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Membrane switching: key player in *Vibrio cholerae* and *Uronema marinum* interaction

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To date, it is estimated that there are 2.9 million cases of cholera each year, resulting in 95,000 deaths. In an attempt to remedy this situation, the WHO has set an ambitious target: to eliminate cholera from high-risk areas by 2030. One of the reasons explaining the difficulty to eradicate this pathogen is that this bacterium is able to thrive in nutrient-poor environments, and particularly in phosphate-depleted areas.

While the vast majority of marine waters are limited by essential nutrients such as inorganic phosphate, coastal areas are subject to phosphate waves. In addition, global warming is expected to exacerbate nutrient deficiency in surface ocean. To survive in phosphate-limited environment, *V. cholerae* regulates genes belonging to the Pho regulon and remodels its membrane using the PhoBR two-component regulatory system. In the environment, bacteria are under constant predation pressure from heterotrophic protists such as *Uronema marinum*, a ciliate that graze on bacteria, thereby controlling their population. Previous studies have shown that *V. cholerae* is able to escape digestion by protists and that bacteria that have passed through these predators are more infectious to humans.

This study aims to explore the modification induced by phosphate depletion in *V. cholerae* membrane using ‘omics’ approaches and how this affects its interaction with one of its main predators.

We have identified two new glycolipids, an amino acid-based lipid in addition to a modification of the LPS structure in *V. cholerae* membrane under phosphate-limited conditions. We have shown that *V. cholerae* grown in phosphate-deficient conditions is more captured and in greater quantities by the ciliate than those grown in a phosphate-rich medium. Our results suggest that the activation of PhoBR system may promote the concentration of *V. cholerae* in heterotrophic protists compartments, leading to the emergence of potential hyper-infectious outbreak.

Mots-Clés: *Vibrio cholerae*, Lipid remodeling, Protist predation, Phosphorus limitation

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Influence of feed on the gut microbiota of Grey partridges

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In recent decades, agriculture has intensified to meet growing demand and boost productivity. So-called conventional farming involves increased use of pesticides, which are now omnipresent in the environment. Due to their wide distribution and lack of specificity, non-target organisms, such as farmland birds, can be exposed to them in a variety of ways (water, food, etc.). Today, these individuals are exposed to sub-lethal doses of many pesticides, recognised as a major factor in the decline of birds. On the other hand, organic farming, which excludes pesticides, limits this exposure and can promote biodiversity. The effects of pesticides on the physiology of birds are well documented, but the role of the gut microbiota as a modulator remains little studied. As ingestion is the main path of exposure, the gut microbiota may be directly affected. Involved in the absorption of nutrients, its composition and diversity could be altered, with negative or beneficial effects on immunity. It is therefore essential to gain a better understanding of the factors influencing these interactions. The aim of this study was to assess the impact of a feeding with or without pesticides on the gut microbiota and physiology of captive Grey partridges (*Perdix perdix*), by comparing two groups fed either organic or conventional grain. Cloacal samples were taken at the beginning and end of the experiment to analyse the microbial diversity and taxonomic composition of each individual. Alpha diversity, reflecting individual microbial diversity, was assessed using species richness and the Shannon index. Beta diversity, comparing communities between individuals, was analysed using the Bray-Curtis and Jaccard indices. The results show that microbial diversity did not differ according to feed, but decreased over time in females. Microbial communities' structure was not influenced by feed, although a slight diversification was observed at the end of the experiment in males fed on conventional grain. These results reveal complex interactions between feed, microbiota and physiology, underlining the importance of continuing research to better understand these relationships, both for wildlife and for human health.

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Mots-Clés: Bacterial communities, Microbiota, Agricultural practices, Specialist farmland birds

One for all: Impact of phosphorus limitation on membrane remodelling, prey-predator interactions and methane oxidation

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Methane (CH₄) is a potent greenhouse gas that accounts for approximately 20–30% of global warming, second only to carbon dioxide (CO₂). Consequently, it is one of the leading targets in short-term global warming mitigation. The only known natural way to filter methane are by methane-oxidising bacteria (methanotrophs). Protists such as amoebae and ciliates have been recognised as grazers of methanotrophs. Yet, the interplay between nutrient stress, especially phosphorus, an essential macronutrient for bacterial activity, and methanotroph susceptibility to predation is poorly characterised.

