2010

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Abstract
Coral reef biodiversity has declined over the past few decades and despite recent significant scientific interest in reefs in light of noticeable effects of climate change on this fragile habitat there is still much undiscovered about the true diversity of the symbiont community. There is also much to ascertain about underlying holobiont mechanisms relating to beneficial properties resident bacteria possess to help their hosts defend themselves from pathogenic xenobacteria or how symbionts provide the holobiont and other reef organisms with nutrition by utilising the carbon and nitrogen cycles. These are some of the questions addressed in this literature review. It is concluded that research has built up a large quantity of data about bacterial diversity and functions but information is still lacking about archaea and viruses. It is also concluded that projects always open up the way for new research as the complexity of the ecosystem expands the more knowledge we gain about it, demonstrating how much we have still to discover if science is to aid declining reefs in future.

Introduction

Rosenberg *et al.* (2007) have described coral reefs as "the largest structures made by living creatures". Biogenic activities over millennia has lead reefs to evolve to become a complex, productive, yet fragile marine ecosystem with a biodiversity to rival that of a terrestrial rainforest (Rohwer *et al.* 2002, Allen and Steene, 2007). Microorganisms are critically important components of scleractinians and research has provided ample evidence that corals support mutualistic symbiotic relationships with autotrophic *Symbiodinium* dinoflagellates, commonly referred to as zooxanthellae (Ben-Haim and Rosenberg, 2004) to continue their hermatypic (reef building) host's ecological success (Ritchie, 2006). These are quite a diverse group, spanning several different clades in the *Symbiodinium* genus (Rowan *et al.* 1997).

Zooxanthellae inhabit host tissue cells to form a symbiosome: a host membrane which encompasses the endosymbiont, separating it from host cytoplasm (Wakefield and Kempft, 2001). Carbohydrates pass from symbiont to host through these membranes and it has been proposed that coralline amino acids stimulate this transfer (Smith *et al.*, 2005). Zooxanthellae occupy the primary producer niche, fixing carbon dioxide to release energy and oxygen for use by the host and gaining carbon dioxide, nitrogenous compounds and shelter in return (Kushmaro and Kramarsky-Winter, 2004; Smith *et al.*, 2005). This activity has culminated in reefs having very high productivity despite residing in oligotropic waters of little nutritive value (Coffroth, 1990). In addition to *Symbiodinium*, corals support diverse and abundant bacterial (Rohwer, 2001, 2002), archaeal (Kellogg, 2004) and viral communities (van Oppen *et al.*, 2009).

This review focuses mainly on distinctive roles of coral associated microorganisms which benefit the well-being and survival of the holobiont — this being the "complex symbiosis between the coral animal, endobiotic alga and an array of microorganisms" (Bourne *et al.*, 2009) — as nutritive and defensive aids in the reef (Figure 1). Moreover, it will review how climate and pathogens influence the holobiont. Some theories on the projected roles of bacteria in coral evolution, such as the probiotic hypothesis and the hologenome theory by Rosenberg and his colleagues (Reshef *et al.*, 2006, Rosenberg *et al.*, 2007) are discussed.

Populations within the coral colony

Within individual coral colonies, bacteria have been isolated from the skeleton (Shashar *et al.*, 1994), tissues (Bourne and Munn, 2005), the lipid-rich surface mucus microlayer (Paul *et al.*, 1986; Kellogg, 2004) and surrounding seawater (Ritchie and Smith, 2004; Rohwer, 2002). Within each site there is a unique community that varies between species and structural layers (Ritchie and Smith, 2004; Rohwer, 2002). Experiments have demonstrated that specific associations remain in place regardless of space and time; for example, shown by isolating identical species separated by 10 km for one study (Rohwer, 2001). In contrast, the result of one study has suggested that archaea are not host species specific (Kellogg, 2004). Unfortunately, this is the only research to have looked at archaeal diversity in corals so there is a considerable gap in research in this area which could reaffirm this information.

