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Characterization of Lactic Acid Bacteria Isolated from the Intestines of Common Carp of West Azarbaijn, Iran

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Abstract: The natural presence of lactic acid bacteria in fish may be of great interest in producing fermented fish products worldwide. The aim of this study was to characterization of Lactic Acid Bacteria (LAB) isolated from the intestines of various samples of the Common Carp (*Cyprinus carpio*) of commercial farms in West Azarbaijan, Iran. All isolates were gram-positive, catalase-positive bacilli that did not produce gas from glucose, H and L test positive in O/F medium. All isolates were able to growth at 15 and 45°C. This isolates were divided into genera of the *Lactobacillus* by sugar fermentation patterns. *Lactobacillus fermentium* was the dominant member of population of lactic acid bacteria that isolated from the intestines, accounting for 10% of the isolates.

Key words: Common carp, genus *Lactobacillus*, intestines, West Azarbaijan, Urmia, Iran

INTRODUCTION

Microflora of the intestinal tract is an integral part of the whole living organism. A great number of endogenous and exogenous factors determining the number and species composition of microbe populations and affecting physiological and biochemical features of the microorganisms themselves influence it. Many different bacteria get into an organism from the environment. However, due to the natural selection, only those bacteria survive, which find favourable living conditions in that organism. Lactic Acid Bacteria (LAB) are widely distributed in various animal intestines (Devriese *et al*., 1987; Mitsuoka, 1980; Sakata *et al*., 1980) and some LAB because probiotics have played an important role in beneficial functions for industrial animals (Perdigon *et al*., 1995). There have been several reports by Mitsuoka (1990) Perdigon *et al*. (1995) and Salminen and Wright (1998a) of LAB occurring among the major microbial populations in animal intestines. It is well established that some LAB improve the intestinal microflora and promote the growth and health of animals (Mitsuoka, 1990; Perdigon *et al*., 1995). Most probiotics contain single or multiple strains of LAB and are part of the natural microflora of many animals; they are generally regarded as safe and may display antagonistic activities against pathogenic bacteria (Byun *et al*., 1997; Garriga *et al*., 1998). The intestinal microflora, especially LAB, may influence the growth and health of fish. However, few studies have reported the composition of intestinal LAB flora in fish. Lactic Acid Bacteria (LAB) are characterized as Gram-positive, usually non-motile, nonsporulating bacteria that produce lactic acid as a major or sole product of fermentative metabolism. Kandler and Weiss (1986) have classified *Lactobacillus* isolates from temperate regions according to their morphology, physiology and molecular characters. Schleifer (1987) classified LAB based on the molecular characteristics. LAB from food and their current taxonomical status have been described by many (Huber *et al*., 2004; Ringø and Gatesoupe, 1998; Salminen and Von Wright, 1998b). Ringø and Gatesoupe (1998) have prepared a review of the LAB present in fish intestine. Taxonomic studies on LAB from poikilothermic animals are rare (Al-Harbi and Uddin, 2004; Asfie *et al*., 2003; Huber *et al*., 2004; Ringø and Gatesoupe, 1998).

The aim of the study was to characterization of Lactic Acid Bacteria (LAB) isolated from the intestines of various samples of the Common Carp (*Cyprinus carpio*) of commercial farms in West Azarbaijan, Iran.

MATERIALS AND METHODS

Fish and experimental conditions: The investigated 20 individuals adults Common Carp belonged to 4 commercial farms of West Azarbaijan of Iran (every farm 5 individuals). The fish transferred to the saloon for intensive culture of fish and were put into 4 tanks

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(100 L, Poly Vinyl Chloride). The flow rate of water was approximaly 4 L min^{-1}. The water temperature was measured 17±1°C during the whole trail.

Isolation of LAB: The fish were starved for 48 h before sampling and were sacrificed with a blow to the head. They opened aseptically and their whole intestines were removed. The intestines were dissected and their contents were collected by carefully scraping using a rubber spatula. Each time fish of 1 farm were sampled and the intestine content of each fish was weighed. One gram of the intestine content was homogenized with 9 mL of sterile saline and vortexed for 1 min in stomacher. Subsequently, dilution series were prepared from the homogenate in sterile saline from 10^{-1}-10^{-10} and pour plated on MRS agar plates. The plates were incubated anaerobically at 37°C for 48-72 h. MRS agar and broth were used for enumeration and culture of LAB (De Man *et al*., 1960). 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 (Kandler and Weiss, 1986; Sharpe *et al*., 1979). The used tests were: Gram reaction; production of catalase and cytochrome oxidase; growth at 15 and 45°C in 1 week; acid production from carbohydrates (1% w v^{-1})-D-fructose, D-galactose, D-xylose, mannose, raffinose, lactose, surbitol, salicin, glucose, melezitose, sucrose, ribose, arabinose, melebise, cellobiose, manitol, inolin, maltose and trehalose in MRS broth devoid of glucose and beef extract with chlorophenol red as indicator; production of acid and gas from 1% glucose (MRS broth without beef extract); H and L test in O/F medium.

RESULTS AND DISCUSSION

Counts of LAB were 10^{7}-10^{8} cfu g^{-1} in the intestines of the common carp. The LAB isolates were classified into the genera *Lactobacillus* based on their morphology and biochemical characters (Sharpe *et al*., 1979). Of the cultures, 10% in adult fish belonged to the genus *Lactobacillus*. The predominant *Lactobacillus* sp. was further classified to the species level (Kandler and Weiss, 1986). The differentiating characteristics of *Lactobacillus* sp. are given in Table 1 and 2. All isolates *Lactobacillus* sp. were Gram-positive, facultatively anaerobic, catalase-positive bacilli that did not produce gas from glucose, H and L test positive in O/F medium. All isolates were able to growth at 15 and 45°C. Strain showed variation in their sugar fermentation pattern. The results of the carbohydrate fermentation tests were positive reactions for most sugars. But fermentation test showed mannose-negative. The species identified showed above 80% or more similarity to the ATCC type cultures. Only tests that gave reproducible results were included in the classification scheme. The species identified was *L. fermentium* that was the dominant member of population of lactic acid bacteria that isolated from the intestines, accounting for 10% of the isolates. It is interesting to note that majority of the *Lactobacillus* sp. that have been isolated from adult fish were those species, which were commonly found on meat, animals and human (Kandler and Weiss, 1986). There were a few reports of isolation of LAB from fresh and seawater fish (Azizpour, 2009; Balcázar *et al*., 2007; Jankauskine, 2000; Yimin *et al*., 1999; Cone, 1982). However, Maugin and Novel (1994) found that *Lactococcus* was the major flora isolated from fish. The occurrence of typical lactobacilli as described by Kandler and Weiss (1986) were rare in fish and prawn. In our studies, we attempted to classify LAB on the basis of the available classification schemes. However, further studies are needed in order to include other atypical *Lactobacillus* cultures in the classification scheme.

Table 1: Differentiating characteristics of *Lactobacillus* sp.

	Growth at						
Lactobacillus sp.	15°C	45°C	Gram reaction	Production of catalase	Cytochrome oxidase	Indole production	H and L test in O/F medium
L. fermentium	+	+	+	+	+	-	+

Table 2: Sugar fermentation pattern

	Sugar fermentation						
Lactobacillus sp.	Mannose	Raffinose	Salicin	Lactose	Surbitol	Xylose	Trehalose
L. fermentium	+	+	+	+	+	+	+

	Glucose	Melezitose	Sucrose	Ribose	Arabinose	Melebiose	Cellobiose	Manitol
L. fermentium	+	+	+	+	+	+	+	+

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