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## **Food and Functionalities**

Emeline Roux

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Dossier de candidature  
pour l’Habilitation à Diriger des Recherches / Application form for  
Habilitation to Supervise Research

Aliments et Fonctionnalités / Food and Functionalities

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Soutenance le 29 novembre 2024 devant le jury composé de / Defense on  
November 29, 2024 with the jury composed of:

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Merci à tous ceux qui m'ont fait confiance, m'ont fait progresser, je précise que cette liste n'est pas exhaustive et j'espère ne pas avoir oublié trop de monde... Au CNAM (Jacques Nicolas et Catherine Billaud and Co), à Grignon (Thierry Chardot et tous les collègues de Chimie Biologique), à Créteil, à Strasbourg, à Nancy (merci beaucoup, Clarisse, Annie et Magali, merci à tous), à Rennes (Yves Le Loir, Hélène Falentin, Sergine Even, Eric Guédon et tout le personnel du STLO ; Dominique Lavenier, Jacques Nicolas, Pierre Peterlongo, et tous les Symbiose, GenScale, Dyliss et GenOuest au pays des bisounours ; Gaëlle Boudry et David Val-Laillet et tous les NuMeCan ; Claire Piquet-Pellorce et tout le bel UFR SVE ; Ludovic Paquin avec qui c'est tellement plus facile de travailler et de partager la gestion d'une formation).

La vie est faite de hasards et de rencontres.

A toute ma famille.

(...)

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## Abbreviations

ABIES: École doctorale ABIES Agriculture, alimentation, biologie, environnement et santé (Doctoral School Agriculture, food, biology, environment and health)

bac: baccalauréat (A-level; bac+5 meaning 5 years of studies after baccalauréat)

BECV: Biologie, Environnement et Chimie du Vivant (Biology, environment and life chemistry)

BMCP: Biologie moléculaire, cellulaire et physiologie (Life sciences, Molecular and cellular biology and physiology)

BUT: Bachelor Universitaire de Technologie (University bachelor of technology)

Calbinotox: Composés Alimentaires: Biofonctionnalités et Risques Neurotoxiques (Research team on food compounds: Biofunctionalities and Neurotoxic Risks)

CCC: Centre Culinaire Conseil (culinary advice centre)

CFIA: Carrefour des Fournisseurs des Industries Agro-alimentaires (professional exhibition in the food industry in Rennes, sectors: Ingredients & intermediate food products, Equipment & Processes and Packaging)

CM: Cours Magistraux (amphitheatre lectures)

CNAM: Conservatoire National des Arts et Métiers (national conservatory of arts and crafts)

CNU: Conseil National des Universités (French National Universities Council)

CRCT: Research time and thematic conversion

DEA: Diplôme d'Études Approfondies (master degree in research)

DRACI: Développement et Recherche en Art Culinaire Industrialisé (development and research in the industrial culinary arts, a professional bachelor's degree)

EAT: Contrôle du comportement alimentaire (Control of eating behavior, a team from the NuMeCan institute)

ENVA: École Nationale Vétérinaire d'Alfort (Alfort National Veterinary School)

ENSIA / AgroParisTech: École Nationale Supérieure des Industries agricoles et Alimentaires (National School of Agricultural and Food Industries, that joined AgroParisTech - Institute for Life and Environmental Sciences and Industries in 2007)

FEBS: Federation of European Biochemical Societies

FROMTIQ: Fromagerie - technologie, innovation, qualité (cheesemaking - technology, innovation, quality, a speciality of the LP I3A)

GB: Génie Biologique (biological engineering, a IUT department)

GB2A: Génie Biologique Agro-Alimentaire (a sub-division of the GB department of the IUT: biological engineering in agronomy and the food industry)

GBS: Génie Biologique Santé (a sub-division of the GB department of the IUT: health bioengineering)

I2BC: Institute for Integrative Biology of the Cell (CNRS, Gif-sur-Yvette)

I3A: Industrie Agro-Alimentaire, Alimentation (a national LP label: Food industry and nutrition)

IAA: Industrie Agro-Alimentaire (Food Industry)

IAB: Industries Agroalimentaires et Biologiques (Food and biological industries, one of two options in the DUT GB2A)

IGDR: Institut de Génétique et Développement de Rennes (Rennes Institute of Genetics and Development)

IMT: Institut Mines-Telecom

IN: Ingénierie Nutraceutique (an NSA Master's programme dedicated to nutraceutical engineering)

INA P-G / AgroParisTech: Institut national agronomique Paris-Grignon (National Agronomic Institute Paris-Grignon), that became AgroParisTech - Institute for Life and Environmental Sciences and Industries in 2007

INRA / INRAE: Institut National de la Recherche Agronomique / Institut National de Recherche pour l'Agriculture, l'alimentation et l'Environnement (French National Institute for Agronomic Research - National Research Institute for Agriculture, Food and the Environment since 2020 following institutional fusions)

Inria: Institut national de recherche en informatique et en automatique (French National Institute for Research in Digital Science and Technology)

INSERM: Institut national de la santé et de la recherche médicale (National Institute of Health and Medical Research)

IRSET: Institut de recherche en santé, environnement et travail (Institute for Health, Environment and Work Research)

ITS: 16S-23S intergenic region

IUP: Institut Universitaire Professionnalisés (professional University institutes)

IUT: Institut Universitaire de Technologie (University institute of technology)

L1 / L2 / L3: Licence 1<sup>ère</sup>, 2<sup>ème</sup> et 3<sup>ème</sup> années (Bachelor's first, second and third years)

LAB: Lactic Acid Bacteria

LD: Lipid Droplet

LEGGO: association Légumineuses à Graines du Grand Ouest (Leguminous with seeds from the West part of France)

LP: Licence Professionnelle (professional bachelor degree)

M1 / M2: Master 1<sup>ère</sup>, 2<sup>ème</sup> années (Master's first and second years)

MIAMSA: Innovation et science des aliments (an NSA Master's programme dedicated to innovation and food science)

MLST: Multi Locus Sequence Typing

N-B: Nancy-Brabois (IUT location)

NSA: Nutrition et Sciences des Aliments (nutrition and food science, a national master's degree)

NuMeCan: Nutrition, Métabolismes et Cancer (research institute for Nutrition, Metabolisms and Cancer)

NutriS: Nutrition en Santé (an NSA master's degree programme dedicated to health nutrition)

ONT: Oxford Nanopore Technologies

PAI: Produits Alimentaires Intermédiaires (Intermediate Food Products)

PAT: Plan Alimentaire Territorial (Territorial Food Plan)

PB2P: Protéolyse et Biodisponibilité des Protéines et Peptides (Proteolysis and bioavailability of proteins and peptides, a former UR AFPA laboratory team)

PEEL: Pôle entrepreneuriat étudiant de Lorraine (Lorraine student entrepreneurship center)

PL: Phospholipid

PPP: Projet Personnel et Professionnel (Personal and Professional Project)

QSP2A: Qualité et sécurité en production agro-alimentaire (quality and safety in food production, a speciality of the LP I3A)

RNCP: Répertoire National des Certifications Professionnelles (French National Directory of Professional Certifications)

SFCA: Service Formation Continue et Alternance (Continuing education and work-linked training)

SFM : Société Française de Microbiologie (French Microbiology Society)

STLO: Science et Technologie du Lait et L'Œuf (Science and Technology of milk and eggs)

TAG: Triacylglycerol

TD: Travaux Dirigés (supervised exercises in small groups: from 28 students at the IUT to a maximum of 40 students at the University of Rennes in L1; note: 1h30 of TD is equivalent to 1h of CM in our teaching duties)

TP: Travaux Pratiques (laboratory practice in small groups: from 14 students at the IUT to a maximum of 20 students at the University of Rennes in L1; note: until 2009, 1h30 TP was equivalent to 1h TD in our teaching duties, today 1h TP counts as 1h TD)

UE: Unité d'Enseignement (teaching unit)

UFA: Université Franco-Allemande (French-German University)

UFR SVE: Unité de Formation et de Recherche Sciences de la Vie et de l'Environnement (Life and Environmental Sciences Faculty)

UHP / UL: Université Henri Poincaré / Université de Lorraine (University Henri Poincaré / University of Lorraine since 2012 following institutional fusions)

UMR : Unité Mixte de Recherche (mixed research unit)

UR1 / UR: Université Rennes1 / Université de Rennes (University of Rennes1 / University of Rennes since 2023 following institutional fusions)

UR AFPA: Unité de Recherche Animal et Fonctionnalités des Produits Animaux (Research unit on animals and animal product functionalities)

## Glossary - Foreword

Or when I imagined my German colleague, Hans-Jürgen, reading this manuscript and trying to understand the fine details of French higher education. You have to admit, it's pretty tricky! The French system of education (post-baccalaureate), I have been involved in (...):

- CNAM: Conservatoire National des Arts et Métiers. The CNAM was founded in 1794 to improve national industry. This establishment specializes in lifelong higher education to promote professional experience. When I was a lecturer at CNAM (during my thesis), lectures and supervised sessions were given on Thursday and Friday evenings, and practical work took place on Saturdays. It graduates technicians (equivalent of 2 years of studies after the baccalaureate) and engineers (equivalent of 5 years of studies after the baccalaureate).
- IUP: Institut Universitaire Professionnalisés (professional University institutes). IUP were a component of universities, which existed from 1992 to the mid-2000s, providing technological and professional education in 2 years: year 3 and year 4 after the Baccalaureate. One third of the training was organized in a professional environment. Half of the courses were to be given or supervised by professionals not affiliated to the University. Graduates were awarded the title of "Ingénieur maître" (master engineer). With the Bachelor's-Master's-Doctorate reform (Bologna process), IUPs (bac+4) have been replaced by Master's degrees (bac+5).
- IUT: Institut Universitaire de Technologie (University Institute of Technology). Delivers a technician's diploma (DUT) after 2 years of study (directly after the baccalaureate). The DUT is a professional diploma that combines theory and practice (a lot), with projects, internships and/or work-study programmes for rapid professional integration. IUTs were created in 1966, and the biology department in Nancy was one of the first four IUTs created on an experimental basis. Since 2021, IUTs offer a 3-year diploma: the Bachelor Universitaire de Technologie (BUT).
- LP: Licence Professionnelle (professional bachelor degree). This one-year degree aims to provide skilled workers (3 years of studies after the Baccalaureate) who can quickly become operational. It was created in November 1999. Since 2021, several LPs have been integrated into BUT (such as the LP I3A QSP2A at the IUT de Nancy-Brabois, specialized in quality management in the food industry), while others have remained independent (such as the LP DRACI at the University of Rennes, specialized in food innovation).
- University: 3 years to obtain a Bachelor's degree (from L1 to L3) then 2 years to obtain a master's degree (M1 and M2).

# 1. CURRICULUM VITAE

## 1.1. Personal details

(...)

Current administrative status: Associate Professor, University of Rennes.

Research unit: NuMeCan - Nutrition, Metabolisms and Cancer - Inserm U1241 - INRAE U1341 -  
Université de Rennes

## 1.2. Professional background and positions

(...)

## 1.3. Education and qualifications

(...)

## 1.4. Overview of main responsibilities and activities

### 1.4.1. Teaching

#### *Education management*

(...)

#### *International cooperation*

(...)

### 1.4.2. Research

19 A-rank publications; 5 papers in international and 17 national conferences.

Co-supervision of 3 theses, 10 Master2 internships, 5 Master1 internships.

