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Characterization of *Lactobacillus* from perch (*Perca fluviatilis*) intestines of Aras Dam, Iran

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ABSTRACT

Lactobacilli bacteria are gram positive non-spore forming, catalase-negative and filamentous bacteria that mainly ferment different carbohydrates into lactate and acetate. Fifty perch (perca fluviatilis) hunted from Aras Dam, Iran, randomly. The samples were transported to the Microbiology Laboratory, under sterile conditions in the vicinity of ice and after dissection according to sterile method 1 gram of stool was sampled from the anterior part of intestine and cultured in MRS agar-plates. After phonotypical and biochemical identity of bacteria, for distinction of lactobacillus species molecular characterization, 16S rDNA gene was amplified and PCR products were sequenced. Results of sequencing about 3 isolated bacteria indicated that this species has been Lactobacillus plantarum and the founding's corresponding to produce results from biochemical tests and determine cutting pattern for this species.

Key words: Perch, Lactobacillus plantarum, Aras Dam, bacteria, Iran

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INTRODUCTION

The perch, Perca fluviatilis is a freshwater species inhabiting different ecosystems varying from artificial ponds to brackish water. The perch especially adults are known to prefer slow-slowing rivers, deep lakes and ponds avoids cold, fast-flowing waters but may penetrate into but not breed in such waters. Normally they are found lying close to or amongst obstacles in the water. Larval perch chose habitats to increase survival. For this purpose, the larval perch move from littoral zone to the open water and then return to littoral zone after a few weeks (22). The perch may also stay in pelagic zone for several months (7). Stomach contents analysis of perch indicates that they go through two major ontogenetic shifts during their life span. The larval perch feed on protozoan, phytoplankton, rotifers and copepod nauplii and switch to macro invertebrates at intermediate sizes and then to piscivory at larger sizes (13, 20). Although, piscivory may occur at early stages under certain conditions (5).

Studies showed that the perch show trophic polymorphisms influenced by habitat and resource use. The overall food composition of different perch populations is usually dissimilar and is dependent on the specific lake, the species composition and the availability of the feed base. It has been observed that the diet structure of individuals inhabiting littoral and pelagic habitats change within the same lake (2). Lactobacilli bacteria are gram positive,

without spore, catalase-negative and filamentous bacteria that mainly ferment different carbohydrates into lactate and acetate. Different types of amino acids, vitamins and minerals are essential for their growth (16). Lactobacilli have important ecological roles in the gastrointestinal tract that includes production of antimicrobial substances, immune response enhancement and increase in nutrient availability and use of non- digestible carbohydrates (1, 12). The aim of this study was to investigate lactobacillus plantarum in the intestine of perch (Perca fluviatilis) in Aras Dam by PCR Method.

MATERIALS AND METHODS

During this research, 50 perch (Perca fluviatilis) hunted from Aras dam randomly, in 2012 spring. The samples were transported to the Microbiology Laboratory under sterile condition and in the vicinity of ice. After dissection according to sterile method, 1 gram of stool was sampled from the intestine content and homogenized with 9 cm^3 of sterile saline and vortexes for 1 min in stomacher. Subsequently, dilution series were prepared from the homogenate in sterile saline from 10^{-1} to 10^{-10} and pour plated on MRS agar plates. To prepare the best conditions for growth of the lactobacilli incubated in an anaerobic jar with gas pack type C at 37^{oC} for 48-72 hours. MRS agar and broth were used for enumeration and culture of LAB (9). Plates that had 25-250 colonies were counted. After recording the results, well isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plate and transferred to MRS broth.

Identification of the bacterial strains: The cultures were identified according to their morphological cultural, physiological and biochemical characteristics (16). The used tests were gram reaction, production of catalase and cytochrome oxidase growth at 15 and 45^{oC} in 1 week acid production from carbohydrates (1% w/v), D-galactose, surbitol, lactose, arabinose, mannitole, melezitoze, melibioze maltose, raffinose, salicin and trehalose in MRS broth devoid of glucose and beef extract with chlorophenole red as indicator, production of acid and gas from glucose (MRS-broth without beef extract) H and L test in O/F medium (8).

Amplification of 16S rDNA: Total chromosomal DNA was extracted from overnight broth cultures of the various strains according to the method reported by Atashpaz et al. (2010), and subsequently quality of extracted DNA was evaluated using gel monitoring apparatus (G: Box ^{TM} Version 06-2d.1e, Syngene, Cambridge, UK) and Spectrophotometric Method (NanoDrop 1000 Spectrophotometer, NanoDrop Technologies, Wilmington, DE, USA) (3). Amplification of the16S rDNA was carried out using the primer pair reported earlier (17):

16F 5'-AGAGTTTGATCMGGCTCAG-3'
16R-5'-TACCTTGTTAGGACTTCACC-3'

PCR amplification was performed using master mix (Ampliqon, Herlev, Denmark), 0.4 µm primer, 40 ng chromosomal DNA and the final volume was reached to the 25µL. The cycling program was as follow: denaturation at 95°C for 4 min, 32 cycle of 94°C, 50 sec, 59°C, 50 sec, 72°C for 80 sec and a final extension was performed 5 min in 72°C.

Sequencing and analysis of PCR-amplified 16S rDNA: PCR purified products were sequenced by Macrogene (Korea) having used Chromas Bioanformatic software, the determined sequences were compared with the sequences deposited in NCBI GenBank as 16S rDNA gene of different Lactobacillus species.

