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ORIGINAL ARTICLE

# Investigation of bacteria with polyketide synthase genes and antimicrobial activity isolated from South China Sea sponges

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**Keywords**
antimicrobial activity, bacteria, fosmid library, PCR, phylogenetic analysis, polyketide synthase, sponge.

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## Abstract

**Aims:** To obtain bacteria with PKS (polyketide synthase) genes and antimicrobial activity from sponges.

**Methods and Results:** Eighteen bacteria with KS (ketosynthase) genes were identified by polymerase chain reaction (PCR) screening of 98 isolates from South China Sea sponges, *Stelletta tenuis*, *Halichondria rugosa*, *Dysidea avara* and *Craniella australiensis*. 16S rRNA gene-based Blast analysis indicated that 15 isolates belonged to the phylum *Firmicutes*, among which 14 isolates were closely related to genus *Bacillus*, and 1 to *Staphylococcus lentus*. Two isolates were identified as actinomycetes, and one as *Alcaligenes* sp. in the phylum *Proteobacteria*. The 18 KS domains belong to trans-AT type I PKS and match PKS of marine bacterial symbionts. The 18 bacteria exhibited broad-spectrum antimicrobial activities against fungi, gram-positive and gram-negative bacteria. A 21·8-kb PKS gene cluster fragment containing five modules was isolated from the *Staphylococcus lentus* isolate A75 by screening of a fosmid library.

**Conclusions:** The PKS gene diversity and different antimicrobial spectra indicate the potential of bacteria associated with South China Sea sponges for diverse polyketide production.

**Significance and Impact of the Study:** Combined with bioactivity assay the PKS gene-based approach can be applied to efficient screening of strains of pharmaceutical value and the prediction of related compounds.

## Introduction

Marine organisms are an important source of natural products (Hill 2006; Blunt *et al.* 2007), and most of the marine natural products are structurally diverse and have unusual bioactivities (Hill 2003, 2004, 2005, 2006; Blunt *et al.* 2007, 2008). The availability of biomass is a limiting factor for isolating marine natural products. Therefore, alternative and ecologically sound sources of marine natural products are needed. Marine micro-organisms have contributed to the majority of marine natural products. Many marine organisms especially invertebrates, such as tunicates, bryozoans and sponges, have symbiotic relationships with micro-organisms. There is increasing evidence that many metabolites, in particular polyketides and nonribosomal peptides, are not produced by the marine animals themselves but by their associated bacteria (Kobayashi and Ishibashi 1993; Jensen and Fenical 1994; Piel 2006). Thus, marine organism-associated microbes have attracted more attention as potential sources of marine natural products in order to solve the supply shortage.

Sponges as one of the oldest marine animals and a major component of marine invertebrates are rich sources of potentially valuable natural products such as polyketides, nonribosomal peptides and alkaloids with antibacterial, antifungal, antiviral and anti-inflammatory activities (Friedrich *et al.* 2001; Proksch *et al.* 2002; Hill 2003, 2004; Müller and Müller 2003; Blunt *et al.* 2007), but the exact origin of these compounds is still under investigation (Zheng *et al.* 2000; Lee *et al.* 2001). Sponges often

 

harbour diverse micro-organisms (Vacelet and Donadey 1977; Hentschel *et al.* 2006; Li and Liu 2006b; Li *et al.* 2006a, 2007a; Thiel *et al.* 2007), which have been proposed to supply nutrients, stabilize sponge skeletons, protect the sponge host from bio-fouling or predation (Lee *et al.* 2001), and be involved in the synthesis of some sponge-derived bioactive compounds (Dunlap *et al.* 2007). The study of metabolite-related genes of micro-organisms associated with sponges will give insight into the origin of sponge-derived natural products.

Polyketides, comprising a large and structurally diverse family of bioactive natural products, are one of the most important classes of marine natural compounds (Hill 2003, 2004, 2005). The common feature of complex polyketides is the involvement of large polyfunctional polyketide synthases (PKS) in which the ketosynthase (KS) domain is present in each module and exhibits the highest degree of conservation among all domains. Therefore, the KS domain is especially well suited for phylogenetic analyses of PKS gene diversity (Moffitt and Neilan 2003; Ginolhac *et al.* 2004; Kim and Fuerst 2006; Fieseler *et al.* 2007).

