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The effect of lactic acid bacteria administration on growth, digestive enzyme activity and gut microbiota in Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) fry

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Abstract

The aims of this study was to assess the effect of two lactic acid bacteria (LAB), *Lactobacillus curvatus* and *Leuconostoc mesenteroides*, originally isolated from gastrointestinal (GI) tract of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*), respectively, on growth, survival and digestive enzyme (amylase, lipase and protease) activities and the population level of LAB in the GI tract. The treatments included 10 different groups; control, separate supplements of *L. curvatus* and *Leu. mesenteroides* at three different counts [2×10^9, 5×10^9 and 9×10^9 colony forming units (CFU) per gram food] and three combinations of the two LAB ($2 \times 10^9 + 2 \times 10^9$, $5 \times 10^9 + 5 \times 10^9$ and $9 \times 10^9 + 9 \times 10^9$ CFU per gram food). The bacteria used in this study were added in lyophilized form to chopped Chironomidae. In the beluga study, highest specific growth rate, survival and improved intestinal enzyme activities were noted in the rearing group fed 9×10^9 *L. curvatus* per gram food. In Persian sturgeon, the inclusion level of 2×10^9 *Leu. mesenteroides* had similar positive effect. The ability of LAB to colonize the digestive tract seems to involve host specificity, and our bacteriological results are relevant to initiate future probiotic studies in sturgeons and future directions will be discussed.

KEY WORDS: beluga, digestive enzymes, growth, LAB, Persian sturgeon, probiotic

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Introduction

Sturgeon is one of the oldest anadromous or potamodromous family (*Acipenseridea*) among the bony fishes with 28 species (Bahmani *et al*. 2001; Asadi *et al*. 2006), and the most important genera includes; *Acipenser*, *Huso*, *Scaphirhynchus* and *Pseudoscaphirhynchus* (Bemis *et al*. 1997; Bahmani *et al*. 2001; Askarian *et al*. 2009). Of the six species, living in the Caspian Sea are beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*) two of them (Bahmani *et al*. 2001), and nowadays, these two species are mentioned as endangered species because of over fishing, loss of habitat and decrease of water quality according to IUCN list (www.iucnredlist.org).

Some information is available on the microbiota on external surface (skin, gills and fins) and in the gastrointestinal (GI) tract of sturgeon (Shenavar Masouleh *et al*. 2006; Delaedt *et al*. 2008; Huys *et al*. 2008; Akrami *et al*. 2009; Askarian *et al*. 2009; Ghanbari *et al*. 2009). However, to our knowledge, there is no information available about the use of probiotics in sturgeon aquaculture. The term probiotics is constructed from the Latin word *pro* (for) and the Greek word *bios* (life) (Zivkovic 1999) and was created in the 1950s by Kollath (1953). Lilley & Stillwell (1965) used this term to denote bacteria that promote the health of other organisms, but the definition of a probiotic used in aquaculture differs greatly depending on the source (Gram & Ringø 2005; Merrifield *et al*. 2010). Generally, probiotics offer potential alternatives by providing benefits to the host primarily via the direct or indirect modulation of the intestinal microbiota, enhanced immune system and growth, stimulate enzyme activity and improved disease resistance. As described by numerous authors, several lactic acid bacteria (LAB) strains have been used as probiotic in aquaculture to increase the immune enhancement, disease resistance, modulate the gut microbiota and competitive exclusion of pathogens through the production of inhibitory compounds (Gatesoupe 1991, 1999, 2008; Gildberg *et al*. 1997; Gildberg & Mikkelsen 1998; Ringø & Gatesoupe 1998; Phianphak *et al*. 1999; Ringø & Birkbeck 1999; Robertson *et al*. 2000; Nikoskelainen *et al*.

2001, 2003; Panigrahi *et al*. 2004; Ringø 2004; Ringø *et al*. 2005, 2010; Balcazar *et al*. 2006, 2007, 2008; Merrifield *et al*. 2010). Furthermore, there are several reports available about the influence of probiotics on digestive enzyme activity in fish (Tovar *et al*. 2002; Tovar-Ramirez *et al*. 2004; El-Haroun *et al*. 2006; Wache *et al*. 2006; Ghosh *et al*. 2008; Suzer *et al*. 2008; Saenz de Rodriganez *et al*. 2009). This topic is highly relevant to evaluate as the intestinal tract, where digestion and absorption take place, is very important for fish. The first aim of this study was therefore to investigate the effect of two LAB, *Lactobacillus curvatus* and *Leuconostoc mesenteroides* originally isolated from the GI tract of beluga and Persian sturgeon, respectively (Askarian *et al*. 2009), on growth, survival and digestive enzyme (amylase, lipase and protease) activities.