Recent research has reported that methanotrophs such as *Methylosinus trichosporium* OB3b can remodel their membrane lipids in P-deficient conditions by replacing phospholipids with glycolipids. However, the ecological role of this process, in particular, resistance or susceptibility to protozoan predation, is still unknown, while global warming is expected to exacerbate nutrient deficiencies in surface waters. We hypothesise that phosphorus-driven lipid remodelling in methanotrophs influences their susceptibility to protist predation, altering predator-prey dynamics and impacting methane oxidation in nutrient-limited environments.

To test this, we will use a variety of interaction experiments to observe the ingestion, growth, and digestion of prey inside protozoa predators. In addition, this study focuses on methanotrophs in greater depth by examining membrane lipid remodelling of different strains in phosphorus-limited conditions using LC/MS.

By gaining a greater understanding of methanotrophic bacteria and their trade-off mechanisms in nutrient-limited conditions, this research seeks to predict how these limitations modify the bacterial membrane, predation by heterotrophic protists and ultimately their ability to oxidize methane.

Mots-Clés: Methanotrophs, Phosphorus limitation, Lipid remodelling, Protist predation, Methane Oxidation

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Understanding OsHV-1 μ Var transmission dynamics in Pacific oysters through a mechanistic and stochastic SWEIRD Model

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Since the rise of oyster farming in the 17th century, French aquaculture has been recurrently impacted by emerging pathogens. After successive cultivation failures of native and introduced species, the Pacific oyster (*Magallana gigas*) has become the dominant species since the 1970s. However, the emergence in 2008 of a highly virulent variant of the Ostreid herpesvirus type 1 (OsHV- 1 μ Var) has led to recurrent mass mortality events in spats, with rates reaching 100% in some production sites. This still ongoing crisis threatens the long-term viability of the industry and underscores the urgent need to better understand the viral transmission dynamics to inform disease management strategies.

To this end, we developed a compartmental epidemiological model tailored to the transmission dynamics of OsHV- 1 μ Var in *M. gigas*. Building on the classical SEIR framework, our SWEIRD model introduces a viral water compartment to capture environmental transmission and explicitly accounts for phenotypic host tolerance by distinguishing tolerant and non-tolerant individuals through separate epidemiological pathways. Model parameters were estimated from targeted experimental trials measuring incubation periods, infectious durations, and viral shedding rates in both tolerant and non-tolerant oysters, as well as virus decay in seawater. These parameters were implemented using stochastic processes to reflect inter- and intra-individual variability. Simulations revealed distinct epidemic dynamics between tolerant and non-tolerant pathways and highlighted key temporal patterns of mortality and environmental contamination. Moreover, an Approximate Bayesian Computation (ABC) approach, based on a validation dataset, was used to refine parameter distributions and improve the model's fit to observed dynamics. In addition, a sensitivity analysis identified the most influential parameters driving infection peaks and transmission speed. Our integrative approach offers a robust framework to explore mollusc pathogens transmission dynamics and provides a basis for future spatial modeling and in situ data interpretation during upcoming field sampling.

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Mots-Clés: Epidemiology, Virus, Modelling, Oyster, OsHV, 1

Effects of environmental conditions on mutualistic interactions between the ichthyotoxic microalgae *Prymnesium parvum* and a bacterial community.

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The haptophyte, *Prymnesium parvum*, produces ichthyotoxins that can significantly disrupt biodiversity and affect coastal economies reliant on fishing, aquaculture and tourism. Interactions between microalgae and associated bacterial communities play a key role in influencing phytoplankton metabolism and physiology. This study focuses on understanding how environmental conditions shape mutualistic interactions between *P. parvum* and its associated bacteria. To investigate the physiology and metabolism of both the algae and bacteria within the holobiont concept, co-cultures of *P. parvum* and a synthetic bacterial community were performed under various nutrient-limited conditions in photobioreactors. Culture monitoring, metabarcoding, metatranscriptomic and metabolomic analyses were conducted to assess the dynamics of both the algal and microbial communities. The results show that depending the nutrient conditions, the interactions can range from neutral to beneficial, enhancing both the growth of microbial communities and the harmful capacity of *P. parvum*.

These findings pave the way for further functional analyses and metabolic networks. Understanding the interactions between *P. parvum*, bacteria and environment contributes to a more comprehensive view of the ecological impacts of *P. parvum* blooms.

