | | | | 0,24 | 0,26 | | | | |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium | | | | | | | | 0,37 | | | | |
| | | | | Flavobacterium | | | | 0,39 | 0,34 | 0,44 | 0,57 | | 0,45 | 0,55 | 0,47 | 0,52 |
| | Sphingobacteriia | Sphingobacteriales | Sphingobacteriaceae | Sphingobacterium | | | | | | | | | | | | 0,37 |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | | | | 1,48 | | 1,69 | 1,58 | 1,92 | 1,70 | | 1,65 | 1,70 |
| | | | Paenibacillaceae | Paenibacillus | | | | | 0,94 | | | 1,33 | | 1,52 | | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | | 1,17 | 1,02 | 1,23 | 1,33 | 1,46 | 1,22 | | 1,20 | |
| | | | Lachnospiraceae | Butyrivibrio | | | | | | | | | | 0,47 | | |
| | | | | Lachnoclostridium | | | | | | | | | | | | 0,33 |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | | | | | | | 1,50 | | | | | |
| | | Rhizobiales | Aurantimonadaceae | Aurantimonas | | | | | | | 1,96 | 2,50 | | | | |
| | | | Bartonellaceae | Bartonella | | | | | | | 1,97 | | | | | 1,34 |
| | | | Beijerinckiaceae | Chelatococcus | | | | | | | 2,28 | 2,18 | | | | |
| | | | Bradyrhizobiaceae | Bosea | | | | | 1,34 | 0,00 | 4,00 | 3,80 | 1,81 | | | 2,29 |
| | | | | Bradyrhizobium | | | 4,33 | 1,67 | 1,62 | 1,76 | 4,56 | 4,26 | 2,04 | 1,71 | 1,86 | 2,78 |
| | | | Hyphomicrobiaceae | Devosia | | | | | | | 3,02 | | | | | |
| | | | | Hyphomicrobium | | | | | | | 2,24 | 2,19 | | | | 1,42 |
| | | | | Prosthecomicrobium | | | | 1,27 | 1,31 | 1,46 | 4,21 | 4,07 | 1,73 | | | 2,24 |
| | | | Methylobacteriaceae | Methylobacterium | | | | 1,48 | | 1,77 | 3,96 | 3,79 | | | | 2,37 |
| | | | | Microvirga | | | | | 1,05 | | 2,88 | 2,74 | 1,39 | | | |
| | | | Methylocystaceae | Pleomorphomonas | | | | | 0,92 | 0,98 | 2,62 | 2,52 | 1,17 | | | 1,60 |
| | | | Phyllobacteriaceae | Aminobacter | | | | | | | 2,54 | | | | | |
| | | | | Mesorhizobium | | | | 1,47 | 1,48 | 1,64 | 4,27 | 3,97 | 1,82 | | | 2,49 |
| | | | Rhizobiaceae | Rhizobium | | | | 1,84 | 1,97 | 2,12 | 5,55 | 5,22 | 2,48 | | | 3,15 |
| | | | Xanthobacteraceae | Azorhizobium | | | | | 0,85 | | 2,54 | 2,42 | 1,16 | | | 1,50 |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas | | | | | | | 1,28 | 1,25 | | | | |
| | | | Rhodobacteraceae | Labrenzia | | | | 0,87 | 0,86 | | 2,54 | 2,38 | 1,16 | | | 1,46 |
| | | | | Pannonibacter | | | | | | | 2,18 | | | | | |
| | | | | Paracoccus | | | | | | | | | | 0,71 | | |
| | | | | Rhodovulum | | | | | | 1,10 | | | | | | |
| | | | | Rhodobacter | | | | | | | | | | | 0,70 | |
| | | Rhodospirillales | Acetobacteraceae | Roseomonas | | | | | | | 1,38 | 1,36 | | | | |
| | | | Rhodospirillaceae | Azospirillum | | | | 1,08 | 1,07 | 1,20 | 2,16 | 2,23 | 1,31 | | | 1,53 |
| | | | | Inquilinus | | | | | | | | | | | 0,48 | |
| | | | | Rhodospirillales bacterium URHD0088_family | Rhodospirillales bacterium URHD0088_genus | | | | | | 1,13 | | | | | |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | | | | 1,42 | 1,46 | 1,63 | 2,78 | 2,74 | 1,70 | | | 1,98 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | | | | | | 2,87 | | 2,08 | 2,56 | 2,48 | | |
| | | | | Azohydromonas | | | | 4,62 | 4,02 | | | | | | 4,53 | 3,83 |
| | | | | Bordetella | | | | | | | 1,45 | 1,94 | | | | |
| | | | Aquabacterium_family | Aquabacterium | | | | | | | | | 3,83 | | | |
| | | | Aquincola_family | Aquincola | | | | 5,11 | 4,45 | | | | 4,84 | | | |
| | | | Burkholderiaceae | Burkholderia | | | | 3,91 | 3,43 | 4,33 | 2,89 | 3,49 | 3,86 | 4,68 | 4,17 | 3,92 |
| | | | | Ralstonia | | | | 3,17 | 2,83 | 3,52 | | | | | | |
| | | | Comamonadaceae | Acidovorax | | | | 5,54 | 4,91 | 6,13 | | 3,86 | 5,26 | 5,58 | 6,23 | 5,31 |
| | | | | Caldimonas | | | | | 3,73 | | | | | | 4,51 | 3,86 |
| | | | | Comamonas | | | | | 4,43 | | | | | | 5,23 | 4,81 |
| | | | | Pelomonas | 73,68 | 88,67 | 54,07 | 16,78 | 14,69 | 18,38 | 6,42 | 9,56 | 15,56 | 16,74 | 22,37 | 18,32 |
| | | | | Pseudorhodoferax | | | | 3,76 | | 4,19 | | | | | | |
| | | | | Variovorax | | | | | 3,61 | | | | 3,81 | | 4,39 | |
| | | | Ideonella_family | Ideonella | | | 25,32 | | | | 2,94 | | | | | 5,32 |
| | | | Methylibium_family | Methylibium | | | | 7,27 | 6,30 | 7,94 | 3,13 | 4,52 | 6,63 | 6,64 | 7,53 | 6,22 |
| | | | Oxalobacteraceae | Herbaspirillum | | | | 3,02 | | | | | | | | |
| | | | | Janthinobacterium | | | | | 2,49 | | | | | | | |
| | | | | Massilia | | | | 2,70 | 2,44 | 3,04 | 1,65 | 2,04 | 2,71 | 2,83 | 3,00 | 2,70 |
| | | | Polyangium brachysporum_genus_family | Polyangium brachysporum_genus | | | | 4,31 | 3,74 | 4,72 | | | 4,03 | 4,08 | 4,59 | |
| | | | Rhizobacter_family | Rhizobacter | | | | 6,36 | 5,56 | 6,99 | | | 5,89 | | 6,70 | 5,37 |
| | | | Rubrivivax_family | Rubrivivax | | | | 4,61 | 4,10 | 5,06 | | | 4,36 | | 4,77 | |
| | | | Sphaerotilus_family | Sphaerotilus | | | | 4,10 | 3,63 | 4,53 | | 2,86 | 3,91 | 3,82 | | |
| | | | Thiomonas_family | Thiomonas | | | | | | | | | | | 3,10 | |
| | | Neisseriales | Neisseriaceae | Neisseria | | | | | | | 0,83 | | | | | |
| | | Rhodocyclales | Rhodocyclaceae | Thauera | | | | 1,86 | 1,69 | 2,03 | | 1,44 | 1,82 | 1,86 | 1,96 | |
| | Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio | | | | | | | | | | | | 0,97 |
| | | Myxococcales | Cystobacteraceae | Hyalangium | | | | | | | | 0,44 | | | | |
| | | | Myxococcaceae | Myxococcus | | | | | | | 0,52 | | | | | |
| | | | Polyangiaceae | Chondromyces | | | | | | | | | | 0,37 | 0,29 | |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Marinobacter | | | | | | | | | | 0,92 | | |
| | | Enterobacterales | Enterobacteriaceae | Escherichia | | | | | 0,75 | | | | | | | 0,91 |
| | | | | Enterobacter | | | | 0,91 | | | 0,95 | | | | 0,86 | |
| | | | | Serratia | | | | | | 0,91 | | 0,92 | 0,87 | | | |
| | | Oceanospirillales | Halomonadaceae | Halomonas | | | | | | | 0,92 | | | | | |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | | | 8,33 | 2,72 | 2,66 | 3,05 | 3,63 | 3,97 | 3,10 | 2,84 | 2,75 | 3,19 |
| | | Vibrionales | Vibrionaceae | Vibrio | | | | | | | 1,27 | | | | | |
| | | Xanthomonadales | Xanthomonadaceae | Lysobacter | | | | | | | | | | | | 1,12 |
| | | | | Xanthomonas | | | | 1,12 | 0,98 | 1,25 | | 1,30 | 1,18 | 1,38 | 1,13 | |
| Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | Mycoplasma | 26,32 | 11,33 | 4,20 | | | | | | | | | |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | | | | | | | | | | 0,26 | | |
| | | | | Penicillium | | | | | | | 0,25 | | | | | |
| | | | Herpotrichiellaceae | Exophiala | | | | | | | | 0,29 | | | 0,17 | |
| | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | | | | 0,21 | | 0,23 | | | 0,24 | | | |
| | | | Ophiocordycipitaceae | Tolypocladium | | | | | | | | | | | | 0,12 |

## *Table 6.8: Proportion of each genus during the 2017 kinetic in LB (sample I)*

| | | | | | D 7 | | | D 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Nocardiaceae | Nocardia | | 0,53 | | | 0,71 | |
| | | | | Rhodococcus | | | | 0,68 | | 0,68 |
| | | Micrococcales | Microbacteriaceae | Microbacterium | | | 0,47 | | | 0,53 |
| | | | Micrococcaceae | Arthrobacter | | 0,52 | | 0,62 | 0,57 | |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis | | | | 0,59 | | 0,61 |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 29,75 | 2,48 | 20,03 | 2,93 | 2,60 | 2,98 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | 0,61 | 0,72 | | |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Flavobacterium | | 0,49 | | 0,50 | 0,51 | 0,48 |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 1,74 | 1,40 | 1,58 | 1,58 | 1,47 | 1,55 |
| | | | Paenibacillaceae | Paenibacillus | | | | | 1,17 | 1,12 |
| | | Lactobacillales | Lactobacillaceae | Lactobacillus | | | | 0,61 | | 0,59 |
| | | | Streptococcaceae | Streptococcus | | | | | 0,72 | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | | | 1,19 | 1,34 | 1,55 | 1,32 |
| | | | Lachnospiraceae | Blautia | | 0,33 | | | | |
| | Negativicutes | Selenomonadales | Veillonellaceae | Pelosinus | | | | | 1,99 | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Asticcacaulis | | | | | | 1,40 |
| | | | | Brevundimonas | | 1,33 | | | 1,44 | |
| | | | | Caulobacter | | | | 1,67 | | |
| | | Rhizobiales | Aurantimonadaceae | Aureimonas | | | | 1,86 | | |
| | | | Bartonellaceae | Bartonella | | | | | | 1,50 |
| | | | Bradyrhizobiaceae | Bosea | | | | | | 2,48 |
| | | | | Bradyrhizobium | | 1,70 | | 2,64 | 2,15 | 2,67 |
| | | | Hyphomicrobiaceae | Prosthecomicrobium | | | | 1,97 | 1,52 | |
| | | | Methylobacteriaceae | Methylobacterium | | | | 2,68 | 2,36 | 2,83 |
| | | | Methylocystaceae | Pleomorphomonas | | | | 1,47 | | 1,57 |
| | | | Phyllobacteriaceae | Mesorhizobium | | 1,69 | | 2,54 | 2,15 | 2,63 |
| | | | Rhizobiaceae | Agrobacterium | | 1,67 | | 2,36 | 2,05 | 2,44 |
| | | | | Rhizobium | 1,68 | 2,25 | 1,78 | 3,33 | 2,87 | 3,51 |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas | | | | 1,31 | | |
| | | | Rhodobacteraceae | Loktanella | | | 0,84 | | | |
| | | | | Paracoccus | | 1,35 | | 1,63 | 1,56 | 1,66 |
| | | Rhodospirillales | Acetobacteraceae | Acetobacter | | 1,31 | | 1,49 | 1,47 | 1,50 |
| | | | Rhodospirillaceae | Azospirillum | | 1,77 | 1,38 | 2,18 | 2,13 | 2,14 |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter | | 5,67 | 3,48 | 5,22 | 5,58 | 4,72 |
| | | | | Croceicoccus | | 3,73 | | 3,45 | 3,72 | 3,11 |
| | | | | Erythrobacter | 2,37 | 6,76 | 4,11 | 6,26 | 6,76 | 5,74 |
| | | | | Porphyrobacter | 1,94 | 5,53 | 3,41 | 5,08 | 5,46 | 4,62 |
| | | | Sphingomonadaceae | Citromicrobium | | 3,52 | | | | 3,04 |
| | | | | Novosphingobium | 3,90 | 12,13 | 7,16 | 11,03 | 11,76 | 9,74 |
| | | | | Sphingobium | | 6,61 | 4,07 | 6,25 | 6,55 | 5,65 |
| | | | | Sphingomonas | 3,98 | 10,98 | 6,70 | 10,34 | 10,74 | 9,81 |
| | | | | Sphingomonas-like bacterium B12_genus | | 2,32 | | 2,12 | 2,27 | 1,96 |
| | | | | Sphingopyxis | | 5,08 | 3,13 | 4,73 | 5,06 | 4,43 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | | | | | 0,97 | |
| | | | | Azohydromonas | | 1,62 | | | | |
| | | | | Bordetella | | 0,00 | | | | 0,93 |
| | | | Burkholderiaceae | Burkholderia | 9,83 | 2,52 | 7,18 | 2,34 | 1,97 | 2,36 |
| | | | | Ralstonia | 40,15 | | 26,29 | | | |
| | | | Comamonadaceae | Acidovorax | | 2,29 | | | | |
| | | | | Pelomonas | 3,24 | 4,17 | 2,35 | 1,61 | 0,89 | 1,77 |
| | | | Methylibium_family | Methylibium | | 2,19 | | | | |
| | | | Oxalobacteraceae | Herbaspirillum | | 1,83 | | | | |
| | Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio | | 0,78 | | 0,86 | 0,89 | 0,85 |
| | Gammaproteobacteria | Enterobacterales | Enterobacteriaceae | Escherichia | | | | | 0,82 | |
| | | | | Enterobacter | | | | | | 0,87 |
| | | | | Salmonella | | | | 0,57 | | |
| | | | | Serratia | 1,13 | 0,73 | 0,98 | | | |
| | | Pseudomonadales | Moraxellaceae | Acinetobacter | | | | | 0,80 | |
| | | | Pseudomonadaceae | Pseudomonas | | 2,43 | 2,97 | 2,97 | 2,62 | 3,00 |
| | | Vibrionales | Vibrionaceae | Vibrio | | | | | 0,96 | 1,04 |
| | | Xanthomonadales | Xanthomonadaceae | Xanthomonas | | | | | 0,92 | |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | | | | 0,31 | 0,28 | |
| | Saccharomycetes | Saccharomycetales | Saccharomycetaceae | Zygosaccharomyces | | | | 0,17 | | 0,18 |
| | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | 0,29 | 0,28 | 0,28 | | | |

### *Table 6.9: Proportion of each genus during the 2017 kinetic in R2A (sample I)*

| | | | | | D 7 | | | D 28 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 |
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Corynebacterium | 0,57 | | | | | |
| | | | Mycobacteriaceae | Mycobacterium | | | | | 0,85 | |
| | | | Nocardiaceae | Nocardia | | | | 0,43 | | |
| | | | | Rhodococcus | 0,60 | 0,55 | 0,58 | 0,20 | | |
| | | Micrococcales | Microbacteriaceae | Microbacterium | 0,52 | | 0,47 | 0,48 | 0,68 | |
| | | | Micrococcaceae | Arthrobacter | | 0,53 | | | | 0,60 |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis | | | | 0,18 | 0,54 | |
| | | | | Lentzea | 0,24 | | 0,25 | | | |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 2,46 | 2,35 | 2,35 | 2,72 | 2,60 | 18,25 |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | | | | 0,44 | 0,61 | |
| | | | Prevotellaceae | Prevotella | 0,48 | 0,47 | 0,51 | | | |
| | | | Porphyromonadaceae | Porphyromonas | | | | 0,10 | | |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium | 0,37 | | | | 0,32 | 0,33 |
| | | | | Flavobacterium | | 0,43 | 0,47 | 0,46 | | |
| | Sphingobacteriia | Sphingobacteriales | Sphingobacteriaceae | Pedobacter | | | | 0,13 | | |
| Cyanobacteria | Cyanobacteria | Nostocales | Tolypothrichaceae | Tolypothrix | 0,29 | | | | | |
| | | Synechococcales | Leptolyngbyaceae | Leptolyngbya | | | | | 0,15 | |
| | | | Synechococcaceae | Synechococcus | | | | 0,14 | | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | 1,47 | 1,35 | 1,38 | 1,51 | 1,44 | 1,67 |
| | | | Paenibacillaceae | Paenibacillus | | 1,00 | 1,03 | 0,76 | 0,52 | |
| | | Lactobacillales | Lactobacillaceae | Lactobacillus | 0,55 | 0,48 | 0,53 | 0,55 | 0,55 | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | 1,29 | 1,15 | 1,16 | 1,27 | 1,22 | 1,27 |
| | | | Lachnospiraceae | Dorea | | | | 0,10 | | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Asticcacaulis | | | 1,30 | | | |
| | | | | Brevundimonas | | | | 0,46 | | |
| | | | | Caulobacter | 1,46 | 1,51 | | 1,04 | 1,60 | 1,19 |
| | | Rhizobiales | Aurantimonadaceae | Aureimonas | | | | 0,54 | 0,00 | |
| | | | Bradyrhizobiaceae | Bradyrhizobium | 1,82 | 1,91 | 1,87 | 2,30 | 2,29 | |
| | | | Hyphomicrobiaceae | Prosthecomicrobium | | | | 1,48 | 1,40 | |
| | | | Methylobacteriaceae | Methylobacterium | | | | 1,57 | 2,25 | |
| | | | Methylocystaceae | Pleomorphomonas | | | | | | |
| | | | Phyllobacteriaceae | Mesorhizobium | | 1,83 | 1,81 | 2,13 | 2,08 | |
| | | | Rhizobiaceae | Agrobacterium | 1,79 | 1,93 | 1,86 | 2,05 | 1,95 | |
| | | | | Rhizobium | 2,41 | 2,57 | 2,48 | 2,91 | 2,78 | 2,04 |
| | | | Xanthobacteraceae | Azorhizobium | | | | 0,45 | | |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas | | | | | 0,60 | |
| | | | Rhodobacteraceae | Paracoccus | 1,41 | 1,49 | 1,42 | 1,76 | 1,54 | |
| | | | | Ruegeria | | | | | | 0,91 |
| | | Rhodospirillales | Acetobacteraceae | Acetobacter | | 1,42 | 1,41 | 0,47 | 0,65 | |
| | | | Rhodospirillaceae | Azospirillum | | | 1,93 | 2,13 | 2,13 | 1,72 |
| | | | | Inquilinus | 1,04 | 1,09 | | | | |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter | 5,96 | 6,08 | 6,11 | 5,10 | 5,26 | 3,55 |
| | | | | Croceicoccus | 3,85 | 3,94 | 4,03 | 3,34 | 3,45 | 2,40 |
| | | | | Erythrobacter | 7,11 | 7,40 | 7,37 | 6,18 | 6,38 | 4,33 |
| | | | | Porphyrobacter | 5,86 | 6,06 | 6,06 | 4,90 | 5,08 | |
| | | | Sphingomonadaceae | Citromicrobium | 3,60 | 3,74 | 3,75 | 3,20 | 3,28 | |
| | | | | Novosphingobium | 12,79 | 13,17 | 12,99 | 10,92 | 11,28 | 7,69 |
| | | | | Sphingobium | 6,97 | 7,23 | 7,14 | 6,14 | 6,44 | 4,48 |
| | | | | Sphingomonas | 11,58 | 11,94 | 11,86 | 10,29 | 10,74 | 7,46 |
| | | | | Sphingomonas-like bacterium B12_genus | 2,50 | 2,55 | 2,58 | 2,11 | 2,19 | |
| | | | | Sphingopyxis | 5,26 | 5,44 | 5,43 | 4,63 | 4,75 | 3,36 |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | 1,16 | | | | | |
| | | | | Bordetella | | | | 0,78 | 0,39 | 2,43 |
| | | | Burkholderiaceae | Burkholderia | 2,25 | 2,12 | 2,12 | 2,47 | 2,35 | 6,68 |
| | | | | Ralstonia | | | | | | 23,11 |
| | | | Comamonadaceae | Pelomonas | 1,82 | 0,75 | 0,98 | 2,14 | 1,32 | 2,04 |
| | | | Oxalobacteraceae | Herbaspirillum | 1,61 | 1,47 | 1,45 | 1,72 | 1,71 | |
| | | | | Massilia | 0,96 | | | 0,62 | 0,45 | |
| | Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio | 0,82 | 0,78 | 0,87 | 0,81 | 0,77 | |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Marinobacter | | 0,58 | | 0,45 | 0,59 | |
| | | | | Paraglaciecola | 0,51 | | | | | |
| | | Enterobacterales | Enterobacteriaceae | Enterobacter | | | | | | 1,02 |
| | | | | Serratia | 0,77 | 0,68 | 0,72 | 0,72 | 0,71 | |
| | | Pseudomonadales | Moraxellaceae | Acinetobacter | 0,84 | | | 0,28 | 0,37 | |
| | | | Pseudomonadaceae | Pseudomonas | 2,49 | 2,48 | 2,47 | 2,87 | 2,80 | 3,31 |
| | | Vibrionales | Vibrionaceae | Vibrio | 1,00 | | | 0,66 | | |
| | | Xanthomonadales | Xanthomonadaceae | Xanthomonas | 1,01 | 0,97 | 0,98 | 0,66 | 0,51 | |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus | | 0,29 | | 0,10 | 0,29 | |
| | | Chaetothyriales | Herpotrichiellaceae | Exophiala | 0,22 | | | 0,17 | | |
| | Sordariomycetes | Hypocreales | Nectriaceae | Fusarium | 0,29 | 0,28 | 0,27 | | 0,14 | |
| | | | Ophiocordycipitaceae | Hirsutella | | | | | | 0,15 |

***Table 6.10: Sum up of the different genera found in the 2017 kinetic from Pool 2.*** *Red: only found in LB (28 genera), blue: only found in R2A (35 genera), green: found in both media (98 genera)*

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Actinobacteria | Actinobacteria | Actinomycetales | Actinomycetaceae | Actinomyces |
| | | Bifidobacteriales | Bifidobacteriaceae | Bifidobacterium |
| | | Corynebacteriales | Corynebacteriaceae | Corynebacterium |
| | | | Gordoniaceae | Gordonia |
| | | | Mycobacteriaceae | Mycobacterium |
| | | | Nocardiaceae | Nocardia |
| | | | | Rhodococcus |
| | | Micrococcales | Cellulomonadaceae | Actinotalea |
| | | | | Cellulomonas |
| | | | Intrasporangiaceae | Janibacter |
| | | | Microbacteriaceae | Microbacterium |
| | | | Micrococcaceae | Arthrobacter |
| | | | Promicromonosporaceae | Promicromonosporaceae bacterium W15_genus |
| | | Propionibacteriales | Nocardioidaceae | Mumia |
| | | | | Nocardioides |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis |
| | | | | Lentzea |
| | | Streptomycetales | Streptomycetaceae | Streptomyces |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides |
| | | | Prevotellaceae | Prevotella |
| | | | Porphyromonadaceae | Porphyromonas |
| | Chitinophagia | Chitinophagales | Chitinophagaceae | Sediminibacterium |
| | Cytophagia | Cytophagales | Cytophagaceae | Rhodonellum |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium |
| | | | | Flavobacterium |
| | Sphingobacteriia | Sphingobacteriales | Sphingobacteriaceae | Pedobacter |
| | | | | Sphingobacterium |
| bacterium YEK0313_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus |
| Cyanobacteria | Cyanobacteria | Nostocales | Tolypothrichaceae | Tolypothrix |
| | | Synechococcales | Leptolyngbyaceae | Leptolyngbya |
| | | | Synechococcaceae | Synechococcus |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus |
| | | | Paenibacillaceae | Paenibacillus |
| | | Lactobacillales | Lactobacillaceae | Lactobacillus |
| | | | Streptococcaceae | Streptococcus |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium |
| | | | Lachnospiraceae | Blautia |
| | | | | Butyrivibrio |
| | | | | Dorea |
| | | | | Lachnoclostridium |
| | | | | Oribacterium |
| | | | Peptococcaceae | Desulfosporosinus |
| | Negativicutes | Selenomonadales | Veillonellaceae | Pelosinus |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Asticcacaulis |
| | | | | Brevundimonas |
| | | | | Caulobacter |
| | | | | Phenylobacterium |
| | | Rhizobiales | Aurantimonadaceae | Aureimonas |
| | | | | Aurantimonas |
| | | | Bartonellaceae | Bartonella |
| | | | Beijerinckiaceae | Beijerinckia |
| | | | Bradyrhizobiaceae | Afipia |
| | | | | Bosea |
| | | | | Bradyrhizobium |
| | | | | Rhodopseudomonas |
| | | | Chelatococcaceae | Chelatococcus |
| | | | Hyphomicrobiaceae | Devosia |
| | | | | Hyphomicrobium |
| | | | | Prosthecomicrobium |
| | | | Methylobacteriaceae | Methylobacterium |
| | | | | Microvirga |
| | | | Methylocystaceae | Pleomorphomonas |
| | | | Phyllobacteriaceae | Aminobacter |
| | | | | Mesorhizobium |
| | | | Rhizobiaceae | Agrobacterium |
| | | | | Kaistia |
| | | | | Rhizobium |
| | | | Rhodobiaceae | Amorphus |
| | | | Xanthobacteraceae | Azorhizobium |