The increased interest during the last decade in LAB in the GI tract of fish is related to the fact that LAB often produce bacteriocins and other chemical compounds that may inhibit colonization of pathogenic bacteria in the GI tract (Ringø *et al*. 2005; Ringø 2008; Merrifield *et al*. 2010). However, even if these bacteria produce antimicrobial compounds, they might not be applicable as probiotics in aquaculture if they are not able to adhere to and colonize gut mucus. Finally, we addressed the issue as to whether dietary supplement of LAB modulate the population level of LAB in the GI tract and evaluate whether host specificity is involved.

Methods and materials

Experimental design

The present investigation was carried out at the International Sturgeon Research Institute, Gilan Province, Iran. Three thousand fry of Persian sturgeon (*A. persicus*) and beluga (*H. huso*) with mean weight and length of 40.30 ± 2.06 mg and 12.01 ± 0.90 mm and 50.10 ± 1.03 mg and 13.00 ± 0.90 mm, respectively, were used. Sixty 20-L fibre glass tanks (100 fish per tank) supplied with fresh water were used and the experiment lasted for 50 days. Ten treatments with three replicates were used for each species. Mean temperature, pH and oxygen level during the experiment were 17 °C, 7.1 and 8.1 mg L^{-1} for Persian sturgeon and 15 °C, 7.1 and 8.6 mg L^{-1} for beluga.

Preparation of the feed

In this study, two species of LAB were used. The *Leu. mesenteroides* strain showed 99% similarity to accession no AB362705 and was originally isolated from the GI tract of Persian sturgeon (Askarian *et al*. 2009), while *L. curvatus* (similarity = 98% to accession no. AY204891) was originally isolated from the GI tract of beluga (Askarian *et al*. 2009). The bacteria were added at different counts in lyophilized form to chopped Chironomidae (blood worms collected daily from natural environment) and immediately fed to the respective tanks. The viability of freeze-dried bacteria was determined by plate counting on MRS agar and was 2.8×10^9 CFU g^{-1} for *Leu. mesenteroides* and 1.2×10^{13} CFU g^{-1} for *L. curvatus*. The fish were fed approximately 6% of their body weight every day and a detailed description the 10 different treatments presented in Table 1.

Specific growth rate and feed conversion ratio

Sampling was carried out seven times (every week) during the experiment. Thirty fry from each treatment were randomly sampled for determination of specific growth rate (SGR) as described by Kissil *et al*. (2001).

$$\mathrm{SGR} = (\ln W_2 - W_1)\ (\mathrm{g})/(t_2 - t_1)\ (\mathrm{day})$$

Enzymatic determinations

At the end of the experiment after 50 days, were 30 fishes from each treatment group transferred to laboratory for enzymatic determination. The head and tail of fish were cut off and the remaining part homogenized on ice in an electric homogenizer (Heidolph instruments, Schwabach, Germany)

Table 1 Experimental treatments applied in studies with beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*)

Treatment no.	1	2	3	4	5	6	7	8	9	10
Type and counts of bacteria (CFU g^{-1} food)	A $10^9 \times 2$	A $10^9 \times 5$	A $10^9 \times 9$	B $10^9 \times 2$	B $10^9 \times 5$	B $10^9 \times 9$	A + B $10^9 \times 2 + 10^9 \times 2$	A + B $10^9 \times 5 + 10^9 \times 5$	A + B $10^9 \times 9 + 10^9 \times 9$	Control

A – *Lactobacillus curvatus* was originally isolated from the digestive tract of beluga (Askarian *et al.* 2009); B – *Leuconostoc mesenteroides* was originally isolated from the digestive tract of Persian sturgeon (Askarian *et al.* 2009).

as described elsewhere (Furné *et al*. 2005). Thereafter, were the homogenates centrifuged at 30 000 ***g*** for 30 min at 4 °C in a Kontron centrifuge model Centrikon H-401 (Zurich, Switzerland) and the supernatant collected and frozen at −80 °C (Furné *et al*. 2005). Protease, amylase and lipase activities were determined according to the methods described by Furné *et al*. (2005).

