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Research article

Antibacterial activity of *Streptomyces* spp. against *Aeromonas hydrophila* causing hemorrhagic disease in intensively cultured striped catfish

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Abstract

Antibiotic-resistant bacteria are becoming more common due to the overuse of antibiotics in aquaculture. Alternative antibiotic solutions are fascinating and under the consideration in many countries. This study's objective was to isolate and identify certain actinomycete isolates with inhibitory action against the hemorrhagic disease-causing *Aeromonas hydrophila* in intensively cultivated catfish. This investigation isolated ten actinomycete isolates from sediment and water samples of catfish ponds in Vinh Long province. Using the diffusion well method, the study identified all isolates with antogonisttic activity against *A. hydrophila*. In particular, two isolates, BCA1.2 and BCA1.5, presented the highest inhibitory activity with inhibitory diameter zones of 11±1.0 mm and 10±1.0 mm, respectively. Two actinomycete isolates, BCA1.2 and BCA1.5, had their growth affected by pH, temperature, and NaCl. The findings revealed that two of these isolates showed optimum growth in a pH range of 7.0–8.0 and at 37°C. Two isolates, BCA1.2 and BCA1.5, survived well on medium containing a high salt concentration of NaCl ranging from 3% to 7% and were able to utilize different sources of carbon. They produced a wide range of hydrolytic extracellular enzymes, such as cellulase, amylase, protease, lipase, and chitinase. Isolates BCA1.2 and BCA1.5 were identified as *Streptomyces* based on morphological, cultural, physiological, and biochemical characteristics and 16S rRNA gene sequencing.

Keywords: *Aeromonas hydrophila,* Hemorrhagic disease, Striped catfish, Antibacterial activity, Actinomycetes.

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INTRODUCTION

Bacterial species belonging to the *Aeromonas* spp., such as *Aeromonas veronii*, *A. sobria*, *A. bestiarum*, *A. caviae*, *A. salmonicida*, and *A. dhakensis*, are considered one of the most serious fish pathogens for aquaculture in many countries (Majtán et al., 2012; Turska-Szewczuk et al., 2013; Alghabshi et al., 2018; Soto-Rodriguez et al., 2018; Li et al., 2020; Xue et al., 2022). Among them, *A. hydrophila* has been recognized as the most noxious for aquatic animals (Marinho-Neto et al., 2019). *A. hydrophila* infection is responsible for tremendous economic loss of a variety of cultured fish in the United States (Hossain et al., 2014), as well as in Asia (Mazumder et al., 2021). In Vietnam, several fish species, including tilapia, channel catfish (*Ictalurus punctatus*), and carp (common carp and grass carp), which are the principal species raised in Northern Vietnam, have been reported to be affected by *A. hydrophila* (Nhinh et al., 2021).

Striped catfish (*Pangasianodon hypophthalmus*) is one of the species of freshwater catfish with high economic value that is popularly farmed in Vinh Long province of the Mekong Delta, Vietnam (Phan et al., 2009). However, in recent years, the intensive farming of striped catfish at high densities and the management of the pond environment have not been strictly controlled, so disease problems occur more often and cause damage to farmers (Le and Cheong, 2010). In striped catfish, previous studies revealed that hemorrhagic diseases caused by *A. hydrophila* (Crumlish et al., 2010) cause great damage to fish farming. A recent study by Hoa et al. (2021) reported that *A. hydrophila* was found in the Mekong Delta in 11% of larvae, 30% of fry, and 30% of seemingly healthy striped catfish.

Currently, there is no commercial vaccine to prevent hemorrhagic disease caused by an *A. hydrophila* infection in the striped catfish in Vietnam. Therefore, to prevent and control disease during culture, fish farmers often use a variety of antibiotics during treatment (Phu et al., 2015). *Aeromonas* has become more resistant to antibiotics as a result of drug overuse, and the presence of antibiotic residues in aquatic products poses a concern for human health (Cabello et al., 2013; Okocha et al., 2018). To decrease the amount of antimicrobial residues in aquaculture and, subsequently, the consequences on food safety, it is advised to utilize antibiotic alternatives such as probiotics (Hoseinifar et al., 2018), phage therapy (Liu et al., 2022), and essential oils (Souza et al., 2019). Until now, several studies have demonstrated that the use of probiotics in aquaculture is safe for the host and effective against infectious illnesses (Kaktcham et al., 2017).