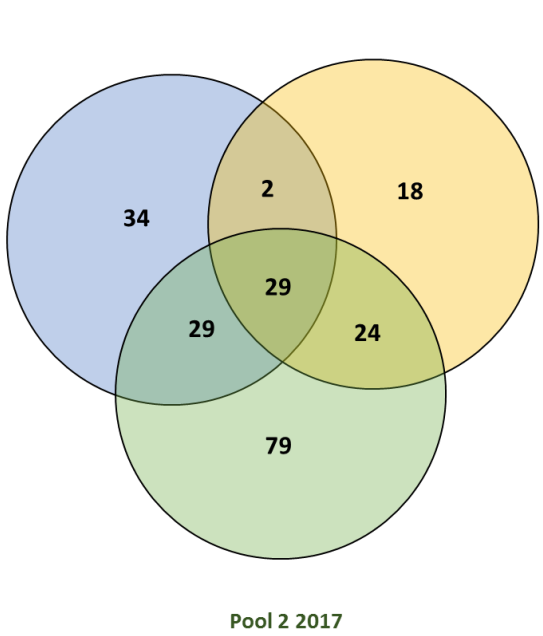
| | | | |
|---|---|---|---|
| | Rhodobacterales | Hyphomonadaceae | Hyphomonas |
| | | | Oceanicaulis |
| | | Rhodobacteraceae | Labrenzia |
| | | | Loktanella |
| | | | Pannonibacter |
| | | | Paracoccus |
| | | | Rhodobacter |
| | | | Rhodovulum |
| | | | Ruegeria |
| | Rhodospirillales | Acetobacteraceae | Acetobacter |
| | | | Roseomonas |
| | | Rhodospirillaceae | Azospirillum |
| | | | Inquilinus |
| | | Rhodospirillales bacterium_family | Rhodospirillales bacterium URHD0088_genus |
| | Sphingomonadales | Erythrobacteraceae | Altererythrobacter |
| | | | Croceicoccus |
| | | | Erythrobacter |
| | | | Porphyrobacter |
| | | Sphingomonadaceae | Citromicrobium |
| | | | Novosphingobium |
| | | | Sphingobium |
| | | | Sphingomonas |
| | | | Sphingomonas-like bacterium B12_genus |
| | | | Sphingopyxis |
| Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter |
| | | | Azohydromonas |
| | | | Bordetella |
| | | Aquabacterium_family | Aquabacterium |
| | | Aquincola_family | Aquincola |
| | | Burkholderiaceae | Burkholderia |
| | | | Ralstonia |
| | | Comamonadaceae | Acidovorax |
| | | | Caldimonas |
| | | | Comamonas |
| | | | Pelomonas |
| | | | Pseudorhodoferax |
| | | | Variovorax |
| | | Ideonella_family | Ideonella |
| | | Leptothrix_family | Leptothrix |
| | | Methylibium_family | Methylibium |
| | | Oxalobacteraceae | Collimonas |
| | | | Herbaspirillum |
| | | | Janthinobacterium |
| | | | Massilia |
| | | Polyangium brachysporum_family | Polyangium brachysporum_genus |
| | | Rhizobacter_family | Rhizobacter |
| | | Rubrivivax_family | Rubrivivax |
| | | Sphaerotilus_family | Sphaerotilus |
| | | Thiomonas_family | Thiomonas |
| | Neisseriales | Chromobacteriaceae | Andreprevotia |
| | | Neisseriaceae | Neisseria |
| | Nitrosomonadales | Sterolibacteriaceae | Sulfuritalea |
| | Rhodocyclales | Zoogloeaceae | Azoarcus |
| | | | Uliginosibacterium |
| | | | Thauera |
| Deltaproteobacteria | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio |
| | Myxococcales | Archangiaceae | Cystobacter |
| | | | Hyalangium |
| | | | Stigmatella |
| | | Myxococcaceae | Myxococcus |
| | | Polyangiaceae | Chondromyces |
| Epsilonproteobacteria | Campylobacterales | Campylobacteraceae | Campylobacter |
| | | Helicobacteraceae | Helicobacter |
| Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Alteromonas |
| | | | Marinobacter |
| | | | Paraglaciecola |
| | | Pseudoalteromonadaceae | Pseudoalteromonas |
| | Enterobacterales | Enterobacteriaceae | Candidatus Blochmannia |
| | | | Escherichia |
| | | | Enterobacter |
| | | | Salmonella |
| | | | Serratia |
| | Oceanospirillales | Halomonadaceae | Halomonas |
| | Pseudomonadales | Moraxellaceae | Acinetobacter |
| | | Pseudomonadaceae | Pseudomonas |
| | Vibrionales | Vibrionaceae | Vibrio |
| | Xanthomonadales | Xanthomonadaceae | Lysobacter |
| | | | Pseudoxanthomonas |
| | | | Rhodanobacter |
| | | | Xanthomonas |

| | | | | |
|---|---|---|---|---|
| Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | Mycoplasma |
| Ascomycota | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus |
| | | | | Penicillium |
| | | Chaetothyriales | Clavicipitaceae | Cladophialophora |
| | | | Herpotrichiellaceae | Exophiala |
| | Saccharomycetes | Saccharomycetales | Saccharomycetaceae | Zygosaccharomyces |
| | Sordariomycetes | Hypocreales | Hypocreaceae | Metarhizium |
| | | | | Trichoderma |
| | | | Nectriaceae | Fusarium |
| | | | Ophiocordycipitaceae | Hirsutella |
| | | | | Tolypocladium |
| | | Sordariales | Madurella_family | Madurella |

# Appendix 5: Comparison between the kinetics from Pool 1 2015, Pool 2 2015 and Pool 2 2017

***Figure 6.2: Comparison between the genera obtained after the 2015 kinetics in Pools 1 and 2 and after the 2017 kinetics from Pool 2.*** *A) Venn diagram: 29 genera common to all, 29 common to pools 1 and 2 2017, 24 genera common to Pool 2 2015 and 2017, 2 genera common to pools 1 and 2 in 2015, 34 genera only in Pool 1, 18 genera only in Pool 2 2015, and 79 genera only in Pool 2 2017; B) Legend of the table; C) Table with all the genera present during the kinetics and representing the Venn diagram*

A)




B)

| |
|---|
| Pool 1 |
| Pool 1 / Pool 2 2017 |
| Pool 1 / Pool 2 2015 |
| Pool 2 2015 |
| Pool 2 2015/2017 |
| Pool 2 2017 |
| All 3 |

C)

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Actinobacteria | Actinobacteria | Actinomycetales | Actinomycetaceae | Actinomyces |
| | | Bifidobacteriales | Bifidobacteriaceae | Bifidobacterium |
| | | Corynebacteriales | Corynebacteriaceae | Corynebacterium |
| | | | Gordoniaceae | Gordonia |
| | | | Mycobacteriaceae | Mycobacterium |
| | | | Nocardiaceae | Nocardia |
| | | | | Rhodococcus |
| | | Micrococcales | Cellulomonadaceae | Actinotalea |
| | | | | Cellulomonas |
| | | | Intrasporangiaceae | Janibacter |
| | | | Microbacteriaceae | Agromyces |
| | | | | Cryocola |
| | | | | Leifsonia |
| | | | | Microbacterium |
| | | | Micrococcaceae | Arthrobacter |
| | | | | Micrococcus |
| | | | Promicromonosporaceae | Promicromonosporaceae bacterium W15_genus |
| | | Propionibacteriales | Nocardioidaceae | Mumia |
| | | | | Nocardioides |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis |
| | | | | Lentzea |
| | | Streptomycetales | Streptomycetaceae | Streptomyces |
| Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides |
| | | | Prevotellaceae | Prevotella |
| | | | Porphyromonadaceae | Porphyromonas |
| | | | Rikenellaceae | Rikenella |
| | Chitinophagia | Chitinophagales | Chitinophagaceae | Sediminibacterium |
| | Cytophagia | Cytophagales | Cytophagaceae | Dyadobacter |
| | | | | Microscilla |
| | | | | Rhodonellum |
| | | | | Runella |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium |
| | | | | Chryseobacterium or Bergeyella |
| | | | | Flavobacterium |
| | Sphingobacteriia | Sphingobacteriales | Sphingobacteriaceae | Pedobacter |
| | | | | Sphingobacterium |
| bacterium YEK0313_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus |

| | | | | |
|---|---|---|---|---|
| Cyanobacteria | Cyanobacteria | Nostocales | Aphanizomenonaceae | Nodularia |
| | | | Hapalosiphonaceae | Mastigocladus |
| | | | Nostocaceae | Anabaena |
| | | | | Cylindrospermum |
| | | | | Nostoc |
| | | | Tolypothrichaceae | Tolypothrix |
| | | Oscillatoriales | Cyanothecaceae | Cyanothece |
| | | Synechococcales | Leptolyngbyaceae | Leptolyngbya |
| | | | Synechococcaceae | Synechococcus |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus |
| | | | Paenibacillaceae | Paenibacillus |
| | | | Staphylococcaceae | Staphylococcus |
| | | Lactobacillales | Lactobacillaceae | Lactobacillus |
| | | | Streptococcaceae | Streptococcus |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium |
| | | | Lachnospiraceae | Blautia |
| | | | | Butyrivibrio |
| | | | | Dorea |
| | | | | Lachnoclostridium |
| | | | | Oribacterium |
| | | | | Roseburia |
| | | | Peptococcaceae | Desulfosporosinus |
| | Negativicutes | Selenomonadales | Veillonellaceae | Pelosinus |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Asticcacaulis |
| | | | | Brevundimonas |
| | | | | Caulobacter |
| | | | | Phenylobacterium |
| | | Rhizobiales | Aurantimonadaceae | Aurantimonas |
| | | | | Aureimonas |
| | | | Bartonellaceae | Bartonella |
| | | | Beijerinckiaceae | Beijerinckia |
| | | | | Methylocapsa |
| | | | Bradyrhizobiaceae | Afipia |
| | | | | Bosea |
| | | | | Bradyrhizobium |
| | | | | Rhodopseudomonas |
| | | | Brucellaceae | Ochrobactrum |
| | | | | Pseudochrobactrum |
| | | | Chelatococcaceae | Chelatococcus |
| | | | Hyphomicrobiaceae | Blastochloris |
| | | | | Devosia |
| | | | | Hyphomicrobium |
| | | | | Prosthecomicrobium |
| | | | Methylobacteriaceae | Methylobacterium |
| | | | | Microvirga |
| | | | Methylocystaceae | Methylocystis |
| | | | | Methylopila |
| | | | | Pleomorphomonas |
| | | | Phyllobacteriaceae | Aminobacter |
| | | | | Mesorhizobium |
| | | | Rhizobiaceae | Agrobacterium |
| | | | | Kaistia |
| | | | | Rhizobium |
| | | | Rhodobiaceae | Afifella |
| | | | | Amorphus |
| | | | | Parvibaculum |
| | | | Xanthobacteraceae | Azorhizobium |
| | | | | Starkeya |
| | | | | Xanthobacter |
| | | Rhodobacterales | Hyphomonadaceae | Hyphomonas |
| | | | | Oceanicaulis |
| | | | Rhodobacteraceae | Labrenzia |
| | | | | Loktanella |
| | | | | Pannonibacter |
| | | | | Paracoccus |
| | | | | Rhodobacter |
| | | | | Rhodovulum |
| | | | | Ruegeria |
| | | Rhodospirillales | Acetobacteraceae | Acetobacter |
| | | | | Komagataeibacter |
| | | | | Roseomonas |
| | | | Rhodospirillaceae | Azospirillum |
| | | | | Inquilinus |
| | | | | Magnetospira |
| | | | | Oceanibaculum |
| | | | | Rhodovibrio |
| | | | | Skermanella |
| | | | | Thalassobaculum |
| | | | Rhodospirillales bacterium_family | Rhodospirillales bacterium URHD0088_genus |
| | | Sphingomonadales | Erythrobacteraceae | Altererythrobacter |
| | | | | Croceicoccus |
| | | | | Erythrobacter |
| | | | | Porphyrobacter |
| | | | Sphingomonadaceae | Citromicrobium |
| | | | | Novosphingobium |
| | | | | Sphingobium |
| | | | | Sphingomonas |
| | | | | Sphingomonas-like bacterium B12_genus |
| | | | | Sphingopyxis |

| | | | | |
|---|---|---|---|---|
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter |
| | | | | Azohydromonas |
| | | | | Bordetella |
| | | | Aquabacterium_family | Aquabacterium |
| | | | Aquincola_family | Aquincola |
| | | | Burkholderiales bacterium JOSHI_001_family | Burkholderiales bacterium JOSHI_001_genus |
| | | | Burkholderiaceae | Burkholderia |
| | | | | Ralstonia |
| | | | Comamonadaceae | Acidovorax |
| | | | | Caldimonas |
| | | | | Comamonas |
| | | | | Hydrogenophaga |
| | | | | Hylemonella |
| | | | | Limnohabitans |
| | | | | Pelomonas |
| | | | | Polaromonas |
| | | | | Pseudorhodoferax |
| | | | | Ramlibacter |
| | | | | Rhodoferax |
| | | | | Variovorax |
| | | | | Verminephrobacter |
| | | | Ideonella_family | Ideonella |
| | | | Leptothrix_family | Leptothrix |
| | | | Methylibium_family | Methylibium |
| | | | Oxalobacteraceae | Collimonas |
| | | | | Herbaspirillum |
| | | | | Janthinobacterium |
| | | | | Massilia |
| | | | Polyangium brachysporum_family | Polyangium brachysporum_genus |
| | | | Rhizobacter_family | Rhizobacter |
| | | | Rubrivivax_family | Rubrivivax |
| | | | Sphaerotilus_family | Sphaerotilus |
| | | | Thiomonas_family | Thiomonas |
| | | Neisseriales | Chromobacteriaceae | Andreprevotia |
| | | | | Chromobacterium |
| | | | | Leeia |
| | | | | Pseudogulbenkiania |
| | | | Neisseriaceae | Neisseria |
| | | Nitrosomonadales | Sterolibacteriaceae | Methyloversatilis |
| | | | | Sulfuritalea |
| | | Rhodocyclales | Zoogloeaceae | Azoarcus |
| | | | | Uliginosibacterium |
| | | | | Thauera |
| | Deltaproteobacteria | Desulfuromonadales | Geobacteraceae | Geobacter |
| | | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio |
| | | Myxococcales | Archangiaceae | Cystobacter |
| | | | | Hyalangium |
| | | | | Stigmatella |
| | | | Kofleriaceae | Haliangium |
| | | | Myxococcaceae | Myxococcus |
| | | | Polyangiaceae | Chondromyces |
| | Epsilonproteobacteria | Campylobacterales | Campylobacteraceae | Campylobacter |
| | | | Helicobacteraceae | Helicobacter |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Alteromonas |
| | | | | Marinobacter |
| | | | | Paraglaciecola |
| | | | Pseudoalteromonadaceae | Pseudoalteromonas |
| | | Enterobacterales | Enterobacteriaceae | Candidatus Blochmannia |
| | | | | Enterobacter |
| | | | | Escherichia |
| | | | | Klebsiella |
| | | | | Salmonella |
| | | | | Serratia |
| | | | Erwiniaceae | Pantoea |
| | | Oceanospirillales | Halomonadaceae | Halomonas |
| | | Pseudomonadales | Moraxellaceae | Acinetobacter |
| | | | Pseudomonadaceae | Pseudomonas |
| | | Vibrionales | Vibrionaceae | Vibrio |
| | | Xanthomonadales | Xanthomonadaceae | Lysobacter |
| | | | | Pseudoxanthomonas |
| | | | | Rhodanobacter |
| | | | | Xanthomonas |
| Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | Mycoplasma |
| Ascomycota | Dothideomycetes | Dothideales | Saccotheciaceae | Aureobasidium |
| | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus |
| | | | | Penicillium |
| | | | Trichocomaceae | Talaromyces |
| | | Chaetothyriales | Clavicipitaceae | Cladophialophora |
| | | | Herpotrichiellaceae | Exophiala |
| | Saccharomycetes | Saccharomycetales | Saccharomycetaceae | Zygosaccharomyces |
| | Sordariomycetes | Hypocreales | Cordycipitaceae | Torrubiella |
| | | | Hypocreaceae | Metarhizium |
| | | | | Trichoderma |
| | | | Nectriaceae | Fusarium |
| | | | | Neonectria |
| | | | Ophiocordycipitaceae | Hirsutella |
| | | | | Ophiocordyceps |
| | | | | Tolypocladium |
| | | Sordariales | Madurella_family | Madurella |

# Appendix 6: Supplemental figures of the Article "Direct meta-analysis reveals microbial diversity in the pool of the core of a nuclear reactor"

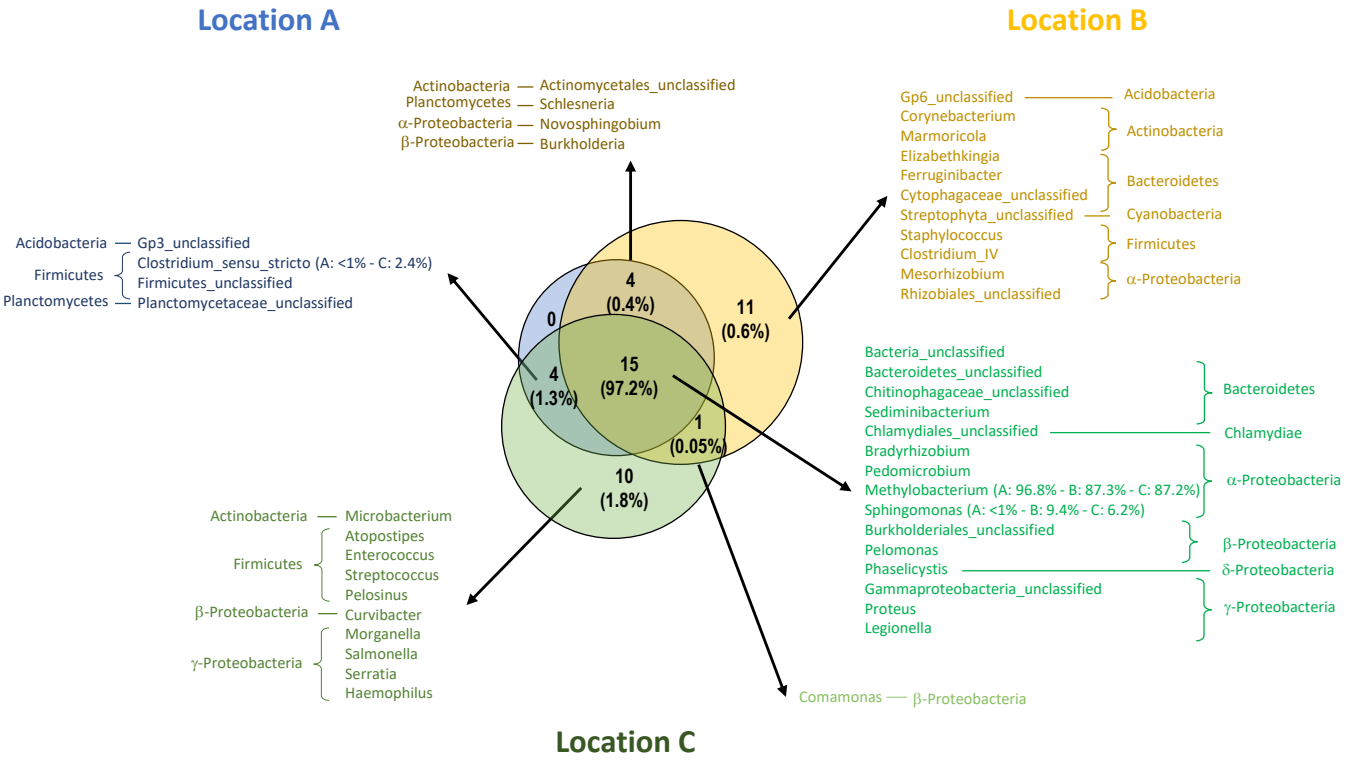



***Figure 6.3: Microbial communities present in the water of the cooling pool of the reactor's core during a shutdown, analyzed by meta-amplicomics 16SrRNA, at the taxonomic level genus.*** *To compare each location within each other, a subsampling at 28977 was performed.*

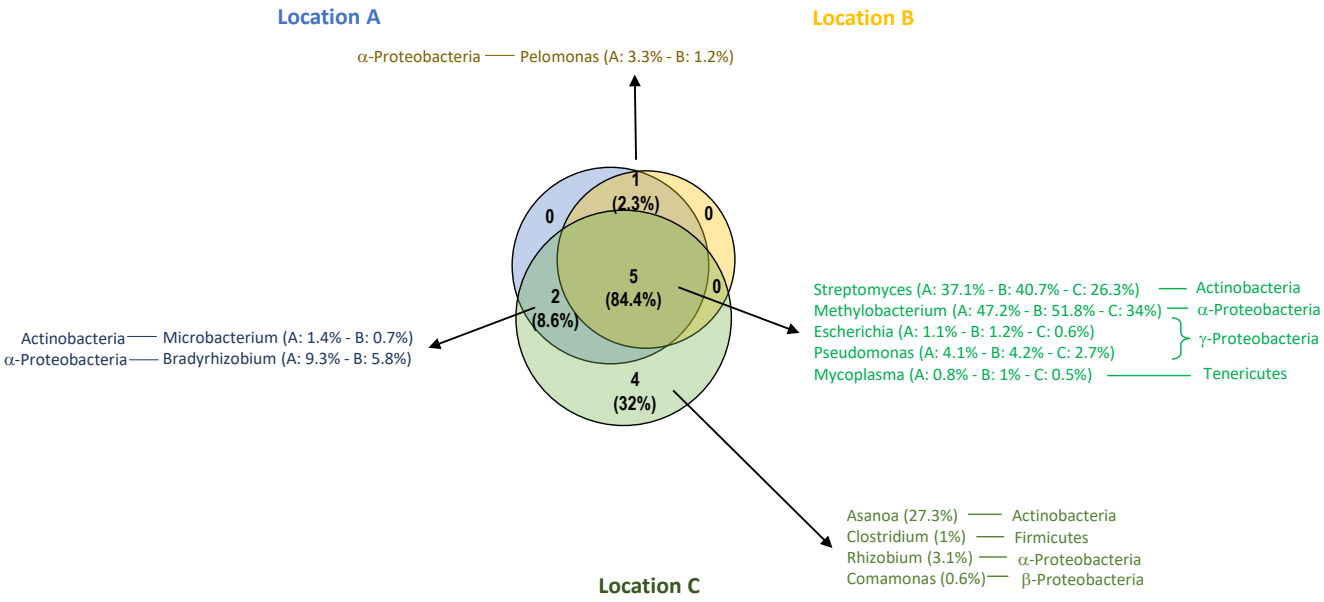
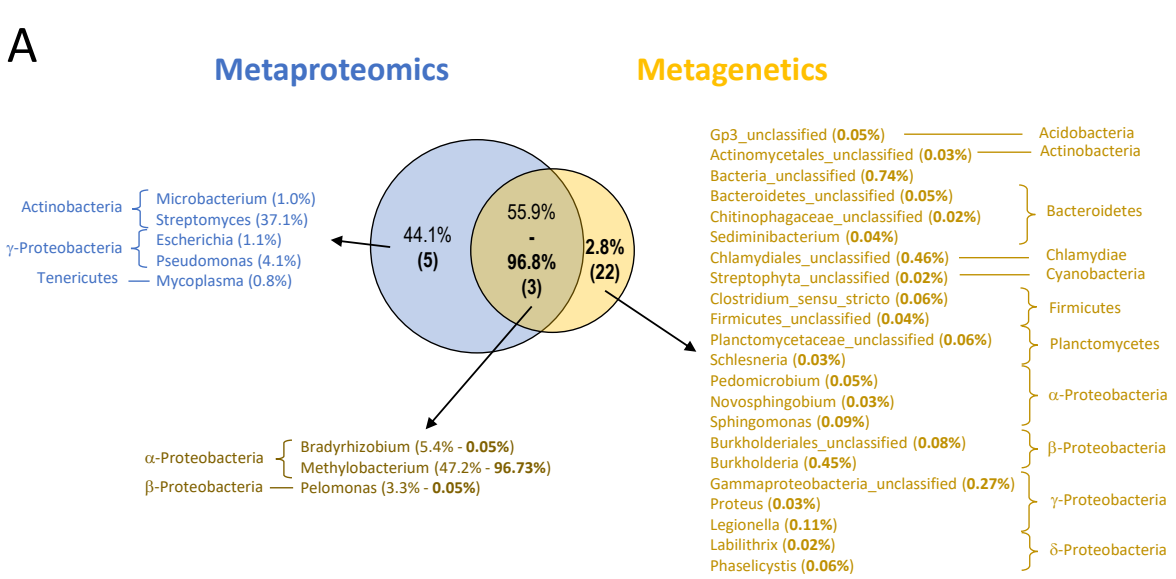
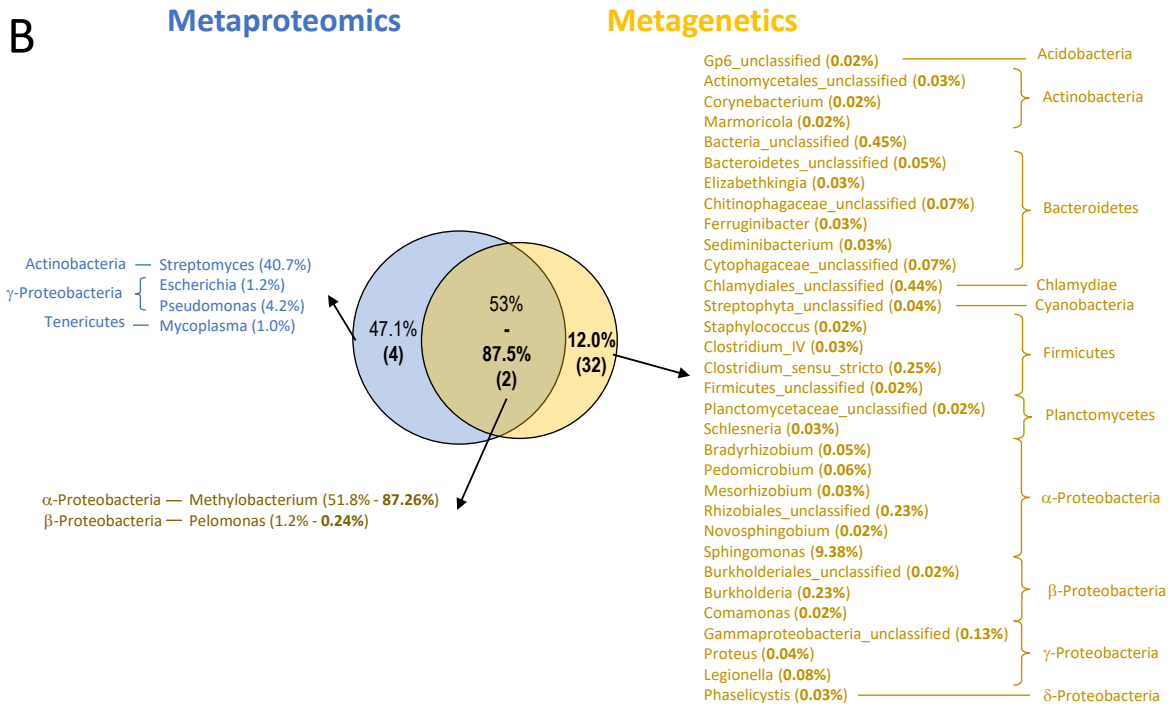



***Figure 6.4: Microbial communities present in the cooling pool of the reactor's core assessed during a reactor shutdown, analyzed by metaproteomics, at the taxonomic level genus.*** *The number of identified genera is indicated on the Venn diagram. In brackets the abundance of each genus or the cumulated abundance.*

A


Metaproteomics
Metagenetics
Actinobacteria
Microbacterium (1.0%)
Streptomyces (37.1%)
γ-Proteobacteria
Escherichia (1.1%)
Pseudomonas (4.1%)
Tenericutes
Mycoplasma (0.8%)
44.1%
(5)
55.9%
-
96.8%
(3)
2.8%
(22)
α-Proteobacteria
Bradyrhizobium (5.4% - 0.05%)
Methylobacterium (47.2% - 96.73%)
β-Proteobacteria
Pelomonas (3.3% - 0.05%)
Gp3_unclassified (0.05%)
Acidobacteria
Actinomycetales_unclassified (0.03%)
Actinobacteria
Bacteria_unclassified (0.74%)
Bacteroidetes_unclassified (0.05%)
Chitinophagaceae_unclassified (0.02%)
Sediminibacterium (0.04%)
Bacteroidetes
Chlamydiales_unclassified (0.46%)
Chlamydiae
Streptophyta_unclassified (0.02%)
Cyanobacteria
Clostridium_sensu_stricto (0.06%)
Firmicutes_unclassified (0.04%)
Firmicutes
Planctomycetaceae_unclassified (0.06%)
Planctomycetes
Schlesneria (0.03%)
Pedomicrobium (0.05%)
Novosphingobium (0.03%)
Sphingomonas (0.09%)
α-Proteobacteria
Burkholderiales_unclassified (0.08%)
Burkholderia (0.45%)
β-Proteobacteria
Gammaproteobacteria_unclassified (0.27%)
Proteus (0.03%)
Legionella (0.11%)
γ-Proteobacteria
Labilithrix (0.02%)
Phaselicystis (0.06%)
δ-Proteobacteria

B


Metaproteomics
Metagenetics
Actinobacteria
Streptomyces (40.7%)
γ-Proteobacteria
Escherichia (1.2%)
Pseudomonas (4.2%)
Tenericutes
Mycoplasma (1.0%)
47.1%
(4)
53%
-
87.5%
(2)
12.0%
(32)
α-Proteobacteria
Methylobacterium (51.8% - 87.26%)
β-Proteobacteria
Pelomonas (1.2% - 0.24%)
Gp6_unclassified (0.02%)
Acidobacteria
Actinomycetales_unclassified (0.03%)
Corynebacterium (0.02%)
Marmoricola (0.02%)
Actinobacteria
Bacteria_unclassified (0.45%)
Bacteroidetes_unclassified (0.05%)
Elizabethkingia (0.03%)
Chitinophagaceae_unclassified (0.07%)
Ferruginibacter (0.03%)
Sediminibacterium (0.03%)
Cytophagaceae_unclassified (0.07%)
Bacteroidetes
Chlamydiales_unclassified (0.44%)
Chlamydiae
Streptophyta_unclassified (0.04%)
Cyanobacteria
Staphylococcus (0.02%)
Clostridium_IV (0.03%)
Clostridium_sensu_stricto (0.25%)
Firmicutes_unclassified (0.02%)
Firmicutes
Planctomycetaceae_unclassified (0.02%)
Schlesneria (0.03%)
Planctomycetes
Bradyrhizobium (0.05%)
Pedomicrobium (0.06%)
Mesorhizobium (0.03%)
Rhizobiales_unclassified (0.23%)
Novosphingobium (0.02%)
Sphingomonas (9.38%)
α-Proteobacteria
Burkholderiales_unclassified (0.02%)
Burkholderia (0.23%)
Comamonas (0.02%)
β-Proteobacteria
Gammaproteobacteria_unclassified (0.13%)
Proteus (0.04%)
Legionella (0.08%)
γ-Proteobacteria
Phaselicystis (0.03%)
δ-Proteobacteria

