Functional Gene-Guided Discovery of Type II Polyketides from Culturable Actinomycetes Associated with Soft Coral Scleronephthya sp

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Abstract

Compared with the actinomycetes in stone corals, the phylogenetic diversity of soft coral-associated culturable actinomycetes is essentially unexplored. Meanwhile, the knowledge of the natural products from coral-associated actinomycetes is very limited. In this study, thirty-two strains were isolated from the tissue of the soft coral Scleronephthya sp. in the East China Sea, which were grouped into eight genera by 16S rDNA phylogenetic analysis: Micromonospora, Gordonia, Mycobacterium, Nocardioides, Streptomyces, Cellulomonas, Dietzia and Rhodococcus. 6 Micromonospora strains and 4 Streptomyces strains were found to be with the potential for producing aromatic polyketides based on the analysis of KS (ketoacyl-synthase) gene in the PKS II (type II polyketides synthase) gene cluster. Among the 6 Micromonospora strains, angucycline cyclase gene was amplified in 2 strains (A5-1 and A6-2), suggesting their potential in synthesizing angucyclines e.g. jadomycin. Under the guidance of functional gene prediction, one jadomycin B analogue (7b, 13-dihydro-7-O-methyl jadomycin B) was detected in the fermentation broth of Micromonospora sp. strain A5-1. This study highlights the phylogenetically diverse culturable actinomycetes associated with the tissue of soft coral Scleronephthya sp. and the potential of coral-derived actinomycetes especially Micromonospora in producing aromatic polyketides.

Introduction

Corals are considered as the rainforests of the oceans. Coral-derived natural products span a wide range of chemical classes (e.g. prostaglandins, diterpenes, alkaloids and steroids) [1] and display a variety of biological activities (e.g. antitumor, anti-inflammatory and antibacterial activities) [2,3,4,5]. Actinomycetes are widely distributed in marine habitats including the sea surface, water column, marine snow, sediments and marine organisms [6,7,8,9,10,11,12]. Excitingly, many previously unknown actinomycete taxa have been successfully isolated from marine habitats [7,13,14,15]. Meanwhile, novel and unique natural products have been increasingly recovered from marine actinomycetes [6,7,16,17,18,19,20]. It has been demonstrated that some compounds originally isolated from marine invertebrates are in fact produced by microorganisms associated with invertebrates [21]. Actinomycetes are frequent components of symbiotic communities in invertebrates [6]. Since coral-associated actinomycetes could play important role in protecting coral host [22], the actinomycetes associated with corals may be involved in the synthesis of natural products isolated from corals. Investigating the coral-associated actinomycetes facilitates to reveal the true origin of biologically active substances, and therefore, is significant for solving the supply problem in marine drug development. However, to date, related reports on coral-associated actinomycetes are still scarce and mainly limited to stony corals [23,24,25]. Novel compounds with biological activity have been extracted from soft corals [2,3,4,5], so, it is significant to investigate the soft coral-associated actinomycetes regarding their diversity as well as their potential in secondary metabolite biosynthesis.

Generally, traditional activity-based screening of microbial strains and valuable natural products has its inherent limitation because some natural products cannot be synthesized under the normal condition or the compound yield is very low. With the increasing knowledge of biosynthesis gene cluster for synthesizing natural products, functional gene-based analysis provides a useful approach for predicting natural products [26]. Gene-based analysis has been previously applied in predicting type I polyketide biosynthesis in marine Actinobacteria [27]. However, type II polyketide biosynthesis has been rarely concerned. Aromatic polyketides, which are synthesized by type II polyketide synthase (PKS), exhibit a wide array of biological activities including antibacterial, antitumor, antiviral and enzyme inhibitory activities [28], and afford some of the most common antibiotics and anticancer drugs currently in clinical use, e.g. tetracyclines and anthracyclines. Type II PKS consists of three or more enzymes that act in an iterative manner. The core module in all type II PKS gene clusters is composed of ketoacyl-synthase (KS), chain length factor (KSₐ), chain extension factor (KSₐ’), and acyl carrier protein (ACP). Moreover, cyclase is responsible for the cyclization of aromatic polyketides. Thus, KSₐ
and cyclase gene can be used as makers for the screening of type II polyketide compounds.

