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Properties of *Enterococcus faecalis*, a new probiotic bacterium isolated from the intestine of snakehead fish (*Channa striatus* Bloch)

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The present study aimed to isolate and characterize the lactic acid bacteria (LAB) from the intestine of snakehead (*Channa striatus*) fingerling to be used as a new probiotic in aquaculture. The total colony count of bacteria in the fish intestine was 2.1 × 10^6 cfu/g. Five LAB were isolated from the intestine of twenty fish and one of these isolates, LAB-4 was identified as *Enterococcus faecalis* by conventional and molecular techniques. Probiotic properties showed that this LAB could grow from pH 3 to 8, but the best growth was observed at pH 7. *E. faecalis* grew at 0.15 and 0.3% bile salt concentrations, from 15to 45°C and at 4% NaCl in de Man Rogosa and Sharp (MRS) broth. This bacterium showed *in vitro* inhibitory activity against three fish pathogens viz., *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Shewanella putrefaciens*. Antibiotic sensitivity tests indicated that *E. faecalis* was resistant instantly to: streptomycin, gentamycin and kanamycin, intermediate to tetracycline, and sensitive to chloramphenicol, amoxicillin and ampicillin antibiotics. Moreover, significantly (*P*<0.05) improved survival of fish was observed when fed with *E. faecalis*-fortified diet in an *in vivo* challenge test using *A. hydrophila*. Based on the results, it can be concluded that *E. faecalis* is a promising probiotic for snakehead fish against pathogenic infestation.

Key words: Isolation, characterization, probiotic, *Enterococcus faecalis*, snakehead fish.

INTRODUCTION

Since the use of antibiotics has negative effects on animals and environment, several alternative strategies such as probiotic bacteria have been suggested (Lauzon et al., 2008; Pan et al., 2008). The use of lactic acid bacteria (LAB) as main probiotics can control potential pathogens in aquaculture (Ringø and Gatesoup, 1998;

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Aly et al., 2008; Kim and Austin, 2008). Some LABs are normal microbiota in the gastrointestinal (GI) tract of healthy aquaculture animals that can be used as probiotic (Kim and Austin, 2008). Probiotics can prevent the growth of harmful bacteria by colonization in the gut and produce organic acids and antimicrobial compounds (Ruiz-Moyano et al., 2008; Das et al., 2010). In addition, probiotic bacteria appears to have a wide variety of benefits such as lactose digestion, resistance to enteric pathogens, anti-colon cancer effect, small bowel bacteria overgrowth, allergy, immune system modulation and reduction in serum cholesterol to the host (Cebeci and Gurakan, 2003; Salminen et al., 2004). Some properties such as acid and bile salt tolerance, antibacterial activity against pathogens and antibiotic susceptibility are important tools to be investigated, when selecting potential probiotic bacteria (Cebeci and Gurakan, 2003; Balcázar et al., 2008; Pan et al., 2008). Furthermore, challenge tests have been suggested as a golden standard to be included when evaluating probiotics (Aly et al., 2008) and the resistance to enteric pathogens (Cebeci and Gurakan, 2003).

Snakehead (*Channa striatus*) is a popular food fish in Southeast Asian countries (Jais et al., 2002; Rahman et al., 2012, 2013; Muntaziana et al., 2013).

As there is less information accessible on the bacterial community in the gastrointestinal tract of fish (Navarrete et al., 2009; Zhou et al., 2009; Wu et al., 2012) and no information available on bacteria in the intestine of snakehead, the first aim of the present study was to isolate and identify LAB from the intestine of snakehead fingerlings. The second aim was to evaluate the characteristics of an isolated LAB. As *Aeromonas hydrophila*, a common freshwater fish pathogen is causing high mortality in different life stages of fish (Aly et al., 2008; Rengpipat et al., 2008); the third aim of the present study was to show if dietary supplementation of a LAB had any effect in a challenge study using *A. hydrophila*.

MATERIALS AND METHODS

Sampling

A total of 60 healthy snakehead fingerling fish (*Channa striatus*) with the average weights of 5.0-6.0 g were collected over three times from a fish farm in Seri Kembangan, Selangor, Malaysia (3.0333° N, 101.7167°E), and transferred to the Aquatic Animal Health Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The fishes were maintained in a fiberglass tank (1000 l) at UPM and after 14 days of collection, twenty fish were randomly selected, anesthetized with tricaine methanesulphonate (100 mg/l) (Sigma, Chemical Co. St. Louis, MO, USA), disinfected with alcohol (70%), and dissected under antiseptic conditions. The intestines were removed and homogenized in a sterile saline solution (0.85% v/w), as described elsewhere (Rengpipat et al., 2008).

