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Diversified collection of novel sponge-associated actinobacteria of Ha Tien sea, Kien Giang province, Vietnam

Tran Vu Phuong[1], Ngo Thi Phuong Dung[2], Cao Ngoc Diep[3]
[1,2,3]*Biotechnology R&D Institute, Can Tho University, Can Tho City, Vietnam*

Abstract– Marine actinobacteria are the most economically and biotechnologically valuable prokaryotes receiving much attention for their capacities of antibiotics and enzyme inhibitors. A total of 142 endophytic actinomycetes was isolated from 44 samples of 5 different sponge species in Ha Tien Sea, Vietnam. Due to the antimicrobial activity, 21 isolates were selected for 16S rDNA sequencing. Of identified isolates 13 were characterized as Streptomyces spp. and the remainders belonged to other genera as Microbacterium, Rhodococcus and Gordonia. The antimicrobial activity and the amplifying genes coding for polyketide synthetase (PKS) and nonribosomal peptide synthetase (NRPS) showed that isolates purified from sponges in Ha Tien sea had broad-spectrum antimicrobial activity, mainly against gram-positive bacteria as Bacillus cereus and Staphylococcus aureus. Microbacterium tumbae was also found as a rare actinomycete displayed the antifungal activity. These new cultures can be employed as bioactive resources against pathogens, particularly in relation to food-borne diseases and human health.
Keywords: Actinobacteria, antimicrobial activity, marine sponge, nonribosomal peptide synthetase, polyketide synthetase.

I. INTRODUCTION

Actinobacteria are a diverse group of filamentous Gram-positive soil bacteria with a high G+C (Guanin + Cytosin) content and are abundantly distributed in the environment (such as soil, freshwater, seawater etc.) with strongly biological activities against a majority of phytopathogens including fungi and oomycetes, and thus, actinobacteria have become a sort of potential resource to prevent plant fungal diseases [1]. It has become increasingly difficult to find actinobacteria with a great capacity to produce new antibiotics [2], and as a result, infectious diseases associated with multidrug resistance are rapidly spreading, around the globe. At this juncture, the isolation of new actinobacteria from extreme environments has become a hotspot among researcher and scientific community over the past 20 years [3]. These actinobacteria from extreme environments were found to exhibit a unique source of novel biologically active compounds [2]. The rare actinobacteria have the ability to produce active metabolite, which has the property of diverse, unique, unprecedented, and occasionally complicated compounds usually with low toxicity. Among these unusual marine environments to be explored as niches for novel microbes are sponges, which host hundreds of different bacterial groups within their mesohyl tissue. Sponges are constantly filtering bacteria from the water column and are home to a diversity of microbial symbionts [4]. The remarkable biotechnological potential of actinomycetes for drug discovery has been observed since the 1960s, and since that time, actinomycetes have been responsible for more than 70% of all antibiotics discovered [5]. Marine actinobacteria are the most economically and biotechnologically valuable prokaryotes. Representative genera of marine actinobacteria include Actinomadura, Aeromicrobium, Dietzia, Gordonia, Marinophilus, Micromonospora, Nonomuraea, Rhodococcus, Saccharomonospora, Saccharopolyspora, Salinispora, Streptomyces, Solwaraspora, Williamsia, Verrucosispora and several others [6]. New genera within the Streptomycetaceae family continue to be isolated from marine-derived sediments and sponges and have shown as a promising source of novel metabolites [7]; however, few complete genome sequences have been published [8]. Streptomyces, the largest genus of actinobacteria, can produce a variety of beneficial antibiotics, industrial enzymes, and bioactive secondary metabolites including antibacterial, herbicides, and fungicides [9-10]. Approximately, two-thirds of all known natural antibiotics used in medicine and agriculture were belonged to Streptomyces [1]. The production of antibiotics by Streptomyces may play an important role in the bio-control of pathogens [11]. Moreover, Streptomyces spp. have been extensively studied due to their dominance, ease of isolation, enhancement of plant growth, and improvement of crop productivity [12]. The method applied to study the distribution of PKS and NRPS biosynthetic systems was suggested in a collection of wild-type actinomycetes isolated from tropical soil samples [13]. The antimicrobial activity and amplifying genes coding for PKS-I, PKS-II and NRPS showed that endophytic actinomycetes isolated from medicinal plants in Panxi plateau performed as valuable reservoirs of novel bioactive compounds [14].

