Exploring fungi-bacteria interaction in PAH contaminated coastal sediment

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THÈSE

UNIVERSITE DE PAU ET DES PAYS DE L'ADOUR

École doctorale 211 : Sciences Exactes et leurs Applications

Présentée et soutenue le 23 Novembre 2021

par **Joyce ÁLVAREZ BARRAGÁN**

pour obtenir le grade de docteur
de l'Université de Pau et des Pays de l'Adour
Spécialité : Doctorat en Physiologie & Biologie des organismes – Populations – Interactions

EXPLORING FUNGI-BACTERIA INTERACTION IN PAH CONTAMINATED COASTAL SEDIMENT

Exploration des interactions *Fungi-Bacteria* dans des sédiments côtiers contaminés par les HAP

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UNIVERSITÉ DE PAU ET DES PAYS DE L'ADOUR

A mi mamá, Virginia

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Vers la France pour être mon pays d'accueil.

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To all the family that act as friends and all the friends that act as family, this is yours.

To fungi, for being.

Sommarie

General introduction

Sediments occupy around 70% of the area of the planet, making them the biggest ecosystem on Earth. Beside their extension, several factors vary between sediment locations that can go from the coastal sediments to the bottom of the deep sea; this confers sediments a variety of physicochemical characteristics that include salinity, oxygen concentration, particle size, content of organic matter and many more. With all of these heterogenic characteristics, it is not surprising to find a vast microbial diversity inhabiting these ecosystems. The microorganisms that live in sediments and their interactions play a key role in several geochemical processes including sulphur, nitrogen and carbon cycling. Nevertheless, the dynamics of microorganisms and their interactions are constantly affected by the input of hydrocarbon contamination due to natural or anthropogenic sources.

From the hydrocarbons that enter in the marine environment, polycyclic aromatic hydrocarbons (PAHs) are pollutants of main concern due to their toxic, mutagen and carcinogenic potential. Moreover, the transformation products of incomplete degradation of PAHs (generally by physical or chemical processes) are more toxic than parent PAHs, and lead to critical cellular effects. For this reason, the microbial biodegradation that involves the transformation of PAHs in carbon dioxide and water has become an alternative to cleaning PAHs polluted sediments.

Several microorganisms have been identified and characterized for their capacity to tolerate and degrade PAHs. From these microorganisms, fungi and bacteria have been studied, and the pathways they use to degrade PAHs have been described. Fungi degrade PAHs extracellularly using ligninolytic monooxygenase enzymes or intracellularly via P450 cytochrome. Whereas, bacteria have PAHs degradation pathways that change depending on the presence of oxygen (aerobic pathway) or the absence of it (anaerobic pathway). Nevertheless, fungi and bacteria have their limitations. To date, there is no report of fungi that can mineralize completely PAHs to carbon and water, and bacteria cannot degrade high molecular weight PAHs efficiently. This is why there is interest to focus on the interaction of fungi and bacteria together for the complete biodegradation of PAHs. In hydrocarbon-polluted sediments, fungi are likely primary degraders of high molecular weight hydrocarbons via secreted extracellular enzymes, and work synergistically with hydrocarbon-degrading bacteria. The interaction of fungi and bacteria give one another special advantages to overcome PAHs pollution. It is described that bacteria can improve fungal reproduction and affect their metabolism. Besides, fungi facilitate bacterial growth and shape bacterial communities. Moreover, fungi and bacteria collaborate for PAHs degradation. Fungal mycelia mobilize intracellularly entrapped PAHs making them bioavailable for bacterial degradation and bacteria use fungal hyphae as paths for dispersion. The dispersion of bacteria by fungal mycelia is highly relevant in sediments where PAHs accumulate

creating hydrophobic patches that are more resistant to degradation. However, no information exists on the effect of fungal transport of bacteria in PAHs contaminated marine sediments.

In this context, the work presented here shows the effort made to understand fungal communities and their interactions with bacterial communities from PAHs contaminated coastal sediments; making an emphasis on the impact of PAHs on fungal and bacterial diversity. To accomplish this, a bibliographic synthesis of the current knowledge is presented, including the role of fungi in marine environment, their potential to biodegrade PAHs and their interactions with bacterial communities for PAHs degradation (Chapter I). The results of the thesis research are presented in chapters in scientific publication format, already submitted or published, starting with the impact of PAHs contamination on fungal and bacterial communities diversity from coastal sediments and their inter-kingdom interactions. These interactions were evaluated constructing co-occurrences networks between fungi and bacteria from coastal sediment with no contaminated samples and comparing them with networks constructed with contaminated samples (Chapter II). Following, it was done the isolation of fungal cultivable diversity of coastal sediments, which were evaluated of PAHs removal capacity, and the presence of the genes related to PAHs degradation (Chapter III). Finally, two fungi selected for their lowest and highest removal capacities were used to analyze fungal selectivity over bacterial communities and their bacterial translocation potential through contaminated sediment gaps in order to find biomarkers of fungal and bacterial interactions in PAHs contaminated sediments (Chapter IV). The analysis of results and further perspectives of thesis research are presented in the last chapter (Chapter V). The comprehension and elucidation of fungal and bacterial interactions in PAHs contaminated sediments presented might give important knowledge for further implementation of bioremediation strategies to mitigate the effect of PAHs in contaminated environments.

Introduction générale

Les sédiments représentent environ 70 % de la superficie de la planète, ce qui en fait le plus grand écosystème sur Terre. Plusieurs facteurs varient en fonction de leur emplacement, allant de la côte jusqu'aux abysses, conférant aux sédiments diverses caractéristiques physico-chimiques, comme la salinité, la concentration en oxygène, la taille des particules, la teneur en matière organique et bien d'autres. Il n'est alors pas surprenant de trouver une vaste diversité microbienne habitant ces écosystèmes. Les micro-organismes qui vivent dans les sédiments et leurs interactions jouent un rôle clé dans plusieurs processus biogéochimiques, notamment dans les cycles du soufre, de l'azote et du carbone. Néanmoins, la dynamique des micro-organismes et leurs interactions sont constamment affectées par l'apport de contaminations aux hydrocarbures, provenant de sources naturelles ou anthropiques.

Parmi les hydrocarbures entrant dans le milieu marin, les hydrocarbures aromatiques polycycliques (HAP) sont des polluants préoccupants en raison de leur potentiel toxique, mutagène et cancérigène. De plus, les produits de transformation de la dégradation incomplète des HAP (généralement par des processus physiques ou chimiques) sont plus toxiques conduisant à des effets cellulaires critiques. Pour cette raison, la biodégradation microbienne qui implique la transformation des HAP en dioxyde de carbone et en eau, est devenue une alternative à la remédiation des sédiments pollués par les HAP.

Plusieurs microorganismes ont été identifiés et caractérisés pour leur capacité à tolérer et à dégrader les HAP. Parmi ces microorganismes, des champignons et des bactéries ont été étudiés et leurs voies de dégradation ont été décrites. Les champignons dégradent les HAP de manière extracellulaire à l'aide d'enzymes monooxygénases ligninolytiques ou de manière intracellulaire via le cytochrome P450. Alors que les bactéries ont des voies de dégradation des HAP qui changent en fonction de la présence (voie aérobie) ou absence (voie anaérobie) d'oxygène. Néanmoins, les champignons et les bactéries ont leurs limites. À ce jour, il n'y a aucune étude rapportant que les champignons sont capables de minéraliser complètement les HAP en dioxyde de carbone et en eau, alors que les bactéries ne peuvent pas dégrader efficacement les HAP de haut poids moléculaire. Ainsi, l'interaction entre champignons et bactéries mérite d'être examinée pour obtenir la biodégradation complète des HAP. Dans les sédiments pollués par des hydrocarbures, les champignons sont probablement des dégradeurs primaires de molécules de poids moléculaire élevé via des enzymes extracellulaires, travaillant en synergie avec les bactéries dégradant les hydrocarbures. L'interaction entre champignons et bactéries donne des avantages particuliers pour surmonter la présence de HAP. Il est décrit que les bactéries peuvent améliorer la reproduction des champignons et affecter leur métabolisme. Par ailleurs, les champignons facilitent la croissance bactérienne et façonnent les communautés bactériennes. De plus,

les champignons et les bactéries collaborent pour la dégradation des HAP. Les mycéliums fongiques mobilisent les HAP piégés à l'intérieur de la cellule, les rendant biodisponibles pour la dégradation bactérienne, alors que les bactéries utilisent les hyphes fongiques comme voies de dispersion. La dispersion des bactéries par les mycéliums fongiques est très pertinente dans les sédiments où les HAP s'accumulent créant des zones hydrophobes plus résistantes à la dégradation. Cependant, aucune information n'est disponible sur l'effet du transport fongique des bactéries dans les sédiments marins contaminés par les HAP.

Dans ce contexte, les travaux présentés ici montrent l'effort fourni pour comprendre les communautés fongiques et leurs interactions avec les communautés bactériennes issues des sédiments côtiers contaminés par les HAP ; mettant l'accent sur l'impact des HAP sur la diversité fongique et bactérienne. Pour ce faire, une synthèse bibliographique des connaissances actuelles est présentée, incluant le rôle des champignons en milieu marin, leur potentiel de biodégradation des HAP et leurs interactions avec les communautés bactériennes pour la dégradation des HAP (Chapitre I). Les travaux de thèse sont présentés dans des chapitres au format de publication scientifique, déjà soumis ou publiés, en commençant par l'impact de la contamination par les HAP sur la diversité des communautés fongiques et bactériennes des sédiments côtiers et leurs interactions. Ces interactions ont été évaluées en comparant des réseaux de co-occurrences entre champignons et bactéries de sédiments côtiers contaminés par des HAP à ceux obtenus de sédiments non contaminés (Chapitre II). Ensuite, il a été fait l'isolement de la diversité fongique cultivable des sédiments côtiers qui ont été évalués de la capacité d'élimination des HAP et de la présence des gènes liés à la dégradation des HAP (Chapitre III). Enfin, deux champignons sélectionnés pour leurs capacités d'élimination des HAP, l'un ayant la plus faible et l'autre la plus élevée, ont été utilisés pour étudier les interactions champignon-bactéries. Le potentiel de translocation bactérienne des champignons à travers des obstacles de sédiments contaminés a été examiné afin de i) montrer la sélectivité fongique sur les communautés bactériennes et ii) déterminer des biomarqueurs des interactions fongiques et bactériennes dans les sédiments contaminés par les HAP (chapitre IV). Les principales conclusions issues des résultats obtenus et les perspectives de recherche de thèse sont présentées dans le dernier chapitre (Chapitre V). La compréhension et l'élucidation des interactions entre champignons et bactéries dans les sédiments contaminés par les HAP apportent des connaissances importantes pour la mise en œuvre de stratégies de bio-remédiation visant à atténuer l'effet des HAP dans les environnements contaminés.

Chapter I: Bibliographic synthesis

I.1. Marine sediments

Marine ecosystems consist of two very different compartments; the water column and the sediment matrix (Glud, 2008). Marine sediments cover the ~70% of the Earth's surface and comprise the largest single ecosystem on Earth in area. The size of this ecosystem allows a variety of environments from tidally exposed areas, where temperature, salinity, and sediment water content fluctuate greatly, to the greatest depths of the oceans, where no light penetrates, temperatures approach freezing, environmental variables are largely invariant, and pressure exceeds 100 times that experienced at sea level (Snelgrove, 2001). Furthermore, sediments composition is very heterogeneous according to their source of origin (Figure I.1) which are divided in two: lithogenic and biogenic. The lithogenic

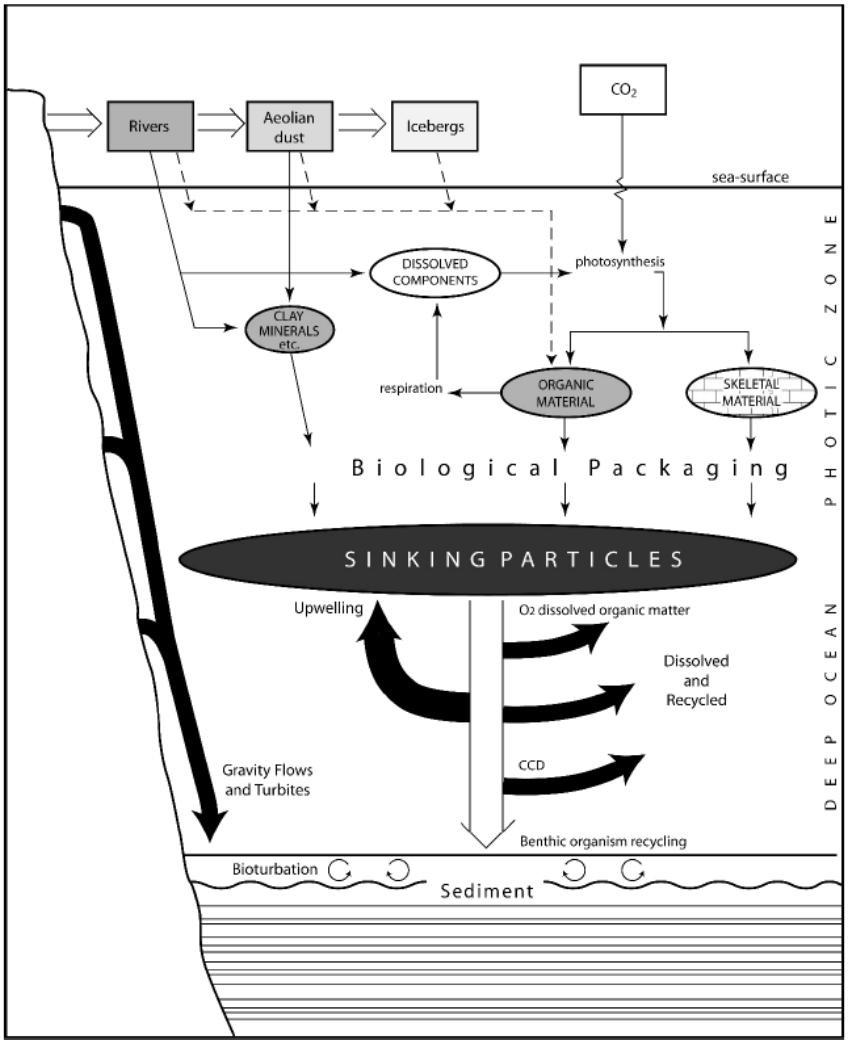

Figure I.1. Formation of marine sediments, sources and depositional pathways (Maslin and Swann, 2006).

component is composed of fragments of rocks from different sizes that can go from microscopic clays to large boulders. The biogenic component originates in surface waters and dwells down to deposit; it is composed at least 30% of skeletal debris from microorganisms rich in calcium carbonate, biogenic silica and organic matter (Maslin and Swann, 2006). With all these heterogeneity of physicochemical parameter, it is not surprising that marine sediments host a wide diversity of microorganisms.

It is estimated that subsea floor constitute almost five-sixths of the total biomass of the planet and one-third of its living biomass (Hoshino et al., 2020; Kallmeyer et al., 2012; Whitman et al., 1998). They play a key role in geochemical processes cycling sulphur, nitrogen, iron and organic or inorganic carbon (Parkes et al., 2014). Being sediments the reservoir of the largest pool of organic carbon on the planet (Parkes et al., 2014), it is highly relevant to investigate and analyse the microbial communities inhabiting this ecosystem and their interactions. From these interactions, fungi and their associated bacteria have a key role in the carbon cycling and minerals (Verrecchia, 2000), highlighting their importance for these ecosystems.

I.2. Diversity of fungi and role in marine sediments

I.2.1. Fungal diversity in marine ecosystems

Fungi occur in different marine environments that include the water bodies in the ocean known as marine-derived fungi (Babu et al., 2010; Birolli et al., 2018; Campeão et al., 2017; Vieira et al., 2018), to coastal sediments (Elshafie et al., 2007; Simister et al., 2015; Babu et al., 2010; Amer et al., 2019), mangrove (Ghizelini et al., 2018), deep sea sediments (Redóu et al., 2014; Xu et al., 2014; Edgcomb et al., 2011; Zhang et al., 2014; Campeão et al., 2017) and even in high artic sediments (Zhang et al., 2015). Fungi can be obligate marine fungi if they grow and reproduce exclusively in marine or estuarine environment (Kohlmeyer and Kohlmeyer 1979), to consider a fungus marine, its growth and reproduction in marine habitat has to be demonstrated (Shaerer et al., 2007). Nevertheless, there are fungi exclusive from these habitats; several families are shared with soil and other environments.

Some fungal population in sediment has been described and analysed (Table I.1), but part of their large diversity remains unknown. As first approach, cultivation was the main tool to characterize and identify fungi in marine ecosystems. These studies showed prevalence in strains of the phylum Ascomycota (Elshafie et al., 2007; Simister et al., 2015; Redóu et al., 2014; Ahumada-Rudolph et al., 2016; Mouton et al., 2012; Ravalet et al., 2000; Babu et al., 2010; Amer et al., 2019; Chikere and Azubuike, 2014; Birolli et al., 2017; Ghizelini et al., 2018). In addition, the phylum Basidiomycota was also found and isolated (Redóu et al., 2014; Ahumada-Rudolph et al., 2016; Mouton et al., 2012; Vieira et al., 2018) and even some strains of Zygomycota (Ravalet et al., 2000; Babu et al., 2010; Chikere and Azubuike, 2014). Nevertheless, with new technologies emerging for sequencing and identification of microorganisms, the exploration of deeper fungal diversity study has been possible. As in culture studies, Ascomycota result in the prevalent phylum (Xu et al., 2014; Zhang et al., 2014; Zhang et al., 2015). In less proportion, Zygomycota (Zhang et al., 2014; Zhang et al., 2015) and Basidiomycota phyla (Xu et al., 2014; Edgcomb et al., 2011; Zhang et al., 2014; Zhang et al., 2015) have been found. More recently, fungi from the phylum Chrytidiomycota have been found and described as one of the most abundant fungal phylum in marine environments (Gleason et al., 2011; Longcore and Simmons, 2020) (Table I.1). The fungi belonging to Chrytridiomycota are zoosporic fungi; they have singular characteristics including motile spores and association with plankton (Grossart et al., 2016). The finding and classification of new phyla of fungi suggest that more research has to take place to map and evaluate the diversity of this Kingdom and their role in sediment ecosystems.

Table I.1. Identified fungi in marine environments.

Phylum	Genera and specie	Analysis	Source and site	Reference
Ascomycota	*Aspergillus niger, Aspergillus terrus, Aspergillus nidulans, Cladosporium cladosporoides, Penicillium, Pacelomyces, Aspergillus ochraceus, Alternaria alternata, Penicillium chrysogenum*	Isolation and culture	Coastline from Gulf of Oman	Elshafie et al., 2007
Ascomycota Basidiomycota	*Acremonium, Aspergillus, Cordyceps, Eurotium, Exophilia, Cladophialophora, Cladosporium, Fusarium, Microascus, Oidiodendron, Paecilomyces, Penicillium, Phialophora, Purpureocillium, Sarocladium, Bjerkandera, Sistotrema, Trametes*	Culture and enrichment	Deep subseafloor from Cantebury Basin, New Zealand	Rédou et al., 2015
Ascomycota Basidiomycota	*Trichoderma harzianum, Trichoderma deliquescens, Penicillium crustosum, Talaromyces atroroseus, Rhodotorula mucilaginosa*	Isolation and culture	Marine sediments from Aysén Providence, Chile	Ahumada-Rudolph et al., 2019
Ascomycota Basidiomycota	*Acremonium, Aspergillus, Eurotium amstelodami, Paraconiothyrium cyclothyrioides, Penicillium, Trichoderma, Yarrowia lipolytica, Rhodotorula mucilaginosa*	Isolation and culture	Marine sediments from Sta. Helena Bay, South Africa	Mouton et al., 2012
Ascomycota Zygomycota	*Neurospora, Emerichella, Sacharamyces, Aspergillus, Penicillium, Verticillium, Alternaria, Cephalosporium, Cladosporium, Curvularia, Drechslera, Nigrospora, Fusarium, Ascochyta, Mucor, Rhizopus*	Isolation and culture	Coastal marine water and sediments from South-East Coast, India	Babu et al., 2010
Basidiomycota	*Ustilaginomycetes, Basidiomycetes*	DNA analysis from 18S rRNA gene	Deep sea sediments from Peru	Edgcomb et al., 2011
Ascomycota Basidiomycota Zygomycota	*Cryptococcus, Candida, Trichosporon, Rhodotorula, Aspergillus, Alternaria, Cladophialophora, Cladosporium, Fusarium, Geomyces, Trichoderma, Galactomyces, Sterigmatomyces, Basidiomycete, Dipodascus, Guehomyces, Horteae, Sporobolomyces, Eurotium, Hypocrea, Leptospheria, Mortierella, Phoma, Xeromyces, Rhizoscyphus*	Clones identified by ITS gene	Deep sea sediments from East Indian Ocean	Zhang et al., 2014
Ascomycota	*Penicillium*	Isolation and culture	Sediments ranging between 0.5 to 1m from Alexandria, Egypt	Amer et al., 2019
Ascomycota (62 OTUs) Basidiomycota (26 OTUs) Glomeromycota (1 OTU) Unkwnown (21 OTUs) Zygomycota (1 OTU) Chrytridiomycota (2 OTUs)		DNA extraction and 454 pyrosequencing ITS1F-ITS4	Marine sediments from High Artic Kongsfjorden, Svalbard	Zhang et al., 2015

I.2.2. Role and importance of fungi in marine sediments

The active ecological role of fungi has been overlooked considering that sediment microbial communities are connected by synergistic, antagonistic or neutral relationships. Fungal microbiome plays a role in the overall coordination and whole microbial communities, controlling the structure of sediment microbial functional networks (Booth et al., 2019).

In the marine habitat, fungi interact and associate with almost all marine organisms (Ainsworth et al., 2017; Yarden, 2014). In the water column, fungi from Chytridiomycota phylum parasite phytoplankton, diatoms and cyanobacteria contributing to the cycling of organic compounds known as "mycoloop" (Frenken et al., 2017; Haraldsson et al., 2018; Kagami et al., 2007) (Figure I.2). They modify the amount and composition of the dissolved organic carbon liberated by phytoplankton impacting the structures of bacterial communities (Senga et al., 2018) and sediment composition. This is highly relevant because photosynthetic phytoplankton convert dissolved organic carbon to organic matter producing oxygen creating marine webs for other fungi and heterotrophic bacteria (Worden et al., 2015).

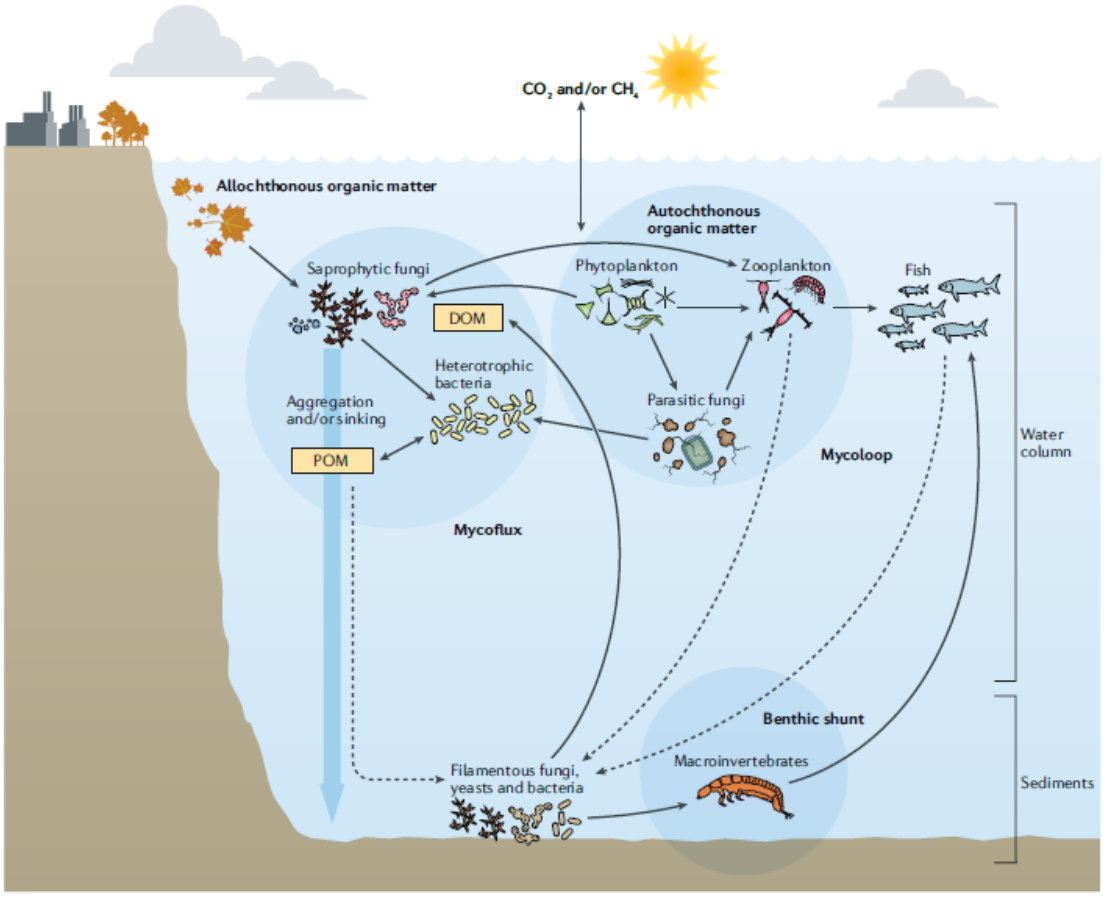

Figure I.2. Fungi and their ecological role in marine ecosystem (Grossart and Rojas-Jimenez, 2016).

Fungi have been also found to dominate ocean sediments, particularly in rich-organic matter sediments (Orsi et al., 2013a). In sediments, fungi have been found actively engaged in processing a range of different organic matter types including lipids, proteins, and carbohydrates via specific hydrolases (Orsi et al., 2013b). Deeper down in subsurface sediments, fungal gene expression was associated with growth, division, and sporulation, catalytic activities, and the synthesis of antimicrobial products (Pachiadaki et al., 2016). These data suggest nutrient recycling and cross-feeding interactions between fungi and other components of the microbial communities (Gutiérrez et al., 2011).

In the deep-sea sediments, fungi trap soil and humid material particles acting as a binding agent using polysaccharides (Aspiras et al., 1971; Tisdall, 1991) to form microaggregates (Kandeler et al., 1999; Molope and Page, 1986). This process trap and cycles the dissolved and particulate organic matter (Verdugo et al., 2004) helping microorganisms like bacteria or other fungi to remain protected in certain particle size (Suberkropp and Weyers, 1996). The presence of large formation of aggregates with fungal filamentous networks sequestrate and store carbon (Bailey et al., 2002). Fungi in the deep sea sediments have a close relationship with chemoautotrophic prokaryotes involved in iron oxidation (Bengtson et al., 2014). It is suggested that this association, chemoautotrophs fix the dissolved carbon from fluids and build biomass used by fungi for their metabolism. In turn, fungi metabolize carbohydrates producing CO_2 making it available for chemoautotrophs (Bengtson et al., 2014).

The most studied fungi are aerobic but anaerobic fungi are also known to habit in lakes, landfilled sites (McDonald et al., 2012), and deep-sea (Nagano and Nagahama, 2012). Anaerobic fungi have no mitochondria and are unable to produce energy with anaerobic/aerobic respiration (O'Fallon et al., 1991; Yarlett et al., 1986). As a substitute, they contain organelles called hydrogenosomes that are able to metabolise glucose to cellular energy. This metabolic pathway lead as residues H_2, CO_2, acetylate, formate, lactate and ethanol (Brul and Stumm, 1994; Yarlett et al., 1986; Khejornsart and Wanapat 2010) that are profited by other organisms. For example, the sub-seafloor is the habitat of anaerobic communities that use formate and hydrogen as electron sources (Orcutt et al., 2011).

Fungi are thriving, abundant, active, and functioning components of the oceans, from surface sunlit water to deep subsurface sediments and crust, influencing marine biogeochemical cycles in multiple ways. Nevertheless, these cycles and their equilibrium are susceptible to disturbance provoked by pollution, being oil splits and hydrocarbon contamination persistent in these environments. Studying alterations that hydrocarbon pollution cause to fungal diversity apport crucial information to evaluate their impact and develop environmental recovery strategies.

I.3. Degradation of PAHs in marine sediments

I.3.1. PAHs definition and occurrence in marine sediments

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of hazardous organic chemicals consisting of two or more fuzzed rings (Figure I.3). In nature, they are formed during forest fires, volcanic eruptions, or by plant and bacterial reactions (Wilson and Jones, 1993). PAHs formation result also from incomplete combustion of organic materials (through pyrolysis and pyrosynthesis) like coal, tar, oil and gas, automobile exhaust, tobacco and smoked food, either during industrial and other human activities or during geothermal reactions associated with the production of fossil-fuels and minerals, (Ghosal et al., 2016). An estimated 1.3 million tonnes of petroleum enters the marine environment each year (National Research Council (US) Committee on Oil in the Sea, 2003). Acute pollution incidents cause great public concern, notably ~600,000 tonnes of crude oil released after the Deepwater Horizon explosion in the Gulf of Mexico (Crone and Tolstoy, 2010) and approximately 5.74 million tonnes have been lost as a result of tanker incidents between 1970 and 2017 (Oil Tanker spill statistics, 2017). The impact of hydrocarbons, especially PAHs, on wildlife and fisheries may be long-lasting (McGenity et al., 2012).

