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Diversity and Chemical Defense Role of Culturable Non-Actinobacterial Bacteria Isolated from the South China Sea Gorgonians

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The diversity of culturable non-actinobacterial (NA) bacteria associated with four species of South China Sea gorgonians was investigated using culture-dependent methods followed by analysis of the bacterial 16S rDNA sequence. A total of 76 bacterial isolates were recovered and identified, which belonged to 21 species of 7 genera, and *Bacillus* was the most diverse genus. Fifty-one percent of the 76 isolates displayed antibacterial activities, and most of them belonged to the *Bacillus* genus. From the culture broth of gorgonian-associated *Bacillus methylotrophicus* SCSGAB0092 isolated from gorgonian *Melitodes squamata*, 11 antimicrobial lipopeptides including seven surfactins and four iturins were obtained. These results imply that *Bacillus* strains associated with gorgonians play roles in coral defense mechanisms through producing antimicrobial substances. This study, for the first time, compares the diversity of culturable NA bacterial communities among four species of South China Sea gorgonians and investigates the secondary metabolites of gorgonian-associated *B. methylotrophicus* SCSGAB0092.

Key words: Gorgonian-associated bacteria, diversity, *Bacillus methylotrophicus*, lipopeptides, antimicrobial activity

Interactions between epibiotic marine bacteria and their host organisms are known to play a significant role in marine ecosystems, but this association has received little attention [8]. Coral surfaces often harbor diverse microbial communities, and it appears that these microorganisms are not generally detrimental to their coral hosts [5]. For instance, microorganisms found on coral surfaces may provide the host with protection from pathogens [24]. To date, many bioactive compounds isolated from corals are characterized to play important roles in protecting the colonies against grazing, feeding, and marine biofouling [4]. However, it is recognized that symbiotic bacteria may be the true producers [6]. Accordingly, coral-associated microorganisms are suggested to be a possible first line of chemical defense for corals, possibly through productions of bioactive substances [24, 27].

The secondary metabolites of gorgonian-associated bacteria and their possible chemical defense role for their hosts remain unclear for the large part. Aiming to assess the antibacterial potential of gorgonian-associated bacteria, we investigated the diversity of culturable non-actinobacterial (NA) bacteria associated with four species of healthy South China Sea gorgonians, and screened bioactive bacterial strains through antimicrobial tests. In order to further get a clear understanding of the bioactive substances produced by these strains, we selected the antibacterial bacterium *Bacillus methylotrophicus* SCSGAB0092 isolated from gorgonian *Melitodes squamata* to study its secondary metabolites. In this paper, we describe the isolation, identification, and screening of marine gorgonian-associated NA bacteria, and also the isolation and structure elucidation of secondary metabolites from gorgonian-associated *B. methylotrophicus* SCSGAB0092.

Materials and Methods

Gorgonian-Associated NA Bacterial Isolation and Identification

Four species of South China Sea gorgonians (*Dichotella gemmacea*, *Melitodes squamata*, *Muricella flexuosa*, and *Subergorgia suberos*) were used for NA bacterial isolation. Detailed information of the gorgonian sampling site, sample collection, and treatment was given

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by Zhang *et al*. [33]. After the gorgonian samples were thoroughly homogenized with a sterile mortar with the addition of 2 volumes of sterile seawater, a 10-fold dilution was made and 0.1 ml of the resulting solution was plated on four different media (M1–M4) plates. The compositions of the four media were as follows (g/l): M1: glucose (4), yeast extract (4), malt extract (5); M2: mannitol (2), L-asparagine (0.1), $CaCO_3$ (2), K_2HPO_4 (0.5), $MgSO_4$ (0.1), $FeSO_4$ (0.001), vitamin B_1 (0.001), vitamin B_6 (0.001), vitamin lactoflavin (0.001), nicotinic acid (0.001), biotin (0.001), phenylalanine (0.001), alanine (0.0003); M3: peptone (5), yeast extract (3), NaCl (5); M4: typtone soy (15). All media contained 20 g of agar and 1 L of seawater, and were adjusted to pH 7.0. The inoculated plates were incubated at 30°C for 1–4 weeks until the morphology of the bacteria could be distinguished. In order to inhibit the growth of fungi, 0.05 g/l streptomycin and potassium dichromate (50 ml of 1 g/l sterilized potassium dichromate in 1 L of sterilized media) were added to the media.

