Phylogenetic Diversity of Bacterial Community Associated with the Marine Sponge *Halichondria nigrocutis* Collected off Southwest Indian Coast

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**ABSTRACT**

This study aimed to evaluate the bacterial communities associated with the marine sponge *Halichondria nigrocutis* present in Indian waters by both cultivation and cultivation-independent techniques. Results using cultivation method showed that bacteria belonging to *Bacillus*, *Acinetobacter* and *Vibrio* spp. to be the predominant groups. Metagenomic study of sponge-associated bacteria by cultivation independent approach, involving cloning and sequencing of the 16S rDNA gene generated sequences that were subjected to phylogenetic analysis. Results demonstrated the community structure to be represented by the phyla *Proteobacteria* (alpha-, gamma- and delta-classes), *Cyanobacteria*, *Actinobacteria* and *Firmicutes*, with isolates belonging to alpha-proteobacterial group to be predominating. To our knowledge this study appears to be the first to record cultivable and uncultivable bacterial groups associated with *H. nigrocutis* from Indian waters.

**Key words:** *Halichondria nigrocutis*, Marine sponge, Phylogenetic diversity, Sponge-associated bacteria

Sponges classified in the phylum Porifera are primitive metazoans that inhabit marine and freshwater habitats. Of the estimated 15,000 sponge species described so far (Hooper and van Soest, 2002), >486 sponges are recorded from the Indian waters (Thomas, 1998). Sponges are sessile filter feeders and possess efficient capacity to filter out large volumes of water per day through their aquiferous channel system, as a result of which they capture vast amount of planktons and microbes that form the key components of their natural diet (Hentschel et al. 2003; Webster and Taylor, 2012). It is well documented that some of the microbes can associate themselves as ‘symbionts’ capable of growing in the microenvironment of the sponge mesohyl reaching a density of $10^7$ - $10^9$ depending on the sponge type (Hentschel et al. 2003; 2006; Imhoff and Stöhr 2003; Simister and Deines 2012; Webster and Taylor 2012). Studies show the bacteria associated with sponge to be host specific, although their role in the life history of sponge is unclear. In recent years, the study of sponge-bacterial symbiosis has gained considerable interest as the bioactive secondary metabolites produced by sponges are thought to be a result of compounds biosynthesized by specific sponge associated microbial groups rather than being of sponge origin. Many of the bioactive molecules have proven to be pharmacologically important as anticancer, antiviral and anti-inflammatory molecules (Laport *et al*. 2009; Sagar and Minneman, 2010; Kim and Dewapriya 2012). While several of these compounds have been successfully approved for use in treating diseases, there are many in pipeline that are undergoing preclinical/clinical trials for various diseases (Laport *et al*. 2009; Hill and Fenical 2010; Sagar *et al*. 2010).

In India, the sponge fauna is dominated by the species belonging to the class *Demospongiae* (Venkataraman and Wafer 2005). Although the bacterial communities associated with Demosponge species have been recorded from several parts of the world (Taylor *et al*. 2007), there are very few such studies published for species in Indian waters. Further, the sponges belonging to the class Demospongiae are known to produce a large number of secondary metabolites that are being used as natural therapeutics (Faulkner 1998). Hence, the purpose of this study was to document the bacterial community and diversity in the marine sponge *Halichondira nigrocutis* (class *Demospongiae*, order *Halichondrida*, family *Halichondridae*) found in the intertidal zone of West Coast of India.

**MATERIALS AND METHODS**

**Sample collection:** Specimens of the marine sponge *H. nigrocutis* collected during the lowest low tide from the intertidal zone of Majaahalli beach, Karwar, Karnataka, India (latitude 14° 56' 23.74" N and longitude 74° 04'
30.77° E) were cut into individual pieces, transferred to a sterile plastic bag containing seawater, and transported to the laboratory on ice for further processing. Each sponge sample was rinsed with filtered and autoclaved seawater until they were visibly free of debris. The sponge surface was then disinfected with a rapid wash with 70% ethanol followed by immediate dipping in autoclaved and filtered seawater. Fragments (1-cm³) of the specimen were aseptically cut and used for the isolation of bacteria while, few sponge fragments of the same size was immediately frozen on dry ice and stored at -80°C for DNA extraction.

