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Isolation and characterization of actinomycetes from white leg shrimp (*Litopenaeus vannamei)* ponds antagonistic to *Vibrio parahaemolyticus*

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ABSTRACT

Marine microorganisms are a source of useful bioactive agents and are helpful in developing a strategy to use specific bacteria strains isolated from polluted shrimp farm to treat marine aquaculture diseases. Among them, actinomycetes have gained special importance as they have a major role in the recycling of organic matter as well as the production of pharmaceuticals, enzymes, antimicrobial agents, immune-modifiers and vitamins. The eight actinomycetes isolates were recovered from shrimp pond sediment and were identified using 16s rDNA sequence as *Actinomadura geliboluensis* (2 isolates) and *Streptomyces* sp. (6 isolates). These isolates were then investigated for their ability to produce antibiotic compounds, enzymes as well as for pathogenicity markers. The eight isolates showed strong enzymatic production with amylase, chitinase, gelatinase, amylase, and antimicrobial activity against *Vibrio parahaemolyticus*. However, only 2 isolates were non-hemolytic (γ hemolytic) (PH 13 and PH 15). The minimum inhibition concentration (MIC) of the isolates PH 13 and PH 15 on *V. parahaemolyticus* were at the concentration of 1:524 and 1:1048, respectively. Although PH 13 had higher antimicrobial activity, PH 15 showed increased enzymatic activity by producing cellulose and amylase. Furthermore, the results of hydrophobicity assay suggested these isolates are promising candidate probiotic for shrimp farming. Results from this study provide a first insight on characteristics of marine actinomycetes isolates recovered from shrimp ponds sediments in Vietnam.

Keywords: Actinomycetes, *Actinomadura geliboluensis*, *Streptomyces* sp., enzymes, antimicrobial.

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INTRODUCTION

Vibrionaceae has been consistently identified as one of the dominant families in the natural intestinal flora of wild and farmed penaeid shrimp and be considered as an obligate pathogen. However, as a result of the rapid change of shrimp culture worldwide, reports of disease and mortality in shrimp caused by Vibrio began to spread. *V. harveyi* was one of the first and is considered as one of the most important bacterial pathogens in aquaculture, affecting a wide range of cultured marine organisms (Austin and Zhang, 2006), later joined by *V. parahaemolyticus* and others (Corteel and Decamp, 2016). Recently, a new emerging disease known as Acute Hepatopancreatic Necrosis Disease (AHPND) caused by a specific bacterium strain of the *Vibrio parahaemolyticus*, has been a major issue of concern for economic loss in the shrimp farming industry in the world (De Schryver et al., 2014; Zorriehzahra and Banaederakhshan, 2015; Dong et al., 2017; Velázquez-

Lizárraga et al., 2019). The possible trend is using specific bacterial strains isolated from intensive shrimp farms to increase the efficiency of environmental remediation in those farming areas (Kumar et al., 2009; Gracia-Bernal et al., 2015), in which actinomycetes are microbes group being studied for decomposing organic compounds and inhibiting the proliferation of pathogens like *Vibrio parahaemolyticus* and *V. harveyi*, in the pond (Dharmaraj, 2011).

Actinomycetes are Gram-positive mycelial bacteria, ubiquitous in soil and especially significant for their role in the recycling of organic matter and producing bioactive compounds (Srinivasan et al., 1991), and most isolates shown to produce bioactive compounds. Several studies suggested that the isolation of actinomycetes from marine sediment may be valuable for the isolation of novel actinomycetes with the potential to yield useful new products (Goodfellow and Haynes, 1984; You et al., 2005; Das et al., 2010; Gracia-Bernal et al., 2015). In another hand, Actinomycetes are well known as producers of many extracellular enzymes with polymer-degrading properties, including chitinase, lipase and cellulase (Gupta et al., 1995). Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer (Hasan et al., 2006). Actinomycetes degrade starch and casein and produce of antimicrobial agents against both gram negative and gram positive bacteria (Barcina et al., 1987; Pisano et al., 1992; Cabello et al., 2013), especially proven to be antagonistic to *Vibrio spp.* pathogenic to shrimps (Gracia-Bernal et al., 2015). The aims of this study were to study the characteristics of actinomycetes isolates that were recovered from intensive shrimp ponds antagonistic to *V. parahaemolyticus*.