Mots-Clés: microalgae, cocultures, bacteria, interactions, nutrient limitation

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High metabolic versatility and phenotypic heterogeneity in a marine non-cyanobacterial diazotroph

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In a marine environment generally lacking in bioavailable nitrogen, access to atmospheric nitrogen is becoming a key issue. Nitrogen fixation is reserved for diazotrophs possessing a key enzyme, nitrogenase. This enzyme presents two problems: its high energy cost and its sensitivity to oxygen. Cyanobacteria have been widely studied, but it's only recently that a large diversity of non-cyanobacterial diazotrophs bacteria (NCDs) has been discovered. This work is based on a high-throughput sequencing study combined with a genetic and physiological characterization of an NCD, *Vibrio diazotrophicus*, as well as on cell-scale quantification of nitrogenase expression, in order to determine the adaptive strategies of this NCD when growing under nitrogen-deficient conditions. We were able to demonstrate that *V. diazotrophicus* has great metabolic versatility, able of assimilating a wide range of organic and inorganic nitrogen sources. In addition, a quantitative epifluorescence microscopy approach revealed posttranscriptional regulation of nitrogenase expression, as well as modulation by *V. diazotrophicus* of the proportion of cells expressing nitrogenase and its intensity. This modulation is dependent on ammonium concentration and is regulated by the transcriptional regulator NtrC. Finally, we were able to demonstrate that this phenotypic heterogeneity in nitrogenase expression is found in other marine NCDs, suggesting that it is a conserved trait. This study lifts the veil on the physiology of NCDs and their importance in the biogeochemical cycle of marine nitrogen. Related publication : Crétin, P., Mahoudeau, L., Joubelin-Delavat, A., Paulhan, N., Labrune, E., Verdon, J., ... & Delavat, F. (2025). High metabolic versatility and phenotypic heterogeneity in a marine non-cyanobacterial diazotroph. *Current Biology*.

Mots-Clés: Diazotrophy, nitrogenase, NCD, phenotypic heterogeneity

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Microbial plankton networks complexity increases under river run-off impact

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In estuarine ecosystems, river run-off strongly influences community composition and species interactions. Although microorganisms play key roles in the functioning of these ecosystems, microbial planktonic community structure and its interaction patterns under river influence still remain poorly understood and their multisite and temporal variabilities are often overlooked. The microorganisms’ small size and the difficulty to culture them under controlled conditions make the direct observation of interactions challenging. Co-occurrence networks, inferred from eDNA data and amplicon sequencing, have been commonly used to uncover potential interactions. To gain further insights into the spatiotemporal variability of microbial associations (bacteria and protists) across estuarine gradient influenced by run-off, impacted and non-impacted waters were sampled fortnightly over a 3 years period (2020-2023) across four French ecosystems. Sample-specific networks were built and their topological properties were analyzed with eight metrics. Network’s structure varied among ecosystems and along the in-off shore gradients of each site. Despite pronounced spatial (between sites) and seasonal variability, a consistent pattern emerged, notably revealing a decrease in microbial networks complexity along the land-sea gradient. This greater network complexity in freshwater influenced waters might imply a higher potential for cooperation and/or competition for resources among microorganisms in highly disturbed ecosystems. Distinct microbial taxa were found to be linked either to impacted or non-impacted networks, underlying their major role in ecological interactions in estuaries.

Mots-Clés: network, co, occurrence, estuary, planktonic

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Genomic Characterization of Marine *Vibrio* Species for the Development of Novel Diagnostic Tools

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The genus *Vibrio* comprises numerous marine bacteria, including several species recognised as important pathogens with significant implications for both human health and aquaculture. In particular, marine molluscs are highly susceptible to infections caused by specific *Vibrio* taxa. The development of accurate species-level diagnostic tools remains a major challenge, largely due to the substantial intraspecific genetic diversity and the limited availability of complete and curated genome assemblies in public repositories. This taxonomic complexity hinders both pathogen identification and disease management strategies in marine ecosystems.

To address these limitations, we sequenced and assembled 115 complete genomes from strains representing 42 distinct *Vibrio* species. The genomes were obtained by long-read sequencing and assembled using a dedicated pipeline optimised for long-read data, ensuring high contiguity and completeness. Notably, eight of these genomes represent the first complete assemblies available for their respective species. Various methodologies are currently used for bacterial identification, including sequence-based approaches and MALDI-TOF mass spectrometry.

