



Bacterial strategies for biogeochemical transformations: diversity, mechanisms, regulations and environmental drivers

François Thomas

► To cite this version:

François Thomas. Bacterial strategies for biogeochemical transformations: diversity, mechanisms, regulations and environmental drivers. Bacteriology. Sorbonne Université, 2021. <tel-04092426>

HAL Id: tel-04092426

<https://hal.science/tel-04092426v1>

Submitted on 9 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

Mémoire en vue de l'obtention de l'
Habilitation à Diriger des Recherches (HDR)

présenté et soutenu publiquement par

Dr. François THOMAS

Chargé de recherches, CNRS

le 20 décembre 2021

**Bacterial strategies for biogeochemical transformations:
diversity, mechanisms, regulations and environmental drivers**

devant le jury composé de

Dr Laurence Blanchard, CR CNRS

Pr. Magali Remaud-Simeon, PR INSA Toulouse

Pr. Thomas Schweder, PR University of Greifswald

Pr. Alain Dufour, PR Université de Bretagne Sud

Dr. Raphaël Lami, MC Sorbonne Université

Rapportrice

Rapportrice

Rapporteur

Examineur

Examineur

Laboratoire de Biologie Intégrative des Modèles Marins
UMR8227 (CNRS-Sorbonne Université)
Station Biologique de Roscoff
Place George Teisier, 29680 Roscoff (France)

Contents

Contents	3
Acknowledgements	4
Abbreviations	5
Foreword	6
Research activities	7
1 <i>Bacterial contribution to mangrove food webs</i>	7
2 <i>Defense reaction in brown algae</i>	8
3 <i>The alginolytic system in <i>Zobellia galactanivorans</i></i>	10
4 <i>Sulfur-oxidizing bacteria in contrasted environments</i>	14
5 <i>Effect of plants on terrestrial pollutant-degrading bacteria</i>	18
6 <i>Bacterial conversion of macroalgal biomass</i>	21
6.1 Detailed characterization of enzymes and metabolic pathways	22
6.2 Regulatory controls in alga-degrading bacteria	24
6.3 Diversity and environmental drivers of alga-degrading bacteria	26
Research project	28
References	32
Annex: Curriculum Vitae	35

Acknowledgements

En premier lieu, je souhaite remercier chaleureusement Laurence Blanchard, Alain Dufour, Raphaël Lami, Magali Remaud-Simeon et Thomas Schweder d'avoir accepté d'évaluer mes travaux et projets de recherche. C'est un véritable honneur de vous compter comme membres de mon jury d'HDR.

J'exprime toute ma reconnaissance aux différents mentors qui m'ont permis de m'épanouir en tant que scientifique depuis 16 ans : Andrea Alfaro, Catherine Leblanc, Philippe Potin, Mirjam Czjzek, Gurvan Michel, Stefan Sievert et Aurélie Cébron. J'ai énormément appris à vos côtés (et j'apprends toujours !). Vous avez su guider mes pas dans le monde scientifique en me laissant développer mon indépendance et en respectant mes choix. C'est une chance d'avoir pu travailler avec chacun.e d'entre vous. Un merci tout particulier à Mirjam, responsable de l'équipe Glycobiologie Marine, qui reste une source d'inspiration quotidienne par sa bienveillance et sa positivité à toute épreuve.

Mes sincères remerciements vont également à tous les collègues des différentes institutions que j'ai fréquentées, certains devenus des amis, dont la liste est bien trop longue pour figurer ici et risquerait de ne pas être exhaustive. Aucun des travaux présentés dans ce manuscrit n'aurait pu aboutir sans vous. Sans citer tout le monde, j'adresse toute ma gratitude à Tristan Barbeyron qui restera mon modèle de microbiologiste et à Murielle Jam sans qui le laboratoire ne tournerait pas. Un grand merci à tous les membres passés et présents de l'équipe Glycobiologie Marine dont je partage le quotidien avec plaisir. La diversité de nos approches est une force particulièrement stimulante.

Je remercie énormément les personnes que j'ai eu le plaisir de (co-)encadrer, qui m'ont fait confiance pour les guider et qui ont largement contribué au succès de mes travaux ces dernières années. Une pensée toute particulière pour Anissa Dieudonné, Maéva Brunet et Magda Dudek, qui furent respectivement mes premières expériences d'encadrement d'un Master 2, d'une thèse et d'un post-doctorat. J'ai certainement développé mes compétences à votre contact ! Je remercie également Nolwen Le Duff, super assistante-ingénieure, dont l'efficacité et l'organisation légendaires ont permis d'initier et finaliser de nombreux projets.

Je souhaite remercier tous les collaborateurs nationaux et internationaux qui ont contribué à mes recherches passées et actuelles, pour leur excellence scientifique, leur investissement sans faille et leur ouverture d'esprit. Je n'oublie pas tous les personnels des services communs (plateformes, administration, services techniques) dont le travail est indispensable pour le fonctionnement quotidien des laboratoires.

Un merci immense à mes parents, mon frère, ma famille et mes ami.e.s (dont beaucoup rencontré.e.s au travail !) qui m'ont toujours soutenu, dans les moments de joie comme dans ceux de doute. C'est bon de vous avoir dans ma vie.

Enfin, à mon mari Jérôme : mon meilleur allié, mon meilleur supporter, mon meilleur critique, mon meilleur pilier, ma meilleure bouée. Mon meilleur tout court.

Abbreviations

AUS	Alginate Utilization System
CAZyme	Carbohydrate-Active enzyme
CBM	Carbohydrate-Binding Module
ChIP-seq	Chromatin immuno-precipitation coupled to high-throughput sequencing
DOC	Dissolved Organic Carbon
EMSA	Electrophoretic Mobility Shift Assay
FCSP	Fucose-Containing Sulfated Polysaccharides
GC-MS	Gas Chromatography coupled to Mass Spectrometry
HISH-SIMS	Halogenated probe In Situ Hybridization coupled to Secondary Ion Mass Spectro.
nano-SIMS	Nano-scale Secondary Ion Mass Spectrometry
OTU	Operational Taxonomic Unit
PAH	Polycyclic Aromatic Hydrocarbons
PHE	Phenanthrene
PL	Polysaccharide Lyase
PUL	Polysaccharide Utilization Locus
qPCR	quantitative PCR
ROS	Reactive Oxygen Species
RT-PCR	Reverse Transcription coupled to PCR
SBR	Station Biologique de Roscoff
SIP	Stable Isotope Probing
SOX	Sulfur-Oxidizing Bacteria
WT	Wild-Type strain

Foreword

Microorganisms have colonized virtually all environments on Earth, and feature an incredibly diverse array of metabolic capabilities. Due to this extreme versatility, they appear as key players of biogeochemical processes, controlling fluxes of organic matter and energy in their respective ecosystems. As a microbiologist with a strong background in molecular biology and biochemistry, I use interdisciplinary approaches to gain insights into which environmental bacteria can perform these processes, how they do it, and how it varies depending on the conditions. My research is therefore centered on the molecular dissection of metabolic pathways, their regulation and the diversity of bacteria involved.

Throughout my career, I investigated multiple aspects of microbiology in marine and terrestrial environments, including mangrove estuaries, salt marshes, polluted soils, deep-sea hydrothermal vents and algal beds (Figure 1). I find all these ecosystems fascinating, yet under-investigated, especially in light of the fact that they provide important ecological and economical functions to society. This career path gave me the unique opportunity to investigate contrasted ecosystems and multiple organisms (macroalgae, terrestrial plants, heterotrophic and chemolithotrophic bacteria). It also allowed me to work on a broad range of scales, from molecules to cells, organisms and ecosystems. The overarching theme of my research is to bridge detailed molecular understanding of metabolic processes to broader microbial ecology questions. Although it is not always easy to be part of both worlds, I find this integrative approach particularly exciting and much needed to approach a full picture of our microbial planet.

In this manuscript, I chose to summarize each of my diverse research experiences, including work dating to before my PhD. Indeed, I am convinced that my current and future projects were strongly influenced by the concepts and techniques I acquired since I first stepped into a research laboratory, 16 years ago. Each of these experiences surely made me who I am as a scientist, and probably also as a person.

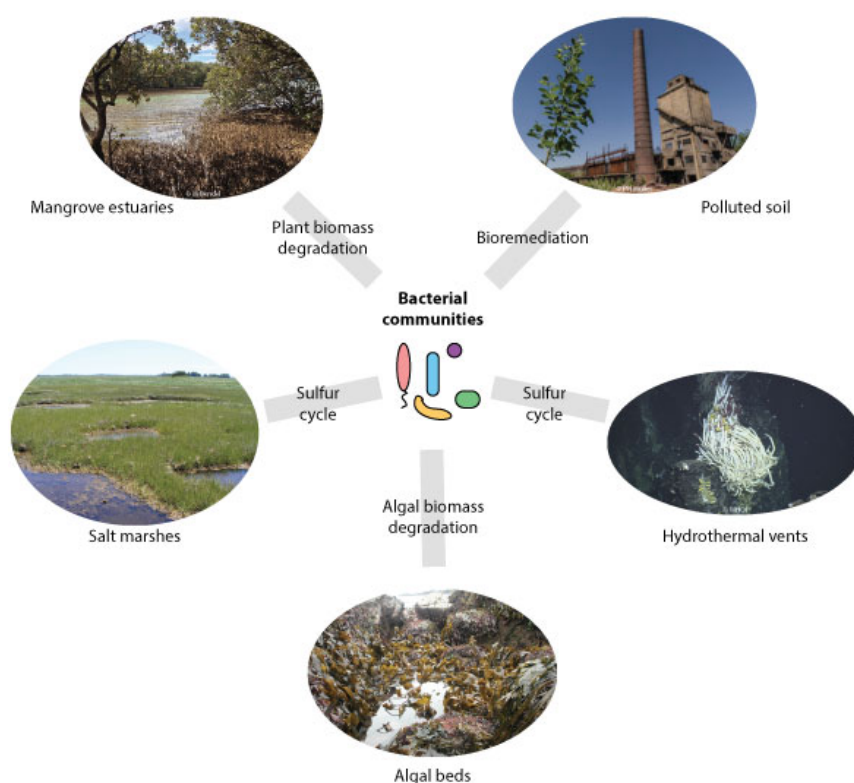


Figure 1: Overview of the diversity of environments and bacterial processes I studied.

Research activities

1 Bacterial contribution to mangrove food webs

My first experience in academic research dates back to 2005, at the end of my engineering degree in Biochemistry and Biotechnologies from INSA Lyon. I joined the Earth and Oceanic Sciences group at the Auckland University of Technology (New Zealand), for a 6-month internship supervised by Dr. Andrea Alfaro.

During this project, I investigated the contribution of bacterial degradation to the fate of plant biomass in the transfer of organic matter within estuaries colonized by mangroves. The investigated estuary (Matapouri, New Zealand), hosted a large variety of available carbon sources to fuel trophic chains, notably derived from plants, macroalgae, phytoplankton and bacteria. However, the individual contributions of each of these potential sources were unclear, hindering the global understanding of the ecosystem functions, a pre-requisite to efficient conservation strategies. In this context, we collected all available sources of organic matter in the field, analyzed their isotopic signatures and specific fatty acid biomarkers, and compared them to those of the dominant fauna. The overarching principle was "You are what you eat", i.e. the molecular composition of a consumer organism reflects that of its main food sources. This work showed that all consumers depended on several primary sources [1], implying that none of the potential food sources were obligatory for the survival of the dominant organisms studied. In addition, we showed the crucial role of bacterial metabolism for trophic transfers within the estuary food web (Figure 2). Indeed, through their degradation activity, bacteria contributed to the transfer of the plant biomass to superior trophic levels [1, 2]. This work led to the publication of two articles in Estuarine, Coastal and Shelf Science [1] and in Estuaries and Coasts [2].

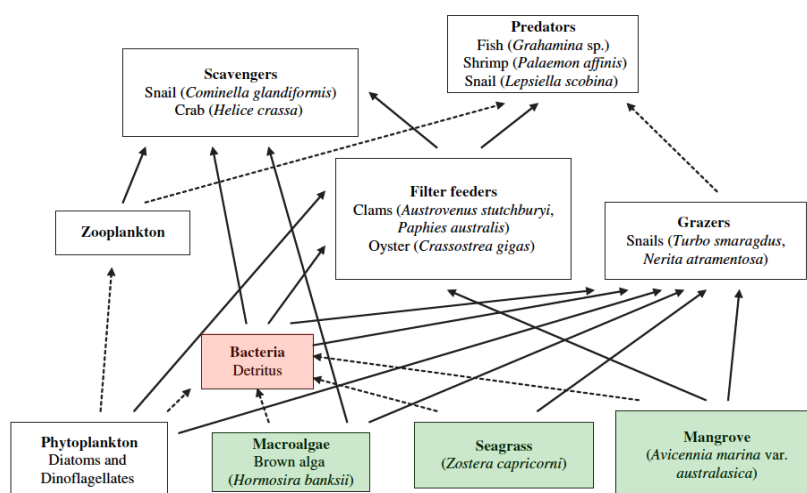


Figure 2: Diagram of the food web at Matapouri Estuary based on fatty acid biomarkers, stable isotopes, and field observations. Through their degradation activities, heterotrophic bacteria (in red) contribute to the transfer of plant and macroalgal biomass (in green) to upper compartments. Modified from [1].

2 Defense reaction in brown algae

From 2006 to 2008, I studied defense reactions in brown algae in the Algal Defense group (UMR7139, Station Biologique de Roscoff SBR), under the supervision of Dr. Catherine Leblanc and Dr. Philippe Potin.

The main scientific question was to assess the existence of distance signaling mechanisms in brown algae during defense reactions following biotic (e.g. bacterial attack or grazing by herbivores) or abiotic stresses (e.g. UV, dessication, etc.). Together with Opisthokonts (animals and fungi) and Archaeplastida (land plants, green and red algae), brown macroalgae are one of the few eukaryotic lineages that have evolved complex multicellularity [3, 4]. Although distant in the eukaryotic tree of life, these three lineages feature similar local immune responses [5]. Besides local immune responses, terrestrial plants and animals are known to feature distance signaling mechanisms, activated upon stress. This comprises two types of responses: (i) a systemic response that protects a part of the organism that has not yet been reached by the aggression, via intra-individual signaling and (ii) the emission of infochemicals that can be perceived by neighboring organisms and prime defense reactions should a future attack occur, via inter-individual signaling. However, such mechanisms were unknown in brown algae, despite the potential implications in terms of the evolution of innate immunity in eukaryotes.

To test the existence of distance signaling in brown algae, my work focused on the kelp *Laminaria digitata*, for which local defense reactions had already been studied [6–9]. We used a combination of tools to characterize the multi-faceted response to stress at the molecular level, including the release of reactive oxygen species (luminometer, ROS-sensitive probes), the transcription of specific genes (quantitative RT-PCR), the induction of defense enzymes (native extractions and enzymatic measurements) and the emission of volatile oxidative compounds (GC-MS). The results notably showed that brown algae emit soluble chemical signals in the seawater following abiotic stress [10, 11] or when they perceive fragments of their cell wall (oligo-alginates) that mimic an attack by heterotrophic bacteria or grazers [10]. Furthermore, we showed that unstressed neighboring algae can perceive some of these molecules, leading to a "warned state" and potentiating subsequent defense reactions [12]. For example, algae that had previously perceived a warning signal from a stressed individual displayed a faster regulation of ROS emission and a greater induction of the expression of defense-related genes compared to naive algae (Figure 3). This inter-individual communication was reminiscent of the priming effect previously known in terrestrial plants [13] and had never been demonstrated in marine algae before.

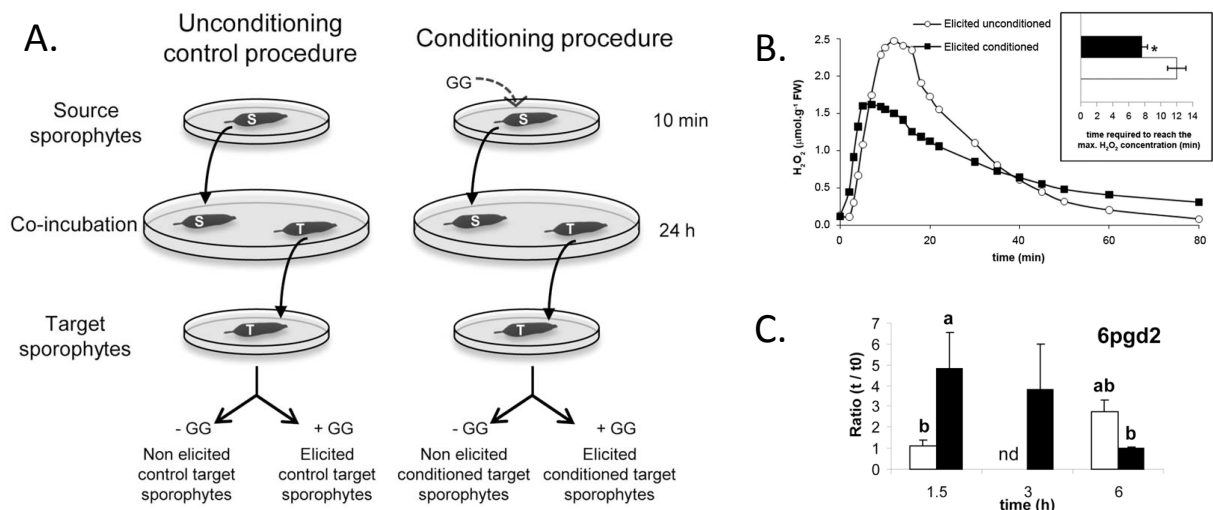


Figure 3 (previous page): Testing the existence of inter-individual signaling in brown algae. **A.** Experimental set-up. ‘Source’ *Laminaria digitata* sporophytes (S) were either elicited by application of oligoalginates (GG) in filtered seawater, either handled in the same way without elicitation for control procedure. Control and conditioned ‘target’ sporophytes (T) were co-incubated with non-elicited or elicited source algae, respectively. After 24 hours, the defense responses of each target sporophyte were tested by a subsequent GG elicitation. **B.** GG-induced oxidative burst in conditioned and unconditioned *L. digitata* sporophytes. Sporophytes were elicited with GGs in seawater and the concentration of H_2O_2 was recorded. Inset: Means and standard errors ($n = 3$) of the time required to reach the maximum of H_2O_2 concentrations in the medium after elicitation. **C.** Change in transcript levels of the *6pgd2* defense-related gene in conditioned and unconditioned *L. digitata* sporophytes after elicitation with GGs. Modified from [12].