**Bacterial Isolation, PCR and Sequencing:** Sponge associated bacteria were isolated as described previously (Santavy et al. 1990). Approximately, 1g of the sponge tissue was aseptically removed and transferred to tube containing 99 ml of sponge dissociation medium, soaked for 20 min and homogenized. A tenfold serial dilution was made and spread plated on Zobell marine agar 2216 medium (HiMedia, Mumbai). All inoculated plates were incubated at ambient temperature (28 ± 1°C) for a week. Colonies developed on plates were enumerated on the seventh day and recorded as colony forming units (CFU). Bacteria showing distinct colony morphology were picked and purified on Zobell marine agar. The bacterial isolates were identified using a series of biochemical tests (Holt et al. 2000; Bain and Shewan, 1968).

Molecular confirmation of the isolates was done by PCR amplification of the 16s rDNA gene using specific primers. Briefly, each purified isolate was suspended in 50 μl of colony lysis solution (10 mM Tris·HCl, pH 7.5, 10 mM EDTA and 50 μg/ml of proteinase-K) and incubated at 55 °C for 15 min followed by proteinase-K treatment at 80 °C for 10 min. The mixture was centrifuged at 15,000 × g at 4°C for 15 min and the supernatant containing the genomic DNA was used as template for PCR. Amplification of the 16S rDNA gene was carried out with eubacterial specific primers 27F (5’- AGA GTT TGA TCC TGG  CTC AG -3’) and 1492R (5’- GGT TAC CTT GGT  ACG ACT T- 3’). PCR amplification was performed in a total volume of 50 μl reaction mixture consisting of 100 ng of template DNA, 1x PCR buffer (10 mM Tris·HCl, pH 8.3; 50 mM KCl; 2.5 mM MgCl₂; 0.01% gelatin), 0.2 mM of dNTP mix, 10 pmol of each of forward and reverse primer, 1.5 U of Taq DNA polymerase (Bangalore GeNei, Bangalore). The PCR cycling conditions consisted of an initial delay at 94 °C for 10 min, followed by 35 cycles of 94 °C for 1 min, 54 °C for 2 min, and 72 °C for 2 min and a final extension step of 72 °C for 10 min. PCR products were separated by electrophoresis on a 1.5% (w/v) agarose gel, stained with 0.5 μg/ml ethidium bromide and bands were visualized under UV-transilluminator (Herolab, Germany).

Purification of the PCR products was done using QIAquick PCR purification kit (QIAGEN, Germany). The purified PCR products were cloned using the Strataclone® Cloing kit according to manufacturer’s instructions (Strataclone, USA). The clones were sequenced using the M13 forward and reverse primers, ABI Big Dye Terminator Kit Version 3.1 and 3100 Genetic Analyser (PE Applied Biosystems, Foster City, California) by M/s. Bangalore GeNei, Bangalore.

**Total DNA extraction from sponge, PCR and 16S clone library construction:** Total community DNA was extracted from sponge tissue as described previously (Webster et al. 2001). Freeze dried sponge tissue (1.5g) was ground in liquid nitrogen and suspended in 5 ml of TE buffer (10 mM Tris-Cl and 1 mM EDTA, pH 8.0) containing 50 mg/ml lysozyme and incubated at 30 °C for 30 min. Guanidinium thiocyanate buffer (60% guanidinium thiocyanate, 20 ml of 100 mM EDTA and 5 ml of 10% Sarkosyl) was added and the mixture vortexed for 5 min. Samples were transferred to ice, and ammonium acetate was added to a final concentration of 2.5 M. The supernatant was transferred to a fresh tube and an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, vortexed and centrifuged at 10,000 × g for 10 min. The aqueous phase containing DNA was transferred to a fresh tube and precipitated by adding 2-3 volumes of isopropanol followed by centrifugation at 14,000 × g for 10 min. The pellet was washed with 1 ml of 95% ethanol and vacuum dried. Finally, the dried pellet was dissolved in 100 μl of 1x TE buffer (pH 8.0) and stored at -20°C until further use. Two micro litres of the DNA extracted from marine sponge was taken for determining the DNA concentration using NanoDrop ND-1000 spectrophotometer (NanoDrop, USA).
**PCR and clone library construction:** PCR using eubacterial primers and cloning of purified products for sequencing was carried out by the protocol outlined above for culturable bacteria.