Many natural polyketide compounds have been isolated from sponges (Fontana *et al.* 1998; Okada *et al.* 2002; Huang *et al.* 2004), but the investigation of marine microbial PKS genes is still in its infancy. Although some progress has been made by both culture-independent (Piel *et al.* 2004a,b; Fieseler *et al.* 2007) and -dependent approaches (Kim *et al.* 2005; Schirmer *et al.* 2005; Kim and Fuerst 2006), there is still a lack of knowledge of the marine microbial PKS gene diversity and the biological function associated with the PKS genes. Compared with a culture-independent approach, a culture-dependent strategy can provide valuable insight into the origin of sponge-derived natural products, and cultivable bacteria have contributed significantly to the understanding of sponge–microbe association. The China Sea has become an important source of marine natural compounds since 2001 (Blunt *et al.* 2007), and investigations of marine microbial PKS and nonribosomal peptide synthetase (NRPS) genes have been initiated (Jiang *et al.* 2007, 2008; Zhang *et al.* 2007, 2008). The aim of this study was to obtain bacteria of pharmacological interest from sponge in the South China Sea by an integrated approach of polymerase chain reaction (PCR) screening of PKS gene and bioassay of antimicrobial activity.

## Materials and methods

### Sponge-associated micro-organisms and bioassay of antimicrobial activity

Among 422 bacteria isolated from four South China Sea sponges, *Stelletta tenuis* (A), *Halichondria rugosa* (B), *Dysidea avara* (C) and *Craniella australiensis* (D), 98 bacteria belonging to the *Firmicutes*, *Proteobacteria* and actinomycetes (Li and Liu 2006b; Li *et al.* 2007b) were selected randomly for PKS genes screening. Antimicrobial bioassays were carried out by the paper-disc method using six indicator species, *Escherichia coli* (AS 1·3373), *Staphylococcus aureus* (AS 1·2465), *Pseudomonas fluorescens* (AS 1·55), *Bacillus subtilis* (AS 1·3343), *Candida albicans* (AS 2·2086) and *Aspergillus niger* (AS 3·5487) from the Chinese Biodiversity Information Center, Chinese Academy of Sciences. Isolated bacteria were cultured in medium containing 10 g $l^{-1}$ peptone, 5 g $l^{-1}$ beef extract prepared with artificial sea water (ASW; Li and Liu 2006b) at 37°C for 24 h. Microbial indicators, except for *A. niger*, were first cultured in the same medium as before using distilled water instead of ASW at 37°C for 18–24 h and then spread on plates with the same medium containing 2% agar. *Aspergillus niger* was cultured in medium containing 30 g $l^{-1}$ sucrose, 2 g $l^{-1}$ $NaNO_3$, 1 g $l^{-1}$ $K_2HPO_4$, 0·5 g $l^{-1}$ $MgSO_4{\cdot}7H_2O$, 0·5 g $l^{-1}$ KCl, 0·01 g $l^{-1}$ $FeSO_4$ at 28°C for 7 days and then spread on plates with the same medium containing 2% agar. Forty microlitre of the isolate cultures was pipetted onto 5-mm paper discs and placed on the surface of the indicator plate medium. After incubation at 28°C for 3–4 days for indicator *A. niger* and 37°C for 1 day for the other five indicators, the diameter (mm) of the clear inhibition zone produced around each paper disc was measured. The tests were repeated thrice with duplicates in every repetition. The final value of the zone diameter was an average of six results.

