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ISOLATION, IDENTIFICATION OF ANTIMICROBIAL ACTIVITY-POSSESSING MARINE ACTINOMYCETES AND FUNGI FROM SAMPLES COLLECTED AT THE BACH LONG VY TO LY SON ISLANDS

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SUMMARY

The marine environment has been known as a resource containing a lot of compounds with antibiotic, anti-tuberculosis and antiviral activities. Many of the marine microorganisms have been found to live in sediment, sand, surface or within the body of other living organisms such as sponges, soft coral, mollusk, algae… Natural products from marine actinomyces and fungi are considered an important source for the discovery of novel compounds, of their rich secondary metabolites. From 38 samples collected at 22 coordinates and different depths in the sea from Bach Long Vy island - Hai Phong to Ly Son island - Quang Ngai on the marine research vessel "Oparin Academik", 40 actinomycetes and 20 fungal strains were isolated. The crude extracts of 60 isolates were primary screened for their antimicrobial activity against 7 pathogenic microorganisms by the Bioassay method in a 96-well tray. As a result, 53/60 strains were resistant at least 1 tested microorganisms, 22/60 isolates against 3 or more the test microorganisms, respectively. In particular, there were 4 isolates (three actinomyces G817, G819, G824 and fungus OM01) with the strongest resistance to four or more tested strains, which were then studied further. The results of phenotypic and molecular identification by 16S rRNA sequences for G817, G819, G824 and 18S rRNA sequence for OM01 showed that OM01 was *Penicillium citrinum*; G817, G819 strains belonged to *Salinispora arenicola*, while G824 was identified as *Pseudonocardia carboxydivorans*. The promising candidate isolates were analyzed in a phylogenetic tree based on MegaX software.

Keywords: Antimicrobial activity, *Penicillium* sp., *Pseudonocardia carboxydivorans*, Oparin 7th, *Salinispora arenicola*.

INTRODUCTION

The ocean is the largest habitat on the planet, and microorganisms are the most abundant type of life with many diverse forms. These microorganisms (viruses,

bacteria, archaea...) play an important role in the biogeochemical cycles of basic elements. They exist not only in a planktonic state, but also in the association with other marine organisms such as animals, plants, algae, etc. (Pita *et al* 2018; Chananan *et al*, 2023). Many studies are focused on secondary substances produced by marine microorganisms has achieved remarkable achievements. Among them, many secondary compounds with intricate chemical structures and good biological activities have been discovered, that may play the role to the production of novel drugs or leads. Since the past few decades, scientists around the world have been attracted by the diversity of marine microorganisms and the natural products they produce with various activities such as antibiotic activities such as antiviral, antibacterial, antifungal, immunosuppressive, anti-inflammatory, anti-tumor… (Banakar *et al*., 2019). Marine actinomycetes are evaluated as an important source in the production of antibiotics in which *Streptomyces* is the predominant actinomycete (Luzhetskyy *et al*., 2007). However, only a small amount marine fungi has been studied. Marine fungi are also considered a potential source of microbial origins due to their secondary metabolites diversity and promising biological activity, particularly those associated with other organism (sponge, deep sea sediments, debris, marine plants, invertebrates and vertebrates, etc. (Tisthammer, 2016; Frank, 2023).

MATERIALS AND METHODS

Chemicals

Chemicals used for the environment were supplied from Hidia (India), Sigma - Aldrich (USA), Fisher Scientific, Duc Giang (Vietnam). Total DNA isolation kit of Madison (USA), Dream Tag PCR Master mix of Thermo Scientific (Korea), Fisher Scientific standard DNA indicator, primer pairs for amplifying 16S rRNA gene (16sF: 5'-GAGTTTGATCCTGGCTCAG) -3'; 16sR: 5'-AAGGAGGTGATCCAACC-3') and 18S rRNA gene NS3F (5'-GCAAGTCTGGTGCCAGCAGCC-3') và NS8R (5'-TCCGCAGGTTCACCTACGGA-3').