Streptomyces species are Gram-positive, aerobic, filamentous bacteria and belong to the *Streptomycetaceae* family (Dehnad et al., 2010). *Streptomyces* can grow in different environments, such as soil (Hamid et al., 2020), sediment (Chakraborty et al., 2015), and marine (Tenebro et al., 2021). By far, *Streptomyces*' ability to produce bioactive secondary metabolites, such as antibiotics and other antifungals, antivirals, antitumorals, antihypertensives, and immunosuppressants, is its most desirable characteristic (de Lima Procópio et al., 2012). Around two-thirds of all naturally occurring antibiotics are produced by the genus *Streptomyces* (Mohanraj and Sekar, 2013). The genus is capable of producing around 150,000 more beneficial chemicals than all *Streptomyces* secondary metabolites discovered to date, according to the review by Lacey

and Rutledge (2022). Many antibiotics, for instance, neomycin (Adinarayana et al., 2004), chloramphenicol (Sekurova et al., 2016), streptomycin (Ohnishi et al., 2008), cypemycin (Claesen and Bibb, 2010), grisemycin (Claesen and Bibb, 2011), bottromycin (Vior et al., 2020), and chloramphenicol (Sekurova et al., 2016) are all derived from the genus *Streptomyces*.

Streptomyces has been the subject of various studies that have looked for novel bioactive substances or novel activities (Quinn et al., 2020). The *Streptomyces* species with antibacterial action against bacterial pathogens in striped catfish aquaculture, however, have not been the subject of recent studies. *Streptomyces* species have been found to be promising sources of probiotics that can be used in aquaculture (Hu et al., 2021). Until now, there have been some studies using beneficial bacteria, such as lactic acid bacteria (Turnip et al., 2018) or *Bacillus* (Ran et al., 2012; Romanova et al., 2020), capable of antimicrobial activity against pathogenic bacteria in catfish. However, there are no studies on *Streptomyces* with inhibitory activity against pathogenic bacteria on Pangasius in the Mekong Delta, Vietnam. Therefore, the investigation was accomplished to isolate and select some indigenous *Streptomyces* with antibacterial activity against *A. hydrophila*, which causes hemorrhagic disease in intensively cultured striped catfish in Vinh Long province.

MATERIALS AND METHODS

Sediment and water sample collection

Sediment and water samples used for isolating *Streptomyces* were collected in striped catfish farming ponds of different localities in Long Ho, Tra On, and Mang Thit districts, Vinh Long province (Figure 1). Sludge samples were collected at five sites in the pond, then mixed together to ensure uniformity, while water samples were collected in areas where fish are normally fed (Qin *et al.*, 2016).

Figure 1 Sampling locations for actinomyces isolation (●)

Isolation of *Streptomyces*

Streptomyces were isolated on Gause I medium (soluble starch 20 g/l, K_2HPO_4 0.5 g/l, $MgSO_4.7H_2O$ 0.5 g/l, NaCl 0.5 g/l, KNO_3 0.5 g/l, $FeSO_4$ 0.01 g/l, agar 20 g/l, pH = 7.0 to 7.4) (Hu et al., 2021). This work was fulfilled according to Wang et al. (2012) with some modifications. In brief, 90 ml of sterile distilled water was combined with 10 g of sludge or 10 ml of water, and the mixture was shaken at 150 rpm for 24 hours. Then, the sample was serially diluted up to 10^6. The sample from each dilution (100 µl) was spread onto the surface of the Gause I. For 5–7 days, the dishes were incubated at 30°C. Then, presumptive *Streptomyces* colonies (typically round, small, opaque, and compact) and frequently pigmented (gray, brown, blue, white, pink, or other colors) were selected and subcultured many times until the colonies were pure.

Antimicrobial activity of *Streptomyces* isolates against *A. hydrophila*

Antibacterial activity against *A. hydrophila* was tested by the agar-well diffusion method of Abussaud et al. (2013) with some modifications. The experimental steps are as follows:

Preparation of A. hydrophila: a test tube holding 5 ml of sterile 0.85% NaCl physiological saline solution was filled with a loopful of bacterial colonies. Next, the bacterial suspension was spread on MHA (Mueller-Hinton Agar, Merck, Germany). The bacterial density used in the study was 10^8 CFU/ml, determined by comparison with Macfarland standard tubes. Finally, a 6-mm col tip was used for making wells on the agar surface.

Preparation of Streptomyces: *Streptomyces* isolates were grown in liquid Gause I medium and were shaken at 150 rpm for 4 days. The supernatant was collected after the culture had been centrifuged at 8,000 rpm for 5 minutes at 4°C. Each well was filled with the supernatant (80 µl), and the plates were then incubated at 30°C for 24 hours. The inhibition zones around the wells were observed and compared to those of the control plate (without *Streptomyces*). The antagonistic activity of each actinomycete was performed in triplicate.

Morphological and cultural characteristics

Two isolates, BCA1.2 and BCA1.5, displayed the highest inhibitory activity against *A. hydrophila* and were chosen for testing morphological, cultural, and biochemical characteristics. The International *Streptomyces* Project (ISP) description was used to identify the size, shape, margin, color of the substrate and aerial mycelium, and diffusible pigments of *Streptomyces* (Shirling and Gottlieb, 1966). The spore chain's morphology was studied under a light microscope. The surface morphology of the spores was examined using a scanning electron microscope (SEM).