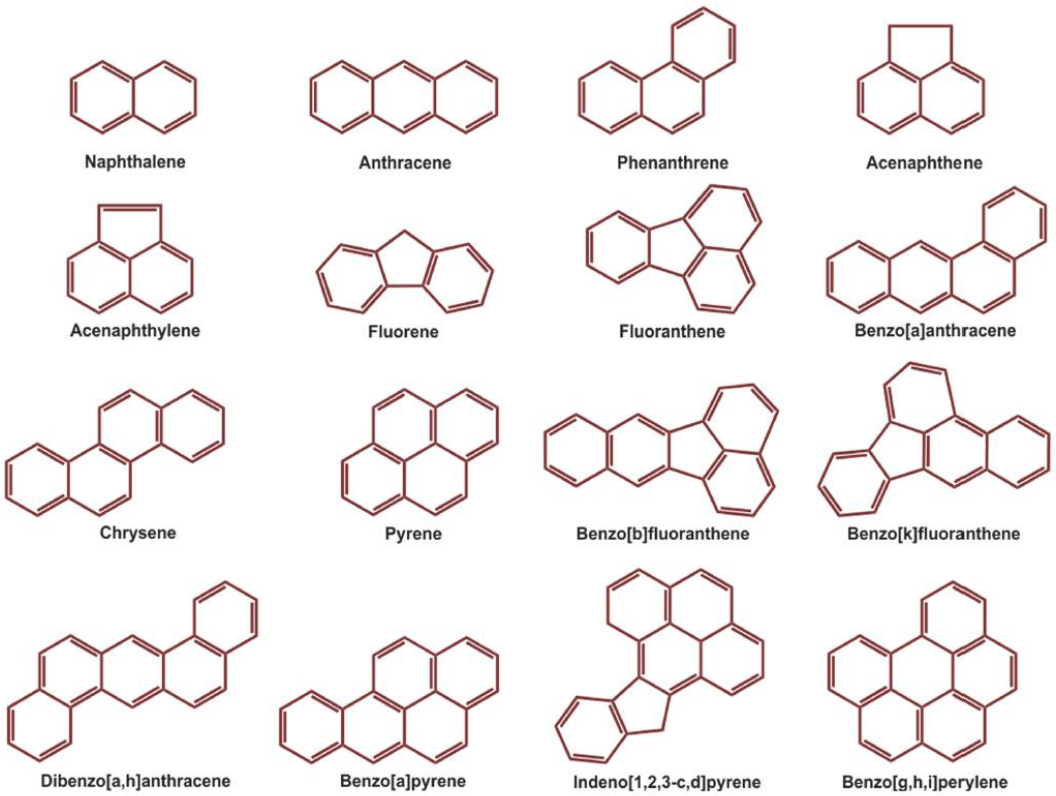

Figure I.3. Structure of some 16 PAHs listed by the High Priority Pollutants by the Environmental Protection Agency (EPA) (Ghosal et al., 2016).

Due to their hydrophobic nature, most PAHs in aquatic and terrestrial ecosystems bind to particles in soil and sediments, rendering them less available for biological uptake, and they also bio-accumulate in food chains (Sanglard et al., 1986). The more rings PAHs have in their structure, recalcitrance increases. Depending on their hydrophobicity, PAHs tend to associate with both suspended particulate materials (SPM) at the water column as well as button sediment, which is recognized as the destination where some contaminants will be deposited and remain for long period of time (Cardoso et al., 2016) (Figure I.4).

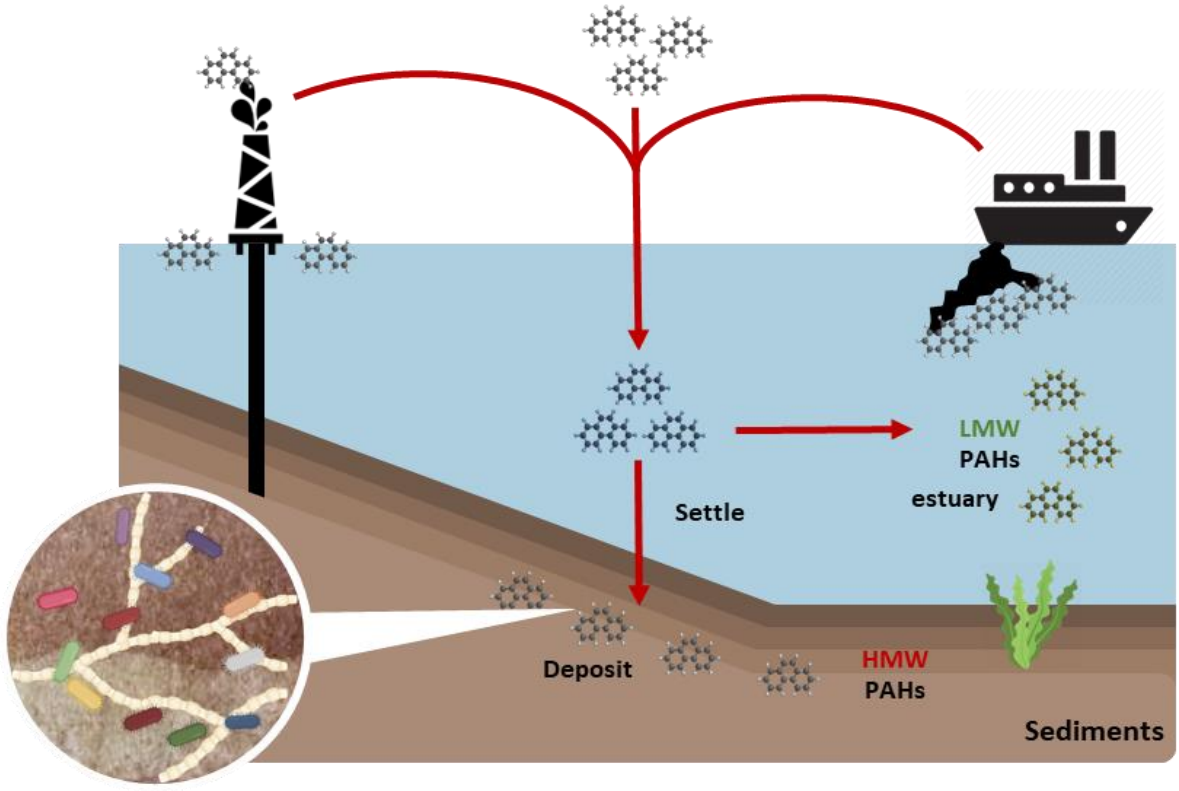

Figure I.4. PAHs input, fate and deposit in marine ecosystems (adapted from Cardoso et al., 2016)

PAHs occur in various ecosystems and are priority pollutants due to their potential toxicity, mutagenicity, and carcinogenicity. Low-molecular-weight PAHs are acutely toxic and most high-molecular-weight PAHs are mutagenic and carcinogenic (Santarelli et al., 2008). In addition, the transformation products of PAHs are more toxic than parent PAHs and lead to critical cellular effects (Schnitz et al., 1993). Consequently, cleaning of PAHs-polluted places has been thought to be one of the most essential alternatives for restoring environmental damage (Ghosal et al., 2016). Biodegradation of crude oil to carbon dioxide and water is the major process by which hydrocarbon-contaminated environments are remediated.

1.3.2. Fungi in PAHs degradation

Fungi appear to play an important role in cycling anthropogenic sources of carbon. Fungi were found to dominate benthic communities impacted by oil and included taxa known to degrade hydrocarbons (Bik et al., 2012) due to their high tolerance to these compounds (Nasrawi, 2012). There are several genera linked to hydrocarbon degradation in sediments (Walker and Colwell, 1975) such as: *Cladosporium*, *Cunninghamella*, *Penicillium*, *Fusarium*, *Alternaria*, and *Aspergillus* (Nasrawi, 2012; Passarini et al., 2011; Steliga et al., 2012) (Table I.2).

Table I.2. Fungi detected in contaminated marine environments

Phylum	Genera and specie	Analysis	Source and site	Contamination	Reference
Ascomycota	*Fusarium, Scopulariopsis, Aspergillus*	Isolation and culture	Beaches from Gulf of Mexico, Mexico	Crude oil	Simister et al., 2015
Ascomycota Zygomycota	*Aspergillus, Cladosporium, Coniothyrium, Curvularia, Fusarium, Getrichum, Gliocladium, Monocillum, Penicillium, Phialophora, Scopulariopsis, Trichoderma, Absidia, Gongronella, Mucor, Rhizomucor*	Isolation and culture	Sediments	Pyrene	Ravelet et al., 2000
Basidiomycota	*Tinctoporellus, Marasmiellus, Peniophora*	Isolation and culture	Marine derived from Brazilian sponges	Pyrene Benzo(α)pyrene	Vieira et al., 2018
Ascomycota Microsporidia	-	Illumina sequencing	Seawater from Foz do Amazonas Barreirinhas, Brazil	Benzene, Toluene, Ethylbezenes, Xylene, PAHs, Alkanes	Campeão et al., 2017
Ascomycota Zygomycota	*Aspergillus, Candida, Penicillium, Rhizopus, Saccharomyces, Cladosporium*, Fusarium*, Mucor** *just in sediments	Isolation and culture	Water and sediments from Rivers State, Nigeria	Crude oil	Chikere and Azubuike, 2014
Ascomycota	*Cladosporium*	Isolation and culture	Marine derived from Sao Paulo State Coast, Brazil	Anthracene Acenaphthene Phenanthrene Pyrene Anthraquinone Fluorene Fluoranthene	Birolli et al., 2018
Ascomycota	*Aspergillus, Penicillium*	Isolation and culture	Mangrove sediments from Sepetiba Bay and Guanabara Bay, Brazil	Crude oil	Ghizelini et al., 2019

Fungal PAHs degradation mainly involves monooxygenase enzymes that are intracellular or extracellular. Intracellular PAHs degradation has been characterized in non-ligninolytic fungi, in which cytochrome P450 monooxygenase oxidize PAHs to epoxides and dihydrodiols (Cerniglia and Sutherland, 2010) (Figure I.5). The result of this degradation pathway create secondary compounds that act as potent carcinogens, some times more potent than the same PAHs (Cerniglia and Sutherland, 2010). For example, degradation that include photochemical reactions can turn PAHs into nitro-PAHs which are carcinogenic (Finlayson-Pitts and Pitts, 1997). In comparison, the extracellular PAHs degradation (characterized in white rot fungi) involves the production of oxidative extracellular enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase that transform PAHs in simpler molecules that are used by other organisms as carbon source (Figure I.5). This is why oxidation on PAHs by ligninolytic enzymes represent a better strategy for cleaning contaminated PAHs environment. In addition, ligninolytic enzymes present other advantages: i) as they are secreted extracellularly they are able to diffuse toward immobile PAHs, and ii) they are able to transform a wide range of substrates thanks to their broad substrate specificity (Cerniglia and Sutherland, 2010). Fungi mineralize PAHs by a combination on ligninolytic enzymes, cytochrome P450 monooxygenase, and epoxide hydrolases (Bezalel et al., 1997). However, most fungi are not able to use PAHs as sole carbon source but they are able to co-metabolize them in more oxidized products making them more available for further degradation by other organisms like bacteria (Ghosal et al., 2016).

I.3.4. Bacteria in PAHs degradation

The microbial response to an oil spill at sea is dependent on numerous factors, including the oil composition and degree of weathering, as well as environmental conditions, particularly temperature and nutrient concentrations (Duran and Cravo-Laureau 2016). There are some typical patterns; the most notable is the large increase in abundance of *Alcanivorax* spp., which degrade straight-chain and branched alkanes (Coulon et al., 2007; Harayama et al., 2004; Head et al., 2006; Kostka et al., 2011; McGenity et al., 2012; McKew et al., 2007; Teramoto et al., 2009; Yakimov et al., 2007) and has a multitude adaptations to access oil and to survive in open marine environments (Sabirova et al., 2008; Schneiker et al., 2006) , followed by *Cycloclasticus* spp., which degrade PAHs (Cui et al., 2008; Harayama et al., 2004; Head et al., 2006; Teira et al., 2007; Yakimov et al., 2007) with key enzymes including alkane hydroxylases and three cytochrome P450-dependent alkane monooxygenases (Schneiker et al., 2006).

Another bacteria include strains affiliated to *Acinetobacter* spp., which are commonly isolated from oil-contaminated marine environments (Ron and Rosenberg, 2010). They also have a diverse array of alkane hydroxylase systems enabling them to metabolize both short- and long-chain alkanes (van Beilen and Funhoff, 2007; Rojo, 2009). *Alcanivorax* spp. are sometimes outcompeted by *Thalassolituus* spp. in temperate environments (McKew et al., 2007), which is classified as obligate hydrocarbon-degrading bacteria reaching 90% of the microbial community near an oil spill (Yakimov et al., 2007). Due to the incidence in the environment *Alcanivorax* spp. is considered as potential producer of antibacterial molecules (McGenity et al., 2012). Additionally, two NaCl-dependent hydrocarbon-degrading from marine environment have been isolated: *Planomicrobium alkanoclasticum* MAE2 (Engelhardt et al., 2001) and *Yeosuana aromativorans* GW1-1 (Kwon et al., 2006), which degrade alkanes and PAHs respectively. Other genera, including *Acinetobacter*, *Marinobacter*, *Pseudomonas* and *Rhodococcus* spp. (Duran, 2010; Gerdes et al., 2005; Vila et al., 2010; Yu et al., 2005), contribute to hydrocarbon degradation.

Sediments add to the complexity of identifying the main hydrocarbonoclastic microbes, but nearly all of the above genera are detected in the aerobic zone of marine sediments and presumed to be active in hydrocarbon degradation. Microbial communities from coastal sediments vary more from one location to another than those from open waters, and have much greater community evenness (Zinger et al., 2011). Moreover, in sediments, cells are much more concentrated, resulting in a greater likelihood of interactions, which becomes even more prevalent in biofilms where cells are more densely packed (McGenity et al., 2012).

Bacterial PAHs degradation involves two different pathways according to the oxygen availability and presence. In the aerobic catabolism, the initial step involves the oxidation of PAHs to dihydrodiol by a multicomponent enzyme system. This hydroxylated intermediate may then be processed to a central intermediate catechol or protocatechuate, which is cleaved through the ortho or meta cleavage type of pathways. In polynuclear aromatic compounds like PAHs, there is sequential cleavage of the rings via dihydroxylation and cleavage leading to more intermediates and ultimate conversion to intermediates of the tricarboxylic acid cycle (van Der Meer et al., 1992; Kanaly and Harayama, 2000) (Figure I.5). Comparatively, under anoxic (anaerobic) conditions where molecular oxygen is not present, requires alternative terminal electron acceptors like nitrate (NO_3^-), sulphate (SO_4^{2-}), manganese (IV), CO_2 and Ferric iron (Fe^{3+}) etc. (Meckenstock et al., 2004). It has been reported that Sulphate-reducing, denitrifying, and methanogenic bacterial communities contribute to the anaerobic degradation pathway of PAHs (Haritash and Kaushik, 2009) (Figure I.5). As in the aerobic pathway, the anaerobic metabolism requires prior PAH molecule activation. The activation via enzymatic pathways has been described involving a direct carboxylation or a methylation followed by the addition of fumarate. In both cases, another activation step occurs via coenzyme A before the β-oxidation metabolism (Duran and Cravo-Laureau, 2016; Meckenstock et al., 2016).

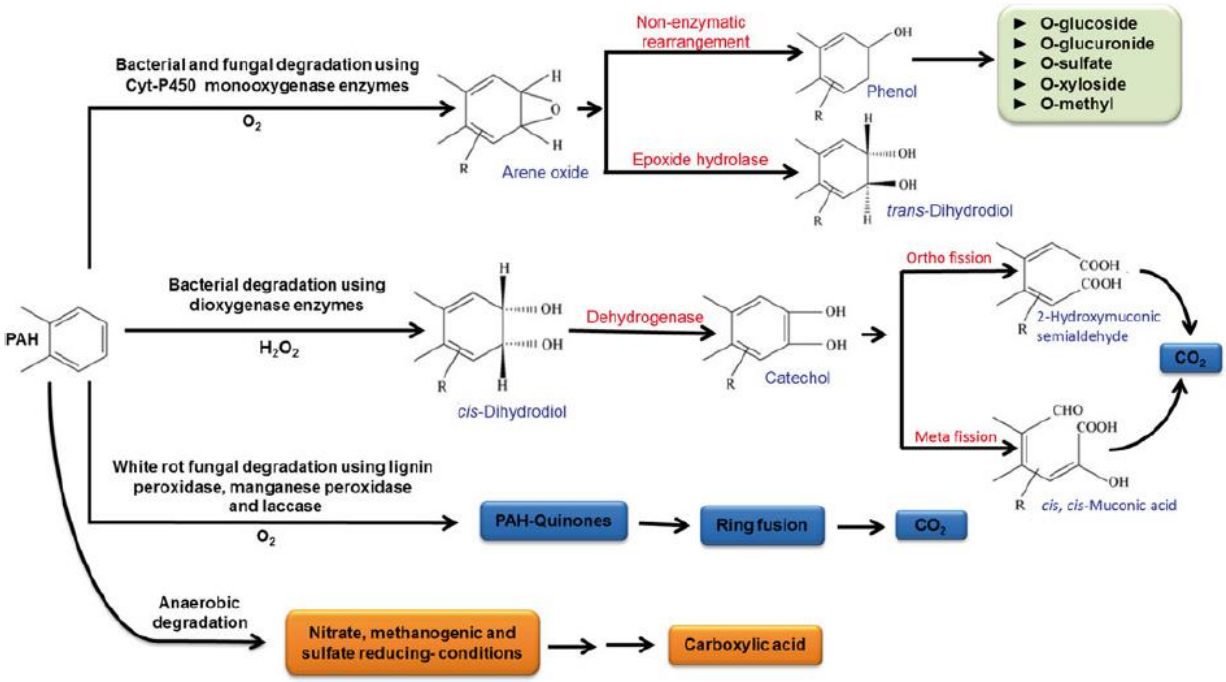

Figure I.5. PAHs degradation pathway for bacteria or fungi (Shahsavari et al., 2015).

I.4. Challenges of PAHs biodegradation in marine sediments

As described previously, sediments have unique physical-chemical characteristics, which drive to particular challenges related to PAHs degradation. Sediments are thus hazardous environments where microbial processes involved in PAHs depletion take place.

I.4.1. Oxygen concentration

Thermodynamically, O_2 represents the most favourable abundant electron acceptor available for PAH degradation. However, seawater contains little O_2, rapidly depleted by microbial activity, generally only extends a few millimetres or centimetres into the sediment. Even in near-surface coastal sediments, oxygen is usually rapidly removed due to aerobic respiration and this thus facilitates anaerobic respiration which uses a sequential series of electron acceptors providing decreasing energy yield (NO^{3-}, Mn^{4+}, Fe^{3+}, SO_4^{2-}, and CO_2), and the formation of characteristic zones of NH^{4+}, Mn^{2+}, Fe^{2+}, S^{2-} (often as metal sulphides) and CH_4 with increasing depth (Parkes et al., 2007).

Biodegradation of hydrocarbons in anoxic marine sediments is slower than in oxic zones (Shin, 2000) and aerobic respiration is considered as primary mechanism of hydrocarbon degradation, even in marine sediments (McGenity et al., 2012). The complete mechanism of PAHs depletion in marine ecosystems is not fully understood, but it is known that aerobic and anaerobic microorganisms coexist and create chemostatic interactions (Gerrits and Gottschal, 1993; Wimpenny and Abdollahi, 1991). In these interactions, the result of respiration of one organism act as electron acceptor of other microorganism in phototroph-heterotroph interactions (Sauze et al., 2017), which could be a pathway in PAHs biodegradation. Further studies are required to obtain more information on the metabolism of PAHs in anoxic environments including all the interacting organisms.

I.4.2. Salinity

The effect of salinity on microbial hydrocarbonoclastic activity depends on the variation amplitude of salt concentrations (Martins and Peixoto, 2012). In some cases, very low concentration of salt reduce hydrocarbon degradation meanwhile optimal biodegradation results in moderate salinity ranges (Díaz et al., 2002). In contrast, high salt concentrations constitute a stressful agent for most of the known living organisms (Kargi and Dincer; Woolard and Irvine, 1994).

Hypersaline sediments tend to accumulate high amount of organic matter resulting in higher amounts of hydrocarbons entrapped and attached to it (Javor, 1989). Thus, the increment in salinity reduces

PAHs bioavalability that in turn decreases its biodegradation. Nevertheless, several hydrocarbonoclastic halophilic and halotolerant microorganisms have been isolated from chronically hydrocarbon impacted sites close to petroleum industry facilities, most of them affiliated to the δ-proteobacteria class (Bruns and Berthe-Corti, 1999; Gauthier et al., 1992; Le Borgne et al., 2008; Mnif et al., 2009; Nicholson and Fathepure, 2004; Sei and Fathepure, 2009; Yakimov et al., 1998). Among the halophilic fungi, *Fusarium lateritium* and *Drechslera* sp. were shown to metabolize crude oil, with degradation efficiency improved more than thrice if petroleum was an additional source of carbon and energy (Obuekwe et al., 2005).

Chemically speaking, hydrocarbons are less bioavailable in saline environments than in non-saline. This is the consequence of the "salting out effect" where soluble salts reduce hydrophobic organic compounds solubility in water, the higher the salts concentration in aqueous phase the higher the tendency of organic compounds to be adsorbed in the solid matrix of sediments (Means, 1995; Turner and Rawling, 2001; Mackay et al., 2006). It is suggested that PAHs sorption to the sediment particles increases in the presence of salts, not only because the salting out effect on such hydrocarbons but also because of the salting out effect on the organic phase of the sediment, turning entrapped water in sediments, a better solvent for polyaromatics (Means, 1995; Turner and Rawling, 2001).

Moreover, high concentrations of salt not only decrease the access to hydrocarbons for microorganisms but alter the concentration of oxygen. In high salinity environments, the oxygen solubility decreases directly proportional with the rise of salts (McGenity, 2010; Mnif et al., 2009). This is why, to improve the environmental recovery strategies in marine sediments, an effort should be done to understand the mechanisms of PAHs degradation during anoxic metabolism. A strategy to increase the solubility of hydrophobic compounds used by microorganisms include the production of emulsifying agents which have been reported to be produced by several hydrocarbon-degrading halophiles (Al-Mallah et al., 1990; Margesin and Schinner, 2001; Martínez-Checa, F. Toledo, 2002; Yakimov et al., 1998). Emulsifiers affect the bioabailability of hydrocarbons, which is another of the challenges of marine sediments.

I.4.3. Bioavailability and coefficient of partition

The extent of sorption of non-polar molecules (as PAHs) to the solid matrix has a major influence on its transport and fate in environment. In soil/sediment-water systems, where the organic matter content is significant, the sorption of non-polar molecules occurs largely by partition. This means that one part goes to the solid organic matter and the rest in the water column (Chiou et al., 1998; Kile and Chiou, 1995; Stuer-Lauridsen and Pedersen, 1997), this distribution is called soil/sediment-water

distribution coefficient (Kd). The Kd of PAHs is strongly dependent on the organic matter content in soil/sediments. As more organic matter is present in the solid phase, more PAHs are attached to it (Kile and Chiou, 1995), creating hydrophobic patches with less bioavailability. The accumulation of PAHs increase as more non-polar molecules and organic matter enter in the environment, thus explain the difficulties in the creation of bioremediation strategies.

I.5. Fungi and bacteria interactions in PAHs degradation

It has been shown that fungi and bacteria collaboration improve degradation of PAHs (Li et al., 2008). Mycelia have been found to mobilize entrapped PAHs via vesicle-bound cytoplasmic transport ("hyphal pipelines"; (Furuno et al., 2012) making PAHs bioavailable and thus more amenable for bacterial degradation (Fester et al., 2014; Schamfuß et al., 2013) (Figure I.6). Fungal hyphae grow across air-water interfaces, bridge air-filled soil pores, as well as into pore soils with a diameter as little as 2 μm^2 and even penetrate rock matrices (Bornyasz et al., 2005). In addition, fungal mycelia has been shown to be used as paths ("fungal highway" or "fungal translocation networks") for the active dispersal of bacteria (Simon et al., 2015) (Figure I.6). It has been demonstrated that mycelia-based bacterial dispersal stimulates contaminant biodegradation in situations where chemicals and/or bacteria are heterogeneously distributed (Worrich et al., 2016).

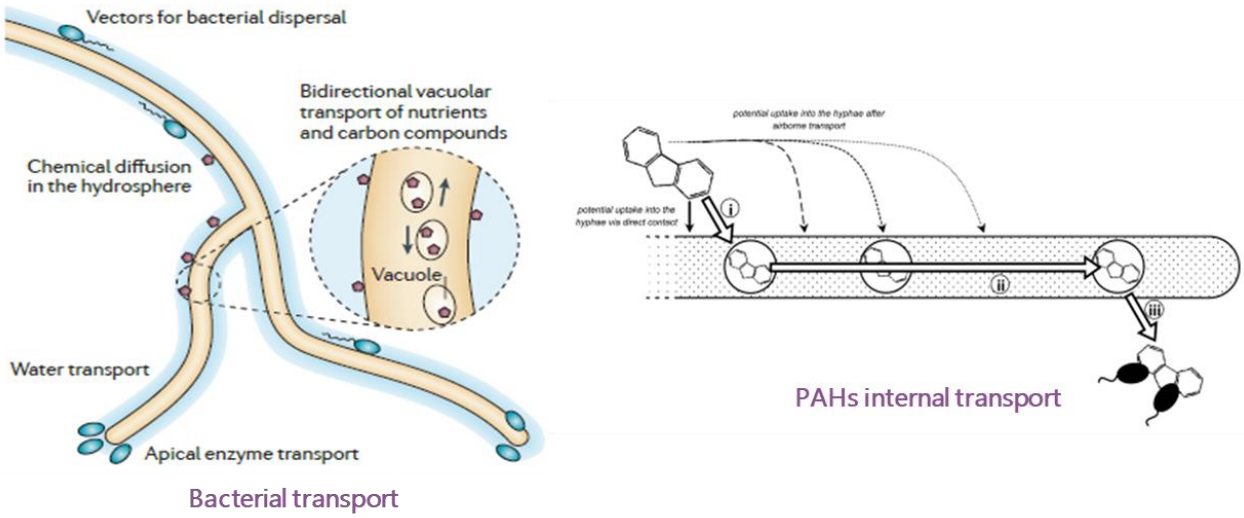

Figure I.6. Fungal mycelia as vectors of motile bacteria dispersion and intracellular transport of PAHs via vacuoles (Furuno et al., 2012; Harms et al., 2011)**.**

Fungi are known to facilitate bacterial growth, alter bacterial community structures or promote their activity in the hyphosphere by providing sugars and other sources of energy (Ingham et al., 2011; Koele et al., 2009; Zhang et al., 2018a). Mycelia also have a great influence on the structure of the surrounding via physical, biological and biochemical processes and often share their microhabitats with bacteria. Bacteria that interact with fungi extracellularly affect fungal development and conidia production. Effects of bacteria on fungal growth, measured by biomass, hyphal branching patterns, elongation, morphology, and density, have been documented (Frey-Klett et al., 2011). Bacteria that

colonize the fungal surroundings (hyphosphere) might be essential for the complete depletion of PAHs (Harms and Wick, 2006) (Figure I.7). In oil-polluted sediments, fungi are likely primary degraders of high molecular weight hydrocarbons via secreted extracellular enzymes and work synergistically with oil-degrading bacteria (McGenity et al., 2012).

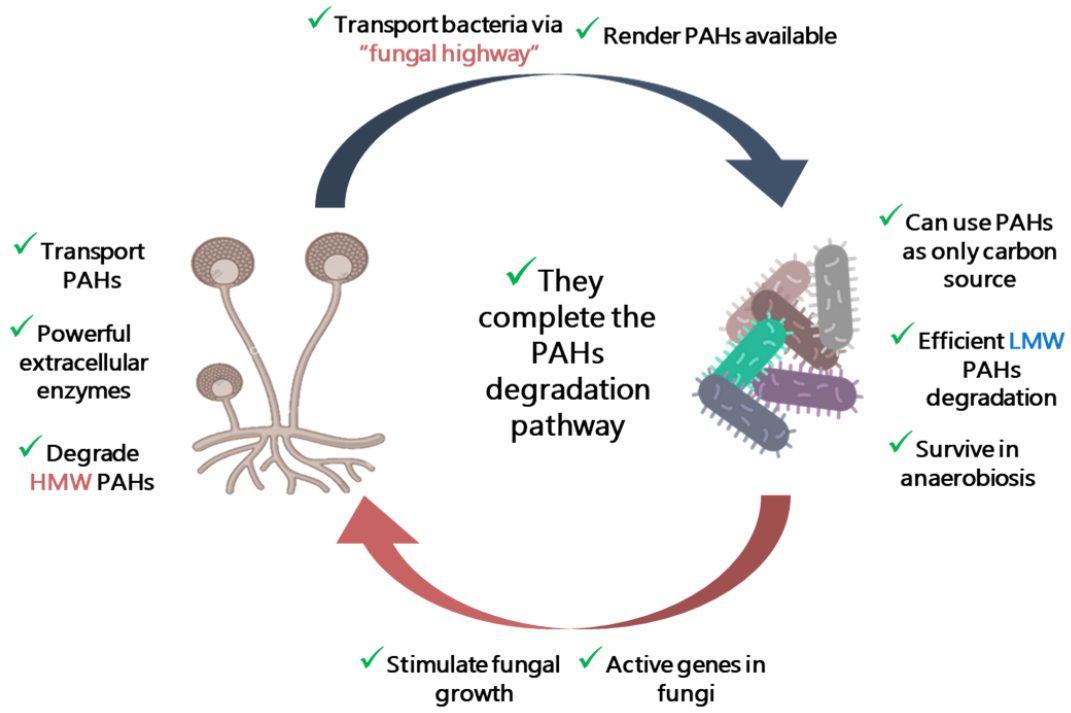

Figure I.7. Fungi and bacteria advantages and interactions in PAHs degradation.

