



Bacterial Communities Associated With Healthy and Diseased (Skeletal Growth Anomaly) Reef Coral *Acropora cytherea* From Palk Bay, India

Raju Rajasabapathy^{1*}, Kesava Priyan Ramasamy², Balakrishnan Manikandan³, Chellandi Mohandass³ and Rathinam Arthur James^{1*}

¹ Department of Marine Science, Bharathidasan University, Tiruchirappalli, India, ² Department of Marine Systems, Tallinn University of Technology, Tallinn, Estonia, ³ Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, India

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*Correspondence:

Raju Rajasabapathy
sabajuly06@gmail.com
Rathinam Arthur James
james.msbd@gmail.com

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Skeletal growth anomalies (SGA) are easily recognizable disease in reef-forming corals which drain energy from the host and end up with mortality. We collected mucus samples of healthy and SGA affected colonies of *Acropora cytherea* from Palk Bay, the southeast coast of India and investigated the taxonomic composition of mucus-associated bacterial communities using full-length 16S rRNA gene nanopore sequencing. Metagenomic analysis revealed the dominance of Proteobacteria, Firmicutes, and Actinobacteria in both healthy and diseased samples. The proportion of Proteobacteria in diseased samples was almost doubled compared to the healthy one, whereas Acidobacteria (fourth dominant phyla in healthy sample) and Actinobacteria were significantly low in diseased samples. Planctomycetes, Cyanobacteria, and Chloroflexi were some of the other major phyla found in healthy samples which were low in diseased corals. Kruskal-Wallis test indicated high number of putative pathogenic bacteria group viz. *Staphylococcus*, *Salmonella*, *Klebsiella*, and *Escherichia* in diseased samples compared to healthy coral. The corals perhaps have acquired potential pathogens belong to the phylum Proteobacteria during SGA formation.

Keywords: coral reef, *Acropora cytherea*, growth anomalies, bacterial community, nanopore sequencing

INTRODUCTION

Coral diseases become a rising issue over several decades, yet fundamental knowledge of their causes is not known in many cases. Skeletal growth anomalies (SGAs) are a group of coral diseases characterized by reduced colony growth, absence of polyp formation, decreased skeleton density and reduced numbers of zooxanthellae (Cheney, 1975; Bak, 1983) and sources confirm that SGAs are not transmissible (Peters et al., 1986). SGA on coral reefs was first witnessed in *Madrepora kauaiensis* from Hawaii (Squires, 1965) since then it has been reported in several

species of reef-building corals including *Acropora*, *Montipora*, *Platygyra*, *Turbinaria*, and *Porites* (Burns and Takabayashi, 2011; Irikawa et al., 2011; Williams, 2013; Ng et al., 2015; Tavakoli-Kolour et al., 2015; Hussain et al., 2016). Though the etiology of many coral diseases remains unknown, majority of the diseases reported are recognized or suspected to be of microbial origin (Richardson and Aronson, 2002). However, previous studies on reef coral *Acropora cytherea* revealed that the formation of SGA is potentially intensified by environmental stresses such as increased sea temperature (Irikawa et al., 2011). While hitherto no report of microbial community variations from healthy and SGA affected *A. cytherea* are available.

Microorganisms are important diverse component of the coral tissues as well as surface mucus layer and plays a major role in nutrient cycling (Shashar et al., 1994; Ainsworth et al., 2010; Thompson et al., 2014), response to environmental stress (Bourne et al., 2008), coral health (Bourne et al., 2016; Peixoto et al., 2017) and defense against diseases (Reshef et al., 2006; Ritchie, 2006; Rosenberg et al., 2007; Kvennefors et al., 2012). Coral-associated microbial communities are host-specific (Rohwer et al., 2002; Medina, 2011) even if the same coral species from geographically different locations (Rohwer et al., 2001). Yet, many reports revealed that healthy and diseased corals have different microbial associations (Reis et al., 2009; de Castro et al., 2010; Sere et al., 2013). A substantial number of coral diseases i.e., coral bleaching, white-band syndrome, white-plague, white-pox, were demonstrated to have microbial origins (Harvell et al., 2007). In recent years, culture-independent and metagenomics techniques paved way to identify a wide array of microorganisms that are associated with coral surface mucus and tissues of healthy and diseased corals. To date, only few studies are available on microbial community composition from SGA affected reef corals *Platygyra carnosus* (Chiu et al., 2012; Ng et al., 2015). For better understanding the future health of coral reefs, it is important to be aware of the microbial communities that are associated with corals and how they shift with response to a disease or environmental stress.

