Isolation and characterization of an antibacterium against *Vibrio harveyi* 11593 from a mixed pond with *penaeus japonicus* bate, *portunus trituberculatus* and *ruditapes philippinarum* in China

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Abstract. Aquaculture diseases, particularly Vibriosis, are becoming a pressing concern due to incurred aquacultural loss in China. Commercial antibiotics may be used to control it, but its increased antibiotic resistance to prominent pathogenic bacteria has become a prevalent problem nationwide, and a global threat to public health. Probiotics are recommended instead because they are healthy, environment-friendly, and capable of maintaining productivity. An antibacterium against *Vibrio harveyi* 11593 was isolated from a mariculture pond with shrimp, crabs, and shellfish in China. The bacterium, E14, has an inhibitory zone diameter (DIZ) of 24.5 ± 0.5 mm. The strain was identified as *Bacillus pumilus* based on morphological observation, conventional biochemical tests, and 16S rDNA sequence analysis. The gram-positiveand motile bacterium is around 1.10–1.32 μm × 0.67–0.83 μm in size. Optimized conditions for antimicrobial substance production of *B. pumilus* E14 require that it be cultured for 26 h at 28 °C, with an initial pH of 7.0 in 100 mL/500 mL LB with 3% NaCl. The *B. pumilus* E14 cultures were confirmed to be safe and efficacious and actually worked to protect the host animal in shrimp larvae (*Penaeus chinensis*) culture. The *B. pumilus* E14 obtained in this study strengthened the strain's defense against aquaculture disease and made a good candidate for an alternative probiotics and benefit to sustainability of aquaculture.

1. Introduction

Today, the aquaculture is honoured as one of the fastest growing food-producing sectors in the world, posting an average annual growth rate of 8.9% since 1970 [1]. According to FAO statistics in 2013, world food fish aquaculture production attained another all-time high of 70.5 million tonnes. As the largest aquaculture producer in the world, China has produced 43.5 million tonnes of food fish in 2013 [2].

However, aquaculture is currently suffering from grave economic loss due to infectious diseases of microbial origin, which result in high mortality rates and lesions [3]. Vibriosis is one of the biggest causes of aquaculture loss in China [4]. It is caused by bacteria, mainly of the genus *Vibrio*, such as *Listonella anguillarum* (formerly *Vibrio anguillarum*), *Vibrio ordalii*, *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio salmonicida* [5-7]. These bacteria have been identified as major reasons for most common disease outbreaks in fish and shellfish. Among them, *V. harveyi* is considered an acute pathogen of ocean fish and invertebrates, particularly penaeid shrimp [8].

Thus, controlling Vibriosis is vital to maintaining aquatic productivity and meeting ever-growing human needs. The disease can be controlled using various methods, notably commercial antibiotics. However, commercial antibiotics used for disease treatment produces ill-natured effects, including toxicity to reared organisms and the release of chemical residues into the environment, which cause risks to animal and human health [9].

These factors prompted the development of new dietary supplementation strategies, such as using health- and growth-promoting compounds, including probiotics, prebiotics, synbiotics, phytobiotics, and other functional dietary supplements[10]. Application of probiotics in aquaculture seems relatively recent, but there remains an increasing enthusiasm in environment-friendly treatments. Probiotics have become quite common, and their treatment may broaden a spectrum for greater nonspecific disease protection [11, 12].

At present, there exists an increasing need to identify new effective antibacteria with few undesirable side effects for shrimp and other sea farming organisms. Hence, aquaculture disease control research has steered toward the discovery of probiotics for prevention and treatment of microbial infections.

The co-culture mode in a mixed pond with *Penaeus japonicus* Bate, *Portunus trituberculatus* and *Ruditapes philippinarum* polycultures is one of the most common and fastest-growing mariculture modes in the east coast of China. A comparison of the bacterial community structure between the co-culture and control pond has been reported, demonstrating the richer diversity of the bacterial communities in co-culture ponds [13]. Samples collected from this common model can serve as a no-rejection-effect candidate for the protection of animals in an aquaculture system. In this study, we aimed to isolate and screen antibacteria against a well-known fish pathogen, such as *V. harveyi*. We then identified the antimicrobial strain and optimized various parameters for maximum antibacterial ability.

2. Material and methods

2.1. Sample collections

The sediments sample was collected from a seawater pond with *Penaeus japonicus* Bate, *Portunus trituberculatus Ruditapes philippinarum* by the scoop with the long handle at 25~30℃ in mid-July, 2012. The pond was one of ten artificial ponds, each pond is about 200×50×1.0 m^3 and located in Ganyu County, Lianyungang city, Jiangsu province, eastern China (34o58'23"W-119o11'36"E). *2.2 Medium*

2216E: Seawater 1000 mL with tryptone 5g, yeast extract 1g, FePO4 0.1g, and Agar 20g (optional). pH 7.0-7.2.

