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ORIGINAL ARTICLE

Isolation and molecular identification of *Lactobacillus plantarum* from intestinal samples of silver carp

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The intestines of most animals contain bacterial species called lactobacilli. *Lactobacillus plantarum* is a microorganism that settles in the intestinal environment and inhibits the activities of useless microorganisms and pathogens (Andanil et al., 2012). Lactic acid bacteria are the most important probiotic microorganisms including various bacteria such as lactobacilli and *Bifidobacterium* (Marco et al. 2006). Lactobacilli are gram-positive, non-motile, spore-less and catalase negative bacilli that convert different sugars into lactate and acetate (Guy et al., 2014). *L. casei* and *L. plantarum* also have good antagonistic impacts on *Staphylococcus aureus* and *Escherichia coli* (Salehi, 2013). Probiotics can stabilize microbial balance in the intestinal environment by improving their health and usefulness. In addition, probiotics play an important role in maintaining the consumers' health through the synthesis of some essential nutrients (Behnsen et al., 2009; Mohammadian et al., 2014). The bacterium was identified by a molecular technique using a primer designed from the 16S rRNA gene to perform PCR reaction for the detection of *Lactobacillus* genera (Nour, 1998). The intestines from 50 fish specimens were sampled, among which 28 positive samples of *L. plantarum* were identified and isolated after staining and testing by PCR (Lotfi et al. 2010).
Keywords: *Lactobacillus plantarum*; silver carp; probiotic

Introduction

The silver carp *Hypophthalmichthys molitrix* is native to China and eastern Siberia that has been globally introduced for fish farming. The main habitat of this fish species is the Amur River. Silver carp performs mass spawning when water temperature reaches 21-23°C and the river is flooding and turbid. There will be no spawning activity whatsoever in still water, hence, the only means to induce spawning in this species is to inject pituitary hormone in artificial propagation .The number of eggs is between 1500,000 and 200,000, with a diameter of 0.7-1 mm. The eggs are hatched after 1.5 days and the larvae feed on the yolk sac, after which they feed on algae of a few microns and a small percentage of zooplankton, hence, the fish is called phytophagous as well. Male silver carps mature in their habitat at 4-6 and females at 5-6 years of age. The propagation and rearing of these fish together with grass carp, common carp, and bighead carp have considerably gained ground in most countries. In Iran, rearing of this species is of a particular importance due to its feeding on phytoplankton naturally produced in farming pounds. These fish hibernate, are sound-sensitive being attracted by sounds and jump out of the water up to 1.5 m. Each part of animals' body contains a unique collection of microorganisms that reside at different parts of the body as normal flora playing important roles in maintaining one's health and well-being. These microorganisms show very diverse functions including maintaining balancing immune system and metabolic activities, reducing the risk of cancer, helping digestive activities, preventing diabetes and obesity, etc. Human vaginal flora contains a dynamic and complex microbial flora in which the coexistence of different microorganisms maintains an individual's health. Several studies have shown that lactobacilli exist in the intestines of healthy animals. Lactobacilli are the major members of lactic acid bacteria (Lotfi et al., 2010; Izadi et al., 2011). Lactobacilli are the most important probiotic microorganisms including various bacteria of lactobacilli and *Bifidobacterium* species (Marco et al. 2006). Lactobacilli are gram-positive, non-motile, spore-less, and catalase negative bacilli that convert different sugars into lactate and acetate (Gatesoupe, 2008). Most lactobacilli are safe and may be antagonistic to pathogenic bacteria (Mir Dawoody et al., 2011; Jean Guy et al, 2014; Mohammadian et al., 2014). Some lactobacilli have so far been identified to possess antimicrobial properties. For instance, *L. casei* is able to inhibit the growth of *Helicobacter pylori*, which can be used as a live probiotic organism in nutrition to reduce the risk of *H. pylori* pathogen. L. *casei* and *L. plantarum* also have a good antagonistic effect on *Staphylococcus aureus* and *Escherichia coli* (Salehi, 1392) .The most important activity of probiotics in the digestive tract of fish is through the improvement of food intake through the production of extracellular enzymes and vitamins. The results of various experiments showed that growth, weight gain, specific growth rate, feed intake efficiency, protein efficiency ratio, and protein gain were higher in probiotic-fed fish species. Other important influences of probiotics include reduction of disease incidence and prevalence, immune system boost, and antiviral activity (Behnsen et al., 2013). DNA was sequenced using specified primers by PCR. The bacterium was identified by a molecular

technique using a primer designed from the 16S rRNA gene for the detection of *Lactobacillus* genera via PCR reaction (Nour, 1998). After sequencing, *Lactobacillus*-specific primers registered in the NCBI gene bank were used, followed by designing a primer specific to the bacterium (Kwon et al., 2004).

