Coral-Bacterial Communities before and after a Coral Mass Spawning Event on Ningaloo Reef

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Abstract

Bacteria associated with three coral species, Acropora tenuis, Porites damicornis and Tubastrea faulkneri, were assessed before and after coral mass spawning on Ningaloo Reef in Western Australia. Two colonies of each species were sampled before and after the mass spawning event and two additional samples were collected for P. damicornis after planulation. A variable 470 bp region of the 16 S rRNA gene was selected for pyrosequencing to provide an understanding of potential variations in coral-associated bacterial diversity and community structure. Bacterial diversity increased for all coral species after spawning as assessed by Chao1 diversity indicators. Minimal changes in community structure were observed at the class level and data at the taxonomical level of genus incorporated into a PCA analysis indicated that despite bacterial diversity increasing after spawning, coral-associated community structure did not shift greatly with samples grouped according to species. However, interesting changes could be detected from the dataset; for example, the Proteobacteria increased in relative abundance after coral spawning and particularly the Roseobacter clade was found to be prominent in all coral species, indicating that this group may be important in coral reproduction.

Introduction

Corals exhibit a range of reproductive strategies, which include both sexual and asexual propagation. Brooding coral species show internal fertilization and expel well-developed larvae at various times of the year, usually over the summer months. Most corals however reproduce during annual spawning events, by broadcast spawning their gametes for external fertilization [1]. Mass spawning is a well-known phenomenon occurring worldwide and involves the synchronous release of gametes from benthic invertebrates including scleractinian corals. The timing of coral mass spawning depends on the geographical location, and usually occurs in summer, once a year over a few nights following the full moon [1]. Coral reproduction is regulated by several life processes such as gamete production, fertilization, planktonic larval dispersal, larval settlement, post-settlement growth, and survival. Disruption in these early life stages can result in compromised or failed recruitment and profoundly affect the distribution and survival of corals [2].

A stimulation of microbial processes within reef waters after episodic spawning events has previously been reported [3,4]. After a coral mass spawning event on the Great Barrier Reef (GBR), bacterial abundances in reef water increased 2-fold and remained elevated for three days, before declining to below pre-spawning values [4]. The input of large quantities of particulate organic matter in the form of degrading gametes enhance pelagic and benthic autotrophic and heterotrophic activities [5], and can result in rapid oxygen depletion in the water column [6].

Microbes in coral reef ecosystems have been extensively studied with regard to their role in coral health and disease [7], coral antimicrobial properties [8] and their involvement in the biogeochemical cycling of nutrients [9,10]. Furthermore microbes have been suggested to co-evolve with their coral host [7] and to benefit the coral in adapting to environmental changes in the ecosystem [11]. Previous studies suggested that bacterial communities in corals are distinct from those inhabiting the surrounding seawater [12] and that some corals harbour specific bacteria species, despite temporal or geographical separation [13,14]. Conversely, other studies showed that bacterial consortia varied with location [15] and time [16], indicating that coral-microbial community structures may be either a result of environmental drivers [16,17] or species- and site specific [18]. Understanding the acquisition, maintenance and successional changes of microbial communities through different coral life stages is fundamental to understanding the functional roles these partnerships have in overall coral health.

Energy demanding physiological processes such as reproduction affect the corals metabolism which may impact its numerous microscopic partners (including Symbiodinium, Bacteria, Archaea, Fungi and viruses which form a functionally relevant mutualistic relationship with coral known as the coral holobiont) [14]. Coral reproduction itself as well as the environmental changes associated with the large scale ecological event of coral mass spawning could potentially influence coral bacterial associates. If corals acquire bacteria according to their specific requirements in different life
stages, reproduction accomplished colonies might rid themselves of bacteria associated with and important to reproduction and recruit alternative bacteria populations more suitable for the time after spawning. Coral bacteria might also change due to corals releasing large quantities of beneficial bacteria with their gametes (spawners) or planula larvae (brooders), and the re-colonization with new bacteria; or corals may simply return to pre-spawning bacterial populations as observed for temperature stressed and bleached corals [19].