MATERIALS AND METHODS

Actinomycetes isolates collection

Actinomycetes isolates were recovered from from 7 farms located in two main intensive white leg shrimp (*Litopenaeus vannamei)* farming in Thua Thien Hue province, Central Vietnam. At each farm visit, 10 g of sediments from waste water pond were collected, each sample was pre-treated for selective isolation of actinomycetes according to Laskshmi et al. (2008). First, samples were heat treated at 50 to 60°C for 1 h in the temperature control incubator (Yihder LM-4200, Germany). After that, the sediment were mixed with $CaCO_3$ (with the ratio 10:1, respectively) and incubated at 28°C for a week. The pre-treated samples were serially diluted, vortexed and streaked onto M media agar plates (soluble starch 15 $g.L^{-1}$, NaNO3 1 $g.L^{-1}$, K_2HPO_4 0.5 $g.L^{-1}$, $MgSO_4.7H_2O$ 0.5$g.L^{-1}$, $FeSO_4.7H_2O$ 0.01$g.L^{-1}$, agar 20 $g.L^{-1}$) and incubated at 28°C for 7 days (Zheng et al., 2012). Supplemented with 1% anti-fungal agent Mystrep (Biostad, India) and antibacterial compound Kanamycin (Nam Khoa, Vietnam) to prevent the growth of fungal and Gram negative bacteria. A single colony on M Agar with specific characteristic of actinomycetes were sub-cultured on Marine Actinomycete Growth (MAG) agar (starch 10 $g.L^{-1}$, yeast extract 4 $g.L^{-1}$, pepton 20 $g.L^{-1}$, agar 25 $g.L^{-1}$ and sea water). Slant cultures of the different isolates grown on MAG medium were inoculated in 20 ml seed medium. The tubes were incubated in the reciprocal shaker (Yihder LM-4200, Germany) at ×10 g for 7 days at room temperature and were used for this study. All isolates were then identified by 16S rDNA sequencing before storage in cryo-preservative in commercially prepared glass beads (Technical Service Consultant Ltd, UK) at -70°C.

Identification and phylogenetic analysis

The genomic DNA of recovered actynomyces isolates was extracted by microbial DNA isolation kit (Tiangen Biotech Co. Ltd, China). The 16S rDNA gene fragment was amplified by PCR using the general-purpose primers of prokaryotic 16S rDNA: upstream primer 5–AGAGTTTGATCCTGGCTCAG–3', and downstream primer 52–AAGGAGGTGATCCAGCCGCA–32 (Wang et al., 2009). The 50 µl PCR reaction consisted of 25 µl Master Mix (Thermo scientific, UK) which contained 0.625 u Taq DNA Polymerase,75nM Tris-HCl (pH8.8 at 25°C), 20 mM (NH4)2SO4, 1.5 mM $MgCl_2$, 0.01% Tween 20, 0.2 mM each of dNTP, 10 pmol of each primer (the forward and reverse primer), 200 ng of template DNA and nuclease-free water to volume. The thermo-cycling condition consisted of an initial denaturation step at 94°C for 3 min, 35 cycles of 94°C for 30 s, 55°C for 60 s, 72°C for 90 s and 5 min for final extension at 72°C. An internal control (no DNA template) was included. The PCR amplification was conducted in Bio-Rad thermal cycler (MyCycler, Bio-Rad, USA). The expected product was 1500bp. The PCR products were purified by Quiagen test kit following the manufacturer protocol and sent to the laboratory of Bioneer Sequencing Team (Republic of Korea) for sequencing to confirm the identity of the bacteria.

Antimicrobial activity test

Bacterial preparation

Vibrio parahaemolyticus recovered from AHPND diseased shrimp of *Litopanaeus vannamei* were used to test the antagonistic potential of actinomycete isolates. These cultures were obtained from the laboratory of Fish Pathology, Faculty of Fisheries, Hue University of Agriculture and Forestry. A single colony of *Vibrio parahaemolyticus* on TCBS agar was sub-cultured in 10 mL of TSB (Tryptone Soya Broth, Oxoid, UK) with 2% NaCl added at 28°C for 1 day to achieve exponential growth. The bacterial suspension was centrifuged at x 110 g for 15 min, cell pellet was resuspended and adjusted by 0.86% sterile saline to give an optical density (OD_{600}) value of 1 which corresponds to approximately 10^9 $cfu.ml^{-1}$. The viable colony counts were performed using the Miles and Misra method (Miles et al., 1938) and then 10-fold serial dilutions performed to give 1×10^5 $cfu.ml^{-1}$ for the antimicrobial test.

