MOBILOME

Project title: Vesicles and viruses produced by marine thermophilic microorganisms: physiology and detoxification.

Institute: UBO, IUEM
Laboratory: LM2E, UMR 6197

Supervisors: Dr Anne Godfroy and Dr Claire Geslin

- e-mail: Anne.Godfroy@ifremer.fr and e-mail: claire.geslin@univ-brest.fr

5 Publications


Project (in english)

Abstract

Mobile genetic elements (MGEs) such as viruses, plasmids, vesicles ..., which collectively represent the mobilome, interact with cellular organisms from all three domains of life, including those thriving in the most extreme environments. While efforts have been made to better understand deep-sea vent microbial ecology, our knowledge of the mobilome associated with prokaryotes inhabiting deep-sea hydrothermal vents remains limited. During this PhD project we will focus on the abyssal mobilome by studying new viruses and vesicles associated with thermophilic Bacteria and Archaea present in deep-sea hydrothermal vents. The production of MVs is a universal mechanism for cell-cell communication that has been only recently appreciated. Membrane vesicles (MVs) in Bacteria, as well as exosomes and ectosomes in eukaryotes can transfer toxins, quorum sensing agents, pathogenicity factors, RNA and possibly DNA. The production of MVs is a poorly understood mechanism for archaea and thermophilic bacteria. In this context, we want to investigate interactions between MVs with viruses and analyze the regulations carried out. Moreover, hydrothermal vents are characterized by the presence of heavy metals (cadmium, copper, zinc...). The potential role of MVs as a detoxifying mechanism against heavy metals will be also searched. Many MGEs are already available in our laboratory (in-house collections). These collections will be enriched by new genetic elements found during the PhD work. These studies can have important implications with the discovery of new genes and new biological functions.

Scientific context

Viruses exist wherever life is found. Viruses are by far the most abundant ‘lifeforms’ in the oceans and are the
reservoir of most of the genetic diversity in the sea. They infect the three domains of life and are detected in the most extreme environments. Deep-sea hydrothermal vents represent one of the most extreme environments on Earth. These ecosystems are characterized by very high pressure, the lack of solar energy and the prevalence of chemosynthesis. Our knowledge of the viral diversity associated to the prokaryotic microorganisms inhabiting the deep sea hydrothermal vents is still limited. Only few studies have focused on viral abundance and impact on microbial mortality within these ecosystems. A limited number of viruses were isolated and characterized from these extreme environments (Lossouarn et al., 2015a).

LM2E is specialized in the study of viruses isolated from deep sea hydrothermal vents. Two viruses, PAV1 and TPV1, associated to hyperthermophilic anaerobic Archaea, Thermococcales, have been described in our laboratory. They represent the only two marine hyperthermophilic viruses isolated (Geslin et al., 2003 et 2007; Gorlas et al., 2012). Recently, we wished to widen our study to other hyperthermophilic and anaerobic Archaea occupying the same habitats (deep sea vents). We have preferentially target the order of Methanococcales, autotrophic hyperthermophilic Archaea producing methane, for which no virus was described and published. The characterization of the first virus isolated from a marine hyperthermophilic methanogen is under progress in our laboratory.

In order to deepen our knowledge on the viral diversity of marine hydrothermal microorganisms, we have extended our investigation to the bacterial order Thermotogales. They share the same ecological niche as the Thermococcales and are metabolically close. We isolated and characterized the first virus infecting bacteria from this order, Marinitoga piezophila virus 1 (MPV1). Surprisingly, we found that MPV1 virions carry not only the viral DNA but preferentially package a plasmid of 13.3 kb (pMP1) also carried by M. piezophila. This ‘ménage à trois’ highlights potential relevance of selfish genetic elements in facilitating lateral gene transfer in the deep-sea biosphere (Lossouarn et al., 2015b).

In this context, the aims of the PhD work will be to continue the characterization of new viruses isolated from marine thermophilic bacteria and archaea and also to study membrane vesicles produced by the same micro-organisms. Mobile genetic elements (MGEs), such as viruses, plasmids, membrane vesicles... (collectively referred as a mobilome) have a potential to be powerful agents that drive evolution and adaptation of their cellular hosts in the extreme marine environments, and comparative studies of these elements from hyper/thermophilic Archaea and Bacteria will help us understand the dynamics of the microbial communities in the deep biosphere. Moreover, these MGEs constitute a vast reservoir of new genes and new biological functions.

- **Aims of the PhD project**

The novelty and originality of the PhD project is notably based on the study of membrane vesicles produced by marine and thermophilic micro-organisms. The production of MVs is a universal mechanism for cell-cell communication that has been only recently appreciated. Membrane vesicles (MVs) in Bacteria, as well as exosomes and ectosomes in eukaryotes can transfer toxins, quorum sensing agents, pathogenicity factors, RNA and possibly DNA. The production of MVs is a poorly understood mechanism for archaea and thermophilic bacteria

![Figure 1 Various possible physiological roles of bacterial membrane vesicles in the environment.](image)

Soler, ISME J, 2014.