Palk Bay is a fringing reef, 25–30 km long and 300 m wide, located on the southeast coast of India. The fringing reef runs parallel to land in east to west direction with an average water depth of 9 meters (Ravindran et al., 2012). In Palk Bay region, scleractinian diversity studies showed a reduction in species richness from >60 species (Pillai, 1971) to 34 species in 40 years of time (Venkataraman and Rajan, 2013). The dominant genera recorded along the Palk Bay regions were *Porites* sp., *Favia* sp., *Acropora* sp., *Platygyra* sp., *Goniastrea* sp., *Favites* sp. and *Siderastrea* sp. (Marimuthu et al., 2016). Earlier studies documented various diseases including bleaching, black band, white band, white spot, pink spot, white plaque and yellow band diseases (Thinesh et al., 2011). During a coral survey in the Palk Bay region in 2018, we observed SGA affected colonies of *A. cytherea* for the first time. With the aim of characterizing bacterial community composition, we collected healthy and the SGA affected *A. cytherea* colonies from Palk Bay region. This investigation presents the first comprehensive metagenomic analysis (using nanopore sequencing) on the comparison of bacterial communities

between healthy and SGA affected *A. cytherea* colonies from Palk Bay, southeast coast of India.

MATERIALS AND METHODS

Study Area and Sample Details

Samples were collected from healthy and SGA reef coral *A. cytherea* from Palk Bay region, Tamil Nadu during June 2018 (Figure 1A). The SGA affected colonies are characterized by poorly developed branches with a small portion of the colony showing abnormal skeletal mass (≤ 5 cm diameter) (Figure 1B). Mucus samples were collected from the abnormal portion of the colony using sterile syringes. Approximately 10 cm² area of the tissue was sampled for mucus in both healthy and SGA affected corals. Collected samples were transported to the laboratory in ice and stored at -20°C until DNA extraction.

DNA Extraction and PCR Amplification

Total DNA was extracted from both the mucus samples using a DNA isolation kit (Bogar Biobee) following the manufacturer's protocol. PCR amplification of 16S rRNA gene was performed using consensus primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Lane, 1991). PCR products were purified by using Montage PCR Clean-up kit according to manufacturer's instructions. The quality, quantity (approx. 100 ng/ μl) and formulation of the PCR product were checked using Qubit Fluorometer 3.0.

Sequencing and Bioinformatics Analysis

Library was prepared using 1 μg of the purified DNA template. The library preparation was carried out using NEB End repair/dA-tailing (EXP-PBC001) and ligation sequencing kit (SQK-LSK109) as per the manufacturer's protocol. Libraries were purified using AMPure XP beads and loaded on to the Nanopore MinION SpotON flow cell (R9.4.1). Reads containing raw reads were base called using MinKNOW (Oxford Nanopore Technologies). The raw reads were further quality checked using FastQC Version 0.11.8 (Andrews, 2010). Further, poor quality reads were trimmed using Trimmomatic with default parameters (Bolger et al., 2014). The taxonomic abundance profile of healthy and diseased coral was classified using Kraken classification tool (Wood and Salzberg, 2014) in OmicsBox server¹. The raw sequence data obtained from this study are available from the GenBank BioProject under the accession number PRJNA576005.

Statistical Analysis

Alpha diversity measures (Chao-1, Shannon and Simpson indices) were calculated using EstimateS v9.1.0 (Colwell, 2013) followed by Principal coordinate analysis (PCoA) and Kruskal-Wallis test based on the classified species in healthy and diseased samples (PAST software 3.26). Rarefaction analysis was performed by plotting the number of species against the total number of reads using EcoSim700

