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Diversity of Lactic Acid Bacteria in the Gastrointestinal Tracts of Reared Beluga (*Huso huso*) and Persian Sturgeon (*Acipenser persicus*): A Comparative Study

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Abstract: The composition of lactic acid bacteria (LAB) in intestine of two species of sturgeon, beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*), was analyzed. LAB in the gastrointestinal (GI) tract of the two sturgeon species was not similar as LAB population levels in beluga was significantly higher than Persian sturgeon. Six strains of lactic acid bacteria isolated from the GI tract of beluga and Persian sturgeon were characterised by 16S rDNA. Two species of LAB including *Enterococcus seriolicida* and *Leuconostoc mesenteroides* were isolated from GI tract of Persian sturgeon and the predominant species was *L. mesenteroide*. Furthermore, *Lactobacillus curvatus*, *Lactococcus raffinolactis*, *Lactococcus lactis* and *Streptococcus* sp. were isolated from the GI tract of beluga and the counts of *L. curvatus* was significantly higher in the GI tract of beluga than other species.

Key words: Beluga, Persian sturgeon, lactic acid bacteria, gastrointestinal tract

INTRODUCTION

The chondrostean fishes comprised of the sturgeons and paddlefishes, are anadromous and potamodromous species of the Northern Hemisphere. The evolutionary history goes back to 100 million years, apparently originated as a group in fresh water in the early Triassic Period and includes 28 species, of which 6 species inhabit the Caspian basin (Bahmani, 1998; Barton *et al*., 2000). Beluga sturgeon (*Huso huso*) and Persian sturgeon (*Acipenser persicus*) are anadromous species endemic to the waters of Caspian Sea and rivers following into it. Different aspects about ecology and biology of this primitive bony fish are well studied (Bahmani, 1998). Sturgeon culture is an important industry and technologies for commercial culture of various sturgeon species have been established over the last 20-30 years to provide meat and caviar for human consumption plus recreational purpose (Willims *et al*., 1990).

It is known that resident microbiota play an important role in host health and physiology (Hooper *et al*., 2001; Yan and Polk, 2002; Tuohy *et al*., 2003; Hagi *et al*., 2004). Bacteria belonging to the LAB are characterized as Gram-positive, usually nonmotile, nonsporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism. Members of this group containing both rods (lactobacilli and carnobacteria) and cocci (streptococci) and they are generally catalase-negative and they usually lack cytochromes.

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During the last decade, it has been suggested that the intestinal microbiota of fish is more complex than earlier believed (Ringø and Gatesoupe, 1998; Asfie *et al*., 2003; Hagi *et al*., 2004; Burr *et al*., 2005; Gram and Ringø, 2005) and that several species of LAB are part of the natural intestinal microbiota of healthy fish (Ringø, 2004). It has been reported that the intestinal LAB of fish are highly variable and that LAB seems to change in the aqueous environment (Gatesoupe, 1999; Irianto and Austin, 2002b; Hagi *et al*., 2004).

A better understanding of the many factors affecting the population level of LAB in the digestive tract of fish may be of interest for commercial aquaculture. From an Iranian point of view, more information is required about the different techniques and physiological conditions of sturgeon culture which can contribute to a better management and development of the fish with regards to the presence of LAB in the digestive tract. Therefore, the present study, conducted for the first time in Iran, focuses on isolation of LAB in the GI tract of two species of sturgeons cultured under identical conditions.

MATERIALS AND METHODS

Fish and Rearing Conditions

This project was conducted at International Sturgeon Research Institute at Gilan province in Iran. Thirty juvenile beluga and 30 juvenile Persian sturgeon, weight between 90 to 1000 g (Table 1), were reared in 20 square fiberglass tanks (three fishes from each weight group and each species, were kept in one tank) separately, volume 500 L, supplied with fresh water at 10°C, pH 7.3, 7 ppm dissolved oxygen and 0.1 L sec^{-1} discharge. Fish were fed commercial dry pellets (45% protein, 14% fat and 10% carbohydrate, Chine Co., Iran). Fishes were acclimated for 15 days before experimental start and fasted 24 h before sampling.

