MEDWELL PUBLISHING
Research Journal of Biological Sciences 4 (3): 324-326, 2009
ISSN: 1815-8846

Biochemical Characterization of Lactic Acid Bacteria Isolated from Rainbow Trout (*Oncorhynchus mykiss*) of West Azarbaijn, Iran

K. Azizpour
Department of Marine Biotechnology, Artaemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran

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 adult and young Rainbow Trouts (*Oncorhynchus mykiss*) of commercial farms in West Azarbaijan, Iran. All isolates were Gram-positive, catalase-positive bacilli and cocci that did not produce gas from glucose, H and L test positive in O/F medium. Most isolates were able to growth at 15 and 45°C. This isolates were divided into 2 groups by sugar fermentation patterns. Strain in the group 1 were placed in the clusters of the genera *Lactobacillus* and strain in the group 2 were placed in the clusters of the genera *Enterococcus*. *Lactobacillus plantarum* was the dominant member of population of lactic acid bacteria that isolated from the intestines, accounting for 9% of the isolates.

Key words: Lactic acid bacteria, rainbow trout, genus *Lactobacillus*, intestines, West Azarbaijan, Iran

INTRODUCTION

Microflora of the intestinal tract s 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 (Perdigon *et al*., 1995; Salminen and Wrightm, 1998) 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 (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 Huber *et al*. (2004), Ringø and Gatesoupe (1998) and Salminen and von Wright (1998). Ringo 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 adult and young Rainbow Trouts (*Oncorhynchus mykiss*) of commercial farms in West Azarbaijan, Iran.

MATERIALS AND METHODS

Fish and experimental conditions: The investigated 41 individuals adults and young Rainbow Trouts (*Oncorhynchus mykiss*) belonged to 5 commercial farms of West Azarbaijan of Iran: 1st farm (10 individuals: 5 adults and 5 young fish), 2nd farm (8 individuals:

5 adults and 3 young fish), 3rd farm (3 individuals: 3 adults), 4th farm (10 individuals: 5 adults and 5 young fish) and 5th farm (10 individuals: 5 adults and 5 young fish). The fish transferred to the saloon for intensive culture of fish and were put into 9 tanks (300 L, Poly Vinyl Chloride), comprising 2 groups (adult and young fish). 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, glucose, inolin, surbitol, lactose, maltose, D-mannose, raffinose, salicin, sucrose, trehalose and D-xylose 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

The LAB isolates were classified into the genera *Enterococcus* and *Lactobacillus* based on their morphology and biochemical characters (Sharpe *et al*., 1979). Table 1 shows the distribution of different genera of LAB in adult and young fish. Of the cultures, 9% in adult fish belonged to the genus *Lactobacillus* and 8% belonged to the genus *Enterococcus*. 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 2. All isolates *Lactobacillus* sp. were Gram-positive, catalase-positive bacilli that did not produce gas from glucose, H and L test positive in O/F medium. Most isolates were able to growth at 15 and 45°C. Strain showed variation in their sugar fermentation pattern. 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. plantarum* (2 isolates). *Lactobacillus plantarum* was the dominant member of population of lactic acid bacteria that isolated from the intestines, accounting for 9% 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 (Balcázar *et al*., 2007; Cone, 1982). *L. plantarum* have been isolated from herring, Arctic krill and chilled channel catfish fillets (Hagi *et al*., 2004; Schroder *et al*., 1979; Spanggaard *et al*., 2000). 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

Table 1: The percentage distribution of different genus of LAB in adult and young fish samples

	Lactic acid bacteria (%)	
Sample	*Enterococcus*	*Lactobacillus*
Adult fish (23)	8	9
Young fish (18)	6	0

Table 2: Differentiating characteristics of *Lactobacillus* sp.

	Growth at (°C)												
Lactobacillus sp.	15	45	Gram reaction		Production of catalase		Cytochrome oxidase		Indole production		H and L test in O/F medium		
L. plantarum	+	+	+		+		+		-		-		
	Sugar fermentation												
Lactobacillus sp.	Mannose	Raffinose	Salicin	Lactose	Surbitol	Xylose	Trehalose	Glucose	Maltose	Sucrose	Galactose	Fructose	Inolin
L. plantarum	+	+	+	+	+	+	+	+	+	+	+	+	+

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.

ACKNOWLEDGEMENT

This resaerch was supported by Artaemia and Aquatic Animals Research Institute under research project code A/009/2008. The author would like to thank A. Tukmechi for technical assistance.