In this study, we compared the performance of MALDI-TOF, MLSA, and whole genome sequencing for the identification of *Vibrio* species. Using comparative genomics, we delineated the *Vibrio* core genome and identified a set of candidate species-specific genomic markers. These loci were systematically validated against a comprehensive dataset of 6,904 publicly available *Vibrio* genomes from 51 species, with particular emphasis on clades containing mollusc pathogens. Our analysis revealed novel markers with high discriminatory power and interspecies specificity, enabling robust taxonomic resolution across the genus.

We propose a novel single-gene diagnostic marker that allows accurate and consistent identification of *Vibrio* species, offering a streamlined alternative to current multi-locus approaches. In addition to its diagnostic potential, this marker provides a strong phylogenetic signal, supporting more accurate classification within the genus. This work also allowed us to formulate recommendations for the identification and diagnosis of *Vibrio* bacteria, contributing to the development of next-generation molecular tools for monitoring *Vibrio* diversity in marine ecosystems.

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Mots-Clés: Vibrio, genomic, diagnostic, ONT

Manila clam microbiota response to *Vibrio tapetis*, temperature and antibiotics at tissue level

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Vibrio tapetis is the causative agent of the Brown Ring Disease (BRD) on the Manila Clam, *Ruditapes philippinarum*, which lead to a significant decrease of the Manila clam production in Northwestern Europe. While the pathogen leads to higher BRD at 14°C, at 21°C clam immunity seems stimulated. Research on Manila clam microbiota is relatively recent and most of them have focused on the digestive gland microbiota, but none has ever compared several tissues under a microbial challenge. The objective of this study was to improve our understanding of the interactions between Manila clam microbiota, a clam pathogen and environmental factors. In a nested-design lab experiment, we studied the potential effect of *V. tapetis*, temperature increase and antibiotics (ATB) on clam health and microbiota diversity. Microbiota from three tissues (digestive glands DG, extrapallial fluids EPF, and hemolymph HLPH) were analyzed over a two-month period.

During the experiment, the proportion of ill clams remained relatively low. The three tissues exhibited specific microbiota: both fluids exhibited relatively similar microbiota (dominated by α and γ -*Proteobacteria*, *Flavobacteriales* and *Spirochaetales*), but were very different from the DG (largely dominated by *Mycoplasmatales* followed by α -*Proteobacteria*). Despite high variability between individuals, significant impacts on β -diversity were highlighted for all the tested parameters. At 14°C, *V. tapetis* exposures were associated with an increase in *Crocinitomicaceae*, which did not persist at 21°C. Therefore, the temperature might partially mitigated some *V. tapetis* effects. However, temperature increase was also a structuring factor including by increasing *Nitrospirales* in fluids. ATB exposure modified clam microbiota and made clams more sensitive to both the *V. tapetis* challenge and the temperature increase. Our results suggest that pathogens and environmental parameters can modify microbiota without influencing clam health.

Mots-Clés: Manila clam, microbiota, *Vibrio tapetis*, tissue, scale analysis, temperature, antibiotic, metabarcoding

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Assessment of a microbiome engineering strategy using Lactic Acid Bacteria as bioprotective cultures to delay the spoilage of Gilthead Seabream (*Sparus aurata*) fillets

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The Mediterranean gilthead seabream (*Sparus aurata*) is a widely consumed seafood product, with global production reaching 354,920 tonnes in 2022, primarily from aquaculture (FAO, 2024). However, seabream is microbiologically sensitive and highly perishable, which leads to significant food waste. Following slaughter, seabream flesh becomes vulnerable to microbial colonization, leading to the development of a specific endogenous microbiota.

Some bacteria play a role in spoilage, degrade the product quality by generating metabolites that alter its sensory characteristics. Biopreservation is a soft preservation technic which seeks to modulate the microbiota of food product to slow down spoilage or pathogen growth. This technic involves inoculating the product with microorganisms or metabolites that exhibit antimicrobial activity (Passerini *et al.*, 2021).

In the frame work of the European project Foodguard, five protective lactic acid bacteria (LAB) were tested on seabream fillets stored under modified atmosphere packaging at 8°C during 11 days. During these shelf-life experiments, LAB strains effects on the microbial community and seabream quality were assessed on 110 samples using: classical microbiological enumeration techniques, 16S amplicons sequencing approach, biochemistry analysis and sensory evaluations conducted by an expert panel.