Isolation of gut bacteria

Bacterial analyses were carried out at the end of experiment, after 50 days of feeding. The fish were starved for 24 h before sampling to clear their alimentary tracts (Bairagi *et al*. 2002). The head and tail of 30 fishes, 3 × 10 fish from each tank treated separately, were cut off, and the remaining parts were thoroughly rinsed three times with sterile saline (0.85% w/v), transferred to betadine (a mixture of povidone–iodine and detergent used to disinfect skin) solution (0.01% v/v) for 15 min, and washed three times with sterile saline to remove non-adherent bacteria. Three replicates consisting of 10 fish from each tank were suspended in 10 mL sterile saline. Pooled samples of 10 fish were used to avoid individual variations in the gut microbiota (Spanggaard *et al*. 2000; Ringø *et al*. 2006). The suspended samples were homogenized with an electric homogenizer. The homogenates were serially diluted with sterile saline; 0.1 mL of the appropriate dilutions spread on the surface of triplicate plates of tryptic soy agar added 5% glucose (TSAg), MRS and lactic agar and incubated at 30 °C under aerobic conditions for 2 days. Preliminary identification of the gut bacteria was carried out by light microscopy (CH3-BH-PC; Olympus, Tokyo, Japan) and standard biochemical tests (Gram-staining, oxidase, nitrate reduction, catalase tests, aerobic and anaerobic growth). LAB strains from each treatment were identified based on CO_2 production from glucose, production of NH_3 from arginine, growth at different temperatures (15 and 45 °C), different pH (4.2 and 9.6) and their ability to grow in different concentrations of NaCl (6.5% w/v, 10% w/v and 18% w/v) in MRS broth, as described elsewhere (Sujaya *et al*. 2001; Thapa *et al*. 2006) for determination of *Leu. mesenteroides* and *L. curvatus*.

Statistical analysis

All results are presented as the average and standard deviation and one-way ANOVA was performed to determine the significant difference ($P < 0.05$) between parameters according to Ribeiro *et al*. (1999) and Nikoskelainen *et al*. (2003).

Results

The SGR of Persian sturgeon (*A. persicus*) displayed highest value in treatment 4 (supplemented 2×10^9 *Leu. mesenteroides*) while lowest value was noted in treatment 3 (supplemented with 9×10^9 *L. curvatus*) (Fig. 1). SGR was significant different ($P < 0.05$) between these two treatments.

The results of the beluga (*H. huso*) study revealed maximum and minimum SGR in treatment 3 and 6 (supplemented 9×10^9 *Leu. mesenteroides*), respectively (Fig. 1). No significant difference ($P > 0.05$) was observed in SGR between treatment 1, 2 and 3 fed *L. curvatus*.

The highest and lowest survival of Persian sturgeon was noted in treatment 4 and 3, respectively (Fig. 2). Furthermore, treatment 4, 5 and 6 fed only *Leu. mesenteroides*

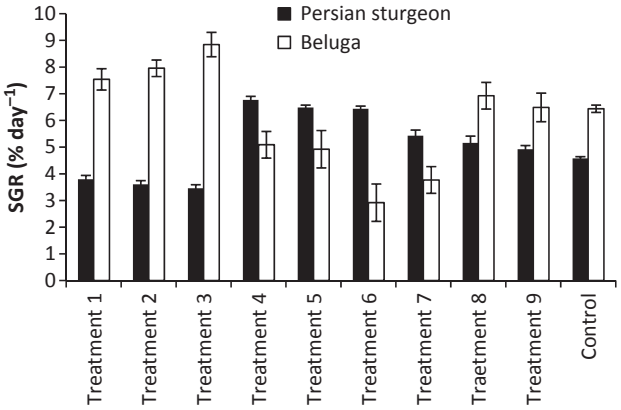

Figure 1 Specific growth rate (SGR) of Persian sturgeon and beluga. Each value represents the mean value ± SD of 30 fry from each treatment.