(...)

# 2. TEACHING ACTIVITIES

(...)

### 3. RESEARCH ACTIVITIES

#### 3.1. Context and general direction

Throughout my career, I've always worked on **food and its functionalities**, from its technical and bio-functionalities to, more recently, the link between food and health through the study of intestinal microbiota (Figure 2).

(...)

19 publications in A-ranked international journals; 5 international conferences with published proceedings, 17 national conferences.

Keywords: proteolytic system, lactic acid bacteria, *Streptococcus thermophilus*, bioactive peptides, bacterial genome sequencing, Oxford Nanopore Technologies (MinION), bioinformatics analyses, microbiota.

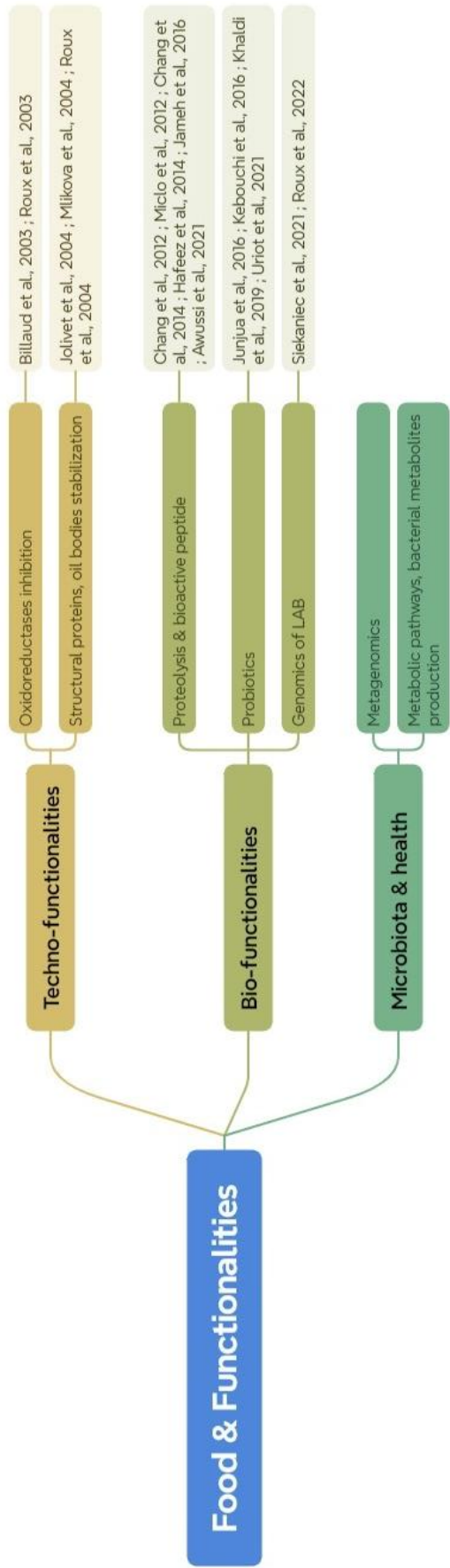


Figure 1: Main research topics addressed since 2000 and publications associated (LAB: Lactic Acid Bacteria)

## 3.2. Food & techno-functionalities (2000 - 2003)

### 3.2.1. Study of the inhibition of purified apple polyphenoloxidase activity by L-cysteine.

Effect of heating conditions in the presence or absence of glucose or fructose; Food enzymatic browning (oxidoreductase activities) and food non-enzymatic browning (Maillard reaction products) interactions

DEA internship, CNAM, Chair of Industrial and Food Biochemistry, Paris, under the supervision of Jacques Nicolas and Catherine Billaud.

Some redox enzymes are well known for their role in altering the organoleptic qualities of food. Lipoxygenase, for example, is responsible for developing the cardboard taste of beer, while polyphenoloxidases cause browning of certain fruits during slicing. Polyphenoloxidases are copper metallo-enzymes. They catalyse the oxidation of diphenols into quinones. Secondary polymerization of the quinones then produces the brown products of enzymatic browning.

We measured the effect of an amino acid taken alone, or heated in the presence of sugars, on the activity of an oxidoreductase. We used a model medium: purified apple polyphenoloxidase and the products of a reaction between glucose (or fructose) and cysteine. Three techniques were used in parallel: polarography (to measure the consumption of an enzyme substrate: oxygen), UV-visible spectrophotometry (appearance of a coloured primary product) and high-performance liquid chromatography (detection of secondary compounds formed). Measurements of oxygen consumption, one of the enzyme substrates, showed no inhibition of enzyme activity by cysteine alone. On the other hand, good inhibition of polyphenoloxidase activity was measured in the presence of Maillard reaction products (Billaud *et al.*, 2003). An equation is given for the inhibition rate as a function of substrate and inhibitor concentrations. It reflects a mixed mode of inhibition (neither purely competitive, non-competitive or uncompetitive) (Roux *et al.*, 2003). Furthermore, experiments involving incubation of the enzyme and Maillard reaction products, followed by dialysis, have shown reversible inhibition, certainly linked to the chelating power of Maillard reaction products towards metals.

Consequently, we could envisage, during the malting of barley in the brewing industry, a natural, oriented production of Maillard reaction products with a strong inhibitory activity. This would improve beer preservation.

(...)

### 3.2.2. Oleosins: new emulsifiers of plant origin. Comparison of lipid bodies extracted from plants (*A. thaliana*) and yeasts (*Y. lipolytica*)

PhD, INRA, Grignon, UMR (mixed research unit) Chimie Biologique, under the supervision of Dr. Thierry Chardot (DR INRA).

Note: in 2002, thanks to a grant from the FEBS (Federation of European Biochemical Societies), I spent two months in the biochemistry laboratory of the Technische Universität, Graz, Austria, under the supervision of Prof. Günther Daum and Dr. Karin Athenstaedt. There I studied the lipid body of the yeast *Yarrowia lipolytica*.

Surfactant molecules are commonly used in industry for their emulsifying, softening, wetting or detergent properties. Europe's oilseed crops are mainly exploited for their oil. However, "waste" oilcake from the oil industry represents a potential reservoir of functional proteins, in particular oleosins. The latter are located on the surface of plant lipid reserve globules, the oleosomes, and feature a hydrophobic central segment with the longest known sequence of contiguous hydrophobic amino acids (72 amino acids) and hydrophilic N- and C-terminal ends and therefore have a structural role (Figures 3 and 4). Due to the presence of oleosins, oleosomes do not coalesce even during seed reimplantation upon germination.

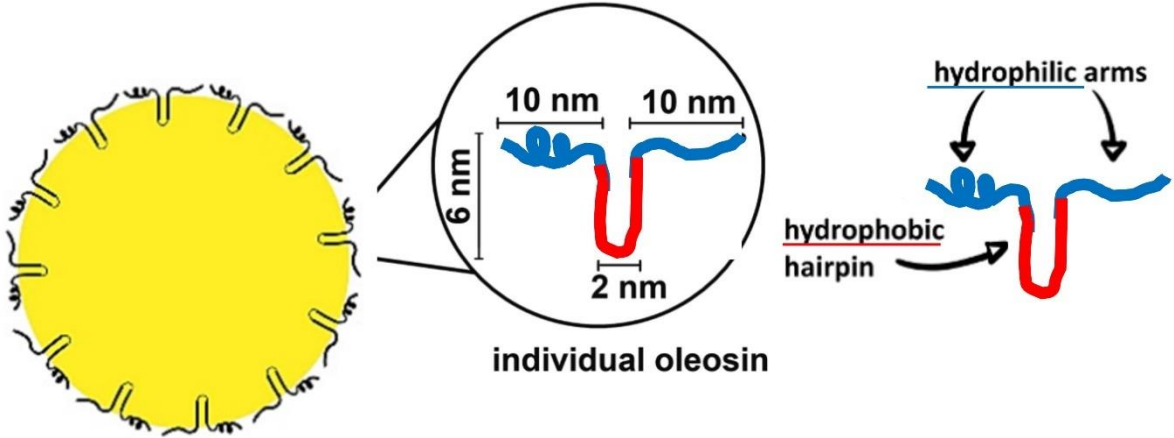


Figure 2: Schematic representation of oleosomes and oleosins, adapted from Plankensteiner *et al.* (2024)

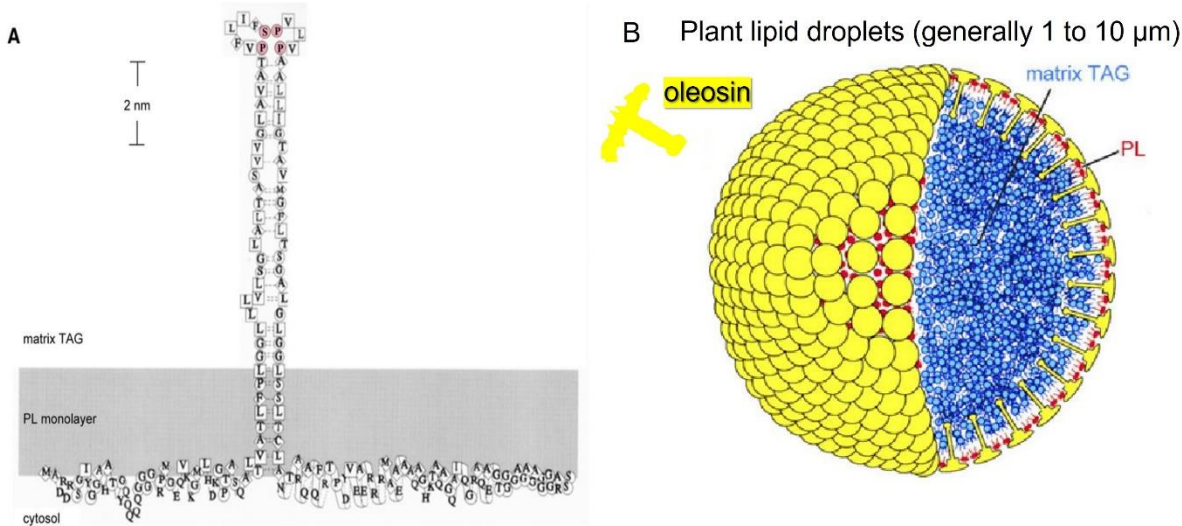


Figure 3: Plant oleosins and oleosome, a review from Huang, 2018

**A**, Model of an oleosin molecule on the surface of a lipid droplet (LD) (Tzen and Huang, 1992). Hydrophobic indices of amino acids: square - very hydrophobic (4.5–3.8), diamond - hydrophobic (2.8–1.8), circle - amphipathic (20.4 to 21.6); no enclosure for hydrophilic (23.2 to 24.5) residues. Hairpin with a Pro knot at the loop (S and P in pink). **B**, Simplified model of oleosome. Oleosin (yellow), PLs (red), and TAGs (blue).

As a first step, we studied the lipid and protein composition of lipid reserve storage organelles: plant oleosomes (model plant *Arabidopsis thaliana*) (Jolivet *et al.*, 2004) and the lipid bodies of a yeast, *Yarrowia lipolytica* (Mlícková *et al.*, 2004) (Figure 5). Although yeasts have storage organelles similar to those of plants, the protein pattern is very different: unlike plant oleosomes, yeast lipid bodies have a much more complex protein pool, but no equivalent to oleosins.