### Genomic DNA extraction

The *Firmicutes* and *Proteobacteria* bacteria were subjected to centrifugation at 10 000 rev $min^{-1}$ for 10 min at 4°C and the pellet was suspended in 100 $\mu$l of artificial calcium-/magnesium-free seawater (CMFSW; 31·6 g NaCl, 0·75 g KCl, 1·0 g $MgSO_4$, 2·4 g Tris-HCl, 20 mmol $l^{-1}$ EDTA, $H_2O$ 1 l, pH 7·0). Then 150 $\mu$l of cell-break buffer (50 mmol $l^{-1}$ Tris, 5 mmol $l^{-1}$ EDTA, 1% sodium dodecyl sulfate or SDS, pH 11·5) and 150 $\mu$l of proteinase K reaction buffer (50 mmol $l^{-1}$ Tris, 15 mmol $l^{-1}$ $CaCl_2$, pH 3·0) were added. After incubation with proteinase K (10 mg $ml^{-1}$) at 55°C for 30 min, genomic DNA was extracted sequentially with Tris-phenol (pH 8·0), Tris-phenol/chloroform/isoamyl alcohol (25 : 24 : 1) and chloroform/isoamyl alcohol (24 : 1) and precipitated with isopropyl alcohol. In the case of actinomycetes, the total DNA was extracted according to the modified phenol–chloroform method (Li *et al.* 2006a). RNA was removed by RNase (10 mg $ml^{-1}$; Sigma). The genomic DNA was stored at −20°C before PCR amplification.

### 16S rRNA gene sequencing and BLAST analysis

Amplification of 16S rDNA was performed in a Master Cycler Gradient (Hybaid, Middlesex, UK) with the primers 27f (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-CTACGGCTACCTTGTTACGA-3′) (Polz *et al.* 1999). PCR was carried out as follows: initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 2 min; and a final extension of 10 min at 72°C. PCR products were purified by electrophoresis in a 1·5% (wt/vol) agarose gel and recovered using a gel purification kit (Shenergy Biocolor Bioscience & Technology Company, Shanghai, China). The purified PCR products were ligated into the pUCm-T vectors (Shenergy Biocolor Bioscience & Technology Company), and transformed into competent *E. coli* DH5α. 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 colour-based recombinant selection. Positive clones were identified by PCR amplification with pUCm-T vector primer pairs T7 (5′-TAATACGACTCACTATAGGG-3′) and M13 (5′-CAGGAAACAGCTATGACC-3′) using 55°C as the annealing temperature.

16S rDNA sequences were determined with an ABI 3730 automatic capillary DNA sequencer (AGRF, Brisbane, Australia) with primers T7 and M13. 16S rDNA sequences were matched with sequences in the GenBank database using the BLAST search programme (http://www.ncbi.nlm.nih.gov/). The 16S rRNA gene sequences were deposited in GenBank with accession numbers EF370046–49, EF 370051–55, DQ274113, DQ274116, DQ091004, DQ277986, DQ091005–7, DQ180135 and DQ180138.

### KS gene screening and phylogenetic analysis

The primers listed in Table 1 were used to amplify the KS domain genes of PKS gene clusters. PCR was performed using the following amplification parameters: initial denaturation at 94°C for 5 min; then 35 cycles of 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min; and a final extension at 72°C for 10 min. The products were assessed by gel electrophoresis and amplicons of the correct size (700 bp) were gel purified and cloned into pUCmT vectors. The sequences of the cloned inserts were determined using T7 or M13 primers. Deduced amino acid sequences of KS domains were used as queries to search related proteins in the nr protein database at NCBI using the BLASTP algorithm with default parameters. A phylogenetic tree based on the amino acid sequences of KS domains was constructed using the multiple sequence alignment tools in CLUSTALX and MEGA package. Neighbour-joining method was adopted with 1000 bootstrap reiterations. The KS gene sequences were deposited in GenBank under the accession numbers EF622022–EF622038, and EF513145.

### Fosmid library construction and PKS gene cluster screening

A Copy Control Fosmid Library Production Kit (Epicentre, WI, USA) was used to construct a fosmid library of *Staphylococcus lentus* isolate A75 following the manufacturer's recommendation. Recombinant colonies were transferred into 96-well plates. The PKS-containing clones were detected by PCR with primers GCf and GCr (Schirmer *et al.* 2005) under the following conditions: 94°C for 5 min; 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, 35 cycles; 72°C for 10 min. PKS gene cluster-positive clones were selected for sequencing. After treatment with restriction enzymes *Pst*I, *Pvu*I, *Eco*72I, *Bsp*I, *Msp*I, *Hind*III, *Xba*I and *Bsp*120I, the fragments were subcloned into pUCm-T vectors and sequenced using T7 and M13 primers. The PKS cluster sequence was assembled from the sequences of the subclones.

