Antibacterial activity, antibiotic susceptibility and probiotic use of lactic acid bacteria (LAB) in Persian sturgeon (*Acipenser persicus*)

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Abstract

Growth behavior of five lactic acid bacteria previously isolated from Persian sturgeon (*Acipenser persicus*), were evaluated at different pH, temperature and salt concentrations. Also, antibacterial activity of extracellular products (ECPs) of this LAB were assessed to *Aeromonas hydrophila*. Further, their antibiotic susceptibility was determined with some commonly used antibiotics in aquaculture. In an in vivo work the effect of *Lactococcus lactis* as the supplementary diet was evaluated on growth performance of Persian sturgeon for a period of 56 days. Strong growth of all LAB were seen at 20 and 30˚C, as well as 40 and 80 g L-1 NaCl. The LAB ECPs exhibited varied results of antagonism to the *A. hydrophila* with maximum activity observed at temperature between 25 and 30˚C. Also, the higher antagonistic activity was observed for ECPs of *W. cibaria*, *P. pentosaceus*, and *L. lactis* at pH 9. Both *W. cibaria* and *E. faecalis* were resistance to oxytetracycline, erythromycin, trimethoprim sulfamethoxazol, enrofloxacin, florfenicol, and flumequine, while *L. lactis* was sensitive to oxytetracycline, erythromycin, enrofloxacin, florfenicol. These results showed that use of *L. lactis* can act as a positive probiotic in Persian sturgeon feed via improvement of fish growth performance, feed efficiency and fish health.

Keywords: sturgeon, health, *Lactococcus lactis*, probiotic.

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Introduction

Nowadays, a high request for consumption of aquaculture production has been significantly increased together with a remarkable decrease in the wild aquatic animal stock resources. Appllication of new commercial aquatic animal species and aquaculture methods are generally raised some new diseases and economical problems (Oidtmann, Thrush, Denham & Peeler 2011). Therefore, there is a highly demand for new methods for prevention and treatments of fish diseases in aquaculture industry in order to increase the cost-benefit for the farmers.

Application of antibiotics for treatment and control of fish diseases can cause a harmful effect for both the environment and consumer. The residual of chemical substances such as antibiotics could be appeared as some drug resistances in both animal and human bacterial flora. Hence to improve the optional tools for prevention of fish diseases it should be deflected to find some new health managements such as enhancing the husbandry, the water quality, decreasing the fish densities, improveding food efficacy, vaccines, probiotics and immunostimulants (Sharifuzzaman & Austin 2009). Although vaccines and chemotherapeutic treatments have been used for protection of fish from bacterial diseases but sometimes need more therapeutics to prevent or treat fish infections because vaccination process is frequently incapable in immature fish (Balcazar, Vendrell, Blas, Ruiz-Zarzuela, Múzquiz & Gironés 2008).

Intensive sturgeon culture are exposed to stressful conditions and culture may result in incidences of some diseases, which have caused acute economic

losses (Soltani & Kalbassi 2001; Khoshbavar-Rostami, Soltani & Hassan 2006,2007; Yang & Li 2009; Meng, Xiao & Zeng 2011). Persian sturgeon, *Acipenser persicus*, is one of the most important commercial fish species in the South Caspian Sea.

The beneficial effects of some probiotics, prebiotics, immunostimulants, and vitamin have been demonstrated in a number of previous studies in sturgeon (Falahatkar, Soltani, Abtahi, Kalbassi, & Pourkazemi 2006; Jalali, Ahmadifar, Sudagar & Takami 2009; Akrami, Abdolmajid, Abbas & Abdolmohammad 2009; Jafaryan et al. 2010; Hoseinifar et al. 2011a,b,c; Askarian, Kousha, Salma & Ringø 2011) However, there is no information for the dietary effect of *lactococcus lactis* as a probiotic in sturgeon. Also, despite isolation and in some contents characterization of some lactic acid bacteria (LAB) from alimentary tract of sturgeons have reported, there is no information regarding the physiological responses of this LAB to environmental and chemical variables. The aim of the present study was to assess the growth behavior of five species of LAB consisting of Lactococcus garvieae, Weissella cibaria , Pediococcus pentosaceus, *Lactococcus lactis* and Enterococcus facealis isolated from Persian sturgeon fingerling intestine at different salt concentrations, temperatures and pHs. Also, the antibacterial activity of the extracellular products of this LAB was evaluated to a virulent strain of *Aeromonas hydrophila*. The antibiotic resistance profiles of these LABs were also tested using some commonly used antibiotics in the aquaculture. Moreover, potential use of *Lactococcus lactis* (JF831150) was assessed on some growth performance, haematological and nonspecific immune response parameters of Persian sturgeon.