Physiological and biochemical characteristics

The impact of pH, temperature, and NaCl on the growth of the two chosen actinomycetes isolates (BCA1.2 and BCA1.5) was tested. Different incubation temperatures (15, 25, 37, and 45°C) and pH values (4.0, 5.0, 6.0, 7.0, 8.0, and 9.0) were examined using ISP-2 media. Salinity tolerance tests were accomplished with ISP-2 supplemented with 1, 3, 5, 7, and 10% of NaCl. Actinomycetes isolates were then streaked across the plates, which were subsequently incubated at 30°C for 5-7 days. Daily growth measurements were taken of the aerial and substrate mycelia.

The actinomyces isolates were examined for oxidase, catalase, urease, gelatin liquefaction, starch hydrolysis, nitrate reduction, H_2S production, methyl red, and Voges-Proskaeur (Waksman, 1961; Prescott et al., 1991; Collins et al., 1995).

Utilization of carbon sources consisting of glucose, fructose, sucrose, mannitol, rhamnose, xylose, raffinose, inositol, and arabinose were tested on carbon utilization agar (ISP-9) supplemented with 1% carbon sources. Growth was measured seven days after the plates were incubated at 28 °C using glucose as a positive control and carbon source-free media as a negative control (Nonomura, 1974).

Production of extracellular hydrolytic enzymes

Two isolates, BCA1.2 and BCA1.5, displayed the highest inhibitory activity against *A. hydrophila* and were selected for the presence of different enzymes like cellulase, amylases, proteases, lipases, and chitinases.

Cellulase production: The cellulase enzyme production of actinomyces was determined according to Kasana et al.(2008). On a basal salt agar medium that contained 0.5% carboxymethyl cellulose, the actinomycetes isolates were inoculated (CMC). After incubation at 28°C for 7 days, the plates were then flooded with Gram's iodine. The appearance of a clean zone denotes that the isolated organisms produced cellulase successfully. In every experiment, plates without the CMC (non-substrate) were used as controls.

Amylase activity: In the case of amylase production, actinobacterial isolates were cultivated on starch-agar media (starch 20 g/l, peptone 5 g/l, beef extract 3 g/l, and NaCl 3%) and incubated for 7 days at 30°C. The appearance of a clear zone around the colonies after the plates were flooded with iodine solution, indicating the presence of amylase-positive isolates (Elmansy et al., 2018).

Protease production: Protease activity was tested according to Deepthi et al. (2012) with some modifications. The skim milk agar medium (peptone 5 g/l, NaCl 3%, and skim milk powder 10 g/l, pH = 7.0) was streaked with an actinobacterial isolate. A clear zone formed around the colonies, and the isolate was shown to have protease activity.

Lipase production: Lipolytic activity was examined according to Ramnath et al. (2017) with some modifications. In brief, the actinomyces isolate was inoculated in Tween 20 agar (peptone 10.0 g/l, NaCl 5.0 g/l, $CaCl_2.2H_2O$ 0.1 g/l). Following a 20-minute autoclave, 10 ml of Tween-20 were added to the medium, and the pH was then brought down to 7.0. After 7 days of incubation at 30ºC, lipase activity was found looking for a whitish halo caused by the growth of calcium crystals.

Chitinase activity: The chitinolytic property of the actinomycetes was determined according to Nagpure et al. (2014). The isolates were spot-inoculated in the middle of the agar medium (K_2HPO_4 0.7 g/l, KH_2PO_4 0.3 g/l, $MgSO_4$ 0.5 g/l, $FeSO_4$ 0.01 g/l, $ZnSO_4$ 0.001 g/l, $MnSO_4$ 0.001 g/l, $(NH_4)_2SO_4$ 0.25 g/l, yeast extract 1.0%, and 1.0% colloidal chitin). The plates were then flooded with an aqueous solution containing 0.1% Congo red for 40 minutes after 7 days of incubation at 28°C. The chitinase enzyme was identified when clearance zones appeared around the colony.

Molecular identification of *Streptomyces* isolates

Streptomyces were identified by PCR with primers 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3' (Heuer *et al.*, 1997) for amplifying 16S rRNA gene fragments. The PCR reaction mixture consisted of sterilized water, 1X PCR buffer, $MgCl_2$ (25 mM), 0.1 mM of each dNTPs, forward and reverse primers (10 µM each), *Taq* DNA polymerase (2.5 unit/µl), and template DNA. PCR products (1,500 bp) were sequenced at Macrogen Company, Korea (www.macrogen.com). The phylogenetic tree was constructed using MEGA X (Molecular Evolutionary Gentics Analysis) software based on the neighbor-joining algorithm (Saitou and Nei, 1987) with a bootstrap value of 1,000 replications (Tamura, 2013).

Data analysis

The difference in antagonistic activity of the treatments was analyzed by one-way ANOVA with the Turkey test using Minnitab.20 software at a 5% significance level.

