Effect of lactic acid bacteria and the potential probiotic *Hafnia alvei* on growth and survival rates of narrow clawed crayfish (*Astacus leptodactylus* Esch., 1823) stage II juveniles

Didinen B.I.[1*]; Bahadır Koca S.[1]; Metin S.[1]; Diler O.[1]; Erol K.G.[2]; Dulluc A.[1]; Koca H.U.[1]; Yigit N.O.[1]; Ozkok R.[2]; Kucukkara R.[2]

Received: June 2013 Accepted: May 2016

Abstract

The aim of this study was to screen potential probiotic bacteria against *Aeromonas hydrophila* and determine the effects of antagonistic bacteria and a commercial product containing lactic acid bacteria on the survival and growth of stage II *Astacus leptodactylus* juveniles. For this purpose, a total of 110 bacterial strains were isolated from adult, stage II crayfish juveniles and rearing water screened for antagonistic activities against *A. hydrophila* with well diffusion agar assay. *Hafnia alvei* strain from stage II crayfish juveniles displayed the inhibition zone (10mm) against *A. hydrophila*. The experiment was conducted in a completely randomized design with four treatments for 60 days: (I) crayfish fed with live food without probiotics (control group); (II) crayfish fed with live food enriched with lactic acid bacteria (0.015 gL^{-1}); (III) crayfish fed with live food enriched with *Hafnia alvei* (10^6 CFU mL^{-1}); (IV) crayfish fed with control diet and *H. alvei* added to rearing water (10^6 CFU mL^{-1}). As a result of this study, lactic acid bacteria and *Hafnia alvei* applications did not positively affect growth and survival of stage II *A. leptodactylus* juveniles. In the future, studies on screening potential probiotic bacteria should be used *in vitro* and *in vivo* tests. In addition, it will be useful to investigate the lactic acid bacteria and *Bacillus* spp. from indigenous microflora of crayfish.

Keywords: *Astacus leptodactylus*, *Aeromonas hydrophila*, Lactic acid bacteria, *Hafnia alvei*, Growth, Survival

1-Suleyman Demirel University, Egirdir Fisheries Faculty, Çünür, Isparta, Turkey
2- Fisheries Research Institute, Egirdir, Isparta, Turkey
*Corresponding author's email: behirediden@hotmail.com

Introduction

Astacids are considered valuable fishery resources, as there are no other commercially important species of Crustacea found in the fresh waters of Turkey. The only native crayfish species found in Turkey is *Astacus leptodactylus* Eschscholtz, 1823. Astacid culture is mainly based on semi-extensive systems in which stage II juveniles are stocked in natural earthen ponds or in artificial ponds. However, mortality of juveniles of Astacids is more than in warm water crayfish (e.g., cambarids) (Mazlum, 2007). One of the most important factors affecting survival and growth of these juveniles is nutrition. There have been a few studies describing nutrition (Cilbiz, 2011; Koca *et al.*, 2011), and feed additives (Mazlum *et al.*, 2011; Safari *et al.*, 2014; Safari *et al.*, 2015) for *A. leptodactylus*. There are a few studies on the effects of probiotic bacteria on growth and survival of fresh water crayfish (Didinen *et al.*, 2008; Sajedi-Raad *et al.*, 2010; Ambas *et al.*, 2013; Mona *et al.*, 2015). Hovewer, there is no published study on the effects of selected or commercial probiotic bacteria on growth and survival of stage II *A. leptodactylus* juveniles.

Lactic acid bacteria (LAB) are the most commonly applied probiotics in terrestrial animal nutrition, and their use as probiotics has been proposed for aquatic species (Gatesoupe, 1991; Ringo and Gatesoupe, 1998; Gatesoupe, 2002). Lactic acid bacteria are used as probiotics in aquaculture because of their properties such as low or no virulence, outcompeting harmful bacteria by producing antimicrobial substances, adherence capacity to colonize the digestive tract and competing with pathogens for adhesion sites (Farzanfar, 2006; Mota *et al.*, 2006).