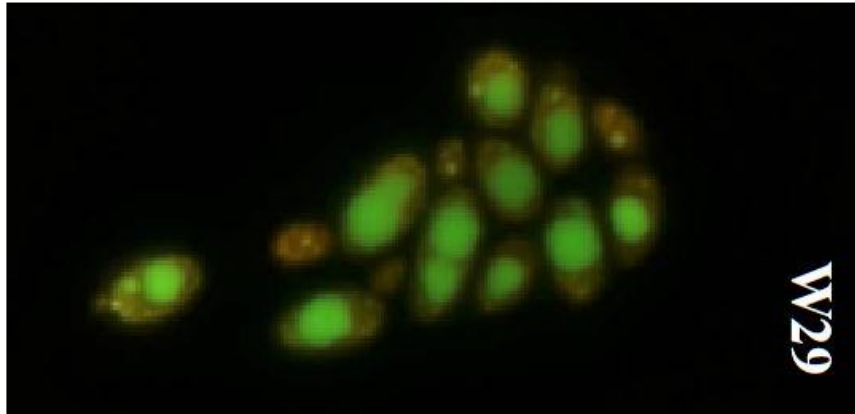


Figure 4: Picture of yeasts *Yarrowia lipolytica* W29 after 20 h of growth on minimum medium enriched with 5% oleic acid (microscopy, Nile red fluorescence). Lipid bodies appear green.

We cloned and expressed two isoforms of *Arabidopsis thaliana* oleosins in *E. coli* and *Yarrowia lipolytica*. Oleosins expressed in yeasts were addressed to the oil bodies. Furthermore, oleosins expressed in bacteria were purified under denaturing conditions. By experimental and model studies, we postulate an  $\alpha$ -helix organisation of the central domain. Surface tension studies showed that purified recombinant oleosins were inserted at water / oil interfaces and decrease efficiently the interfacial tension (better than  $\beta$ -casein). They were also inserted in PLs monolayer on water / air interface. Therefore, as postulated, plant oleosins have properties of an oil / water emulsifier (Roux *et al.*, 2004).

(...)

### 3.3. Food & bio-functionalities (2005 - 2020)

#### 3.3.1. Characterization of the surface proteolytic system of *S. thermophilus* and production of bioactive peptides

Université de Lorraine, Nancy, UR AFPA laboratory, team PB2P

Note: The PB2P team of the UR AFPA laboratory has split off to merge with other UR AFPA team members in a new "Calbinotox" project team in 2016 (host team on January 1, 2018).

The Challenges of the PB2P/Calbinotox team are the following: Our team focuses on the impact on consumer health of bioactive molecules derived from proteins (biologically active peptides), notably derived from milk, or from dietary fatty acids (derived from omega-6 fatty acids) in the presence of GRAS or even probiotic bacteria, with the brain as the target organ, via the gut-brain axis.

Here's a non-exhaustive list of the main activities I took part in as part of the team.

The probiotic yoghurt bacteria *S. thermophilus*, auxotrophic for some amino acids, is dependent on its proteolytic system to fulfil its nutritional needs. Strains possess zero or a single PrtS (subtilisin-like serine protease) protease, covalently anchored to the cell wall; an Ami oligopeptide transport system, composed of one to three oligopeptide-affine proteins (Ami A1, A2 and A3), two permeases (Ami C and D) forming a membrane pore and two ATPases (Ami E and F) supplying energy to the system; a pool of intracellular peptidases that hydrolyze oligopeptides into free amino acids (Figure 6). We studied the ability of *S. thermophilus* strains to generate (bioactive) peptides by hydrolysis of bovine caseins. These peptides were identified by LC-MS/MS in collaboration with STLO, INRA Rennes (Miclo *et al.*, 2012; Chang *et al.*, 2014; review Hafeez *et al.*, 2014). Moreover, we were able to show that the *S. thermophilus* 4F44 strain also expressed a unique, free extracellular form of PrtS (Chang *et al.*, 2012). The existence of this free form is due to a deficiency in the activity of sortase A, which anchors the protease to the cell wall. We were able to demonstrate this by constructing a mutant strain deleted from its *srtA* gene using the natural transformation technique, based on a quorum sensing mechanism (Awussi *et al.*, 2021). This free form of the protease was purified and in turn used to generate (bioactive) peptides from bovine caseins. Finally, we showed that the adhesion capabilities of the mutant strain lacking the *srtA* gene were unaffected and thus that the PrtS protease played no role in adhesion in the intestinal digestive tract (Kebouchi *et al.*, 2016). In addition, by studying the genome of the *S. thermophilus* LMD-9 strain, a new peptide transport system was discovered. Expression of this transport system was confirmed by RT-PCR. A mutant in which the new transport system was inactivated was constructed and specificity studied. This OTS cluster is involved in the transport of di- and tripeptides (Jameh *et al.*, 2016).

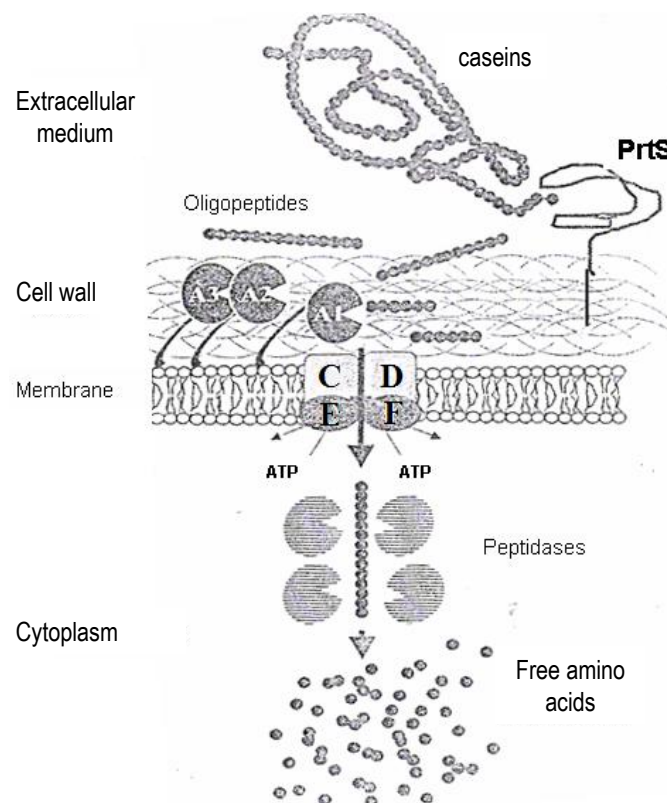


Figure 5: Schematic representation of the proteolytic and oligopeptide transport systems in *S. thermophilus* (Monnet, 2006). PrtS represents the wall-anchored surface protease. The transport system consists of the Ami proteins (A1-A2-A3; C, D, E and F).

### 3.3.2. Probiotic bacteria

#### *Some probiotic characteristics and phylogeny of 30 strains of S. thermophilus*

The anti-inflammatory properties (at INRA Jouy-en-Josas, Dr. Jean-Marc Chatel, Micalis), adhesion capacities and resistance to digestive tract stress of 30 *S. thermophilus* strains from our collection were studied. In addition, we established a phylogenetic tree of our strains using the molecular biology method of Multi Locus Sequence Typing (MLST), with partial PCR amplification and sequencing of 8 “housekeeping” genes. The tree was constructed using the “Neighbor Joining” method. Most of our *S. thermophilus* strains resist well to stress and have promising anti-inflammatory properties, however, we could not demonstrate a link between the genetic proximity of the strains (Figure 7) and their probiotic properties (Figure 8) (Junjua *et al.*, 2016). In this study, I supervised the entire design and analysis by MLST and the construction of the phylogenetic tree. The study of these probiotic properties was pursued as part of Ophélie Uriot's thesis (Uriot *et al.*, 2021).

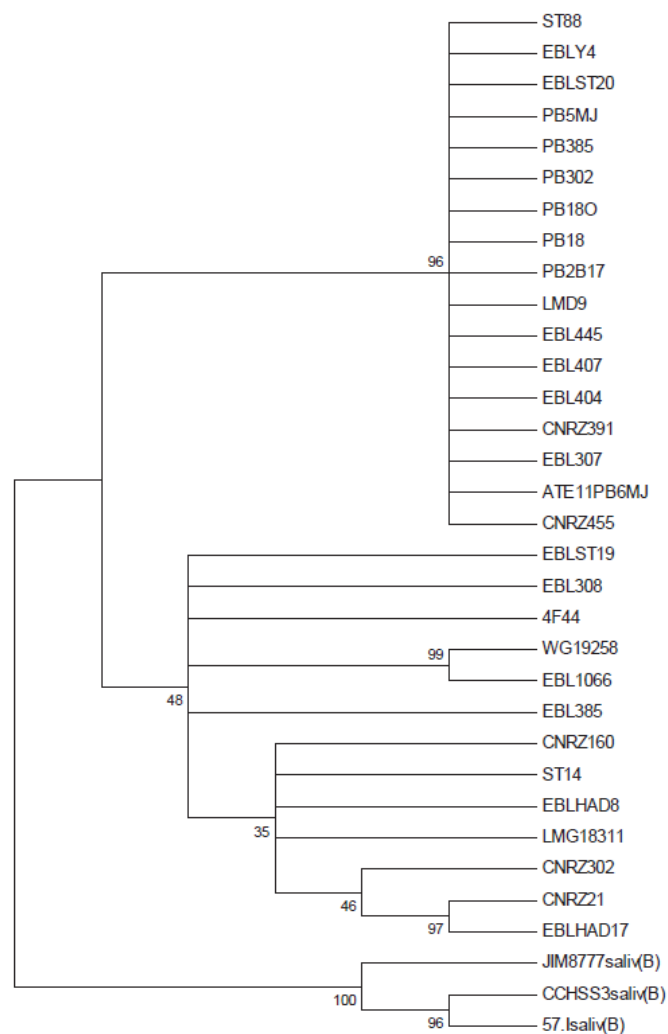


Figure 6: Phylogenetic tree of the 30 *S. thermophilus* strains.

It was established from concatenated sequence of the housekeeping genes *ddlA*, *pepO*, *glcK*, *ilvC*, *thrS*, *tkk*, *dnaE*, *pyrE* and *serB*, using the Neighbor Joining Method and rooted using the corresponding sequences of *S. salivarius* JIM8777, CCHSS3 and 57.I (Junjua *et al.*, 2016).