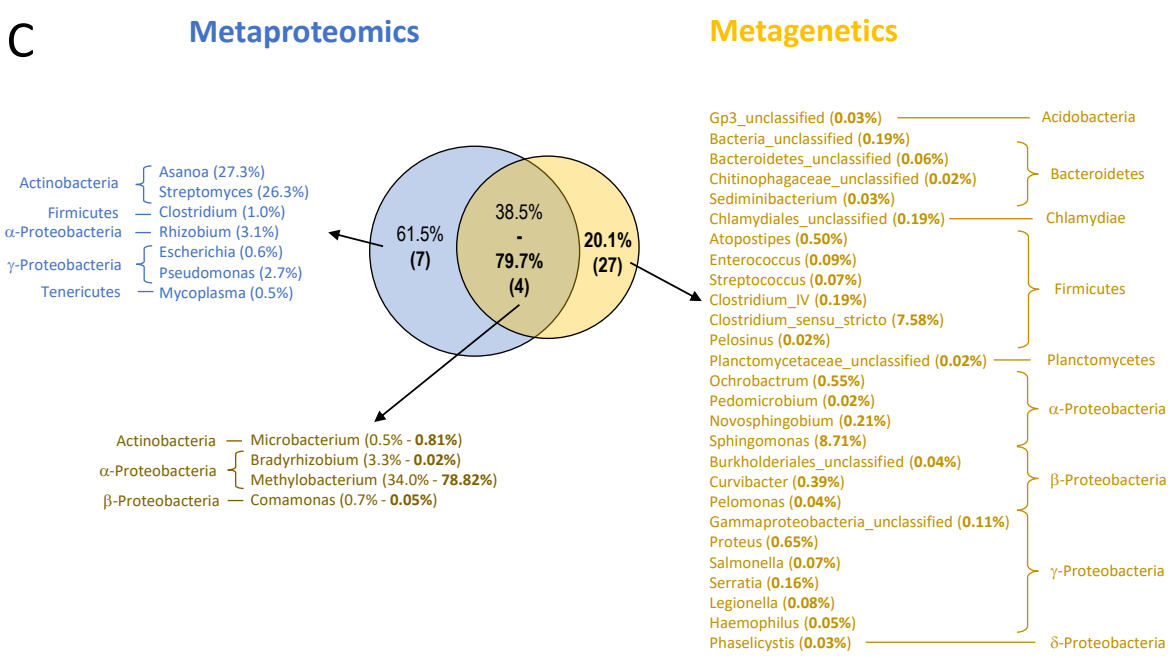



***Figure 6.5: Comparison of metaproteomic and metagenetic analysis of microbial communities present in the cooling pool of the reactor's core at shutdown, at genus level, at the different sampling locations: (A) Loc. A, (B) Loc. B, and (C) Loc. C.*** *Abundance, in %, and number of genera, in brackets. Bold numbers refer respectively to metagenetic analysis.*

# Appendix 7: Supplemental data from Pool 2 scrubs

***Table 6.11: Metagenetics data of each genus present in the 8 different scrubs.*** *In red: proportion higher than 0.016%.*

| | | | | | | Scrub 1 | Scrub 2 | Scrub 3 | Scrub 4 | Scrub 5 | Scrub 6 | Scrub 7 | Scrub 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | Genus | OTU | % | % | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Actinomycetales_unclassified | Actinomycetales_unclassified | Otu43 | | | 0,708 | | 3,244 | | | |
| | | | | | Otu50 | | | 0,469 | | | | | |
| | | | | | Otu75 | | | | 0,971 | | | | |
| | | | Dermabacteraceae | Helcobacillus | Otu74 | | | | 1,408 | | 0,930 | | |
| | | | Microbacteriaceae | Mycetocola | Otu46 | 4,543 | 5,846 | | | | 1,288 | 4,877 | 0,614 |
| | | Micrococcales | Micrococcaceae | Nesterenkonia | Otu101 | | | 0,085 | | | | | |
| | | Rubrobacterales | Rubrobacteraceae | Rubrobacter | Otu124 | 0,613 | | | 0,874 | | | | |
| Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Otu12 | | | 9,324 | | | | 0,066 | |
| | | | | | Otu13 | | | 8,503 | | | | | |
| | | | | | Otu16 | | | 7,290 | | | | | |
| | | | | | Otu20 | | | 3,214 | | | | | |
| | | | | | Otu24 | | | 1,721 | | | | | |
| | | | | | Otu34 | | | 1,003 | | | | | |
| | | | | | Otu38 | | | 0,895 | | | | | |
| | | | | | Otu83 | | | 0,159 | | | | | |
| | | | | | Otu92 | | | 0,128 | | | | | |
| | | | | | Otu90 | | | 0,123 | | | | | |
| | | | | | Otu95 | | | 0,108 | | | | | |
| | | | | | Otu105 | | | 0,069 | | | | | |
| | | | | | Otu115 | | | 0,056 | | | | | |
| | | | | | Otu55 | | | | | | | | 3,116 |
| | | | | | Otu274 | | | 0,005 | | | | | |
| Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Porphyromonadaceae_unclassified | Otu285 | | 0,089 | | | | | | |
| | Chitinophagia | Chitinophagales | Chitinophagaceae | Sediminibacterium | Otu21 | | | 2,986 | 4,612 | | | 1,560 | |
| Chloroflexi | Chloroflexi_unclassified | Chloroflexi_unclassified | Chloroflexi_unclassified | Chloroflexi_unclassified | Otu22 | | | 3,260 | | | | | |
| | Ktedonobacteria | Ktedonobacterales | Ktedonobacteraceae | Ktedonobacter | Otu41 | | | 0,749 | | | | | |
| Deinococcus-Thermus | Deinococci | Thermales | Thermaceae | Meiothermus | Otu72 | | | 0,195 | | | | | |
| | | | | Thermaceae_unclassified | Otu23 | | | 2,409 | | | | | |
| Firmicutes | Bacilli | Bacillales | Bacillales_Incertae_Sedis_XII | Exiguobacterium | Otu86 | | | | | | 4,292 | | |
| | | Lactobacillales | Enterococcaceae | Melissococcus | Otu9 | 2,501 | 9,699 | | | | | | |
| | Clostridia | Clostridia_unclassified | Clostridia_unclassified | Clostridia_unclassified | Otu14 | | | 7,300 | | | | 0,417 | |
| | | Clostridiales | Clostridiaceae | Clostridium_sensu_stricto | Otu15 | 45,840 | 45,084 | 3,909 | 31,117 | 58,016 | 56,652 | 41,301 | 43,790 |
| | | | | | Otu32 | 5,360 | | | | 2,107 | 7,010 | | |
| | | | | Clostridium_IV | Otu107 | | 0,753 | | | | | | |
| | | | | Clostridiaceae_1_unclassified | Otu61 | | | | | | 0,572 | | 2,457 |
| | | | Clostridiales_unclassified | Clostridiales_unclassified | Otu51 | 3,420 | | 0,223 | 2,427 | | 3,863 | 3,888 | 2,366 |
| | Negativicutes | Veillonellales | Veillonellaceae | Veillonellaceae_unclassified | Otu66 | | | | | 1,980 | | | |
| Planctomycetes | Planctomycetacia | Planctomycetales | Planctomycetaceae | Zavarzinella | Otu30 | | | 1,152 | 0,291 | | | | |
| | | | | | Otu69 | | | 0,228 | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Alphaproteobacteria | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Otu35 | | | 0,988 | | | | | |
| | | Rhizobiales | Bradyrhizobiaceae | Bradyrhizobiaceae_unclassified | Otu80 | 0,204 | | | | | | | |
| | | | Brucellaceae | Brucellaceae_unclassified | Otu79 | | | 0,044 | | | | 1,450 | 3,071 |
| | | | | Ochrobactrum | Otu47 | 10,516 | 8,857 | | | | | | |
| | | | Methylobacteriaceae | Methylobacterium | Otu1 | | | 6,305 | | | | 1,428 | |
| | | | | | Otu54 | | | 0,244 | | | | | |
| | | Rhodobacterales | Rhodobacteraceae | Rhodobacteraceae_unclassified | Otu36 | | | 0,213 | | | | 7,887 | |
| | | | | | Otu71 | | | | | | | 1,736 | |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | Otu6 | | | 7,170 | 19,320 | 2,338 | | | 6,938 |
| | | | | | Otu39 | | 14,172 | | | | | | |
| | | | | Sphingomonadaceae_unclassified | Otu57 | | | | | 2,465 | | | |
| | Betaproteobacteria | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Otu98 | | | 0,097 | | | | | |
| | | Burkholderiales | Alcaligenaceae | Alcaligenes | Otu87 | | | | | | | 1,208 | |
| | | | Burkholderiaceae | Ralstonia | Otu28 | | | 0,031 | 0,097 | | | 0,088 | 0,114 |
| | | | | Wautersia | Otu70 | | | | | 0,084 | 5,651 | | |
| | | | Comamonadaceae | Comamonadaceae_unclassified | Otu11 | | | 9,653 | | | | | |
| | | | | Curvibacter | Otu104 | | | 0,964 | 9,320 | 13,166 | | 25,989 | 16,219 |
| | | | | Diaphorobacter | Otu49 | 0,102 | | | 8,883 | | 8,512 | | |
| | | | | Pelomonas | Otu17 | 3,063 | 5,004 | 6,964 | | 2,064 | | | 4,026 |
| | | | Oxalobacteraceae | Herminiimonas | Otu58 | | | 0,139 | 5,631 | 1,095 | | 0,549 | 1,342 |
| | | Rhodocyclales | Rhodocyclaceae | Rhodocyclaceae_unclassified | Otu127 | | 0,709 | | | | | | |
| | Gammaproteobacteria | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Otu18 | | | 6,097 | | | | 1,406 | |
| | | | | | Otu42 | | | 0,711 | | | | | |
| | | | | | Otu122 | | | 0,523 | | | | | |
| | | | | | Otu77 | | | 0,177 | | | | | |
| | | Enterobacterales | Enterobacteriaceae | Enterobacteriaceae_unclassified | Otu8 | 8,627 | 4,606 | | 1,553 | 3,602 | 4,721 | 0,769 | 1,365 |
| | | | | | Otu56 | | | | | | | 1,142 | |
| | | | Morganellaceae | Morganella | Otu76 | | | 0,164 | | 1,475 | | | 6,642 |
| | | | | Proteus | Otu33 | 15,212 | 5,182 | 1,077 | 12,184 | 8,363 | 6,509 | 4,240 | 5,710 |
| | | Legionellales | Coxiellaceae | Aquicella | Otu37 | | | 0,905 | | | | | |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonadaceae_unclassified | Otu10 | | | | 1,311 | | | | |
| | Deltaproteobacteria | Myxococcales | Myxococcales_unclassified | Myxococcales_unclassified | Otu29 | | | 1,203 | | | | | |
| | | | | | Otu116 | | | 0,056 | | | | | |
| | Hydrogenophilalia | Hydrogenophilales | Hydrogenophilaceae | Hydrogenophilus | Otu63 | | | | | | | | 2,229 |

*Table 6.12: OTUs number obtained for each scrub after Illumina MiSeq V4V5 sequencing and OCToPUS analysis.*

| Scrub | OTUs number |
|---|---|
| **1** | 12 |
| **2** | 11 |
| **3** | 48 |
| **4** | 15 |
| **5** | 13 |
| **6** | 11 |
| **7** | 18 |
| **8** | 15 |

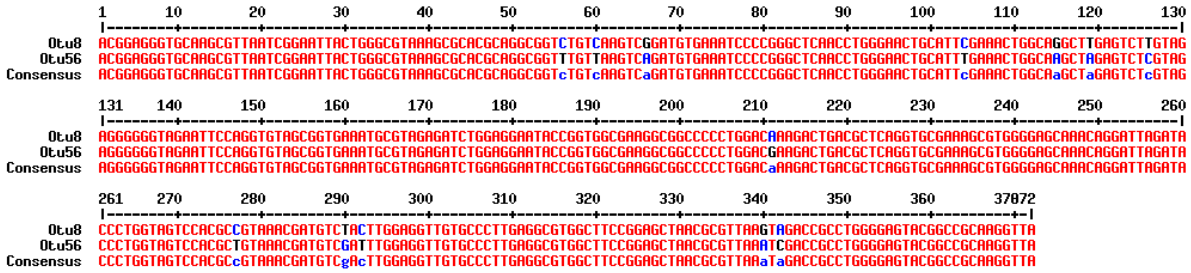



*Figure 6.6: Sequence homology between OTUs 8 and 56, both identified as "Enterobacteriacea_unclassified". Homology obtained using MultAlin (http://multalin.toulouse.inra.fr/multalin/multalin.html)*

# Appendix 8: Pool 1 – metagenetics results 2015 sampling

***Table 6.13: Diversity present in Pool 1 in the different analyzed samples from 2015.*** *In red: OTUs with a proportion higher than the 0.016% cut-off*