**Phylogenetic analysis of 16S rDNA sequences:** The partial 16S rDNA sequences were initially de-replicated by comparing all the sequences in a data set to each other, using the ALIGN program (http://blast.ncbi.nlm.nih.gov/bl2seq/wblast2.cgi) of NCBI. Only one representative sequence having >98% similarity was taken for further analysis. Sequences were also checked for chimeras using the program CHECK CHIMERA of the Ribosomal Database Project-II (RDP-II) website (http://rdp.cme.msu.edu/). Chimeric sequences were not included in the analysis. To identify known sequences with a high degree of similarity, the clone sequences were matched to sequences in the RDP-II database using the SEQMATCH program and with sequences in GenBank database using Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Based on the percentage similarity of unknown clone sequences to representative bacterial sequences in RDP–II and GenBank databases, the sequences were further classified up to their genus (93-96% match) and species (97-100% match) levels or considered as a related type (86-92% match) (Stackebrandt & Goebel, 1994). Phylogenetic and the neighbour joining phylogenetic trees were constructed using MEGA version 4 (Tamura et al. 2007). The robustness of inferred tree topologies was analysed by bootstrapping using 1000 replicates. The 16S rDNA sequence of *Fusobacterium* sp. (AY953256) was included as an out group to root the tree.

**RESULTS**

**Culture-based studies:** The bacterial population associated with *H. nigrocutis* was estimated to be 6.9 x 10^7 CFU/g. Based on morphological and biochemical characterisation, the cultivatable bacteria from *H.nigrocutis* were identified as *Vibrio*, *Bacillus* and *Acinetobacter* spp. Further, sequence analysis of 16S rRNA gene of representative morphotypes revealed the cultivable bacteria to belong to two phylogroups: *Firmicutes* represented by *Bacillus* spp. with a sequence similarity of 90-99% and *Gamma-proteobacteria* represented by *Vibrio* spp. and *Acinetobacter* spp. having a sequence similarity of 91-98%. The phylogenetic affiliations of these isolates are presented in Fig. 1.

![Fig. 1. Neighbour-joining phylogenetic tree constructed from analysis of 566 bp of 16S rDNA sequences of culturable bacteria from the marine sponge *Halichondria nigrocutis*. Numbers at the nodes are percentages indicating the bootstrap values based on Maximum Composite Likelihood analysis from 1,000 replicates. Only values >50% are shown. The scale bar represents 0.02 substitutions per nucleotide position.](image-url)
Phylogenetic analysis of 16S rDNA sequences: The unculturable bacteria associated with *H. nigrocutis* was investigated based on the 16S rDNA PCR yielding an amplicon of ~950 to 995 bp. 80 representative clones that were sequenced and analysed yielded 37 independent sequence profiles which affiliated the microbial sequences to 6 bacterial groups: alpha-proteobacteria, gamma-proteobacteria, delta-proteobacteria, cyanobacteria, actinobacteria and firmicutes (Fig. 2). The dominant species was represented by the alpha-proteobacterial group (n=25; 67.6%), followed by gamma-proteobacteria, actinobacteria and firmicutes (n=3; 8.1% each), cyanobacteria (n=2; 5.4%) and delta-proteobacteria (n=1; 2.7%). Most of the sequences obtained from *H. nigrocutis* were found to be closely related to bacterial sequences associated with sponges of distant taxonomic and geographical origin. Within the alpha-proteobacterial cluster a large number (n=16) of sequences were closely related (>95%) to...
Table 1: *Halichondria nigrocutis* associated bacterial 16S rDNA clones and their closest match in GenBank

