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Characterization of Actinomyces Strains Isolated from Mangrove Forests in Vietnam

Nguyen Bao Trang, Pham Hong Quynh Anh,
Keo Phommavong, Nguyen Quang Huy*

Faculty of Biology, VNU University of Science, 334 Nguyen Trai, Hanoi, Vietnam

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Abstract: 61 actinomycete strains were isolated by culture techniques in mangrove forests in Cat Ba, Hai Phong and Xuan Thuy, Nam Dinh. The 31 isolates (50.82%) showed the antibacterial activity with at least one of test microorganisms including *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus*, in which two strains SCA N2.2 and GI H1.3 had strongest antibacterial activity. Two strains growed at optimal temperature at 37ºC. Strain SCA N2.2 could grow in the medium with 3% NaCl concentration while GI H1.3 strains growed in the medium without NaCl.

Based on morphology, color of colony, biological characteristic and 16S rDNA sequence , GI H1.3 strain and SCA N2.2 strain were classified to *Actinomadura* genus and *Streptomyces* genus, and were considered as *Actinomadura glauciflava*_AB1846 and *Streptomyces griseoincarnatus*_AB184207, respectively.

Keywords: Actinomyces, antimicrobial, isolation, mangrove forests, 16S rDNA .

1. Introduction

Nowadays, antibiotic resistant pathogenic microorganisms are increasing continuously. That's not only the inappropriate use of antibiotics in human medicine, but also the overuse of that in agriculture. In the last three decades, even though pharmacological industries have produced a number of new antibiotics, resistance to these drugs of microorganisms has increased [1]. Because of this problem, there is need to discover new drugs against these drug resistant pathogens. Many scientists and pharmaceutical industry have concentrated on the isolation of actinomycetes from different habitats to screen antimicrobial activity served for medicine and agriculture [2, 3].

Mangrove forests are large ecosystems and they make up over a quarter of the total coastline in the world. Due to the presence of rich source of nutrients mangroves are called the homeland of microbes. The mangrove environment is more and more appreciate as an exceptional reservoir of naturally bioactive compounds. These compounds have structure of chemical features not found in naturally terrestrial products [4]. One of microorganism groups in mangrove forests is the

*Corresponding author. Tel.: 84-904263388
Email: nguyenquanghuy@vnu.edu.vn

actinomycetes. The actinomycetes population density is more in terrestrial soils than in marine sediments. In the past, the research work was mainly concentrated on common habitats of actinomycetes. Actinomycetes living in harsh environmental conditions (including extreme high and low temperatures, extremely high or low pH, high salt concentrations etc.) have received relatively little attention from the microbiologists. The mangrove environment is a potent source for the isolation of antibiotic producing actinomycetes [5, 6]. Vietnam has a large mangrove area and is one of 16 countries where have high biodiversity in the world. Thus, we decided to isolate actinomycetes with antimicrobial activity in mangrove forest in Vietnam.

2. Material and Method

2.1. Material

The sludge samples were collected in mangrove areas in Cat Ba, Hai Phong and Xuan Thuy National Park, Nam Dinh.

Tested microorganisms including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923*, Bacillus subtilis* ATCC 23857*, and Bacillus cereus* ATCC 14579 were provided by the VNU-Institute of Microbiology and Biotechnology.

Isolated media: Gause I (GI) containing starch 20g**,** KNO_3 1g**,** $MgSO_4.7H_2O$ 0.5g**,** K_2HPO_4 0.5g, $FeSO_4$ 0.01g**,** NaCl 0.5g**,** agar 20g; and starch casein agar (SCA) including starch 20g, casein 0.3g, KNO_3 2g, $MgSO_4.7H_2O$ 0.05g, K_2HPO_4 2g, $FeSO_4.7H_2O$ 0.01g, NaCl 2g, $CaCO_3$ 0.02g, agar 20g, pH 7.

Antibacterial test medium: Luria Bertani Agar including peptone 15 g, yeast extract 5g, agar 18g and water 1 liter.