With the aim to reveal the diversity of culturable Actinobacteria associated with soft coral and screen the actinomycetes with the potential for synthesizing type II polyketides, actinomycetes were isolated from the tissue of soft coral Scleronephthya sp. in the East China Sea. The isolates were tested for their potential in producing aromatic polyketides by the detection of KS₄ and cyclase gene. Finally, type II polyketide compound was identified in the fermentation broth of Micromonospora sp. strain A5-1 under the guidance of functional gene prediction.

**Methods**

**Ethics Statement: N/A**

This study was approved by Shanghai Jiao Tong University, China.

**Sample collection and isolation of actinomycetes**

Soft coral Scleronephthya sp. was collected from Zhao’an Bay (23°53’N; 117°10’E) in the East China Sea. The sample was stored at −20°C until analysis. Coral tissue was rinsed three times with sterile artificial seawater (ASW) (1.1 g CaCl₂, 10.2 g MgCl₂·6H₂O, 31.6 g NaCl, 0.75 g KCl, 1.0 g Na₂SO₄, 2.4 g Tris-HCl, 0.02 g NaHCO₃, 1 L distilled water, pH 7.6) to remove the microbes loosely attached on the surface, and then aseptically grinded using a pestle and a mortar. Six types of media were used for isolating coral-associated actinomycetes [7,10,12,29] (Table S1). All media were supplemented with K₂Cr₂O₇ (50 μg ml⁻¹) to inhibit the growth of fungi, and with nalidixic acid (15 μg ml⁻¹) to inhibit fast-growing Gram-negative bacteria. Actinomycetes were isolated by serial dilution on agar plates in triplicate at 28°C for 3–6 weeks. The colonies bearing distinct morphological characteristics were picked up and transferred onto freshly prepared plates until pure cultures were obtained.

**Genomic DNA extraction**

A single colony was transferred to a 5-ml microtube with 1 ml of liquid medium from which the isolate was originally picked up. The cultures were incubated for 3–5 days at 28°C with shaking at 180 rpm. Microbial cells were collected by centrifugation and genomic DNA was extracted as described by Li and De Boer [30].

**PCR amplification of 16S rRNA gene**

The universal bacterial primers 27F (5’-GAGTTTGATCCTGGCTCAG-3’) and 1500R (5’-AGAAAGGAGGTGATCCAGCC-3’) were used for the amplification of 16S rRNA gene [31]. The PCR was carried out in a 20 μl PCR mixture including 10 μl Taq Premix (Takara, Dalian, China), 0.5 μl 27F (10 μM), 0.5 μl 1500R (10 μM) and 5% DMSO. Cycling conditions were as follows: initial denaturation at 94°C for 3 min, 30 cycles of 94°C for 45 s, 60°C for 1 min, and 72°C for 1 min, and a final extension of 10 min at 72°C.

**PCR amplification of KS₄ and angucycline cyclase gene**

The degenerate primers IIPF6 (5’-TSGGCTGCTCAGYGC-SATC-3’) and IIPR6 (5’-TGGAACCCCGGAABCCCGCT-3’) were employed to amplify the KS₄ gene of PKS II [32]. The PCR was carried out in a 20 μl PCR mixture including 10 μl Taq Premix, 0.8 μl IIPF6 (25 μM), 0.8 μl IIPR6 (25 μM) and 5% DMSO. Cycling conditions were as follows: initial denaturation at 95°C for 3 min, 30 cycles of 95°C for 35 s, 55°C for 40 s, and 72°C for 2 min, and a final extension of 10 min at 72°C.