LB with 3% NaCl: Distilled water 1000 mL with tryptone 10 g, yeast extract 5g, NaCl 30 g and Agar 20 g (optional). pH 7.0-7.2.

YPG: Distilled water 1000 mL with tryptone 5g/L, glucose 5g,yeast extract 5g, pH 7.0-7.2.

PPM: Distilled water 1000 mL with tryptone 20g, glucose 18 g, KNO3 5 g, NaCl 1g, pH 7.0-7.2.

NYPD: Distilled water 1000 mL with glucose 10g, beef extract 8g, yeast extract 5g, pH 7.0-7.2.

NA: Distilled water 1000 mL with tryptone 20g, beef extract 1g, yeast extract 2g, NaCl 5 g, pH 7.0-7.2.

CM: Distilled water 1000 mL with tryptone 20 g, beef extract 3g, pH 7.0-7.2.

Above reagents were from commercial sources: tryptone, yeast extract, beef extract, Oxoid; NaCl, FePO4, KNO3, Sinopharm Chemical Reagent Co., Ltd; Agar, Biosharp.

All medium was made suitable for sterilization in the autoclave at 121℃ for 30 min.

2.2. Bacterial isolate

The sediments sample 5 g with seawater sterilized 45 ml were cultured on 120 rpm shaker at 28℃ for 2 h. Then microorganisms were isolated by dilution plate technique described by Ping Shen [14]. The culture was 10-fold serial diluted to 10^{-1}~10^{-7} with seawater sterilized and then inoculated into 2216E agar plates to culture at 28 °C for 3 days and to identify different strains. Single colonies were picked based on its morphology, color, size, and streaked onto 2216E agar plates several times until the morphology of colonies was uniform.

2.3. Selection of antimicrobial strains to inhibit in vitro growth of V. harveyi 11593

The ability of isolated bacteria to inhibit the growth of *V. harveyi* 11593 was detected by agar well diffusion assay [15]. Agar plates were previously coated with 100 μL of *V. harveyi 11593*, which corresponded to 5 x 10^7 cfu/mL. , 6-mm diameter wells were made in the agar plates by a sterilized borer, then 50 μL E14 cutured solution was poured into the wells and incubated at 28℃ for 24 h, and diameter of inhibitory zone (DIZ) was measured for the antimicrobial activity. This test was done in triplicate. A species with the largest DIZ was finally selected for further study.

2.4. Determination for appropriate dose-volume of antimicrobial strain against V. harveyi 11593

The Oxford cup method was used in this test. The broth of antagonistic bacterial strains and *V. harveyi 11593* were 10-fold serial diluted to 5.0 × 10^7 CFU/mL with sterile seawater respectively. Then two 2216E agar plates were previously spread with 100 μL dilution of *V. harveyi 11593*. Five oxford cups

(inside diameter, 6 mm; outer diameter, 8 mm; height, 10 mm) were put vertically on every plate. The center cup was poured into 100 μL sterile 2216E as control, and four cups around the center were poured into defferent volume (20, 40, 60 or 80 μL in one plate and100, 120, 140 or160 μL in the other) of antagonistic bacterial strain dilution. All the plates were incubated at 28℃ for 24 h and The DIZ was measured for the antimicrobial activity. The test was repeated three times.

2.5. Identification of antimicrobial strain

The identification of the isolated strain was assessed by morphological observation, conventional biochemical tests and 16S rRNA gene analysis. The biochemical tests were determined with bacterial biochemical tube trace identification kit (Hangzhou Tianhe Microoganism Reagent Co., Ltd.) according to the manufacturer's instructions. Morphological observation was carried out using the Gram-staining and Nikon eclipse 90i microscope (Japan). Two oligonucleotides, based on the report of Dunbar et al. [16], were used to amplicate 16S rDNA of the isolate: 27F -AGAGTTTGATCCTGGCTCAG and 1492R-TACCTTGTTACGACTT (synthesised by Shanghai Sangon Biotech engineering co., LTD). Polymerase chain reaction (PCR) was performed with a T100 thermal cycler (Bio-Rad) using a 50 μL containing: 2.5 U Taq DNA polymelase, 1 × PCR buffer, 200 μM each dNTP, 20 pmol each primer, and 1 μl template DNA extract. The PCR conditions used were: initial denaturation at 95℃ for 5 min, followed by 30-cycles: 94℃ for 1 min, at 52℃ for 1 min, at 72℃for 2 min and with a final elongation step at 72℃ for 5 min. The reagents in this part were purchased from Takara. The 16S rDNA amplified by PCR was sequenced by Shanghai Sangon Biotech engineering co., LTD. The analysis of nucleotide sequences was performed at http://rdp.cme.msu.edu/seqmatch. Sequences were aligned using the program CLUSTAL neighbor-joining and a phylogenetic tree was made by using the MEGA 5.10 program.

