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ORIGINAL PAPER

16S rDNA clone library-based bacterial phylogenetic diversity associated with three South China Sea sponges

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Abstract Culture-independent molecular techniques, 16S rDNA clone library alongside RFLP and phylogenetic analysis, were applied to investigate the bacterial diversity associated with three South China Sea sponges, *Stelletta tenui*, *Halichondria rugosa* and *Dysidea avara*. A wide bacterial diversity was detected according to total genomic DNA-based 16S rDNA clone library, abundant clones with low identify with sequences retrieved from database were found as well as uncultured sponge symbionts. The phylogenetic analysis shows that the bacterial community structure of *Stelletta tenui* is similar to that of *Halichondria rugosa* comprising gamma-*Proteobacteria* and *Firmicutes*. Whereas, alpha-*Proteobacteria*, gamma-*Protebacteria*, *Bacteroidetes* and uncultured sponge symbionts were found in sponge *Dysidea avara*, suggesting that *Dysidea avara* has the highest bacteria diversity among these sponges. A specific sponge–microbe association is suggested based on the difference of bacterial diversity among these three sponges from the same geography location and the observed sponge species-specific bacteria.

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Introduction

Recently, much attention has been given to marine animal-associated microorganisms in order to find the true producer of biological active compounds and understand the sponge–microbe associations (Hill 2004; Piel 2004). As a filter feeder, sponge is able to filter thousands of liters of water 1 day, which make sponge harbor large numbers of diverse bacteria in its tissue. In general, for high-microbial-abundance sponge, microorganisms can contribute up to 40–70% of the sponge body volume exceeding microorganisms in seawater by 2–4 orders of magnitude (Hentschel et al. 2006). In sponge associated microbial diversity study, culture-dependent or microscopy-based methods have been used (Hentschel et al. 2001; Olson et al. 2000; Vacelet and Donadey 1997). Recently, molecular investigations on sponges have revealed a wider range of sponge-associated microbes including members of *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria* and *Archaea* (Friedrich et al. 1999; Hentschel et al. 2006, 2002; Hill 2004; Li et al. 2006; Webster and Hill 2001; Wichels et al. 2006).

It is estimated that there are about 5,000 species sponges in the South China Sea (Zhang et al. 2003), but, to date, rare study has been involved (Li et al. 2006) and little is known about their microbial communities. In the case of sponges *Stelletta tenui*, *Halichondria rugosa*, *Dysidea avara*, the associated microbial diversity has rarely assessed.

In our previous study on sponge *Stelletta tenui* by transmission electron micrographs (TEM) (Li et al. 2005), diverse microorganisms were observed within the sponge body such as mesohyl, cell and the inner cavity. The same phenomenon has been observed for *Halichondria rugosa* and *Dysidea avara*. The community structure of the predominant bacteria associated with sponges *Stelletta tenui*, *Halichondria rugosa*, *Dysidea avara* and *Craniella australiensis* have also been investigated by using DGGE fingerprint-based molecular method (Li et al. 2006). 16S rDNA-DGGE fingerprinting has been proved to be particularly useful as an initial investigation for distinguishing between communities (Taylor et al. 2005; Webster et al. 2004; Wichels et al. 2006). However, DGGE-based method is suitable for the predominant bacteria that comprise 1% or more of cells within a given sample. If we want to assess the all-around sponge-associated bacterial community, some molecular methods such as 16S rDNA clone library should be introduced. Therefore, in the present study, culture-independent 16S rDNA clone library-based molecular strategy alongside RFLP (Restriction Fragment Length Polymorphism) and phylogenetic analysis were used to investigate the bacterial community associated with three South China Sea sponges *Stelletta tenui*, *Halichondria rugosa* and *Dysidea avara* from the same geography location.

Materials and methods

Sponge sample

Sponges identified as *Stelletta tenui*, *Halichondria rugosa* and *Dysidea avara* were collected by scuba diving at a depth of about 20 m in the South China Sea around Sanya Island in November 2002 (Li et al. 2006). The seawater temperature is about 20°C. Sponges were enclosed in sterile bags immediately and transferred to laboratory on ice. Sponge samples were cut into small pieces and stored at –20°C before DNA extraction.