In a subsequent study, we also discovered that local defense reactions triggered a systemic response within stressed *L. digitata* sporophytes [14]. We showed that a signal produced at the attacked site could be transported towards distant zones where the stress has not been applied. This systemic response allows a global protection of the algal specimen after a local attack, including the emission of ROS, the increase in the activity of defense-related enzymes and a stronger resistance to herbivory (Figure 4). Again, this mechanism was reminiscent of systemic immune responses of land plants and animals [15] but yet uncharacterized in marine algae.

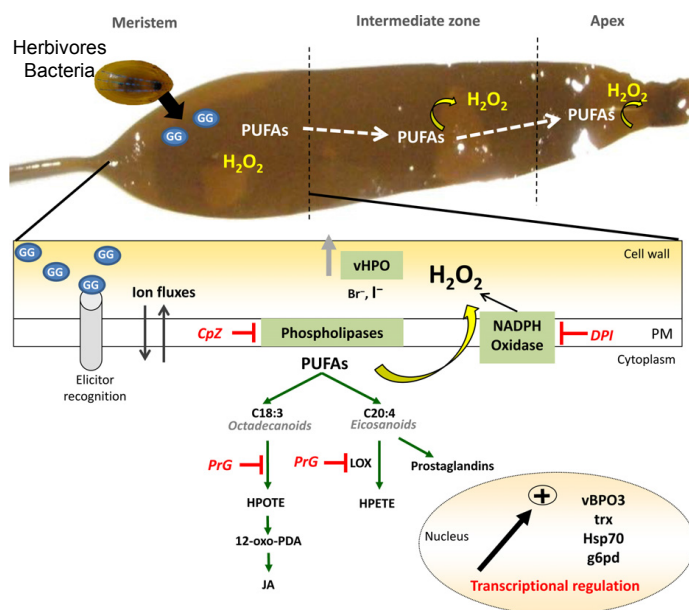


Figure 4: Hypothetical model for the systemic propagation of defense signals in *Laminaria digitata*. Insert below: the recognition of oligoalginates (GG) elicitors by algal cells triggers the production of signaling molecules, which activate either defense-specific biochemical pathways or general cell metabolism. PUFAs, polyunsaturated fatty acids; LOX, lipoxygenase; vHPO, vanadium-dependent haloperoxidase; VHCs, volatile halocarbons; HPOTE, hydroperoxy-octadecatetraenoic acid; HPETE, hydroperoxy-eicosatetraenoic acid; 12-oxo-PDA, 12-oxo-phytodienoic acid; JA, jasmonic acid; PM, plasma membrane; inhibitors – PrG, propyl gallate; CpZ, chlorpromazine-HCl; DPI, diphenylene iodonium; g6pd, glucose-6-phosphate dehydrogenase; trx, thioredoxin; hsp 70, heat shock protein; vBPO3, vanadium-dependent bromoperoxidase 3. Modified from [6].

Altogether, this work on distance signaling led to the publication of five articles in *New Phytologist* [14], *PLoSOne* [12, 16], *Chembiochem* [10] et *Atmospheric Chemistry and Physics* [11]. Overall, it suggests that kelps have evolved defense strategies that are similar to other multicellular Eukaryotes: detect the attack; locally trigger immediate defense reactions; induce gene-regulated defense responses in the zone of attack; protect the individual as a whole (i.e. systemic defense through a vascular system); and warn neighboring individuals or distal parts of the same individuals by volatile signaling (i.e. priming effect). As the ancestors of multicellular eukaryotes were all unicellular organisms, these elaborate strategies should have emerged independently at least three times in the evolution of Eukaryotes, in the Opisthokonta (animals), the Archaeplastida (land plants), and the Stramenopiles (brown algae). Furthermore, these defense reactions likely play an important role in the interactions of macroalgae with other organisms in their environment. This led me to question the ways by which algae-associated bacteria could attack their host, a topic that I decided to investigate during my PhD.

3 The alginolytic system in *Zobellia galactanivorans*

From 2008 to 2011, I performed my doctoral research in the Marine Glycobiology group (UMR7139, Station Biologique de Roscoff) under the direction of Dr. Gurvan Michel and Dr. Mirjam Czjzek. I used the marine bacterium *Zobellia galactanivorans* as a model organism to understand the bacterial degradation of alginate, one of the main components of brown algal extracellular matrices.

Alginate is a linear anionic polysaccharide (Figure 5) consisting of two uronic acid monomers, α -L-guluronate (G) and β -D-mannuronate (M), that can be arranged either as homopolymeric blocks (polyG and polyM) or mixed blocks with alternating monomers (polyMG). Alginate can account for up to 40% of the dry weight of brown algae [17]. As a major fraction of the extracellular matrix in brown algae, it is also one of the first compounds encountered by algae-degrading bacteria during the attack of algal tissues. Furthermore, alginate represents ca. 5% of the dissolved organic carbon (DOC) released by brown algae into seawater via exudation [18]. Therefore, alginate constitutes an abundant resource for heterotrophic bacteria in coastal environments.

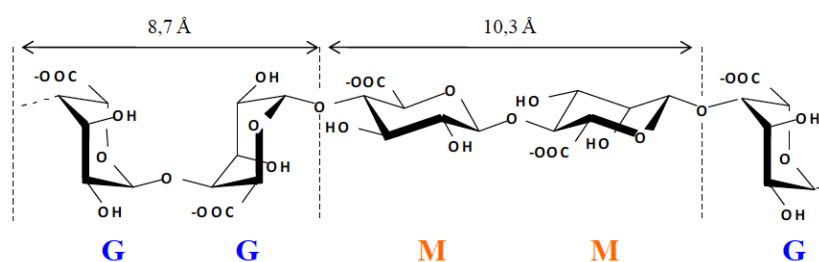


Figure 5: Chemical structure of alginate, comprising α -L-guluronate (G) and β -D-mannuronate (M)

I studied the degradation of alginate by the flavobacterial strain *Zobellia galactanivorans* Dsij^T, which over the years has become a model for the degradation of algal polysaccharides [19]. *Z. galactanivorans* belongs to the phylum *Bacteroidetes* (family *Flavobacteriaceae*) that is recognized for its ability to degrade complex and high-molecular weight organic compounds such as polysaccharides and proteins [20, 21]. *Z. galactanivorans* was initially isolated from a healthy red alga in Roscoff [22], and later shown to degrade a large number of algal polysaccharides from red and brown algae. Before the start of my PhD work, expert annotation of *Z. galactanivorans* genome revealed it encoded a large number of proteins dedicated to polysaccharide degradation [23], such as carbohydrate-active enzymes (CAZymes) and sulfatases. Yet, the exact degradation pathway of alginate and its regulation was still unknown.

First, I used bioinformatic predictions to select candidate genes potentially involved in alginate catabolism in *Z. galactanivorans*. Targeted transcriptomic approaches (end-point and quantitative RT-PCR, Northern Blot) showed that these candidate genes were grouped mainly in two operons dedicated to alginate catabolism (Figure 6A), with two additional genes isolated in the genome. Altogether, they formed a complex Alginate Utilization System (AUS).

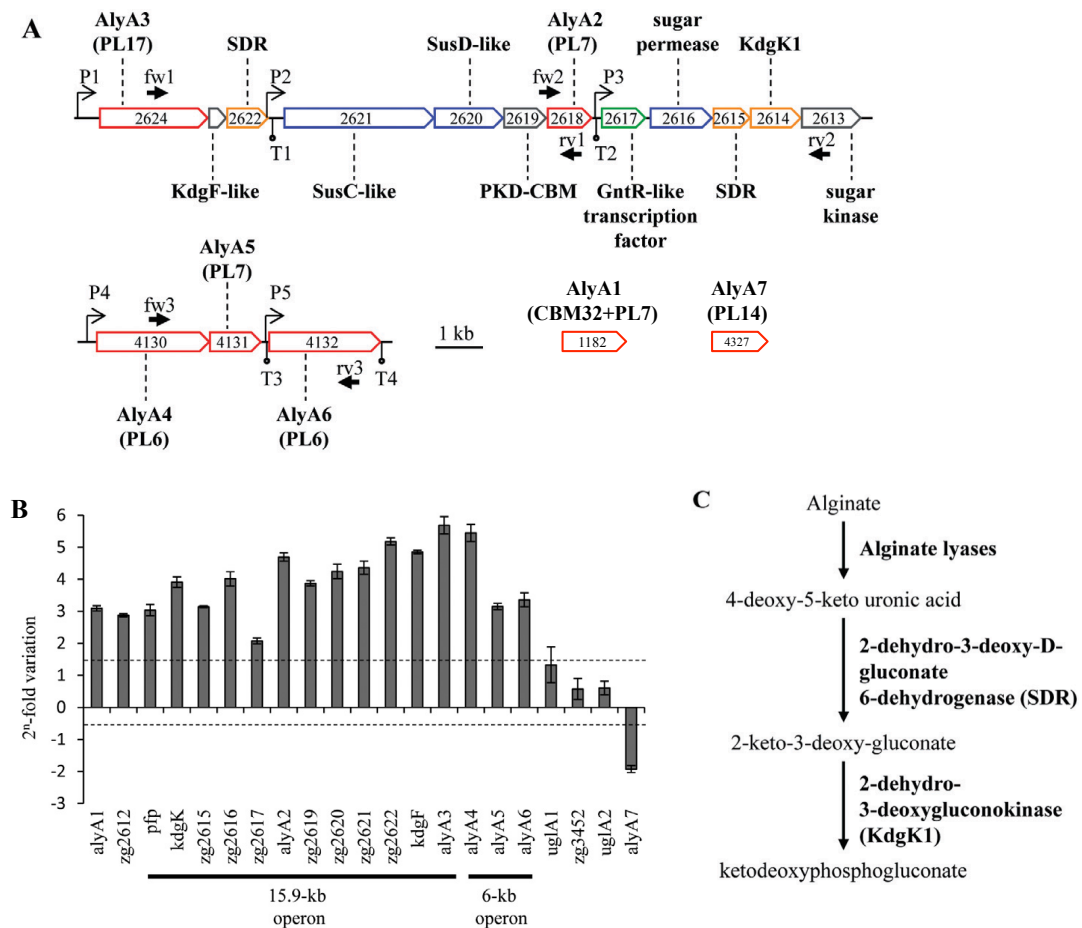


Figure 6: A. Genetic organization of the alginolytic system of *Z. galactanivorans*, comprising two operons and two isolated genes. Predicted promoters (P1-P5) and Rho-independent terminators (T1-T4) are depicted. **B.** Differential expression of genes from the alginolytic system in the presence of alginate compared to glucose (mean \pm s.e.m., $n=3$). **C.** Alginate catabolic pathway. Adapted from [24].

The longest of these operons featured the typical organization of a Polysaccharide Utilization Locus (PUL), a hallmark of polysaccharide degradation by terrestrial and human gut *Bacteroidetes* that had previously not been empirically validated in marine representatives. PULs encode a suite of proteins necessary for the assimilation of a specific polysaccharide [25]. This includes (i) CAZymes for the breakdown of the substrate, (ii) modification enzymes catalyzing the removal of substituents (e.g. acetylases, sulfatases), (iii) membrane proteins favoring binding of the substrate to cell surface, (iv) transporters for oligosaccharides and (v) transcriptional regulators that control the PUL expression depending on substrate availability. This organization was first described in the gut symbiont *Bacteroides thetaiotaomicron*, with the characterization of the Starch Utilization System (Sus) that became the paradigm for PUL systems [26, 27]. Here, I could show that *Z. galactanivorans* possessed a PUL dedicated to alginate utilization, with seven alginate lyases and five downstream enzymes that process the substrate to the central metabolism (Figure 6C). Targeted transcriptomics further showed that the expression of all the genes (except for *alyA7*) was induced in the presence of alginate (Figure 6B), demonstrating that *Z. galactanivorans* can sense the nature of the available carbon sources in its medium and adapt its metabolism.

To better understand the regulation mechanisms controlling polysaccharide degradation in *Z. galactanivorans*, I performed a genome-wide transcriptomic approach using DNA microarrays. The results allowed to identify potential signaling pathways responding to the presence of alginate

and to detect additional genes that might play a yet unknown role in algal polysaccharide degradation [28]. In addition, this global transcriptomic analysis was performed in parallel on bacteria cultivated with other polysaccharides from brown and red algae. Data indicated that transcriptomic profiles were more similar when using substrates from the same algal groups, although their chemical structure was highly different (Figure 7). This suggested the existence of two distinct transcriptomic programs, triggered by bacteria depending on the type of algal biomass available in their environment.

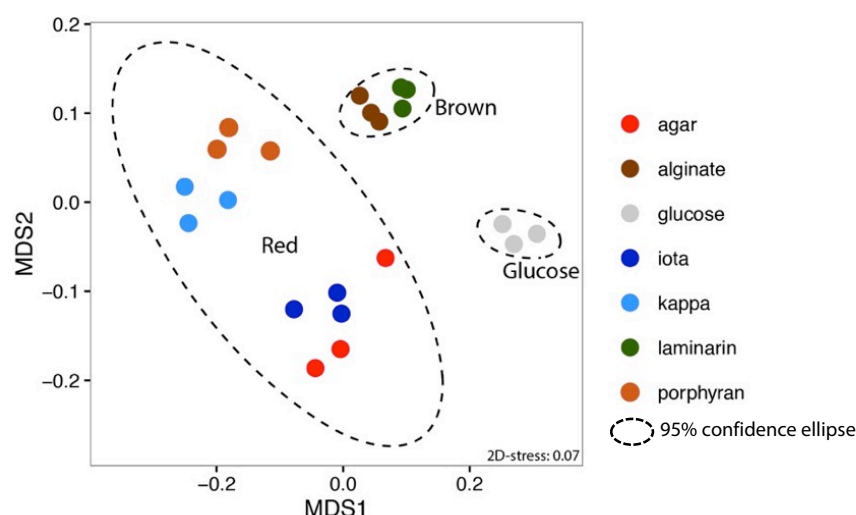


Figure 7: Ordination of genome-wide expression data obtained for *Z. galactanivorans* on polysaccharides from brown (alginate, laminarin) and red algae (agar, porphyran, iota-carrageenan, kappa-carrageenan), and glucose used as a control condition. Modified from [28].

I complemented these transcriptomic approaches with functional genomics, to strengthen our understanding of alginate catabolism by validating the predicted functions of genes within the alginolytic system. I applied a medium-throughput cloning and expression strategy on 35 target genes. Among them, 14 recombinant proteins were successfully expressed in *Escherichia coli*, and seven were produced at larger scale and purified to test their enzymatic functions. Using this strategy, I validated experimentally the complementary activities of 4 alginate lyases, one kinase and one dehydrogenase, acting sequentially to breakdown alginate into simple compounds (Figure 6C). Furthermore, I characterized the biochemical and structural properties of two alginate lyases AlyA1 and AlyA5 in detail [29]. Notably, I measured their kinetic parameters, assessed the influence of temperature and pH on their activity, and identified their mode of action by analyzing their degradation products using NMR and mass spectrometry via a collaboration with glycochemists. Although both enzymes belong to the same polysaccharide lyase family (PL7), they feature different modes of action. AlyA1 is an endo-guluronate lyase cleaving glycosidic bonds between two adjacent G residues. By contrast, AlyA5 is an exolytic oligo-alginate lyase that cleaves unsaturated, G, or M residues from the non-reducing end of alginate oligosaccharides. The 3D-structure of both recombinant enzymes was solved using molecular replacement after X-ray diffraction, highlighting the molecular determinants of these differences in terms of mode of action and substrate recognition (Figure 8). Indeed, AlyA1 has an open-cleft topology that can accommodate long molecules, agreeing with its endolytic mode of action. By contrast, two insertions in AlyA5 sequence block the catalytic cleft, giving rise to pocket topology that explains its exolytic mode of action.

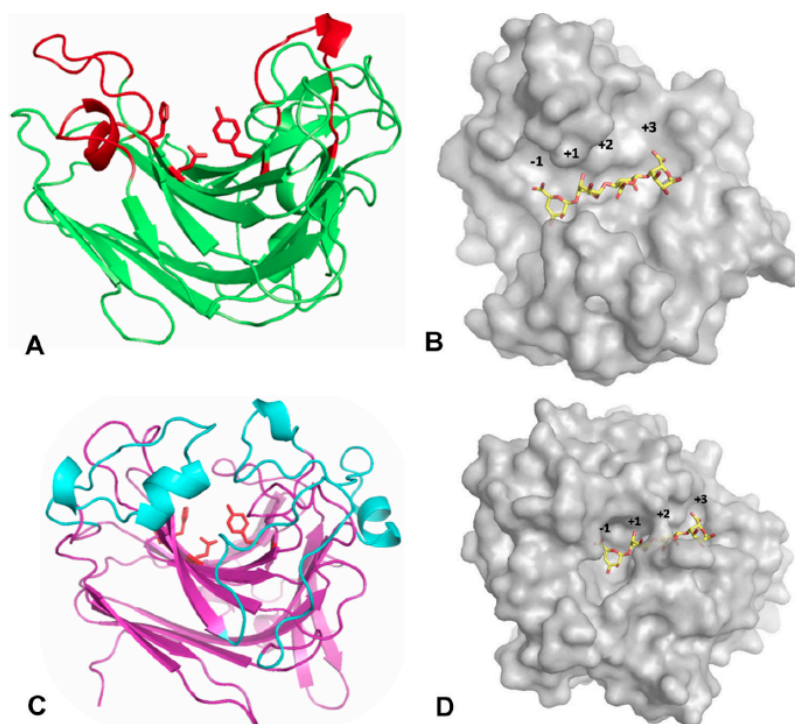


Figure 8: Crystallographic structures of alginate lyases AlyA1 (**A**, **B**) and AlyA5 (**C**, **D**) from *Z. galactanivorans*. Ribbon representations (**A**, **C**) show a similar β -jelly roll fold for both proteins, but with two insertions in AlyA5 (highlighted in blue) that close the catalytic cleft on one side. In the surface representations (**B**, **D**), an alginate oligosaccharide was superimposed to illustrate the open-cleft topology of AlyA1 (endolytic enzyme) compared to the pocket topology for AlyA5 (exolytic enzyme). Adapted from [29].