This research was studied to investigate the culturable diversity of actinobacteria from sponges of the Ha Tien Sea, Vietnam and to analyse genes coding for PKS-I, PKS-II, and NRPS actinobacteria as well as to explore the potential use of these newly sponge-associated actinobacteria as a novel source of bioactives.

II. MATERIALS AND METHODS

2.1 Sample collection

A total of 5 different sponge species was collected by scuba diving from the Ha Tien sea at a depth of 0.5 – 1.0 m from surface water, including 4 sites: Dam island (10◦13′81N; 104◦49′56'90"E), Heo island (10◦17′89"N; 104◦53′18'E), Nghe island (10o02'96" N; 104o55'62" E) and Nui Den (10◦37′50" N; 104◦44′58" E). The samples were placed into plastic bags and transported to the laboratory using ice box and stored at -20 ºC until analysis.

2.2 Isolation of Actinobacteria

Starch Casein Agar medium [15] was used for the isolation of sponge-associated actinobacteria. It was supplemented with Aginalxic (0,5 mg/L) and Nystatin (0,5 mg/L) to inhibit fungi and Gram-negative bacteria. Sponge samples were rinsed with sterile natural seawater to remove the microbes loosely attached on the surface. Subsequently, a few tissue cubes were excised from different sections (including cortex and endosome) of the sponge samples. They were cut into pieces and aseptically ground using sterilized pestles and mortars. Actinobacteria were isolated by means of serial dilution and plating techniques. The inoculated plates were incubated at 28 ºC for 3–6 weeks. The colonies bearing distinct morphological characteristics were picked up and transferred to freshly prepared media until pure cultures were obtained.

2.3 Screening assays for antibacterial activity

The bioactivity of bacterial isolates was examined [16-17]. The pathogenic bacteria including Edwardsiella ictaluri, Bacillus cereus, Escherichia coli, Salmonella enterica and Candida albicans, were provided by College of Aquaculture and Fisheries, Biotechnology Research and Development Institute (Can Tho University) and Can Tho Center for Technology, Standard, Quality (Department of Science and Technology, Can Tho City). The liquid cultures were grown with shaking at 150 rpm for 7–14 days depending on their growth rate at 30 ºC. The broth was centrifuged in 50 mL falcon tubes at 4,193xg for 15 min at room temperature (28-32 ºC); Megafuge 1.0R, Heraeus) and the supernatant was stored at 4 ºC. The bacterial and fungal test organisms were plated in Mueller Hinton Agar medium and Potato Dextrose Agar medium, respectively. Antimicrobial extract was added to the wells, then the plates were incubated at 4 ºC for 2 h for diffusion of antimicrobial extract and observed for the zones of inhibition at 28 ºC for 48 h.

2.4 The Agar well diffusion method

The active isolates were cultured by the method given in the previous step. The supernatants were used for testing extracellular antimicrobial activity by the agar well diffusion method. By using a sterile cork borer, wells were punctured in appropriate agar medium previously seeded with one of the test organisms. One hundred microlitre of the culture supernatants were added to each well. The plates were then incubated at 4 ºC for at least 2 h to allow the diffusion of crude extracts followed by incubation for 24 h at 37 ºC for bacteria and 48 h at 28 ºC for yeast. The diameters of inhibition zones were monitored and measured [18].

2.5 Genomic DNA Extraction

To prepare cultures for the extraction of genomic DNA from the isolates, 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 ºC with shaking at 180 rpm. Bacterial cells from these cultures were collected by centrifugation and genomic DNA was extracted [19].

2.6 16S rDNA Gene Amplification and Sequencing

Amplification of 16S rDNA by PCR was carried out using the universal primers 27F [20] and 1492R [21]. The 50 µL reaction mixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 µM of each deooxynucleotide triphosphate, 500 nM of each primer (Fermentas) and 20 ng DNA. The thermocycling profide was carried out with an initial denaturation at 95 ºC (5 min) followed by 30 cycles of denaturation at 95 ºC (30 s), annealing at 55 ºC (30 s), extension at 72 ºC (90 s) and a final extension at 72 ºC (10 min) in C1000 Thermal Cycler (Bio-Rad). Aliquots (10 µL) of PCR products were electrophoresed and visualized in 1% agarose gels using standard electrophoresis procedures.