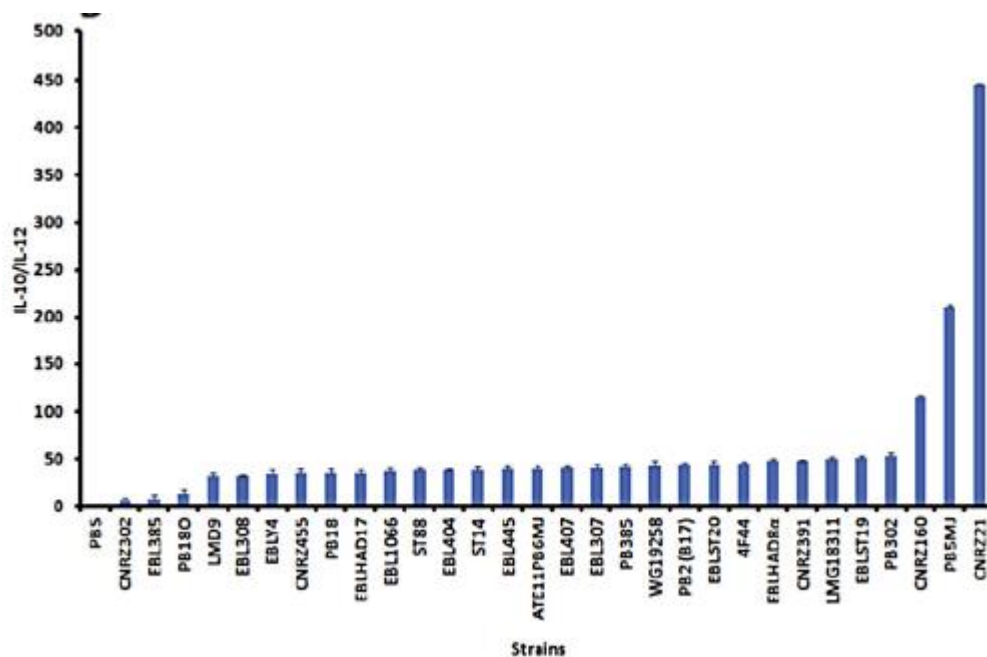


Figure 7: Production of cytokines after co-incubation of the human PBMC cells with the 30 *S. thermophilus* strains: IL-10/IL-12 ratio. (Junjua *et al.*, 2016)

#### *Identification of lactic acid bacteria in traditional Algerian and Tunisian fermented milks and study of some probiotic characteristics*

Collaboration with research institutes in Tunisia (Dr. El Hatmi Halima, Institut supérieur de biologie appliquée de Medenine, Tunisia) and Algeria (Dr. Karima Kharroub, INATAA, Université Frères Mentouri Constantine, Algeria).

Two students (M2 and PhD) harvested traditional fermented products in their countries and isolated the lactic acid bacteria present. I trained them in molecular biology techniques: extraction of bacterial DNA, partial PCR amplification of 16S rRNA and the 16S-23S intergenic region (ITS), bioinformatics analysis of the sequences obtained to identify the bacterial species isolated, and MLST classification. The adhesion capacity and resistance to bile salts and acid pH of 4 *S. macedonicus* strains isolated from Algerian milk were then studied. These *S. macedonicus* strains are highly resistant to the stresses encountered in the digestive tract within a food matrix, and also display good adhesion capacities (Khaldi *et al.*, 2019; PhD Khaldi, 2020).

(...)

#### *Health promoting properties of S. thermophilus*

In 2018, I joined the MicroBio team at STLO, under the supervision of Dr. Eric Guédon (DR INRA). *S. thermophilus* is a species with very little genetic divergence due to the recent emergence of the species (around 10,000 years ago), yet phenotypic heterogeneity of our strains has been demonstrated in our previous work. To understand the genetic determinants of the observed traits, we have made sequenced the genomes of five of our *S. thermophilus* strains by the PacBio sequencer (Pacific Biosciences). The strains were chosen for their adhesion capacity, variable acid and/or bile stress resistance (after Junjua *et al.*, 2016). Thanks to the expertise of STLO's MicroBio team, I was able to annotate the raw sequence data and then begin to study intra-“category” similarities and inter-“category” differences using bioinformatics methods. As part of this CRCT, I also studied the

“health” properties of 79 *S. thermophilus* strains through a bibliographic and bioinformatics study (Figure 9) (Roux *et al.*, 2022).

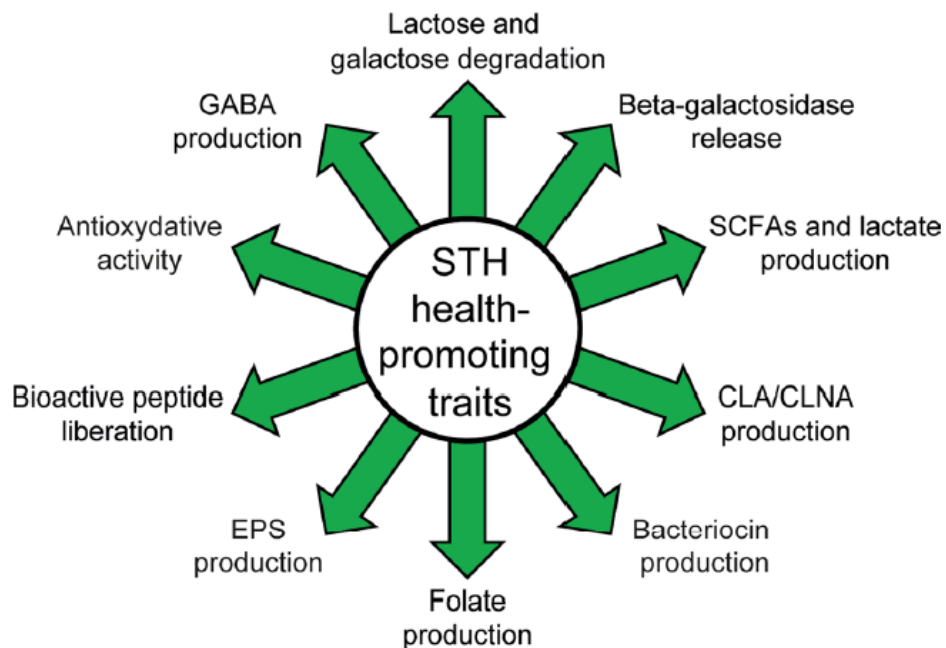


Figure 8: Health-promoting properties of *S. thermophilus*.

SCFAs, short chain fatty acids; CLA, conjugated linoleic acid; CLNA, conjugated linolenic acid; EPS, exopolysaccharides; GABA, gamma-aminobutyric acid (Roux *et al.*, 2022).

### 3.4. From Nanopore sequencing to microbiota & health (since 2021)

(...)

#### 3.4.1. Nanopore principle

The principle of the ONT is the following: “All Oxford Nanopore sequencing devices (MinION on Figure 10) use flow cells which contain an array of tiny holes — nanopores — embedded in an electro-resistant membrane. Each nanopore corresponds to its own electrode connected to a channel and sensor chip, which measures the electric current that flows through the nanopore. When a molecule passes through a nanopore, the current is disrupted to produce a characteristic ‘squiggle’. The squiggle is then decoded using basecalling algorithms to determine the DNA or RNA sequence in real time (Figure 11)” (<https://nanoporetech.com/platform/technology>, consulted on July 5, 2024).

The main limitation of Nanopore technology is (was) its error rate, which was between 5% and 10% when we started this project in 2018. Systematic errors are (were) due to homopolymers (succession of identical nucleotides), since the speed of passage through the pore is (was) not constant.

Improvements in physics (type of sensors at pore level, now with a double reading head at membrane level; lower, but now more constant, speed), chemistry (improved library preparation kits) and bioinformatics (monthly updates of the Guppy basecalling software) have resulted in error rates of less than 1% in 2024, approaching those of PacBio HiFi.



Figure 9: MinION device  
(ONT, <https://nanoporetech.com/products/sequence/minion>)

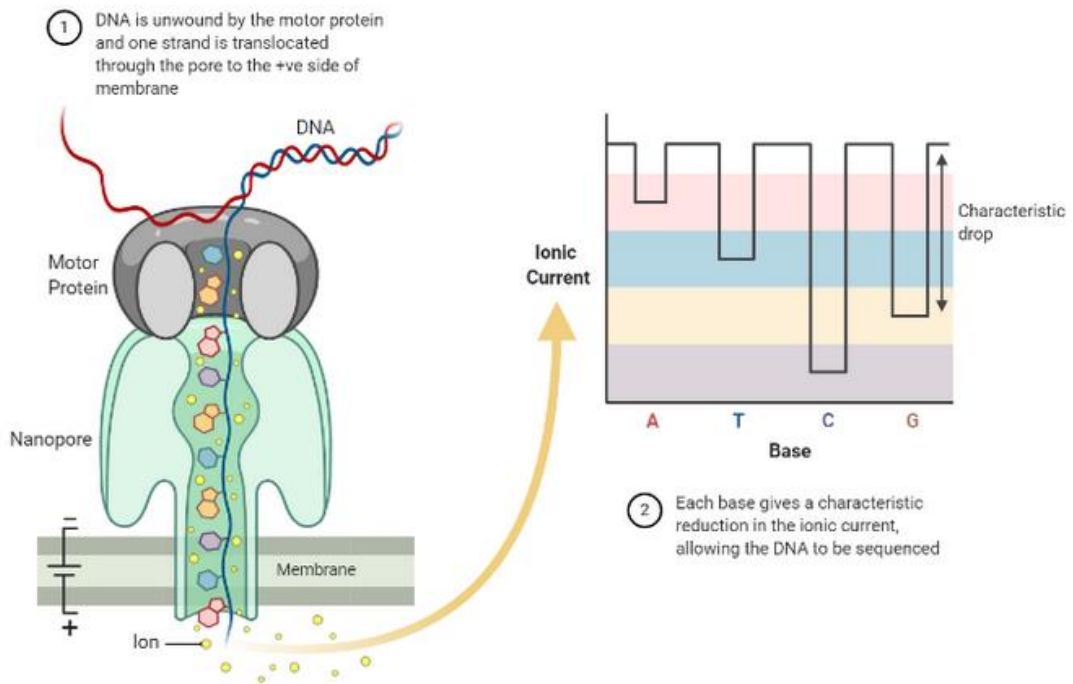


Figure 10: Very simplified Nanopore sequencing principle  
(source Biorender)

### 3.4.2. A rapid method to differentiate bacterial strains

- September 2018 - August 2021:

We therefore decided to launch a project with my Calbinotox team in Nancy, the STLO MicroBio team and the GenScale team. The idea was to develop a rapid method for bacterial identification at strain level, based on a few DNA sequences obtained on a MinION. This project had the advantage of being useful to the 2 biology teams and of combining my skills in microbiology and molecular biology with my interest in bioinformatics. Thanks to contacts from STLO and the GenScale team, I learned quickly about ONT technology by being hosted on the I2BC (Institute for Integrative Biology of the Cell, CNRS) platform in Gif-sur-Yvette and at Genoscope in Evry, two leading specialists in sequencing technologies in France. I was then able to implement this technology at STLO (INRAE, Rennes) and even train colleagues at IGDR (Institute of Genetics and Development from Rennes), since I was the first in Brittany to have this Nanopore technology and the necessary expertise. Our strain identification project was well evaluated and, in parallel with my Inria delegation, we obtained an INRAE-Inria thesis grant for a PhD student in bioinformatics. This project was called "TherMin".

As the biologist on the project, I also had to optimize the DNA extraction step to favour the extraction of long fragments, which is not a trivial problem, and carry out all the sequencing; but I also trained in bioinformatics to make the most of the MinION sequencing data, and assembled 30 new *S. thermophilus* genomes myself (Illumina short reads hybrid assembly and my Nanopore long reads,

Unicycler software <https://github.com/rrwick/Unicycler>, Wick *et al.*, 2017). One of my genome assemblies has even become the reference genome for *S. thermophilus* (CIRM-65; [https://www.ncbi.nlm.nih.gov/datasets/genome/GCF\\_903886475.1/](https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_903886475.1/)).

