DISTRIBUTION OF PKS-I, PKS-II AND NRPS GENES IN 23 ACTINOBACTERIAL STRAINS SELECTED FROM SPONGES IN THE HA TIEN SEA, VIETNAM

Tran Vu Phuong[1,*], Huynh Van Tien[1], Cao Ngoc Diep[1], Ha Thanh Toan[1]

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 198 endophytic actinomycetes was isolated from 44 samples of 5 different sponge species in Ha Tien Sea, Vietnam. Twenty - three actinobacterial isolates against at least two of the five tested bacteria with moderate to high resistance were selected and characterized by the genetic sequencing method. They were identified to be belonged to 4 families: Actinomycetaceae, Microbacteriaceae, Nocardiaceae, and Gordoniaceae. The difference of present rate of genes including PKS-I, PKS-II and NRPS gene, was found as follows: 16/23 strains (with 69.6%), 14/23 strains (with 67.8%), 11/23 strains (with 47.8%) respectively, three strains had three PKS-I, PKS-II and NRPS genes, especially *Gordonia bronchialis* HD2.5a strain (Gordoniaceae) was the best strain in the resistance with 4/5 human diseases microbes. 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, non - ribosomal peptide synthetase, polyketide synthetase.*

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1. INTRODUCTION

Most currently marketed antibiotics are natural products of microbial origin and >120 of the most important medicines in use today are obtained from terrestrial microorganisms [1 - 3]. The epidemiology of antibiotic - resistant bacteria and the need for confirmed test results [4]; leading to the global concern that we may soon be facing a post - antibiotic era with reduced capabilities to combat microbes. Therefore, a concerted worldwide search for new antibiotics from microbial origin is on - going, with focus on the potential of marine and terrestrial bacteria as sources for novel metabolites with interesting biological and pharmaceutical properties [5, 6]. Actinomycetes are a group of aerobic, branched, unicellular Gram - positive bacteria with high percentage of G+C (70%) in their genetic material. This bacterium has many important roles in various industries because of its ability to produce a few diverse metabolite compounds. These metabolite compounds have benefits such as antibiotics, antifungal, antiviral, anticancer, enzymes, immune suppressants and other compounds that are beneficial in industry [7]. It has become increasingly difficult to find actinobacteria with a great capacity to produce new antibiotics [8]. These actinobacteria from extreme environments were found to exhibit a unique source of novel biologically active compounds [8]. The rare actinobacteria could produce active metabolite, which has the property of diverse, unique, unprecedented, and occasionally complicated compounds usually with low toxicity. Sponges are constantly filtering bacteria from the water column and are home to a diversity of microbial symbionts [9]. The remarkable biotechnological potential of actinomycetes for drug discovery has been observed since the 1960s and since that time,

[1] Institute Of Food and Biotechnology, Can Tho University, Vietnam
[*] Email: tvuphuong@ctu.edu.vn

actinomycetes have been responsible for more than 70% of all antibiotics discovered [2]. Marine actinobacteria are the most economically and biotechnologically valuable prokaryotes.

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 [10]. 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 [11].

This research was conducted 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 gene actinobacteria as well as to explore the potential use of these newly sponge associated actinobacteria as a novel source of bioactives.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 5 different sponge species were collected by scuba diving from the Ha Tien Sea at a depth from 0.5 m to 1.0 m under 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 (10°02'96" N; 104°55'62" 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 an ice box and stored at -20°C until analysis.

2.2. Isolation of Actinobacteria

Starch Casein agar medium [12] 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 to 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°C 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 [13, 14]. The pathogenic bacteria including *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhimurium* 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°C. The broth was centrifuged in 50 mL falcon tubes at 4,193 xg for 15 min at room temperature (28 - 32°C); Megafuge 1.0 R, Heraeus) and the supernatant was stored at 4°C. 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°C for 2 hours for diffusion of antimicrobial extract and observed for the zones of inhibition at 28°C for 48 hours.

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 microliters of the culture supernatants were added to each well. The plates were then incubated at 4°C for at least 2 hours to allow the diffusion of crude extracts followed by incubation for 24 hours at 37°C for bacteria and 48 hours at 28°C for yeast. The diameters of inhibition zones were monitored and measured [15].

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°C with shaking at 180 rpm. Bacterial cells from these cultures were collected by centrifugation and genomic DNA was extracted [16].

