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SHORT COMMUNICATION

Inter- and intra-habitat bacterial diversity associated with cold-water corals

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The discovery of large ecosystems of cold-water corals (CWC), stretching along continental margins in depths of hundreds to thousands of meters, has raised many questions regarding their ecology, biodiversity and relevance as deep-sea hard-ground habitat. This study represents the first investigation that explicitly targets bacterial diversity from distinct microbial habitats associated with the cosmopolitan reef-building coral *Lophelia pertusa*, and also compares natural (fjord) and controlled (aquarium) conditions. Coral skeleton surface, coral mucus, ambient seawater and reef sediments clearly showed habitat-specific differences in community structure and operational taxonomic unit (OTU) number. Especially in the natural environment, bacterial communities associated with coral-generated habitats were significantly more diverse than those present in the surrounding, non-coral habitats, or those in artificial coral living conditions (fjord vs aquarium). These findings strongly indicate characteristic coral–microbe associations and, furthermore, suggest that the variety of coral-generated habitats within reef systems promotes microbial diversity in the deep ocean.

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Introduction

Cold-water coral (CWC) reefs have become increasingly recognized for their potential to locally enhance faunal biodiversity in the deep ocean (Freiwald *et al.*, 2004; Roberts *et al.*, 2006). Microbial diversity and function within these ecosystems, have so far been only poorly understood. As evidenced by earlier investigations on warm-water coral (WWC) reefs, the coral holobiont (host animal plus all associated microorganisms) is a complex system containing diverse, abundant and active microbial representatives of all three domains of life (Rohwer *et al.*, 2002; Wegley *et al.*, 2004; Wild *et al.*, 2004; Bourne and Munn, 2005). Several microbial habitats and respective niches, such as those directly generated by the coral (skeleton, tissue and mucus), but also ambient seawater and reef sediments, are known to be available for WWC reef-associated microorganisms (see Rosenberg *et al.*, 2007, and references therein).

Recent studies on microbial communities in CWC reefs have also provided initial indications of coral–microbe associations. While Yakimov *et al.* (2006) showed that living specimens of the stony CWC *Lophelia pertusa* in the Mediterranean harbor, specific bacterial communities that are different from those of dead coral material or sediments, another investigation even showed the spatial stability of CWC–microbe associations with an Antarctic soft coral across an environmental impact gradient (Webster and Bourne, 2007). Characteristic bacterial assemblages were also found in an Alaskan octocoral, but exhibited minimal influence of transient water-column microbes (Penn *et al.*, 2006).

This work contributes the first high-resolution molecular fingerprinting analyses of bacterial communities associated with the cosmopolitan reef-building CWC, *Lophelia pertusa* (L., 1758). Using the Automated Ribosomal Intergenic Spacer Analysis (ARISA, which targets the 16S–23S rRNA-gene spacer length polymorphism) and multivariate

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statistics, this study examines the relationships between bacterial community structure and distinct microbial habitats in a CWC, such as skeleton surface and mucus, associated with living coral specimens from both natural (fjord) and controlled (aquarium) environments. We tested the null hypothesis that the microbial diversity of CWC-generated habitats reflects that of the ambient seawater or of proximal reef sediments, and therefore contains no 'coral-specific' bacterial signatures.

Materials and methods

A total of 12 coral fragments (5–12 cm in length) derived from three living colonies (white phenotype) from the Langenuen fjord (near Bergen, Norway) were collected in December 2006 at 59°56′5″N, 05°28′5″E, 167 m water depth, by the remotely operated vehicle *Aglantha* (Institute for Marine Research, Bergen, Norway). Seawater (2 × 2 l) was sampled nearby at 250 m depth with 5l-Niskin bottles mounted on a conductivity-temperature-depth rosette sampler and reef associated surface sediments (0–5 cm depth) were collected at 175 m depth using a Van–Veen grab (59°52′9″N, 05°31′5″E) in October 2006.

The aquarium samples used for comparative analyses comprised 12 living coral fragments (5–12 cm in length) and seawater (2 × 1 l), which were obtained from a flow-through aquarium with fjord bottom-water retrieved from about 100 m water depth off Bergen (University of Bergen, Norway; located about 30 km from the Langenuen fjord) in October 2006. The aquarium corals originated from three coral colonies (white phenotype) collected earlier in the Langenuen fjord (59°52′9″N, 05°31′5″E) at 120 m depth in July 2006, and further maintained under conditions adjusted to their natural environment (Wild *et al.*, 2008).

