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Compositional shifts in bacterial communities associated with the coral *Palythoa caribaeorum* due to anthropogenic effects

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ABSTRACT

Corals harbor abundant and diverse prokaryotic communities that may be strongly influenced by human activities, which in turn compromise the normal functioning of coral species and predispose them to opportunistic infections. In this study, we investigated the effect of sewage dumping on the bacterial communities associated with the soft coral *Palythoa caribaeorum* at two sites in the Brazilian coast. We observed a dominance of bacterial species classified as human pathogens at sites exposed to untreated sewage discharge. The microbial diversity of undisturbed sites was more homogeneous and diverse and showed greater abundance. In addition, bacterial communities differed substantially between the exposed and undisturbed areas. The microbial community associated with the samples collected from the exposed sites revealed the anthropogenic effect caused by organic matter from untreated sewage dumping, with an abundance of pathogenic bacterial species.

1. Introduction

Corals of the Zoantharia order is particularly common in tropical and subtropical regions, where they constitute the most abundant benthic component of coral reefs. They belong to the class Anthozoa and subclass Hexacorallia and are sessile marine cnidarians. Because most species are colonial and lack skeleton, the taxon is often considered as an intermediate between hard corals (Scleractinia) and sea anemones (Actiniaria) (Irei et al., 2015).

The microbial species biodiversity in corals and sponges involved in symbiosis is very similar; thousands of prokaryotic operational taxonomic units (OTUs) and thousands of viral types have already been described (Li et al., 2014). Advances in techniques and methodologies have provided a great insight into taxonomic inventory surveys, thus enabling us to determine host specificity between bacterial species and coral hosts (Sunagawa et al., 2010). However, there is a lack of knowledge about how variations in temporal, spatial, and mainly, environmental conditions and different coral species could affect prokaryote–host relationship. There are several possible roles of prokaryotes in coral and sponges holobionts (host and its associated microbiota), including immunology (Work and Aeby, 2014), host resilience (Raina et al., 2013), host health and disease resistance (Krediet et al., 2013a; Krediet et al., 2013b), nutrient uptake and cycling (Ceh et al., 2013; Lema et al., 2012; Lesser et al., 2004; Yang et al., 2013), and pathogenesis and disease. Several articles have been published focusing on coral disease syndromes after the most prominent cause of global reef decline was related to pathogens (Pratte, 2013; Richardson, 2012; Rosenberg et al., 2007b).

Several studies report a close association between microorganisms and zoanthid hosts. The role of zoanthid-associated bacteria in nitrogen cycling and their nitrogenase activity have already been described. Nitrogen-fixing bacteria belonging to Vibrionaceae were previously isolated from zoanthids (Shieh and Lin, 1992); in addition, cyanobacteria, Rhizobiales, *Rhodobium* sp., and some other bacterial groups involved in the steps of nitrogen metabolism such as ammonia oxidation (*Nitrospira* sp. and *Nitrosococcus* sp.) were recovered from zoanthids. The dinoflagellate *Symbiodinium* has also been found to be associated with Zoanthidea; this microorganism provides an energy reserve by fixing carbon for the host. Similarly, the isolation of hemolytic bacteria producing palytoxin was described; this suggests the participation of this microorganism in host chemical defense. Diverse actinobacterial genera have already been described to be associated with zoanthids. Considering that many of them are antibiotic producers, they produce bioactive compounds that protect the zoanthid host against pathogens (Sun et al., 2014). Some independent cultivation approaches strongly suggest that the two most dominant bacterial phyla associated with zoanthid

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Palythoa samples are Acidobacteria and Proteobacteria (O'Connor-Sánchez et al., 2014).

Long-term monitoring suggests that globally declining coral cover is mainly caused by overfishing, climate change, and disturbances due to river runoff enriched with nutrients and sediments (D'Angelo and Wiedenmann, 2014). In coastal waters, sewage discharge is an important pollution source that can cause elevated concentrations of nutrients (Reopanichkul et al., 2009). This in turn favors nutrient-limited, r-selected, and potentially pathogenic taxa, facilitating their establishment in coral ecosystems (Zaneveld et al., 2016). In addition, sewage introduces many microbial taxa that link human pathogens and the coral bacterial community (Closek et al., 2014). Climatic and anthropogenic stressors lead to shifts in the composition of the bacterial community of coral reefs, thus compromising normal functioning of coral species and predisposing them to opportunistic infection (Bally and Garrabou, 2007; Harvell et al., 1999; Harvell et al., 2002; Lesser, 2007).

Zoanthids often dominate sites where stress conditions lead to a decrease in scleractinian corals and may occupy >50% of the reef surface area. *Palythoa caribaeorum* is one such zoanthid and a key species in the functioning of coral reefs in northeastern Brazil (Francini-Filho et al., 2013), where it frequently dominates disturbed reefs because of its physiological tolerance, high reproductive rates (Silva et al., 2015), and production of mucus that protects it against desiccation (Denny, 1989). Information regarding the composition of the bacterial community can help to monitor the health of the host as it tends to change before noticeable symptoms appear in the host colonies. The present study aims to assess the effects of direct sewage discharge on the bacterial community associated with the zoanthid *P. caribaeorum* by pyrosequencing the partial 16S rRNA gene in specimens collected from two contrasting reef sites.