As an example of the fungal bacterial relations described above, it has been reported that the enzyme laccase sometimes is produced by fungi during interspecific interactions with bacteria (White and Boddy, 1992). Also laccase activity has been reported to increase when cultures of the white-rot fungi *T. versicolor* and *P. ostreatus* are co-cultured with the bacteria *E. magnusii*, *B. subtillis* and *E. coli* (Baldrian, 2004). Since laccase participates in the degradation of some organic pollutants, the result of the interaction between fungi and bacteria demonstrates to be beneficial for PAHs biodegradation, increasing the degradation rate of benzo(a)pyrene in co-cultures compared with single cultures of 1.2 and 3.5-fold order of magnitude (Baldrian, 2004). As a result, the comprehension and seek of fungal and bacterial interactions in PAHs degradation has been carried on, especially on soil microorganisms but is poorly understood in marine environments.

Many fungi detected in marine environments have been already characterized from soil or plant habitats, even when those marine samples were collected from locations far from obvious terrestrial inputs. As previously described, to date the vast majority of fungi identified from marine environments belong to the Ascomycota phylum, also found in soil. Beside this coincidence, fungi specific from

sediments have been found indicating that this ecosystem is the reservoir for unexplored fungi and bacteria interactions. Moreover, the capacity of fungal hyphae to transport bacteria in marine ecosystems has not yet been explored. In this habitats, bacterial transport along hyphae find different challenges as water saturation or salt concentration. Particularly, in marine sediments contaminated with oil PAHs accumulate due to their hydrophobicity. This reduces their bioavailability but also create hydrophobic patches that represent physical barriers for bacterial translocation.

We hypothesized that fungal and bacterial interactions are occurring in PAHs contaminated coastal sediments that allows translocation of bacteria through hydrophobic patches. These fungi and bacteria interactions probably involve the selection of bacteria by fungi. In order to gain more information about fungal and bacterial interactions from PAHs contaminated sediments, the aims of this work are i) analyze the impact of PAHs contamination in fungal and bacterial communities interactions in sediments, ii) scrutinize the cultivable diversity of fungi from sediments, their tolerance to PAHs and determine the presence of genes encoding for the extracellular ligninolytic enzymes, and iii) verify if there are fungal selectivity of bacterial communities from sediments under PAHs contamination looking for biomarkers of fungal bacterial interaction. The comprehension and elucidation of fungal-bacterial interactions in PAHs contaminated sediments might give important knowledge useful for further implementation of bioremediation strategies to mitigate the effect of PAHs in contaminated environments.

Chapter II. Fungal-bacterial co-occurrence in PAHs contaminated coastal marine sediment: network analysis

II.1. Chapter introduction

Sediments have heterogenic physicochemical characteristics allowing them to be the reservoir of different microbial communities. From these, fungal and bacterial communities are important in the geochemical cycles that regulate sedimentary ecosystems. As expected, these communities share not just space but also relate metabolically creating inter-kingdom interactions. These fungal and bacterial interactions include mutualism antagonism, or synergism. The nature of their dynamic change according to environmental factors. Accordingly, the input of PAHs pollution in sediments, alter the fungal and bacterial interactions shaping microbial community structures. In this chapter, the interaction between fungal and bacterial communities inhabiting PAHs contaminated coastal sediments was evaluated. The interactions were evaluated constructing co-occurrence networks, based on rRNA gene meta-barcoding data, assembled between fungal and bacterial pairs of communities. It is suggested that organisms that co-occur may share similar ecological characteristics (Williams et al., 2014) making this analysis a tool to elucidate microbial interactions. Furthermore, the effect of PAHs on the diversity of fungal bacterial interactions was also evaluated comparing co-occurrence networks with PAHs contaminated to those from no contaminated samples. This chapter is presented in the format of scientific publication and will be submitted in the journal Environmental Science and Pollution Research

Chapter II. Fungal-bacterial co-occurrence in PAHs contaminated coastal marine sediment: network analysis

II.2. Fungal-bacterial network in PAHs contaminated coastal marine sediment

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II.2.1. Abstract

Fungal microbiome interacts with the other biotic components in coastal sediment playing a key role in the overall coordination of the whole microbial community. These interactions are affected by human activities, such as the constant affluence of polycyclic aromatic hydrocarbons (PAHs). Although fungi and bacteria interactions have been found to play a key role in PAHs bioremediation in soil, the effect of PAHs on fungal diversity and their specific interactions with bacteria in coastal sediments are yet to be investigated. The understanding of fungal bacterial interactions under PAHs contamination is critical for further bioremediation regarding the important fungal diversity observed in coastal sediment. Here, we investigated the fungal bacterial co-occurrence, and the effect of PAHs in their interactions, in microbial communities from coastal sediments contaminated or not with PAHs. The study confirms the central role that fungi have in coordinating interaction networks with bacteria. The effect of PAHs was obvious on fungi-bacteria network as the abundance and diversity of fungi and bacteria decreased, but the links between the remaining microorganisms were stronger. The network from PAHs contaminated sediments showed that fungi from Chrytridiomycota and Ascomycota phyla have a strong co-occurrence with facultative anaerobic and anaerobic bacteria affiliated to the families Prolixibacteraceae, Fusobacteriaceae and Desulfobulbaceae, not previously described. These results suggest that fungi promote bacterial anaerobic metabolisms, which are important to cope with the presence of PAHs in sediments. Our study reveals the importance of fungal bacterial interactions in coastal sediments paving the way for future studies to fully understand fungal role in coastal sediment.

Keywords: fungal-bacteria interaction, Illumina sequencing, fungal and bacteria diversity, hydrocarbon contamination, co-occurrence network, microbial community

II.2.2. Introduction

Coastal marine sediments are a niche of several interacting microorganisms including algae, archaea, bacteria and fungi. Several studies have shown the crucial role of bacteria and fungi in biogeochemical processes including carbon, nitrogen and sulfur cycling (Amend et al., 2019; Worden et al., 2015).

In sediment, fungi have shown an active role interacting between them and microbial communities (Amend et al., 2019). Fungal microbiome plays a role in the overall coordination of the whole microbial communities, controlling the structure of sediment microbial functional networks (Booth et al., 2019b). Transcriptomic analyses have demonstrated that fungi and bacteria react to the presence of each other and respond differently depending on the interacting partners (Deveau et al., 2015, 2018).

The presence of polycyclic aromatic hydrocarbons (PAHs) also plays an important role in bacterial community composition and organization (Muckian et al., 2007; Duran and Cravo-Laureau, 2016). Because bacteria are important interacting partners with fungi, the changes that occur in bacterial communities have an impact in the complete microbial structure (Schulz-Bohm et al., 2017). Fungi play an important role in cycling anthropogenic carbon sources. Several fungal genera have been linked to PAHs degradation in sediments (Walker and Colwell, 1975) including *Cladosporium*, *Cunninghamella*, *Penicillium*, *Fusarium*, *Alternaria*, and *Aspergillus* (Nasrawi, 2012; Passarini et al., 2011; Steliga et al., 2012) from the phylum Ascomycota (Álvarez-Barragán et al., 2021; Xu et al., 2014; Zhang et al., 2015), some other genera from other phyla as Basidiomycota and Chrytridiomycota.

In PAHs contaminated sediments, fungi are likely primary degraders of high molecular weight hydrocarbons via secreted extracellular enzymes. Fungi work synergistically with PAHs-degrading bacteria (McGenity et al., 2012), fungi and bacteria interactions being beneficial for both partners in PAHs contaminated environments. Mycelia have been found to mobilize entrapped PAHs via vesicle-bound cytoplasmic transport (Furuno et al., 2012) making them available for bacterial metabolism. In addition, fungal mycelia are used by bacteria as paths for their active dispersal (Simon et al., 2015), providing sugar and other sources of energy promoting activity in exchange (Boer et al., 2005). In turn, bacteria also affect fungal development (hypha branching patterns, elongation, morphology and density) as well as their reproduction by stimulating spore generation (Frey-Klett et al., 2011).

As fungal and bacterial interactions are crucial for the functioning of marine sediment environment, the aim of this study is to explore the fungi-bacteria interactions in coastal marine sediments by developing a network approach comparing microbial communities from various geographical locations. We hypothesize that the presence of PAHs contamination will affect the fungi-bacteria interactions resulting in the alteration of network properties. In order to validate the hypothesis, the

network constructed form microbial data of PAHs-contaminated sediment was compared with that obtained from un-contaminated sediments.

II.2.3. Material and methods

Coastal sediments from different marine sites were incubated in microcosms mimicking tidal cycles (Stauffert et al., 2013a) or oxygen fluctuations (Terrisse et al., 2017; Vitte et al., 2013), with or without hydrocarbon addition. Sediments were collected and DNA was extracted for microbial characterization. DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio) according to manufacturer's instructions used for later PCR amplifications.

Bacterial 16S rRNA genes were amplified targeting the V4-V5 hypervariable region with the primers 515F (5'-GTGYCAGCMGCCGCGGTA-3') and 928R (5'-CCCCGYCAATTCMTTTRAGT-3') as previously described (Wang and Qian, 2009). For the identification of fungal communities, the 18S rRNA genes were amplified using the primers FF390 (5'-CGATAACGAACGAGACCT-3') and FR1 (5'-AICCATTCAATGGT-AIT-3') that target the V7-V8 region as described in (Chemidlin Prévost-Bouré et al., 2011). (2021). All PCR mixtures were performed in triplicate in a volume of 25 µL using AmpliTaq Gold 360 Master Mix (Applied Biosystems). The reaction conditions for bacteria amplification include an initial denaturation step at 95°C for 10 minutes, followed by 35 cycles with denaturation step at 95°C for 30 s, hybridization step at 60°C for 30 s, and elongation step at 72°C for 40 s. For fungi, the conditions were: 95°C for 10 minutes, 35 cycles with denaturation step at 95°C for 30 s, hybridization step at 50°C for 45 s, and elongation step at 72°C for 30 s. Sequencing was carried out at the Genotoul platform (Toulouse, France) with Illumina-MiSeq. The 2 x 250 paired-end sequencing gave a full length 411 bp fragment with a 87 bp overlap for the V4-V5 region and a 450 bp fragment with a 150 bp overlap for the V7-V8 region.

To process and delimitate the amplicon sequence variants (ASVs) the open source software Qiime2 (Bolyen et al., 2019) was used for all sequences. The two datasets were first demultiplexed and dada2 was used to control reads quality and infer amplicon sequence variants (ASVs) (Callahan et al., 2017). Default settings were used except that reverse reads were truncated to 230 base pairs before merging. The taxonomy was assigned to the ASVs with a fixed sequence similarity threshold of 97% using the SILVA database release 132 for 16S and 18S sequences (Quast et al, 2013). Then, the datasets were filtered to remove mitochondria, chloroplasts and unassigned (at the domain level) sequences. Phylogenetic trees were constructed using FastTree (Price et al., 2010) in the Qiime2 phylogeny plugin. The dataset encompassed 66 samples. Both dataset were deposited in the NCBI Sequence Read Archive

(SRA) database, the bacterial dataset was assigned with the BioProject ID: PRJNA762061 (SUB10353467). The fungal dataset were assigned with the BioProject ID: PRJNA762024 (SUB10342418).

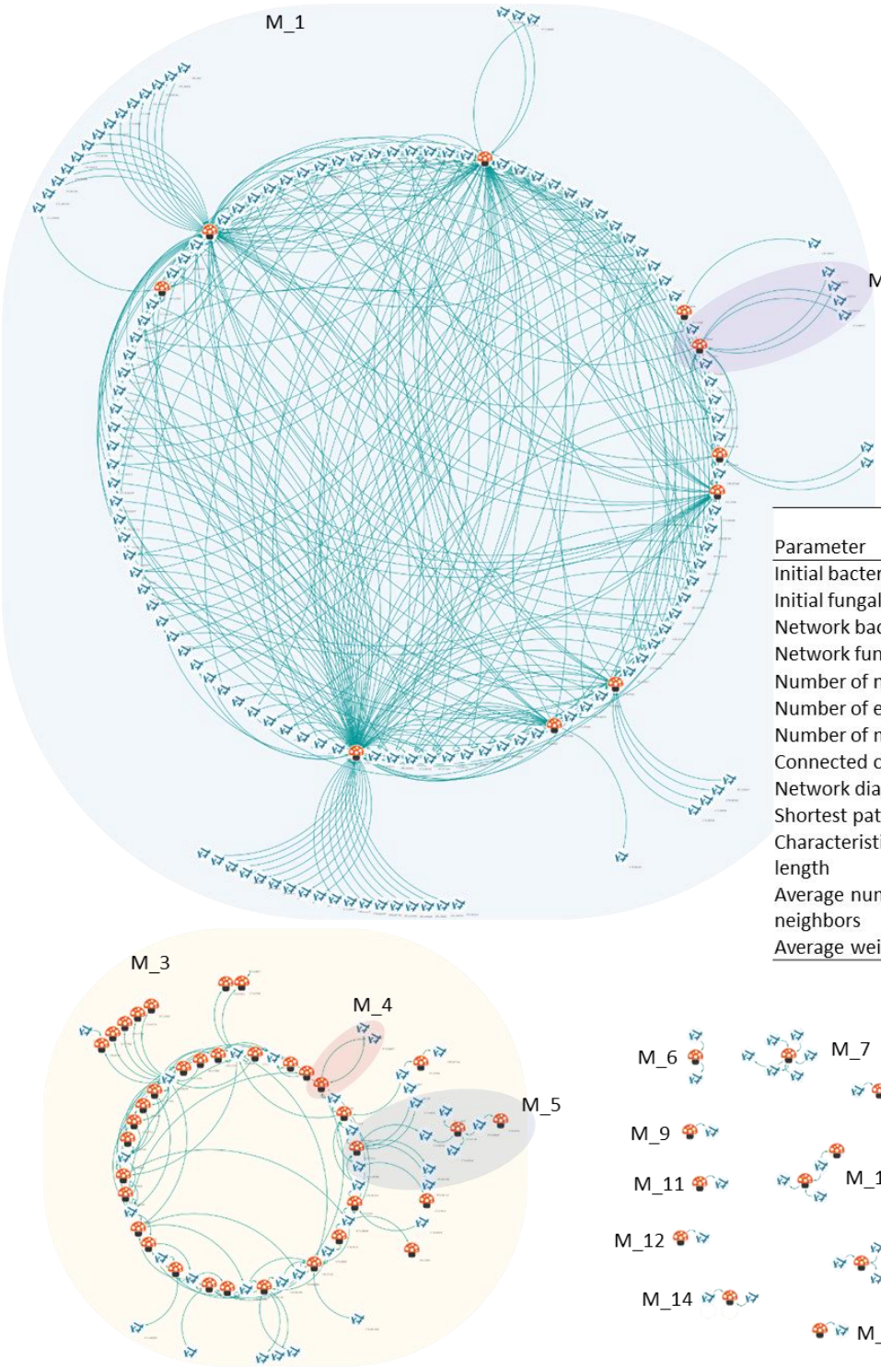
Co-occurrence network analysis between bacterial and fungal ASVs was inferred from an undirected co-occurrence network. Each network was computed individually using the CoNet (v1.1.1.beta) plugin within Cytoscape (v3.5.1) software (Oesper et al., 2011). The detailed use of Cytoscape software can be found at the manufacturer's website (http://manual.cytoscape.org/en/ stable/). In each network, co-occurrence positive interactions were identified using Spearman correlation coefficient > 0.7 and a statistical significance (*p*-value) < 0.05 for general PAH contaminated network and a Spearman correlation coefficient > 0.75 and a statistical significance (*p*-value) < 0.005. Modularity was computed using ModuLand 2.0 package included in Cytoscape software. Network characterization for comparisons between contaminated and uncontaminated microbial communities in sediments was performed using a set of overall network topological indices (i.e., average node connectivity, average path length, average clustering coefficient and modularity).

II.2.4. Results and discussion

II.2.4.1. Are fungi and bacteria co-occurring in PAHs contaminated coastal sediments?

In order to get a first insight on how fungi and bacteria are co-occurring in PAHs contaminated coastal sediments, a co-occurrence network was constructed with 9382 microbial ASVs (8551 bacteria, 831 fungi at 97% cutoff) from 51 samples of PAHs contaminated coastal sediments. Only strong correlations were retained by performing the network using Spearman correlation coefficient > 0.7 and a *p*-value < 0.05. The resulting network consisted in 256 nodes (ASVs) and 487 edges composing 15 modules (Figure II.1). The network was dominated mainly by bacterial ASVs (198) corresponding to the 77% of the nodes, indicating that bacteria are the main component structuring microbial communities, as previously reported in PAHs contaminated marine sediments (Jeanbille et al., 2016). Co-occurrence between fungi and bacteria has been extensively studied in soils (Banerjee et al., 2016; Bell et al., 2014; de Menezes et al., 2015) but in sediments, bacterial associations have been considered mainly with eukaryotes (Jeanbille et al., 2016) overlooking interaction with fungi. Our analysis showed that strong co-occurrences occurs between fungi and bacteria in PAHs contaminated coastal sediments suggesting that fungal and bacterial interactions play a crucial ecological role in contaminated coastal environments.

Modularity analysis revealed a complex structure of 15 modules that represent highly connected ASVs. Two main modules were identified for including the higher number of nodes and edges (M_1 and M_3, Figure II.1). The module with the higher amount of ASVs was dominated by bacterial ASVs (M_1, Figure 1) while the second module was mainly confined by fungal ASVs (M_3, Figure II.1). Such observations suggested that modules dominated by fungi have probably different ecological roles than modules dominated by bacteria. The other modules were smaller containing less than 10 nodes. These co-occurrences might reflect ASVs performing similarly or complementary functions, but they may also be caused by shared preferred environmental conditions (Steele et al., 2011). In agreement with previous works (Chaffron et al., 2010; Freilich et al., 2010; Faust and Raes, 2012), each module represents probably a specific ecological and/or functional niche.



Parameter	Network Spearman 0.7
Initial bacterial ASVs	8551
Initial fungal ASVs	831
Network bacterial ASVs	198
Network fungal ASVs	58
Number of nodes	256
Number of edges	487
Number of modules	15
Connected components	12
Network diameter	13
Shortest paths	14876 (22%)
Characteristic path length	3.406
Average number of neighbors	3.805
Average weight	0.75

Figure II.1: Fungal-bacteria co-occurrence network in microbial community from coastal marine sediment. The co-occurrence network is based on Spearman positive correlations. Each node corresponds to fungal (red) or bacterial (blue) ASVs with a schematic icon corresponding to kingdom taxonomical classification. Edge lines represent significant co-occurrence relationships between nodes with a Spearman correlation of 0.7 and a *p*-value > 0.05. Modularity is represented with colours and characters (M_1 to M_15). The network parameters are indicated in the Table.

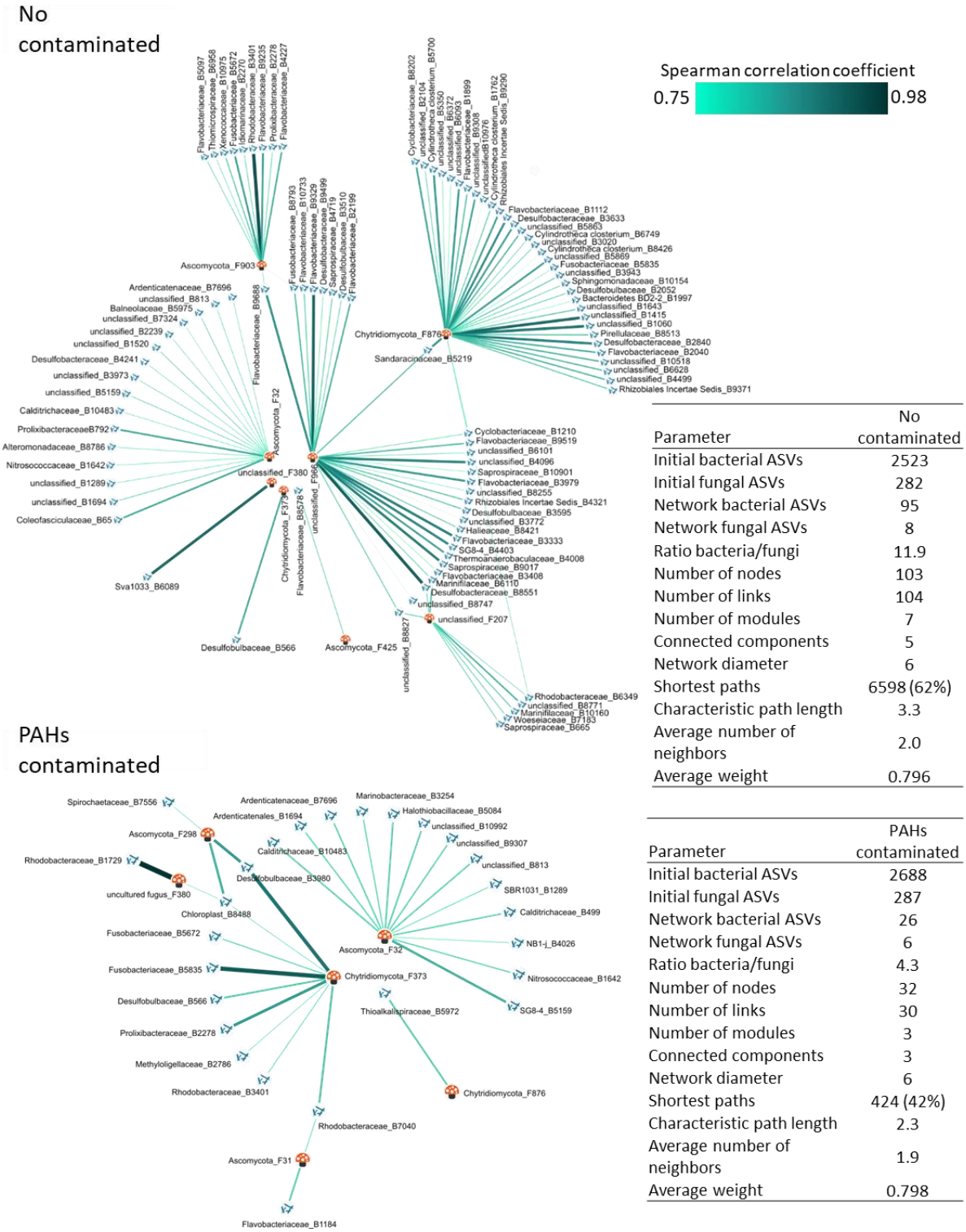
II.2.4.2. How PAHs contamination affects the microbial community?

In order to evaluate the effect of PAHs contamination in sediment fungal bacterial interactions, two co-occurrence networks were constructed using a set of 35 samples separated by no contaminated (17 samples) and PAHs contaminated (18 samples). The networks were constructed by data sets containing 2523 bacterial ASVs and 282 fungal ASVs for the no contaminated, and 2688 bacterial ASVs and 287 fungal ASVs for the PAHs contaminated. The networks were constructed with significant Spearman correlation coefficient > 0.75 and a *p*-value < 0.005 to select the strongest relations (Figure 2). The resulting networks had a different number of bacterial nodes, being more abundant in no-contaminated sediments, even though both networks had a similar number of fungal nodes. The different amount of bacterial nodes modifies the ratio bacteria to fungi that correspond to 11.9 for no contaminated network and 4.3 for PAHs contaminated network. Such result suggested that the presence of PAHs reduce the fungi-bacteria interactions, affecting mainly the bacteria diversity probably by the selection of PAHs tolerant bacteria. In agreement with previous reports in soil, hydrocarbon contamination had an effect on the structure of the fungal-bacterial communities (Bell et al., 2014). Both networks have similar average weight but they present shortest path significant differences (Figure II.2), showing less connections and more specific links for PAHs contaminated network. Thus, this observation suggested that there are specialist fungi-bacteria associations in PAHs contaminated sediments. The links observed in the PAHs-contaminated network probably play an important role in PAHs degradation, or at least these links insure a PAHs tolerance. Moreover, the strong links in this PAHs-contaminated network highlights the importance of fungal-bacterial interactions, suggesting that both microorganisms cooperate to overcome PAHs contamination.

In order to analyze the interactions between fungi and bacteria, the relative important members of each network with a Spearman correlation coefficient > 0.82 were identified (Table II.1). The main fungal phyla present in both networks correspond to Ascomycota and Chytridiomycota, which are commonly observed in coastal marine sediment environments (Álvarez-Barragán et al., 2021; Elshafie et al., 2007; Gleason et al., 2011). In no contaminated sediments, the ASV Chytridiomycota F876 was found to be associated with cyanobacteria. This was not surprising as Chrytridiomicota are known as parasites of cyanobacteria (Rasconi et al., 2009; Sønstebø and Rohrlack, 2011). Moreover, in the no-contaminated network, Chytridiomycota F876 and Chytridiomycota F373 ASVs were associated with sulphate-reducing bacteria (Desulfobacteraceae), which are anaerobic bacteria inhabiting marine sediments (Rothermich et al., 2002). It is likely that fungi perform the first step in the degradation of complex organic matter (Boer et al., 2005), providing then molecules consumed by sulphate-reducing bacteria, which are usually found in coastal marine sediments playing a key role in the degradation of organic matter (Jørgensen, 1982).

The network was also composed by one ASV corresponding to the Ascomycota phylum, genus *Penicillium* which is commonly found in coastal sediments (Amer et al., 2019) and was linked with Fusobacteriaceae, Flavobacteriaceae and Rhodobacteraceae bacterial families. For PAHs contaminated sediments network, only the Chytridiomycota ASV F373 was found to be linked with three bacterial families: Desulfobulbaceae, Prolixibacteraceae and Fusobacteriaceae. In comparison with the no contaminated network, the PAHs contaminated network showed less Chytridiomycota members, which is in accordance with previous work reporting that members of the Chytridiomycota phylum are sensitive to hydrocarbon (Ventorino et al., 2018) having less resilience capacity compared to other fungi (Lemmel et al., 2021). For example, they produce un-walled spores (Longcore and Simmons, 2020) that are more susceptible to environmental changes.

All bacteria associated with fungi in PAHs-contaminated network belong to taxa recognized as anaerobic or facultative anaerobic (Ruan, 2013). Such result suggested that anaerobic metabolism of PAHs has a critical role in structuring the microbial network in contaminated sediment. Anaerobic members have been described within the Chytridiomycota phylum, further supporting the importance of the anaerobic metabolism in microbial assemblage (Rezaeian et al., 2004) indicating that fungi-bacteria interactions are possible under anaerobic conditions. Such result highlights the importance of the Chytridiomycota phylum and their interacting bacteria in PAHs metabolism that have not been yet explored. Noteworthy, most of the ASVs were not affiliated to bacterial genera indicating that most are unknown bacteria, indicating that an effort is required to identify these bacteria associated to fungi.



Parameter	No contaminated
Initial bacterial ASVs	2523
Initial fungal ASVs	282
Network bacterial ASVs	95
Network fungal ASVs	8
Ratio bacteria/fungi	11.9
Number of nodes	103
Number of links	104
Number of modules	7
Connected components	5
Network diameter	6
Shortest paths	6598 (62%)
Characteristic path length	3.3
Average number of neighbors	2.0
Average weight	0.796

Parameter	PAHs contaminated
Initial bacterial ASVs	2688
Initial fungal ASVs	287
Network bacterial ASVs	26
Network fungal ASVs	6
Ratio bacteria/fungi	4.3
Number of nodes	32
Number of links	30
Number of modules	3
Connected components	3
Network diameter	6
Shortest paths	424 (42%)
Characteristic path length	2.3
Average number of neighbors	1.9
Average weight	0.798

Figure II.2. Fungal-bacteria co-occurrence networks in microbial community from no contaminated and PAHs contaminated coastal marine sediments. The co-occurrence network is based on Spearman positive correlations. Each node represents fungal (red) or bacterial (blue) ASVs with a schematic icon corresponding to kingdom taxonomical classification. Edge lines represent significant co-occurrence relationships between nodes with a Spearman correlation of 0.75 and a *p*-value > 0.005. Edge size is proportional to the Spearman correlation coefficient. The network parameters are indicated in the Tables.

Table II.1. Fungal-bacterial interactions from network analysis from PAHs contaminated sediments with a Spearman's correlation above 0.82 and *p*-value < 0.005.