**Sequencing and phylogenetic analyses**

The PCR products were purified using Agarose Gel DNA Purification Kit (Takara, Dalian, China) and sequenced on an ABI 3730 automated sequencer by Beijing Genomic Institute (Shenzhen, China). The gene sequences obtained were proofread using Chromas, version 1.62 (Technelysium). The nucleotide sequences were matched with published sequences in NCBI using the BLAST search program (http://www.ncbi.nlm.nih.gov/). For KS₄ and cyclase gene, translated protein sequences were derived from nucleotide sequences using the ORF FINDER available at the NCBI (http://www.ncbi.nlm.nih.gov/projects/orf/gorf/). The deduced amino acid sequences were used as queries to search the related proteins in the nr protein database using the BLASTP algorithm. For 16S rRNA gene and KS₄ multiple sequence alignment was performed using CLUSTALX. Phylogenetic tree was constructed using Mega 4 [34]. The consistency of the trees was verified by bootstrapping (1,000 replicates) for parsimony.

**Nucleotide sequence accession numbers**

16S rRNA, KS₄ (PKS II) and angucycline cyclase gene sequences from the soft coral-derived actinomycete isolates were deposited in the GenBank database under the following accession numbers: JN627163–JN627194, JN627195–JN627204 and JQ943912–JQ943913.

**Fermentation and chemical identification**

Strain A5-1 was inoculated in 25 ml flask using GYMM medium (20 g glycerol, 10 g yeast extract, 4 g malt extract, 10 g mannitol, 1 liter ASW) at 28°C, 180 rpm in the dark for 3 weeks, and then transferred to a 250 ml Erlenmeyer flask containing 100 ml of D-galactose-L-isoleucine medium [35]. The culture was incubated at 28°C, 180 rpm in the dark for 45 days. On the fifteenth day, ethanol was added to a final concentration of 6% (v/v) to induce the synthesis of jadomycin [35].

After mycelium was removed by filtration, the fermentation broth was concentrated with 100 ml of acetic ether (EtOAc) and concentrated in vacuo. EtOAc extract was dissolved in methanol for HPLC-DAD analysis on an Agilent 1200 (Agilent Technologies, USA) series with an on-line Diode Array Detector (DAD/UV) and a C₁₈ RP-column (Eclipse XDB-C₁₈ 5 μm, 4.6x150 mm). Ultraviolet absorption was compared with that of jadomycins according to their maximum absorption wavelength (λ<sub>max</sub>) [36].

For LC-QTOF-MS analysis, the methanol solution of strain A5-1 extract was detected on an ultra performance liquid & quadrupole time of flight mass spectrometry (UPLC-QTOF-MS Premier, Waters Corporation, USA). The analytes were separated on a C₁₈ RP-column (ACQUITY BEH C₁₈ 1.7 μm, 2.1x100 mm, Waters Co.), with linear gradient elution from H₂O (1% formic acid) to 35% H₂O/Mecn (1% formic acid). Total ions chromatography (TIC) and mass spectrum of selected ion were acquired in positive electro-spray ionization mass spectrum (ESI-MS) mode.

In the case of ¹H NMR analysis, the EtOAc extract was dried in vacuo and then dissolved in CD₃OD. Proton nuclear magnetic
resonance (1H NMR) spectrum was recorded on an AVANCE III 400 spectrometer (400 MHz, Bruker).

Results

Recovery and phylogenetic diversity of coral Scleronephthya sp.-associated actinomycetes

After incubation for 6 weeks, 32 isolates were recovered. Based on the BLAST analyses of 16S rRNA gene sequences, these 32 isolates were assigned to Actinobacteria with 98–100% similarity to their nearest relatives in the GenBank database, including 8 genera: Micromonospora (8 isolates), Gordonia (8 isolates), Mycobacterium (6 isolates), Nocardioides (3 isolates), Streptomyces (4 isolates), Cellulomonas (1 isolate), Dietzia (1 isolate) and Rhodococcus (1 isolate) (Table 1; Figure 1), which indicated that Micromonospora and Gordonia are relatively dominant among the culturable actinobacterial community in the tissue of the soft coral Scleronephthya sp..

Four strains (Gordonia sp. strain A5-14, Rhodococcus sp. strain A2-19, Micromonospora sp. strains A1-11 and A5-2) share high homology with relatives derived from marine sediments. Eight strains (Mycobacterium poriferae strains A1-12, A1-17, A3-1, A3-11 and A5-20, Micromonospora sp. strains A5-1, A6-2 and A6-10) show high similarity to relatives isolated from marine sponges.

