

Chapter 2

REVIEW OF LITERATURE

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 - 2.2. Sponges
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2.1. Coral Reef Ecosystems

Coral reefs are tropical, shallow water ecosystems, largely restricted to the area between the Tropics of Cancer and Capricorn. Lying between latitudes 30⁰N and 30⁰S and they form only about 0.2% of earth's ocean area (Kleypas, 1997). These carbonate structures are found mostly in warm, shallow (2-70 m), well-lighted zone of tropical seas with salinity of 32-35 and temperature above 20°C. The reefs are classified based on the location and form. The table below lists the different types of reefs.

The term 'coral' refers to coelenterates secreting a massive calcareous skeleton, particularly of the order Scleractinia (Class: Anthozoa). Many groups of extinct and extant organisms have aggregated to form reefs for over 3.5 billion years (Wood, 1998). They rank as among the most biologically productive and diverse of all natural ecosystems, their high productivity stemming from their efficient biological recycling, high retention of nutrients and their structure which provides habitat for a vast array of other organisms. Also, as substantial topographic structures, coral reefs protect coastlines from erosion and help create sheltered harbors. Reefs and their associated carbonate sediments are also important as storehouses of organic carbon and as regulators of atmospheric carbon dioxide, which in turn could influence climate and sea-level fluctuations (Opdyke, 1992). Thus, their influence is global and multifaceted.

Oceans	Types of Coral Reefs		
	Atoll	Barrier Reef	Fringing Reefs
Pacific Ocean	American Samoa, China, Cooks, Fed. St. Micronesia, Fiji, French Polynesia, Hawaii & other U.S. Is., Kiribati, Marshalls, New Caledonia, Palau, Papua New Guinea, U.K. (Pitcairn), Solomons, Taiwan, Tokelaus, Tuvalu, Vanuatu	American Samoa, E. Australia, Cooks, Fed. St. Micronesia, Fiji, French Polynesia, Guam, Hawaii & other U.S. Is., New Caledonia, N. Marianas, Palau, Papua New Guinea, Solomons, Tokelaus, Tonga, Wallis & Futuna	E. Australia, China, W. Columbia, Cooks, W. Costa Rica, Ecuador, Fed. St. Micronesia, Fiji French Polynesia, Guam, Hawaii & other U.S. Is. Japan, New Caledonia, Niue, N. Marianas, Palau, Papua New Guinea, U.K. (Pitcairn), Solomons, Taiwan, Tonga, Vanuatu, Wallis & Futuna, W. Samoa
Atlantic Ocean	Bahamas, Belize, Brazil, E. Columbia, Cuba, Mexico, Venezuela	Anguilla, Antigua & Barbuda, Bahamas, Barbados, Belize, Bermuda, British Virgins, E.Columbia, Cuba, Dominican Republic, Grenada, Guadeloupe, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat & St. Kins Nervis, Netherlands Antilles, Nicaragua, Puerto Rico, St. Martin & St. Barthelemy, St. Vincint, Trinidad & Tobago, Turks & Caicos, U.S.A. Atlantic/Gulf Coast, U.S. Virgins, Venezuela.	Anguilla, Antigua & Barbuda, Bahamas, Barbados, Belize, Bermuda, Brazil, British Virgins, Caymans, E. Columbia, E. Costa Rica, Cuba, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat & St. Kins Nervis, Netherlands Antilles, Nicaragua, E. Panama, Puerto Rico, St. Lucia, St. Martin & St. Barthelemy, St. Vincint, Trinidad & Tobago, Turks & Caicos, U.S. Virgins, Venezuela
Indian Ocean	Bahrain, Reunion, Saudi Arabia, Sudan, W. Australia, Chagos, India, Indonesia Maldives, Seychelles	Bahrain, Madagascar, Mauritius, Qatar, Reunion, Saudi Arabia, Somalia, Sudan, W. Australia, Comoros, India, Indonesia.	Bahrain, Djibouti, Egypt, Ethiopia, Iran, Israel, Jordan, Kenya, Kuwait, Madagascar, Mauritius, Mozambique, N. Yemen, Oman, Qatar, Reunion, Saudi Arabia, Somalia, Sudan, W. Australia, Burma, Comoros, India, Indonesia, Sri Lanka.