2.2. Experimental method

The samples were isolated by the Vinogradski method [6]. For each collected sample, 1g of sample was suspended in 9 ml of water with NaCl (9.0 g/L) then incubated in an shaker incubator at 28 ºC with shaking at 200 rpm for 30 min. The supernatant liquid from the dissolved soil sample was diluted up to 10^{-5} and vortexed at maximum speed. Then, 0.1 ml of each diluted sample from 10^{-1} to 10^{-5} were spread on the Petri plates with SCA and GI media. Next, the Petri plates were incubated at 28 ºC for 4 to 7 days. After that, colonies look like actinomycetes were selected. Then, each isolate was repeated streaking on plates with two medium GI or SCA for purity colonies actinomycetes [6]. In order to prove obtained strains were Actinomycetes, the sporophore and morphology of isolated strains were observed by the cultures coverslip method using light microscope.

Antimicrobial activity of strains was determined using Kirby-Bauer disk diffusion method [7].

The 16S rDNA coding gene was sequenced in VNU-Institute of Microbiology and Biotechnology. The results were compared with the reference species sequences on Database DDBJ/EMBL/GenBank using BLAST Search software. Phylogenetic tree was done by software Clustal X 1.83.

3. Results and discussions

3.1. Isolation of actinomycete strains

The collected samples were enriched, diluted and spread on GI and SCA agar medium plates. After 4 to 7 days of incubation at 30 ^oC, the plates appeared the different colonies including bacteria, fungi and actinomycete colonies.

Based on the morphological characteristics including colony color, surface, mycelium type, pigment production and sporophore, 61 actinomycetes strains were isolated (34 strains were isolated on GI medium, 27 strains were isolated on SCA medium) from mangrove forests in Cat Ba, Hai Phong and Xuan Thuy,

Nam Dinh. The number of actinomycetes strain on SCA medium was lower than that in GI medium because on the SCA medium, the microorganism used organic nitrogen source easily, so they growed rapidly and occupied the habitat of actinomycetes. Some actinomycete colonies appeared in the plates from both Hai Phong and Nam Dinh samples. This indicates that some strains of actinomycetes have widely distributed in nature. Similar finding was reported by Lam et al., that the marine actinomycetes are widely distributed in various marine ecosystems [8].

The collection of isolates was diverse with respect to growth pattern, aerial and hyphae and pigments. Excessive to moderate pigment production was also the isolates. Colony color ismostly color of aerial mycelium. The pigment production of colonies is substrate mycelium for rooting deeply in the environment to absorb nutrient. According to Shirling and Gottlie [9], 61 strains isolated were divided into 7 groups, including brown, green, grey, yellow-orange, purple, red, and white (Table 1).

Table 1. Colony colors of isolated strains

Color	Brown	Green	Grey	Yellow - orange	Purple	Red	White
Number of sample	4	1	11	17	8	5	15
Rate (%)	6.56	1.64	18.03	27.87	13.12	8.19	24.59

According to Table 1, the yellow-orange group was predominating among the isolated strains, at 27.87%. This result was consistent with the research in mangrove in Vietnam before. Notably, most of yellow-orange colonies had antimicrobial activity.

3.2. Screening of actinomycetes strains for antimicrobial activity

In this study, a total of 61 isolated actinomycetes were screened for their antibacterial activity against test pathogen. Among the tested isolates, 31 strains (50.82%) showed the antibacterial activity with at least one of test microorganisms including *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus* (data not show).

One selected strain that resisted all Gram (+) bacteria (GI H1.3) and which had the antibacterial activity with both negative and Gram (+) bacteria (SCA N2.2) were used for next experiments. The morphology of two strains was showed in Figure 1. Both strains could not produce pigment, colony colour was grey with SCA N2.2 and white with GI H1.2.

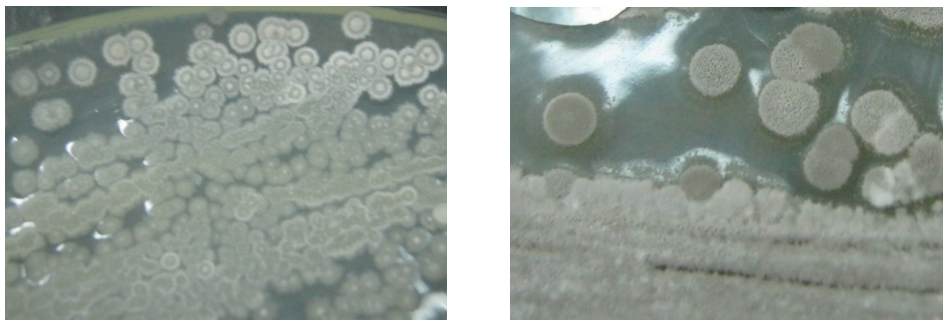

Figure 1. Morphology of GI H1.3 strain (left side) and SCA N2.2 strain (right side) under light microscope (× 40).

