African Journal of Biotechnology Vol. 10(59), pp. 12717-12721, 3 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.565
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Predominant lactic acid bacteria isolated from the intestines of silver carp in low water temperature

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Accepted 12 August, 2011

The composition of intestinal lactic acid bacteria (LAB) in silver carp (*Hypopthalmichthys molitrix*) in Gheshlaghdam Lake was analyzed based on morphology and biochemical tests in December 2009 to March 2010. Most isolates were Gram-positive, non motile and catalase-negative bacilli that did not produce gas from glucose. Growth at 15°C was positive for all isolates, while it was positive for some isolates at 45°C.The results of the carbohydrate fermentation tests were positive reactions for most sugars. The results show that in winter, the predominant LAB were *Lactococcus plantarum, Lactococcus raffinolactis* and *Lactococcus lactis*, respectively.

Key words: Silver carp, lactic acid bacteria, intestine, Gheshlaghdam Lake.

INTRODUCTION

Lactic acid bacteria (LAB) are Gram-positive, non-sporulating and catalase negative rods or cocci that ferment various carbohydrates mainly to lactate and acetate. Various amino acids, vitamins and minerals are essential for their growth (Kandler and Weiss, 1986). Accordingly, they are commonly associated with nutriatious environments like foods, decaying material and the mucosal surface of the gastrointestinal and urogenital tract (Kandler and Weiss, 1986; Walstra et al., 1999). Various authors have shown that LAB are also part of the normal intestinal flora of fish (Ringø and Gatesoupe, 1998), with majority of the Lactobacillus species inhibiting the intestinal tract. It has been postulated that *lactobacilli* have several promoting effects, including the prevention of diarrhea and intestinal infections (Isolauri et al., 1991; Biller et al., 1995), alleviation of inflamematory bowel disease (Sartor, 2004), production of antimicrobial substances or bacteriocins against undesirable pathogens (Bernet et al., 1994; Servin, 2004), and regulation of gastrointestinal immunity (Christensen et al., 2002). In addition, it has been reported that they exert beneficial effects such as suppressing colon cancer, decreasing serum cholesterol and aiding in digestion or absorption of feed ingredients and synthesis of vitamins (Pereira and Gibson, 2002; Rafter et al., 2007).

LABs are widely distributed in various animal intestines (Devrise et al., 1987; Sakata et al., 1980). They are also the biological basis for the production of great multitude of fermented foods (Lasagno et al., 2002). The most important contribution of these bacteria to fermented products is to preserve the nutritive qualities of the raw material and inhibit the growth of spoilage and pathogenic bacteria (Mattila et al., 1992). There have been several reports (Mitsuoka, 1990; Salminen and Wright, 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 (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.

LABs are characterized as Gram-positive, usually non-motile, non-sporulating bacteria that produce lactic acid as a major or unique 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 authors