Table 1: Coral reefs of the world

2.2. Sponges

Sponges originated in the Pre-cambrian era, represent the very base of metazoan evolution and can be regarded as the oldest animal phylum still alive (Wilkinson, 1984). These multi-cellular animals belong to phylum Porifera (pore bearers) and possess relatively little in the way of differentiation and coordination of tissues. They are classified into three taxonomic classes namely Calcarea (calcareous sponges), Hexactinellida (glass sponges) and Demospongiae (demosponges). An estimate of 15000 species (Hooper and van Soest, 2002) inhabit a wide variety of marine and fresh water habitats and are found throughout the tropical, temperate and polar regions (Figure 1). Of all the described classes of sponges, the Class Demospongiae is the most abundant, forming 85% of the sponges (Hooper and van Soest, 2002).

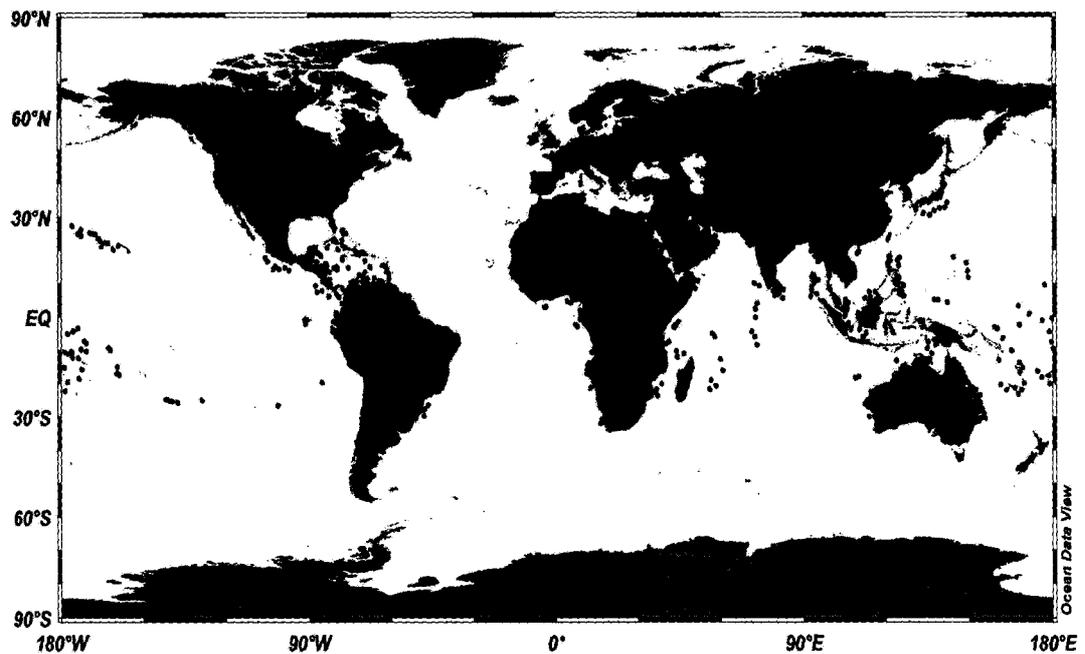
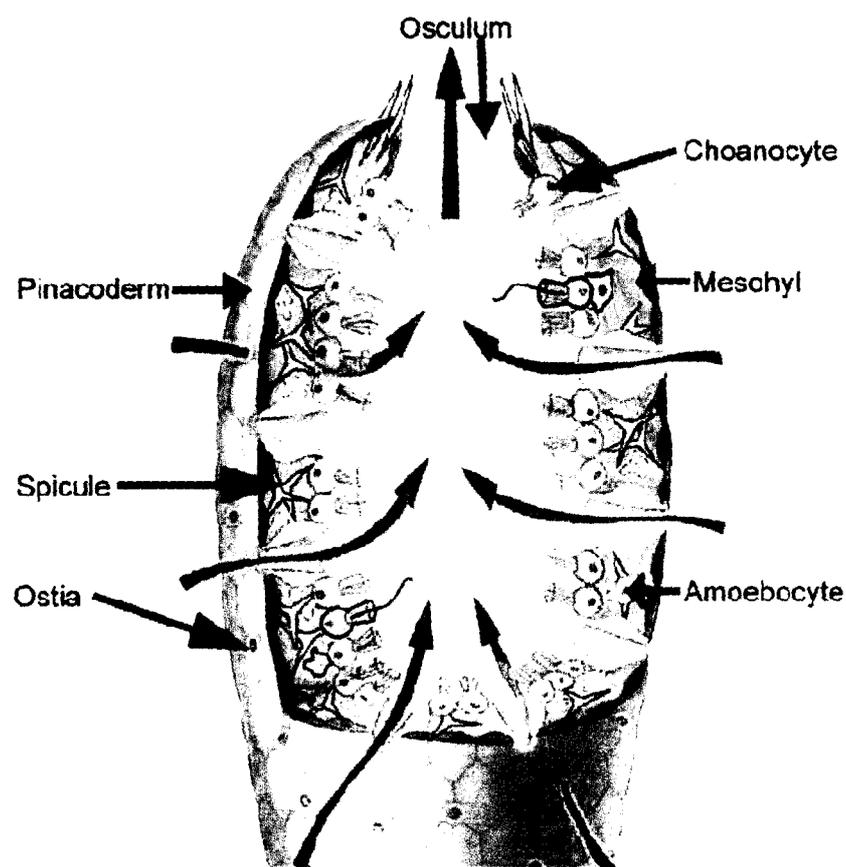