Sampling was conducted not only from juvenile sturgeons but also from larva stage. For this purpose, 90 beluga and 90 Persian sturgeon larva were sampled two times during larva rearing including: at the first of mixed feeding with weight of 50±1.01 mg in beluga and 40±1.20 mg in Persian sturgeon and at the end of larva rearing with weight of 345±10.31 mg in beluga and 103±8.23 mg in Persian sturgeon.

Media

In this study, tryptic soy agar medium (TSA) supplemented with 5% glucose (D(+) - glucose monohydrate) (Riedel-dehaën, Germany) (TSAg), MRS agar medium (Lactobacilli MRS Broth (Difco) supplemented with 1.2% agar) and lactic agar medium (20 g tryptone peptone (Difco), 5 g yeast extract, 2.5 g gelatin, 5 g glucose, 5 g lactose, 5 g sucrose, 4.0 g NaCl, 1.5 g sodium acetate, 0.5 g ascorbic acid, pH 6.8 and diluted to 1000 mL) were used for isolation of Total Viable Counts (TVC) and Lactic Acid Bacteria (LAB) in the digestive tract of beluga and Persian sturgeon.

Table 1: Number and weight of sampled juvenile sturgeons in each species

	Average of weight (g)		No. of sample	
Weight (g)	Beluga	Persian sturgeon	Beluga	Persian sturgeon
100>	91±2.01	91±2.01	3	3
100-200	138±1.23	138±1.23	3	3
200-300	227±1.23	227±1.23	3	3
300-400	312±1.76	312±1.76	3	3
400-500	431±1.34	431±1.34	3	3
500-600	511±2.06	511±2.06	3	3
600-700	633±1.45	633±1.45	3	3
700-800	741±1.56	741±1.56	3	3
800-900	831±1.72	831±1.72	3	3
900-1000	961±1.72	961±1.72	3	3

Isolation of Intestinal Bacteria from Juvenile Beluga and Persian Sturgeon

The bacteriological sampling of the two fish species was carried out in February 2007. Three fishes of each species and weight group (Table 1) were sampled. Sturgeons were sampled after anesthesia in 140 mg L^{-1} buffered solution of tricaine methane sulphonate (MS_{222}), which induced the cessation body and opercular movements within 4-8 min after exposure. The skin was washed with 70% ethanol before opening the ventral surface with sterile scissors. The gastrointestinal (GI) tract of each fish was divided into four sections; oesophagus, stomach, proximal-and distal intestine and 1 g of each sections were washed three times with sterile saline (0.85% (w/v) and suspended in 10 mL of it. The suspension was serially diluted to 10^{-7} and 0.1 mL of the solution was spread in triplicate on TSAg and MRS agar plates. The plates were incubated at 30°C under aerobic and aerobic conditions for two day. Colonies were divided according to their macroscopic morphology in each plate and the number of them were counted. After enumeration, 600 colonies from beluga and 400 colonies from Persian sturgeon were removed and subcultured until purity and stored at -80°C. Then primitive identification of LAB bacteria was carried out according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1999) by using different tests and media as catalase, oxidase, nitrate reduction, Sulfide-Indole-Motility (SIM) agar (Merck, Germany), O.F. Basal medium (Merck, Germany) , lactic agar medium, considering their growth under different temperature (10 and 45°C) and finally Gram staining. After these procedures, colonies that their characterizations are similar to LAB bacteria according to Bergey's key including 40 colonies from beluga and 30 colonies from Persian sturgeon were selected.