2. Materials and methods

2.1. Sample collection

Samples of *P. caribaeorum* were obtained during the summer (March 2015) from two locations in the state of Alagoas, northeast Brazil: Ponta Verde coral reef (9°66′32″S 35°69′82″W), located in an urban area and constantly exposed to untreated sewage dumping from illegal connections to storm sewers, and Sereia sandstone reef (9°56′52″S 35°64′49″W), an area less exposed to effluent (Fig. 1). At each location, fragments of approximately 10 cm^2 from three different and apparently healthy colonies of *P. caribaeorum* were sampled, placed in sterile plastic bags, stored on ice during transport to the laboratory, and frozen at −20 °C until DNA extraction was performed. All specimens were collected in the morning during low tide from water-filled pools. Three physicochemical parameters (pH, temperature, and salinity) were measured using a multiparameter probe (model Hanna HI 9828), and information about bathing conditions based on thermotolerant coliform counting was obtained from Instituto do Meio Ambiente, Brazil. Permission for this study was obtained from the regulatory institution Instituto Brasileiro do Meio Ambiente (license no. L. 32723-1).

2.2. DNA extraction

All samples were washed with sterile seawater prior to DNA extraction to remove loosely attached bacteria from the surface of the sample. The samples were frozen in liquid nitrogen and grinded using pestle and mortar. Next, 600 μl of CTAB extraction buffer (4 g CTAB, 16.4 g NaCl, 20 ml 1 M Tris-HCl, 8 ml 0.5 M EDTA, and 200 ml distilled water) was added to the pellets followed by vortex. After incubation at 65 °C for 1 h, the tubes were centrifuged at 14,000 rpm for 10 min. The supernatants were then transferred to tubes on ice and extracted with an equal volume of phenol/chloroform. Genomic DNA was precipitated with 0.3 M sodium acetate (pH 5.2) and isopropanol and collected by centrifugation at 14,000 rpm for 15 min. DNA was resuspended in Milli-Q ultrapure water and stored at −20 °C.

2.3. Purification and quantitation

After extraction, the genomic DNA was purified using the PowerClean® DNA Clean-Up Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. Purity and concentration of the extracted DNA were verified using the L-Quant 2 spectrophotometer (Loccus do Brasil Ltda.; wavelength: 230, 260, and 280 nm). Extracted genomic DNA was sent to Molecular Research DNA Laboratory (Shallowater, TX, USA) for pyrosequencing.

2.4. PCR amplicon library preparation for pyrosequencing

Bacterial 16S rRNA gene was amplified using primers 27F (3′-AGAGTTTGATCMTGGCTCAG-5′) and 1492R (5′-TACCTTGTTACGACTT-3′). After the initial 25 cycles, another 5 cycles were performed so that each sample's barcode sequences and A and B adapters were included. After the PCR, all the amplicons produced from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Amplicon libraries were bound to the beads under favorable conditions with only one fragment per bead, and the beads were emulsified in a PCR mixture in oil. The amplicons were sequenced using the GS FLX titanium Roche platform (454 pyrosequencing technology) and reagents according to the manufacturer's instructions.

2.5. 16S rRNA amplicon sequence analysis

The sequences obtained were analyzed according to the UPARSE + Qiime protocol proposed by the Brazilian Microbiome Project (Pylro et al., 2014), with some modifications. Briefly, reads were assigned to respective samples according to their barcodes, and primer sequences were removed in the demultiplex step. The sequences were filtered according to quality (maximum error expected = 1.0) and trimmed to 250 bp. No ambiguous bases were included. Sequences were dereplicated and simultaneously checked for chimeras using a *de novo* approach and later checked again with UCHIME (Edgar et al., 2011) against 16S "Gold" database (reference database in the Broad Microbiome Utilities). Reads with ≥97% similarity were grouped into OTUs using the USEARCH

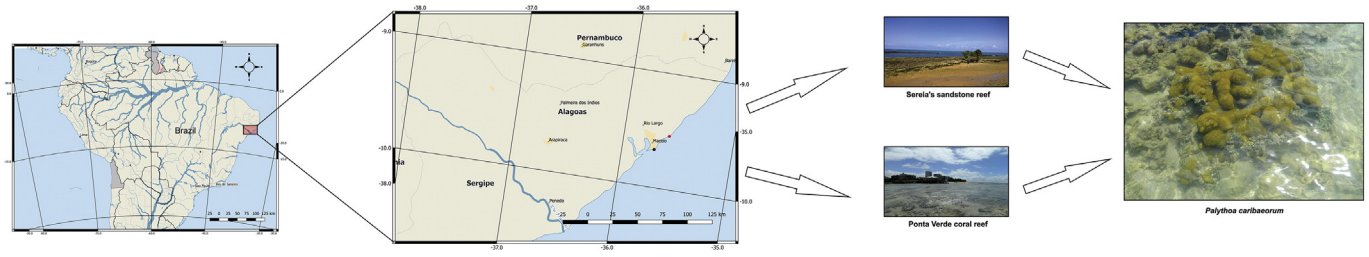

Fig. 1. Locations of the metagenomic sampling sites. Black circle indicates Ponta Verde coral reef and red circle indicates Sereia sandstone reef. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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