Figure 2: World distribution of sponges associated with coral reefs (Dots in black)

Sponges are sedentary filter-feeding organisms, characterized by an unusual body plan built around a system of water canals and chambers. They possess an elaborate aquiferous system comprising of the inhalant openings called the ostia and the exhalant openings called the osculum. The basic body plan consists of three different layers namely pinacoderm, mesohyl and choanoderm. The outer pinacoderm is made of epithelial cells known as pinacocytes. The middle mesohyl, which is the extensive layer of connective tissue consisting of phagocytic cells called the amoebocytes. The inner most layer called the choanoderm consists of the flagellated cells called the choanocytes which line the canals. Thus, despite such simple body plan, the sponges are remarkable in pumping large volumes of water (upto $24 \text{ m}^3 \text{ kg}^{-1} \text{ sponge day}^{-1}$) through their aquiferous system. They ingest particles of sizes between 5 and 50 μm through the cells of mesohyl and the pinacoderm and microparticles of size 0.3 to 1 μm via the cells of the choanocyte chambers (Vogel, 1977) (Figure 2).



Bacterial Diversity: Evolution and Speciation in a Model Host-Parasite System of the genus on the sponges and their associated bacteria

Figure 2.2: Schematic diagram of a partially sectioned sponge

Sponges play key functional roles that influence the coral survival and coral reef geology and ecology. Sponges can increase coral survival by binding live corals to the reef frame (Wulff and Buss, 1979; Wulff, 1984). Sponges also shelter juvenile spiny lobsters (Butler et al, 1995) and numerous invertebrate and algal symbionts (Beebe, 1928; Ribeiro et al, 2003; Cerrano et al. 2000, 2004). Among marine invertebrates, sponges are the most prolific phylum, with regard to presence of novel pharmacologically active compounds (Thomas et al, 2010). Recently, the spicules of the sponges have been discovered as effective light-collecting optical fibers Muller et al, (2009). Sponges have been the focus of much recent interest due to two other reasons namely production of biologically active secondary metabolites and their close association with a wide array of microorganisms (Taylor et al, 2007).