Isolation of LAB from the fish intestine

Homogenized intestine samples were serial diluted (up to 10^{-4}) and 0.1 ml of each dilution was spread onto triplicate tryptic soy agar (TSA) (Sigma, USA) plates and incubated at 30°C for 48 h to count the colony forming units (cfu) of bacteria. The homogenized stomach samples were also immersed in de Man Rogosa and Sharp (MRS) broth (Sigma, USA) and incubated at 30°C for 24 h. After incubation, 0.1 ml of the cultured broth was spread onto MRS agar containing bromo-cresol purple (0.17 g/l, Sigma, USA) (Rengpipat et al., 2008). The plates were incubated at 30°C for 48 h under anaerobic conditions (anaerobic jar, Oxoid, USA). Colonies of yellow appearance were transferred to MRS agar and sub-cultured three times to obtain pure colonies (Nguyen et al., 2007; Kopermsub and Yunchalard, 2010).

Antagonistic effect test for the selection of LAB

Primary antibacterial activity of the isolates was studied by disc diffusion technique using cell-free cultured broth to select one isolate with the highest inhibitory activity against *A. hydrophila*, a procedure previously suggested by Aly et al. (2008). *A. hydrophila* was cultured in Tryptic Soy Broth (TSB, Sigma, USA), incubated at 30°C for 24 h and then streaked on TSA plates. Bacterial cells of the cultured MRS broths of five LAB isolates were precipitated at 4°C and 8586 g for 5 min (Eppendorf, 5810R, Germany). Sterile discs were immersed in the supernatants, air dried, and placed on TSA plates. The plates were incubated at 30°C for 24-48 h to observe inhibition zones (Lauzon et al., 2008).

Identification of selected LAB by conventional and molecular techniques

The carbohydrate fermentation pattern of the most promising LAB isolate from the antagonistic test was determined using an API kit (50 CH, API 50 CHL medium, bioMérieux, France) to identify the selected LAB (Aly et al., 2008). Further identification of the LAB isolate was carried out using 16S rRNA gene sequencing as described by Pond et al. (2006). Briefly, the genomic DNA of the isolates was extracted using a DNA extraction kit (Genomic DNA Mini kit, Genaid, bioMérieux, France). Polymerase chain reaction (PCR) was used to amplify the 16S rRNA of the extracted DNA using the primers pAF 5' AGA GTT TGA TCC TGG CTC AG 3' as forward and phR 5' AAG GAG GTG ATC CAG CCG CA 3' as reverse primers. The purified products were sequenced by NHK Sequencing Service Laboratory in South Korea (NHK Bioscience Solutions SDN BHD) using the specific primers (pAF and phR). In 16S rRNA gene sequencing, approximately 1500 bp was analyzed by BioEdit software and then compared with BLAST data from GenBank in the National Center for Biotechnology Information.

Probiotic properties

pH tolerance

Acid tolerance of the selected bacterium at different pH levels was investigated. MRS broths with different pH levels; 2, 3, 4, 5, 6, 7 and 8 were prepared using 1% HCl (Sigma, Chemical Co. St. Louis, MO, USA) and 1 N NaOH (Sigma, USA), and distributed into 25 ml bottles. The broth media and the control bottles were autoclaved at 121°C for 15 min and soon after cooling, they were inoculated with an overnight culture (30 µl) of the selected strain in the MRS broth followed by incubating at 30°C. Optical density at 600 nm (OD_{600}) was measured by a spectrophotometer (Shimadzu, UV-1601, Japan) after 2, 4 and 8 h of incubation. The viability of the isolate was also controlled by duplicate inoculation on MRS agar plates as described elsewhere (Balcázar et al., 2008; Kim and Austin, 2008).

Bile salt tolerance

Bile salt tolerance was tested in MRS broth with 0, 0.15 and 0.3% (w/v) Oxgall bile salt (Sigma, USA). Duplicate bottles (25 ml medium) of MRS broth containing different concentrations of filtered bile salt were inoculated by 30 µl of the cultured strain and incubated at 30°C. Growth rate was assessed by measuring OD_{600} after 0, 2, 4 and 8 h post-incubation (Balcàzar et al., 2008; Kim and Austin, 2008).

Growth at different NaCl concentrations

Growth rate of the LAB strain at different sodium cloride concentrations was determined in MRS broth by adding 0, 1, 2, 3 and 4% NaCl (Sigma, USA). The duplicate bottles (25 ml medium) containing different levels of NaCl were inoculated with 30 µl cultured bacterium and incubated at 30°C. OD_{600} was measured after 0, 2, 4, 8, 16 and 24 h of incubation as described by Kim and Austin (2008).