A. hydrophia is a pathogenic bacterium for freshwater crayfish (Jiravanichpaisal *et al.*, 2009). In addition, Wasow *et al.* (2004) reported that the incidence of plague in *A. leptodactylus* was accompanied by bacteremia caused by *Pseudomonas fluorescens*, *A. hydrophila* and *Aeromonas caviae*. However, there is no study looking for probiotic bacteria effective against this pathogen in *A. leptodactylus*.

The aim of this study was to isolate potential probiotic bacteria strains from healthy narrow clawed crayfish (*A. leptodactylus*) and rearing water based on antimicrobial activities against *A. hydrophila*. This study also evaluated the impact of the potential probiotic and a commercial probiotic containing lactic acid bacteria on the growth and survival of stage II *A. leptodactylus* juveniles.

Materials and methods

Isolation of potential probiotic bacteria

The samples were taken from hepatopancreas, gills and intestine of adult crayfish and the whole body of stage II crayfish juveniles; rearing water of adult and juvenile crayfish at

Suleyman Demirel University, Egirdir Fisheries Faculty, Aquaculture Unit. The samples were homogenized, serially diluted in phosphate buffered saline (PBS, Sigma), plated onto Tryptic Soy Agar (TSA, Merck) and Plate Count Agar (Merck) and incubated for 2–3 day at an ambient temperature of approximately 25 °C. Each isolate was stored at −80 °C in Tryptic Soy Broth (TSB, Merck) with 15% (v/v) glycerol.

Inhibitory activity of the isolates

All bacteria isolated from the samples were tested for antagonistic activity by a well diffusion agar assay (WDAA) against *A. hydrophila*. Target organisms were grown in 4mL TSB for 2 d at 25°C, and 10 µL of each culture was mixed into 10 mL of melted TSA (43.5–44°C). After solidifying and drying for 15–20 minutes, wells were punched (diameter, 3 mm) and 10 µL of a 2 day old potential probiotic bacterial culture (approx. 10^8–10^9 CFU mL^{-1}) grown in TSB at 25°C was added to wells in triplicates. Plates were incubated at 25°C for one day and observed for clearing zones around the wells. Strains causing clearing zones in the WDAA were tested once more in TSA to ensure that the antagonistic activity was stable after storage and sub-culture (Hjelm *et al.*, 2004).

Identification of potential probiotic bacterium

Identification of antagonistic bacterium was based on the following criteria: Gram reaction, catalase reaction (3% H_2O_2), motility, oxsidase reaction, ability to metabolize glucose by oxidation and/or fermentation in OF basal medium supplemented with 1.5% glucose, growth at 41^{o}C in TSB, methyl red test, fermentation of carbohydrates (cellobiose, maltose, mannose, trehalose, xylose, raffinose, salicin, sorbitol, lactose). Additional tests were performed using API 20 E system (bioMérieux) (Holt *et al.*, 1994; Austin and Austin, 2007).

Pathogenicity test of potential probiotic bacteria on stage II crayfish juveniles

The juveniles were distributed into groups of 25 animals each in 10 L plastic troughs containing 5 L of ground water. Animals were challenged by immersion at the final concentration of 10^7CFU mL^{-1} with *H. alvei* for a period of 2 days. The experiment was carried out in triplicate and juvenile mortality was monitored for 14 days (Austin *et al.*, 1995).

Production of stage II A. leptodactylus juveniles for experiment

In this study for the production of stage II juvenile crayfish, females carrying fertilized eggs were obtained from Egirdir Lake. For adaptation, oviferous females were placed into circular fiberglass tanks (bottom area 1.5 m^2, volume 400 L) at a rate of 15 individuals per tank. The tank system was placed inside a room with natural photoperiod, and each tank was supplied with continuous aeration. A static system was used and 30% of the water in each tank was exchanged every

day. Water temperature inside the tank ranged from 17 to 20^{o}C. Dissolved oxygen ranged from 5 to 7 mg L^{-1}, while the pH ranged from 7 to 7.6 during production for stage II juveniles.

Experimental groups

The experiment was conducted in a completely randomized design with four treatments for 60 days: (I) crayfish fed with live food without probiotics (control group); (II) crayfish fed with live food enriched with commercial probiotics (0.015 gL^{-1}); (III) crayfish fed with live food enriched with potential probiotics (10^6 CFU mL^{-1}); (IV) crayfish fed with control diet and potential probiotics added to rearing water (10^6 CFU mL^{-1}).