Results show that three LAB strains significantly reduced the presence of seabream spoilage-associated bacteria such as *Brochothrix*, *Pseudomonas*, and members of the *Enterobacteriaceae* family. Sensory analyses confirmed the beneficial effects of these strains, which helped maintain the fillet quality for up to 8 days of storage. Multi-omics analyses were conducted to correlate microbiota composition and dynamics with biochemical profiles, as well as sensory scores and attributes, thereby revealing biologically relevant and robust molecular signatures.

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Mots-Clés: LAB, biopreservation, seabream, multiomics

Coastal microbiome dynamics and anthropogenic pressures: new insights from environmental DNA

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Human activities and climate change have significant impacts on the functioning of coastal ecosystems. Analyzing the dynamics of microbial communities and species offers valuable insights into these changes. Environmental genomics, particularly the use of environmental DNA (eDNA) to assess microbial biodiversity in water or sediment samples, has opened new avenues for evaluating these impacts. These approaches now enable the development of innovative tools for environmental monitoring and management through the study of coastal microbiomes. River inputs and the materials they transport contribute to variability in the composition of micro-eukaryotic communities in estuarine sediments (ECOMINE project). These inputs create gradients in community composition (ROME) and drive potential interactions among microorganisms (ROME/BIOcean5D). Such variations help identify bacterial bioindicators of anthropogenic pressures, which can be applied across Europe to assess freshwater inputs, even in offshore marine ecosystems (OBAMA-Next project). At the same time, new monitoring systems are being developed to detect health, environmental, and marine biodiversity risks (ROME, Ostreobila, BIOcean5D). Ancient sedimentary eDNA analysis allows us to assess ecosystem states prior to significant human influence, across centuries and throughout the Anthropocene. This approach reveals irreversible shifts in species and community structures (PALMIRA), as well as biodiversity losses linked to pollution or intensive industrial activities like mining (HISTOMINE). The link between biodiversity and human-induced pollution is currently being studied at the European scale. Using paleogenetic techniques, researchers aim to identify the effects of eutrophication, urbanization, industrialization, aquaculture, and coastal artificialization. These studies also examine ecosystem resilience (BIOcean5D, TREC, PaleoBreizh) and assess the impact of ecological restoration and biodiversity conservation efforts in marine protected areas (SeaMap). Ultimately, this research aims to define microbial bioindicators of pollution across Europe (TREC, CONTRAST), with potential applications for the sustainable management and conservation of coastal ecosystems.

Mots-Clés: Coastal ecology, Estuaries, Pollution, Paleogenomics, Environmental genomics

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Towards the modeling of cross-feeding interactions between the toxic microalga *Prymnesium parvum* and its microbiome under nutrient stress conditions

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Prymnesium parvum is a toxin-producing haptophyte microalga known to cause harmful algal blooms (HABs) with severe ecological impact. However, the molecular and ecological mechanisms underlying its toxicity remain unclear. An emerging hypothesis suggests that environmental stressors, such as nutrient limitation, can alter toxin production not only directly, but also indirectly, by reshaping the surrounding microbiome. This, in turn, may modulate biotic interactions with the host, thereby influencing its physiology and capacity for toxin release.

In this study, we aim to explore the role of the microbiome in supporting *P. parvum* growth by uncovering cross-feeding interactions under different nutrient conditions. These interactions, involving metabolite exchanges between organisms, were shown to play a critical role in shaping microbial community structure and function. One way of exploring cross-feeding interactions is through the use of the constraint-based metabolic modeling framework. This approach relies on the reconstruction of genome-scale metabolic models (GEMs), which represent the network of biochemical reactions an organism can carry out, including the metabolites it produces, consumes, and exchanges with its environment. GEMs enable predictions of metabolite secretion profiles and potential cross-feeding exchanges between interacting species, helping to identify prototroph–auxotroph relationships under variable nutrient conditions.

Notably, *P. parvum* has been identified as an auxotroph for B12 vitamin (cobalamin), a complex yet essential cofactor for multiple vital processes. A recent co-culture experiment involving *P. parvum* and a synthetic community of 15 bacterial species comprising B12 producers showed sustained algal growth in the absence of exogenous B12, indicating potential cross-feeding of B12 or its precursors.