16S rDNA clone library construction

In order to investigate the sponge associated bacterial diversity wholly, different parts of a sponge were mixed together for total genomic DNA extraction. The mixed sponge pieces were split and mortared as small as possible, washed twice with TE solution (pH 8.0), and then total genomic DNA was extracted and stored according to the method of Li et al. (2006). Small sponge pieces were dipped in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) and blended to slurry, the slurry was ground in a mortar on ice. Cells were collected by centrifugation at 10,000 rpm for 5 min at 4°C and incubated with lysozyme (10 mg/ml, ChemSonic) at 37°C for 30 min followed by the treatment of 10% SDS and proteinase K (10 mg/ml, Merck) at 55°C for 30 min. Genomic DNA was extracted orderly with tris–phenol (pH 8.0), tris–phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), precipitated by absolute ethyl alcohol, RNA was removed by RNase (10 mg/ml, Sigma). The genomic DNA was stored at –20°C before 16S rRNA gene PCR amplification.

Amplification of ca.1,500 bp 16S rDNA was performed in Master Cycler Gradient (HYBAID, UK) with the eubacterial primers 27f (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-CTACGGCTACCTTGTTACGA-3′) (Polz et al. 1999). The amplification program was as follows: initial denaturation at 94°C for 3 min, 30 cycles of 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min, and a final extension of 10 min at 72°C. PCR products were analyzed by electrophoresis in 1% (wt/vol) agarose gel and recovered using a gel purification kit (Shenergy Biocolor Bioscience & Technology Company, China).

The obtained purified PCR products were ligased into the pUCmT vector (Shenergy Biocolor Bioscience and Technology Company, China) and transformed into $CaCl_2$-competent *Escherichia coli* DH5α. The positive recombinants were screened on X-Gal (5-bromo-4-chloro-3-indoly-β-D-galactopyranoside)–IPTG (isopropyl-β-D-thiogalactopyranoside)–ampicillin–tetracycline indicator plates by color-based recombinant selection. Positive clones were identified by PCR amplification with pUCmT vector primer pairs T7 (5′-TAATACGACTCACTATAGGG-3′) and M13 (5′-CAGGAAACAGCTATGACC-3′) using the same program as 16S rDNA amplification.

RFLP, 16S rDNA sequencing and phylogenetic analysis

The PCR-amplified products of positive recombinants were digested with the restriction enzymes *Csp*6I and *Hin*fI (Bioasia Biotechnology Company, China) for 4 h at 37°C, electrophoresed in 2% agarose gels at 80 V for 50 min and stained with ethidium bromide. Clones with similar banding patterns were grouped together, one representative clone from each group was chosen for partial 16S rDNA sequencing using ABI3730 DNA Sequencer (USA) with primer 1492r in Bioasia Biotechnology Company, China. The obtained sequences of ca. 600 bp 16S rDNA were compiled and aligned

using the CLUSTALX program. Finally, sequences were checked for chimera formation (CHECK_CHIMERA online analysis of Ribosomal Database Project II) and then compared to sequences retrieved from database by using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al. 1990) to determine approximate phylogenetic positions. The phylogenetic tree was generated by neighbor-joining method with 1,000 resampling bootstrap analysis using Mega II software (Li and Liu 2006).

Results

After mixing a sponge's different parts together, total genomic DNA was successfully extracted (Fig. 1), consequently, about 1,500 bp 16S rDNA was amplified by PCR (Fig. 2). From the 16S rDNA clone library of sponge *Stelletta tenui*, a number of 124 positive clones were selected and analyzed with RFLP resulting in 11 and 14 banding patterns with *Csp*6I and *Hin*fI, respectively (Fig. 3). Based on Fig. 3, 20 representative clones were chosen for sequencing and submitting to GenBank, the obtained 20 accession numbers and the BLAST result are shown in Table 1. In the case of sponge *Halichondria rugosa*, 112 positive clones were selected, 12 representative clones were sequenced according to RFLP patterns (data not shown), and the BLAST result is shown in Table 2. One hundred and four clones of the 16S rDNA library of sponge *Dysidea avara* were compared by RFLP, 20 representative clones were chosen for 16S rDNA sequencing and BLAST analysis (Table 3). The phylogenetic tree is shown in Fig. 4 according to the obtained ca. 600 bp 16S rDNA sequences of the total 52 representative clones.