Total genomic DNA was extracted from all selected bacterial isolates as described by Li and De [15]. From the genomic DNA, nearly full-length 16S rRNA gene sequences were amplified by polymerase chain reaction using primers 27F (5'-GAGTTTGAT CCTGGCTCAG-3') and 1525R (5'-AGAAAGGAGGTGATCCAGCC-3'). DNA sequencing of the selected bacterial strains was carried out by Invitrogen (China). Sequences were corrected using Sequencher, and the most similar sequences in GenBank were found using BLAST (Basic Local Alignment Search Tool) searches. When the top three matching BLAST hits were from the same species and were 97% similar to the query sequence, this species name was assigned to the selected isolate [29].

Screening for Antimicrobial Strains

The antimicrobial activities of 76 isolated gorgonian-associated NA bacteria were determined by a double-layer technique [31]. These strains were grown on seawater medium M3 plates, which were allowed to grow for 5–7 days depending on the growth rate of the various strains. The agar blocks containing the cells were then excised and placed on the assay plates spread with five indicator bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and two marine bacteria (*Pseudoaltermonas piscida* UST010620-005 and *Micrococcus luteus* UST950701-006). The two marine indicator bacteria were cultured on medium M3 plates at 30°C for 24–48 h, and other three indicator bacteria were cultured on LBA (peptone 10 g/l, yeast extract 5 g/l, NaCl 10 g/l, agar 20 g/l, pH 7.0) plates at 37°C overnight. The antibacterial activity was expressed as the diameter of the growth inhibition zone (in millimeter). All the indicator microorganisms were from the South China Sea Institute of Oceanology, Chinese Academy of Sciences.

Preparation and Analysis of Marine Bacterial Crude Extracts

Prior to scale-up fermentation, further tests were performed on the antimicrobial active strains to compare the effects of different growth media on secondary metabolite production. The strains were inoculated in 6 seawater fermentation media (M5–M10) and incubated for 5 days at 30°C. The compositions of the media were as follows (g/l): M5: peptone (10), yeast extract (5), NaCl (10); M6: starch (10), yeast extract (4), peptone (2), $Fe_2(SO_4)_3{\cdot}4H_2O$ (0.04), KBr (0.1); M7: glucose (15), soy meal (15), cornsteep powder (5), $CaCO_3$ (2); M8: starch (10), yeast extract (2), glucose (10), glycerol (10), cornsteep powder (2.5), peptone (2), $CaCO_3$ (3); M9: trehalose (10), glycerol (6), yeast extract (3), $FeSO_4$ (0.01), $CaCO_3$ (2); M10: starch (5), trehalose (2), soya peptone (3), fish flour (2). All media contained 1 L of seawater and were adjusted to pH 7.0. Each culture broth was centrifuged (3,220 ×*g*, 15 min) to pellet the cells and the cell-free supernatant was extracted twice with an equal volume of ethyl acetate. The solvent extracts were combined and evaporated to dryness under reduced pressure at 45°C to yield crude extracts, and each crude extract obtained was weighed [19]. All prepared crude extracts were analyzed by using an analytical RP-HPLC column (Inertsil ODS-SP, 5 μm, 4.6×150 mm) with a photodiode array detector. The crude extracts were eluted using a linear gradient of water : methanol (from 95:5 to 0:100) in 35 min, and then 100% methanol for 10 min at a flow rate of 1.0 ml/min. The chemical compositions of the extracts were initially deduced from the UV absorption characteristics.

Bacterial Strain Cultivation and Scale-Up Fermentation

Based on the analysis of crude extracts by HPLC, the most appropriate bacterial strain *B. methylotrophicus* SCSGAB0092 and medium used for scale-up fermentation were selected. An inoculum of bacteria strain from an overnight cultured plate was inoculated into a 500-ml Erlenmeyer flask containing 150 ml of sterile seed liquid medium and cultured on a rotary shaker (200 rpm) at 30°C for 2 days. Then, 7.5 ml of the seed culture was transferred into 500-ml Erlenmeyer flasks containing 150 ml of scale-up fermentation medium and cultivated on a rotary shaker (200 rpm) at 30°C for 5 days. Both seed medium and scale-up fermentation medium were medium M6.