The following actinobacteria-specific primers were used for the amplification of actinobacterial 16S rRNA gene fragment [22]. Cycling conditions were as follows: initial denaturation at 95 ºC for 4 min, 30 cycles of 95 ºC for 45 s, 68 ºC for 45 s, and 72 ºC for 1 min, and a final extension of 5 min at 72 ºC.
S-C-Act-0235-a-S-20 (5′-CGCGGCCTATCAGCTTGTTG 3′), and
S-C-Act-0878-a-A-19 (5′-CCGTACTCCCCAGGCGGGG-3′)

2.7 Sequence Analysis

The 16S rRNA gene sequences were compared with those from the type strains available in NCBI (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST) [23]. For the phylogenetic analysis, multiple sequence alignment was performed using CLUSTALX, version 1.81. Phylogenetic tree was constructed using Mega 7.0 [24]. The consistency of the trees was verified by bootstrapping (1000 replicates) for maximum likelihood.
Detection and Analysis of PKS-I, PKS-II, and NRPS
PCR primers and amplifications were carried out and analysed [13-14].

2.8 Statistical analysis

The experimental results were analysed as a two-way ANOVA with the isolates and with levels of diameters of inhibition zones. All analyses were conducted using the programme MSTATC, Minitab 16. The data were considered significantly different at $p < 0.01$. Duncan test at $P = 0.01$ was used to differentiate between statistically.

III. RESULTS and DISCUSSION

Characterization and detection of PKS-I, PKS-II, and NRPS sequences of wild-type sponge-actinomycetes
The morphological examination of the sponges was identified at College of Natural Science, Can Tho University. Genera of sponges were characterized including Hexactinosa sp., Leucosolenia sp., Verticillitida sp., Lithonia sp. and Haliclona sp.. A total of 142 isolates of actinomycetes was purified from 44 sponge samples collected at 4 sites. The numbers of samples and isolates (samples/isolates) were obtained at Dam island, Nghe island, Heo island and Nui Den as follow: 15/72, 11/33, 10/19 and 8/18, respectively. Almost their colonies have round-shaped; milky, white clear and yellow, entire or loabate margin; diameter size of these colonies varied from 0.2 to 3.0 mm and all of them have Gram-positive. Eighty-nine of 142 tested isolates could produce antimicrobial active metabolites inhibiting at least one of the test pathogens. Over fifty percent isolates were capable of inhibiting the growth of Gram-positives, 35/142 isolates were actively against Candida albicans and 21 isolates showed activity against one among five following pathogens including Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Candida albicans (Table 1).

Table 1 Antimicrobial activity of sponge-derived actinomycetes

No	Actinobacterial isolates	Inhibition zone diameter [D = d1 - d2] (mm)				
		Bacillus cereus	Staphylococcus aureus	Escherichia coli	Salmonella typhimurium	Candida albicans
01	H27d	16.0 c	ND	8.0 b	13.0 b	15.0 b
02	HD28p	14.0 d	ND	5.0 e	10.0 d	14.0 bc
03	ND7c	21.0 a	ND	6.0 d	12.0 c	22.0 a
04	ND6c	17.0 bc	ND	14.0 a	14.0 a	22.0 a
05	N5c	ND	14.0 a	ND	ND	13.7 c
06	N5d	ND	8.0 d	ND	ND	ND
07	N6a	14.3 d	ND	ND	ND	ND
08	N7b	9.3 hi	ND	ND	ND	ND
09	N8b	11.0 fg	ND	ND	ND	ND
10	N8c	9.0 hi	ND	ND	ND	ND
11	N8d	14.0 d	ND	ND	ND	ND
12	N8e	8.0 ij	ND	ND	ND	ND
13	N9c	10.0 gh	ND	ND	ND	ND
14	N9d	11.0 fg	ND	ND	ND	ND
15	N9f	6.0 k	ND	ND	ND	ND
16	N9h	14.0 d	ND	ND	ND	ND
17	H10a	10.0 gh	ND	ND	ND	ND
18	N10a	7.0 jk	12.0 b	ND	ND	8.0 f