	Fungal taxonomy	Fungal key	Bacterial key	Bacterial taxonomy
No contaminated	Unclassified	F966	B3333	Epsilonbacteraeota, Campylobacteria, Campylobacterales, Thiovulaceae, Sulfurimonas
			B9688	Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Actibacter
			B8551	Proteobacteria, Deltaproteobacteria, Desulfobacterales, Desulfobacteraceae, Desulfosarcina
			B4096	Proteobacteria, Gammaproteobacteria, Gammaproteobacteria Incertae Sedis
			B3979	Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae
			B9017	Bacteroidetes, Bacteroidia, Chitinophagales, Saprospiraceae
			B4008	Acidobacteria, Thermoanaerobaculia, Thermoanaerobaculales, Thermoanaerobaculaceae, Subgroup 10
			B4403	Planctomycetes, Phycisphaerae, MSBL9, SG8-4
			B9329	Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Lutibacter
	Chytridiomycota, Incertae Sedis, Chytridiomycetes, Lobulomycetales, Lobulomycetaceae	F876	B9290	Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiales Incertae Sedis, Anderseniella
			B2840	Proteobacteria, Deltaproteobacteria, Desulfobacterales, Desulfobacteraceae
			B5700	Cyanobacteria, Oxyphotobacteria, Chloroplast, Cylindrotheca closterium
			B8202	Bacteroidetes, Bacteroidia, Cytophagales, Cyclobacteriaceae
			B6093	Cyanobacteria, Oxyphotobacteria, Chloroplast
			B3633	Proteobacteria, Deltaproteobacteria, Desulfobacterales, Desulfobacteraceae, Desulfococcus
			B1112	Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Eudoraea
			B1060	Proteobacteria, Gammaproteobacteria
			B1415	Cyanobacteria, Oxyphotobacteria, Chloroplast
	Dikarya, Ascomycota, Pezizomycotina, Eurotiomycetes, Eurotiales, Aspergillaceae, *Penicillium*	F903	B5672	Fusobacteria, Fusobacteriia, Fusobacteriales, Fusobacteriaceae, Psychrilyobacter
			B3401	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
			B4227	Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Lutimonas
			B9235	Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Winogradskyella
	Chytridiomycota, Incertae Sedis, Chytridiomycetes, Lobulomycetales, Lobulomycetaceae	F373	B566	Proteobacteria, Deltaproteobacteria, Desulfobacterales, Desulfobulbaceae
PAHs contaminated	Chytridiomycota, Incertae Sedis, Chytridiomycetes, Lobulomycetales, Lobulomycetaceae	F373	B5835	Fusobacteria, Fusobacteriia, Fusobacteriales, Fusobacteriaceae, Propionigenium
			B3980	Proteobacteria, Deltaproteobacteria, Desulfobacterales, Desulfobulbaceae
			B2278	Bacteroidetes, Bacteroidia, Bacteroidales, Prolixibacteraceae
	Dikarya, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreales	F298	B3980	Proteobacteria, Deltaproteobacteria, Desulfobacterales, Desulfobulbaceae
	Unclassified	F380	B1729	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae

II.2.5. Conclusion

Fungi occur in PAHs contaminated coastal sediments interacting with diverse bacterial populations creating assemblages allowing to both, fungi and bacteria, to survive in hazardous conditions. PAHs contamination alters the fungi-bacteria interactions, resulting in the selection of the stronger interactions. Interestingly, the fungal Chytridiomycota phylum was found to interact with anaerobic bacteria, which suggest that anaerobic metabolism of PAHs has a central role in the microbial community structure. Further studies are required to fully understand these interactions and their functional role in response to PAHs contamination.

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Chapter II. Fungal-bacterial co-occurrence in PAHs contaminated coastal marine sediment: network analysis

II.3. Chapter principal results and discussion

This chapter focused on showing the importance of fungi-bacteria interaction in the microbial community assemblages inhabiting PAHs-contaminated sediments. It has been shown that fungi have a central role coordinating bacterial communities in coastal sediments (Roberts et al., 2020). Moreover, it has been reported that the microbial communities inhabiting sediments are sensible to PAHs contamination (Jeanbille et al., 2016). Thus it is suggested, that the effect of the interaction between fungi and bacteria results beneficial for both partners and allow them to overcome the PAHs pollution hazardous conditions. In our study, co-occurrences between fungi and bacteria in the presence or absence of PAHs were analysed in order to determine the effect of these pollutants on inter-Kingdom interactions. The study is focused on coastal sediments as they have unique characteristics (salinity, oxygen concentration, water saturation, etc.) that in consequence, allow them to have unique fungal and bacterial interacting communities, not yet explored. In this chapter, we showed that fungi have a key role in driving the bacterial community assemblage when exposed to PAHs, fungi ASVs constituting the main nodes linked with several bacterial edges that connected the complete network. Moreover, strong co-occurrence between anaerobic bacteria and the fungal phylum Chytridiomycota was observed, revealing the importance of this fungal phylum recently found abundant in marine environment (Gleason et al., 2011). This observation suggests that marine sediments are the reservoir of unique microbial communities with probably specific interactions. The role that members of the Chytridiomycota phylum play in PAHs degradation has not been yet described, but the evidence that they are associated with anaerobic bacteria suggests that they promote anaerobic metabolism for PAH degradation. The role of the associated anaerobic bacteria, particularly in PAHs co-metabolism still needs to be elucidated. The results presented here represent a first approach to understand fungal and bacterial interactions in coastal sediments under PAHs contamination. Further analyses are required to obtain more information for a better understanding of the fungi-bacteria interactions in marine coastal ecosystems. In order to bring light on fungal-bacteria interaction, fungi from coastal sediments were isolated and characterized for their tolerance toward PAHs (Chapter III). Two of these fungi were then used as translocation networks in fungal-bacterial interaction experiments in order to describe the bacterial communities interacting with fungi in presence of PAHs (Chapter IV).

Chapter III. Fungi in PAH-contaminated marine sediments: Cultivable diversity and tolerance capacity towards PAH

III.1. Introduction

In the previous chapter (Chapter II), a co-occurrence network analysis was performed to evaluate the interactions with fungi and bacteria communities from PAHs contaminated coastal sediments, and the impact that PAHs pollution have in these interactions. The results of these analyses showed the role that fungi have on coastal sediments coordinating the structure of microbial communities and the relevance of fungi and bacteria interactions toward PAHs pollution. Considering the importance that fungi have over the population of bacterial communities and the possible metabolism of PAHs, this chapter (Chapter III) is dedicated to the isolation of fungi from coastal sediments that will enable to further evaluate fungal bacterial interactions (Chapter IV). In order to have a wide view of the cultivable fungal diversity inhabiting coastal sediments, 85 fungi were isolated on seawater media contaminated with PAHs. The fungal isolates were then identified using the ribosomal internal transcribed spacer (ITS), which allows the evaluation of cultivable diversity belonging to these phyla, including the possibility of new species. Following this, the fungi were tested for their ability to tolerate and remove four PAHs from the media (phenanthrene, pyrene and fluoranthene, and benzo[a]pyrene) with gas chromatography technique. Furthermore, a scrutiny of the genes that encode enzymes related to PAHs extracellular degradation was performed searching for manganese peroxidase, lignin peroxidase and laccase genes. Considering the importance for fungal bacterial interactions the capacity that fungi have to internalize PAHs, two fungi were selected and the intracellular uptake and transport of pyrene along the hyphae was analysed by microscopic observations.

Microscopic analyses presented in this work were performed in Helmholtz Centre for Environmental Research - UFZ, Germany, with the collaboration of Lukas Y. Wick and his teamwork. The results obtained are presented in scientific publication format and were published in January 2021 in the *Marine Pollution Bulletin* under the title “Fungi in PAH-contaminated marine sediments: Cultivable diversity and tolerance capacity towards PAH”.

III.2. Fungi in PAH-contaminated marine sediments: Cultivable diversity and tolerance capacity towards PAH

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Fungi in PAH-contaminated marine sediments: Cultivable diversity and tolerance capacity towards PAH

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ABSTRACT

The cultivable fungal diversity from PAH-contaminated sediments was examined for the tolerance to polycyclic aromatic hydrocarbon (PAH). The 85 fungal strains, isolated in non-selective media, revealed a large diversity by ribosomal internal transcribed spacer (ITS) sequencing, even including possible new species. Most strains (64%) exhibited PAH-tolerance, indicating that sediments retain diverse cultivable PAH-tolerant fungi. The PAH-tolerance was linked neither to a specific taxon nor to the peroxidase genes (LiP, MnP and Lac). Examining the PAH-removal (degradation and/or sorption), *Alternaria destruens* F10.81 showed the best capacity with above 80% removal for phenanthrene, pyrene and fluoranthene, and around 65% for benzo[*a*]pyrene. *A. destruens* F10.81 internalized pyrene homogenously into the hyphae that contrasted with *Fusarium pseudoygamai* F5.76 in which PAH-vacuoles were observed but PAH removal was below 20%. Thus, our study paves the way for the exploitation of fungi in remediation strategies to mitigate the effect of PAH in coastal marine sediments.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are important pollutants threatening the marine environment due to their toxicity (Duran and Cravo-Laureau, 2016). Although the more spectacular input of PAHs in marine environments is due to accidental oil spills, the main source remains on natural oil seeps (Duran and Cravo-Laureau, 2016). PAHs accumulate in sediments because of their hydrophobicity constituting a chronic contamination (Rothermich et al., 2002). Their fate in the environment depends on biotic and abiotic factors (Duran and Cravo-Laureau, 2016).

Many microorganisms including archaea, bacteria, algae and fungi are able to degrade PAHs (Duran and Cravo-Laureau, 2016; Bordenave et al., 2008; Guermouche M'rassi et al., 2015; Haritash and Kaushik, 2009). In the last years, the interest on PAHs removal and biodegradation by fungi has increased (Mineki et al., 2015; Morales et al., 2017). The fungal removal of PAHs consists in three main processes: two oxidation processes involving extracellular peroxidases (lignin peroxidase, manganese peroxidase and laccase; Chen et al., 2001; Scheel et al., 2000), and membrane attached monooxygenases (cytochrome P450; Črešnar and Petrič, 2011; Syed et al., 2010), and absorption and storage of PAHs in lipid vacuoles (Verdin et al., 2005). The ability of fungi to use PAHs as sole carbon and energy sources has been described (Rafin et al., 2000). However, it has been reported that most of fungi require co-metabolism with another carbon source for PAH degradation (Cerniglia et al., 1986).

Fungi have been found in all marine habitats (Orsi et al., 2013), revealing their high diversity (Jones, 2000). Ascomycota and Basidiomycota are the main fungal phyla found in marine environments as described for soil ecosystems (Clemente et al., 2001; Field et al., 1992; Godoy et al., 2016; Li et al., 2008; Mineki et al., 2015; Potin et al., 2004; Valentín et al., 2006). Although fungi of terrestrial origin have been isolated from marine ecosystems (Li and Wang, 2009), recent molecular analysis revealed specific fungal sequences suggesting the existence of novel species of marine fungi (Amend et al., 2019; Grossart and Rojas-Jimenez, 2016). Fungi isolated from marine habitats exhibit similar morphological characteristics to their terrestrial counterparts (Méjanelle et al., 2000). However, they might possess particular properties to survive in marine environments (Amend et al., 2019), particularly in PAHs contaminated sediments (Greco et al., 2018). Such properties, as salinity tolerance and the capacity to degrade and accumulate PAHs, less bioavailable due to adsorption solid materials, remain to be explored (Bonugli-Santos et al., 2015; Bugni and Ireland, 2004; Trincone, 2010).

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This study aimed to explore the cultivable marine fungi, recovered from oil-contaminated saline sediments, for their PAH-tolerance capacity. For this purpose fungal strains were isolated from various marine coastal environments, characterized and identified by ITS sequence analysis, and their features explaining the PAH tolerance examined.

2. Material and methods

2.1. Culture media

The culture media used in this study were based on the seawater minimal medium (swMM; Brito et al., 2006), which composition was as follow: KCl 0.75 g/L, $CaCl{\cdot}2H_2O$ 1.47 g/L, NH_4Cl 1.5 g/L, $MgSO_4{\cdot}7H_2O$ 6.64 g/L, NaCl 20 g/L, Na_2CO_3 0.265 g/L, 1 mL of trace elements solution (H_3BO_3 300 mg/L, $FeSO_4{\cdot}7H_2O$ 1.1 g/L, $CoCl_2{\cdot}6H_2O$ 190 mg/L, $MnCl_2{\cdot}2H_2O$ 50 mg/L, $ZnCl_2$ 42 mg/L, $NiCl_2{\cdot}6H_2O$ 24 mg/L, $Na_2MoO_4{\cdot}2H_2O$ 2 mg/L), 1 mL of vitamin solution (biotine 2 mg/L, ρ-aminobenzoate 10 mg/L, thiamine 10 mg/L, pantothenate 5 mg/L, pyridoxamine 50 mg/L, vitamin B_{12} 20 mg/L, nicotinate 20 mg/L), and 100 μL of phosphate buffer 50 mM. The pH was adjusted with HCl to 6.5. Chemicals were purchased from Sigma Aldrich (Germany).

The malt dextrose agar (MDA) and malt dextrose (MD) media, in which distillated water was exchanged by swMM (MDAsw and MDsw respectively) to keep salinity conditions, were used for the isolation and for maintaining fungal strains.

2.2. Selection and conservation of fungal strains

Oil polluted sediment collected from different coastal areas were used as inoculum for the isolation of fungal strains with the ability to degrade PAHs. Each sample was inoculated directly in MDAsw and incubated for 5 days. Also, dilutions at 10^{-1}, 10^{-2} and 10^{-3} were performed taking 100 mg of each source.

The isolated fungal strains were conserved as conidia and mycelia in glycerol at −70 °C. Fungi were inoculated in MDsw grown until conidia overwhelmed cultures. Mycelia and conidia were recovered from the flask and then dispatched in at least 3 Eppendorf tubes (100 mg of biomass each) for each strain. After addition of 1 mL glycerol (30% solution), the tubes were frozen and kept at −70 °C until use. In order to check viability one tube with mycelia was tested after 7 days of storage by inoculating MDsw culture.

2.3. Fungi identification sequencing and phylogenetic analysis

Fungi were harvested from MDAsw cultures from 10 days of incubation and DNA was extracted using the QUIAGEN DNeasy® UltraClean® Microbial Kit (Cat. No. 12224-40) following the manufacturer instructions. The identification was based on ITS sequences, which were amplified using the primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) that amplify the ITS1, 5.8S and ITS2 region of the rRNA genes operon. The amplified region allows the identification at the species level and even at the subspecies level (Fajarningsih, 2016). The PCR reaction mix was prepared with 1 μL of extracted DNA in 9.5 μL of DEPC-treated water, 1 μL of each primer (20 μM), 12.5 μL AmpliTaq Gold 360 Master Mix 2× (Thermo Fisher Scientific, USA). The amplification was performed through 35 cycles of 95 °C (30 s), 55 °C (30 s) and 72 °C (1 min), with a previous activation start of 95 °C (10 min) and final extension step at 72 °C (10 min). ITS amplified fragments were sequenced at the Eurofins platform (France).

Sequence data were edited using Chromas Pro version 1.34. For identification, fungal ITS rRNA sequences were compared with NCBI (National Centre for Biotechnology Information; http://www.ncbi.nlm.nih.gov) database as previously described (Giloteaux et al., 2010). Fungal ITS sequences in this study and reference sequences from GenBank were edited and aligned using CLUSTAL-W (Thompson et al., 2003) as described (Bruneel et al., 2008). The aligned sequences were imported into MEGA 3.1 (Kumar, 2004) for creating Neighbour-joining (NJ) trees based on pairwise genetic distances. The quality of the branching patterns for NJ was assessed by bootstrap resampling of the data sets with 1000 replications and rooted to *Rhizopus oryzae* CBS 112.07^T (NR 103595.1) and *Trametes versicolor* CFMR FP-135156-Sp^T (NR 154494.1). The sequences determined in this study have been submitted to the ITS NCBI database and assigned Accession nos. MT889820 to MT889904.

2.4. Fungal tolerance to PAHs

The tolerance to hydrocarbons was tested by inoculating and cultivating the fungi in swMM supplemented with 25 mg/L of each fluoranthene, phenanthrene, pyrene and 5 mg/L of benzo[*a*]pyrene as only carbon source. The analytical grade PAHs (Aldrich Chemical Co) were added to the media as solution in acetone. Fungal strains were inoculated in the plates and incubated at 20 °C in darkness during 15 days in order to maintain culture condition closer to that observed in the environment. The capacity of fungi to grow and develop conidia was considered as tolerance while in absence of development the strain was classified as no-tolerant.

2.5. Fungal PAHs removal rates

Between 80 and 100 mg of mycelia and conidia were recovered of MDAsw plates and inoculated in 80 mL flasks with 30 mL of MDsw (1% MD). Fluoranthene, phenanthrene, pyrene and benzo[*a*]pyrene were then added from a stock solution prepared in acetone that contain 20 mg/L of each hydrocarbon. Samples were set for 1 h before incubation to let acetone evaporate. An un-inoculated flask was used as abiotic control and PAHs concentration reference. Cultures were incubated in darkness for 20 days at 20 °C with gentle shaking at 80 rpm, in order to maintain culture condition closer to that observed in the environment. Hydrocarbons were extracted after incubation adding 30 mL of ethyl acetate and shacked for 15 min at 600 rpm. The recovery yield was estimated to be about 98% of the initial concentration using the abiotic controls as reference. Chrysene was used as internal standard during extraction in a concentration of 10 mg/L. Two milliliters of organic phase was pulled in a glass vial for its analysis in Gas Chromatography equipped with Flame Ionization Detector (GC-FID) (Agilent Technologies®, Network 6850 GC System) with a capillarity C18 reverse column (30 m * 0.25 mm * 0.25 μm). For the analysis, 1 μL was injected with a split ration of 1/50 using helium as carrier gas. Column temperature ramp settle from 200 to 240 °C with stepped temperature increase of 5 °C/min and held during 1 min at 240 °C. Flame ionization detector was settled at 290 °C.

The removal capacities (degradation and/or sorption) for selected strains (*Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76 strains exhibiting the highest and the lowest removal capacities, respectively) was determined in triplicate with an incubation period of 15 days at with gentle shaking at 80 rpm in order to maintain culture condition closer to that observed in the environment. PAHs extraction was performed as above described. *Phanerochaete chrysosporium* strain was used as reference for PAH-removal capacity, which often serves as reference for the comparison of PAH-removal capacities even between strains from different phyla, as it is the fungi the most studied in PAH-degradation (Cao et al., 2020). A one-way of analysis of variance (ANOVA) was used to assess the significance of PAH-removal differences between samples with a significance level of $p < 0.05$.

2.6. PCR detection of peroxidase and laccase genes

The presence of genes encoding for enzymes known to be related to PAHs degradation: laccase (lac), manganese peroxidase (MnP1, MnP2, MnP3) and lignin peroxidase (LiP1, LiP2, LiP3, LiP4, LiP5, LiP6) was checked by PCR amplification. *Phanerochaete chrysosporium*, an effective PAH degrader (Bamforth and Singleton, 2005; May et al., 1997), was

Table 1
Primers used for the amplification of fungal peroxidases genes.

Gene[a]	Sequence (5′-3′)[b]	Tm (°C)	Size (nt)	Reference
LiP1	LIG1u (F): GCCGCAATTTCTCTTGCTCTTTCCA LIG1d (R): TACATCGAACCACGCGCACGAGATT	57	179/126	Broda et al. (1995)
LiP2	LIG2u (F): CATCGCAATTTCGCCCGCCATGGAGGA LIG2d (R): ACCTTCTGAACGAATGGCTTCTGGAGC	57	222/179	Broda et al. (1995)
LiP3	LIG3u (F): TATTGCCATCTCTCCTGCTATGGAGGCC LIG3d (R): ATGTTAGGGTGGAAGTTGGGCTCGATG	57	179/126	Broda et al. (1995)
LiP4	LIG4u (F): GTGCGCCTGGTTCCCCATTCTGCAG LIG4d (R): AATTGGTCTCGATAGTATCGAAGAC	57	350/222	Broda et al. (1995)
LiP5	LIG5u (F): GGTCTCGATCGAGGAGAAGGTAATGATC LIG5d (R): TTGCCCCGACGGCGTGCACAC	57	350/222	Broda et al. (1995)
LiP6	LIG6u (F): GACCTGCTCGAACGGCAAGGTCGTCC LIG6d (R): CATGATAGAACCATCGGCGCCTCGC	57	350/222	Broda et al. (1995)
MnP1	mnp1-f (F): CAGACGGTACCCGCGTCACC mnp1-r (R): AGTGGGAGCGGCGACATCAC	60	246/123	Bogan et al. (1996)
MnP2	mnp2-f (F): CCGACGGCACCCGCGTCAGC mnp2-r (R): CGAGCGGGAGCGGCGACGCC	60	≈900	Bogan et al. (1996)
MnP3	mnp3-f (F): CCGACGGTACCAAGGTCAAC mnp3-r (R): AGCGGCAGCGGCGACGCGAC	60	≈900	Bogan et al. (1996)
Lac	Lac (F): CACTGGCACGGNTTCTTCCA Lac (R): GTGACTATGATACCAGAANGT	52	246/123	D'Souza et al. (1996)

[a] LiP, lignin peroxidase; MnP, manganese peroxidase; Lac, laccase.
[b] (F), forward; (R), reverse.

used as positive control for the presence of the peroxidase genes. The sequences of the primers and the Tm for the amplification of the different genes are presented in Table 1. The reaction mix was prepared with 1 μL of extracted DNA in 9.5 μL of DEPC-treated water, 1 μL of each primer (20 μM), 12.5 μL AmpliTaq Gold 360 Master Mix 2× (Thermo Fisher Scientific, USA). The amplification was performed through 35 cycles of 95 °C (30 s), Tm (Table 1, 45 s) and 72 °C (45 s), with a previous activation start of 95 °C (10 min) and final extension step at 72 °C (10 min). Peroxidase genes amplified fragments were sequenced at the Eurofins platform (France).

2.7. PAHs internalization and transport

The capacity to internalize and transport PAHs through hyphae was examined for selected strains (*F. pseudonygamai* F5.76 and *A. destruens* F10.81). The experimental setup consisted on an empty petri dish with two MDAsw cubes over a crystal slide with a separation of 6 mm between them. One of the cubes contained pyrene at 30 mg/L while the other no. The fungi were inoculated in the cube with pyrene and incubated for 7 days in darkness at 20 °C. The transport of PAHs was evaluated inside the mycelia that reach the cube without pyrene using an epifluorescence microscope (Nikon, Eclipse E600) with DAPI light filter (excitation 345 nm, emission 485 nm) for PAH detection (fluorescence wavelengths range from 210 to 380 nm) (Verdin et al., 2005).

3. Results and discussion

3.1. Identification of fungal strains Isolated from coastal sediments

In total, 85 fungal strains were isolated from PAHs contaminated coastal sediments in seawater media containing malt dextrose agar (swMDA). The strains were identified with the complete ITS sequence (including ITS1, 5.8S rRNA gene, and ITS2 regions), which provide accurate identification of fungi species even at the subspecies level (Fajarningsih, 2016). The phylogenetic analysis showed that 83 strains belong to the Ascomycota Phylum and two strains belong to the Basidiomycota Phylum (Figs. 1 and 2). Such result was not surprising since fungi belonging to Ascomycota have been found prevalent in marine sediments (Babu et al., 2010; Birolli et al., 2018; Ravelet et al., 2000) and other environments (Reyes-César et al., 2014). The 85 fungal strains fall into six different Orders: Capnodiales (59 strains), Eurotiales (14 strains), Trichosphaeriales (1 strain), Hypocreales (2 strains), Pleosporales (7 strains) and Polyporales that belong to Basidiomycota Phylum (2 strains).

The isolated strains affiliated to Eurotiales included strains belonging to *Talaromyces* (*T. helicus*), *Aspergillus* (*A. fumigatus* and *A. chevalieri*), and *Penicillium* (*P. glandicola*, *P. crustosum*, and *P. bialowiezense*) genera (Fig. 1). *Talaromyces* and *Aspergillus* genera are known for their ability of PAH degradation in soil (Fayeulle et al., 2019), while *Aspergillus* genera, especially *A. fumigatus*, has been detected in oil-contaminated mangrove sediments (Ghizelini et al., 2019). The isolated strains related to the *Nigrospora* genus (Trichosphaeriales), *N. rubi* and *N. gorlenkoana*, are described for the first time in marine sediments. The presence of these strains in the sediments might be explained by plant material entering into the sea by air transportation or runoff, as they are known to be associated with plants (Hao et al., 2020). Similarly, the strains affiliated to the Hypocreales, *Fusarium pseudonygamai* (plant pathogen), *Lecanicillium longisporum* and *Akanthomyces muscarius* (entomopathogens) have been described only in soil so far (Ansari and Butt, 2012; Bashyal et al., 2016; Danilovich et al., 2020). Regarding the Pleosporales, the strain F1.72, closely related to *Neosulcatispora strelitziae* and *Phaeosphaeria podocarpi*, recently described fungal species (Crous et al., 2014, 2016), represents probably also a novel fungal species. However, further analysis, including multi-locus based phylogeny, is required to characterize the strain. Two other strains were closely related to species within the Pleosporales, *Alternaria destruens* and *Epicoccum poae*, which have been isolated from plants (Kumar and Kaushik, 2013; Chen et al., 2017). So far, these strains have not been shown to exhibit hydrocarbon degradation capacity. The strains affiliated to the Polyporales were related to *Trametes versicolor* and *Bjerkandera adusta* that are known to be able to degrade hydrocarbon (Lladó et al., 2012; Andriani et al., 2016).

All the Capnodiales were affiliated to two complexes of the *Cladosporium* genus (Fig. 2) defined by a multi-locus phylogeny (Schubert et al., 2007). Among the Cladosporioides complex, the isolated strains were affiliated to species known to be associated with human and animals diseases such as *C. crousii*, *C. welwitschiicola*, *C. austroafricanum*, *C. pini-ponderosae*, and *C. puyae* (Sandoval-Denis et al., 2016), and with marine organisms such as *C. colombiae* (Ravi Theja and Chandra, 2020). Similarly, the isolated strains belonging to the Herbarum complex, *C. rhusicola*, *C. subcinereum*, *C. angustiherbarum* have been described involved in human and animals infections (Sandoval-Denis et al., 2016), while *C. allicinum* was found associated with marine organisms (Poli et al., 2020; Bovio et al., 2019) and several strains related to *C. herbarum* have been described for their ability to degrade PAH in marine sediment (Marco-Urrea et al., 2015; Xiao et al., 2020). Noteworthy, the strain D16.68 is the more distant from *Cladosporium* species (Fig. 2) suggesting that it might represent a novel species within the *Cladosporium* genus, but further phylogenetic analysis based on multi-locus are required to elucidate the taxonomic position. Although *Cladosporium* has been already reported in saline environments (Zalar et al., 2007), in

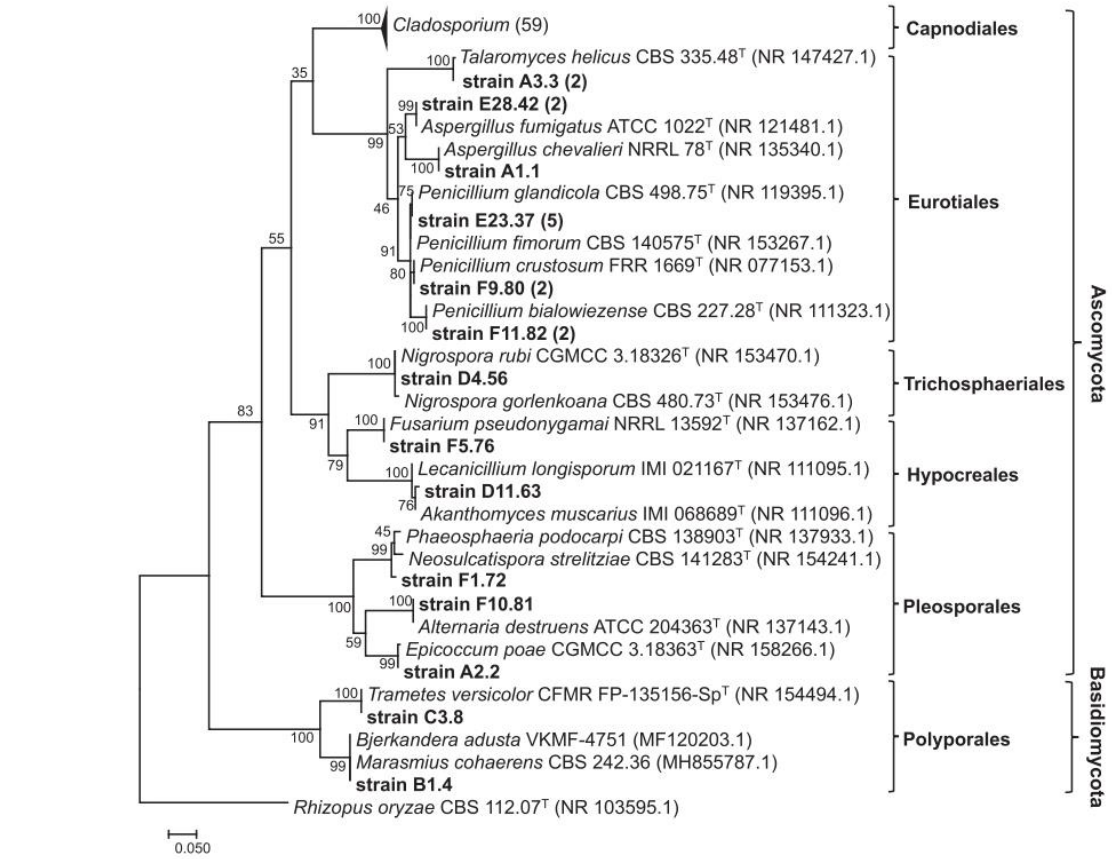

Fig. 1. Phylogenetic tree, based on ITS sequences, showing the positions of fungal strains isolated from oil contaminated coastal marine sediments. The Neighbour joining phylogenetic tree was rooted with the ITS sequence of *Rhizopus oryzae* CBS 112.07^T (NR 103595.1). The scale bar corresponds to 0.05 substitutions per nucleotide position. Percentages of 1000 bootstrap re-sampling that supported the branching orders in each analysis are shown above or near the relevant nodes. For the isolates, the number of isolated strains is indicated in parenthesis. For the type strains, the accession number is indicated in parenthesis.