Finally, I evaluated the taxonomic and environmental distribution of this complex alginolytic system using comparative genomics on all sequenced prokaryotic genomes available at that time (93 *Archaea* and 1743 *Bacteria*). I identified clusters of genes homologous to the AUS only in 39 bacterial species, belonging only to the two phyla *Bacteroidetes* and *Proteobacteria* [24]. The majority of these clusters was found in marine bacteria (31/39), mostly isolated from coastal regions. Surprisingly, AUS were also detected in human gut bacteria. Molecular phylogenies inferred for all homologous proteins in the AUS revealed that their extent distribution likely results from multiple horizontal gene transfers. I showed that the AUS initially emerged in ancestral marine *Bacteroidetes* and was transferred several times independently to marine *Proteobacteria*. The spatial proximity of marine bacteria sharing the same ecological niche (algae-associated bacteria) likely favored these gene exchanges. In addition, the presence of AUS in gut bacteria from Japanese people likely results from lateral transfers from a marine *Bacteroidetes*. Such a transfer could be explained by the traditional consumption of brown algae in Asian cuisine (e.g. Kombu, Wakame), and thus of alga-associated bacteria, favoring the contact with gut bacteria. This finding was reminiscent of a similar transfer (discovered by the group a few years before) of a marine gene to gut bacteria from Asian people, allowing the latter to use the red algal polysaccharide porphyran [30]. Therefore, food-enabled transfer of genetic material from marine bacteria to gut symbionts might be more frequent than anticipated and shape the human microbiome depending on particular substrates available in the diet.

Overall, this interdisciplinary doctoral work allowed deciphering the molecular mechanisms of alginate degradation in a marine bacterium, unveiled potential regulation pathways and showed conserved pathways in phylogenetically and ecologically distant bacteria. It also gave new insights into algae-bacteria interactions, highlighting how well equipped *Z. galactanivorans* is to degrade algal compounds. This work led to the publication of six articles in *Environmental Microbiology* [24], *The Journal of Biological Chemistry* [29, 31], *Frontiers in Microbiology* [20, 28] and *Journal of Microbiological Methods* [32].

4 Sulfur-oxidizing bacteria in contrasted environments

From 2012 to 2014, I was a postdoctoral researcher at the Woods Hole Oceanographic Institution (USA), in the Laboratory for Microbial Ecology and Physiology led by Dr. Stefan Sievert. My research focused on natural communities of sulfur-oxidizing bacteria (SOX) in two contrasted marine environments: salt marshes colonized by the halophytic plant *Sporobolus alterniflorus* (formerly *Spartina alterniflora*) and deep-sea hydrothermal vents.

SOX bacteria are chemolithotrophs that can oxidize reduced sulfur compounds (e.g. H_2S) into sulfate or elemental sulfur while fixing carbon [33]. This process is usually coupled to the reduction of oxygen or nitrate. Therefore, SOX bacteria are important players of the carbon, sulfur and nitrogen cycles in the environment. This is especially true in habitats where reduced sulfur species are abundant and concentrations of oxygen are low, such as salt marsh sediments and hydrothermal vents [34]. Despite this crucial role for nutrient cycling and energy flow, the drivers of SOX bacteria diversity and functions in these environments were still understudied.

Salt marshes are highly productive coastal ecosystems found in intertidal areas and vegetated by salt tolerant non-woody plants. Abundant plant-derived organic matter fuels anaerobic respiration through sulfate reduction, leading to a sustained production of sulfide available for SOX bacteria [35]. I first evaluated the diversity of SOX bacteria in salt marsh sediments using high-throughput sequencing of the 16S rRNA gene, showing a complex assemblage dominated by *Gammaproteobacteria* from the orders *Chromatiales* and *Thiotrichales* [36, 37]. The results further showed that salt marsh restoration strategies such as sand amendments, currently envisioned to mitigate the effects of sea-level rise, could drastically modify the bacterial communities with a decrease of the relative abundance of SOX bacteria to the profit of iron-oxidizers [37].

To assess the parameters controlling sulfur oxidation in salt marshes, I then sequenced and quantified functional genes and transcripts involved in two sulfur-oxidizing metabolic pathways: (i) the *sox* pathway that completely oxidizes H_2S to sulfate and (ii) the *rdsr* pathway where elemental sulfur accumulates as an intermediate before being processed by a reverse-acting dissimilatory sulfite reductase. The results revealed potential interactions between particular SOX taxonomic groups and roots of the dominant salt marsh plant *S. alterniflorus* [36]. A common pool of SOX species dominated by *Gammaproteobacteria* was active on roots, in the rhizosphere and in the sediment, with a global prevalence of the *rdsr* pathway over the *sox* pathway (Figure 9). However, the activity of bacteria using the *sox* pathway increased drastically on roots (Figure 9, bottom right panel), together with an enrichment in sulfur-oxidizing *Epsilonbacteraeota*.

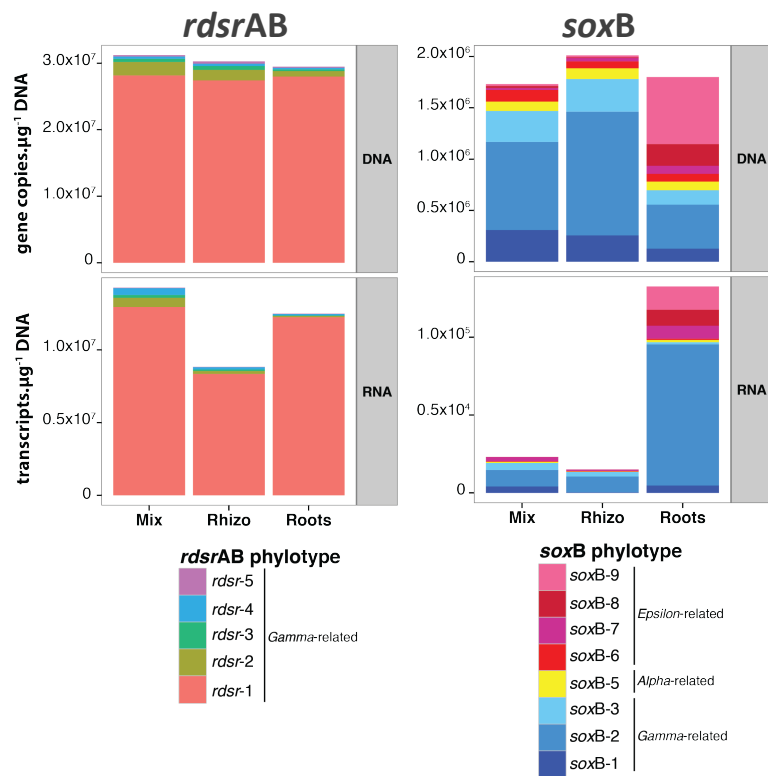


Figure 9: Variations in the abundance of genes and transcripts for several phylotypes of *rdsrAB* and *soxB*, involved in two distinct sulfur oxidation pathways. Three types of samples were analyzed: the raw sediment (Mix), the sediment directly in contact with roots of *S. alterniflorus* (i.e. the rhizosphere, Rhizo) and the roots themselves (Roots). Adapted from [36].

To better understand these potential plant-microbe interactions, we investigated the effect of sediment oxygenation by plants on the SOX bacterial communities. Indeed, *S. alterniflorus* can direct oxygen to the anoxic sediment via its root system [38]. Around roots, SOX bacteria might therefore use oxygen as an electron acceptor, replacing nitrate otherwise used in anoxic sediments. Plants of *S. alterniflorus* were grown under controlled laboratory conditions in rhizoboxes, i.e. mesocosms where the front plate is transparent and removable, allowing direct visualization and sampling of the rhizosphere compartment (Figure 10A). Planar optodes (2D oxygen sensors) were placed directly in contact with the sediment to follow in real-time the *in situ* concentration of oxygen in the rhizosphere. At the end of the experiment, sediment and root samples with a known history of oxygen concentration were collected (Figure 10B), to study the composition and activity of bacterial communities using high-throughput sequencing of the 16S rRNA gene and functional microarrays (GeoChip). I showed a decrease of the richness and diversity of the bacterial communities in oxic conditions, suggesting that oxygen diffusion from the roots selects for a fraction of the resident community from the anoxic sediment. Furthermore, the presence of oxygen in the rhizosphere correlated with a lower expression of two genes from the *sox* pathway (Figure 10C and D). Although low oxygen concentrations can favor sulfur oxidation, we therefore suggested that high concentrations found around the roots might inhibit SOX bacteria.

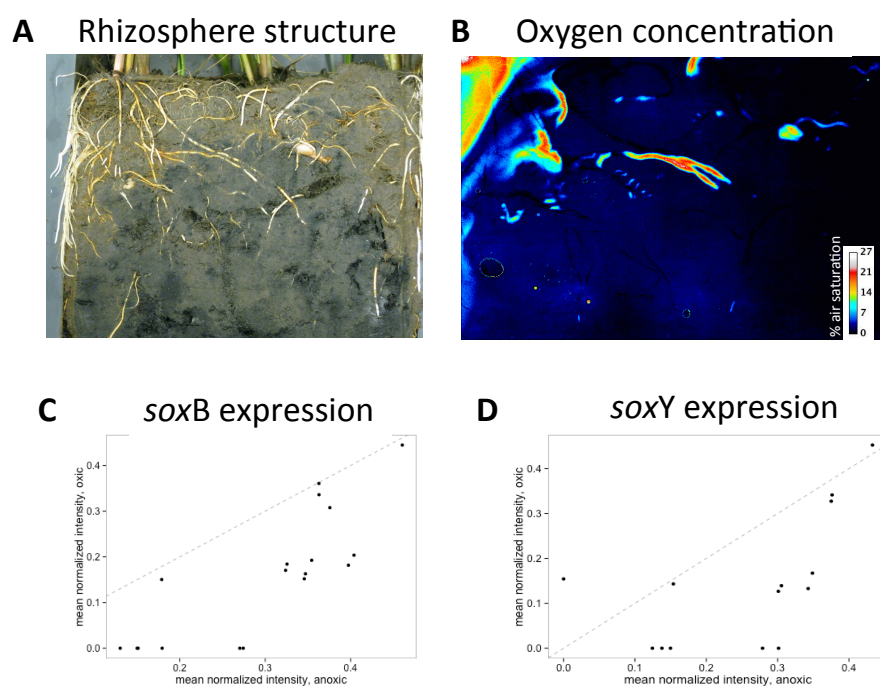


Figure 10: Influence of oxygen concentration around *S. alterniflorus* roots and the expression of genes from the sox pathway. **A.** Structure of the rhizosphere in rhizoboxes. **B.** Oxygen concentration in the sediment, measured using planar optodes on the same set-up as in panel A. Dark regions are anoxic, colored regions feature higher oxygen concentrations. **C., D.** Comparison of expression levels of the genes *soxB* and *soxY* in samples from oxic (Y-axis) or anoxic (X-axis) areas. Most points fall below the 1:1 line (dotted line), suggesting a higher expression in anoxic conditions. Unpublished results.

During this first post-doctoral contract, I also had the chance to collaborate on an additional project aimed at characterizing the primary productivity of subseafloor deep-sea hydrothermal vents, where high sulfide concentrations from vent fluids fuel intense chemolithotrophic activity of SOX bacteria [39]. During a research cruise on vents from the East Pacific Rise, we incubated subseafloor fluids under *in situ* conditions in isobaric gas-tight reactors [40]. We tested the effect of temperature and additions of nitrate, oxygen or hydrogen (potential electron acceptors in vent systems) on the composition and carbon fixation rates of bacterial communities [41]. My main contribution to this project was to show that sulfur-oxidizing *Epsilonbacteraeota* (e.g. *Sulfurimonas*, *Thioreductor*, etc.) dominated the communities in all conditions (Figure 11A). Subsequent single-cell-based activity measurements using isotopic tracing and chemical imaging via nanoscale secondary ion mass spectrometry (nano-SIMS) confirmed that *Epsilonbacteraeota* were the major autotrophs in these environments (Figure 11B). This study revealed a very active population of SOX-bacteria with high carbon fixation rates, significantly impacting biogeochemical cycling in the deep ocean.

Overall, the work I performed during this postdoctoral experience gave insights into the diversity of SOX bacteria, the impact of abiotic factors and biotic interactions on their activity, and their role for biogeochemical cycling. This work led to the publication of 5 articles in *PNAS* [41], *Frontiers in Microbiology* [36, 42], *PLoS one* [37] and *Deep-Sea Research Part I* [40], and of one outreach article in *Science Journal for Kids* [43].

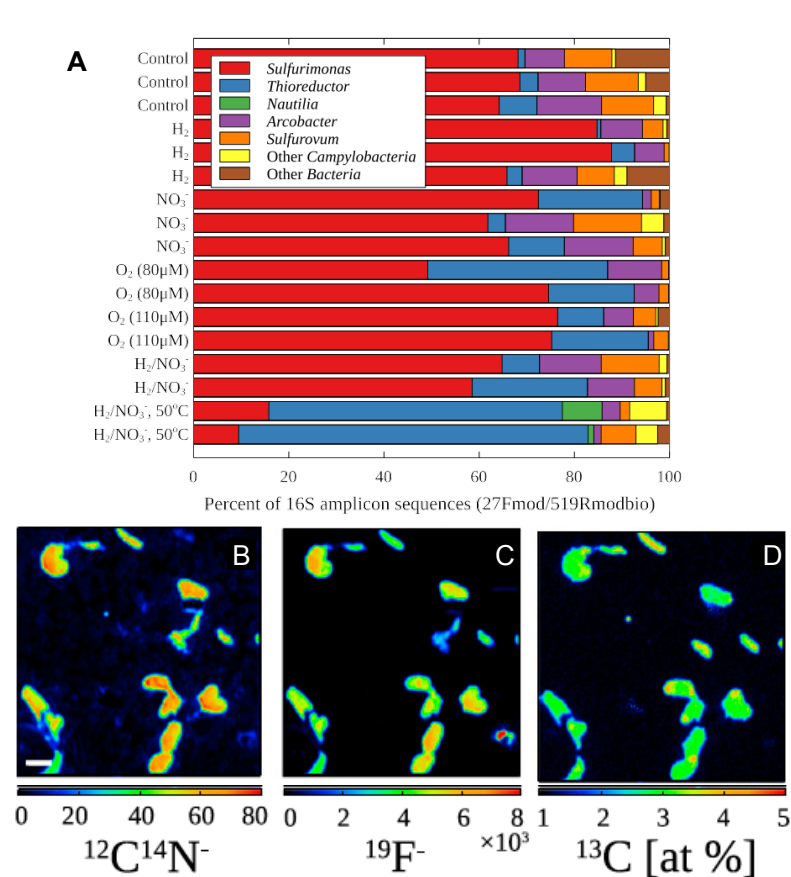


Figure 11: A. Community composition obtained at the end of 24-hours incubations of subseafloor fluids with various amendments of potential electron acceptors. All the genera shown belong to *Epsilonbacteraeota*. **B-D.** Metabolic activity of *Epsilonbacteraeota* cells from subseafloor fluids as quantified by nanoSIMS. The three images show the same field of view with different signals. Signal for $^{12}\text{C}^{14}\text{N}^-$ (**B**) was used as an indicator of total biomass, signal for $^{19}\text{F}^-$ (**C**) reveals cells successfully hybridized with a fluor-containing probe targeting *Epsilonbacteraeota*, and ^{13}C enrichment (**D**) shows carbon fixation from $\text{NaH}^{13}\text{CO}_3^-$. All cells showing a significant carbon fixation gave a positive signal with the *Epsilonbacteraeota* probe, confirming the crucial role of these autotrophic SOX bacteria. Adapted from [41].

5 Effect of plants on terrestrial pollutant-degrading bacteria

From 2014 to 2015, I was a postdoctoral researcher at the Interdisciplinary Laboratory for Continental Ecosystems (LIEC, UMR7360, Nancy, France), working on an ANR project led by Dr. Aurélie Cébron. I studied the diversity and metabolic pathways of soil bacteria involved in the degradation of polycyclic aromatic hydrocarbons (PAH), which are toxic organic compounds frequently polluting soils following industrial activities [44]. One of the main goals of the project was to investigate the effect of the presence of plants PAH-degrading bacteria, as a potential remediation strategy [45].

I first compared the short-term (0-10 days) temporal dynamics of PAH concentrations and degrading bacterial communities in PAH-polluted soil either without plants or with the ryegrass *Lolium multiflorum* growing in newly-designed microcosms (Figure 12). For the planted condition, both bulk soil (not attached to roots) and rhizospheric soil (directly adhering to roots) were collected. All soil samples were used to (i) estimate the quantity and quality of dissolved organic compounds using ion-exchange chromatography, (ii) quantify degradation rates of the model PAH phenanthrene (PHE) using reverse-phase chromatography, (iii) characterize the resident (DNA level) and active (RNA level) bacterial communities using qPCR and 16S RNA gene metabarcoding, and (iv) identify potential PAH degraders by sequencing clone libraries of PAH-degrading genes [46].