RESULTS

Actinomycete isolation

The study isolated ten actinomyces-like isolates (designated BCA1.2, BCA1.5, BCA1.6, BCA2.3, BCA2.4, BCA2.5, BCA2.6, BTAB4, BTAB5, NCA2.1) on Gause I medium from four samples of water and five of sludge in intensive catfish culture ponds in Long Ho, Tra On, and Mang Thit districts, Vinh Long province. The origins of actinomycete isolates are detailed in Table 1.

Table 1 Actinomyce isolates from sediment and water samples of different striped catfish ponds in Vinh Long province

Source isolation	Number of samples	Number of isolates	Isolate's name	Place of isolation
Sediment pond	2	4	BCA1.2, BCA1.5 BCA1.6, and BCA2.3	Long Ho District
Water pond	2	1	NCA2.1	
Sediment pond	2	2	BCA2.4 and BCA2.5	Mang Thit District
Water pond	2	0		
Sediment pond	2	3	BCA2.6, BTAB4, and BTAB5	Tra On District
Water pond	2	0		
Total	**12**	**10**		

Morphological characteristics of actinomycete isolates on Gause I medium are summarized in Table 2. This investigation indicated that the actinomycete isolates grow slowly. The colonies appeared about 5–7 days after incubation on Gause I medium. The colors of actinomycete colonies are diverse, such as creamy (Figure 2a), gray (Figure 2b), white (Figure 2c), purple (Figure 2d), greenish-white (Figure 2e), and green (Figure 2f). All isolates have a round shape and an irregular margin with radioactive circles. They are capable of diffusing pigment on the Gause I medium (Figure 2c).

Table 2 Morphological characteristics of actinomyce isolates on Gause I medium

Isolate	Aerial mycellium	Substrate mycellium	Soluble pigment	Melanoid pigment
BCA1.2	White	Pale yellow	Pale yellow	None
BCA1.5	Gray	White	None	None
BCA1.6	White	White	None	None
BCA2.3	Gray white	White	None	None
BCA2.4	White	Dark yellow	Dark yellow	None
BCA2.5	White	White	None	None
BCA2.6	Purple white	Purple	None	None
BTAB4	Brown	Brown	None	None
BTAB5	Blue white	Green	None	None
NCA2.1	Green	Dark blue	None	None

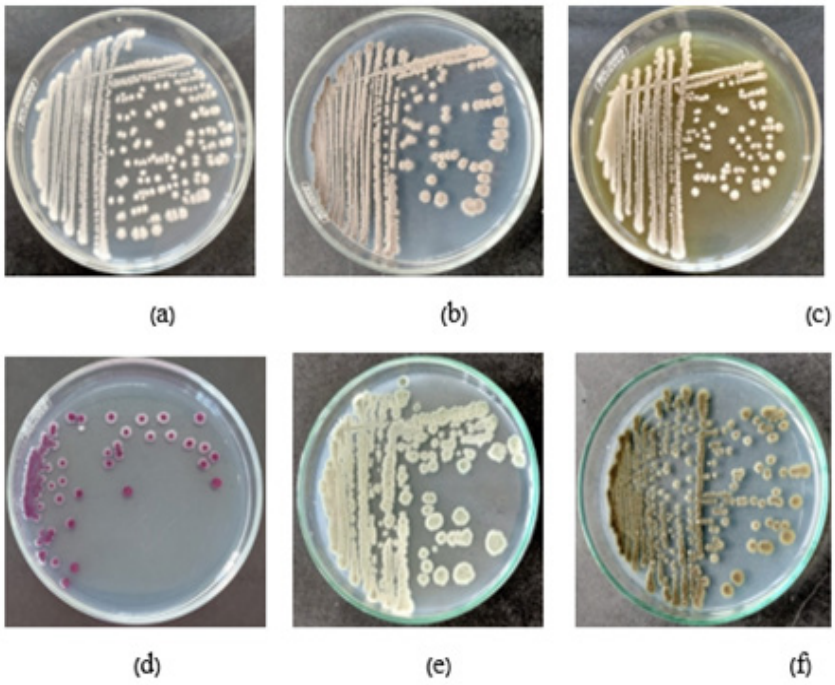

Figure 2 Colony color of isolated actinomycetes isolates on the Gause I medium a) Creamy (isolate BCA1.6), (b) Grayish-white (isolate BCA2.3), (c) White (isolate BCA1.2), (d) Purple-white (isolate BCA2.6), (e) Greenish-white (isolate BTAB5), (f) Green (isolate NCA2.1)