Growth at different temperature levels

Growth of the selected LAB strain was evaluated at nine different temperatures, viz., 10, 15, 20, 25, 30, 35, 40, 45 and 50°C. 30 µl of an overnight MRS broth culture was transferred to duplicate MRS broth bottles and incubated at 30°C. OD_{600} was measured after 0, 4, 8, 16 and 24 h of incubation according to Balcázar et al. (2008).

Antibacterial activity against three fish pathogens

Three freshwater fish pathogens; *Aeromonas hydrophila, Pseudomonas aeruginosa* (obtained from the pure stock kept at Aquatic Animal Health Unit, Faculty of Veterinary Medicine, UPM, Malaysia) and *Shewanella putrefaciens* (ATCC-49138, Lot: 4987125) were used to test the antibacterial potential of the LAB; *in vitro* growth inhibition of the target bacteria. This was tested using disc diffusion and well diffusion techniques previously described by Balcázar et al. (2008). The pathogenic bacteria were cultured in TSB and incubated at 30°C for 24 h. Subsequently, 30 µl of the culture with 10^3 cfu/ml cells were spread onto duplicate TSA plates. The selected LAB strain was cultured in MRS broth at 30°C for 18 h. The cells were harvested by centrifugation at 7155 g and 4°C (Eppendorf, 5810R, Germany) for 5 min and the supernatant was used for antibacterial activity by the disc and well diffusion methods.

Antibiotic sensitivity test

Antibiotic sensitivity of the selected strain were tested against eight common antibiotics [gentamycin (GM, 10 µg), streptomycin (S, 10 µg), amoxicillin (AMX, 25 µg), tetracycline (TE, 30 µg), chloramphenicol (C, 30 µg), ampicillin (AM, 10 µg), erythromycin (E, 15 µg) and kanamycin (K, 30 µg)] using disc diffusion technique (Akinjogunla et al., 2010). Fifty µl of the 24 h broth cultured strain were spread on MRS agar and subsequently, antibiotic Bio-discs (bioMérieux, France) were placed on duplicate plates using the Oxoid Disc Dispenser System (USA). The plates were incubated at 30°C for 24-48 h to measure the inhibition zones (Kim and Austin, 2008). The interpretations of zone sizes were expressed based on the standard table of the Kirby-Bauer Test (Bauer et al., 1966).

Experimental design in challenge test

In total, 120 snakehead fingerlings with an average weight of 6.5 ± 0.3 g were randomly distributed into 12 aquaria each (45 × 30 × 30 cm) containing 10 fish. The experiment was set up with a completely randomized design in treatments, each of which was triplicated. Four treatments were used: (T_c) LAB was not included in the diet and the fish were not injected with the pathogen, (T_1) LAB was supplemented to the diet at 10^7 cfu/g and the fish were not injected with the pathogen, (T_2) LAB was included in the diet; similar level as for T_1 and the fish were injected (10^7 cfu/ml) with the pathogen and (T_3) LAB was included in the diet; similar level for T_1 and the fish were not injected with the pathogen. To prepare experimental diets, the LAB was cultured in MRS broth (Sigma, USA) and incubated at 30°C for 18 h. The LAB was harvested using refrigerated centrifuge (Eppendorf 5810R, Germany) at 4°C and 1207 g for 30 min. The bacterial pellet was washed twice with sterile saline solution and adjusted at 10^7 cfu/ml based on optical density and total plate count of the LAB during 24 h. The prepared suspension was mixed with commercial feed by adding 200–300 ml distilled water per kg diet; dried at room temperature (25°C); stored in sterile plastic bags and placed in refrigerator at 4°C. The LAB-fortified diet preparation was repeated every two weeks during the five-week feeding trial. Commercial dry feed (MAY FISH FEED LTD SDN BHD) was served as a basis of the experimental diet. Proximate composition of the diet including dry matter (DM), crude protein (CP), crude fiber (CF), lipid and ash were analyzed according to AOAC (2000) and were 92.56, 33.81, 3.12, 7.73 and 3.7%, respectively. The experimental fish were acclimatized for two weeks prior to use for the experiment. All fish were fed twice (10 am and 4 pm) daily at the rate of 20 g/kg of estimated biomass for five weeks.