2.3. Microbial Association with Sponges

2.3.1. International Scenario:

2.3.1.1. Introduction

Sponge-associated microorganisms were first observed using light microscopy in sections of sponge tissues by Feldmann (1933). The advent of electron microscopy and its application opened up a new avenue in sponge microbiology. Studies on sponge associated bacteria date back to the seventies. The pioneering work of Vacelet and colleagues (1977), shed light on the presence of bacteria using electron microscopy, though it was known that microorganisms are ingested by the sponges for nutrition (Reiswig, 1971, 1975; Bergquist, 1978). This was followed by examination of bacterial cells in the sponge tissue using microscopic, immunological and autoradiographic techniques (Wilkinson, 1978a, b, c; 1984; Wilkinson et al, 1984; Simpson, 1984). Based on these studies, it was established beyond doubt that many sponges harbour abundant bacteria. Their efficiency in clearing bacteria (at least 95% removed) and other small plankton from the

water column (Reiswig, 1971; Pile, 1997), with increased water clarity has an obvious advantage of filtering out the pathogens. Further, the filter feeding properties of the sponges help in the transfer of microorganisms to the mesohyl region of the sponges, which may either be ingested by the phagocytic cells or become established as sponge-specific microbiota (Kennedy et al, 2007) which thrive both extracellularly and intracellularly (Haygood et al, 1999; Sumich, 1992; Althoff et al, 1998; Friedrich et al, 1999). Host organisms such as sponges provide a unique set of environmental conditions for microbial colonization that is very much different from the surrounding sea water. Thus marine organisms function as habitat islands allowing allopatric speciation of microbes living in physically separated hosts (Begon et al, 1996; Powledge, 2003). The surfaces or internal spaces of the sponges are more nutrient-rich than sea water and therefore sponges offer nourishment and a safe habitat to their associated microorganisms (Bultel-Ponce et al, 1999). About 50-60% of the biomass of the sponges is composed of bacteria (Vacelet and Donadey, 1977; Usher et al, 2004) that is equivalent to 10^8 - 10^{10} bacteria per gram wet weight of sponge (Hentschel et al, 2006). All the sponges may not contain the same levels of microorganisms (Hentschel et al, 2006). 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 (ii) Small populations of specific bacteria occurring intracellularly (iii) Populations of non-specific bacteria resembling those in the surrounding seawater. Among the sponges, the association of many demosponges with microorganisms is well recognized (Hentschel et al, 2003, 2006; Hill, 2004; Imhoff and Stohr, 2003).

Diversity

Sponge associated microbial diversity may in part be explained by the varying physical, chemical, and biological conditions within the sponge host, which may affect microbial ecology (e.g., number of species supported in the system; relative abundance) and evolution (e.g., specialization through niche

partitioning). These are obligate mutual associations which may exist since the Precambrian, before the evolution of the extant sponge orders and classes took place.

Culture-Dependent Studies

Since 1970s, majority of studies investigating the bacterial communities associated with corals have focused on the culturable bacteria. Earliest studies based on culturing techniques concluded that sponge-associated bacteria were similar to that of the ambient water (Bertrand and Vacelet 1971; Madri et al, 1971). It was only a decade later that the idea of bacteria being specifically associated with members of the phylum Porifera understood and was first given by the isolation of a unique bacterium from the sponge *Verongia aerophoba* by Wilkinson et al, (1981). A community of morphologically diverse bacteria has been found to be associated with various marine sponges through cultivation (Santavy et al, 1990; Olson et al, 2000; Hentschel et al, 2001; Webster and Hill, 2001). This was followed by other studies which reiterated the presence of sponge-specific bacteria which were not obtained from the surrounding seawater (Wilkinson, 1978a, b; Wilkinson et al, 1981, 1984; Santavy and Colwell, 1990; Althoff et al, 1998). Also, immunological and microscopic studies of Wilkinson and colleagues (1981; 1984) suggested the symbiotic relationship between bacteria and sponges. Each of the three domains of life, Bacteria, Archaea and Eukarya are known to inhabit in the sponges (Santavy and Colwell, 1990; Douglas, 1994; Preston, 1996; Larkum et al, 1987). The advent of molecular techniques made possible the characterization and confirmation of sponge-specific clusters or sponge-derived bacterial groups (Hentschel et al, 2002). Sponges belonging to different orders, occupying highly dissimilar habitats, harbour similar members of microbial communities (Fiesler et al, 2004; Lafi et al, 2005; Hill et al, 2006; Thiel et al, 2007). Marine Actinomycetes related to the *Salinospora* group previously reported only from marine sediments were isolated from the Great Barrier Reef marine sponge *Pseudoceratina clavata* and South China Sea sponge,