Media

The media used in microbiological research was referenced by Stanley and Holt (1989) with an improvement of the Department of Pharmaceutical Chemistry and Pharmacology, College of Pharmacy, University of Illinois Chicago, USA: A1 (g/L): soluble starch: 10, yeast extract: 4, peptone: 2, instant ocean: 30, agar: 15; A1+ (g/L): soluble starch: 10, yeast extract: 4, peptone: 2, instant ocean: 30, $CaCO_3$: 1.5 mL of 20 mg/mL $FeSO_4$, 5 mL of 8 mg/mL KBr, agar agar: 15; M1 (g/L): soluble starch: 1, yeast extract: 0.4, peptone: 0.2, instant ocean: 30, agar: 15; SWA (g/L): instant ocean: 30, agar: 15; SCA: instant ocean: 10 g/L, CaCO3: 2mg/L, $FeSO_4.7H_2O$: 10 mg/L; $MgSO_4.7H_2O$: 50 mg/L; casitone 300 mg/L; K_2HPO_4: 2 g/L; KNO_3: 2 g/L; agar: 15; NZSG (g/L): soluble starch: 20, yeast extract: 5, glucose: 10, NZ amine A: 5, instant ocean: 30, agar: 15; PMDA (g/L): potatoes extract: 30, dextrose: 20, malt extract: 10; instant ocean: 30, agar: 15; PDA (g/L): potatoes extract: 30, dextrose: 20; instant ocean: 30, agar: 15; ISP1 (g/L): casiton: 5, yeast extract: 3, instant ocean: 30, agar: 15; ISP2 (g/L): soluble starch: 5, yeast extract: 2, glucose: 10, malt extract: 10, instant ocean: 30, agar: 15. All add water to 1000 mL.

Sampling

Samples were collected in May 2021, according to the regulations of the Oparin Academik vessel. The samples were taken by hoisting to the bottom of the sea at different coordinates by SCUBA diving and pickaxe. The samples were placed in 50 mL falcon tubes or plastic boxes filled with sterilized seawater. All sample boxes must be labeled with the site name, the date and time of the sample collection and other information. After being taken, the samples were then brought to the laboratory on the vessel for preparation of microbial isolates.

Isolation of microorganisms

Weighed 0.5 g of marine samples into a falcon tube and dissolved in 4.5 mL of sterile distilled water. For necessary samples that must be preserved, gently rolling a saline moistened cotton swab across their skin or tissue surface. After mixing, a 1.5 mL aliquot of suspension was added to two tubes, one tube of each paired set was subjected to heat shock (60°C, 8 minutes). The homogeneous suspension was further diluted 10 times and 30 µL of the inoculum was spread evenly on the 9 prepared media. Plates were incubated in an incubator at 28 - 30°C for 7 to 60 days. The form of colonies and mycelium was observed on medium surface every day. Separated actinobacteria-like or fungi-like colonies were picked and subcultured on A1 medium for actinobacteria and PDA for fungi (Qinyuan *et al*., 2016, Dayarathne *et al*., 2020).

Generating crude extract from fermented culture

The isolates were cultured in 1000 mL conical flasks containing 500 mL of A1+ for actinomycetes and PDA medium for fungi, at 28 - 30°C, 170 rpm. After 7 days of culture, the culture was extracted with 300 mL ethyl acetate (5 times x 15 min). The extract was then evaporated under reduced pressure (250 mbar, heating bath at 45°C) to remove the solvent to obtain the crude extract (Carroll *et al*., 2020).

Bioassay

The antimicrobial activity of crude extracts was determined by the multiple dilution method of Andrews (2001). Those crude extracts were evaluated against Gram negative bacteria (*Escherichia coli* ATCC25922 (E.C), *Pseudomonas aeruginosa* ATCC27853 (P.A), *Salmonella enterica* ATCC13076 (S.A)), and three Gram positive bacteria (*Enterococcus faecalis* ATCC29212 (E.F), *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC 14579 (B.C)) and one yeast *Candida albicans* ATCC10231 (C.A)). The results of this method are expressed as minimum inhibitory concentration (MIC).