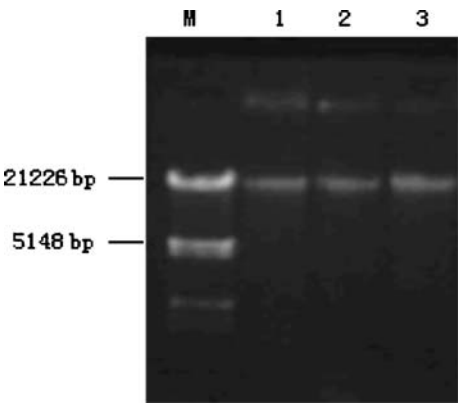

Fig. 1 Sponge total genomic DNA. Line 1, *Stelletta tenui*; line 2, *Halichondria rugosa*; line 3, *Dysidea avara*

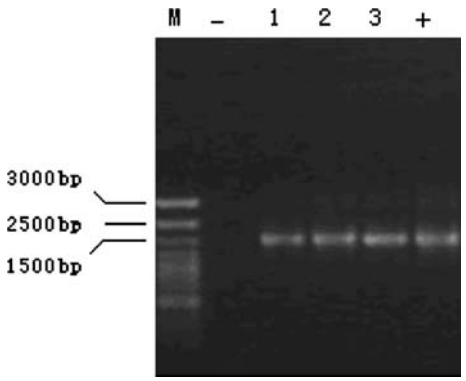

Fig. 2 Sponge associated bacterial 16S rDNA product amplified by PCR. Line 1, *Stelletta tenui*; line 2, *Halichondria rugosa*; line 3, *Dysidea avara*. –, Negative control; +, positive control with *Escherichia coli*

Based on Tables 1–3, abundant clones with low identify below 96% to the closest relative, for example S-clone 15, S-clone 94, S-clone 155, S-clone 66, H-clone 58, H-Clone195, H-clone 292, H-clone 11, D-clone 46 and D-clone 135, were found in the 16S rDNA clone libraries. H-Clone 195 matches an uncultured bacterium clone ARKCH4-16 with a 92% similarity. In particular, D-clone 38 and D-clone 98 match uncultured sponge symbionts RSWS10 and RSWS 18 with 98% similarity. The results of the phylogenetic affiliation in Fig. 4 show that the first dominant group of these sponges associated bacterial clone libraries is gamma-*Proteobacteria* (36/52), the second dominant bacteria are phyla *Bacteroidetes* (6/52) and *Firmicute* (6/52), the third dominant bacteria are alpha-*Proteobacteria*.

The revealed bacteria associated with sponge *Stelletta tenui* are regrouped to two phyla, *Proteobacteria* comprising *Psychrobacter* sp., *Marinomonas* sp., *Pseudomonas* sp., *Oceanisphaera* sp., *Oceanisphaera litoralis* and *Firmcutes* including *Bacillus* sp., *Sporosarcina* sp. The 16S rDNA clone library is dominated by gamma-*Proteobacteria Psychrobacter* sp. (12/20).

In the 16S rDNA clone library of sponge *Halichondria rugosa*, *Proteobacteria* and *Firmicutes*, mainly gamma subclass of the *Proteobacteria* comprising *Psychrobacter* sp., *Moellerella wisconscensis*, *Marinomonas* sp., *Pseudomonas* sp., *P. halodenitrificans* and *Bacillus* sp. of phylum *Firmicutes*, were found. *Psychrobacter* sp. bacteria make up the majority of the detected gamma-*Proteobacteria* as in sponge *Stelletta tenui*. H-clone 195, H-clone 241, H-clone 272, H-clone 38 and H-clone 331 have >98% identity to the bacteria previously retrieved from sea environment.

As for sponge *Dysidea avara*, the library is mainly composed of sequences belonging to *Proteobacteria* comprising gamma subdivisions such as *Psychrobacter* sp.,

