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Full Length Research Paper

Bacteria associated with the coral *Echinopora lamellosa* (Esper 1795) in the Indian Ocean - Zanzibar Region

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Infectious diseases are now known to have major effects on the structure and function of coral reef ecosystems throughout the world. The number of recognized coral diseases has increased dramatically. The problem was first recognized in the Caribbean in the early 1970's but has now been reported to affect coral communities worldwide. There is little information regarding bacteria associated with diseased corals in the Indian Ocean. However, one of the most common disease signs observed is a rapid loss of tissue leaving exposed white skeleton in contact with compromised tissue, followed by necrosis. These signs have been referred to as white plague in the Caribbean. Similar signs have been observed in the Indo-Pacific and are referred to as white syndrome. The pathogens associated with these disease signs depend on the species and geographic location of the corals. In the Caribbean, the disease was associated with *Aurantimonas coralicida* and in the Red Sea with *Thalassomonas loyaeana*, both newly described species. During exploratory surveys in the reefs near Zanzibar in the Indian Ocean, mucus samples were collected from healthy and apparently diseased *Echinopora lamellosa* (with signs of white syndrome) colonies. Samples were plated on two solid media: GASW (a nonspecific medium) and TCBS (*Vibrio* selective medium). Growth on TCBS was only found with diseased samples. Culturable isolates were characterized using metabolic profiling. A relatively high prevalence of Class Gamma Proteobacteria was found with diseased samples compared with healthy samples and *Vibrio* species were well represented in diseased samples.

Key words: Disease, coral reef, echinopora, Indian Ocean, white syndrome.

INTRODUCTION

The continual decline of coral reefs throughout the world has been widely documented by scientists since the mid-1990s (Bruckner, 2006; Gochfeld et al., 2006). Coral diseases were first reported in the 1970s (Antonius, 1973) on reefs in the Caribbean and in the Florida Keys and current research strongly suggest lack of recovery of disease-impacted reefs (Garrison et al., 2003).

While it is believed that the Caribbean Region is a "disease hot spot" (Weil et al., 2006), the number of reports on the distribution of coral diseases across the Indo-Pacific is also increasing (Rosenberg et al., 2004; Weil, 2004; Weil et al., 2006; Harvell et al., 2007).

Although ongoing studies provide data on abundance as well as distribution of catastrophic diseases, little is known about environmental factors that promote pathogenic infections of corals (Voss et al., 2006). It is generally believed that climate warming plays an important role in reef degradation (Harvell et al., 1999; Rosenberg et al., 2002; Ainsworth et al., 2007; Harvell et al., 2007; McClanahan et al., 2004).

To date, many coral diseases have been recognized worldwide, but the causative agents for most of them have yet to be identified (Garrison et al., 2003; Weil et al., 2006). Following Koch's postulates, primary pathogens for some of the common coral reef diseases have been described. White plague in Florida is caused by newly described gram-negative bacterium *Aurantimonas coralicida* (Denner et al., 2003), but similar signs are associa-

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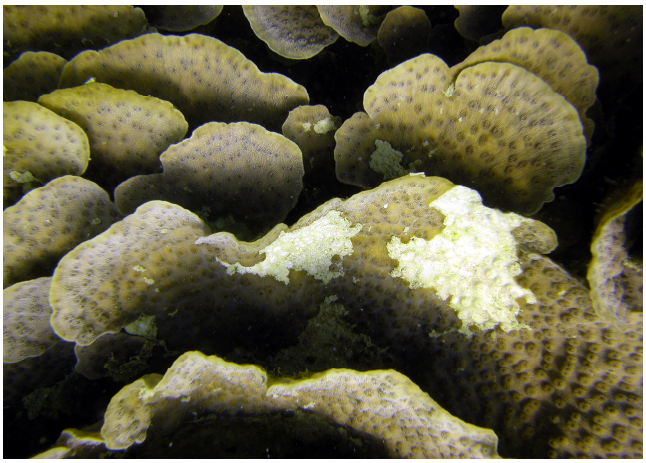

Figure 1. *Echinopora lamellosa* demonstrating white syndrome signs. Indian Ocean near Zanzibar Island.

Table 1. Bacteria taxonomic groups associated with healthy and diseased *Echinopora lamellosa* samples.