Antimicrobial activity

The antagonism assay of actinomycetes was conducted by using the agar-diffusion method (Garcia-Bernal et al., 2015). The actinomycetes strains were grown on starch casein agar for 7 days at 30°C. Take 100 µl of Vibrio suspension which was prepared above plated on the TSA (Tryptone Soya Agar, Oxoid, UK) with 2% NaCl added. After 30 min, four 5.5 mm plugs of the seventh culture day of actynomyces isolates on M2 agar were made by a cork borer (5.5 mm diameter) and placed on these TSA plates. The plates will be then incubated at 30°C for 24h and the diameter the zone of inhibition were measured for determining the antimicrobial activity.

The minimum inhibitory concentration assays of the 8 isolates were conducted in the 96-well Immulon™ 4HBX plates

(ThermoScientific, Maine, USA) using a modification of the method described by Ferrari et al. (2000). Briefly, 150 µl of the Vibrio suspension of 10^5 cfu.ml^{-1} (prepared in 2.3.1) was inoculated into each well and then 150 µl of serially diluted cell free actinomyces suspension (by centrifuged in the cooling centrifuge (Digisystem, Germany) at ×110 g for 15 min), and diluted in PBS (from 1:2 to 1:2048) was added in each well containing bacteria. The 96-well was then incubated at 30°C for 24 h under aerobic condition. Each tray should be sealed in a plastic bag or with a tight-fitting plastic cover before incubated to prevent drying. After that the absorbance was measured at OD_{450} using a micro-plate reader (Biotek Synergy HT). A viable test was performed by plating 10 µl of each solution in 96-well plate into TSA plates with 2% NaCl added. These plates were incubated at 30°C overnight. The concentration of the actinomycete that was MIC end point was one having visible growth of bacteria on the agar plate.

Hydrophobicity and enzyme production assays

The Actinomycetes isolates recovered from the sediment samples above were used to test the hydrolytic (amylase, gelatinase, lipase, cellulase and chitinase) activity. Slant cultures of the different isolates grown on MAG medium were used for this study.

Hydrophobicity activity

Hydrophobicity test was performed using the Congo red method and the Bacterial adherence to hydrocarbons (BATH) test as described by Garcia-Bernal et al. (2015). The isolates with a reddish color on TSA plates containing 1% sodium chloride and 0.03 Congo red were considered positive for the test. By contrast isolates with a translucent to white color were negative. The BATH test was determined by percent hydrophobicity followed the method and formula of Garcia-Bernal et al. (2015).

Amylase production

The isolates were spot inoculated onto Starch Agar medium of Harigan and Maccance (1972) (peptone 10 g.L^{-1}; Beef Extract 10 g.L^{-1}, Starch 5 g.L^{-1}, agar 20 g.L^{-1} and 1000 mL of sea water; pH=7.2) and incubated for 4 to 5 days at room temperature (28 ± 2°C). The production of amylase was tested by flooding the plates with iodine solution. Un-hydrolyzed starch formed a blue color and amylolytic colonies developed a clear zone around them.

Lipase production

Production of lipase was tested on Tributyrin Agar medium *(*Rhodes, 1959) (Peptone 5 g.L^{-1}, Beef Extract 3 g.L^{-1}, Tributyrin 10 ml, Agar 20 g.L^{-1}, and 1000 nl of seawater; pH 7.2). The isolates were spot inoculated onto plates and incubated for 4 to 5 days at room temperature. Lipase production was detected by the appearance of halo zone around the colony.

Gelatinase production

Fraziers gelatin agar medium (peptone 5 g.L^{-1}, beef extract 3 g.L^{-1}, gelatin 2 g.L^{-1}, agar 20 g.L^{-1}, and 1,000 mL of seawater; pH 7.0) were used for detection of gelatinase activity. The isolates were spot inoculated onto plates and incubated for 4-5 days at room temperature. The plates were flooded with tannic acid solution and the colonies with halo zone were noted as positive (Fraziers, 1926; Whaley et al., 1982).