2.6. 16S rRNA Gene Amplification and Sequencing

Amplification of 16S rDNA by PCR was carried out using the universal primers 27F [17] and 1492R [18], implementation consists of the following components and processes: The 50 µL reaction mixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 µM of dNTP, 500 nM of each primer (Fermentas) and 20 ng DNA. The following programs: 5 min at 95°C and 30 cycles of denaturizing for 30 s at 95°C, annealing for 2 min at 55°C, extension for 90 s at 72°C and a final extension for 10 min at 72°C 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 [19]. Cycling conditions were as follows: Initial denaturation for 4 min at 95°C for and 30 cycles of denaturizing of 45 s at 95°C, annealing of 45 s at 68°C, 1 min at 72°C and a final extension of 8.5 min at 72°C.

S-C-Act-0235-a-S-20(5′-CGCGGCCTATCAGCT TGTTG 3′) and

S-C-Act-0878-a-A-19(5′-CCGTACTCCCCAGGC GGGG-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) [20]. For the phylogenetic analysis, multiple sequence alignment was performed using CLUSTALX, version 1.81. Phylogenetic trees were constructed using Mega 7.0 [21]. The consistency of the trees was verified by bootstrapping (1000 replicates) for maximum likelihood [10, 11].

2.8. Statistical analysis

The experimental results were analyzed as 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.

3. RESULTS AND DISCUSSION

A total of 198 isolates of actinomycetes were 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.

Table 1. Antimicrobial activity of 23 sponge-derived actinomycete isolates

No	Ampersand Isolates	Inhibition zone diameter [D = d_1 - d_2] (mm)				
		Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Candida albicans*
1	ND1.1a	15.0 ^{f}	-	14.0 ^{a}	-	23.0
2	ND1.3b	14.0 ^{g}	-	-	14.0 ^{d}	16.0 ^{h}
3	ND1.5a	17.0 ^{d}	-	-	11.0 ^{g}	12.0 ^{k}
4	ND1.7a	21.0 ^{a}	-	9.0 ^{d}	12.0 ^{f}	23.7 ^{b}

No	Ampersand Isolates	Inhibition zone diameter [D = d_1 - d_2] (mm)				
		Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Candida albicans*
5	ND1.7b	13.0 [h]	-	14.0 [a]	-	25.7 [a]
6	ND2.6c	17.0 [d]	-	9.0 [d]	14.0 [d]	21,7 [d]
7	ND2.7c	21.0 [a]	-	12.0 [b]	12.0 [f]	22.0 [d]
8	HD1.2c	9.0 [i]	-	7.0 [f]	10.0 [h]	26.0 [a]
9	HD1.3d	15.0 [f]	-	8.0 [e]	19.0 [b]	14.0 [j]
1	HD1.3e	20.0 [b]	-	11.0 [c]	15.0 [c]	19.0 [e]
11	HD1.5c	17.0 [d]	-	9.0 [d]	22.0 [a]	-
12	HD1.6a	21.0 [a]	-	8.0 [e]	4.0 [l]	18.0 [f]
13	HD2.1a	14.0 [g]	-	5.0 [h]	19.0 [b]	18.0 [f]
14	HD2.3a	13,0 [h]	-	14.0 [a]	6.0 [k]	16.0 [h]
15	HD2.3b	20.0 [b]	-	6.0 [g]	15.0 [c]	-
16	HD2.3c	18.0 [c]	-	6.0 [g]	9.0 [i]	26.0 [a]
17	HD2.4a	17.0 [d]	-	7.0 [f]	9.0 [i]	17.0 [g]
18	HD2.5a	15.0 [f]	-	12.0 [b]	14.0 [d]	14.0 [j]
19	HD2.5d	15.0 [f]	-	-	14.0 [d]	12.0 [k]
2	HD2.7d	16.0 [e]	-	4.0 [i]	13.0 [e]	15.0 [i]
21	HD2.8p	14.0 [g]	-	11.0 [c]	10.0 [h]	14.0 [j]
22	HD2.9a	7.0 [k]	-	-	12.0 [f]	14.0 [j]
23	N10b	7.0 [k]	12.0 [a]	-	-	-
24	Control	8.0 [j] Ampicillin	12.0 [a] Ampicillin	7.0 [f] Tetracycline	8.0 [j] Tetracycline	10.0 [l] Fluconazole
	CV%	2.05	5.89	2.45	3.74	1.43

Note: Sample collection site letters, isolation sample numbers, and isolate species number digits. Means within a column followed by the same letter/s are not significantly different at 99%; Standard deviations must be presented together with means 1.43~5.89. D = diameter of inhibition zone of isolates, d1 = diameter of inhibition zone, d2 = diameter of well, ND: not detected.

