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SHORT COMMUNICATION

Microbial communities associated with the invasive Hawaiian sponge *Mycale armata*

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Microbial symbionts are fundamentally important to their host ecology, but microbial communities of invasive marine species remain largely unexplored. Clone libraries and Denaturing gradient gel electrophoresis analyses revealed diverse microbial phylotypes in the invasive marine sponge *Mycale armata*. Phylotypes were related to eight phyla: Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Acidobacteria, Chloroflexi, Crenarchaeota and Firmicutes, with predominant alphaproteobacterial sequences (>58%). Three Bacterial Phylotype Groups (BPG1—associated only with sequence from marine sponges; BPG2—associated with sponges and other marine organisms and BPG3—potential new phylotypes) were identified in *M. armata*. The operational taxonomic units (OTU) of cluster BPG2-B, belonging to Rhodobacteraceae, are dominant sequences of two clone libraries of *M. armata*, but constitute only a small fraction of sequences from the non-invasive sponge *Dysidea* sp. Six OTUs from *M. armata* were potential new phylotypes because of their low sequence identity with their reference sequences. Our results suggest that *M. armata* harbors both sponge-specific phylotypes and bacterial phylotypes from other marine organisms.
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Marine invasive species pose a serious threat to the world's oceans. Symbiotic microbes can dramatically impact ecosystem function by changing the phenotype of their hosts (Gerardo *et al.*, 2006). However, microbial communities within invasive marine species remain relatively unknown. The marine sponge *Mycale armata* (family Mycalidae) is a recent, unintentionally introduced sponge species, becoming invasive and the most abundant sponge species in the Hawaiian reef ecosystem. In Kaneohe Bay (Oahu, Hawaii), this species overgrows and kills native coral lagoon–patch reef communities (Supplementary Figure 1). To reveal microbial features of *M. armata*, molecular methods were applied to investigate its microbial communities.

Sponge samples were collected along the shores of Coconut Island in August 2004 and May 2005, situated within Kaneohe Bay and surrounded by 64 acres of coral reef. Samples of sea water and sponges (invasive and non-invasive) were collected and processed according to the method of Gao *et al.* (2008). Total genomic DNA was used as a PCR template for the amplification of 16S rRNA genes for library construction and denaturing gradient gel electrophoresis analysis (Gao *et al.*, 2008; Zhu *et al.*, 2008). Band patterns of microbial communities varied in different sponge species collected at the same time, but bands were similar in samples of the same sponge species collected at different times (Figure 1). Seven major bands were consistently present in samples of *M. armata* collected in both years. Two bands (1 and 2), which were common to *M. armata* and other non-invasive sponges (*Dysidea* sp., *Gelliodes fibrosa*, *Tedania* sp. and *Scopalina* sp.), and two other, unique bands (3 and 4) were detected in the samples of *M. armata* collected in both years. The denaturing gradient gel electrophoresis band patterns suggested that *M. armata* contained microbial phylotypes present in the other Hawaiian sponges, but also harbored unique microbial phylotypes and these phylotypes seemed to have little temporal changes.

To reveal microbial composition in *M. armata*, four clone libraries were constructed from the samples of *M. armata* (A and B for samples collected in August 2004 and May 2005, respectively), *Dysidea* sp. (C) and sea water (D). Clone sequences were de-replicated first and then grouped into the same operational taxonomic unit (OTU) using a percent sequence identity of greater than 97%. The percentage coverage and diversity of the four libraries are summarized in Supplementary Table 1. Analysis of clone sequences identified 24 and 19