Live food culture and probiotic enrichment

Live food organisms (*Daphnia magna* and Chrinomid larvae) were cultured in outdoor tanks (700 L). *Chlorella* sp. was introduced in culture tanks fertilized with horse manure (200 gL^{-1}). *Daphnia magna* and Chrinomid larvae were grown in culture tanks containing *Chlorella sp*. culture at a cell density of $2x10^5$ cells mL^{-1}.

The probiotic (consisting of *Lactobacillus plantarum, L. delbrueckii* sub sp. *bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidium, Streptococcus salivarius* subsp. *thermophilus, Enterococcus faecium, Aspergillus oryza, Candida pintolopesii* and Ascorbic acid) was used according to manufacturer's instructions.

The commercial probiotic and potential probiotic culture was added to live food (*Daphnia magna* and Chrinomid larvae) tanks every day at the final concentrations of 0.015 gL^{-1} and 10^6 CFU mL^{-1}, respectively.

Experimental conditions

The experiment was performed using *A. leptodactylus* Stage II juvenile at the Aquaculture Department of Egirdir Fisheries Faculty, Suleyman Demirel University. A total of 450 juveniles (initial weight, carapax and total length were 0.035±0.003 g, 6.25±0.24 mm and 12.13±0.29 mm, respectively) were stocked into each fiberglass tank (3x0.5x0.5 m, bottom area 1.5 m^2) filled with 300 L of ground water. The experiment groups were run in triplicates. Several bricks (27.5x17.5x13 cm) with 32 holes were placed in each tank as shelter. The tank system was housed inside a room with natural photoperiod and each tank was supplied with continuous aeration. A static system was used and 30% of the water in each tank was exchanged every day. The calcium concentration of water during the production of juveniles was 156 mgL^{-1}. Water temperature and dissolved oxygen ranged from 19 to 21^{o}C, and 6 to 7 mgL^{-1}, respectively during the experiment. The juveniles were fed live food ad libitum every 24 h.

Measurements

The measurements of the carapax, total length, and weight of the animals were

performed both at the beginning (100 individuals) and at the end of the experiment (all individuals). The number of surviving juveniles in each tank was counted at the end of the experiment.

The specific growth rate (SGR, % day^{-1}) and survival rate were calculated as follows:

SGR (% day^{-1})=$100(\ln W_t - \ln W_0)/t$, where W_0 and W_t are the initial mean weight and final mean weights, and t is the time in days (t=60 day)

Survival rate (SR, %) = [(final number of crayfish/initial number of crayfish) ×100]

Statistical analysis

The final carapax and the total length, weight, specific growth rate, and survival rate of *A. leptodactylus* juveniles were analyzed with one way analysis of variance (ANOVA) to determine if significant differences occurred among the probiotic treatments and control group. Duncan's multiple-range test was used to compare differences among individual means. The data were analyzed with SPSS 11.05. All statistical computations were performed at the probability level of $p<0.05$.

Results

Antagonistic activities against *A. hydrophila* of 110 bacterial isolates were tested by the well diffusion agar assay. *H. alvei* strain isolated from stage II crayfish juveniles was displayed inhibition zone (10mm) against *A. hydrophila*. *H. alvei* didn't showed pathogenic effects on crayfish juveniles. Phenotypic characteristics of *Hafnia alvei* was given in Table 1.

Table 1: Phenotypic characterization of *Hafnia alvei* isolated from juvenile crayfish.