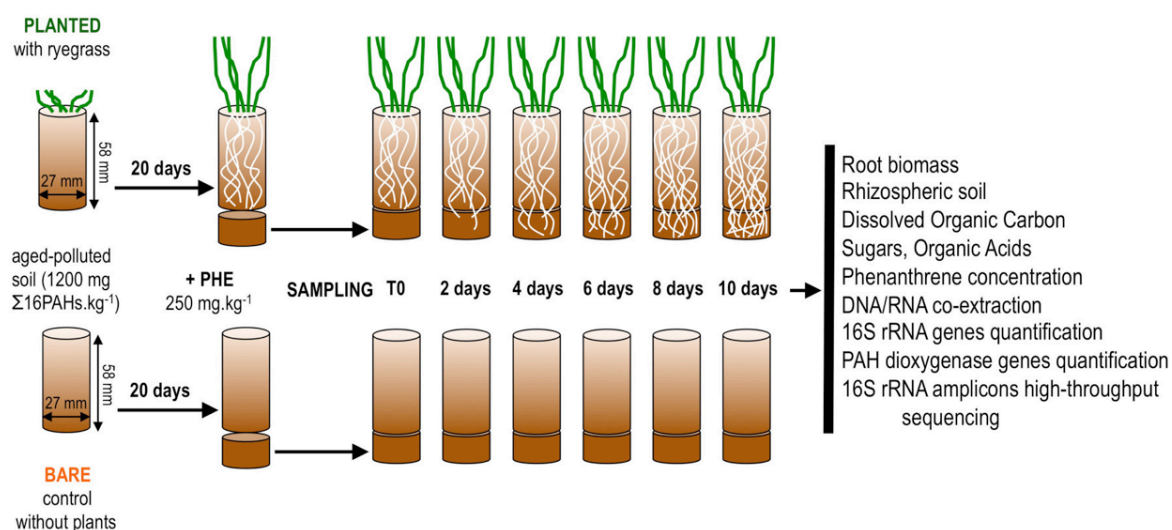


Figure 12: Microcosm set-up and analyses. Microcosms consisted of 2 compartments filled with contaminated soil from a former coking site. Ryegrass seeds were sown in the first compartment and plants were grown until they reached the bottom of the first compartment. A second compartment containing soil spiked with fresh PHE was then added. At the time of sampling, a scalpel was used to detach the second compartment and collect the soil after a known time of contact with actively growing roots. Control microcosms without plants were prepared in the same way. Figure from [46].

The results showed that colonization of contaminated soil by actively growing roots provided new sources of organic substrates, notably labile carbohydrates such as mannitol, trehalose, sucrose, glucose, xylose, mannose and fructose. These compounds might be released by plant root exudation. In parallel, bacterial species richness increased in planted soil compared to bare soil (Figure 13A), contrasting from what is commonly shown in non-contaminated soils where richness or diversity indices are usually lower in the presence of plants. This discrepancy could be due to the oligotrophic nature of contaminated soils, where nutrients are scarce and carbon

sources are either too recalcitrant or not bio-available. In these extreme environments, plant-derived labile compounds likely offer new substrate niches to favor the development of inactive bacterial species, leading to the observed increase in richness. Interestingly, the presence of ryegrass slowed down the degradation of PHE, suggesting that PAH-degrading bacteria might consume plant-derived labile substrates preferentially over PHE. In addition, plants also induced shifts in the composition of total bacterial communities (Figure 13B). In particular, different potential PAH-degraders were detected depending on the presence of ryegrass. In bare microcosms, major potential PHE utilizers included *Sphingomonadales* representatives. Their relative abundance within the active community (at the RNA level) strongly decreased in planted soil, while other PAH-degraders emerged, such as *Pseudomonas*, *Ralstonia* and *Variovorax*. Cultivation approaches using the same soil led to the isolation of 13 PAH-degrading strains, all affiliated to *Pseudomonas* [47].

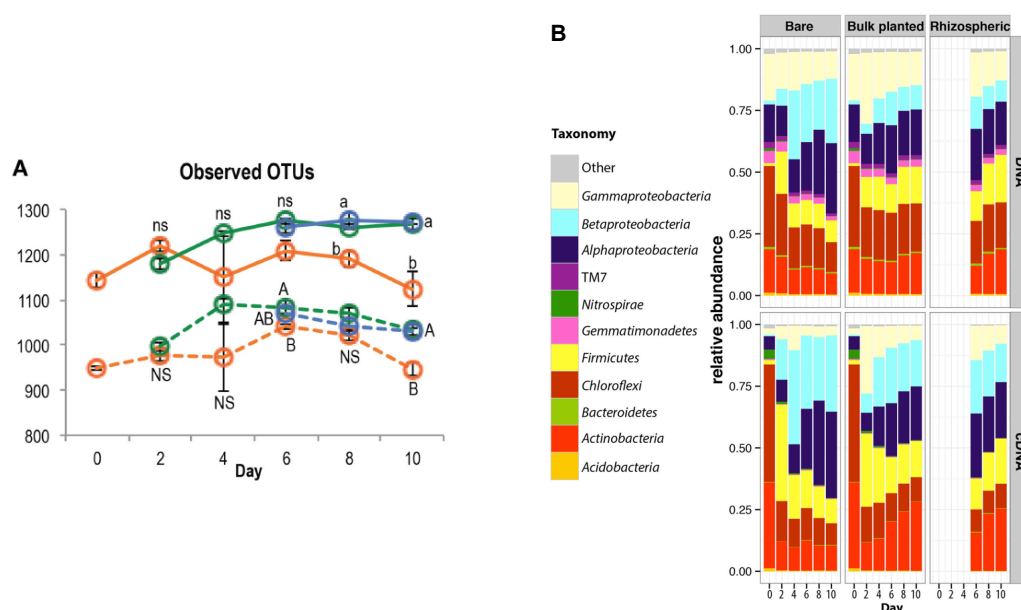


Figure 13: Short-term effect of plants on the bacterial community of PAH-contaminated soil. **A.** Species richness in bare (orange), bulk planted (green), and rhizospheric soil (blue), based on DNA or cDNA libraries (plain and dotted line, respectively). The effect of sample type was tested separately for each time point and nucleic acid using ANOVA followed by *post-hoc* Tukey test, and letters denote group with significant difference ($P < 0.05$, lower case for DNA, upper case for cDNA).

To better resolve the identity and metabolic pathways of PAH-degrading bacteria in contaminated soils, I conducted a stable isotope probing approach (SIP) combined with metagenomics [48]. Briefly, ^{13}C -labeled phenanthrene was spiked into contaminated soil with or without ryegrass, using the microcosms described above (Figure 12). Soil was retrieved after 10 days for total DNA extraction. By separating DNA according to its buoyant density, we could trace the isotope ^{13}C into DNA and specifically detect PAH-assimilating bacteria from the natural communities using both 16S rRNA gene metabarcoding and shotgun metagenomics. Again, ^{13}C -PHE dissipation was 2.5-fold lower in ryegrass-planted conditions than in bare soil, confirming the negative effect of plants on PAH degradation on a short term (10 days). Metabarcoding revealed a diverse set of PAH-degraders belonging to *Proteobacteria*, *Actinobacteria* and *Firmicutes* that was influenced by the presence of ryegrass (Figure 14A). For example, *Sphingomonas* were the dominant PAH-degraders in bare soil but they were outcompeted by *Sphingobium* and *Micrococcaceae* in planted soil. Functional gene annotation of shotgun metagenomics data allowed reconstruction of complete pathways with several routes for PHE

catabolism (Figure 14B). Notably, *Sphingomonadales* were the major taxa performing the first steps of PHE degradation in both conditions, suggesting their critical role to initiate in situ PAH remediation. Yet, the contribution of *Sphingomonadales* to the pool of PAH-degrading genes decreased in planted soil, confirming that other PAH-degraders become active in this condition. Hence, we proposed that PHE-degraders could act in a consortium, whereby complete PHE mineralization is achieved through the combined activity of taxonomically diverse co-occurring bacteria performing successive metabolic steps. By performing the first steps of degradation, *Sphingomonadales* might increase PAH bioavailability and open new substrate niches for other PAH-degraders. Finally, genes involved in carbohydrate metabolism were more abundant in planted than bare soil, further highlighting the potential effect of plant-derived labile compounds on the metabolism of the PAH-degrading bacterial community.

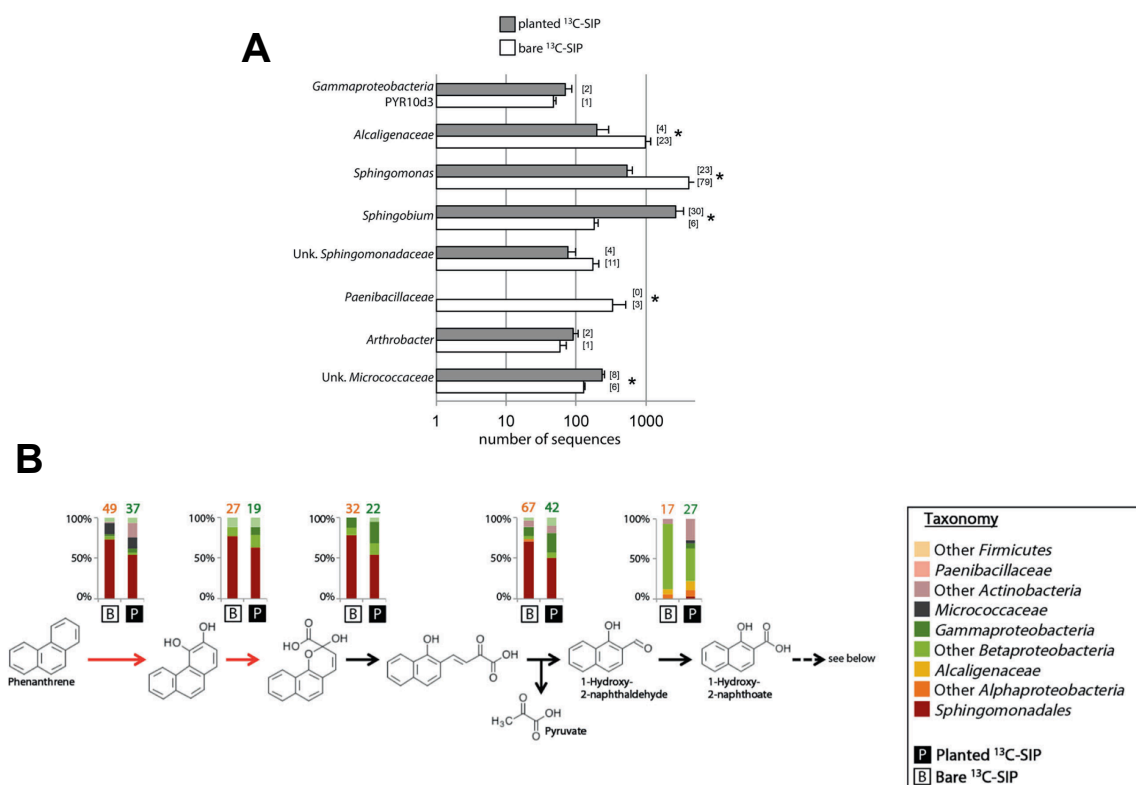


Figure 14: Active PAH-degraders are revealed by analyzing ¹³C-enriched DNA fractions from soil spiked with ¹³C-PHE. **A.** Abundance of taxonomic groups significantly enriched in ¹³C in bare (white) and planted conditions (gray). Values are depicted on a log-scale (mean ± s.e.m., n = 3). Values in bracket beside each bar represent the number of OTUs for each taxon. Differences between bare and planted condition were tested using Welch's t-test (*, P < 0.05). **B.** Reconstruction of the first step of the PHE catabolic pathway. The number of genes identified in the two conditions is added in orange (bare soil, B) and green (planted soil, P) above bar graphs with the taxonomic affiliation of identified genes. Adapted from [48].

Overall, this work gave insights into the diversity and metabolic pathways of PAH-degrading consortia in historically contaminated soils. We showed that plant growth induces clear shifts in the major PAH-degraders and has a short-term negative impact on PAH dissipation (<10 days). Yet, plant-assisted organic pollutant remediation is a balance between the positive effect of rhizodeposits on microbial abundance vs. negative effect due to substrate competition, suggesting plants might be beneficial in a longer term. This work led to the publication of 4 articles in *The ISME Journal* [48], *Frontiers in Microbiology* [46], *International Biodeterioration and Biodegradation* [47] and *Canadian Journal of Microbiology* [49].

6 Bacterial conversion of macroalgal biomass

Since 2015, I am a permanent CNRS Researcher (section n°20) in the Marine Glycobiology group of the Laboratory for Integrative Biology of Marine Models (LBI2M, UMR8227, Station Biologique de Roscoff, France). Elaborating on a theme that started fascinating me during my PhD, I now focus my research on the bacterial conversion of macroalgal biomass.

Green, red and brown macroalgae (collectively known as seaweeds) are dominant primary producers in coastal regions, often locally exceeding phytoplankton and other benthic carbon fixers [50]. Seaweeds thus represent an important reservoir of organic matter and are considered a global carbon sink [51]. The composition of macroalgal biomass is unique and consists of >50% of polysaccharides that differ from those known in terrestrial plants by the nature of their monosaccharide units and the presence of sulfated motifs and other substituents [52]. For example, brown algae contain alginate, laminarin and fucose-containing sulfated polysaccharides (FCSP), red macroalgae contain carrageenans, agars and porphyrans, and green macroalgae contain ulvans. Turnover of this biomass is mostly mediated by marine heterotrophic bacteria that can colonize macroalgae and access, degrade and remineralize the algal compounds. Bacterial breakdown is therefore a crucial bottleneck to re-inject the large pool of organic matter from macroalgae in the marine carbon cycle [53], by (i) making it accessible for higher trophic levels, (ii) liberating dissolved or particulate matter for local recycling or further export and (iii) eventually influencing how much carbon is sequestered. Because they control the fluxes of carbon and energy both locally and between adjacent zones, microbial processes for the degradation of macroalgae are of critical importance for coastal ecosystem functions. The role of microorganisms as recyclers of macroalgal biomass is even more relevant in today's context of global change, since their action can influence the carbon balance in coastal ecosystems already threatened by human activities and pollution [54]. Besides their ecological importance, macroalga-degrading bacteria are also a vast reservoir of novel enzymatic activities to produce high-value compounds with potential applications in biotechnology, agriculture, food, feed, medicine and cosmetics [55–57].

Studies of the mechanisms underlying the interactions of these bacteria with macroalgae and their degradation pathways are therefore crucial to understanding coastal ecosystems' nutrient cycles and discovering novel enzymatic functions. Building on my interdisciplinary background in environmental microbiology, molecular biology and biochemistry, my research questions are:

1. Who are the algae-degrading bacteria in marine environments?
2. How do changing habitats, spatial heterogeneities or temporal variations in polysaccharide availability shape the community assemblage and influence the activity of algae-degrading bacteria?
3. What are the genes involved in polysaccharide degradation and how do they evolve? Are they shared between microorganisms performing similar functions in contrasted niches?
4. What proteins do these genes encode? What are their biochemical functions in the metabolic process?
5. What are the regulatory events, controlling the expression of these genes and/or the activity of the proteins, to fine-tune the microbial response to the available substrates?

Over the past six years, I tackled these questions by developing three complementary research axes as described below. This work led to the publication of 11 articles in *Environmental Microbiology* [23, 58, 59], *Nature Communications* [60], *The ISME Journal* [61], *Nucleic Acids Research* [62], *Glycobiology* [63], *Environmental Microbiology Reports* [64], *Advances in Botanical Research* [65], *Journal of Phycology* [66] and *IJSEM* [67].

6.1 Detailed characterization of enzymes and metabolic pathways

The goal of the first research axis is to obtain a detailed characterization of bacterial enzymes and metabolic pathways involved in the degradation of algal compounds, with a focus on polysaccharides. For this work, I study the flavobacterium *Zobellia galactanivorans* Dsij^T as a model of algae-degrading bacteria, as already presented above (see §3).

To deepen the understanding of the alginolytic pathway in *Z. galactanivorans*, I first questioned the biological role of the alginate lyase AlyA1 [58]. In contrast with the other alginate lyase genes in *Z. galactanivorans*, *alyA1* does not belong to an operon (see Figure 6 above) and is only present in a fraction of the available genomes from other marine alginolytic bacteria. In addition, this enzyme is the only alginate lyase from *Z. galactanivorans* that is secreted in soluble form in the medium and possesses a carbohydrate-binding module (CBM). These original properties suggested a complex evolutionary history and unique role of AlyA1 for brown algal biomass degradation. Using phylogenies, I showed that *alyA1* was likely acquired recently by a limited number of Flavobacteria (including *Z. galactanivorans*) through a horizontal gene transfer from marine Actinobacteria. To elucidate the biological role of AlyA1 *in vivo*, a mutant strain deleted for the *alyA1* gene was produced using a novel mutagenesis protocol that I participated to implement in the laboratory thanks to collaboration with Pr. McBride (Univ. Wisconsin, Milwaukee). This mutation had no effect on the growth rate in liquid medium with alginate as a sole carbon source. However, the alginolytic activity detected in spent medium and the concentration of alginate degradation products were much lower for the mutant strain compared to the wild-type strain (WT). Furthermore, the mutant strain grew slower on alginate gels, without liquefying the gel as typically observed for the WT (Figure 15A). Cultivation experiments in microcosms containing fresh tissues of the brown alga *Laminaria digitata* as a sole carbon source showed that the mutant strain had a slower growth and released less degradation products compared to the WT (Figure 15B).