### Analysis of sequence similarities and PKS domains

Amino acid sequences were deduced from the nucleotide sequences by the ExPASy Molecular Biology Server. The putative amino acid sequences were predicted by PKS/NRPS (nonribosomal peptide synthetase)-software (http://btisnet.nii.res.in/~zeeshan/webpages/nrpsall.html)

**Table 1** Primers used for polymerase chain reaction screening of beta-ketosynthase domains

| Primer | Sequence(5′-3′) | Reference |
|---|---|---|
| GBf | RTRGAYCCNCAGCAICG | Kim and Fuerst 2006 |
| GBr | VGTNCCNGTGCCRTG | |
| GCf | GCSATGGAYCCSCARCARCGSVT | Schirmer *et al.* 2005 |
| GCr | GTSCCSGTSCCRTGSSCYTCSAC | |
| KSDPQQf | MGNGARGCNNWNSMNATGGAYCCNCARCANMG | Fieseler *et al.* 2007 |
| KSHGTGr | GGRTCNCCNARNSWNGTNCCNGTNCCRTG | |
| KSI1f | 5′-GCI ATGGAYCCICARCARMGIVT-3′ | Schirmer *et al.* 2005 |
| KSI2r | 5′-GTICCIGTICCRTGISCYTCIAC-3′ | |

and aligned with other known sequences using the BLAST programme (http://ncbi.nlm.nih.gov/blast). The obtained PKS gene cluster was deposited in GenBank with accession number EU165038.

## Results

By PCR screening, 18 of the 98 bacterial isolates from South China Sea sponges, *S. tenuis*, *H. rugosa*, *D. avara* and *C. australiensis* were found to be KS domain-positive. The phylogeny of the 18 isolates is depicted in Table 2. 16S rRNA gene-based BLAST analysis indicated that 15 of the isolates belonged to the phylum *Firmicutes*, among which 14 isolates were closely related to genus *Bacillus*, and 1 to *Staphylococcus lentus*. Two isolates were identified as actinomycetes, and one as *Alcaligenes* sp. in the phylum *Proteobacteria*.

According to BLAST analysis based on the deduced amino acid sequences, most of the KS domains (12/18) matched those of *Bacillus* with identity above 97% (Table 2). In Fig. 1, sequences of various modular PKS types, including NRPS-PKS, *trans*-acyl transferase (*trans*-AT) PKS, *cis*-acyl transferase (*cis*-AT) PKS and FAS (fatty acid synthase), were selected as references, and the representatives of type II PKS were selected as out-groups. The 18 KS domain sequences were grouped into *trans*-AT type I PKS, and closely related to PKS of marine bacterial symbionts (Fig. 1). No KS domains of these sponge-associated bacteria exhibited a close relationship with FAS or PKS-like FAS.

As shown in Table 3, all of the 18 bacteria with KS genes exhibited antimicrobial activity, and most of the bacteria were active against multiple indicator organisms suggesting broad-spectrum antimicrobial activities. For instance, isolates A75 and B63 exhibited antimicrobial activities against five indicators including fungi, Gram-positive and Gram-negative bacteria.

A PKS gene cluster fragment of 21·8 kb in length (GenBank No. PKS-A75 EU165038) was found by PCR screening of the fosmid library of *S. lentus* isolate A75. A search (http://btisnet.nii.res.in/~zeeshan/webpages/nrpsall.html) yielded five modules as shown in Fig. 2. Module one includes the T, KS and acyl carrier protein (ACP) domains, module two the KS domain, module three the KS, AT, KR and two ACP domains, module four the KS and AT domains and five the KS and KR domains. The T domain serves as the peptidyl carrier domain that covalently binds the substrate via a thioester linkage, the ACP domain with a phosphopantetheinyl arm that carries out unit loading and the AT domain is for carboxylic acid unit selection. The KS domain is for decarboxylative condensation. The function of the ketoreductases (KR) domain is for the postextensional modification of a growing chain (Minowa *et al.* 2007). The results of the homology search of amino acid sequences of NRPS-PKS domains in the GenBank