MIC defines *in vitro* levels of susceptibility or resistance of test microbial strains to the antibiotic or agents used. Streptomycin (Sigma) and cycloheximide (Merck) were used as positive controls for bacteria and yeast. The crude extract was initially diluted in DMSO at a decreasing concentration range: 256, 128, 64, 32, 16, 8, 4 and 2 µg/mL for the number of experiments. The final test microbial concentration of 2 x 10^5 CFU/mL per each well. All plates were incubated at 37°C with shaking at 120 rpm. After 24 h, read the MIC value as the value at the well with the lowest concentration of antimicrobial agent that completely inhibits the visible growth of a test microbial. Three independent trials were performed in

triplicate for each assay (Hadacek, Greger, 2000; Andrew, 2001).

Extraction of genomic DNA and identification of the isolated actinomycetes and fungi

Total DNA was extracted by DNA isolation kit of Madison (USA). Fungal cells were mechanically disrupted by liquid nitrogen grinding before using DNA extraction kit. The PCR was carried out in a 25 µL mixed volume containing 10 µL sterile ddH_2O, 12.5 µL PCR Master mix, 1.0 µL primer at 10 pmol/µL concentration for each primer, 0.5 µL (concentration of approximately 50 ng/µL) of total DNA. The thermal cycles of PCR were: 94^oC / 2 minutes, (94^oC / 1 min, 58^oC (for actinomyces) or (62^oC for fungi) / 1 min 72^oC / 1min 20 s) × 30 cycles, 72^oC / 8 minutes and kept the sample at 8^oC. Estimated product size is about 1.5 kb for actinomyces and 1.3 kb for fungi. PCR products were purified using Invitrogen's purification kit. The PCR products were sequenced by Bioscience's ABI PRISM 3100 automated sequencer (Sambrook, Russell, 2000). Gene sequences were analyzed by BioEdit version v.7.2 and compared with the genes in NCBI database by multiple sequence alignment using CLUSTALW program. Phylogenetic tree was performed using the Maximum Likelihood method in program MEGAX software (Kumar, 2008).

RESULTS AND DISCUSSION

Collection of samples and isolation of microorganisms

The research team commits that using samples in this study complied with international guidelines and considered the conservation of marine resources.

Total 38 samples were collected at 22 different coordinates along from Bach Long Vy island - Hai Phong province to Ly Son island - Quang Ngai province on the marine research vessel "Oparin Academik". The samples included 7 mollusks, 11 seaweeds, 6 sponges, 6 soft corals, 2 marine animals, 1 echinoderm and 5 sediments. Eighty strains have been isolated with different colony morphology and color, including 40 actinomycetes and 20 fungi strains. Mycelium colour and diffusible pigment of actinomycetes and fungi isolates were determined on the basis of morphological characterization. The detail results are illustrated in Table 1.

After fermentation at volume of 500 mL, the cultures were extracted with ethyl acetate solvent (5 times) to obtain crude residues.

Table 1. Collection of samples and isolation of microorganisms summary report.

No.	Color and morphology of colonies	Sample	Media	Heat shock/ Non heat	Strain name
1	Light gray, rough colonies brown base, size 2.5 mm	Sediment	ISP2	Heat shock	G811
2	Orange, solidly grown to 1.5 mm	Sponge	A1	Heat shock	G812