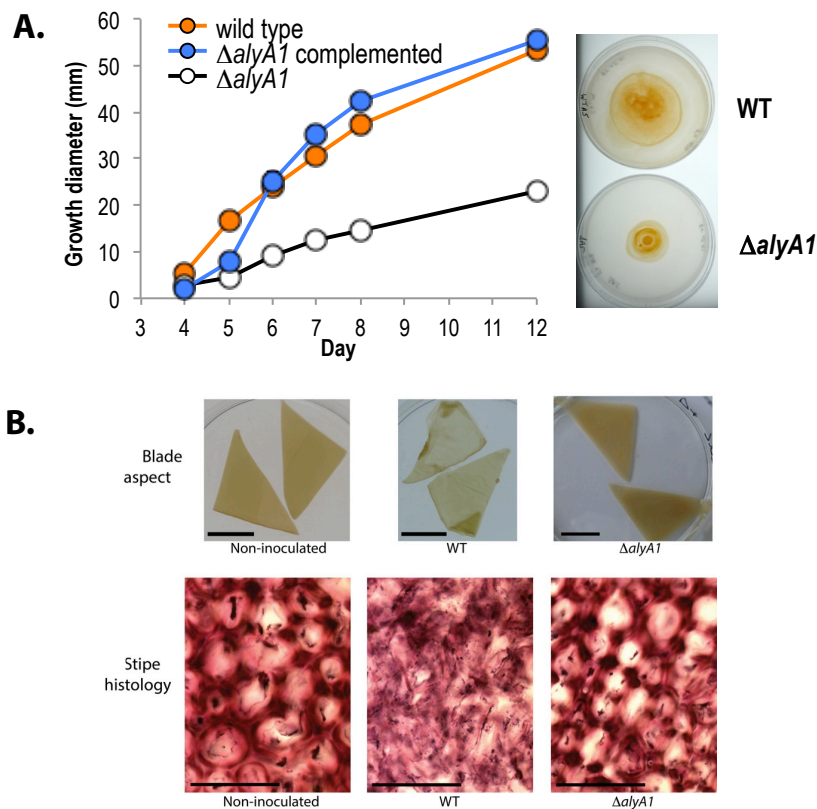


Figure 15: Effect of the *alyA1* deletion in *Z. galactanivorans*. **A.** Growth of WT, *alyA1* mutant, and complemented mutant strains on plates containing alginate gels as the sole carbon source. Pictures on the right show the extent of growth after 14 days. **B.** Aspects of algal tissues after incubation with WT or mutant strain in microcosms. Note the degradation of algal pieces with WT but not with *alyA1* mutant. Scale bars for blade aspect: 1 cm. Scale bars for stipe histology: 10 μ m. Adapted from [58].

Since the algal pieces had been collected from the field and were not axenic, I analyzed the bacterial communities growing in the microcosms using 16S rRNA gene Illumina metabarcoding. The results showed that growth of the WT strain favored the development of other bacterial genera that were not detected in non-inoculated microcosms or in microcosms inoculated with the *alyA1* mutant strain. Overall, these results showed that AlyA1 is essential for optimal attack of alginate in solid matrices, especially in the natural context of brown algal cell walls, and that the function of this enzyme is not redundant with other alginate lyases. Acquisition of *alyA1*-like genes could allow bacteria to act as pioneers for the degradation of polysaccharides embedded within algal extracellular matrices, releasing soluble fragments that become available for other opportunistic members of the microbial communities. In addition, the demonstrated role of AlyA1 for the degradation of fresh algal tissues might inspire future optimization of algal biomass refinery strategies.

The research performed in this first research axis is also illustrated by the recent characterization of the enzyme AlyA3 [63], another alginate lyase from *Z. galactanivorans* encoded within the large alginolytic operon (see Figure 6 above). Through a collaboration between many members of the Marine Glycobiology group, the Cristal'O platform (Station Biologique de Roscoff) and the Norwegian University of Science and Technology, we demonstrated that this enzyme from the PL17 family is an exolytic oligo-alginate lyase highly specific of M stretches. Its activity is complementary to that of the previously characterized AlyA1 and AlyA5, highlighting the absence of redundancy within the AUS. The crystallographic structure solved by Dr. Mirjam Czjzek (PDB 7BJT and 7BM6) showed that compared to the only other structure within PL17 (Alg17c, PDB 4OJZ), AlyA3 contains an inserted flexible loop at the entrance of the catalytic groove, likely involved in substrate recognition, processivity and turnover.

Another example of the work performed in this first research axis is the discovery of a complete catabolic pathway for carrageenans, which are major red algal polysaccharides. Carrageenans are sulfated galactans comprising a linear backbone of D-galactose alternatively linked in β -1,4 and α -1,3. The residue linked in α is either a D-galactose-6-sulfate or a 3,6-anhydro-D-galactose (a bicyclic sugar found exclusively in red algae) [52]. Although carrageenans represent an abundant substrate in coastal habitats and are used in the food, feed and cosmetic industries for their gelling and biological properties [68], their catabolism by heterotrophic marine bacteria was largely unknown. To fill this gap, a collaborative project led by Dr. Gurvan Michel involved many members of the Marine Glycobiology group together with collaborators from INRA Jouy-en-Josas, University of Wisconsin and Genoscope. This study performed mostly on *Z. galactanivorans* allowed the first description of a complete degradation pathway for carrageenans in a marine bacterium [60]. This catabolic pathway is encoded in a complex regulon and conserved in the *Bacteroidetes* phylum. As for the AUS described previously, most of the genes of the carrageenolytic system belong to a PUL. A notable exception was the absence of genes encoding SusC- and SusD-like proteins, which participate to substrate binding and import and were hitherto hallmarks of the PUL architecture [25, 69]. Within this project, my main contribution was to create and phenotype mutant strains of *Z. galactanivorans*, in order to validate the biological role of the newly discovered catabolic pathway *in vivo*. Deletion mutants were obtained for 4 genes of the PUL (Figure 16A) and a double mutant *dagA3/dagB*, and tested for their growth on a minimum medium containing carrageenan as a sole carbon source. For example, growth is abolished for the strain deleted for the transcription factor *cgrA* but restored by ectopic complementation (Figure 16B). Furthermore, transcriptomics and comparative

genomics identified two candidate genes distal from the PUL, encoding a SusCD-like system (ZG3580 and ZG3581) that might be involved in carrageenan degradation. I obtained a double mutant $\Delta 3580/3581$, which showed a slower growth on carrageenan compared to the WT strain (Figure 16C). Combined with biochemical analyses, these results allowed confirming the function of ZG3580 and ZG3581 in carrageenan catabolism and extending the general definition of PULs [60].

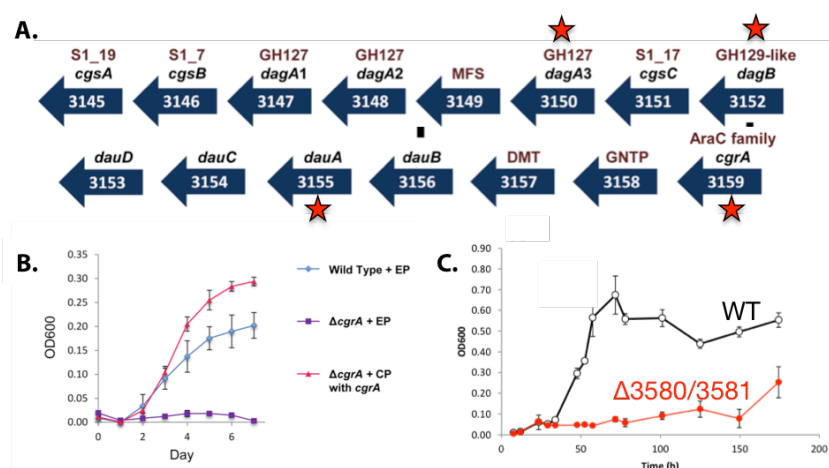
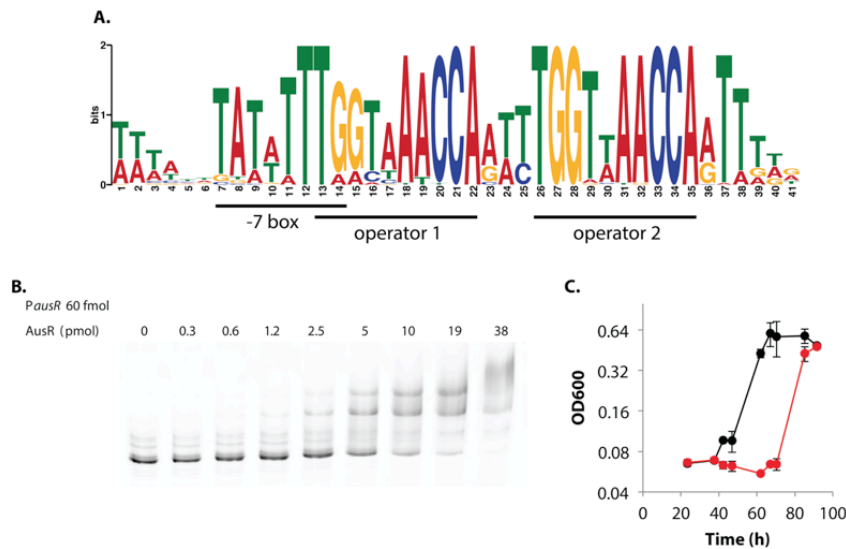


Figure 16: **A.** Carrageenan utilization locus discovered in *Z. galactanivorans*. Red stars denote genes for which mutant strains were obtained. **B.** Growth of the WT and *cgrA*-deleted strains on carrageenan as a sole carbon source and energy source. EP: empty plasmid. CP: complementation plasmid. **C.** Growth of WT and *zg3680/zg3581* double mutant strain on carrageenan as a sole carbon and energy source. Adapted from [60].

6.2 Regulatory controls in alga-degrading bacteria

The second axis of my current research aims at understanding the regulation mechanisms involved in the degradation of algal polysaccharides by marine bacteria. To date, most of the knowledge on the regulation of PUL expression comes from the study of a few intestinal *Bacteroides* that degrade plant- or host-derived polysaccharides [70]. By contrast, very little is known on how marine Flavobacteria perceive the available substrates and adapt their metabolism. This second axis of my research is best illustrated by a recent study [62] conducted within the RegAl project that I coordinated. Together with a Master student and a postdoctoral researcher whom I supervised, we investigated the regulation of the Alginate Utilization System (AUS, presented in §3) in *Z. galactanivorans*. We showed that the AUS expression is strongly and rapidly induced when alginate is added to the growth medium. Alginate is consumed in two phases: the polymer is first degraded in oligosaccharides that accumulate in the medium before being assimilated in a second phase. We showed that this regulation depends on the transcriptional repressor AusR (Figure 17), a FadR-like protein of the GntR family encoded within the alginate PUL. An *ausR* deletion mutant had a delayed growth on alginate, confirming its biological function. We further demonstrated that AusR binds to promoters upstream several AUS genes on simple, double or triple copies of a palindromic operator whose consensus sequence is [TGGXXAACCA]. This binding is inhibited in the presence of alginate oligosaccharides.



Combining genetic, genomic, transcriptomic and biochemical data, we proposed the first model of the regulation for a PUL in marine bacteria (Figure 18). In the absence of alginate, the expression of most genes from the AUS is strongly repressed by the fixation of AusR on its operators, blocking recognition by RNA polymerase. This control is less strict for two genes encoding the secreted alginate lyases AlyA1 and AlyA7, allowing a basal expression of these enzymes. Upon alginate addition, these extracellular enzymes degrade the polymer and release metabolic intermediates that act as effectors of the AusR regulator. This inhibits the formation of AusR/DNA complexes and relaxes the transcriptional repression of the AUS. In addition, the results also evidenced decoupling of expression levels within the AUS, suggesting other fine-tuning regulation mechanisms such as a potential riboswitch. Overall, this study was the first characterization of a PUL regulator in marine bacteria. We detected similar AusR operator sequences in most alginate PULs from other marine bacteria, highlighting the conservation of this regulatory pathway and its relevance to macroalgal degradation in the environment.

Figure 18: Model of the regulation of alginate utilization in *Z. galactanivorans*. Red arrows depict the repression of AUS promoters by AusR in the absence of alginate. qPCR-estimated repression levels are indicated. Red ellipses indicate the number of AusR operators, with their distance to the -7 box. Genes and proteins colored in grey are those with low or no repression by AusR, allowing their basal expression in the absence of alginate. From [62].

6.3 Diversity and environmental drivers of alga-degrading bacteria

The first two axes presented above take advantage of the model bacterium *Z. galactanivorans* to gain detailed insights into the molecular mechanisms underlying macroalgal biomass conversion. To complement this approach, the third axis focuses on environmental studies of the diversity and ecology of macroalga-degrading bacteria in coastal habitats, as illustrated by two recent examples [59, 61].

Within the ALGOSIP project that I coordinated, we developed isotopic tracing to explore the diversity of marine bacteria degrading macroalgae, using Stable Isotope Probing (SIP) combined with high-throughput DNA sequencing and chemical imaging via nanoSIMS [61]. SIP is a powerful method to trace the utilization of labeled substrates into the cellular material of consuming organisms. It allows identifying active microbial degraders of a specific compound within complex natural communities [71–73]. Although largely used to study bacteria degrading pollutants or terrestrial plant compounds, it had never been applied to the bacterial degradation of macroalgal biomass. We first optimized a ^{13}C -labeling protocol for the kelp *Laminaria digitata* [65], and obtained alginate with a significant isotope ratio of ca. 3.6 (compared to 1.1 in control alginate). To detect the main alginolytic bacteria in a coastal environment, natural seawater sampled from an algal bed in Roscoff was amended either with natural or ^{13}C -enriched alginate. After 48 h, elemental analyses coupled to isotopic ratio mass spectrometry (EA-IRMS) showed a bulk enrichment $\delta^{13}\text{C} = 273\text{‰}$ in cells from microcosms amended with ^{13}C -alginate, evidencing carbon acquisition from alginate by some members of the bacterial community. NanoSIMS coupled to the hybridization of halogenated probes (HISH-SIMS) was performed to visualize this incorporation of ^{13}C at the single-cell level into specific taxa. The results showed that most alginate incorporators belonged to *Gammaproteobacteria* and *Flavobacteriia* (Figure 19), with carbon assimilation rates ranging from 0.14 to 27.50 $\text{fg C } \mu\text{m}^{-3} \text{ h}^{-1}$.

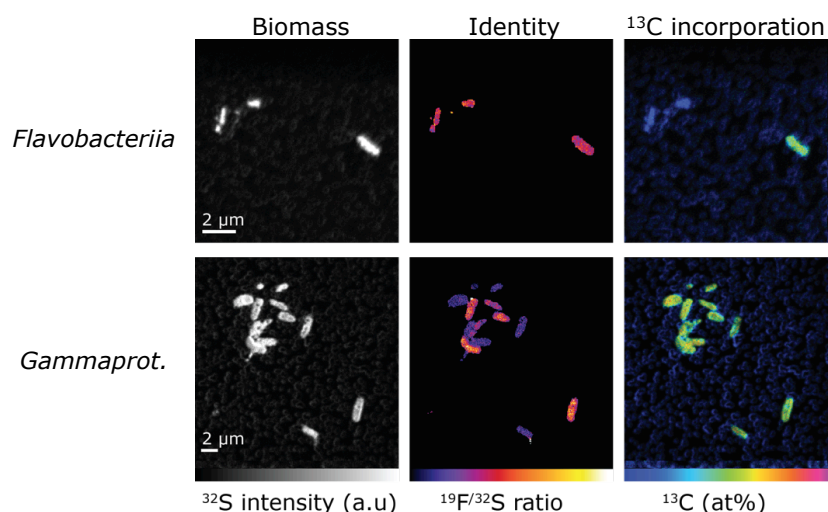


Figure 19: HISH-SIMS analysis of bacterial cells from natural seawater microcosms amended with ^{13}C -enriched alginate. Samples were hybridized with ^{19}F -labeled probes specific for *Flavobacteriia* (top row) or *Gammaproteobacteria* (bottom row). The ^{32}S sulfur signal was used to detect all cells, and significant ^{13}C enrichment reveals alginate incorporation at the single-cell level. Modified from [61].

We used DNA-SIP to precise the identity of these alginate incorporators, based on the assumption that DNA from cells that have incorporated ^{13}C from alginate should also be enriched in ^{13}C , and therefore have a greater buoyant density. Total DNA was extracted from the microcosms and fractionated by ultracentrifugation on density gradients (from "heavy" to "light" DNA). Each fraction was analyzed by metabarcoding of the 16S rRNA gene. The results showed that only a few initially rare *Flavobacteriaceae* (e.g. *Wenyngzhuangia*) and *Alteromonadales* (e.g. *Colwellia*) incorporated ^{13}C from alginate into their biomass, accounting for most of the carbon assimilation based on bulk isotopic measurements. These taxa had a copiotrophic behavior,

rapidly becoming dominant (ca. 25%) in the bacterial communities after alginate addition. Finally, we screened a fosmid metagenomic library built using DNA from alginate-enriched microcosms and found two alginolytic clones. Sequence analysis revealed that both clones originated from *Alteromonadales* members, confirming their important role as alginate degraders *in situ*. Overall, this study highlighted the high degree of niche specialization in heterotrophic communities and may help constraining the quantitative role of alga-degrading bacteria in coastal ecosystems.

