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Short Communication

Antibacterial Activity of Lactic Acid Bacteria (LAB) Against Significant Fish Pathogens

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ABSTRACT

Fifty (50) Nile tilapia (*Oreochromis niloticus*) and 50 Common carp (*Cyprinus carpio*) were screened for lactic acid bacteria (LAB) using specific media.The antibacterial activity of LAB was estimated against pathogenic bacteria (*Aeromonas cavie* and *Pseudomonas fluorescence*). The results indicated that the recovered LAB isolates from intestine of Nile tilapia and Common carp were 13 (26%) and 4 (8%), respectively. The isolated species were *Lactococcus lactis, Lactobacillus animalis, Lactobacillus plantarum, Lactobacillus fermentum* and *Lactobacillus raffinolactis* recovered from Nile Tilapia intestine, whereas *Lactococcus lactis, Lactobacillus animalis, Lactobacillus plantarum* and *Lactobacillus acidophilus* were isolated from Common carp. LAB isolates had an antibacterial effect against *Pseudomonas fluorescence* and *Aeromonas cavie*.

Key words: *Aeromonas*, Antimicrobial activity, Fish, LAB, *Pseudomonas*

INTRODUCTION

Aquaculture has an important role in the development of many national economies and plays a key role in rural development. Aquaculture also plays an important role in meeting the increasing demand for aquatic animal production (Haylor and Bland, 2001). Disease out breaks is recognized as a significant constraint for aquaculture production and trade, affecting both the economic development and socioeconomic revenue of the sector in many countries (Walker and Subasinghe, 2000). It has been estimated that 10% of fish losses in aquaculture is due to disease and more than 50% of this is due to bacterial agents. According to many reports, lactic acid bacteria (LAB) are normal flora in gastrointestinal (GI) tract of healthy animals like mammals and aquaculture animals (Nikoskelainen *et al.*, 2001). (LAB) are characterized as Gram - positive, usually non-motile, non spore forming bacteria that produce lactic acid as a major or a sole product of fermentative metabolism. (Kandler *et al*, 1986).

Physiological and biochemical characteristics of 84 strains isolated from intestines of beluga and Persian sturgeon revealed that these strains can be categorized into 2 metabolic groups; facultative and obligate hetero-fermentative. The most common presumptive lactobacilli species were *Lactobacillus sakei* and *Lactobacillus plantarum*. (Ghanbari *et al*., 2009). (LAB) are known microorganisms that have probiotic properties. They can produce inhibitory compounds such as lactic acid, hydrogen peroxide, diacetyl, acetaldehyde and bacteriocin. These compounds are able to inhibit the growth of harmful microorganisms (Ringo and Gatesoupe, 1998 and Gatesoupe, 1999). Lactobacilli are present in the intestines of various fish species at larval, fry and fingerling stages inhabiting ponds. They provide information on the changes in their composition as a function of the season of the year and life-stage of the fish. However, it was discussed that some human activities like artificial feeding in ponds would have had an effect on the bacterial composition and load in some fish, like carp *(Cyprinuscarpio*) which showed the highest content of LAB in the intestines (Kvasnikov *et al.*, 1977). Dietary administration of *Lactobacillus* spp. enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides* (Son *et al*., 2009), *Epinephelus bruneus* and Nile tilapia (*Oreochromis niloticus*) (Ngamkala *et al*., 2010). Therefore, the antibacterial activity of some LAB isolated from fish was evaluated against some bacterial fish pathogens such as *Aeromonas cavie* and *Pseudomonas fluorescence.*

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Table 1: Prevalence of isolated LAB from freshwater fishes

Fish species	No. of examined fish	% of isolation		Organs	LAB species
		No	%		
Oreochromis niloticus Nile tilapia	50	13	26	Intestine	*L. lactis, L. animalis, L. plantarum, L. fermentum, L. raffinolactis*
Cyprinus carpio Common Carp	50	4	8		*L. lactis, L. animalis, L. plantarum, L. acidophilus*

MATERIALS AND METHODS

Samples

Fifty (50) Nile tilapia (*Oreochromisniloticus*) and 50 Common carp (*Cyprinuscarpio*) with 20±3 g body weight were randomly collected from the production ponds of Central Lab for Aquaculture Research in Abbassa, Abu-Hammad, Sharkia Governorate, Egypt. Fishes were transported to Fish Health dry Lab for microbiological isolation.