2.6. Optimization of conditions for producing the maximum active substanse by B. pumilus E14

The effect of medium (pH 7.0 -7.2) on antibacterial activity of *B. pumilus* E14 was determined by incubation at 160 rpm in different medium (LB with 3% NaCl, PPM, NYPD, YPG, NA and CM) with triangular flask. The effect of temperature on antibacterial production by *B. pumilus* E14 in optimal medium was tested by incubation at different temperatures (18℃, 22℃, 28℃, 32℃ and 40℃). The effect of pH on antimicrobial production was detected by inoculating *B. pumilus* E14 in medium adjusted to different pH values (5.0，6.0，7.0，8.0 and 9.0), and cultured at optimal temperature. The effect of medium volume in 500 mL triangular flask on antibacterial activity of B. pumilus was determined by incubation at medium volumes (50 mL, 75 mL, 100 mL, 125 mL and 150 mL), and cultured at optimal temperature and optimal pH value. Antibacterial activity was analysed using samples from the 24 h culture. The test was repeated three times.

2.7. The growth curve and the antibacterial activity by B. pumilus E14 at growing stage

The growth curve and the antibacterial activity by B. pumilus E14 at growing stage was studied in LB with 3% NaCl with optimized pH and temperature. Samples were fetched out every 2h, from 0 to 36h, followed by measurement of the optical density at 600 nm and the antibacterial activity. The test was repeated three times.

2.8. Feeding test of shrimp larvae (Penaeus chinensis) with strain E14

To demonstrate that the strain E14 cultures are safe and effective to be used in aquaculture, we do the following work based on Fu [17] and modified. E14 and *V. harveyi* 11593 were cultured in LB incubated at 28℃ for 24h and diluted to 5.0 × 107 CFU/mL with sterile seawater. The shrimp larvae (*Penaeus chinensis*) were obtained from a private shrimp farmer in Lianyungang, China and acclimatized in the laboratory for 3 days in PVC tanks before experimentation. Then 240 vibrant shrimp larvae with 10.0 ± 2.0 mm length were selected and divided randomly into 4 groups marked A,B,C and D, and each group was sub-grouped into 3 sub-groups named I, II, III. Group A, 50 mL sterile saline and 10 mL *B. pumilus* E14; Group B, 50 mL sterile saline and 10 mL *V. harveyi* 11593; Group C, 40 mL sterile saline and 10 mL *B. pumilus* E14 and 1 mL *V. harveyi* 11593； Group D, 60 mL sterile saline (control). The survived shrimp larvae were counted every 8 hours in the following 48 hours, during which no other feed was added.

2.9. Statistic analysis

SPSS statistical 17.0 was use in the data analyzing , and Duncan's multiple test was applied to distinguished a significant difference among samplesat the level of $p<0.05$.

3. Results

3.1. Bacterial isolates and screening

Sixteen bacterial isolates were obtained from sediments samples based on color, morphology, size, edge uniformity, and clone transparency. Eleven strains named E1-2, E2-1, E3-1, E3-2, E3-3, E5-2, E7, E11, E12, E13-2-2 and E14, respectively, were verified by agar well diffusion assay to inhibit the growth of *V. harveyi* 11593. Their DIZs were listed, and their significant differences marked in table 1. Strains E14 and E13-2-2 exhibited the top two maximum antimicrobial activities, which did not show any significant difference, but were significantly different from those of other strains. Strain E14 showed a clearer and more visible DIZ (data not shown) than E13-2-2. Strain E14 was thus selected for further research.

Table 1. The diameter of inhibitory zone from eleven isolated strains against *V. harveyi* 11593.

Strian number	Diameter Of Inhibitory Zone /mm				Strian number	Diameter Of Inhibitory Zone /mm			
	I	II	III	Mean		I	II	III	Mean
E13-2-2	18.0	17.0	16.0	17.0[a]	E7	11.0	10.0	12.0	11.0[bc]
E14	16.0	16.5	17.0	16.5[a]	E3-3	10.0	10.5	10.5	10.3[cd]
E12	12.0	12.0	12.5	12.2[b]	E5-2	11.0	11.0	9.0	10.3[cd]
E2-1	11.5	12.0	12.5	12.0[b]	E3-2	10.5	8.0	9.0	9.2[d]
E1-2	11.0	12.5	12.0	11.8[b]	E3-1	7.0	6.5	7.5	7.0[e]
E11	12.0	11.5	11.0	11.5[bc]					

a, b, c, d, e: significant differences at the 5% level.