Intraperitoneal injection

Intraperitoneal injection was used to introduce *A. hydrophila*. Briefly, *A. hydrophila* was cultured in TSB and incubated at 30°C for 18 h. The cultured broth was centrifuged (Eppendorf, 5810 R, Germany) at 1006 g and 4°C for 30 min and pellet bacteria were washed two times with sterile saline solution. Then, the concentration of *A. hydrophila* was adjusted to 10^7 cfu/ml by total plate count (TPC) and optical density. After two weeks of feeding with or without LAB-fortified diet, a 0.1 ml aliquot of *A. hydrophila* was injected to T1 (fish fed LAB in the diet) and T3 (no addition of LAB in the diet) in the morning before feeding. As in the control, 0.1 ml sterile water was injected to T2 (with LAB and no pathogen) and Tc (no LAB and no pathogen) to make uniform condition for injection stress (Aly et al., 2008; Abdel-Tawwab and Ahmad, 2009). The challenge experiment was terminated two weeks after injection.

Investigation of infected fish in challenge test

During the challenge test, dead fish were dissected. The anatomy of internal organs; intestine, liver, kidney, spleen and abdomen were investigated to study the symptoms of infection. In addition, infected organs were cultured (by swab) on TSA plates and incubited at 30°C for 24 h and sub cultured two times. Three to five pure colonies from infected organs of each treatment were Gram stained and API-20E kit (bioMérieux, France) tested. This was conducted to confirm *A. hydrophila* infection according to Rengpipat et al. (2008).

Statistical analysis

The statistical analysis was conducted to compare the quantitative results in probiotic properties and *in vivo* challenge test by the

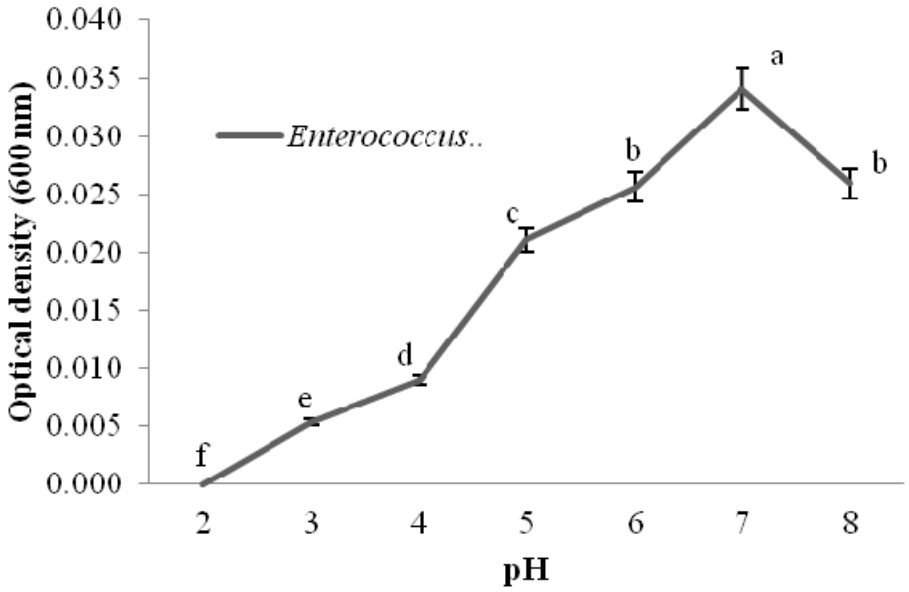

Figure 1. pH tolerance of *E. faecalis* after 2 h of incubation at different pH. Vertical bars indicate ± SE. Means with the same letter are not significantly different ($P > 0.05$).

analysis of variance (ANOVA) using the SAS program (Version 8.2). Duncan's multiple range test was performed to determine the differences among the treatment means (α = 0.05) (SAS, 2001).

RESULTS

Isolation, selection and identification of LAB

Plate counts of bacteria indicated that LAB were a minor part of the microbiota in the stomach of snakehead as they accounted for only 12.2% of the total bacterial count of 2.1×10^6 cfu/g in the intestine. Five yellow colonies of LAB coded as LAB-1 to LAB-5 were isolated from the intestine of the snakehead fingerlings. The isolates were Gram-positive, catalase- and oxidase- negative and were short rod or cocco- bacilli shaped. The antibacterial test, LAB-4 showed a significantly higher ($P< 0.05$) inhibition zone against *A. hydrophila* than the other LAB. Based on this criterion, strain LAB-4 was selected for further identification and probiotic characterization. 16S rRNA gene sequence analysis of LAB-4 showed that the bacterium was closely related to *E. faecalis* (100% similarity) with accession no. HM579789.

pH tolerance

pH tolerance of *E. faecalis* showed that the growth rate of this strain significantly ($P < 0.05$) changed when grown at different pH; 2 to 8 (Figure 1). There was no growth and viability at pH 2 after 2 h incubation, but the strain grew well at pH 7.