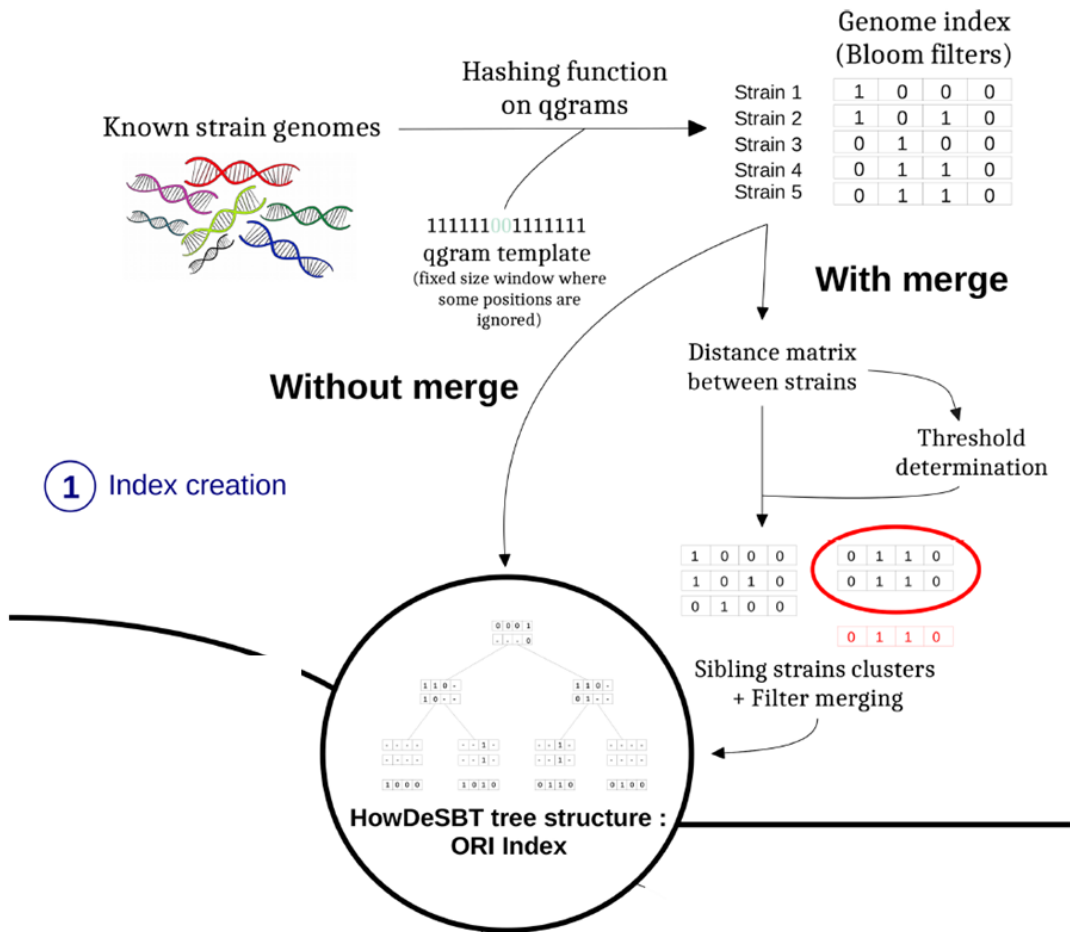
We have succeeded in developing a tool, ORI, which can be used to differentiate bacteria at strain level, or between sibling strains if the strains are too close genetically. Although I don't code, I was able to provide all the data necessary for the development of the ORI tool, as well as beta-test and challenge it, since I am now a bioanalyst.

The principle of ORI is the following: (1) the creation of the index containing the reference genomes of the interest species and (2) the query of this index with long reads from Nanopore sequencing in order to identify the strain(s). Spaced seeds which introduce don't care positions in kmers that won't be disturbed by sequencing errors were used. We've added a "merge" option that allows the assignment of a group of strains that are very/too close genetically (Figures 12 and 13).

(<https://github.com/gsiekaniec/ORI>) (Siekaniec *et al.*, 2021b; PhD Siekaniec, 2021).

We have obtained very good identification results, whether on single or mixed strains (up to 6 genomes tested) of *S. thermophilus*, *S. pyogenes* or lactobacilli (Siekaniec *et al.*, 2021a).

(...)



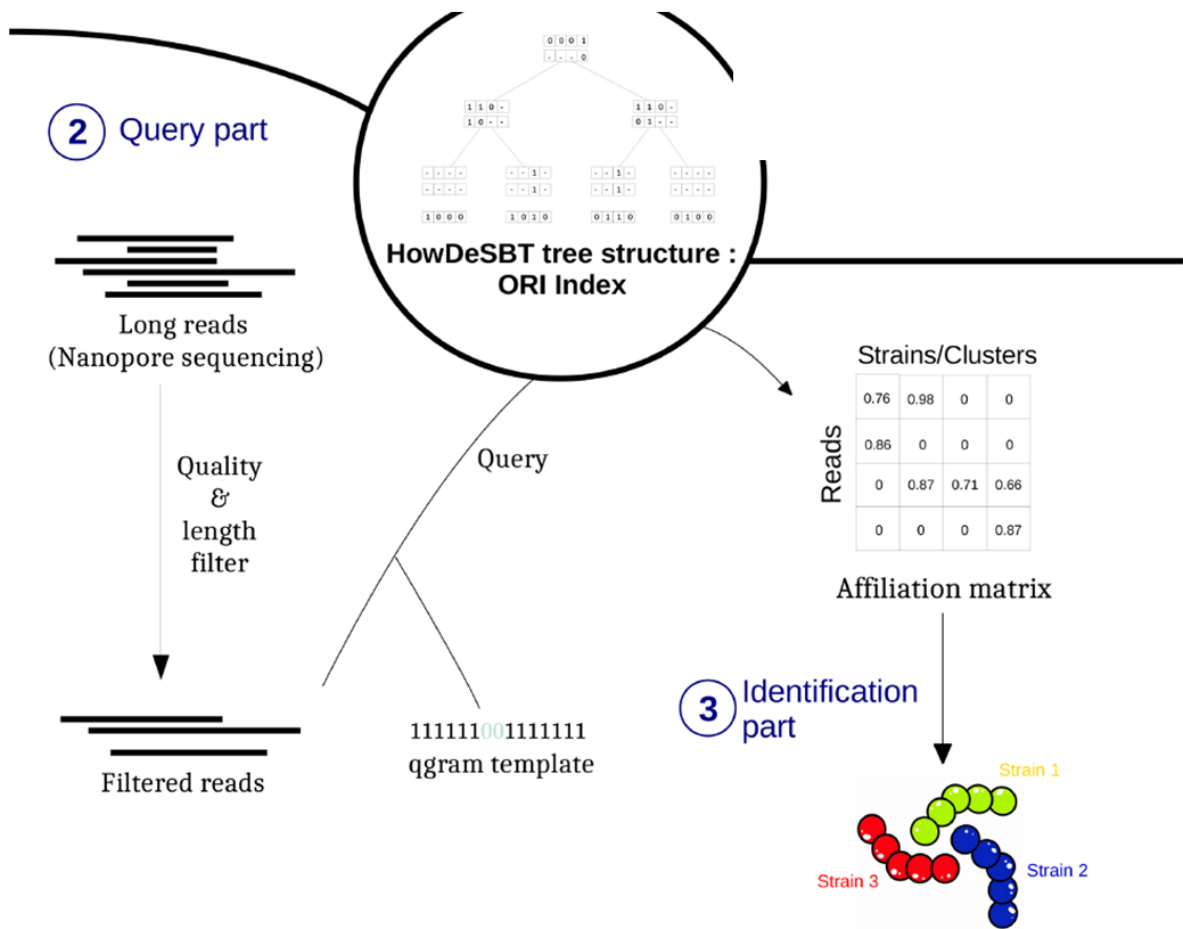


Figure 11: Overview of the ORI method in three steps (1) genome indexing, (2) query the index from filtered reads, and (3) identification of strains (Siekaniac *et al.*, 2021b)

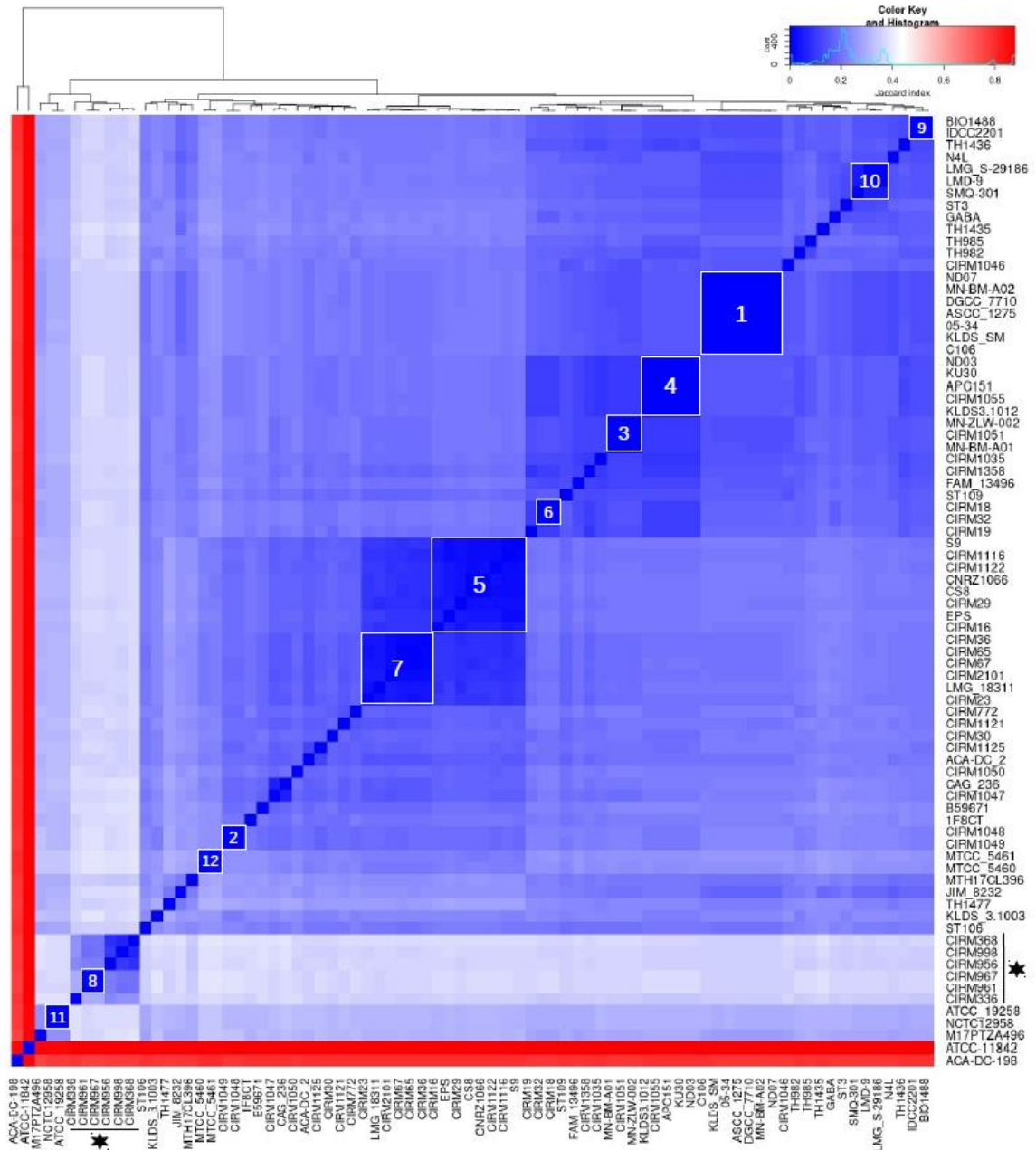


Figure 12: Heatmap of the Jaccard distance for 77 *S. thermophilus* strains + *S. macedonicus* ACA-DC 198 + *L. delbrueckii* subsp. *bulgaricus* ATCC 11842. The clusters of sibling strains are represented by white squares numbered from 1 to 12 (Siekaniac *et al.*, 2021b)

### 3.4.3. Microbiota characterization

(...)