Material and Methods

Microorganisms and Culture Conditions

Lactococcus garvieae (JF831155), Weissella cibaria (JF831160), Pediococcus pentosaceus (JF831149), *Lactococcus lactis* (JF831150), and Enterococcus faecalis (JF831161) were originally isolated from intestine of Persian sturgeon (*Acipenser persicus*) (Soltani, Pourkazemi, Ahmadi, Taherimirghead, Merrifield & Masouleh 2013). *A. hydrophila* (A4) strain was originally isolated from diseased Persian Sturgeon (Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Iran). LAB strains were grown on MRS broth (Merck, Germany) incubated for overnight at 30 °C before analysis. *A. hydrophila* in TSB (Merck, Germany) incubated for overnight at 25 °C ahead of examination.

Growth Ability at Different Salt Concentration, Temperature, and pH

Growth ability of LAB at different environmental conditions was performed following method described by Reddy (2007), with slight modifications. The growth behavior of LAB was assessed at five different temperatures of 4, 10, 20, 30, and 40°C by inoculating into 5 mL MRS broth (Merck, Germany) incubated for 24 h.

Striled MRS broth (Merck, Germany) with different pHs values of 3.7, 4.5, 5, and 6.7 (adjusted with 1N HCL) (Merck, Germany) and pH 8 and 9 (adjusted with 1N NaOH) (Merck,Germany) were used to evaluate the LAB growth. A volume of 100µl of overnight culture was inoculated to broth tubes maintained at different pH incubated at 30°C for 24 h. Also, the LAB growth was assessed at four different salinity consisting of 40, 80, 120 and 300 g L-1 NaCl prepared in MRB broth (Merck, Germany) inoculated with 100µl of 24 h culture of each LAB incubated at 30°C for 24h. The growth behavior of LABs was measured by turbidimetric assay.

Antibacterial Activity of LAB Extracellular Products (ECPs)

The agar well diffusion assay was used to detect the possible antimicrobial activity of the LAB supernatant cultures according to procedure described by Casla, Requena & Gómez (1996) with slight modifications. Briefly, wells of 5 mm diameter of TSA (Merck,Germany) were loaded with 50 µl of the LAB culture supernatants obtained by centrifugation (Hettich,Germany) at 10,000 ×g for 10 min at 4°C, filtered through a 0.2 µm millipore filter (Whatman®, Schleicher & Schuell) buffered at

pH 6.7. The supernatant of each LAB isolate was prepared with four levels of pH of 6.7, 7.0, 8.0, and 9.0. Lawns of the *A. hydrophila* were prepared by inoculating (ca 108cells/well) in 15 mL of the soft-overlay (7.5 g L-1 agar) medium. The antibacterial activity of LAB ECPs against *A. hydrophila* (108 cells/well) was also examined at four different temperatures of 4, 25, 30, and 60°C provided at pH 6.7 for 30 min using well diffusion agar and zones of growth inhibition were measured after an overnight incubation.

Antibiotic Resistance Profiles

The antibiotic resistance profiles were measured following methods described by Bello, Cocolin, Zeppa, Field, Cotter & Hill (2012), with slight modifications. The LAB were grown in MRS broth (Merck,Germany) for an overnight at 30 °C. A volume of 20 mL of TSA agar (Merck, Germany) was inoculated with revitalized LAB strains (1% v/v) and permitted to solidify. Antibiotic resistance pattern was determined by the disk diffusion method using oxytetracycline (30 µg/disc), erythromycin (15 µg /disc), enrofloxacin (5 µg/disc), florfenicol (30 µg/disc), flumequine (30 µg/disc), and trimethoprim -sulfamethoxazol (200 µg/disc)(Padtan-Teb, Iran).The plates were incubated at 30 °C for 24 h and the zone of inhibition was measured.