Extraction and Isolation of Compounds

A total of 40 L of fermentation broth was extracted by the process mentioned above to yield 10.5 g of crude gum. The crude gum was subjected to silical gel column chromatography followed by stepwise elution with $CHCl_3$-MeOH (100:0, 99:1, 98:2, 95:5, 90:10, 80:20, 60:40, 0:100), and to give eight fractions (I–VIII) by TLC. Fraction V was subjected to Rp-18 reverse-phase column chromatography, eluting with MeOH/H_2O (v/v from 30:70 to 100: 0) to give five subfractions (V-1 to V-5). One third of subfraction V-4 (677.1 mg) was repeatedly purified on a semi-preparative RP-HPLC column (Phenomenex Gimini 5μ C18 110A) with 82% MeCN/H_2O (0.01% CF_3COOH) at a flow rate of 3 ml/min to yield compounds **1** (18.6 mg, t_R = 20.5 min), **2** (9.7 mg, t_R = 40.5 min), **3** (35.5 mg, t_R = 27.0 min), **4** (4.0 mg, t_R = 16.5 min), **5** (3.6 mg, t_R = 26.0 min), **6** (18.5 mg, t_R = 28.5 min), and **7** (3.6 mg, t_R = 45.0 min). Fraction VIII was applied to a Sephadex LH-20 column eluted with $CHCl_3$–MeOH (1:1) and then to Rp-18 reverse-phase column chromatography eluting with MeOH/H_2O (v/v from 60:40 to 100: 0) to give two subfractions (VIII-1 and VIII-2). Subfraction VIII-2 (150 mg of 2424.1 mg) was further purified on a semi-preparative RP-HPLC column (YMC-Pack, ODS S-5μ 250×10 mm i.d.), and eluted with 70% CH_3OH/H_2O at a flow rate of 3 ml/min to give compounds **8** (22.3 mg, t_R = 22.3 min), **9** (31.0 mg, t_R = 53.4 min), **10** (14.6 mg, t_R = 60.4 min), and **11** (5.0 mg, t_R = 30.2 min).

Identification of Compounds

The structures of the compounds were determined by spectroscopic analysis. ^1H, and ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker AV-500 MHz NMR spectrometer with TMS as the internal standard. High-resolution electrospray ionization mass spectrometry (HRESIMS) and electrospray ionization mass spectrometry (ESIMS)

were separately obtained on a Bruker maXis mass spectrometer and Bruker amaZonSL mass spectrometer (Bruker Daltonics, Inc., Bremen, Germany).

RESULTS

Diversity and Distribution of Gorgonian-Associated NA Bacteria

A total of 76 NA bacterial strains were isolated and purified from the four species of gorgonians, *D. gemmacea*, *M. squamata*, *M. flexuosa*, and *S. suberos*. As identified by bacterial 16S rDNA sequences, the 76 isolates in these gorgonians belonged to 21 species of seven genera: *Bacillus*, *Staphylococcus*, *Halobacillus*, *Geobacillus*, *Jeotgalicoccus*, *Psychrobacter*, and *Vibrio*. *Bacillus* was the most diverse genus with 54 isolates belonging to 15 species, and *Staphylococcus* was the second most diverse genus with 12 isolates. The rest of the bacterial genera occurred as one to three isolates.

The distribution of NA bacteria in the four species of gorgonians is shown in Table 1. Among the 21 bacterial species, *B. methylotrophicus* and *S. equorum* were the most abundant species, which could be recovered from all the four gorgonian species. Whereas *B. altitudinis* was only isolated from gorgonians *D. gemmacea* and *M. squamata*, *B. stratosphericus* was only isolated from gorgonians *M. squamata* and *M. flexuosa*, and *B. tequilensis* was only recovered from gorgonians *D. gemmacea* and *S. suberosa*. The rest of the bacterial species were rare in these gorgonians.

Antimicrobial Active Strains

All the 76 NA bacterial isolates were tested against five indicator bacteria. Thirty-nine isolates belonging to 14 species displayed antibacterial activity against at least one bacterium (Table 2). Most of the antibacterial isolates exhibited moderate or strong activity against Gram-positive bacteria including *S. aureus* and *M. luteus*. Six isolates exhibited moderate or strong activity against at least three indicator bacteria. Among the antibacterial isolates, five strains identified as *Bacillus tequilensis* SCSGAB0078, *Bacillus amyloliquefaciens* SCSGAB0082, *Bacillus pumilus* SCSGAB0102, *Bacillus methylotrophicus* SCSGAB0092, and *Geobacillus stearothermophilus* SCSGAB0115 were selected to culture in different media for the subsequent screening.