Using culture and molecular techniques, holobiont biodiversity has been shown to be large. Tissues are dominated by gamma-proteobacteria whilst mucus is dominated by alpha-proteobacteria, which include *Roseobacter* (Munn, 2004), *Spongiobacter* (Bourne *et al.*, 2008) and the majority of phototrophs (Bourne and Munn, 2005). This pattern is conserved across different colonies of the same

species, and these results are supported by other authors such as Rohwer *et al.* (2002).

This picture of biodiversity has come from a mix of methodologies. Culture has revealed a large bacterial diversity despite the majority being unculturable even when using plate media dilution methods. In various areas of microbiology culture leads to several genera being unrepresented or underrepresented in environmental samples (Giovannoni and Rappe, 2000). In recent years, molecular methods which bypass culturing have been developed and there have been advances in metagenomic methods which generate mass genetic information about environmental samples. Now, using analyses by 16S rDNA, Polymerase Chain Reactions (PCR), and Denaturing Gradient Gel Electrophoresis (DGGE), more representative population information has been acquired (e.g. Bourne and Munn, 2005; Kellogg, 2004). It is now realised that there are more alpha-proteobacteria than previously thought since they are better identified with molecular methods and highly abundant in marine waters, whilst gamma-proteobacteria are more easily cultured on solid media but less well represented in clone libraries (Munn, 2004).

Naturally associated bacterial communities play beneficial and detrimental roles in the holobiont; for example, isolates have antimicrobial properties to act as antagonists against opportunistic pathogens (Nissimov *et al.*, 2009; Harder *et al.*, 2002; Kelman *et al.*, 2009). There is also sufficient evidence to suggest that bacteria could act as alternative sources of nutrition when nutrients are scarce and that some residents possess the ability to fix nitrogen or carbon for consumption (Shashar *et al.*, 1994; Ducklow and Mitchell, 1979).

The role of coral associated microorganisms in pathogen defence
The symbiotic community is stable in optimal conditions, but environmental fluctuations trigger bacterial community shifts. One example is temperature dependent bleaching. Elevated water temperatures as a result of El Niño events cause expulsion of zooxanthellae from tissues, making the host open to subsequent infection from pathogens such as *Vibrio*. One mechanism for expulsion is the reduced capacity of bacteria to secrete antimicrobials (Frias-Lopez *et al.*, 2002) while other work reviews the idea of expulsion as a result of photoinhibition (Smith *et al.*, 2005) or as an adaptive reaction to gain more suitable symbionts for the prevailing conditions (Rosenberg *et al.* 2007).

Hermatypic cnidarians require the ability to regulate symbiont populations to maintain their health and protect themselves from pathogens in their surroundings (Kelman *et al.*, 2009). Many bacteria associated with coral have been found to possess antimicrobial properties, particularly those in the mucus layer which is the primary barrier between coral tissues and surrounding seawater where pathogenic xenobacteria are more prevalent. For the past three decades, disease on the coral reefs have been increasing (Gochfeld and Aeby, 2008) due to climate change, ocean warming, ocean acidification, and leaching from sewage and fertilisers which enhance microbe reproduction by providing an energy source. Reefs worldwide are declining at a rate of 1% a year with over 70% infected by disorders such as Aspergillosis in sea fan corals, or Black Band Disease in reef building scleractinians (Bruno and Selig, 2007); however, only five out of 29 currently documented ailments have had their causative pathogen(s) identified (Garrison *et al.*, 2003). Besides reef dwellers, 0.5 billion humans also rely on reef biodiversity for food, tourism and biotechnology (Bourne *et al.*, 2009) and this proportion is expected to double in the next 50 years (Smith *et al.* 2005). From an economical point of view, continued study

of coralline immunity and resistance mechanisms, especially in light of current biodiversity decline, is increasingly vital as the projected future of the reefs gets bleaker.

The coral mucus microlayer as an interface has particularly been focussed on whilst researching pathogenic resistance, defined as the "natural or acquired ability of an organism to... resist the effects of an antagonistic agent" (Mullen *et al.* 2004). This microlayer has a range of defensive mechanisms; for example, phagocytes in amoebocyte cells in the mesogleal layer between epidermal tissues engulf and destroy microorganisms (Gochfeld and Aeby, 2008; Mullen *et al.* 2004). One example is increased amoebocyte density in *Aspergillus* infected *Gorgonia* sea fan tissues, whereas healthy tissues have lower densities, supporting an idea that these cells and phagocytotic processes are utilised as an immune response (Mydlarz *et al.*, 2008).