19	N10d	12.0 ef	ND	ND	ND	ND
20	N11c	ND	5.0 e	ND	ND	ND
21	H23e	6.0 k	10.0 c	ND	ND	12.0 d
22	Control	8.0 ij Ampicilin	12.0 b Ampicilin	7.0 c Tetracyclin	8.0 e Tetracyclin	10.0 e Fluconazole

Means within a column followed by the same letter/s are not significantly different at $p < 0.01$
D = diameter of inhibition zone of isolates, d1 = diameter of inhibition zone, d2 = diameter of well, ND: not detected

A total of 21 isolates was chosen for identification due to their antimicrobial activity. The fragments of 600-620 bp 16S rRNA were obtained from PCR and sequencing. Homology searches of 16S rRNA gene sequence of selected strains in GenBank by BLAST (Table 2) revealved that they had similarity to sequences of genus Streptomyces (13 isolates) and other genera including Microbacterium (3 isolates), Rhodococcus (4 isolates), and Gordonia (1 isolate).

Table 2. Phylogenetic affiliation of isolates on the basis of 16S rRNA gene sequences by using BLAST programme in the GenBank database based on sequences similarity

Taxonomic group and strain	Closest species relative	Similarity (%)
Actinomycetaceae		
N5c	Streptomyces recifensis strain WZS121 (MH497607)	99
	Brevibacterium sediminis strain N2 (MH732973)	99
N5d	Streptomyces chumphonensis strain HQA999 (MH041238)	99
	Streptomyces tateyamensis strain 18I (MG009024)	99
N6a	Streptomyces qinglanensis strain G1_G14LB (MH517409)	100
	Streptomyces sp. strain WMMA1687 (KY015039)	100
N7b	Streptomyces griseoaurantiacus strain XY173 (MH432690)	100
	Streptomyces albidoflavus strain HQA017 (KT758349)	100
N8b	Streptomyces griseoincarnatus (MG1951451)	100
	Streptomyces variabilis strain N4 (MH362710)	100
N8c	Streptomyces rochei strain SG-J10 (MH7346050	100
	Streptomyces sampsonii strain AND24 (MH727710)	100
N8d	Streptomyces flaveolus strain ADIP1 (KF732809)	99
	Streptomyces olivaceus strain HQA933 (MH044533)	99
N8e	Streptomyces fradiae strain 5406MeiHua (EF063502)	99
	Streptomyces rochei strain SG-J10 (MH734605)	99
N9d	Streptomyces griseoaurantiacus strain XY173 (MH4326900	100
	Streptomyces albidoflavus strain HQA017 (KT758349)	100
N9f	Streptomyces olivaceus strain HQA933 (MH044533)	100
	Streptomyces pactum strain DBT124 (KU195411)	100
N9h	Streptomyces coelicolor strain DSM 40233 (KY820720)	99
	Streptomyces sampsonii strain NRRL B12325 (KY820696)	99
N10a	Streptomyces variabilis strain AC31 (KX845578)	100
	Streptomyces coelicoflavus strain RA 10 (KJ995819)	100
H10a	Streptomyces chumphonensis strain HQA999 (MH041238)	100
	Streptomyces tateyamensis strain 18I (MG009024)	100
Microbacteriaceae		
ND7c	Microbacterium tumbae strain C3 (MG958700)	100
	Microbacterium kyungheense strain MK (MF3734980)	100
H23e	Microbacterium binotii Rup 1 (LC389374)	99
	Microbacterium neimengense, strain Marseille-AA00152 (LT223596)	99
HD28p	Microbacterium tumbae strain C3 (MG958700)	100
	Microbacterium kyungheense strain MK (MF373498)	100
Nocardiaceae		
N9c	Rhodococcus pyridinivorans strain AI4 (MH707179)	99
	Rhodococcus sp. strain BSRT1-1 (MH105079)	99
N10d	Rhodococcus pyridinivorans strain AI4 (MH7071790	100
	Rhodococcus sp. strain BSRT1-1 (MH105079)	100

ND6c	Rhodococcus hoagii strain AL01 (MF928189)	100
	Rhodococcus equi strain TRB132 (KX981343)	100
H27d	Rhodococcus hoagii strain AL01 (MF928189)	100
	Rhodococcus equi strain TRB132 (KX981343)	100
Gordoniaceae		
N11c	Gordonia bronchialis strain CS6.2 (KU597101)	99
	Gordonia alkanivorans strain OSL (MH142370)	99