Bile salt tolerance

Three bile salt concentrations (0, 0.15 and 0.3%) were studied to find out the tolerance of *E. faecalis* after 2, 4 and 8 h of incubation. This bacterium not only showed viability but also exhibited proliferation in all three concentrations for all incubation times (Figure 2). As bile salt concentration increased, the growth rate of *E. faecalis* significantly ($P < 0.5$) decreased after 2 h of incubation. A similar trend was also observed after 4 and 8 h post-incubation.

Growth in different NaCl concentrations and temperature levels

E. faecalis showed good viability and growth rates in 0 to 4% NaCl after 4, 8, 16 and 24 h incubation. However, the growth decreased with increasing NaCl concentrations. Moreover, the growth rate of *E. faecalis* was significantly ($P < 0.05$) increased with increasing temperature up to 30°C, but decreased at 40°C. No growth was observed at 10 and 50°C and the viability was observed to be nil at 50°C.

Antibacterial activity test

Results from the disc diffusion technique showed that *E. faecalis* significantly ($P < 0.05$) inhibited *in vitro* growth of *A. hydrophila* and *S. putrefaciens,* but had no impact on *P. aeruginosa* growth. *E. faecalis* showed higher inhibi-

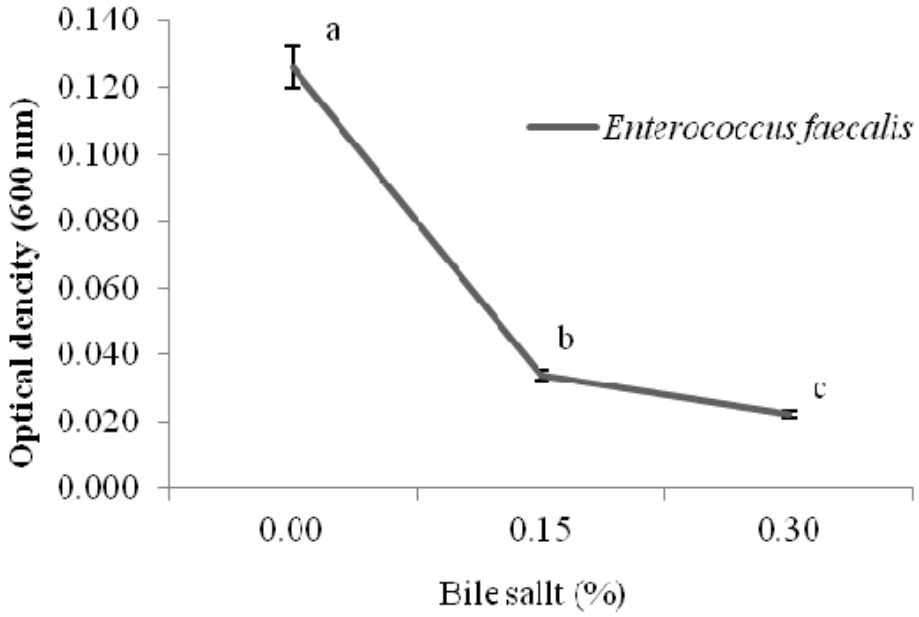

Figure 2. Bile salt tolerance of *E. faecalis* after 2 h incubation in different concentrations. Vertical bars indicate ± SE. Means with the same letter are not significantly different ($P > 0.05$).

tory activity against the three pathogens when tested by the well diffusion technique and a significant ($P < 0.05$) higher effect was noted against *A. hydrophila* as compared to *P. aeruginosa* and *S. putrefaciens* (Figure 3). The inhibition zones against three pathogenic bacteria by using the well diffusion method was significantly ($P < 0.05$) higher than the results of the disc diffusion method.

Antibiotic sensitivity test

With respect to antibiotic susceptibility profiles test, *E. faecalis* was found to be resistant (R) to tetracycline (TE), streptomycin (S), gentamycin (GM) and kanamycin (K), intermediate (I) to erythromycin (E) and sensitive (S) to chloramphenicol (C), amoxicillin (AMX) and ampicillin (AM)

Effect of *A. hydrophila* challenge test on experimental fishes

All fish in treatment group T_3 (without the supplementation of LAB to the diet and injected with *A. hydrophila*) were dead in the three replicate tanks after 48 h (Table 1). The survivability of snakehead fingerlings was 100% for the control group (Tc) and treatment group T_2 (LAB-fortified diet without injection of *A. hydrophila*). Treatment group (T_1) fed with *E. faecalis* and injected with the pathogen showed 56.6% mortality at 48 h after injection; afterwards no mortality was observed. Statistical analysis of the survival rate of fish fed fortified diet with *E. faecalis* and exposed to *A. hydrophila* (T_1) was significantly ($P < 0.05$) improved as compared to fish fed non LAB-fortified diet but exposed to *A. hydrophila* (T_3). In the latter group, 100% mortality was observed 48 h post-injection.