No.	Color and morphology of colonies	Sample	Media	Heat shock/ Non heat	Strain name
3	Orange, firm growth to black, size 1 mm	Sediment	M1	Non Heat	G813
4	White, dry, round colonies size about 1 mm	Sponge	M1	Non Heat	G814
5	Orange, firm growth to black, size 1 mm	Seaweed	M1	Non Heat	G815
6	Opaque yellow, spores are pale yellow, size 0.5 mm	Seaweed	M1	Non Heat	G816
7	Orange, solidly grown size 1 mm	Soft coral	M1	Non Heat	G817
8	Pale pink colonies, size about 1.5 mm	Sponge	ISP1	Heat shock	G818
9	Orange colonies, size 1.5 mm	Mollusk(snail)	A1	Heat shock	G819
10	Pale orange, size about 1.5 mm	Seaweed	A1	Heat shock	G820
11	Lemon yellow, diffuse, size 1 mm	Mollusk(snail)	A1	Heat shock	G821
12	Pale milky yellow, slightly yellow, 1 mm	Mollusk(snail)	A1	Heat shock	G822
13	Opaque white, tiny, about 0.5 mm	Sponge	Isp1	Heat shock	G823
14	Milky white, about 1-2 mm in size	Sponge	Isp2	Heat shock	G824
15	Gray, rough colonies brown base, size 0.5 mm	Sediment	A1	Heat shock	G825
16	Light gray, rough colonies brown base, size 2.5 mm	Sediment	SCA	Heat shock	G826
17	Gray white, size 1-2 mm	Mollusk	PMDA	Heat shock	G827
18	Milky white,tiny, size 1-2 mm	Sponge	SCA	Heat shock	G828
19	Opaque white, diffuse, size 0.5 mm	Sponge	SCA	Heat shock	G829
20	Yellow orange to black, tough, size 0.5 mm	Sponge	A1	Heat shock	G830
21	Opaque white, tough, 0.5mm	sea animals	M1	Non Heat	G831
22	Yellow-orange, size 1 mm	Sponge	Isp2	Heat shock	G832

No.	Color and morphology of colonies	Sample	Media	Heat shock/ Non heat	Strain name
23	Opaque white, size 2.5 mm	Soft coral	M1	Non Heat	G833
24	Pale pink, diffuse, 2 mm	Seaweed	M1	Non Heat	G834
25	Pale opalescent, diffusely growing, size 1mm	Sediment	ISP1	Heat shock	G835
26	Yellow, filamentous, 1.5 mm	Sponge	M1	Heat shock	G836
27	Yellow colonies, size 2 mm	Seaweed	A1	Heat shock	G837
28	Milky white colonies, size 1.5 mm	echinoderms	Isp2	Heat shock	G838
29	Pale opaque white, size 1 mm	Seaweed	Isp2	Heat shock	G839
30	White opaque, mucilaginous, size 1.5 mm	Seaweed	SWA	Non Heat	G840
31	Pale opaque white, size 1mm	Seaweed	A1	Heat shock	G841
32	Pale orange bacteria, mucus, size 1.5 mm	Sponge	M1	Non Heat	G842
33	Pale orange bacteria, mucus, size 1.5 mm	Sediment	SWA	Non Heat	G843
34	Colonies are yellow, filamentous, size 1.5 mm	Mollusk	PDA	Heat shock	G844
35	Gray-white, diffuse, size 2 mm	Sediment	A1	Heat shock	G845
36	Milky white colonies, size 1.5 mm	Soft coral	Isp2	Heat shock	G846
37	Rough gray-white, diffuse, size 2.5 mm	Seaweed	PDA	Heat shock	G847
38	Pale pink, mucilaginous, 0.5 mm	Sponge	Isp1	Heat shock	G848
39	Orange, hardy, size 1.5 mm	Soft coral	SWA	Non Heat	G849
40	Light gray-white, diffuse, size 1.5 mm	Seaweed	SWA	Non Heat	G850
41	Dark moss green powdery, wrinkled surface, white thin edges	Mollusk	ISP1	Heat shock	OM01
42	Light moss green powdery, wrinkled surface, white thin edges	Sediment	A1	Heat shock	OM02