Isolation of Intestinal Bacteria from Beluga and Persian Sturgeon Larvae

Ninety sturgeon larvae from each species were used for bacteriological investigations. Larvae (selected from rearing thanks) was washed three times with sterile saline (0.85% (w/v) NaCl) and put into 0.01% Iodine solution for 15 min and thereafter washed three times using with sterile saline. Thereafter, larvae were homogenized in 5 mL of sterile saline by using an electric homogenizer (Heidolph Instruments). The suspension was diluted to 10^{-7} and 0.1 mL of the solution was spread in triplicate onto TSAg and MRS agar plates. The plates were incubated at 30°C under aerobic and aerobic conditions for 2 days. Colonies were divided according to their macroscopic morphology in each plate and the number of them were counted. After enumeration, 600 colonies from beluga and 400 colonies from Persian sturgeon were randomly removed and subcultured until purity and stored at -80°C. Then primitive identification of LAB bacteria was carried out according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1999) and colonies that their characterizations are similar to LAB bacteria according to Bergey's key including 30 colonies from beluga and 20 colonies from Persian sturgeon were selected.

Identification of Lactic Acid Bacteria (LAB) by 16S rRNA Gene Sequencing

Identification of LAB species by PCR was carried out. Seventy colonies from beluga and 50 colonies from Persian sturgeon were used and suspended in 50 μL of distilled water and directly used as the PCR template. PCR was performed using an iCycler. The reaction mixture using Takara Ex Taq (Takara Bio, Otsu, Japan) was as follows: 1 μL of template solution, 2 μL of 10×Ex Taq buffer, 2 μL of dNTP mixture, 0.8 μL of each primer (10 pmol μL^{-1}), 0.1 μL of Ex Taq polymerase and 13.3 μL of sterilized distilled water. The 16S rRNA gene was amplified by PCR by using bacteria specific primers. The primer sequences were 5'-AGAGTTTGATCCTGGCTCAG-3' (10F, corresponding to positions 8-27 of the *Escherichia coli* 16S rRNA gene) and 5'-GGTTACCTTGTTACGACTT- 3' (1,500R, corresponding positions 1510-1492 of *E. coli* 16S rRNA gene). After 9 min preheat at 95°C, amplification was performed by means of 35 cycles of 1 min of denaturing at 95°C, 1 min at 50°C for primer annealing and 2 min at 72°C for primer extension. PCR

products were purified using a Gene Clean III Kit. Purified fragments were sequenced using CEQ2000 Dye Terminator Cycle Sequencing with Quick Start Kit and CEQ2000XL DNA Analysis System (Beckman Coulter). The same primer (10 F) used for PCR amplification was used as a sequencing primer. We determined approximately 400 bases at the 5' end. The sequences derived from isolates were used for BLAST search (National Center for Biotechnology Information).

Other primers that were used to determine the full sequence of 16S rDNA, included:

- 500R (5'-GTATTACCGCGGCTGCTGCTGG-3')
- 800R (5'-CATCGTTTACGGCGTGGAC-3')
- 1100R (5'-TTGCGCTCGTTGCGGGACT-3')
- 1400R (5'-ACGGGCGGTGTGTACAAG-3')
- 1000F (5'-GTCCCGCAACGAGCGCAAC-3')

RESULTS

Total Viable Counts of Bacteria

TVC in the digestive tract of beluga, in all weight groups from 100 to 1000 g, were 1.3×10^8 cfu g^{-1} GI and approximately 1.1×10^8 g^{-1} GI in Persian sturgeon (Table 2, 3).

TVC of bacteria in beluga larvae (8.2×10^7 cfu g^{-1} GI) and Persian sturgeon (5.2×10^7 cfu g^{-1} GI) larvae were significantly lower than that observed in the other weighting groups. Significant difference ($p<0.05$) in TVC was observed between Persian sturgeon and beluga in larval stages compared to the other weight groups.