Characteristics of *Hafnia alvei*			
Gr staining	**-**	**Acid production from:**	
Oxidase	-	Glucose[a]	+
Catalase	+	Mannitol[a]	+
O/F Test	+/+	Inositol[a]	-
Motility	+	Rhamnose[a]	+
Growth at 41°C	+	Saccharose[a]	-
β-alactosidase(ONPG)[a]	+	Mellibiose[a]	-
Arginine dihydrolase [a]	-	Amygdalin[a]	-
Lysine decarboxylase[a]	+	Arabinose[a]	+
Ornithine decarboxylase[a]	+	Cellobiose	+
Production of H_2S[a]	-	Maltose	+
Urease [a]	-	Mannose	+
TDA[a]	-	Trehalose	+
Production of indole[a]	-	Xylose	+
VP[a]	+	Raffinose	-
Gelatinase [a]	-	Salicin	-
NO_3 reduction[a]	+	Sorbitol	-
Methyl red	+	Lactose	-

[a] Performed to API 20 E

The end of the experiment, final weights, total body lengths, specific growth rates and survival rates of crayfish juveniles was ranged 0.50-0.57 g, 26.49–28.3 mm, 3.02 - 3.08%, 18.32-26.04% in all groups, respectively. The survival rates of the groups treated with *H. alvei* was found relatively high levels. However, there was no significant differences between survival and growth parameters of all groups (p>0.05) (Table 2).

Table 2: Growth parameters, survival and mortality rates of the crayfish in the end of experiment.

	Groups			
Parameters	**I (Control)**	**II**	**III**	**IV**
Total length (mm)	28.3±5.4	26.49±4	26.54±4.34	27.02±4.04
Carapax length (mm)	14.44±2.69	13.49±1.97	13.22±2.21	13.77±1.97
Final weight (g)	0.57±0.31	0.50±0.26	0.53±0.25	0.55±0.27
SGR (% day^{-1})	3.08±0.0028	3.02±0.0028	2.96±0.0021	3.06±0.056
Survival rate (%)	23.87±2.07	18.32±5.88	25.4±6.22	26.04±6.55

In each parameter was not shown non-significant difference between groups (p>0.05)

Discussion

In this study, *H. alvei* strain isolated from stage II *A. leptodactylus* juveniles showed the antagonistic property towards *A. hydrophila*. There is only one study on probiotic bacteria effective against bacterial pathogens in fresh water crayfish. Ambas *et al.* (2015) found that *Bacillus mycoides* A10 and *Shewanella* sp. A12 isolated from gastrointestinal tract (GIT) of *Cherax cainii* inhibited growth of pathogen *Vibrio mimicus* and *Vibrio cholerae*, respectively. In adddition, a few reports about selection of potential probiotic bacterial strains in shrimp have been documented. Gullian *et al.* (2004) reported inhibitory effects of *Vibrio* P62, *Vibrio* P63 and *Bacillus* P64 isolated from the hepatopancreas of healthy wild shrimp (*Penaeus vannamei)* against *V. harveyi* (S2). Rattanachuay *et al.* (2007) noted *Pseudomonas sp.* isolated from water samples of intensive shrimp ponds showed high antagonistic activity against shrimp *Litopenaeus vannamei* pathogen *V. harveyi*. Balcázar *et al.* (2007) demonstrated *in vitro* antagonistic effects of *V. alginolyticus* UTM 102, *B. subtilis* UTM 126, *Roseobacter gallaeciensis* SLV03, and *P. aestumarina* SLV22 from the gastrointestinal tract of adult shrimp *L. vannamei* against the shrimp-pathogenic bacterium, *Vibrio parahaemolyticus* PS-017. Liu *et al.* (2014) also found that *Bacillus* sp. obtained from the gastrointestinal tract of healthy *L. vannamei* displayed antimicrobial activity against aquatic pathogens *A. hydrophila*, *A. sobria* and *A. caviae*.

One of the most important features of a probiotic is that it must not be pathogenic or toxic to its host (Kersarcodi-Watson *et al.*, 2008). *H. alvei* did not cause any harmful effects to crayfish stage II juveniles upon challenge even at a dose of 10^{-7} cells mL^{-1} introduced by immersion in this study. Capkin and Altinok (2009)

noted antagonistic effect of *Enterobacter cloacae* isolated from rainbow trout intestinal microflora against *Yersinia ruckeri* and it is safe for rainbow trout. Burbank *et al*. (2011), the two *Enterobacter* sp. (C6-6 and C6-8) also identified as non-pathogenic to rainbow trout, inhibit *Flavobacterium psychrophilum in vitro*.