Fish and rearing condition

Apparently healthy Persian sturgeon (*Acipenser persicus*) weighing 114±5.47g were acclimated to the rearing conditions for 2 weeks at the International Sturgeon Research Institute, Guilan Province, Iran prior to the experiment. The fish were randomly divided into 4 groups each group containing 15 fish. Water quality parameters of freshwater consisting of temperature, dissolved oxygen content, and pH were 20.48± 1.02 ˚C, 6.62±0.43 mg L-l, and 6.8-7.25, respectively.

Preparation of feed with the *Lactococcus lactis* and feeding fish

Preparation of feed with the *Lactococcus lactis* (JF831150) was according to Aly, Mohamed & John (2008 b) with slight modification. Briefly, the bacterium was inoculated in MRS broth (Merck,-Germany) incubated for 24 h at 30 ˚C and centrifuged at 5000 g for 10 min at 4 ˚C,. The bacterial cells were then washed three times with sterile saline to obtain a suspension of ca 1010 cells mL-1. The bacterial suspension was the sprayed into the commercial food [Biomar, 470 g kg-1 protein, 140 g kg-1 lipid, 81 g kg-1 ash , 31 g kg-1 cellulose/fibre, 8.8 g kg-1 phosphorous, 23.4 g kg-1 calcium, 2.7 g kg-1 sodium, vitamin A 7500(Ui kg-1), vitamin D3 1500(Ui kg-1), copper 1.6 (mg kg-1), Magnesium 12.6 (mg kg-1), Zinc 78.6(mg kg-1), Iodine 1.9 (mg kg-1), and ethoxyquin 1.9 (mg kg-1)] slowly, mixing part by part in a drum mixer to give experimental diets containing *L. lactis* at 106, 107, and 108 cfu g-1 diets. The pellets were air-dried at 25 ˚C under sterile conditions for 12 h, packed and stored in a refrigerator (4˚C) until used within 3 days. The viability of the incorporated bacterial cells in the feed was assessed by CFU counting via spreading onto MRS agar (Merck, Germany). Three groups of fish (each group containing 15 fish) were fed with diets containing 106 (Treatnebt one=T1), 107 (Treatnebt one=T2), and 108 (Treatnebt one=T3) cfu g-1 of *Lactococcus lactis* for 56 days. The forth group was considered as control . Fish were fed 3% biomass per day provided in equal rations at 09.00 and 17.00 h.

Growth performance and carcass composition

Growth performance was assessed in terms of condition factor (CF), specific growth rate (SGR), feed conversion ration (FCR), protein efficiency ration (PER), hepatosomatic index (HSI), weight gain (WG), initial body weight (IBW), and feed efficiency (FE). The calculations were performed using the following formulae (Merrifield, Dimitroglou, Bradley, Baker & Davies 2009 a):

CF = W×100 / L3; SGR= (LnFW-LnIW) × 100/T; FCR= feed intake/ (Wf-Wi); PER=(Wf-Wi)/PI; HSI= LW × 100/W; WG= (Wf-Wi); IBW = (Wf - Wi) × 100 / BWi ; × 100 (; FE= [(Wf-Wi)/FI]×100, where W is the weight in g, FW is the final weight, IW is the initial weight, L is the length in mm, T

is the duration of feeding (in days), WG, is the wet weight gain, LW is the liver weight, FI is the feed intake and PI is the protein ingested. Fish were analysed according to AOAC (1995) protocols to determine carcass composition.

Sample collection and analysis

Fish samples and analysis were collected to procedures described by Aly, Ahmed, Ghareeb & Mohamed 2008a) and Harikrishnan, Kim, Kim, Balasundaram & Heo (2011) with slight modification. After 8 weeks feeding, nine fish were randomly collected from each treatment and blood samples were obtained from caudal vein after fish being anaesthetized with solution of tricaine methane-sulfonate (MS-222, Sigma Chemical Co., USA). The sera samples were pooled and preserved at -20 ˚C or used immediately for analysis. Haematocrit levels (expressed as % packed cell volume: % PCV), white and red blood cells (WBC: ×103 mm-3 and RBC: ×106 mm-3), haemoglobin (Hb: g dL-1), mean corpuscular volume (MCV: fl), mean corpuscular haemoglobin (MCH:Pg), mean corpuscular haemoglobin concentration (MCHC: %) and leukocytes were determined as lymphocytes (LYM: %), monocytes (MON:%), eosinophils (EOS;%), and neutrophils (NEU:%) following standard methods described by Merrifield et al. (2009a) and Rawling, Merrifield & Davies (2009). The serum lactate dehydrogenate (LDH: IU mL-1), alanine aminotransferase (ALT: IU mL-1), aspartate aminotransferase (AST: IU mL-1) activities, glucose (mM), and concentration of total protein (g L-1) were determined using an autoanalyzer (Biotecnica Instruments, Italy). Also, serum lysozyme activity was determined according to Sahoo, Mahapatra, Saha, Barat, Sahoo, Mohanty, Gjerde, Ødegard, & Salte (2008). Alternative complement activity (ACH50) was assayed using the method of Matsuyama, Tanaka, Nakao & Yano (1988). The level of IgM was measured using analysis kits (Binding Site Company, www.bindingsite.co.uk) in MININEPH auto analyzer (Binding Site, UK).