Notably, significant differences in the total number of isolates were observed among the 6 different media (Figure S1). M5 produced the highest recovery with 10 isolates, followed by M1 (8

Table 1. Actinomycetes and those with PKS II gene from the soft coral Scleronephthya sp.

| Genus            | Strain (NCBI accession no.) | Most closely related strain (NCBI accession no.) | Identity (%) | PKS II |
|------------------|-----------------------------|-------------------------------------------------|--------------|--------|
| Cellulomonas      | A5-19 (JN627163)            | Cellulomonas sp. (EU303275)                       | 98           | –      |
| Dietzia          | A1-8 (JN627164)             | D. maris (GQ870425)                              | 99           | –      |
| Gordonia         | A1-3 (JN627165)             | G. paraffinivorans (NR_028832)                   | 99           | –      |
|                  | A1-10 (JN627166)            | G. paraffinivorans (NR_028832)                   | 99           | –      |
|                  | A4-4 (JN627167)             | G. paraffinivorans (NR_028832)                   | 99           | –      |
|                  | A4-16 (JN627168)            | G. paraffinivorans (NR_028832)                   | 99           | –      |
|                  | A4-20 (JN627169)            | G. laeacae (GU727686)                            | 99           | –      |
|                  | A5-8 (JN627170)             | G. bronchialis (FJ536306)                        | 99           | –      |
|                  | A5-9 (JN627171)             | G. alkanivorans (AY995556)                       | 99           | –      |
|                  | A5-14 (JN627172)            | Gordonia sp. (DQ448772)                         | 99           | –      |
| Mycobacterium    | A1-12 (JN627173)            | M. poriferae (NR_025235)                        | 99           | –      |
|                  | A1-17 (JN627174)            | M. poriferae (NR_025235)                        | 99           | –      |
|                  | A3-11 (JN627175)            | M. poriferae (NR_025235)                        | 99           | –      |
|                  | A3-11 (JN627176)            | M. poriferae (NR_025235)                        | 99           | –      |
|                  | A5-20 (JN627177)            | M. poriferae (NR_025235)                        | 99           | –      |
|                  | A5-7 (JN627178)             | M. duvalii (NR_026073)                          | 100          | –      |
| Rhodococcus      | A2-19 (JN627179)            | Rhodococcus sp. (GQ871747)                      | 99           | –      |
| Nocardioides     | A1-2 (JN627180)             | Nocardioides sp. (FJ171223)                      | 99           | –      |
|                  | A5-6 (JN627181)             | Nocardioides sp. (FJ171223)                      | 98           | –      |
|                  | A6-8 (JN627182)             | Nocardioides sp. (FJ171223)                      | 99           | –      |
| Streptomyces     | A4-1 (JN627183)             | S. variabilis (EU841659)                        | 99           | +      |
|                  | A4-2 (JN627184)             | S. variabilis (EU841659)                        | 100          | +      |
|                  | A4-3 (JN627185)             | S. variabilis (EU841659)                        | 99           | +      |
|                  | A6-1 (JN627186)             | S. variabilis (EU841659)                        | 99           | +      |
| Micromonospora   | A1-11 (JN627187)            | Micromonospora sp. (EU214967)                    | 99           | –      |
|                  | A1-15 (JN627188)            | Micromonospora sp. (EU531460)                    | 99           | +      |
|                  | A5-2 (JN627189)             | Micromonospora sp. (EU214980)                    | 99           | +      |
|                  | A5-13 (JN627190)            | M. purpureochromogenes (FJ486489)                | 100          | +      |
|                  | A5-1 (JN627191)             | Micromonospora sp. (GU002071)                    | 99           | +      |
|                  | A6-2 (JN627192)             | Micromonospora sp. (GU002071)                    | 99           | +      |
|                  | A6-9 (JN627193)             | Micromonospora sp. (EU531460)                    | 100          | +      |
|                  | A6-10 (JN627194)            | Micromonospora sp. (GQ863921)                    | 99           | –      |