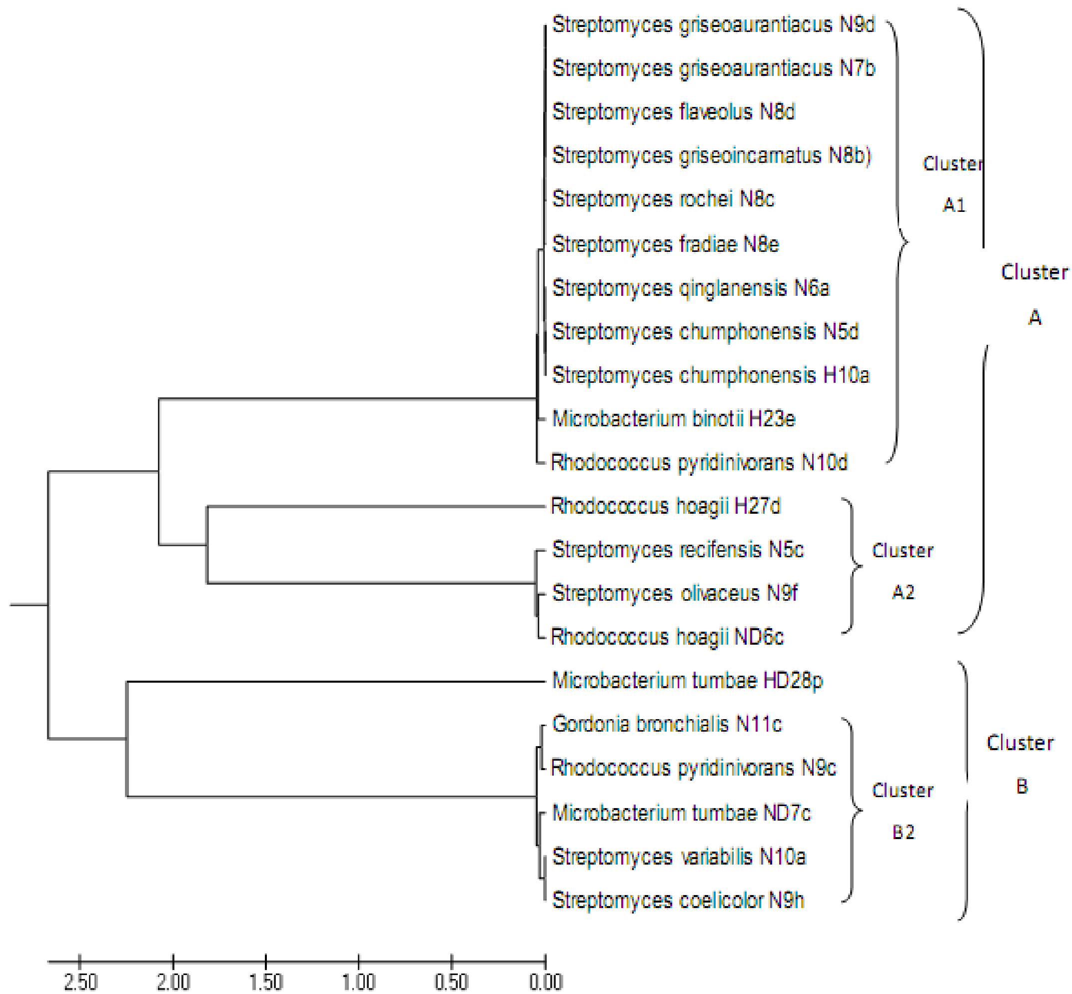

Figure 1. The UPGMA phylogenetic tree of partial 16S rRNA gene sequences of actinobacteria isolated from sponges of the Ha Tien Sea and closely related type strains. Numbers in the figure refers to percentage bootstrap values which were calculated for 1000 replicates. Bar, 0.02 was per nucleotide position.