Alternatively, mucus secretes acidic mucopolysaccharides which repel or trap bacteria, preventing damage (Mullen *et al.* 2004). Furthermore, mucus selects for bacteria which antagonise and inhibit opportunistic agents colonising the mucus by a 10 fold amount than if the microlayer were not present (Ritchie, 2006), highlighting how vital mucus is to scleractinian survival.

In stressful periods, tissue and mucoid communities become colonised by pathogenic xenobacteria if host defences are impaired. For example, host bacteria prevent epizootic colonisation by *Vibrio* spp. (this genus is responsible for bacterial induced bleaching) by secreting antimicrobial allochemicals (Ritchie, 2006; Harder, 2003; Slattery, 1995). Conversely, especially when temperature increases enough to stimulate zooxanthellae expulsion, it has been discovered that antimicrobial secretions from apparently healthy tissue and mucus decreases, elevating holobiont susceptibility (Ritchie, 2006). It is likely that there are innumerable immunity mechanisms, but better diagnostic tools need to be developed to accurately interpret the various dynamics within the holobiont.

There are a few theories attempting to explain zooxanthellae expulsion during bleaching events and how this theoretically contributes to coral evolution. One of the first theories was the adaptive bleaching hypothesis (ABH), first put forward by Buddemeier and Fautin (1993) to propose how corals may be at less future risk than authors such as Hoegh-Guldberg (1999) later suggested. They proposed that coral expel zooxanthellae in order to obtain more thermally tolerant symbionts in their place, creating "ecospecies" with polymorphic combinations of host and symbionts to adapt to fluctuating conditions. This moves the model of a single host and *Symbiodinium* unit on to a multi-symbiont complex model (Buddemeier *et al.*, 2004). There is convincing evidence to support this theory. Experiments on *Symbiodinium* clades A, B and C established C to be most ubiquitous in Caribbean coral *Montastrea annularis* in deeper waters (Rowan *et al.*, 1997); however, in natural bleaching events C isolates decreased in shallow waters with low irradiance and in deep waters with high irradiance since these conditions were the "upper limit of *Symbodinium* C's adaptive zone" pushing their physiologies to the point of expulsion (Rowan *et al.*, 1997). Conversely, clades A and B and in later research new clade D (Baker *et al.*, 2004) possessed the greatest thermotolerance and were present in post bleaching corals in higher proportions than C (Rowan *et al.*, 1997), supporting adaptation predictions. Unfortunately, there is a price for thermotolerance. Whilst corals with clade D exhibit greater photosynthetic activity at bleaching temperatures of 28-35ºC, at lower temperatures they had less activity compared with C, which was most beneficial under normal conditions (Rowan *et al.*, 2004). Hence, the price is

reduced energy expenditure for growth (Little *et al.*, 2004) or photosynthesis (Rowan *et al.*, 2004, Sotka and Thacker, 2005). Sotka and Thacker (2005) have criticised the ABH for not considering post bleaching associations, indicating that corals revert back to original states with start proportions of clade C. They also state that since there was no genetic change there was no real adaptation occurring. ABH is logical since only with some form of adaptation do organisms survive changes over time (Sotka and Thacker, 2005). What remains to be seen is whether corals can adapt to climate change events such as El Niño over years rather than decades.