Table 1. Distribution of non-actinobacteria in four species of gorgonians.

Bacterial species (strain number, accession number, and sequence similarity in GenBank)	Number of bacterial isolates			
	Dichotella gemmacea	*Melitodes squamata*	*Subergorgia suberosa*	*Muricella flexuosa*
Bacillus altitudinis (SCSGAB0018, JX315293, 100%)[a]	6	1		
B. amyloliquefaciens (SCSGAB0082, JX315304, 99%)[d]			1	2
B. arbutinivorans (SCSGAB0126, JX315315, 99%)[c]			1	
B. aryabhattai (SCSGAB0090, JX315306, 100%)[b]	2	1	1	
B. cereus (SCSGAB0095, JX315309, 99%)[b]		1		
B. cibi (SCSGAB0106, JX315312, 99%)[b]		1	1	
B. endophyticus (SCSGAB0048, JX315299, 99%)[d]				1
B. licheniformis (SCSGAB0041, JX315297, 99%)[d]				1
B. megaterium (SCSGAB0051, JX315300, 99%)[d]				1
B. methylotrophicus (SCSGAB0092, JX315308, 99%)[b]	2	2	1	3
B. pumilus (SCSGAB0102, JX315311, 98%)[b]		3	1	
B. subtilis (SCSGAB0128, JX315316, 99%)[c]	1		1	
B. stratosphericus (SCSGAB0061, JX315301, 99%)[d]		1		6
B. tequilensis (SCSGAB0078, JX315303, 99%)[c]	9		1	
B. vallismortis (SCSGAB0089, JX315305, 99%)[d]	1			1
Geobacillus stearothermophilus (SCSGAB0115, JX315313, 99%)[b]	1	1		
Halobacillus naozhouensis (SCSGAB0116, JX315314, 97%)[c]		2	1	
Jeotgalicoccus halophilus (SCSGAB0012, JX315292, 97%)[a]	1		1	
Psychrobacter faeculis (SCSGAB0010, JX315290, 99%)[a]	2			
Staphylococcus equorum (SCSGAB0091, JX315307, 99%)[b]	3	5	3	1
Vibrio cholera (SCSGAB0063, JX315302, 97%)[d]				1
Total number of bacterial species/isolates	10/28	10/18	11/13	9/17

Strains marked by a letter "a", "b", "c", and "d" were isolated from gorgonian *Dichotella gemmacea*, *Melitodes squamata*, *Subergorgia suberosa*, and *Muricella flexuosa*, respectively.

Table 2. Antibacterial activities of bacteria isolated from South China Sea gorgonians.

Bacterial strains	Diameter of the growth inhibition zone (mm)				
	BS	SA	EC	ML	PP
Bacillus altitudinis SCSGAB0018		14.0		12.5	
B. amyloliquefaciens SCSGAB0082	9.0	9.0		20.0	
B. cereus SCSGAB0095	11.0				
B. cibi SCSGAB0106		12.0			
B. licheniformis SCSGAB0041		13.0		19.0	
B. methylotrophicus SCSGAB0092		20.0	11.0	19.0	13.0
B. pumilus SCSGAB0102	11.0	11.0		12.0	11.0
B. stratosphericus SCSGAB0061				10.0	
B. subtilis SCSGAB0128		19.0			
B. tequilensis SCSGAB0078	15.0	11.0		17.0	
B. vallismortis SCSGAB0089		11.0		17.0	
Geobacillus stearothermophilus SCSGAB0115		11.0	9.0	17.0	
Jeotgalicoccus halophilus SCSGAB0012	13.5				
Staphylococcus equorum SCSGAB0091		14.0		13.0	

Relatively high antimicrobial activity: zone of inhibition greater than 12 mm.
Indicator bacteria, EC: *Escherichia coli*; SA: *Staphylococcus aureus*; BS: *Bacillus subtilis*; ML: *Micrococcus luteus*; PP: *Pseudoaltermonas piscida.*