In another project, we collaborated with a benthic ecology team from the SBR to investigate the diversity and succession of kelp-associated bacteria *in situ*. This team studied the dynamics and decomposition processes of massive accumulations of detached kelps that frequently occur following storms in coastal regions [74]. Our colleagues mimicked such a natural accumulation by enclosing kelp fragments in cages submerged in the Bay of Morlaix, with regular sampling (0-6 months). They showed that 85% of the algal biomass was lost after 6 months. To complement their analyses, we used samples from the same experiment to characterize the temporal succession of bacteria associated with degrading kelp tissues. We first showed a strong increase in the cultivable bacterial counts during the first two weeks [66]. In parallel, we evidenced a decrease in the proportion of bacteria resistant to iodine, one of the defense compounds in kelps. These results based on cultivation methods suggested important shifts in bacterial communities during algal degradation. To better characterize the temporal succession, community composition was analyzed by 16S rRNA gene metabarcoding during a Master 2 internship that I co-supervised. We used newly-validated primers that avoid amplification from plastid DNA, a frequent bias in plant-associated microbiome studies [64]. The results showed the succession of three types of microbiota on detached kelp biomass [59]. First, the resident community had a very low diversity and was dominated by *Alpha*- and *Gammaproteobacteria* (Figure 20A and B). During the first six weeks, kelp fragments were colonized by *Bacteroidia* (including *Flavobacteria*), reminiscent of their specialization towards the utilization of complex algal polysaccharides. This corroborates their potential role as pioneers of algal biomass degradation. Other bacteria were favored after 11 weeks (e.g. *Acidimicrobiia*). They could be opportunists, feeding on simple compounds released by pioneers during kelp degradation. Metabolic inference from taxonomic data further revealed significant variations in the estimated CAZyme profile. For example, the abundance of predicted endolytic enzymes active on polysaccharides (cellulase, endo-xylanase) decreased over time, while that of exo-enzymes more active on oligosaccharides (galactosidase, glucosidase) increased (Figure 20C). Overall, this study evidenced major shifts in the composition of kelp-associated microbiota during algal decay. It highlighted potential metabolic interactions between pioneer and opportunistic bacteria, which could influence the fate of carbon in coastal ecosystems.

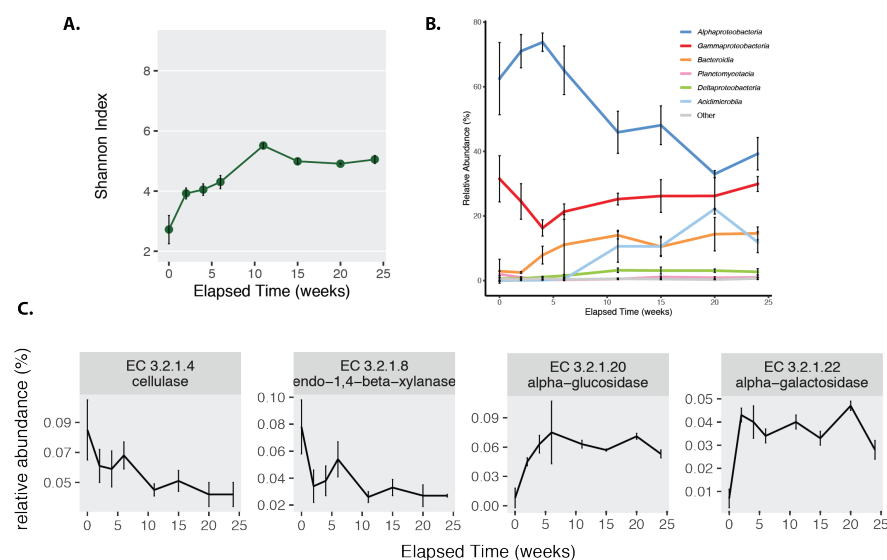


Figure 20: Taxonomic and functional analysis of bacterial communities associated with kelp during *in situ* degradation. **A.** Shannon diversity index. **B.** Class-level taxonomic composition. **C.** Selection of predicted enzymatic functions with significant variations over time. Modified from [59].

Research project

In the next four years, I wish to pursue my investigations on the **mechanisms, regulations and ecological implications of macroalgal biomass degradation** by marine heterotrophic bacteria, building on the solid expertise we have acquired in the Marine Glycobiology group. So far, I mostly studied the degradation of pure, soluble algal polysaccharides (e.g. alginate, carrageenan, agar). Although powerful, these studies do not represent the reality of what is expected in natural conditions, where bacteria encounter complex mixes of substrates either dissolved in the seawater or embedded within algal tissues. A rapidly growing body of literature [75–79], including some of my own research [61], shows that only a fraction of marine bacteria are specialists for the degradation of complex organic matter. Acting as pioneers for the degradation, these specialist degraders fuel the growth of heterotrophic microbial communities by producing metabolites that become substrates for secondary consumers. Recently, several frameworks were proposed to conceptualize these networks of trophic interactions [75, 80, 81], as summarized in [Figure 21](#).

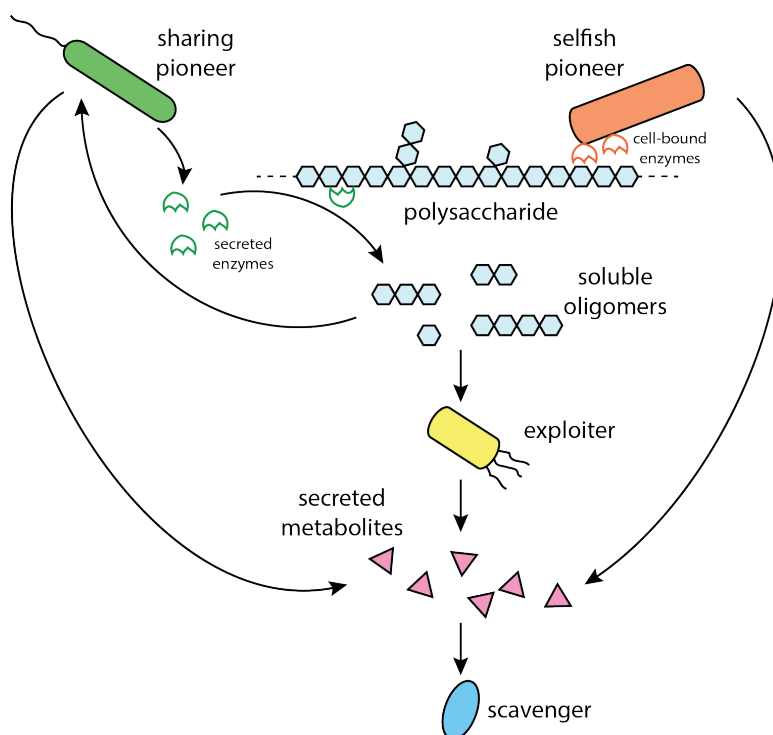


Figure 21: Conceptual scheme of potential trophic interactions within heterotrophic bacterial communities. Two types of pioneer bacteria can grow on complex polymers. Selfish pioneers release little or no degradation products to the external environment thanks to coupled degradation by cell-bound enzymes and direct uptake of large oligomers by dedicated transporters. Sharing pioneers use secreted enzymes to degrade the substrate, leading to the production of soluble oligomers in the environment. Exploiters can grow on these soluble oligomers, but not on the intact polymer. Scavengers benefit from metabolites secreted by other species. Not drawn to scale. Freely inspired from [75, 81].

Therefore, **pioneer alga-degrading bacteria** play a central role in coastal ecosystems by initiating macroalgal biomass recycling. In this context, my research will aim at unraveling the strategies of pioneer bacteria mediating macroalgal breakdown in coastal environments. I will tackle the following questions, combining studies on the model pioneer bacteria *Z. galactanivorans*, collections of isolated alga-associated bacteria and natural communities:

- 1) What are the pathways used by pioneer bacteria to **degrade intact, fresh seaweed biomass**?
- 2) How do pioneer bacteria integrate the presence of **multiple, complex substrates**?
- 3) How do pioneer bacteria **interact with other members** of the bacterial communities?

To date, most studies used soluble, single algal polysaccharides or at best autoclaved crushed tissues as substrates [82]. To understand how bacteria can initiate the breakdown of intact algal tissues, we will use *Z. galactanivorans* as a model pioneer species, since we previously showed it was able to completely degrade kelp biomass [58]. This work already started within the ALGAVOR ANR project that I coordinate (2018-2023). Maéva Brunet, a PhD student whom I co-supervise, compares the responses of *Z. galactanivorans* grown on different brown algae as sole carbon sources (*Laminaria digitata*, *Fucus vesiculosus* and *Ascophyllum nodosum*) to that obtained on purified substrates (alginate, FCSP and maltose). RNA-seq transcriptomic data were obtained in each condition. Between 5 to 25% of the genes are differentially expressed by cells grown on fresh algal biomass compared to pure substrates, about half of them of unknown function. The upregulated pathways might represent yet **overlooked genetic determinants of the pioneer behavior** in marine bacteria, and an untapped source of original activities. They could target novel substrates or help bacteria to cope with algal defenses and compete with other members of the bacterial community. Much work remains to be done in the upcoming years to **unveil their biological functions** through bacterial physiology, genetics and biochemical characterization. For example, we started characterizing a new PUL that was one of the most induced loci in the presence of brown macroalgae compared to pure substrates. Preliminary biochemical assays suggest it might target FCSP containing galacturonic acids, for which no specific enzymes are known to date. Furthermore, we will evaluate the distribution of these pioneer genetic determinants in the sequenced genomes of isolated marine bacteria and in metagenomic dataset, to gain insights into the **evolution and ecological niches of pioneer bacteria**.

I will also pursue the elucidation of **regulation mechanisms** involved in the degradation of algal biomass, for which knowledge is still limited. Previous research showed that a given polysaccharide not only induces the expression of PULs dedicated to its degradation, but also of additional genes distal to PULs [28, 60]. Often, the function of these genes is not related to the degradation of the triggering substrate. This suggests the existence of cross-regulations, whereby a given substrate can influence the expression of metabolic pathways not directly involved in its utilization. This hitherto unknown **cross-regulation processes** might allow bacteria to optimize their growth in their natural environment, when degrading multiple substrates contained in macroalgal biomass. In the upcoming years we will focus on potential cross-regulations involved in the utilization of alginate and carrageenan in *Z. galactanivorans*, for which PULs have been well characterized previously [24, 60, 62]. Each PUL encodes a transcription factor that controls its expression (AusR and CgrA, respectively). We will test two hypotheses: (H1) a PUL-encoded regulator controls the expression of distal genes involved in complementary metabolic pathways linked to the degradation of other substrates and/or interaction with macroalgae; (H2) non-target substrates can influence the expression of a PUL. First, we will establish the network of genes controlled by each regulator (i.e. its regulon). We will produce mutant strains of *Z. galactanivorans* where AusR or CgrA are fused to a FLAG-tag, followed by anti-FLAG chromatin immuno-precipitation coupled to high-throughput sequencing (ChIP-seq). This will allow mapping the AusR and CgrA binding sites onto the genome, and obtaining the complete list of genes controlled by these two regulators. The control of AusR and CgrA on selected genes distal to their respective PUL will be validated experimentally by EMSA. The expression levels of these distal genes in absence and presence of the inducing substrate will be monitored on the WT and strains deleted for AusR and CgrA (already available in the group), to quantify the effect of cross-regulations. Finally, we will evaluate the potential cross-regulation effects of other algal substrates that co-occur with alginate and carrageenan in macroalgae (e.g. laminarin and floridean starch in brown and red algae, respectively). We will assess if and how pioneer bacteria can **prioritize the**

utilization of substrate mixes by catabolic repression, using two scenarios. First, we will compare *Z. galactanivorans* responses on media containing both substrates (alginate + laminarin, carrageenan + starch) to those obtained with each substrate separately. Second, we will study the kinetics of metabolic transition, by adding a second substrate to cultures pre-adapted to a first substrate. In both scenarios, we will measure the growth rates and yields, the activity of the regulators and the co-regulation of distal genes. Overall, this project should provide unprecedented knowledge on cross-regulations that allow marine bacteria to rapidly **fine-tune their metabolism** depending on the multiple, diverse, complex and variable substrates found in macroalgal biomass, reminiscent of what is known in gut *Bacteroides* [83, 84]. I already submitted a proposal to the SAD call of the Brittany Region (under review, expected answer December 2021), which could provide funding for a 2-years postdoctoral researcher to work on this project.

Another axis of my research during the upcoming years will focus on the characterization of **trophic interaction networks** between pioneer alga-degrading bacteria and secondary consumers. I will assess how metabolic interactions (e.g cross-feeding, public good sharing) can shape the assemblage and succession of bacterial communities involved in macroalgal biomass utilization. This will first be tested using **synthetic communities**, in which we will co-culture strains with a known behavior on pure substrates or fresh algae. During the ongoing ALGAVOR ANR project, my PhD student Maéva Brunet has already started developing this approach with simple synthetic communities containing only 2 partners. The preliminary results suggest that *Z. galactanivorans* (model of pioneer bacteria) can favor the growth of some *Tenacibaculum* strains (model of secondary consumer) on brown algal biomass. We will scale up this approach in the future. To consider interactions that are relevant in a natural context, we will take advantage of available culture collections of macroalga-associated bacteria isolated at the same time from the same environment. For example, the Marine Glycobiology group has access to a vast collection of 324 bacterial strains isolated from the brown alga *A. nodosum* [85]. Among them, we recently sequenced the genomes of 50 selected *Flavobacteriaceae* strains during the IDEALG project. Additional strains from other taxa (*Alphaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*) will be sequenced within the SEABIOZ ANR project in which I am a partner (2020-2024). Efforts to establish the catabolic profile of each strain on a collection of mono-, oligo- and polysaccharides are ongoing, and already revealed marked discrepancies even between closely related strains [67]. This genomic and physiological information will be integrated to **assign strains to predicted functional ecotypes** (e.g. pioneers, exploiters, scavengers), as inspired by a recent study of chitin-degrading bacterial communities [86]. The predictions will be experimentally tested by monitoring the growth of strains from different ecotypes co-cultured in multipartite synthetic communities. To validate the pioneer behavior of strains, cell-free supernatant from predicted pioneers grown on fresh macroalgae or pure polysaccharides will be used as substrates for predicted exploiters and scavengers, with parallel analysis of the metabolites released and consumed by each group. This will give access to the hierarchical structure of trophic interaction networks among co-occurring strains and help understand the impact of individual pioneer degraders on macroalga-associated bacterial communities. A complementary approach to these synthetic community experiments will build on the SIP protocol I recently developed for macroalgal biomass [61, 65]. Indeed, access to ¹³C-labeled macroalgae opens the way for unprecedented **time-resolved studies of carbon flow within natural microbial communities**. Labeled algal biomass will be provided as substrate to natural communities and samples will be collected at different time points. This will allow tracing the successive transfers of ¹³C along the trophic interaction network, hypothesizing that pioneer cells should be isotopically enriched first, followed by secondary consumers. This approach could be tested both on algal tissues and

purified substrates, to differentiate bacteria that can initiate the attack of intact algae from those more relevant to the utilization of dissolved compounds released through algal exudation or decay. A potential limitation of this time-resolved SIP approach could be the expected dilution of the isotopic signal downstream the trophic network, which would necessitate high initial isotopic enrichment of the substrate to still find significant signatures in late consumers. The current labeling protocol with ^{13}C -sodium bicarbonate only yielded algae with a maximum isotopic ratio of 3.6 ^{13}C atom percent, which might need to be optimized by growing algae under $^{13}\text{CO}_2$ atmosphere or performing multi-isotopic labeling (e.g. ^{13}C , ^{15}N , ^{18}O).

To conclude, the research I propose for the next four years implements a **multi-scale approach, from natural bacterial communities** in macroalgae-dominated habitats **to detailed mechanistic understanding** of processes for the degradation of fresh algal biomass. This fundamental knowledge will provide unprecedented insights into the **metabolic, regulatory and ecological strategies** of the yet understudied group of pioneer bacteria that specialize in macroalgae breakdown, a current black box in coastal ecosystem functioning. Furthermore, anticipated findings could **impact future industrial and biotechnological applications** towards the valorization of algal biomass.