The anatomy of dead (infected) fish in group T_3 showed hemorrhage in kidney, spleen, eye and abdominal muscles in all fishes. In addition, swollen abdomen with yellowish liquid was observed. The results of the challenge test confirmed that *A. hydrophila* is capable of inducing mortality in snakehead fish, but the survival of fish fed with LAB supplemented diet was significantly improved.

DISCUSSION

Total colony count of bacteria in intestine

The results of the present study showed a low population level of culturable bacteria (2.1×10^6 cfu/g) in the intestine of snakehead. This level is higher than that reported in the foregut (7×10^3 to 7×10^4 cfu/g), midgut (4×10^3 cfu/g) and hindgut (4.5×10^4 to 4.5×10^5 cfu/g) of Atlantic cod *(Gadus morhua* L.) (Ringø et al., 2006). Furthermore, the current study showed that LAB was a minor part of microbiota in snakehead intestine. Ringø et al. (2006) described that the gut microbiota of fish are less diversed than in terrestrial animals.

pH and bile salt tolerance

Kim and Austin (2008) described that one of the most important criteria for characterization of probiotic bacteria

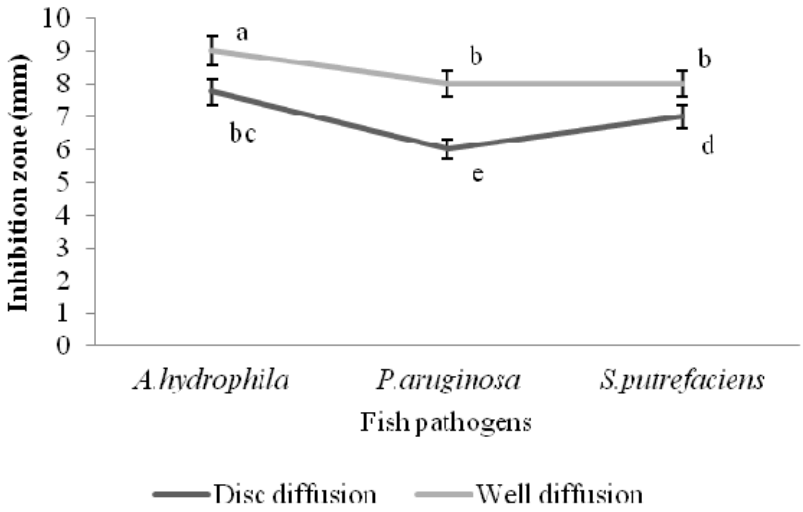

Figure 3. Comparison of disc and well diffusion techniques in antagonistic effect test with *E. faecalis* against pathogenic bacteria. Vertical bars indicate ± SE. Means with the same letter are not significantly different ($P > 0.05$).

Table 1. Effect of four experimental groups: with and without *E. faecalis* in diet, and with and without pathogen injection on fish survival (means ± SE).

Treatment	Survival (%)
Tc	100 ± 4.51^{a}
T_1	53.4 ± 3.32^{b}
T_2	100 ± 4.77^{a}
T_3	0 ± 0.01^{c}

SE: Standard error, Tc: fish fed without *E. faecalis* in the diet and without pathogen injection, T_1: fish fed with *E. faecalis* in the diet and with pathogen injection, T_2: fish fed with *E. faecalis* in the diet and without pathogen injection, T_3: fish fed without *E. faecalis* in the diet with pathogen injection. Means with the same letter in columns are not significantly different ($P > 0.05$), ($n = 30$).

is their tolerance to acidic conditions. The results of the present study displayed that *E. faecalis* was able to grow at pH from 3 to 8. This result is in agreement with Cebeci and Gurakan (2003) and Nguyen et al. (2007) who reported the viability of *L. plantarum* at pH 4 to 10 and Balcázar et al. (2008) reported the growth activity of *L. fermentum* and *L. plantarum* at pH 2.5 to 6.5.

Bile salt tolerance has been suggested as an important criterion for probiotic bacteria to grow and survive in fish intestine (Balcázar et al., 2008). The results of the present study are in agreement with Cebeci and Gurakan (2003), Nguyen et al. (2007) and Balcàzar et al. (2008), who reported the tolerance of *Lactobacillus* species to different bile salt concentrations. Probiotics that tolerate at low pH and bile salt levels are able to pass through the stomach and then colonize and grow in the intestine as well as survive there in stress conditions (Cebeci and Gurakan, 2003).