3.2. Determination for appropriate dose-volume of the antagonistic bacterial strain E14 against V. harveyi 11593

The appropriate dose-volume of the antagonistic bacterial strain E14 against *V. harveyi* 11593 was determined using the Oxford cup method (figure 1). The DIZ increased with the E14 dose-volume. The maximum DIZ (23.7 ± 0.5 mm) appeared at 80 μL (6.0 × 107 CFU/mL) and had no significant differences with those at 100, 120, 140, and 160 μL. Hence, 80 μL was was defined as the optimized dose-volume.

3.3. Classical and phylogenetic identification of antimicrobial strain E14

To know more detail about this strain, morphological, biochemical, and genetic analyses were carried out as described in the methodology. Gram-staining showed that strain E14 was a gram-positive rod approximately 1.10–1.32 μm × 0.67–0.83 μm in size (figure 2a). The flagella (figure 2b) in strain E14 was also reported with alveolate. For the genetic characterization of strain E14, PCR was performed to amplify 16S rRNA gene as described above. The 16S rDNA sequence (1512 bp) of strain E14 was aligned for comparison with available sequences from GenBank, and the nucleotide sequence was deposited (accession number JX987716). Sequences of strain E14 shared the greatest similarity with those of *Bacillus pumilus* (identical to the *B. pumilus* MCCC 1A08208 and *B. pumilus* MCCC 1A06858). A phylogenetic tree based on the bacterial 16S rDNA sequences showed the close relationship of E14 with *B. pumilus* (figure 3).

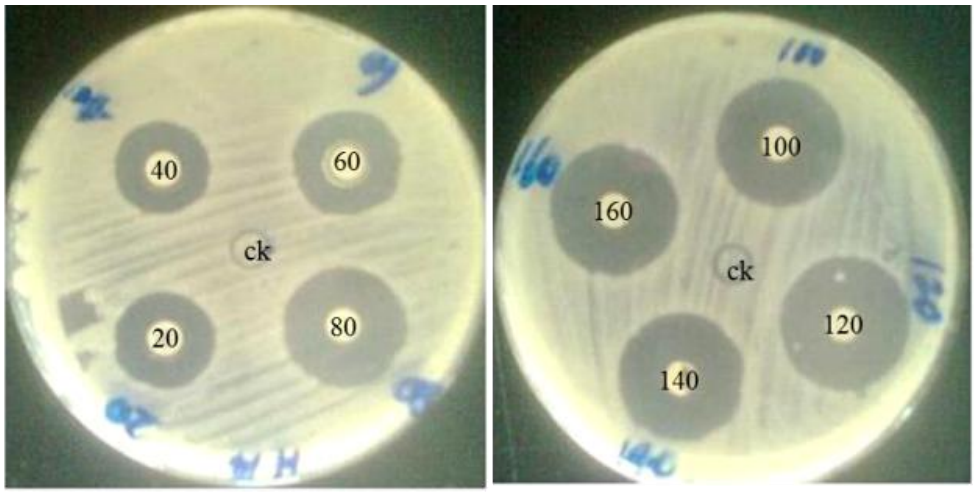

Figure 1. The effect of volume of antagonistic bacterial E14 to *V. harveyi* 11593 . The number in the plates means the volume of E14 (μL); ck, sterile 2216E medium.

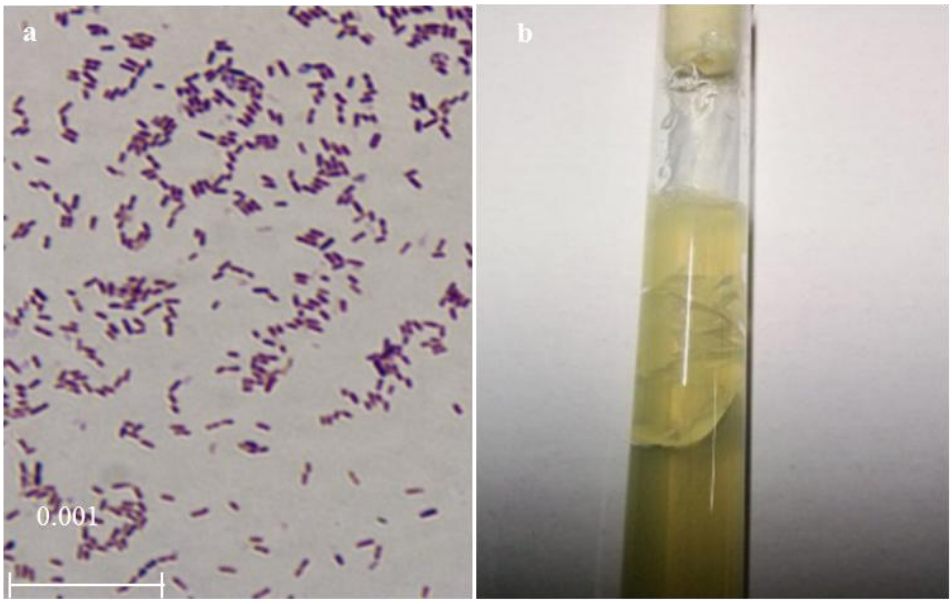

Figure 2. The morphological characteristics of strain E14. a, Gram staining; b, flagella motility.