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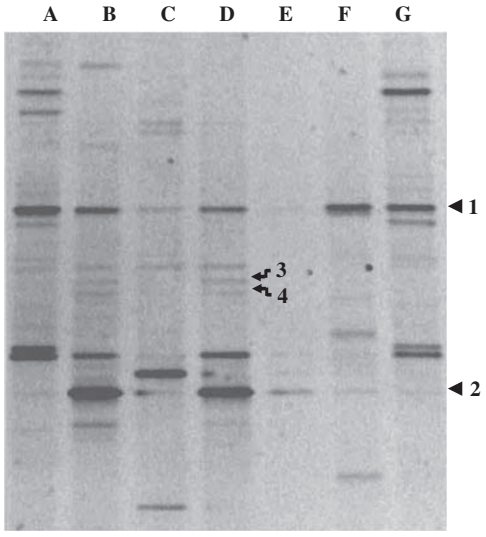

Figure 1 Denaturing gradient gel electrophoresis (DGGE) banding profiles of bacterial 16S rRNA genes PCR-amplified from total DNA extracted from sponges using the primers GC-BAC341 and BAC521. Lanes A and G, *Dysidea* sp.; lane C, *Gelliodes fibrosa*; lanes B and D, *Mycale armata*; lane E, *Tedania* sp. and lane F, *Scopalina* sp. Lanes D–G, samples collected at Kaneohe bay in August 2004. Lanes A–C, samples collected in May 2005 from the same location. PCR products were concentrated and prepared as described by Gao *et al.* (2008).

OTUs from *M. armata* libraries A and B, respectively. Additional 27 and 15 OTUs were identified from clone libraries of *Dysidea* sp. (C) and sea water (D), respectively. Sequences of 16S rRNA gene clones from *M. armata* libraries were related to members of Proteobacteria (classes Alpha-, Beta-, Delta- and Gammaproteobacteria), Bacteroidetes, Actinobacteria, Cyanobacteria, Firmicutes, Chloroflexi, Acidobacteria and Crenarchaeota (Figure 2; Supplementary Figures 2 and 3). Alphaproteobacteria were a dominant microbial group, comprising 61.3% and 58.0% of clone sequences from libraries A and B, respectively. Although alphaproteobacterial sequences have been observed in 16S rRNA gene libraries constructed from over 12 marine sponges collected from several ocean provinces (Hentschel *et al.*, 2006; Taylor *et al.*, 2007; Thiel *et al.*, 2007a, b; Zhu *et al.*, 2008), this is only the second report of alphaproteobacterial dominance in the 16S rRNA clone library, after the report on *Halichondria panicea* (Wichels *et al.*, 2006). The three classes (Alpha-, Gamma- and Deltaproteobacteria) and three phyla (Actinobacteria, Cyanobacteria and Bacteroidetes) were found in both *M. armata* clone libraries (A and B). Sequences of chloroplasts and the phylum Verrucomicrobia were found exclusively in *Dysidea* sp. whereas members of Actinobacteria, Crenarchaeota and Deltaproteobacteria were present only in one (A or B) library of *M. armata* (Figure 2). Fewer phyla were identified in the seawater library, but unclassified bacterial and euryarchaeote sequences (Supplementary Figure 2) were present only in the seawater library.

Three Bacterial Phylotype Groups (BPGs) were identified from OTUs derived from *M. armata* and *Dysidea* sp. (Figure 2). The first group (BPG1) included 10 OTUs affiliated only with sponge-derived 16S rRNA bacterial sequences. Of this group, three OTUs of cluster BPG1-D were members of Gammaproteobacteria and found in the samples of *M. armata* collected in both years. Three OTUs of clade BPG1-B were closely clustered with betaproteobacterial sequences identified in Mediterranean sponge *Tethya aurantium* (Thiel *et al.*, 2007b) and the Antarctic sponges *Latrunculia apicalis* and *M. acerata* (Webster *et al.*, 2004). The OTUs of two clusters BPG1-C and BPG1-D (Figure 2) were phylogenetically related to gammaproteobacterial sequences from the Hawaiian sponges *Suberites zeteki* (Zhu *et al.*, 2008) and the Great Barrier Reef sponge *Rhopaloeides odorabile* (Webster *et al.*, 2001), respectively.