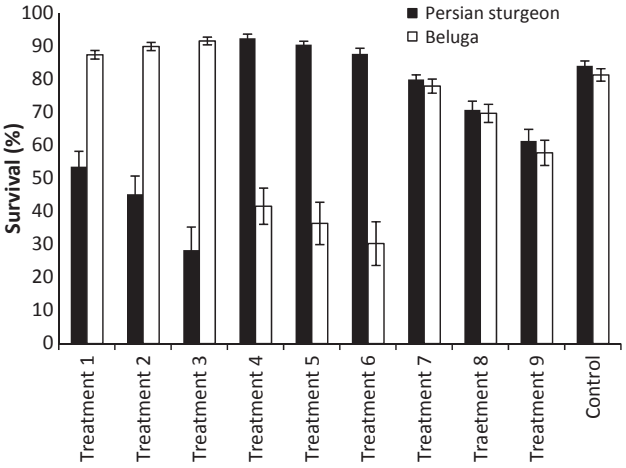

Figure 2 Survival (%) of Persian sturgeon and beluga. Each bar represents mean ± SD. $N = 300$ in each treatment.

showed significant higher ($P < 0.05$) survival compared to the other treatments. According to Fig. 2 highest and lowest survival of beluga were noticed in treatment 3 and 6, respectively, and the difference between these two treatments was significantly different ($P < 0.05$). The three treatments (1–3) of beluga fed *L. curvatus* had a significantly ($P < 0.05$) higher survival compared to the other treatment groups.

Amylase, lipase and protease activities in Persian sturgeon and beluga are showed in Fig. 3. Highest enzymatic activities in Persian sturgeon were detected in treatment 4 while lowest activities was observed in treatment 3. Generally, the enzyme activities in treatments fed *Leu. mesenteroides* (group 4–6) were significantly ($P < 0.05$) higher compared to the other treatments.

In beluga, maximum amylase, lipase and protease activities were noticed in treatment 3 while lowest enzyme activities were noticed in the control group, fish fed control diet for 50 days (Fig. 3). The enzyme activities in three treatments (1–3) fed *L. curvatus* were significantly higher than the other treatments.

Total viable counts (TVC) of aerobic gut bacteria in Persian sturgeon and beluga are showed in Table 2. In all the treatment groups fed LAB for 50 days, the population level of bacteria per g increased from the initial sampling, 4.2×10^4 TVC g^{-1} (log = 4.62) in Persian sturgeon and 6.9×10^4 TVC g^{-1} (log = 4.84) in beluga, to approximately 5.4×10^4 TVC g^{-1} (log = 4.73) in Persian sturgeon and around 8.2×10^4 TVC g^{-1} (log = 4.91) in beluga, respectively. However, the TVC levels in experimental groups of Persian sturgeon and beluga fed LAB were more or less similar to that of control fish after 50 days of feeding indicating that LAB feeding does not affect the population level of the adherent aerobic gut microbiota.

Generally, higher population levels of intestinal LAB were observed in Persian sturgeon fed *Leu. mesenteroides*, treatment groups 4–6, compared to fish fed *L. curvatus* (treatment groups 1–3), the LAB mixture and control fish (Table 2). In contrast to these results, highest LAB levels in beluga were noticed when the fish were fed *L. curvatus*, treatment groups 1–3. In fish fed a mixture of the two LAB strains, generally higher LAB levels were observed in beluga compared to that of Persian sturgeon. However, the population levels of LAB in Persian sturgeon and beluga fed a LAB mixture were more or less similar to that noticed in the control group after 50 days of feeding (Table 2). The ratio of LAB versus TVC (%) in the GI tract of Persian sturgeon and beluga is showed in Table 2. Compared to the approximate value of 0.80% at initial sampling, prior to experimental start, and values of