Statistical analysis

One-Way ANOVA (SPSS 17.0; SPSS Inc., Chicago, IL, USA) and Duncan's test were conducted to find significant differences between treated and control trials at $P<0.05$ level.

Results

Bacterial growth behaviar

All LAB isolates were able to grow at 20 and 30˚C while no growth occurred at 4, 10 and 40˚C. Also, all LAB isolates were able to grow at salinites 4, and 8 g L-1 (w/v) NaCl with no growth at 12 and 30 g L-1 (w/v) NaCl. Furthermore, both *W. cibaria* and *E. faecalis* were able to grow at pH 3.7-9, while *L. lactis* and *L. garvieae* were able to grow at pH 5-9 and pH 6-9, respectively. Interstingly the P. pentosacus could not grow above pH 7.

Antibiotic susceptibility

Both *W. cibaria* and *E. faecalis* were resistance to the examined antibiotics while *L. lactis* exhibited sensitivity to oxytetracycline (23mm), erythromycin (22mm), enrofloxacin (21mm) and florfenicol (18mm). *L. garvieae* was also sensitive to all of used antibiotic (13-26mm) except erythromycin. *P. pentosaceus* exhibited sensitive only to oxytetracycline (11mm).

Anti- *Aeromonas hydrophila* activity of ECPs

The maximum antagonistic activity of 9-10 mm of ECPs against *A. hydrophila* were observed at temperature between 25 and 30˚C for the ECPs of *P. pentosaceus*, *L. lactis*, *E. faecalis* while *W. cibaria* showed moderate activity (7-8mm) at the examined temperatures and *L. garvieae* showed highest activity(10mm) at 30˚C. The highest antagonistic activities (9-10mm) were observed for the ECPs of *W. cibaria*, *P. pentosaceus*, and *L. lactis* at pH 9 while *L. garvieae* and *E. faecalis* showed moderate activity (7-8mm) at the examined pH.

Haematological and immunological parameteres

Haematocrit levels (%PCV), MCHC, MON remained unaffected in the experimental groups. WBC and RBC counts were significantly higher in T3 than control. On the other hand, the highest HB,

Table 1 Changes of hematological parameters of *Acipenser persicus* fed *Lactococcus lactis* (JF831150) for 56 days

Parameters	Control	T1	T2	T3
WBC ($\times10^3$ mm-3)	16.5±1.74^a	20.2±1.62^{ab}	20.3±0.64^{ab}	26.4±3.20^b
RBC ($\times10^6$ mm-3)	0.50±0.08^a	0.63±0.00^{ab}	0.68±0.05^{ab}	0.72±0.05^b
Haemoglobin (g dl^{-1})	3.50±0.09^a	3.66±0.14^{ab}	4.07±0.22^b	4.13±0.14^b
Haematocrit (% PCV)	27.9±0.72	27.2±1.55	25.7±1.20	25.4±1.09
Mean Corpuscular Volume (ft)	589±113^b	431±28^{ab}	379±21.5^a	356±32.7^a
Mean Corpuscular Haemoglobin Concentration (%)	73.4±11.86	58±2.85	60.9±8.04	57.9±5.16
Mean Corpuscular Haemoglobin (Pg)	12.6±0.59^a	13.5±0.26^{ab}	15.9±1.49^{ab}	16.4±1.25^b
Lymphocytes (%)	82±1.52^b	78.7±1.85^b	68±2.30^a	67±1.15^a
Monocytes (%)	1.66±1.20	1.66±1.20	3.66±0.88	0.66±0.33
Neutrophils (%)	11.7±1.76^a	16.7±0.66^b	19.3±0.88^{bc}	21.3±0.88^c
Eosinophils (%)	4.66±1.76^{ab}	3±1.52^a	9±1.15^{bc}	11±0.57^c