Cellulase production

The isolates were spot inoculated onto Cellulose Agar medium of Riviere*,* (1961) (cellulose powder 5 g.L^{-1}, yeast extract 0.5 g.L^{-1}, $NaNO_3$ 1 g.L^{-1}, casein hydrolysate 0.5 g.L^{-1}, agar 20 g.L^{-1} g, and 1,000 ml of seawater; pH 6.8) and incubated for 4 to 5 days at room temperature. Cellulase production was detected by the appearance of halo zone around the colony.

Chitinase test

The isolates will spot inoculated onto Chitin Agar medium *(*Holding and Collee*,* 1971) and incubated for 4 to 5 days at room temperature. Chitinase production will be detected by the appearance of halo zone around the colony.

Haemolysis activity

Apparently healthy shrimp (*Litopenaeus vannamei)* (30 shrimp) were collected from a farm located at Phong Dien District*,* brought to the laboratory of Fish Pathology, and cultured in the 120 L fibre tank in the wet lab of Faculty of Fisheries, Hue University of Agriculture and Forestry (HUAF) for a week, prior to use. Shrimp used in this test were between 25-30 g and health checks of shrimp prior to getting blood were performed by PCR of 3 shrimp for checking viral diseases (WSSV, YHD, IHHNV). In addition, sampling the hepatopancreas of 3 shrimp directly onto tryptone soya agar (TSA, Oxoid) were carried out to check for bacterial growth.

After that, the shrimps were surface sterilized by washing using ice-cold freshly prepared sodium hypochlorite solution (2000 ppm) followed by 70% ethanol. Haemolymph (1 ml) was then collected aseptically from the rostral sinus using a sterile capillary tube and transferred into a sterile eppendorf tube containing 200 µl shrimp anticoagulant solution (Song and Hsieh, 1994).

The hemolysis of actymomycetes were performed by observing hemolysis zone on prawn blood agar (Chang et al., 2000). One ml hemolymph of was mixed with 130 J11 3% (w/v) Rose Bengal stain prepared in shrimp anticoagulant solution (3% W/V) in order to stain the hemocytes. Nutrient Agar (Himedia, India) with 1.5% NaCl was prepared and its temperature allowed to drop to 45 - 50°C after autoclaving. Rose Bengal stained hemolymph (1 ml) was added to 15 ml of the medium with gentle shaking for proper mixing. The medium was poured into a petri dish and the plate rotated clockwise and anti-clock wise so as to ensure thorough mixing and even spreading of haemocytes throughout. After surface drying, the plate was observed for the stained intact hemocytes. Actinomycete strains were inoculated on to the prawn blood agar plate. The plates were incubated for 48 h at 28 ± 2°C and observed for hemolysis around the colonies. Hemolysis was confirmed by microscopic observation of the lysed hemocytes around the colony.

Ethical considerations

The Animal Ethics Committee of HUAF is going to be established in 2020 so formal ethical approval was not required or obtained at the time this study was conducted. In the absence of a regulatory framework for formal ethical approval, the work for hemolytic test was conducted according to the ethical standards of the UK Home Office, based on training received by the first author at the Institute of Aquaculture, University of Stirling, Stirling, UK.

RESULTS

Identification of actinomycetes

There were 8 isolates which were recovered from seven intensive shrimp pond in two communes. Among these 8 isolates, there were two different morphological type colonies growing on M agar plates (Table 1).

The isolates were confirmed by their morphology by observing their characteristic under the microscope. Appearance of actinomycete like colonies on isolation media are shown in Figure 1. Morphology of representative actinomycetes isolates was visually observed and their aerial and substrate mycelium were described in Table 1.

All isolates are Gram positive. Colony characteristics of the actinomycetes isolates are shown in Table 1. All isolates were found to grow slowly on agar media. The colonies were then purified and slant culture onto MAG medium.

In which, the isolates of PH 11, PH 12, PH 13, DH 11, DH 12, DH 13 were long filamentous with branches. The colonies of these isolates on M1 and Marine actinomyces agar were flat, off white and irregular with 2-4 mmm diameters. By contrast, the morphology of isolates of PH 14, and PH 15 were cluster and branches. On the M1 medium and marine actinomyces agar, these isolates grew deep into agar, the colonies were opaque, solid with 1-2 mm diameters.

Table 1. Colony morphology of the isolates on two kinds of medium.