Table 2: The Mean of total viable count of bacteria from gastrointestinal tract of juvenile (n = 30 for each species) and larva (n = 90 for each species) of beluga under aerobic and anaerobic condition

Weight (g)	Average of weight (g)	Total viable count (TVB) (cfu g^{-1} intestine)
100>	91±2.01	1.2×10^8
100-200	138±1.23	1.3×10^8
200-300	227±1.23	1.3×10^8
300-400	312±1.76	1.4×10^8
400-500	431±1.34	1.4×10^8
500-600	511±2.06	1.4×10^8
600-700	633±1.45	1.4×10^8
700-800	741±1.56	1.4×10^8
800-900	831±1.72	1.4×10^8
900-1000	961±1.72	1.3×10^8
Average of TVB (cfu g^{-1} intestine)		1.3×10^8
Larvae (cfu $larvae^{-1}$)	50±1.72	8.2×10^7

Table 3: The mean of total viable count of from gastrointestinal tract of juvenile (n = 30 for each species) and larva (n = 90 for each species) of Persian sturgeon under aerobic and anaerobic condition

Weight (g)	Average of weight (g)	Total viable count (TVB) (cfu g^{-1} intestine)
100>	95±1.10	8.61×10^8
100-200	149±1.34	1.10×10^8
200-300	243±2.41	1.10×10^8
300-400	310±1.54	1.10×10^8
400-500	421±2.31	1.10×10^8
500-600	524±1.98	1.10×10^8
600-700	645±2.03	1.10×10^8
700-800	767±1.89	1.10×10^8
800-900	875±2.18	1.10×10^8
900-1000	978±1.86	1.10×10^8
Average of TVB (cfu g^{-1} intestine)		1.10×10^8
Larvae (cfu $larvae^{-1}$)	40±2.43	5.20×10^7

Table 4: The mean of lactic acid bacteria isolated from gastrointestinal tract of juvenile (n = 30) and larva (n = 90) of Persian sturgeon and beluga

Weight (g)	Beluga (cfu g^{-1} intestine)	Persian sturgeon (cfu g^{-1} intestine)
100>	6.50×10^6	4.00×10^6
100-200	7.60×10^6	5.10×10^6
200-300	7.70×10^6	5.20×10^6
300-400	8.30×10^6	5.80×10^6
400-500	8.20×10^6	5.70×10^6
500-600	8.30×10^6	5.80×10^6
600-700	7.90×10^6	5.40×10^6
700-800	8.50×10^6	6.00×10^6
800-900	8.00×10^6	5.50×10^6
900-1000	7.50×10^6	5.00×10^6
Average (cfu g^{-1} intestine)	7.90×10^6	5.40×10^6
Larvae (cfu $larvae^{-1}$)	4.40×10^6	2.20×10^6

Table 5: Total counts of LAB in different part of gastrointestinal (GI) tract of juvenile beluga and Persian sturgeon

Species GI	Esophagus (cfu g^{-1} esophagus)	Stomach (cfu g^{-1} stomach)	Proximal intestine (cfu g^{-1} intestine)	Distal intestine (cfu g^{-1} intestine)
Beluga	9.4×10^5	2.8×10^6	3.2×10^5	3.8×10^6
Lactococcus lactis	1.1×10^5	1.4×10^5	1.0×10^5	1.4×10^5
Lactococcus raffinolactis	1.2×10^5	1.6×10^5	1.0×10^5	6.1×10^5
Lactobacillus curvatus	4.8×10^5	2.5×10^6	1.2×10^5	3.0×10^6
Streptococcus sp.	2.3×10^5	-	-	-
Persian sturgeon	1.7×10^5	5.4×10^5	1.1×10^5	4.6×10^6
Leuconostoc mesenteroides	1.7×10^5	5.4×10^5	1.1×10^5	3.8×10^6
Enterococcus seriolicida	-	-	-	8.1×10^5

Detection of Lactic Acid Bacteria (LAB)

As shown in Table 4, the total counts of LAB in the beluga intestinal tract were 7.9×10^6 cfu g^{-1} GI and 5.8×10^6 cfu g^{-1} GI in Persian sturgeon in all weight groups from 100 to 1000 g. No significant difference was detected between different weight groups.