An UPGMA phylogenetic tree (Figure 1.) of these isolates described the two clusters. Cluster A had 15 strains including 2 smaller clusters as cluster A1 with 11 strains (Streptomyces griseoaurantiacus N9d, Streptomyces griseoaurantiacus N7b, Streptomyces flaveolus N8d, Streptomyces griseoincarnatus, Streptomyces rochei N8c, Streptomyces fradiae N8e, Streptomyces qinglanensis N6a, Streptomyces chumphonensis N5d, Streptomyces chumphonensis H10a, Microbacterium binotii H23e, Rhodococcus pyridinivorans N10d) while cluster A2 with 4 strains (2 were Streptomyces recifensis N5c and Streptomyces olivaceus N9f and 2 were Rhodococcus hoagii H27d and Rhodococcus hoagii ND6c). Cluster B had 2 smaller clusters including cluster B1 with only 1 strain (Microbacterium tumbae HD28p) and cluster B2 with 5 strains (2 were Streptomyces variabilis N10a and Streptomyces coelicolor N9h, 1 was Microbacterium tumbae ND7c, 1 was Gordonia bronchialis N11c, and 1 was Rhodococcus pyridimivorans N9c).

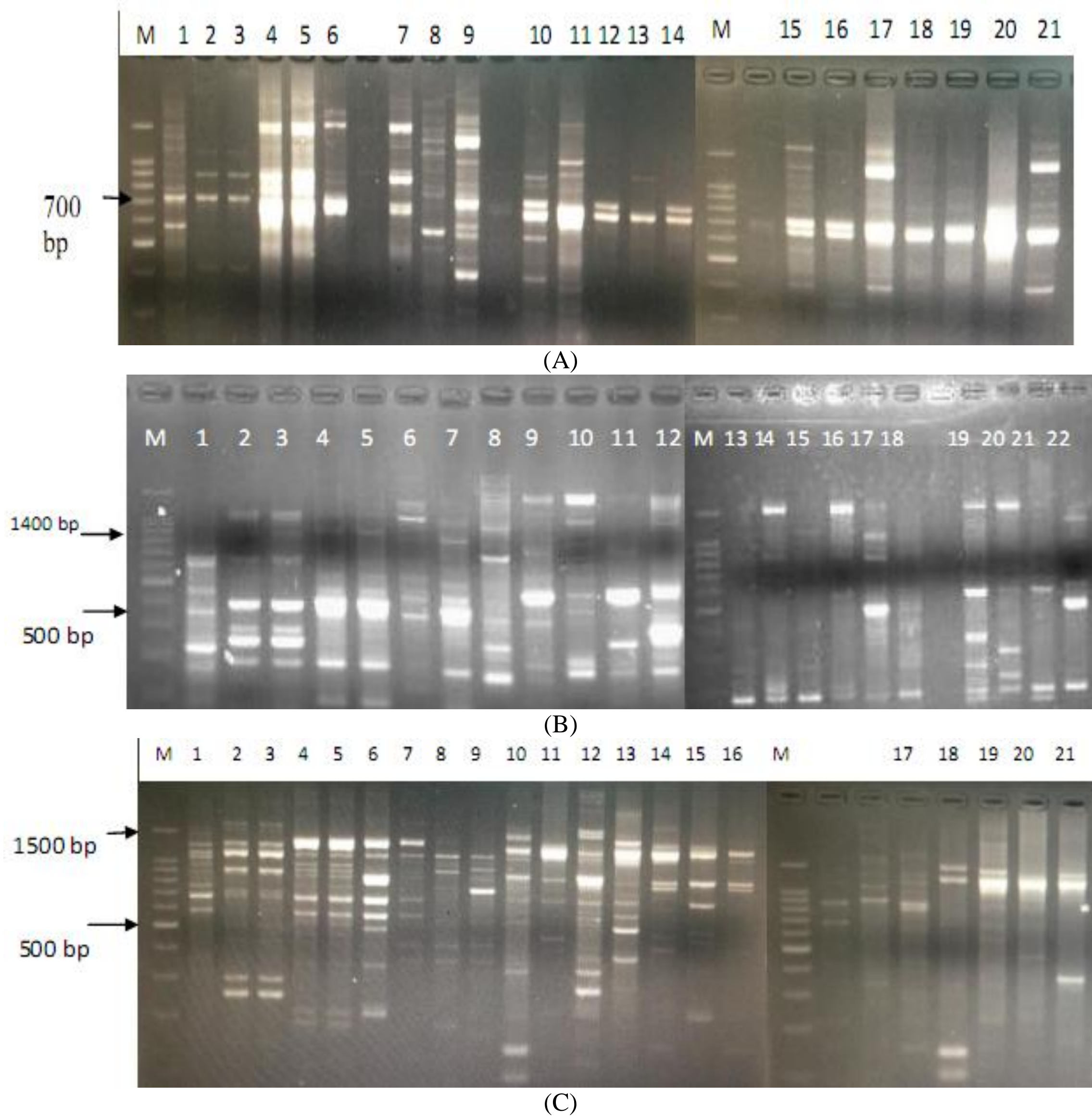

Figure 2. Agarose gel electrophoresis of PCR products from DNA isolated from representative actinomycete strains: (A) Selective amplification of 700–800 bp fragments using primers A3F/A7R specific for NRPS adenylation sequences; (B) Selective amplification of the 1200–1400 bp fragments using K1F/K2R and K1F/M6R; (C) Specific primers for PKS-I ketosynthase and methyl-malonyl-CoA transferase sequences. The numbering in the figure refers to the codes of actinobacterial isolates.

Note: M: Ladder 100 bp, 1: HD28P, 2: H23e 3: N9d, 4: H27d, 5: ND6c, 6: ND7c, 7: N5c, 8: N9f, 9: N8b, 10: N10a, 11: N5d, 12: N8d, 13: H10a, 14: N8e, 15: N10d, 16: N11c, 17: N9c, 18: N9h, 19: N7b, 20: N8c, 21: N6a

Table 3 Distribution of NRPS, PKS-I, and PKS-II in actinomycetes

No	Taxa	NPRS (A3F/A7R)	NPS-I (K1F/K2R)	(K1F/M6R)	NPS-II (KSα/ KSβ)