Basal diet without bacteria (Control), basal diet containing 106 CFUg^{-1} *Lactococcus lactis* (JF831150) (T1), basal diet containing 10^7 CFUg^{-1} *Lactococcus lactis* (JF831150) (T2) and basal diet containing 108 CFUg^{-1} *Lactococcus lactis* (JF831150) (T3).

Table 2 Changes in some biochemical and immunological parameters of *Acipenser persicus* fed *Lactococcus lactis* (JF831150) for 56 days

Parameters	Control	T1	T2	T3
Lysozyme(U mL^{-1})	7.16±0.72^a	14.3±5.36^{ab}	19±5.56^{ab}	28.3±7.31^b
Protein(g L^{-1})	26.2±1.70	28.50±0.80	23.8±1.80	23.4±4.30
Alternative complement activity (U mL^{-1})	26±1.15^a	42.7±11.2^{ab}	53.3±5.23^b	58±7.54^b
Lactate dehydrogenase (IU mL^{-1}	1432±398	1512±112	1303±53.6	1174±42.9
IgM (mg dl^{-1})	7.5±0.55	10.8±4.25	11±2.36	15.1±2.80
Alanine aminotransferase (IU mL^{-1})	4.66±2.02	2.33±1.33	2.66±1.20	1.33±0.33
Aspartate aminotransferase (IU mL^{-1})	514±84.9	546±17.1	473±33.8	404±43.2
Glucose(mM)	3.44±0.27	3.74±0.62	2.66±0.17	2.85±0.47

Basal diet without bacteria(Control), basal diet containing 10^6 CFUg^{-1} *Lactococcus lactis* (JF831150) (T1), basal diet containing 10^7 CFUg^{-1} *Lactococcus lactis* (JF831150) (T2) and basal diet containing 108 CFUg^{-1} *Lactococcus lactis* (JF831150) (T3).

MCH, NEU and EOS were obtained in T3, while the lowest levels were obtained in control (P<0.05). Moreover, the MCV and LYM levels were significantly decreased in T3, while the highest levels were obtained in control (Table 1).

The total protein, LDH, IgM, ALT, AST, and glucose of the experimental groups were not significantly different from the control. Also, lysozyme, and ACH50 increased significantly in groups compared to control one (Table 2).

Growth performance of fish

The growth performance of fish fed with 3 levels of *L. lactis* is shown in Table 4. The highest growth rate was obtained in T3, while the lowest one was obtained in T1 (P<0.05). The best FCR was obtained in T3 (0.96), while the highest one was obtained in T1 (1.11). The CF and HSI of the experimental groups were not significantly different from the control. Also, the highest SGR was obtained in T3 (1.46), while the lowest one was obtained in T1 (1.28) (P < 0.05). The highest PER was obtained in T3 (0.48), while the lowest one was obtained in T1 (0.42) (P < 0.05). In addition, the highest FE was obtained in T3 (103), while the lowest one was obtained in T1 (89.92) (P<0.05) (Table 3).

The highest moisture was obtained in T2, while the lowest one was obtained in the control (P<0.05). The crude protein content was significantly higher in control than experimental groups. The highest total lipid was obtained in T2, while the lowest one was obtained in control. Meanwhile, the lowest and the highest ash-content were significantly obtained in the control and T1, respectively (Table 4).