RESULTS AND DISCUSSION

During this research about 50 perch (perca fluviatilis) (200±10 g) hunted from Aras dam, 7 samples were positive for lactobacillus and after biochemical tests 4 cases of Lactobacillus plantarum were detected (Table 1). According to the FAO/WHO guidelines (10) DNA-DNA hybridization is the Gold Standard Method to identification of strains. However, this methodology is laborious and costly which requires a large collection of reference strains. The use of DNA sequences encoding 16S rDNA has been suggested as a suitable substitute (19). Therefore, to confirm the lactobacillus in the genus level, PCR amplification of 16S rDNA gene was performed. Figure 1 represents the experimental profiles of PCR reaction. As shown in Fig. 1, significant inhibition of the amplification was observed. And as expected, fragments (~1500 bp) correspondences to the full length of 16S rDNA in lactobacilli species were obtained. Results of 16S rDNA sequencing that had produced by forward and reverse primer by use of BLAST (b12seq) and chromas

Table 1: Differentiating characteristics of Lactobacillus fermentum

Lactobacillus sp.	*L. fermentum*	*L. plantarum*
Growth (˚C)		
15	-	+
45	+	-
Gram reaction	+	+
Production of catalase	-	-
Acid and gas from glucose	+	-
NH_3 from arginine	+	-
Sugar fermentation		
Arabinose	+	
Raffinose	+	+
Salicin	+	+
Lactose	+	
Sorbitol	+	
Mannitol	+	+
Trehalose	+	
Melezitose	-	
Mannose	+	+
Melibiose	+	+

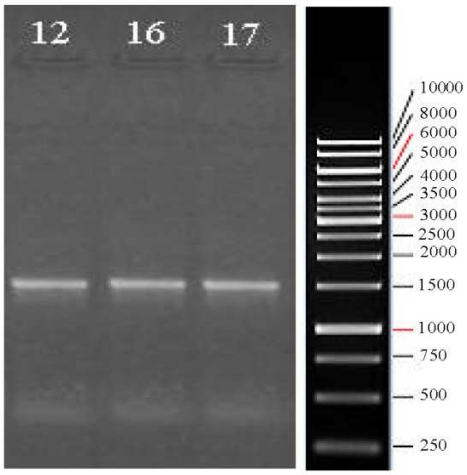

Fig. 1: PCR product (~1500 bp) of 16S rDNA gene for 3 putative isolates. Lane P1-P3 represents the PCR patterns

Bioinformatics Software analyzed and aligned. Results of sequencing about isolated bacteria indicated that this species has been Lactobacillus plantarum and the founding's corresponding to produce results from biochemical tests and determine cutting pattern for this species.

Lactobacilli are commonly found in foods like dairy products, beverages, fruits, vegetables and fermented meat. They are also important members of the intestinal and urogenital micro biota of humans and animals. Several lactobacillus strains are now being used as probiotics in commercially available food products. Strains which are studied thoroughly and are accepted as probiotics using established criteria belong to the species L. rhamnosus L. acidophilus, L. casei, L. reuteri and L. fermentum (21).

Lactobacilli are reported for the first time from the intestines of Eurpopean eel (A. anguila), perch (P. fluviatilis), Rudd (S. erithrophthalmus), Ruffe (G.cernuus), Bleak (A. alburnus), Silver bream (B. bjoerkna), orfe (L. cephalus)and Somnul (S. glanis) and African catfish (C. gariepinus). The two first species, commonly named eel and perch are highly valuable species for aquaculture (15, 18). In the study of Kvasnikov, common Lactobacilli such as L. plantarum, L. casei, L. leichmannil, L. acidophilus, L. fermentum, L. cellobiosus and L. buchneri were isolated from fish like Ciprinus carpio, Hypophthalmichtus molitrix and Aristichthus nobilis living in river (6).

During this research about 50 perch (Perca fluviatilis) (200±10 g) hunted from Aras dam, 7 samples were positive for lactobacillus and after biochemical tests 4 cases of L. plantarum were detected. In the study of Ghanbari et al.

(2009), number of lactobacilli in the intestine of Acipenser persicus and Huso huso was about $10^{5.3}$ to $10^{6.4}$ CFU g^{-1} (11). The main isolated lactobacillus was L. sakei and L. plantarum . In the study of Azizpour (2009) for biochemical identification of lactic acid bacteria in rainbow trout, 9% of bacteria isolated from intestinal content belonged to lactobacillus and 8% to enterococcus (4). Identified species of Lactobacillus were L. plantarum that was the dominant acid lactic bacterial population (5%).

In a survey by Jankauskiene (2002) to study the defense mechanism based on the frequency of lactobacilli bacteria in the gut micro flora of carp fish, in the contents of the gut and intestinal wall of 65 carps, lactobacillus bacteria was found in the gut contents of 42 carps (64.61%) and the intestinal wall of 22 carps (33.84%) (14). Microflora studies in different parts of intestine showed the existence of lactobacilli in the contents of anterior part of intestine of 24 carps, middle section of 25 carps and posterior part of 25 carps and in the microflora of intestinal wall of 7,11 and 7 carps, respectively. A number of very frequent of lactobacilli in all three sectors and the middle part of the wall have been identified (14). Researchers (2006), in study of presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system, the most common presumptive lactobacilli species were L. alimentarius and L. sakei. It is necessary however to use molecular techniques to have more reliable information on the identity and diversity of Lactobacilli species in fish (6).

CONCLUSION

In this study for molecular characterization, 16S rDNA gene was amplified. Results of 16S rDNA sequencing about isolated bacteria indicated that this species has been Lactobacillus plantarum and the founding's corresponding to produce results from biochemical tests and determine cutting pattern for this species.

Considering that so far no studies on lactobacilli in the intestine of perch (Perca *fluviatilis*) intestine in Aras dam had been done so during this review researchers were able to isolate L. fermentum and L. plantarum from the intestine of these fish in Aras dam for the first time.

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