Actinomycetaceae					
01	Streptomyces recifensis N5c	+	ND	+	ND
02	Streptomyces chumphonensis N5d	+	ND	ND	ND
03	Streptomyces ginglanensis N6a	ND	ND	+	ND
04	Streptomyces grigeoincamatus N7b	+	+	+	ND
05	Streptomyces grigeoincamatus N8b	+	ND	ND	ND

06	Streptomyces rochei N8c	+	+	+	ND
07	Streptomyces flaveolus N8d	+	+	+	ND
08	Streptomyces fradiae N8e	+	ND	ND	ND
09	Streptomyces griseoaurantiacus N9d	+	+	+	ND
10	Streptomyces olivaceus N9f	ND	ND	ND	ND
11	Streptomyces coelicolor N9h	ND	ND	+	ND
12	Streptomyces variabilis N10a	+	+	+	ND
13	Streptomyces chumphonensis H10a	+	ND	ND	ND
Microbacteriaceae					
14	Microbacterium tumbae ND7c	+	+	+	ND
15	Microbacterium binotii H23e	+	+	+	ND
16	Microbacterium tumbae HD28p	+	ND	+	ND
Nocardiaceae					
17	Rhodococcus hoagii ND6c	+	ND	+	ND
18	Rhodococcus hoagii H27d	+	ND	+	ND
19	Rhodococcus pyrridinivorans N10d	+	ND	ND	ND
20	Rhodococcus pyrridinivorans N9c	+	ND	ND	ND
Gordoniaceae					
21	Gordonia bronchialis N11c	+	+	ND	ND

+: detected, ND: not detected

Isolates were screened for the presence of NRPS, PKS-I, and PKS-II sequences by specific amplification of total DNA. All three pathways were detected at a high frequency in the Streptomyces, Microbacteriaceae, Nocardiaceae and Gordoniaceae populations (Table 3). The detection levels of PKS-I sequences varied according to the specificity of the pair of primers used, K1F/K2R or K1F/M6R. However, the ratios between both pairs remained quite similar in the different actinomycetes groups whereas PKS-II sequences with primers KSα/KSβ did not identify strain among 21 strains (Figure 2.). The research findings on the diversified collection of sponge-associated actinobacteria isolated from the Ha Tien sea, Kien Giang province of Vietnam indicate the important sources for searching such potential resources of biological active compounds.