No.	Color and morphology of colonies	Sample	Media	Heat shock/ Non heat	Strain name
43	White cotton, thin edges of colonies.	Seaweed	PMDA	Heat shock	OM03
44	Pale moss green, wrinkled surface, white thin edges	Seaweed	ISP2	Heat shock	OM04
45	Pale yellowish brown, smooth surface, white thin edges	Seaweed	SWA	Heat shock	OM05
46	Yellowish green, smooth surface, white thin edges	sea animals	PDA	Heat shock	OM 06
47	Pale green, smooth surface, white thin edges	Sediment	PMDA	Heat shock	OM 07
48	Yellowish green, smooth surface, white thin edges	Soft coral	SWA	Heat shock	OM 08
49	Dark moss color, surface wrinkled, deeply ingrained in the medium, white thin edges	Mollusk (snail)	SWA	Heat shock	OM 09
50	Moss gray, wrinkled surface, deeply ingrained in the medium, white thin edges	Mollusk	PMDA	Heat shock	OM 10
51	Dark mossy, deeply ingrained in the medium, white thin edges	Seaweed	PMDA	Heat shock	OM 11
52	Dark moss color, surface wrinkled, deeply ingrained in the medium, white thin edges	Sponge	ISP2	Non Heat	OM 12
53	Dark brown, wrinkled surface, deeply ingrained in the medium, white thin edges	Sponge	SCA	Heat shock	OM 13
54	Dark brown, deeply rooted in the medium, with thin edges	coral	SCA	Non Heat	OM 14
55	White, wrinkled surface, deeply infiltrated into the medium, thin colony edges	Soft coral	PDA	Non Heat	OM 15
56	White-yellow, wrinkled surface, deeply rooted in the medium, thin edge	Mollusk (snail)	PMDA	Heat shock	OM 16
57	Gray, wrinkled surface, convex on the medium, thin edges	Seaweed	SWA	Non Heat	OM 17
58	Mossy, surface with a white cotton layer, white thin edges	Soft coral	ISP1	Heat shock	OM 18
59	Mossy, wrinkled surface, white cottony edges	Seaweed	PMDA	Heat shock	OM 19
60	Yellow, wrinkled surface, deeply ingrained in the medium, white thin edges	Seaweed	PMDA	Heat shock	OM 20

Screen the *in vitro* antimicrobial activity of extracts

The results (Table 2) showed that 53/60 isolates had inhibitory activity from 1 to 4 strains of test microorganisms, of which 20 isolates showed antibacterial activities against at least 3 strains of test microorganisms. In which, 9/60 strains exhibited antagonistic activity against 4 tested microorganisms with MIC values equal to or even lower than the positive control. Our research results were similar in some studied to find compounds from marine microorganisms that have the great ability to fight pathogenic bacteria or yeast. Thus, marine actinomycetes and marine fungi are among the good candidates (Vaibhav *et al*., 2014).

Table 2. MIC values of EtOAc extract of 60 strains.

No.	Isolates	Gram +			Gram -			Yeast
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
		MIC (µg/mL)						
1	G811	128	-	128	-	-	-	128
2	G812	256	256	128	-	-	-	128
3	G813	128	-	-	-	-	-	-
4	G814	256	-	-	-	-	-	128
5	G815	-	-	-	-	-	-	128
6	G816	-	-	-	-	-	-	-
7	G817	16	16	32	-	-	-	8
8	G818	128	128	-	-	-	-	64
9	G819	16	64	128	-	-	-	16
10	G820	256	-	256	-	-	-	-
11	G821	128	64	32	-	-	-	128
12	G822	-	-	-	-	-	-	128
13	G823	128	64	-	-	-	-	-
14	G824	8	16	32	-	-	-	16
15	G825	-	-	128	-	-	-	64
16	G826	256	-	256	-	-	-	128
17	G827	32	-	128	-	-	-	256
18	G828	-	-	-	-	-	-	128
19	G829	32	-	128	-	-	-	128
20	G830	32	-	256	-	-	-	128
21	G831	-	-	256	-	-	-	128
22	G832	-	-	-	-	-	-	256
23	G833	128	256	64	-	-	-	128
24	G834	-	-	-	-	-	-	128
25	G835	64	-	256	-	-	-	128