Total counts of culturable LAB counts in beluga larvae (mean weight 50 mg) (4.4×10^6 cfu g^{-1} GI) and Persian sturgeon larvae (mean weight 40 mg) (2.2×10^6 cfu g^{-1} GI) was significantly ($p<0.05$) lower than that observed in the other weight groups.

The percentages of LAB compared with TVC values were 6.1 and 5.2% for beluga and Persian sturgeon, respectively. Furthermore, the percentages of LAB in larvae of beluga and Persian sturgeon were 5.3 and 4.2%, respectively.

Population Level of LAB in Different Sections of the Gastrointestinal Tract

According to Table 5 the maximum counts of LAB were isolated from distal intestine in beluga (3.8×10^6 cfu g^{-1} GI) and Persian sturgeon (4.6×10^6 cfu g^{-1} GI). The counts of LAB in proximal intestine of beluga (3.2×10^5 cfu g^{-1} GI) and esophagus of Persian sturgeon (1.7×10^5 cfu g^{-1} GI) were significantly lower in comparison to other sections ($p<0.05$).

The percentages of LAB in distal intestine, compared with total LAB counts in other parts of GI, were approximately 48 and 96% for beluga and Persian sturgeon, respectively.

Composition of LAB in the Gastrointestinal Tract of Sturgeon and Identification of LAB by 16S rDNA

Seventy LAB colonies from beluga and 50 LAB colonies from Persian sturgeon were selected as described before and 16S rDNA analysis was performed. Fifty LAB colonies from beluga were identified as *Lactobacillus curvatus*, 10 LAB colonies as *Lactococcus raffinolactis* , 6 LAB colonies as *Lactococcus lactis* and 4 LAB colonies as *Streptococcus* sp. Furthermore 40 LAB colonies from Persian sturgeon were identified as *Leuconostoc mesenteroides* and 10 LAB colonies as *Enterococcus seriolicida* (Table 5, 6).

Table 6: Closest relative of isolated lactic acid bacteria from beluga and Persian sturgeon

Species	Sequence identity (%)	Strain No.	Accession No.
Lactobacillus curvatus	98-99	YMRS3	AY204891
Lactococcus lactis	98-99	ATCC 19257	M58836
Lactococcus raffinolactis	98-99	NCDO 617	X54261
Leuconostoc mesenteroides	98-99	NRIC 1539	AB023246
Enterococcus seriolicida	98-99	ATCC 49156	AJ387923

Table 7: Total counts of LAB species in larva beluga and Persian sturgeon larva

Species	Total No. (cfu larva^{-1})
Beluga	
Lactococcus lactis	1.0×10^{6}
Lactococcus raffinolactis	1.2×10^{6}
Lactobacillus curvatus	1.9×10^{6}
Streptococcus sp.	3.1×10^{5}
Persian sturgeon	
Leuconostoc mesenteroides	1.9×10^{6}
Enterococcus seriolicida	3.2×10^{5}

The numbers and compositions of LAB in the intestinal tract of two species of sturgeon (beluga and Persian sturgeon) were examined. Four species of LAB including *Lactobacillus curvatus*, *Lactococcus raffinolactis*, *Lactococcus lactis* and *Streptococcus* sp. were isolated from GI of juvenile beluga.

As shown in Table 5, *L. curvatus* and *Streptococcus* sp. had significantly the maximum (6.1×10^{6} cfu g^{-1} GI) and minimum (2.3×10^{5} cfu g^{-1} GI) counts of LAB that isolated from gastrointestinal tract in juvenile beluga ($p<0.05$). No significant difference was detected between counts of *L. lactis* and *Streptococcus* sp. in beluga ($p>0.05$). *Streptococcus* sp. was isolated from esophagus but other species were isolated from all parts of GI tract of juvenile beluga.