Another two linked hypotheses developed later are the probiotic hypothesis and Rosenberg's subsequent hologenome hypothesis. The probiotic hypothesis suggests that corals expel resident zooxanthellae unable to survive suddenly adverse conditions to open up a niche for fitter zooxanthellae to colonise the host and enable normal activities to resume (Reshef *et al.*, 2006). This theory is similar to ABH but more inclusive as it includes all resident microorganisms rather than just zooxanthellae. Although this allows more flexible and rapid adaptation to changing conditions than genetic mutation (Rosenberg *et al.*, 2007) it might still be months before favourable symbionts colonise, making it increasingly likely for a host to die from bleaching or an alternative disease, for temperature stimulates lysis or expulsion of resident bacteria, archaea and viruses unable to survive or release antimicrobial chemicals to defend themselves. This hypothesis is supported by microbial diversity associated with coral tissues, some of which confer protective potential. The fact that corals appear to defend themselves using antimicrobials to develop immunity is seen by the current absence of *Vibrio shiloi.* Before 2004, this was a prevalent bacterium associated with bleaching but now it is not found (Reshef *et al.*, 2006). Community change associated with altered environmental conditions help explain reef survival through the ages (Rosenberg *et al.*, 2007)

Rosenberg *et al.* developed the hologenome hypothesis in 2007 from the probiotic hypothesis. It was proposed that host genes interact with resident microorganism genes to create a hologenome which is "the combined genomes of the holobiont" (Rosenberg *et al.*, 2007). The microbial genomes change at a faster rate than host cells alone, conferring a more rapid adaptive potential to the holobiont. The authors proposed that genetic information could be passed along by symbionts via abundance change in associated microorganisms, introduction of new microbes from the outside, mutation, horizontal gene transfer and selection acting on the existing population. Rosenberg and colleagues wanted to apply this theory to higher organisms which also use symbionts for species survival, which has attracted harsh correspondences from Leggat *et al.* (2007). who believe the hologenome hypothesis has "oversimplified the host symbiont relationship along with the diversity of roles and conserved acclimation mechanisms" which account for outside changes. There is some support for the hologenome theory with work by Ritchie (2006) using ultraviolet sterilised mucus layers to demonstrate the importance of residential bacteria in structuring the overall holobiont community. For the moment, while this seems like an elegant idea the theory is likely to remain divisive until future research clarifies more about the dynamics of interactions in the holobiont.

The role of bacteria in coral nutrition
Coral reefs produce a surplus quantity of carbon and are a massive primary producing ecosystem despite residing in oligotrophic ocean stretches of poor nutritive value (Shashar *et al.*, 1994). This carbon comes from a variety of sources, with the majority assimilated from photosynthetic products of symbiotic

zooxanthellae located in the gastrodermal cell layer (Kushmaro and Kramarsky-Winter, 2004). Additional carbon sources include suspension feeders and bacterial aggregates on detritus particles in surrounding seawater which become trapped in the mucoid layer and ingested by mesentery filaments which transfer the matter toward the mouth (Goldberg, 2002). These aggregates account for up to 23% of bacterial activity on atoll reefs (Kushmaro and Kramarsky-Winter, 2004). There is considerable evidence to suggest that bacterial growth on the mucus surfaces enhance the probability of particle aggregation (Sorokin, 1973) and the likelihood that mucus acts as a trap in order to create these aggregations which are used as a source of food for the coral, given that the highest densities of aggregates have been isolated from around the polyp mouths. These aggregates are also a food source for suspension feeders which ingest dissolved expelled mucus from water surrounding corals (Kushamaro and Kramarsky-Winter, 2004; Wild *et al.*, 2004). Copious volumes up to 4.8 litres per day of fixed carbon gets released as mucus and is used to trap particulates and ultimately release these to surrounding waters in such volumes that "mucus can dominate suspended matter around the reefs" (Wild *et al.*, 2004). Moreover, the carbon to nitrogen content ratio lends reasonable support to an idea that dissolved mucus in ambient seawater is a food source. The mucus layer is therefore an important carbon source aiding scleractinian metabolism (Gochfeld and Aeby, 2008) and providing the ecosystem with energy in a tightly controlled cycling system to ensure that as few nutrients as possible escape to the open ocean.