Medium	Isolates	
	PH 11, PH 12, PH 13, DH 11, DH 12, DH 13	**PH 14, PH 15,**
M1 medium	Flat, off white, irregular, dry, 2-3mm	Grown deep into agar, opaque, solid, 1-2mm
Marine actinomycetes agar	Off white, dry, irregular, 3-4mm	Grown deep into agar, opaque, solid, 1-2mm
Gram stain	Gram positive, filamentous, branches, evenly length	Gram positive, cluster and branches

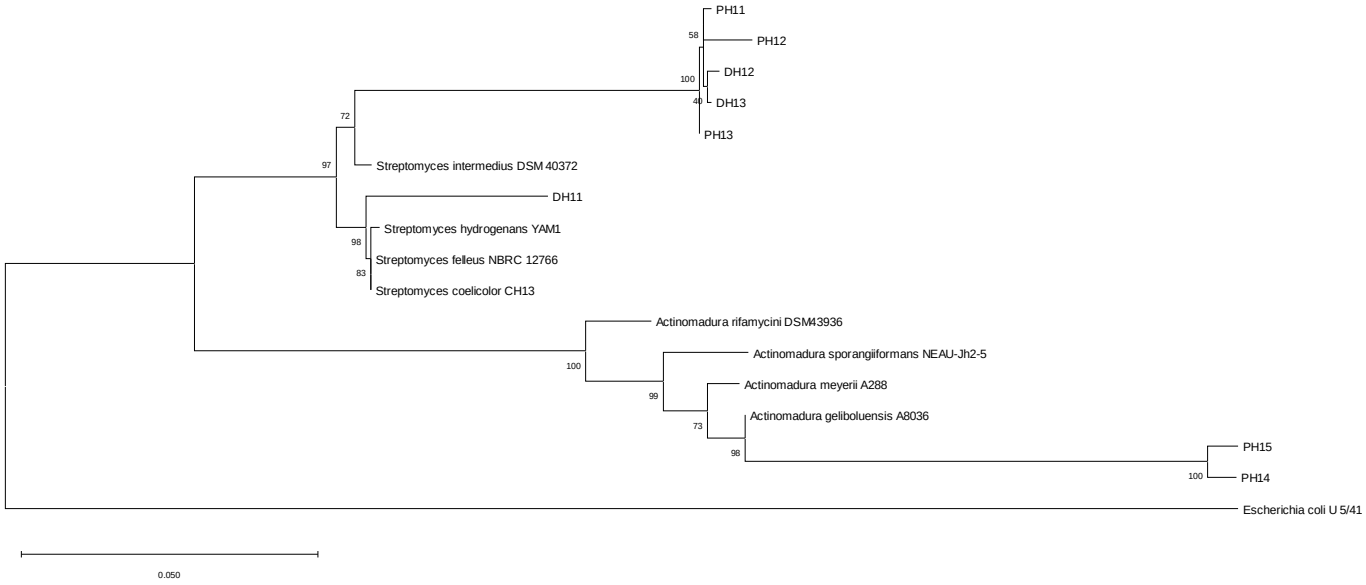

Figure 1. Phylogenetic tree of the isolates of 8 actinomycetes isolates.

16S rDNA sequence and phylogenetic analysis

PCR amplification of 16S rDNA yielded a single amplicon of 1500bp for all the isolates. Phylogenetic trees based on the 16S rRNA sequence data of 8 isolates was presented in Figure 1.

Sequencing of the PCR amplicon provided from robust 1214 nucleotides sequence when compared against the partial genome sequence of *Actinomadura* on Gene Bank database, which matched 93% genetic similarity *Actinomadura geliboluensis* strain A8306 (Figure 1). Therefore, PH14 and PH 15 were identified as *A. geliboluensis*

The 16S rRNA nucleotide sequence of PH 11, PH 12, PH 13, DH 11, DH 12 and DH 13 was determined (Figure 1). The result of the homology search with

GeneBank database using the BLAST system showed that the 16S rRNA nucleotide sequence of these isolates had a highest identity of 95.5% with that of *Streptomyces* sp.

Enzymes production

All the isolates did not show lipase activity but produced gelatinase (Table 2). The isolates of Actinomadura PH 14, PH 15 and Streptomyces DH 11, DL 13 produced four enzymes consisting of cellulose, amylase, gelatinase, and chitinase. Two isolates produced 3 enzymes including cellulose, amylase, and gelatinase (PH 13), or chitinase (DH12). The isolate of PH 11 and PH 12 only produced 2 enzymes (cellulase and gelatinase) (Table 2).