For sub-sampling, all selected coral fragments were maintained in *in-situ* water for not more than 30 min before thorough rinsing with sterile, 0.2-μm-filtered seawater. Freshly produced mucus (up to 0.3 ml per fragment) was collected directly from polyps using sterile syringes after induction of mucus excretion through air exposure. Skeleton surfaces (partially covered by coenenchyme; Beuck *et al.*, 2007) were then sampled using sterile scalpel blades by scraping two distinct, 1-cm^2 patches per coral fragment. Scraping was carried out both on the corallite directly surrounding a living polyp and on the skeletal part most distant to all polyps present, yielding a mixture of surface plaques and calcareous particles. Skeleton surface scrapings and sediments (1 g per DNA extraction) were directly used for DNA extraction, whereas mucus and seawater samples were first concentrated onto sterile 0.2 μm polycarbonate filters (Millipore, Eschborn, Germany). Immediate processing of samples was carried out at all steps to minimize biases that may be introduced during retrieval and maintenance of corals during sampling.

Total community DNA was extracted and purified with Ultra Clean Soil DNA kits (MoBio, Carlsbad, CA, USA). Bacteria-specific ARISA (in triplicate PCR) using normalized DNA quantities of 22 ng per reaction for all samples, subsequent data formatting and binning were carried out as described elsewhere (Cardinale *et al.*, 2004; Hewson and Fuhrman, 2006). Non-metric multidimensional scaling (NMDS) and the following statistical tests (Bonferroni-corrected) were implemented in *R* (version 2.5.0). Between-group variation was tested by pairwise analysis of similarities (ANOSIM). Within-group variation (scatter in the NMDS ordination plot) was compared by dispersion analysis, that is, by evaluating whether the difference between sample location and the group centroid was significant, using pairwise Wilcoxon–Mann–Whitney tests after ensuring that an overall Kruskal–Wallis test was significant at $P<0.05$. Operational taxonomic unit (OTU) numbers were compared by pairwise Wilcoxon–Mann–Whitney tests. Redundancy analyses (RDA) and variation partitioning were carried out as described elsewhere (Ramette, 2007). ARISA and statistical analyses were carried out twice independently. As consistent conclusions were obtained twice, only one set of results is presented here.

Results and discussion

Molecular fingerprinting of bacterial communities from samples of coral skeleton surface, coral mucus, ambient seawater and reef sediments retrieved freshly from the field site (hereafter named 'fjord samples') clearly showed significant differences in

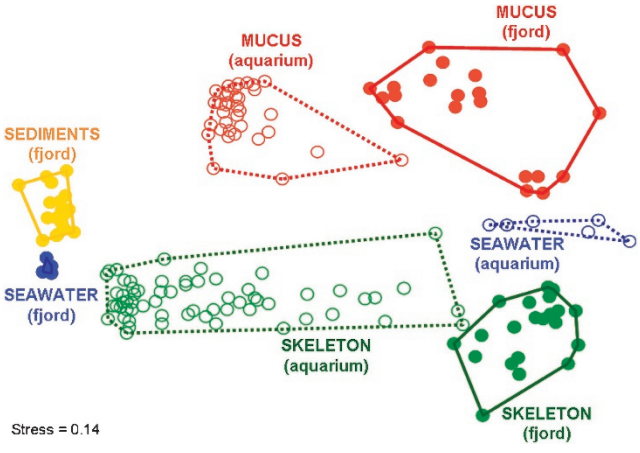

Figure 1 NMDS ordination plot (Bray–Curtis distance matrix) of ARISA profiles for coral-derived samples. ARISA was carried out on samples from coral skeleton surface, coral mucus, seawater, and sediments from natural (fjord) and controlled (aquarium) conditions. The proximity between samples in the plot corresponds to high-community similarity, and the quality of the ordination is indicated by a low-stress value.