3.3. Influence of some environmental factors

3.3.1. Influence of NaCl concentration on antimicrobial activity

The strains were isolated in mangrove areas so NaCl concentration importantly impacts on antimicrobial activity of the selected strains. The optimal NaCl concentration of SCA N2.2 strains was 3%, while GI H1.3 strain growed in media without NaCl and decreased with increasing NaCl concentration (Table 2). This indicates that NaCl concentration had different influence on antimicrobial activity for different strains. Especially in SCA N2.2, the activity against *E.coli* began to appear in high NaCl concentration, which is 2%.

Table 2. Influence of NaCl concentration on antimicrobial activity of two selected strains

NaCl concentration (%)	Antimicrobial activity (D-d, mm)							
	GI H1.3 strain				SCA N2.2 strain			
	B. subtilis ATCC 23857	*S. aureus* ATCC 25923	*B. cereus* ATCC 14579	*E. coli* ATCC 25922	*B. subtilis* ATCC 23857	*S. aureus* ATCC 25923	*B. cereus* ATCC 14579	*E. coli* ATCC 25922
0	27.1 ± 1.2	23.2 ± 1.1	16.0 ± 0.6	0	29.3 ± 1.4	24.5 ± 1.2	0	0
1	15.2 ± 0.6	11.1 ± 0.4	14.8 ± 0.5	0	31.1 ± 1.4	25.2 ± 1.2	0	0
2	14.5 ± 0.7	10.2 ± 0.3	9.4 ± 0.4	0	34.0 ± 1.6	30.2 ± 1.4	0	20.2 ± 1.1
3	0	0	0	0	35.2 ± 1.6	40.1 ± 1.8	0	24.8 ± 1.2
4	0	0	0	0	31	30.5 ± 1.2	0	15.5 ± 0.6

Table 3. Influence of temperature on antimicrobial activity of two selected strains

Temperature (ºC)	Antimicrobial activity (D-d, mm)	
	GI H1.3 strain	SCA N2.2 strain
25	17.2 ± 0.7	22.1 ± 0.3
30	17.0 ± 0.4	22.7 ± 0.5
37	26.6 ± 0.2	26.3 ± 0.4

3.3.2. Influence of temperature on antimicrobial activity

The determination of the temperature effect was carried out with a series of temperature from 25 ºC to 37 ºC. The optimal temperature for antimicrobial activity of the selected strains is 37 ºC (Table 3).

3.4. 16S rDNA coding gene sequencing

Compared with other sequences in Genebank, 16S rRNA gene sequence of GI H1.3 strain was 99,8% homologous (1447/1450bp) with *Actinomadura glauciflava*_AB1846, 99,7% homologous (1446/1450bp) with *Actinomadura glauciflava*_AB18461, 99,2% homologous (1439/1450bp) with *Actinomadura mexicana*_AF277195 and *Actinomadura citrea*_AJ420139. Based on this result, it was confirmed that GI H1.3 strain belongs to the *Actinomadura* genus and is considered as *Actinomadura glauciflava* GI H1.3 (Fig. 2).

Compared with other sequences in gene bank, 16S rDNA gene sequence of SCA N2.2 strain was 100% homologous with *Streptomyces labedae*_AB184704, *Streptomyces griseoincarnatus*_AB184207 as well as *Streptomyces vinaceus*_AB184763, *Streptomyces erythrogriseus*_AB18460 and *Streptomyces variabilis*_DQ442551, 99,7% homologous (1447/1450 bp) with *Streptomyces griseorubens*_AB184139. Based on this result, it was confirmed that SCA N2.2 strain belongs to

the *Streptomyces* genus and is considered as *Streptomyces griseoincarnatus* SCA N2.2 (Fig. 3). *Streptomyces griseourbens* was strain meditaed delignification of paddy straw for improved enzymatic saccharification yields [10].