Almost all their colonies have round shapes; milky, white clear and yellow, entire, or lobate margin; diameter size of these colonies varied from 0.2 to 3.0 mm and all of them have Gram - positive. Twenty - three of 198 tested isolates could produce antimicrobial active metabolites inhibiting at least two of the test pathogens. Over fifty percent isolates could inhibit the growth of Gram - positives, 20/198 isolates were actively against *Candida albicans,* and 21 isolates showed activity against two among five following pathogens including *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Candida albicans* (Table 1).

Based on the result from table 1, 23 isolates were chosen to analyze for PCR technique and sequencing. The result showed that 23 selected isolates belonged to the families Actinomycetaceae 65.2% (15 strains), Microoccupied 8.7% (2 strains), Nocardiaceae 21.7% (5 strains), Gorgoniaceae 4.4% (1 strain), respectively (Figure 1).

Family Actinomycetaceae, genus *Streptomyces* with 15 strains (65.2%) was the most prominent genus, this result is suitable with the [22] result' (occupied over 95% species in class Actinomycetales) and family Gordoniaceae had the lowest ratio (4.4%) (1 strain).

A total of 23 isolates were chosen for identification due to their antimicrobial activity. The fragments of 600 - 620 bp 16S rRNA were obtained from PCR, antimicrobial activity and presence of biosynthetic gene sequences, the detection levels of PKS-I and PKS-II and NRPS sequences by specific amplification of total DNA: Type I (PKS-I), polyketide synthase type II (PKS-II) and nonribosomal peptide synthetase (NRPS) with PCR (Figure 2, 3, 4).

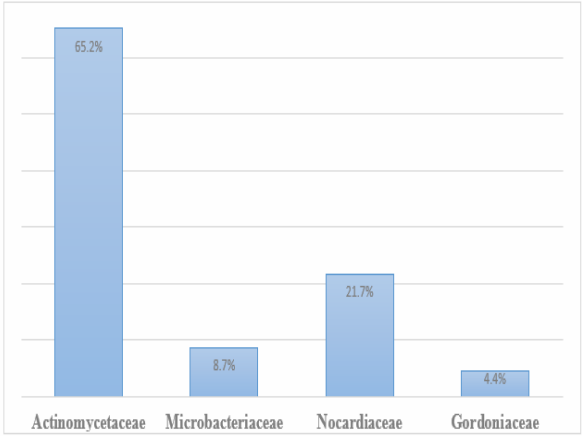
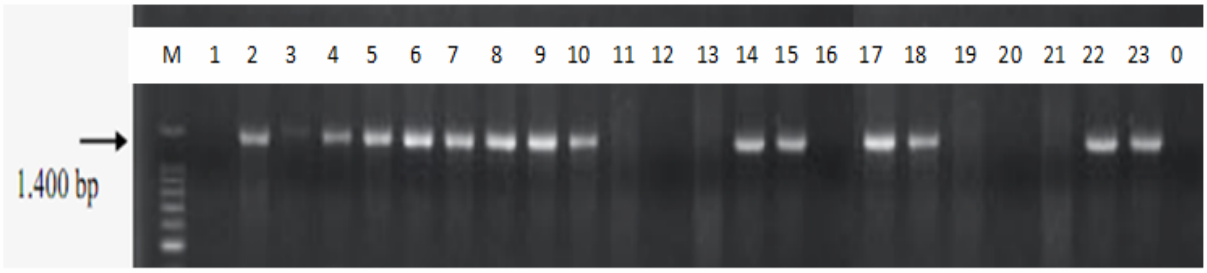


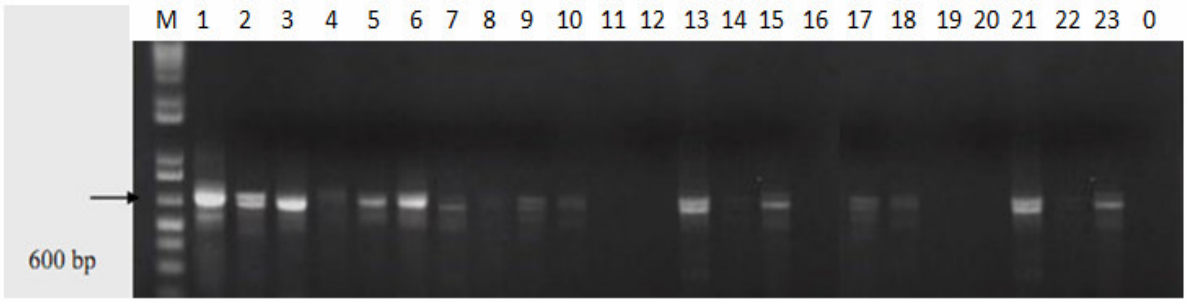
Figure 1. Ratio of the Actinomycete strains were selected to sequence

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.