This study investigated the diversity and community structure of coral-associated bacterial communities before and after a coral mass spawning event. Three coral species were assessed: the broadcast spawning coral _A. tenuis_ (which participated in the synchronous event), the brooding coral _P. damicornis_ (additional samples were collected for this species after its respective reproductive event); and the ahermatypic coral _T. falkneri_. _T. falkneri_ does not associate with the algal symbiotic partner _Symbiodinium_ which has previously been suggested to be involved in structuring coral microbial communities [20,21]. Like _P. damicornis_, the ahermatypic coral _T. falkneri_ broods and releases planulae and was intended to serve as an ahermatypic example. However no reproductive activity was observed through the study time (one month of observation) and the timing of reproduction for this species is unknown for the Ningaloo Reef system. Bacterial diversity was assessed by a 16 S rRNA gene pyrosequencing approach allowing for large-scale exploration of taxonomic diversity. This study is the first to investigate the dynamics of coral-microbial associates before and after coral reproductive stages.

**Methods**

**Sample Site and Sample Collection**

Three coral species, _P. damicornis_, _A. tenuis_ and _T. falkneri_ were used in this study. Two replicated colonies per coral species were tagged and sampled on a reef flat (5–6 m water depth) near Coral Bay (23° 07’S, 113° 07’E), Ningaloo Reef, Western Australia. For each species, two pieces were collected (one from each replicate colony) two days before and two days after the coral mass spawning event in March 2009. Two additional _P. damicornis_ colonies were removed from the reef structure and kept in an open plastic container (80×50×50 cm) on the reef flat during the day and assessed for reproductive activity on the beach at night time. The container was kept in knee deep water to maintain the ambient water temperature and returned to the reef at sunrise. _P. damicornis_ released their planulae one week after the mass spawning event and were sampled on the reef two days after the last reproductive activity.

Two similar sized coral nubbins (approximately 2 cm in size) were removed from two coral colonies of each species using a bone clipper. Coral nubbins were placed immediately into individual, sterile zip-lock plastic bags under water and rinsed 3 times with artificial seawater (0.2 μm filtered and autoclaved) on the surface and placed on ice. The coral samples were air brushed with 2 ml of ASW to remove the coral tissue including the associated microbial communities from the coral skeleton and the tissue slurry aliquoted into cryovials. All samples were stored at −80°C until required for analysis. Samples were processed within one hour of sampling. Permits for this study were provided by the Department of Environment and Conservation.

**DNA Extraction and PCR and Sequencing Preparation**

Frozen tissue samples from all sampled corals were aseptically transferred to 1.5 ml Eppendorf tubes and total genomic DNA extracted using the MO BIO PowerPlant DNA Isolation Kit as per the manufacturer’s instructions (MO BIO Laboratories, CA, USA). Extracted DNA was quantified using a GeneQuant Pro spectrophotometer (Amersham Pharmacia Biotech) and stored at −20°C until required.

A 470 bp region of the 16S ribosomal RNA gene (16 S rRNA) including the variable regions 1–3 was selected for tag pyrosequencing using the bacterial forward primer 63 F which included the primer A adaptor on the 5’ end along with a unique 8 bp barcode (5’- CCATCTCATCCTCGGTGTGTCCGACT-CAGNNNNNNNCAGGCCCTAACACATGCAAGTC -). The bacterial reverse primer 533 R with the primer B adaptor on the 5’end (5’- CCTATCCCCGTGTGCCCTTGCG-CAGGTCTCAGTTACCCGGGCTGTGGCACC-). All amplifications were run under the following conditions: 1x QIagen PCR Buffer (Qiagen, Germany), 1 U of HotStarTaq DNA Polymerase (Qiagen), 200 μM of each deoxynucleotide triphosphate (dNTP), 25 pmole of each primer and MilliQ water up to 50 μl. Equal volumes of DNA (20 ng total) from each sample were used as template to generate PCR amplicons (tags). Thermocycling conditions for the amplification consisted of an initial ‘enzyme activation’ at 95°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, followed by a final extension step of 72°C for 10 min. A total of 5 PCRs were performed for each sample and the replicate PCR’s pooled to generate more than 1 μg of template DNA. PCR products were purified using the MO Bio PCR purification kit as per the manufacturer’s instructions. Note: three of the _P. damicornis_ (before, after spawning and after planulation) and one of the _T. falkneri_ samples (after spawning) failed to amplify and were excluded from subsequent analysis. The amount of DNA in each sample was quantified using the Quant-iT PicoGreen assay (Invitrogen, Carlsbad, CA). All samples with their respective bar codes (10 samples in total) were pooled in equimolar amounts for 454 pyrosequencing on a Roche GS-FLX system at the Australian Genome Research Facility (AGRF) Brisbane, Australia.

