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Investigating symbiont densities and localization in hydrothermal vent and cold seep mytilids using fluorescence hybridization techniques and image analysis

S. Halary (1,3), V. Riou (2), G. Frébourg (1), T. Boudier (3), F. Gaill (1), S. Duperron (1)

(1) UMR 7138, Systématique, Adaptation, Evolution, Université Pierre et Marie Curie, Paris, France, (2) Departamento de Oceanografia e Pescas, Universidade dos Açores, Portugal, (3) Laboratoire d'imagerie intégrative, Institut Curie, Orsay, France

Bacterial symbiosis is essential for the adaptation and survival of metazoans inhabiting chemosynthesis-based ecosystems in the deep-sea. In the last two decades, many cases of multiple endosymbiosis have been described in mytilid species from cold seeps and hydrothermal vents, mainly involving methane- and/or sulfide-oxidizing bacteria. How symbiont diversity, abundances and interactions are affected by the host and the environment are not yet understood in multiple symbioses. Classical tools such as TEM and clone libraries do not yield reliable quantitative estimates, and new approaches are needed to better describe the structure and dynamics of symbiotic bacterial populations.

First, we investigated variability of bacterial densities in the dual symbiotic vent mussel *Bathymodiolus azoricus* in relation to environmental parameters such as methane and sulfide concentrations. We developped specific software to quantify densities of bacteria on FISH-hybridized gill sections, and to perform statistical comparisons between specimens collected at different vent sites as well as specimens kept in the lab under starvation conditions. Similar techniques were then applied to the small, *Bathymodiolus*-related mussel *Idas* sp. from cold seeps in the Mediterranean, which was recently shown to harbour 6 bacterial symbionts. We compared symbiont diversity and densities between specimens collected within carbonate crusts and at the tip of vestimentiferan chitinous tubes, and thus likely experiencing distinct living conditions.

Besides that, the high symbiont diversity in *Idas* sp. might result from the presence of additional extracellular symbionts. To assess which symbionts occur within or outside of host cells, we coupled FISH identification of bacteria with Transmission Electron Tomography.

Developping tools allowing for symbiont quantification and structural analysis of symbiotic associations will improve our understanding of interactions between host, symbionts and their environment. In the future, functional aspects of symbioses will be tackled by coupling these techniques with investigation of mussel and bacterial gene expression patterns.