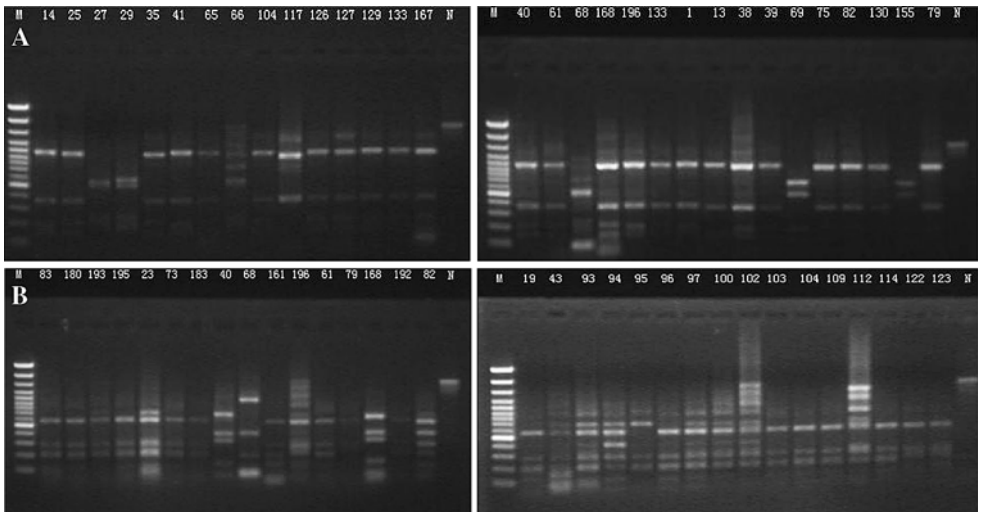

Fig. 3 RFLP patterns of *Stelletta tenui* associated bacterial 16S rDNA clones digested with *Csp*6I (**A**) and *Hin*fI (**B**), respectively

Table 1 Sequence similarity of clone from sponge *Stelletta tenui* to the closest relative in GenBank

Clone No.	Accession No.	Closest relative and its accession number	Similarity (%)
S-Clone 6	DQ236251	*Pseudomonas* sp. CL-2 (AY017062)	98
S-Clone 10	DQ236252	*Psychrobacter psychrophilus* isolate CMS 32 (AJ748270)	100
S-Clone 13	DQ236253	*Psychrobacter psychrophilus* isolate CMS 32 (AJ748270)	99
S-Clone 15	DQ236254	*Shewanella pacifica* KMM 3590 (AF500076)	92
S-Clone 55	DQ236257	*Psychrobacter* sp. 9-2 (AY382586)	100
S-Clone 81	DQ236260	*Psychrobacter psychrophilus* isolate CMS 32 (AJ748270)	99
S-Clone 83	DQ236261	*Psychrobacter psychrophilus* isolate CMS 32 (AJ748270)	100
S-Clone 85	DQ236262	*Psychrobacter maritimus* type strain Pi2-20T (AJ609272)	99
S-Clone 87	DQ236263	*Psychrobacter* sp. (AY382586)	99
S-Clone 89	DQ236264	*Oceanisphaera litoralis* type strain DSM 5406 (AJ550470)	96
S-Clone 94	DQ236265	*Marinomonas* sp. BSi20101 (DQ007439)	92
S-Clone 117	DQ236266	*Psychrobacter maritimus* (AJ609272)	99
S-Clone 155	DQ236267	*Psychrobacter luti* strain LMG 21276 (AJ430828)	95
S-Clone 176	DQ236268	*Psychrobacter psychrophilus* isolate CMS 32 (AJ748270)	99
S-Clone 191	DQ236269	*Psychrobacter* sp. (AY382586)	99
S-Clone 196	DQ236270	*Psychrobacter psychrophilus* isolate CMS 32 (AJ748270)	99
S-Clone 27	DQ236255	*Sporosarcina* sp. GIC9 (AY439261)	97
S-Clone 29	DQ236256	*Sporosarcina* sp. GIC9 (AY439261)	97
S-Clone 66	DQ236258	*Bacillus* sp. cryopeg_7 (AY660699)	96
S-Clone 68	DQ236259	*Sporosarcina* sp. GIC9 (AY439261)	97

Table 2 Sequence similarity of clone from sponge *Halichondria rugosa* to the closest relative in GenBank