HPLC Analysis of Crude Extracts

Five bioactive strains (SCSGAB0078, SCSGAB0082, SCSGAB0102, SCSGAB0092, SCSGAB0115) were inoculated in six different marine broth media to offer 30 culture broths, and then the broths were extracted with ethyl acetate to offer 30 crude extracts. All the crude extracts were analyzed by HPLC. By analyzing and comparing these chromatograms, it was effortless to find that most of the crude extracts showed only a few peaks, while several crude extracts showed a fair amount of peaks, which implied significant differences between these crude extracts. However, we also observed that several chromatograms were similar, and the main peaks in these chromatograms shared similar UV absorption characteristics, which meant these crude extracts might contain structural analogs (Fig. 1). To confirm the structures of the analogs, we selected one bacterium, *B. methylotrophicus* SCSGAB0092, as the representative for scale-up fermentation.

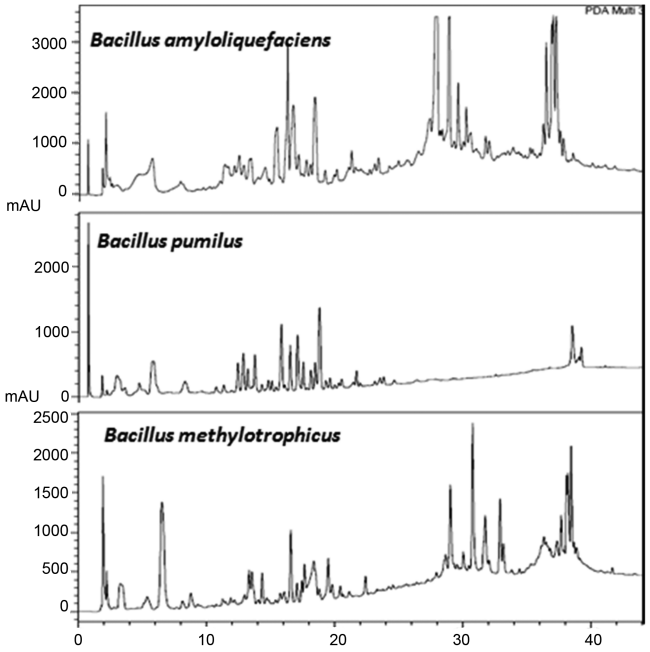

Fig. 1. RP-HPLC chromatograms of the crude extracts yielded from A1BFe medium inoculated with *Bacillus amyloliquefaciens* SCSGAB0082, *Bacillus pumilus* SCSGAB0102, and *Bacillus methylotrophicus* SCSGAB0092, respectively.

Identification of Compounds

From the crude gum extracted from the scale-up fermentation broth of *B. methylotrophicus* SCSGAB0092, two lipopeptide mixture subfractions V-4 and VIII-2 (surfactins and iturins), and 11 pure compounds (compounds **1**–**11**) including 7 surfactin compounds and 4 iturin compounds were obtained (Fig. 2, Table 3). The structural elucidations of these metabolites were based on spectral analyses and comparison with published data.

High-resolution ESI-MS or ESI-MS of **1**–**7** (Table 3) exhibited that the molecular formulas of **1**–**7** were $C_{51}H_{89}N_7O_{13}$, $C_{53}H_{93}N_7O_{13}$, $C_{52}H_{91}N_7O_{13}$, $C_{50}H_{87}N_7O_{13}$, $C_{53}H_{93}N_7O_{13}$, $C_{53}H_{93}N_7O_{13}$, and $C_{54}H_{95}N_7O_{13}$, respectively. The NMR data (500 MHz, DMSO-d_6) of **1**–**7** were greatly similar, which meant that **1**–**7** were analogs. In the ^{1}H NMR spectra of **1**–**7**, respectively, signals of seven NH protons at δ_H 7.0–8.5 and seven -NHCH- methine protons