In the present study, addition of *H. alvei* to rearing water and live food did not affect growth and survival of *A. leptodactylus* juveniles. Similarly, Ambas *et al.* (2013) also reported that dietary supplementation of *B. mycoides* (A10) and *Shewanella* sp. isolated and selected from *Cherax tenuimanus* had no significant impact on growth and survival of the marron. Rattanachuay *et al.* (2007) also noted that the addition of the potential probiotic *Pseudomonas sp.* to rearing water in shrimp did not affect growth in shrimp. In contrast, Balcázar *et al.* (2007), evaluated four bacterial strains isolated from the gastrointestinal tract of adult shrimp (*L.vannamei)*, *V. alginolyticus* UTM 102, *B. subtilis* UTM 126, *R. gallaeciensis* SLV03, and *P. aestumarina* SLV22 for potential use as probiotics for shrimp. Feeding shrimp with diets containing all potential probiotics showed the best growth in comparison with the control groups. Gullian *et al.* (2004) also reported that shrimps *Penaeus vannamei* fed with probiotic (*Vibrio* P62, *Vibrio* P63 and *Bacillus* P64) showed significantly higher weight (p<0.05) than the control group. In addition, use of dietary *S. haliotis* D4, *B. cereus* D7 and *A. bivalvium* D15 isolated from the gut of shrimp has improved growth of white shrimp (*L. vannamei*) (Hao *et al.*, 2014).

In this study, growth and survival of crayfish juveniles were not affected by feeding lactic acid bacteria enriched live food for 60 days. There is only one study on lactic acid bacteria in crayfish. Didinen *et al.* (2008) also reported that feeding lactic acid bacteria did not affect survival and growth in crayfish juveniles (average 15 g). However, the positive effects of lactic acid bacteria on growth of shrimp were noted in previous studies. Venkat *et al.* (2004) pointed out significantly higher growth performance in *Macrobrachium rosenbergii* post larvae fed *Artemia* bioencapsulated *L. sporogenes* over the control group. Similarly, Dash *et al.* (2014) indicated that *L.plantarum* can be used to improve growth in *M. rosenbergii*. Javadi *et al.* (2011) reported that the use of commercial probiotic containing different lactic acid bacteria in *P. indicus* led to a significant increase in growth parameters. Ajitha *et al.* (2004) noted that growth was superior in *Penaeus (Fenneropenaeus) indicus* fed lactic acid bacteria (*L. acidophilus, S. cremoris, L. bulgaricus* and *L. bulgaricus*) in comparison to the control group. Kongnum and Hongpattarakere (2012) also reported that use of *L. plantarum* supplemented diet in *Litopenaeus vannamei* improved relative growth rate (% RGR).

As a result of this study, *H. alvei* strain isolated from stage II narrow clawed crayfish juveniles displayed antimicrobial activity against crayfish

pathogen *A. hydrophila*. However, *H. alvei* and the commercial probiotic containing lactic acid bacteria have not shown positive effect on growth parameters and survival in stage II *A. leptodactylus* juveniles. In the future further studies should be used *in vitro* (tolerance to high bile and low pH conditions, hydrophobicity, antibiotic susceptibility) and *in vivo* [total haemocytes counts (THC), proportion of granular cells (GC) and determination of bacterial load in the haemolymph] tests. In addition, it will be useful to investigate the lactic acid bacteria and *Bacillus* species from indigenous microflora of narrow clawed crayfish in these studies.

Acknowledgments

This study was supported by a grant from Scientific and Technological Research Council of Turkey (TUBITAK) (Project no: TOVAG-106 O 353)

References

Ajitha, S., Sridhar, M., Sridhar, N., Singh, I.S.B. and Varghese, V., 2004. Probiotic effect of lactic acid bacteria against *Vibrio alginolyticus* in *Penaeus (Fenneropenaeus) indicus* (H. Milne Edwards). *Asian Fish Sciences*, 17, 71-80.

Ambas, I., Buller, N. and Fotedar, R., 2015. Isolation and screening of probiotic candidates from marron, *Cherax cainii* (Austin, 2002) gastrointestinal tract (GIT) and commercial probiotic products for the use in marron culture. *Journal of Fish Diseases*, 38(**5**), 467-476.