<table>
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<tr>
<th>Clone</th>
<th>Accession no.</th>
<th>Nucleotides compared (bp)</th>
<th>Closest match in GenBank (% identity, accession number, sequence)</th>
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alphaproteobacterial symbionts associated with the marine sponge, *H. okadai* and the marine coral *Diploria strigosa*. The alpha-proteobacterial clones TSN19, TSN23 and TSN39 had 98-99% affiliation to the marine sponge *Hymeniacidon flavia* bacterial clone, HM100953. Clone TSN65 was closely affiliated to the clones associated with the Cnidarian *Acropora pruinosa* (98%) and Deep sea octacoral (95%). Among the gamma-proteobacteria, the clone TSN71 matched closest to clone HQ241784 associated to host *Tsitsikamma favus*, a marine sponge endemic to the coast of South Africa. The Delta-proteobacteria sequence (TSN60) had affiliation (97-98%) with the unculturable proteobacterial clones associated with Cnidaria *Muricea elongata* and *Acropora pruinosa*. Cyanobacterial clones (TSN43, TSN58) had close species level affiliations to uncultured *Synechococcus* sp. from oligotrophic oceanic waters and to a clone associated with the coral *D. strigosa*. Actinobacteria clone (TSN35) showed near complete identity (99%) to uncultured actinobacterium clones derived from marine eukaryotes, water and sediments. *Firmicutes* (TSN32, TSN54 and TSN67) had sequence similarity (>94%) with a culturable *Clostridium* clone (AB470963) associated with a *Montipora* a marine coral species. Considering the criterion that sequences with <93% similarity to already available bacterial sequences are used for the proposal of a new genus and >97% for the proposal of a new species (Stackebrandt and Goebel, 1994), 4 alpha-proteobacterial (TSN18, TSN30, TSN34, TSN52), 2 gamma-proteobacterial (TSN40, TSN70) and 2 actinobacterial (TSN7, TSN8) clones associated with *H. nigrocutis* were seen to be novel at the genus level while the remaining could be considered novel at the species level (Table 1). The sequences have been submitted to GenBank and assigned accession numbers (Table 1).

**DISCUSSION**

This is the first study of the bacterial communities associated with the marine sponge *H. nigrocutis*. The bacteria associated with *H. nigrocutis* included those belonging to the phyla *Proteobacteria* (alpha-, beta- and gamma-classes), *Cyanobacteria*, *Firmicutes* and *Actinobacteria*. The largest cluster was represented by the *Proteobacterial* group which is consistent with data derived from other sponge-associated 16S rRNA gene libraries (Webster and Taylor 2012). Among the uncultured *Proteobacterial* group associated with *H. nigrocutis*, alpha-proteobacteria was the most predominant, followed by Gamma- and Delta proteobacteria. Proteobacterial groups (alpha-, beta-, gamma-, delta-classes) have been reported for several sponge species such as *Aplysina cavernicola* (Thomas et al. 2003), *Rhopaloeides odorabile* (Webster et al. 2001; Webster and Hill 2001), *Theonella swinhoei* (Schmidt et al. 2000) and *H. panacea* (Wichels et al. 2006). Interestingly, most of the alpha-proteobacteria sequences analysed in this study were closest to uncultured alpha-proteobacterial clones associated with the Japanese marine sponge *H. okadai* and the Caribbean coral *Diploria strigosa*. The results are in agreement with an earlier study by Taylor et al. (2007) and a recent analysis by Sunagawa et al. (2010) wherein they report a number of monophyletic 16S rRNA clusters to contain exclusively sponge- and coral-derived sequences. The culturable bacterial community associated with *H. nigrocutis* consisted of *Firmicutes* and Gamma-proteobacteria.