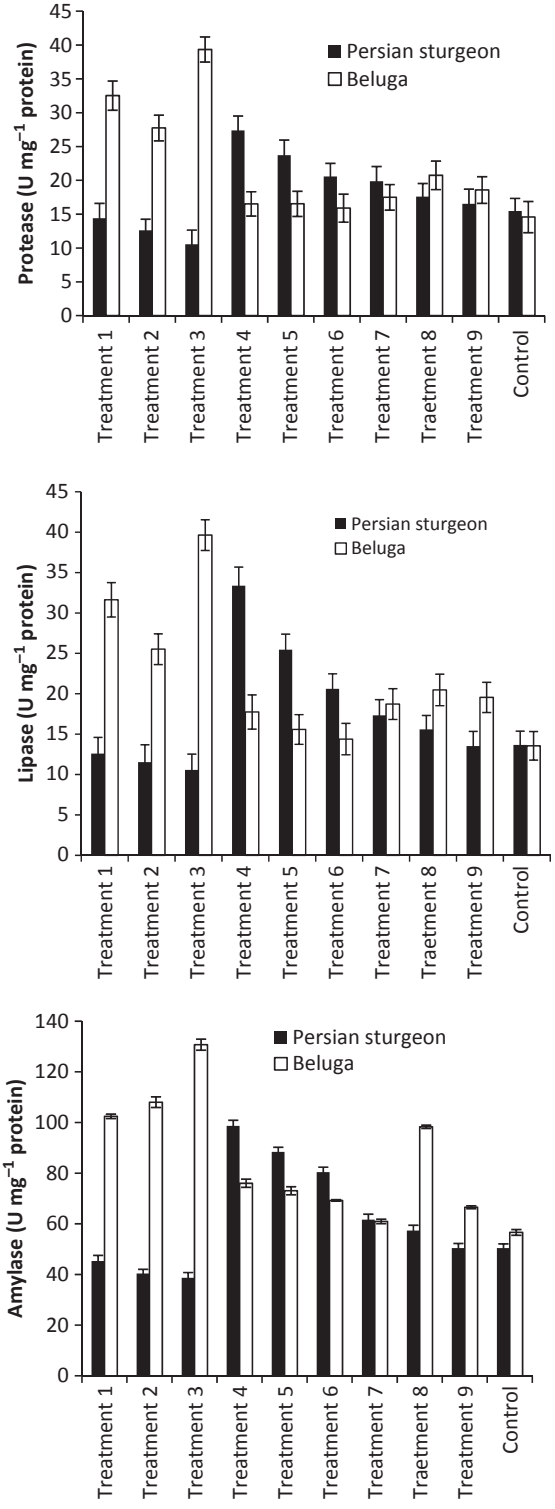

Figure 3 Mean amylase, lipase and protease activities in Persian sturgeon and beluga. Each bar represents mean value ± SD of 30 fry from each treatment.

Table 2 Total viable counts (TVC g^{-1}), total lactic acid bacteria (LAB g^{-1}), ratio of LAB versus TVC (%) and numbers of *Lactobacillus curvatus* and *Leuconostoc mesenteroides* strains isolated from the digestive tract of Persian sturgeon and beluga

	Start	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Control
Persian sturgeon											
TVC	4.2×10^4 ± 5.6×10^3	5.3×10^4 ± 8.5×10^3	5.3×10^4 ± 7.5×10^3	5.4×10^4 ± 7.3×10^3	5.7×10^4 ± 8.7×10^3	5.6×10^4 ± 9.3×10^3	5.6×10^4 ± 6.9×10^3	5.3×10^4 ± 7.9×10^3	5.5×10^4 ± 8.7×10^3	5.1×10^4 ± 5.8×10^3	5.3×10^4 ± 7.1×10^3
LAB	340 ± 12	220 ± 11	210 ± 14	190 ± 19	840 ± 28	720 ± 31	700 ± 25	460 ± 19	340 ± 18	310 ± 16	320 ± 19
Ratio of LAB/TVC (%)	0.81	0.41	0.38	0.35	1.47	1.28	1.25	0.86	0.61	0.60	0.60
L. curvatus	0	2	2	4	0	0	0	2	3	3	2
Leu. mesenteroides	70	55	50	40	470	480	540	180	130	105	110
Beluga											
TVC	6.9×10^4 ± 8.1×10^3	8.2×10^4 ± 6.9×10^3	8.4×10^4 ± 7.1×10^3	8.1×10^4 ± 6.5×10^3	8.1×10^4 ± 8.2×10^3	8.2×10^4 ± 7.1×10^3	8.1×10^4 ± 7.3×10^3	8.2×10^4 ± 6.7×10^3	8.1×10^4 ± 9.1×10^3	8.0×10^4 ± 8.0×10^3	8.2×10^4 ± 7.9×10^3
LAB	540 ± 21	720 ± 19	770 ± 24	1070 ± 31	550 ± 24	420 ± 19	360 ± 18	600 ± 22	690 ± 23	615 ± 20	612 ± 32
Ratio of LAB/TVC (%)	0.78	0.87	0.91	1.32	0.67	0.50	0.44	0.72	0.85	0.76	0.74
L. curvatus	200	500	620	950	230	160	140	240	340	280	270
Leu. mesenteroides	11	36	31	20	44	45	47	24	28	31	24