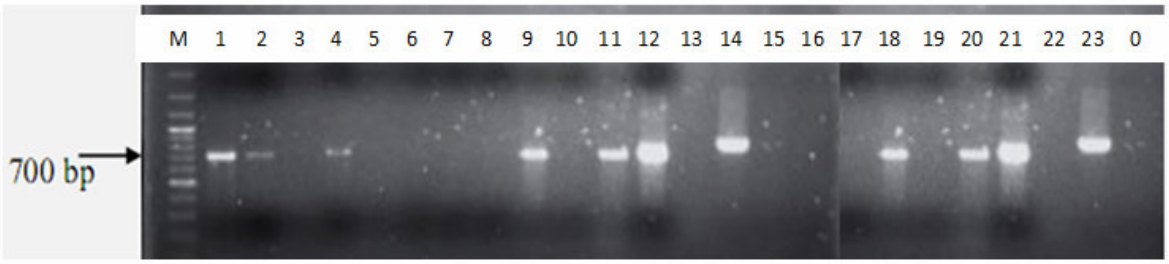
(M: Ladder 100 bp, 1: N10b, 2: HD2.9a, 3: HD2.8p, 4: HD2.7d, 5: HD2.5d, 6: HD2.5a, 7: HD2.4a, 8: HD2.3c, 9: HD2.3b,10: HD2.3a, 11: HD2.1a, 12: HD1.6a, 13: HD1.5c, 14: HD1.3e, 15: HD1.3d, 16: HD1.2c, 17: ND2.7c, 18: ND2.6c, 19: ND1.7b, 20: ND1.7a, 21: ND1.5a, 22: ND1.3b, 23: ND1.1a, 0: Negative Control)

Figure 2. Agarose gel electrophoresis of PCR products from DNA isolated from representative actinomycete strains: Selective amplification of the 1200 - 1400 bp fragments using primers for NKS-I gene of 23 selected actinomycete strains



(M: Ladder 100 bp plus, 1: HD2.1a, 2: HD2.3a, 3: HD2.3b, 4: HD2.3c, 5: HD2.4a, 6: HD2.5a, 7: HD2.5d, 8: HD2.7d, 9: HD2.8p,10: HD2.9a, 11: ND2.7c, 12: ND2.6c, 13: ND1.7b, 14: ND1.7a, 15: ND1.5a, 16: ND1.3b, 17: ND1.1a, 18: HD2.1c, 19: HD1.3d, 20: HD1.3e, 21: HD1.5c, 22: HD1.6a, 23: N10b, 0: Negative Control).

Figure 3. Agarose gel electrophoresis of PCR products from DNA isolated from representative actinomycete strains: Selective amplification of the 600 bp fragments using primers for NKS-II gene of 23 selected actinomycete strains



(M: Ladder 100 bp plus, 1: ND2.7c, 2: ND2.6c, 3: ND1.7b, 4: ND1.7a, 5: ND1.5a, 6: ND1.3b, 7: ND1.1a, 8: HD1.2c, 9: HD1.3d,10: HD1.3e, 11: HD1.5c, 12: HD1.6a, 13: HD2.1a, 14: HD2.3a, 15: HD2.3b, 16: HD2.3c, 17: HD2.4a, 18: HD2.5a, 19: HD2.5d, 20: HD2.7d, 21: HD2.8p, 22: HD2.9a, 23: N10b, 0: Nagative control)

Figure 4. Agarose gel electrophoresis of PCR products from DNA isolated from representative actinomycete strains: Selective amplification of 700 - 800 bp fragments using primers for NRPS adenylation sequences of 23 selected actinomycete strains

Homology searches of 16S rRNA gene sequence of selected in GenBank by BLAST (Table 2) revealved that they had similarity to sequences of genus *Streptomyces* (15 strains) and 3 families including Microbacteriaceae (*Microbacterium tumbae*) (2 strains), Nocardiaceae (*Rhodococcus*) (5 strains) and Gordoniaceae (*Gordonia bronchialis*) (1 strain).

According to [23], the diversity of actinobacteria in *Xestospongia muta* and *Xestospongia testudinaria* were very high. The 106 actinobacteria isolates were isolated from 7 genera of *Hymeniacidon perleve* sponge [24]. Identified 181 species of actinomycete with 3 genera from 5 species of sponge in China [25]. Analysis of the diversity of microbes from 2 strains of Red sea sponges (*Hyrtios erectus* and *Amphimedon* sp.), showed that 35 species of actinomycete in 4 genera. In our study, 198 actinomycete isolates isolated from sponges in Ha Tien Sea, 23 isolates were identified having high activity against human disease microbes.