Hymeniacidon perleve (Sun et al, 2010; Kim et al, 2005). Novel Actinobacteria was obtained from the sponge *Xestospongia muta* from the Pacific and Atlantic Oceans suggesting that these bacteria could be true symbionts of the sponges (Montalvo et al, 2005). However, the diversity of the culturable microbial communities examined in two sponge species- *Pseudoceratina clavata* and *Rhabdastrella globostellata* showed the presence of Firmicutes and Alphaproteobacteria (Lafi et al, 2005). Interestingly, Pimentel-Elardo, (2003), reported the presence of culturable Planctomycetes from marine sponges which grew slowly on oligotrophic media and failed to grow on nutrient-rich media.

Culture-Independent Studies

Culture-based techniques are inadequate for studying bacterial diversity owing to the fact that in many environmental samples about 99% of the bacteria cannot be cultured using traditional techniques (Rappe and Giovannoni, 2003). More recent studies on association of bacteria, archaea, and cyanobacteria with sponges were based mainly on culture-independent molecular methods, especially 16S rRNA approach (Preston et al, 1996; Hentschel et al, 2002; Fieseler et al, 2004; Thiel et al, 2007). This molecular approach has revealed a high diversity of environmental and symbiotic bacteria undiscovered by cultivation methods. The 16S rRNA clone libraries obtained from sponges of different phylogenetic and geographical origin have revealed complex and sponge-specific bacterial communities (Hentschel et al, 2002; Hill et al, 2006). Fourteen monophyletic sponge-specific clusters belonging to Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Nitrospira and Proteobacteria were obtained (Hentschel et al, 2002). Taylor et al, (2004) identified three types of sponge-associated bacteria by DGGE analysis of three sponges, *Cymbastela concentrica*, *Callyspongia* sp. and *Stylinos* sp.: 'specialists'- found on only one host species, 'sponge associates'- found on multiple hosts but not in seawater, and 'generalists' from multiple hosts and seawater. Recently a great diversity of unique and previously unrecognized microorganisms

associated with sponges has been revealed using culture-independent molecular methods such as 16S rRNA clone library analysis, DGGE and FISH. These techniques have been used in investigating the bacterial diversity in different geographic regions such as Western Australia, China Sea, Mediterranean, Caribbean, Red Sea and Antarctica (He et al, 2006; Li et al, 2007; Li and Liu, 2006; Meyer and Kuever, 2008; Muscholl-Silberhorn et al, 2008; Taylor et al, 2004; Thiel et al, 2007; Meyer and Kuever, 2008). Alphaproteobacteria has been identified as an important group in deep-water sponges (Olson et al, 2002). Numerous investigations on bacterial diversity based on culture-independent methods have been conducted widely (Hinde et al, 1999; Althoff et al, 1998; Burja et al, 1999; Burja and Hill, 2001; Webster and Hill, 2001; Webster et al, 2001, Thiel et al, 2007). Nested-PCR method revealed high diversity of Actinobacteria (ten genera) associated with the marine sponge, *Hymeniacidon perleve* and an unidentified sponge from South China Sea (Xin et al, 2008). Bacterial diversity in the breadcrumb sponge, *Halichondria panacea* showed the presence of specific *Roseobacter* group (Wichels et al, 2006). Diversity of microbes associated with the Antarctic sponges showed that bacterial communities clustered within the Proteobacteria and Bacteroidetes (Webster et al, 2004). Denaturing gradient gel electrophoresis (DGGE) analysis of *Aplysina cavernicola* revealed the presence of the Alphaproteobacteria, Gammaproteobacteria and the phylum Bacteroidetes (Thoms, 2003). Fieseler (2004) suggested the status of a new candidate phylum, named "Poribacteria", to acknowledge the affiliation of the new bacterium with sponges in addition to Planctomycetes, Verrucomicrobia, and Chlamydia while studying the bacterial diversity in sponges of Bahamas such as *Aplysina fistularis*, *Aplysina insularis*, *Aplysina lacunosa*, *Verongula gigantea*, and *Smenospongia aurea*. Demospongiae have been studied by 16S rRNA clone libraries and sequencing (Turque et al, 2008). PCR-DGGE community analysis showed the presence of Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria in *Dysidea avara* and only Gammaproteobacteria in *Craniella australiensis* which are sponges from the South China Sea (Le et al, 2006). Complex nitrogen cycling has been

reported in the sponge *Geodia barrette* from west coast of Norway. Ammonia oxidizing archaea and Candidatus *Scalindua brodae* which are known to be associated with the nitrogen cycle has been reported from this sponge (Hoffmann et al, 2009).