¹<https://www.biobam.com/omicsbox/>

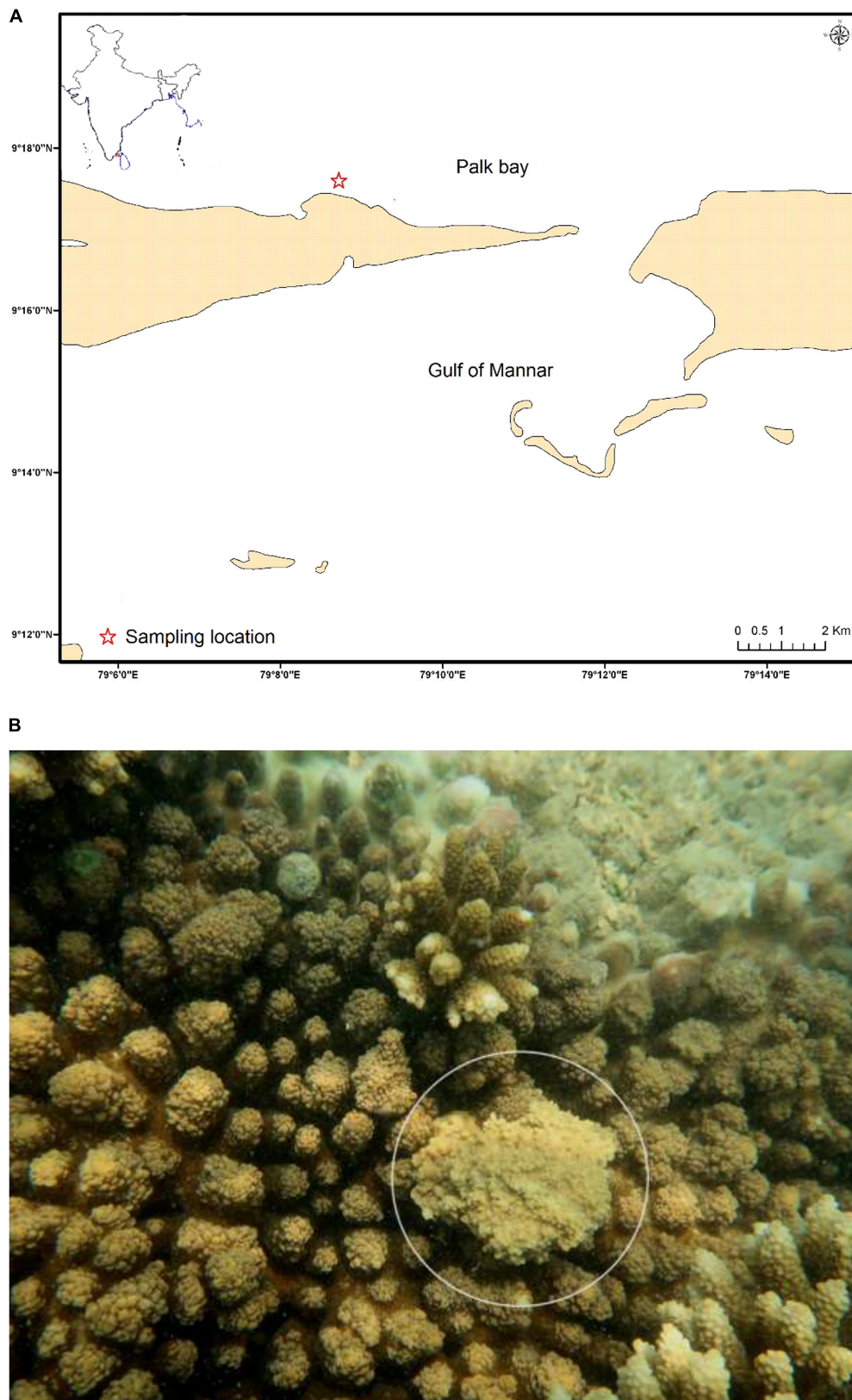


FIGURE 1 | (A) Sampling location of the Palk Bay coral reef ecosystem, Southeast coast of India (Asterisk indicates the sampling site); **(B)** Reef coral *Acropora cytherea* (white circle indicates the SGA).

(Gotelli and Entsminger, 2004). The community composition between healthy and diseased was also tested using non-parametric Wilcoxon rank-sum test, p -value was calculated using GraphPad 8.2.1 Software (GraphPad Int, United States). The significant level was determined as $p < 0.05$.

RESULTS AND DISCUSSION

Skeletal growth anomalies (SGA) is an easily identifiable disease on the basis of irregular or spherical protuberant masses, reduced growth rates and decreased skeletal density (Burns and Takabayashi, 2011) and has been reported in several scleractinian corals, including 11 species of *Acropora* (Irikawa et al., 2011). However, no reports have been recorded in the aspect of microbial contribution or microbial role in the SGA affected

Acropora reef corals. This study provides detailed amplicon metagenomic analysis into the coral mucus associated bacterial communities based on 16S rRNA gene of the healthy and diseased coral *A. cytherea*.