Ambas, I., Suriawan, A. and Fotedar, R., 2013. Immunological responses of customised probiotics-fed marron, *Cherax tenuimanus*,(Smith 1912) when challenged with *Vibrio mimicus*. *Fish and Shellfish Immunology*, 35(**2**), 262-270.

Austin, B. and Austin, D., 2007. Bacterial fish pathogens: Diseases of farmed and wild fish, 4th edn, UK: Springer-Praxis.

A**ustin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, J. and Griffith D.R.W., 1995.** A probiotic reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordali*. *Journal of Fish Diseases*, 18, 93-96.

Balcázar, J.L., Rojas-Luna, T. and Cunningham, D.P., 2007. Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp (*Litopenaeus vannamei*) following immersion challenge, with *Vibrio parahaemolyticus*. *Journal of Invertebrate Pathology*, 96, 147-150.

Burbank, D.R., LaPatra, S.E., Fornshell, G. and Cain, **K.D., 2012.** Isolation of bacterial probiotic candidates from the gastrointestinal tract of rainbow trout *Oncorhynchus mykiss* (Walbaum) and screening for inhibitory activity against

Flavobacterium psychrophilum. *Journal of Fish Diseases*, 35(11), 809-16.

Capkin, E. and Altinok, I., 2009. Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis disease. *Journal of Applied Microbiology*, 106, 1147–1153.

Cilbiz, M., 2011. Effect of the different dietary calcium levels on growth, survival rate and moulting of the fresh water crayfish (*Astacus leptodactylus*, esch., 1823). Master Thesis. Suleymen Demirel University, Graduate School of Natural and Applied Sciences, Isparta-TURKIYE.

Dash, G., Raman, R.P., Prasad, K.P., Makesh, M., Pradeep, M.A. and Sen, S., 2014. Evaluation of *Lactobacillus plantarum* as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture*, 432, 225-236.

Didinen, B.I., Bahadir Koca, S., Diler, Ö., Koca, H. U., Dulluc, A., Ekici, S., Gulle, İ. and Özkok, R., 2008. Effect of dietary supplementation commerical probiotic (protexin) on growth and survival of narrow-clawed crayfish (*Astacus leptodactylus* Esch.) The symposium on interactions between social economic and ecological objectives of inland commercial and recreational fisheries and aquaculture. Turkey. Antalya.

Farzanfar, A., 2006. The use of probiotics in shrimp aquaculture. *International Journal of Environmental Health Research*, 48, 149–158.

Gatesoupe, F.J., 1991. The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatilis*, and their dietary value for larval turbot, *Scophthalmus maximus*. *Aquaculture*, 96, 335–342.

Gatesoupe, F.J., 2002. Probiotic and formaldehyde treatments of *Artemia nauplii* as food for larval pollock, *Pollachius pollachius*. *Aquaculture*, 212, 347–360.

Gullian, M., Thompson, F. and Rodriguez, J., 2004. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannemei*. *Aquaculture*, 233, 1-14.

Hao, K., Liu, J.Y., Ling, F., Liu, X. L., Lu, L., Xia, L. and Wang, G.X., 2014. Effects of dietary administration of *Shewanella haliotis* D4, *Bacillus cereus* D7 and *Aeromonas bivalvium* D15, single or combined, on the growth, innate immunity and disease resistance of shrimp, *Litopenaeus vannamei*. *Aquaculture*, 428, 141-149.

Hjelm, M., Bergh, O., Riaza, A., Nielsen, J., Melcheiorsen, J., Jensen, S., Duncan, H., Ahrens, P., Birkbeck, H. and Gram, L., 2004. Selection and identification of autochthonous potential

probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. *Systematic and Applied Microbiology*, 27, 360-371.

Holt, J.G., Krieg, N.R. and Sneath, P.H.A., Staley JT, Williams ST, 1994. Bergey's manual of determinative bacteriology. 9th edn. Baltimore: Williams &Wilkins.