Table 2. Phylogenetic affiliation of 23 isolates on the basis of 16S rRNA gene sequences by using BLAST programme in the GenBank database based on sequences similarity

No	Taxonomic group and strain	Closest species relative	Nucleotide number (bp)	Similarity (%)
	Actinomycetaceae			
1	ND1.1a	*Streptomyces coelicolor* ND1.1a	606	100
2	ND1.3b	*Streptomyces griseoaurantiacus* ND1.3b	604	100
3	ND1.5a	*Streptomyces ramulosus* ND1.5a	608	100
4	ND1.7a	*Streptomyces tateyamensis* ND1.7a	602	100
5	ND1.7b	*Streptomyces ambofaciens* ND1.7b	603	100
6	HD1.2c	*Streptomyces recifensis* HD1.2c	605	100
7	HD1.3d	*Streptomyces olivaceus* HD1.3d	605	100
8	HD1.3e	*Streptomyces althioticus* HD1.3e	603	100
9	HD1.5c	*Streptomyces tateyamensis* HD1.5c	604	99.83
10	HD1.6a	*Streptomyces flaveolus* HD1.6a	604	100
11	HD2.1a	*Streptomyces chumphonensis* HD2.1a	609	100
12	HD2.3b	*Streptomyces olivaceus* strain HD2.3b	602	100
13	HD2.3c	*Streptomyces coelicolor* HD2.3c	606	100
14	HD2.9a	*Streptomyces qinglanensis* HD2.9a	604	100
15	N10b	*Streptomyces variabilis* N10b	609	100
	Microbacteriaceae			
16	ND2.7c	*Microbacterium tumbae* ND2.7c	618	100
17	HD2.8p	*Microbacterium tumbae* HD2.8p	610	100
	Nocardiaceae			
18	ND2.6c	*Rhodococcus hoagii* ND2.6c	606	100

No	Taxonomic group and strain	Closest species relative	Nucleotide number (bp)	Similarity (%)
19	HD2.3a	*Rhodococcus hoagii* HD2.3a	604	100
20	HD2.4a	*Rhodococcus rhodochrous* HD2.4a	603	100
21	HD2.5d	*Rhodococcus pyridinivorans* HD2.5d	604	100
22	HD2.7d	*Rhodococcus hoagii* HD2.7d	613	100
	Gordoniaceae			
23	HD2.5a	*Gordonia bronchialis* HD2.5a	604	100

The result presenting at figure 5 showed that 23 actinobacterial strains described two clusters: Cluster A had 14 strains, and cluster A divided two small clusters: Cluster A1 had 12 strains including 10 *Streptomyces* strains, *Microbacterium tumbae* HD2.8p strain and *Rhodococcus rhodochrous* HD2.4a strain. Cluster A2 had two strains: *Rhodococcus hoagii* HD2.7d strain and *Rhodococcus hoagii* ND2.6c strain. Cluster B had 9 strains and this cluster divided two small clusters: Cluster B1 had 4 strains with *Microbacterium tumbae* ND2.7c strain located in the separate clusterand three *Streptomyces coelicolor* HD2.3c (originated from Dam island), *Streptomyces coelicolor* ND1.1a strain (from Den mountain) and *Streptomyces variabilis* N10b strain (from Nghe island), this result showed that three strains had other originsfar from many kilometers, but they had genetic relationship nearly. Cluster B2 had 5 strains including *Rhodococcus rhodochrous* HD2.4a strain in separate cluster, two strains *Streptomyces recifensis* HD1.2c strain and *Streptomyces olivaceus* HD1.3d strain (in smaller cluster of cluster B2) and two strains: *Rhodococcus hoagii* HD2.3a strain and *Rhodococcus pyridinivorans* HD2.5d strain in smaller cluster in cluster B2, however all of five strains in cluster B2 had original site (Dam island), this result showed that they had close genetic relationship.

These actinobacteria strainshad genetic relationship closely even though they isolated from the sites far from Dam island to Nui Den, however many strains isolated at one site, but they were located in two separate clusters in the phylogenetic tree. These changes were based on the analyses of sequences of actinobacteria strainswith diversity of sponge - associated actinobacteria and seawater condition [26].