Antimicrobial activity of *Streptomyces* against *A. hydrophila*

The results revealed that all actinomycete isolates had antagonistic activity against *A. hydrophila* (Figure 3). Among them, 2/10 (20%) isolates exhibited strong inhibitory activity, 4/10 (40%) isolates showed moderate inhibitory ability, and 4/10 (40%) isolates showed weak inhibitory activity. The results in Figure 3 showed that isolate BCA1.2 has the strongest antagonistic activity with an inhibition diameter zone of 11±1.0 mm, while isolates BCA2.4 and BCA2.6 have the lowest prohibitory activity with an inhibition diameter zone of 3±0.0 mm

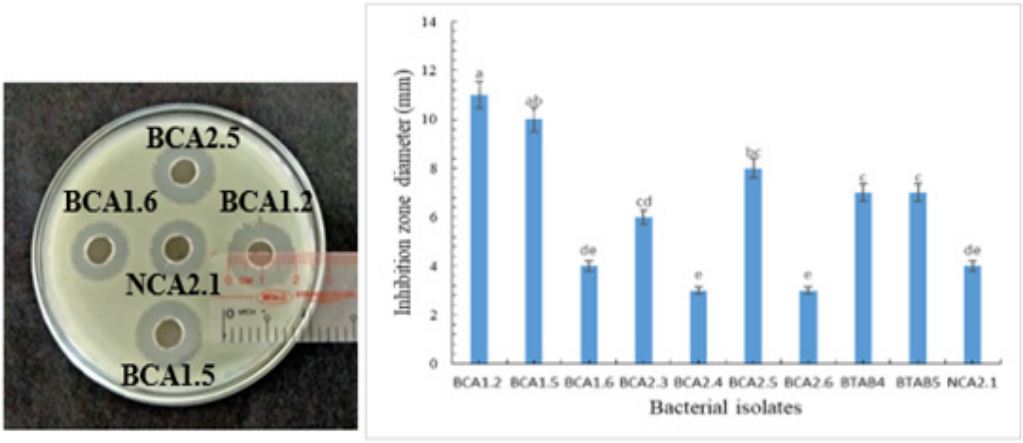

Figure 3 Antibacterial activity of actinomycete isolates against *A. hydrophila*. Means followed by the same letter are not significantly different

Morphological characteristics

The morphological features of two isolates, BCA1.2 and BCA1.5, were presented in Table 3. The findings showed that two isolates, BCA1.2 and BCA1.5, were rough in appearance on the ISP-2 medium. SEM analysis of the morphology of the spore chains and the surface of the spores revealed that two isolates, BCA1.2 and BCA1.5, had spore chains with a short-rod form and a smooth surface **(Table 3)**.

Table 3 Morphological characteristics of two isolates, BCA1.2 and BCA1.5, on ISP-2 medium

Characteristics	Isolate BCA1.2	Isolate BCA1.5
Colony diameter (mm)	2-4	1-3
Colony margin	Filamentous, radical circle	Filamentous
Colony elevation	Convex	Flat
Spore surface	Smooth	Smooth
Spore chain morphology	Long spore chain	Long spore chain

Cultural characteristics

Cultural characteristics of two isolates, BCA1.2 and BCA1.5, were detailed in Table 4. The results demonstrated that both iolates could grow on all the ISP media. The isolate BCA1.2 can grow well on all media but shows weak growth on IS-7 medium. Meanwhile, isolate BCA1.5 can grow well on all media but shows poor growth on IS-4 medium.

Table 4 Cultural characteristics of two isolates, BCA1.2 and BCA1.5, on ISP-2 medium

Characteristics	Growth		A. mycelium color		S. mycelium color		Soluble pigment	
	BCA1.2	BCA1.5	BCA1.2	BCA1.5	BCA1.2	BCA1.5	BCA1.2	BCA1.5
ISP-2	++	++	Brown	Brown	White	Brown	None	None
ISP-3	++	++	Grey	Grey	Grey	Grey	None	None
ISP-4	++	+	White	White	White	White	None	None
ISP-5	++	++	Pink	Pink	Pink	Pink	None	None
ISP-6	++	++	Blue	Blue	Blue	Blue	None	None
ISP-7	+	++	Pale yellow	Pale yellow	Pale yellow	Pale yellow	None	None

+: weak growth, ++: moderate growth, +++: strong growth, A. mycelium color: aerial mycelium color, S. mycelium color: substrate mycelium color

Physiological and biochemical characteristics

The findings showed that isolates BCA1.2 and BCA1.5 developed the best at 37°C, with a pH in the range of 7.0–8.0, and had the ability to survive concentrations of NaCl up to 7.0% (Table 5). Physiological characteristics of two isolates, BCA1.2 and BCA1.5, are summarized in Table 5.