***Echinopora lamellosa* samples**		
taxonomic groups	**Diseased**	**Healthy**
Gamma Proteobacteria	86%	64%
Alpha Proteobacteria	5%	1%
Beta Proteobacteria	2%	1%
Actinobacteria	1%	32%
Bacilli	0%	1%

ted with *Thallasomonas loyaeana* in the Indo-Pacific region (Rosenberg et al. 2006). A fecal enteric bacterium in humans - *Serratia marcenscens* is associated with white pox (Patterson et al., 2002), bacterial bleaching with *Vibrio shilonii* (Kushmaro et al., 2001) and sea fans aspergillosis with *Aspergillus sydowi* (Smith et al., 1996; Geiser et al., 1998; Smith et al., 1998; Smith and Weil, 2004). Recently, *Vibrio carchariae* was identified as the putative pathogen of white band type II (Gil-Agudelo et al., 2006). Additionally, it has been widely accepted that black band disease is caused by a bacterial consortium (Richardson et al., 1997; Bythell et al., 2002; Richardson, 2004).

This study provides a preliminary approach to characterize bacteria associated with *Echinopora lamelosa* showing classic signs of white syndrome. The major goal of this project was to compare the bacteria composition in mucus layers from healthy and diseased *Echinopora*.

Diversity of coral-associated bacteria has been studied previously from healthy corals (Rohwer et al., 2001; Rohwer et al., 2002; Frias-Lopez et al., 2002) and those showing disease signs (Pantos et al., 2003).

However, this is the first study regarding bacterial composition of diseases affecting corals in Tanzania-Zanzibar region. From the data obtained in this research, we examined differences in microbial communities between healthy and diseased coral colonies.

MATERIALS AND METHODS

The scleractinian coral *E. lamellosa* (Esper, 1795) (Figure 1) belongs to a family Faviidae and is a very common and widely distributed coral in the Indian Ocean reefs (Veron, 2002).

Mucus samples from healthy and diseased *Echinopora* were collected from colonies in Morogo (6º 11.418 S and 39º 07.926 E) and Aquarious (6º13.574 S and 39º 08.814 E) reefs, 5-7 km to the west of Stone Town (Figure 2), the capital of the Zanzibar Island. These were well developed and complex fringing reefs with a high abundance and diversity of corals. The reefs had a steep slope and went down to 22 m deep ending on a gentle sandy slope or sandy plains.

Samples were collected in April 2006. Sterile syringes were used to collect the mucus from surface mucopolysaccharide layers of healthy and diseased *E. lamellosa*. A syringe was slowly drawn along the healthy and diseased coral tissue surface with steady, gentle suction applied.

Coral mucus was then plated on non-selective GASW media (glycerol artificial seawater agar, Smith and Hayasaka, 1982) and a *Vibrio* selective TCBS (Difco, thiosulphate citrate bile salts sucrose agar), as some *Vibrio* species have been linked to coral diseases (Breitbart et al., 2005; Ben-Haim and Rosenberg, 2003). Plates were incubated at 30ºC to obtain individual colonies. Isolates demonstrating different cell morphologies were replated to produce pure cultures.

Metabolic profiling was used to characterize the surface microbiota. Several Biolog Micro plates (GN/GP) were used to test the ability of each isolate to utilize a preselected panel of 95 different carbon sources. Each isolate was then suspended in prewarmed, sterile ASW (Artificial Sea Water), within the density range specified for later identification. Then, cell suspensions were inoculated into Biolog Plates, 150 ul per well. Sodium Thioglycolate (anti-capsule agent) was added to improve the reactions when the organisms were inoculated. Plates were incubated for 3 days to allow the metabolic pattern to form and were then read on an automated microplate reader. If an adequate match was found, a characterization of the isolate was made using the Micro Log 3 System.

Using aseptic techniques, each bacterial strain was preserved in a 2.0 ml cryogenic vial containing 50% glycerol and 50% GASW for future study to obtain 16S DNA sequences, and stored at -80C.

RESULTS

In all, 578 bacterial strains were isolated from diseased *Echinopora* colonies and 99 from healthy colonies. To compare the microbial communities associated with the mucopolysacharide layer of clinically healthy and diseased (white syndrome) areas in coral colonies, bacterial strains were divided into groups defined primarily in terms of ribosomal RNA. Six major taxonomic groups comprised bacterial communities found in *Echinopora* samples: Alpha Proteobacteria, Beta Proteo-bacteria, Gamma Proteobacteria, Actinobacteria, Bacilli and Flavo-bacteria. Our results demonstrate distinct differrences in the abundance of bacterial groups between mucus from healthy and diseased colonies. The overall comparison of bacterial composition is presented in Table