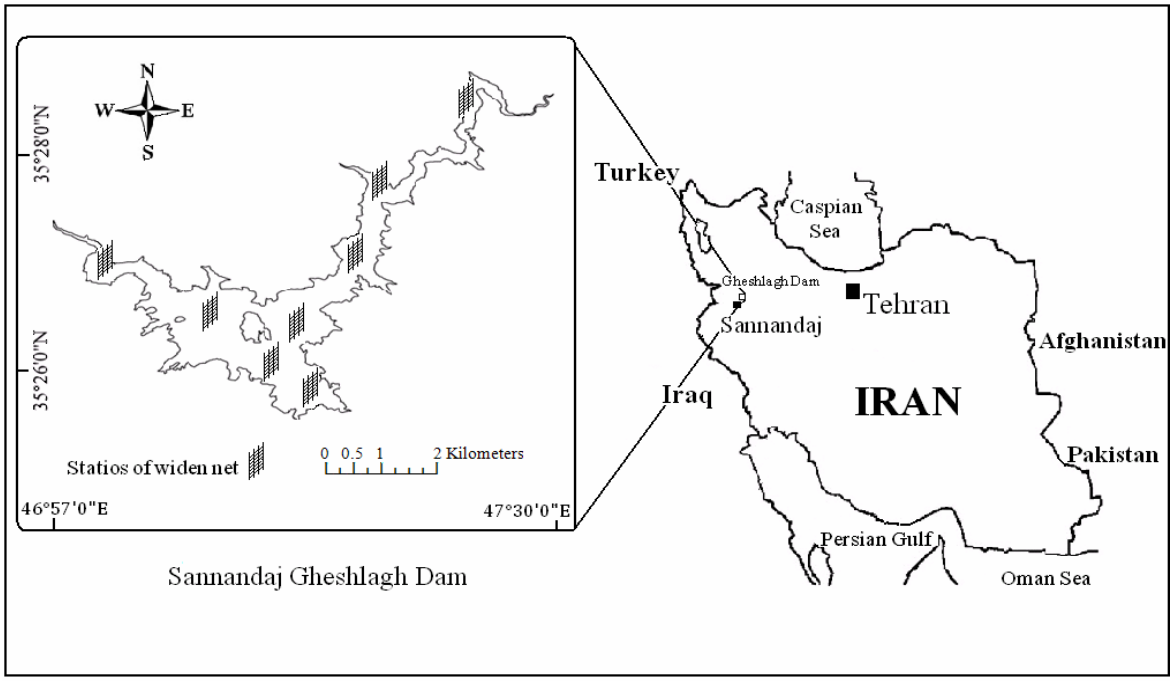

Figure 1. Geographical location of Gheshlaghdam Lake.

(Huber et al. 2004; Ringø and Gatesoupe, 1998; Salminen and Von Wright, 1998). Ringø and Gatesoupe (1998) 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). A previous study by Hagi (2004) indicated that the predominant LAB composition in fish intestine was changed seasonally.

The aims of this study were to characterize the dominant lactic acid bacteria (LAB) isolated from the intestines of various samples of the silver carp (*Hypophthalmichthys molitrix*) in Gheshlaghdam lake in Kurdistan province, Iran, in winter and to make a survey of the presence of LAB in the intestinal content of fresh water fish, silver carp, from a lake under the wild condition basically to make a bank collection for spread using this bacteria in food products especially in fish food, as a probiotic. The results suggest that seasonal isolation of LAB would lead to successful addition of various probiotic LAB.

MATERIALS AND METHODS

Fish and experimental conditions

During winter, three times sampling were done, once per month in 2009 to 2010. In each sampling, five individuals adults silver carp (mean weight 1.2 kg) belonging to the Gheshlaghdam Lake were transferred to 500 L fiberglass indoor tank without water flow and with continuous aeration. The water temperature was 5 ± 2°C during the whole trail.

Experiment location

Kurdistan province, with an area of 28203 km^2, is one of the western provinces of Iran, adjacent to West Azarbaijan, Zanjan, Hamedan, and Kermanshah provinces and having more than 230 km of shared border with Iraq. The geographical coordinates of the Province are from 34° 44' to 36° 30' of northern latitude and from 42° 31' to 48° 16' of eastern longitude. The capital of the province is Sanandaj, which is 1373 m above sea level. Gheshlaghdam Lake is 15 km far from Sanandaj (Figure 1), with water temperatures varying between 4 to 6°C in winter.

Isolation of lactic acid bacteria

For the isolation of LAB, first, the fish were opened aseptically and their whole intestines were removed. The intestines were dissected and their contents were collected separately by carefully scraping using a rubber spatula. 1 g of the intestine content was homogenized with 9 ml of sterile normal saline and mixed for 1 min. Subsequently, dilution series were prepared in sterile saline from 10^{-1} to 10^{-10}. Samples were plated on to de Man- Rogosa and Sharp (MRS) agar (Merck).The plates were incubated anaerobically at 37°C for 48 to 72 h. Approximately 20 well grown colonies were picked from each plate for future examination.

Identification of the lactic acid bacteria spp.

Classification of the isolates was based on the established methods using important biochemical and morphological observation and tests previously described (Buller, 2004; Kazaki et al., 1992; Kandler and Weiss, 1986). The selected isolates were examined

Table1. Differentiating characteristics of *Lactobacillus* species isolated from the intestine of silver carp.

	L. plantarum	*L. raffinolactis*	*L. lactis*
Growth at 10°C	+	+	+
Growth at 45°C	-	-	+
Gram staining	+	+	+
Beta haemolytic	-	-	-
Urea	-	-	-
Motility	-	-	-
Oxidase	-	-	-
Indole	-	-	-
Citrate	-	-	-
Gelatin	-	-	-
Mannose	+	-	+
Raffinose	-	+	-
Salicin	+	+	+
Lactose	+	+	+
Sorbitol	+	-	-
Xylose	+	-	-
Trehalose	+	-	+
Glucose	+	-	+
Melezitose	+	+	-
Sucrose	+	-	-
Ribose	+	-	+
Arabinose	-	-	-
Melibiose	-	+	-
Cellobiose	-	+	+
Mannitol	+	+	+
Maltose	+	+	+
Arginine hydrolase	-	-	+
VP	+	+	+
Gas from glucose	+	-	-
Cfu/g	8- 9 × 10^3	5 - 6 × 10^3	2.9 - 4 × 10^1

microscopically for cellular morphology and Gram stain phonotype. Catalase activity was tested by spotting colonies with 3% hydrogen peroxide; grown at 10 and 45°C in MRS broth. Fermentation of different sugar was determined by API 50 CH (Biomerieux); production of acid and gas from 1% glucose (MRS broth without beef extract); production of ammonia from arginine; indole production in tryptone broth; Methyl red and Voges-Proskauer test in methyl-red and Voges-Proskauer (MRVP medium).