All actinomycetes isolates were positive with Congo red and hydrophobicity percent of these isolates were 52.1 to 82.4% (Table 2).

In vitro hemolytic and microbial activity of actinomycetes isolates

All 8 actinomycetes isolates showed the antimicrobial activity against *Vibrio parahaemolyticus.* However, only 2 isolates Streptomyces sp. PH 13 and *A. geliboluensis* PH 15 were non-hemolytic (γ hemolytic). Six out of 8 isolates were hemolytic (β heamolytic) (Table 3). The MIC results of 8 recovered isolates were presented in Table 3. PH13 and PH 15 had the MIC at the diluted concentration of 1:512 and 1:1024 (Table 3).

Table 2. Enzymatic production and hydrophobicity test of actinomycetes isolates.

Actinomycete isolates	Cellulase	Amylase	Lipase	Gelatinase	Chitinase	Hydrophobicity test	
						Congo Red	Bath (%)
PH 11	+	-	-	+	-	+	54.2
PH 12	+	-	-	+	-	+	75
PH 13	+	+	-	+	-	+	82.4
PH 14	+	+	-	+	+	+	66.6
PH 15	+	+	-	+	+	+	80
DH 11	+	+	-	+	+	+	64.5
DH 12	+	-	-	+	+	+	67.7
DH 13	+	+	-	+	+	+	52.1

(+): positive; (-): negative.

Table 3. Antimicrobial and hemolytic activity of 8 actinomycetes isolates.+

Isolate	Diluted concentration of actinomycetes suspension											Inhabitation zone (mm)	Heamolysis
	1:1	1:2	1:4	1:8	1:16	1:64	1:128	1:256	1:512	1:1024	Control		
PH 11	2	+	+	+	+	+	+	+	+	+	+	2-4	β
PH 12	-	+	+	+	+	+	+	+	+	+	+	3-5	β
PH 13	-	-	-	-	-	-	-	-	-	+	+	4-6	γ
PH 14	-	+	+	+	+	+	+	+	+	+	+	16-18	β
PH 15	-	-	-	-	-	-	-	-	+	+	+	10-12	γ
DH 11	-	-	-	-	-	-	-	-	+	+	+	10-12	β
DH 12	-	+	+	+	+	+	+	+	+	+	+	2-4	β
DH 13	-	-	-	-	-	-	-	-	+	+	+	14-16	β

(+): bacteria grow after 24 hours; (-): no growth.

DISCUSSION

In the present study, 8 actinomycetes isolates were recovered from the sediment of intensive shrimp ponds. The selective screening of these samples resulted in 2 actinomycete isolates (PH13 and PH 15). While the isolate of PH 15 was identified as *A. geliboluensis*, the PH 13 isolate was *Streptomyces* sp.

The isolate of *Streptomyces* sp. was also previous recovered from shrimp ponds (Chau et al., 2016), however *Actinomadura geliboluensis* was first recovered from the sediment of intensive shrimp pond in Thua Thien

Hue. Both *Streptomyces* sp. and *A. geliboluensis* were recovered from shrimp ponds sediment in Phong Hai by M 1 and Marine actinomycetes agar.

The genus *Actinomadura* was proposed by Lechevalier and Lechevalier (1970). *A. geliboluensis* sp. were isolated from soil and assigned as a novel actinobacterium because of their enzyme production and antimicrobial activity (Sazak et al., 2012). In previous study, actinomycete isolates were isolated from shrimp ponds sediments in Thua Thien Hue by starch casein agar (SCA) and AIM medium, resulted of 50 isolates of actinomycete grown on plates and 1 strain was identified as *Streptomycete* sp. A1 (Chau et al., 2011). However, in this study we only recovered 8 actynomyces isolates from sediments of intensive shrimp ponds. This could be the increasing of chemical used in shrimp farming over the past five years; especially shrimp farms are applying tarpaulin to reduce bacteria generally (Phuoc pers.comm.).

Addition, the isolation medium also affects to the number of actinomyces isolates that are recovered from sediments. Zhang and Zhang (2011) reviewed that the number of non-actinomycetes colonies was predominant in Gause's No.1 medium while the number of actinomycetes was abundant in the ZSSE medium. All the isolates were slow growing on MAG medium for approximately 7 to 8 days.