Figure 2. Map showing geographical location of Zanzibar and the collection sites

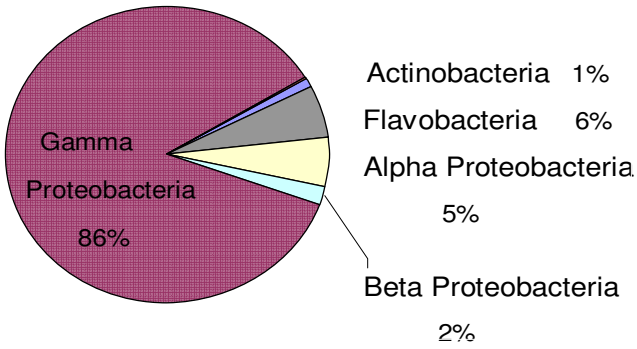

Figure 3a. Bacteria taxonomic groups associated with diseased *Echinopora lamellosa* cultured on GASW media

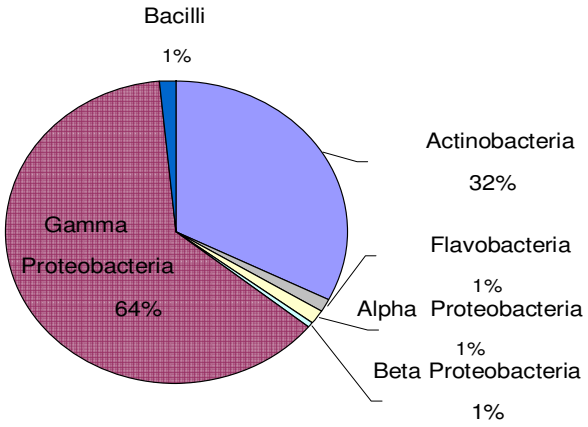

Figure 3b. Bacteria taxonomic groups associated with healthy *Echinopora lamellosa* cultured on GASW media

1 and Figure 3a/ 3b.

Both diseased and healthy coral mucus samples, plated on GASW media had the highest percentage of Gamma Proteobacteria (86% diseased and 64% healthy). It is important to note that although this taxonomic group appears to be dominant in both samples, a substantially higher proportion occurred in diseased coral mucus. Similarly, isolates cultured on TCBS from diseased *Echinopora* mucus was largely composed of Gamma Proteobacteria (99%). In contrast, there were no bacteria colonies isolated from healthy mucus coral samples plated on TCBS.

Additionally, samples from healthy *Echinopora* were distinguished from diseased by a larger composition of Actinobacteria (32%). This metabolic group represented only 1 % in diseased samples.

Taxonomic breakdown into bacterial species was examined in order to provide more detailed information about

Table 2. Dominant species representing healthy and diseased *Echinopora lamellosa* samples (TCBS and GASW).

Dominant Bacteria species	TCBS Diseased	GASW Diseased	GASW Healthy
Vibrio spp.	53%	56%	60%
Aeromonas spp.	34%	25%	29%

bacteria associated with the mucus from healthy and diseased *Echinopora* colonies. Samples obtained from diseased tissues, cultured on GASW were well represented by two species: *Vibrio* spp. - 56% and *Aeromonas* spp. -25%. Similar results were found from diseased coral mucus plated on TCBS: *Vibrio* spp.-53% and *Aeromonas* spp.-34%. Our study shows that the same species appear to be dominant in healthy samples as well: *Vibrio* spp.-60% and *Aeromonas* spp.-29% (Table 2).

Microbial investigation reveled that the only metabolic type observed in all diseased *Echinopora* samples was *V. mediterranei*. Whether this indicates that this species is strongly associated with the coral disease requires further research.

DISCUSSION

Since coral reefs are in significant decline and the degradation produced by new diseases and syndromes continues to increase, there has been a considerable number of different studies performed recently (Weil et al., 2006; Ainsworth et al., 2007; Harvell et al., 2007). While microbial communities associated with coral diseases have been reported previously (Pantos et al., 2003; Breitbart et al., 2005; Sekar et al., 2006), this is the first research to explore mucus bacterial composition of white syndrome affecting corals in Tanzania-Zanzibar.

Although, there have been a few studies on coral reef degradation in this region (Horrill et al., 1994; Johnstone et al., 1998; Obura et al., 2004; Obura, 2005), microbiological studies have not been commonly reported (Harvell et al., 2007). Bacteria-induced bleaching was found in Zanzibar (Rosenberg and Ben-Haim 2002). Black band, white band and yellow band have also been documented in Zanzibar (McClanahan et al., 2004; Harvell et al., 2007). Recently, reef surveys conducted off the coast of Zanzibar revealed white syndrome signs affecting corals species (Weil and Jordan-Dalgreen, 2005). According to Weil and Jordan-Dalgreen (2005), white syndrome signs were found on several *Echinopora* colonies in the reefs of Zanzibar and Mnemba. Additionally, previous research (McClanahan et al., 2004) reports *Echinopora* colonies affected by an outbreak of white syndrome in the Indian Ocean.