Streptomyces sp. isolated from sponges at Egypt sea, which had high resistance ability to *Staphylococcus aureus* and *Candida albicans*, they also against to *Enterococcus faecalis*, *Leishmania major*, *Trypanosoma brucei* [27]. Besides, *Streptomyces* sp. isolated from *Halichondria panicea*sponge having antimicrobial activity [28, 29], *Streptomyces* sp. Againstto *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. In our results showed that *Streptomyces* sp. had high resistance activity to *Bacillus cereus* and *Salmonella typhimurium*. In addition, *Brevibacterium* sp. isolating from *Callyspongia*sponge identified to against *Escherichia coli*and *Staphylococcus aureus*from 2 compounds: 6-hydroxymethyl-1-phenazine-carboxamide and 1,6-phenazinedimethanol [30]. *Brevibacterium* sp. also isolated *Neopetrosia exigua*sponge in Mauritius sea, East Africa [31]. *Microbacterium* sp. isolated *Erylus discophorus*sponge [32] and *Microbacterium* sp. also isolated *Spongia* sp. sponge at Portugal sea [33], *Microbacterium* sp. identified to against to *Staphylococcus aureus* [26].

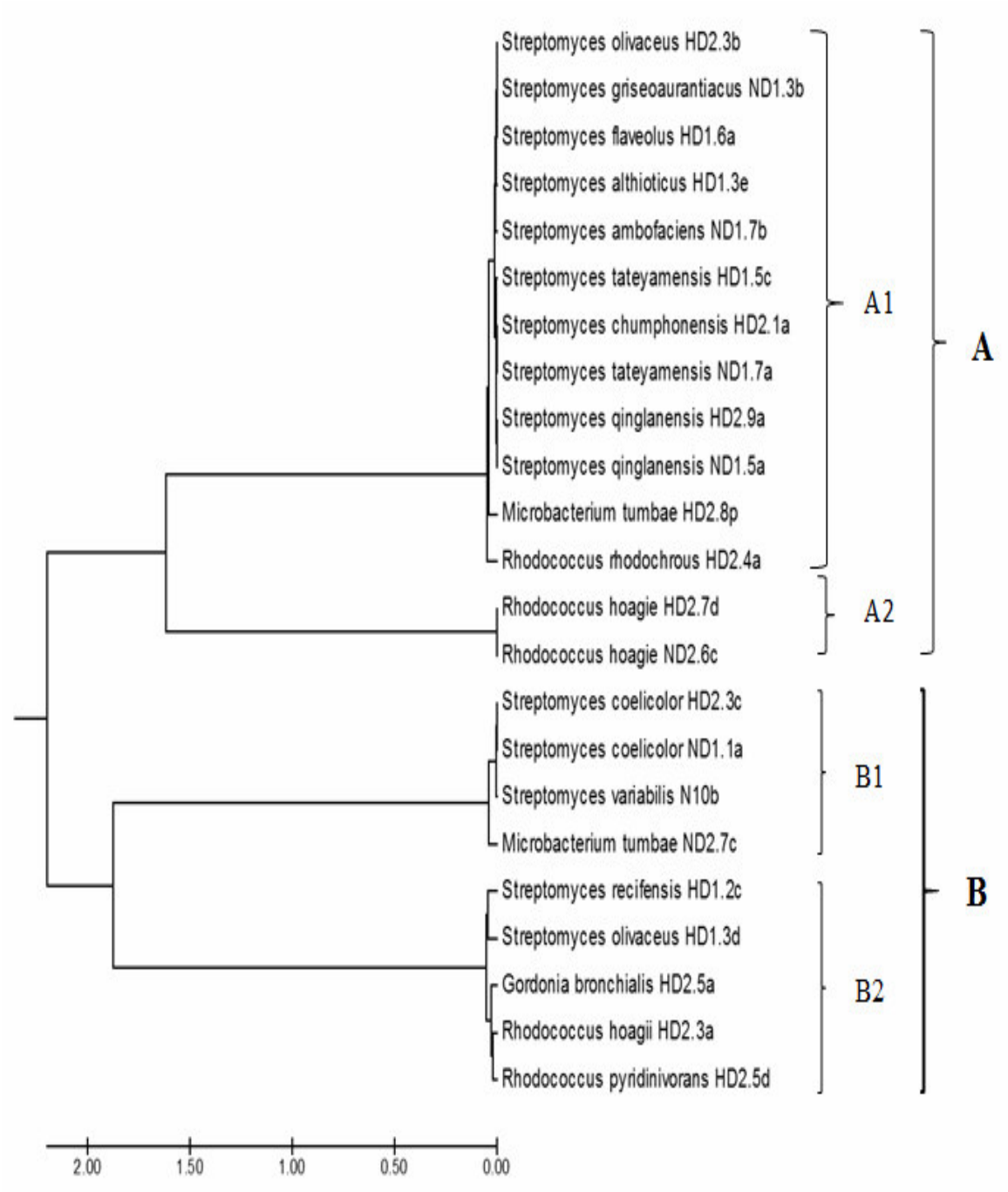


Figure 5. The maximum likelihood phylogenetic tree of partial 16S rRNA gene sequences of 23 actinobacteria isolates with primers (SC-Act-0235-aS-20 and SC-Act-0878-aA-19) isolated from sponges of the Ha Tien Sea and closely related type strains. Numbers in the figure refer to percentage bootstrap values in the MEGA 11 program which were calculated for 1000 replicates and nucleotide spacing