Another element of coral nutrition that has been under recent investigation is nitrogen fixation. Nitrogen is a critical component of any organism's makeup since by being part of the protein structure nitrogen is involved with most biochemical processes in an organism (Francis *et al.*, 2007). In the ocean, nitrogen is primarily available as gaseous dinitrogen which needs to be fixed to form ammonium in order to be made available to living organisms (Olson *et al.*, 2009). This procedure can only be carried out by some bacteria and archaea which possess the nitrogen fixing enzyme nitrogenase (Olson *et al.*, 2009). The key gene for nitrogenase, *nif*H, has been isolated from bacteria in the skeleton and tissues of coral *Favia favus* (Shashar *et al.*, 1994). Skeletal endolithic microorganisms contribute approximately 50-65% of nitrogen required by the host scleractinian (Rosenberg *et al.*, 2007; Kushmaro and Kramarsky-Winter, 2004); therefore, like carbon, multiple sources are necessary to make up the additional fixation requirements in this nitrogen limited ecosystem (Shashar *et al.*, 1994).

The mucus microlayer contains higher proportions of nitrogen than seawater (Ducklow and Mitchell, 1979), which would confer an advantage for suspension feeders and supports the above ideas that corals fix nitrogen and other matter not just for themselves but also to provide nutrition to the reef food chain.

One bacterial group involved with nitrogen fixation are symbiotic cyanobacteria, which have been identified in large volumes in *Montastrea cavernosa* epithelial tissue layers to carry out photosynthetic dependent nitrogen fixation (Lesser *et al.*, 2004). This location makes them "an integrated member of the coral holobiont" (Olson *et al.*, 2009) so they are likely to be permanent coral residents. Since 2004, Olson *et al.* (2009) have verified that cyanobacteria are not the only coralline diazotrophs. Phylogenetic analysis of two *Montipora* species has revealed that only three of 62 isolated sequences originated from cyanobacteria. The majority (37 out of 62 sequences) of these were gamma-proteobacteria, some of which were closest related to vibrios which can be pathogenic (Ben-Haim *et al.*, 1999) in diseased coral, or symbiotic in healthy colonies (Koren and Rosenberg, 2006). It was also

established that alpha- and delta-proteobacteria possess *nifH*, further enhancing the diversity of associates capable of nitrogen fixation (Olson *et al.*, 2009). Significant experiments such as this have revealed that the nitrogen cycle and the roles that microbes have in it are more complex than previously imagined. Moreover, fixation is far more prevalent than previously recognised and again highlights the importance of efficiency in reef ecosystem nutrient cycling.

Another biogeochemical cycle involving marine bacteria is the sulphur cycle. There is considerable evidence that bacteria reduce dimethysulphoniopropionate (DMSP) to dimethylsulphide (DMS), both of which have been uncovered in high concentrations within scleractinians (Jones *et al.*, 2007) since the conversion is executed by dinoflagellates and pyrmnesiophytes (Raina *et al.*, 2009) which are coccolithophores which can induce algal blooms. In very new research, corals are considered to "have an integral role in sulphur cycling in oligotrophic reef waters" (Raina *et al.*, 2009) but more work needs to be done to verify this. DMSP reducers are thought to provide nutrition for symbionts and structure their communities (Bourne *et al.*, 2009). Raina *et al.* (2009) also discovered that at least 10 strains of coral associated bacteria possess the gene *dddD* which degrades DMSP to DMS and acrylic acid. The majority of these bacteria were gamma-proteobacteria which included *Spongiobacter* and *Pseudomonas* related microbes. This was the first study to look at sulphur degradation in coral reefs, but research by authors such as Ledyard *et al.* (1993) had already found that *Pseudomonas* degrades DMSP. Raina was the first to show that isolated coral *Spongiobacter* was involved in sulphur degradation and was also very dominant, making up over 50% of clones in the study. Considering the diversity and specificity of coral associates between species it was not that surprising that some new microbe with *dddD* would be discovered. It is feasible to imagine that, in different coral families, different strains will carry out identical vital tasks for survival not just for the reef ecosystem but also to carry on global climate regulating processes such as cloud formation (Jones *et al.* 2007).

Conclusion

This review has described some of the beneficial roles played by bacterial communities associated with scleractinian corals. In particular it has been shown that gamma-proteobacteria are dominant in immunity and nutrition whilst alpha-proteobacteria take secondary place, which is unsurprising considering gamma-proteobacteria make up a greater proportion of the community (Rohwer *et al.*, 2002).