Clone No.	Accession No.	Closest relative and its accession number	Similarity (%)
H-Clone11	DQ274120	*Moellerella wisconsensis* (AM040754)	96
H-Clone38	DQ274121	*Psychrobacter psychrophilus* (AJ748270)	99
H-Clone58	DQ274122	*Marinomonas sp.* BSi20101 (DQ007439)	91
H-Clone59	DQ274123	*P. halodenitrificans* (X90868)	98
H-Clone60	DQ274124	*Pseudomonas* sp. SKU (AY954288)	97
H-Clone95	DQ274125	*Moellerella wisconsensis* (AM040754)	97
H-Clone195	DQ274126	Uncultured bacterium clone ARKCH4-16 (AF468266)	92
H-Clone241	DQ274127	*Bacillus* sp. cryopeg_7 (AY660699)	99
H-Clone272	DQ274128	*Psychrobacter maritimus* (AJ609272)	100
H-Clone292	DQ274129	*Psychrobacter psychrophilus* (AJ748270)	95
H-Clone323	DQ274130	*Bacillus psychrophilus* (D16277)	99
H-Clone331	DQ274131	*Psychrobacter* sp. A1-1 (AJ871596)	98

Table 3 Sequence similarity of clone from sponge *Dysidea avara* to the closest relative in GenBank

Clone No.	Accession No.	Closest relative and its accession number	Similarity (%)
D-Clone10	DQ274134	*Psychrobacter cibarius* (AY639872)	99
D-Clone21	DQ274135	*Psychrobacter cibarius* (AY639872)	100
D-Clone25	DQ274136	*Oceanisphaera koreensis* (DQ190440)	97
D-Clone46	DQ274140	Uncultured Arctic sea ice bacterium (AY165598)	92
D-Clone47	DQ274141	*Psychrobacter fozii* (PJO430827)	99
D-Clone52	DQ274142	*Psychrobacter cibarius* (AY639872)	99
D-Clone84	DQ274145	*Psychrobacter* sp. St1 (AF260715)	99
D-Clone135	DQ274152	Uncultured *Pseudoalteromonas* sp. (AY664362)	95
D-Clone142	DQ274153	*Psychrobacter* sp. X 159 (DQ191162)	97
D-Clone148	DQ274154	*Psychrobacter psychrophilus* (AJ748270)	98
D-Clone122	DQ274148	*Paracoccus* sp. MBIC4036 (AB025192)	97
D-Clone125	DQ274149	*Paracoccus* sp. MBIC4036 (AB025192)	97
D-Clone132	DQ274151	Uncultured bacterium (AJ514433)	97
D-Clone38	DQ274138	Uncultured sponge symbiont RSWS10 (AF434940)	98
D-Clone98	DQ274147	Uncultured sponge symbiont RSWS18 (AF434946)	98
D-Clone01	DQ274132	*Bizionia paragorgiae* (AY651070)	97
D-Clone39	DQ274139	*Arenibacter latericius* (AF052742)	98
D-Clone79	DQ274144	*Bizionia paragorgiae* (AY651070)	100
D-Clone91	DQ274146	*Bizionia paragorgiae* (AY651070)	99
D-Clone128	DQ274150	*Bizionia paragorgiae* (AY651070)	98

Oceanisphaera koreensis, *Pseudomonas* sp. and alpha subclass such as *Paracoccus* sp., and *Bacteroidetes* including *Bizionia paragorgiae*, *Arenibacter latericius* and uncultured sponge symbiotic bacterium, for example, D-Clone 38 matches an unidentified uncultured sponge symbiont. In particular, D-Clone 46 with 92% similarity to uncultured Arctic sea ice bacterium was observed in the library.

Through the comparison of bacterial diversity among sponges *Stelletta tenui*, *Halichondria rugosa* and *Dysidea avara*, it is suggested that the bacterial diversities of the South China Sea sponges are great. The bacterial community structure of *Stelletta tenui* comprising gamma-*Proteobacteria* and *Firmicutes* is similar to that of *Halichondria rugosa*. However, the bacterial diversity of *Stelletta tenui* seems higher than that of *Halichondria rugosa* according to Tables 1 and 2 and Fig. 4. Whereas, alpha-*Proteobacteria*, gamma-*Protebacteria*, *Bacteroidetes* and uncultured sponge symbionts were found in sponge *Dysidea avara*, the later two species were absent in *Stelletta tenui* and *Halichondria rugosa*, which means that *Dysidea avara* has the highest bacteria diversity among the three tested sponges.