Table 3 Growth performance, protein efficiency ratio, hepatosomatic index, condition factor, food conversion ratio, and feed efficiency of *Acipenser persicus* fed *Lactococcus lactis* (JF831150) for 56 days

Parameters	Control	T1	T2	T3
Initial weight (g)	120±3.21	117±5.13	108.7±3.28	111±6
Final weight (g)	257±2.68^{c}	239±4.07^{ab}	226±1.79^{a}	252±6.61^{bc}
Weight gain (g)	137±0.52^{b}	122±1.50^{a}	118±2.42^{a}	140±0.74^{b}
Initial body weight (%)	115±3.55^{ab}	105±5.44^{a}	109±5.27^{a}	127±6.06^{b}
Condition factor	0.37	0.37	0.35	0.39±0.02
Feed Conversion Ratio	0.98^{b}	1.11±0.01^{a}	1.15±0.02^{ab}	0.96^{ab}
Hepatosomatic Index	1.77±0.15	2.12±0.18	1.65±0.04	1.80±0.37
Specific growth rate (% day^{-1})	1.36±0.02^{ab}	1.28±0.04^{a}	1.31±0.04^{ab}	1.46±0.04^{c}
Protein efficiency ration (%)	0.47^{b}	0.42^{a}	0.40^{a}	0.48^{b}
Feed efficiency	101.0±0.38^{b}	89.9±1.10^{a}	86.9±1.78^{a}	103.0±0.54^{b}

Basal diet without bacteria (Control), basal diet containing 10^{6} CFUg-1 *Lactococcus lactis* (JF831150) (T1), basal diet containing 10^{7} CFUg^{-1} *Lactococcus lactis* (JF831150) (T2) and basal diet containing 108 CFUg^{-1} *Lactococcus lactis* (JF831150) (T3).

Table 4 Body proximate composition after 8 weeks feeding of *Acipenser persicus* fed containing *Lactococcus lactis* (JF831150)

Composition (g kg^{-1})	Initial	Control	T1	T2	T3
Moisture	837	732±1.1^{a}	766±4.3^{c}	770±1.5^{c}	749±2.3^{b}
Crude protein	124	241±0.5^{c}	190±4.7^{a}	184±2.3^{a}	212±4^{b}
Total lipid	17.6	17.5± 0.2^{c}	26.3±1.2^{c}	28.3±0.6^{c}	22.6±1.4^{b}
Ash	12.5	9.6±0.6^{a}	12.3±0.3^{b}	11.6±0.8^{ab}	11.3±0.8^{ab}

Basal diet without bacteria(Control), basal diet containing 106 CFUg-1 *Lactococcus lactis* (JF831150) (T1), basal diet containing 107 CFUg-1 *Lactococcus lactis* (JF831150) (T2) and basal diet containing 108 CFUg-1 *Lactococcus lactis* (JF831150) (T3).

Discussion

The in vitro growth of LAB at 20-30˚C together with their resistance to acidity and alkaline conditions is correlated to the natural environmental condition of the fish intestine suggesting the isolated LAB are the representatives of the bacterial flora that are resident in the intestine of Persian sturgeon. For instance, the in vitro ability of some LAB including *E. faecalis*, *P. pentosaceus*, and *W. cibaria* to grow at low pH of 3.7-4.5 is similar to the fish intestine condition that is an acidic environment. However, these results showed that all of isolated LAB had the best growth in almost neutral pH condition which is preferable for almost all LAB. Similar results have been reported by Holt, Krieg, Staley & Willams (1994) and Balcazar et. al (2008) who assessed the environmental responses of some LAB such as *E. faecalis*, *L. lactis*, *L. garviae* and *P. pentosaceus* in the in vitro condition.

The results of present study also showed that the extracelluar products of most of isolated LAB showed a maximum inhibitory activity to *A. hydrophila* at 20-30˚C indicating an optimum temperature for the production of the highest ECPs. However, *L. lactis* showed the highest antimicrobial activity against *A. hydrophila* similar to the results obtained by Balcazar et al. (2008) who demonstrated a strong inhibitory activity by *L. lactis* CLFP 101 against both *A. hydrophila* and *A. salmonicida*. As the best inhibitory activity due to *L. lactis* occurred at optimum physiological temperature of *A. hydrophila* then it is possible to recommend *L. lactis* as a suitable potential probiotic against motile *Aeromonas* septicemia caused by *A. hydrophila* in Persian sturgeon (Soltani & Kalbassi 2001). Such anti- *A. hydrophila* may be in part due to production of organic acids, hydrogen peroxide, carbon dioxide, acetic acid, bacteriocins, diacetyl, acetaldehyde, ethanol, and low molecular weight antimicrobial compounds e.g reuterin from some LAB (Balcazar, Vendrell, Blas, Ruiz-Zarzuela, Gironés & Múzquiz 2007; Lee & Salminen 2009). In addition, all examined LAB were able to keep their anti-*A. hydrophila* activities at pH 7 to 9, a property that may be a benefit tool to pass

the gastrointestinal tract of fish. Such a character is an important factor for a bacterial strain to become a suitable probiotic because the high rate of its viability and its capacity of colonization in the fish intestine is necessary (Goktepe, Juneja & Ahmedna 2006; Lee & Salminen 2009).