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Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence (ca.1,400 bp) of actinomycetes from the tissue of soft coral Scleronephthya sp.. The sequences obtained in this work are marked by black dot. The scale bar represents the number of base substitutions per nucleotide position.
isolates), M4 (6 isolates), M6 (5 isolates), M3 (2 isolates) and M2 (1 isolate). Additionally, the actinomycete diversity recovered from the different media varied (Figure S1). For example, M1 and M5 yielded the highest diversity with 5 genera, followed by M6 (3 genera), M4 (2 genera), M2 (1 genus) and M3 (1 genus). As expected, the combination of 6 media achieved a better recoverability of coral-associated actinomycetes.

The potential for producing type II polyketides based on functional gene analysis

The presence of KS<sub>a</sub> gene was detected in two of the eight genera, Streptomyces (4 strains) and Micromonospora (6 strains) (Table 1). Based on BLAST analyses, the KS<sub>a</sub> sequences from four Streptomyces strains show high (98.4–98.8%) sequence similarity to their BLAST matches, whereas, the KS<sub>a</sub> sequences from six Micromonospora strains share relatively lower (<89.4%) homology with previously reported sequences.

A phylogenetic tree was generated using 10 KS<sub>a</sub> amino acid sequences obtained in this study and 17 reference sequences retrieved from GenBank (Figure 2). Reference sequences related to biosynthetic pathways help to group the obtained sequences into different clusters representing different chemotypes. As shown in Fig. 2, KS<sub>a</sub> sequences from 6 Micromonospora strains are separated into three major phylogenetic divisions. For example, sequences from strains A5-1 and A6-2 fall into a cluster with angucycline ketosynthase sequences, and show the closest evolutionary relationship with Jad A (AAB36562) which is involved in the biosynthesis of jadomycin B (Table 2). Sequences of strains A5-2, A6-9 and A5-13 are clustered in a group together with relative Lac 31 (ABX71114) associated with the biosynthetic pathway of lactonamycin. Interestingly, the unique KS<sub>a</sub> sequence from strain A1-15 is clearly separated from any known sequence involved in characterized pathways. After the phylogenetic analysis, 32 strains were tested for the presence of angucycline cyclase gene which is involved in the aromatization of angucycline. The target band of
approximately 650 bp was successfully amplified in *Micromonospora* sp. strains A5-1 and A6-2. This result indicates that these two *Micromonospora* strains have the potential in producing angucycline compounds such as jadomycin.

The identification of a novel analogue of jadomycin B in the fermentation broth of *Micromonospora* sp. strain A5-1

Among *Micromonospora* sp. strains A5-1 and A6-2 with potential to produce jadomycin B or its analogues, strain A5-1 was selected

Figure 3. HPLC of the EtOAc extract of *Micromonospora* sp. strain A5-1 fermentation broth (UV spectra of selected peaks at $t_R$ 5.22 min show similar absorption as jadomycins).

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| Strain               | NCBI accession no. | Top BLAST match (NCBI accession no.)                                      | Identity (%) |
|----------------------|--------------------|---------------------------------------------------------------------------|--------------|
| *Micromonospora* sp. A1-15 | JN627200 | *Streptomyces* sp. SirexAA-E 3-ketoacyl synthase gene (CP002993)           | 85.8         |
| *Micromonospora* sp. A5-1  | JN627196 | *Micromonospora* sp. SAUK6030 type II polyketide synthase-like gene (GQ118939) | 86.9         |
| *Micromonospora* sp. A5-2  | JN627201 | *Streptomyces venezuelae* jadomycin polyketide synthase (jadA) gene (AF126429) | 85.8         |
| *Micromonospora* sp. A5-13 | JN627202 | *Micromonospora aurantiaca* ATCC 27029 3-ketoacyl synthase gene (CP002162) | 88.2         |
| *Micromonospora* sp. A6-2  | JN627203 | *Micromonospora* sp. SAUK6030 type II polyketide synthase-like gene (GQ118939) | 86.7         |
| *Micromonospora* sp. A6-9  | JN627204 | *Micromonospora aurantiaca* ATCC 27029 3-ketoacyl synthase gene (CP002162) | 85.8         |
| *Streptomyces* sp. A4-1   | JN627199 | *Streptomyces* sp. JS-14 ketosynthase gene (GU373728)                       | 98.7         |
| *Streptomyces* sp. A4-2   | JN627198 | *Streptomyces* sp. JS-14 ketosynthase gene (GU373728)                       | 98.8         |
| *Streptomyces* sp. A4-3   | JN627197 | *Streptomyces* sp. JS-14 ketosynthase gene (GU373728)                       | 98.5         |
| *Streptomyces* sp. A6-1   | JN627195 | *Streptomyces* sp. JS-14 ketosynthase gene (GU373728)                       | 98.4         |
Figure 4. TIC of the EtOAc extract of *Micromonospora* sp. strain A5-1 fermentation broth (the peak with $t_R$ at 4.18 min is putative jadomycin analogue).