Growth in different NaCl concentrations and temperatures

E. faecalis in the present study showed high potential proliferation in an environment up to 4% NaCl. Nguyen et al. (2007) reported that *L. plantarum* PH04 could grow at 6% NaCl and at temperatures between 25 and 45°C. Kim and Austin (2008) reported that two probiotic carnobacteria strains isolated from rainbow trout intestine were able to grow in up to 15% (w/v) NaCl and at temperatures ranging from 10 to 37°C. The growth ability of *Carnobacterium* strains isolated from brown trout (*Salmo trutta*) was limited in 8% NaCl but they grew at temperatures between 4 and 45°C (Gonzalez et al., 2000). Similar results were also reported by Samelis et al. (1994) and Thapa et al. (2006). The results of this study showed that *E. faecalis* could grow within a wide range of temperature (15 - 45°C).

Antibacterial and antibiotic susceptibility tests

The selected strain, *E. faecalis* showed *in vitro* growth inhibition against the three tested fish pathogens, especially *A. hydrophila* and these results are in accordance with Rengpipat et al. (2008), who reported inhibition activity against *A. hydrophila* using cell-free cultured broths of five LAB. Kim and Austin (2008) demonstrated antibacterial ability of *Carnobacterium* strains (isolated from rain-

bow trout intestine) against *A. hydrophila* and *A. salmonicida*. Antibiotic susceptibility test can indicate resistance or sensitivity to specific antibiotics. LAB showing resistance to specific antibiotics indicates that these bacteria can be included in the diet at the same time if antibiotic treatment is required. Antibiotic resistance is an advantageous capacity as the intestinal microbiota can quickly recover after antibiotic treatment (Cebeci and Gurakan, 2003; Kim and Austin, 2008).

Challenge test

The challenge test indicates that snakehead fish was infected readily by *A. hydrophila* but the survival was improved when they were fed with dietary *E. faecalis*. All infected fish showed hemorrhage in internal organs with swollen abdomen. Similar observations were also reported by Rengpipat et al. (2008) and Aly et al. (2008) in their studies with sea bass (*Lates calcarifer*) and Nile tilapia (*Oreochromis niloticus*), respectively. According to Abdel-Tawwab and Ahmad (2009), the number of *A. hydrophila* cells were declined after an artificial challenge in fish with Spirulina (*Arthrospira platensis*) and that bacterial numbers were lower in the liver and kidney of fish treated with probiotic than the control. Therefore, *E. faecalis* can be used as a high potential probiotic to inhibit *A. hydrophila* activity in snakehead fish culture.

Conclusion

The present study revealed that *E. faecalis* has potential probiotic properties. In addition, it suggests that *E. faecalis* is a safe alternative to antibiotics to inhibit *A. hydrophila* activity in snakehead fish culture.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Abdel-Tawwab M, Ahmad MH (2000). Live Spirulina (*Arthrospira platensis*) as a growth and immunity for Nile tilapia, *Oreochromis niloticus* (L.), challenged with pathogenic bacteria *Aeromonas hydrophila.* Aquacult. Res. 40(9):1-10.

Akinjogunla OJ, Inyang CU, Akinjogunla VF (2010). Bacterial species associated with anatomical parts of fresh and smoked bonga fish (*Ethmalosa fimbriata*): Prevalence and Susceptibility to Cephalosporins. J. Microbiol. 6:87-97.

Aly SM, Abd-El-Rahman AM, John G, Mohamed MF (2008). Characterization of some bacteria isolated from *Oreochromis niloticus* and their potential use as probiotics. Aquaculture 277(1-2):1-6.

Balcázar JL, Vendrell D, de Blas I, Ruiz-Zarzuela I, Muzquiz JL, Girones O (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquacult. 278(1-4):188-191.

Bauer AW, Kirby WM, Sherris JC, Turck (1966). Antibiotic susceptibility testing by a standard single disc method. Am. J. Clin. Pathol. 45:493-496.

Cebeci A, Gurakan C (2003). Properties of potensial probiotic *Lactobacillus plantarum* strains. Food Microbiol. 20(20):511-518.

Das S, Ward LR, Burke C (2010). Screening of marine *Streptomyces* spp. for potential use as probiotics in aquaculture. Aquacult. 305(1-4):32-41.

Gonzalez CJ, Encinas JP, Garcia-Lopez ML, Otero A (2000). Characterization and identification of lactic acid bacteria from freshwater fishes. Food Microbiol. 17:383-391.