The present study revealed the importance of marine actinomycetes as a potent source of antimicrobial compounds. In this study, although 4 isolates proved to be inhibitory to *V. parahaemolyticus*, but only 2 isolates were non-hemolytic in which, the isolate of PH13 showed more antimicrobial activity than PH 15. The β hemolytic isolates could produce toxic secondary metabolites to shrimp (Garcia-Bernal et al., 2015). These two γ hemolytic isolates are the most promising probiotic candidates because they are active against Vibrio and should not be either pathogenic or able of producing toxic substances that may harm shrimp. Lakshmi et al. (2008) reported that actinomycete isolates were obtained from marine sediments were non-pathogenic to shrimps and there are no reports of marine actinomycetes as pathogens in culture systems. However, toxicity tests in the final host are important to clarify that these isolates are innocuous (Arafah et al., 2013). Lakshmi et al. (2008) suggested that actinomycetes could inhibit the proliferation of *Vibrio* in aquaculture systems by the application of actinomycetes under rearing conditions. However, in Thua Thien Hue there were no reports that actinomycetes can strongly reproduce in shrimp ponds. The antimicrobial activity can be further studied by testing with other *Vibrio spp.* The measure of antimicrobial compounds produced by the actinomycetes could be different from one medium to another (Jose and Jebakumar, 2013). Previous study showed *Streptomyces sp.* were recovered from shrimp ponds in Thua Thien Hue also had high activity against *V. harveyi* and *V. parahaemolyticus* (Chau et al., 2016).

Actinomycetes are Gram-positive mycelial bacteria, ubiquitous in soil, are well known as producers of many extracellular enzymes with polymer-degrading properties, including chitinase (Gupta et al., 1995). All the isolates were tested for their cellulose, lipase, amylase, gelatinase and chitinase activities, enzymatic activities of the isolates were different. The two promising probiotic candidates (PH 13 and PH 15) showed strong extracellular enzymatic activities. These isolates produced cellulose, amylase, gelatinase and chitinase. In aquaculture systems, these enzymes may help shrimp with improving food digestion, better growth rate, and water quality by degrading fecal matter and uneaten food in hatchery tanks and grow-out ponds (Bermudez-Brito et al., 2012)

Chitin is widespread in both terrestrial and aquatic environments, as a component of invertebrate exoskeletons, fish scales, and cell walls of many fungi. Only cellulose is more globally abundant as a biological polymer (Shahidi et al., 1999; Zaku et al., 2011). Actinomycetes are one of the known cellulose producers has attracted considerable research interests (Arunachalam et al., 2010). The lack of enzymes production in other isolates maybe dependent on the isolation medium. When screening the enzymatic activity of some actinomyces strains on SCA medium Lakshmi et al. (2008) revealed that actinomyces showed a potential of wide range enzymes production, which may be the result of natural selection of microorganisms to survive competitive in the environment (Das, 2012). Sharma (2014) reported that the sources from which the bacteria were isolated can affect to the biological function of actinomycetes.

In addition, hydrophobic activity is considered as one of the factors related to the attachment of microorganisms to the host gastrointestinal tract (Bezkorovainy, 2001). In this study, the hydrophobicity percentage of PH 13 and PH 15 were 82.4 and 80% respectively showed these isolates were strong hydrophobic and they could attach to the gut of shrimp (Garcia-Bernal et al., 2015). The adhesion capacity to the gastrointestinal tract of these strains has been considered as one of the most important criteria to select the novel candidate probiotic isolates. Although, an egg white lysozyme (HEWL) and Gold nanoparticles (AuNP) are proposed as immunostimulants that stimulate immune responses in shrimp against *V. parahaemolyticus* infection (Tello-Oleaa et al,. 2019; Woraprayote et al., 2020). However, actinomycetes is still a potential probiotic candidate for preventing the infection of *V. parahaemolyticus* in shrimp.

Conclusion

This study showed the potential in using actinomycetes isolates as a probiotic for shrimp farming, however,

further study *in vivo* condition should be conducted to clarify which of these isolates actually have the best probiotic effect and could be applied effectively for prevention the infection of *V. paraheamolyticus* in shrimp.

Declaration of competing interest

All authors approved the manuscript, this submission and declared no known conflicts of interest associated with this publication.

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