| Phylum | Class | Order | Family | Genus | OTU | Sample A scrub | Sample B filter | Sample B scrub 1 | Sample B scrub 2 | Sample C filter | Sample C scrub | Sample D filter | Sample D scrub 1 | Sample D scrub 2 | Sample E filter |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acidobacteria | Acidobacteria_unclassified | Acidobacteria_unclassified | Acidobacteria_unclassified | Acidobacteria_unclassified | Otu273 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,020 | 0,000 |
| | Acidobacteria_Gp3 | Acidobacteria_Gp3_unclassified | Acidobacteria_Gp3_unclassified | Acidobacteria_Gp3_unclassified | Otu163 | 0,000 | 0,020 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu428 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Gp3 | Gp3_unclassified | Gp3_unclassified | Otu77 | 0,000 | 0,123 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Acidobacteria_Gp4 | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified | Otu222 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Gp4 | Gp4_unclassified | Gp4_unclassified | Otu127 | 0,000 | 0,044 | 0,000 | 0,000 | 0,000 | 0,000 | 0,151 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu78 | 0,000 | 0,112 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu249 | 0,000 | 0,000 | 0,000 | 0,000 | 0,020 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu352 | 0,000 | 0,013 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu282 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Aridibacter | Aridibacter_unclassified | Otu55 | 0,000 | 0,228 | 0,000 | 0,000 | 0,000 | 0,000 | 0,058 | 0,000 | 0,002 | 0,000 |
| | | | | | Otu235 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu256 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu53 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu359 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu429 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Blastocatella | Blastocatella_unclassified | Otu33 | 0,000 | 0,496 | 0,000 | 0,000 | 0,031 | 0,000 | 4,097 | 0,000 | 0,508 | 0,000 |
| | Acidobacteria_Gp6 | Gp6 | Gp6_unclassified | Gp6_unclassified | Otu44 | 0,000 | 0,402 | 0,049 | 0,000 | 0,000 | 0,000 | 0,281 | 0,000 | 0,079 | 0,000 |
| | | | | | Otu153 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,052 | 0,000 | 0,011 | 0,000 |
| | | | | | Otu390 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu421 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Actinobacteria | Actinobacteria | Acidimicrobiales | Acidimicrobiales_unclassified | Acidimicrobiales_unclassified | Otu158 | 0,000 | 0,021 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,007 | 0,000 |
| | | | | | Otu332 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu315 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Actinomycetales | Actinomycetales_unclassified | Actinomycetales_unclassified | Otu221 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,784 |
| | | | | | Otu288 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,017 | 0,000 | 0,000 |
| | | | Actinomycetaceae | Actinomyces | Otu330 | 0,000 | 0,000 | 0,000 | 0,000 | 0,017 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu437 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 | 0,000 |
| | | | Brevibacteriaceae | Brevibacterium | Otu241 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,025 | 0,000 |
| | | | Corynebacteriaceae | Corynebacterium | Otu103 | 0,107 | 0,002 | 0,000 | 0,000 | 0,005 | 0,000 | 0,045 | 0,000 | 0,000 | 4,422 |
| | | | | | Otu204 | 0,142 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,037 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu194 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,103 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu303 | 0,000 | 0,000 | 0,000 | 0,000 | 0,020 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Dermabacteraceae | Brachybacterium | Otu166 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,014 | 0,000 |
| | | | Microbacteriaceae | Microbacteriaceae_unclassified | Otu12 | 0,000 | 0,000 | 16,626 | 0,000 | 0,000 | 0,000 | 0,006 | 0,000 | 0,000 | 2,482 |
| | | | | | Otu226 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,765 |
| | | | | | Otu484 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,075 |
| | | | | Microbacterium | Otu17 | 4,616 | 1,190 | 5,044 | 17,907 | 0,025 | 0,281 | 0,031 | 1,735 | 3,558 | 0,000 |
| | | | Micrococcaceae | Micrococcus | Otu146 | 0,273 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 | 0,001 | 0,000 |
| | | | | Rothia | Otu170 | 0,202 | 0,002 | 0,000 | 0,000 | 0,020 | 0,000 | 0,035 | 0,000 | 0,000 | 0,000 |
| | | | Mycobacteriaceae | Mycobacterium | Otu28 | 0,000 | 0,028 | 0,000 | 0,223 | 0,000 | 0,000 | 0,056 | 0,000 | 2,036 | 0,000 |
| | | | | | Otu178 | 0,000 | 0,021 | 0,000 | 0,038 | 0,000 | 0,000 | 0,054 | 0,043 | 0,308 | 0,000 |
| | | | Propionibacteriaceae | Propionibacteriaceae_unclassified | Otu371 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,261 |
| | | | | Propionibacterium | Otu105 | 0,688 | 0,005 | 0,012 | 0,187 | 0,042 | 0,002 | 0,144 | 0,006 | 0,029 | 0,970 |
| | | | Pseudonocardiaceae | Pseudonocardia | Otu366 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Bifidobacteriales | Bifidobacteriaceae | Bifidobacterium | Otu403 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,187 |
| | | Gaiellales | Gaiellaceae | Gaiella | Otu295 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Rubrobacterales | Rubrobacteraceae | Rubrobacter | Otu362 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,280 |
| Armatimonadetes | Chthonomonadetes | Chthonomonadales | Chthonomonadaceae | Chthonomonas/Armatimonadetes_gp3 | Otu233 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 |
| Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Otu25 | 0,000 | 0,000 | 0,000 | 0,807 | 0,026 | 0,000 | 0,000 | 0,000 | 2,936 | 0,000 |
| | | | | | Otu31 | 0,000 | 0,804 | 0,094 | 0,000 | 0,000 | 0,000 | 0,408 | 0,084 | 0,774 | 0,000 |
| | | | | | Otu51 | 0,000 | 0,251 | 0,000 | 0,000 | 0,000 | 0,000 | 0,926 | 0,000 | 0,108 | 0,765 |
| | | | | | Otu29 | 0,000 | 0,799 | 0,000 | 0,000 | 0,000 | 0,000 | 0,128 | 0,000 | 0,187 | 0,000 |
| | | | | | Otu39 | 0,000 | 0,455 | 0,000 | 0,000 | 0,000 | 0,000 | 0,146 | 0,000 | 0,106 | 0,000 |
| | | | | | Otu54 | 0,000 | 0,266 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu65 | 0,000 | 0,173 | 0,073 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu59 | 0,000 | 0,201 | 0,007 | 0,000 | 0,000 | 0,000 | 0,016 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu64 | 0,000 | 0,179 | 0,040 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu230 | 0,122 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,018 | 0,000 |
| | | | | | Otu205 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,087 | 0,000 | 0,035 | 0,000 |
| | | | | | Otu287 | 0,000 | 0,002 | 0,011 | 0,041 | 0,000 | 0,000 | 0,045 | 0,000 | 0,004 | 0,000 |
| | | | | | Otu286 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,045 | 0,015 | 0,000 | 0,000 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Otu100 | 0,000 | 0,065 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu106 | 0,000 | 0,061 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu348 | 0,050 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu134 | 0,000 | 0,043 | 0,000 | 0,003 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 |
| Otu212 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,030 | 0,000 |
| Otu223 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,028 | 0,000 |
| Otu323 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,013 | 0,013 | 0,000 |
| Otu145 | 0,000 | 0,026 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu151 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu209 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 |
| Otu164 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu169 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu306 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,015 | 0,000 | 0,000 |
| Otu232 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 | 0,000 |
| Otu181 | 0,000 | 0,016 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu184 | 0,000 | 0,015 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu186 | 0,000 | 0,015 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu336 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,010 | 0,000 |
| Otu418 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu203 | 0,000 | 0,013 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu216 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu229 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu215 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu239 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu244 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu260 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu272 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu255 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu254 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu274 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu320 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu389 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,008 | 0,000 | 0,000 |
| Otu284 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu406 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 |
| Otu307 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu328 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu337 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu373 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu408 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu410 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu383 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu395 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu399 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu398 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu409 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu411 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu427 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu432 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu435 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu442 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu445 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu455 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu464 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu489 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu504 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu508 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu507 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu536 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu537 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu574 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu599 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Otu602 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteroidetes | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Otu129 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,039 | 0,000 | 0,000 | 2,612 |
| | | | | | Otu68 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,103 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu110 | 0,000 | 0,059 | 0,000 | 0,000 | 0,000 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu107 | 0,000 | 0,060 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu117 | 0,000 | 0,050 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu228 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Cytophagia | Cytophagales | Cytophagales_unclassified | Cytophagales_unclassified | Otu49 | 0,000 | 0,269 | 0,000 | 0,000 | 0,000 | 0,000 | 0,212 | 0,000 | 0,190 | 0,000 |
| | | | | | Otu140 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,202 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu142 | 0,000 | 0,028 | 0,000 | 0,000 | 0,000 | 0,000 | 0,101 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu135 | 0,000 | 0,032 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu360 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,029 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu291 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Cytophagaceae | Spirosoma | Otu283 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,018 | 0,000 |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium | Otu311 | 0,000 | 0,000 | 0,000 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Cloacibacterium | Otu118 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 3,247 |
| | | | | Flavobacterium | Otu174 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 1,232 |
| | | | | | Otu522 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | Sphingobacteriia | Sphingobacteriales | Sphingobacteriales_unclassified | Sphingobacteriales_unclassified | Otu545 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Chitinophagaceae | Chitinophagaceae_unclassified | Otu245 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,070 | 0,000 | 0,000 | 0,000 |
| | | | | Lacibacter | Otu119 | 0,000 | 0,045 | 0,000 | 0,000 | 0,000 | 0,000 | 0,093 | 0,000 | 0,019 | 0,000 |
| | | | | Parasegetibacter | Otu407 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Sediminibacterium | Otu248 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Saprospiraceae | Saprospiraceae_unclassified | Otu10 | 0,000 | 4,719 | 0,068 | 0,000 | 0,010 | 0,000 | 3,357 | 0,000 | 1,800 | 0,000 |
| | | | | Haliscomenobacter | Otu85 | 0,000 | 0,095 | 0,000 | 0,000 | 0,000 | 0,000 | 0,052 | 0,026 | 0,020 | 0,000 |
| | | | Sphingobacteriaceae | Sphingobacteriaceae_unclassified | Otu130 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 2,519 |
| | | | | Mucilaginibacter | Otu41 | 2,379 | 0,000 | 0,000 | 0,000 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Pedobacter | Otu349 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,317 |
| Chlamydiae | Chlamydiia | Chlamydiales | Chlamydiales_unclassified | Chlamydiales_unclassified | Otu426 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 | 0,000 |
| | | | | | Otu447 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu480 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu503 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu509 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Parachlamydiaceae | Parachlamydiaceae_unclassified | Otu481 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Neochlamydia | Otu530 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| Chloroflexi | Caldilineae | Caldilineales | Caldilineaceae | Litorilinea | Otu308 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Chloroflexia | Chloroflexales | Oscillochloridaceae | Oscillochloris | Otu448 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Thermomicrobia | Sphaerobacterales | Sphaerobacteraceae | Sphaerobacteraceae_unclassified | Otu262 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,021 | 0,000 | 0,000 |
| | | | | Nitrolancea | Otu176 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Cyanobacteria/Chloroplast | Chloroplast | Chloroplast | Bacillariophyta | Bacillariophyta_unclassified | Otu1 | 0,000 | 59,421 | 2,896 | 3,362 | 0,087 | 0,000 | 30,624 | 0,007 | 36,520 | 43,142 |
| | | | | | Otu549 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,037 |
| | | | | | Otu341 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,012 | 0,000 |
| | | | | | Otu531 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu479 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu513 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu519 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu520 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu538 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu540 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu543 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu544 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu572 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu573 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu594 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu598 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu600 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu595 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu597 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu596 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Streptophyta | Streptophyta_unclassified | Otu70 | 1,602 | 0,013 | 0,138 | 0,268 | 0,047 | 0,075 | 0,179 | 0,042 | 0,115 | 6,177 |
| | Cyanobacteria | Cyanobacteria_unclassified | Cyanobacteria_unclassified | Cyanobacteria_unclassified | Otu99 | 0,000 | 0,068 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu299 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Deinococcus-Thermus | Deinococci | Thermales | Thermaceae | Thermus | Otu401 | 0,000 | 0,000 | 0,000 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Firmicutes | Bacilli | Bacillales | Bacillales_unclassified | Bacillales_unclassified | Otu382 | 0,000 | 0,003 | 0,000 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu300 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Bacillales_Incertae_Sedis_XI | Gemella | Otu155 | 0,000 | 0,023 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Bacillaceae | Anoxybacillus | Otu329 | 0,000 | 0,000 | 0,000 | 0,000 | 0,017 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Bacillus | Otu381 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Listeriaceae | Brochothrix | Otu343 | 0,000 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,014 | 0,000 |
| | | | Staphylococcaceae | Staphylococcus | Otu90 | 0,282 | 0,006 | 0,000 | 0,000 | 0,059 | 0,000 | 0,569 | 0,037 | 0,000 | 2,575 |
| | | | Thermoactinomycetaceae_2 | Planifilum | Otu264 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,578 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lactobacillales | Carnobacteriaceae | Granulicatella | Otu219 | 0,000 | 0,000 | 0,046 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Enterococcaceae | Enterococcus | Otu112 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 1,586 |
| | | | Streptococcaceae | Lactococcus | Otu361 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,029 | 0,000 | 0,000 | 0,000 |
| | | | | Streptococcus | Otu115 | 0,000 | 0,055 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,009 | 0,000 | 0,000 |
| | | | | | Otu319 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,041 | 0,000 | 0,000 | 0,000 |
| | | | Leuconostocaceae | Leuconostoc | Otu470 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Clostridia | Clostridiales | Lachnospiraceae | Lachnospiraceae_unclassified | Otu301 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,429 |
| | | | Peptoniphilaceae | Peptoniphilus | Otu259 | 0,098 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Ruminococcaceae | Ethanoligenens | Otu175 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 1,232 |
| | | | Clostridiales_Incertae_Sedis_XI | Anaerococcus | Otu201 | 0,145 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Finegoldia | Otu446 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 |
| | Negativicutes | Selenomonadales | Veillonellaceae | Veillonella | Otu160 | 0,231 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,080 | 0,000 | 0,000 | 0,933 |
| Gemmatimonadetes | Gemmatimonadetes | Gemmatimonadales | Gemmatimonadaceae | Gemmatimonas | Otu91 | 0,000 | 0,028 | 0,000 | 0,000 | 0,007 | 0,000 | 0,557 | 0,000 | 0,129 | 0,000 |
| | | | | | Otu154 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu380 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Hydrogenedentes | Candidatus_Hydrogenedens | Candidatus_Hydrogenedens_unclassified | Candidatus_Hydrogenedens_unclassified | Candidatus_Hydrogenedens_unclassified | Otu269 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,056 | 0,000 | 0,000 | 0,000 |
| Nitrospirae | Nitrospira | Nitrospirales | Nitrospiraceae | Nitrospira | Otu13 | 0,000 | 3,482 | 0,000 | 0,000 | 0,017 | 0,000 | 0,136 | 0,000 | 0,000 | 1,344 |
| | | | | | Otu603 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Planctomycetes | Planctomycetia | Planctomycetales | Planctomycetaceae | Planctomycetaceae_unclassified | Otu120 | 0,000 | 0,005 | 0,049 | 0,000 | 0,000 | 0,000 | 0,301 | 0,000 | 0,113 | 1,120 |
| | | | | | Otu38 | 0,000 | 0,666 | 0,044 | 0,000 | 0,000 | 0,000 | 0,017 | 0,000 | 0,019 | 0,000 |
| | | | | | Otu147 | 0,000 | 0,025 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,634 |
| | | | | | Otu34 | 0,000 | 0,587 | 0,066 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu86 | 0,000 | 0,090 | 0,015 | 0,000 | 0,000 | 0,000 | 0,411 | 0,015 | 0,089 | 0,000 |
| | | | | | Otu43 | 0,000 | 0,414 | 0,055 | 0,000 | 0,000 | 0,000 | 0,041 | 0,000 | 0,091 | 0,000 |
| | | | | | Otu191 | 0,000 | 0,015 | 0,051 | 0,000 | 0,000 | 0,000 | 0,113 | 0,000 | 0,022 | 0,000 |
| | | | | | Otu111 | 0,000 | 0,002 | 0,000 | 0,000 | 0,023 | 0,000 | 0,016 | 0,000 | 0,143 | 0,000 |
| | | | | | Otu124 | 0,000 | 0,046 | 0,000 | 0,000 | 0,000 | 0,000 | 0,023 | 0,000 | 0,104 | 0,000 |
| | | | | | Otu104 | 0,000 | 0,076 | 0,028 | 0,000 | 0,000 | 0,000 | 0,021 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu84 | 0,000 | 0,095 | 0,021 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu136 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,014 | 0,009 | 0,075 | 0,000 |
| | | | | | Otu81 | 0,000 | 0,110 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu250 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,043 | 0,000 | 0,024 | 0,000 |
| | | | | | Otu101 | 0,000 | 0,065 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu268 | 0,000 | 0,004 | 0,032 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu214 | 0,000 | 0,017 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu188 | 0,000 | 0,015 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu195 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu227 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu237 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu276 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu246 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu265 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu289 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu285 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu325 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu434 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 | 0,000 |
| | | | | | Otu376 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu377 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu392 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu420 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu443 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu444 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu463 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu477 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Gemmata | Otu88 | 0,000 | 0,086 | 0,019 | 0,000 | 0,000 | 0,000 | 0,675 | 0,000 | 0,069 | 0,000 |
| | | | | | Otu83 | 0,000 | 0,099 | 0,014 | 0,000 | 0,009 | 0,000 | 0,276 | 0,000 | 0,071 | 0,000 |
| | | | | | Otu526 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,060 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu335 | 0,000 | 0,005 | 0,000 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu207 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu356 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Pirellula | Otu217 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Schlesneria | Otu27 | 0,000 | 0,914 | 0,000 | 0,000 | 0,000 | 0,000 | 0,041 | 0,000 | 0,196 | 0,000 |
| | | | | | Otu132 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,047 | 0,089 | 0,000 |
| | | | | Singulisphaera | Otu298 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Zavarzinella | Otu152 | 0,000 | 0,013 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,059 | 0,000 |
| | | | | | Otu423 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu267 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified | Otu108 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,031 | 0,000 | 0,147 | 0,000 |
| | | | | | Otu190 | 0,000 | 0,017 | 0,000 | 0,000 | 0,000 | 0,000 | 0,058 | 0,000 | 0,033 | 0,000 |
| | | | | | Otu114 | 0,000 | 0,053 | 0,023 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,020 | 0,000 |
| | | | | | Otu182 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,042 | 0,000 |
| | | | | | Otu193 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu405 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 | 0,000 | 0,007 | 0,000 |
| | | | | | Otu198 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu379 | 0,000 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu202 | 0,000 | 0,013 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu266 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu439 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 | 0,000 |
| | | | | | Otu346 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu369 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu386 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu413 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu441 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu452 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Alphaproteobacteria | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Otu42 | 0,000 | 0,171 | 0,007 | 0,000 | 0,121 | 0,000 | 2,915 | 0,000 | 0,820 | 0,000 |
| | | | | | Otu20 | 0,000 | 1,860 | 0,010 | 0,000 | 0,000 | 0,000 | 0,231 | 0,000 | 0,240 | 0,000 |
| | | | | | Otu71 | 0,000 | 0,059 | 0,000 | 0,000 | 0,000 | 0,000 | 1,013 | 0,000 | 0,077 | 0,000 |
| | | | | | Otu57 | 0,000 | 0,326 | 0,011 | 0,000 | 0,000 | 0,000 | 0,182 | 0,000 | 0,400 | 0,000 |
| | | | | | Otu45 | 0,000 | 0,415 | 0,026 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,075 | 0,149 |
| | | | | | Otu87 | 0,000 | 0,087 | 0,000 | 0,000 | 0,000 | 0,000 | 0,213 | 0,000 | 0,030 | 0,000 |
| | | | | | Otu122 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,140 | 0,000 | 0,114 | 0,000 |
| | | | | | Otu82 | 0,000 | 0,105 | 0,000 | 0,000 | 0,000 | 0,000 | 0,146 | 0,000 | 0,007 | 0,000 |
| | | | | | Otu67 | 0,000 | 0,164 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,015 | 0,000 |
| | | | | | Otu97 | 0,000 | 0,070 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu251 | 0,000 | 0,008 | 0,000 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,024 | 0,000 |
| | | | | | Otu270 | 0,000 | 0,009 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,020 | 0,000 |
| | | | | | Otu263 | 0,000 | 0,000 | 0,000 | 0,000 | 0,028 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu302 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,018 | 0,000 |
| | | | | | Otu344 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,009 | 0,000 |
| | | | | | Otu247 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu422 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu475 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Caulobacterales | Caulobacteraceae | Brevundimonas | Otu6 | 0,000 | 0,004 | 0,019 | 49,090 | 7,565 | 0,000 | 0,000 | 0,000 | 9,779 | 0,000 |
| | | | | | Otu23 | 0,000 | 0,343 | 0,000 | 0,000 | 0,018 | 0,000 | 2,389 | 0,030 | 1,275 | 0,000 |
| | | | | | Otu510 | 0,000 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu569 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu424 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu506 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 |
| | | | | Phenylobacterium | Otu322 | 0,000 | 0,005 | 0,000 | 0,000 | 0,012 | 0,000 | 0,031 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu278 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 |
| | | Rhizobiales | Rhizobiales_unclassified | Rhizobiales_unclassified | Otu339 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu425 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu535 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | | | | | Otu534 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | | | Rhizobiales_incertae_sedis | Phreatobacter | Otu261 | 0,000 | 0,009 | 0,029 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Beijerinckiaceae | Beijerinckiaceae_unclassified | Otu271 | 0,000 | 0,000 | 0,000 | 0,000 | 0,026 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Bradyrhizobiaceae | Afipia | Otu19 | 0,000 | 0,083 | 7,328 | 1,109 | 0,000 | 0,601 | 0,068 | 0,000 | 0,063 | 0,000 |
| | | | | | Otu532 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,157 | 0,000 |
| | | | | Bosea | Otu46 | 0,000 | 0,018 | 0,008 | 0,000 | 0,000 | 0,000 | 0,087 | 0,000 | 0,096 | 0,000 |
| | | | | Bradyrhizobium | Otu208 | 0,000 | 0,000 | 0,000 | 0,121 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Brucellaceae | Ochrobactrum | Otu141 | 0,000 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 | 0,056 |
| | | | Hyphomicrobiaceae | Hyphomicrobiaceae_unclassified | Otu210 | 0,000 | 0,054 | 0,000 | 0,000 | 0,000 | 0,000 | 0,085 | 0,000 | 0,023 | 0,000 |
| | | | | | Otu79 | 0,000 | 0,108 | 0,005 | 0,000 | 0,000 | 0,000 | 0,008 | 0,000 | 0,019 | 0,000 |
| | | | | | Otu171 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Hyphomonas | Otu113 | 0,000 | 0,030 | 0,000 | 0,000 | 0,000 | 0,000 | 0,386 | 0,000 | 0,000 | 0,000 |
| | | | | Pedomicrobium | Otu60 | 0,000 | 0,203 | 0,000 | 0,000 | 0,000 | 0,000 | 0,047 | 0,000 | 0,000 | 0,000 |
| | | | Methylobacteriaceae | Methylobacterium | Otu4 | 0,166 | 0,001 | 4,601 | 13,920 | 1,976 | 0,065 | 0,000 | 46,859 | 1,178 | 1,325 |
| | | | | | Otu7 | 0,000 | 0,005 | 14,170 | 0,000 | 0,041 | 0,000 | 0,000 | 1,655 | 16,999 | 0,000 |
| | | | | | Otu157 | 0,249 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,083 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu391 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu511 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 |
| | | | | | Otu533 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 |
| | | | Rhizobiaceae | Rhizobiaceae_unclassified | Otu279 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,052 | 0,000 | 0,000 | 0,000 |
| | | | | Rhizobium | Otu197 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,970 |
| | | | Rhodobiaceae | Rhodobiaceae_unclassified | Otu74 | 0,000 | 0,129 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Xanthobacteraceae | Xanthobacter | Otu150 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Rhodobacterales | Rhodobacteraceae | Rhodobacteraceae_unclassified | Otu26 | 0,000 | 0,275 | 0,033 | 0,000 | 0,014 | 0,000 | 3,866 | 0,000 | 2,499 | 0,000 |
| | | | | | Otu231 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,765 |
| | | | | | Otu125 | 0,000 | 0,045 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Rhodobacter | Otu16 | 0,000 | 3,212 | 0,000 | 0,000 | 0,000 | 0,000 | 3,107 | 0,000 | 0,371 | 0,000 |
| | | | | Rubellimicrobium | Otu402 | 0,000 | 0,000 | 0,000 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rhodospirillales | Acetobacteraceae | Acetobacteraceae_unclassified | Otu61 | 0,000 | 0,195 | 0,042 | 0,000 | 0,000 | 0,000 | 0,080 | 0,000 | 0,061 | 0,000 |
| | | | | Otu93 | 0,000 | 0,075 | 0,000 | 0,000 | 0,000 | 0,000 | 0,062 | 0,000 | 0,038 | 0,000 |
| | | | | Otu148 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,023 | 0,000 | 0,000 | 0,000 |
| | | | | Otu220 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Reyranella | Reyranella_unclassified | Otu133 | 0,000 | 0,040 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,019 | 0,000 |
| | | | | Otu156 | 0,000 | 0,023 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Rhodospirillaceae | Rhodospirillaceae_unclassified | Otu98 | 0,000 | 0,070 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,052 | 0,000 |
| | | | | Otu168 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,021 | 0,000 |
| | | | | Otu351 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,011 | 0,000 |
| | | | | Otu384 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Rhodospirillales_unclassified | Rhodospirillales_unclassified | Otu350 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,007 | 0,000 |
| | | | | Otu277 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu416 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Rickettsiales | Rickettsiaceae | Rickettsiaceae_unclassified | Otu385 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu417 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Sphingomonadales | Erythrobacteraceae | Porphyrobacter | Otu14 | 0,000 | 0,330 | 0,015 | 0,000 | 0,000 | 0,000 | 0,755 | 0,000 | 0,361 | 0,000 |
| | | Sphingomonadaceae | Blastomonas | Otu18 | 0,000 | 2,472 | 0,000 | 0,000 | 1,692 | 0,000 | 2,556 | 0,000 | 0,025 | 4,478 |
| | | | Hephaestia | Otu514 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu521 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | | | Novosphingobium | Otu374 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,009 | 0,952 |
| | | | Sphingobium | Otu275 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Sphingomonadaceae_unclassified | Otu22 | 0,000 | 1,800 | 0,061 | 0,000 | 0,000 | 0,002 | 1,370 | 0,000 | 1,550 | 0,000 |
| | | | | Otu47 | 0,000 | 0,064 | 0,017 | 0,000 | 0,000 | 0,000 | 0,611 | 0,000 | 0,219 | 0,000 |
| | | | | Otu566 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu564 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu565 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu567 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu451 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu353 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu462 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu515 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu568 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu571 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu576 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu578 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu582 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu523 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | | | | Otu524 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | | | | Otu529 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,001 | 0,000 | 0,000 |
| | | | Sphingomonas | Otu2 | 87,789 | 1,600 | 47,204 | 12,642 | 0,003 | 85,505 | 4,398 | 49,174 | 6,747 | 1,511 |
| | | | | Otu73 | 0,000 | 0,004 | 0,628 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Sphingopyxis | Otu69 | 0,000 | 0,022 | 0,000 | 0,000 | 0,000 | 0,000 | 0,184 | 0,000 | 0,188 | 0,000 |
| | | | | Otu318 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu400 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | SAR11 | Candidatus_Pelagibacter | Candidatus_Pelagibacter_unclassified | Otu577 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Betaproteobacteria | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Otu30 | 0,000 | 0,729 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,050 | 0,000 |
| | | | | Otu165 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 | 0,000 | 0,045 | 0,000 | 0,000 | 0,000 |
| | | | | Otu206 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,032 | 0,000 |
| | | | | Otu338 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,035 | 0,000 | 0,000 | 0,000 |
| | | | | Otu305 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,015 | 0,000 |
| | | | | Otu334 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu461 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Burkholderiales | Burkholderiales_unclassified | Burkholderiales_unclassified | Otu15 | 0,000 | 0,454 | 0,061 | 0,000 | 0,004 | 0,000 | 22,630 | 0,032 | 2,017 | 0,000 |
| | | | | Otu40 | 0,000 | 0,563 | 0,000 | 0,000 | 0,166 | 0,000 | 0,452 | 0,000 | 0,283 | 1,325 |
| | | | | Otu601 | 0,000 | 0,172 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Burkholderiales_incertae_sedis | Burkholderiales_incertae_sedis_unclassified | Otu581 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 |
| | | Burkholderiaceae | Burkholderia | Otu367 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,027 | 0,000 | 0,000 | 0,000 |
| | | | Burkholderia | Otu469 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Limnobacter | Otu5 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,423 | 0,000 |
| | | | Ralstonia | Otu72 | 0,000 | 0,000 | 0,012 | 0,000 | 0,124 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu516 | 0,000 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Comamonadaceae | Comamonadaceae_unclassified | Otu3 | 0,000 | 0,001 | 0,000 | 0,000 | 87,363 | 0,000 | 0,000 | 0,002 | 0,002 | 0,000 |
| | | | | Otu8 | 0,000 | 0,009 | 0,008 | 0,000 | 0,188 | 0,000 | 0,004 | 0,001 | 0,118 | 0,168 |
| | | | | Otu453 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu472 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu473 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu518 | 0,000 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu517 | 0,000 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu590 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu589 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Oxalobacteraceae | Massilia | Otu37 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 |
| | Gallionellales | Gallionellaceae | Sideroxydans | Otu131 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,095 | 0,000 | 0,000 | 0,000 |
| | Hydrogenophilales | Hydrogenophilaceae | Hydrogenophilus | Otu388 | 0,000 | 0,000 | 0,000 | 0,020 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Methylophilales | Methylophilaceae | Methylophilus | Otu327 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Neisseriales | Neisseriaceae | Snodgrassella | Otu144 | 0,314 | 0,000 | 0,000 | 0,116 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 |
| | Rhodocyclales | Rhodocyclaceae | Rhodocyclaceae_unclassified | Otu258 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Gammaproteobacteria | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Otu32 | 0,000 | 0,636 | 0,000 | 0,000 | 0,012 | 0,000 | 2,676 | 0,000 | 0,863 | 1,717 |
| | | | | Otu35 | 0,000 | 0,562 | 0,000 | 0,000 | 0,000 | 0,000 | 0,058 | 0,000 | 0,097 | 0,000 |
| | | | | Otu75 | 0,000 | 0,150 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,133 | 0,000 |
| | | | | Otu128 | 0,000 | 0,036 | 0,000 | 0,000 | 0,000 | 0,000 | 0,085 | 0,000 | 0,098 | 0,000 |
| | | | | Otu126 | 0,000 | 0,045 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,016 | 0,000 |
| | | | | Otu290 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,049 | 0,000 | 0,000 | 0,000 |
| | | | | Otu161 | 0,000 | 0,021 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,011 | 0,000 |
| | | | | Otu280 | 0,000 | 0,000 | 0,000 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu185 | 0,000 | 0,016 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu368 | 0,000 | 0,001 | 0,015 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu211 | 0,000 | 0,013 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu225 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu253 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu321 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu375 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu393 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu419 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu488 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu505 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu575 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Chromatiales | Chromatiaceae | Rheinheimera | Otu172 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,045 | 0,000 |
| | Enterobacteriales | Enterobacteriaceae | Enterobacter | Otu238 | 0,113 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,029 | 0,000 | 0,000 | 0,000 |
| | | | Escherichia/Shigella | Otu89 | 0,000 | 0,000 | 0,014 | 0,062 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Legionellales | Coxiellaceae | Aquicella | Otu109 | 0,000 | 0,066 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu326 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu440 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Legionellaceae | Legionella | Otu121 | 0,000 | 0,034 | 0,000 | 0,000 | 0,000 | 0,000 | 0,320 | 0,000 | 0,085 | 0,000 |
| | | | | Otu466 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,008 | 0,000 | 0,039 | 0,000 |
| | | | | Otu137 | 0,000 | 0,030 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu378 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,025 | 0,000 | 0,000 | 0,000 |
| | | | | Otu200 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,003 | 0,000 |
| | Oceanospirillales | Oceanospirillaceae | Marinomonas | Otu149 | 0,267 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Pasteurellales | Pasteurellaceae | Pasteurellaceae_unclassified | Otu364 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,029 | 0,000 | 0,000 | 0,000 |
| | | | Haemophilus | Otu187 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Pseudomonadales | Moraxellaceae | Acinetobacter | Otu102 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,015 | 0,167 | 0,000 | 0,026 | 1,344 |
| | | | | Otu236 | 0,000 | 0,000 | 0,002 | 0,033 | 0,026 | 0,000 | 0,033 | 0,026 | 0,011 | 0,000 |
| | | | Enhydrobacter | Otu243 | 0,000 | 0,000 | 0,011 | 0,028 | 0,000 | 0,008 | 0,000 | 0,010 | 0,018 | 0,000 |
| | | | Moraxellaceae_unclassified | Otu296 | 0,000 | 0,000 | 0,025 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Psychrobacter | Otu179 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,042 | 0,000 | 0,000 |
| | | Pseudomonadaceae | Pseudomonas | Otu196 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,970 |
| | | | | Otu138 | 0,139 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,034 | 0,000 |
| | | | | Otu478 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Xanthomonadales | Sinobacteraceae | Nevskia | Otu11 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,012 | 0,000 |
| | | Xanthomonadaceae | Stenotrophomonas | Otu9 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 13,390 | 0,000 | 0,000 | 0,031 | 2,743 |
| | | | | Otu579 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu580 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 |
| Deltaproteobacteria | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified | Otu66 | 0,000 | 0,174 | 0,000 | 0,000 | 0,000 | 0,000 | 0,180 | 0,000 | 0,143 | 0,000 |
| | | | | Otu50 | 0,000 | 0,268 | 0,000 | 0,000 | 0,000 | 0,000 | 0,074 | 0,000 | 0,052 | 0,000 |
| | | | | Otu76 | 0,000 | 0,125 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,017 | 0,000 |
| | | | | Otu292 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,033 | 0,000 | 0,000 | 0,000 |
| | | | | Otu281 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu316 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu345 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu397 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu414 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu449 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Bdellovibrionales | Bdellovibrionaceae | Bdellovibrio | Otu358 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,029 | 0,000 | 0,000 | 0,000 |
| | | | | Otu467 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 |
| | | | | Otu342 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu347 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu357 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Vampirovibrio | Otu139 | 0,000 | 0,028 | 0,000 | 0,000 | 0,000 | 0,000 | 0,062 | 0,000 | 0,081 | 0,205 |
| | | | | Otu95 | 0,000 | 0,072 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu234 | 0,000 | 0,011 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu242 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu340 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu355 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu365 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu396 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu542 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Myxococcales | Myxococcales_unclassified | Myxococcales_unclassified | Otu92 | 0,000 | 0,096 | 0,000 | 0,000 | 0,000 | 0,000 | 0,083 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu96 | 0,000 | 0,070 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu293 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu314 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu317 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu412 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu415 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu476 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Labilitrichaceae | Labilithrix | Otu294 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Myxococcaceae | Aggregicoccus | Otu370 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Sandaracinaceae | Sandaracinus | Otu177 | 0,000 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,083 | 0,000 | 0,000 | 0,616 |
| | | | | | Otu240 | 0,000 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Verrucomicrobia | Spartobacteria | Spartobacteria_unclassified | Spartobacteria_unclassified | Spartobacteria_unclassified | Otu24 | 0,000 | 1,468 | 0,061 | 0,000 | 0,000 | 0,000 | 1,194 | 0,000 | 0,457 | 0,000 |
| | Subdivision3 | Subdivision3_unclassified | Subdivision3_unclassified | Subdivision3_unclassified | Otu48 | 0,000 | 0,290 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | Prosthecobacter | Otu80 | 0,000 | 0,107 | 0,000 | 0,000 | 0,000 | 0,000 | 0,693 | 0,000 | 0,028 | 0,000 |
| Archaea-Thaumarchaeota | Nitrosopumilales | Nitrosopumilaceae | Nitrosopumilus | Nitrosopumilus_unclassified | Otu485 | 0,000 | 0,000 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| unknown_unclassified | unknown_unclassified | unknown_unclassified | unknown_unclassified | unknown_unclassified | Otu539 | 0,000 | 0,001 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

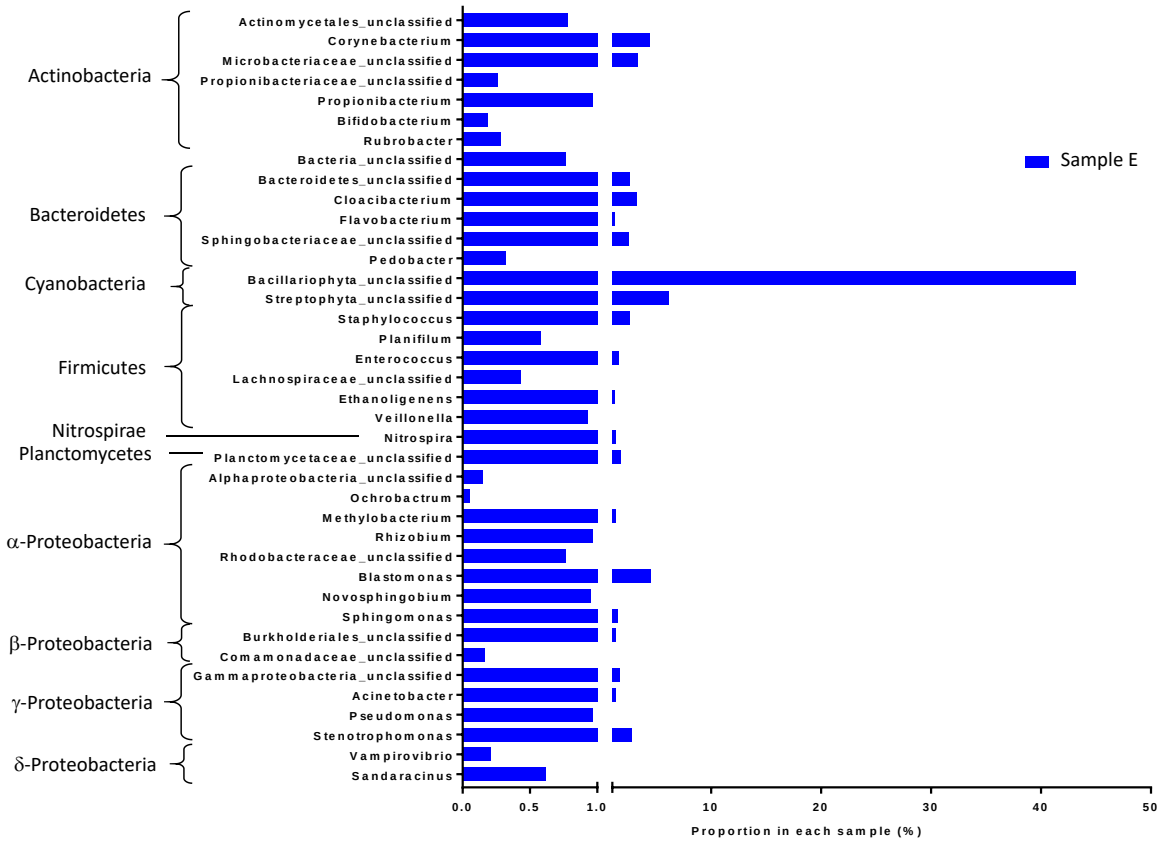



***Figure 6.7: Diversity at the genus level from the water sample E water after filtration analyzed using metagenetics.*** *A 0.016% cut-off was used*


***Figure 6.8: Diversity at the genus level from the samples collected at the northern wall analyzed using metagenetics.*** *A 0.016% cut-off was used.*

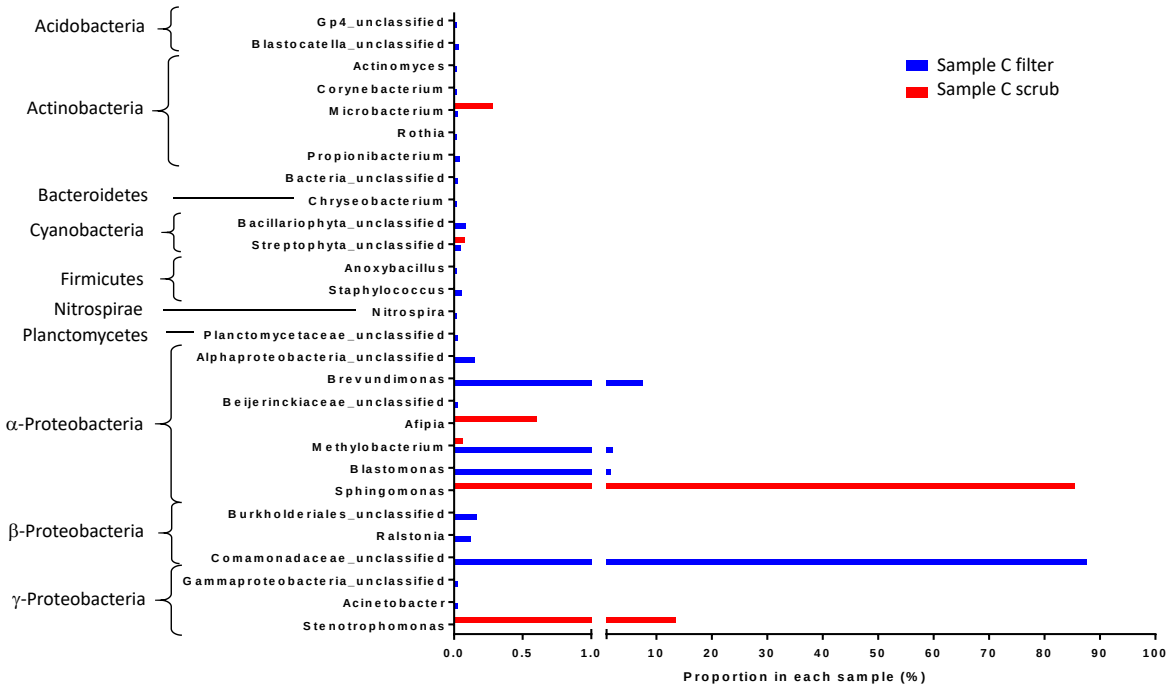



*Figure 6.9: Diversity at the genus level from the samples collected at the bottom western wall analyzed using metagenetics. A 0.016% cut-off was used.*