A problem with coral microbiology is that, even though there have been significant leaps forward in research, it is a comparatively young field with the number of scientists much lower than those working in human health. For example, *Vibrio cholera*e in humans is well understood, but by comparison barely anything is known about similar *Vibrio* spp. coral (Bourne *et al.*, 2009). There are gaps in a range of research areas, for example in establishing archaea and virus diversity and their functions in the holobiont, since most research is focussed on bacteria. However, high quality research over the past few decades has established the general bacterial community structure and made some headway into their roles both beneficial and antagonistic. It has been conclusively demonstrated that reef biodiversity is in decline and their plight highlights the need to expand knowledge on areas such as holobiont health, disease and nutrition. There could be additional research looking at antagonistic and mutualistic interactions in resident host bacteria which might influence community structure and functions which is justification for further research on coral bacteria in a dissertation project.

References

Allen, G.R., and Steene, R., Indo-Pacific Coral Reef Field Guide, Singapore, Tropical Reef Research, 1-15

Baker, A.C. (2004) Symbiont diversity on coral reefs and its relationship to bleaching resistance and resilience. In *Coral Health and Disease*, Rosenberg, E., and Loya, Y. (eds), New York, Springer-Verlag, 177-194

Ben-Haim, Y., Banim, E., Kushmaro, A., Loya, Y., and Rosenberg, E. (1999) Inhibition of photosynthesis and bleaching of zooxanthellae by the coral pathogen *Vibrio shiloi. Environmental Microbiology* **1:** 223-229

Ben-Haim, Y., and Rosenberg, E. (2004) Temperature regulated bleaching and tissue lysis of *Pocillopora damicornis* by the novel pathogen *Vibrio coralliilyticus*. In *Coral Health and Disease*, Rosenberg, E., and Loya, Y. (eds), New York, Springer-Verlag, 301-324

Bourne, D.G., and Munn, C.B. (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Environmental Microbiology* **7:** 1162-1174

Bourne, D.G., Iida, Y., Uthicke, S., and Smith Keune, C. (2008) Changes in coral associated microbial communities during a bleaching event. *The ISME Journal* **2:** 350-363

Bourne, D.G., Garren, M., Work, T.M., Rosenberg E., Smith, G.W., Harvell, C.D. (2009) Microbial disease and the coral holobiont. *Trends in Microbiology* **17:** 554-562

Brown, B.E., and Bythell, J.C. (2005) Perspectives on mucus secretion in reef corals. *Marine Ecology Progress Series* **296:** 291-309

Bruno, J.F., and Selig, E.R. (2007) Regional decline of coral cover in the Indo-Pacific: Timing, Extent and Subregional Comparisons. *PLoSONE* **2;** 8; e711. doi:10.1371/journal.pone.0000711 [cited 5th April 09]

Buddemeier, R.W., and Fautin, D.G. (1993) Coral bleaching as an adaptive mechanism, a testable hypothesis. *Bioscience* **43:** 320-326

Buddemeier, R.W., Baker, A.C., Fautin, D.G., and Jacobs, J.R. (2004) The Adaptive hypothesis of bleaching. In *Coral Health and Disease*, Rosenberg, E., and Loya, Y (eds), New York, Springer-Verlag, 427-444

Coffroth, M.A. (1990) Mucus sheet formation on poritid corals: an evaluation of coral mucus as a nutrient source on reefs. *Marine Biology* **105:** 39-49

Ducklow, H.W., and Mitchell, R. (1979) Bacterial populations and adaptations in the mucus layers on living corals. *Limnology and Oceanography* **24:** 715-725

Francis, C.A., Beman, J.M., and Kuypers, M.M.M. (2007) New processes and players in the Nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *The ISME Journal* **1:**19-27

Frias-Lopez, J., Zerkle, A.L., Bonheyo, G.T., and Fouke, B.W. (2002) Partitioning of bacterial communities between seawater and healthy, Black Band diseased, and dead coral surfaces. *Applied and Environmental Microbiology* **68:** 2214-2228

