SPATIAL AND TEMPORAL ANALYSIS OF BACTERIAL DIVERSITY ASSOCIATED WITH THE MEDITERRANEAN GORGONIAN *PARAMURICEA CLAVATA*

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Abstract
We combined terminal-restriction fragment length polymorphism (T-RFLP) and clone library analyses to investigate the diversity and the spatio-temporal changes of bacterial assemblages associated with the gorgonian *Paramuricea clavata*. We found a clear difference between the bacterial communities during winter and summer while T-RFLP profiles were highly similar between *P. clavata* populations separated by hundreds of kilometres. Sequencing data from 16S rDNA clone libraries demonstrated the presence of distinct phylogenetic taxa during summer and winter, with an increased diversity in the warm season. In the context of recurrent gorgonian diseases and mortalities, this picture of the structure of bacterial communities will be helpful to monitor the effects of thermal anomalies on the resident microbial flora.
Keywords: Bacteria, Cnidaria, Mortality, Western Mediterranean, Global Change

Introduction
Mass mortality outbreaks in coastal ecosystems have considerably increased during the past few decades affecting in particular marine invertebrates [1]. In the Northwestern (NW) Mediterranean, recurrent mass mortalities have affected benthic macroinvertebrate species such as gorgonians, sponges and bryozoans. The environmental driver of these large-scale events was an increase in seawater temperature during temperature anomalies in summer [2]. From diseased *P. clavata* colonies, we previously isolated a *Vibrio coralliilyticus* strain that showed thermodependent virulence during controlled infection experiments in aquaria, supporting the involvement of bacterial pathogens in gorgonian die-offs [3]. We hypothesized that variations in specific composition of bacteria associated to *P. clavata* at elevated temperature could be used to detect potential pathogens prior to apparition of disease signs, and might help identify causal agents. However, composition and dynamics of the natural microbial communities living in association with temperate gorgonians are unknown. The main aim of this study was to establish a baseline for diversity and abundance of the bacterial community of *P. clavata*.

Material and methods
Three sampling sites were chosen along the NW Mediterranean coast: Riou Island (gulf of Marseilles), Medes Island (Catalan coast) and Scandola (NW Corsica). For each site, apical branch tips of 3 *P. clavata* colonies were collected at a depth of 20 m, in march 2007, july 2007 and february 2008. Three liters of seawater were also collected and filtered for analysis of planktonic bacteria. For T-RFLP analysis, bacterial 16S rDNA was amplified from total genomic DNA by PCR using fluorescent-labeled universal primers. PCR products were digested by restriction endonucleases *Cfo*I and *Msp*I and separated on an ABI 3130 Genetic Analyzer (Applied Biosystems). The terminal restriction fragments (T-RFs) data collection for each sample was converted into a binary matrix which was used for metric multidimensional scaling (MDS) analyses. Clone libraries were generated by amplification of 16S rDNA using unlabeled primers and subsequent cloning in pGEM-T vector (Promega). Sequences were compared to 16S GenBank database using the Blast program.

Results and discussions
The T-RFLP profiles of bacterial 16S rDNA obtained from three independent *P. clavata* colonies sampled at the same site were highly similar, suggesting that gorgonians maintain a conserved microbial population. Comparison of profiles obtained from Riou, Medes and Scandola further indicated that bacterial communities were very similar between geographically remote *P. clavata* populations. In agreement with a bacterial-gorgonian interaction, no overlap was found between T-RFLP profiles of bacterial communities of *P. clavata* and those of the surrounding seawater. Seasonality in bacterial diversity was investigated by the analysis of *P. clavata* colonies sampled at the three study sites in the winter and the summer of 2007. T-RFLP data showed that species composition differed greatly between the two seasons. Furthermore, no significant differences in bacterial communities were found between samples from winter 2007 and winter 2008. Ordination by MDS supported clustering of bacterial assemblages by seasons, and confirmed that *P. clavata* colonies from different areas of the NW Mediterranean harbor closely related microbial flora (Fig. 1).

Bacterial 16S rDNA clone libraries were derived from *P. clavata* colonies sampled in winter and summer at the Riou site. The winter library was dominated by sequences of a unique bacterium (100% of the 88 clone sequences) affiliated with gamma-proteobacteria. The highest identity scores were obtained with *Spongiobacter*-related sequences (92%), previously retrieved from marine sponges. In summer, a larger diversity of bacteria was detected and clone sequences were dominated by members of the Firmicutes (*Paenicillus sp.* and *Propionibacterium sp.*, accounting for 86% of the 92 clone sequences). Although the role of associations between bacteria and *P. clavata* is unknown, understanding how they change through time may represent a potential indicator of the gorgonian health status. Studies on tropical species of corals and gorgonians have demonstrated shifts in the microbial populations of affected colonies prior to visual signs of disease [4]. Thus, changes in the normal microbial community could serve as an early signal of stressful environmental conditions. We are currently looking for bacterial associations in different sympatric gorgonians (*Eunicella singularis*, *Eunicella cavolinii* and *Corallium rubrum*) to evaluate if related bacteria appear in different hosts. A similar pattern of host-microbe associations might reveal common mechanisms through which bacterial communities and gorgonian health are interlinked.

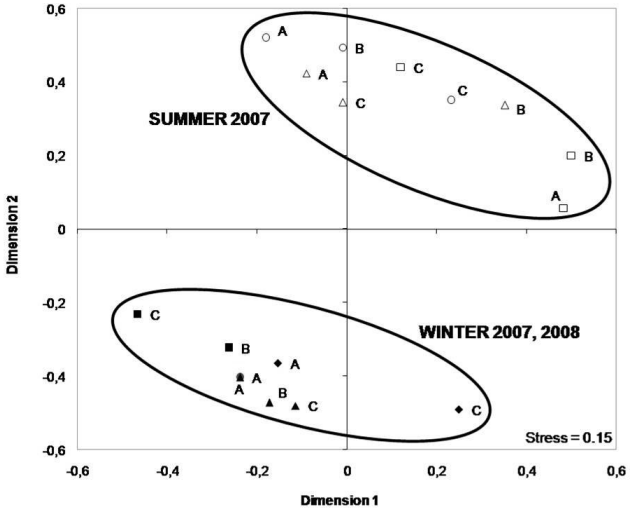

Fig. 1. MDS representation plot of the distances between T-RFLP profiles of Riou (square, diamond), Medes (triangle) and Scandola (circle) samples collected in summer (white) or winter (black)

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