Discussion

In general, the partial sequences-based phylogenetic analyze is largely consistent with that using most of the 16S rRNA gene (Rath et al. 1998). The partial 16S rRNA gene fragment is able to distinguish most phylotypes from their relatives and determine phylogenetic relationships among the clones (Cebron et al. 2004; Li et al. 2006; Li and Liu 2006; Muyzer et al. 1993). So, in this study, clones were partially sequenced to obtain ca. 600 bp 16S rDNA sequences for phylogenetic analysis. In addition, bacterial diversity associated with the respective part of a sponge was not involved in this investigation, because this study attempted to investigate the all-round bacterial community associated with a sponge and compare the difference of bacterial diversity among different sponges. For this purpose, different parts of a sponge were mixed together for total genomic DNA analysis to get more comprehensive bacterial diversity of a sponge.

Based on our study by TEM (Li et al. 2005), microorganisms are mainly distributed in the mesohyl and inner cavity of sponge although a few are found in sponge cells. According to Taylor et al. (2004), sponge-associated bacteria can be divided into two groups: 'generalists' and 'specialists', the former mainly come from the sea water and distribute mainly in the inner cavity or sponge surface, the later are sponge-host specific bacteria and distribute mainly in the sponge mesohyl or cells. It is well known that *Protebacteria* make up the main bacterial community in the sea water (Imhoff 2001), several studies have reported that *Protebacteria* are important bacterial community components associated with sponge from different location (Hentschel et al. 2006; Friderich et al. 2001; Thomas et al. 2003). The similar conclusion that *Protebacteria* especially gamma-*Proteobacteria* are the dominant bacteria in sponge can be drawn according to

Fig. 4 Phylogenetic tree based on about 600 bp of 16S rRNA gene sequences from sponges *Stelletta tenui*, *Halichondria rugosa* and *Dysidea avara* clone libraries. The numbers at the nodes are percentages indicating the levels of bootstrap support based on a neighbor-joining analysis of 1,000 resampled data sets. Scale bar represents 0.02 substitutions per nucleotide position

this study and our previous results (Li et al. 2006; Li and Liu 2006). Because the tested sponges were sampled in winter, it is not surprise that there is apparent dominance of *Psychrophilic* sp. bacteria, e.g., 12/20 clones for sponge *Stelletta tenui*, 7/20 clones for *Dysidea avara*. The similar clones such as D-Clone 142 and H-Clone 272, H-Clone 38, S-Clone 55 and H-Clone 331 among the three tested sponges also suggest the possible effect of seawater environment on the 'generalists' bacterial community. Besides 'generalists' bacteria, 'specialists' bacteria for sponges have been revealed (Taylor et al. 2004). Similarly, some potential sponge specific bacteria, especially uncultured sponge symbionts, were observed in this study. As shown in Fig. 4, alpha-*Proteobacteria* D-Clone 122, 125, 132, and *Bacteroidetes* D-clone 01, 39, 79, 91, 98, 128 are specific for sponge *Dysidea avara*. Based on the difference of bacterial diversity among these three sponges from the same geography location and the observed sponge species-specific bacteria, a specific sponge–microbe association is suggested despite the presence of some possible 'generalists' bacteria from the sea water. This suggestion is also supported by our previous study on another South China Sea sponge *Craniella austrialiensis* (Li and Liu 2006).

As reported in our previous study on the bacterial community associated with these sponges using DGGE fingerprint-based method (Li et al. 2006), only predominant *Proteobacteria* were detected for sponges *Stelletta tenui* and *Halichondria rugosa*, while *Proteobacteria* and *Firmicutes* were found in the present study. For sponge *Dysidea avara*, the revealed bacterial community based on 16S rDNA library is similar to that by PCR-DGGE. Basically, 16S rDNA clone library-based approach outgoes 16S rDNA PCR-DGGE in getting higher bacterial diversity when the library is big enough to cover the 16S rDNA genetic diversity. However, clone library-based approach is generally restricted by the selected clone numbers and the sponge part sampling, it is possible that some bacteria remain undetected. Even so, in this paper, based on the extracted total genomic DNA after mixing the sponge different parts together, the expected higher bacterial diversity was achieved. Analysis of the cloned 16S rDNA libraries shows the presence of abundant bacteria with low identity below 98% to their closest relatives, for instance, 8/20 clones for *Stelletta tenui*, 2/12 clones for *Halichondria rugosa* and 7/20 clones for *Dysidea avara*, and uncultured sponge symbionts such as D-clone 38, D-clone 98, which suggests potential unknown bacterial resource in these sponges.