REFERENCES

Al-Harbi, A.H. and M.N. Uddin, 2004. Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus*×*Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. Aquaculture, 229: 37-44.

Asfie, M., T. Yoshijima and H. Sugita, 2003. Characterization of the goldfish fecal microflora by the fluorescent *in situ* hybridization method. Fish Sci., 69: 21-26.

Balcázar, J.L., I. de Blas, I. Ruiz-Zarzuela, D. Vendrell, A.C. Calvo, I. Márquez, O. Gironés and J.L. Muzquiz, 2007. Changes in intestinal microbiota and humoral immune response following probiotic administration in brown trout (*Salmo trutta*). Br. J. Nutr., 97: 522-527.

Byun, J.W., S.C. Park, Y. Benno and T.K. Oh, 1997. Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). J. Gen. Applied Microbiol., 43: 305-308.

Cone, D.K., 1982. A possible marine form of *Ichthyobodo* sp. on haddock, *Melanogrammus aeglefinus* (L.), in the North-west Atlantic Ocean. J. Fish Dis., 5: 479-485.

De Man, J.C., M. Rogosa and E. Sharpe, 1960. A medium for the cultivation of lactobacilli. J. Applied Bacteriol., 23: 130-135.

Devriese, L.A., A. Kerckhove, R. Kilpper-Balz and K.H. Schleifer, 1987. Characterization and identification of *Enterococcus* sp. isolated from the intestines of animals. Int. J. Syst. Bacteriol., 37: 257-259.

Garriga, M., M. Pascual, J.M. Monfort and M. Hugas, 1998. Selection of lactobacilli for chicken probiotic adjuncts. J. Applied Microbiol., 84: 125-132.

Hagi, T., D. Tanaka, Y. Iwamura and T. Hoshino, 2004. Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. Aquaculture, 234: 335-346.

Huber, I., B. Spanggaard, K.F. Appel, L. Rossen, T. Neilson and L. Gram, 2004. Phylogenetic analysis and *in situ* identification of the intestinal microbial community of rainbow trout (*Oncorhynchus mykiss* Walbaum). J. Applied Microbiol., 96: 117-132.

Kandler, O. and N. Weiss, 1986. Microbiology of Mesu: A Traditional Fermented Bamboo Shoot Product. Bergey's Manual of Systematic Bacteriology. In: Sneath P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.). Baltimore: Williams and Wilkins, 2: 1209-1234.

Maugin, S. and G. Novel, 1994. Characterization of lactic acid bacteria isolated from seafood. J. Applied Bacteriol., 76: 616-625.

Mitsuoka, T., 1980. A Color Atlas of Anaerobic Bacteria. 1st Edn. Tokyo University Press, Tokyo, pp: 20-29.

Perdigon, G., S. Alvarez, M. Rachid, G. Aguero and N. Gibbato, 1995. Immune system stimulation by probiotics. J. Dairy Sci., 78: 1597-1602.

Ringø, E. and F.J. Gatesoupe, 1998. Lactic acid bacteria in fish: A review. Aquaculture, 160: 177-203.

Sakata, T., H. Sugita, T. Mitsuoka, D. Kakimota and H. Kadota, 1980. Isolation and distribution of obligate anaerobic bacteria from the intestines of freshwater fish. Bull. Jpn. Soc. Sci. Fish, 46: 1249-1255.

Salminen, S. and A. von Wright, 1998. Lactic acid Bacteria Microbiology and Functional Aspects. 2nd Edn. New York: Marcel Dekker Inc., pp: 180-193.

Schleifer, K.H., 1987. Recent changes in the taxonomy of lactic acid bacteria. FEMS Microbiol. Rev., 46: 201-203.

Schroder, K., E. Clausen, A.M. Sandberg and J. Raa, 1979. Identification of Lactic Acid Bacteria. Advances in Fish Science and Technology. In: Connel, J.J. (Ed.). Farnham, England: Fishing Newsbook Ltd, pp: 480-483.

Sharpe, M.E., T.F. Fryer and D.G. Smith, 1979. Identification of Lactic Acid Bacteria. Identification Methods for Microbiologists. In: Gibbs, E.M. and F.A. Skinner (Eds.). London: Academic Press, pp: 233-259.

Spanggaard, B., I. Huber, J. Nielsen, T. Nielsen, K.F. Appel and L.A. Gram, 2000. The microflora of rainbow trout intestine: A comparison of traditional and molecular identification. Aquaculture, 182: 1-15.