**Sequence and Statistical Analyses**

The sequence fasta and quality files were extracted from the raw sff output from the 454 sequence run and the sequence tag and its associated quality scores were removed. The python script split_libraries.py from the QUIME pipeline [22] was used to remove poor quality (<25) and short sequences (<150 bp), remove the primer and barcode, and add a sample identifier to the header of each sequence. The fasta file was checked for chimeric sequences against a chimera-free database of 16 S rRNA gene sequences (Green Genes 29/11/10 release) using UCHIME [23]. All sequences that were identified as potential chimeras were removed. Homopolymer sequence errors were corrected using ACACIA (pers.com. Dr. Gene Tyson) resulting in a chimera and error-free fasta file. The number of reads per sample was quantified for each of the previous steps (see Table 1). The number of chimeric-free and error-free reads was normalised to 475 reads per sample to allow comparative diversity analysis between all samples. No significant differences ($P = 0.01$; 1000 permutations) were observed between the raw, cleaned and normalised datasets when PCA analysis was performed on the relative abundance of the dominant OTUs and correlation between datasets was assessed by Procrustes rotation [24]. Therefore all analysis reported in this study was conducted on the randomly subsampled and normalised dataset. Sequences were clustered using uchclust [25] to obtain groups of sequences at both the 90% and 97% similarity levels. These groups represent operational taxonomic units (OTUs) defined at an approximate...
were consistently present in coral samples.

Bacterial class-level data were obtained using taxonomic assignments with the Greengenes database, and were then grouped into operational taxonomic units (OTUs) at 97% similarity, which is a common level for bacterial community analysis. This approach allowed for the identification of the most abundant OTUs across all coral species and time points. Sequences were amplified for the V3-V4 region of the 16S rRNA gene. Phylogenetic affiliations were assigned through the QIIME pipeline using GreenGenes and BLAST (0.75 e-value) to determine the taxonomic annotations of the OTUs.

Results

Samples collected from A. tenuis, P. damicornis, and T. faulkneri were used to investigate the bacterial communities before and after mass spawning. A principal component analysis was performed to compare bacterial species diversity across coral species and time points. Replicate samples were highly similar for each time point, with only minor variations observed between samples within each species. The pyrosequencing dataset was deposited in the NCBI Sequence Read Archive (SRA) database with the accession number (pending).

An analysis of similarities (ANOSIM) was performed to compare bacterial species diversity between coral samples. A test run on R, using the package “vegan” identified significant differences in bacterial communities between coral species; the significance was computed by the permutation of the group membership, with 10,000 replicates and Bray-Curtis distance as a distance measure.

Discussion

Three coral species, A. tenuis, P. damicornis, and T. faulkneri, were examined to explore shifts in coral-associated bacterial assemblages before and after a coral mass spawning event. Bacterial diversity increased after reproductive activity for both coral species, A. tenuis after coral spawning and P. damicornis after planulae release, as indicated by Chao 1 index.

No major shifts in coral bacterial communities were observed at the class level through the coral reproduction event. This observation was consistent for all coral species which demonstrated similar microbial communities with only minor variations in abundances between bacterial classes. A more precise taxonomic assignment at the genus level (97% similarity) indicated similarities between A. tenuis and T. faulkneri in both, proportions and identities of their bacterial communities. P. damicornis however displayed differences in bacterial composition compared to the other two coral species. Only seventeen percent of the most abundant OTUs were shared between all coral species investigated, and were associated with the γ-Proteobacteria, Rhodobacteraceae, and Rhodospirillales and the γ-Proteobacteria Shewanellaceae, Pseudoalteromonas and Sienostrophomonas (Fig. 3).

Table 1. Sampling times and statistical diversity parameters.

| Samples/Species | A. tenuis (1) | A. tenuis (2) | P. damicornis | T. faulkneri (1) | T. faulkneri (2) | A. tenuis (1) | A. tenuis (2) | P. damicornis | P. damicornis | T. faulkneri (1) |
|-----------------|--------------|--------------|--------------|----------------|----------------|--------------|--------------|--------------|--------------|----------------|
| Sampling time   |              |              |              |                |                |              |              |              |              |                |
| before coral mass spawning | 932 | 1031 | 595 | 1598 | 1247 | 2008 | 1566 | 492 | 475 | 1966 |
| after coral mass spawning |              |              |              |                |                |              |              |              |              |                |
| High quality seqs. | 475 | 475 | 475 | 475 | 475 | 475 | 475 | 475 | 475 | 475 |
| Rarefied seqs.   |              |              |              |                |                |              |              |              |              |                |
| OTU0.05          | 140 | 181 | 54 | 359 | 222 | 357 | 272 | 63 | 127 | 473 |
| Chao 0.05        | 148.606 | 208.991 | 71.038 | 377.421 | 220.389 | 412.396 | 269.426 | 111.446 | 242.625 | 503.815 |

