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Coral mucus bacteria as a source for antibacterial activity

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Abstract. In the oligotrophic marine environment there are ecological niches rich in nutrients and diverse in bacterial populations. One such niche is the coral surface mucus layer. Interactions amongst microorganisms found in coral mucus may be symbiotic or competitive; competing over space and food. It has been hypothesized that the microbial communities found on the coral surface may play a role in the coral's defense, possibly through the production of antimicrobial substances. To find potentially active compounds produced by coral-mucus bacteria, over 200 selected microorganisms isolated from the mucus layer of a number of coral species were grown using agar plating technique. Screening for antibacterial substances was performed using overlay and drop techniques, and antibacterial activity was tested against indicator microorganisms. Results indicate that more than 20% of cultivable mucus-associated bacteria originating from hard corals demonstrated antibacterial activity. Isolates obtained from hard corals demonstrated higher percentage of activity than soft corals' isolates. Isolates related to the genera *Vibrio* and *Pseudoalteromonas* demonstrated high activity against both gram-positive and gram-negative bacteria. These results demonstrate the existence of microorganisms with antibacterial activity on the coral surface, indicating that they may play a role in protecting the coral host against pathogens.

Key words: Antibacterial activity, Coral mucus, Mucus-associated bacteria.

Introduction

The coral mucus layer serves as an ecological niche rich in nutrients and diverse in bacterial populations. However, the exact role of these microorganisms in the coral holobiont is yet to be determined. Such interactions may be symbiotic or competitive; competing over space and food. Rohwer et al. (2002) hypothesized that the microbial community found on a coral's surface may play a role in the coral's defense mechanism, possibly by occupying niches or through the production of antimicrobial metabolites.

Various studies have reported antimicrobial activity of extracts from marine organisms such as sponges (Kelman et al. 2001), soft corals (Harder et al. 2003, Kelman et al. 1998) and scleractinian corals (Geffen and Rosenberg 2005, Koh 1997). Kelman et al. (2006) reported that the majority of extracts from six soft corals demonstrated high antimicrobial activity against marine bacteria isolated from surrounding seawater, as opposed to extracts from six stony corals exhibiting little or no antimicrobial activity. Ritchie (2006) found that 20% of cultured bacteria from the mucus layer of the coral *Acropora palmata* displayed antimicrobial activity, and that a novel mucus-mediated medium was found to be selective for isolates that produce antibiotics. Thus, the presence of microbial populations on the mucus surface of various invertebrates may play a part in their defense strategy.

Material and Methods

Samples were collected from coral mucus layer, as well as from sea water and sediment around the corals, from the Gulf of Eilat. Among coral samples collected were seven hard corals (*Platygyra sp.*, *Porites sp.*, *Fungia granulosa*, *Favia sp.*, *Stylophora sp., Pocillopora sp.* and *Turbinaria sp.*) and two soft corals (*Rhytisma fulvum fulvum* and *Xenia sp.*). Mucus samples were collected from apparently healthy corals from the upper portion of the coral colony or polyp, and the collection procedure was carried out as described previously by Barneah et al. (2007). Serial dilutions were performed using autoclaved artificial sea water followed by spreading over marine agar plates at 50% and 10% concentrations.

Isolates were screened for antibacterial activity using two techniques: overlay with soft agar and drop technique. Screening was performed against common indicator bacteria, including *Bacillus cereus*, *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus*, as well as the marine pathogen *Vibrio coralliilyticus*. The first method is a modification of the overlay technique described by Geis et al. (1983), and involved pouring soft agar mixed with indicator bacteria over marine agar plates containing 5-week old colonies of marine isolates from coral mucus. For this method, only *B. cereus*

and *E. coli* served as indicator bacteria. Antibacterial activity was defined by measuring the radius of the inhibition zone of the growth of indicator bacteria around the colony after 12 hours of incubation. The drop technique involved incubation of marine isolates in 100% marine broth for 72 hours at 26°C with agitation (120 rpm). After sufficient growth, 10 µl of the marine isolates were placed on agar plates containing 120 µl of an overnight culture of indicator bacteria spread as a lawn, followed by incubation for 12 hours at the optimal temperature for that indicator. Isolates obtained from sea water and sediment were used as negative controls for antibacterial screening, using the drop technique. Supernatant, produced by filtration of marine isolate cultures through sterile 0.2 µm filters, was also tested for antibacterial activity using this technique. Activity was detected by the formation of an inhibition zone where the drop was placed, and recorded qualitatively based on degree of transparency.

Isolates' DNA was extracted and 16S rRNA gene fragments were PCR amplified using 8f and 1512r primers. Sequences were then compared with the GeneBank database (NCBI Blast), and a Phylogenetic analysis was performed using MEGA 3.1 software.

Results

Over 20% of cultivable bacteria isolated from mucus demonstrated activity against indicator bacteria. Seventy eight isolates originating from hard corals were screened for antibacterial activity using the overlay technique. Nineteen of these isolates (24.3%) demonstrated activity against indicator bacteria. The percentage of active isolates from each coral species, as determined using the overlay technique, is presented in Fig. 1. The highest percentage of active isolates originated from *Pocillopora sp.* and *Platygyra sp.* (44% and 38%, respectively).