References

1. Alfaro AC, Thomas F, Sergeant L, Duxbury M. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuar Coast Shelf Sci* 2006; **70**: 271–286.
2. Alfaro AC, Dewas SE, Thomas F. Food and habitat partitioning in grazing snails (*Turbo smaragdus*), Northern New Zealand. *Estuaries and Coasts* 2007; **30**: 431–440.
3. Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, et al. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* 2010; **465**: 617–21.
4. Kloareg B, Badis Y, Cock JM, Michel G. Role and evolution of the extracellular matrix in the acquisition of complex multicellularity in eukaryotes : a macroalgal perspective. *Genes* 2021; **12**: 1059.
5. Cosse A, Leblanc C, Potin P. Dynamic defense of marine macroalgae against pathogens: from early activated to gene-regulated responses. *Adv Bot Res* 2007; **46**: 221–266.
6. Küpper FC, Müller DG, Peters AF, Kloareg B, Potin P. Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of sporophytes of *Laminariales*. *J Chem Ecol* 2002; **28**: 2057–2080.
7. Küpper FC, Gaquerel E, Cosse A, Adas F, Peters AF, Müller DG, et al. Free fatty acids and methyl jasmonate trigger defense reactions in *Laminaria digitata*. *Plant Cell Physiol* 2009; **50**: 789–800.
8. Küpper FC, Gaquerel E, Boneberg E-M, Morath S, Salaun J-P, Potin P. Early events in the perception of lipopolysaccharides in the brown alga *Laminaria digitata* include an oxidative burst and activation of fatty acid oxidation cascades. *J Exp Bot* 2006; **57**: 1991–1999.
9. Küpper FC, Kloareg B, Guern J, Potin P. Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiol* 2001; **125**: 278–291.
10. Goulitquer S, Ritter A, Thomas F, Ferec C, Salaün J-P, Potin P. Release of volatile aldehydes by the brown algal kelp *Laminaria digitata* in response to both biotic and abiotic stress. *Chembiochem* 2009; **10**: 977–982.
11. McFiggans G, Bale CSE, Ball SM, Beames JM, Bloss WJ, Carpenter LJ, et al. Iodine-mediated coastal particle formation: an overview of the Reactive Halogens in the Marine Boundary Layer (RHAMBLE) Roscoff coastal study. *Atmos Chem Phys* 2010; **10**: 2975–2999.
12. Thomas F, Cosse A, Goulitquer S, Raimund S, Morin P, Valero M, et al. Waterborne signaling primes the expression of elicitor-induced genes and buffers the oxidative responses in the brown alga *Laminaria digitata*. *PLoS One* 2011; **6**: e21475.
13. Heil M, Ton J. Long-distance signalling in plant defence. *Trends Plant Sci* 2008; **13**: 264–272.
14. Thomas F, Cosse A, Le Panse S, Kloareg B, Potin P, Leblanc C. Kelps feature systemic defense responses : insights into the evolution of innate immunity in multicellular eukaryotes. *New Phytol* 2014; **204**: 567–576.
15. Kachroo A, Robin GP. Systemic signaling during plant defense. *Curr Opin Plant Biol* 2013; **16**: 527–533.
16. Ritter A, Cabioch L, Brillet-Guéguen L, Corre E, Cosse A, Darteville L, et al. Herbivore-induced chemical and molecular responses of the kelps *Laminaria digitata* and *Lessonia spicata*. *PLoS One* 2017; **12**: 1–17.
17. Kloareg B, Quatrano RS. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanogr Mar Biol An Annu Rev* 1988; **26**: 259–315.
18. Newell R, Lucas M, Velirnirov B, Seiderer L. Quantitative significance of dissolved organic losses following fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). *Mar Ecol Prog Ser* 1980; **2**: 45–59.
19. Lami R, Grimaud R, Sanchez-Brosseau S, Six C, Thomas F, West NJ, et al. Marine bacterial models for experimental biology. *Established and emerging marine organisms in experimental biology*. 2021. in press
20. Thomas F, Hehemann J-H, Rebuffet E, Czjzek M, Michel G. Environmental and gut *Bacteroidetes*: the food connection. *Front Microbiol* 2011; **2**: 93.
21. Kirchman DL. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol Ecol* 2002; **39**: 91–100.
22. Barbeyron T, L'Haridon S, Corre E, Kloareg B, Potin P. *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from a red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2001; **51**: 985–97.
23. Barbeyron T, Thomas F, Barbe V, Teeling H, Schenowitz C, Dossat C, et al. Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: Example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij^T. *Environ Microbiol* 2016; **18**: 4610–4627.
24. Thomas F, Barbeyron T, Tonon T, Génicot S, Czjzek M, Michel G. Characterization of the first alginolytic operons in a marine bacterium: from their emergence in marine *Flavobacteriia* to their independent transfers to marine *Proteobacteria* and human gut *Bacteroides*. *Environ Microbiol* 2012; **14**: 2379–94.
25. Grondin JM, Tamura K, Déjean G, Abbott DW, Brumer H. Polysaccharide Utilization Loci: Fuelling microbial communities. *J Bacteriol* 2017; **199**: e00860-16.
26. Anderson KL, Salyers AA. Genetic evidence that outer membrane binding of starch is required for starch utilization by *Bacteroides thetaiotaomicron*. *J Bacteriol* 1989; **171**: 3199–3204.
27. Anderson KL, Salyers AA. Biochemical evidence that starch breakdown by *Bacteroides thetaiotaomicron* involves outer membrane starch-binding sites and periplasmic starch-degrading enzymes. *J Bacteriol* 1989; **171**: 3192–3198.
28. Thomas F, Bordron P, Eveillard D, Michel G. Gene expression analysis of *Zobellia galactanivorans* during the degradation of algal polysaccharides reveals both substrate-specific and shared transcriptome-wide responses. *Front Microbiol* 2017; **8**: 1808.

29. Thomas F, Lundqvist LCE, Jam M, Jeudy A, Barbeyron T, Sandström C, et al. Comparative characterization of two marine alginate lyases from *Zobellia galactanivorans* reveals distinct modes of action and exquisite adaptation to their natural substrate. *J Biol Chem* 2013; **288**: 23021–37.
30. Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 2010; **464**: 908–12.
31. Hehemann J-H, Correc G, Thomas F, Bernard T, Barbeyron T, Jam M, et al. Biochemical and structural characterization of the complex agarolytic enzyme system from the marine bacterium *Zobellia galactanivorans*. *J Biol Chem* 2012; **287**: 30571–84.
32. Thomas F, Barbeyron T, Michel G. Evaluation of reference genes for real-time quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. *J Microbiol Methods* 2011; **84**: 61–6.
33. Ghosh W, Dam B. Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea. *FEMS Microbiol Rev* 2009; **33**: 999–1043.
34. Sievert SM, Kiene RP, Shulz-Vogt HN. The Sulfur Cycle. *Oceanography* 2007; **20**: 117–123.
35. Howarth RW. The ecological significance of sulfur in the energy dynamics of salt marsh and coastal marine sediments. *Biogeochemistry* 1984; **1**: 5–27.
36. Thomas F, Giblin AE, Cardon ZG, Sievert SM. Rhizosphere heterogeneity shapes abundance and activity of sulfur-oxidizing bacteria in vegetated salt marsh sediments. *Front Microbiol* 2014; **5**.
37. Thomas F, Morris JT, Wigand C, Sievert SM. Short-term effect of simulated salt marsh restoration by sand-amendment on sediment bacterial communities Franc. *PLoS One* 2019; 1–18.
38. Holmer M, Gribsholt B, Kristensen E. Effects of sea level rise on growth of *Spartina anglica* and oxygen dynamics in rhizosphere and salt marsh sediments. *Mar Ecol Prog Ser* 2002; **225**: 197–204.
39. Sievert SM, Hügler M, Taylor CD, Wirsén CO. Sulfur Oxidation at Deep-Sea Hydrothermal Vents. *Microbial Sulfur Metabolism*. 2008. pp 238–258.
40. McNichol J, Sylva SP, Thomas F, Taylor CD, Sievert SM, Seewald JS. Assessing microbial processes in deep-sea hydrothermal systems by incubation at in situ temperature and pressure. *Deep Res Part I Oceanogr Res Pap* 2016; **115**: 221–232.
41. McNichol J, Stryhanyuk H, Sylva SP, Thomas F, Musat N, Seewald JS, et al. Primary productivity below the seafloor at deep-sea hot springs. *Proc Natl Acad Sci* 2018; **115**: 6756–6761.
42. Signori CN, Thomas F, Enrich-Prast A, Pollery RCG, Sievert SM. Microbial diversity and community structure across environmental gradients in Bransfield Strait, Western Antarctic Peninsula. *Front Microbiol* 2014; **5**: 1–12.
43. Sievert SM, McNichol J, Thomas F. How do deep-sea hot spring ecosystems work? *Sci J for Kids* 2018; 1–5.
44. Samanta SK, Singh O V, Jain RK. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *Trends Biotechnol* 2002; **20**: 243–248.
45. Khan S, Afzal M, Iqbal S, Khan QM. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. *Chemosphere* 2013; **90**: 1317–1332.
46. Thomas F, Cébron A. Short-term rhizosphere effect on available carbon sources, phenanthrene degradation, and active microbiome in an aged-contaminated industrial soil. *Front Microbiol* 2016; **7**: 1–15.
47. Thomas F, Lorgeoux C, Faure P, Billet D, Cébron A. Isolation and substrate screening of polycyclic aromatic hydrocarbon degrading bacteria from soil with long history of contamination. *Int Biodeterior Biodegradation* 2016; **107**: 1–9.
48. Thomas F, Corre E, Cébron A. Stable isotope probing and metagenomics highlight the effect of plants on uncultured phenanthrene-degrading bacterial consortium in polluted soil. *ISME J* 2019; **13**: 1814–1830.
49. Bourceret A, Leyval C, Thomas F, Cébron A. Rhizosphere effect is stronger than PAH concentration on shaping spatial bacterial assemblages along centimeter-scale depth gradients. *Can J Microbiol* 2017; **63**: 881–893.
50. Duarte CM, Middelburg JJ, Caraco N. Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences Discuss* 2005; **2**: 1–8.
51. Smith S V. Marine macrophytes as a global carbon sink. *Science* 1981; **211**: 838–840.
52. Ficko-Blean E, Hervé C, Michel G. Sweet and sour sugars from the sea: the biosynthesis and remodeling of sulfated cell wall polysaccharides from marine macroalgae. *Perspect Phycol* 2014; **2**: 51–64.
53. Mann KH. Ecology of coastal waters: a systems approach. 1982. University of California Press, Berkeley.
54. van der Loos LM, Eriksson BK, Falcão Salles J. The macroalgal holobiont in a changing sea. *Trends Microbiol* 2019; **27**: 635–650.
55. Jung KA, Lim SR, Kim Y, Park JM. Potentials of macroalgae as feedstocks for biorefinery. *Bioresour Technol* 2013; **135**: 182–190.
56. Poblete-Castro I, Hoffmann SL, Becker J, Wittmann C. Cascaded valorization of seaweed using microbial cell factories. *Curr Opin Biotechnol* 2020; **65**: 102–113.
57. Trincone A. Enzymatic technologies for marine polysaccharides. 2019. CRC Press, Boca Raton, USA.
58. Zhu Y, Thomas F, Larocque R, Li N, Duffieux D, Cladière L, et al. Genetic analyses unravel the crucial role of a horizontally acquired alginate lyase for brown algal biomass degradation by *Zobellia galactanivorans*. *Environ Microbiol* 2017; **19**: 2164–2181.
59. Brunet M, de Bettignies F, Le Duff N, Tanguy G, Davoult D, Leblanc C, et al. Accumulation of detached kelp biomass in a subtidal temperate coastal ecosystem induces succession of epiphytic and sediment bacterial communities. *Environ Microbiol* 2021; **23**: 1638–1655.
60. Ficko-Blean E, Préchoux A, Thomas F, Rochat T, Larocque R, Zhu Y, et al. Carrageenan catabolism is encoded by a complex regulon in marine heterotrophic bacteria. *Nat Commun* 2017; **8**.

61. Thomas F, Le N, Wu DT, Cébron A, Uroz S, Leroux C, et al. Isotopic tracing reveals single-cell assimilation of a macroalgal polysaccharide by a few marine Flavobacteria and Gammaproteobacteria. *ISME J* 2021; 10.1038/s41396-021-00987-x
62. Dudek M, Dieudonné A, Jouanneau D, Rochat T, Michel G, Sarels B, et al. Regulation of alginate catabolism involves a GntR family repressor in the marine flavobacterium *Zobellia galactanivorans* Dsij^T. *Nucleic Acids Res* 2020; **48**: 7786–7800.
63. Jouanneau D, Klau LJ, Larocque R, Jaffrennou A, Duval G, Le Duff N, et al. Structure–function analysis of a new PL17 oligoalginate lyase from the marine bacterium *Zobellia galactanivorans* Dsij^T. *Glycobiology* 2021; **cwab058**.
64. Thomas F, Dittami SM, Brunet M, Le Duff N, Tanguy G, Leblanc C, et al. Evaluation of a new primer combination to minimize plastid contamination in 16S rDNA metabarcoding analyses of alga-associated bacterial communities. *Environ Microbiol Rep* 2020; **12**: 30–37.
65. Thomas F, Le Duff N, Leroux C, Darteville L, Riera P. Isotopic labeling of cultured macroalgae and isolation of ¹³C-labeled cell wall polysaccharides for trophic investigations. *Adv Bot Res* 2020; **95**: 1–17.
66. de Bettignies F, Dauby P, Thomas F, Gobet A, Delage L, Bohner O, et al. Degradation dynamics and processes associated with the accumulation of *Laminaria hyperborea* (Phaeophyceae) kelp fragments: an in situ experimental approach. *J Phycol* 2020; **56**: 1481–1492.
67. Barbeyron T, Thiébaud M, Le Duff N, Martin M, Corre E, Tanguy G, et al. *Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria from the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended description of the genus *Zobellia*. *Int J Syst Evol Microbiol* 2021. in press
68. Kraan S. Algal Polysaccharides , Novel Applications and Outlook. 2012.
69. Hemsworth GR, Dejean G, Davies GJ, Brumer H. Learning from microbial strategies for polysaccharide degradation. *Biochem Soc Trans* 2016; **44**: 94–108.
70. McKee LS, La Rosa SL, Westereng B, Eijssink VG, Pope PB, Larsbrink J. Polysaccharide degradation by the *Bacteroidetes*: mechanisms and nomenclature. *Environ Microbiol Rep* 2021; **10.1111/17**.
71. Radajewski S, Ineson P, Parekh NR, Murrell JC. Stable-isotope probing as a tool in microbial ecology. *Nature* 2000; **403**: 646–9.
72. Dumont MG, Murrell JC. Stable isotope probing — linking microbial identity to function. *Nat Rev Microbiol* 2005; **3**: 499–504.
73. Musat N, Musat F, Weber PK, Pett-Ridge J. Tracking microbial interactions with NanoSIMS. *Curr Opin Biotechnol* 2016; **41**: 114–121.
74. Krumhansl KA, Scheibling RE. Production and fate of kelp detritus. *Mar Ecol Prog Ser* 2012; **467**: 281–302.
75. Pontrelli S, Szabo R, Pollak S, Schwartzman J, Ledezma-Tejeda D, Cordero OX, et al. Hierarchical control of microbial community assembly. *bioRxiv* 2021; doi: 10.1101/2021.06.22.449372.
76. Datta MS, Sliwerska E, Gore J, Polz M, Cordero OX. Microbial interactions lead to rapid micro-scale successions on model marine particles. *Nat Commun* 2016; **7**: 1–7.
77. Hehemann J-H, Arevalo P, Datta MS, Yu X, Corzett CH, Henschel A, et al. Adaptive radiation by waves of gene transfer leads to fine-scale resource partitioning in marine microbes. *Nat Commun* 2016; **7**: 12860.
78. Enke TN, Datta MS, Schwartzman J, Cermak N, Schmitz D, Barrere J, et al. Modular assembly of polysaccharide-degrading marine microbial communities. *Curr Biol* 2019; **29**: 1528–1535.
79. Yu X, Polz MF, Alm EJ. Interactions in self-assembled microbial communities saturate with diversity. *ISME J* 2019; **13**: 1602–1617.
80. Cavaliere M, Feng S, Soyer OS, Jiménez JI. Cooperation in microbial communities and their biotechnological applications. *Environ Microbiol* 2017; **19**: 2949–2963.
81. Arnosti C, Wietz M, Brinkhoff T, Hehemann J-H, Probandt D, Zeugner L, et al. The biogeochemistry of marine polysaccharides: sources, inventories, and bacterial drivers of the carbohydrate cycle. *Ann Rev Mar Sci* 2021; **13**: 9.1-9.28.
82. Zhu Y, Chen P, Bao Y, Men Y, Zeng Y, Yang J, et al. Complete genome sequence and transcriptomic analysis of a novel marine strain *Bacillus weihaiensis* reveals the mechanism of brown algae degradation. *Sci Rep* 2016; **6**: 38248.
83. Rogers TE, Pudlo NA, Koropatkin NM, Bell JSK, Moya Balasch M, Jasker K, et al. Dynamic responses of *Bacteroides thetaiotaomicron* during growth on glycan mixtures. *Mol Microbiol* 2013; **88**: 876–890.
84. Tuncil YE, Xiao Y, Porter NT, Reuhs BL, Martens EC, Hamaker BR. Reciprocal prioritization to dietary glycans by gut bacteria in a competitive coexistence. *MBio* 2017; **8**: 1–17.
85. Martin M, Barbeyron T, Martin R, Portetel D, Michel G, Vandenbol M. The cultivable surface microbiota of the brown alga *Ascophyllum nodosum* is enriched in macroalgal-polysaccharide-degrading bacteria. *Front Microbiol* 2015; **6**: 1–14.
86. Pollak S, Gralka M, Sato Y, Schwartzman J, Lu L, Cordero OX. Public good exploitation in natural bacterioplankton communities. *Sci Adv* 2021; **7**: eabi4717.

Annex: Curriculum Vitae

François Thomas, Ph.D.