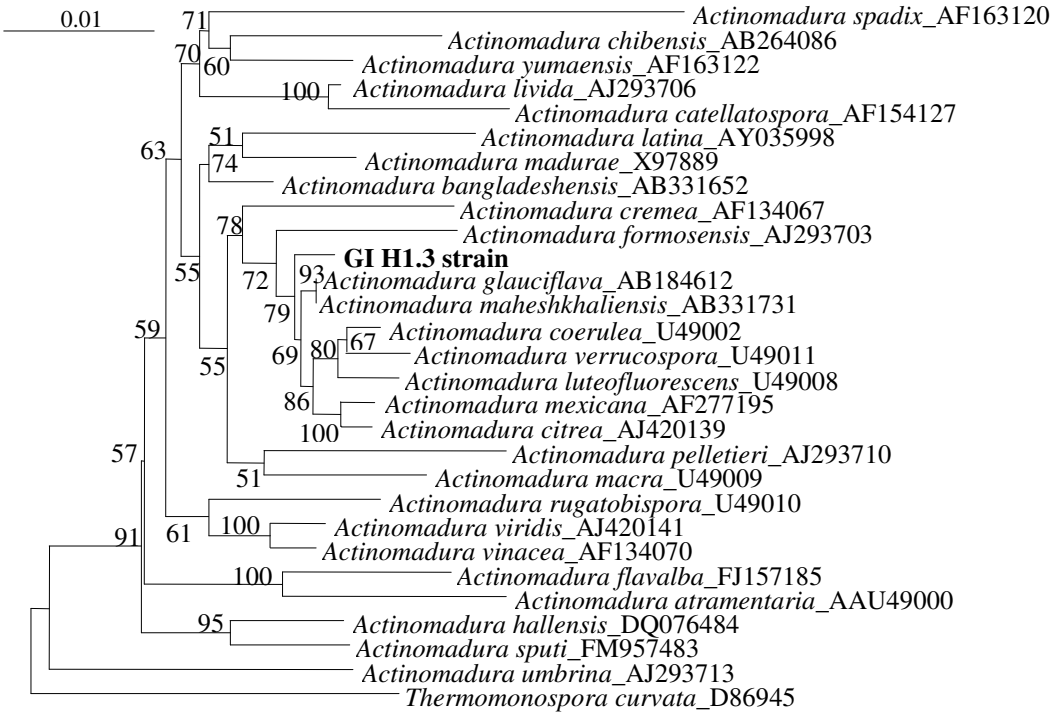

Figure 2. Phylogenetic tree of GI H1.3 strain based on 16S rDNA gene sequences.

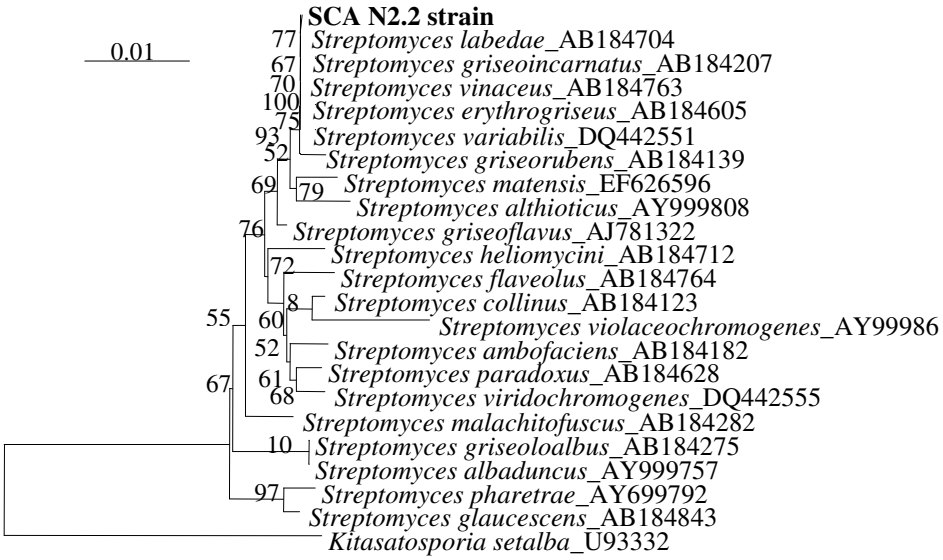

Figure 3. Phylogenetic tree of SCA N2.2 strain based on 16S rDNA gene sequences.