The results of South China Sea sponges reported in this paper expand the knowledge of the phylogenetic diversity of sponge associated bacterial communities. It is suggested that the isolation efficiency of sponge-associated bacteria can be improved under the guidance of the revealed bacterial diversity by culture-independent molecular strategy such as 16S rDNA library (Li and Liu 2006). Therefore, the revealed bacterial diversity in this study is valuable for these sponges associated bacterial isolation and cultivation.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Cebron A, Coci M, Garnier J (2004) Denaturing gradient gel electrophoretic analysis of ammonia-oxidizing bacterial community structure in the lower seine river: impact of Paris wasterwater effluents. Appl Environ Microbiol 70:6726–6737

Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplyina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence in situ hybridization (FISH). Mar Biol 134:461–470

Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U (2001) Temporal variation of the microbial community associated with the Mediterranean sponge *Aplysina aerophoba*. FEMS Microbiol Ecol 38:105–113

Hentschel U, Schael M, Wagner M, Fieseler L, Gernert C, Hacker J (2001) Isolation and phylogenetic anaysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. FEMS Microbiol Ecol 35:305–312

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microbial Ecol 55:167–177

Hill RT (2004) Microbes from marine sponges: a treasure trove of biodiversity for natural products discovery. In: Bull AT (ed) Microbial diversity and bioprospecting. ASM Press, pp 177–190

Imhoff JF (2001) True marine and halophilie anoxygenic phototrophic bacteria. Arch Microbiol 176:243–254

Li Z, Liu Y (2006) Marine sponge *Craniella austrialiensis*-associated bacterial diversity revelation based on 16 S rDNA library and biologically active Actinomycetes screening, phylogenetic analysis. Lett Appl Microbiol 43:410–416

Li Z, Qin E, Jiang Q (2005) Distribution characteristics of sponge *Stelletta tenui* associated microorganisms by TEM. J Fish China 29(1):38–42

Li Z, He L, Wu J, Jiang Q (2006) Bacterial community diversity associated with four marine sponges from the South China Sea based on 16S rDNA-DGGE fingerprinting. J Exp Mar Biol Ecol 329:75–85

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel

electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Olson JB, Lord CC, McCarthy PJ (2000) Improved recoverability of microbial colonies from marine sponge samples. Microb Ecol 40:139–147

Piel J (2004) Metabolites from symbiotic bacteria. Nat Prod Rep 21:519–538

Polz MF, Harbison C, Cavanaugh CM (1999) Diversity and heterogeneity of epibiotic bacterial communities on the marine nematode *Eubostrichus dianase*. Appl Environ Microbiol 65:231–240

Rath J, Wu KY, Herndl GJ, DeLong EF (1998) High phylogenetic diversity in a marine-snow-associated bacterial assemblage. Aquat Microb Ecol 14:262–269

Taylor MW, Schupp PJ, Nys R, de Kjelleberg S, Steinberg PD (2005) Biogeography of bacteria associated with the marine sponge *Cymbastela concentrica*. Environ Microbiol 7:419–433

Taylor MW, Schupp PJ, Dahllöf I, Kjelleberg S, Steinberg PD (2004) Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. Environ Microbiol 6:121–130

Thomas C, Horn M, Wagner M, Hentschel U, Proksch P (2003) Monitoring microbial diversity and natural product profiles of the sponge *Aplysina cavernicola* following transplantation. Mar Biol 142:685–692

Vacelet J, Donadey C (1997) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Biol Ecol 30:301–314

Webster NS, Hill RT (2001) The culturable microbial community of the Great Barrier Reef sponge *Rhopaloeides odorabile* is dominated by an alpha-*Proteobacterium*. Mar Biol 138:843–851

Webster NS, Negri AP, Munro MHG, Attershill CN (2004) Diverse microbial communities inhabit Antarctic sponges. Environ Microbiol 6:288–300

Wichels A, Würtz S, Döpke H, Schütt C, Gerdts G (2006) Bacterial diversity in the breadcrumb sponge *Halichondria panicea* (pallas). FEMS Microbiol Ecol 56:102–118

Zhang W, Xue S, Zhao QY, Zhang XY, Li JH, Jin MF, Yu XJ, Yuan Q (2003) Biopotentials of marine sponges from China oceans: past and future. Biomol Eng 20:413–419