The main objective of this study was to characterize and compare bacteria associated with surface mucopolysaccharide layers from healthy *E. lamellosa* and diseased colonies, demonstrating white syndrome signs. This approach to the study revealed that *E. lamellosa* mucus layers were mainly represented by the taxonomic group Gamma Proteobacteria, with its distinctly higher abundance in diseased coral samples. This supports previous research (Ritchie and Lewis, 2005) that also documents higher Gamma Proteobacteria composition in diseased mucus coral samples (*M. capitata)* compared to healthy from regions in Hawaii.

Other investigations have reported Gamma Proteobacteria as a prominent bacteria group in coral samples (Beleneva et al., 2004; Breitbart et al., 2005; Ritchie and Lewis, 2005). It is important to note that members in this class are common important pathogens (*Salmonella*, *Yersinia*, *Vibrio*, *P. aeruginosa)*.

Differences associated with microbial communities between diseased (white-plague like) and non-diseased corals (*Montastraea annularis)* have been previously reported in the Caribbean region (Pantos et al., 2003). According to Pantos et al. (2003) changes observed in coral microbial flora may serve as a bioindicator of environmental stress and disease.

Although an identification of the bacterial strains responsible for white syndrome signs need to be further researched, this preliminary approach strongly indicates that *Vibrio* species play an important role in diseased coral samples from Zanzibar. Luna et al. (2007) also reports *Vibrio* species as distinctly more abundant in diseased compared to healthy corals (rapid tissue necrosis). The microbial community associated with tumors (*P. compressa*) contained more culturable *Vibrio* spp. than the microbes associated with healthy colonies (Breitbart et al., 2005). These results agree with those reported by others (Ritchie and Smith, 1998; Kushmaro et al., 2001; Ben-Hain et al., 2003; Breitbart et al., 2005), where *Vibrio* spp. have been suggested or found to be putative pathogens producing coral diseases.

In our study, *V. mediterranei* was only found in diseased mucus samples. This is very suggestive that *V. mediterranei* might be causing white syndrome signs in *E. lamellosa*, or that it is an opportunistic bacteria. Koch's postulates need to be tested and fulfilled to prove this hypothesis.

The presence of *Vibrio spp*. in the mucopolysacchcaride layers seem to be strongly correlated with diseased coral colonies (Gil-Agudelo et al., 2006). According to Kushmaro et al. (1997) *V. shiloni* is responsible for bacterial bleaching in the Mediterranean Sea. *Vibrio coralliiticus* infects and kills *Pocillopora damicornis* in the Indo-Pacific (Ben-Haim et al., 2003). There is some evidence the Caribbean yellow band disease is caused by *Vibrio* as well (Cervino et al., 2001). In summery, *Vibrio* is considered as one of the most important coral pathogens (Gil-Agudelo et al., 2006).

Additionally, a relatively large percentage of Actinobacteria only in healthy coral samples might be related to the

ability of this group of microorganisms to produce a wide range of antibiotics against other pathogenic bacteria (Caundliffe, 2006).

Our results agree with those presented by Luna et al. (2007) regarding bacteria communities cultured on TCBS from healthy and diseased samples. Luna et al. (2007) demonstrated a distinctly higher bacterial composition on TCBS from diseased coral samples compared to healthy. Based upon the data we obtained, there was also a high abundance of bacteria on TCBS plates from diseased coral colonies, while isolates from healthy coral colonies did not grow on TCBS. As reported by Luna et al. (2007) these isolates were mainly *Vibrio spp.*

Although many questions remain to be answered, our results highlight the need for future study concerning bacteria associated with coral diseases in the Indian Ocean. Integrated research will lead to a better understanding of potential impacts of coral reef diseases and their lack of recovery worldwide. Until causative agents are identified, and the role of different factors affecting coral reefs understood, an effective management approach appears to be very difficult (Harvell et al., 2004).

Conclusions

This research provides new information about microbial populations in coral colonies demonstrating white syndrome signs in Zanzibar Region. The bacteria communities associated with diseased *E. lamellosa* are distinctly different from those associated with healthy. Characterizing and comparing the bacteria composition in healthy and diseased coral samples is an essential step to understand changes associated with coral diseases. Finally, there is a need for additional studies on *V. mediterranei*, as this species may be responsible for white syndrome signs in *E. lamellosa* in Zanzibar Region.

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