Methods

A total of 50 specimens from each sample were equally collected from fish farming centers at different locations. Then, the fish intestines were sampled, cultured in MRS broth medium, and placed in an anaerobic liner with microaerophiliic conditions. After the incubation period, the bacteria were examined in the presence of opacity. The growth medium was sampled again and incubated in MRS agar medium, after which the colony was stained with Gram staining. The catalase, oxidase, motility, and indole tests were performed following the determination of Gram-positive bacilli. If the bacilli were Gram-negative, the colony was cultured again for PCR reaction. Following PCR reaction, the gene was confirmed using electrophoresis to observe specific bands according to the marker. When the desired bands appeared, positive samples (with the desired base pairs or the same specific gene) were identified according to the protocol. A total of 28 positive samples of *L. acidophilus* were reported out of 50 specimens (Figures 1-4).

Statistical analysis

After determining the type of lactobacilli, SPSS software was used for statistical analysis. There was a significant correlation between the number of positive samples and total collected samples ($p<0.05$).

Findings

The main composition of lactobacilli from 50 fish samples studied are presented in Table 1.

Table 1. Composition of lactobacilli from 50 fish samples.

Total samples	*L. plantarum*	Other bacteria
50	28	23

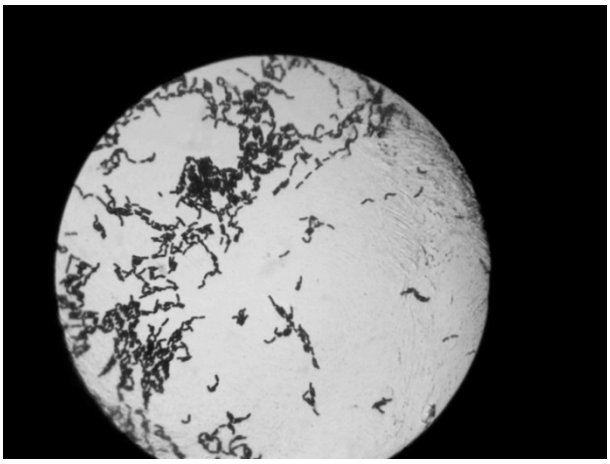

Figure 1. Gram-positive bacilli

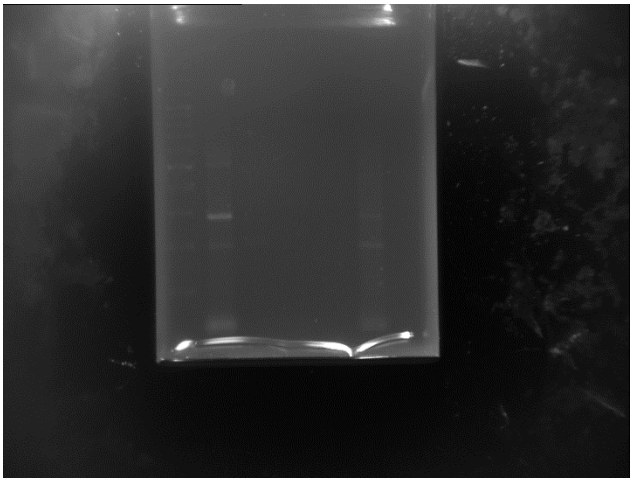

Figure 2. A specific PCR output.

Discussion and conclusion

Among studies conducted in various countries, Diaz et al. (2013) examined and identified probiotic *Lactobacillus* species isolated from dolphin's digestive tract. They found a symbiosis between *Lactobacillus* bacteria and the dolphin's digestive tract. Also, Jafarian et al. (2009) focused on the effects of dietary probiotics on rainbow trout larvae. They observed that probiotic bacilli considerably improved growth parameters in the larvae.

Norfat et al. (2010) also investigated antagonistic effects of probiotic bacteria on intestinal pathogenic bacteria. Andy et al. (2012) studied antagonistic activity of probiotic bacteria on rainbow trout nutrition. The results showed that fish fed dietary probiotics were more disease-resistant. Kabiri et al. (2010) conducted an *in vitro* study on inhibition of K562 chronic myeloid leukemia using cytoplasmic extracts of lactobacilli isolated from common carp's intestine. They suggested that the use of cytoplasmic extracts from *Lactobacillus* bacteria isolated from common carp's intestine had anti-tumor effects similar to human-derived lactobacilli. Esmaili et al. (2011) investigated the effects of bacilli extracted from beluga's intestine on biochemical resistance of larval carp and showed that probiotics could reduce stress and cortisol levels to cope with diseases.

All of the above evidence suggest that lactobacilli species are common in different societies. Given that there is evidence of the vital role of lactobacilli in promoting animal health through the prevention of various diseases and increased survival, these beneficial bacteria can be easily incorporated in animal diets.

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