By leveraging the metabolic modeling framework, we aim to predict B12 biosynthesis and release capacity within this synthetic community, and potential cross-feeding interactions with *P.*

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parvum under varying nutrient conditions. Moreover, our approach aims to facilitate the design of minimal synthetic consortia optimized for algal support under nutrient stress conditions. We discuss how this modeling framework can guide community member selection, generate testable hypotheses concerning microbial interactions and growth outcomes, and ultimately improve our understanding of microalgal-microbiome interactions in ecologically relevant contexts.

Mots-Clés: constraint based metabolic modeling, cross feeding interactions, vitamin B12, holobiont system

Impact of model foods enriched with quorum sensing autoinducers on the intestinal microbiota : search for a proof of concept in rats

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In developed countries, consumption of foods rich in live bacteria is becoming more popular, driven by interests for lightly preserved foods like smoked fishes, new fermented products like lacto-fermented vegetables, and food preservation with protective bacteria. Such foods are considered healthy, although the underlying mechanisms are not fully understood, with possible involvement of probiotic delivery, anti-nutrients removal, microbiota modulation, or bacterial metabolite enrichment.

We hypothesize that the benefits of bacteria-enriched foods result from their content of type 1 or type 2 autoinducers (AI-1 & AI-2), signalling molecules used by bacteria for intercellular communication.

We selected, within relevant starters or bioprotective bacteria, bacterial strains that exhibit the highest AI productions (as quantified by mass spectrometry) when grown on milk ultrafiltrate. Using these strains, we generated model foods enriched in AI which *in vitro* digestibility test and

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in vivo impact on the composition of the microbiota (metabarcoding) and on the inflammatory state of the intestinal mucosa (RT-qPCR) following 8 days-supplementation of recently weaned rats, have been assessed.

Hafnia alvei CIRM-BIA 1832 and *Lactococcus lactis* CIRM-BIA 242 exhibited the highest potential for AI production. Both AIs underwent partial degradation during *in vitro* test (approximately 30% and 70% for AI-1 and AI-2). Feeding AIs enriched food had no significant impact on rat growth nor food consumption. No significant differences were observed in both ileal and colonic microbiota features (alpha and beta diversities indexes, relative abundances of main families and genera). Conversely, AI-1 supplementation resulted in a significant decrease in PON-2 expression (by 8), and increases in that of TNFa (by 4) and IL1b (by 1.7), in the ileal mucosae of rats.

Finally, AI content in food constitutes a possible vector for interaction with the mucosal homeostasis of the host's intestine. The health implications of the proinflammatory changes observed remain to be established.

Lachnospirales, a new digestive and symbiotic partner for *Rimicaris exoculata* and *Rimicaris chacei* ?

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In deep-sea hydrothermal vent - deprived of light - primary energy production is ensured by chemosynthetic microbial communities. These microorganisms are free-living in the environment or forming associations with metazoan hosts which are called holobionts. Among them, two endemic shrimp *Rimicaris exoculata* and *Rimicaris chacei* from the Mid-Atlantic Ridge (MAR) have been well described. Even if these two species exhibit very contrasting biological traits, they harbor similar microbial communities in various organs. One is found in the cephalothoracic cavity, another in the foregut and the last one colonizes the midgut. The midgut symbiotic community is mainly composed of *Candidatus* Microvillspirillaceae, long and thin unicellular filamentous organisms that are inserted between the microvilli of the intestinal epithelium in the ectoperitrophic space. Recent studies have also revealed the presence of a second major lineage acquired during juvenile metamorphosis: *Lachnospirales*. We decided to study these bacteria from shrimp from two sites (TAG and Snake Pit) using a combination of three approaches: Fluorescent *in situ* Hybridization (FISH), metabarcoding and metagenomics. Firstly, a specific FISH probes targeting *Lachnospirales* was developed, tested and optimized. It was used to detect and identify cocobacilli and filamentous *Lachnospirales* inside the midgut tube of adult and juvenile specimens of *Rimicaris exoculata* and *Rimicaris chacei*. To complement these data, an analysis of two MAGs (Metagenomic Assembled Genomes) has revealed a new lineage of *Lachnospirales*, whose metabolism seems to be close to that of the *Candidatus* Microvillspirillaceae with which they co-occur. *Lachnospirales* are mixotrophs, capable of degrading complex polymers and supplying nutrients to their host. Finally, to better characterize these lineages, an analysis of taxonomic diversity (metabarcoding) during the life cycle of the two species on the two MAR sites was carried out. This analysis enabled us to formulate hypotheses concerning the acquisition and transmission strategies of these symbionts within the holobiont.

