PROJECT « Lucinid clams as model organisms for chemosynthetic symbioses: an integrative appproach »

Objectives and scientific hypotheses

Beneficial host-microbe interactions are virtually universal, and the field of biology has reached a consensus that beneficial microbes far outnumber pathogens in our bodies and in our environment. The importance of mutualistic symbioses in past and present evolution of Eukaryotes is now widely recognized. Although the importance of bacterial mutualistic symbioses is now widely recognized (e.g. human microbiome), knowledge of the molecular mechanisms at work for the establishment and functioning of symbiosis is still limited. Most of these animal- or plant-associated microbiomes are enormously diverse making the complex functional interplay between partners almost impossible to disentangle. A few models of bacterial symbiosis have provided the first details on host-microbe interactions, due to their natural simplicity (e.g. legumes andrhizobia , bobtail squid and Vibrio , or several insect-bacteria associations). In these symbioses, the establishment and maintenance of the association heavily relies on cell communication between partners, recruiting various biochemical signals and receptors, including on the host side those related to immune response and apoptosis. Dramatic shifts in metabolism are also apparent in cells directly involved in endosymbiosis (bacteriocytes). The establishment of a mutualistic endosymbiosis implies delicately balanced relationships between eukaryotic cells and bacteria, at the boundary between the "simple" phagotrophic predation/digestion of bacteria by eukaryotes and the cell disease/disruption provoked by pathogenic bacteria. Understanding the pathways enabling these balanced interactions, and the biomolecules allowing it, is therefore of foremost importance for fundamental research in eukaryote/prokaryote interactions within both a functional and evolutionary framework. To reach a comprehensive understanding of these processes we need to extend the diversity of model systems in which the molecular interplay between host and symbiont is finely characterized. Chemosymbiotic bivalves are ideal for this purpose due to the natural simplicity of their association with sulfur-oxidizing bacteria. We expect to find both common and unique ways to establish and maintain the host-symbiont association, compared to presently known models of beneficial and pathogenic associations.

Lucinidae is the most species-rich bivalve family alive today. Their reduced digestive tract and feeding apparatus attest to the importance of their symbiotic source of nutrition. Each species so far investigated hosts a single species of gammaproteobacterial sulfur-oxidizing bacteria in specialized cells of the gill epithelia. Lucinids are widely distributed in nature, from shallow to deep marine waters, and from temperate to tropical habitats. In these symbioses, the bacteria fix CO_2 into organic carbon, which they pass on to their host for nutrition, however, the exact mechanism of nutritional transfer are still poorly understood. Lucinids are the only animal hosts of chemosynthetic symbionts that have been successfully raised in the laboratory after experimentally induced spawning. In the wild, *Loripes. orbiculatus* and *Lucinoma borealis* also produce gelatinous egg masses that contain the developing larvae. The possibility to adress the same questions using two distinct species that are living together is also an unique oportunity to develop new biological models.

The main biological questions we intend to address in this pHD-project are:

1- how does the host select his partner? or in other words, how the host is able to distinguish the “good” guy from the “bad” guy? Indeed the implementation of a symbiotic relation involves the exchange of signaling molecules between partners and if different recognition pathways have been identified in some species, very few data are available in the case of mollusc species.

2- who is controlling who and how? Innate immune system is the main line of defense, triggering diverse humoral and cellular activities via signal transduction pathways and protecting the

host from invading pathogens. Endocellular symbiosis implies that symbionts somehow by-pass the defense mechanisms of the host. However, the molecular mechanisms and responses as well as the chemical molecules involved in the communication between the host and the bacteria remain poorly understood, particularly in invertebrate intracellular symbiosis.

3 : does the microbiota contribute to the symbiose establisment and/or regulation ?

.Lucinids larvae are aposymbiotic with respect to the gill symbiont found in adults, but they are unlikely to be completely sterile. They associate with commensal microbial communities ('microbiomes'), for example in their digestive tract, which could affect their health during development and as adults. Our goals are to understand the development of the *L. orbiculatus* and *L. borealis* microbiomes, by following the evolution of microbiota from the apo-symbiotic stages (eggs and larvae) to the stage of gill colonization by symbionts and during the growth of individuals and then better understand the potential interactions between *L. orbiculatus* and *L. borealis*, and their microbiome, including the gill symbionts, during development from embryos to adults

Methodology :

To achieve our objectives, we will combine both field population survey and exprimental designs and will apply a multi-disciplinary approach coupling transcriptomic, metabolomic, metabarcoding and imaging tools.

1- Fieldwork .