26	G836	128	-	-	-	-	-	-
27	G837	-	-	256	-	-	-	64
28	G838	-	-	-	-	-	-	256
29	G839	128	-	256	-	-	-	128
30	G840	-	-	-	-	-	-	128
31	G841	-	-	-	-	-	-	256
32	G842	128	256	256	-	-	-	64
33	G843	-	-	-	-	-	-	-
34	G844	-	-	256	-	-	-	128
35	G845	-	-	-	-	-	-	256
36	G846	256	-	-	-	-	-	64
37	G847	-	-	256	-	-	-	128
38	G848	-	-	-	-	-	-	-
39	G849	128	256	64	-	-	-	64
40	G850	-	-	-	-	-	-	-
41	OM01	16	32	32	-	-	-	16
42	OM02	128	-	-	-	-	-	64
43	OM03	256	-	-	-	-	-	-
44	OM04	256	-	-	-	-	-	-
45	OM05	128	-	-	-	-	-	-
46	OM 06	256	-	-	-	-	-	-
47	OM 07	256	128	64	-	-	-	-
48	OM 08	128	-	-	-	-	-	-
49	OM 09	128	-	-	-	-	-	128
50	OM 10	-	-	-	-	-	-	-
51	OM 11	-	-	-	-	-	-	-
52	OM 12	256	-	-	-	-	-	-
53	OM 13	-	-	-	-	-	-	64
54	OM 14	128	-	-	-	-	-	-
55	OM 15	-	-	-	-	-	-	256
56	OM 16	-	-	-	-	-	-	64
57	OM 17	-	-	-	-	-	-	-
58	OM 18	256	-	256	-	-	-	-
59	OM 19	128	256	256	-	-	-	-
60	OM 20	128	256	256	-	-	-	-
Streptomycin		256	256	128	32	256	128	-
Cyclohexamide								32

(-): not active. Streptomycin is used as reference antibiotics for bacteria and cyclohexamide for yeast. MIC values are the means of average of three trials which did not show any variation.

According to the MIC values in Table 2, the 4 strains with the highest antimicrobial activity were: G817, G819, G824 and OM01. All four strains were active against *Enterococcus faecalis* with MIC_{G817} = 16 µg/mL, MIC_{G819} = 16 µg/mL, MIC_{G824} = 8 µg/mL, MIC_{OM01} = 16 µg/mL; inhibited *Stapphylococus* at MIC_{G817} = 16 µg /mL, MIC_{G819} = 64 µg/mL MIC_{G824} = 16 µg/mL, MIC_{OM01} = 32 µg/mL; were against *Bacillus cereus* at MIC_{G817} = 32 µg/mL, MIC_{G819} = 128 µg/mL, MIC_{G824} = 32 µg/mL, MIC_{OM01} = 32 µg/mL. In addition, 4 strains were also exhibited strong anti-yeast activity inhibit *Candida albicans* ATCC10231 with MIC_{G817} = 8 µg/mL, MIC_{G819} = 16 µg/mL, MIC_{G824} = 16 µg/mL, MIC_{OM01} = 16 µg/mL. These 4 strains selected for further research in the next steps.

Identification of the four isolates

Three actinomycetes strains G817, G819, G824 were cultured for 14 days at 30ºC on starch casein agar (SCA) and OM01 was raised on PDA medium for 10 days at 30ºC. The morphology of the four isolates is shown in Figure 1. These features are consistent with the taxonomy of the genera, *Salinispora*, *Pseudonocardia* and the genus *Penicillium*.

A B C D

Figure 1. Colonies of four promising strains G817(A), G819(B), G824(C) and OM01(D).

Four isolates G817, G819, G824, OM01 were identified based on amplification and sequencing of the rRNA gene (16S region for actinomycetes, 18S region for fungi). Gene sequences were analyzed and processed using Bioedit software. The nearly complete 16S rRNA gene sequence of strains G817 (1395 bp), G819 (1405 bp), G824 (1413 bp) and nearly complete 18S rRNA gene sequence of strains OM01 (1160 bp) were determined and compared with corresponding sequences in Genbank database by Blast program.