The percentages of *L. curvatus*, *L. raffinolactis*, *L. lactis* and *Streptococcus* sp. compared with total counts of LAB were about 79.7, 15.2, 4.9 and 2.9%, respectively in beluga.

According to Table 5, *Leuconostoc mesenteroides* (4.5×10^{6} cfu g^{-1} GI) had significantly the maximum counts of LAB that isolated from gastrointestinal tract of Persian sturgeon ($p<0.05$) in comparison to *Enterococcus seriolicida*. The percentages of *L. mesenteroides* and *E. seriolicida* were about 87.8 and 12.2%, respectively.

The results revealed that all four species of LAB including *Lactobacillus curvatus* (1.9×10^{6} cfu larva^{-1}), *Lactococcus raffinolactis* (1.2×10^{6} cfu larva^{-1}), *Lactococcus lactis* (1.0×10^{6} cfu larva^{-1}) and *Streptococcus* sp. (3.1×10^{5} cfu larva^{-1}) were also isolated from beluga in larva stage. Furthermore, *Leuconostoc mesenteroides* (1.9×10^{6} cfu larva^{-1}) and *Enterococcus seriolicida* (3.2×10^{5} cfu larva^{-1}) were isolated From Persian sturgeon in larva stage (Table 7). The results described in the previous sections and this section suggested that LAB composition in Larva and juvenile of beluga and Persian sturgeon followed the same pattern.

DISCUSSION

During the last two decades several studies have demonstrated the presence of lactic acid bacteria (LAB) in the digestive tract of fish (Ringø, 2004). However, the present study is the first one showing the presence of LAB in the gastrointestinal (GI) tract of beluga and Persian sturgeon. Based on our results and that of Ringø (2004) we put forward the hypothesis that LAB are more frequently occurring in the GI tract of fish than previous believed. However, one should bear in mind that LAB are not dominant in the normal intestinal microbiota of fish. On the one hand, it is possible to maintain an artificially high population level of LAB in the fish gut by a regular intake with food containing the bacteria (Ringø, 2004). With respect to sturgeon farming and especially at larval stages such treatment

should be further considered to improve health and quality of sturgeon in culture. On the other hand, there are a growing number of studies reporting the importance of lactic acid bacteria as well as of other bacteria in the prevention of fish diseases (Gram and Ringø, 2005). The importance of a stable gut microbiota might have some application as the gut is one of the major infection routes in fish (Birkbeck and Ringø, 2005), especially when vaccination is not yet fully operational.

It is known that the intestinal microbial flora of fish changes in parallel with environmental changes (Holben *et al*., 2002; Hagi *et al*., 2004). The intestinal microbial flora of fish would also be expected to vary among fish species. However, no systematic examination has previously been reported on the difference in LAB compositions among fish species living in the same environment (Sugita *et al*., 1994; Al-Harbi and Uddin, 2004).

Total viable counts and total LAB counts in beluga and Persian sturgeon larvae were significantly lower in comparison to adult of two fishes and LAB has the lowest population in gastrointestinal tract of beluga and Persian sturgeon. Similar results has been also reported by Hagi *et al*. (2004).

The presence of LAB in different part of GI has been demonstrated in different fish species (Ringø, 2004). The results of present study revealed that distal intestine had the maximum counts of LAB in beluga and Persian sturgeon and based on present results we put forward the hypothesis that distal intestine is the best site for isolating LAB in the two species of sturgeon.

Two species of LAB *Enterococcus seriolicida* and *Leuconostoc mesenteroides were* isolated from GI tract of Persian sturgeon in this study. The count of *L. mesenteroides* was significantly higher than other species.