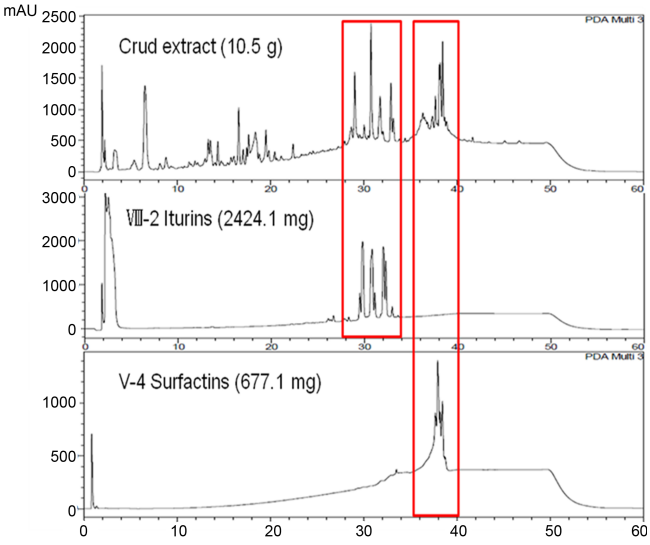

Fig. 2. Comparison of RP-HPLC chromatograms of the crude extract of *B. methylotrophicus* SCSGAB0092, iturins mixture and surfactins mixture.

at δ_H 3.9–4.8 indicated the presence of seven amino acid units; signals of one methine proton at δ_H 5.0–5.1 and long-chain alkyl methylene protons at δ_H 1.1–1.4 indicated the presence of a β-hydroxyl fatty acid chain; and the β-H, γ-H, and δ-H signals of seven amino acid units were also observed at δ_H 1.3–3.0 and δ_H 0.7–0.9. In the ^{13}C NMR spectra of **1–7**, respectively, ten carbonyl signals at δ_C 169–180, seven -NH-CH methine signals at δ_C 49–60, one oxygenated methine at δ_C 69–73, and other methines, methylenes, and methyls at δ_C 10–45 were observed. All the NMR data of **1–7** showed great similarity to those of surfactins [3, 28]. Thus, combined with the molecular formulas, NMR spectral data, and comparison with literatures [3, 28], **1–7** were inferred to be surfactins.

High-resolution ESI-MS of compounds **8–11** (Table 3) showed that the molecular formulas of **8–11** were $C_{48}H_{74}N_{12}O_{14}$, $C_{50}H_{78}N_{12}O_{14}$, $C_{50}H_{78}N_{12}O_{14}$, and $C_{49}H_{76}N_{12}O_{14}$, respectively. The NMR data (500 MHz, DMSO-d_6) of **8–11** showed close similarity, which meant that **8–11** had analogous structures. In the ^{1}H NMR spectra of **8–11**, respectively, seven NH proton signals at δ_H 6.8–8.7 and eight -NHCH- methine proton signals at δ_H 4.0–4.6 indicated the presence of eight amino acid units; signals at δ_H 6.6–7.1 showed the presence of one *para*-substituted benzene ring; signals of long-chain alkyl methylene protons at δ_H 1.1–1.3 and terminal methyl protons at δ_H 0.8–1.0 indicated the presence of a β-amino fatty acid chain; and β-H and γ-H signals of amino acid units were observed at δ_H 1.3–3.0. In the ^{13}C NMR spectra of **8–11**, respectively, twelve carbonyl signals at δ_C 170–185; signals of a *para*-substituted benzene ring at δ_C 115–156; eight -NHCH- methine signals at δ_C 50–65; and other methines, methylenes, and methyls at δ_C 10–45 were observed. All the NMR data of **8–11** showed great similarity to those of iturins [10]. Furtheremore, 2D NMR spectra including HSQC, HMBC, and ^{1}H-^{1}H COSY of **8** further confirmed the sequence of amino acid units of **8** as iturin. Therefore, **8–11** were inferred to be iturins.

DISCUSSION

Coral-associated bacteria were believed to be a possible first line of defense [27]. Although there are a few reports about the diversity of bacteria in gorgonian corals [8, 9], this paper is the first report to compare the diversity of culturable non-actinobacterial bacterial communities among four species of South China Sea gorgonians, and to investigate the secondary metabolites of gorgonian-associated

Table 3. Molecular weight and type of compounds **1–11**.