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associated with the \(\alpha\)-Proteobacteria *Rhodobacteriaceae*, *Roseobacter*, and *Rhodospirillales* and the \(\gamma\)-Proteobacteria *Shewanella*, *Pseudoalteromonas* and *Stenotrophomonas*. Corals have previously been reported to harbour specific bacteria which differ from bacterial communities in the seawater [12] and the aforementioned bacteria groups seem to represent a consistent and important component in coral-

![Figure 1. Bacterial 16 S rRNA gene sequences retrieved from three coral species before (b), after (a) coral spawning and after planulation (**a**). Replicate samples were pooled and dominant affiliations were grouped at the class level. The similarity tree was done using the neighbour-joining method and the Bray-Curtis algorithm (\(n = 1000\) replications). Note: due to failure in amplification *P. damicornis* and *T. faulkneri* are represented by one sample per sampling point and one sample after coral spawning, respectively. doi:10.1371/journal.pone.0036920.g001](#)

![Figure 2. Principal component analysis (PCA) of 16 S rRNA gene sequences, showing non-pooled coral samples grouped into OTUs>97% identity, before, after coral spawning and after planulation. doi:10.1371/journal.pone.0036920.g002](#)
bacterial associations with *A. tenuis*, *P. damicornis* and *T. faulkneri*, whereas other bacterial groups not found to be consistently dominant are likely to vary between coral species.

Investigating the taxonomic assignment at the genus level revealed a specific partition for bacterial classes associated with corals. For example within the family Sphingomonadaceae, the genus *Erythrobacter* was present in *A. tenuis* and in *T. faulkneri* only, whereas *Novosphingobium* was only represented in *P. damicornis*. Increased retrieval of sequences related to the genera *Erythrobacter* and *Pseudoalteromonas* potentially highlight their significance in *A. tenuis* reproduction; both genera are commonly known to associate with corals [13,14,27] and can inhibit the growth of the coral pathogen *Vibrio coralliilyticus* [28]. Furthermore, *Pseudoalteromonas* has previously been shown to induce coral settlement [29] and to possess antimicrobial properties [8,30].

Interestingly all bacteria types affiliated with the class *ß*-Proteobacteria either increased or remained unchanged in relative proportion of retrieved sequences after reproduction in the corals *A. tenuis* and *P. damicornis* compared to pre-spawning samples. This suggests that *ß*-Proteobacteria may be important in coral reproduction including possible implications for the survival and increase of fitness in coral larvae. Previous work reported the genus *Roseobacter* to amongst the first acquired bacteria in early developing stages of the coral *Pocillopora meandrina* [31,32]. *Roseobacter* clade affiliated

### Table 2. OTU’s (grouped at 97% identity) of the most abundant bacteria from three coral species before (b), after (a) coral spawning and after planulation (a*); sequences of proportions >1% were included and numbers represent percentages of sequence affiliations.