Antimicrobial activity and presence of biosynthetic gene sequences

The production of antimicrobial activity by the sponge isolates was evaluated against a test panel of four bacteria (B. cereus, S. aureus, E. coli, and S. typhymurium) and one yeast (C. albicans). There was 61.5% of the Streptomyces showing the antimicrobial activity against at least one of the test strains, mostly the Gram positive strains of B. cereus and S. aureus (14.5%). The activity levels against E. coli, S. typhymurium, and C. albicans were 15% and 33.3%, respectively. Four strains of family Nocardiaceae showed the antimicrobial activity against E. coli, S. typhymurium, and C. albicans were 50% and 50%, respectively. Three strains of family Microbacteriaceae showed their antimicrobial activity against B. cereus, S. aureus, E. coli, S. typhy and C. albicans were 100%, 33.3%, 66.6%, 66.6% and 100%, respectively. In contrast, the antimicrobial activity was lowly found among representatives of the family Gordoniaceae (4.3%) (Table 4).

Table 4. PCR detection of PKS-I and NRPS biosynthetic sequences and antimicrobial activities in wild-type sponge-actinomycetes

Taxonomic group	Total strain	NRPS	NPS-I		Total active strain	Antimicrobial test panel activity				
		A3F/A7R	K1F/K2R	F1F/M6R		BC	SA	EC	ST	CA
Streptomycetaceae	13	10	5	8	13	11	2	0	0	2
Microbacteriaceae	3	3	2	3	3	3	1	2	2	3
Nocardiaceae	4	4	ND	2	2	0	0	2	2	2
Gordoniaceae	1	1	1	ND	1	1	0	0	0	0
Total	21	18	8	13	19	15	3	4	4	7

Antimicrobial test panel including BC: Bacillus cereus, SA: Staphylococcus aureus, EC: Escherichia coli, ST: Salmonella typhimurium, CA: Candida albicans; ND: not detected

The approaches discussed in this study help to identify isolates with a potential talent. A rapid method to fingerprint strains using PCR products obtained from metabolic screens was also investigated as an alternative characterization tool to be applied, not only in the study of distinct isolates among ribosomally related groups but also in the

identification of enriched populations with different biosynthetic potentials for the production of antimicrobial compounds. In recent years, scanning the genes encoding polyketide synthases and nonribosomal peptide synthetases that synthesize most of the biologically active polyketide and peptide compounds have been broadly applied to assess the biosynthetic potential of culturable microorganisms and culture-independent samples [25-26]. However, the results from previous studies and from this present research suggest that for the culturable actinomycetes, the antimicrobial potential maybe only assessed by screening of antimicrobial activity against the desired indicator organisms. The species of Stretomyces occupied and showed some antimicrobial activity against gram-positive bacteria but the occurrence of strains of Microbacterium tumbae as M. tumbae ND7C had many antimicrobial activity ability against gram-positive bacteria and gram-negative bacteria as well as yeast. The isolates of Microbacterium sp. and Rhodococcus sp. purely obtained from marine sponge were also found [27] to have the antimicrobial activity against Staphylococcus aureus (gram-positive bacteria) and Enterococcus faecalis (gram-negative bacteria) but not against Candida albicans. The isolation and diversity of actinobacteria from marine sponges have previously reported that actinobacteria are major components of the microbial communities of Xestospongia muta and X. Testudinaria [28]. In another study [29], 106 actinomycete strains were isolated representing seven genera from the sponge Hymeniacidon perleve. Additionally, 181 culturable actinomycetes affiliated with three genera were recovered from five sponges offshore China [30]. The microbial diversity of two Red Sea sponges, Hyrtios erectus and Amphimedon sp., was inspected using cultivation and cultivation-independent analyses. Focused cultivation on actinobacteria yielded 35 actinomycetes represented by four genera [31]. In the present study, 21 newly defined strains of actinomycete isolated from sponges of the Ha Tien sea were achieved performing the antimicrobial activity against pathogenic microbes on human. Of which, genus Streptomyces with over 50% of the tested strains were characterized.

IV. CONCLUSION

In conclusion, the culturable diversity of sponge-associated actinobacteria from the Ha Tien Sea was established. Streptomyces isolates were found as the predominant strains showing antibacterial activity. Besides, Microbacterium tumbae performed as a rare actinomycete which displayed antifungal activity. It is indicated that marine sponge are a potent source of endophytic actinomycetes with wide biological activity against pathogenic fungi as well as Gram-positive and Gram-negative bacteria. This makes it a promising application of such newly functional sponge-associated actinomyces as a novel source of bioactives.

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