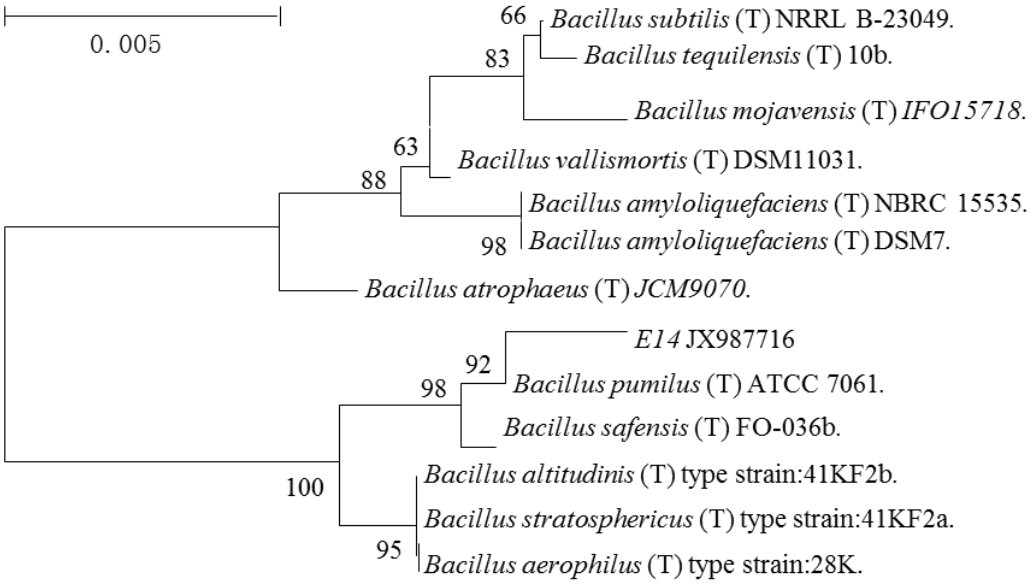

Figure 3. A phylogenetic tree analysis based on the bacterial 16S rDNA sequences of E14 and related members of the genus *Bacillu* . Numbers at nodes are levels of bootstrap support based on neighbor-joining analyses of 1000 replications. The scale bar represents 0.005 substitutions per nucleotide position.

The physiological characteristics of strain E14 are shown in table 2. Strain E14 exhibited physiological characteristics very similar to those of *B. pumilus*. Considering the results of morphological, biochemical, and genetic analyses, the isolated strain E14 was identified and named as *B. pumilus* E14.

3.4. Optimization of conditions for producing the maximum active substanse by B. pumilus E14

The effects of culture mediums, temperature, initial pH of medium, and medium volumes rate were designed to determine conditions that produce the maximum antibacterial ability of *B. pumilus* E14 (figure 4). All seven media were available for *B. pumilus* E14 (figure 4a), but antimicrobial activities with DIZ 24.5 ± 0.5 mm in LB, 2216E, and PPM showed significant differences with those of NYPD, YPG, CM, and NA. No significant difference was available in the antimicrobial activities of LB, 2216E, and PPM. LB is a widely used medium; thus, LB with 3% NaCl was adopted as the optimal medium for *B. pumilus* E14 cultivation.

Table 2. Phenotypic characteristics of strain E14 in conventional biochemical tests.

Item	E14	Item	E14	Item	E14	Item	E14
Gelatin liquefaction	+	amylolysis	-	lipid hydrolysis	-	Proteolysis （Casein）	+
Glucose	+	Cellobiose	-	Mannitol	-	Sulfuretted hydrogen	-
Lactose	-	Galactose	+	Sobitol	-	Propylene hydrochloric acid	-
Maltose	-	Inositol	-	Phenylalanine	-	Editpotassium cyanide test	+
Fructose	+	Arbaitol	-	Urea	-	Editpotassium cyanide control	+
Mannose	+	Rhamnose	-	Tartrate	+	Arginine dihydrolase	-
Xylose	-	Raffinose	-	Acetamide	+	Arginine dihydrolase control	-