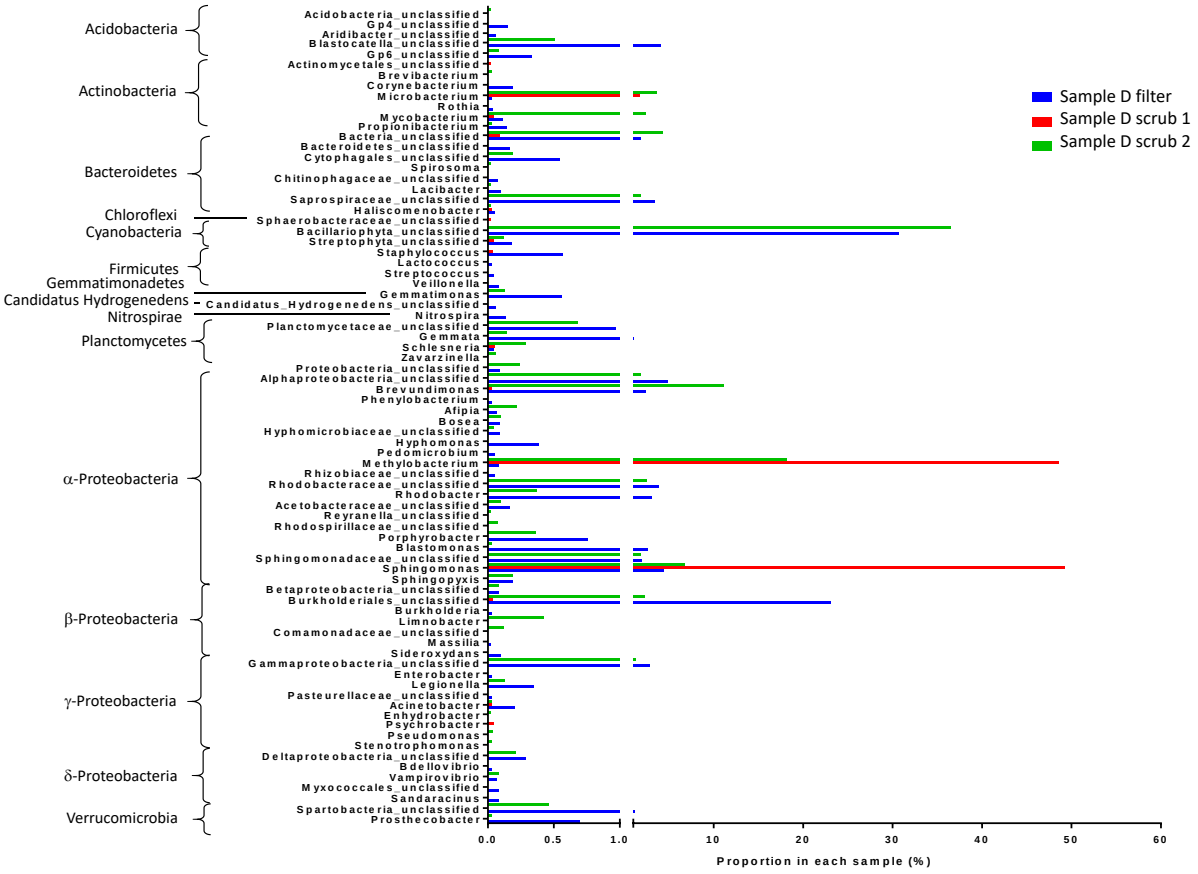



*Figure 6.10: Diversity at the family level from the samples collected at the top western wall analyzed using metagenetics. A 0.016% cut-off was used.*

# Appendix 9: Pool 1 – metagenetics results 2017 sampling

***Table 6.14: Diversity present in Pool 1 in the different analyzed samples from 2017.*** *In red: OTUs with a proportion higher than the 0.016% cut-off*

| Phylum | Class | Order | Family | Genus | OTU | Sample A | Sample B | Sample C | Sample D | Sample E | Sample F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Acidobacteria | Acidobacteria_Gp3 | Acidobacteria_Gp3_unclassified | Acidobacteria_Gp3_unclassified | Acidobacteria_Gp3_unclassified | Otu163 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Gp3 | Gp3_unclassified | Gp3_unclassified | Otu77 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Acidobacteria_Gp4 | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified | Otu222 | 0,000 | 0,000 | 0,503 | 0,000 | 0,000 | 0,000 |
| | | Gp4 | Gp4_unclassified | Gp4_unclassified | Otu249 | 0,000 | 0,000 | 0,044 | 0,000 | 0,000 | 0,000 |
| | | | Aridibacter | Aridibacter_unclassified | Otu55 | 0,054 | 0,000 | 0,094 | 0,107 | 0,000 | 0,000 |
| | | | | | Otu53 | 0,008 | 0,004 | 0,993 | 0,000 | 0,000 | 0,005 |
| | | | | | Otu359 | 0,000 | 0,000 | 0,017 | 0,000 | 0,000 | 0,002 |
| | | | Blastocatella | Blastocatella_unclassified | Otu33 | 0,036 | 0,000 | 0,048 | 0,000 | 0,000 | 0,000 |
| | Acidobacteria_Gp6 | Gp6 | Gp6_unclassified | Gp6_unclassified | Otu44 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu390 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu153 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| Actinobacteria | Actinobacteria | Actinomycetales | Corynebacteriaceae | Corynebacterium | Otu194 | 0,000 | 0,000 | 0,000 | 0,000 | 0,147 | 0,000 |
| | | | Microbacteriaceae | Microbacterium | Otu17 | 0,010 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 |
| | | | Micrococcaceae | Micrococcus | Otu146 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Mycobacteriaceae | Mycobacterium | Otu178 | 0,030 | 0,006 | 0,009 | 0,000 | 0,000 | 0,000 |
| Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Otu52 | 0,010 | 0,000 | 1,001 | 0,199 | 0,000 | 0,011 |
| | | | | | Otu39 | 0,000 | 0,019 | 0,050 | 0,032 | 0,777 | 0,000 |
| | | | | | Otu51 | 0,216 | 0,000 | 0,000 | 0,172 | 0,000 | 0,000 |
| | | | | | Otu29 | 0,032 | 0,000 | 0,000 | 0,107 | 0,000 | 0,000 |
| | | | | | Otu54 | 0,139 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu145 | 0,040 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu336 | 0,000 | 0,000 | 0,034 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu304 | 0,000 | 0,000 | 0,027 | 0,000 | 0,000 | 0,005 |
| | | | | | Otu297 | 0,000 | 0,000 | 0,028 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu239 | 0,000 | 0,017 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu244 | 0,012 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu459 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu465 | 0,000 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu205 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu64 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu458 | 0,000 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu460 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 |
| | | | | | Otu223 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu501 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu493 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu557 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu255 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu550 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu497 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 |
| | | | | | Otu499 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,004 |
| | | | | | Otu512 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu230 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 |
| | | | | | Otu556 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 |
| | | | | | Otu586 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu587 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu588 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu592 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteroidetes | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Otu68 | 0,422 | 0,000 | 0,675 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu159 | 0,036 | 0,000 | 0,091 | 0,000 | 0,000 | 0,006 |
| | | | | | Otu110 | 0,077 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu129 | 0,042 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu107 | 0,000 | 0,000 | 0,012 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu117 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Cytophagia | Cytophagales | Cytophagales_unclassified | Cytophagales_unclassified | Otu49 | 0,210 | 0,000 | 0,026 | 1,938 | 0,000 | 0,000 |
| | | | | | Otu140 | 0,089 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu324 | 0,046 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu372 | 0,022 | 0,000 | 0,015 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu142 | 0,020 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Cytophagaceae | Hymenobacter | Otu213 | 0,000 | 0,017 | 0,051 | 0,000 | 0,000 | 0,000 |
| | Sphingobacteriia | Sphingobacteriales | Chitinophagaceae | Lacibacter | Otu119 | 0,238 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Saprospiraceae | Haliscomenobacter | Otu85 | 0,585 | 0,000 | 0,041 | 0,000 | 0,000 | 0,000 |
| | | | | Saprospiraceae_unclassified | Otu10 | 0,073 | 0,000 | 0,000 | 0,473 | 0,000 | 0,000 |
| Chlamydiae | Chlamydiia | Chlamydiales | Chlamydiales_unclassified | Chlamydiales_unclassified | Otu482 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu471 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 |
| | | | Parachlamydiaceae | Parachlamydiaceae_unclassified | Otu559 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu585 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| Chloroflexi | Thermomicrobia | Sphaerobacterales | Sphaerobacteraceae | Sphaerobacteraceae_unclassified | Otu262 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Cyanobacteria | Chloroplast | Chloroplast | Bacillariophyta | Bacillariophyta_unclassified | Otu1 | 1,993 | 0,006 | 0,030 | 0,380 | 0,000 | 0,000 |
| Firmicutes | Bacilli | Bacillales | Listeriaceae | Listeria | Otu491 | 0,000 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Staphylococcaceae | Staphylococcus | Otu90 | 0,000 | 0,004 | 0,000 | 0,000 | 2,353 | 0,000 |
| | | Lactobacillales | Enterococcaceae | Enterococcus | Otu112 | 0,000 | 0,000 | 0,000 | 0,000 | 4,391 | 0,000 |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium_IV | Otu551 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Clostridium_sensu_stricto | Otu58 | 0,063 | 0,037 | 0,054 | 3,023 | 15,735 | 0,010 |
| | | | | | Otu143 | 0,004 | 0,000 | 0,003 | 0,297 | 2,143 | 0,000 |
| | | | | | Otu167 | 0,000 | 0,000 | 0,000 | 0,125 | 1,450 | 0,002 |
| | Negativicutes | Selenomonadales | Acidaminococcaceae | Phascolarctobacterium | Otu192 | 0,000 | 0,000 | 0,000 | 0,246 | 0,000 | 0,000 |
| Deinococcus-Thermus | Deinococci | Deinococcales | Trueperaceae | Truepera | Otu436 | 0,000 | 0,000 | 0,008 | 0,000 | 0,000 | 0,000 |
| Gemmatimonadetes | Gemmatimonadetes | Gemmatimonadales | Gemmatimonadaceae | Gemmatimonas | Otu431 | 0,016 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Nitrospirae | Nitrospira | Nitrospirales | Nitrospiraceae | Nitrospira | Otu13 | 0,079 | 0,000 | 0,000 | 0,923 | 0,000 | 0,000 |
| Planctomycetes | Planctomycetia | Planctomycetales | Planctomycetaceae | Planctomycetaceae_unclassified | Otu162 | 0,000 | 0,011 | 0,014 | 0,380 | 0,000 | 0,000 |
| | | | | | Otu214 | 0,091 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu120 | 0,081 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 |
| | | | | | Otu101 | 0,058 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu331 | 0,038 | 0,000 | 0,000 | 0,000 | 0,000 | 0,006 |
| | | | | | Otu136 | 0,000 | 0,000 | 0,020 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu86 | 0,000 | 0,015 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu454 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu111 | 0,000 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu34 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu463 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu43 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Gemmata | Otu83 | 0,000 | 0,045 | 0,033 | 0,612 | 0,000 | 0,015 |
| | | | | | Otu207 | 0,000 | 0,000 | 0,055 | 0,000 | 0,000 | 0,000 |
| | | | | Schlesneria | Otu27 | 0,014 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Proteobacteria | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified | Otu313 | 0,000 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu438 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,008 |
| | | | | | Otu457 | 0,000 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 |
| | Alphaproteobacteria | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Otu42 | 0,085 | 0,004 | 0,073 | 0,250 | 0,000 | 0,007 |
| | | | | | Otu57 | 0,056 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu71 | 0,032 | 0,000 | 0,019 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu20 | 0,040 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu251 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Caulobacterales | Caulobacteraceae | Brevundimonas | Otu23 | 5,270 | 10,880 | 3,695 | 1,896 | 0,000 | 0,134 |
| | | | | | Otu424 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu6 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Caulobacter | Otu310 | 0,010 | 0,000 | 0,024 | 0,000 | 0,000 | 0,007 |
| | | | | Phenylobacterium | Otu322 | 0,004 | 0,009 | 0,019 | 0,000 | 0,000 | 0,005 |
| | | | | | Otu278 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rhizobiales | Bradyrhizobiaceae | Afipia | Otu19 | 0,018 | 0,000 | 0,000 | 0,380 | 0,000 | 0,000 |
| | | | | Otu532 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | Bosea | Otu46 | 0,528 | 0,700 | 1,524 | 0,682 | 0,000 | 0,245 |
| | | | Bradyrhizobium | Otu208 | 0,000 | 0,000 | 0,000 | 0,000 | 0,987 | 0,000 |
| | | Brucellaceae | Brucellaceae_unclassified | Otu199 | 0,000 | 0,000 | 0,000 | 0,232 | 0,000 | 0,000 |
| | | | Ochrobactrum | Otu141 | 0,000 | 0,000 | 0,000 | 0,478 | 0,000 | 0,000 |
| | | Hyphomicrobiaceae | Hyphomicrobiaceae_unclassified | Otu210 | 0,016 | 0,000 | 0,000 | 0,473 | 0,000 | 0,000 |
| | | | Pedomicrobium | Otu60 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Methylobacteriaceae | Methylobacterium | Otu404 | 0,000 | 0,006 | 0,012 | 0,000 | 0,000 | 0,002 |
| | | Phyllobacteriaceae | Mesorhizobium | Otu218 | 0,014 | 0,000 | 0,050 | 0,000 | 0,000 | 0,019 |
| | | Rhizobiaceae | Rhizobium | Otu94 | 0,530 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Xanthobacteraceae | Xanthobacteraceae_unclassified | Otu123 | 0,127 | 0,021 | 0,275 | 0,264 | 0,000 | 0,042 |
| | Rhodobacterales | Rhodobacteraceae | Rhodobacteraceae_unclassified | Otu26 | 0,645 | 0,000 | 1,507 | 2,244 | 0,000 | 0,005 |
| | | | | Otu125 | 0,024 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Rhodobacter | Otu16 | 0,488 | 0,000 | 0,000 | 0,352 | 0,000 | 0,000 |
| | Rhodospirillales | Acetobacteraceae | Acetobacteraceae_unclassified | Otu220 | 0,000 | 0,000 | 0,000 | 0,195 | 0,000 | 0,000 |
| | | | | Otu61 | 0,016 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu93 | 0,010 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Roseomonas | Otu593 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | Reyranella | Reyranella_unclassified | Otu474 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu133 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 |
| | | Rhodospirillaceae | Niveispirillum | Otu387 | 0,000 | 0,000 | 0,014 | 0,000 | 0,000 | 0,000 |
| | | | Skermanella | Otu224 | 0,000 | 0,000 | 0,049 | 0,000 | 0,000 | 0,002 |
| | Sphingomonadales | Erythrobacteraceae | Erythrobacteraceae_unclassified | Otu312 | 0,000 | 0,000 | 0,024 | 0,000 | 0,000 | 0,000 |
| | | | Porphyrobacter | Otu14 | 1,265 | 22,108 | 7,019 | 6,574 | 1,408 | 1,521 |
| | | | | Otu552 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Sphingomonadaceae | Blastomonas | Otu18 | 0,000 | 0,011 | 0,014 | 0,000 | 0,000 | 0,000 |
| | | | Sphingomonadaceae_unclassified | Otu47 | 2,515 | 1,503 | 1,138 | 1,887 | 0,000 | 0,086 |
| | | | | Otu22 | 1,244 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu353 | 0,012 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Sphingomonas | Otu2 | 1,000 | 2,620 | 0,052 | 0,000 | 1,029 | 0,220 |
| | | | | Otu56 | 0,054 | 0,131 | 0,952 | 0,172 | 0,000 | 0,011 |
| | | | | Otu73 | 0,155 | 0,501 | 0,614 | 0,000 | 0,000 | 0,041 |
| | | | Sphingopyxis | Otu69 | 0,014 | 0,009 | 0,665 | 0,000 | 0,000 | 0,000 |
| Betaproteobacteria | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Otu490 | 0,000 | 0,006 | 0,003 | 0,000 | 0,000 | 0,000 |
| | | | | Otu334 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu486 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu584 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | Burkholderiales | Alcaligenaceae | Alcaligenes | Otu183 | 0,000 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 |
| | | Burkholderiales_unclassified | Burkholderiales_unclassified | Otu40 | 0,171 | 0,000 | 0,279 | 0,134 | 0,000 | 0,030 |
| | | | | Otu15 | 0,591 | 0,000 | 0,830 | 0,153 | 0,000 | 0,005 |
| | | | | Otu601 | 0,129 | 0,000 | 0,321 | 0,121 | 0,000 | 0,000 |
| | | | | Otu546 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Otu553 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 |
| | | Burkholderiaceae | Burkholderia | Otu469 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 |
| | | | | Otu555 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,002 |
| | | | Limnobacter | Otu5 | 76,476 | 21,277 | 65,071 | 67,812 | 60,210 | 76,475 |
| | | | | Otu363 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,025 |
| | | | | Otu554 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,002 |
| | | | | Otu570 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | | Otu583 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | Ralstonia | Otu72 | 0,000 | 0,000 | 0,000 | 0,023 | 0,000 | 0,000 |
| | | Comamonadaceae | Comamonadaceae_unclassified | Otu8 | 0,121 | 33,989 | 0,085 | 0,705 | 2,731 | 0,466 |
| | | | | Otu548 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Oxalobacteraceae | Massilia | Otu37 | 0,000 | 0,000 | 2,202 | 0,000 | 0,000 | 0,002 |
| | Gallionellales | Gallionellaceae | Sideroxydans | Otu131 | 0,006 | 0,017 | 0,154 | 0,000 | 0,000 | 0,000 |
| | Rhodocyclales | Rhodocyclaceae | Methyloversatilis | Otu252 | 0,000 | 0,000 | 0,040 | 0,000 | 0,000 | 0,000 |
| | | | Rhodocyclaceae_unclassified | Otu258 | 0,000 | 0,015 | 0,038 | 0,000 | 0,000 | 0,000 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gammaproteobacteria | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Otu32 | 0,065 | 0,032 | 0,164 | 0,250 | 0,378 | 0,000 |
| | | | | | Otu75 | 0,016 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu161 | 0,000 | 0,009 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu560 | 0,000 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | Chromatiales | Chromatiaceae | Rheinheimera | Otu172 | 0,018 | 0,000 | 0,000 | 0,144 | 0,000 | 0,000 |
| | | | Ectothiorhodospiraceae | Ectothiorhodospiraceae_unclassified | Otu354 | 0,000 | 0,000 | 0,021 | 0,000 | 0,000 | 0,000 |
| | | Enterobacteriales | Enterobacteriaceae | Enterobacter | Otu238 | 0,000 | 0,000 | 0,000 | 0,088 | 0,000 | 0,000 |
| | | | | Escherichia/Shigella | Otu89 | 0,583 | 0,004 | 0,002 | 0,797 | 0,000 | 0,000 |
| | | | | Morganella | Otu547 | 0,000 | 0,004 | 0,000 | 0,172 | 0,000 | 0,000 |
| | | | | Proteus | Otu116 | 0,000 | 0,013 | 0,015 | 0,501 | 3,971 | 0,000 |
| | | Legionellales | Coxiellaceae | Aquicella | Otu433 | 0,000 | 0,000 | 0,000 | 0,037 | 0,000 | 0,000 |
| | | | | | Otu109 | 0,000 | 0,011 | 0,010 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu326 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 |
| | | | | Coxiella | Otu483 | 0,000 | 0,007 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | Legionellaceae | Legionella | Otu137 | 0,000 | 0,000 | 0,003 | 0,000 | 0,000 | 0,002 |
| | | Pseudomonadales | Moraxellaceae | Moraxellaceae_unclassified | Otu180 | 0,000 | 0,000 | 0,072 | 0,000 | 0,000 | 0,000 |
| | | | | Perlucidibaca | Otu21 | 0,000 | 0,166 | 7,987 | 0,000 | 0,000 | 0,002 |
| | | | Pseudomonadaceae | Pseudomonas | Otu138 | 0,000 | 0,000 | 0,000 | 0,000 | 2,290 | 0,000 |
| | | Xanthomonadales | Sinobacteraceae | Nevskia | Otu11 | 2,436 | 2,067 | 0,614 | 3,959 | 0,000 | 20,529 |
| | | | | | Otu309 | 0,000 | 0,000 | 0,026 | 0,000 | 0,000 | 0,000 |
| | | | | Sinobacteraceae_unclassified | Otu36 | 0,004 | 3,595 | 0,051 | 0,000 | 0,000 | 0,007 |
| | Deltaproteobacteria | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified | Otu292 | 0,000 | 0,000 | 0,027 | 0,000 | 0,000 | 0,000 |
| | | Bdellovibrionales | Bacteriovoracaceae | Peredibacter | Otu257 | 0,000 | 0,000 | 0,038 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu468 | 0,000 | 0,000 | 0,005 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu591 | 0,000 | 0,000 | 0,002 | 0,000 | 0,000 | 0,000 |
| | | | Bdellovibrionaceae | Bdellovibrio | Otu502 | 0,006 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | Vampirovibrio | Otu95 | 0,000 | 0,009 | 0,016 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu139 | 0,008 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | | | | | Otu242 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,005 |
| | | Myxococcales | Myxococcales_unclassified | Myxococcales_unclassified | Otu92 | 0,018 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| Verrucomicrobia | Opitutae | Opitutales | Opitutaceae | Opitutus | Otu541 | 0,004 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 |
| | Spartobacteria | Spartobacteria_unclassified | Spartobacteria_unclassified | Spartobacteria_unclassified | Otu450 | 0,000 | 0,000 | 0,000 | 0,000 | 0,000 | 0,007 |
| | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | Prosthecobacter | Otu80 | 0,125 | 0,000 | 0,059 | 0,000 | 0,000 | 0,000 |
| candidate_division_WPS-1 | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | Otu394 | 0,000 | 0,000 | 0,013 | 0,000 | 0,000 | 0,000 |
| unknown_unclassified | unknown_unclassified | unknown_unclassified | unknown_unclassified | unknown_unclassified | Otu500 | 0,000 | 0,000 | 0,000 | 0,014 | 0,000 | 0,000 |

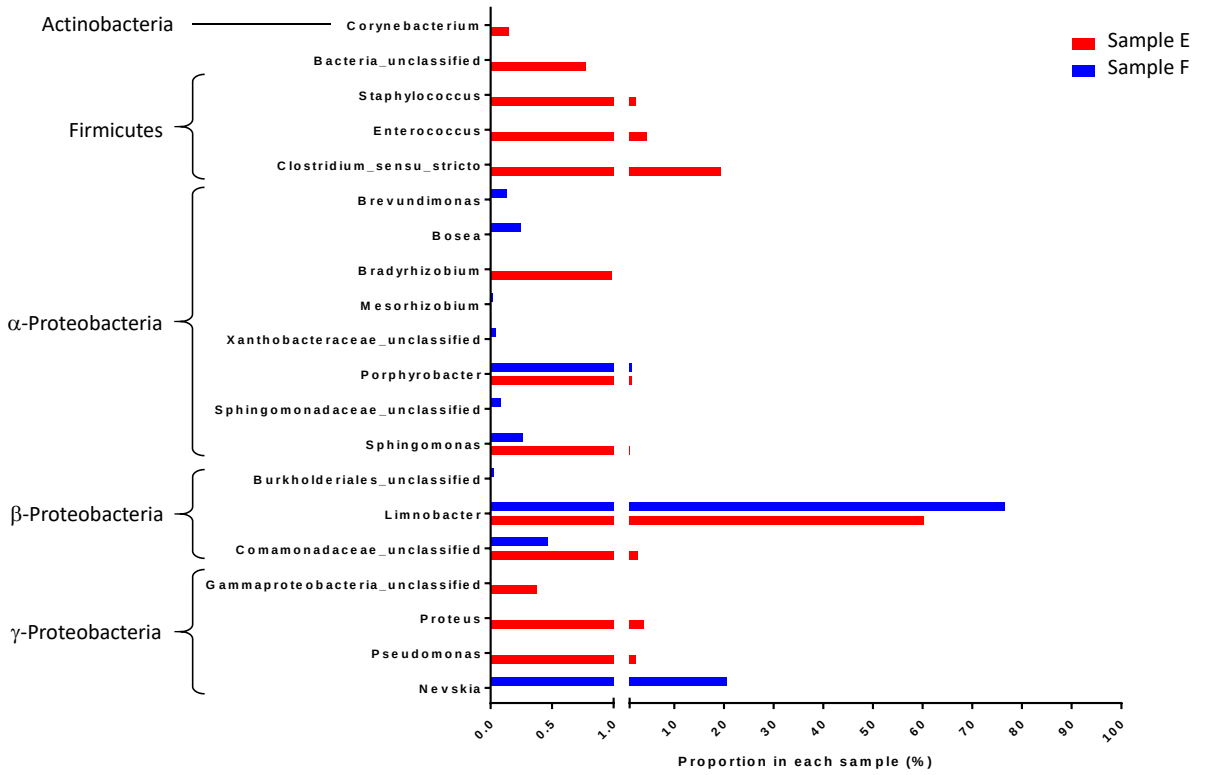



*Figure 6.11: Diversity observed at the family level from the samples collected at the middle of the pool using metagenetics. A 0.016% cut-off was used.*

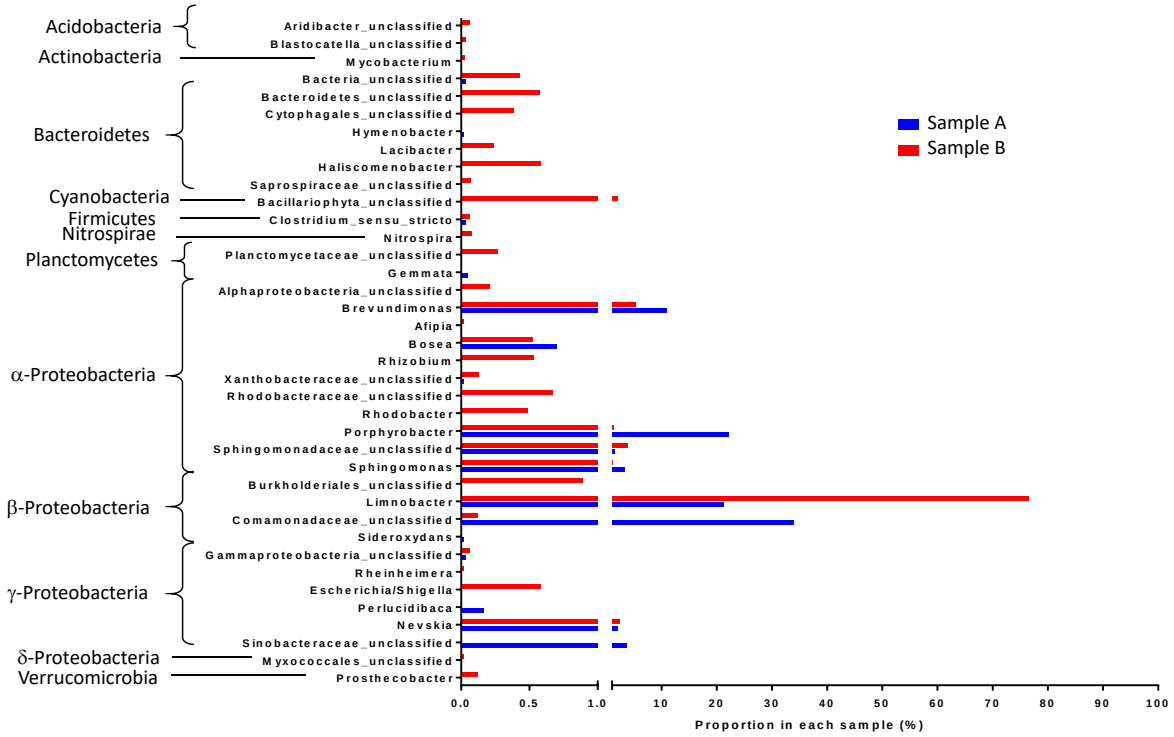



*Figure 6.12: Diversity observed at the family level from the samples collected at the northern wall using metagenetics. A 0.016% cut-off was used.*

