

Chapter 6

SUMMARY AND CONCLUSION

Sponges belong to the phylum Porifera that has been estimated to comprise at least 15,000 extant species with 85% belonging to class Demospongiae. These sessile metazoans are filter feeders capable of turning over many thousands of litres of water per day. Prokaryotic microorganisms, as well as nano and pico-eukaryotes, are the most important diet of these sponges (Pile et al, 1997; Ribes et al, 1999). Sponge-microbe association involves a diverse range of heterotrophic bacteria, cyanobacteria, dinoflagellates, diatoms etc. These microbes colonize all the three cell layers of the sponges (intercellular and intracellular matrix) and occupy up to 60% of the sponge volume and this underlines the ecological relevance of sponge-associated microbiota for tropical coral reef ecosystems. Three types of bacterial association have been proposed based on the studies carried out: (i) abundant populations of sponge-specific microbes in the sponge mesohyl (**Type I**) (ii) small populations of specific bacteria occurring intracellularly (**Type II**) and (iii) populations of non-specific bacteria resembling those in the surrounding seawater (**Type III**). With the advent of molecular tools, few precise and general insights into the microbial composition could be gained. Among the sponges, the association of demosponges with microorganisms is explored the most and few basic questions have been answered (Hentschel et al, 2002, 2006; Webster and Hill, 2002). Congruent results of these studies are 1) the presence of sponge-specific clusters or sponge-derived bacterial groups (Hentschel et al, 2002) and 2) sponges belonging to different orders, occupying highly dissimilar habitats, harbouring similar members of microbial communities.

Molecular techniques revealed a common microbial signature in many sponges that is phylogenetically complex yet highly sponge-specific and distantly different from marine planktonic bacteria. Fourteen monophyletic sponge-specific clusters belonging to Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Nitrospira and Proteobacteria were obtained (Hentschel et al, 2002). As common patterns are beginning to emerge, so are the exceptions.

Collectively the microbes confer upon their hosts the potential to exploit an impressive metabolic repertoire including photosynthetic carbon fixation, nitrification, anaerobic metabolism, bioluminescence and secondary metabolite production.

India has 486 species of sponges distributed in vast coral reefs. In spite of the vast diversity, studies on sponge-associated microbes are recent and are restricted to biotechnological aspects - as a source for drug discovery (Thakur and Anil, 2000; Anand et al, 2006; Selvin et al, 2004, 2009). Also, studies on the diversity and function of sponge-associated microbes from Indian waters are very limited (Selvin et al, 2009).

Diversity is a key issue within both eukaryote and prokaryote ecology. In spite of India having vast marine biodiversity, studies on interaction/relationship between eukaryote and prokaryote are limited. In this thesis, an attempt has been made to explore this relationship between microbial diversity and host specificity using marine sponge-bacterial association. This study represents the first comprehensive analysis of bacteria associated with two species of sponges viz. *Dysidea granulosa* and *Sigmadocia fibulata* (Class: Demospongiae) inhabiting the coral reefs of Kavaratti (Lakshadweep) and Gulf of Mannar, both regions are known for the most prolific sponge populations with respect to diversity and biomass. Salient findings of this study are listed below:

- Bacterial abundance in the two species of sponges was in the order of 10^{7-8} CFU g⁻¹ of sponge tissue (w/w) which was 2-3 order higher than

that of ambient water, irrespective of the media and methods used. This high abundance of associated bacteria indicates their overall importance.

- Abundance of bacteria showed both spatial and temporal variations in both sponge species. High recoverability of sponge-associated bacteria was in 10-25% nutrient agar media.
- Gammaproteobacteria was the most predominant group in both species of sponges and ambient water. In the sponges, *Vibrio* was the dominant genus present. Other associated bacteria were, *Bacillus*, *Micrococcus* and *Staphylococcus*. These groups showed spatial and temporal variability in occurrence and abundance and can be grouped as type III (Cosmopolitan).
- Type II bacteria were *Pseudovibrio denitrificans* and *Ruegeria* sp. (Alphaproteobacteria).
- Two novel species have been isolated from the sponge *S. fibulata* from Kavaratti
 1. *Formosa* sp. (93% similarity with the nearest culturable neighbour)

Phylum: Bacteroidetes; Class: Flavobacteriales
 2. *Kiloniella laminariae* (91% similarity with the nearest culturable neighbour)

Phylum: Proteobacteria; Class: Alphaproteobacteria;

Family : Kiloniellales
- 16S rDNA PCR - RFLP analysis revealed 48 different phlotypes in sponge. Culturable bacteria belonged to 4 different phyla ie, Phylum Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. In Proteobacteria, both Alpha and Gamma groups were present.
- Denaturing Gradient Gel Electrophoresis (DGGE) finger print-based analysis revealed statistically different banding patterns of bacterial

communities in both the species of sponges with *D. granulosa* having the greater diversity (Shannon-Wiener diversity index- 3.1).