**Table 2** Similarity of 16S rDNA sequences and beta-ketosynthase domain amino acid sequences to their relatives in GenBank

| Strain | 16S rDNA accession no. | Closest relative (accession no.) | Identity (%) | KS gene accession no. | Closest relative (accession no.) | Identity (%) |
|---|---|---|---|---|---|---|
| A11 | DQ274113 | *Alcaligenes* sp. L981 (AY371437) | 98 | EF622022 | *Bacillus subtilis* W168 PKSX (AAA85144) | 98 |
| A45 | DQ274116 | *Bacillus sporothermodurans* (U49080) | 95 | EF622023 | *Bacillus subtilis* complete genome section9 (Z99112) | 98 |
| A75 | DQ091004 | *Staphylococcus lentus* SSH39 (AB219154) | 100 | EF622024 | *Bacillus subtilis* BA7 (CAA78479) | 96 |
| B17 | EF370046 | *Bacillus subtilis* BCRC 17366 (EF433402) | 100 | EF622029 | *Bacillus subtilis* W168 PKSX (AAA85144) | 94 |
| B18 | DQ277986 | *Bacillus subtilis* MO2 (AY553095) | 100 | EF622030 | *Bacillus subtilis* PKSJ (P40806) | 97 |
| B19 | EF370047 | *Bacillus subtilis* AU25 (EF032688) | 100 | EF622031 | *Bacillus subtilis* PKSX (AAA85144) | 95 |
| B22 | EF370048 | *Bacillus* sp. MHS007 (DQ993316) | 100 | EF622032 | *Bacillus subtilis* strain 168 (NP389598) | 97 |
| B27 | EF370049 | *Bacillus subtilis* MO2 (AY553095) | 100 | EF622033 | *Bacillus subtilis* strain 168 (NP389601) | 99 |
| B63 | EF370051 | *Bacillus amyloliquefaciens* (AY620954) | 100 | EF622034 | *Bacillus amyloliquefaciens* FZB42 (YP001421792) | 99 |
| B114 | EF370052 | *Bacillus subtilis* CGMCC1869 (EF159949) | 100 | EF622025 | *Bacillus subtilis* strain 168 (NP389600) | 98 |
| B131 | EF370053 | *Bacillus subtilis* BZ15 (AY162133) | 100 | EF622026 | *Bacillus subtilis* PKSP (E69679) | 99 |
| B144 | EF370054 | *Bacillus subtilis* AU25 (EF032688) | 99 | EF622027 | *Bacillus subtilis* strain 168 (NP389598) | 97 |
| B145 | EF370055 | *Bacillus subtilis* WL-6 (DQ198162) | 100 | EF622028 | *Bacillus subtilis* strain 168 (NP389600) | 97 |
| C51 | DQ091005 | *Bacillus vallismortis* (AB021198) | 99 | EF622035 | *Bacillus subtilis* strain 168 (NP389600) | 91 |
| C77 | DQ091006 | *Bacillus vallismortis* (AB021198) | 100 | EF622036 | *Bacillus subtilis* BA7 (CAA78479) | 91 |
| C89 | DQ091007 | *Bacillus vallismortis* (AB021198) | 99 | EF513145 | *Bacillus subtilis* strain 168 (NP389600) | 91 |
| DA20 | DQ180135 | *Actinomycetales* bacterium H07 (AY944257) | 100 | EF622037 | *Bacillus subtilis* PKSJ (P40806) | 97 |
| DA23 | DQ180138 | *Streptomyces* sp. FXJ23 (AY314785) | 99 | EF622038 | *Bacillus subtilis* PKSJ (P40806) | 97 |


 