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Figure 5. Mass spectrum of selected ion at $t_R$ 4.18 min in TIC.

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for fermentation to test the gene prediction since the two strains belong to the same species. Only 10 mg EtOAc extract of the fermentation broth of *Micromonospora* sp. strain A5-1 was obtained because *Micromonospora* sp. strain A5-1 grew very slowly and the biomass was very low.

Jadomycin B displays 5 UV absorptions: 212 nm, 238 nm, 280 nm, 312 nm and 520 nm [36]. In the EtOAc extract of fermentation broth of *Micromonospora* sp. strain A5-1, one peak (retention time \( t_R \) at 5.22 min, Figure 3) shows similar UV profiles as that of jadomycins except the absorption band over 350 nm which is contributed by the substructure of \( \beta \)-quinone. The result suggests the existence of jadomycin B analogue with one keto function reduction in the fermentation broth of *Micromonospora* sp. strain A5-1.

Jadomycin B shows mass to charge \( m/z \) at 306 and 550 in ESI mass spectrum which are assigned as key fragmentation ion [phenanthroviridin+H]\(^+\) and pseudomolecular ion [jadomycin B+H]\(^+\) [37]. In this study, TIC of the EtOAc extract of *Micromonospora* sp. strain A5-1 fermentation broth shows one \( m/z \) 566 with \( t_R \) at 4.18 min (Figure 4), which is 16 amu more than that of pseudo-molecular ion of jadomycin B. So, the 14 amu corresponding to methylene should be added to the keto reduction derivative of jadomycin B. In the mass spectrum (Figure 5), the key fragmentation ion at \( m/z \) 322 instead of that at \( m/z \) 306 of jadomycins supports the change in phenanthroviridin. Based on the spectral data analysis and comparison with jadomycin B, the putative structure of target compound corresponding to the peak with \( t_R \) at 4.18 min in Figure 4 should be 7b, 13-dihydro-7-O-methyl jadomycin B. The possible MS fragmentations are shown in Figures 5 & 6. Meanwhile, this assignment is also supported by the \( ^1H \) NMR data (Figure 7), which are consistent with that of jadomycin B [36].

Figure 6. Suggested fragmentation process of selected ion at \( t_R \) 4.18 min in TIC.
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Discussion

The phylogenetic diversity of culturable actinomycetes associated with coral *Scleronephthya* sp.

Studies on sponge-associated actinomycetes indicate that medium exhibits significant effect on the diversity of Actinobacteria recovered [12,38]. So, in order to gain a better recoverability of coral-associated actinomycetes, six different media were used in this study. Similarly, medium-dependent recovery efficiency was observed. Taking the dominant *Micromonospora* for example, it was recovered from only 3 types of media. Moreover, not any one medium can recover all 8 genera, suggesting the necessity of combining different media to increase the recovery rate of cultured actinomycetes.