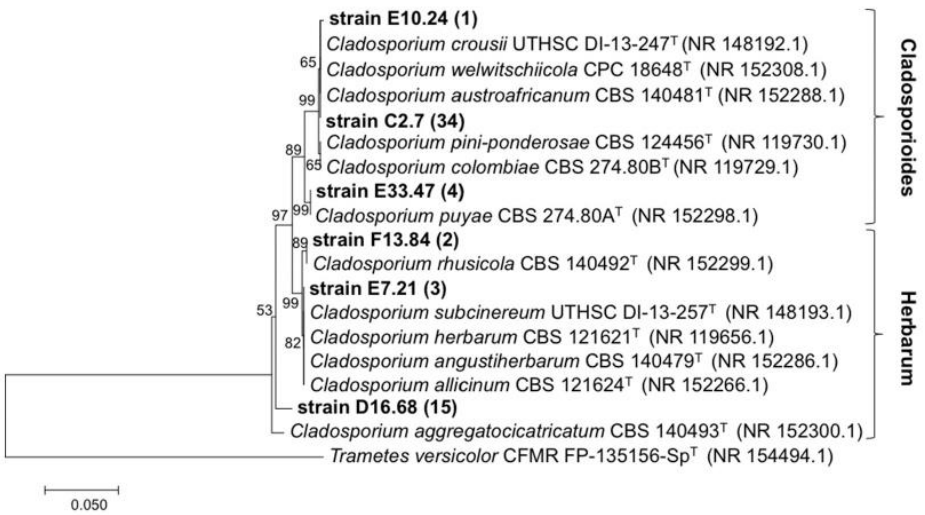

Fig. 2. Phylogenetic tree, based on ITS sequences, showing the positions of fungal strains isolated from oil contaminated coastal marine sediments within the *Cladosporium* genus. The Neighbour joining phylogenetic tree was rooted with the ITS sequence of *Trametes versicolor* CFMR FP-135156-Sp^T (NR 154494.1). The scale bar corresponds to 0.05 substitutions per nucleotide position. Percentages of 1000 bootstrap re-sampling that supported the branching orders in each analysis are shown above or near the relevant nodes. For the isolates, the number of isolated strains is indicated in parenthesis. For the type strains, the accession number is indicated in parenthesis.

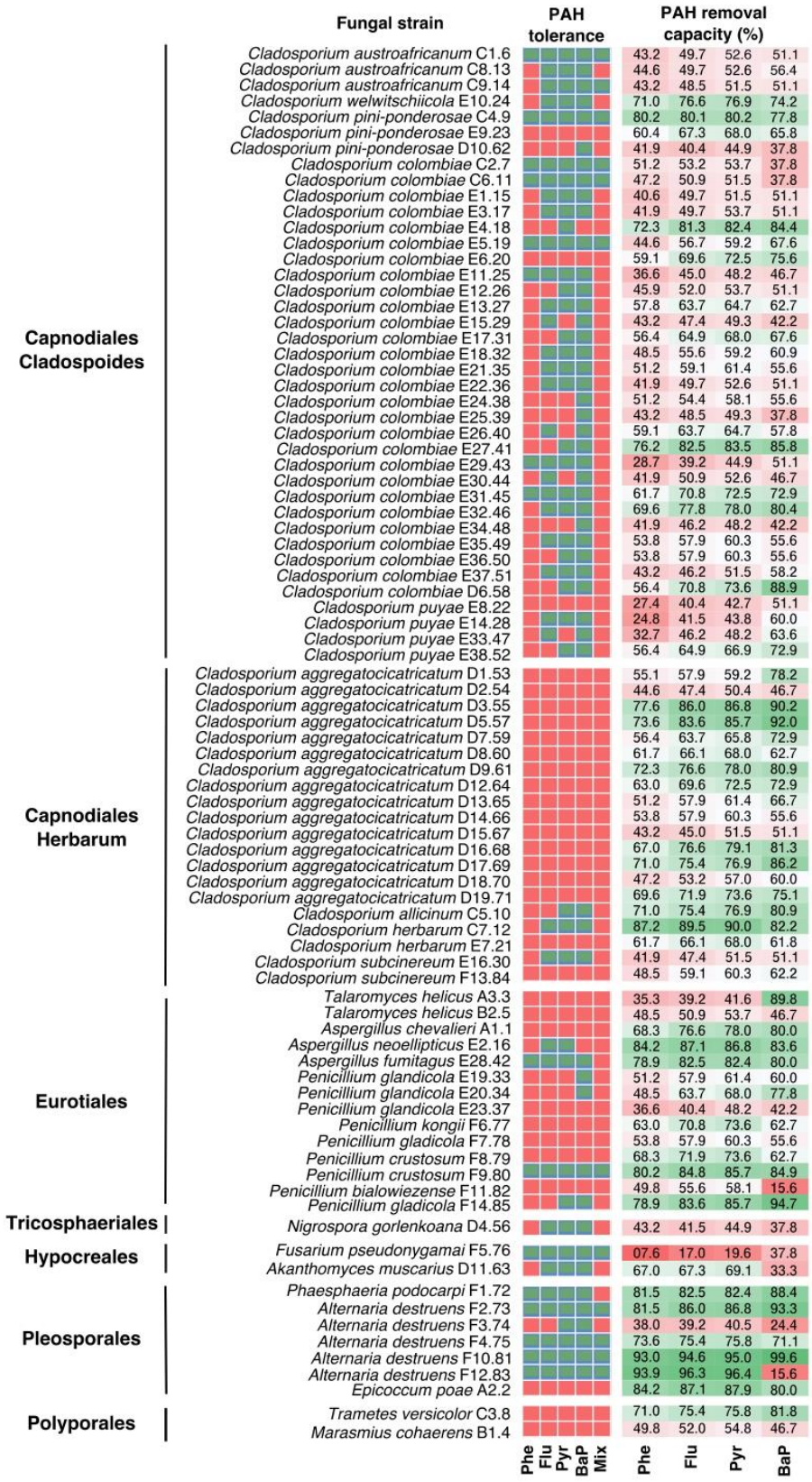
Fungal strain
PAH tolerance
PAH removal capacity (%)
Phe Flu Pyr BaP Mix
Phe Flu Pyr BaP
Capnodiales Cladospoides
Cladosporium austroafricanum C1.6 43.2 49.7 52.6 51.1
Cladosporium austroafricanum C8.13 44.6 49.7 52.6 56.4
Cladosporium austroafricanum C9.14 43.2 48.5 51.5 51.1
Cladosporium welwitschiicola E10.24 71.0 76.6 76.9 74.2
Cladosporium pini-ponderosae C4.9 80.2 80.1 80.2 77.8
Cladosporium pini-ponderosae E9.23 60.4 67.3 68.0 65.8
Cladosporium pini-ponderosae D10.62 41.9 40.4 44.9 37.8
Cladosporium colombiae C2.7 51.2 53.2 53.7 37.8
Cladosporium colombiae C6.11 47.2 50.9 51.5 37.8
Cladosporium colombiae E1.15 40.6 49.7 51.5 51.1
Cladosporium colombiae E3.17 41.9 49.7 53.7 51.1
Cladosporium colombiae E4.18 72.3 81.3 82.4 84.4
Cladosporium colombiae E5.19 44.6 56.7 59.2 67.6
Cladosporium colombiae E6.20 59.1 69.6 72.5 75.6
Cladosporium colombiae E11.25 36.6 45.0 48.2 46.7
Cladosporium colombiae E12.26 45.9 52.0 53.7 51.1
Cladosporium colombiae E13.27 57.8 63.7 64.7 62.7
Cladosporium colombiae E15.29 43.2 47.4 49.3 42.2
Cladosporium colombiae E17.31 56.4 64.9 68.0 67.6
Cladosporium colombiae E18.32 48.5 55.6 59.2 60.9
Cladosporium colombiae E21.35 51.2 59.1 61.4 55.6
Cladosporium colombiae E22.36 41.9 49.7 52.6 51.1
Cladosporium colombiae E24.38 51.2 54.4 58.1 55.6
Cladosporium colombiae E25.39 43.2 48.5 49.3 37.8
Cladosporium colombiae E26.40 59.1 63.7 64.7 57.8
Cladosporium colombiae E27.41 76.2 82.5 83.5 85.8
Cladosporium colombiae E29.43 28.7 39.2 44.9 51.1
Cladosporium colombiae E30.44 41.9 50.9 52.6 46.7
Cladosporium colombiae E31.45 61.7 70.8 72.5 72.9
Cladosporium colombiae E32.46 69.6 77.8 78.0 80.4
Cladosporium colombiae E34.48 41.9 46.2 48.2 42.2
Cladosporium colombiae E35.49 53.8 57.9 60.3 55.6
Cladosporium colombiae E36.50 53.8 57.9 60.3 55.6
Cladosporium colombiae E37.51 43.2 46.2 51.5 58.2
Cladosporium colombiae D6.58 56.4 70.8 73.6 88.9
Cladosporium puyae E8.22 27.4 40.4 42.7 51.1
Cladosporium puyae E14.28 24.8 41.5 43.8 60.0
Cladosporium puyae E33.47 32.7 46.2 48.2 63.6
Cladosporium puyae E38.52 56.4 64.9 66.9 72.9
Capnodiales Herbarum
Cladosporium aggregatocicatricatum D1.53 55.1 57.9 59.2 78.2
Cladosporium aggregatocicatricatum D2.54 44.6 47.4 50.4 46.7
Cladosporium aggregatocicatricatum D3.55 77.6 86.0 86.8 90.2
Cladosporium aggregatocicatricatum D5.57 73.6 83.6 85.7 92.0
Cladosporium aggregatocicatricatum D7.59 56.4 63.7 65.8 72.9
Cladosporium aggregatocicatricatum D8.60 61.7 66.1 68.0 62.7
Cladosporium aggregatocicatricatum D9.61 72.3 76.6 78.0 80.9
Cladosporium aggregatocicatricatum D12.64 63.0 69.6 72.5 72.9
Cladosporium aggregatocicatricatum D13.65 51.2 57.9 61.4 66.7
Cladosporium aggregatocicatricatum D14.66 53.8 57.9 60.3 55.6
Cladosporium aggregatocicatricatum D15.67 43.2 45.0 51.5 51.1
Cladosporium aggregatocicatricatum D16.68 67.0 76.6 79.1 81.3
Cladosporium aggregatocicatricatum D17.69 71.0 75.4 76.9 86.2
Cladosporium aggregatocicatricatum D18.70 47.2 53.2 57.0 60.0
Cladosporium aggregatocicatricatum D19.71 69.6 71.9 73.6 75.1
Cladosporium allicinum C5.10 71.0 75.4 76.9 80.9
Cladosporium herbarum C7.12 87.2 89.5 90.0 82.2
Cladosporium herbarum E7.21 61.7 66.1 68.0 61.8
Cladosporium subcinereum E16.30 41.9 47.4 51.5 51.1
Cladosporium subcinereum F13.84 48.5 59.1 60.3 62.2
Eurotiales
Talaromyces helicus A3.3 35.3 39.2 41.6 89.8
Talaromyces helicus B2.5 48.5 50.9 53.7 46.7
Aspergillus chevalieri A1.1 68.3 76.6 78.0 80.0
Aspergillus neoellipticus E2.16 84.2 87.1 86.8 83.6
Aspergillus fumitagus E28.42 78.9 82.5 82.4 80.0
Penicillium glandicola E19.33 51.2 57.9 61.4 60.0
Penicillium glandicola E20.34 48.5 63.7 68.0 77.8
Penicillium glandicola E23.37 36.6 40.4 48.2 42.2
Penicillium kongii F6.77 63.0 70.8 73.6 62.7
Penicillium gladicola F7.78 53.8 57.9 60.3 55.6
Penicillium crustosum F8.79 68.3 71.9 73.6 62.7
Penicillium crustosum F9.80 80.2 84.8 85.7 84.9
Penicillium bialowiezense F11.82 49.8 55.6 58.1 15.6
Penicillium gladicola F14.85 78.9 83.6 85.7 94.7
Tricosphaeriales
Nigrospora gorlenkoana D4.56 43.2 41.5 44.9 37.8
Hypocreales
Fusarium pseudonygamai F5.76 07.6 17.0 19.6 37.8
Akanthomyces muscarius D11.63 67.0 67.3 69.1 33.3
Pleosporales
Phaesphaeria podocarpi F1.72 81.5 82.5 82.4 88.4
Alternaria destruens F2.73 81.5 86.0 86.8 93.3
Alternaria destruens F3.74 38.0 39.2 40.5 24.4
Alternaria destruens F4.75 73.6 75.4 75.8 71.1
Alternaria destruens F10.81 93.0 94.6 95.0 99.6
Alternaria destruens F12.83 93.9 96.3 96.4 15.6
Epicoccum poae A2.2 84.2 87.1 87.9 80.0
Polyporales
Trametes versicolor C3.8 71.0 75.4 75.8 81.8
Marasmius cohaerens B1.4 49.8 52.0 54.8 46.7

Fig. 3. PAHs tolerance and removal capacities of isolated fungal strains. PAHs tolerance corresponds to the capacity of the fungal strains to grow (green) or not (red) on solid seawater minimal medium in the presence of different PAHs and PAHs mixture. PAHs removal capacity, determined in liquid cultures containing a mixture of PAHs, corresponds to the percentage of PAHs eliminated after 20 days of fungal growth. The color gradient follows to the removal capacity from low (red) to high (green). Phe, phenanthrene; Flu, fluoranthene; Pyr, pyrene; BaP, benzo[*a*]pyrene; Mix, mixture of the 4 PAHs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hydrocarbon contaminated sediments (Ravelet et al., 2000) showing as well resistance to metals (Shao and Sun, 2007), it was surprising to obtain mainly strains of this genus. It is likely that members of the *Cladosporium* genus are well adapted to the culture conditions imposed during the screening procedure. The cultural approach owns some limitations. Indeed, the development of conidia is controlled by different factors (Tan et al., 1995), such as the presence of PAHs (Zafra et al., 2015), influencing the selection of cultivable strains. In order to overcome such limitations, the application of different culture conditions will enlarge the diversity of isolated strains.

Despite the limitations inherent of the cultivable approach, a large diversity of cultivable fungi was obtained from hydrocarbon-contaminated marine sediments, spanning 11 fungal genera. The isolated strains included not only strains affiliated to Orders which members were isolated from marine sediments (Mouton et al., 2012; Ravelet et al., 2000) showing the capacity to degrade PAHs (Fedorak et al., 1984; Simister et al., 2015), but also some isolated strains yet not described in marine sediments, nor for their tolerance to the presence of PAHs. Thus, our study shows that a large fungal diversity remains hidden in marine sediments, which represent a metabolic potential for the development of remediation strategies for the mitigation of the effect of PAHs.

3.2. PAHs tolerance and removal capacities of the fungal isolated strains

Most of the isolated fungal strains (54 strains, 64%) were able to grow in the presence of at least the presence of one PAH showing their tolerance to hydrocarbons (Fig. 3). Among them, 61% tolerate the presence of benzo[*a*]pyrene, 52% pyrene and 45% fluoranthene. Few fungal strains were able to develop in presence of phenanthrene either alone (19%) or in mixture with other PAHs (14%). Similar results showing high tolerance of fungal strains to pyrene, and low tolerance to phenanthrene and PAHs mixture have been reported in the same range of concentrations (Lee et al., 2014). Toxic effects on fungal growth have been observed with phenanthrene (Lisowska, 2004) and metabolites produced from PAHs mixture (Lundstedt et al., 2003). Interestingly, the tolerance capacity is consistent with the phylogeny (Fig. 3), the members of the same Order showing similar tolerance patterns. Noteworthy, the two groups Cladosporioides and Herbarum within the Capnodiales Order showed distinct tolerance capacities, which further support the classification into two distinct groups.

In order to assess the PAHs removal capacity (degradation and/or sorption) of fungal isolated strains, maltose and dextrose were added as extra carbon source, since fungi have been shown to have low ability to use PAHs as sole carbon source (Harrison, 2009). In these conditions, fungal strains belonging to the Pleosporales Order showed the most efficient removal capacities while strains affiliated to the Hypocreales Order showed the lowest removal capacity (Fig. 3). In the Pleosporales Order, 85% of the strains showed removal capacity above 70%. The Cladosporioides group of the Capnodiales Order exhibited the less number of strains with removal capacities above 70%. The comparison of the removal capacities of the isolated strains showed two main clusters (Fig. 4) separating the strains with high removal capacities from those with low removal capacities. Interestingly, members of the same species showed divergent removal capacities. Such discrepancies have been described (Lee et al., 2014), strains from the same species showing different metabolic capacities.

The analysis also showed that pyrene and fluoranthene (4 rings PAHs) clustered together, further confirmed by strong correlation between pyrene and fluorentene removal capacities (Pearson coefficient: 0.996, R^2: 0.993), indicating that they were removed by almost a similar pattern of fungal strains (Fig. 3). The benzo[*a*]pyrene (5 rings PAH) and phenanthrene (3 rings PAH) were apart indicating that the patterns of fungal strains able to remove them were different. Such observations highlighted that the removal capacity depends also on the PAH structure as previously suggested (Ghosal et al., 2016).

In order to further characterize the genetic PAH degradation potential of the isolated fungal strains, the presence of genes encoding manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Lac), known to participate in the degradation of PAHs (Ghosal et al., 2016), was examined in their genomes by PCR (Fig. 4). All strains possess at least one of these genes, the LiP2 being the most distributed (82/85 strains, 96%) among the isolated fungal strains (Fig. 4). Noteworthy, when the LiP2 gene was not present, the strain possessed the MnP2 gene. Almost all strains (80/85 strains, 94%) possessed at least a manganese peroxidase gene, MnP2 gene being the most detected (75/85 strains, 88%). Such results were not surprising since most of the peroxidase enzymes are known to be produced in marine environment (Bonugli-Santos et al., 2015). Surprisingly, the Lac gene, found in many marine fungal species (Ben Ali et al., 2020; D'Souza-Ticlo et al., 2009), was detected in only 4 strains, which exhibited the most genetic potential possessing more than 5 of the targeted genes. However, since various types of Lac genes have been described in fungi (Moreno et al., 2017; Yang et al., 2016), the primers used to detect the presence of Lac genes are probably not well suited for recovering the entire Lac gene diversity. The use of primers targeting broader Lac gene diversity or targeting at least Lac gene detected within the Ascomycota phyla (the major phyla of the isolated strains) is required to better define the presence of Lac genes in the isolated strains. Similar observations can be drawn for the LiP and MnP genes indicating that further efforts are needed for in depth characterization of the genetic potential of the isolated strains.

Interestingly, the genetic potential of *Alternaria destruens* F10.81, exhibiting the highest PAH removal capacity, was different to that of *Fusarium pseudonygamai* F5.76, showing the lowest removal capacity, by just the presence of the LiP1 gene. Although it cannot be excluded that

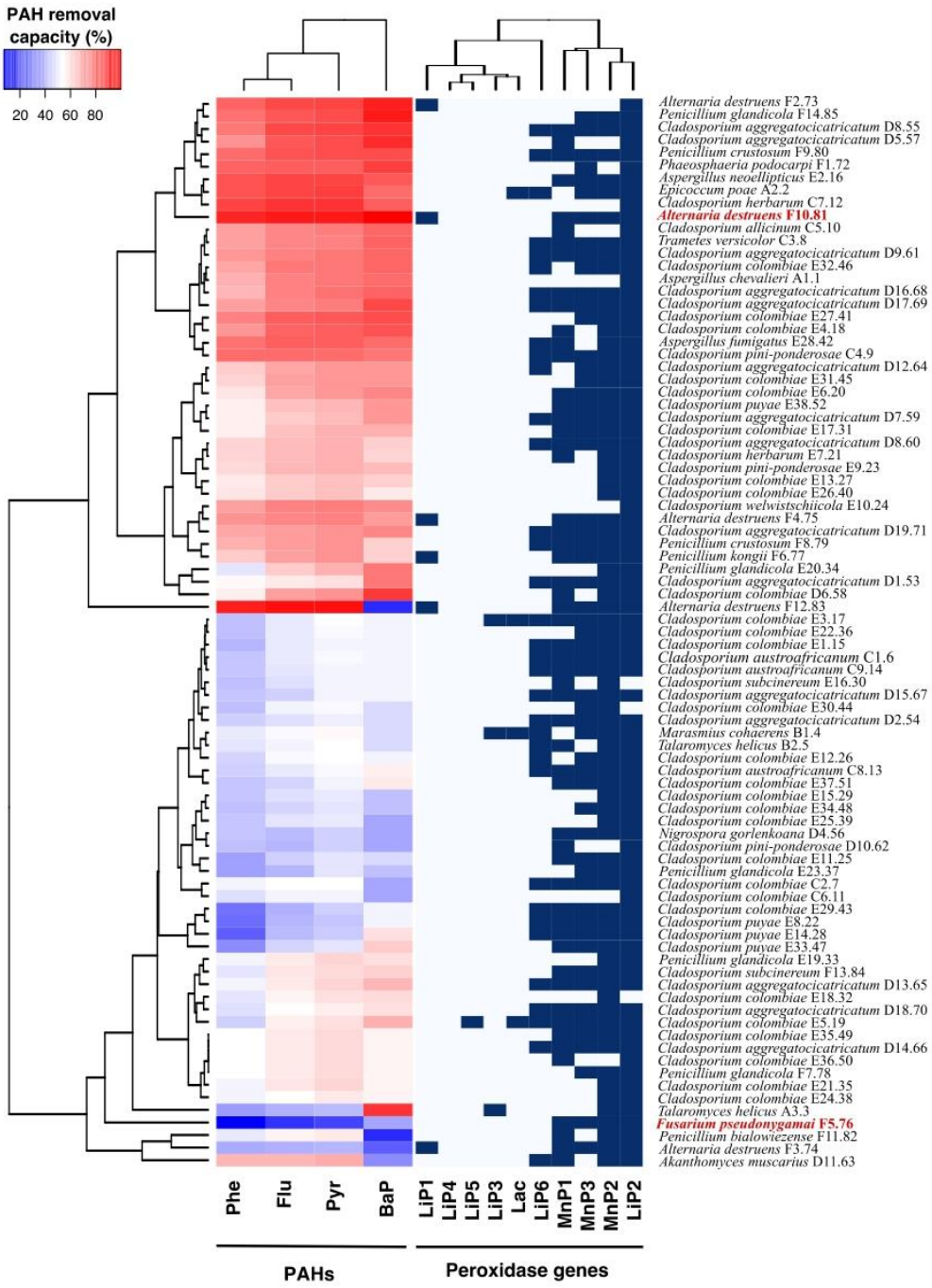

Fig. 4. Comparison of PAHs removal capacity of the isolated fungal strains and their genetic potential. The heatmap is based on similarity index calculated from removal capacity data shown in Fig. 3. Phe, phenanthrene; Flu, fluoranthene; Pyr, pyrene; BaP, benzo[*a*]pyrene. The genetic potential corresponds to the presence (dark blue) of peroxidase genes assessed by PCR targeting manganese peroxidase (MnP1-3), lignin peroxidase (LiP1-6) and laccase (Lac). The absence of the genes is indicated in pale blue. The fungal strains selected for further analysis, corresponding to the highest and lowest removal capacities, are highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the expression of the genes might be controlled by different regulation mechanisms in both strains, such observation suggested that the presence of the LiP, MnP and Lac genes was not linked with the PAH-removal capacity. In the same way, Pearson correlation analysis was unable to establish correlation between the genetic profiles (LiP, MnP and Lac) and PAH-removal capacity of the isolated fungal strains, but confirmation by determining the activity of the enzymes would be required in order to conclude on the involvement of the enzymes in PAH-removal.

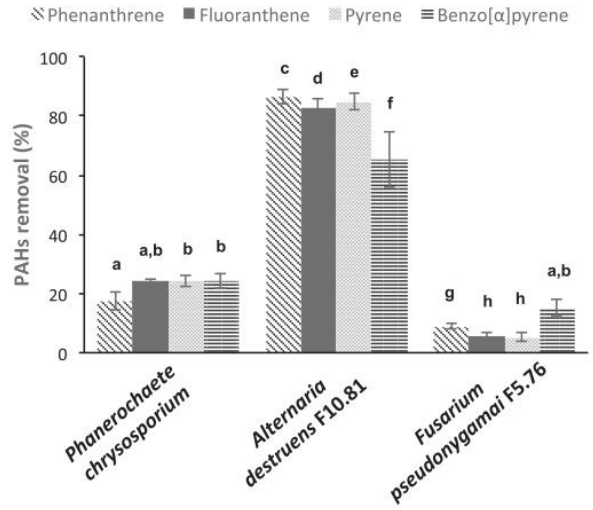

Fig. 5. PAHs removal capacities of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76 compared to that of the reference strain *Phanerochaete chrysosporium*. Means of tree replicates are presented. The bar indicates SD. In each hydrocarbon removal test, mean followed by the same letter do not differ statistically by the Turkey test at 5%.

Anyway, considering the observed genetic potential and despite the inherent bias of the molecular tools used in our study, these results suggested that other mechanisms are probably involved in PAH removal. Thus, further studies are required for elucidating whether the PAH-removal potential of the isolated strains involves degradation and/or sorption mechanisms. The mechanisms described so far involve monooxygenase genes (Cerniglia, 1997; Cerniglia and Sutherland, 2010), particularly the intracellular P450 monooxygenase gene that implies the internalization of PAH into fungal cells (Cerniglia, 1997). In order to determine whether the internalization of PAH and the hyphae PAH transport are mechanisms involved in PAH removal, the *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76 strains were selected, because they exhibited the highest and lowest PAH removal capacities respectively, for further characterization.

3.3. PAH removal characterization of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76

The removal capacity of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F576 was compared with that of *Phanerochaete chrysosporium*, which is the most studied fungi for PAH-degradation (Cao et al., 2020). It serve often as control fungi even for comparing PAH-removal capacity from strains belonging to different phyla (Cao et al., 2020), because it exhibit the capacity to degrade a broad range of organic compounds (Deschler et al., 1998; Duran et al., 2002), including several PAHs (Pointing, 2001). Under our conditions *P. chrysosporium* presented low rates of PAHs removal (<30%), just above to that exhibited by *F. pseudoygamai* F5.76 and around 3 times less to that

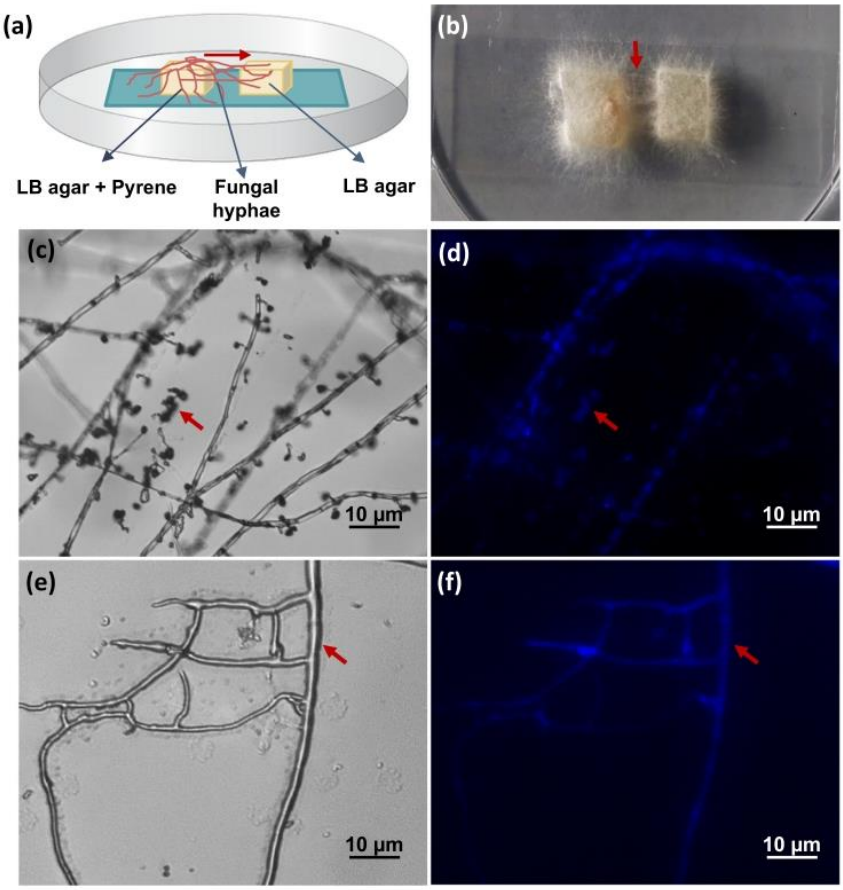

Fig. 6. PAHs internalization in the hyphae of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76. (a) Experimental schema for the detection of internal transport of PAHs along the mycelia. Fungi were inoculated in a 1 cm^3 cube of solid seawater minimal media with 10% LB and 20 mg/L of pyrene. The red arrow indicates the direction of the hyphae growth. (b) Macroscopic observation showing the colonization of *Fusarium pseudonygamai* F5.76 of a piece of media from the other. The red arrow indicates the hyphae forming bridges between the two pieces of media. Observation of *Fusarium pseudonygamai* F5. 76 hyphae after colonization by (c) light microscopy and by (d) fluorescence after exposing to DAPI light. The red arrows show the storage of PAHs into conidia. Observation of *Alternaria destruens* F10.81 hyphae after colonization by (e) light microscopy and by (f) fluorescence after exposing to DAPI light. The red arrows show the homogeneous distribution of PAHs into the hyphae. The microscopic observations were performed at a magnification of 160×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observed for *A. destruens* F10.81 (Fig. 5). In fungi, gene regulation involves complex control mechanisms as those observed for peroxidases genes. It is known that in most fungal strains the LiP, MnP and Lac genes are expressed during the idiophase, the fungal secondary phase, when nitrogen is limited and under the control of complex regulation signals (Junghanns et al., 2005; Kamitsuji et al., 2004; Knop et al., 2015; Duran et al., 2002; Solé et al., 2012), although the expression of MnP genes have been observed under high nitrogen content in fungal genera such as *Pleurotus* and *Trametes* (Kaal et al., 1995; Janusz et al., 2013; Stajić et al., 2006). Thus, the differences observed in removal capacities between the fungal strains are probably due to the medium composition and culture conditions.