CNRS Researcher
Marine Glycobiology group, UMR8227
Station Biologique de Roscoff, 29680 Roscoff (FR)

Phone: +33-256-452-148

E-mail: francois.thomas@sb-roscoff.fr

EDUCATION

2011	Ph.D. Microbiology and Biochemistry Pierre et Marie Curie University, Paris 6
2006	Biochemistry and Bio-engineering "Agregation" Highly competitive professional teaching qualification for secondary and higher education level Ecole Normale Supérieure, Cachan
2005	Master of Engineering in Biochemistry and Biotechnologies National Institute for Applied Sciences, Lyon

RESEARCH EXPERIENCE

2015 – present	CNRS Researcher (CR Classe Normale) Marine Glycobiology research group, Station Biologique, Roscoff (France)
2014 – 2015	Postdoctoral Investigator – with Dr. A. Cébron LIEC, UMR7360 CNRS-Université de Lorraine (France) <i>Organization of microbial communities and fate of pollutants in the rhizosphere</i>
2012 – 2014	Postdoctoral Investigator – with Dr. Stefan Sievert Sievert Lab for Microbial Ecology and Physiology, WHOI (USA) <i>The role of sulfur oxidizing bacteria in salt marsh carbon and nitrogen cycling</i>
2011- 2012	Research and Teaching Assistant – with Dr. Pascal Claquin FRE3484 BioMEA, Université de Caen - Basse Normandie (France) <i>Characterization of extracellular polymeric substances in coastal ecosystems</i>
2008-2011	Doctoral work – with Dr. Gurvan Michel and Dr. Mirjam Czjzek Marine Glycobiology research group, Station Biologique, Roscoff (France) <i>Alginate degradation by the marine bacterium <i>Zobellia galactanivorans</i></i>
2006-2008	Research Assistant - with Dr. Catherine Leblanc and Dr. Philippe Potin Algal Defense research group, Station Biologique, Roscoff (France) <i>Distance signaling in the defense responses of the brown alga <i>Laminaria digitata</i></i>
2005	Undergraduate Research Assistant – with Dr. Andrea C. Alfaro AUT, Earth and Oceanic Research Institute (New Zealand) <i>Trophic interactions within estuarine food webs using lipid biomarkers and stable isotopes</i>

TEACHING EXPERIENCE

2020	Lecture (2 h) for Master 2 "Microbiology, Environment, Health"
2011-2012	Research and Teaching Assistant (72 h at Master's level) Université de Caen-Basse Normandie. Fields: Cell biology, Genetics, Immunology, Physiology
2008-2011	Teaching assistant (192 h at Bachelor and Master's level) Ecole Normale Supérieure de Cachan. Fields: Microbiology, Biochemistry

SUPERVISING EXPERIENCE (period 2015-2021)

Nov 2018-Dec 2021	Maéva Brunet's PhD thesis (Sorbonne Université) 60%
May-Jul 2021	Maël Garnier's Bachelor internship (Univ. Poitiers) ~ 50%
Feb-Jul 2019	Manon Choulot's Master 2 internship (Ecole de Biologie Industrielle, Cergy) ~ 50%
Apr-Jun 2019	Jacky Ame's DUT internship (IUT Génie Biologique, Clermont-Ferrand) ~ 50%
Apr-Jun 2019	Manon Thiébaud's DUT internship (IUT Génie Biologique Laval) ~ 50%
Feb-Aug 2018	Marion Urvoy's Master 2 internship (INSA Toulouse) ~ 30%
Feb-Aug 2018	Maéva Brunet's Master 2 internship (INSA Toulouse) ~ 50%
Aug-Nov 2018	Santi Anggraeni's scientific exchange (PhD thesis, Dresden University) ~ 30%
Jun 2017- Nov 2018	Magda Dudek's postdoctoral project ~ 100%
Jan 2017- Jun 2017	Anissa Dieudonné's Master 2 internship (Univ. Bretagne Occidentale) ~ 70%
Sept. 2016- Dec. 2016	Niels Zevenbergen's Master 2 internship (Wageningen Univ.) ~ 100%

FINANCED AND COORDINATED PROJECTS (period 2015-2021)

2018-2023	ALGAVOR project (283 k€) Agence Nationale de la Recherche <i>Ecological and metabolic strategies of specialist bacteria mediating macroalgae breakdown</i>
2018-2020	ALGOSIP interdisciplinary project (44 k€) Défi ISOTOP, Mission pour l'Interdisciplinarité du CNRS <i>Metagenomic exploration of the diversity of macroalgae-degrading bacteria</i>
2017-2018	Internal research grant, UMR8227 (10 k€) <i>Succession and role of epiphytic bacteria during the degradation of Laminaria hyperborea</i>
2016-2018	Stratégie d'Attractivité Durable, Région Bretagne (116 k€) <i>Regulation of the alginate catabolism in the algae-degrading bacteria Zobellia galactanivorans</i>
2015-2016	Internal research grant, UMR8227 (5 k€) <i>Kinetic study of alginate degradation by Zobellia galactanivorans</i>

COLLECTIVE DUTIES

- Elected member of the UMR8227 Lab Council
- Member of the Teaching Council of the Station Biologique de Roscoff
- Member of the temporary Council of OSU Stations Marines
- Animation of a working group on "Ecology and Evolution of heterotrophic bacteria", UMR8227 (2016-2019)
- Animation of a working group on "Fate of organic matter in marine ecosystems", SBR (2016-present)
- Member of the jury for Sébastien Bridel's PhD defense (2020)
- Member of the jury for Pierre-Yves Mocaër's PhD defense (2019)
- Member of the annual thesis committee for N. Nguyen (MARUM-MPI, 2021-present)
- Member of the annual thesis committee for X. Li (INSA Toulouse, 2021-present)
- Member of the annual thesis committee for F. de Bettignies (ED227, 2016-2019)
- Member of the annual thesis committee for U. Guyet (ED227, 2016-2019)
- Invited Member of the annual thesis committee for E. Grigorian (ED227, 2017-2020)
- Reviewer for 12 articles during 2018-2021 (e.g. ISME J, Env Microbiol, Nat Comm Biol, Mol Ecol)

OUTREACH ACTIVITIES (period 2015-2021)

2020-21	Creation of outreach activities on microbiology, for schools and adults Member of working task to prepare the 150 th anniversary of Station Biologique de Roscoff
2019	Animation of a hands-on practical class on uses of marine polysaccharides for a primary school, a maritime fair and Science Festival

- 2018 Blog post (<http://laplaneterevisitee-corse.mnhn.fr/fr/carnet-bord/carnet-bord-scientifiques/reveler-invisibles>)
 Science Festival, Station Biologique de Roscoff
 Publication of an outreach paper: Sievert SM, McNichol J & Thomas F (2018), "How do deep-sea hot spring ecosystems work?", Science Journal for Kids
- 2017 Science Festival, Station Biologique de Roscoff
 Blog article (<https://web.who.edu/darklife/category/dark-life-2017/>)
 Animation of a hands-on practical class on uses of marine polysaccharides for a group of high school students
- 2016 Science Festival, Station Biologique de Roscoff
 Meeting with a group of high school students (Programme Horizon Mer)
 Meeting with artists interested on algae for a new exhibition center in Morlaix
- 2015 Science Festival, Station Biologique de Roscoff
 Presentation of scientific career for PhD students (Journées de rentrée, ED515)

PEER-REVIEWED PUBLICATIONS

underlined: F. Thomas as corresponding author; *Both authors contributed equally; supervised students and postdocs are in blue

- Barbeyron T, Thiébaud M, Le Duff N, Martin M, Corre E, Tanguy G, Vandenbol M & Thomas F (2021) *Zobellia roscoffensis* sp. nov. and *Zobellia nedashkovskayae* sp. nov., two flavobacteria from the epiphytic microbiota of the brown alga *Ascophyllum nodosum*, and emended description of the genus *Zobellia*. *International Journal of Systematic and Evolutionary Microbiology*, 71: 004913.
- Jouanneau D, Klau LJ, Larocque R, Jaffrennou A, Duval G, Le Duff N, Roret T, Jeudy A, Aachmann FL, Czjzek M & Thomas F^s (2021) Structure-function analysis of a new PL17 oligoalginate lyase from the marine bacterium *Zobellia galactanivorans* Dsij^T. *Glycobiology*, doi: 10.1093/glycob/cwab058.
- Thomas F, Le Duff N, Wu TD, Cébron A, Uroz S, Riera P, Leroux C, Tanguy G, Legeay E, Guerquin-Kern JL (2021). Isotopic tracing reveals single-cell assimilation of a macroalgal polysaccharide by a few marine Flavobacteria and Gammaproteobacteria. *The ISME Journal*, doi: 10.1038/s41396-021-00987-x.
- Brunet M, de Bettignies F, Le Duff N, Tanguy G, Davoult D, Leblanc C, Gobet A & Thomas F (2021) Accumulation of detached kelp biomass in a subtidal temperate coastal ecosystem induces succession of epiphytic and sediment bacterial communities, *Environmental Microbiology*, 23: 3, 1638-1655.
- Dudek M^{*}, Dieudonné A^{*}, Jouanneau D, Rochat T, Michel G, Sarels B & Thomas F (2020) Regulation of alginate catabolism involves a GntR family repressor in the marine flavobacterium *Zobellia galactanivorans* Dsij^T, *Nucleic Acids Research*, 48: 14, 7786-7800.
- de Bettignies F, Dauby P, Thomas F, Gobet A, Delage L, Bohner O, Loisel S & Davoult D (2020) Degradation dynamics and processes associated with the accumulation of *Laminaria hyperborea* (Phaeophyceae) kelp fragments: an in situ experimental approach, *Journal of Phycology*, 56: 6, 1481-1492.
- Thomas F, Le Duff N, Leroux C, Darteville L & Riera P (2020) Isotopic labeling of cultured macroalgae and isolation of ¹³C-labeled cell wall polysaccharides for trophic investigations, *Advances in Botanical Research*, 95, 1-17.
- Thomas F, Dittami S, Brunet M, Le Duff N, Tanguy G, Leblanc C & Gobet A (2020) Evaluation of a new primer combination to minimize plastid contamination in 16S rDNA metabarcoding analyses of alga-associated bacterial communities, *Environmental Microbiology Reports*, 12: 1, 30-37.
- Thomas F, Morris JT, Wigand C & Sievert SM (2019) Short-term effect of simulated salt marsh restoration by sand-amendment on sediment bacterial communities, *PLoS One*, 14 (4), e0215767
- Thomas F, Corre E & Cébron A (2019) Stable isotope probing and metagenomics highlight the effect of plants on uncultured phenanthrene-degrading bacterial consortium in polluted soil, *The ISME Journal*, 13, 1814-1830.
- McNichol J, Stryhanyuk H, Sylva SP, Thomas F, Musat N, Seewald JS & Sievert SM (2018) Primary productivity below the seafloor at deep-sea hot springs, *PNAS*, 26, 6756-6761.
- Ficko-Blean E^{*}, Préchoux A^{*}, Thomas F, Rochat T, Larocque R, Zhu Y, Stam M, Génicot S, Jam M, Calteau A, Viart B, Ropartz D, Pérez-Pascual D, Correc G, Matard-Mann M, Stubbs KA, Rogniaux H, Jeudy A, Barbeyron T, Médigue C, Czjzek M, Vallenet D, McBride MJ, Duchaud E & Michel G (2017) Carrageenan catabolism is encoded by a complex regulon in marine heterotrophic bacteria. *Nature Communications*, 8: 1, 1685.

- Thomas F**, Bordron P, Eveillard D & Michel G (2017) Gene expression analysis of *Zobellia galactanivorans* during the degradation of algal polysaccharides reveals both substrate-specific and shared transcriptome-wide responses. **Frontiers in Microbiology** 8, 1808.
- Bourceret A, Leyval C, **Thomas F** & Cébron A (2017) Rhizosphere effect is stronger than PAH concentration on shaping spatial bacterial assemblages along centimetre-scale depth gradients. **Canadian Journal of Microbiology**, 63: 11, 881-893.
- Zhu Y*, **Thomas F***, Larocque R, Li N, Duffieux D, Cladière L, Souchaud F, Michel G & McBride MJ (2017) Genetic analyses unravel the crucial role of a horizontally acquired alginate lyase for brown algal biomass degradation by *Zobellia galactanivorans*. **Environmental Microbiology**, 19: 6, 2164-2181.
- Ritter A, Cabioch L, Brillet-Guégan L, Corre E, Cosse A, Darteville L, Duruflé H, Fasshauer C, Goulitquer S, **Thomas F**, Correa JA, Potin P, Faugeron S & Leblanc C (2017) Herbivore-induced chemical and molecular responses of the kelps *Laminaria digitata* and *Lessonia spicata*. **PLoS one**, 12:3.
- Barbeyron T, **Thomas F**, Barbe V, Teeling H, Schenowitz C, Dossat C, Goesmann A, Leblanc C, Glöckner FO, Czejek M, Amann R & Michel G (2016) Habitat and taxon as driving forces of carbohydrate catabolism in marine heterotrophic bacteria: example of the model algae-associated bacterium *Zobellia galactanivorans* Dsij^T. **Environmental Microbiology**, 18:12, 4610-4627.
- Thomas F** & Cébron A (2016) Short-term rhizosphere effect on available carbon sources, phenanthrene degradation, and active microbiome in an aged-contaminated industrial soil. **Frontiers in Microbiology**, 7:92.
- Thomas F**, Lorgeoux C, Faure P, Billet D & Cébron A (2016) Isolation and substrate screening of polycyclic aromatic hydrocarbon degrading bacteria from soil with long history of contamination. **International Biodeterioration and Biodegradation**, 107, 1-9.
- McNichol J, Sylva SP, **Thomas F**, Taylor CD, Sievert SM & Seewald JS (2016) Assessing microbial processes in deep-sea hydrothermal systems by incubation at in situ temperature and pressure. **Deep Sea Research Part I**, 115, 221-232.
- Signori CN*, **Thomas F***, Prast AE, Pollery RCG & Sievert SM (2014) Microbial diversity and community structure across environmental gradients in Bransfield Strait, Western Antarctic Peninsula. **Frontiers in Microbiology**, 5:647.
- Thomas F**, Giblin AE, Cardon ZG & Sievert SM (2014) Rhizosphere heterogeneity shapes abundance and activity of sulfur-oxidizing bacteria in vegetated salt marsh sediments. **Frontiers in Microbiology**, 5:309.
- Thomas F**, Cosse A, Le Panse S, Kloareg B, Potin P & Leblanc C (2014) Kelps feature systemic defense responses: insights into the evolution of innate immunity in multicellular eukaryotes. **New Phytologist**, 204:3, 567-576.
- Thomas F**, Lundqvist LCE, Jam M, Barbeyron T, Sandström C, Michel G & Czejek M (2013) Comparative characterization of two marine alginate lyases from *Zobellia galactanivorans* reveals distinct modes of action and exquisite adaptation to their natural substrate. **The Journal of Biological Chemistry**, 288:32, 23021-37.
- Thomas F**, Barbeyron T, Tonon T, Czejek M & Michel G (2012) Characterization of the first alginolytic operons in a marine bacterium: From their emergence in marine *Flavobacteriia* to their independent transfers to marine *Proteobacteria* and human gut *Bacteroides*. **Environmental Microbiology**, 14:9, 2379-94.
- Hehemann JH, Correc G, **Thomas F**, Bernard T, Barbeyron T, Jam M, Helbert W, Michel G & Czejek M (2012) Biochemical and structural characterization of the complex agarolytic system from the marine bacterium *Zobellia galactanivorans*. **The Journal of Biological Chemistry**, 287:36, 30571-84.
- Thomas F**, Hehemann JH, Rebuffet E, Czejek M & Michel G (2011) Environmental and gut *Bacteroidetes*: the food connection. **Frontiers in Microbiology**, 2:93.
- Thomas F**, Barbeyron T & Michel G (2011) Evaluation of reference genes for real time quantitative PCR in the marine flavobacterium *Zobellia galactanivorans*. **Journal of Microbiological Methods**, 84, 61-66.
- Thomas F**, Cosse A, Goulitquer S, Raimund S, Morin P, Valero M, Leblanc C & Potin P (2011) Waterborne signaling primes the expression of elicitor-induced genes and buffers the oxidative responses in the brown alga *Laminaria digitata*. **PLoS one**, 6:6.

- McFiggans G, Bale CSE, Ball SM, Beames JM, Bloss WJ, Carpenter LJ, Dorsey J, Dunk R, Flynn MJ, Furneaux KL, Gallagher MW, Heard DE, Hollingsworth AM, Hornsby K, Ingham T, Jones CE, Jones RL, Kramer LJ, Langridge JM, Leblanc C, LeCrane JP, Lee JD, Leigh RJ, Longley I, Mahajan AS, Monks PS, Oetjen H, Orr-Ewing AJ, Plane JMC, Potin P, Shillings AJL, **Thomas F**, von Glasow R, Wada R, Whalley LK & Whitehead JD (2010). Iodine-mediated coastal particle formation: an overview of the Reactive Halogens in the Marine Boundary Layer (RHAMBLE) Roscoff coastal study. *Atmospheric Chemistry and Physics*, 10, 2975–2999.
- Goulitquer S, Ritter A, **Thomas F**, Ferec C, Salaün JP & Potin P (2009) Release of volatile aldehydes by the brown algal kelp *Laminaria digitata* in response to both biotic and abiotic stress. *Chembiochem*, 10, 977-982.
- Alfaro AC, Dewas SE & **Thomas F** (2007) Food and habitat partitioning in grazing snails (*Turbo smaragdus*), northern New Zealand. *Estuaries and Coasts*, 30, 431-440.
- Alfaro AC, **Thomas F**, Sergeant L & Duxbury M (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine and Coastal Shelf Science*, 70, 271-286.

Résumé

Je suis un microbiologiste avec une solide expérience en biologie moléculaire et biochimie et un engagement constant pour la recherche interdisciplinaire. Je m'intéresse tout particulièrement à la compréhension des processus bactériens impliqués dans diverses transformations biogéochimiques importantes pour le fonctionnement des écosystèmes. Mes recherches abordent plusieurs questions complémentaires pour déchiffrer la diversité des bactéries impliquées, les voies métaboliques utilisées, leurs mécanismes moléculaires, leurs régulations et leurs déterminants environnementaux. Au cours de ma carrière, j'ai eu la chance d'étudier des processus variés dans des environnements contrastés, et sur une large gamme d'échelles. Dans ce mémoire, je résume mes travaux sur la contribution bactérienne au réseau trophique d'une mangrove, la diversité et les fonctions de bactéries sulfo-oxydantes de marais littoraux et d'environnements hydrothermaux profonds, la dégradation bactérienne de polluants organiques dans les sols terrestres, et les interactions bactéries-macroalgues impliquées dans la dégradation de la biomasse algale. Construisant sur le spectre de concepts et techniques abordés dans ma carrière, le projet de recherche que je propose pour les quatre prochaines années poursuivra l'étude des mécanismes, régulations et implications écologiques de la dégradation de la biomasse des macroalgues par les communautés de bactéries marines hétérotrophes.

Abstract

I am a microbiologist with a strong background in molecular biology and biochemistry, and a consistent commitment to interdisciplinary research. I am particularly interested in understanding the bacterial processes involved in diverse biogeochemical transformations that influence ecosystem functioning. My research tackles several complementary questions to decipher the diversity of bacteria involved in these processes, the metabolic pathways they use, their molecular mechanisms, their regulations and environmental drivers. Along my career path, I had the unique opportunity to study various processes in contrasted environments, and at a broad range of scales. In this manuscript, I summarize my work on the bacterial contribution to mangrove food webs, the diversity and functions of sulfo-oxidizing bacteria in salt marshes and deep-sea hydrothermal vents, the bacterial degradation of pollutants in terrestrial soils, and the bacteria-macroalgae interactions involved in the degradation of algal biomass. Building on this spectrum of concepts and techniques, the research framework I propose for the next four years will pursue the investigation of the mechanisms, regulations and ecological implications of macroalgal biomass degradation by heterotrophic marine bacterial communities.