The presence of PKS-I, PKS-II and NRPS genes in all of *Streptomyces,* Microbacteriaceae, Nocardiaceae and Gordoniaceae (Figure 6 and table 3).

Table 3. The presence of PKS-I, PKS-II and NRPS genes of 23 selected actinomycete strains

No	Taxa	Gen PKS-I K1F/M6R (1.200 - 1.400 bp)	Gen PKS-II KSαF/KSαR (600 bp)	Gen NRPS A3F/A7R (700 - 800 bp)
	Actinomycetaceae			
1	*Streptomyces coelicolor* ND1.1a	+	+	-
2	*Streptomyces griseoaurantiacus* ND1.3b	+	-	-
3	*Streptomyces ramulosus* ND1.5a	-	+	-
4	*Streptomyces tateyamensis* ND1.7a	-	+	+
5	*Streptomyces ambofaciens* ND1.7b	-	+	-
6	*Streptomyces recifensis* HD1.2c	-	+	-
7	*Streptomyces olivaceus* HD1.3d	+	-	+
8	*Streptomyces althioticus* HD1.3e	+	-	-
9	*Streptomyces tateyamensis* HD1.5c	-	+	+
10	*Streptomyces flaveolus* HD1.6a	-	-	+
11	*Streptomyces chumphonensis* HD2.1a	-	+	-
12	*Streptomyces olivaceus* strain HD2.3b	+	+	-
13	*Streptomyces coelicolor* HD2.3c	+	-	-
14	*Streptomyces qinglanensis* HD2.9a	+	+	-
15	*Streptomyces variabilis* N10b	**+**	**+**	**+**
	Microbacteriaceae			
16	*Microbacterium tumbae* ND2.7c	+	-	+
17	*Microbacterium tumbae* HD2.8p	+	-	+
	Nocardiaceae			
18	*Rhodococcus hoagii* ND2.6c	+	-	+
19	*Rhodococcus hoagii* HD2.7d	+	-	+
20	*Rhodococcus hoagii* HD2.3a	**+**	**+**	**+**
21	*Rhodococcus rhodochrous* HD2.4a	+	+	-
22	*Rhodococcus pyridinivorans* HD2.5d	+	+	-
	Gordoniaceae			
23	*Gordonia bronchialis* HD2.5a	**+**	**+**	**+**

Ghi chú: "+": detected; "-": no detected

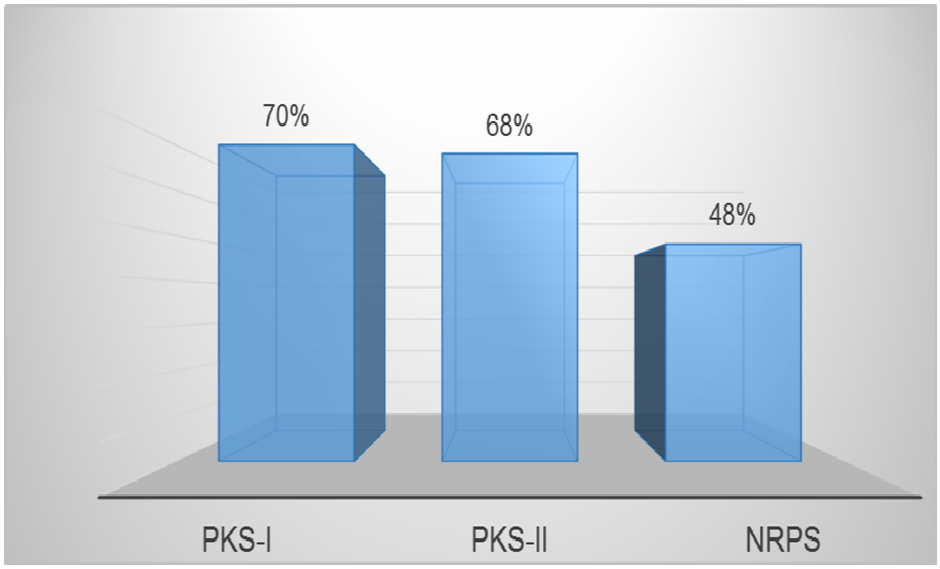


Figure 6. Percent of quantity strains presented in three kinds of primers for detection of PKS-I, PKS-II and NRPS gene