- PCR-16S rDNA analysis of both sponges revealed diverse and complex bacterial assemblages represented by 8 phyla - Acidobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, Firmicutes, Proteobacteria, Spirochaetes and Thermotogae.
- In Proteobacteria, Alpha, Gamma, Delta and Epsilon classes were present.
- Type I bacteria - Cyanobacteria was recorded only in sponge *S. fibulata*
- Proteobacteria, Chloroflexi and Firmicutes were the major phyla associated with both the sponges. However, some sponge associates could be considered as transient fauna due to variation observed.
- The temporal and spatial variability in abundance of sponge bacteria and Type III diversity depended on availability of the substrates in water and host as well as bacterial abundance and diversity of water. Selective elimination of the host also should be considered.
- Ecological aspects of sponge–microbes were delved for the first time. Metabolic function, activity and benefit to the sponge were inferred with the knowledge of the taxonomic nature of sponge-associated microbes.
- Although the exact relationship between sponge and bacterial association could not be established due to the complexity of live host association, the following circumstantial hypothesis could be drawn on nutrition and defense mechanisms, based on the results obtained:
 1. To the coral reef sponge, the harboured microbial populations are highly active and capable of mineralizing a wide variety of compounds which provide nutrition to the hosts. Amylase and phosphatase were the most expressed enzymes.

2. Sponges or the associated bacteria are capable of producing bioactive compounds to deter pathogenic microbial attack and at the same time would support beneficial microbes and rely heavily on the production of chemicals as a form of defense. Defense was associated with the antibacterial activity. Majority of sponge-associated bacteria exhibited defense mechanism against gram negative and gram positive bacteria.
3. To the bacteria, sponge proved a safe abode and food. The versatile capacity (hydrolytic activity and utilization of simple molecules) of the sponge would have added advantage to the microbes to compete in host by increasing its production rate so that even if a few get ingested, a constant number can be maintained, and in case not ingested, it can provide nutrients for the sponge by hydrolysis of complex molecules.
4. Sponge-microbe association seems to be more of commensalism/mutualism (mutual sharing and not true symbiosis) as most of the species were cosmopolitan with a few phyla being host associated or host specific.

Conclusions and Future Directions

- The two sponge species are conducive platform for the enrichment of bacteria. 16S rDNA based molecular and culture-based approaches used for describing the community associated with *D. granulosa* and *S. fibulata* were valuable in revealing the large diversity of bacteria associated with these sponges. This work provides an excellent resource of several candidate bacteria for production of novel pharmaceutically important compounds.
- Currently very little information exists on the spatial distribution of associated bacteria within the sponge. Different microbial profiles are likely to exist between exterior and interior tissue samples. Hence, knowledge of the location of associated species in the sponge may

provide information about the preferred microenvironment of a particular strain, which can be used to design cultivation experiments as these organisms are still regarded as mostly uncultivable and underrepresented.

- Understanding the variability and specificity of sponge-bacterial association will help to understand the bacterial population dynamics.
- The presence of Archaea in sponges has been reported lately and the diversity and functional aspects are yet to be studied. Similarly, the potential role of phages and protozoa in regulating microbial communities within the sponge is a new area to be investigated.
- Sponge-bacteria contributed to their nutrient cycling and defense. Many fundamental aspects of metabolism and evolution of symbionts are yet to be understood. The application of metagenomics would provide access to sponge microbiota as a rich source for novel enzymes and metabolites. It would also open new avenues for sustainable production of biotechnologically relevant compounds and lay the ground work for future studies on the role of these diverse microbes in ecology, evolution, and development of marine sponges.
- However, construction and handling of even larger libraries will be necessary to cover the complex microbial diversity and function. The improvement in high throughput sequencing capacities will make environmental genomics analyses of sponge micro-biota a manageable task.
- The single cell genomic approach reveal link between function and phylogeny of bacteria associated with sponges by cloning the entire gene clusters from single bacterial cell of known phylogenetic origin.

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