It is likely that the seawater medium with high nitrogen content as well as the culture conditions used in our study limited the removal capacities of *P. chrysosporium* (Singh and Chen, 2008) and *F. pseudoygamai* F5.76. In contrast, *A. destruens* F10.81 exhibited removal rates above 80% for all PAHs except for benzo[*a*]pyrene (65% removal; Fig. 5). Such higher PAH removal capacity of *A. destruens* F10.81 suggested that either its genes involved in PAH removal respond to different regulation signals than the other two strains or the PAH removal was performed by other mechanisms. For example, the expression of LiP, MnP and Lac genes has been observed under high nitrogen content in some fungal species (i.e. *Pleurotus ostreatus* and *Trametes trogii*) and even under both high and low nitrogen content for *Dichomitus squalens*, while for other fungal species, such as *P. chrysosporium*, the peroxidase genes are expressed under nitrogen limitation (Janusz et al., 2013; Stajić et al., 2006). The expression of genes involved in PAH-removal even in high nitrogen content might be an asset for the fungal saprotrophic life-style in marine environments where secreted enzymes, such as peroxidases, are likely to be lost by rapid diffusion in the aquatic environment (Richards et al., 2012).

Possible PAH removal has been described through biosorption mechanisms, which include adsorption onto cell surface (Raghukumar et al., 2006) and absorption into the cell (Verdin et al., 2005; Yang et al., 2013). Several studies have demonstrated the capacity of fungi to uptake PAHs (Deng et al., 2010; Wu et al., 2009) and also to transport them along the fungal hyphae (Furuno et al., 2012; Schamfuß et al., 2013). Both strains, *Fusarium pseudoygamai* F5.76 and *Alternaria destruens* F10.81, were able to uptake and transport pyrene (Fig. 6). Clear pyrene containing vacuoles were observed in *F. pseudoygamai* F5.76 (Fig. 6c, d) while pyrene was homogeneously distributed in *A. destruens* F10.81 (Fig. 6e, f) suggesting that the fungal strains have developed different strategies for PAH uptake. It has been demonstrated that the vacuoles serves for possible storage of PAHs as carbon source and for PAH transport along the hyphae allowing the distribution of PAH within the mycelia network (Darrah et al., 2006; Furuno et al., 2012). Consistently, pyrene was also accumulated into conidia in *F. pseudoygamai* F5.76 (Fig. 6c, d), which represents carbon source reserve for the development of conidia as previously reported (Allaway et al., 1997; Bago et al., 2002). In contrast, the homogenous pyrene distribution in *A. destruens* F10.81 (Fig. 6e, f) suggested a diffusion mechanism. Such different pyrene uptake mechanism probably explains the highest removal capacities of *A. destruens* F10.81 in comparison to *F. pseudoygamai* F5.76 (Fig. 5). However, further studies are required to determine whether higher pyrene absorption or internal degradation by monooxygenase (i.e. cytochrome P450) are the underlying physiological mechanisms of PAH removal in *A. destruens* F10.81.

4. Conclusion

The exploration of the cultivable fungal diversity of hydrocarbon-contaminated coastal sediments revealed that coastal sediment hide fungal diversity yet unexplored for their metabolic potential, especially regarding PAH removal capacity. A large proportion of the isolated strains (48%), dispatched within 6 fungal genera, exhibited PAH-tolerance with a removal capacity (degradation and/or sorption) above 60%. Such diversity in PAH-removal capacity represents a functional potential for ecosystem recovery exploitable for bioremediation treatments (Harms et al., 2011). However, the mechanism underlying the PAH-removal capacity (degradation and/or sorption) is unclear because it is probably not related to the presence of extracellular peroxidase genes (LiP, MnP and Lac) and it is strain specific. The comparison of two isolated strains exhibiting contrasted removal capacities showed different PAH-uptake behaviour suggesting that the mechanisms by which fungi perform PAH-uptake might determine the efficiency of PAH-removal. *Alternaria destruens* F10.81, the most efficient PAH-remover (above 80%) was able to internalize pyrene homogenously into the hyphae that contrasted with the behaviour of *Fusarium pseudoygamai* F5.76 in which PAH-vacuoles were observed but exhibiting a PAH-removal capacity below 20%. It is likely that *Alternaria destruens* F10.81 owns features well adapted to PAH-contaminated coastal sediments, which represent potential for the development of a bioremediation process. However, further studies are required to understand the PAH-removal mechanism in order to manage fungal resources to mitigate the effects of PAH contamination.

CRediT authorship contribution statement

Joyce Álvarez-Barragán: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Cristiana Cravo-Laureau:** Conceptualization, Investigation, Resources, Formal analysis, Supervision, Writing – original draft. **Lukas Y. Wick:** Conceptualization, Formal analysis, Supervision, Writing – original draft, Funding acquisition. **Robert Duran:** Conceptualization, Investigation, Resources, Formal analysis, Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter III. Fungi in PAH-contaminated marine sediments: Cultivable diversity and tolerance capacity towards PAH

III.3. Chapter principal results and discussion

As seen in Chapter II, fungi and bacteria have networks of interactions in PAHs polluted coastal sediments. In order to further analyse these interactions, 85 fungi from coastal sediments were isolated in seawater media to keep salinity conditions. The identification, based on the ITS gene sequence, revealed that 83 (97.6%) isolates were members of the phylum Ascomycota and 2 (2.4%) of the phylum Basidiomycota. Among the 85 fungi, 48% of the isolated strains exhibited PAH – tolerance with a removal capacity above 60%. Such results demonstrate that sediments are an important reservoir of PAH - tolerant fungi that has not yet been completely explored. The capacity of fungi to tolerate PAHs was not correlated with the presence of ligninolytic enzymes genes that are related to extracellular degradation of PAHs, suggesting other strategies for PAHs removal, such as intracellular degradation via cytochrome P450 or PAHs storage. Surprisingly, the storage of PAHs was also observed to occur differently between fungi, the fungus with the highest removal rates (*Alternaria destruens* F10.81) store PAHs in the cytoplasm while the fungus with the lowest removal rates (*Fusarium pseudonygamai* F5.76) store PAHs in vacuoles. Further analysis must be done to determine whether these strategies are related to removal capacities or to different PAHs metabolisms. The fungal uptake of PAHs is important not only for decreasing pollutant content in the environment (decreasing environmental impact), but also for the translocation of PAHs via the fungal hyphae resulting in PAHs bioavailable for degrading bacteria in other locations (Furuno et al., 2012). Moreover, it is known that fungi transport not just PAHs but also bacteria that travel along the zone surrounding the hyphae (the “hyphosphere”) for distant locations (Banitz et al., 2013). The potential of the two selected fungi (*Alternaria destruens* F10.81 and *Fusarium pseudoygamai* F5.76) to translocate single bacteria and the possible selection of bacteria within a microbial community by fungi is evaluated in the following chapter (Chapter IV).

Chapter IV: Marine fungal translocation network transport aerobic/anaerobic bacteria from PAHs contaminated sediments

IV.1. Chapter introduction

Considering the uptake of PAHs by fungi as a factor that aid bacteria in PAHs degradation (Banitz et al., 2013), two fungi (*Alternaria destruens* F10.81 and *Fusarium pseudoygamai* F5.76) were chosen for their high and low PAHs removal capacity (described in Chapter III). Furthermore, studies in soil have demonstrate that fungal hyphae can be used by PAHs tolerant bacteria to translocate from one site to another (see "fungal highway" in Chapter I), allowing bacteria to reach unless unaccessible locations (Kohlmeier et al., 2005). As sediments are water-filled environments, PAHs accumulate in hydrophobic patches, which are less accessible to reach by for PAHs degrading bacteria. For this reason, in this chapter (Chapter IV) is evaluated the capacity of fungal hyphae to be used as translocation networks by bacteria through PAHs contaminated sediments (mimicking PAHs polluted environment), which has not yet been described. First, to explore the bacteria transport aptitudes required for the translocation via fungal hyphae, eight bacteria were isolated and challenged to use fungal networks to transport through gaps with different hydrophobicities (air, wet sand, PAHs contaminated sediment and wax) in media containing PAHs. Then, to evaluate bacterial selectivity by fungal mycelia from *A. destrins* F10.81 and *F. pseudoygamai* F5.76 used by bacteria as translocation networks through a PAHs contaminated gap, the diversity of bacterial communities was analyzed. The selectivity of bacteria by fungal translocation networks in sediments is for the first time described. The findings described are congruent with previous studies, which demonstrate bacterial selectivity along with fungal translocation networks (Zhang et al., 2018). To further characterize the selectivity of bacteria by fungal translocation networks, analysis that allow the targeting of specific biomarkers related to fungal and bacterial interactions were carried on. The results from the analysis showed the transport of aerobic and anaerobic bacteria, which was unexpected and suggest the creation of oxic and anoxic niches during the fungal development. To corroborate this hypothesis, analyses of oxygen depletation around the hypha were additionally performed. The information gather in this chapter, give an insight of fungal and bacterial interactions and transport under PAHs contamination stress.

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Chapter IV: Marine fungal translocation network transport aerobic/anaerobic bacteria from PAHs contaminated sediments

IV.2. Marine fungal translocation network transport aerobic/anaerobic bacteria from PAHs contaminated sediments

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IV.1. Abstract

This work present the first evaluation of bacterial transport along fungal mycelia through hydrophobic patches under conditions mimicking marine coastal sediments. The capacity of *Alternaria destruens* F10.81 and *Fusarium pseudoygamai* F5.76 strains to transport isolated bacteria through different gaps (air, water, sand, sediment) in PAHs containing media was first examined, revealing that PAHs tolerance and bacterial motility are required features for transport. The characterization of bacterial communities collected along the fungal mycelia showed that mycelia transport preferential bacteria indicating fungal selectivity relied by specific fungal and bacterial interactions occurring along the hyphosphere. *Spirochaeta litoralis*, known as strictly anaerobic bacteria was identified among the four specific bacterial strains transported by *Alternaria destruens* F10.81. Such result, suggests the formation of anoxic micro-niches during the hyphal development, which was confirmed by observing oxygen depletion around the hyphae during the fungal development. We thus advocate that aerobic bacteria are first transported at the oxic front of mycelia growth and then anaerobic bacteria are transported following the oxygen depletion by fungal growth. The results presented here show new insight of fungal-bacteria interactions in PAHs contaminated sediments paving the way for new questions related to unexplored inter-kingdom interaction mechanisms.

Keywords: fungal translocation network, fungal-bacterial interactions, hyphal development, oxygen consumption, bacterial diversity, anaerobic bacteria

IV.2. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are of major concern for the environment because they are toxic and resilient due their hydrophobic nature (Duran and Cravo-Laureau, 2016). They tend to bind to particles in soil and sediments (Sanglard et al., 1986), creating patches heterogeneously distributed and decreasing their bioavailability (Worrich et al., 2016). Microorganisms and their interactions play a key role in PAHs degradation (Duran and Cravo-Laureau, 2016; McGenity et al., 2012). Among these interactions, fungal bacterial interactions (FBI) have been demonstrated to stimulate PAHs degradation (Worrich et al., 2016).

Bacteria co-exist with fungi as free living organisms, or in the fungal intracellular environment (obligate endobacteria) or both (facultative endobacteria) (Olsson et al., 2017). The number of bacteria able to stablish symbiotic interactions with fungi seems to be limited (Baschien et al., 2009; Lyons et al., 2005; Olsson et al., 2017; Scheublin et al., 2010; Simon et al., 2015; Warmink et al., 2009) indicating a selectivity in FBI. Bacteria associate directly with the fungal hyphae (Baschien et al., 2009; Cuong et al., 2011) living in the hyphosphere, the volume influenced by the hyphae (Olsson et al., 2017). Bacteria colonizing the hyphosphere are essential for the depletion of PAHs (Harms and Wick, 2006; Kohlmeier et al., 2005)

The FBI in the hyphosphere has been demonstrated to be synergetic and mutualistic where both partners get benefit (Olsson et al., 2017). Bacteria influence the metabolism and physiology of fungi (Barron, 2003; Frey-Klett et al., 2011; Netzker et al., 2015), while fungal hyphae transfer nutrients, water and pollutants to bacteria (Banitz et al., 2013; Furuno et al., 2012; Leveau and Preston, 2008; Wick et al., 2007; Worrich et al., 2017). In addition, fungal mycelia has been demonstrated to be used as translocation networks (fungal highways) for the dispersion of bacteria (Abeysinghe et al., 2020; Kohlmeier et al., 2005; Simon et al., 2015), particularly in soil allowing bacteria to colonize different niches and crossing obstacles (Bornyasz et al., 2005; Worrich et al., 2016). The bacteria dispersion via mycelia has been shown to stimulate PAHs degradation when pollutants or bacteria are heterogeneously distributed (Kohlmeier et al., 2005; Worrich et al., 2016). Translocation networks have been largely studied in soils but no attention has been paid on translocation networks in marine sediments because it is assumed that the translocation of bacteria is not limited in aquatic environments (Grossart et al., 2001). Nevertheless, bacteria have to face different obstacles for dispersion in aquatic environments including hydrophobic patches formed by hydrocarbons (Fu et al., 1994), plastics (Biermann et al., 2020) and organic matter aggregates (Maggi and Tang, 2015). It has been demonstrated that fungi extend their mycelia through marine sediments providing space and support for bacteria (Booth et al., 2019). In marine ecosystems a large diversity of fungi has been

described, the vast majority of fungi belonging to the Ascomycota and Basidiomycota phyla (Álvarez-Barragán et al., 2021; Xu et al., 2014; Zhang et al., 2015). We hypothesized that to overcome obstacles, bacteria can use fungi as translocation networks in marine environments through FBI probably selecting bacteria. We setup a specific experimental design for testing i) the capacity of bacteria to use fungal translocation networks to overcome patches formed by PAHs contaminated sediments, ii) the specificity and selectivity of the FBI.

IV.3. Material and methods

IV.3.1. Media and sediments

To mimic marine conditions, seawater minimal media (swMM) was used with the following composition: KCl 0.75 g/L, CaCl·2H2O 1.47 g/L, NH4Cl 1.5 g/L, MgSO4·7H2O 6.64 g/L, NaCl 20 g/L, Na2CO3 0.265 g/L, 1 mL of trace elements solution (H3BO3 300 mg/L, FeSO4·7H2O 1.1 g/L, CoCl2·6H2O 190 mg/L, MnCl2·2H2O 50 mg/L, ZnCl2 42 mg/L, NiCl2·6H2O 24 mg/L, Na2MoO4·2H2O 2mg/L), 1mL of vitamin solution (biotine 2 mg/L, ρ-aminobenzoate 10 mg/L, thiamine 10 mg/L, pantothenate 5 mg/L, pyridoxamine 50 mg/L, vitamin B12 20 mg/L, nicotinate 20 mg/L), and 100 µL of phosphate buffer 50 mM. The pH was adjusted with HCl to 6.5. Chemicals were purchased from Sigma Aldrich (Germany).

IV.3.2. Fungal selection

Two fungi of high and low PAH-removal capacity (*A. destruens* F10.81 and *F. pseudonygamai* F5.76 respectively) were isolated from a heavily PAH contaminated coastal sediment (Etang de Berre, France) previously described (Álvarez-Barragán et al., 2021). Fungi were mantained in MDA (malt dextrose agar) elaborated with swMM to keep salinity conditions (MDAsw).

IV.3.3. Bacteria isolation

Sediments from Etang de Berre were inoculated directly in LB media prepared with swMM (LBsw) to keep salinity. After 48h of growht, eight bacteria with different phenotipic colony formation were selected and reinoculated in separated plates of LBsw media until pure cultures were obtained. Bacteria were evaluated for swimming and swarming motility and their capacity of growth in 30 mgL-1 of pyrene as only carbon source. DNA from bacteria was extracted from isolated colonies in swLB media using the QUIAGEN DNeasy® UltraClean® Microbial Kit (Cat. No. 12224-40) following the manufacturer instructions. Bacteria were identified by the 16S gene amplifying a consensus sequence with the forward primer 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse primer 1387r (5′-

GGG CGG WGT GTA CAA GGC-3') with an approximate length of 1,300 bp (Marchesi et al., 1998). The PCR reaction mix was prepared with 1 µL of extracted DNA in 9.5 µL of DEPC-treated water, 1 µL of each primer (20 µM), 12.5 µL AmpliTaq Gold 360 Master Mix 2X (Thermo Fisher Scientific, USA). The amplification was performed through 35 cycles of 95 °C (45 s), 58 °C (45 s) and 72 °C (1 min), with a previous activation start of 95 °C (10 min) and final extension step at 72 °C (10 min). Amplified fragments were sequenced at the Eurofins platform (France). Sequence data were edited using Chromas Pro version 1.34. For identification, 16S rRNA sequences were compared with NCBI (National Centre for Biotechnology Information; http://www.ncbi.nlm.nih.gov) database. The sequences determined in this study have been submitted to the NCBI database and assigned Accession numbers: OK128316 to OK128323.

IV.3.4. Experimental system

Bioessays were assesd by placing in Petri dishes (5 of diameter) fulled with swMM containing 10% of swLB media and 30 mgL-1 of pyrene, letting separation gaps of 2 mm that create two proportions: 1.4 and 3.4 cm (Figure 1A-a). The gaps were filled either with sterile wet sand, sterile sediment, wax or let empty (emulating air pores) to mimic spatially distinct barriers as in heterogenic contaminated sediment (Figure 1B). The barriers were chosen due to the differences of hydrophobic and hydrophilic characteristics.

Actively growing mycelia was picked up from PDA dishes and inoculated at 3 mm from the border in the bigger side and it was let growh until the hypha grow 1 cm long (48 hours) (Figure 1A-b). Bacteria (pregrown for 24 h in liquid swLB cointaining 30mgL-1 pyrene) were inoculated close the micelia growht (Figure 1A-c). The setups were incubated in darkness for 10 days until the mycelia growht beyond the barriers (Figure 1A-d). Bacterial translocation along hyphae was assessed by microscopic observation. All experiments were carried out in triplicate.

IV.3.5. Bacterial communities transported by translocation networks

The same setups previously descrived were used with *F. pseudonygamai* F5.76 and *A. destruens* F10.81 and glass fibbers (abiotic control) as translocation networks and a sediment gap was placed. To evaluate the transport and selection of bacterial communities along the translocation networks, sediments from Etang the Berre or Canal Vieil were used as inoculums. The experimental system was as previously described. The sampling was performed at four points: in the inoculum, after the inoculum, in the gap and after the gap (Figure 1C). Fungal growht and bacterial colonies were observed after incubation time (Figure 1D) and all samples were collected at the same moment and placed in independent Eppendroff tubes. To asses the cultuvable bacterial community in the media used,

sediment from both sources was inoculated in Petri dishes without translocation network and recovered in the same collection times. All samples were performed in triplicate.

IV.3.6. DNA extraction and Illumina Miseq sequencing

DNA from the setups at each sampling point and from samples of sediments from Etang de Berre and Canal Vieil were extracted with PowerSoil DNA Isolation Kit (MoBio Laboratories) according to the manufacturer's recommendations. PCR amplification of the V3-V4 hypervariable regions (344F–801R) with fused MiSeq adapters and heterogeneity spacers in a 25-µl PCR. The hypervariable region of the 16S rRNA genes were performed using the bacteria primers 344F-V3V4 (5'-ACGGRAGGCAGCAG-3') and 801R-V3V4 (5'-TACCAGGGTATCTAATCCT-3'). The reverse and forward primers included Illumina adapters. Before amplification, the concentration of DNA was determined with a Quant-It kit in microplate in order to normalize the following amplification. Amplifications were performed in triplicate using AmpliTaq Gold 360 Master Mix (Applied Biosystems) to prepare 25 µL reactions containing 2 ng of DNA of Bacteria. Cycling conditions were: an initial denaturation step at 95°C for 10 minutes, followed by 35 cycles with denaturation step at 95°C for 30 s, hybridization step at 60°C for 30 s, and elongation step at 72°C for 40 s or 30 s, after which a final elongation step at 72°C for 10 minutes was performed. Sequencing by synthesis was carried out at the Genotoul platform (Toulouse, France) with Illumina-MiSeq. The 2x 250 paired-end sequencing gave a full length ~460 bp for the V3-V4 region.

IV.3.7. Sequence processing and ASV delimitation

The open source software Qiime2 (Bolyen et al., 2019) was used for 16S. Sequencing resulted in 2985157 raw reads. The dataset was first demultiplexed and dada2 (Callahan et al., 2016) was used to control reads quality and infer amplicon sequence variants (ASVs) (Callahan et al., 2017). Default settings were used except that reverse reads were truncated to 230 base pairs before merging. This denoising step resulted in a 1699056 trimed sequences dataset. The taxonomy was assigned to the ASVs with a fixed sequence similarity threshold of 97% using the SILVA database (Quast et al., 2012) release 132 for 16S sequences. Then, the dataset was mitochondria, chloroplasts and unassigned (at the domain level) filtered. Bioinformatics treatment resulted in 1882 prokaryotic ASVs. Phylogenetic trees were constructed using FastTree (Price et al., 2010) in the Qiime2 phylogeny plugin. The phylogenetic tree was made ultrametric for the following analyses using the "chronos" function in the R package ape (Paradis et al., 2004) with branch lengths representing relative time and all tips equidistant from the root. The dataset encompassed 84 samples. The complete dataset was deposited in the NCBI Sequence Read Archive (SRA) database and is available under the BioProject ID PRJNA761592 (Submission ID: SUB10342502).

IV.3.8. Statistics

Statistical analysis of ASV richness of each sample and diversity index Shannon were calculated comparing translocation networks and sampling points in R software version 4.0.3 (http://www.r-project.org/). Unconstrained non-metric multidimensional scaling (NMDS) for sampling points and translocation networks was performed using Bray-Curtis distances in the metaMDS function (k=3) of the vegan package 2.5-6. Differences of translocation network effects on the microbial communities were tested using permutational multivariance analysis of variance (PERMANOVA) with 9999 permutations. Statistical differences in relative abundance of phylums between same groups were using two sample homoscedastic *t*-test, two-taile. To identify the significant abundant bacterial ASVs from both sediments between translocation networks after the gap, finear discriminant analysis effect size (LEfSe) analysis was applied, the analysis was run in Hutlab Galaxy (https://huttenhower.sph.harvard.edu/galaxy/).

IV.3.9. In vivo mapping of oxygen concentration in the mycopshere:

A plannar optode sensor foil (SF-RPSu4, PreSens, Regensburg, Germany) and a VisiSens TD Detector Unit DU02 (PreSens, Germany) were used for time-lapse optical mapping of oxygen concentration in the mycopshere of *A. destruens F10.81* and *F. pseudonygamai F5.76* (cf. SI and Figure 5A for the calibration procedure of the sensor foils and calibration data). Shortly, a sensor foil (1 cm × 1 cm) was glued to the bottom of a Petri dish (µ-Dish 35 mm, low, Ibidi, Gräfelfing, Germany) using silicon glue. The Petri dish was kept in the dark to let the silicon glue cure overnight. After that a circular agarose pad (Ø: 18 mm; h: 0.7 mm, swMM + 10% of swLB media + 30 mg L^{-1} of pyrene) was prepared as described by Young et al. (2012) and placed on top of the sensor foil. A circular agarose pad (Ø: 10 mm) with the fungal inoculum was then placed at 1 mm distance from the oxygen sensing pad. An observation area (1.8 × 2.5 mm, 4 mm from the fungal inoculum) was chosen in the oxygen monitoring pad to monitor oxygen dynamics during fungal habitat colonization. Specifically, images of the observation area were taken by the detector unit (exposure 100000 µm, gain 10) with a 1 h imaging interval during the 96-hour incubation. Time-lapse oxygen mapping experiments were performed in triplicate each for *A. destruens F10.81* and *F. pseudonygamai F5.76*. Another, identical setup was used to map the spatial oxygen and mycelial distribution of fungus *A. destruens F10.81* after 48 h incubation of the whole oxygen monitoring area (1 cm × 1 cm). To screen for oxygen in the 1 cm x 1 cm sensor foil, 30 spots (cf. 1.8 × 2.5 mm per spot) were imaged by the VisiSens TD Detector Unit as follows: the position of VisiSens TD Detector Unit was fixed while the position of the Petri dish was precisely shifted using a Nikon microscope stage (Nikon AZ100, Tokyo, Japan) controled by the microscope imaging softerware (large image accuisition, NIS-ELEMENTS, Basic Research, Nikon). After that, microscopic images were immediately taken to visualize mycelial distribution in the direct vicinity of the sensor

foil using the Nikon microscope (AZ100, Tokyo, Japan). Briefly, bright-field images were taken by the Nikon micrscope with 5× objective and 3× microsope magnification (a total magnification of 15 ×, 1.78 × 2.22 mm per image) with LED illumination (exposure time 30 ms). Images taken by the VisiSens TD Detector Unit (spatial O_2 information) and Nikon microscope (mycelial distribution) were grouped separately, and then the size of the two grouped images were stadardized to 1.87 µm per pixel using ImageJ. The standardized images were then overlaied in ImageJ with 50% opacity to combine the spatial oxygen information and the mycelial distribution in the microcosm.

IV.4. Results and discussion

IV.4.1. Bacterial requirements for translocation in fungal networks

In order to determine the needed characteristics of bacteria able to use the fungal highway as translocation network, eight bacteria isolated from coastal sediments were tested (Table IV.1). Seven bacteria were able to tolerate the presence of pyrene, while the bacterium B2 sensitive to pyrene, served as control for PAHs growth capacity (Table IV.1). All bacteria were motile (swimming or swarming) except the bacterium B3 selected as control for motility (Table IV.1).

The mycelia of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76, with high and low removal PAHs capacity respectively (Álvarez-Barragán et al., 2021), were used as translocation networks to cross different physical barriers (Figure IV.1B). The bacterial transport along the translocation networks was observed only with motile bacteria able to tolerate the presence of pyrene (Table IV.1). These results showed that motility is a crucial characteristic of bacteria for transport along the translocation networks, as previously reported for isolated bacteria (Kohlmeier et al., 2005). But information with complex bacterial communities is still scarce (Zhang et al., 2018b). Although bacteria were transported through the gaps irrespective of the hydrophobicity, only three bacteria were transported through the air gap. Such observation was surprising since several studies reported the capacity of motile bacteria to cross air gaps through fungal translocation networks (Furuno et al., 2012; Kohlmeier et al., 2005; Simon et al., 2015). It is likely that fungal networks require a physical support for bacterial translocation (Table IV.1).