Mots-Clés: Symbiosis, Holobiont, Lachnospirales, Rimicaris spp, Hydrothermal vent, Metage-

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FISH, a new tool for in-situ preservation of RNA in tissues of deep-sea mobile fauna

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Accessing the metabolic functioning of deep-sea animals in situ remains a technological challenge as the recovery time of samples is incompatible with the short lifespan of such molecules as mRNAs. Tools able to preserve RNA in situ exist, but they are incompatible with the study of mobile fauna. Here, we describe a new sampling tool, named FISH (fixer in situ of homogenized substrates), implemented on a submersible and equipped with a number of new specific features to collect and preserve in situ tissue of mobile fauna. Connected to the suction pump of a submersible, the FISH sampler incorporates a sampling bowl to which two bottles of a preservative reagent are attached, a suction hose, and a support containing a motor connected to the sampling bowl by a magnetic coupling system. We used the deep-sea hydrothermal shrimp *Rimicaris exoculata* from the Mid-Atlantic Ridge as a model to test the suitability of our new tool. The FISH sampler was compared to two other sampling methods, which use a metatranscriptomic approach targeting microbial communities associated with cephalothorax symbionts. RNA quality, gene assignment, and taxonomic and gene function diversity showed differences between in situ and on-board preservation of tissues. Of the alternative sampling methods tested, the suction sampler was clearly not suitable for RNA-based studies, while pressurized recovery showed results closer to the sample quality obtained with FISH sampling. The FISH sampler has therefore demonstrated to be a cost-effective and reliable tool to efficiently preserve RNA recovered from deep-sea environments.

Mots-Clés: technological development, mRNA, abyss, in situ fixation, symbionts, tool

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Norovirus genetic diversity in a watershed scale upstream of oyster-farming sites: the contribution of passive sampling

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Introduction and objectives

Norovirus contamination of oysters is a public health and economic issue for the oyster industry. This human pathogen originates from human wastewater. Detecting it upstream of oyster-farming sites would make it possible to prevent oyster contamination. The objectives of this study were to use passive sampling on the scale of a watershed to identify input channels, and to analyze the genetic diversity of the contamination using targeted metagenomics.

Materials and methods

Passive samplers using nylon membran were immersed for 48 h at twelve selected sites in the watershed, including two in oyster farming areas, where oysters were also positioned for 18 months. Nucleic acids were extracted from both matrices (membran and oyster) and norovirus were detected by RT-qPCR. Two regions of the genome of genogroups I and II were amplified: the capsid (VP1) and the polymerase (RdRp) following an adapted protocol. Amplicons were sequenced using Illumina MiSeq. An automated, standardized targeted metagenomics analysis workflow was adapted and optimized to reconstruct and identify norovirus sequences.

Results, discussion and conclusion

Noroviruses were identified in 15% of membranes (50/334) and 41% of oysters (23/56). Sequencing revealed a high degree of genetic diversity. Six genotypes were identified for norovirus of the GI-VP1 genogroup (GI.1, GI.2, GI.3, GI.4, GI.5 and GI.6), nine for noroviruses GII-VP1 (GII.1, GII.2, GII.3, GII.4, GII.6, GII.7, GII.12, GII.13 and GII.17), and three for GI-P-type noroviruses (GI.P1, GI.P2, GI.P4). Genotyping carried out on oysters and membranes showed complementarity between the two matrices, greater genetic diversity in the winter period, and inter-site variability.

For the first time, genotyping using passive samplers was possible. This innovative study demonstrates the benefits of passive sampling for diagnosing the geographical distribution of norovirus contamination in a watershed. However, it needs to be crossed with other data for a more detailed characterization of contamination pathways (knowledge of wastewater networks, river flow, marine currents).