| OTU’s                                                                 | *A. tenuis* | *P. damicornis* | *T. faulkneri* |
|-----------------------------------------------------------------------|-------------|-----------------|----------------|
|                                                                       | b          | a               | a*             |
| Bacteria; Acidobacteria; Acidobacteria; Acidobacteriaceae, unclassified | 14         | 7               | 3              |
| Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales, unclassified, unclassified | 1          |                 |                |
| Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales, unclassified, unclassified | 1          |                 |                |
| Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales, unclassified | 4          | 7               | 4              |
| Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales, unclassified | 2          |                 |                |
| Bacteria; Bacteroidetes; Flavobacteria; Sphingobacteriales, unclassified, unclassified | 2          |                 |                |
| Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillaceae | 45         | 16              | 7              |
| Bacteria; Firmicutes; Clostridia; Clostridiales; Incertae Sedis XII, unclassified | 2          |                 |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Rhizobiales, unclassified, unclassified | 1          |                 |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Rhizobiales; Rhodomicrobium | 2          | 2               | 1              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Rhodobacteriales, Roseobacter | 6          | 8               | 12             |
| Bacteria; Proteobacteria; ß-Proteobacteria; Rhodobacteriales; Silicibacter | 1          |                 | 1              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Rhodospirillales, unclassified, unclassified | 3          | 4               | 2              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Sphingomonadales; Sphingomonadaceae; Erythrobacter | 10         | 18              | 1              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Sphingomonadales; Sphingomonadaceae; Novosphingobium | 2          | 1               | 2              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Sphingomonadales; Sphingomonas | 1          |                 |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales, unclassified, unclassified | 4          | 5               | 6              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Alteromonadaceae, unclassified | 1          | 1               | 3              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Alteromonadaceae; Astaquariibacter | 4          | 4               | 2              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Alteromonadaceae; Alteromonas | 2          |                 | 4              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Colwelliaceae, unclassified | 1          | 1               |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Colwelliaceae; Thalassomonas | 6          | 2               |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Incertae sedis 7, unclassified | 2          | 4               |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Shewanellaceae, Shewanella | 7          | 6               | 1              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Alteromonadales; Pseudalteromonadaceae; Pseudoalteromonas | 2          | 7               | 1              |
| Bacteria; Proteobacteria; ß-Proteobacteria; Enterobacteriales; Enterobacteriaceae, unclassified | 2          |                 |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Enterobacteriales; Enterobacteriaceae; Shigella | 22         | 10              |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Oceanospirillales; Oceanospirillaceae, unclassified | 3          | 2               |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Oceanospirillales; Oceanospirillaceae; Oceanospirillum | 1          | 1               |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter | 33         | 53              | 27             |
| Bacteria; Proteobacteria; ß-Proteobacteria; Vibrionales; Vibrionaceae, unclassified | 1          |                 |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Vibrionales; Vibrionaceae; Vibrio | 7          | 2               |                |
| Bacteria; Proteobacteria; ß-Proteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas | 1          | 9               | 18             |
| Unclassified Bacteria                                                                 | 4          | 4               | 1              |

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bacteria species are abundant and diverse in seawater and various metabolic functions have been reported for this taxon [33], including antibiotic properties against coral pathogens [34]. In the present study Roseobacter affiliated sequences are prominent in all coral species and represent the only bacteria type increasing after spawning as well as after planulation (Fig. 3), possibly providing antimicrobial activity against potentially pathogenic bacteria for coral compromised after energy demanding life stages such as spawning [35]. These findings support the idea that Roseobacter affiliated bacteria may be specifically related to the process of reproduction in brooding as well as in spawning corals.

This is the first study to directly compare shifts in coral bacterial associations before and after spawning. Coral species displayed similar classes of bacteria, though at the genus level, small differences in associated bacterial communities were observed. Abundant OTUs potentially represent bacteria which play a role during coral reproduction since they specifically appeared before or after coral spawning or in distinctly high numbers in between sampling times. This study lays the groundwork for future research investigating potentially important functional roles of the identified bacterial groups, and their implication in coral reproduction and the early establishment of corals.

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Author Contributions

Conceived and designed the experiments: JC DGB. Performed the experiments: JC RMS. Analyzed the data: JC JBR DGB. Contributed reagents/materials/analysis tools: DGB MvK. Wrote the paper: JC JBR DGB MvK.