Field populations of *L. orbiculatus* and *L. borealis* will be sampled at a regular time scale in the Rocoff sea-grass bed and standard environmental monitoring will be operated at each sampling session to estimate physico-chemical parameters (mainly pH, oxygen, sulfide, nitrogen, salinity, temperature,....). Microbiota from the surrounding enviornment of both species will be also analysed to determine the diversity of bacteria present in the sediment and to compare this diversity with the lucinids microbiota. Isotopic signatures for carbon and nitrogen will be also followed.

2- Laboratory exeriments :

2-1 :Understanding of symbiont depuration :

Because aposymbiotic adult stages have never been observed in nature, we will produce those individuals in laboratory conditions. We will keep both species in tanks containing sediments to allow them to stay buried and aerated sea-water (control condition). Individuals will be separated into two populations: one kept in the control condition and another one in which the natural activity of sulfatereducing bacteria in sediment will be maintained by providing the bacteria with a source of carbon to stimulate sulfide production. We will keep those populations for at least one year and will regularly sample individuals for transcriptomic (RNAseq), metabolomic, isotopic, microbiota and imaging analysis.

2-2 : Possibility to induce symbiont recolonization :

We will set up experiments consisting in the exposure of aposymbiotic individuals to gill extracts containing symbionts and symbionts purified from tissues but also by adding of sulfur pulses or organic substrates. Translocation experiment using aposymbiotic individuals obtained in the laboratory to the field will be perfoemd using caging experiments. Individuals will be then sampled along a time scale (up to 6 months period) after translocation. Symbiont content and activity will be determined and subsequent host transcriptome, metabolites profiling and microbiota analyses will be done if symbiont re-acquisition (from aposymbiotic) is observed.

2-3 : Symbiosis acquisition via larvae :

Egg mass (aposymbiotic) will be sampled in the field and preserved in aquaria until the larvae release to monitor the process of the symbiont establishment and thus identify at which developmental stage the gill symbiosis is acquired but also to collect aposymbiotic pediveligers that could be used in various experimental infection assays. Symbiont content and activity will be determined and subsequent host transcriptome, metabolites profiling, microbiota and imaging analyses will be performed. In parallele, protocols for their maintenance in laboratory conditions until the reproductive stage will be set up in the beginning of the project to allow a complete control of the life cycle.

In this project, all transcriptomic analysis will be conducted using RNAseq approach coupled with quantitative PCR. Imaging analysis (mainly FISH) will be used especially in the understanding of symbiont depuration and acquizition step to get a better picture of tissue change and symbiont localization. Microbiota will be obtained by metabarcoding sequencing and metabolite profiling will help to identify specific chemical compounds involved in the chemical mediation of the symbiosis between he host and the symbiont. A global analysis (modelisation) will be conducted to obtain an integrative vision of the symbiotic relationship

Project suitabilty with the « Institut de l'Océan » : This project aims to develop a new biological model for the study of symbiosis in marine mollusks. It is based on a multi-disciplinary approach combining genomics, transcriptomics, metabolomics and microbiota studies and is based on an approach in both natural and experimental populations. It also proposes to master the complete biological cycle of the two species studied in order to allow a mastery of the different phases in the process of symbionts acquisition. The symbotic models studied are also emblematic of seagrass beds, an ecosystem particularly affected by global changes, and understanding their function could represent a new model to study the impacts of these changes on these ecosystems. This project will also allow the development of new collaborations between the two partners of the project who each bring specific and complementary skills.

The UMR7144 partner (A. Tanguy), bring the genomic data (host and symbiont genomes of both species are under assemblage) and has strong competence in transcriptomic analysis. The natural sea-grass bed present in front of the marine station will guarantee the permanent availability of the two species. An experimental platform (CRBM) at the marine Station will allow to manage the experimental part of the project. The partner UMR7245 (S. Duperron) bring a strong expertise in the characterization of metabolites that participate and regulate the interactions of microorganisms with each other, with their environment, or with their host, has a strong competence in microbiota analysis and has an imaging analysis platform to perform all the imaging work planned in the project (FISH labelling, immuno-histology,...). In this phD project, other specific competences present in the two laboratories are available and will be used to carry out this project (data analysis). The phD student will share his/her time between both laboratories.

References associated to the project : Pales Espinosa et al., 2013. J. Exp. Mar. Biol. Ecol 448 (2013) 327–336) ; Duperron et al., (2020). Front Public Health. The model being new for both partner, very few references directly link to the model are available but both partners have publicaion on other symbiotic model species.

Candidate profile : the candidate should have competence in genomic (transcriptomic and metabarcoding) analysis and in statistics and informatic. An interest for laboratory and experimental work and basic knowledge in symbiotic models will be appreciated.