The second group (BPG2) contained 14 OTUs related to 16S rRNA bacterial sequences from both sponges and other marine organisms, such as corals and squids. Seven OTUs of cluster BPG2-B belonged to Alphaproteobacteria and were present in all samples of *M. armata* and *Dysidea* sp. (Figure 2). These OTUs were predominant among clone sequences from two *M. armata* libraries, but contributed only to a small fraction of clone sequences from *Dysidea* sp. The OTUs of clusters BPG2-C and BPG2-E were members of Alpha- and Gammaproteobacteria, respectively, and were found in the samples of *M. armata* collected in both years (Figure 2). Interestingly, two OTUs (MA17 and MB73) of cluster BPG2-C were closely related to *Pseudovibrio denitrificans* isolated from sea water in Taiwan (Shieh *et al.*, 2004) and to the *P. denitrificans*-like clone sequences from 11 marine sponges (Enticknap *et al.*, 2006; Hentschel *et al.*, 2006; Taylor *et al.*, 2007) with 98% and 99% sequence identity, respectively, in 16S rRNA gene sequences. Because of potential function of the *P. denitrificans*-like phylotypes in host nitrogen metabolism (Webster and Hill, 2001; Enticknap *et al.*, 2006), these OTUs are a promising target for future exploration of their physiological function in *M. armata*. The last group (BPG3), including six OTUs, comprised potentially new bacterial phylotypes because they branched into new clusters, without immediate phylogenetic neighbors (for example, MA47 and MA43). The remaining OTUs were the nonspecific sequences because of their phylogenetic affiliation with 16S rRNA gene sequences from sea water, sediments and other environments (for example, MB12 and DC31).

Finally, most OTUs of Bacteroidetes, Acidobacteria, Firmicutes, Actinobacteria, Chloroflexi and Crenarchaeota from *M. armata* and *Dysidea* sp. displayed close phylogenetic affiliations with sequences derived from sea water or sediments, instead of from marine sponges (Supplementary Figure 2). None of the cyanobacterial sequences from *M. armata* and *Dysidea* sp. were related to the