Archaeobacteria are also found to be associated with the sponges. The presence of Archaea (group I crenarchaeotes and group II euryarchaeotes) was reported for the first time in a sponge inhabiting the Great Barrier Reef, *Rhopaloeides odorabile* using 16S ribosomal RNA community analysis (Webster, 2001). A molecular analysis of archaeal communities in eight sponges collected along the coast of Cheju Island, Korea using terminal-restriction fragment length polymorphism (T-RFLP) in conjunction with sequencing analysis of 16S rDNA clones. The terminal-restriction fragment (T-RF) profiles showed that each sponge had a simple archaeal community represented by a single major peak of the same size except for one unidentified sponge (Lee, 2003).

Temporal variability in the composition of sponge-associated bacterial communities by several investigators is minor suggesting a seemingly stable relationship between sponge and the bacteria (Friedrich et al, 2001; Webster and Hill, 2001; Taylor et al, 2004). It was shown that bacterial community composition in the marine sponge *Cymbastela concentrica* from Australia was reported to be different between temperate and tropical environments while very similar over 500 km separated temperate regions (Taylor et al, 2007). As information on normal microbiota is important to understanding the significance of microbes in structuring healthy aquatic ecosystems a study of the comparison of bacterial communities of the wild and captive sponge *Clathria prolifera* from Chesapeake Bay was carried out by Isaacs et al, (2009). The study showed that the bacterial communities differed significantly in the two thus restating the significant effect of aquaculture on bacterial community composition. As the sponge-microbe association can also be pathogenic numerous work has been carried out on diseased sponges (Vacelet and Gallissian, 1978; Webster et al, 2002, 2008; Olson et

al, 2006; Cervino et al, 2006). Such studies showed that the microbial community associated with diseased and healthy sponges had distinct differences at all taxonomic levels which are significant information as sponges have ecologically significant role.

Biotechnological Relevance

Bacteria associated with sponges are a rich source of antimicrobial compounds (Stierle et al, 1988; Shigemori et al, 1992). Even though sponge-associated prokaryotes are difficult to culture (Amman et al, 1995; Schmidt et al, 2000) many researchers have reported the isolation and structural elucidation of antimicrobial metabolites from sponge-associated bacteria (Bultel-Ponce et al, 1998; Elyakov et al, 1991; Unson et al, 1994). *Pseudoalteromonas maricaloris* isolated from Great Barrier Reef sponge *Fascaplysinopsis reticulata* was a source of two brominated chromopeptides which showed moderate toxicity to the eggs of sea urchin (Blunt et al, 2009; Speitling et al, 2007). *Vibrio* sp., isolated from marine sponge produced a new antibiotic trisindole which showed potential antibacterial activity against *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Kobayashi and Kitagawa, 1994, Kobayashi et al, 1994, Braekman and Dalozze, 2004). Potential bacterial strains from *Haliclona* sp. exhibited antibacterial activity against the pathogenic bacteria. The active strains showed similarity to *Vibrio parahaemolyticus*, *Pseudoalteromonas* and Alphaproteobacteria (Radjasa et al, 2007). Wide range of chemical classes of compounds (eg. Terpenoid, alkaloid, peptides and polyketides) were isolated from sponge-associated microbes with an equally wide range of biotechnological applications (eg; anticancer, antibacterial, antifungal, antiviral, anti-inflammatory and antifouling) (Blunt et al, 2005; Blunt et al, 2006; Fusetani 2004; Keyzers et al, 2005; Matsunaga and Fusetani, 2003; Moore, 2006; Piel, 2004, 2006). More novel bioactive metabolites are obtained from sponges each year than any other marine taxon (Blunt et al, 2006; Munro et al, 1999). The occurrence of important metabolites within the sponge-

associated bacteria opens up the possibility of continuous supply of biologically active compounds by laboratory cultivation of the producer.