While waiting for the associate professor position to open, we recruited, at the beginning of 2021, while I was still on an Inria delegation, an M2 trainee together (Gaëlle Boudry and myself) to initiate the collaboration. The subject we have decided to develop was as follows: “Study of the intestinal

microbiota of a newborn piglet using MinION sequence analysis and reconstruction of metabolic networks”.

So, for the past 3 years, I have been working on characterising the intestinal microbiota of piglets (human model) and reconstructing metabolic networks to predict the production of metabolites by the microbiota and thus gain a better understanding of host-microbiota interactions, in particular in a context of dysbiosis. This project, which is of great interest to the NuMeCan Institute, draws on GenScale's expertise in processing large-scale genomic data (assembly, genome reconstruction, taxonomic assignment, genome indexing), as well as Dyliss' expertise (another bioinformatics team at IRISA) in reconstructing metabolic networks from previously identified/reconstructed genomes. Although I'm now affiliated to the NuMeCan EAT team, I'm still working as an external collaborator in the GenScale team. My office is still located on the IRISA site, which makes it much easier to interact with colleagues from the GenScale and Dyliss teams, with whom I co-supervise bioinformatics masters and PhD student to carry out my research.

Thanks to improvements in sequencing technologies and a drastic reduction in sequencing costs in recent years, the idea is to use the Nanopore technology to sequence all the DNA present (metagenomics) in a colic sample. This metagenomics sequencing provides much more information than a conventional Illumina short-read sequencing of the DNA coding for rRNA16S (a ribosomal subunit with highly conserved sequences in which universal primers hybridise and highly variable sequences that enable taxonomic assignment). Taxonomic assignment after amplification and partial 16S sequencing is based on around 500 nucleotides only, whereas a bacterial genome is around Mb in size (around 2 Mb for *S. thermophilus*, 4 Mb for *E. coli*). In this way, 16S RNA gives access to a reliable affiliation at family or bacterial genus level at best. We know, for example, that there are strains of *E. coli* that are pathogenic, commensal or even probiotic. It turns out that it is not so much the bacterial taxa that are important, but rather the enzymes they express through the genes contained in their genome. It therefore seems important to describe bacteria at genome level.

To launch this project, we capitalized on a research programme, Holopig, carried out at INRAE by both the NuMeCan-EAT and GenPhySE (Toulouse) laboratories, for which extra samples of colon content were still available. The aim of this Holopig project was to demonstrate the role of enterobacteria in the development and maturation of the intestinal barrier in the first days of life. Piglets were treated with colistin (an antibiotic targeting Gram-negative bacteria, including enterobacteria). The colic contents of control and treated piglets (without enterobacteria) were sequenced (16S RNA) and analysed by metabolomics (nuclear magnetic resonance and mass spectrometry) (Beaumont *et al.*, 2023). These relatively simple samples (microbiota from the first few days of life, still poorly differentiated) (Figure 14), with strong contrasts between the control and treated samples (with or without Gram negative bacteria) and for which we had metabolomics data, were ideal to start our development of a microbiota functional analysis pipeline based on metagenomics data.

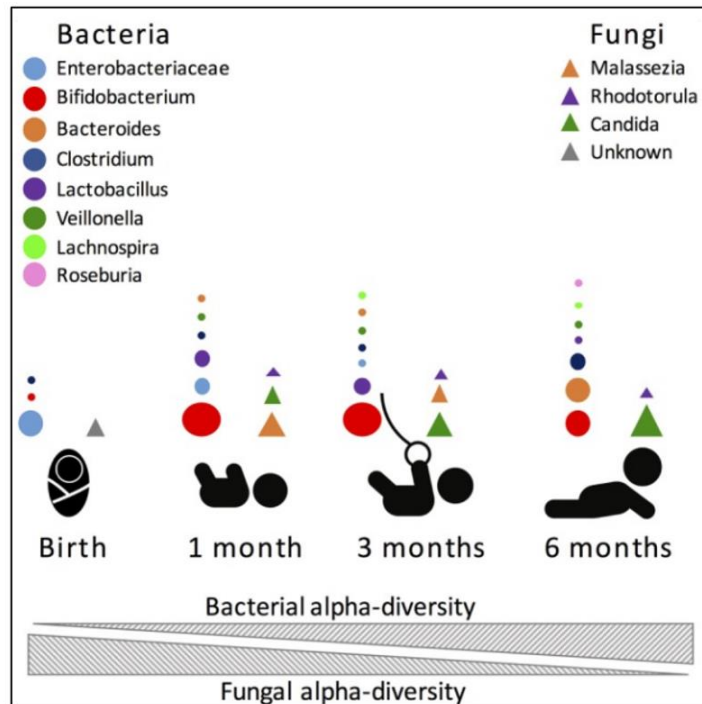


Figure 13: The bacterial and fungal composition of the intestinal microbiota from birth to 6 months (Laforest-Lapointe and Arrieta, 2017). Enterobacteria, which are very present at birth, decrease sharply as bacterial diversity increases

Therefore, during Rania Ouazhrou M2 training (2021), Oxford Nanopore sequencing technology, was used to sequence total DNA, extracted from a seven-day-old piglet gut microbiota sample, to identify the bacterial species present and to predict their metabolite production. Since, the reconstruction of metabolic networks requires complete and well annotated genomes, an approach based on the identification of the closest reference genomes was chosen (tools: Kraken2 followed by ORI) and thus allows to overcome the high error rate of the ONT and low genomes coverage. A total of 22 different genus and species corresponding to 54 genomes were identified with an abundance >0.5% of reads, 17 were Grams- bacteria and 37 were Grams+. Genome annotations were harmonized (tool: Egg-nog-mapper) and metabolic pathways were predicted using Pathway-tools (Figure 15). The abundance of bacterial species in the sample corresponded to what was expected for a porcine intestinal microbiota. Indeed, the genus *Lactobacillus* was the most abundant in the sample. Thus, we were able to simulate the dominant strains in our sample as well as possible. For *E. coli* (enterobacteria), the species of interest for our study, three reference strains, representative of the sample, were chosen.

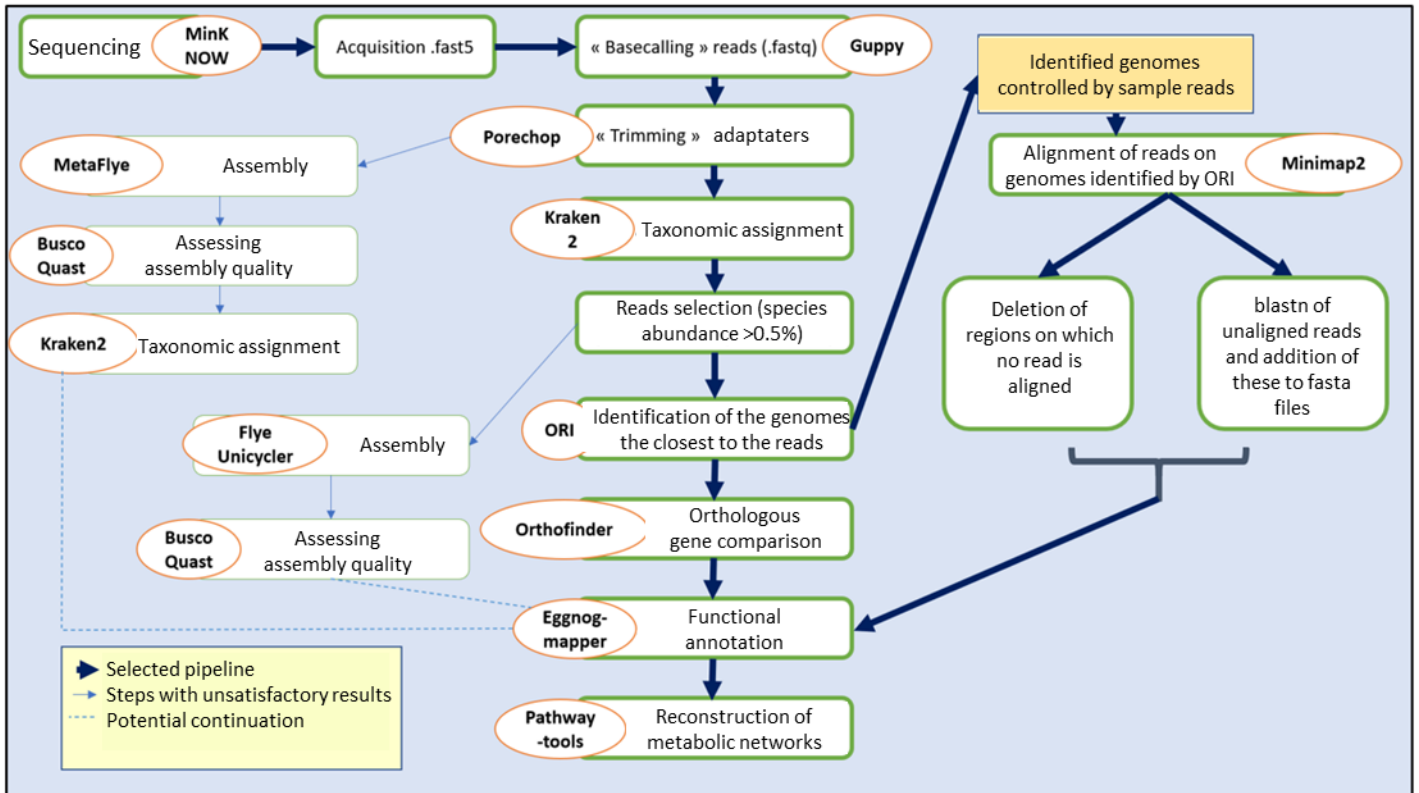


Figure 14: Schematic representation of the proposed pipeline for analysing an intestinal microbiota: from Nanopore sequencing to the reconstruction of metabolic networks.

Bolded boxes and arrows represent the stages followed (middle and right), non-bolded boxes and arrows (left) represent stages explored but which did not yield satisfactory results (internship report by Rania Ouazahrou, 2021)

The method for identifying the genomes corresponding to the sample reads was improved during Jacky Ame's internship (M2, 2022) and 5 new control samples and 5 treated samples were analysed. Meanwhile, a M1 student, Moana Aulagner, worked on improving the reconstruction of metabolic networks on the control sample treated as part of Rania's internship. This work was continued with the arrival of Lune Angevin at the beginning of 2024 in M2, co-supervised by Anne Siegel and Jeanne Got from the Dyliss team, who improved the "reconstruction of metabolic networks" part and applied it to all the 11 samples sequenced in Nanopore.

(...)

A doctoral contract has now been obtained to continue and improve the work with Lune Angevin (see PhD project in appendix 2).