A number of probiotics have been incorporated into aquatic animal feeds to increase the growth performances (Ghosh, Sen, & Ray 2003; Son, Chang, Wu, Guu, Chi & Cheng 2009; Merrifield et al. 2009a,b; Abd El-Rhman, Khattab, & Shalaby 2009; Askarian et al. 2011).

Manipulation of intestinal microflora via probiotics plays an important mechanism for increase of growth performances and survival (Vendrell, Balcazar, Blas, Ruiz-Zarzuela, Girones & Muzquiz 2008). However, probiotics are also effectively important of feed with detoxification and production of some hydrolytic enzymes such as amylase, protease and some vitamins like biotin as well as vitamin B12 (El-Haroun, Goda & Chowdhury 2006). In the present study, after 8 weeks administration of the supplemented diets had a significant increase in final weight, FCR, SGR, PER, and FE of Persian sturgeon. This result is in agreement with Askarian et al. (2011) after feeding Persian sturgeon (*Acipenser persicus*) and Beluga (*Huso huso*) with Chironomidae incorporated with Lactobacillus curvatus and Leuconostoc mesenteroides for 50 days. Mechanisms of the improvement of growth performances have not completely clear but production of some vitamins like vitamin k, and B12 (Martens, Barg, Warren & Jahn 2002) as well as extracellular enzymes such as esterase, protease, and amylase (Azokpota, Hounhouigan, Nago & Jakobsen 2006) could have important role. Administration of probiotics enriched diets helps to improve feed utility and digestion of proteins, as well as increase the digestibility of feed resulting in increase of growth and FE (Lara-Flores, Olvera-Novoa, Guzma´n-Me´ndez & Lo´pez-Madrid 2003). The results presented in this study revealed a significant increase in the lipid content compare to control. On the contrary, in Nile tilapia, *Oreochromis niloticus* (L.), fed with commercial probiotics including Bacillus subtilis and Bacillus licheniformis increase protein and decrease lipid contents (El-Haroun et al. 2006). Moreover, Merrifield et al. (2009 a,b) also reported that commercial probiotics such as B. subtilis, B. licheniformis, and Enterococcus faecium had no effect on carcass factors. Variation in lipid and protein contents in fish could be due to their synthesis and stock in muscles (Abdel-Tawwab, Khattab, Ahmad & Shalaby 2006). Improvement of immunity using dietary enriched probiotic could be attributed to the fish species, genetic, difference in consumption time and quantity of probiotics supplementation diet, and probiotic origins (Nikoskelainen, Ouwehand, Bylund, Salminen & Lilius 2003; Panigrahi, Kiron, Puangkaew, Kobayashi, Satoh & Sugita 2005; Salinas, Cuesta, Esteban. & Meseguer 2005; Kim & Austin 2006; Pieters, Brunt, Austin & Lyndon 2008; Son et al. 2009). The present study revealed that administration of probiotic in diets significantly enhanced the WBC, RBC, haemoglobin, MCH, neutrophils and eosinophils. Immunocompetent cells are important for assessment of fish health and their activation and proliferation is stimulated by probiotics enriched diets (Irianto & Austin 2002; Brunt & Austin 2005). Leukocytes are the sources of lysozymes production (Akrami Ghelichi & Ahmadifar 2011), and in this study a significant increase in WBC population was correlated with the enhancement of lysozyme level in fish sera. The present study also indicates that probiotics diet could decrease the Haematocrit levels (%PCV) similar results seen Abd El-Rhman et al. (2009) who feed Nile tilapia with *Micrococcus luteus* and *Pseudomonas* species as probiotics for 90 days. Also, in this study, alternative complement activity (ACH50) was enhanced significantly in fish fed with the probiotic supplementation diet after 8 weeks. Similar results were seen in works reported by Panigrahi, Kiron, Kobayshi, Puangkaew, Satoh& Sugita (2004); Son et al. (2009) ; Harikrishnan et al. (2011) and Geng et al. (2011) used some probiotics in the diets of rainbow trout (*Oncorhynchus mykiss*), grouper (*Epinephelus coioides*), rock bream (*Oplegnathus fasciatus*), cobia (Rachycentron canadum) and tilapia (*Oreochromis niloticus*).