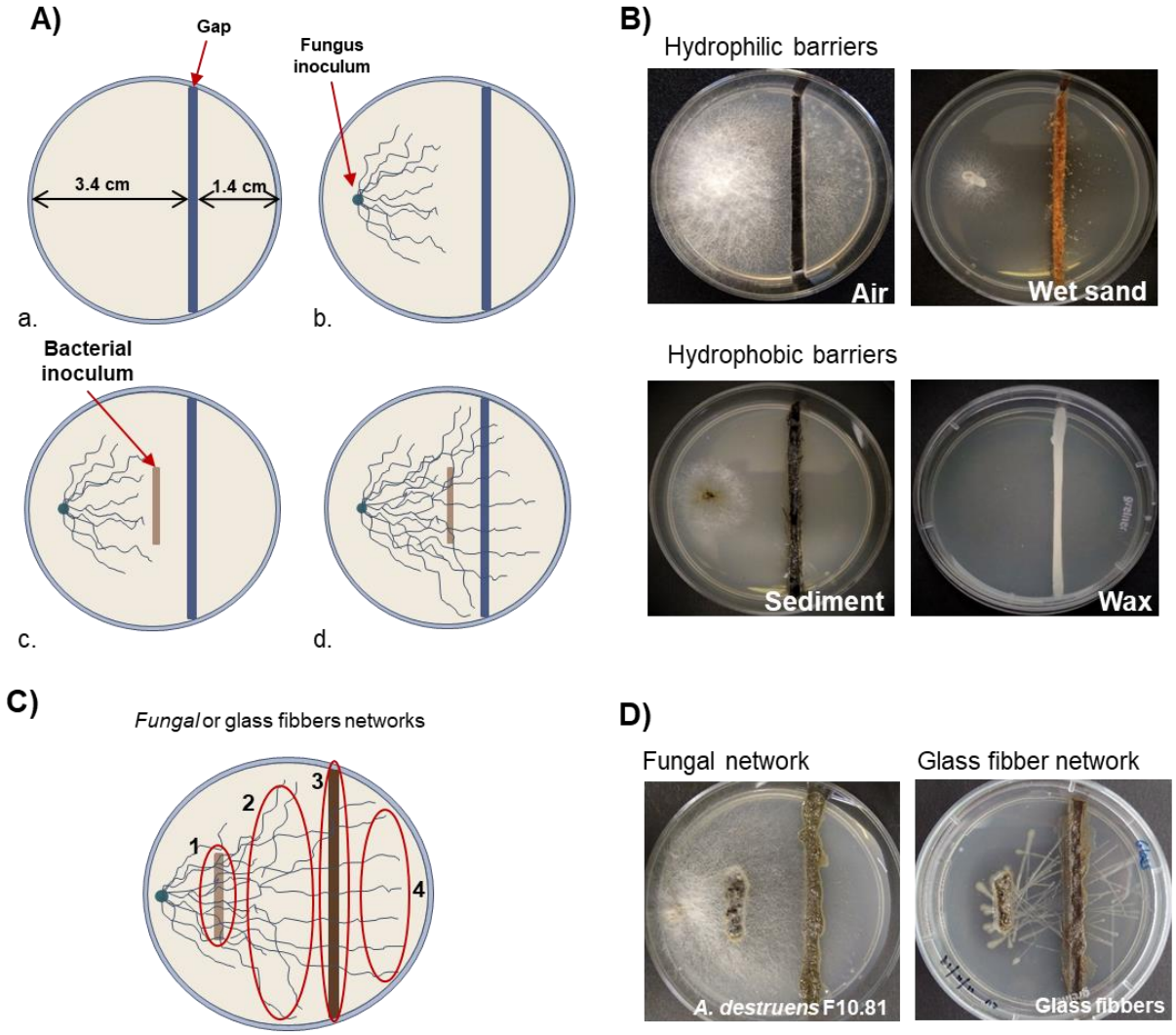

Figure IV.1. Experimental design for bacteria (A, B) and bacterial communities (C, D) transport through translocation networks. A) Isolated bacteria translocated through physical barriers (air, wet sand, sediment, and wax) a. before inoculation, b. 48h after fungus inoculation, c. the bacteria inoculation, d. 10 days after bacterial inoculation, the presence of bacteria was observed after the gap. B) Pictures showing the different gaps used. C) Bacterial communities transport through fungal or glass fibbers translocation networks. The sampling points are indicated: sediment inoculum (1), before the gap (2), sediment gap (3), and after the gap (4). D) Pictures showing the experimental device after incubation in presence of fungi and glass fibbers.

Indeed, motile bacteria were translocated through hydrophilic and hydrophobic gaps in both fungal translocation networks, which may be explained by the capacity of fungi to modify their surface hydrophobicity (Unestam and Sun, 1995). The main difference between *A. destruens* F10.81 and *F. pseudonygamai* F5.76 translocation networks relies in the capacity of bacterium B8 to cross hydrophobic gaps with *F. pseudonygamai* F5.76 whereas it crosses the hydrophilic wet sand gap with *A. destruens* F10.81. The mechanism modifying fungal surface involves the production of various hydrophobins (Wösten et al., 1994) probably explaining the differences observed between fungal translocation networks. As *A. destruens* F10.81 and *F. pseudonygamai* F5.76 were able to translocate bacteria, they were used to study translocation of microbial communities.

Table IV.1. Bacterial characteristics and their translocation capacities through the hyphae of *F. pseudonygamai* F5.76 (F) and *A. destruens* F10.58 (A).

	Bacteria identification	Bacterial motility and growth in PAHs			Transport in fungal bridge through gap***							
		Growth on pyrene**	Swimming* (cm - 16h)	Swarming* (cm -16h)	Air gap		Wet sand gap		Sediment gap		Wax gap	
					F	*A*	*F*	*A*	*F*	*A*	*F*	*A*
B1	*Pseudomonas* sp.	+	3.5 ± 0.4	7.4 ± 1.2	-	-	+	+	+	+	+	+
B2	*Bacillus thuringiensis*	-	2.8 ± 0.3	-	-	-	-	-	-	-	-	-
B3	*Bacillus cereus*	+ +	-	-	-	-	-	-	-	-	-	-
B4	*Vibrio anguillarum*	+ +	3.8 ± 0.2	-	-	-	-	+	+	+	+	+
B5	*Vibrio anguillarum*	+ +	3.5 ± 0.3	-	+	+	+	+	+	+	+	+
B6	*Pseudomonas* sp.	+	4.1 ± 0.2	8.1 ± 0.1	-	+	+	+	+	+	+	+
B7	*Bacillus subtilis*	+ +	4.7 ± 0.3	2.8 ± 0.8	-	+	+	+	+	+	+	+
B8	*Exiguobacterium* sp.	+ +	1.9 ± 0.2	-	-	-	-	+	+	-	+	-

* Cultured along 16h at 25 °C in swimming and swarming media; ** Sea water minimal media added with 30 mg/L of pyrene as unique carbon source; *** sw-MM added with 10% LB and 30 mgL-1 of pyrene; *F* – *F. pseudonygamai* F5.76; *A* – *A. destruens* F10.58.

IV.4.2. Bacterial selection by fungal translocation network

Specific experimental design was setup allowing the analysis of bacterial transport from the inoculum and at different sampling points through translocation networks crossing a sediment gap (Figure 1C). Two different sediments (Canal Vieil and Etang de Berre) were selected as inoculum in order to determine the specificity of the fungi-bacteria interaction. The bacterial communities collected on the sampling points on the fungal translocation networks (*A. destruens* F10.81 and *F. pseudonygamai* F5.76) were compared to those obtained with a glass fibber translocation network control. The sequencing of the bacterial communities provided 2985157 reads of high quality sequences, after trimming the obtained 1699056 sequences were distributed within 1882 ASVs (Table IV.2).

For the Canal Vieil sediment, the inoculum showed similar Shannon index (H) irrespective of the presence or not of a translocation network (Table IV.2). In contrast, for Etang de Berre sediment, the Shannon index (H) of the inoculum was significantly higher (*t*-test, *p*-value < 0.05) for *F. pseudonygamai* F5.76, lower for glass fibbers (*t*-test, *p*-value < 0.05), and similar for *A. destruens* F10.81. Thus, the presence of translocation networks had different effect according to the origin of the microbial community. Indeed previous studies have shown that the microbial community composition is dependent on physical-chemical parameters (Stauffert et al., 2013), which probably affect the bacterial population able to be translocated. For *A. destruens* F10.81 translocation network, the Shannon index (H) was significantly different (*t*-test, *p*-value < 0.05) along the sampling points only for Etang de Berre sediment (Table 2). For *F. pseudonygamai* F5.76 translocation network, the Shannon index (H) was significantly lower (*t*-test, *p*-value < 0.05) in the gap and after the gap compared to the inoculum. For glass fibbers translocation network, the Shannon index (H) was significantly lower (*t*-test, *p*-value < 0.05) only for after the gap with Etang de Berre sediment. Thus, the nature of the translocation network affect the diversity of microbial communities along the sampling points suggesting a selection of bacterial communities related to fungi-bacteria interaction. Consistently, several studies have demonstrated fungal-bacterial interactions (FBI) involving complex molecular mechanisms (Deveau et al., 2018; Schmidt et al., 2016), which might results on bacterial community selection (Zhang et al., 2018b). In order to characterize the FBI and demonstrate bacterial selection, the structure of the bacterial community was analysed.

Table IV.2. Alpha diversity indices of the translocated bacterial communities obtained at the different sampling points transported along different translocation networks

Indices	Canal Vieil													
	In situ	Cultivable[1]	*A. destruens* F10.58				*F. pseudonygamai* F5.76				Glass fiber			
			Inoculum[2]	Before[2]	Gap[2]	After[2]	Inoculum[2]	Before[2]	Gap[2]	After[2]	Inoculum[2]	Before[2]	Gap[2]	After[2]
Reads	32 071 ± 1 839	37 147 ± 3 504	27 007 ± 2 796	41 234 ± 3 659	35 335 ± 1 367	37 752 ± 7 157	16 819 ± 12 089	32 294 ± 3 416	33 735 ± 4 715	38 393 ± 5 366	35 395 ± 5 536	35 264 ± 7 549	32 790 ± 1 520	38 183 ± 3 282
Trimmed sequences	11 107 ± 1 186	16 415 ± 1 442	13 563 ± 2 287	24 005 ± 2 965	21 142 ± 941	23 301 ± 3 487	6 295 ± 5 035	16 605 ± 3 743	18 554 ± 3 233	27 196 ± 2 457	17 680 ± 4 013	22 502 ± 4 570	18 459 ± 858	23 127 ± 3 501
Richness (ASV)	260.67 ± 76.22	116 ± 43.49	102.33 ± 14.04	26 ± 15.87	31.33 ± 8.5	35 ± 20.42	81.67 ± 51.29	41.33 ± 4.51	43.67 ± 35.8	17.67 ± 8.96	114.67 ± 23.12	48.67 ± 13.5	41.67 ± 8.96	42.33 ± 24.21
Shannon (H)	**4.06 ± 0.84**	**2.86 ± 0.42**	**2.82 ± 0.35**	**1.15 ± 0.66**	**1.54 ± 0.32**	**1.46 ± 0.31**	**3.32 ± 0.44**	**1.43 ± 0.5**	**1.35 ± 0.13**	**0.22 ± 0.2**	**2.76 ± 0.49**	**1.09 ± 0.22**	**1.56 ± 0.38**	**1.62 ± 0.93**

Indices	Etang de Berre													
	In situ	Cultivable[1]	*A. destruens* F10.58				*F. pseudonygamai* F5.76				Glass fiber			
			Inoculum[2]	Before[2]	Gap[2]	After[2]	Inoculum[2]	Before[2]	Gap[2]	After[2]	Inoculum[2]	Before[2]	Gap[2]	After[2]
Reads	33 049 ± 8 068	40 045 ± 5 648	34 298 ± 9 765	43 494 ± 1 679	45 395 ± 4 033	43 691 ± 17 164	35 170 ± 8 974	39 552 ± 10 649	38 288 ± 7 972	32 874 ± 8 318	33 662 ± 10 307	36 848 ± 5 798	31 662 ± 8 488	33 606 ± 5 086
Trimmed sequences	10 631 ± 2 215	18 188 ± 3 109	14 807 ± 4 102	32 020 ± 1 798	34 536 ± 1 447	26 942 ± 2 904	13 780 ± 2 564	23 353 ± 6 129	22 177 ± 4 660	24 757 ± 7 804	15 703 ± 5 506	24 347 ± 6 580	20 121 ± 4 259	25 040 ± 5 066
Observed	323 ± 40.15	110.33 ± 54.63	109 ± 25.24	28.67 ± 9.61	6.67 ± 5.51	25.67 ± 22.18	129 ± 12.29	64.67 ± 72.28	27.67 ± 4.51	36.67 ± 37.54	74.33 ± 11.37	33.33 ± 14.47	52.67 ± 50.58	38.33 ± 25.11
Shannon (H)	**4.94 ± 0.16**	**2.66 ± 0.27**	**3.14 ± 0.07**	**0.7 ± 0.21**	**0.18 ± 0.09**	**0.23 ± 0.17**	**3.42 ± 0.05**	**1.36 ± 0.92**	**1.31 ± 0.2**	**0.43 ± 0.48**	**2.43 ± 0.32**	**1.32 ± 0.5**	**1.05 ± 0.83**	**0.46 ± 0.24**

Analysis were performed in triplicate; [1] Cultured along 5 days at 25 °C in sea water minimal media added with 10% LB and 30 mg/L of Pyrene; [2] Indicate sampling points in the fungal translocation network setup.

The bacterial communities of the inocula from both Canal Vieil and Etang de Berre sediments were different from those collected along the sampling points (before the gap, in the gap and after the gap) on the translocation networks (Figure IV.2A). For Canal Vieil the only significant difference (PERMANOVA, *p*-value < 0.05) was observed between bacterial communities before and after the gap on the *F. pseudonygamai* F5.76 translocation network (Figure IV.2A). In contrast, for Etang de Berre bacterial communities were not different before and after the gap for fungal translocation networks (PERMANOVA, *p*-value > 0.05) while they were different for the glass fibber translocation network (PERMANOVA, *p*-value < 0.05) (Figure IV.2A). Consistently with alpha diversity analysis, it is likely that the selection of bacteria was dependent on the origin of microbial community and the translocation network.

The composition of bacterial communities from Etang de Berre and Canal Vieil sediments were different (PERMANOVA, *p*-value < 0.001) (Figure IV.2B). Despite the fact that the bacterial communities of the inoculum from Etang de Berre and Canal Vieil were different irrespective of the translocation network, the bacterial communities were similar after the gap with the fungal translocation networks for both sediments (*A. destruens* F10.81, *F. pseudonygamai* F5.76, PERMANOVA, *p*-value > 0.05) (Figure IV.2B). Such observation further supports the selection of bacterial communities by the fungal translocation network, which was consistent with the study showing that fungal networks shape bacterial community structures (Zhang et al., 2018b).

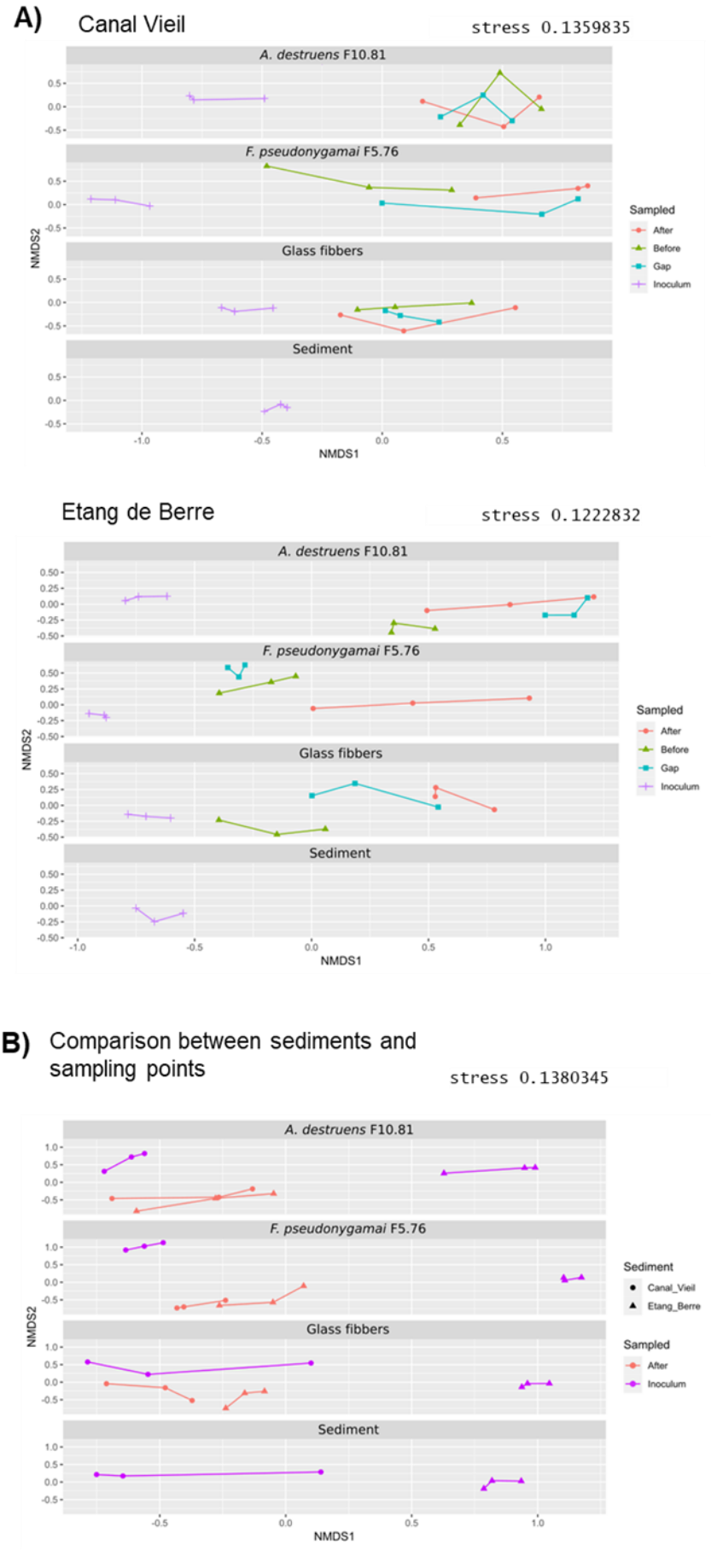

Figure IV.2. Comparison of bacterial communities by non-metric multidimensional scaling (NMDS). The NMDSs, based on Bray-Curtis pairwise dissimilarity, were performed including all samples and then dispatched according to the translocation networks for representation. A) Comparison of bacterial communities from the different sampling points in each translocation networks with sediments from Canal Vieil and Etang de Berre. B) Comparison of bacterial communities obtained from Canal Vieil and Etang de Berre sediments in each translocation networks at inoculum and after the gap sampling points.

Consistently, the initial bacterial community composition of Canal Vieil sediment were different (PERMANOVA, *p*-value < 0.001) to that of the Etang de Berre (Figure IV.3). The bacterial community from Canal Vieil of the cultivable diversity from the sediment was dominated by *Pseudoalteromonas*, *Arcobacter*, *Vribrio* and *Marinomonas* (representing more than 93 ± 2%), while that from Etang de Berre was dominated by *Pseudoalteromonas*, *Arcobacter*, *Oceanospirilum*, *Amphritea*, and *Celeribacter* (representing more than 94 ± 1.4%). Noteworthy, except for *Amphritea* and *Celeribacter*, the members of all the other genera have been described as facultative anaerobes (van Belkum, 2006). The presence of the fungi modified the bacterial composition (PERMANOVA. *p*-values < 0.05) increasing *Labilibacter* and *Arcobacter* abundances from Canal Vieil inoculum while *Celeribacter*, *Spirochaeta* and *Marinifilum* were more abundant in the inoculum from Etang de Berre (Figure IV.3). However, the composition of the inoculum with glass fibbers was unchanged (PERMANOVA, *p*-values > 0.05) in comparison to sediments. This observation suggested that the presence of fungi create specific conditions favoring the growth of such microorganisms under the experimental conditions. It is important to note that most of these dominant genera are known to be motile (van Belkum, 2006), which is a feature that has been shown to be crucial for hyphosphaere colonization (Zhang et al., 2018b). In addition, these genera have probably the capacity to resist to PAHs and grow fast in the used cultured media facilitating the colonization. Indeed, *Amphritea, Arcobacter*, *Celeribacter, Marinifilum, Marinomonas*, *Oceanospirillum, Pseudoalteromonas, Spirochaeta*, and *Vibrio* are known to adopt a K-strategy, fast growing in presence of hydrocarbons (Chronopoulou et al., 2015; Hedlund and Staley, 2001; Lai et al., 2014; Miller et al., 2019; N. NuñAl et al., 2017; Terrisse et al., 2017; Xiong et al., 2015).

The bacterial composition was modified in the sampling points (before, in the gap and after) along the translocation networks in agreement with NMDS. As a result, *Pseudoalteromonas* was found dominant after the gap for all translocation networks. Obviously, *Pseudoalteromonas* was the most adapted to the experimental conditions allowing it to fully cover the hyphosphere. Nevertheless, different genera were observed according to the translocation networks represented at low abundance after the gap (Figure IV.3), which might represent selection of specific genera related to FBI, consistent with alpha diversity observations.

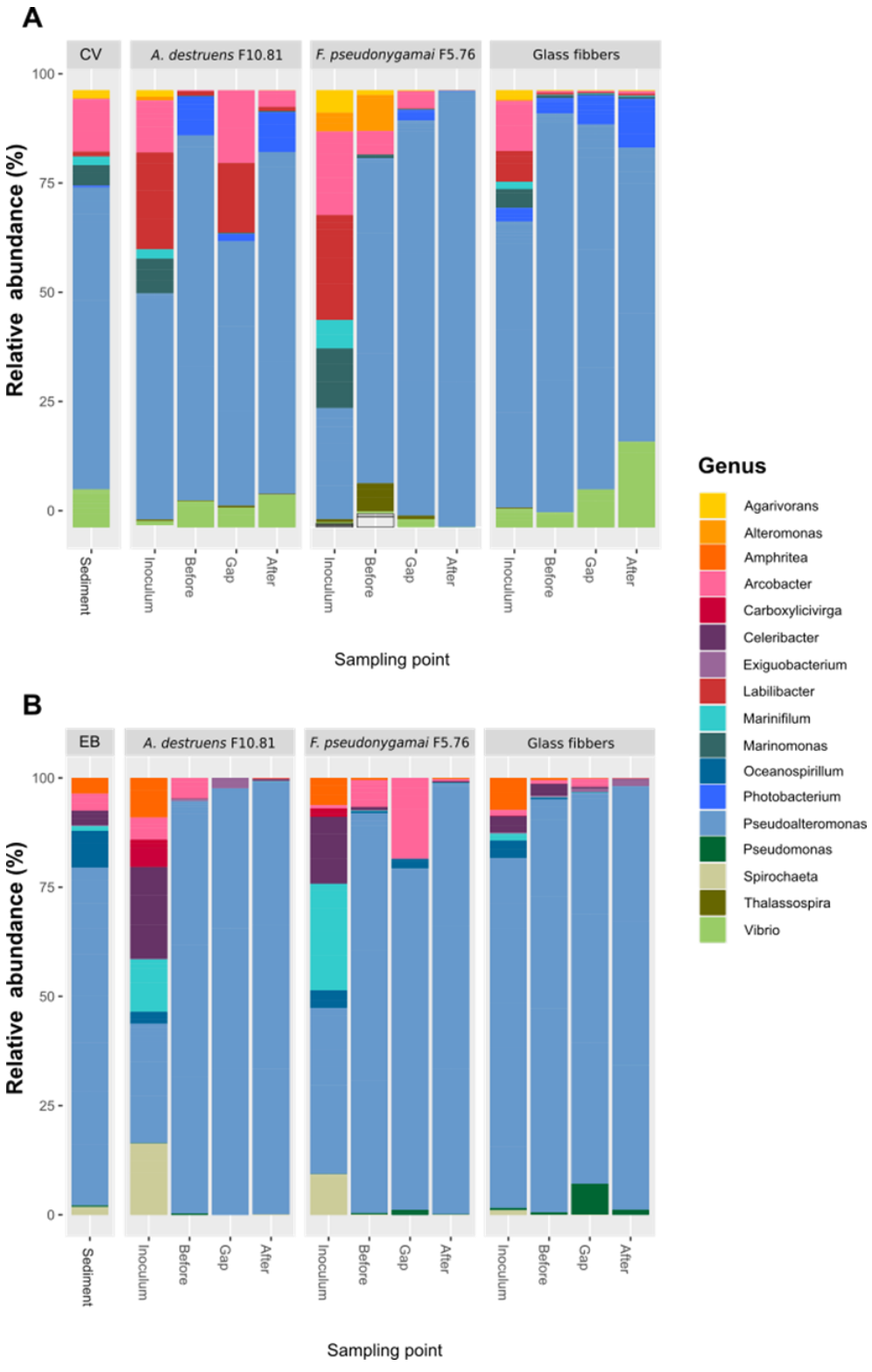

Figure IV.3. Composition of the translocated bacterial communities obtained at the different sampling points with *A. destruens* F10.81, *F. pseudonygamai* F5.76, and glass fibbers translocation networks from sediments of A) Canal Vieil and B) Etang de Berre. The analysis was performed at the genus level applying a threshold similarity of 97% for OTU identification. Only genera with more than 0.1% of relative abundance are presented.

IV.4.3. Specific association between bacteria and fungi

In order to identify the ASVs specifically associated to a translocation network the LEfSe analysis was performed considering both sediments after the gap. LEfSe revealed statistically significant more abundant ASVs for a condition that have been proposed as a biomarker (Segata et al., 2011).

When comparing fungal translocation networks with glass fibbers, eight different biomarkers were identified for glass fibbers and five for *A. destruens* F10.81 (Figure IV.4 A, B). When comparing *A. destruens* F10.81 and *F. pseudonygamai* F5.76 translocation networks, one biomarker was found for *F. pseudonygamai* F5.76 (ASV affiliated to *Pseudomonas* sp.) and six biomarkers for *A. destruens* F10.81 (Figure IV.4C). The different number of specific biomarkers for fungal translocation networks can be explained by their different pyrene removal capacities (Álvarez-Barragán et al., 2021), even if other parameters cannot be excluded. Interestingly four ASVs identified as biomarkers for *A. destruens* F10.81 were found common when comparing either with glass fibbers (Figure IV.4B) or *F. pseudonygamai* F5.76 (Figure IV.4 C), suggesting that they have been specifically selected along the *A. destruens* F10.81 translocation network. These ASVs are related to *Thalassospira* sp., *Spirochaeta litoralis*, *Arcobacter* sp. and *Vibrio* sp. All these genus have been detected in marine sediments and with the presence of hydrocarbons (Hedlund and Staley, 2001; Llobet-Brossa et al., 1998; Paissé et al., 2008; Ye et al., 2019; Zhao et al., 2010), and to the best of our knowledge they have been not described interacting with *Alternaria* sp. The selection of bacteria by fungi has been described to relies on the hyphal production of compounds with either chemio-attractive effect (Deveau et al., 2010) or promoting bacterial growth (Bravo et al., 2013), while fungi receives vitamins from bacteria (Abeysinghe et al., 2020). It was surprising that *Spirochaeta litoralis* was identified as a biomarker for translocation network under our experimental aerobic conditions because it is known as strict anaerobic bacteria (Hespell and Canale-Parola, 1970; Subhash and Lee, 2017). Such observation suggested that anaerobic microniches were created along the fungal translocation network development.

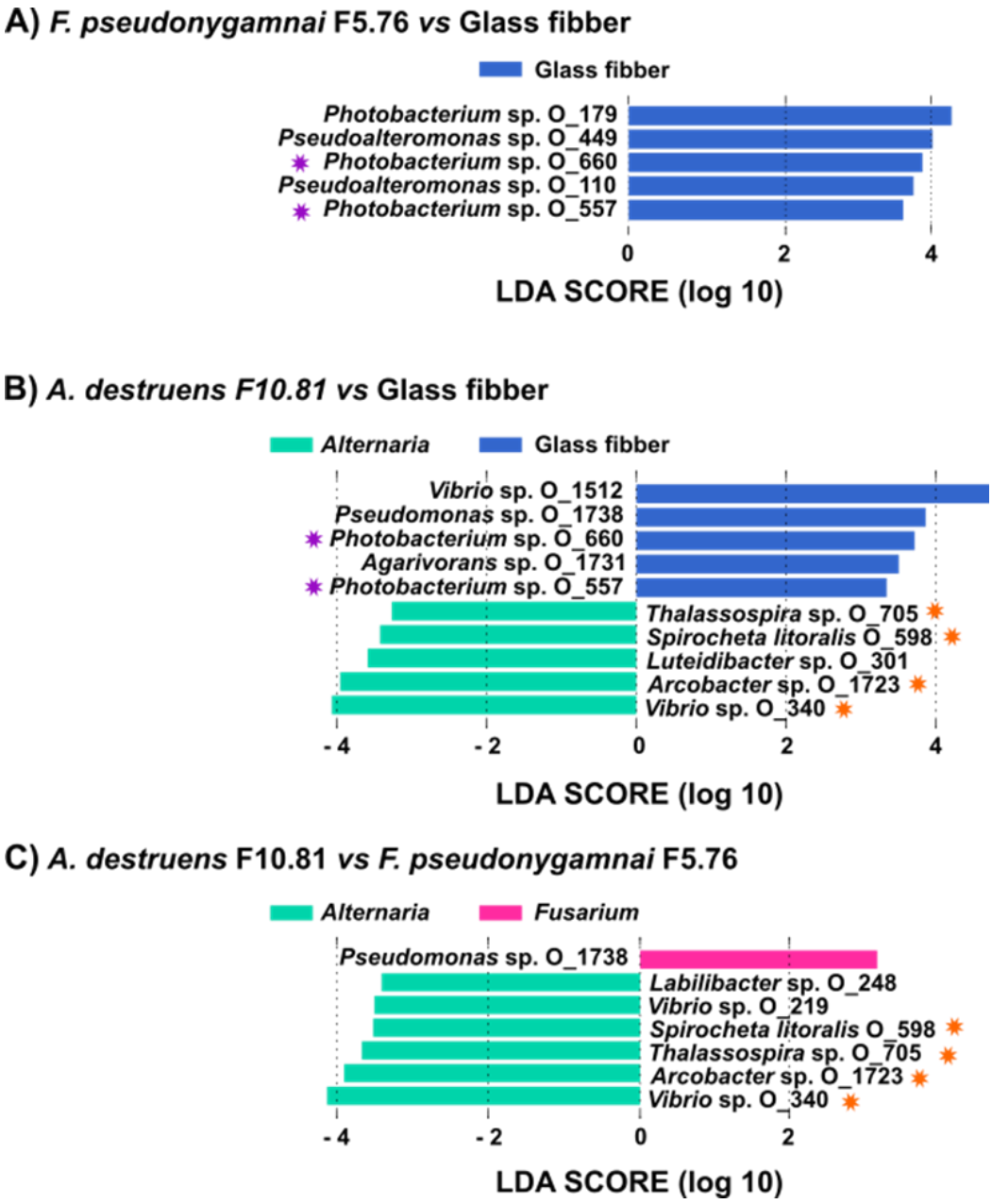

Figure IV.4. Comparison of translocated bacterial communities according to the translocation network. The linear discriminant analysis effect size (LEfSe) identified significant differential abundance distribution of bacterial ASVs, from bacterial communities obtained after the gap, between *A. destruens* F10.81. Hyphae and glass fibbers (A), *F. pseudonygamai* F5.76 hyphae and glass fibbers (B), and between both fungi (C). The analysis was performed considering bacterial communities obtained after the gap with both Etang de Berre and Canal Vieil sediments. The stars indicates ASVs biomarkers identified for *A. destruens* F10.81(orange) and glass fibbers (purple) present in the two comparisons performed for each translocation network.