### 3.4.4. Others

During my 3- years of Inria delegation, I worked as a sort of research engineer on Nanopore sequencing technology. My expertise in Nanopore, as part of a team of bioinformaticians at Inria, has enabled us to develop or strengthen a number of collaborations with teams at INRAE, INSERM and

even IMT (Institut Mines-Telecom) Atlantique. Therefore, besides the major project of my delegation “TherMin”, several collaborations have been set up, including three main ones:

- (i) Sequencing of pathogenic *Streptococcus* genomes, with an INSERM laboratory at Rennes University Hospital (Inserm, CIC 1414), to understand the local temporal evolution of the epidemic linked to *S. pyogenes* strains, in particular *emm* 75. One publication (Devaere *et al.*, 2020) and hosting of a Master 2 bioinformatics student from February to September 2020;
- (ii) Assembling *Vibrio* strains genomes (contaminants in shrimp farming) and phages (PhD Culot, 2020);
- (iii) DNA information archiving, a multidisciplinary project (using Nanopore technology to read back stored information) with the Laboratoire des Sciences et Techniques de l'Information (Lab-STICC, Lorient) and IMT-Atlantique (Brest).

As this last project was and still is important to the GenScale team, I'm going to focus on it. Being the first to acquire Nanopore expertise in Brittany, in early 2019, Dominique Lavenier was contacted by Laura Conde-Canencia, an associate professor at IMT-Atlantique (Lorient), who had started a project on DNA information storage. The pipeline of information storage on DNA is as follows (Figure 16), and the Nanopore is used to read the archived information. I was in charge of the biological part.

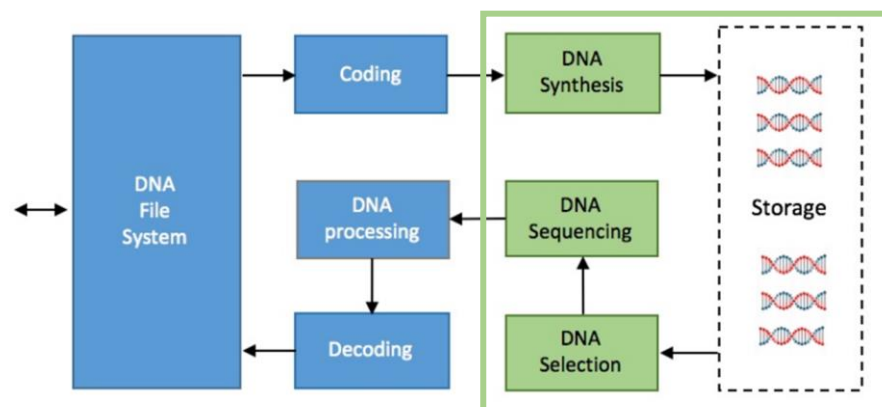


Figure 15: Pipeline used for DNA storage  
(blue boxes: IT; green boxes: biology; source: Dominique Lavenier)

To date, the limiting step in this pipeline is DNA synthesis. There are chemical synthesis processes and more recent enzymatic processes, but DNA synthesis has not exceeded 60 to 200 nucleotides, in order to maintain the accuracy of the sequence (not introducing errors). Currently, DNA synthesis is a long and very costly process. A recent revolution in sequencing technologies has made it possible to re-read cold-stored information (long-term archiving, with infrequent access) at affordable cost and within reasonable times. We're betting that one day, this technological revolution will also affect DNA synthesis methods, and that it will then be simple to generate DNA to store information. In the meantime, we have chosen to assemble DNA fragments of the order of 500 nucleotides to be able to store long information (up to 24 kb to date). To achieve this, Dominique and I have recruited in 2021 a CNRS research engineer in biology (Julien Leblanc) for the dnarXiv project. This project, which began with an Inria exploratory action, has since received a number of other grants (see the Scientific responsibilities - Research project funding section) and other staff have been recruited (PhDs, engineers) in computer science and data security, etc. (Leblanc *et al.*, 2024; poster presentations: Conde-Canencia *et al.*, 2019 ; Lavenier *et al.*, 2019 ; Coatrieux *et al.*, 2022, cf 5.4. National publications).

My main projects and collaborations since 2018, based on Nanopore sequencing technology, are illustrated in Figure 17.

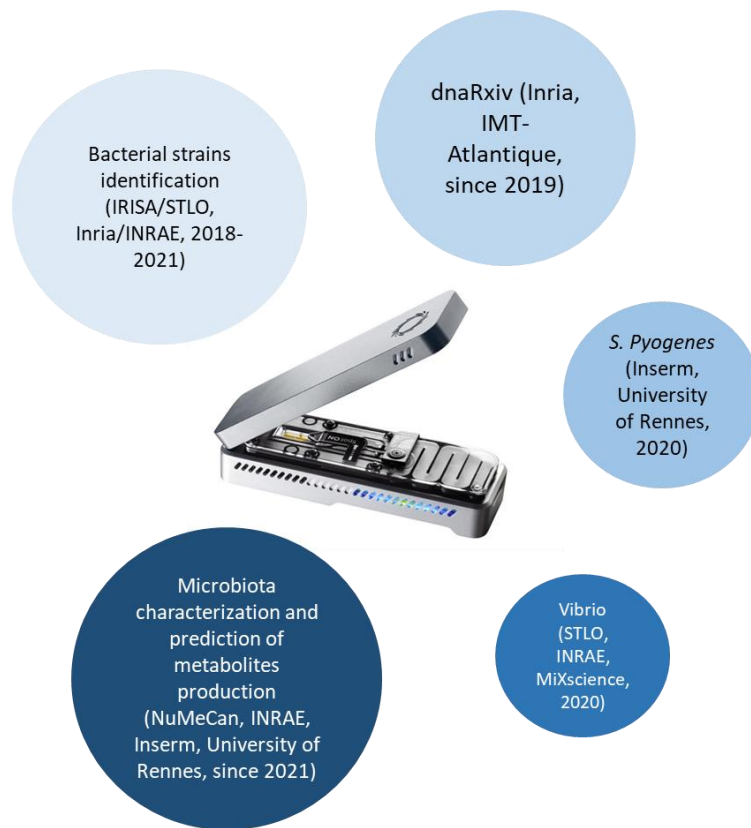


Figure 16: My projects and collaborations with Nanopore technology since 2018

### 3.5. Scientific dissemination and outreach

Popular science

(...)

Services

(...)

### 3.6. Scientific responsibilities

Research project funding

(...)

Other

(...)

## 4. CONCLUSION, RESEARCH PROJECT & PERSPECTIVES

### 4.1. Conclusion

(...)

### 4.2. Project

To date, (too) many bacterial genomes are available in databases. Our approach of identifying strains by matching reads to referenced genomes seems a good idea, in terms of the required coverage and the resulting savings in sequencing time and data production. Lune's PhD thesis (submitted project in Appendix 2) will be co-directed by Pierre Peterlongo (DR Inria, GenScale, expert in genome indexing) and Riccardo Vicedomini (CR CNRS, GenScale, expert in metagenome assembly). The initial focus will therefore be on using sequences to produce genomes that faithfully reflect the composition of the microbiota, either by analogy with reference genomes (indexing, methods based on k-mers) or by *de novo* assembly where possible. Following on from the identification work with ORI, it will be necessary to determine the minimum level of sequencing data required to obtain the best possible picture of the microbiota. The more accurate the genomes, the more reliable the predictions of metabolite production will be. This first part is therefore essential to the successful completion of the project. All this fits in perfectly with the GenScale team's research themes: reducing memory footprint, increasing execution speed.

After this first stage of metagenome reconstruction, we will once again collaborate with researchers from the Dyliss team to make these genomes meaningful. Indeed, our colleagues in the NEM network have emphasized that the overall pool of metabolites produced by the microbiota is probably more important than the bacterial genus present. Dysbiosis certainly needs to be considered at the metabolome level, rather than at the level of bacterial taxa.

The approaches we have adopted so far during Rania's, Moana's and Lune's internships give Boolean results (0 or 1), whereas the biological reality differs in terms of the number of copies of genes depending especially on the abundance of a strain and the systems of regulation of gene expression. Without being able to perfectly match reality, which is too complex, we plan to use new tools from the Dyliss team to adjust our models and introduce more quantitative predictions.

The data from the Holopig project (early life microbiota, well contrasted between 2 populations, well characterized in terms of 16S and metabolome) are ideal for developing and testing the pipeline, by comparing prediction data at pipeline output with measured biological data.

After all this development, our pipeline will be applied to more complex samples with unknown inter-sample variations: in the case of infant nutrition, breastfeeding versus infant formula, the impact of this diet on the intestinal bacterial population is not known exactly.

Our predictions will enable us to better understand the impact of a change in microbiota on the pool of metabolites produced, and thus later on the interactions with the host. This could enable us to propose targeted probiotics to specifically restore deficient metabolic functions. The aim is to improve infant formulas by supplementing them with selected strains of GRAS bacteria.

### 4.3. Perspectives

(...)

### Science and environmental issues

(...)

## 5. PUBLICATIONS AND COMMUNICATIONS

### 5.1. Publications in peer-reviewed journals

19. Leblanc J., Boullé O., **Roux É.**, Nicolas J., Lavenier D. and Audic Y., 2024. *In vitro* construction and long read sequencing analysis of a 24 kb long artificial DNA sequence encoding the Universal Declaration of the Rights of Man and of the Citizen. *BioTechniques*, **76**: 203-215. doi: 10.2144/btn-2023-0109.

18. **Roux É.**, Nicolas A., Valence F., Siekaniiec G., Chuat V., Nicolas J., Le Loir Y. and Guedon E., 2022. The Genomic Basis of the *Streptococcus thermophilus* Health-Promoting Properties. *BMC Genomics*, **23**: 210-232. doi: 10.21203/rs.3.rs-1081849/v1.

17. Awussi A.A., **Roux É.**, Humeau C., Hafeez Z., Maigret B., Chang O.K., Lecomte X., Humbert G., Miclo L., Genay M., Perrin C. and Dary A., 2021. Role of the Sortase A in the Release of Cell-Wall Proteinase PrtS in the Growth Medium of *Streptococcus thermophilus* 4F44. *Microorganisms*, **9**: 2380. [IF<sub>2020-2021</sub> = 3,864]. doi: 10.3390/microorganisms9112380.

16. Siekaniiec G., **Roux É.**, Lemane T., Guédon É. and Nicolas J., 2021. Identification of Isolated or Mixed Strains from Long Reads: A Challenge met on *Streptococcus thermophilus* Using a MinION Sequencer. *Microbial Genomics*, **7**: 1-14. [IF<sub>2020-2021</sub> = 5,237]. doi: 10.1099/mgen.0.000654.

15. Uriot O., Kebouchi M., Lorson E., Galia W., Denis S., Chalancon S., Hafeez Z., **Roux É.**, Genay M., Blanquet-Diot S., and Dary-Mourot A., 2021. Identification of *Streptococcus thermophilus* Genes Specifically Expressed under Simulated Human Digestive Conditions Using R-IVET Technology. *Microorganisms*, **9**: 1113. [IF<sub>2020-2021</sub> = 3,864]. doi: 10.3390/microorganisms9061113.

14. Devaere M., Boukthir S., Moullec S., **Roux É.**, Lavenier D., Faili A. and Kayal S., 2020. Complete genome sequences of two strains of *Streptococcus pyogenes* belonging to an emergent clade of the

genotype *emm89* in Brittany, France. *Microbiol. Resour. Announc.*, **9**: e00129-20. [IF<sub>2020</sub> = 0,877]. doi: 10.1128/MRA.00129-20.