Function

Sponge-microbe interactions appear to be relatively stable, with little variation in time and space (Hentschel et al, 2006). Sponge microbial associations have long been documented, but relatively little is known about the nature of their interactions. Sponges and the microorganisms living within and around them display an array of interactions, from microbial pathogenesis and parasitism to microbes as the major food source for heterotrophic sponges and to mutualistic associations where both partners are benefited (Taylor et al, 2007). These diverse microbial symbionts contribute to primary productivity and nutrient regeneration (Wilkinson and Fay, 1979; Wilkinson, 1983b, 1992; Diaz and Ward, 1997). The microorganisms help in the nutritional processes of the sponge either by intracellular digestion or by translocation of metabolites including nitrogen fixation, nitrification and photosynthesis (Wilkinson and Fay, 1979; Wilkinson and Garrone, 1980). The study on the presence of *nifh* genes affiliated with Proteobacteria and Cyanobacteria detected in the sponges, *Ircinia strobilina* and *Mycale laxissima* emphasizes the role of bacteria in the nutrition of the host in nutrient-limited reef environment (Mohamed et al, 2008). Microbes also help in the stabilization of sponge skeleton and chemical defense against predation and biofouling (Wilkinson et al, 1981; Proksch, 1994; Unson et al, 1994). The overall knowledge on the nutritional requirements of bacteria associated with marine sedentary organisms is poorly understood (Fencel, 1993) and warrants further investigation.

Bacterial associates interact with hosts in many ways. For example, they clean channels from decomposing extraneous organic matter and products of sponge metabolism, and aid in maintaining the filtering capacity of the sponge (Wilkinson and Garrone, 1980; Beer and Ilan, 1998). Bacteria living on the surface of marine invertebrates have been found to produce

chemicals that are having potential antibacterial and antifouling activities. The bacteria associated with marine invertebrates are a rich source of bioactive metabolites. Twenty-nine marine bacterial strains were isolated from the sponge *Hymeniacidon perleve* at Nanji island, and antimicrobial screening showed that eight strains inhibited the growth of terrestrial microorganisms (Zheng, 2005). Antimicrobial activity was found in several isolates, two of which were identified as *Rhodococcus* sp. and *Pseudomonas* sp. by partial 16S rRNA gene sequencing. The recovery of strains with antimicrobial activity suggests that marine sponges represent an ecological niche which harbours a largely uncharacterized microbial diversity and a yet unexploited potential in the search for new secondary metabolites (Chelossi, 2004). The antimicrobial activity of three sponge species was tested against marine benthic bacteria and the presence of epibiotic bacteria on their surfaces was investigated to determine whether there is a correlation between antimicrobial activities and the presence of a bacterial film. Gram-positive and Gram-negative bacteria were equally affected by all the sponge extracts. The encrusting sponge *Crambe crambe* featured the strongest antimicrobial activity in the assays and no bacteria were found on its surface (Becerro, 1994). Antimicrobial compounds of sponge-associated bacteria suggested that microbial symbionts play a critical role in the defense of their host sponge (Bultel et al, 1997; Jensen et al, 1994).

Sponges swirl in large volumes of seawater containing organic particles. As filter feeders, sponges are exposed to pollutants present in waters and accumulated impurities from phytoplankton or other suspended matters. Hence, it is reasonable to believe that some microbes in sponges and/or sponges themselves produce hydrolytic enzymes to convert these organic matters into nutrients. Studies using FISH have shown that bacteria within sponges are metabolically active. The results of previous studies on enzymatic activities of microorganisms isolated from sponges showed that many of them can digest proteins, carbohydrates, and organophosphates, with the activity of alkaline phosphatase being especially notable (Efremova et al, 2002). Species of genus *Cytophaga* were isolated from *Halichondria*

panacea that hydrolyze agar (Imhoff and Truper, 1976). Several *Desulfovibrio* with ability to dehalogenate and degrade brominated compounds were isolated from *Aplysina aerophoba* (Ahn et al, 2003). Metagenomic approaches have also identified several novel enzymes. Compared to studies on natural compounds, studies on enzymes with biotechnological potential from microbes associated with sponges are rare. This increasing interest in this research has improved our knowledge of sponge-microbe interaction. Such studies have revealed only a glimpse of the biodiversity of these microbial communities. Many gaps such as, an indepth understanding of microbial diversity, factors that determine the hosts' specificity and physiology of the association still remain unexplored.