Prior to this study, the investigation of culturable actinomycetes has been mainly focused on stony corals [23,24,25]. In this study, a total of 8 genera were successfully isolated from the soft coral *Scleronephthya* sp., including *Micromonospora*, *Gordonia*, *Mycobacterium*, *Nocardoides*, *Streptomyces*, *Cellulomonas*, *Dietzia* and *Rhodococcus*. The culturable actinomycetes include both common and rare actinomycetes species. Rare actinomycetes derived from marine habitats, such as *Salinispora* [39], *Verrucosispora* [40] and *Micromonospora* [41,42,43], have shown their unique capacity to produce novel natural products. BLAST analyses shows that the isolated actinomycete strains e.g. *Micromonospora*, *Mycobacterium*, *Gordonia* and *Rhodococcus* have closest relatives derived from marine sponges or marine sediments. *Mycobacterium poriferae* was originally isolated from the sponge *Halichondria bowerbanki* [44]. Recently, 11 strains of *M. poriferae* have been isolated from the sponge *Ambichnion queenslandica* and the authors proposed that the isolates may represent a sponge-specific phylotype [45]. It is worth noting that, in this study, 5 strains *M. poriferae* were isolated from the tissue of this soft coral, suggesting that *M. poriferae* are not merely limited in sponges.

The potential of culturable actinomycetes associated with coral *Scleronephthya* sp. in producing type II polyketides

It is proposed that actinomycetes with PKS gene do produce a larger number of new metabolites [26]. In this study, actinomycetes with the potential to produce aromatic polyketides were screened by detecting KS$_a$ and cyclase genes of PKS II. Among the 32 strains actinomycetes, 10 strains from two genera *Streptomyces* and *Micromonospora* yielded positive results. *Streptomyces* is a well-known polyketide producer, so it is not surprising that KS$_a$ gene was identified in all the 4 *Streptomyces* strains. Prior to this study, it was found that most of the *Micromonospora* strains are not potential producers of type II polyketides [26,33]. The known secondary metabolites produced by *Micromonospora* are mainly aminoglycosides, macrolides and enediyines, few aromatic polyketides are known to be produced by *Micromonospora* except...
andhracyclines [46]. In contrast, it is unexpected that the target gene was detected in 6 of 8 Micromonospora strains, indicating that some coral-associated Micromonospora strains have the potential in producing aromatic polyketides.

Early in 1994, it was known that the production of jadomycin B in Sphoophomyces zengela ISP2230 needed to be induced by heat shock, ethanol treatment or phage infection [35]. Apparently, the jadomycin pathway is cryptic and only activated under specific conditions. In this case, natural product discovery strategy based on traditional bioassay is limited. Similarly, the D-galactose-L-isoleucine medium, which is beneficial for producing jadomycin B [35], was used in the fermentation of Micromonospora sp. strain A5-1, followed with ethanol induction [35]. Although jadomycin B was not found in the fermentation broth of Micromonospora sp. strain A5-1, a novel analogue of jadomycin B, i.e., 7b, 13-dihydro-7-O-methyl jadomycin B, was identified, which proved the prediction based on the functional gene screening. This study indicates that gene-based screening may guide the discovery of target metabolites especially those cannot be synthesized under the normal cultivation conditions. However, because Micromonospora sp. strain A5-1 grew very slowly and the yield of target compound was very low, so, in this study, the pure 7b, 13-dihydro-7-O-methyl jadomycin B was not isolated successfully. Alternatively, for the slowly-growing Micromonospora sp with type II polyketides producing potential, the cloning and heterologous expression of related gene cluster is a potential choice for future investigation.

The results from this study indicate that the soft coral tissue harbors diverse actinomycetes, some of which are with potential in synthesizing type II polyketides. This study, together with actinomycetes from stony corals [23,24,25,47], suggests that the diverse culturable coral-associated actinomycetes are important source for marine natural products.

Supporting Information

Table S1 Media used for the isolation of actinomycetes from the soft coral Scleronephthya sp.

(DOC)

Figure S1 The diversity of actinomycetes recovered using six media.

(DOC)

Author Contributions

Conceived and designed the experiments: ZL WS. Performed the experiments: WS YZ. Analyzed the data: WS ZL CP. Wrote the paper: WS ZL CP.

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