13. Khalidi T.E.M., Kebouchi M., Soligot C., Kharroub K., Le Roux Y. and **Roux É.**, 2019. *Streptococcus macedonicus* strains isolated from traditional fermented milks: resistance to gastrointestinal environment and adhesion ability. *Appl. Microbiol. Biotechnol.*, **103**: 2759-2771. [IF<sub>2019</sub> = 3,53]. doi: 10.1007/s00253-019-09651-z.

12. Jameh N., Galia W., Awussi A. A., **Roux É.**, Genay M., Perrin C. and Dary A., 2016. Characterization of a new peptide transport system in *Streptococcus thermophilus*. *Food Res. Int.*, **86**: 34-35. [IF<sub>2015/2016</sub> = 3,182]. doi: 10.1016/j.foodres.2016.04.039.

11. Junjua M., Kechaou N., Chain F., Awussi A. A., Roussel Y., Perrin C., **Roux É.**, Langella P., Bermúdez-Humarán L.G., Le Roux Y., Chatel J.-M. and Dary A., 2016. A large scale *in vitro* screening of *Streptococcus thermophilus* strains revealed strains with a high anti-inflammatory potential. *LWT - Food Sci. Technol.*, **70**: 78-87. [IF<sub>2015/2016</sub> = 2,711]. doi: 10.1016/j.lwt.2016.02.006.

10. Kebouchi M., Galia W., Genay M., Soligot-Hognon C., Lecomte X., Awussi A. A., Perrin C., **Roux É.**, Dary A. and Le Roux Y., 2016. Implication of sortase-dependent proteins of *Streptococcus thermophilus* in adhesion to human intestinal epithelial cell lines and bile salt tolerance. *Appl. Microbiol. Biotechnol.*, **100**: 3667-3679. [IF<sub>2015/2016</sub> = 3,376]. doi: 10.1007/s00253-016-7322-1.

9. Chang O.-K., **Roux É.**, Awussi A. A., Miclo L., Jardin J., Jameh N., Dary A., Humbert G. and Perrin C., 2014. Use of a free form of the *Streptococcus thermophilus* cell envelope protease PrtS as a tool to produce bioactive peptides. *Int. Dairy J.*, **38**: 104-115. [IF<sub>2014</sub> = 2,008]. doi: 10.1016/j.idairyj.2014.01.008.

8. Hafeez Z., Cakir-Kiefer C., **Roux É.**, Perrin C., Miclo L. and Dary A., 2014. Strategies of producing bioactive peptides from milk proteins to functionalize fermented milk products. *Food Res. Int.*, **63**: 71-80. [IF<sub>2014</sub> = 2,818]. doi: 10.1016/j.foodres.2014.06.002.

7. Miclo L.<sup>§</sup>, **Roux É.**<sup>§</sup>, Genay M., Brusseau E., Poirson C., Jameh N., Perrin C. and Dary A., 2012. Variability of hydrolysis of beta-, alpha-s1- and alpha-s2-caseins by 10 strains of *Streptococcus thermophilus* and resulting bioactive peptides. *J. Agric. Food Chem.*, **60**: 554-565. [IF<sub>2012</sub> = 2,906]. doi: 10.1021/jf202176d. <sup>§</sup> co-premiers auteurs

6. Chang O.K., Perrin C., Galia W., Saulnier F., Miclo L., **Roux É.**, Driou A., Humbert G. and Dary A., 2012. Release of the cell-envelope protease PrtS in the growth medium of *Streptococcus thermophilus* 4F44. *Int. Dairy J.*, **23**: 91-98. [IF<sub>2012</sub> = 2,333]. doi: 10.1016/j.idairyj.2011.10.014.

5. Jolivet P., **Roux É.**, D'Andrea S., Davenport M., Negroni L., Zivy M. and Chardot T., 2004. Protein composition of oil bodies in *A. thaliana* ecotype WS. *Plant Physiol. Biochem.*, **42**: 501-509. [IF<sub>2004</sub> = 1,414]. doi: 10.1016/j.plaphy.2004.04.006.

4. Mlickova K., **Roux É.**, Athenstaedt K., D'Andrea S., Daum G., Chardot T. and Nicaud J.-M., 2004. Lipid accumulation, lipid body formation, and acyl coenzyme A oxidases of the yeast *Yarrowia lipolytica*. *Appl. Environ. Microbiol.*, **70**: 3918-3924. [IF<sub>2004</sub> = 3,810]. doi: 10.1128/AEM.70.7.3918-3924.2004.

3. **Roux É.**, Baumberger S., Axelos M. and Chardot T., 2004. Oleosins of *Arabidopsis thaliana*: expression in *Escherichia coli*, purification and functional properties. *J. Sci. Food Agric.*, **52**: 5245-5249. [IF<sub>2004</sub> = 0,871]. doi: 10.1021/jf0355046.

2. Billaud C., **Roux É.**, Brun-Mérimée S., Maraschin C. and Nicolas J., 2003. Inhibitory effect of unheated and heated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction product model systems on polyphenoloxidase from apple. I. Enzymatic browning and enzyme activity inhibition using spectrophotometric and polarographic methods. *Food Chem.*, **81**: 35-50. [IF<sub>2003</sub> = 1,204]. doi: 10.1016/S0308-8146(02)00376-X.

1. **Roux É.**, Billaud C., Maraschin C., Brun-Mérimée S. and Nicolas J., 2003. Inhibitory effect of unheated and heated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction product model systems on polyphenoloxidase from apple. II. Kinetic study and mechanism of inhibition. *Food Chem.*, **81**: 51-60. [IF<sub>2003</sub> = 1,204]. doi 10.1016/S0308-8146(02)00377-1.

## 5.2. Oral international communications

1. **Roux É.**, Miclo L., Chang O.K., Humbert G., Dary A. and Perrin C., septembre 2013. Opportunity to use the cell envelope proteinase Prts of *Streptococcus thermophilus* to produce bioactive peptides. *8<sup>th</sup> NIZO Dairy Conference*, Papendal (Pays-Bas).

## 5.3. International communications by posters

4. Nasri W., Jrad Z., **Roux É.**, Perrin C., Girardet J.-M., Arroum S., Khorchani T. and El Hatmi, H., avril 2014. Isolement et identification des bactéries lactiques du lait de chamelle fermenté spontanément (Isolation and identification of lactic acid bacteria from spontaneously fermented camel milk). *1<sup>er</sup> Congrès International de Technologies Alimentaires et Contrôle Qualité des Aliments (1st International Congress on Food Technology and Food Quality Control)*, Djerba, Tunisie.

3. Daubos N., **Roux É.**, Kobi D., Gutfreund L. and Didierjean L., mai 2005. Yeast discrimination: evaluation of molecular typing tools for yeast strain discrimination and homogeneity checking. *30<sup>th</sup> EBC congress*, Prague (République Tchèque).

2. Billaud S., **Roux É.**, Corbin A. and Nicolas J., septembre 2002. Inhibitory effect of Maillard reaction products prepared from glucose or fructose with L-cysteine on enzymatic browning and activity of polyphenoloxidase from apple and tyrosinase from mushroom. *Polyphenol communications*, Marrakech (Maroc).

1. **Roux É.**, Baumberger S. and Chardot T., septembre 2002. Behaviour of oleosins at oil / water or air / water interfaces. *43<sup>rd</sup> International Conference on the Bioscience of Lipids*, Graz (Autriche).

## 5.4. National publications

(...)

## 6. SUPERVISION

### 6.1. Thesis co-supervision list

3. Since October 2024: Lune Angevin, PhD INRAE NuMeCan and IRISA, Rennes. “Caractérisation fine du microbiote du nouveau-né et prédiction de la production de métabolites en fonction de l’alimentation (lait maternel vs préparations pour nourrissons) pour concevoir des consortia bactériens pour des préparations pour nourrissons plus biomimétiques” (Fine characterization of newborn microbiota and prediction of metabolite production as a function of diet (breast milk vs. infant formula) to design bacterial consortia for more biomimetic infant formulas): microbiology, molecular biology, sequencing, (bio)informatics (co-direction Inria with Pierre Peterlongo and CNRS with Riccardo Vicedomini). I will supervise 40% of this thesis (October 2024-September 2027).

2. December 10<sup>th</sup> 2021: Grégoire Siekaniec, PhD INRAE STLO – Inria GenScale, Rennes. “Caractérisation différentielle de souches d’une espèce bactérienne par séquençage Nanopore (MinION)” (Differential characterization of bacterial strains using Nanopore sequencing (MinION)): microbiology, molecular biology, sequencing, (bio)informatics (co-direction Inria-INRAE with Jacques Nicolas and Eric Guédon). I supervised 30% of this thesis (September 2018 - December 2021).

2 publications:

Siekaniec G., **Roux É.**, Lemane T., Guédon É., and Nicolas J. Bacterial strain identification using a MinION sequencer: meeting the challenge for *Streptococcus thermophilus*. *Microbial Genomics*, **7**: 1-14. doi: 10.1099/mgen.0.000654

**Roux É.**, Nicolas A., Valence F., Siekaniec G., Chuat V., Nicolas J, Le Loir Y. and Guedon E. The Genomic Basis of the *Streptococcus thermophilus* Health-Promoting Properties. *BMC Genomics*, **23**: 210-232. doi: 10.21203/rs.3.rs-1081849/v1

Grégoire did a post-doctorate at the Institut de Cancérologie de l'Ouest in Nantes in the omics data science unit (processing data from breast cancer sequencing) in 2022-2023. He has just started a new post-doctorate in Luxembourg (since May 2024).

1. June 29<sup>th</sup> 2020: Tedj El Moulouk Khaldi, PhD student at the “Institut de la Nutrition, de l’Alimentation et des Technologies Agro-Alimentaires” (INATAA; Institute of Nutrition, Food and food Processing Technologies), University Constantine 1 (UC1), Algeria. “Caractérisation de bactéries lactiques issus de produits laitiers fermentés algériens” (Characterization of lactic acid bacteria from fermented Algerian dairy products): identification of bacterial strains and classification by molecular biology (16S rRNA sequencing, MLST), study of proteolytic activity, capacity to adhere to intestinal cells, potential probiotic effects and generation of bioactive peptides (Algerian supervision by Pr. Karima Kharroub, French co-supervision shared with Pr. Yves Le Roux. Eighteen months scholarship to carry out research at the University of Lorraine (2014-2015). I was responsible for 30% of the supervision of this thesis (February 2014 - defense in June 2020; please note that this thesis is an Algerian thesis which, for various reasons beyond our control (maternity leave, teaching load of Ms. Khaldi who was, and still is, employed in her country, relieved of her teaching duties only during the 18 months spent in France in 2014-2016, etc.), was defended very late).

1 publication:

Khaldi T.E.M., Kebouchi M., Soligot C., Kharroub K., Le Roux Y. and **Roux É.**, 2019. *Streptococcus macedonicus* strains isolated from traditional fermented milks: resistance to gastrointestinal

environment and adhesion ability. *Appl. Microbiol. Biotechnol.*, **103**: 2759-2771. doi: 10.1007/s00253-019-09651-z

To the best of my knowledge, Moulouk is still a university lecturer in Algeria.

## 6.2. Master's 2 internships

(...)

Overall, I have (co-)supervised three PhD students (one thesis defended in 2020, one in 2021, one just started), 10 Master 2 internships, five Master 1 internships, five L3 / LP internships, one L2 internship and ten applied LP tutored projects (duration: 6 weeks).

## 7. SKILLS

### 7.1. Techniques

(...)

### 7.2. Other

Languages

(...)

Computing

(...)

## 8. REFERENCES

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## 9. APPENDIX

(...)