Each value of TVC and LAB represents mean value ± SD of three replicates (10 fry) per treatment.

0.60% and 0.74% in Persian sturgeon and beluga fed the control diet for 50 days, respectively, the highest values (between 1.25% and 1.47%) were noted in Persian sturgeon fed *Leu. mesenteroides*. In contrast to these results, the highest ratio of 1.32% in beluga was demonstrated in fish fed 9×10^9 *L. curvatus*.

Identification of *L. curvatus* and *Leu. mesenteroides* in the digestive tract of Persian sturgeon and beluga showed surprising results (Table 2). In Persian sturgeon, few *L. curvatus* strains were identified while the number of *Leu. mesenteroides* strains were clearly higher in treatment groups exposed only to *Leu. mesenteroides*. In contrast to these results, a completely different situation occurred in beluga, as in this fish species few strains of *Leu. mesenteroides* were identified even when the fish were fed *Leu. mesenteroides*. On the other hand, high level of *L. curvatus* strains was detected when beluga were fed *L. curvatus*. Based on the results presented in Table 2, we suggest that the ability of LAB to colonize the digestive tract of sturgeon seems to involve host specificity. However, when the sturgeons were fed a mixture of the two LAB, treatment 7–9, the population level of *L. curvatus* and *Leu. mesenteroides* was more or less similar to that observed for the control fish.

Discussion

Sturgeons are important contributor to fish production in many countries located around the Caspian Sea. Concerted research efforts have concentrated on optimizing production with eco-friendly alternatives to the therapeutic use of antibiotics. The first studies on screening of probiotic bacteria from aquaculture environments were initiated during the 1980s (Schröder *et al*. 1980; Dopazo *et al*. 1988; Kamei *et al*. 1988; Strøm 1988), has garnered attention for disease prevention in aquaculture (Gatesoupe 1999, 2008; Gomez-Gil *et al*. 2000; Verschuere *et al*. 2000; Irianto & Austin 2002; Balcazar *et al*. 2006; Kim & Austin 2006; Merrifield *et al*. 2010; Sharifuzzaman & Austin 2010). Moreover, probiotics have been attributed with improved food safety in a more environmentally friendly way (Macey & Coyne 2005). Certainly, FAO has now suggested the use of probiotics as a mean of improving the quality of the aquatic environment (Subasinghe *et al*. 2003).

The results of the present study revealed that supplementation of LAB to the food of Persian sturgeon and beluga significantly improved SGR and survival of treatment groups fed LAB originally isolated from the fish species investigated. To our knowledge, improved SGR and survival on the use of probiotics have been reported in two shrimp species

Fenneropenaeus indicus (Ziaei-Nejad *et al*. 2006) and *Penaeus vannamei* (Wang 2007), common carp (*Cyprinus carpio*) (Wang & Xu 2006) and rohu (*Labeo rohita*) (Ghosh *et al*. 2003), red drum (*Sciaenops ocellatus*) (Li *et al*. 2005), Japanese flounder (*Paralichthys olivaceus*) (Taoka *et al*. 2006) and gilthead sea bream (*Sparus aurata*) (Suzer *et al*. 2008). Positive effect of *Bacillus subtilis* on growth and survival of ornamental fishes (*Poecilia sphenops*, *Poecilia reticulata*, *Xiphophorus maculates* and *Xiphophorus helleri*) is also documented (Ghosh *et al*. 2008). The enhanced growth performance might be because of increasing digestive enzyme activity induced by the probiotics, as it has been reported that Gram-positive bacteria, particularly members of the genus *Lactobacillus*, have ability to secrete a wide range of exo-enzymes (Moriarty 1996, 1998; Suzer *et al*. 2008). With respect to SGR and fry survival, it was interesting to notice that Persian sturgeon fed *L. curvatus* originally isolated from beluga (treatment 1–3) and beluga fed *Leu. mesenteroides* (treatment 4–6) originally isolated from Persian sturgeon were significantly lower than that observed for the control group. These findings have not been elucidated but it can be speculated that the bacteria has a detrimental effect on gut morphology, health parameters and the protective gut microbiota. However, to clarify this, additional studies are necessary.