4. Conclusion

61 actinomycete strains were isolated by culture techniques in Cat Ba, Hai Phong and Xuan Thuy, Nam Dinh. Two strains SCA N2.2 and GI H1.3 had strongest antibacterial activity. The optimal condition for SCA N2.2 strains was medium containing 3% NaCl at 37 ºC. On the other hand, the optimal conditions for GI H1.3 was medium without NaCl at 37 ºC.

Based on morphology, color of colony, biological characteristic and 16S rDNA sequence , GI H1.3 and SCA N2.2 strains were poven to belongs to the *Actinomadura* genus and *Streptomyces* genus, and were considered belong to *Actinomadura glauciflava* and *Streptomyces griseoincarnatus*, respectively.

References

[1] Cohen ML, Epidemiology of drug resistance: implications for a post-antimicrobial era, Science 257 (1992) 1050.

[2] Narendra K, Ravi KS, Mishra SK, Singh AK, Pachouri UC, Isolation and screening of soil *Actinomycetes* as source of antibiotics active against bacteria, Inter. J. Microbiol Res 2 (2010) 12.

[3] Subramaniam G, Srinivas V, Prakash B, Arumugam S, Rajendran V, Rupela O, Himabindu K, Krishnamohan K, Rajeev KV, Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion straits in rice. Microbiol. Res 169 (2014) 40.

[4] Carter BK, Biomedical potential of marine natural products, Bioscience 46 (1996) 271.

[5] Sahoo K and Dhal NK, Potential microbial diversity in mangrove ecosystems, Indian J. Marine Scien 38 (2009) 249.

[6] Hayakawa M, Momose Y, Kajiura T, Younazaki T, Tamura T, Hatano K, A selective isolation method for Actinomadura viridis in soils, J.Ferment. Bioeng 79 (1995) 287.

[7] Elie KB, Mohammed AS, Vatch KS, Adele NH, Rabih ST, Salma NT, Screening of selected indigenous plants of Lebanon for antimicrobial activity, J. Ethnopharmacol 93 (2007) 1.

[8] Lam KS, Discovery of novel metabolites from marine actinomycetes, Curr. Opinion. Microbiol 9 (2006) 245.

[9] Shirling EB and Gottlieb D, Methods for characterization of *Streptomyces* species, Int J. Syst. Bacteriol 16 (1966) 313.

[10] Saritha M, Anju A, Surender S, Lata N, *Streptomyces griseourbens* mediated delignification of paddy straw for improved enzymatic saccharification yields, Bioresource Technology 135 (2013) 12.

Đặc điểm sinh học của chủng xạ khuẩn phân lập tại vùng nước ngập mặn tại Việt Nam

Nguyễn Bảo Trang, Phạm Hồng Quỳnh Anh,
Keo Phommavong, Nguyễn Quang Huy

Khoa Sinh học, Trường Đại học Khoa học Tự nhiên, ĐHQGHN, 334 Nguyễn Trãi, Hà Nội, Việt Nam

Tóm tắt: Từ các mẫu đất thu thập tại khu bảo tồn rừng ngập mặn ở Cát Bà, Hải Phòng và Xuân Thủy, Nam Định chúng tôi đã phân lập được 61 chủng xạ khuẩn khác nhau. Trong số các chủng xạ khuẩn phân lập 31 chủng được đánh giá có khả năng kháng lại ít nhất 1 trong 4 chủng kiểm định gồm *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* và hai chủng SCA N2.2 và GI H1.3 có hoạt tính mạnh nhất. Hai chủng này phát triển tối ưu ở nhiệt độ 37^{o}C; trong khi chủng

SCA N2.2 phát triển tối ưu ở môi trường với nồng độ NaCl là 3% thì chủng GI H1.3 lại phát triển tối ưu khi không có muối NaCl.

Dựa vào đặc điểm hình thái, màu sắc khuẩn lạc chủng GI H1.3 được xếp vào chi *Actinomadura* và chủng SCA N2.2 thuộc chi *Streptomyces.* Kết quả giải trình tự 16S rDNA cho thấy chủng GI H1.thuộc về loài *Actinomadura glauciflava* và *Streptomyces griseoincarnatus*, một cách tương ứng với mức độ tương đồng trên 99%.

Từ khóa: Xạ khuẩn, nước ngập mặn, phân lập, kháng khuẩn, 16S rDNA.