References

1. Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, et al. (1984) Mass spawning in tropical reef corals. Science 223: 1186–1188.
2. Albright R, Langdon C (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral Porites astreoides. Global Change Biol 17: 2470–2487.
3. Ghid RN, Eyre BD, Patten N (2008) Biogeochemical responses to mass coral spawning at the Great Barrier Reef: Effects on respiration and primary production. Limnol Oceanogr 53: 1014–1024.
4. Patten NL, Mitchell JG, Middelboe M, Eyre BD, Seuront L, et al. (2008) Bacterial and viral dynamics during a mass coral spawning period on the Great Barrier Reef. Aquat Microb Ecol 50: 209–220.
5. Wild C, Jantzen C, Struck U, Hoegh-Guldberg O, Huettel M (2008) Biogeochemical responses following coral mass spawning on the Great Barrier Reef: pelagic-benthic coupling. Coral Reefs 27: 123–132.
6. Simpson CJ, Cary JL, Masini RJ (1993) Destruction of corals and other reef animals by coral spawn slicks on Ningaloo Reef, Western Australia. Coral Reefs 12: 185–191.
7. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5: 355–362.
8. Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322: 1–14.
9. Olson ND, Ainsworth TD, Gates RD, Takabayashi M (2009) Diazotrophic bacteria associated with Hawaiian Montipora corals: Diversity and abundance in correlation with symbiotic dinoflagellates. J Exp Mar Biol Ecol 371: 140–146.
10. Raina JB, Tapidas D, Willis BL, Bourne DG (2009) Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. Appl Environ Microbiol 75: 3492–3501.
11. Resh LF, Koen O, Loya Y, Zilber-Rosenberg I, Rosenberg E (2006) The coral probiotic hypothesis. Environ Microbiol 8: 2068–2073.
12. Bourne D, Munn C (2005) Diversity of bacteria associated with the coral Pocillopora damicornis from the Great Barrier Reef. Environ Microbiol 7: 1162–1174.
13. Frias-Lopez J, Zerlame AL, Bonheyo GT, Fouke BW (2002) Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. Appl Environ Microbiol 68: 2214–2228.
14. Rohwer F, Segrigan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243: 1–10.
15. Littman RA, Willis BL, Peñler G, Bourne DG (2009) Diversity of coral-associated bacteria differ with location but not species for three Acroporids on the Great Barrier Reef. FEMS Microbiology Letters 68: 152–163.
16. Ceh J, van Keulen M, Bourne DG (2011) Coral-associated bacterial communities on Ningaloo Reef, Western Australia. FEMS Microbiol Ecol 75: 134–144.
17. Hong MJ, Yu YT, Chen CA, Chiang PW, Tang SL (2009) Influence of Species Specificity and Other Factors on Bacteria Associated with the Coral Stylolophora pistillata in Taiwan. Appl Environ Microbiol 75: 7797–7806.
18. Sunagawa S, Woodley CM, Medina M (2010) Threatened Corals Provide Underexplored Microbial Habitats. PLoS ONE 5: e9554.
19. Bourne DG, Idu Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event. ISME J 2: 350–363.
20. Banin E, Khare SK, Naider F, Rosenberg E (2001) Proline-Rich Peptide from the Coral Pathogen Vibrio shiloi that Inhibits Photosynthesis of Zooxanthellae. Appl Environ Microbiol 67: 1536–1541.
21. Raina JB, Dinsead E, Willis BL, Bourne DG (2010) Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? Trends in Microbiology 18: 101–108.
22. Caporaso G, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nature 7: 335–336.
23. Edgar RC, Haas BJ, JC. C, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics.
24. Gower JC, Dijkstra R (2004) Procrustes problems: Oxford University Press.
25. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics. pp 2460–2461.
26. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, et al. (2006) Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench. Composable with ARB. Appl Environ Microbiol. pp 5069–5072.
27. Rohwer F, Bentbar M, Jara J, Azam F, Knowlton N (2003) Diversity of bacteria associated with the Caribbean coral Montastrea franksi. Coral Reefs 20: 85–91.
28. Vizcaino MH, Johnson WR, Kimes ME, Williams K, Torralla M, et al. (2010) Antimicrobial Resistance of the coral pathogen Vibrio corallillichus and Caribbean Sister Phylotypes Isolated from a Diseased Octocoral. Environ Microbiol 59: 646–657.
29. Webster NS, Smith LD, Heyward AJ, Watts JEM, Webb RI, et al. (2004) Metamorphosis of a Scleractinian Coral in Response to Microbial Biofilms. Appl Environ Microbiology 70: 1213–1221.
30. Shnit-Orland M, Kushmaro A (2009) Coral mucus-associated bacteria:a possible first line of defense. FEMS Microbiol Ecol 67: 371–380.
31. Apprill A, Marlow HQ, Marrindale MQ, Rappe MS (2009) The onset of microbial associations in the coral Pocillopora meandrina. ISME J 3: 685–699.
32. Sharp KH, Distel DL, Paul VJ (2011) Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral Porites astreoides. ISME J.
33. Piekarski T, Buchholz I, Schober M, Wagner-Dobler I, Tieden P (2009) Genetic tools for the investigation of Roseobacter clade bacteria. Bmc Microbiol 9.
34. Nissimov J, Rosenberg E, Munn CB (2009) Antimicrobial properties of resident coral mucus bacteria of Oculina patagonica. FEMS Microbiol Letters 292: 210–215.
35. Penesyan A, Tebben J, Lee M, Thomas T, Kjelleberg J, et al. (2011) Identification of the Antibacterial Compound Produced by the Marine Epiphytic Bacterium Pseudovibrio sp. D323 and Related Sponge-Associated Bacteria. Mar Drugs. pp 1391–1402.