It has been documented in a number of aquatic animals that the GI microbiota plays an important role in nutrition (Sakata 1990; Ringø *et al*. 1995; Thompson *et al*. 1999; Verschuere *et al*. 2000; Suzer *et al*. 2008). In addition, some bacteria may participate in the digestion processes of bivalves by producing extracellular enzymes, such as proteases, lipases, as well as providing necessary growth factors (Prieur *et al*. 1990). Similar observations have been reported for the microbiota of adult penaeid shrimp (*Penaeus chinensis*), where a complement of enzymes for digestion and synthesize compounds that are assimilated by the animal (Wang *et al*. 2000). Microbiota may serve as a supplementary source of food, and microbial activity in the digestive tract may be a source of vitamins, essential amino acids and fatty acids (Dall & Moriarty 1983; Sakata 1990).

Various mechanisms have been proposed to explain the beneficial effects of probiotics such as: (i) antagonism towards pathogens, (ii) competitions for adhesion sites, (iii) competition for nutrients, (iv) improvement of water quality, stimulation of host immune responses and (v) enzymatic contribution to digestion. Several studies have documented nutritional effect of algae, probiotic bacteria and *Saccharomyces* on the digestive enzymes of fish and shellfish larvae (Cahu *et al*. 1998; Tovar *et al*. 2002; Tovar-Ramirez *et al*. 2004; Wache *et al*. 2006; Wang & Xu 2006; Ghosh *et al*. 2008; Suzer *et al*. 2008; Saenz de Rodriganez *et al*. 2009). In the present study, the enhancement of SGR and survival in sturgeon fry was simultaneously noticed with increase in digestive enzyme activity. Our results are in agreement with the results of Tovar-Ramirez *et al*. (2004) that reported improved activity of the digestive enzyme, trypsin, amylase and lipase, in European sea bass (*Dicentrarchus labrax*) larva by adding live yeast (*Debaryomyces hansenii*) to the diet. Furthermore, Wang & Xu (2006) showed significant difference ($P < 0.05$) of digestive enzymes activity, protease, amylase and lipase, in common carp by using *Bacillus* sp. as probiotics. In a later study, Suzer *et al*. (2008) reported improved activity of the intestinal enzymes, alkaline phosphatase and leu–ala-peptidase and pancreatic trypsin, amylase and lipase, by using the *Lactobacillus* spp. as probiotic in gilthead sea bream larvae. The enhanced digestive enzyme activities observed in some of the treatment groups in the present study might be attributed to improved gut maturation as previously suggested by Tovar *et al*. (2002) in a study using *D. hansenii* originally isolated from the gut of rainbow trout (*Oncorhynchus mykiss*). In addition to this direct effect, some authors have suggested that the main modes of action and beneficial effects of probiotics are prevention of intestinal disorders and predigestion of antinutrient factors present in the ingredients (Thompson *et al*. 1999; Verschuere *et al*. 2000; Suzer *et al*. 2008). To clarify the mechanisms involved, further sturgeon studies have to be carried out. Moreover, further investigations need to be carried out to clarify why gut enzyme activities were generally lower in Persian sturgeon fed *L. curvatus* originally isolated from beluga (treatment 1–3).

The results of the present study displayed that LAB do not belong to the dominant GI microbiota of sturgeon, as the bacteria only accounted for 0.3–1.4% of the total culturable gut microbiota in all treatments (Table 2). Even though *L. curvatus* and *Leu. mesenteroides* to some extent was detected in the gut of beluga and Persian sturgeon, respectively, at experimental start, their population levels remain unaffected even after 50 days of feeding on the control diet. Based on this finding, we conclude that these bacteria do not belong to the dominant gut microbiota in beluga and Persian sturgeon. Ringø & Gatesoupe (1998) also reported that LAB do not belong to the dominant intestinal microbiota of fish. However, in the present study, the numbers of *L. curvatus* and *Leu. mesenteroides* increased in the gut of beluga (treatments 1, 2 and 3) and Persian sturgeon (treatments 4, 5 and 6), respectively, compared to initial sampling and control after 50 days of feeding (Table 2). It is also worth to notice

that the highest number of LAB was observed in sturgeon groups with highest SGR and gut enzyme activity.