There is scarce information available about the presence of *Enterococcus* in the GI tract of fish as only few studies have isolated the bacteria (Ringø, 2004). The studies that have isolated *Enterococcus* from fish include brown trout (Gonzalez *et al*., 2000), common carp (*Cyprinus carpio*) (Cai *et al*., 1999), turbot (*Scophthalmus maximus* L.) (Toranzo *et al*., 1995), common carp (*Cyprinus carpio*) (Hagi *et al*., 2004), Persian sturgeon (the present study) and Atlantic salmon (*Salmo salar* L.).

Leuconostoc sp. has only been isolated in three studies. Ringø and Strøm (1994) isolated the bacteria from the feces of Arctic charr (*Salvelinus alpinus* L.), where it contributed for approximately 4.5% of the total microbiota when the charr were fed a capelin roe diet. In a later study, Ringø *et al.,* (1998) demonstrated that a *Leuconostoc* sp., which resembled that for *L. mesenteroides* were found, associated with the epithelial mucosa of stomach, small and large intestines when Arctic charr were fed different dietary polyunsaturated fatty acids.

Lactobacillus curvatus, *Lactococcus raffinolactis*, *Lactococcus lactis* and *Streptococcus* sp. were isolated from GI tract of beluga. However, these species were not isolated from the GI tract of Persian sturgeon.

It is documented in several investigations that lactobacilli are part of the native intestinal microbiota of Arctic charr (Ringø, 1993), Atlantic cod (*Gadus morhua* L.) (Strøm and Olafsen, 1990), Atlantic salmon (*Salmo salar* L.) (Ringø *et al*., 2000), brown trout (*Salmo trutta* L.) (Gonzalez *et al*., 2000), common carp (Hagi *et al*., 2004), silver carp (*Hypophthalmichthys molitrix*) (Hagi *et al*., 2004), saithe (*Gadus virens* L.) (Schrøder *et al*., 1980; Strøm, 1988) and beluga (the present study).

The first well-documented study of *Streptococcus* associated with the mucosa of the gastrointestinal tract of fish (salmonids) was reported by Trust and Sparrow (1974). In this study, *Streptococcus* sp. was isolated from proximal and distal intestine. *Streptococcus*-like bacteria was found associated with the epithelial mucosa in stomach and small intestine of Arctic charr fed dietary linoleic acid and linolenic acid (Ringø *et al*., 1998). However, in most of these studies, the bacterial species was isolated from the whole intestinal tract.

There are some reports of causing diseases by genus of *Lactococcus* in fishes. *Lactococcus garvieae* caused diseases in Yellowtail (Kusuda *et al*., 1991) and rainbow trout (Ghittino *et al*., 1995).

Lactococcus piscium was also another species of this genus of LAB that had same effect on rainbow trout (Williams *et al*., 1990). Hagi *et al*. (2004) reported the present of two safe species of this genus including *Lactococcus raffinolactis* and *Lactococcus lactis* that isolated from four cultured freshwater fish species including silver carp (*Hypophthalmichthys molitrix*), common carp, channel catfish (*Ictalurus punctatus*) and deep bodied crucian carp (*Carassius cuvieri*) as same as this research.

The present study showed that the percentage values of LAB compared to total viable counts and based on this observation , LAB is only a minor part of the gut microbiota in sturgeon. Same result has been reported by Hagi *et al*. (2004).

Indigenous LAB are clearly important for fish health, as are the species reported in human beings (Yan and Polk, 2002; Tuohy *et al*., 2003). The predominant LAB discovered in this study may be candidates for use as probiotic bacteria in sturgeons. It is reported that some intestinal bacteria, including LAB isolated from fish intestine, have antibacterial properties (Cai *et al*., 1998; Sugita *et al*., 1998). Experiments using such bacteria as probiotics for fish have recently been reported (Byun *et al*., 1997; Cai *et al*., 1998; Gram *et al*., 1999; Robertson *et al*., 2000; Nikoskelainen *et al*., 2001; Spanggaard *et al*., 2001; Irianto and Austin, 2002a).

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