An increase in complement components in treated

fish sera is because of an increase in WBC population as observed in this study. Also, an increase in level of total IgM has been seen in the treated fish compared to control one (P>0.05) indicating of stimulating of lymphocyte population for IgM production as already reported by other researchers using some teleost fish (Salinas Abelli, Bertoni, Picchietti, Roque, Furones, Cuesta, Meseguer & Esteban 2008; Sun, Yang, Ma & Lin 2010).

Conclusion

In conclusion, the in vitro environmental responses of LAB recovered from the intestine of Persian sturgeon are in correlation with the physiological conditions of fish gastrointestinal tract making it possible to use some of these LABs such as *L. lactis* as a potential probiotic against *A. hydrophila* septicemia. However, examination of the pathogenicity of these LAB bacterial species as probiotic candidates is required prior to judging on their probiotic activity in fish. This is particularly true in case of *L. garvieae* that has become a universal pathogen for many fish species under different environmental conditions. Also, the results of in vitro and in vivo works here in this study clearly show that supplementation of Persian sturgeon feed with *L. lactis* as a native probiotic is able to enhance both fish growth performances and some immunophysiological responses.

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فعالیت ضدباکتریایی، حساسیت آنتی بیوتیکی و استفاده پروبیوتیك باکتری اسید لاکتیك در پرورش تاسماهی ایرانی (*Acipenser persicus*)

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۱ گروه بهداشت و بیماریهای آبزیان دانشکده دامپزشکی دانشگاه تهران. قطب بهداشت و بیماریهای آبزیان دانشگاه تهران

۲ بخش بهداشت و بیماریهای آبزیان موسسه تحقیقات بین المللی تاسماهیان دریای خزر، سازمان تحقیقات، آموزش و ترویج کشاورزی، رشت

چکیده

ارزیابی رفتار رشد ۵ باکتری اسید لاکتیک شناسایی شده از تاسماهی ایرانی در شرایط متفاوت pH ، دمایی و شوری انجام گرفت. همچنین فعالیت ترکیبات ترشحی خارج سلولی باکتری‌های شناسایی شده علیه *Aeromonas hydrophila* و نیز حساسیت آنها نسبت به آنتی بیوتیک‌های غالب مورد مصرف در آبزیان ارزیابی شد. اثر *L. lactis* به عنوان یک مکمل غذایی در یک دوره ۵۶ روزه نیز در شرایط پرورشی تعیین گردید. مطالعات آزمایشگاهی نشان داد که این باکتری‌ها در محدوده دمایی ۳۰-۲۰ درجه سانتی گراد و شوری ۸-۴ ٪ قابلیت رشد دارند. ارزیابی فعالیت ترکیبات ترشحی خارج سلولی این باکتری‌ها نشان داد که این باکتری‌ها در دامنه دمایی ۳۰-۲۵ درجه سانتی گراد از توانایی مقابله با *A. hydrophila* برخوردارند. بیشترین فعالیت آنتاگونیستی ترکیبات خارج سلولی باکتری‌های *P. pentosaceus*، *W. cibaria* و *L. lactis* علیه *A. hydrophila* در pH ۹ بوده است. باکتری‌های *W. cibaria* و *E. faecalis* نسبت به آنتی بیوتیک های اکسی تتراسایکلین، اریترومایسین،تری متوپریم سولفامتوکسازول، انروفلوکساسین، فلورفنیکل و فلومکوئین مقاوم بوده اند درحالیکه باکتری *L. lactis* نسبت به اکسی تتراسایکلین، اریترومایسین، انروفلوکساسین و فلورفنیکل حساسیت نشان داد. نتایج این مطالعه نشان داد که مصرف *L. lactis* می‌تواند در بهبود شاخص‌های رشد، کارآیی غذا و بهداشت تاسماهی ایرانی به عنوان یک پروبیوتیک احتمالی مؤثر باشد.

واژه‌های کلیدی: ماهیان خاویاری، بهداشت، لاکتوکوکوس لاکتیس، پروبیوتیك.

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