Table 5 Physiological characteristics of two isolates, BCA1.2 and BCA1.5, on ISP-9 medium

Isolate	Isolate BCA1.2	Isolate BCA1.5
Growth at different pH values:		
pH 4.0	-	+
pH 5.0	+	+
pH 6.0	++	++
pH 7.0	+++	+++
pH 8.0	++	+++
pH 9.0	++	++
pH 10.0	+	+
Growth at different temperatures:		
15°C	+	+
25°C	++	++
37°C	+++	+++
42°C	+	++
Growth at different NaCl concentrations:		
1% NaCl	+	+
3% NaCl	+++	++
5% NaCl	+++	+++
7% NaCl	+++	++
10% NaCl	+	-

-: no growth; +: weak growth; ++: moderate growth; +++: strong growth

This investigation showed that isolate BCA1.2 could metabolize glucose, fructose, sucrose, mannitol, raffinose, inositol, and arabinose. Among them, glucose, fructose, sucrose, and arabinose presented the highest efficiencies. However, isolated BCA1.2 could not use xylose as its carbon source. Meanwhile, isolate BCA1.5 could use glucose, fructose, sucrose, rhamnose, xylose, raffinose, and arabinose. Among them, glucose and xylose showed the highest efficiency. However, isolated BCA1.5 could not use mannitol or inositol as carbon sources. Besides, the results of biochemical characterization showed that two isolates, BCA1.2 and BCA1.5, were positive for Gram staining, oxidase and catalase reactions, gelatin liquefaction, and starch hydrolysis. However, two isolates, BCA1.2 and BCA1.5, were negative for urease, nitrate reduction, methyl red, Voges-Proskaeur, and H_2S production. The biochemical characteristics of the two isolates, BCA1.2 and BCA1.5, are presented in Table 6.

Table 6 Biochemical characteristics of two isolates, BCA1.2 and BCA1.5, on ISP-9 medium

Isolate		Isolate BCA1.2	Isolate BCA1.5
Gram staining		+	+
Oxidase		+	+
Catalase		+	+
Urease		-	-
Gelatin liquefaction		+	+
Starch hydrolysis		+	+
Nitrate reduction		-	-
Methyl red		-	-
Voges-Proskaeur		-	-
H_2S production		-	-
Growth on carbon source:			
	Glucose	+++	+++
	Fructose	++	+
	Sucrose	+++	+
	Mannitol	+	-
	Rhamnose	+	+
	Xylose	-	++
	Raffinose	+	+
	Inositol	+	-
	Arabinose	++	+

-: no growth; +: weak growth; ++: moderate growth; +++: strong growth

Production of extracellular hydrolytic enzymes

The hydrolytic activity of extracellular enzymes produced by two isolates, BCA1.2 and BCA1.5, is presented in Table 7. In general, two isolates, BCA1.2 and BCA1.5, could produce cellulase, amylase, protease, lipase, and chitinase enzymes. However, isolates of BCA1.2 didn't produce the lipase enzyme.

Table 7 Extracellular hydrolytic enzymes in two isolates, BCA1.2 and BCA1.5

Hydrolytic enzyme	Isolate BCA1.2	Isolates BCA1.5
Cellulase	+	+
Amylase	+	+
Protease	+	+
Lipase	-	+
Chitinase	+	-

-: negative, +: positive

The PCR results showed that all tested actinomycetes were amplified 16S rRNA gene fragments with a size of 1,500 bp (Figure 4).

The BLAST results demonstrated that representative isolate BCA1.2 had a 99.17% similarity to *Streptomyces omiyaensis* strain 13647R (EU741148.1), a 99.06% homology to *Streptomyces* sp. strain HBUM206460 (MT542135), and a 99.06% homology to *Streptomyces fradiae* strain YY69 (MH265962) in the NCBI database. While isolate BCA1.5 showed a 99.21% similarity to *Streptomyces* sp. strain LT3–17 (MT353651.1), *Streptomyces parvulus* strain 2A11 (MN901087), and *Streptomyces tendae* strain AJ2R (KY072953) in the NCBI database. The phylogenetic tree revealed that two isolates were distributed into two distinct groups (Figure 5), in which isolate BCA1.2 and isolate BCA1.5.1 belong to the same cluster.

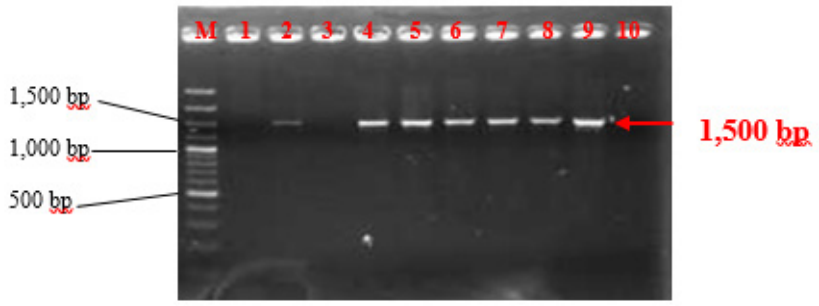

Figure 4 Results of amplification of 16S rRNA gene segments of actinomycete isolates by PCR
M: standard marker 100 bp plus; Lane 1: negative control; Lanes 2–10: isolates NCA2.1, BCA1.6, BCA1.5, BCA1.2, BTAB4, BTAB5l, BCA2.6, BCA2.5, and BCA2.4, respectively.