Isolation of lactic acid bacteria (LAB)

Under complete aseptic condition, samples from intestine, liver, kidney, spleen were inoculated in Tryptic Soy broth and incubated at 30^{o}C for 24 hours (De Man *et al*., 1960). Then, all tubes were subcultured on MRS Agar (DIFCO^{TM} lactobacilli**)** and incubated at 30^{o}C for 48 hours (Ghanbari *et al*., 2009). Suspected colonies of LAB were preserved and transported to the Departmant of Microbiology, Faculty of Veterinary Medicine, Cairo University for identification using API 50 CHL (bioMerieux, France).

Antibacterial activity of LAB in vitro

Antibacterial activity of the isolated LAB was carried out using diffusion method (Gonsales *et al.*, 2006; CLSI, 2013) with a minor modification to study the antibacterial effect of LAB against some pathogenic bacteria in fish. The pathogenic bacteria used in this study (*Aeromonas cavie* and *Pseudomonas fluorescence*) were locally isolated from Nile tilapia and preserved in the strain bank of the Department of Fish Diseases, Central Lab for Aquaculture Research in Abbassa. Briefly, disks of sterile filter paper were immersed in LAB culture (matches 0.5 MacFarland that equals 1.5 X 10^{8}cfu/ml). Suspensions from both pathogenic bacteria were swabbed on Muller Hinton Agar and then discs saturated with LAB were loaded. All plates were incubated at 30^{o}C for 24 hours, after then the inhibition zones were observed.

RESULTS

Table (1) shows the prevalence of isolated LAB from freshwater fishes. The results indicated that the LAB isolates recovered from intestines of Nile tilapia and Common carp were 13 (26%) and 4 (8%), respectively. The recovered species were *Lactococcus lactis, Lactobacillus animalis, L. plantarum, L. fermentum*and *L. raffinolactis* isolated from Nile tilapia, while *Lactococcus lactis, Lactobacillus animalis, L. plantarum* and *L. acidophilus* from Common carp. The antibacterial effect of *Lactobacillus* isolates against *Aeromonas cavie* is less potent than that against *Pseudomonas fluorescence* as shown in Table 2.

Table 2: Inhibition zones (mm) due to antibacterial activity of LAB isolates against *Aeromonas cavie* and *Pseudomonas fluorescence*

Bacterial isolates	Inhibition zone (mm)	
	A. cavie	*P. fluorescence*
1	0	15
2	30	12
3	13	13
4	2.6	0
5	17.5	21.5
6	0	0
7	0	0
8	15	10
9	0	21
10	0	0
11	23	20
12	20	0
13	0	0
14	0	0
15	0	0
16	15	0
17	0	44

DISCUSSION

LAB is those bacteria that ferment sugars getting 50% of lactic acid. Many trials were performed to evaluate LAB as a probiotic supplement in fish feed (Ayo-Olalusi *et al*., 2012). The antibacterial activity of *Lactobacillus spp*. strain RR17 was tested against *Aeromonas spp*., *Vibrio spp*., *Escherichia coli, Pseudomonas spp*. and *Salmonella spp*. isolated from *O. mosambecus* by using agar diffusion assay, it was found that *Lactobacillus spp*. strain RR17 had antagonistic activity against some pathogenic bacterial species (Aly *et al*., 2008; Kim and Austin, 2008; Lara-Flores, 2011). The authors also have demonstrated the positive effects of LAB on the general status of various species of fish. Our results supported the same theory, the percentage of the recovered *Lactobacillus* isolates from intestines of Nile tilapia and Common carp were (26%) and (8%), respectively, and they had an antibacterial effect against *Aeromonas cavie* and *Pseudomonas fluorescence.* It may be owed to the production of bacteriocin which inhibits the growth of Gram-positive and Gram-negative bacteria (Bernet *et al*., 1994; Servin, 2004; Lash *et al*., 2005). Bacteriocins are proteinaceous toxins produced by bacteria that have antibacterial effect. They are typically considered to be narrow spectrum antibiotics, though this has been debated. They are phenomenologically analogous to yeast and paramecium killing factors, and are structurally, functionally, and ecologically diverse (Farkas-Himsley, 1980)

Another explanation is supporting such positive effect, the LAB are significantly reducing the adhesion of some pathogenic bacteria such as *Aeromonas hydrophila, Aeromonas salmonicida, Vibrio anguillarum* and *Yersinia*

ruckeri by competing the adhesion sites and therefore decreasing the ability of pathogens to colonize in GIT of fish (Balcázar *et al*., 2008; Denev *et al*., 2009).

Conclusion

From the present study, it was concluded that LAB as probiotics improve and support good health for host by protection against infections by secretion antibacterial substances against fish pathogens, so it can improve weight gain and feed conversion ratio.

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