In probiotic studies, one can speculate whether the probiont can tolerate the pH of the fish stomach and survive the passage to the intestine. As the pH of stomach in fish is between 3.0 and 4.5 (Ringø *et al*. 2003) and the evacuation time of food is relatively short, it is generally accepted that bacteria such as LAB can survive passage to the intestine. Furthermore, one should bear in mind that *Lactobacillus* and *Leuconostoc* can tolerate relative low pH (4.5).

Difficulties in analysing the complexity of bacterial community by classic methods of cultivation have necessitated the development of molecular methods. To overcome these problems, various methods such as denaturing gradient gel electrophoresis (DGGE), fluorescence *in situ* hybridization and temporal temperature gradient electrophoresis and clone libraries have been developed to circumvent the need for isolation. To the authors' knowledge, only one preliminary study of Siberian sturgeon (*Acipenser baeri*) has investigated the gut microbiota by DGGE (Delaedt *et al*. 2008) using eubacterial primers as described by Muyzer *et al*. (1993). Therefore, in future studies, we recommend using DGGE when evaluating the bacterial gut community in Persian sturgeon and beluga. Furthermore, we recommend that future probiotic studies on sturgeon include the topics: supplementation duration, mucosal immune system, challenge studies and GI morphology evaluation as golden standards.

Nikoskelainen *et al*. (2001) suggested that mucosal adhesion is one of the five important criteria for the selection of probiotics in fish. Whereas some authors postulate that probiotic colonization of intestinal epithelial surface include host specificity (Lin & Savage 1984; Fuller 1986), other have reported the absence of specificity in LAB when binding host intestinal mucus. Gildberg & Mikkelsen (1998) suggested that there seems to be no host specificity between the two strains of *Carnobacterium divergens*, originally isolated from the gut of Atlantic cod (*Gadus morhua* L.) and Atlantic salmon (*Salmo salar* L.) in the GI tract of Atlantic cod fry. Ringø (1999) questioned whether *C. divergens* originally isolated from the gut of Atlantic salmon (Strøm 1988) was able to attach the mucosal surface and colonize the gut of turbot (*Scophthalmus maximus* L.) larvae, and concluded that no host specificity was involved in the gut of turbot larvae at the time of hatching. Two later studies also reported the absence of specificity in LAB when binding host intestinal mucus (Rinkinen *et al*. 2003; Salinas *et al*. 2008). As the fish were starved for 24 h prior to sampling, we suggest that the gut bacteria isolated in the present study probably belong to the autochthonous microbiota. However, one critical comment to this statement is that the sampling procedure is insufficient to distinguish between the autochthonous and allochthonous gut bacteria. However, few strains of *Leu. mesenteroides* were identified in the digestive tract of beluga even when the fish were fed *Leu. mesenteroides* (Table 2). Based on the results presented in Table 2, we put forward the controversial hypothesis that host-specific adherence of *Leu. mesenteroides* in the digestive tract of Persian sturgeon and *L. curvatus* in the GI tract of beluga fry might occur. However, more information concerning the mechanism of action eventually involved in the host-specific adhesion of LAB in sturgeon merits further research.

In the present study, the fry were continuously fed the probiotic diets. However, as the fish were not reverted back to the control diet for a longer period, we cannot conclude that the LAB used in the present study are permanently colonizing the digestive tract. To clarify this additional studies are necessary.

Another aspect on the use of probiotics is dose-dependent studies. According to Merrifield *et al*. (2010), such studies are currently limited and somewhat contradictory. This was also demonstrated in the present study, as supplementation of 9×10^9 *L. curvatus* in the diet to beluga improved the gut enzyme activities, while addition of 2×10^9 *Leu. mesenteroides* enhanced gut activities in Persian sturgeon. To clarify the mechanisms, further studies have to be carried out.

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