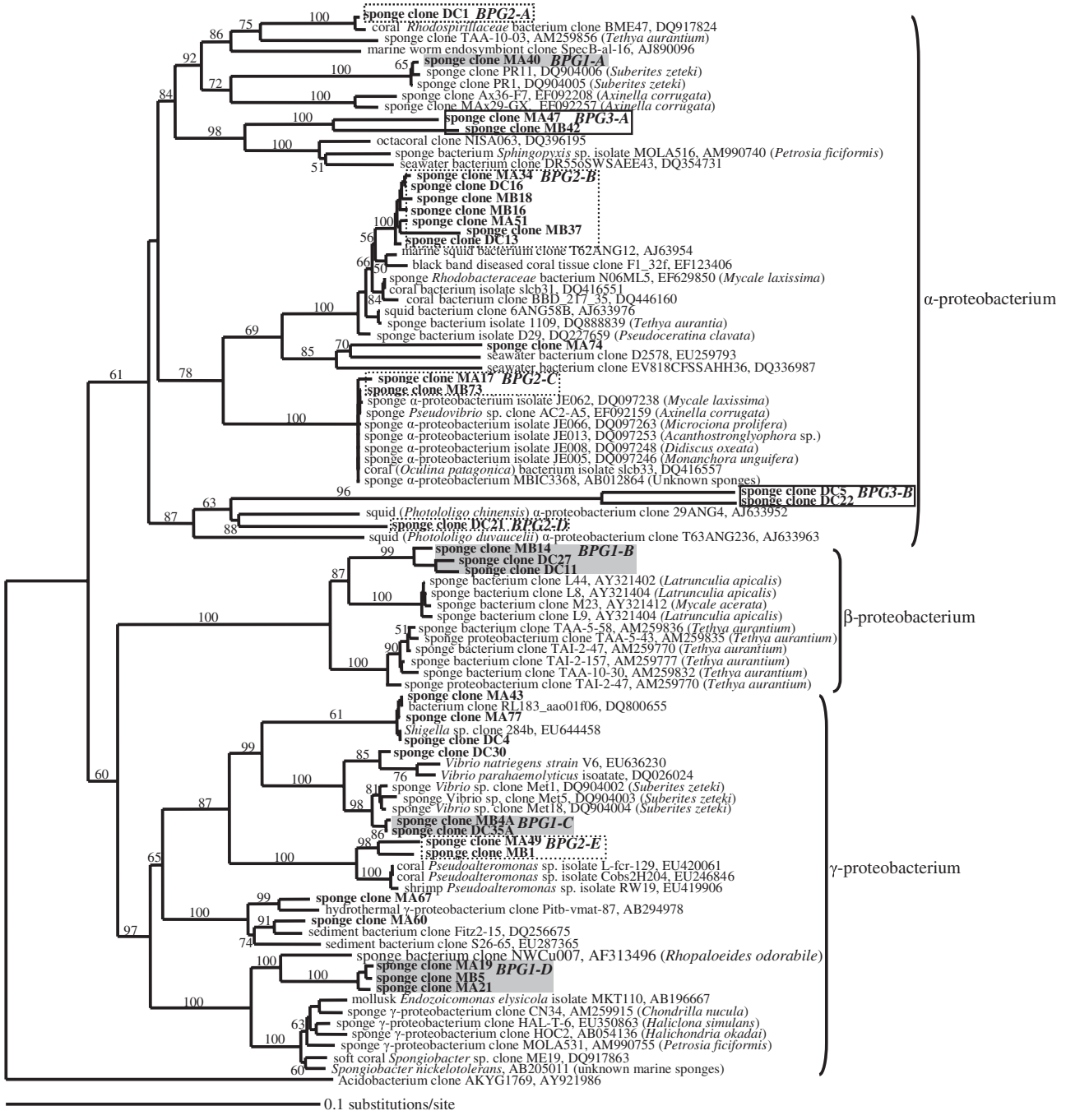

Figure 2 Neighbor-joining phylogenetic tree of proteobacterial 16S rRNA gene sequences derived from the Hawaiian sponges *Mycale armata* and *Dysidea* sp. Clone sequences from the 16S rRNA clone libraries A and B for *M. armata*, library C for Dysidea sp., and library D for sea water are designated with the prefix MA, MB, DC and SW, respectively, and shown in bold. Libraries A, C, and D were constructed from the samples of M. armata, Dysidea sp. and sea water, respectively, were collected in August 2004; library B constructed from the samples of M. armata were collected in May 2005. For matched sequences derived from sponges, the sponge species name is given in parentheses following the sequence accession number. Shaded sequences are OTUs belonging to one of three bacterial phylotype groups (BPG1, BPG2 and BPG3). Numbers above branches indicate bootstrap values of neighbor-joining analysis (>50%) from 1000 replicates.

sponge-specific cyanobacterium *Candidatus Synechococcus spongiarum* (Usher *et al.*, 2004) (Supplementary Figure 3).

Invertebrate endosymbioses have major ecological impacts both on habitat range and on the ecosystems of which they are a part. Symbioses with autotrophic symbionts have allowed several marine species, such as scleractinian corals, pogonophoran and lucinid bivalves, to lead partially or exclusively autotrophic lives in tropical reefs, sulfur-rich habitats and other marine environments (Saffo, 1992). Sponge symbionts are thought to benefit their hosts in many ways (Wang, 2006; Taylor *et al.*, 2007). This study revealed the presence of potentially new

bacterial phylotypes (for example, MA47 and MB42; Figure 2) and sponge–symbiont phylotypes (for example, MA17 and MB73; Figure 2) in sponge *M. armata*. It represents the first report of microbial communities of this invasive marine species. As microbial symbionts have been shown to play an essential role in the invasiveness of their terrestrial hosts (Clay *et al.*, 2005; Lafay and Burdon, 2006; Parker *et al.*, 2007), further study of these OTUs may lead to the understanding of the invasive nature of *M. armata*.

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