Garrison, V.H., Shinn, E.A., and Foreman, W.T. et al. (2003) African and Asian Dust: From desert soils to coral reefs*. Bioscience* **53:** 469-480

Gochfeld, D.J., and Aeby, G.S. (2008) Antibacterial chemical defences in Hawaiian corals provide possible protection from disease. *Marine Ecology Progress series* **362:** 119-128

Goldberg, W.M. (2002) Feeding behavior, epidermal structure and mucus cytochemistry of the scleractinian *Mycetophyllia reesi*, a coral without tentacles. *Tissue and Cell* **34:** 232-245

Harder, T., Lau, S.C.K., Dobretsov, S., Fang, T.K., and Qian, PY. (2003) A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. *FEMS Microbial Ecology* **43:** 337-347

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* **50:** 839-866

Jones, G., Curran, M., Broadbent, A., King, S., Fischer, E., and Jones, R. (2007) Factors affecting the cycling of dimethylsulfide and dimethylsulfoniopropionate in coral reef waters of the Great Barrier Reef. *Environmental Chemistry* **4:** 310-322

Kellogg, C.A. (2004) Tropical archaea: diversity associated with the surface microlayer of corals. *Marine Ecology Progress Series* **273:** 81-88

Kelman, D., Kashman, Y., Hill, R.T., Rosenberg, E., and Loya, Y. (2009) Chemical warfare in the sea: The search for antibiotics from Red Sea corals and sponges. *Pure Applied Chemistry* **81:** 1113-1121

Koren, O., and Rosenberg, E. (2006) Bacteria Associated with Mucus and Tissues of the Coral *Oculina patagonica* in Summer and Winter. *Applied and Environmental Microbiology* **72:** 5254-5259

Kushmaro, A., and Kramarsky-Winter, E. (2004) Bacteria as a source of coral nutrition. In *Coral Health and Disease*, Rosenberg, E., and Loya, Y. (eds), New York, Springer-Verlag, 301-324

Ledyard, K.M., DeLong, E.F., Dacey, J.W.H. (1993) Characterisation of a DMSP degrading bacterial isolate from the Sargasso Sea. *Archives of Microbiology* **160:** 312-318

Leggat, W., Ainsworth, T., Bythell, J., Dove, S., Gates, R., Hoegh-Goldberg, O., Iglesias-Prieto, R. and Yellowlees, D. (2007) The hologenome theory disregards the coral holobiont. *Nature Reviews Microbiology* **5:** doi:10.1038/nrmicro1635-c2

Lesser, M.P., Mazel, C.H., Gorbunov, M.Y., and Falkowski, P.G. (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. *Science* **305:** 997-1000

Little, A.F., van Oppen, M.J.H., and Willis, B.L. (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science* **304:** 1492-1494

Lyons, M.M., Aas, P., Pakulski, J.D., Van Waasbergen, L., Miller, R.V., Mitchell, D.L., and Jeffrey, W.H. (1998) DNA damage induced by ultraviolet radiation in coral-reef microbial communities. *Marine Biology* **130:** 537-543

Mullen, K.M., Peters, E.C., and Harvell, C.D. (2004) Coral resistance to disease. in *Coral Health and Disease*, Rosenberg, E., and Loya, Y. (eds), New York, Springer-Verlag, 377-399

Munn, C.B. (2004) Marine Microbiology Ecology and Applications, Garland Science/BIOS Scientific Publishers, UK, 85-114

Munn, C.B. (2006) Viruses as pathogens of marine organisms – from bacteria to whales. *Journal of the Marine Biological Association* **86:** 453-467

Mydlarz, L.D., Holthouse, S.F., Peters, E.C., and Harvell, C.D. (2008) Cellular responses in sea fan corals: granular amoebocytes react to pathogen and climate stressors. *PLoS1* **3:** e1811

Nissimov, J., Rosenberg, E., and Munn, C.B. (2009) Antimicrobial properties of resident coral mucus bacteria of *Oculina patagonica*. *FEMS Microbial Letters* **292:** 210-215