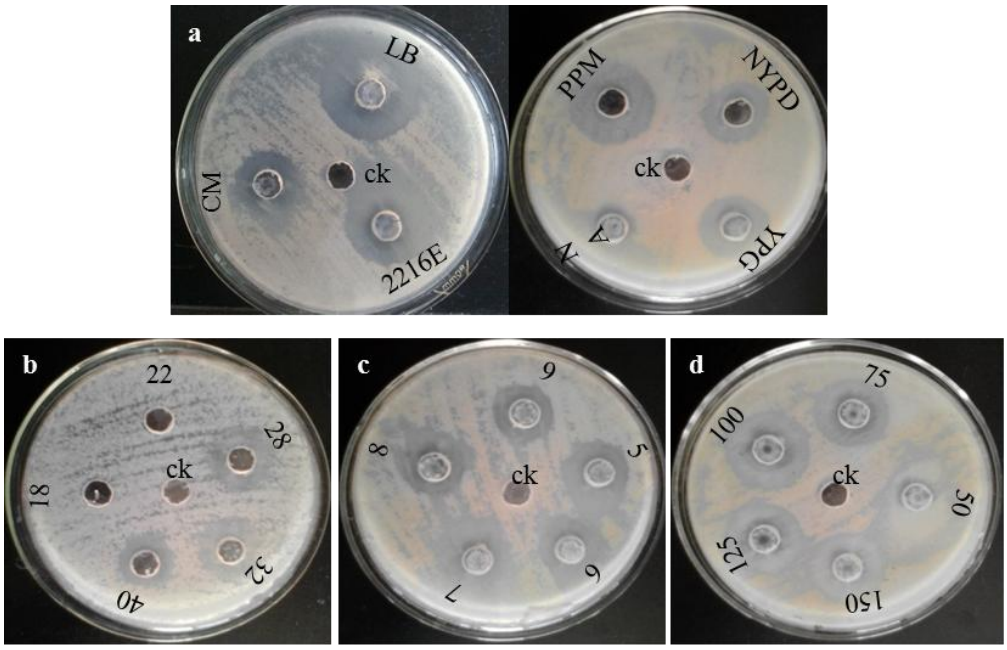

Figure 4. Optimization of various culture parameters for maximum antimicrobial production. a, different medium; b, tempreature/℃; c, pH value; d, medium volume in 500 mL triangular flask/mL.

The optimal temperature for antimicrobial production ranged from 28–40 °C, with no significant difference in antimicrobial activity reported throughout the range. No antimicrobial activity was observed at 18 °C and 22 °C, thus 28–40 °C was recommended as the optimal temperature (figure 4b).

No significant difference in antimicrobial production was observed in the initial pH range of 5.0–9.0 (figure 4c). Thus, the initial pH of 7.0 was chosen because maximum activity was detected there.

Maximum antimicrobial activity was observed at 100 mL/500 mL, which differed significantly from other dose-volumes. Hence, it was chosen as the optimized volume rate for *B. pumilus* E14 cultivation (figure 4d).

3.5. The growth curve and time course of the antibacterial activity by B. pumilus E14

The growth curve and time course of the antimicrobial production of *B. pumilus* E14 is shown in figure 5. Lag phase began before 2h, logarithmic phase at 2–24h and stable phase at 24h, but no decline phase appeared until 36h. During antimicrobial production, inhibitory activity appeared at 8h and increased significantly during cultivation up to 28h; then, it decreased until no inhibitory activity was detected at 36h. Maximum antimicrobial activity was observed at fermentation times ranging from 26–28h (no significant difference was tested within this period). Thus, *B. pumilus* E14 must be cultured for 26 h to achieve maximum production.