A total of 104652 sequences of bacteria were qualified from the OmicsBox tool in which 34998 sequences for the SGA samples and 69654 sequences for healthy samples of the reef coral *A. cytherea*. Sequences were classified into 38 phyla, 71 classes, and 926 genera for the healthy samples, and 34 phyla, 61 classes, and 697 genera for the diseased samples. Based on our study, the bacterial community associated with the healthy coral was dominated by Proteobacteria (31.6%), followed by Firmicutes (23.6%), Actinobacteria (18.0%), Acidobacteria (6.6%), Chloroflexi (5.6%), Bacteroidetes (5.1%), and Planctomycetes (2.1%). SGA affected corals exhibited high diversity of Proteobacteria (57.3%), followed

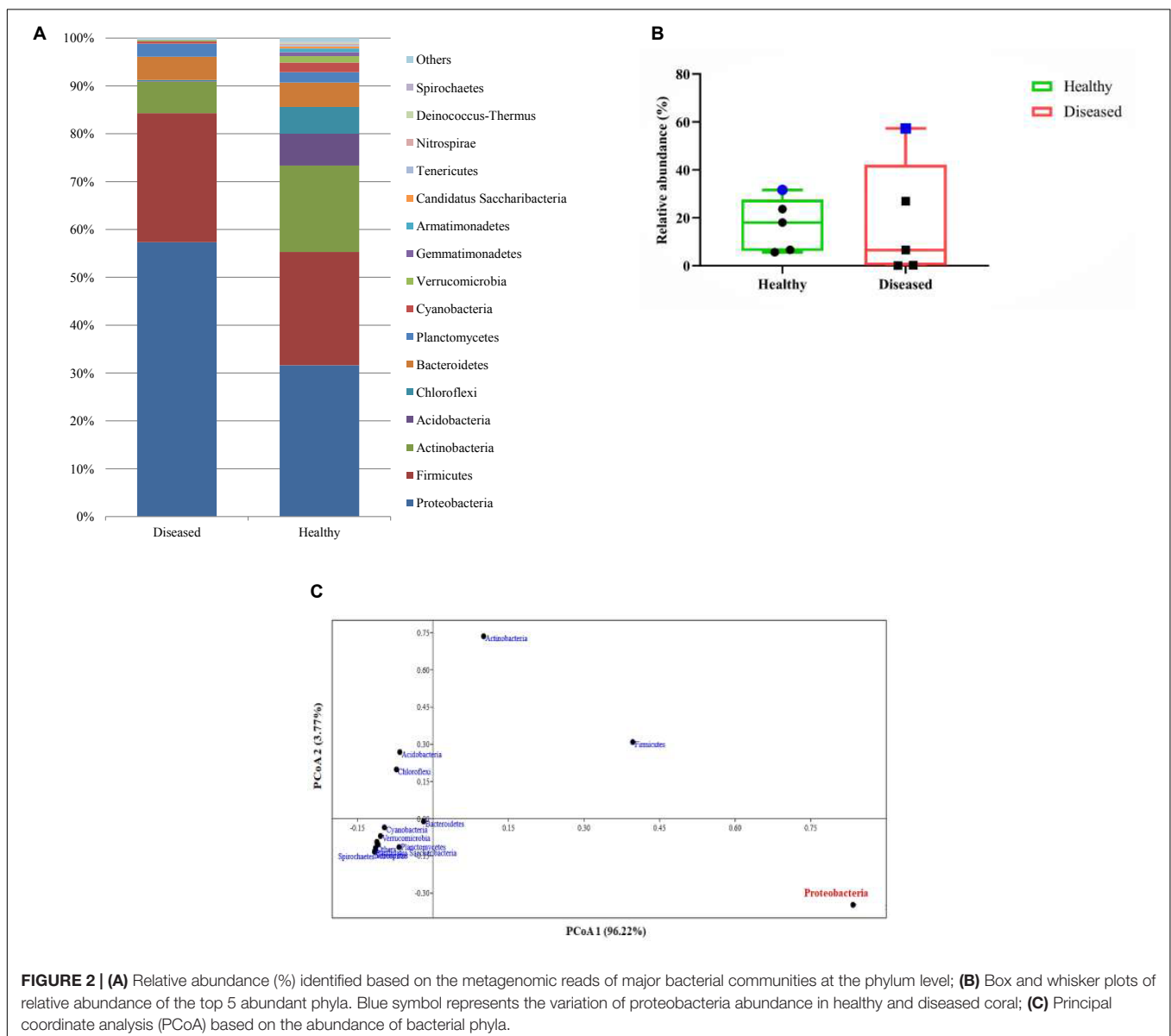
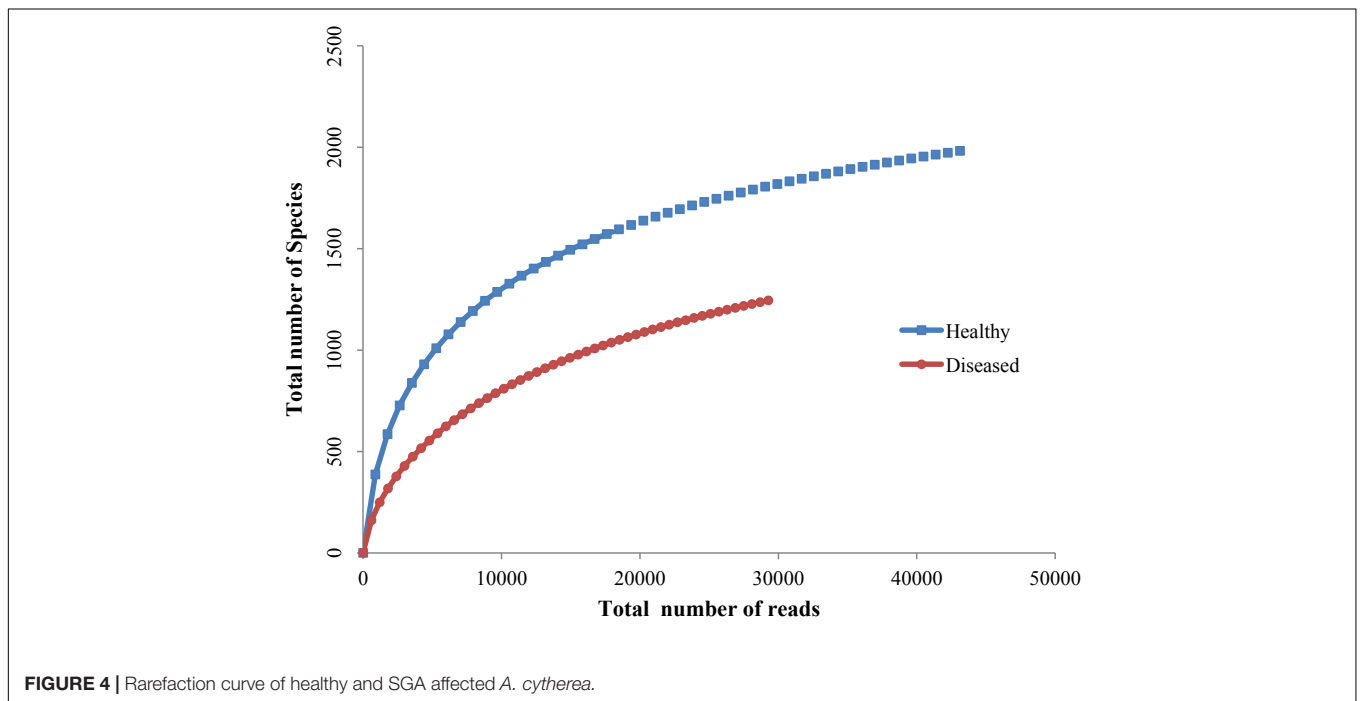
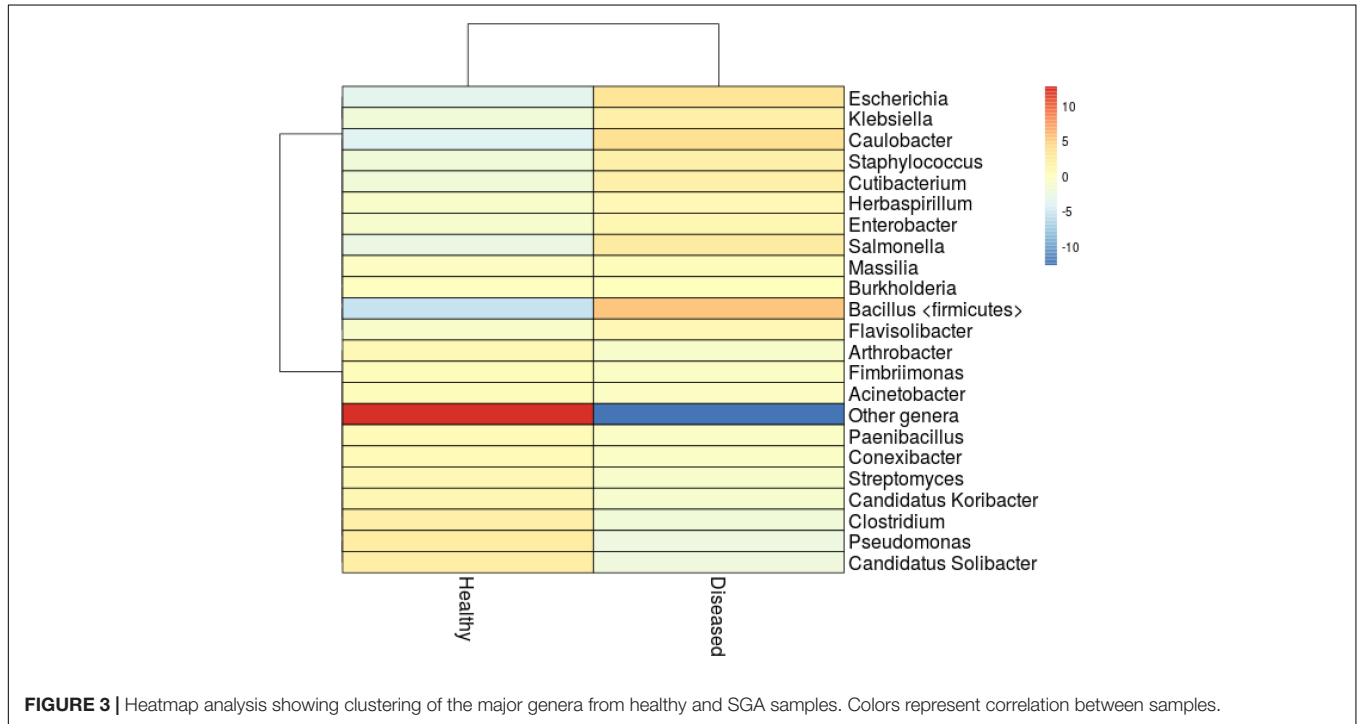