To explore this hypothesis, the oxygen concentration was measured in the hyphosphere during the development of the fungal mycelia. An oxygen depletion was observed during the mycelial growth (Figure IV.5 B) showing that favorable conditions are created for the translocation of anaerobic bacteria along the fungal network.

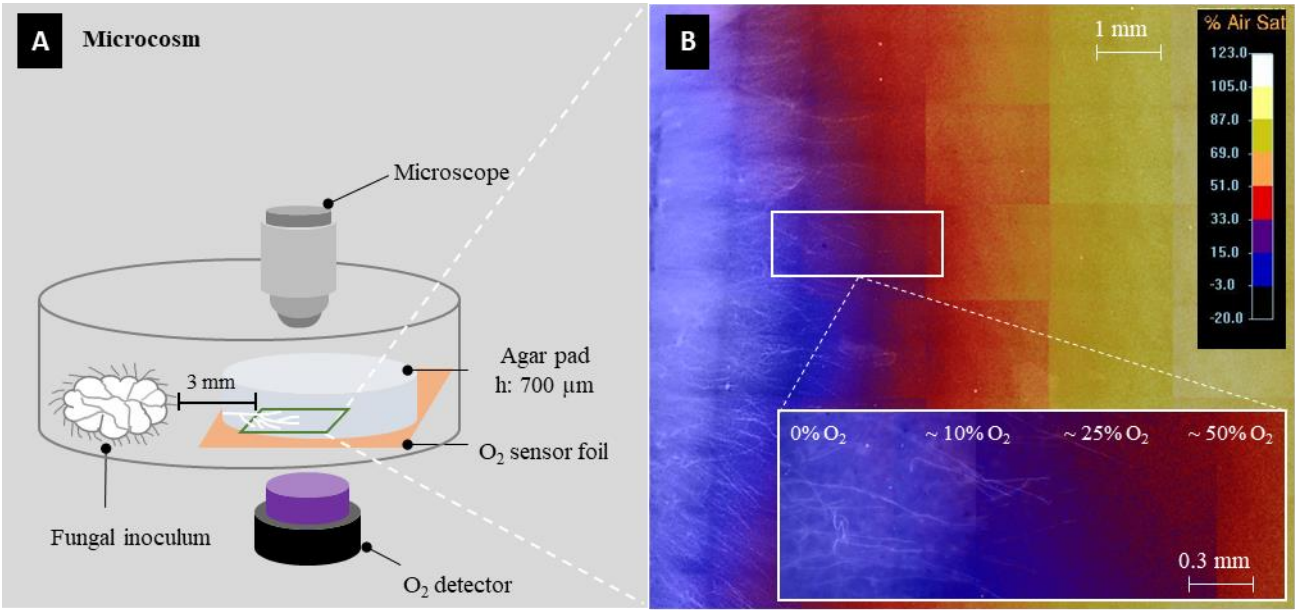

Figure IV.5. Oxygen depletion during fungal growth. A) Schematic view of the microcosm used for mycopshere oxygen mapping and B) spatially distinct oxygen gradient (0%-100%) was observed in the mycopshere of *A. destruens* F10.81. Anoxic environment (indicated by blue colour) was observed in the mycosphere where dense hyphal networks were established.

IV.5. Conclusion

Despite the fact that our experimental conditions selected the fast growing bacteria *Pseudoalteromonas*, our study demonstrated that the fungal translocation network selected specific bacterial populations, especially for *A. destruens* F10.81 while no specific biomarker was identified for *F. pseudonygamai* F5.76. For *A. destruens* F10.81 four high specific biomarkers were identified. Among these biomarkers, the most tricky was the observation of anaerobic bacteria (*Spirochaeta litoralis*). Such result suggested that the oxygen depletion during the hyphal development create anoxic niches allowing the translocation of facultative and anaerobic bacteria. This capacity is of environmental relevance in PAH contaminated coastal sediments allowing anaerobic and aerobic bacteria to be transported through different hydrophobic patches via fungal network. We advocate that aerobic bacteria are transported in the rich oxygen apical hyphae zone while anaerobic bacteria follow after oxygen depletion by fungal growth (Figure IV.6).

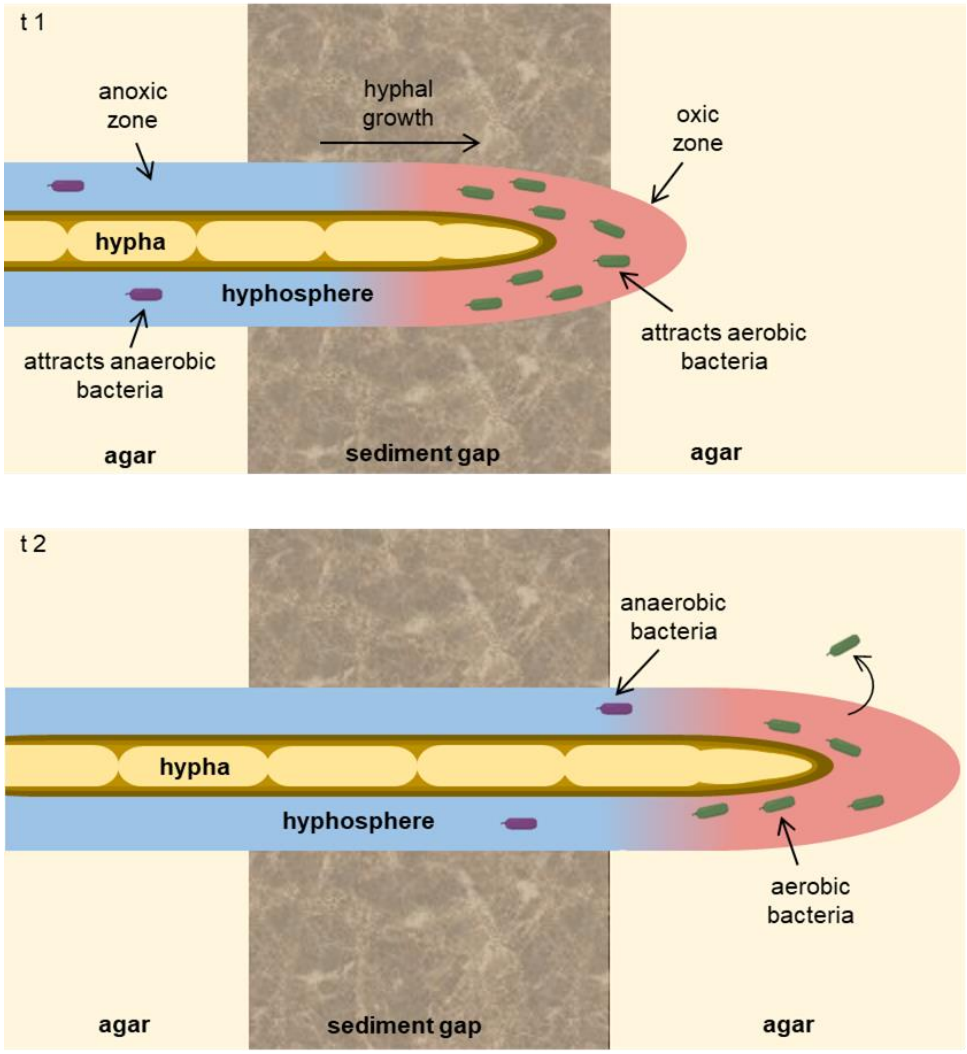

Figure IV.6. Fungal translocation of aerobic and anaerobic bacteria. Schematic representation of the hyphosphere showing oxic (red) and anoxic (blue) zones formed during the hyphal growth (t1 and t2) across the PAHs contaminated sediment gap in which aerobic (green) and anaerobic (purple) translocate.

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Chapter IV. Marine fungal translocation network transport aerobic/anaerobic bacteria from PAHs contaminated sediments

IV.3. Chapter principal results and discussion

In the previous chapter (Chapter III), two fungi were isolated and selected for their low and high PAHs removal capacities (*Fusarium pseudonygamai* F5.76 and *Alternaria destruens* F10.81, respectively). These fungi were consequently used to analyze bacterial transport and selectivity along the fungal mycelia considering the challenges that PAHs contaminated coastal sediments imply. First, the transport via fungal translocation networks of eight bacteria isolates was tested across different gaps (wax, sediment, wet sand and air) mimicking the hydrophobic patches that PAH contamination create in sediments. Such analysis showed that bacteria able to cross the gaps among fungal hyphae needed to be motile and tolerant to PAHs contamination. Furthermore, the selection of bacterial communities according to different fungal translocation networks was assed and evaluated. The bacterial communities obtained from all translocation networks showed mainly dominance of the fast growing bacteria *Pseudoalteromonas* for all networks. Nevertheless, each translocation network has its own bacterial populations demonstrating fungal selectivity for specific bacteria members. Then, deeper analysis of the bacterial communities allowed to associate four bacterial biomarkers to the fungus A. *destruens* F10.81 belonging to *Thalassospira* sp., *Spirochaeta litoralis*, *Arcobacter* sp. and Vibrio sp. So far, these bacteria have not been reported to be associated to fungal partners, nevertheless all the bacteria mentioned have been reported to be associated to PAHs degradation and marine environment. For this reason, further efforts should be done to understand the role of fungal and bacterial interactions in PAHs contaminated sediments.

Finally, in Chapter II, the co-occurrence between fungi and anaerobic bacteria was shown, suggesting that fungi-bacteria interactions are important in the organization of microbial communities. Consecutively in this Chapter IV, we present the detection of the anaerobic bacteria *Spirochaeta litoralis* as specific biomarker of A. *destruens* F10.81 that supports our previous finding. Coupled with this, measurements of the concentration of oxygen around the hypha during its development, confirmed the formation of oxic and anoxic zones that allow the propagation of aerobic and anaerobic bacteria along the fungal translocation network. The results previously described demonstrate that coastal sediments are a source of unexplored interactions and open new questions about fungal and bacterial strategies to overcome PAHs polluted environments, which will be discussed in the Perspectives presented in Chapter V.

Chapter V. Conclusions and perspectives

V.1. Conclusions

Based in the current knowledge of fungal and bacterial interactions under PAHs contamination exposed in Chapter I, with this work we do an effort to understand the fungal and bacterial interactions from coastal sediments considering the particular characteristics of these ecosystems. Supported by chemical, microbiological and molecular techniques, it was possible to make an analysis of the impact of PAHs in the fungal and bacterial diversity dynamics. The analysis present first, the role of fungi as key organisms able create networks of interactions with bacterial communities under PAHs contaminated coastal sediments (Chapter II), suggesting they play a key role in microorganisms dynamics. The diversity of interactions between fungi and bacteria were proved to decrease in number but increase in strength under PAHs pollution, demonstrating the importance of fungal and bacteria co-occurrence for the microbial resilience against pollutants in these ecosystems (Chapter II). Additionally, this analysis showed for the first time the association of anaerobic bacterial communities with facultative anaerobic fungi from the Chytridiomycota phyla. Such result shine light the need of further exploration and understanding of coastal sediments as source of undescribed fungal and bacterial interactions.

In order to understand the role and impact of fungi in PAHs contaminated coastal sediments and to allow us to select two fungi as research models (under laboratory conditions), the cultivable diversity of fungi from coastal sediments was isolated and identified. The fungal diversity was found to be rich in fungi from the phylum Ascomycota (Chapter III) revealing a reservoir of undescribed fungi, calling the attention for further research of new fungal taxa in these ecosystems. Different fungi from coastal sediments result in different degrees of PAHs removal capacities. Considering the toxic effect of PAHs for organisms (Haritash and Kaushik, 2009), the removal of PAHs from the ecosystem might help buffering the environmental impact of these pollutants. Interestingly, the microscopic analysis presented in Chapter III showed two different strategies of removal and storage of PAHs that include cytoplasmatic reserve and vacuole packaging. Further analysis must be performed to know if these different mechanisms affect PAHs degradation and their impact on the fungal metabolism of PAHs molecules. Moreover, the PAHs depletion showed to be independent of the genes of extracellular peroxidases and neither to the capacity of fungi to grow in contaminated media, demonstrating the elasticity of fungal metabolism, which allow them to be highly tolerant to pollutants. To see the impact that different uptake strategies and removal of PAHs might have in the possible associated bacterial communities, two fungi were chosen (*Alternaria destruens* F10.81 and *Fusarium pseudoygamai* F5.76) for studying the fungi-bacteria interactions.

Profiting the capacity of fungi to translocate bacteria from one site to another (Kohlmeier et al., 2005), in Chapter IV the analysis of bacterial communities associated to the two fungal translocation networks chosen in Chapter III is presented. Bacterial communities showed different bacterial translocation characteristics through heterogeneous patches suggesting that fungal translocation might take place in PAHs contaminated coastal sediments. Moreover, different fungal networks shape different bacterial communities during the translocation demonstrating selectivity. Specificity of bacterial translocation through fungal hypha was also demonstrated with the finding of bacterial biomarkers associated to one of the fungus, as a result of the interaction. This work gives the first approach to study fungal and bacterial specific interactions and transport in coastal sediments, but more analysis should be done to understand how these interactions occur. The most surprising result was the transport of anaerobic bacteria described for the first time so far. Analysis of the hyphosphere concentration of oxygen showed that fungal metabolism create niches for aerobic and anaerobic bacteria depending on the hypha stage of development. This information support the previous findings of anaerobic bacteria and facultative anaerobic fungi that co-occur in PAHs contaminated sediments showed in Chapter II.

V.2. Perspectives

The results presented in this work constitute the first basis for understanding fungal-bacterial interaction in PAHs contaminated coastal sediments. The role of fungi as key taxa in coordinating bacterial communities assemblage of selected bacteria was observed in PAHs polluted media. However, it remains unknown whether the selection of bacterial communities from fungal mycelia favours the uptake and degradation of PAHs. Thus, further analyses are required to characterize PAHs removal in fungi-bacteria co-cultures (*Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76 associated with the bacterial communities obtained after the gap). The co-culture analysis will bring light on the capacity of the bacterial communities, selected by fungal mycelia, to aid in the decontamination of PAHs polluted environments. The analysis will be also helpful to describe the possible synergism between fungi and bacteria under hazardous conditions. Such information will provide new insights on the behaviour of fungi-bacteria interactions in coastal sediments, useful information to understand the strategies that these microorganisms use to overcome pollutants as PAHs.

In order to have molecular insight of fungal-bacterial interactions, a molecular approach targeting volatile organic compounds (VOC) that have been reported as drivers of the interactions between fungi and bacteria (Deveau et al., 2018), will allow to understand the communication between fungal and bacterial cells. Transcriptomic analyses have demonstrated that fungi and bacteria react each other, responding differentially according to the interacting partners. Several molecules may be used by microorganisms for mutual detection, most of them being based on small signaling molecules (Scherlach et al., 2013). Also, it has been reported that signaling molecules modify the metabolism of the interacting partner, resulting in growth promotion, enzyme production and enhanced reproduction (Crowe and Olsson, 2001; Fiddaman and Rossall, 1993; Zhao et al., 2011). For example, *Pseudomonas fluorescens* has been shown to induce the production of laccase in *Rhizoctonia solani* in co-cultures mediated by VOCs (Crowe and Olsson, 2001). The whole VOCs pattern in intra-kingdom is still not elucidated.

Moreover, considering the findings of fungal and bacteria anaerobic interactions presented in this thesis, further efforts should be done to elucidate how the presence of PAHs enhance the anaerobic co-metabolism of these pollutants. Studies related to the anaerobic PAHs degradation of fungal-bacterial consortium have been proposed (Elyamine et al., 2021), but not yet realized. The anaerobic PAHs degradation by fungi has recently gain interest as more evidence emerges, new fungal members are classified (Amend et al., 2019) and the PAHs metabolic pathways are elucidated (Aydin et al., 2017).

It is important to highlight that sediments are highly heterogeneous from a location to another, making the possible fungal and bacterial interactions also diverse. As presented in Chapter II, a co-occurrence network approach provided insights gathering information from sediments of different marine ecosystems. In the current work, co-occurrence fungal and bacterial interactions are presented gathering data from sediments of various sites, mainly in France. To take this effort further, the analysis of sediments from other different and numerous locations worldwide will allow to obtain a complete pattern of yet undescribed fungi and bacteria interactions as well as their diversity. Two strategies are proposed to make a robust analysis of co-occurrence network describing fungal and bacterial interactions: i) an *in-situ* sampling and analysis of different sediments from various locations, and ii) a metadata analysis taking advantage of already available information present in databases on the fungal and bacterial diversity in marine coastal sediments.

Several questions remain open about the transport of bacterial along the fungal mycelia in contaminated sediments. Whereas in this work we showed that selected isolated fungi effectively transport bacterial communities along hydrophobic patches in culture media. Thus, the uncultivable fungal communities and their associated bacterial communities were not assessed. In an experimental microbial ecology approach, microcosm experiments might help to mimic as closer as possible marine coastal sediment ecosystem under laboratory conditions in order to further study fungi-bacteria interactions (Figure V.1).

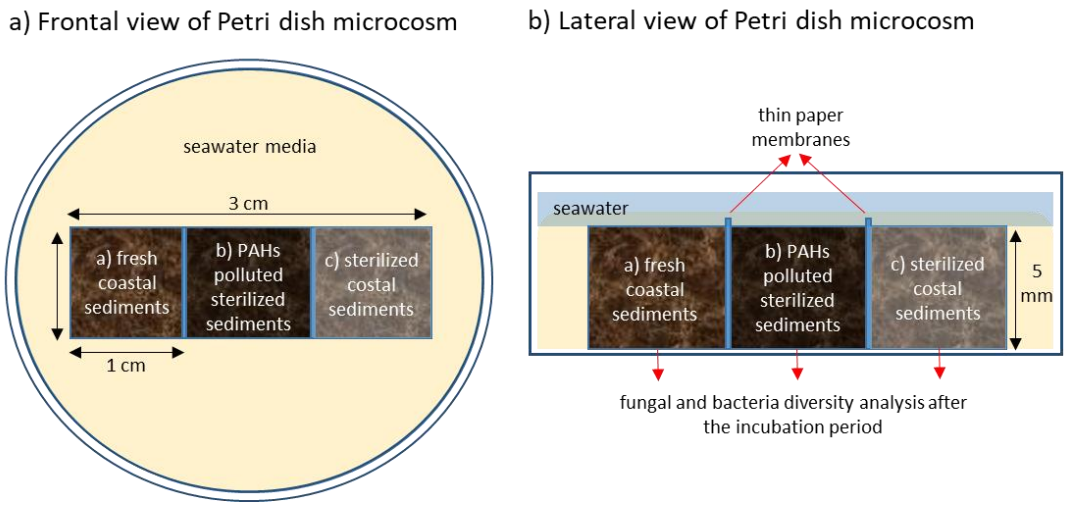

Figure V.1. Schematic representation of the experimental setup designed to analyse bacterial communities transported by fungal translocation network on sediments polluted with PAHs. a) Frontal view: The microcosm consisting a petri dish of 5 cm diameter with a fill-up with seawater media agar (to keep osmolality and offer physical support) except for an empty compartment of 3x1 cm in the centre. **b) Lateral view:** The central compartment is divided with paper membranes that can keep separated sediments with different characteristics. All the sediments come from the same source but received different treatments: a) nothing, b) autoclaved and PAHs input, c) autoclaved. In addition, the complete system is covered with seawater to emulate a water-filled environment.

The results from this experiment will provide an insight of the non-cultivable diversity of bacteria able to be transported along hyphal translocation network without the selective pressure of the media. In addition to this, the microcosm will answer whether bacterial transport along fungal network can occur under water-filled environments.

Finally, all the data recollected from the project here presented, pave the way for implementing new strategies for the recovery of contaminated coastal marine environments, including: i) the development of bioreactors with fungi and bacteria selectively chosen for their capacity to up take and degrade PAHs; ii) the use of communication molecules (COVs) to stimulate fungi-bacteria interaction for enhancing PAHs degradation in situ; iii) the identification of key taxa of fungi and bacteria partners from contaminated marine ecosystems representing potential bioindicators to report the level of pollution and the recovery capacity.

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Resumen

Los sedimentos marinos son medios ambientes heterogéneos con una variedad de características físico-químicas. Son el reservorio de una vasta diversidad microbiana que no ha sido totalmente explorada. Entre las comunidades microbianas, la interacción entre hongos y bacterias juega un papel clave en los procesos biogeoquímicos incluyendo los ciclos del sulfuro, nitrógeno y carbono. Sin embargo, las interacciones entre hongos y bacterias son constantemente alteradas por la presencia de hidrocarburos policíclicos aromáticos (HPAs), los cuáles son tóxicos, mutagénicos y carcinógenos. Los HPAs son moléculas resistentes y persistentes debido a su hidrofobicidad que les permite acumularse en los sedimentos donde la biodegradación es reducida debido a la reducción de su disponibilidad. En este contexto, el objetivo de esta tesis doctoral es profundizar en el entendimiento de las interacciones entre hongos y bacterias en los sedimentos costeros contaminados con HPAs. El análisis de redes de co-occurencia de los sedimentos costeros contaminados y no contaminados con HAPs, basados en las secuencias de los genes rRNA 16S y 18S (para bacterias y hongos, respectivamente), revelo que hay variantes de secuencias amplificadas (VSAs) provenientes de hongos que juegan un papel clave en la organización y ensamblaje de las comunidades microbianas estableciendo interacciones con VSAs de bacterias. La presencia de HPAs afecta dichas interacciones promoviendo un metabolismo anaeróbico. Las interacciones entre hongos y bacterias fueron también investigadas a través del cultivo de diversos hongos, de los cuales fueron aisladas y seleccionadas cepas fúngicas con capacidad de remover HAPs. De éstas cepas, *Alternaria destruens* F10.81 y *Fusarium pseudoygamai* F5.76 dieron evidencia de dos estrategias de captura y transporte intracelular de HAPs que involucran el almacenamiento citoplasmático o vacuolas, respectivamente. Dichos hongos fueron usados a su vez para evaluar la selección y el trasporte de comunidades bacterianas a través de una barrera de sedimento contaminada con HAPs. La diversidad de las comunidades bacterianas, basadas en el análisis de la secuencia de los genes 16S rRNA, revelo que existen bacterias seleccionadas de manera específica por la red de micelio fúngico. A su vez, cuatro biomarcadores específicos para *Alternaria destruens* F10.81 fueron identificados como *Thalassospira* sp., *Spirochaeta litoralis*, *Arcobacter* sp. y *Vibrio* sp. Sorprendentemente, *Spirochaeta litoralis* es una bacteria clasificada como estricta anaerobia sugiriendo la creación de zonas de anaerobiosis derivadas del desarrollo fúngico, lo cuál fue confirmado por la observación del consumo de oxígeno durante el crecimiento de las hifas de *Alternaria destruens* F10.81. Este resultado, fue consistente con la promoción del metabolismo anaeróbico encontrado en los análisis de redes de co-occurencia. Por lo tanto, nuevos conocimientos sobre el papel clave que juegan las interacciones entre hongos y bacterias en la organización de comunidades microbianas de sedimentos marinos costeros contaminados con HAPs son aportados, los cuales representan información útil para desarrollar estrategias de recuperación medioambiental.

Palabras clave: hongo, bacteria, diversidad microbiana, interacciones, red de transporte, hifósfera, HPA, contaminación, biodisponibilidad, sedimentos, aeróbico/anaeróbico.

Abstract

Coastal sediments are heterogeneous environments with a variety of physical-chemical characteristics. They are the reservoir of a vast microbial diversity, yet to be explored. In the microbial communities, the fungi-bacteria interactions play a key role in several biogeochemical processes including sulphur, nitrogen and carbon cycling. Nevertheless, the fungi-bacteria interactions are constantly affected by the input of pollutants, particularly polycyclic aromatic (PAHs) compounds, which are toxic, mutagenic and carcinogenic. PAHs are persistent molecules due to their hydrophobicity accumulating in sediments where their bioavailability for degradation is reduced. In this context, the aim of this PhD thesis is to better understand the fungi-bacteria interactions in coastal sediments contaminated with PAHs. The analysis of fungi-bacteria co-occurrence networks from coastal sediments contaminated or not with PAHs, based on 16S and 18S rRNA barcoding sequences (for bacteria and fungi respectively), revealed that specific fungal amplified sequence variants (ASVs) play a key role in the organization of microbial community assemblages by establishing interactions with bacterial ASVs. The presence of PAHs is affecting these interactions promoting anaerobic metabolism. The fungi-bacteria interactions were further investigated by exploring the fungal cultivable diversity that allowed to isolate, identify, and select fungal strains from marine environments for their capacity to remove PAHs. *Alternaria destruens* F10.81 and *Fusarium pseudoygamai* F5.76 showed different strategies for PAHs uptake that involve either the transport through the cytoplasm or via vacuoles, respectively. These fungal strains were used to evaluate the selection and transport of bacterial communities through PAHs contaminated gap. Diversity analyses of bacterial communities, based on 16S barcoding sequences revealed that specific bacteria were selected by the fungal mycelia network. Moreover, four biomarkers specific to *Alternaria destruens* F10.81 were identified as *Thalassospira* sp., *Spirochaeta litoralis*, *Arcobacter* sp. and *Vibrio* sp. Surprisingly, *Spirochaeta litoralis* is known as anaerobic bacteria suggesting that fungal mycelia development produce anaerobic zones, further confirmed by the observation of oxygen consumption during the hyphal development of *A. destruens* F10.81. Such result was consistent with the promotion of anaerobic metabolism described with the fungi-bacteria co-occurrence network. Thus, new insights on the key role of fungal and bacterial interactions have on the organization of microbial communities in coastal sediments contaminated by PAHs are provided, which represent useful information to develop strategies for environmental recovery.

Keywords: fungi, bacteria, microbial diversity, interactions, translocation network, hyphosphere, PAHs, contamination, bioavailability, sediments, aerobic/anaerobic.

Résumé

Les sédiments côtiers sont des environnements hétérogènes avec une variété de caractéristiques physico-chimiques. Ils sont le réservoir d'une vaste diversité microbienne, encore à explorer. Dans les communautés microbiennes, les interactions champignons-bactéries jouent un rôle clé dans plusieurs processus biogéochimiques, notamment le cycle du soufre, de l'azote et du carbone. Néanmoins, les interactions champignons-bactéries sont constamment affectées par l'apport de polluants, en particulier les hydrocarbures aromatiques polycycliques (HAP), qui sont toxiques, mutagènes et cancérigènes. Les HAP sont des molécules persistantes en raison de leur hydrophobicité, s'accumulant dans les sédiments où leur biodisponibilité pour la dégradation est réduite. Dans ce contexte, l'objectif de cette thèse est de mieux comprendre les interactions champignons-bactéries dans les sédiments côtiers contaminés par les HAP. L'analyse des réseaux de cooccurrence champignons-bactéries à partir de sédiments côtiers contaminés ou non par les HAP, basée sur les séquences des ARNr 16S et 18S (respectivement pour les bactéries et les champignons), a révélé que des variants spécifiques de séquences amplifiées (ASV) fongiques jouent un rôle clé dans l'organisation d'assemblages de communautés microbiennes en établissant des interactions avec des ASV bactériens. La présence d'HAP affecte ces interactions favorisant le métabolisme anaérobie. Les interactions champignons-bactéries ont été étudiées afin d'explorer la diversité fongique cultivable qui a permis d'isoler, d'identifier et de sélectionner des souches fongiques des environnements marins pour leur capacité à éliminer les HAP. *Alternaria destruens* F10.81 et *Fusarium pseudoygamai* F5.76 ont montré des stratégies différentes pour l'absorption des HAP qui impliquent respectivement le transport à travers le cytoplasme ou via des vacuoles. Ces souches fongiques ont été utilisées pour évaluer la sélection et le transport des communautés bactériennes à travers des zones contaminées par les HAP. Des analyses de diversité des communautés bactériennes, basées sur des séquences des gènes 16S ARNr, ont révélé que des bactéries spécifiques étaient sélectionnées par le réseau mycélien fongique. Par ailleurs, quatre biomarqueurs spécifiques à *Alternaria destruens* F10.81 ont été identifiés : *Thalassospira* sp., *Spirochaeta litoralis*, *Arcobacter* sp. et Vibrio sp. De manière surprenante, *Spirochaeta litoralis* est connue comme une bactérie anaérobie suggérant que le développement de mycélium fongique produit des zones anaérobies, confirmée par l'observation de la consommation d'oxygène pendant le développement des hyphes *d'A. destruens* F10.81. Un tel résultat était cohérent avec la promotion du métabolisme anaérobie décrit avec le réseau de cooccurrence champignons-bactéries. Ainsi, de nouvelles informations sur le rôle clé des interactions fongiques et bactériennes sur l'organisation des communautés microbiennes dans les sédiments côtiers contaminés par les HAP sont fournies, et représentent des informations utiles pour développer des stratégies de bioremédiation environnementale.

Mots clés: champignons, bactéries, diversité microbienne, interactions, réseau de translocation, hyphosphère, HAP, contamination, biodisponibilité, sédiments, aérobie/anaérobie.