Jais AMM, Hazliana H, Kamalludin MH, Kader SA, Rasedee A (2002). Effect of haruan (*Channa striatus*) fillet extract on blood glucose and cholestrol concentration and differential white blood cells counts in rats and mice. Paper presented at the Proceedings of the Regional Symposium on Environment and Natural Resources. Hotel Renaissance Kuala Lumpur, Malaysia

Kim DH, Austin B (2008). Characterization of probiotic carnobacteria isolated from rainbow trout (*Oncorhynchus mykiss*) intestine. Lett. Appl. Microbiol. 47(3):141-147.

Kopermsub P, Yunchalard S (2010). Identification of lactic acid bacteria associated with the production of plaa-som, a traditional fermented fish product of Thailand. Int. J. Food Microbiol. 138(3):200-204.

Lauzon HL, Gudmundsdottir S, Pedersen MH, Budde BB, Gudmundsdottir BK (2008). Isolation of putative probionts from cod rearing environment. Vet. Microbiol. 132(3-4): 328-339.

Muntaziana MPA, Amin SMN, Rahman MA, Rahim AA, Marimuthu K (2013). Present culture status of the endangered, *Channa striatus* (Bloch, 1793). Asian J. Anim. Vet. Adv. 8(2):369-375.

Navarrete P, Espejo RT, Romero J (2009). Molecular analysis of microbiota along the digestive tract of juvenile Atlantic salmon (*Salmo salar* L.). Microbial Ecol. 57:550-561.

Nguyen TDT, Kang JH, Lee MS (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. Int. J. Food Microbiol. 113(113): 358-361.

Pan X, Wu T, Zhang L, Song Z, Tang H, Zhao Z (2008). *In vitro* evaluation on adherence and antimicrobial properties of a candidate probiotic *Clostridium butyricum* CB2 for farmed fish. J. Appl. Microbiol. 105:1623-1629.

Pond MJ, Stone DM, Alderman DJ (2006). Comparison of conventional and molecular techniques to investigate the intestinal microflora of rainbow trout (*Oncorhynchus mykiss*). Aquacult. 261(1):194-203.

Rahman MA, Arshad A, Amin SMN (2012). Growth and production performances of threatened snakehead fish, *Channa striatus* at different stocking densities in earthen ponds. Aquacult. Res. 43:297-302.

Rahman MA, Arshad A, Amin SMN, Shamsudin MN (2013). Growth and survival of fingerlings of a threatened snakehead, *Channa striatus* (Bloch) in earthen nursery ponds. Asian J. Anim. Vet. Adv. 8(2):216-226.

Rengpipat S, Rueangruklikhit T, Piyatiratitivorakul S (2008). Evaluation of lactic acid bacteria as probiotic for juvenile seabass(*Lates calcalifer*). Aquacult. Res. 39(2):134-143.

Ringø E, Gatesoupe FJ (1998). Lactic acid bacteria in fish: a review. Aquacult.160(3-4):177-203.

Ringø E, Sperstad S, Myklebust R, Refstie S, Krogdahl Å (2006). Characterisation of the microbiota associated with intestine of Atlantic cod (*Gadus morhua* L.) The effect of fish meal, standard soybean meal and abioprocessed soybean meal. Aquacult. 261:829-841.

Ruiz-Moyano S, Martín A, Benito MJ, Nevado FP, Córdoba MDG (2008). Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in Iberian dry fermented sausages. Meat Sci. 80:715-721.

Salminen S, Wright AV, Ouwehand A (2004). *Lactic Acid Bacteria*, Third ed. Marcel Dekker, Inc., New York.

Samelis J, Maurogenakis F, Metaxopoulos J (1994). Characterisation of lactic acid bacteria isolated from naturally fermented Greek dry salami. Int. J. Food Microbiol. 23:179-196.

SAS Institute Inc (2001). SAS User's Guid: Statistics, SAS Inst. Inc., Cary, NC

Thapa N, Pal J, Tamang JP (2006). Phenotypic identification and technological properties of lactic acid bacteria isolated from traditionally processed fish products of the Eastern Himalayas. Int. J. Food Microbiol. 107:33-38.

Wu S, Tian J, Wang G, Li W, Zou H (2012). Characterization of bacterial community in the stomach of yellow catfish (*Pelteobagrus fulvidraco*). World J. Microb. Biot. 28:2165-2174.

Zhou Z, Shi P, He S, Liu Y, Huang G, Yao B, Ringø E (2009). Identification of adherent microbiota in the stomach and intestine of emperor red snapper (*Lutjanus sebae*) by 16S rDNA-DGGE. Aquacult. Res. 40:1213-1218.