In eukaryotes and bacteria, several studies have shown that membrane vesicles can play a role in detoxification. This phenomenon was first observed in eukaryotic marine organisms such as mollusks and crustaceans which accumu-
late cadmium (Sterling et al., 2007). Storage and excretion of cadmium are performed by MVs as a detoxifying mechanism. Moreover, numerous bacteria produce vesicles containing sulfur. Sulfur is an important element for microbial life present in deep-sea environments and is metabolized by a wide variety of microorganisms, which transiently store sulfur in intracellular vesicles. In our studies, we report the discovery of vesicles containing sulfur (sulfur vesicles) produced by Thermococcus species. We have previously shown that *T. prieurii* produces a virus named TPV1 and harbors also two other extrachromosomal elements: the plasmids pTP1 and pTP2. We have also shown that, in addition to TPV1, *T. prieurii* produces abundant MVs, especially long nanotubes filled with small MVs and sulfur vesicles. We suggest that these dark vesicles accumulate excess of sulfur and transport it outside the host cell as a detoxifying mechanism (Gorlas et al., 2015).

In this context, we want to investigate the interactions between MVs with viruses and analyze the regulations carried out. A focus on the role of vesicles in the physiology of marine and thermophilic microorganisms will be done in this PhD work. Could MVs be used as a detoxifying mechanism? Indeed, hydrothermal vents are characterized notably by the presence of heavy metals (cadmium, copper, zinc...). In order to test if MVs can protect cells against heavy metals, we will study whether addition of cadmium, copper could increase the production of MVs and increase the survival of hyperthermophilic *Archaea* and/or bacterial strains. The study of MVs thus emerges as a new fundamental field of research in cell and evolutionary biology. Further work is now needed to better understand the global impact of MVs on deep-sea hydrothermal vents.

Many MGEs are already available in our laboratory (in-house collections). These collections will be enriched by new genetic elements found during the PhD work. Comparative studies of MGEs from Archaea and Bacteria will help us understand the dynamic genetic network of the microbial communities in the deep biosphere. The study of how microbial populations are modulated by these genetic interactions will become a keystone area. Although the ecological and evolutionary effects of MGEs are not clearly known in deep-sea vents, their production probably plays a major role in the lifestyle of extremophile microorganisms, making analysis of these processes a prerequisite for fully understanding their impact (evolution, adaptation, detoxification) and rationally exploiting their biotechnological potential (transfer of toxins, heavy metals, nucleic acids, etc.).

To our knowledge, there is no equivalent project going on at the national or international level (either in Bacteria, Archaea or Eucaryotes from hydrothermal environments). Studies of interactions between MVs and viruses in microorganisms are in their infancy.

**Methodological approaches:**

LM2E laboratory is a member of networks allowing facilities (platforms) for microscopic, proteomic, genomic and post-genomic researches. We will benefit from these networks in some part for the PhD project. Briefly, on the methodological level, isolates will be cultivated at high temperatures in anaerobic conditions. The presence of viruses and vesicles and their interactions will be checked by transmission electron microscopy and epifluorescence microscopy. Nucleic acids present in these mobile genetic elements will be sequenced and annotated. The successful candidate is expected to conduct innovative research using mass spectrometry (ICP-MS - PSO/Ifremer) in collaboration with E. Ponzevera to address a range of fundamental questions related to the storage and excretion of heavy metals performed by MVs as a detoxifying mechanism in extreme marine environments.

**Candidate profil:**

The candidate will need competences in microbiology, virology, molecular biology and bioinformatics analyses.

**Principal long and international collaborations:**

The LMEE has a long history of fruitful collaboration with Patrick Forterre, head of the laboratory: Molecular Biology of Gene in Extremophiles at the Pasteur institute. In particular these laboratories, with BMGE (University Paris XI, Orsay) have been partners in an ANR project Thermovesicles (2012-2016). The aims of this project are to perform an integrated study on physiological roles of membrane vesicles (MVs) produced by several groups of hyperthermophilic *Archaea* (*Thermococcales, Methanococcales*) and *Bacteria* (*Thermotogales*) isolated from hydrothermal vents. In fine, we want to investigate interactions between MVs with viruses and their possible role in genes transfer between different groups of microbes living in the same environment. These studies will be continued notably in the ERC project (2013-2017) EVOMOBIL in collaboration again with P. Forterre the PI of the project: Co-evolution of viruses, plasmids and cells in archaea: pattern and process.

We have also a fruitful international collaboration with C. Nesbø from Alberta university, Edmonton, Canada notably to sequence and analyse new genomes.