Using primers detected PKS-I gene on 8 strains of *Streptomyces* and all strains of 3 families Microbacteriaceae, Nocardiaceae and Gordoniaceae, with 16/23 strains having the presence of PKS-I gene (69.6%). The presence of PKS-II gene detected on 14/23 strains with KSαF/KSαR primers (60.9%), including 10 species of *Streptomyces*, 3 species of *Rhodococcus* and 1 strain of *Gordonia* (*Gordonia bronchialis* HD2.5a). 11/23 strains were detected when using primers with NRPS gene (47.8%), including 5 species of *Streptomyces*, 2 strains of *Microbacterium*, 3 strains of *Rhodococcus* and *Gordonia bronchialis* HD2.5a.

The result presenting at figure 6 showed that the highest PKS-I gene (occupied 70%) and the lowest NRPS gene (48%). This result also was similar to that of the previous studies about PKS-I, PKS-II and NRPS gene as [34], isolated and select the 41 strains of *Streptomyces* from herbal tree in tropical forest in Van Nam province, China having the ability of resistance to antimicrobial, tumor; among 31.7% strains made cell toxicity to against A549 cell; 29.3% to HL-60 cell, 85.4% to BEL-7404 cell, 90.2% to P388D1 cell and 65.9% strains resisted to *Escherichia coli*, 24.4% to *Staphylococcus aureus*, 31.7% to *Staphylococcus epidermidis*, 12.2% to *Candida albicans* but no strain resisted with *Klebsiella pneumoniae*. When testing the presence of PKS-I, PKS-II and NRPS gene with primers as K1F/M6R, KSαF/KSαRand A3F/A7R, the result showed that detection ratio were PKS-I (34.1%), PKS-II (63.4%) and NRPS (61.0%).

Using primer PKS-I and primer PKS-II to detect the presence of and NPRS gene of 210 actinomycete isolates from 33 other genera [10]. NRPS gene detected 167/210 isolates (79.5%) while 119/210 isolates were detected by PKS-I gene (56.7%). Almost all the species of *Streptomyces* were detected by NRPS and PKS-I gene (97% and 79%). While NRPS and PKS-I gene detected with 91% and 59% actinomycete isolates in Nocardiaceae.

In Viet Nam, studied *Streptomyces cavourensis* YBQ75 strain isolating from C*innamomum cassia* Presl in Yen Bai province, the result showed that this strain resisted to 5 human diseases bacteria as *Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Enterobacter aerogenes, Proteus vulgaris* and the presence of PKS-I, PKS-II and NRPS gene in antibiotic synthesis, *Streptomyces cavourensis* YBQ75 had three of PKS-I, PKS-II and NRPS gene [35]. Our result also showed that three strains *Streptomyces variabilis* N10b, *Rhodococcus*

hoagii HD2.3a and *Gordonia bronchialis* HD2.5a had the presence of three PKS-I, PKS-II and NRPS gene. *Streptomyces variabilis* N10b strain against the *Staphycolococcus aureus* (while 22 strains did not resist with this human disease bacteria), *Rhodococcus hoagii* HD2.3a strain against the human disease microbes as *Bacillus aureus, Esccherichia coli* and *Candida albicans* while *Gordonia bronchialis* HD2.5a strain had high antimicrobial activity with 4 human diseases microbes (except *Staphycolococcus aureus*) as *Bacillus aureus, Esccherichia coli, Salmonell typhymurium* and *Candida albicans*, the results showed that *Gordonia bronchialis* HD2.5a strain (Gordoniaceae) was the best strain in the resistance with 4/5 human diseases microbes while *Streptomyces variabilis* N10b strain only against *Staphycolococcus aureus* and these strains need to be studied the bioactives which were extracted from them as new antibiotics.

4. CONCLUSION

Twenty - three actinomycete strains were selected with high antimicrobial activity, they had at least one in three PKS-I, PKS-II and NRPS gene, three strains had three PKS-I, PKS-II and NRPS gene, especially *Gordonia bronchialis* HD2.5a strain (Gordoniaceae) was the best strain in the resistance with 4/5 human diseases microbes and theyhad high potential for research of bioactive production.

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