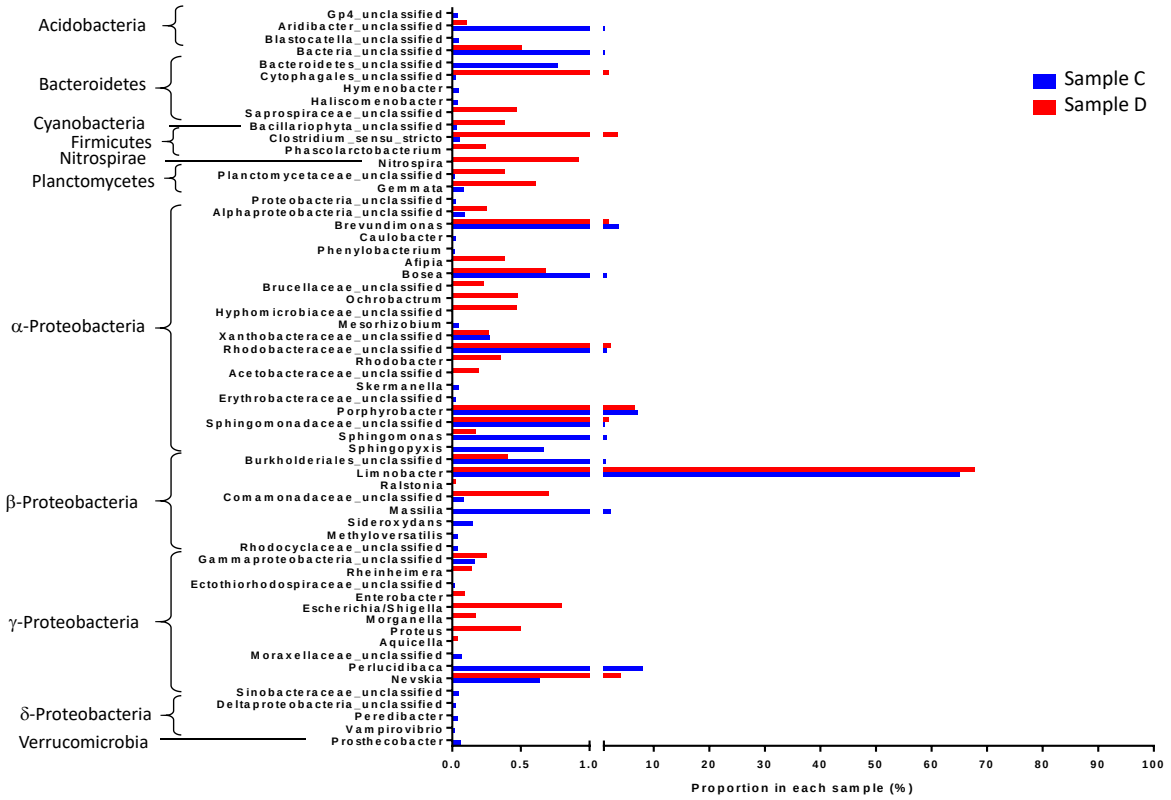



***Figure 6.13: Diversity observed at the family level from the samples collected at the western wall using metagenetics.*** *A 0.016% cut-off was used.*

# Appendix 10: Rarefaction curves obtained in Pool 1 after metagenetics sequencing (2015 and 2017 samplings)

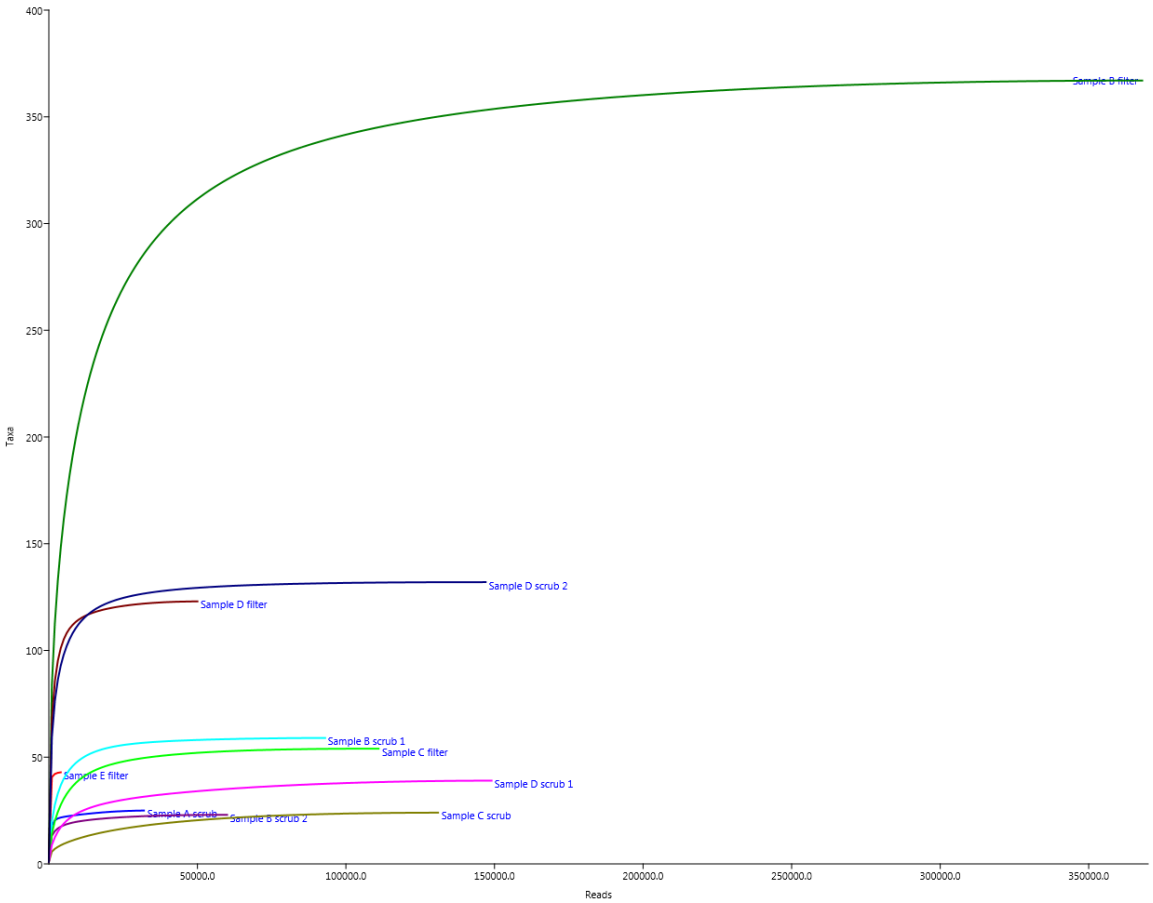



***Figure 6.14: Rarefaction curve of the samples recovered in 2015 (obtained with Past software [278]).*** *The diversity was reached quickly for all samples, except for sample B filter, which exhibited a greater diversity and reached a plateau above 250000 reads and at about 350 OTUs. The other samples reached a plateau faster, below 50000 reads. For sample D filter and scrub 2 the plateau was reached at about 100 OTUs while the other samples reached the plateau below 50 OTUs.*

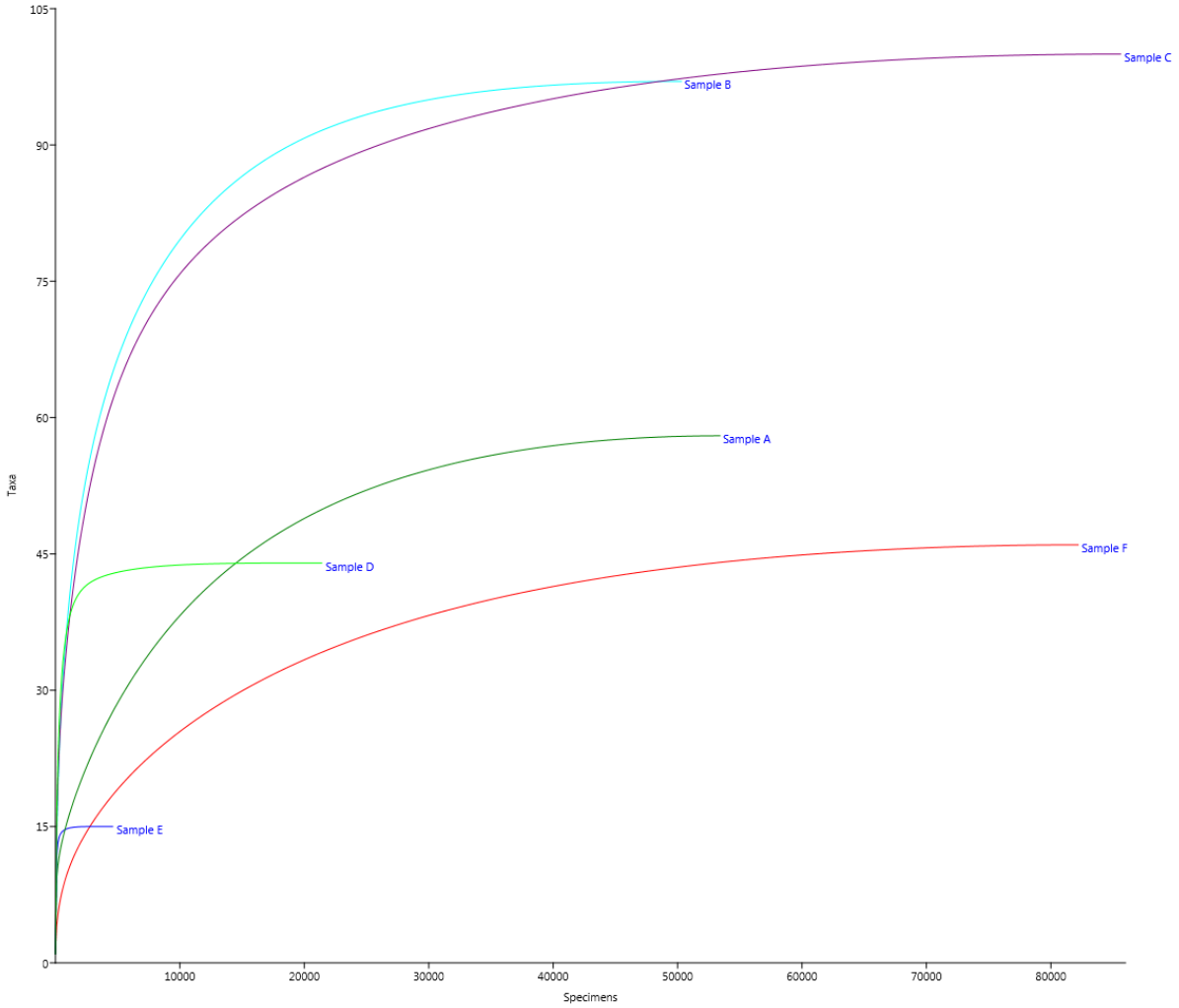
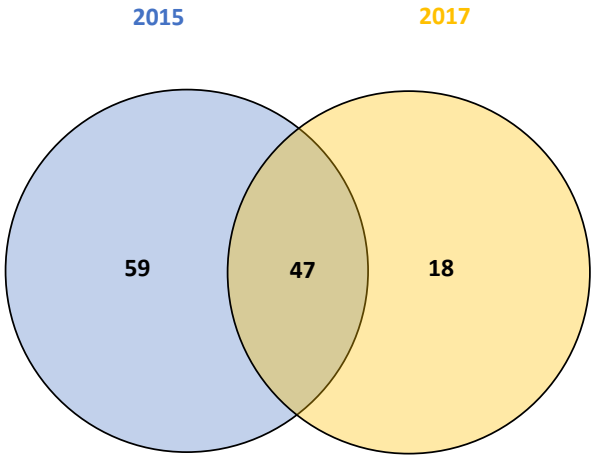



***Figure 6.15: Rarefaction curve of the water recovered in 2017 (obtained with Past software [278]).*** *Samples B and C reached their plateau at 5000 reads at circa 90 OTUs. The other samples were less diverse with a plateau at 50 OTUs or below (15 for TWMP).*

# Appendix 11: Comparison at a 21570 subsampling of the 2015 and 2017 metagenetics analysis

***Figure 6.16: Comparison at a 21570 subsampling of the 2015 and 2017 metagenetic analysis in Pool 1.*** *A) Venn diagram,B) Legend of the table, C) Table of the common and unique genera found in 2015 and 2017. Data from the water, biofilms, and water plus particles were used.*

A)




B)

Pool 1 2015
Pool 1 2017
Common to both

C)

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Acidobacteria | Acidobacteria_Gp3 | Gp3 | Gp3_unclassified | Gp3_unclassified |
| | Acidobacteria_Gp4 | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified |
| | | Gp4 | Gp4_unclassified | Gp4_unclassified |
| | | | Aridibacter | Aridibacter_unclassified |
| | | | Blastocatella | Blastocatella_unclassified |
| | Acidobacteria_Gp6 | Gp6 | Gp6_unclassified | Gp6_unclassified |
| Actinobacteria | Actinobacteria | Acidimicrobiales | Acidimicrobiales_unclassified | Acidimicrobiales_unclassified |
| | | Actinomycetales | Actinomycetaceae | Actinomyces |
| | | | Corynebacteriaceae | Corynebacterium |
| | | | Microbacteriaceae | Microbacteriaceae_unclassified |
| | | | | Microbacterium |
| | | | Micrococcaceae | Micrococcus |
| | | | | Rothia |
| | | | Mycobacteriaceae | Mycobacterium |
| | | | Propionibacteriaceae | Propionibacterium |
| Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified |
| Bacteroidetes | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified |
| | Cytophagia | Cytophagales | Cytophagales_unclassified | Cytophagales_unclassified |
| | | | Cytophagaceae | Hymenobacter |
| | | | | Spirosoma |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium |
| | Sphingobacteriia | Sphingobacteriales | Chitinophagaceae | Chitinophagaceae_unclassified |
| | | | | Lacibacter |
| | | | Saprospiraceae | Saprospiraceae_unclassified |
| | | | | Haliscomenobacter |
| | | | Sphingobacteriaceae | Mucilaginibacter |
| Chloroflexi | Thermomicrobia | Sphaerobacterales | Sphaerobacteraceae | Sphaerobacteraceae_unclassified |
| | | | | Nitrolancea |
| Cyanobacteria/Chloroplast | Chloroplast | Chloroplast | Bacillariophyta | Bacillariophyta_unclassified |
| | | | Streptophyta | Streptophyta_unclassified |
| | Cyanobacteria | Cyanobacteria_unclassified | Cyanobacteria_unclassified | Cyanobacteria_unclassified |
| Firmicutes | Bacilli | Bacillales | Bacillales_Incertae_Sedis_XI | Gemella |
| | | | Staphylococcaceae | Staphylococcus |
| | | Lactobacillales | Carnobacteriaceae | Granulicatella |
| | | | Streptococcaceae | Lactococcus |
| | | | | Streptococcus |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium_sensu_stricto |
| | | | Peptoniphilaceae | Peptoniphilus |
| | | | Clostridiales_Incertae_Sedis_XI | Anaerococcus |
| | Negativicutes | Acidaminococcales | Acidaminococcaceae | Phascolarctobacterium |
| | | Selenomonadales | Veillonellaceae | Veillonella |
| Gemmatimonadetes | Gemmatimonadetes | Gemmatimonadales | Gemmatimonadaceae | Gemmatimonas |
| Hydrogenedentes | Candidatus_Hydrogenedens | Candidatus_Hydrogenedens_unclassified | Candidatus_Hydrogenedens_unclassified | Candidatus_Hydrogenedens_unclassified |
| Nitrospirae | Nitrospira | Nitrospirales | Nitrospiraceae | Nitrospira |
| Planctomycetes | Planctomycetia | Planctomycetales | Planctomycetaceae | Planctomycetaceae_unclassified |
| | | | | Gemmata |
| | | | | Schlesneria |
| | | | | Zavarzinella |

| | | | | |
|---|---|---|---|---|
| Proteobacteria | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified |
| | Alphaproteobacteria | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified |
| | | Caulobacterales | Caulobacteraceae | Brevundimonas |
| | | | | Phenylobacterium |
| | | Rhizobiales | Rhizobiales_incertae_sedis | Phreatobacter |
| | | | Beijerinckiaceae | Beijerinckiaceae_unclassified |
| | | | Bradyrhizobiaceae | Afipia |
| | | | | Bosea |
| | | | | Bradyrhizobium |
| | | | Brucellaceae | Brucellaceae_unclassified |
| | | | | Ochrobactrum |
| | | | Hyphomicrobiaceae | Hyphomicrobiaceae_unclassified |
| | | | | Hyphomonas |
| | | | | Pedomicrobium |
| | | | Methylobacteriaceae | Methylobacterium |
| | | | Phyllobacteriaceae | Mesorhizobium |
| | | | Rhizobiaceae | Rhizobiaceae_unclassified |
| | | | | Rhizobium |
| | | | Rhodobiaceae | Rhodobiaceae_unclassified |
| | | | Xanthobacteraceae | Xanthobacter |
| | | | | Xanthobacteraceae_unclassified |
| | | Rhodobacterales | Rhodobacteraceae | Rhodobacteraceae_unclassified |
| | | | | Rhodobacter |
| | | Rhodospirillales | Acetobacteraceae | Acetobacteraceae_unclassified |
| | | | Reyranella | Reyranella_unclassified |
| | | | Rhodospirillaceae | Rhodospirillaceae_unclassified |
| | | | | Skermanella |
| | | Sphingomonadales | Erythrobacteraceae | Erythrobacteraceae_unclassified |
| | | | | Porphyrobacter |
| | | | Sphingomonadaceae | Blastomonas |
| | | | | Novosphingobium |
| | | | | Sphingomonadaceae_unclassified |
| | | | | Sphingomonas |
| | | | | Sphingopyxis |
| | Betaproteobacteria | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified |
| | | Burkholderiales | Burkholderiales_unclassified | Burkholderiales_unclassified |
| | | | Burkholderiaceae | Burkholderia |
| | | | | Limnobacter |
| | | | | Ralstonia |
| | | | Comamonadaceae | Comamonadaceae_unclassified |
| | | | Oxalobacteraceae | Massilia |
| | | Gallionellales | Gallionellaceae | Sideroxydans |
| | | Hydrogenophilales | Hydrogenophilaceae | Hydrogenophilus |
| | | Neisseriales | Neisseriaceae | Snodgrassella |
| | | Rhodocyclales | Rhodocyclaceae | Methyloversatilis |
| | | | | Rhodocyclaceae_unclassified |
| | Gammaproteobacteria | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified |
| | | Chromatiales | Chromatiaceae | Rheinheimera |
| | | Enterobacteriales | Enterobacteriaceae | Enterobacter |
| | | | | Escherichia/Shigella |
| | | | Morganellaceae | Morganella |
| | | | | Proteus |
| | | Legionellales | Coxiellaceae | Aquicella |
| | | | Legionellaceae | Legionella |
| | | Oceanospirillales | Oceanospirillaceae | Marinomonas |
| | | Pasteurellales | Pasteurellaceae | Pasteurellaceae_unclassified |
| | | | | Haemophilus |
| | | Pseudomonadales | Moraxellaceae | Acinetobacter |
| | | | | Enhydrobacter |
| | | | | Moraxellaceae_unclassified |
| | | | | Perlucidibaca |
| | | | | Psychrobacter |
| | | | Pseudomonadaceae | Pseudomonas |
| | | Nevskiales | Sinobacteraceae | Nevskia |
| | | | | Sinobacteraceae_unclassified |
| | | Xanthomonadales | Xanthomonadaceae | Stenotrophomonas |
| | Deltaproteobacteria | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified |
| | | Bdellovibrionales | Bacteriovoracaceae | Peredibacter |
| | | | Bdellovibrionaceae | Bdellovibrio |
| | | | | Vampirovibrio |
| | | Myxococcales | Myxococcales_unclassified | Myxococcales_unclassified |
| | | | Sandaracinaceae | Sandaracinus |
| Verrucomicrobia | Spartobacteria | Spartobacteria_unclassified | Spartobacteria_unclassified | Spartobacteria_unclassified |
| | Subdivision3 | Subdivision3_unclassified | Subdivision3_unclassified | Subdivision3_unclassified |
| | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | Prosthecobacter |
| candidate_division_WPS-1 | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified |

# Appendix 12: Direct metaproteomics data – Pool 1

***Table 6.15: Proportion of each genus in the water recovered in 2016 and analyzed using direct metaproteomics.*** *Samples A to D were composed of water and particles from the wall.*

| | | | | | Sample A | Sample B | Sample C | Sample D | Sample E |
|---|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | Genus | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Actinomycetales | Nocardiaceae | Rhodococcus | | | 3,70 | | |
| Cyanobacteria | Cyanobacteria | Nostocales | Scytonemataceae | Scytonema | | | 12,50 | 5,26 | |
| Firmicutes | Bacilli | Bacillales | Sporolactobacillaceae | Sporolactobacillus | | | | 2,31 | |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | Brevundimonas | | | | 6,03 | |
| | | Rhizobiales | Phyllobacteriaceae | Hoeflea | | | 8,80 | | |
| | | | Xanthobacteraceae | Xanthobacter | | | | | 19,47 |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | | | | 8,34 | |
| | Betaproteobacteria | Burkholderiales | Comamonadaceae | Acidovorax | | | 10,65 | 9,63 | 11,50 |
| | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Acinetobacter | | 100,00 | | 3,47 | |
| | | | Pseudomonadaceae | Pseudomonas | | | 16,67 | | |
| Bacillariophyta | Bacillariophyceae | Naviculales | Phaeodactylaceae | Phaeodactylum | | | 47,69 | 33,76 | 69,03 |
| | Coscinodiscophyceae | Thalassiosirales | Thalassiosiraceae | Thalassiosira | | | | 31,19 | |

***Table 6.16: Proportion of each genus in the scrubs recovered in 2016 and analyzed using direct metaproteomics***

| | | | | | Sample A | Sample B | Sample C | Sample D |
|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | Genus | % | % | % | % |
| Proteobacteria | Alphaprotobacteria | Caulobacterales | Caulobacteraceae | Caulobacter | 10,98 | 6,12 | 4,40 | 9,28 |
| | | Sphingomonadales | Sphingomonadaceae | Sphingomonas | 80,38 | 91,08 | 95,60 | 83,71 |
| | Betaproteobacteria | Bukholderiales | Comamonadaceae | Acidovorax | 8,64 | 2,80 | | 7,01 |

***Table 6.17: Proportion of each genus in the water recovered in 2017 and analyzed using direct metaproteomics.***

| | | | | | Sample A | Sample B | Sample C | Sample D | Sample E | Sample F |
|---|---|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Mycobacteriaceae | Mycobacterium | 2,75 | | | | 2,08 | |
| | | Streptomycetales | Streptomycetaceae | Streptomyces | 6,58 | | | | | |
| Bacteroidetes | Cytophagia | Cytophagales | Cytophagaceae | Hymenobacter | | | | | 0,63 | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | Bacillus | | 30,37 | | | | |
| | | | Paenibacillaceae | Paenibacillus | | | | | 3,11 | |
| | Clostridia | Clostridiales | Clostridiaceae | Clostridium | 5,21 | | | | 3,78 | |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Erythrobacteriaceae | Porphyrobacter | 1,12 | | | | 1,12 | |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | Achromobacter | | | | 79,80 | | |
| | | | Burkholderiaceae | Limnobacter | 5,21 | | | | 5,56 | |
| | Gammaproteobacteria | Enterobacteriales | Enterobacteriaceae | Escherichia | 62,94 | | | | 39,48 | |
| | | | | Salmonella | | | | | 30,80 | |
| | | Pseudomonadales | Pseudomonadaceae | Pseudomonas | 10,56 | 69,63 | 100,00 | 20,20 | 7,64 | 100,00 |
| | | Xanthomonadales | Sinobacteriaceae | Nevskia | 5,63 | | | | 5,80 | |

***Table 6.18: Proportion of each family in the water recovered in 2017 and analyzed using direct metaproteomics.*** *The number of PSMs differed at each taxonomical level. The proportion of each genus and its corresponding family may thus varies slightly.*

| | | | | Sample A | Sample B | Sample C | Sample D | Sample E | Sample F |
|---|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | % | % | % | % | % | % |
| Actinobacteria | Actinobacteria | Corynebacteriales | Mycobacteriaceae | 2,50 | | | | 2,67 | |
| | | Streptomycetales | Streptomycetaceae | 6,18 | | | | | |
| Bacteroidetes | Cytophagia | Cytophagales | Cytophagaceae | | | | | 2,20 | |
| Firmicutes | Bacilli | Bacillales | Bacillaceae | | 30,88 | | | | |
| | | | Paenibacillaceae | | | | | 4,36 | |
| | Clostridia | Clostridiales | Clostridiaceae | 5,06 | | | | 5,20 | |
| Proteobacteria | Alphaproteobacteria | Sphingomonadales | Erythrobacteraceae | 1,32 | | | | 1,82 | |
| | Betaproteobacteria | Burkholderiales | Alcaligenaceae | | | | 80,00 | | |
| | | | Burkholderiaceae | 10,03 | | | | 12,48 | |
| | Gammaproteobacteria | Enterobacteriales | Enterobacteriaceae | 59,28 | | | | 53,05 | |
| | | Pseudomonadales | Pseudomonadaceae | 9,69 | 69,12 | 100,00 | 20,00 | 9,83 | 100,00 |
| | | Xanthomonadales | Sinobacteraceae | 5,93 | | | | 8,40 | |

*Table 6.19: Proportion of each genus in the scrubs recovered in 2017 and analyzed using direct metaproteomics.*

| | | | | | Sample D scrub 1 | Sample D scrub 2 | Sample C scrub 1 | Sample C scrub 2 | Sample B scrub 1 | Sample B scrub 2 | Sample A scrub 1 | Sample A scrub 2 | Corner H 1 | Corner H 2 | Corner B 1 | Corner B 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | Genus | % | % | % | % | % | % | % | % | % | % | % | % |
| Cyanobacteria | Pleurocapsales_class | Pleurocapsales | Chroococcidiopsidaceae | Chroococcidiopsis | | | 74 | | | | | | | 100 | | |
| Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae | Selenomonas | | | | | | | | | | | | 9,78 |
| Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | | | | | | | | | | | | 51,09 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | Bradyrhizobium | | | | | | | | | | | 100 | |
| | | | Rhizobiaceae | Rhizobium | | 100 | | | | 100 | | | | | | |
| | Betaproteobacteria | Burkholderiales | Burkholederiaceae | Limnobacter | | | | | | | | 100 | | | | |
| | | | Ideonella_family | Ideonella | | | 26 | | | | | | | | | |
| | | Neisseriales | Neisseriaceae | Neisseria | | | | | | | | | | | | 39,13 |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | Alteromonas | | | | 100 | | | | | | | | |
| | | Pseudomonadales | Moraxellaceae | Acinetobacter | | | | | | | 100 | | | | | |
| | | | Pseudomonadaceae | Pseudomonas | 100 | | | | 100 | | | | | | | |

*Table 6.20: Proportion of each family in the scrubs recovered in 2017 and analyzed using direct metaproteomics.* *The number of PSMs differed at each taxonomical level. The proportion of each genus and its corresponding family may thus varies slightly.*

| | | | | Sample D scrub 1 | Sample D scrub 2 | Sample C scrub 1 | Sample C scrub 2 | Sample B scrub 1 | Sample B scrub 2 | Sample A scrub 1 | Sample A scrub 2 | Corner H 1 | Corner H 2 | Corner B 1 | Corner B 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phylum | Class | Order | Family | % | % | % | % | % | % | % | % | % | % | % | % |
| Cyanobacteria | Pleurocapsales_class | Pleurocapsales | Chroococcidiopsidaceae | | | 74,00 | | | | | | | 100,00 | | |
| Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae | | | | | | | | | | | | 16,16 |
| Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | | | | | | | | | | | | 47,47 |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | | | | | | | | | | | 100,00 | |
| | | | Rhizobiaceae | | 100,00 | | | | 100,00 | | | | | | |
| | | Sphingomonadales | Sphingomonadaceae | | | | | | | | | | | | |
| | Betaproteobacteria | Burkholderiales | Burkholederiaceae | | | | | | | | 100,00 | | | | |
| | | | Ideonella_family | | | 26,00 | | | | | | | | | |
| | | Neisseriales | Neisseriaceae | | | | | | | | | | | | 36,36 |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | | | | 100,00 | | | | | | | | |
| | | | Pseudoalteromonadaceae | | | | | | | | | | | | |
| | | Pseudomonadales | Moraxellaceae | | | | | | | 100,00 | | | | | |
| | | | Pseudomonadaceae | 100 | | | | 100,00 | | | | | | | |