Bacterial counts

Total counts and the number of LAB colonies for each isolate were counted with the method described by Buller (2004). The percentages of LAB were compared with total viable counts.

RESULTS

Total colony counts was 5.4×10^7 cfu/g. LAB isolates were classified into the genera *Lactobacillus* and *Lactococcus* based on their morphology and biochemical characters. The differentiating characteristics of LAB species isolated from the intestines of silver carp are shown in Table 1. All isolates were Gram-positive, non-sporulating, facultative anaerobic and catalase negative. The most isolates that were able to grow at 10, but not at 45°C were bacilli that did not produce gas from glucose. The results of the carbohydrate fermentation tests were positive reactions for most sugars. Hydrolysis of gelatin was not positive for isolates. According to the biochemical tests and colony count in pour plates, the number of the predominant LAB species isolate from the intestines of silver carp were in the order *of Lactobacillus plantarum, Lactobacillus raffinolactis* and *Lactococcus lactis.*

DISCUSSION

Fish in all life stages have interactions with bacteria from

the environment. Some relations are detrimental and others are beneficial. Control of pathogen in fish farm should be improved by studying the beneficial bacteria. A growing concern about the high consumption of antibiotic has shown the necessity of alternative methods for disease control. In this study, we confirmed the presence of *Lactobacilli* in the intestine of silver carp. However, Maugin and Novel (1994) found that Lactococcus was the major flora isolated from fish, and Kandler and Weiss (1986) reported that "the occurrence of typical *lactobacilli* is rare in fish and prawn". It is interesting to note that majority of the *Lactobacillus* sp. that have been isolated from adult fish were those species 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, 2009a, b; Balcázar et al., 2007; Jankauskine, 2000; Cai et al., 1999; Cone, 1982).

In this study, we could not find more lactic acid bacteria in the intestinal content of silver carp. This is explained by the influence of season in the lactic acid bacteria population in fish intestines. It has been reported that bacterial microflora of fish intestine changed depending on water temprature and season (Sugita et al., 1989; Al-harbi and Uddin, 2004; Hagi et al., 2004; Bucio, 2006). Highest counts were found in summer and almost absence counts were found in winter. This fact suggests that selection of LAB for fish should be performed seasonally. Previously, Hagi et al. (2004) reported that the predominant LAB in silver carp intestine is *L. raffinolactis*. This result is similar to ours, but probably the discrepancy is due to differences between fish size and water temperatures in Kasumigaura Lake and Gheshlaghdam Lake. However, the results obtained in this study demonstrate that isolates from silver carp could be *L. plantarum*, *L. raffinolactis* and *L. lactis* in winter. This result is considerable for distinguishing the strain of isolate from silver carp intestines by means of molecular techniques.

Conclusion

The ability of this isolates to colonize the intestine of silver carp in winter highlights it as suitable species for widespread use in aquaculture food to minimize pathogen colonization in gastrointestinal tract. In this study, some bacteria were characterized, which may be of interest not only for aquaculture, but also for food preservation.

REFERENCES

Al-Harbi AH, Uddin MN (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, Yoshijima T, Sugita H (2003). Characterization of the goldfish fecal microflora by the fluorescent *in situ* hybridization method.Fish. Sci. 69: 21-26.

Azizpour K, Tokmechi A, Agh N (2009a.). Charactrization of lactic acid bacteria isolated from the intestines of common carp of west Azarbaijan, Iran J. Anim. Veterinary Advances 8(6): 1162-1164

Azizpour K(2009b). Biochemical Characterization of Lactic Acid Bacteria Isolated from Rainbow Trout (*oncorhynchus mykiss*) of West Azarbaijn, Iran. Res. J. Biol. Sci. 4 (3): 324-326.

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

Bernet MF, Brassart D, Neeser JR, Servin AL (1994). Lactobacillus acidophilus LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirolent bacteria. Gut 35: 483-489.

Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL (1995).Treatment of recurrent Clostridium difficile colitis with lactobacillus GG. J. pediatric gastroenterol. nutr. 21: 224-226

Bucio A, Hartermink R, Schrama JW, Verreth J, Rombouts FM (2006). Presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system. Food Microbiol. 23 (5): 476-482.