Recent studies have shown that *Firmicutes* (low G+C Gram positive bacteria) are identified from marine sponges by both cultivation-dependent and cultivation-independent approaches (Webster and Taylor 2012). Based on analysis of denaturing gradient gel electrophoresis, the main cultivable group within the phylum *Firmicutes* is reported to be the *Bacillus* spp. (Taylor et al. 2007). Even in the marine sponges *Aplysina cavernicola* (Hentschel et al. 2001), *S. zeteki* (Zha et al. 2008), *Haliclona simulans* (Phelan et al. 2011) and *H. panacea* (Imhoff and Stohr, 2003) the predominant genus of cultivable bacteria reported was *Bacillus* spp. Marine *Bacillus* spp. has been recognised as a source of many antibiotics such as cyclic peptides, cyclic lipopeptides and novel thiopptides (Nagai et al. 2003). Interestingly, spore formation in *Bacillus* spp. has been shown to be co-regulated with antibiotic production (Marahiel et al. 1993). We surmise that, the association of *Bacillus* and *Clostridium* spp. with *H. nigrocutis* is probably a mutualistic one wherein these bacteria may be playing a critical role in sponge defence against colonization by harmful organisms as also reported earlier (Thakur et al. 2004). Likewise, the association of *Acinetobacter* and *Vibrio* spp. with *H. nigrocutis* is of great interest, because several species of these genera associated with sponges are known to be secondary metabolite producers (Elyakov et al. 1991; Oclarit et al. 1994) and are a recognized source of natural products.
Actinobacteria originally isolated from soil are now being increasingly isolated from seawater and marine sediments. They are well known as producers of secondary metabolites with high pharmacological and commercial application (Jensen et al. 2007; Taylor et al. 2007; Gontang et al. 2010). Sponge-derived actinobacteria have been reported from marine sponges such as Theonella swinhoei, A. aerophoba (Hentschel et al. 2001) Rhopaloeides odorabile (Webster et al. 2001), Xestospongia spp. (Montalvo et al. 2005), Chondrilla nucula (Thiel et al. 2007) and Hymeniacidon perleve (Zhang et al. 2006) and therefore the association of actinobacteria clones with the sponge H.nigrocutis is noteworthy and demands further study. The Cyanobacteria are known to be associated as photosynthetic symbionts with the marine sponges commonly found in shallow water marine environments (Usher et al. 2001; Thacker and Freeman, 2012) wherein both are mutually benefitted. Cyanobacteria has been previously reported to be found in 26 Demospongefiae families (Hentschel et al. 2006) and the association of cyanobacterial clones in the host H. nigrocutis, although a minor component of the clone library, is in agreement with this. Marine Cyanobacteria belonging to the Synechococcus species has been shown to predominate the nutrient-rich gyres of tropical oceanic waters (Moore et al.1995) and implicated in the uptake and utilization of organic nitrogen compounds as a nitrogen source (Moore et al. 2002; Zubkov et al. 2003). The cyanobacterial clones in this study (TSN43, TSN68), was closely related to an uncultured Synechococcus clone (AY125366) shown to be involved in uptake of organic nitrogen in oligotrophic oceanic waters (Zubkov et al. 2003). Therefore, it needs to be seen whether the association of Cyanobacteria associated with H.nigrocutis is truly symbiotic and contributes to any beneficial effects to the host in serving as a source of nutrition.

In this study, surveys of associated microbial communities using the 16S rRNA resulted in the revelation of many novel bacterial species previously not known, within the sponge H.nigrocutis. As the unique bacteria–sponge relationship has been a subject of intense research due to marine organisms being a source of natural products, the novel sequences associated with this sponge await future exploration for elucidating the specific factors involved in sponge–microbe interactions, the functional nature and the putative benefits of these interactions.

ACKNOWLEDGEMENTS

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REFERENCES