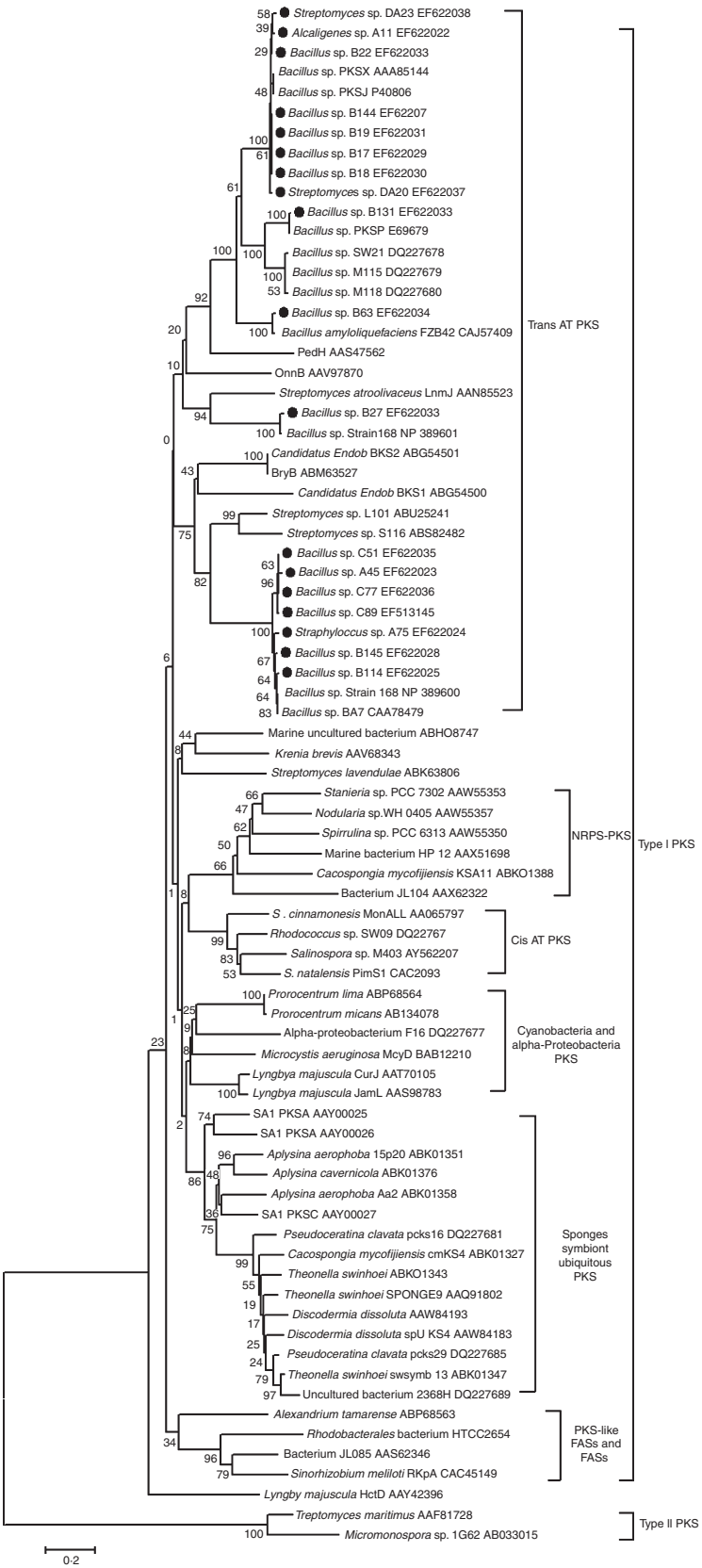


**Figure 1** Neighbour-joining phylogenetic tree of bacteria associated with sponges (indicated by·) and reference sequences based on the amino acid sequences of KS domains. Percentage bootstrap values of neighbour-joining analysis from 1000 resamplings are indicated at the nodes. The scale bar represents 0·2 amino acid substitutions per site.