Javadi, A., Mirzaei, H, Khatibi, S. A., Zamanirad, M., Forogeefard, H. and Abdolalian, I., 2011. Effect of dietary probiotic on survival and growth of *Penaeus indicus* cultured shrimps. *Journal of Animal and Veterinary Advances*, 10, 2271-2274.

Jiravanichpaisal, P, Roos, S, Edsman, L., Liu, H. and Söderhäll, K., 2009. A highly virulent pathogen, *Aeromonas hydrophila*, from the freshwater crayfish *Pacifastacus leniusculus*. *Journal of Invertebrate Pathology*, 101(**1**), 56-66.

Koca, S.B., Yigit, N.O., Dulluç, A., Erol, G., Cılbız, N. and Kucukkara, R., 2011. Appropriate usage level of shrimp waste meal as chitin source for feeding young crayfish (*Astacus leptodactylus* Esch. 1823). *Pakistan Veterinary Journal*, 31(**3**), 207-210.

Kongnum, K. and Hongpattarakere, T., 2012. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. *Fish & Shellfish Immunology*, *32*(**1**), 170-177.

Kersarcodi-Watson, A., Kaspar, H., Lategan, M.J. and Gibson, L., 2008. Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture*, 274, 1–14.

Liu, H., Li, Z., Tan, B., Lao, Y., Duan, Z., Sun, W. and Dong, X., 2014. Isolation of a putative probiotic strain S12 and its effect on growth performance, non-specific immunity and disease-resistance of white shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunology*, 41(**2**), 300-307.

Mazlum, Y., 2007. Stocking density affects the growth, survival, and cheliped injures of third instars of narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 juveniles, *Crustaceana*, 80(**7**), 803-815.

Mazlum, Y., Yılmaz, E., Genç, M.A. and Guner, O., 2011. A preliminary study on the use of manan oligosaccharides (MOS) in freshwater crayfish, *Astacus leptodactylus* Eschscholtz, 1823, juvenile diets. *Aquaculture International*, 19, 111-119.

Mona, M.H., Rizk, E.S.T., Salama, W.M. and Younis, M.L., 2015. Efficacy of probiotics, prebiotics, and immunostimulant on growth performance and immunological parameters of *Procambarus clarkii*

juveniles. *The Journal of Basic and Applied Zoology*, 69, 17-25.

Mota, R., Moreira, J.L., Souza, M., Horta, F., Teixeira, S., Neumann, E., Nicoli, J. and Nunes, A., 2006. Genetic transformation of novel isolates of chicken Lactobacillus bearing probiotic features for expression of heterologous proteins: a tool to develop live oral vaccines. *BMC Biotechnology*, 6, 1–11.

Rattanachuay, P., Kantachote, D. and Suntinanalert, P., 2007. Selection of proteolytic bacteria with ability to inhibit *Vibrio harveyi* during white shrimp (*Litopenaeus vannamei*) cultivation.*Songklanakarin Journal of Science Technology*, 29(**2**), 235-243.

Ringo, E. and Gatesoupe, F.J., 1998. Lactic acid bacteria in fish: a review. *Aquaculture*, 160, 177–203.

Safari, O., Shahsavani, D., Paolucci, M. and Atash, M.M.S., 2014. Single or combined effects of fructo-and mannan oligosaccharide supplements on the growth performance, nutrient digestibility, immune responses and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823. *Aquaculture*, 432, 192-203.

Safari, O., Atash, M.M.S. and Paolucci, M., 2015. Effects of dietary L-carnitine level on growth performance, immune responses and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823. *Aquaculture*, 439, 20-28.

Sajedi-Raad, A., Zamini, A.A., Valipour, A.R. and Bakh, M.H., 2010. The effect of adding probiotic protexin in the diet of freshwater crayfish (*Astacus leptodactylus*) on growth and survival indices. *Journal of Microbiology and Biotechnology*, *2*(**4**), 29-36.

Venkat, H.K, Sahu, N.P. and Jain, K. K., 2004. Effect of feeding lactobacillus-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*, 35, 501-507.

Wasow,T., Bernad, A., Krzywosz, T. and Hul, M., 2004. Some notices on the incidence of crayfish plague in Poland, *Bulletin - VÚRH Vodnany*, 40(**3**), 95-100.