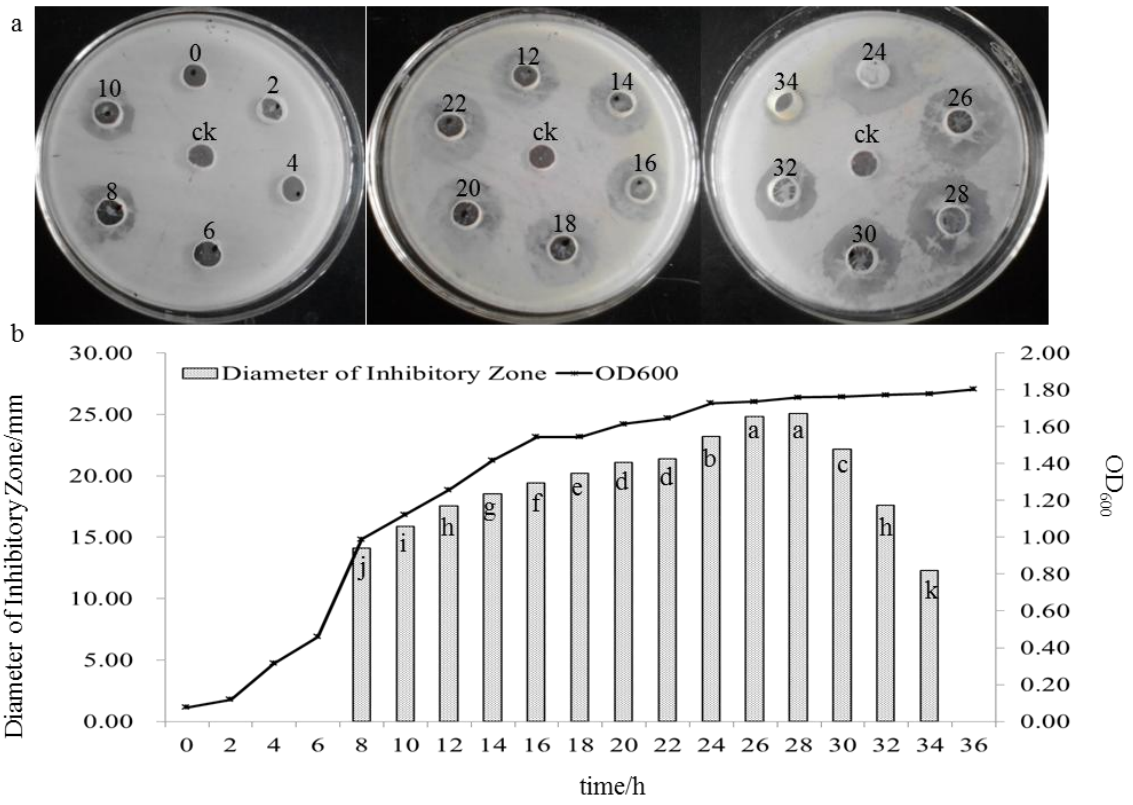

Figure 5. The growth curve and the time course of antibacterial activity by *B. pumilus* E14.
a, Antibacterial activity at the time course； b, Growth curve and Diameter of Inhibitory Zone；
Different letters within a column are significantly different ($p<0.05$) as determined by Duncan's multiple range test.

In summary, the optimized conditions for the maximum production of antimicrobial substance by *B. pumilus* E14 require it to be cultured for 26 h, with an initial pH of 7.0 at 28 °C in 100 mL/500 mL LB with 3% NaCl. The DIZ 24.5 ± 0.5 mm under optimized conditions had no significant difference with the DIZ under non-optimized conditions.

3.6. The preliminary effect of strain E14 on shrimp larvae (Penaeus chinensis) culture

The counts of survived shrimp larvae are shown in table 3. The results demonstrated significant differences in larval survival of shrimp across the 4 groups. The count of larval survival shrimp was A >D >C >B. This means survivability in the 4 groups was *B. pumilus* E14>Control> *B. pumilus* E14 mixed *V. harveyi* 11593>*V. harveyi* 11593.

Almost all larval shrimps survived in the E14 group, but 25.00% died in the *V. harveyi* 11593 group 8 h after cultured. The E14 mixed *V. harveyi* 11593 group also presented significant differences in larval shrimp survival compared to the *V. harveyi* group in the 8~24 h timeframe. The survival rate was 20.00% and 5.00% in the *B. pumilus* E14 mixed *V. harveyi* 11593 group at 24h and 32 h after culture, but 6.67 % and 0.00 % in the *V. harveyi* 11593 group at 24 h and 32 h respectively. At 48 h after culture, survived larval shrimps were only found in the *B. pumilus* E14 group. The significant difference was also observed between the *B. pumilus* E14 group and the control in the 24~48 h period.

Table 3. Comparison of larval survival of shrimp in different treating groups at different times.

Group	A				B				C				D			
Time/ h	I	II	III	Mean	I	II	III	Mean	I	II	III	Mean	I	II	III	Mean
0	20	20	20	20.0	20	20	20	20.0	20	20	20	20.0	20	20	20	20.0
8	20	20	19	19. 7[a]	15	14	16	15.0[c]	18	16	19	17.7[b]	19	19	18	18.7[ab]
16	17	17	16	16.7[a]	8	6	6	6.7[c]	10	8	10	9.3[b]	16	15	13	14.7[a]
24	12	10	12	11.3[a]	2	0	2	1.3[d]	5	4	3	4.0[c]	8	6	7	7.0[b]
32	6	5	5	5.3[a]	0	0	0	0[c]	2	1	0	1.0[c]	3	3	2	2.7[b]
40	2	3	3	2.7[a]	0	0	0	0[b]	0	1	0	0.3[b]	1	0	1	0.7[b]
48	0	0	1	0.3[a]	0	0	0	0[a]	0	0	0	0[a]	0	0	0	0[a]

a, b, c: significant differences at the 5% level.