# Appendix 13: Bacterial viability after irradiation

***Table 6.21: Cell viability after irradiation in NaCl (in %).*** *The viability was calculated after counting colonies and comparing it to the control plates. (-): ND*

| Dose (Gy) | Negative control *Escherichia coli* | Positive control *Deinococcus radiodurans* | CEA-NOB-002 *Micrococcus luteus* | CEA-NOB-006 *Kocuria koreensis* | CEA-NOB-010 *Leifsonia* sp. | CEA-NOB-012 *Gordonia bronchialis* | CEA-NOB-014 *Leifsonia* sp. | CEA-NOB-020 *Nocardia niigatensis* | CEA-NOB-030 *Nocardia niigatensis* | CEA-NOB-031 *Bacillus thuringiensis* | CEA-NOB-032 *Brevibacillus agri* | CEA-NOB-033 *Bacillus* sp. | CEA-NOB-034 *Bacillus thuringiensis* | SKH 154 *Sphingomonas* sp. | SKH 163 *Ralstonia pickettii* | SKH 446 *Pantoea* sp. | SKH 448 *Bacillus* sp. | SKH 450 *Pantoea vagans* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 100 | 100 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 |
| **200** | - | 100 | 106,14 | 24,19 | 72,44 | 50,60 | 100,00 | 29,37 | 35,26 | - | 77,78 | - | - | - | - | - | 110,56 | 95,90 |
| **500** | 0 | 100 | 42,06 | 5,60 | 10,48 | 12,84 | 7,86 | 9,90 | 4,50 | 0,00 | 11,40 | 43,33 | 0,00 | 0,03 | 0,00 | 7,00 | 50,80 | 16,10 |
| **1000** | 0 | 90 | 17,48 | 0,07 | 0,28 | 0,18 | 0,14 | 1,80 | 0,78 | 1,50 | 1,45 | 10,00 | 2,00 | 0,00 | 0,00 | 0,73 | 27,07 | 1,84 |

***Table 6.22: Cell viability after irradiation in liquid LB (in %).*** *The viability was calculated after counting colonies and comparing it to the control plates. (-): ND*

| Dose (Gy) | Negative control *Escherichia coli* | Positive control *Deinococcus radiodurans* | CEA-NOB-002 *Micrococcus luteus* | CEA-NOB-003 *Bacillus* sp. | CEA-NOB-006 *Kocuria koreensis* | CEA-NOB-008 *Bacillus pumilus* | CEA-NOB-010 *Leifsonia* sp. | CEA-NOB-012 *Gordonia bronchialis* | CEA-NOB-014 *Leifsonia* sp. | CEA-NOB-020 *Nocardia niigatensis* | CEA-NOB-030 *Nocardia niigatensis* | CEA-NOB-032 *Brevibacillus agri* | CEA-NOB-033 *Bacillus* sp. | SKH 111 *Pelomonas* sp. | SKH 448 *Bacillus* sp. | SKH 450 *Pantoea vagans* | SKH 497 *Pantoea* sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 |
| **200** | 6,64 | 51,50 | 80,08 | 32,04 | 92,72 | 33,65 | 58,31 | 12,55 | 92,02 | 36,70 | 55,23 | 34,15 | 37,34 | 4,77 | 9,88 | 49,50 | 19,28 |
| **500** | 0,00 | 40,14 | 46,63 | 3,27 | 34,93 | 1,42 | 2,12 | 1,40 | 6,11 | 14,37 | 11,57 | 6,50 | 1,94 | 0,00 | 0,00 | 20,45 | 15,16 |
| **1000** | 0,00 | 24,77 | 37,19 | 0,00 | 3,69 | 0,00 | 0,02 | 0,11 | 0,00 | 0,93 | 0,76 | 0,00 | 0,00 | 0,00 | 0,15 | 14,07 | 2,42 |
| **1500** | 0,00 | 22,65 | 17,72 | 0,00 | 0,64 | 0,00 | 0,00 | 0,09 | 0,00 | 0,53 | 0,23 | 1,22 | 0,00 | 0,00 | 0,00 | 3,23 | 0,43 |

# Appendix 14: Uranium accumulation

*Table 6.23: Cell viability (in %) - Preliminary results*

| | | CEA-NOB-002 | | | | CEA-NOB-006 | | | | CEA-NOB-010 | | | | CEA-NOB-033 | | | | SKH 448 | | | | SKH 450 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 µM | 5 µM | 20 µM | 200 µM | 0 µM | 5 µM | 20 µM | 200 µM | 0 µM | 5 µM | 20 µM | 200 µM | 0 µM | 5 µM | 20 µM | 200 µM | 0 µM | 5 µM | 20 µM | 200 µM | 0 µM | 5 µM | 20 µM | 200 µM |
| + 1h | **Mean** | 55,78 | 56,47 | 35,12 | 34,88 | 22,17 | 41,20 | 49,02 | 59,86 | 15,16 | 43,82 | 29,48 | 36,74 | 33,94 | 79,95 | 38,13 | 49,20 | 81,54 | ND | 33,60 | 63,10 | 91,43 | 90,16 | 70,54 | 54,88 |
| | **Std** | | 12,55 | 11,90 | 16,19 | | 1,82 | 3,17 | 6,61 | | 21,36 | 12,42 | 21,26 | | 19,66 | 25,20 | 6,13 | | ND | 24,72 | 11,11 | | 1,68 | 11,62 | 6,56 |
| + 24h | **Mean** | 78,90 | 67,43 | 38,52 | 67,13 | 24,00 | 55,54 | 42,94 | 38,07 | 61,70 | 48,81 | 28,90 | 20,68 | 17,82 | 41,95 | 30,76 | 71,64 | 82,61 | 71,17 | 45,98 | 31,42 | 76,50 | 85,23 | 27,47 | 22,76 |
| | **Std** | | 2,10 | 20,00 | 24,06 | | 4,99 | 8,20 | 22,06 | | 2,41 | 2,85 | 19,07 | | 12,19 | 0,69 | 17,03 | | 0,19 | 3,97 | 6,68 | | 4,04 | 0,98 | 21,26 |

*Table 6.24: Preliminary results - Uranium recovered per fraction (in nmol) after 1 hour and the recovery % compared to the introduced uranium. Some data were inconsistent with the introduced uranium (e.g. CEA-NOB-002 at 20 µM, duplicate 1 or CEA-NOB-010 at 200 µM, duplicate 1 where the uranium measured in the supernatant was over estimated).*

| | CEA-NOB-002 | | | | | CEA-NOB-006 | | | | | SKH 450 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Micrococcus luteus*** | | | | | ***Kocuria koreensis*** | | | | | ***Pantoea vagans*** | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 1,48 | 1,75 | 0,73 | **3,96** | **83,84** | 2,07 | 1,33 | 1,22 | **4,63** | **98,02** | 0,62 | 2,70 | 0,57 | **3,89** | **82,32** |
| **5-2** | 1,61 | 2,11 | 0,52 | **4,24** | **89,84** | 2,64 | 1,23 | 0,57 | **4,44** | **94,00** | 0,65 | 2,33 | 0,64 | **3,62** | **76,80** |
| **20-1** | 5,81 | 8,77 | 12,68 | **27,26** | **127,26** | 2,62 | 11,07 | 4,95 | **18,64** | **87,00** | 2,58 | 20,54 | 4,77 | **27,89** | **130,19** |
| **20-2** | 4,48 | 12,09 | 32,81 | **49,39** | **230,56** | 3,41 | 9,36 | 4,96 | **17,73** | **82,78** | 2,91 | 11,65 | 4,73 | **19,29** | **90,02** |
| **200-1** | 6,54 | 311,47 | 26,97 | **344,97** | **171,80** | 11,57 | 160,29 | 10,61 | **182,47** | **90,87** | 13,76 | 232,95 | 6,44 | **253,15** | **126,07** |
| **200-2** | 0,63 | 274,55 | 12,34 | **287,52** | **143,18** | 2,95 | 108,29 | 7,84 | **119,09** | **59,31** | 3,70 | 131,70 | 7,85 | **143,25** | **71,34** |

| | SKH 448 | | | | | CEA-NOB-033 | | | | | CEA-NOB-010 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Bacillus sp.*** | | | | | ***Bacillus sp.*** | | | | | ***Leifsonia sp.*** | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 0,39 | 2,03 | 2,97 | **5,38** | **114,06** | 1,51 | 2,51 | 0,46 | **4,48** | **94,82** | 2,21 | 1,59 | 1,90 | **5,69** | **120,59** |
| **5-2** | 1,06 | 1,96 | 1,35 | **4,37** | **92,54** | 1,70 | 2,21 | 0,39 | **4,30** | **91,04** | 3,31 | 1,55 | 0,72 | **5,58** | **118,28** |
| **20-1** | 10,69 | 9,37 | 10,55 | **30,61** | **142,88** | 1,79 | 11,93 | 6,72 | **20,44** | **95,43** | 3,27 | 10,63 | 10,48 | **24,38** | **113,79** |
| **20-2** | 9,26 | 7,76 | 11,53 | **28,55** | **133,28** | 5,21 | 11,29 | 8,89 | **25,39** | **118,50** | 8,12 | 9,20 | 18,70 | **36,02** | **168,13** |
| **200-1** | 17,56 | 166,08 | 12,53 | **196,16** | **97,69** | 7,95 | 275,25 | 13,33 | **296,53** | **147,67** | 6,22 | 567,59 | 42,87 | **616,68** | **307,11** |
| **200-2** | 18,06 | 108,68 | 9,81 | **136,55** | **68,00** | 8,59 | 281,69 | 16,34 | **306,62** | **152,70** | 7,23 | 180,61 | 24,97 | **212,81** | **105,98** |

***Table 6.25: Preliminary results - Uranium recovered per fraction (in nmol) after 24 hours and the recovery % compared to the introduced uranium. Some data were inconsistent with the introduced uranium*** *(e.g. CEA-NOB-002 at 200 µM or CEA-NOB-006 at 20 µM, duplicate 1).*

| | **CEA-NOB-002** | | | | | **CEA-NOB-006** | | | | | **SKH 450** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Micrococcus luteus*** | | | | | ***Kocuria koreensis*** | | | | | ***Pantoea vagans*** | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 3,02 | 1,23 | 1,19 | **5,44** | **115,17** | 3,70 | 1,56 | 0,58 | **5,83** | **123,51** | 1,84 | 2,35 | 0,63 | **4,81** | **101,89** |
| **5-2** | 2,35 | 0,90 | 2,75 | **6,00** | **127,06** | 4,27 | 0,89 | 0,66 | **5,82** | **123,40** | 1,82 | 2,77 | 0,59 | **5,17** | **109,63** |
| **20-1** | 0,82 | 2,76 | 12,67 | **16,26** | **75,89** | 36,09 | 11,36 | 10,60 | **58,05** | **270,97** | 10,56 | 12,79 | 5,17 | **28,51** | **133,08** |
| **20-2** | 0,87 | 3,05 | 14,19 | **18,11** | **84,54** | 16,61 | 6,90 | 7,43 | **30,94** | **144,43** | 11,13 | 13,27 | 7,65 | **32,05** | **149,62** |
| **200-1** | 2,76 | 59,63 | 23,24 | **85,63** | **42,64** | 27,40 | 156,09 | 20,83 | **204,32** | **101,75** | 16,17 | 168,24 | 15,13 | **199,54** | **99,37** |
| **200-2** | 8,61 | 75,62 | 38,77 | **123,00** | **61,25** | 33,12 | 157,27 | 19,85 | **210,25** | **104,70** | 13,43 | 178,42 | 0,01 | **191,86** | **95,55** |

| | **SKH 448** | | | | | **CEA-NOB-033** | | | | | **CEA-NOB-010** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Bacillus sp.*** | | | | | ***Bacillus sp.*** | | | | | ***Leifsonia sp.*** | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 5,39 | 0,82 | 0,42 | **6,63** | **140,49** | 3,01 | 1,76 | 0,48 | **5,26** | **111,39** | 1,31 | 0,99 | 0,38 | **2,69** | **56,99** |
| **5-2** | 4,17 | 1,11 | 0,52 | **5,80** | **122,90** | 4,39 | 1,74 | 0,47 | **6,60** | **139,86** | 1,11 | 1,28 | 0,83 | **3,22** | **68,26** |
| **20-1** | 18,25 | 8,13 | 11,02 | **37,40** | **174,59** | 11,84 | 11,46 | 9,76 | **33,06** | **154,31** | 32,19 | 9,48 | 7,84 | **49,51** | **231,10** |
| **20-2** | 16,85 | 6,63 | 9,45 | **32,94** | **153,74** | 11,94 | 8,39 | 6,36 | **26,69** | **124,58** | 25,88 | 7,56 | 7,40 | **40,83** | **190,60** |
| **200-1** | 41,11 | 121,59 | 19,36 | **182,05** | **90,66** | 21,08 | 148,51 | 13,19 | **182,77** | **91,02** | 43,45 | 174,35 | 15,68 | **233,48** | **116,28** |
| **200-2** | 64,95 | 191,88 | 46,55 | **303,38** | **151,09** | 19,02 | 153,36 | 11,20 | **183,59** | **91,43** | 34,95 | 157,42 | 16,90 | **209,28** | **104,22** |

***Table 6.26: Uranium measured in the controlled tubes.*** *Represents the uranium actually introduced in the bacteria.*

| **Theoretical uranium (µM)** | **Uranium measured (µM)** |
|---|---|
| **5 µM** | 5,18 |
| **10 µM** | 10,33 |
| **50 µM** | 53,88 |
| **500 µM** | 533,18 |
| **1000 µM** | 982,51 |

***Table 6.27: Uranium recovered per fraction (in nmol) after 24 hours and the recovery % compared to the introduced uranium.*** *In grey: negative results measured with the ICP-MS, no uranium was detected in the fraction (replaced by 0 for the balance), in green: results to be re-analyzed.*

| | CEA-NOB-002 | | | | | CEA-NOB-006 | | | | | SKH 450 | | | | | SKH 448 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Micrococcus luteus* | | | | | *Kocuria koreensis* | | | | | *Pantoea vagans* | | | | | *Bacillus sp.* | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 3,64 | 0,67 | 0,10 | **4,41** | **85,20** | 3,41 | 0,32 | 0,02 | **3,75** | **72,48** | 5,96 | 1,27 | 0,44 | **7,67** | **148,22** | 4,62 | 0,11 | 0,01 | **4,73** | **91,49** |
| **5-2** | 3,67 | 0,61 | 0,11 | **4,39** | **84,74** | 3,89 | 0,35 | 0,02 | **4,26** | **82,31** | 4,90 | 2,78 | 0,74 | **8,43** | **162,80** | 4,04 | 0,11 | 0,05 | **4,20** | **81,12** |
| **10-1** | 4,92 | 1,18 | 0,66 | **6,75** | **65,40** | 7,49 | 0,65 | 0,29 | **8,43** | **81,58** | 5,96 | 1,27 | 0,44 | **7,67** | **74,27** | 5,36 | 1,16 | 0,63 | **7,16** | **69,28** |
| **10-2** | 6,49 | 0,96 | 0,72 | **8,17** | **79,11** | 9,30 | 0,38 | 0,24 | **9,92** | **96,03** | 4,90 | 2,78 | 0,74 | **8,43** | **81,57** | 5,23 | 1,04 | 0,51 | **6,78** | **65,64** |
| **20-1** | 0,82 | 2,76 | 12,67 | **16,26** | **75,89** | 36,09 | 11,36 | 10,60 | **58,05** | **270,97** | 10,56 | 12,79 | 5,17 | **28,51** | **133,08** | 18,25 | 8,13 | 11,02 | **37,40** | **174,59** |
| **20-2** | 0,87 | 3,05 | 14,19 | **18,11** | **84,54** | 16,61 | 6,90 | 7,43 | **30,94** | **144,43** | 11,13 | 13,27 | 7,65 | **32,05** | **149,62** | 16,85 | 6,63 | 9,45 | **32,94** | **153,74** |
| **50-1** | 41,86 | 1,86 | 1,65 | **45,37** | **84,20** | 50,00 | -1,32 | 0,98 | **49,66** | **92,16** | 51,05 | 43,90 | 5,10 | **100,05** | **185,67** | 48,11 | -2,52 | 0,33 | **45,91** | **85,21** |
| **50-2** | 43,55 | 18,65 | 1,47 | **63,67** | **118,16** | 40,23 | -1,05 | 0,94 | **40,12** | **74,46** | 47,27 | 51,38 | 4,67 | **103,31** | **191,73** | 56,68 | -0,38 | 0,24 | **56,54** | **104,92** |
| **200-1** | 2,76 | 59,63 | 23,24 | **85,63** | **42,64** | 27,40 | 156,09 | 20,83 | **204,32** | **101,75** | 16,17 | 168,24 | 15,13 | **199,54** | **99,37** | 41,11 | 121,59 | 19,36 | **182,05** | **90,66** |
| **200-2** | 8,61 | 75,62 | 38,77 | **123,00** | **61,25** | 33,12 | 157,27 | 19,85 | **210,25** | **104,70** | 13,43 | 178,42 | 0,01 | **191,86** | **95,55** | 64,95 | 191,88 | 46,55 | **303,38** | **151,09** |
| **500-1** | 62,40 | 454,35 | 15,12 | **531,87** | **99,75** | 68,69 | 460,14 | 9,40 | **538,22** | **100,94** | 11,03 | 1124,37 | 12,30 | **1147,70** | **215,25** | 97,80 | 390,50 | 15,14 | **503,44** | **94,42** |
| **500-2** | 62,41 | 451,77 | 16,33 | **530,50** | **99,50** | 71,88 | 487,20 | 9,50 | **568,59** | **106,64** | 10,46 | 1085,66 | 6,19 | **1102,31** | **206,74** | 119,65 | 389,44 | 16,50 | **525,59** | **98,58** |
| **1000-1** | 14,12 | 1071,13 | 86,34 | **1171,59** | **119,25** | 14,14 | 2809,17 | 10,70 | **2834,01** | **288,45** | 8,78 | 1124,37 | 12,30 | **1145,45** | **116,58** | 8,44 | 1073,23 | 14,95 | **1096,62** | **111,61** |
| **1000-2** | 14,23 | 995,55 | 117,63 | **1127,41** | **114,75** | 17,30 | 2893,83 | 14,82 | **2925,94** | **297,80** | 8,75 | 1085,66 | 6,19 | **1100,60** | **112,02** | 11,98 | 1092,30 | 7,61 | **1111,88** | **113,17** |

| | SKH 497 | | | | | CEA-NOB-032 | | | | | CEA-NOB-033 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Pantoea sp.* | | | | | *Brevibacillus agri* | | | | | *Bacillus sp.* | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 4,07 | 0,45 | 0,13 | **4,65** | **89,87** | 4,24 | 0,11 | 0,01 | **4,36** | **84,27** | 4,86 | 0,04 | 0,28 | **5,18** | **99,99** |
| **5-2** | 4,13 | 0,46 | 0,12 | **4,71** | **91,08** | 4,60 | 0,10 | 0,01 | **4,72** | **91,12** | 4,83 | 0,04 | 0,31 | **5,18** | **100,07** |
| **10-1** | 2,38 | 7,38 | 0,97 | **10,73** | **103,93** | 8,52 | 0,80 | 2,90 | **12,22** | **118,27** | 0,00 | 3,24 | 4,61 | **7,85** | **76,03** |
| **10-2** | 2,66 | 6,60 | 0,97 | **10,22** | **98,97** | 7,49 | 0,80 | 99,92 | **108,21** | **1047,69** | 2,47 | 2,27 | 4,46 | **9,21** | **89,15** |
| **50-1** | 30,01 | 13,67 | 1,84 | **45,52** | **84,48** | 51,03 | 10,16 | 1,26 | **62,45** | **115,90** | 39,22 | -0,40 | 2,32 | **41,14** | **76,34** |
| **50-2** | 34,78 | 16,22 | 1,83 | **52,83** | **98,04** | 50,19 | 0,10 | 1,34 | **51,62** | **95,80** | 38,72 | 9,05 | 1,29 | **49,05** | **91,04** |
| **500-1** | 30,84 | 510,66 | 6,97 | **548,47** | **102,87** | 98,29 | 437,31 | 12,91 | **548,50** | **102,87** | 62,61 | 482,69 | 7,18 | **552,49** | **103,62** |
| **500-2** | 32,52 | 502,57 | 4,64 | **539,74** | **101,23** | 97,10 | 445,42 | 10,73 | **553,25** | **103,76** | 60,94 | 472,73 | 8,10 | **541,77** | **101,61** |
| **1000-1** | 3,51 | 1413,80 | 5,00 | **1422,31** | **144,76** | 25,44 | 1098,78 | 38,43 | **1162,66** | **118,34** | 17,69 | 1109,18 | 10,97 | **1137,84** | **115,81** |
| **1000-2** | 3,23 | 1120,11 | 13,22 | **1136,57** | **115,68** | 37,49 | 1098,86 | 13,58 | **1149,93** | **117,04** | 8,50 | 1045,24 | 10,08 | **1063,82** | **108,28** |

| | CEA-NOB-010 | | | | | CEA-NOB-020 | | | | | CEA-NOB-030 | | | | | CEA-NOB-012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Leifsonia sp.* | | | | | *Nocardia niigatensis* | | | | | *Nocardia niigatensis* | | | | | *Gordonia bronchialis* | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **5-1** | 1,41 | 2,81 | 0,43 | **4,65** | **89,90** | 2,38 | 1,47 | 0,26 | **4,12** | **79,51** | 1,26 | 3,39 | 0,36 | **5,01** | **96,75** | 2,16 | 2,86 | 0,01 | **5,04** | **97,34** |
| **5-2** | 1,89 | 3,56 | 0,46 | **5,91** | **114,13** | 2,52 | 1,71 | 0,36 | **4,59** | **88,78** | 1,25 | 3,82 | 0,37 | **5,44** | **105,11** | 1,54 | 3,49 | 0,03 | **5,06** | **97,82** |
| **50-1** | 11,16 | 42,66 | 1,74 | **55,55** | **103,10** | 17,19 | 28,28 | 2,83 | **48,30** | **89,64** | 5,17 | 51,81 | 2,46 | **59,45** | **110,33** | 5,13 | 48,25 | 2,57 | **55,95** | **103,83** |
| **50-2** | 11,12 | 40,83 | 1,84 | **53,78** | **99,81** | 16,75 | 33,51 | 2,92 | **53,19** | **98,70** | 7,70 | 589,65 | 1,40 | **598,75** | **1111,17** | 11,27 | 47,46 | 2,46 | **61,19** | **113,56** |
| **500-1** | 25,82 | 549,63 | 7,78 | **583,22** | **109,39** | 20,97 | 540,63 | 5,08 | **566,69** | **106,28** | 3,82 | 582,71 | 7,67 | **594,20** | **111,44** | 3,09 | 539,90 | 13,11 | **556,10** | **104,30** |
| **500-2** | 21,66 | 537,55 | 4,02 | **563,24** | **105,64** | 21,56 | 540,15 | 6,42 | **568,13** | **106,56** | 3,65 | 36,84 | 5,56 | **46,04** | **8,64** | 2,90 | 604,34 | 15,08 | **622,32** | **116,72** |

# Appendix 15: Cobalt accumulation

***Table 6.28: Cobalt measured in the controlled tubes.*** *Represents the cobalt actually introduced in the bacteria. The first three concentrations were introduced in CEA-NOB-002, CEA-NOB-006, SKH 450, SKH 448, SKH 497, CEA-NOB-032, and CEA-NOB-033. The last three concentrations were introduced in CEA-NOB-010, CEA-NOB-020, CEA-NOB-030, and CEA-NOB-012.*

| Theoretical cobalt (µM) | Cobalt measured (µM) |
|---|---|
| **5 µM** | 3,50 |
| **50 µM** | 34,47 |
| **500 µM** | 357,63 |
| **5 µM** | 2,85 |
| **50 µM** | 28,66 |
| **500 µM** | 297,66 |

***Table 6.29: Cobalt recovered per fraction (in nmol) after 24 hours and the recovery % compared to the introduced uranium.*** *In grey: negative results measured with the ICP-MS, no uranium was detected in the fraction (replaced by 0 for the balance), in green: results to be re-analyzed.*

| | CEA-NOB-002 | | | | | CEA-NOB-006 | | | | | SKH 450 | | | | | SKH 448 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Micrococcus luteus* | | | | | *Kocuria koreensis* | | | | | *Pantoea vagans* | | | | | *Bacillus sp.* | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **3-1** | 0,00 | 3,24 | 0,08 | **3,32** | **94,82** | 0,05 | 2,47 | 0,24 | **2,76** | **78,85** | 0,05 | 2,54 | 0,11 | **2,70** | **77,20** | 0,00 | 2,66 | 0,01 | **2,67** | **76,32** |
| **3-2** | 0,00 | 2,69 | 0,17 | **2,87** | **81,90** | 0,05 | 2,50 | 0,24 | **2,78** | **79,54** | 0,05 | 2,76 | 0,09 | **2,90** | **82,84** | 0,00 | 2,69 | 0,01 | **2,70** | **77,14** |
| **30-1** | 0,00 | 34,36 | 0,26 | **34,62** | **100,44** | 0,17 | 26,89 | 1,43 | **28,49** | **82,67** | 0,22 | 30,15 | 1,03 | **31,40** | **91,10** | 0,00 | 29,06 | 0,17 | **29,23** | **84,80** |
| **30-2** | 0,00 | 30,09 | 0,68 | **30,77** | **89,26** | 0,21 | 26,35 | 0,98 | **27,54** | **79,92** | 0,19 | 26,77 | 0,51 | **27,47** | **79,70** | 0,00 | 27,95 | 0,17 | **28,12** | **81,60** |
| **300-1** | 0,10 | 331,06 | 7,59 | **338,74** | **94,72** | 1,44 | 289,50 | 5,57 | **296,50** | **82,91** | 0,21 | 296,95 | 2,24 | **299,39** | **83,72** | 0,39 | 308,72 | 1,02 | **310,14** | **86,72** |
| **300-2** | 0,42 | 347,98 | 7,04 | **355,44** | **99,39** | 1,82 | 279,08 | 4,94 | **285,85** | **79,93** | 0,29 | 308,95 | 2,90 | **312,13** | **87,28** | 0,00 | 294,36 | 9,20 | **303,55** | **84,88** |