National Scenario

Studies on the diversity of sponge-associated microbes from Indian waters are limited and most of them deal with bioactive compounds produced by bacteria (Thakur and Anil, 2000; Selvin et al, 2004, 2009). Thakur and Anil (2000) explored the antibacterial activity of the sponge *Ircinia ramosa* and discussed the importance of surface associated bacteria in this function. This study showed an inverse relationship between epibacterial abundance over the sponge surface in nature and antibacterial activity displayed by the sponge extracts in laboratory bioassays. Thakur et al, 2003 investigated the antibacterial activity of the sponge *Suberites domuncula* and the potential basis for epibacterial chemical defence (2003). Further, the antiangiogenic, antimicrobial and cytotoxic potential of the above sponge, *Suberites domuncula* associated bacteria was investigated (Thakur et al, 2005). Selvin and Lipton (2004) studied the secondary metabolites of three sponges namely, *Dendrilla nigra*, *Axinella donnani* and *Clatharia gorgonoides* and found that the former was a potential candidate for harnessing bioactive drugs. This was followed by the evaluation of synthesis of antibacterial agents by the antagonistic *Streptomyces* sp. isolated from marine sponge *Dendrilla nigra* (Selvin et al, 2004). The role of *Dendrilla nigra* associated Actinomycetes in the production of novel antibiotics is well

documented (Selvin et al, 2009). Recently, an attempt has been made by Thomas et al (2010) on the review of marine drugs produced by sponge-microbe association. The production of industrially important enzymes such as amylase, carboxymethylcellulase and protease by bacteria associated from sponges has been reported (Mohapatra and Bapuji, 1998; Mohapatra et al, 2003). Alkalophilic amylase produced by sponge-associated marine bacterium *Halobacterium salinarum* from sponge *Fasciospongia cavernosa* collected from Vizhinjam, peninsular coast was studied in detail by Shanmughapriya et al, (2009). In addition, fungal amylase from *Mucor* sp. associated with marine sponge *Spirastrella* sp. was also characterized (Mohapatra et al, 1998). The functional role of these Actinobacteria in the phosphate accumulation and solubilization was also investigated (Sabaratnam et al, 2010). Feby and Nair (2010) reported the presence of hydrolytic enzymes from bacteria associated from Lakshadweep sponges such as *Sigmatocia fibulata* and *Dysidea granulosa*. Selvin et al, (2007) also probed into the possibility of sponge associated bacteria as potential indicators for heavy metal pollution. Most of the studies conducted in India are on the biotechnological potential of sponge-associated bacteria and studies on the diversity of the sponge associated bacteria are very meager. Recently, Selvin et al, (2009) studied the diversity of culturable heterotrophic bacteria with special emphasis on the Actinobacteria and found that *Micromonospora-Saccharospora-Streptomyces* was the major culturable actinobacterial group.

A central objective of sponge microbiology is to gain a better understanding of the diversity and predictability of sponge-prokaryote associations in addition to its being a treasure trove of bioactive compounds. Hence studies on the diversity of microbes associated and symbiotic with sponges and development of methods to culture them are therefore important to contribute to the future production of new pharmaceuticals. Systematic studies on the temporal and geographical variation of culture dependant and independent diversity of sponge associated bacteria would contribute to augment the existing knowledge on their ecology.

Therefore, a study was carried out on the bacterial diversity of sponges in the coral reef ecosystems of Kavaratti, with the following objectives:

- To study the temporal and geographical variation in bacterial abundance and diversity associated with sponges in coral reefs of Lakshadweep and Gulf of Mannar.
- To understand the phylogeny of sponge-associated bacteria.
- To comprehend the nature of sponge-bacterial association.

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