**Table 3** Antimicrobial activities against indicators

| Strain | Indicator micro-organisms | | | | | |
|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Aspergillus niger* | *Candida albicans* | *Pseudomonas fluorescens* | *Bacillus subtilis* | *Escherichia coli* |
| A11 | ++ | + | ++ | – | + | + |
| A45 | ++ | – | – | ++ | – | – |
| A75 | ++ | ++ | + | + | ++ | – |
| B17 | ++ | + | – | ++ | – | – |
| B18 | + | + | – | +++ | – | – |
| B19 | ++ | + | – | ++ | – | – |
| B22 | + | + | – | +++ | – | – |
| B27 | ++ | +++ | – | + | – | – |
| B63 | ++ | ++ | – | +++ | + | + |
| B114 | +++ | + | – | +++ | – | + |
| B131 | + | – | – | + | – | – |
| B144 | + | +++ | – | ++ | – | – |
| B145 | + | + | – | ++ | – | – |
| C51 | + | – | – | ++ | – | – |
| C77 | – | + | – | + | – | – |
| C89 | + | – | – | ++ | – | – |
| DA20 | – | – | – | + | – | – |
| DA23 | + | – | – | + | + | – |

–, no inhibition; +, inhibition zone ≤2 mm; ++, inhibition zone ≤4 mm; +++, inhibition zone >4 mm.

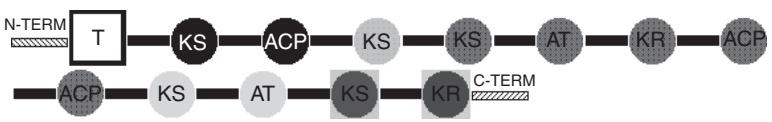


**Figure 2** The putative domains of PKS gene cluster fragment of *Staphylococcus lentus* isolate A75. AT, acyltransferase; ACP, acyl carrier protein; KS, beta-ketosynthase; KR, ketoreductase; T, thiolation domain

**Table 4** Amino acid sequence alignment for PKS domains of *Staphylococcus lentus* isolate A75

| PKS domain | Amino acid sequence position | Identity(%) | Closest relative | Sequence no.* |
|---|---|---|---|---|
| T | 546–570 | 73 | *Bacillus subtilis* | NP389600 |
| KS | 662–1100 | 68 | *B. subtilis* | CAA78479 |
| ACP | 1993–2031 | 74 | *B. subtilis* | NP389600 |
| KS | 2137–2286 | 88 | *B. subtilis* | CAA78479 |
| KS | 3686–3802 | 90 | *B. subtilis* | NP389600 |
| AT | 3898–4009 | 83 | *B. subtilis* | AAF08794 |
| KR | 4623–4700 | 77 | *B. subtilis* | NP389600 |
| ACP | 4743–4802 | 65 | *B. subtilis* | CAA78479 |
| ACP | 4875–4908 | 65 | *B. subtilis* | NP389600 |
| KS | 4983–5271 | 78 | *B. subtilis* | CAA78479 |
| AT | 5391–5504 | 71 | *B. subtilis* | AAF08794 |
| KS | 5990–6401 | 79 | *B. subtilis* | CAA78479 |
| KR | 7025–7113 | 82 | *B. subtilis* | NP389600 |

*Accession numbers from protein databases where the sequences were identified.

database are shown in Table 4. The deduced amino acid sequences showed high identities with known sequences from *B. subtilis* (NP_389600, CAA78479 and AAF08794).

## Discussion

*Bacillus* is well known to produce metabolites with antimicrobial, antifungal and cytotoxic properties. Although *Bacillus* is typically a terrestrial bacterium, it has also been isolated from marine organisms such as soft corals (Kapley *et al.* 2007), and marine sponges (Prokofeva *et al.* 1999; Hentschel *et al.* 2001). The previous investigation of the *in vivo* bacterial communities of the four sponges, *S. tenuis*, *H. rugosa*, *D. avara* and *C. australiensis*, by culture-independent denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene library methods did not show a preponderance of members of the phylum *Firmicutes*, but members of the genus *Bacillus* were detected in a 16S rRNA library (Li and Liu 2006b; Li *et al.* 2006a, 2007a). The high number of *Bacillus* isolates in this study may be owing to the selectivity of the medium.