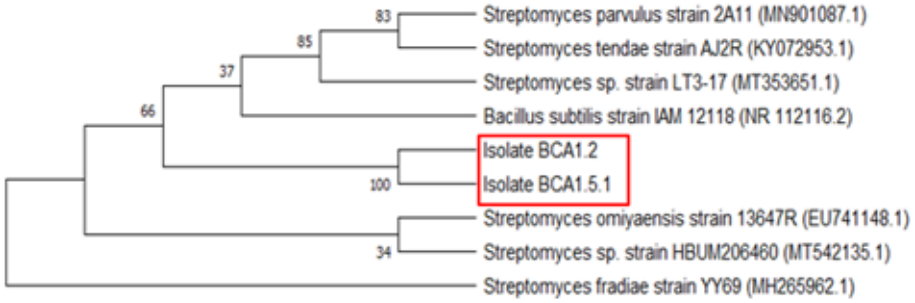

Figure 5 Phylogenetic tree showing the genetic relationship between actinomycete isolates (the numbers at branching points are bootstrap values).

DISCUSSION

According to the findings, 9 out of 10 (90%) actinomycete isolates originated from sediment, and only one (10%) actinomycete isolate was derived from water samples in striped catfish ponds (Table 1). Numerous previous studies were conducted to isolate and characterize the antimicrobial and antifungal activity of actinomyces strains originating from different soils in Vietnam (Thao et al., 2016; Que et al., 2019; Hue et al., 2020; Tram et al., 2021). However, this is the first time that actinomyces isolates have been obtained from the sediments and water of striped catfish ponds in Vinh Long province. The abundant occurrence is due to a fish farming pond that was rich in organic chemicals, which might be the reason for the highest count of *Streptomyces* sp. (Hossain et al., 2014). The ratios of organic matter, total nitrogen, and total phosphorus in fish pond sludge were shown by Phu and Tinh (2012) to be quite high. This result is in agreement with the previous research that indicated that actinomycetes are commonly present in natural environments, but most of them exist in soil (Aouar et al., 2020; Assou et al., 2023). Five actinomycete strains were discovered in the investigation of Meliani et al. (2022) from a sample of the Cheliff River estuary in Mostaganem (Northwest Algeria).

However, no strains of actinomycetes were collected from water samples. The other findings by Deepthi et al. (2012) revealed that twenty-seven isolates were obtained from mangrove soil samples in India. In Vietnam, research results by Hoa et al. (2020) also isolated and screened 61 actinomycetes with antibacterial activity from the marine environment from 80 samples of marine organisms and marine sediments collected from Ly Son islands, Quang Ngai. Also, research results by Hoa et al. (2021) isolated 20 actinomycetes from 15 soil samples in vegetable gardens in Lam Dong province. Recently, research by Anh et al. (2021) isolated 89 strains of actinomycetes with antibiotic activity from cultivated soil in Quoc Oai district (Hanoi). Similarly, Tram et al. (2021) also isolated 18 actinomycetes from orange soil in Ha Giang province.

According to Stanley et al.(1989), colony morphology is one of the first criteria used to classify actinomycetes. This study pointed out that the colony colors of ten actinomycete isolates were diverse (Tables 2 and 3), as previously reported (Canh et al., 2015; Lan et al., 2015; Phuoc et al., 2020). Similarly, previous research reported that different colors and sizes of actinobacteria were isolated (Vijayakumar et al., 2011). According to Nabila and Kannabiran et al. (2018), the colonies of the actinomycete isolates varied in size and colony form, including white, gray, blue, pale orange, and violet. It was also noted that the substrate mycelium was brown, black, and yellow. Another study by Hossain and Rahman (2014) found that *Streptomyces* sp. was found in soil samples in Bangladesh that were off-white, brown, dark brown, yellow, and orange. In Vietnam, Hien et al.(2014) divided 43 actinomycete strains, isolated from eight different soil samples of cultivated land, into seven color groups with different rates, including white (37.2%), gray (22.9%), brown (14.0%), yellow (11.6%), pink (2.3%), purple (2.3%), and blue (4.7%). Based on the color chart of Tresner and Buckus (1963), the recent results of Hanh *et al.* (2021) also showed that the aerial mycelium of actinomycete strains isolated from soil samples in two provinces, Ben Tre and Long An, was classified into five different groups, consisting of gray, red, yellow, green, and white.