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Mots-Clés: Norovirus, passive sampler, contamination, targeted metagenomics

Parallel deployment of passive and grab samplers for variant profiling of SARS-CoV-2 in sewers of two French cities

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Wastewater-based epidemiology emerged as a valuable method to monitor the COVID-19 epidemic and the dynamic of SARS-CoV-2 variants. Because of its ease of deployment and low cost, membrane-based passive sampling is a prime alternative for deploying a monitoring network in wastewater, especially when automatic samplers cannot be used. However, the performance of these strategies for the identification of low-abundance viruses needs to be evaluated. Passive sampling using nylon membranes and grab sampling were carried out in parallel in the sewers of two French cities in April and May 2022, for the detection of norovirus GII and SARS-CoV-2. SARS-CoV-2 sequencing was performed to compare the performance of passive samplers and their paired grab sampler in identifying Omicron sub-lineages. Direct lysis and elution methods from nylon membranes were equally effective for virus recovery and SARS-CoV-2 sequencing. For all sites, the virus concentrations in passive and grab samples were very similar. A near-complete genome coverage at a depth of 30, was obtained for most samples, using ARTIC multiplex PCR (V4.1) and Illumina MiSeq. There was a high proportion of low-frequency mutations for both methods and rare mutations in the S gene were detected, which could reflect the presence of cryptic lineages. Even though a large proportion of BA.2 lineage was detected in sewage, most importantly this study provides the first evidence that the use of passive sampling enables early detection of SARS-CoV-2 variants BA.4 that is, before they are identified in the population.

Mots-Clés: Passive sampling – Wastewater based epidemiology – SARS, CoV, 2 – Omicron variants, norovirus – sequencing

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CRISPR-Cas system diversity in environmental *Campylobacter* species

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Campylobacter species, particularly *C. jejuni* and *C. coli*, are among the leading causes of bacterial gastroenteritis worldwide, transmitted through contaminated food, water, and environmental reservoirs (1). CRISPR-Cas systems play a crucial role in bacterial immunity against mobile genetic elements (MGEs,(2)) and serve as valuable tools for strain typing and source attribution (3). This study explores the diversity and characteristics of CRISPR-Cas systems across a dataset of 2616 *Campylobacter* genomes from various sources, including shellfish and surface water (4,5).

Our analysis revealed that 1530 out of 2616 genomes (58.5%) harbored at least one CRISPR array, with significant differences between species. *C. jejuni* exhibited the highest prevalence of CRISPR-Cas systems, followed by *C. coli*, while most *C. lari* isolates lacked CRISPR arrays, and displayed a distinct distribution of CRISPR types. A total of 1530 CRISPR arrays were identified, with many strains carrying two or more arrays. Type II CRISPR-Cas systems predominated in *C. jejuni* and *C. coli*. Interestingly, Type III CRISPR-Cas systems were as prevalent as Type II in *C. lari*, suggesting functional diversity.

A comparative analysis of CRISPR array composition identified 909 unique arrays and 3169 unique spacers, suggesting high variability. Spacer analysis indicated frequent matches with a few phages, highlighting inter-species encounters. Our findings provide novel insights into the diversity of *Campylobacter* CRISPR-Cas systems and their potential application in source tracking, particularly within a One Health framework.

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2. Barrangou, R.; et al, 2007.
3. Pearson, B.M.; et al 2015
4. Gourmelon, M.; et al 2022
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Mots-Clés: Keywords: Campylobacter, CRISPR, Cas, source attribution, OneHealth

A microbial perspective on animal development and behaviour

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Animals have evolved within the framework of the microbes and are constantly exposed to diverse microbiota. This dominance of the microbial world is forcing all fields of biology to question some of their most basic premises, with developmental biology being no exception. While animals under laboratory conditions can develop and live without microbes, they are far from normal, and would not survive under natural conditions, where their fitness would be strongly compromised. Since much of the undescribed biodiversity on Earth is microbial, any consideration of animal development in the absence of the recognition of microbes will be incomplete. Here, using the ancestral metazoan Hydra as model, I will show that animal development and behaviour may never have been autonomous, rather it requires transient or persistent interactions with the microbial world. I propose that to formulate a comprehensive understanding of embryogenesis and post-embryonic development, we must recognize that symbiotic microbes provide important developmental signals and contribute in significant ways to phenotype production. This offers limitless opportunities for the field of cell and developmental biology to expand.

Mots-Clés: Animal, microbe, Evolution, Hydra, Microbiome, Symbiosis

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