FIGURE 2 | (A) Relative abundance (%) identified based on the metagenomic reads of major bacterial communities at the phylum level; **(B)** Box and whisker plots of relative abundance of the top 5 abundant phyla. Blue symbol represents the variation of proteobacteria abundance in healthy and diseased coral; **(C)** Principal coordinate analysis (PCoA) based on the abundance of bacterial phyla.

by Firmicutes (26.9%), Actinobacteria (6.5%), Bacteroidetes (4.8%) and Planctomycetes (2.7%) (p -value <0.0001). Acidobacteria and Chloroflexi were at lower abundance (0.1 and 0.2% respectively) of total sequences (**Figure 2A**). Among the Proteobacteria, the Gammaproteobacteria sub-group was predominant in both healthy and SGA corals respectively.

Several studies have reported the variations of microbial communities associated between the healthy and reef corals with variety of diseases such as white patch syndrome (Godwin et al., 2012; Wilson et al., 2012; Sere et al., 2013), paling and necrosis (de Castro et al., 2010), dark spot-affected (Kellogg et al., 2014), black band disease (Sato et al., 2010; Miller and Richardson, 2011), and white plague disease (Sunagawa et al., 2009; Cárdenas et al.,



2011; Roder et al., 2014). There are also fewer reports microbial communities of SGA affected reef coral *Platgyra carnosus* (Chiu et al., 2012; Ng et al., 2015) and *Porites compressa* (Breitbart et al., 2005). Overall, all the studies reported the dominance of Proteobacteria which is coinciding with the present report.

Based on the Box-Plot (Figure 2B) and PCoA (Figure 2C) at the phyla level, we found that the Proteobacteria groups were the major contributor to the variations of microbial abundance between the healthy and the diseased coral. This result is in accordance with the previous reports on bacterial communities associated with various diseases (Sunagawa et al., 2009; Sato et al., 2010; Cárdenas et al., 2011; Miller and Richardson, 2011; Sere et al., 2013; Ng et al., 2015). Whereas, members of the Acidobacteria were scarce in the diseased samples which is contradictory to previous reports (Roder et al., 2014; Ng et al., 2015). Within the Proteobacteria, Gammaproteobacteria was identified as the predominant class, which is almost equal to the percentage of Bacilli. These findings contradict previous findings that have revealed the predominance of Alphaproteobacteria in SGA affected corals and other diseases as well (Pantos et al., 2003; Godwin et al., 2012; Ng et al., 2015).