Byun JW, Park SC, Benno Y, Oh TK (1997).Probiotic effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). J. Gen. Appl. Microbiol. 43: 305-308.

Buller NB (2004). Bacteria from fish and other aquatic animals: A practical identification manual.CABI pub. ISBN 0-85199-738-4.

Cai Y, Suyanandana, P Saman P, Benno Y (1999). Classification and characterization of lactic acid bacteria isolated from the intestines of common carp and freshwater prawns. J. Gen. Appl. Microbiol. 45: 177-184.

Christensen HR, Frøkiaer H, pestka J (2002).lactobacilli differentially modulate expression of cytokines and maturation surface markers in marine denderitic cells. Jurnal of immunology 168: 171-178

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

Devrise LA, Kerckhove A, Kilpper-Balz AR, Schleifer KH (1987). Characterization and identification of *Enterococcus* species isolated from the intestines of animals. Int. J. Syst. Bacteriol. 37: 257-259

Dora I, Preira DI, Gibson GR (2002). Cholosterol assimilation by lactic acid bacteria and bifidobacteria isolated from human gut. Appl. Environ. Microbiol. 68:4689-4693.

Garriga M, Pascual M, Monfort JM, Hugas M (1998).Selection of lactobacilli for chicken probiotic adjuncts. J. Appl. Microbiol. 84: 125-132

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

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

Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T (1991). A human Lactobacillus strain (lactobacillus casei sp. Strain GG) promotes recovery from acute diarrhea in children, Pediatrics 88: 90-97

Jankauskine R (2000).The dependence of the species composition of lactoflora in the intestinal tract of carps upon their age. Acta Zool. Lituanica 10 (3): 78-83.

Kazaki M, Uchimaru T, Okaka S (1992). Experimental manual lactic acid bacteria. Asakura-Shoten.Tokyo, pp.30-34

Kandler O, Weiss N (1986). Microbiology of mesu, a traditional fermented bamboo shoot product, In: Bergey's Manual of Systematic Bacteriol. Sneath PHA, Mair NS, Sharpe ME, Holt JG (Eds). Baltimore: Williams, Wilkins 2: 1209 -1234

Lasagno M, Boaleto V, Raya R, Front De Valderz G, Eraso A (2002). Selection of bacteriocin producer strains of lactic acid bacteria from a dairy environment. Microbiologia 25: 37-44

Mattila-Sandholm T, Wirtanen G (1992). Biofilm formation in the

industry: a review. Food Rev. Int. 8 (1992): 573-603.
Maugin S, Novel G (1994). Characterization of lactic acid bacteria isolated from seafood. J. Appl. Bacteriol. 76: 616-625
Mitsuoka T (1990). Intestinal Microbiology, Asakura-shoten, Tokyo. pp; 139-144.
Perdigon G, Alvarez S, Rachid M, Aguero G, Gibbato N (1995). Immune system stimulation by probiotics. J. Dairy Sci. 78: 1597-1602
Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, Karlsson PC, Klinder A, O'Riordan, MO' Sullivan GC, Pool-Zobel B, Rechkemmer G, Roller M, Rowland I, Salvadori M, Thijs H, Van Loo J, Watzl B, Collins JK (2007). Dietary synbiotic reduces cancer risk factors in polypectomised and colon cancer patients" Am. J. Clin. Nutr. Feb. 85(2): 488-96
Ringø E, Gatesoupe FJ (1980). Lactic acid bacteria in fish: A review. Aquaculture 160: 177-203
Sakata T, Sugita H, Mitsuoka T, Kakimota D, Kadota H (1980). Isolation and distribution of obligate anaerobic bacteria from the intestines of freshwater fish. Bull. Jpn.Soc.Sci Fish. 46: 1249-1255
Salminen S,Wright A (1998).Lactic Acid Bacteria. Microbiol. and Functional Aspects, Marcel Dekker, New York, pp. 211-242.
Sartor RB (2004). Therapeutic manipulation of the enteric microflora in inflammatory bowel disease: antibiotics, probiotics and prebiotics. Gastroentrology. 126: 1620-1633
Schleifer KH (1987). Recent changes in the taxonomy of lactic acid bacteria. FEMS. Microbiol. Reviews 46: 201-203
Servin AL(2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol. Reviews 28: 405-440.
Sugita H, Iwata J, Miyajima C, Kubo T, Nuguchi T, Hashimoto K, Deguchi, Y(1989). Change in microflora of puffer fish Fugu niphobles with different water temperature. Mar. Biol. 101: 299-304
Walstra P, Geurts TJ, Noomen A, Jellema,A , Van Boekel, MJS (1999).Daily technology : Principles of milk properties and processes. Marcel Dekker. New york, pp. 727-728.