All the results showed that the strain B. pumilus E14 inhibited the growth of V. harveyi 11593 and lengthened the lives of the larval shrimps which was fed only with bacteria.

4. Discussion

The genus Bacillus is known as one of the major probiotic resources for the production of bacterial pathogens [18-20]. Bacillus species produce biologically active lipopeptides [21], and exhibit anti-HIV [22] and other antibacterial activities [23]. B. subtilis is a common species with excellent antibacterial capabilities [23-28]. An antibacteria against V. harveyi was isolated from a mariculture

pond with shrimp, crabs, and shellfish in China. The strain was identified as B. pumilus based on morphological observation, conventional biochemical tests, and 16S rDNA sequence analysis. The inhibitory zone diameter against V. harveyi of the bacterium, named E14, was 24.5 ± 0.5 mm, thus making it a good candidate for an alternative probiotics agent against V. harveyi. 11593 Interestingly, B. pumilus E14 maybe add a new member to the genus Bacillus for probiotics. More research is needed on this topic.

Strains of B. pumilusare used for enzyme production in an industrial scale and many other fields [29]. B. pumilus is another well-known species with an excellent capability of producing multifarious biological activities [30-34]. B. pumilus E14, which is gram-positive and motile, produces antibacterial substances that can hydrolyze gelatin and casein. The strain E14 tested positive for glucose, mannose, fructose, galactose , tartrate, acetamide and potassium cyanide; whereas it tested negative for lactose, maltose, xylose, cellobiose, inositol, arbaitol, rhamnose, raffinose, mannitol, sobitol, phenylalanine, urea, sulfuretted hydrogen, propylene hydrochloric acid, and arginine dihydrolase. Most of its characteristics were shared with other B.pumilus strains [35], except no amylase and lipid hydrolysis were detected in B. pumilus E14.

The antimicrobial substances produced by strains of genus Bacillus is attributed to mazy genetic regulation, and different culture conditions may induce the different antimicrobial substances. All seven mediums were available for B. pumilus E14, and significant differences among them were reported. Antimicrobial production could be achieved from 28–40 °C. This result implies that B. pumilus E14 is mesophilic, i.e., it is a heat-resistant vegetative cell. B. pumilus E14 is also a neutrophilic species given that maximum activity had been detected at an initial pH of 7.0. Maximum antimicrobial activity was tested at 26–28 h. These results are shared with a previous study, which showed that most Bacillus antimicrobial compounds are produced in the late log phase, possibly due to sporulation [36].

The safety and effectiveness test of E14 used in shrimp larvae（Penaeus chinensis) culture was very limited in this paper. Further evidence is need in the future, for example, expanded culturing volume and sample size of tested shrimp. Additionally, it will also be helpful to reduce observing intervals to every 2 h, especially after 24 h after culture.

The co-culture pond is a complex ecosystem, such that a number of factors (e.g., the introduction of non-native microbial species, input and circulation of nutrients, and human interference) can mediate microbial communities. Competition among microbes for nutrients and space in marine environments is a powerful selection tool for marine microorganisms employed in the production of marine natural products for medical and industrial purposes [37]. The discovery of antimicrobial agents against V. harveyi from a pond with shrimp, crabs, and shellfish in China is dependent on various substances exhibiting different antimicrobial activities. These have been found among certain bacteria due to the specialized role they play in their respective hosts [38]. Bacterium-bacterium antagonism in the environmen may influence its species richness and diversity , thus those antagonisms may become important variables in the ecology of pelagic bacteria, and in bacterium-mediated carbon cycling in the ocean [39]. B. pumilus E14 comes from the co-culture pond; thus, its use may prevent complementarity-mutual exclusiveness and turn it into the dominant bacterial community in the pond.

This strain may also solve problems due to increasing antibiotics, benefit the environment, and ultimately ensure sustainable aquaculture development.

5. Conclusion

The strain E14 antibacterium against Vibrio harveyi 11593 has been obtained from a mixed pond with Penaeus japonicus Bate, Portunus trituberculatus and Ruditapes philippinarum in China. The strain belongs to Bacillus pumilus and has a 24.5 ± 0.5 mm inhibitory zone diameter. The gram-positive, encapsulated, and motile bacterium is around 1.10–1.32 μm × 0.67–0.83 μm in size. B. pumilus E14 also shows protease activity. Optimized conditions for antimicrobial substance production of B. pumilus E14 require that it be cultured for 26 h at 28 °C, with an initial pH of 7.0 in 100 mL/500 mL LB with 3% NaCl.

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