The association of *Vibrio* spp. have been well documented with high abundance in various species of diseased reef corals (Cervino et al., 2004; Sussman et al., 2008; Luna et al., 2010; Ushijima et al., 2016; Hadaidi et al., 2018). Whereas, in our study *Vibrio* spp. represented only 0.14 and 0.18% of the analyzed sequences in healthy and diseased corals respectively. This clearly pointed out that *Vibrio* spp. was not associated with the progress of SGA in *A. cytherea* (present study) and *Platgyra carnosus* as well (Ng et al., 2015).

The association of cyanobacteria (Miller and Richardson, 2011; Hadaidi et al., 2018), *Thalassomonas* (Thompson et al., 2006), *Arcobacter*, *Desulfovibrio* (Hadaidi et al., 2018), *Roseovarius*, *Hoeflea*, and *Cytophaga* (Ng et al., 2015), as main contributors of diseases have been recorded in various coral species. Even though none of the above-mentioned groups of bacteria observed in this study, we identified high abundance of *Staphylococcus* (4.79%), *Salmonella* (5.58%), *Klebsiella* (4.21%), and *Escherichia* (7.98%) in SGA affected corals (Figure 3). These groups were closely related to pathogens of other terrestrial and as well as marine organisms (Ayulo et al., 1994; Wang et al., 2009; Boss et al., 2016), which indicates the role of opportunistic exogenous pathogens. Besides, an increase of *Caulobacter* abundance (8.09%) has also been associated with SGA coral which is comparable to the study of Pootakham et al. (2018) where they reported the predominance of Caulobacterales during bleaching of *Porites lutea*. In addition, A high abundance of *Candidatus Solibacter* and *Candidatus Koribacter* were observed in healthy samples (4.2 and 2.2% respectively) when compared to diseased ones (<0.01%).

Marine bacteria belonging to Actinobacteria are well documented in the aspect of antibiotics or secondary metabolite producers (e.g., Blunt et al., 2013; Abdelmohsen et al., 2014; Kamjam et al., 2017). In this study, Actinobacteria was found to be the predominant phyla with 18.0% in healthy corals whereas its abundance severely affected in the SGA corals with just 6.4%. Reis et al. (2009) in his study revealed

that loss of beneficial microbes (e.g., *Actinobacteria* and *Pseudoalteromonas*), in diseased coral might have resulted in increased susceptibility to diseases by opportunistic bacteria. We also found that the abundance of *Pseudomonas* spp. declined greatly in the diseased sample (Figure 3) which might affect the metabolism of dimethylsulfoniopropionate (DMSP) in *A. cytherea* (Peixoto et al., 2017). This clearly indicated that the natural bacterial communities are gradually replaced by a succession of opportunistic microorganisms including potential pathogens.

Rarefaction analyses of healthy and SGA affected corals indicated that the healthy coral was hosted with more species and also the rarefaction curve reached a clear saturation (Figure 4). A high alpha-diversity (Shannon Index) and richness estimate (Chao1) were observed in both the healthy mucus (5.75 and 2542 respectively) and SGA affected coral (4.55 and 1668 respectively).

CONCLUSION

In conclusion, we established that the bacterial community structure is greatly varied between healthy and SGA affected corals and found Proteobacteria groups were the major contributor to these variations. Several bacterial genera affiliated to putative pathogens were highly recovered and at the same time, beneficial groups such as Actinobacteria were drastically reduced in SGA affected coral. We believe that SGA formation perhaps caused by environmental stress but the coral health potentially aggravated by the shift in the bacterial community. This is the first study to provide detailed metagenomic analysis of bacterial communities associated with SGA affected *A. cytherea* based on 16S rRNA gene amplicon sequencing.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the GenBank BioProject under the accession number PRJNA576005.

AUTHOR CONTRIBUTIONS

RR and BM designed the research work and carried out the sampling. RR and KR performed all the experiments, analyzed all the data, and wrote the entire manuscript. RA and CM contributed in designing the research work and edited the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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