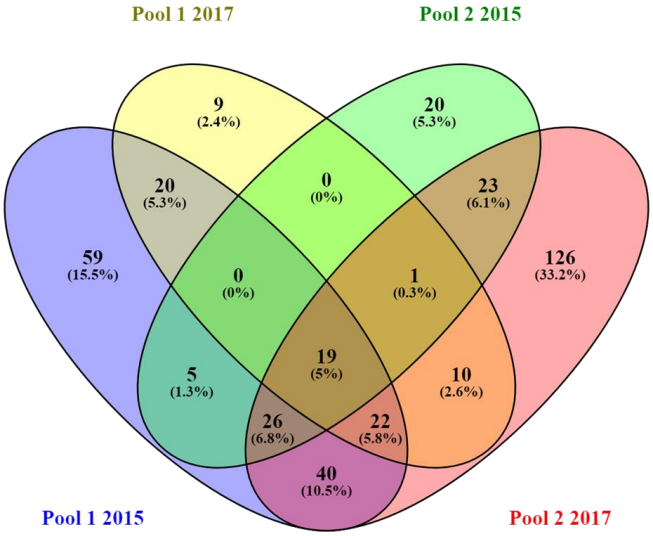
| | SKH 497 | | | | | CEA-NOB-032 | | | | | CEA-NOB-033 | | | | | CEA-NOB-010 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Pantoea sp.* | | | | | *Brevibacillus agri* | | | | | *Bacillus sp.* | | | | | *Leifsonia sp.* | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **3-1** | 0,00 | 2,80 | 0,06 | **2,86** | **81,68** | 0,00 | 2,55 | 0,06 | **2,61** | **74,49** | 0,00 | 2,40 | 0,01 | **2,40** | **68,67** | 0,64 | 2,98 | 0,08 | **3,70** | **136,50** |
| **3-2** | 0,00 | 2,58 | 0,01 | **2,59** | **74,08** | 0,00 | 2,63 | 0,06 | **2,69** | **76,90** | 0,00 | 2,34 | 0,25 | **2,60** | **74,27** | 0,00 | 2,69 | 0,07 | **2,76** | **102,09** |
| **30-1** | | 27,65 | 0,30 | **27,95** | **81,09** | 0,00 | 27,71 | 0,19 | **27,90** | **80,96** | 0,00 | 29,19 | 0,82 | **30,00** | **87,05** | 0,00 | 29,37 | 0,40 | **29,76** | **109,31** |
| **30-2** | 0,00 | 26,39 | 0,45 | **26,85** | **77,89** | 0,00 | 27,56 | 0,19 | **27,76** | **80,53** | 0,00 | 32,48 | 0,29 | **32,77** | **95,06** | 0,00 | 32,79 | 0,35 | **33,14** | **121,70** |
| **300-1** | 0,00 | 310,10 | 1,02 | **311,12** | **86,99** | 0,00 | 283,87 | 3,28 | **287,14** | **80,29** | 0,10 | 308,99 | 4,32 | **313,41** | **87,64** | 0,04 | 291,06 | 2,82 | **293,92** | **103,94** |
| **300-2** | 0,00 | 290,61 | 0,00 | **290,47** | **81,22** | 0,00 | 299,14 | 4,50 | **303,64** | **84,90** | 0,00 | 302,45 | 2,00 | **304,45** | **85,13** | 0,09 | 288,31 | 2,31 | **290,72** | **102,81** |

| | CEA-NOB-020 | | | | | CEA-NOB-030 | | | | | CEA-NOB-012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Nocardia niigatensis* | | | | | *Nocardia niigatensis* | | | | | *Gordonia bronchialis* | | | | |
| | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered | Pellet (nmol) | Supernatant (nmol) | Wash (nmol) | Total (nmol) | % recovered |
| **3-1** | 0,01 | 2,61 | 0,03 | **2,65** | **98,07** | 0,00 | 2,62 | 0,04 | **2,66** | **98,26** | 0,04 | 2,74 | 0,03 | **2,81** | **103,86** |
| **3-2** | 0,01 | 2,86 | 0,05 | **2,92** | **107,97** | 0,00 | 2,85 | 0,04 | **2,89** | **106,92** | 0,04 | 2,80 | 0,03 | **2,87** | **105,99** |
| **30-1** | 0,00 | 30,79 | 0,10 | **30,89** | **113,43** | 0,00 | 26,93 | 0,12 | **27,05** | **99,36** | 0,07 | 28,10 | 0,25 | **28,42** | **104,37** |
| **30-2** | 0,05 | 29,67 | 0,20 | **29,92** | **109,90** | 0,00 | 29,39 | 0,14 | **29,53** | **108,46** | 0,33 | 28,10 | 0,15 | **28,57** | **104,93** |
| **300-1** | 0,65 | 293,60 | 2,67 | **296,91** | **105,00** | 0,13 | 293,28 | 1,93 | **295,34** | **104,44** | 0,83 | 299,46 | 2,12 | **302,41** | **106,94** |
| **300-2** | 0,60 | 293,80 | 7,20 | **301,59** | **106,65** | 0,09 | 292,88 | 1,81 | **294,79** | **104,25** | 0,41 | 284,51 | 1,88 | **286,81** | **101,42** |

## Appendix 16: Microbial diversity: comparison between Pool 1 and Pool 2 (all data combined)

***Figure 6.17: Comparison between the genera obtained after the 2015 and 2017 kinetics in Pools 1 and 2, the metagenetics results from all samples (21570 subsample), and direct phylopetidomics.*** *A) Venn diagram: 19 genera were common to both pools at both sampling point, 59 were only found in Pool 1 in 2015, 9 only in Pool 1 2017, 30 only in Pool 2 in 2015 and 126 only in Pool 2 in 2017; 20 genera were only found in Pool 1 (both in 2015 and 2017) while 23 were only found in Pool 2 (both in 2015 and 2017); in 2015 5 genera were common to both pools while 10 were common in 2017; B) Legend of the table; C) Table with all the genera present in both pools at both sampling time and representing the Venn diagram*

**A)**




**B)**

| |
|---|
| Pool 1 2015 |
| Pool 1 2017 |
| Common to Pool 1 |
| Pool 1 2015 / Pool 2 2015 |
| Pool 2 2015 |
| Pool 2 2017 |
| Common to Pool 2 |
| Pool 1 / Pool 2 2017 |
| Pool 1 2015 / Pool 2 2017 |
| Pool 1 2017 / Pool 2 2017 |
| Pool 1 2015 / Pool 2 |
| Pool 1 / Pool 2 2017 |
| Common to all |

# C)

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Acidobacteria | Acidobacteria_Gp1 | Acidobacteria_Gp1_unclassified | Acidobacteria_Gp1_unclassified | Acidobacteria_Gp1_unclassified |
| | | Gp1 | Gp1_unclassified | Gp1_unclassified |
| | Acidobacteria_Gp2 | Gp2 | Gp2_unclassified | Gp2_unclassified |
| | Acidobacteria_Gp3 | Acidobacteria_Gp3_unclassified | Acidobacteria_Gp3_unclassified | Acidobacteria_Gp3_unclassified |
| | | Gp3 | Gp3_unclassified | Gp3_unclassified |
| | Acidobacteria_Gp4 | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified | Acidobacteria_Gp4_unclassified |
| | | Gp4 | Gp4_unclassified | Gp4_unclassified |
| | | | Aridibacter | Aridibacter_unclassified |
| | | | Blastocatella | Blastocatella_unclassified |
| | Acidobacteria_Gp6 | Gp6 | Gp6_unclassified | Gp6_unclassified |
| Actinobacteria | Actinobacteria | Acidimicrobiales | Acidimicrobiales_unclassified | Acidimicrobiales_unclassified |
| | | | Acidimicrobiaceae | Ilumatobacter |
| | | Actinomycetales | Actinomycetales_unclassified | Actinomycetales_unclassified |
| | | | Actinomycetaceae | Actinomyces |
| | | Bifidobacteriales | Bifidobacteriaceae | Bifidobacterium |
| | | Corynebacteriales | Corynebacteriaceae | Corynebacterium |
| | | | Gordoniaceae | Gordonia |
| | | | Mycobacteriaceae | Mycobacterium |
| | | | Nocardiaceae | Nocardia |
| | | | | Rhodococcus |
| | | Micrococcales | Cellulomonadaceae | Actinotalea |
| | | | | Cellulomonas |
| | | | Dermabacteraceae | Brachybacterium |
| | | | | Helcobacillus |
| | | | Intrasporangiaceae | Janibacter |
| | | | Microbacteriaceae | Microbacteriaceae_unclassified |
| | | | | Agromyces |
| | | | | Cryocola |
| | | | | Leifsonia |
| | | | | Microbacterium |
| | | | | Mycetocola |
| | | | Micrococcaceae | Arthrobacter |
| | | | | Kocuria |
| | | | | Micrococcus |
| | | | | Nesterenkonia |
| | | | | Rothia |
| | | | Promicromonosporaceae | Promicromonosporaceae bacterium W15_genus |

| | | | | |
|---|---|---|---|---|
| | | Micromonosporales | Micromonosporaceae | Asanoa |
| | | Propionibacteriales | Nocardioidaceae | Marmoricola |
| | | | | Mumia |
| | | | | Nocardioides |
| | | | Propionibacteriaceae | Propionibacterium |
| | | Pseudonocardiales | Pseudonocardiaceae | Amycolatopsis |
| | | | | Lentzea |
| | | Rubrobacterales | Rubrobacteraceae | Rubrobacter |
| | | Solirubrobacteriales | Conexibacteraceae | Conexibacter |
| | | Streptomycetales | Streptomycetaceae | Streptomyces |
| Armatimonadetes | Armatimonadetes_gp5_class_incertae_sedis | Armatimonadetes_gp5_order_incetae_sedis | Armatimonadetes_gp5_family_incetae_sedis | Armatimonadetes_gp5 |
| Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified | Bacteria_unclassified |
| Bacteroidetes | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified | Bacteroidetes_unclassified |
| | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides |
| | | | Porphyromonadaceae | Porphyromonadaceae_unclassified |
| | | | | Rikenella |
| | | | Prevotellaceae | Prevotella |
| | | | Porphyromonadaceae | Porphyromonas |
| | Cytophagia | Cytophagales | Cytophagales_unclassified | Cytophagales_unclassified |
| | | | Cytophagaceae | Cytophagaceae_unclassified |
| | | | | Dyadobacter |
| | | | | Hymenobacter |
| | | | | Microscilla |
| | | | | Rhodonellum |
| | | | | Runella |
| | | | | Spirosoma |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | Chryseobacterium |
| | | | | Chryseobacterium or Bergeyella |
| | | | | Elizabethkingia |
| | | | | Flavobacterium |
| | Sphingobacteriia | Sphingobacteriales | Chitinophagaceae | Chitinophagaceae_unclassified |
| | | | | Heliimonas |
| | | | | Lacibacter |
| | | | | Ferruginibacter |
| | | | | Sediminibacterium |
| | | | | Vibrionimonas |
| | | | Saprospiraceae | Saprospiraceae_unclassified |
| | | | | Haliscomenobacter |
| | | | Sphingobacteriaceae | Mucilaginibacter |
| | | | | Pedobacter |
| | | | | Sphingobacterium |
| bacterium YEK0313_phylum | bacterium YEK0313_class | bacterium YEK0313_order | bacterium YEK0313_family | bacterium YEK0313_genus |
| Chlamydiae | Chlamydiae | Chlamydiales | Chlamydiales_unclassified | Chlamydiales_unclassified |
| | | | Parachlamydiaceae | Parachlamydiaceae_unclassified |
| | | | | Neochlamydia |

| | | | | |
|---|---|---|---|---|
| Chloroflexi | Chloroflexi_unclassified | Chloroflexi_unclassified | Chloroflexi_unclassified | Chloroflexi_unclassified |
| | Ktedonobacteria | Ktedonobacterales | Ktedonobacteraceae | Ktedonobacter |
| | | | Ktedonobacterales_unclassified | Ktedonobacterales_unclassified |
| | Thermomicrobia | Sphaerobacterales | Sphaerobacteraceae | Sphaerobacteraceae_unclassified |
| | | | | Nitrolancea |
| Cyanobacteria/Chloroplast | Cyanobacteria | Cyanobacteria_unclassified | Cyanobacteria_unclassified | Cyanobacteria_unclassified |
| | Chloroplast | Chloroplast | Bacillariophyta | Bacillariophyta_unclassified |
| | | | Streptophyta | Streptophyta_unclassified |
| | Cyanobacteria | Nostocales | Aphanizomenonaceae | Nodularia |
| | | | Hapalosiphonaceae | Mastigocladus |
| | | | Nostocaceae | Anabaena |
| | | | | Cylindrospermum |
| | | | | Nostoc |
| | | | Scytonemataceae | Scytonema |
| | | | Tolypothrichaceae | Tolypothrix |
| | | Oscillatoriales | Cyanothecaceae | Cyanothece |
| | | Synechococcales | Leptolyngbyaceae | Leptolyngbya |
| | | | Synechococcaceae | Synechococcus |
| | Pleurocapsales_class | Pleurocapsales | Chroococcidiopsidaceae | Chroococcidiopsis |
| Deinococcus-Thermus | Deinococci | Thermales | Thermaceae | Meiothermus |
| | | | | Thermaceae_unclassified |
| Firmicutes | Bacilli | Bacillales | Bacillales_Incertae_Sedis_XI | Gemella |
| | | | Bacillales_Incertae_Sedis_XII | Exiguobacterium |
| | | | Bacillaceae | Bacillus |
| | | | Paenibacillaceae_1 | Paenibacillus |
| | | | Sporolactobacillaceae | Sporolactobacillus |
| | | | Staphylococcaceae | Staphylococcus |
| | | Lactobacillales | Aerococcaceae | Aerococcus |
| | | | Carnobacteriaceae | Atopostipes |
| | | | | Granulicatella |
| | | | Enterococcaceae | Enterococcus |
| | | | | Melissococcus |
| | | | Lactobacillaceae | Lactobacillus |
| | | | Streptococcaceae | Lactococcus |
| | | | | Streptococcus |
| | Clostridia | Clostridia_unclassified | Clostridia_unclassified | Clostridia_unclassified |
| | | Clostridiales | Clostridiales_unclassified | Clostridiales_unclassified |
| | | | Clostridiaceae | Clostridium_sensu_stricto |
| | | | | Clostridium_IV |
| | | | | Clostridiaceae_1_unclassified |
| | | | Lachnospiraceae | Blautia |
| | | | | Butyrivibrio |
| | | | | Dorea |
| | | | | Lachnoclostridium |
| | | | | Oribacterium |
| | | | | Roseburia |

| | | | | |
|---|---|---|---|---|
| | | | Peptococcaceae_1 | Desulfitobacterium |
| | | | | Desulfosporosinus |
| | | | Peptoniphilaceae | Peptoniphilus |
| | | | Clostridiales_Incertae_Sedis_XI | Anaerococcus |
| | | | Clostridiales_Incertae_Sedis_XIII | Anaerovorax |
| | | | Ruminococcaceae | Ruminococcaceae_unclassified |
| | Firmicutes_unclassified | Firmicutes_unclassified | Firmicutes_unclassified | Firmicutes_unclassified |
| | Negativicutes | Acidaminococcales | Acidaminococcaceae | Phascolarctobacterium |
| | | Selenomonadales | Veillonellaceae | Veillonellaceae_unclassified |
| | | | | Pelosinus |
| | | | | Selenomonas |
| | | | | Sporomusa |
| | | | | Veillonella |
| Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium |
| Gemmatimonadetes | Gemmatimonadetes | Gemmatimonadales | Gemmatimonadaceae | Gemmatimonas |
| Hydrogenedentes | Candidatus_Hydrogenedens | Candidatus_Hydrogenedens_unclassified | Candidatus_Hydrogenedens_unclassified | Candidatus_Hydrogenedens_unclassified |
| Nitrospirae | Nitrospira | Nitrospirales | Nitrospiraceae | Nitrospira |
| Planctomycetes | Planctomycetia | Planctomycetales | Planctomycetaceae | Planctomycetaceae_unclassified |
| | | | | Gemmata |
| | | | | Schlesneria |
| | | | | Singulisphaera |
| | | | | Zavarzinella |
| Proteobacteria | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified | Proteobacteria_unclassified |
| | Alphaproteobacteria | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified | Alphaproteobacteria_unclassified |
| | | Alphaproteobacteria_order_incertae_sedis | Alphaproteobacteria_family_incertae_sedis | Rhizomicrobium |
| | | Caulobacterales | Caulobacteraceae | Asticcacaulis |
| | | | | Brevundimonas |
| | | | | Caulobacter |
| | | | | Phenylobacterium |
| | | Rhizobiales | Rhizobiales_unclassified | Rhizobiales_unclassified |
| | | | Rhizobiales_incertae_sedis | Phreatobacter |
| | | | Aurantimonadaceae | Aurantimonas |
| | | | | Aureimonas |
| | | | Bartonellaceae | Bartonella |
| | | | Beijerinckiaceae | Beijerinckiaceae_unclassified |
| | | | | Beijerinckia |
| | | | | Methylocapsa |
| | | | Bradyrhizobiaceae | Bradyrhizobiaceae_unclassified |
| | | | | Afipia |
| | | | | Bosea |
| | | | | Bradyrhizobium |
| | | | | Rhodopseudomonas |
| | | | Brucellaceae | Brucellaceae_unclassified |
| | | | | Ochrobactrum |
| | | | | Pseudochrobactrum |
| | | | Chelatococcaceae | Chelatococcus |

| | | | |
|---|---|---|---|
| | | Hyphomicrobiaceae | Hyphomicrobiaceae_unclassified |
| | | | Blastochloris |
| | | | Devosia |
| | | | Hyphomicrobium |
| | | | Pedomicrobium |
| | | | Prosthecomicrobium |
| | | Methylobacteriaceae | Methylobacterium |
| | | | Microvirga |
| | | Methylocystaceae | Methylocystis |
| | | | Methylopila |
| | | | Pleomorphomonas |
| | | Phyllobacteriaceae | Aminobacter |
| | | | Hoeflea |
| | | | Mesorhizobium |
| | | Rhizobiaceae | Rhizobiaceae_unclassified |
| | | | Agrobacterium |
| | | | Kaistia |
| | | | Rhizobium |
| | | Rhodobiaceae | Rhodobiaceae_unclassified |
| | | | Afifella |
| | | | Amorphus |
| | | | Parvibaculum |
| | | Xanthobacteraceae | Xanthobacteraceae_unclassified |
| | | | Azorhizobium |
| | | | Starkeya |
| | | | Xanthobacter |
| | Rhodobacterales | Hyphomonadaceae | Hyphomonas |
| | | | Oceanicaulis |
| | | Rhodobacteraceae | Rhodobacteraceae_unclassified |
| | | | Labrenzia |
| | | | Loktanella |
| | | | Pannonibacter |
| | | | Paracoccus |
| | | | Rhodobacter |
| | | | Rhodovulum |
| | | | Rubellimicrobium |
| | | | Ruegeria |
| | Rhodospirillales | Rhodospirillales bacterium_family | Rhodospirillales bacterium URHD0088_genus |
| | | Acetobacteraceae | Acetobacteraceae_unclassified |
| | | | Acetobacter |
| | | | Komagataeibacter |
| | | | Roseomonas |
| | | Reyranella | Reyranella_unclassified |

| | | | |
|---|---|---|---|
| | | Rhodospirillaceae | Rhodospirillaceae_unclassified |
| | | | Azospirillum |
| | | | Inquilinus |
| | | | Magnetospira |
| | | | Oceanibaculum |
| | | | Rhodovibrio |
| | | | Skermanella |
| | | | Thalassobaculum |
| | Sphingomonadales | Erythrobacteraceae | Erythrobacteraceae_unclassified |
| | | | Altererythrobacter |
| | | | Croceicoccus |
| | | | Erythrobacter |
| | | | Porphyrobacter |
| | | Sphingomonadaceae | Sphingomonadaceae_unclassified |
| | | | Blastomonas |
| | | | Citromicrobium |
| | | | Novosphingobium |
| | | | Sphingobium |
| | | | Sphingomonas |
| | | | Sphingomonas-like bacterium B12_genus |
| | | | Sphingopyxis |
| | | | Sphingorhabdus |
| Betaproteobacteria | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified | Betaproteobacteria_unclassified |
| | Burkholderiales | Burkholderiales_unclassified | Burkholderiales_unclassified |
| | | Burkholderiales bacterium JOSHI_001_family | Burkholderiales bacterium JOSHI_001_genus |
| | | Alcaligenaceae | Achromobacter |
| | | | Alcaligenes |
| | | | Azohydromonas |
| | | | Bordetella |
| | | Aquabacterium_family | Aquabacterium |
| | | Aquincola_family | Aquincola |
| | | Burkholderiaceae | Burkholderia |
| | | | Limnobacter |
| | | | Ralstonia |
| | | | Wautersia |
| | | Comamonadaceae | Comamonadaceae_unclassified |
| | | | Acidovorax |
| | | | Caldimonas |
| | | | Comamonas |
| | | | Curvibacter |
| | | | Diaphorobacter |
| | | | Hydrogenophaga |
| | | | Hylemonella |
| | | | Limnohabitans |

| | | | |
|---|---|---|---|
| | | | Pelomonas |
| | | | Polaromonas |
| | | | Pseudorhodoferax |
| | | | Ramlibacter |
| | | | Rhodoferax |
| | | | Variovorax |
| | | | Verminephrobacter |
| | | Ideonella_family | Ideonella |
| | | Leptothrix_family | Leptothrix |
| | | Methylibium_family | Methylibium |
| | | Oxalobacteraceae | Collimonas |
| | | | Herbaspirillum |
| | | | Herminiimonas |
| | | | Janthinobacterium |
| | | | Massilia |
| | | | Undibacterium |
| | | Polyangium brachysporum_family | Polyangium brachysporum_genus |
| | | Rhizobacter_family | Rhizobacter |
| | | Rubrivivax_family | Rubrivivax |
| | | Sphaerotilus_family | Sphaerotilus |
| | | Thiomonas_family | Thiomonas |
| | Gallionellales | Gallionellaceae | Sideroxydans |
| | Hydrogenophilales | Hydrogenophilaceae | Hydrogenophilus |
| | Neisseriales | Chromobacteriaceae | Andreprevotia |
| | | | Chromobacterium |
| | | | Leeia |
| | | | Pseudogulbenkiania |
| | | Neisseriaceae | Neisseria |
| | | | Snodgrassella |
| | Nitrosomonadales | Sterolibacteriaceae | Methyloversatilis |
| | | | Sulfuritalea |
| | Rhodocyclales | Rhodocyclaceae | Rhodocyclaceae_unclassified |
| | | | Azonexus |
| | | | Methyloversatilis |
| | | Zoogloeaceae | Azoarcus |
| | | | Uliginosibacterium |
| | | | Thauera |
| Gammaproteobacteria | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified | Gammaproteobacteria_unclassified |
| | Alteromonadales | Alteromonadaceae | Alteromonas |
| | | | Marinobacter |
| | | | Paraglaciecola |
| | | Pseudoalteromonadaceae | Pseudoalteromonas |
| | Chromatiales | Chromatiaceae | Rheinheimera |

| | | | |
|---|---|---|---|
| | Enterobacteriales | Enterobacteriaceae | Candidatus Blochmannia |
| | | | Enterobacteriaceae_unclassified |
| | | | Enterobacter |
| | | | Escherichia |
| | | | Klebsiella |
| | | Erwiniaceae | Pantoea |
| | | Morganellaceae | Morganella |
| | | | Proteus |
| | | | Salmonella |
| | | | Serratia |
| | Legionellales | Coxiellaceae | Aquicella |
| | | | Coxiella |
| | | Legionellaceae | Legionella |
| | Oceanospirillales | Halomonadaceae | Halomonas |
| | | Oceanospirillaceae | Marinomonas |
| | Pasteurellales | Pasteurellaceae | Pasteurellaceae_unclassified |
| | | | Haemophilus |
| | Pseudomonadales | Moraxellaceae | Moraxellaceae_unclassified |
| | | | Acinetobacter |
| | | | Enhydrobacter |
| | | | Perlucidibaca |
| | | | Psychrobacter |
| | | Pseudomonadaceae | Pseudomonadaceae_unclassified |
| | | | Pseudomonas |
| | Nevskiales | Sinobacteraceae | Nevskia |
| | | | Sinobacteraceae_unclassified |
| | Vibrionales | Vibrionaceae | Vibrio |
| | Xanthomonadales | Xanthomonadaceae | Xanthomonadaceae_unclassified |
| | | | Lysobacter |
| | | | Pseudoxanthomonas |
| | | | Stenotrophomonas |
| | | | Rhodanobacter |
| | | | Xanthomonas |
| Deltaproteobacteria | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified | Deltaproteobacteria_unclassified |
| | Desulfuromonadales | Geobacteraceae | Geobacter |
| | Desulfovibrionales | Desulfovibrionaceae | Desulfovibrio |
| | Bdellovibrionales | Bacteriovoracaceae | Peredibacter |
| | | Bdellovibrionaceae | Bdellovibrio |
| | | | Vampirovibrio |
| | Myxococcales | Myxococcales_unclassified | Myxococcales_unclassified |
| | | Archangiaceae | Cystobacter |
| | | | Hyalangium |
| | | | Stigmatella |
| | | Kofleriaceae | Haliangium |

| | | | | |
|---|---|---|---|---|
| | | | Myxococcaceae | Myxococcus |
| | | | Polyangiaceae | Chondromyces |
| | | | Labilitrichaceae | Labilithrix |
| | | | Phaselicystidaceae | Phaselicystis |
| | | | Sandaracinaceae | Sandaracinus |
| | Epsilonproteobacteria | Campylobacterales | Campylobacteraceae | Campylobacter |
| | | | Helicobacteraceae | Helicobacter |
| Tenericutes | Mollicutes | Mycoplasmatales | Mycoplasmataceae | Mycoplasma |
| Verrucomicrobia | Spartobacteria | Spartobacteria_unclassified | Spartobacteria_unclassified | Spartobacteria_unclassified |
| | Subdivision3 | Subdivision3_unclassified | Subdivision3_unclassified | Subdivision3_unclassified |
| | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | Luteolibacter |
| | | | | Prosthecobacter |
| candidate_division_WPS-1 | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified | candidate_division_WPS-1_unclassified |
| Ascomycota | Dothideomycetes | Dothideales | Saccotheciaceae | Aureobasidium |
| | Eurotiomycetes | Eurotiales | Aspergillaceae | Aspergillus |
| | | | | Penicillium |
| | | | Trichocomaceae | Talaromyces |
| | | Chaetothyriales | Clavicipitaceae | Cladophialophora |
| | | | Herpotrichiellaceae | Exophiala |
| | Saccharomycetes | Saccharomycetales | Saccharomycetaceae | Zygosaccharomyces |
| | Sordariomycetes | Hypocreales | Cordycipitaceae | Torrubiella |
| | | | Hypocreaceae | Metarhizium |
| | | | | Trichoderma |
| | | | Nectriaceae | Fusarium |
| | | | | Neonectria |
| | | | Ophiocordycipitaceae | Hirsutella |
| | | | | Ophiocordyceps |
| | | | | Tolypocladium |
| | | Sordariales | Madurella_family | Madurella |
| Bacillariophyta | Bacillariophyceae | Naviculales | Phaeodactylaceae | Phaeodactylum |
| | Coscinodiscophyceae | Thalassiosirales | Thalassiosiraceae | Thalassiosira |

# Abstract

Biotechnology with microorganisms able to survive high radiation doses and concentrate radionuclides is an attractive technology to decontaminate nuclear effluents with low waste volume. By using two direct and complementary analysis methods, metagenetics and metaproteomics, the complete inventories of microorganisms present in a French nuclear reactor cooling pool (during operation and at shutdown) and in a radioactive $^{60}Co$ sources storage pool have been performed. The microbiota from the two pools were extremely different, with a majority of *Cyanobacteria* in the sources storage pool, and *Proteobacteria* in the reactor pool. Depending on the pools' conditions a change in the microbiota had been observed. *Variovorax* was the main genus identified during the reactor's operation. Some strains of *Variovorax* are able to use $H_2$ as an energy source, conferring them an advantage in nuclear pools. Several microorganisms have been isolated from the reactor pool. Among them 46 bacteria were identified. Their abilities to resist ionizing radiations and to accumulate specific radionuclides were determined. Several genera studied in this work had never been described concerning their ability to resist radiations or accumulate radionuclides. Six strains could entirely remove uranium from a 5 µM solution and up to 50 µM for two strains. This work presents the first complete inventory of a working nuclear reactor cooling pool. This PhD work opens interesting industrial perspectives as the isolated strains could be implemented in a new nuclear effluents decontamination process and fundamental perspectives as their resistance and radionuclides accumulation pathways could be deciphered.

# Résumé

Les biotechnologies basées sur des microorganismes capables de survivre à de fortes doses de rayonnement ionisants et de concentrer les radionucléides constituent une technique attractive pour décontaminer les effluents nucléaires, en particulier du fait du faible volume de déchets générés. En utilisant deux méthodes d'analyses directes et complémentaires que sont la métagénétique et la métaprotéomique, l'inventaire complet de la piscine de refroidissement d'un cœur de réacteur nucléaire (en fonctionnement et à l'arrêt) et d'une piscine de stockage de sources radioactives de cobalt a été réalisé. Les microbiotes sont extrêmement différents entre les deux piscines. Une majorité de *Cyanobacteria* a été identifiée dans la piscine du réacteur alors qu'une majorité de *Proteobacteria* l'a été dans la piscine des sources. Durant le fonctionnement du réacteur, une majorité de *Variovorax* a été identifiée. Certaines espèces de *Variovorax* sont capables d'utiliser du $H_2$ comme source d'énergie, ce qui peut leur donner un avantage dans un environnement nucléaire aquatique. Parmi les microorganismes isolés de la piscine du réacteur, 46 bactéries ont été identifiées. Leur capacité à résister aux rayonnements gamma et à accumuler l'uranium et le cobalt ont été déterminées. Parmi ces bactéries, six souches ont été capables d'accumuler la totalité de l'uranium d'une solution à 5 µM, et deux d'entre elles ont entièrement accumulé l'uranium d'une solution à 50 µM. Ce travail présente le premier inventaire complet d'une piscine de refroidissement d'un cœur de réacteur nucléaire en fonctionnement. Cette thèse présente des perspectives tant sur le plan industriel avec l'utilisation des microorganismes isolés dans un procédé de biodécontamination d'effluents nucléaires que sur le plan fondamental avec l'élucidation des mécanismes développés par ces microorganismes pour résister aux rayonnements et accumuler les radionucléides.