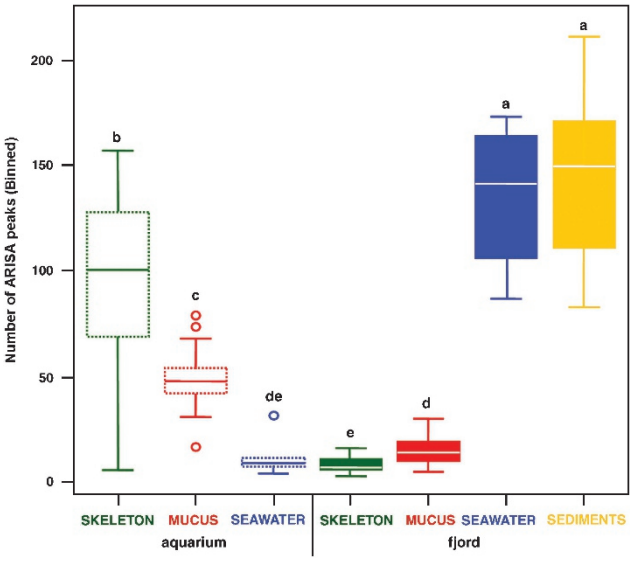

Figure 2 Bacterial OTU number as obtained by ARISA for coral-associated and environmental samples from both natural (fjord) and controlled (aquarium) conditions. The color coding for the different habitats corresponds to that used in Figure 1. The middle line in each box depicts the median of the respective data set. The box width represents 50% of the data, while both whiskers and outliers indicate the distribution of remaining data points, thus representing the overall variation. Different letters above each box denote a significant mean difference in OTU number between respective habitats (Wilcoxon–Mann–Whitney test, $P<0.05$).

community structure and sample dispersion (Figure 1), as well as in OTU number (Figure 2).

Contrary to our null hypothesis, CWC-associated microbial habitats (that is, skeleton surface and mucus, sampled from three coral colonies from one location) clearly exhibited specific bacterial signatures when compared with the surrounding habitats (that is, seawater and sediments; Figure 1; pairwise ANOSIM tests, all $P<0.001$). Bacterial communities from skeleton surface and mucus were even found to be significantly distinct from each other, regardless of the coral origin (fjord or aquarium; all $P<0.001$).

It is interesting that all communities associated with coral-generated microbial habitats (that is, skeleton surface and mucus) showed significantly higher sample dispersion than did communities found in the surrounding environments (that is, seawater and sediments; dispersion analysis, all $P<0.001$). The possibility of CWC-associated habitats offering different niches for bacterial communities could be explained by several factors, such as the availability of stable living conditions, distinct physico-chemical quality of the mucus or skeleton-derived surfaces, or the direct supply of nutritional sources such as CWC-derived organic matter. Furthermore, no community overlap between the respective habitats under different living conditions (fjord vs aquarium) was observed (Figure 1). Whether this indicates abiotic or biotic environmental effects on bacterial communities (that is, through the coral host) is not yet known, but similar findings have been obtained in a study comparing WWC mucus-associated bacteria from reef and aquarium environments (Kooperman *et al.*, 2007).

The OTU numbers differed substantially between the various habitats (Figure 2). In the aquarium, bacterial communities from skeleton surface and mucus yielded significantly higher OTU numbers (medians of 100 and 48, respectively) and greater sample variation than communities from ambient seawater (medians of 9; pairwise Wilcoxon–Mann–Whitney tests, all $P<0.001$). The fjord skeleton surface and mucus, in contrast, showed fewer OTUs (medians of 7 and 14, respectively) and were associated with low variation, whereas samples retrieved from fjord seawater and sediments showed much higher OTU numbers (medians of 142 and 150, respectively) and higher variability (Figure 2).

For the fjord samples, the lower OTU numbers (Figure 2), but higher dispersion within coral-generated habitats (Figure 1), as compared with ambient habitats, seem to be rather counter-intuitive. It may be concluded that coral-generated habitats under natural conditions are associated with rather OTU-poor, but specific, bacterial communities. The selection of such bacterial associates could be induced by nutritional coral–microbe relationships, as coral exudates are known to attract specific, yet diverse populations of organo-heterotrophs (Ritchie and Smith, 2004; Allers *et al.*, 2008), or by specific chemical mediation by the coral host (Kelman *et al.*, 2006; Ritchie, 2006). Our findings are consistent with results from other studies that found specific associations between bacteria and their coral hosts (Rohwer *et al.*, 2002; Bourne and Munn, 2005; Penn *et al.*, 2006; Yakimov *et al.*, 2006; Webster and Bourne, 2007). This indicates that a clear distinction in the bacterial community structure may exist between the different microbial habitats associated with scleractinian CWCs, such as *Lophelia pertusa*.

The comparison between living conditions (fjord vs aquarium) and habitat type (coral-generated vs ambient) as diversity-generating factors showed that each factor contributed 9% to the total ARISA variation (variation partitioning; both $P<0.001$), but showed only 1% of co-variation (that is, the effects of the two factors were not confounded with each other). A large amount of community variation remained unexplained, suggesting that other factors may be likely to be at play. As the variation because of living conditions was of the same amplitude as that caused by habitat types, keeping coral hosts even under the best-controlled conditions may seriously bias microbial diversity analyses. This has important practical implications for future work with coral-associated microbial communities. In conclusion, our data strongly suggest that the variety of coral-generated microbial habitats associated with CWCs promotes microbial diversity in the deep ocean.

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