Piel *et al.* 2004a were the first to investigate a PKS gene cluster obtained from the marine sponge *Theonella swinhoe*, and now PKS gene clusters from more than 25 demosponge species from different oceanic locations near Japan, Australia, Bahamas, France, Netherlands and China

have been investigated, and most of the reported marine PKS are type I PKS (Schirmer *et al.* 2005; Kim and Fuerst 2006; Fieseler *et al.* 2007). Similarly, all the detected PKS in this study belong to type I and show a close relationship with the reported marine PKS. For instance, DQ227678, DQ227679 and DQ227680 were found in *Bacillus* sp. associated with the marine sponge *Pseudoceratina clavata* (Kim and Fuerst 2006); AAY00025, AAY00026 and AAY00027 came from the uncultured bacterial symbionts of sponge *Discodermia dissolute*; ABM63527 was from *Candidatus Endobugula sertula*, an uncultivated bacterial symbiont of the marine bryozoan *Bugula neritina*; AAS47562 was from the bacterial symbiont of *Paederus fuscipes* and AAV97870 was from the symbiont bacterium of *Theonella swinhoe*. Marine type II PKS has been rarely found (Jiang *et al.* 2007, 2008), and there is no report on marine type III PKS to date.

In bacteria, the biosynthesis of polyketide compounds, such as those of the macrolide, polyene or polyether type, usually requires type I PKS, which are large multifunctional enzymes organized as modules. Type I PKS have been of special interest for their modularity and suitability for the creation of customized polyketides (McDaniel *et al.* 1994; Hopwood 1997; Pfeifer *et al.* 2001). A multiplasmid approach employing type I PKS genes has the potential to create thousands of combinations of PKS modules to produce novel polyketides (Xue *et al.* 1999). Bacterial type II and III PKS are heterologous or homologous multi-enzyme complexes carrying a single set of iteratively acting activities that are generally responsible for aromatic polyketides (Malpartida and Hopwood 1984; Funa *et al.* 1999).

Some KS genes are included in the NRPS-PKS group, which includes a certain proportion of current marine PKS genes of cyanobacteria (Ehrenreich *et al.* 2005), *Roseobacter* (Martens *et al.* 2007) and bacteria associated with sponges (Fieseler *et al.* 2007). We also found NRPS genes in seven isolates A11, A75, B27, B114, B131, B144, B145 (Zhang *et al.* 2008), suggesting the presence of this NRPS-PKS gene cluster type in these sponge-associated bacteria.

It has been suggested that the sponge-associated bacteria may play chemical defense roles by releasing biologically active metabolites (Müller *et al.* 2004). The role of sponge-associated bacteria in the chemical defense of sponge hosts is supported by the PKS genes screening and antimicrobial tests in this study. According to the phylogenetic analysis, most of the closest relatives of the obtained KS domains are involved in the production of pharmacologically active polyketides. For example, BKS1 (ABG54500) and BKS2 (ABG54501) from an uncultured microbial symbiont of the marine bryozoan *Bugula neritina*, *Candidatus Endobugula sertula*, were thought to be responsible for bryostatin production (Lopanik *et al.* 2006). BryB (ABM63527), an unusual modular polyketide synthase, was suggested to be involved in bryostatin synthesis by the uncultivated bacterial symbiont of *B. neritina* (Hildebrand *et al.* 2004; Sudek *et al.* 2007). YP001421792 from *Bacillus amyloliquefaciens* FZB42 isolated from a plant rootstalk can accelerate the growth of plants and help plants defend against invading pathogens (Chen *et al.* 2006). PedH (AAS47562) from a sponge was suggested to be related to an enzyme that synthesizes pederin-type polyketides (Piel *et al.* 2004a). LnmJ (AAN85523) was found in *Streptomyces atroolivaceu*, a symbiont of beetles, and this bacterium's PKS gene cluster was found to be involved in the synthesis of an anticancer drug leinamycin (Tang *et al.* 2004).

In summary, combined with bioactivity assays, the PKS gene-based molecular approach can be applied to efficient screening of strains of pharmaceutical value and the prediction of related compounds. The PKS gene diversity and different antimicrobial spectra indicate the chemical diversity of polyketide metabolites. *Firmicutes* especially *Bacillus*, actinomycetes and *Proteobacteria* isolates from sponge in the South China Sea may be potential sources for polyketides production.

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