The result of molecular identification revealed isolates G817, G819 belongs to the *Salinispora arenicola*. Strain G817 has the closest relationship (99.78%) with strain *Salinispora arenicola* CNB-440, accession NR_074502.1. G819 showing the highest levels of similarity with respect to *Salinispora arenicola* strain ATCC BAA-917, accession NR042725 .1 (99.56%). Strain G824 gene sequence exhibited 99.89% identical to strain *Pseudonocardia carboxydivorans* DSM 44104, accession NR119240.1. The comparative sequence analysis revealed that the 18S rRNA sequence of OM01 was highly homologous to *Penicillium malachiteum* CBS 647.95, accession NG_062770.1 (99.3%). The 16S

rRNA and 18S rRNA gene sequences of isolates identified in this study were submitted to GenBank with the following accession numbers: OR884087.1 (*Salinispora arenicola* strain G817), OR880299.1 (*Salinispora arenicola* strain G819), OR883918.1 (*Pseudonocardia carboxydivorans* strain G824) and OR758795.1 (*Penicillium* sp. OM01). The phylogenetic tree was created based on the 16s rRNA gene sequences by MEGAX (Figure 2).

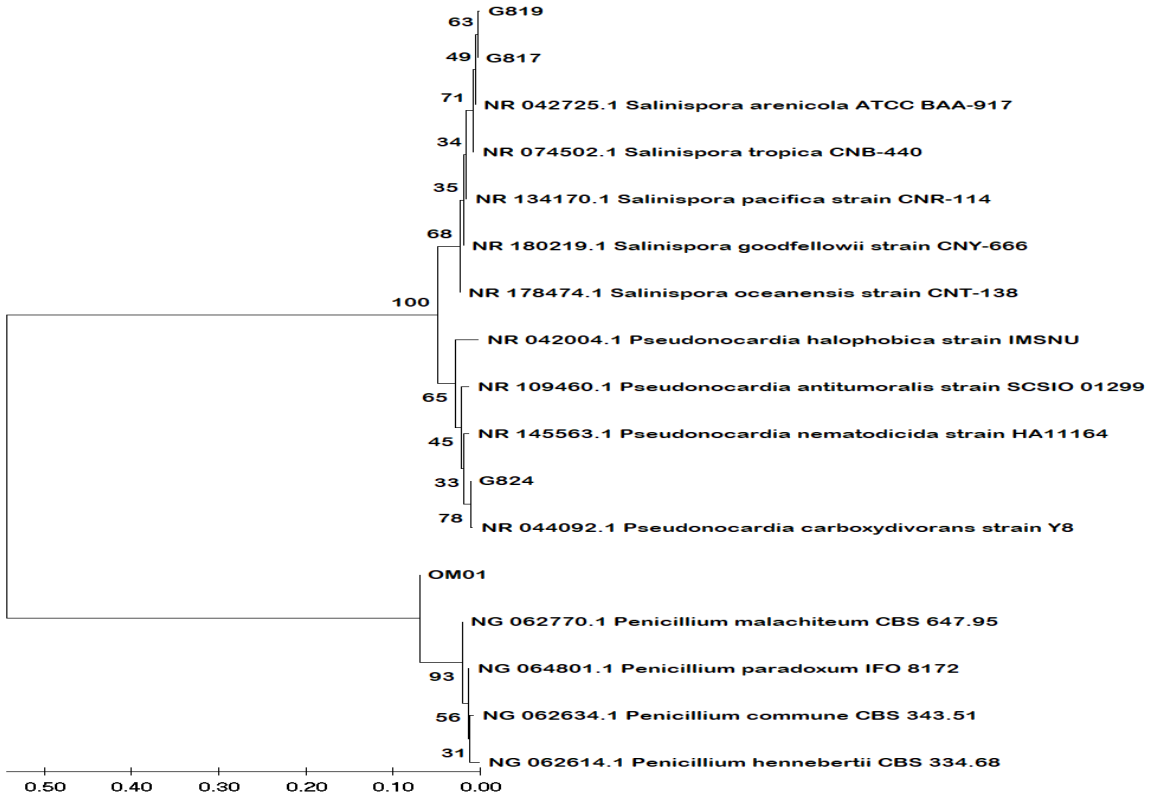

Figure 2. Phylogenetic tree based on 16S rRNA and 18S rRNA gene sequences showing relationships between four studied strains with representative members of genera *Pseudonocardia*, *Salinispora* and genus *Penicillium*.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Nei-Gojobori method and are in the units of the number of synonymous substitutions per synonymous site. This analysis involved 18 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 549 positions in the final dataset.

Evolutionary analyses were conducted in MEGA X (Kumar, 2018).

The exploitation of bioactive compounds from macro-organisms was stunted by conservation issue. Therefore, discovering biologically active secondary compounds in micro-organisms related to macroorganisms-associated is a promising candidate solution. Marine micro-organism resources have the potential as source of natural products with diverse biological functions (Chananan *et al*, 2023).

The genus *Pseudonocardia* has been found to produce some of secondary metabolites with anti-bacterial, anti-fungal and anti-tumor properties. Several antibiotics were produced by some genera in the family *Pseudonocardiaceae* (e.g. rifamycin, erythromycin (Sayed *et al*., 2020) and vancomycin (Yushchuk *et al*., 2020)). A marine derived actinomyces from Avilés submarine Canyon, *Pseudonocardia carboxydivorans* M-227, produced