Number	Molecular weight $[M+H]^+$	Compound type	Cyclic structure	References
1	1,008.67	Surfactin		
2	1,036.6905	Surfactin	$R=C_nH_{2n+1}$ (n=10~13) Val or R—CH—CH_2—CO—Glu—Leu—Leu—Val—Asp—Leu—Leu O	[3, 28]
3	1,022.6718	Surfactin		
4	994.6398	Surfactin		
5	1,036.6887	Surfactin		
6	1,036.6887	Surfactin		
7	1,050.7054	Surfactin		
8	1,043.5449	Iturin		
9	1,071.5773	Iturin	$R=C_nH_{2n+1}$ (n=11~13) R—CH—CH_2—CO—Asn—Tyr—Asn—Gln—Pro—Asn—Ser HN	[10]
10	1,071.5754	Iturin		
11	1,057.5631	Iturin		

B. methylotrophicus SCSGAB0092. It contributes to our knowledge of gorgonian-associated bacteria and their chemical defense roles for their hosts.

Our results showed that *Bacillus* was the most diverse genus in the four gorgonians of *D. gemmacea*, *M. squamata*, *M. flexuosa*, and *S. suberos*. Members of the *Bacillus* genus are often considered microbial factories for the production of a vast array of biologically active molecules [21]. According to our studies, relatively high proportions (70%) of gorgonian-associated NA bacteria belong to the *Bacillus* genus and most of these strains possess antibacterial activities. Besides *B. methylotrophicus* SCSGAB0092, secondary metabolites of other *Bacillus* strains were also analyzed with HPLC. By comparing the chromatograms, we deduced that some of the other *Bacillus* strains also produce similar metabolites as *B. methylotrophicus* SCSGAB0092. Because of the high antagonistic activity, the *Bacillus* genus as the dominant NA bacteria may play a key role in protecting the coral hosts against infectious diseases, which conforms to the hypothesis of Shnit-Orland and Kushmaro [27]. Nevertheless, Shnit-Orland and Kushmaro conjectured that active bacteria might not necessarily secrete metabolites to the environment, but might be active through additional mechanisms, because they found none of the supernatant forms of active cultures of those active bacteria demonstrated antibacterial activity, which was not in concordance with our results. In this study, the secondary metabolites of *B. methylotrophicus* SCSGAB0092 were investigated, and two kinds of antimicrobial lipopeptides identified as surfactins and iturins were isolated from the supernatant of a culture broth of the strain. These findings demonstrate that gorgonian-associated bacteria can produce diverse bioactive substances as a tool to aid the coral holobiont fitness.

To our knowledge, surfactins display hemolytic, antiviral [12], antimycoplasma [30], antibacterial, and antibiofilm [1, 16] activities. Since marine biofilms often induce or enhance the settlement of invertebrates [13, 22], effectively inhibiting the biofilm formation of settlement-inducing bacteria will help protect submerged surfaces from the settlement of macrofoulers [32]. Accordingly, surfactins with antimicrobial and antibiofilm activities may assist the coral holobiont to defend against pathogens and fouling organisms. The biological activities of iturins differ from surfactins. Iturins reveal a strong *in vitro* and *in vivo* antifungal action against a large variety of yeast and fungi but have only limited antibacterial and no antiviral activities [7, 14, 17, 25]. With the antimicrobial activities of lipopeptides, *Bacillus* strains that can efficiently produce these compounds can influence other microbial populations, enabling these bacteria to cope with competing organisms. Furthermore, lipopeptides can act not only as "antagonists" or "killers" by inhibiting pathogen growth but also as "immunostimulators" by reinforcing host resistance potential [21]. It comes to a conclusion that lipopeptides can serve as versatile weapons to protect the hosts from other harmful microorganisms.

On account of their diverse bioacitivities, lipopeptides have also attracted high attentions of researchers from different application fields including agriculture, food [20], petroleum recovery [26], environmental [2, 18], biomedical [23], and cosmetics [11]. Prior to any strain improvement, we prepared a total of 3.1 g of lipopeptides from 10.5 g of crude extract of a 40 L fermentation broth of *B. methylotrophicus* SCSGAB0092. The high yield implies that this strain may be significant for further application research.

In summary, according to this study, bacteria associated with gorgonians, especially *Bacillus* strains, may be the crucial factor in the ecosystem of gorgonians. The biocontrol activities of lipopeptides secreted by *Bacillus* strains contribute to gorgonians regulating the microbial populations that adhere to their surface. Moreover, *B. methylotrophicus* SCSGAB0092 was found to possess potential value in industrial applications for its high yield of lipopeptides.

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