This study demonstrated that all isolates showed inhibitory activity against *A. hydrophila*, a common bacterial pathogen in striped catfish in the Mekong River of Vietnam (Figure 3). These findings are in agreement with previous studies, where it was reported that actinomycete isolates exhibited prohibitory activity against bacterial species in aquaculture (Peng et al., 2022). The findings by Garcia-Bernal et al. (2015) indicated that 5/31 isolated actinomycete strains from marine sediments in Cuba showed antimicrobial activity against three *Vibrio* species. The research by Nabila and Kannabiran (2018) pointed out that about 18% of the actinomycetes isolates revealed antibacterial activity against the selected fish and shellfish bacterial pathogens. In Vietnam, the results of Huong et al. (2020) showed that 8/15 endogenous actinomycetes isolated from medicinal plants in Vietnam were active with tested microorganisms, such as *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Mycrosporum gypseum*. Many studies have shown that actinomycetes have antibacterial activity because they produce many antibiotics during growth, such as erythromycin, streptomycin, rifamycin, and gentamycin (Mahajan and Balachandran, 2012). Also, previous studies revealed that bacteriocins or bacteriocin-like substances were produced by *Streptomyces* species and had

antimicrobial activity against many pathogenic bacteria (Farris et al., 2011; Lee et al., 2014; Hernández-Saldaña et al., 2020; Kurnianto et al., 2021).

Previous studies revealed that nutrient medium, incubation time, temperature, pH, and NaCl are important factors affecting the growth and production of antimicrobial agents by actinomycetes (Gao et al., 2009; Oskay et al., 2011; Akond et al., 2016; Elabbasy et al., 2021). In the study, two isolates, BCA1.2 and BCA1.5, could grow well in ISP2-9 medium (Table 4). This finding is consistent with the study of Nabila and Kannabiran (2018), who found that the isolate *Streptomyces* sp. VITNK9 grew in large numbers on the ISP-3 and ISP-4 agar media. In our investigation, we showed that for two isolates, a pH range of 7.0–8.0 produced the best growth (Table 5). Similarly, Akond et al. (2016) found that the pH ranges of 6.5–8.0 for the isolate *Nocardia* JUBM–35–NS-2 and 6.0–6.5 for the isolate *Streptomyces* JUBM–35–NS-1 produced the best results for growth. However, Kontro et al. (2005) reported that ten *Streptomyces* spp. grew over a broad pH range between 4.0–5.5 and 10.0–11.5 but grew optimally at pH = 7.0 and above. Both actinomycete isolates in this investigation had maximum growth at a NaCl concentration of 3–7% and could survive at a NaCl concentration of 10% (Table 5). At 10% NaCl, however, the growth of isolates BCA1.2 was better than that of isolates BCA1.5 (Table 5). In contrast to strain RL8, which required more than 0.6% salt content in the culture media to develop, strains *Streptomyces* N7 and V4 thrived at salt concentrations ranging from 0 to 10%, according to research by Bernal et al. (2015). The research by Aouar et al. (2020) indicated that all strains were positive for growth in 4% NaCl. However, two *Streptomyces* strains, SO2 and SB1, could grow at 7% NaCl, while no growth was observed for the strain *Streptomyces* SO1.

Besides antimicrobial activity, numerous previous studies demonstrated that actinomycete strains could produce a wide range of extracellular hydrolytic enzymes, such as glucanase, chitinase, protease, lipase, and amylase (Roopan et al., 2019; Al-Dhabi et al., 2020). These findings illustrated that isolate BCA1.2 and BCA1.5, were positive for hydrolytic enzymes, including chitinase, protease, lipase, and amylase (Table 7). Earlier, *Streptomyces* sp. strain A from unexplored mangrove soils was found to have amylase (0.59 mol/ml/min), chitinase (1.356 mol/ml/min), and protease (0.248 mol/ml/min) activities, according to Deepthi et al. (2012). Research by Al-Dhabi et al. (2020) isolated *Streptomyces* sp. Al-Dhabi-49 for the simultaneous production of lipase and protease. Moreover, the production of protease and lipase was influenced by the incubation period, pH values, and culture medium temperature. Recently, according to research by Elabbasy et al. (2021), *Streptomyces canescens* MH7 has been found to be able to produce the extracellular hydrolytic enzymes consisting of glucanase, chitinase, lipase, and protease. The production of various lytic enzymes in this study showed the potential application of two isolates, BCA1.2 and BCA1.5, due to their antifungal activity in the future.

CONCLUSIONS

In conclusion, the study isolated and identified ten actinomycete isolates that had antagonistic activity against *A. hydrophila*. The results revealed that two isolates, BCA1.2 and BCA1.5, grew best in a pH range of 7.0–8.0 and at 37oC. Two isolates, BCA1.2 and BCA1.5, were able to use a variety of carbon sources, and tolerate a high salt concentration of NaCl ranging from 5% to 10%. They produced a variety of extracellular enzymes with hydrolytic activity, including cellulase, amylase, protease, lipase, and chitinase. Using 16S rRNA gene sequencing, two isolates, BCA1.2 and BCA1.5, were determined to be *Streptomyces*. The study's findings point to the potential use of actinomycetes in the future development of probiotics for the prevention of hemorrhagic illness in striped catfish.

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