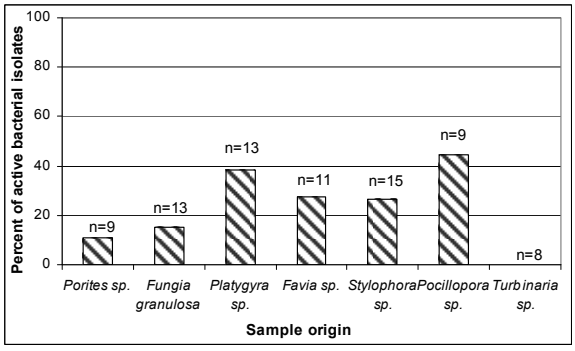

Figure 1: Percentage of active isolates obtained from hard corals. Antibacterial activity was detected using the overlay technique as described in Materials and Methods. n = number of screened bacterial isolates.

146 isolates from soft and hard corals, as well as sea water and sediment, were screened for antibacterial activity using the drop technique. Out of 84 isolates originating exclusively from hard corals, 21 (25%) demonstrated antibacterial activity. However, among 22 isolates obtained from soft corals, only 3 (13.6%) were found to be active. Sediment isolates did not show any antibacterial activity, while only one out of 14 (7%) sea water isolates was found to be active (see Fig. 2). The highest percentage of active isolates originated from *Favia sp.* (42%).

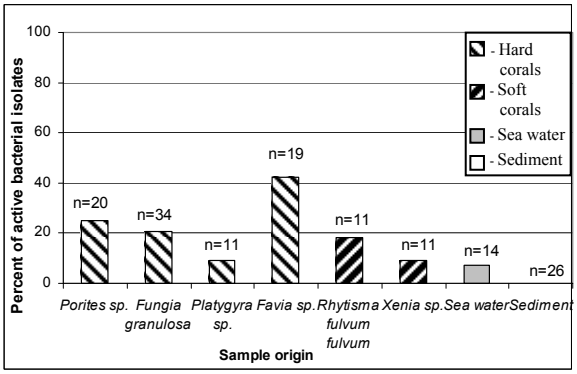

Figure 2: Percentage of active isolates obtained from hard and soft corals, sea water and sediment. Antibacterial activity was detected using the overlay technique as described in Materials and Methods. n = number of screened bacterial isolates.

16S rRNA genes of 46 isolates were sequenced and compared to the NCBI Genebank database. Thirty-eight out of these isolates demonstrated antibacterial activity using either of the screening techniques. Most of the isolates belonged to the *γ-proteobacteria* class (78%), while the rest belonged to the *Firmicutes* (15%) and *Actinomycetales* (7%) phyla. Amongst the *proteobacteria*, isolates related to the genera *Vibrio* and *Pseudoalteromonas* were the most abundant and demonstrated high activity against both gram-positive and gram-negative bacteria.

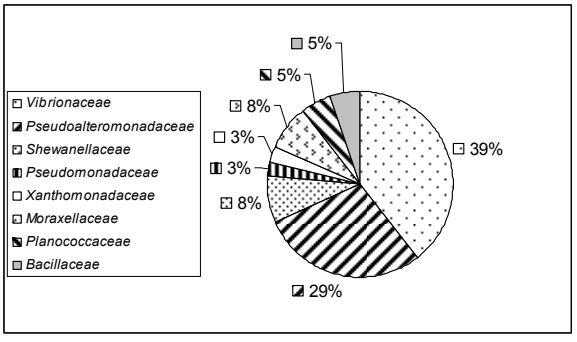

Figure 3: Percent of active isolates from total active isolates sequenced, distributed to each family taxa.

Isolates related to the genera *Shewanella* and *Acinetobacter* only demonstrated activity against gram-positive indicator bacteria. Gram-positive isolates (*Bacillus*, *Planomicrobium*) demonstrated

lower activity, primarily against other gram-positive bacteria. The distribution of active isolates sequenced according to family taxa is presented in Fig. 3.

Discussion

This study tested for antibacterial activity among cultivable coral mucus-associated bacteria and demonstrated that 20-25% of coral mucus cultivable bacteria display antibacterial activity.

Over 230 bacterial isolates obtained from the marine environment of the Gulf of Eilat were screened for antibacterial activity. In general, coral mucus-associated bacteria demonstrated a higher percentage of activity than sea water or sediment isolates. Antibacterial activity detected in isolates originating from hard corals was higher than activity demonstrated by soft coral isolates (25% and 13.64%, respectively). Sea water and sediment isolates demonstrated little or no antibacterial activity.

These results are in agreement with the findings presented by Ritchie (2006), according to which 20% of cultivable bacteria obtained from the mucus layer of the coral *Acropora palmata* demonstrated antimicrobial activity against indicator bacteria. According to Kelman et al. (2006), soft coral extracts were found to be more active than hard coral extracts. The authors also suggested that there are different mechanisms of antimicrobial activity against pathogens involved in scleractinian corals. This also may suggest that production and secretion of antimicrobial compounds by mucus-associated bacteria is part of the scleractinian coral's defense strategy against pathogens (Rohwer et al. 2002; Ritchie 2006; Reshef et al. 2006).

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