Olson, N.D., Ainsworth, T.D., Gates, R.D., and Takabayashi, M. (2009) Diazotrophic bacteria associate with Hawaiian *Montipora* corals: Diversity and abundance in correlation with symbiotic dinoflagellates. *Journal of Experimental Marine Biology and Ecology* **371:** 140-146

Paul, J.H., DeFlaun, M.F., and Jefferey, W.H. (1986) Elevated levels of microbial activity in the coral surface microlayer. *Marine Ecology Progress Series* **33:** 29-40

Raina, J., Tapiolas, D., Willis, B.L., and Bourne, D.G. (2009) Coral-associated bacteria and their role in biogeochemical cycling of sulphur. *Applied and Environmental Microbiology* **75:** 3492-3501

Rappe, M.S., and Giovannoni, S.J. (2003) The Uncultured microbial majority. *Annual Review of Microbiology* **57:** 369-394

Reshef,L., Koren, O., Loya,Y., Zilber-Rosenberg, I., and Rosenberg, E. (2006) The Coral Probiotic Hypothesis. *Environmental Microbiology* **8:** 2068-2073

Ritchie, K.B. (2006) Regulation of microbial populations by coral surface mucus and mucus associated bacteria. *Marine Ecology progress series* **322:** 1-14

Ritchie, K.B., and Smith, G.W. (2004) Microbial Communities of coral Surface Mucopolysaccharide layers. In *Coral Health and Disease*, Rosenberg, E., and Loya, Y. (eds), New York, Springer-Verlag, 301-324

Rohwer, F., Breitbart, M., Jara, J., Azam, F, and Knowlton, N. (2001) Diversity of bacteria associated with the Caribbean coral *Montastrea franksi. Coral Reefs* **20:** 85-91

Rohwer, F., Seguritan, V., Azam, F., and Knowlton, N. (2002) Diversity and distribution of coral associated bacteria. *Marine Ecology Progress Series* **243:** 1-10

Rosenberg, E., Koren, O., Reshef., Efrony, R., and Zilber-Rosenberg, I. (2007) The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology* **169.236.65.21**

Rowan, R., Knowlton, N., Baker, A., and Jara, J. (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleasheing, *Nature* **388:** 265-269

Rowan, R. (2004) Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* **430:** doi:10.1038/430742a

Shashar, N., Cohen, Y., Loya, Y., and Sar, N. (1994) Nitrogen fixation (acetylene reduction) in stony corals: evidence for coral-bacteria interactions. *Marine Ecology Progress Series* **111:** 259-264

Slattery, M., McClintock, J.B., and Heine, J.N. (1995) Chemical defenses in Antarctic soft corals: evidence for antifouling compounds. *Journal of Experimental Marine Biology and Ecology* **190:** 61-77

Smith, D.J, Suggett, D.J., and Baker, N.R. (2005) Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? *Global Change Biology* **11:** 1–11

Sorokin, Y.I. (1973) on the feeding of some scleractinian corals with bacteria and dissolved organic matter. *Limnology and Oceanography* **18:** 380-385

Sotka, E.E., and Thacker, R.W. (2005) Do some corals like it hot? *Trends in Ecology and Evolution* **20:** 59-62

Van Oppen, M.J.H., Leong, J.A. and Gates, R.D. (2009) Coral-virus interactions: A double-edged sword? *Symbiosis* **47**: 1-8

Wakefield, T.S., and Kempf, S.C. (2001) Development of host- and symbiont-specific Monoclonal antibodies and confirmation of the origin of the symbiosome membrane in a Cnidarian–Dinoflagellate symbiosis. *Biology Bulletin* **200:** 127-143

Wegley, L., Yu, Y., Breitbart, M., Casas, V., Kline, D.I., and Rohwer, F. (2004) Coral associated archaea. *Marine Ecology Progress Series* **273:** 89-96

Wild, C., Huettel, M., Klueter, A., Kremb, S.G., Rasheed, M.Y.M., and Jorgensen, B.B. (2004) Coral Mucus Functions as an energy carrier and particle trap in the reef ecosystem. *Nature* **428:** 66-70