branimycins B and C. These antibiotics showed very good antibacterial activities against a variety of microorganisms that cause dangerous diseases (such as *Corynebacterium urealyticum, Clostridium perfringens, Micrococcus luteus, Neisseria meningitidis, Bacteroides fragilis, Haemophilus influenzae, Escherichia coli*) and inhibit drug-resistant bacteria, methicillin-resistant *Staphylococcus aureus* (Alfredo *et al*., 2017).

Recently, there was a very valuable study by Mexican scientists. A member of the marine *Salinispora arenicola* had the ability to inhibit bacterial pathogens growth of *Staphylococcus epidermidis, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacer baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp. was evaluated by cross-streaking plate and supernatant inhibition tests. Ten supernatants inhibited the growth of eight strains of *Staphylococcus epidermidis* from patients. Also, it inhibited the growth of six multi-drug-resistant bacteria (Luis *et al*., 2020).

Research on secondary compounds from marine microbial sources in Vietnam has only been started and received much attention since more than ten years ago, with more and more published studies. In study of Minh *et al*., the extract of the fungus *Penicillium sp*. M30 from Coto island, Viet Nam possessed 10 compounds. In which, 3-acetyl-4-hydroxycinnoline compound was isolated for the first time from the genus *Penicillium*, this compound inhibited 4/7 strains of test microbial with MIC values from 64 - 256 µg/mL; the other compounds also have a broad spectrum of activity against test strains with low MIC values (Minh *et al*., 2019).

CONCLUSION

From 38 samples of sediments and marine organisms collected in the sea from Bach Long Vy island - Hai Phong to Ly Son island - Quang Ngai, 40 actinomycetes and 20 fungal strains were isolated. In which, 3 actinomycetes belonged to *Salinispora arenicola, Pseudonocardia carboxydivorans* and 1 fungal strain *Penicillium sp.* exhibited the highest antibacterial activity against four pathgenic microorganisms. This study indicates the rich marine actinomycetes and fungi diversity of Vietnam's sea and their good biological activity for biopharmaceutical industries.

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mission of the marine research vessel "Oparin Academik" to survey in Vietnam for the 7th time, in order to detect biologically active compound" Project code: QTRU02.10/ 21-22.

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