

Research Article



Studies on Probiotic Analysis in the Gut Region of Fresh Water Fishes in Periyar Lake, Kerala

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ABSTRACT

In the present study, we isolated lactic acid bacteria (LAB) from the intestinal gut of channa (*channa striata*) and Tilapia (*Oreochromis mossambica*). The isolates were identified as *lactobacilli* by standard biochemical characterization. The isolated *lactobacilli* were if subjected to preliminary screening for various probiotic traits such as antibacterial activity Antibiotic susceptibility and auto aggregation among the several isolates. Two *lactobacilli* strains LAB1 and LAB 2 had promising results with higher antibacterial activity and auto aggregation property when compared to other strains of *lactobacilli* the result suggest that the two *lactobacilli* strains could be a potential candidate for a successful biological enhancer probiotic which has antagonistic property against human pathogen.

Keywords: Antibiotic activity, Antimicrobial, Auto aggregation, Biochemical analysis, Hemolysis, Lactic acid bacteria, Periyar lake, Probiotic.

INTRODUCTION

The most commonly used probiotic are Lactic Acid Bacteria (LAB) namely *lactobacilli sp.*, are usually characterized by gram positive, non motile, non sporulating bacteria that produce lactic acid as their main byproduct due to fermentation. The use of probiotic for aquatic animals is increasing with the demand for environment friendly sustainable aquaculture. Early studied, various strategies modulate the composition of the gut microbiota for better growth; digestion, immunity, and disease resistance of the host have been investigated in various livestock as well as in human beings.¹ The role of LAB within the digestive tract of fish has been extensively investigated and reviewed.² Now, researchers are trying to use probiotic bacteria in aquaculture to improve water quality by balancing bacterial population in water and reducing pathogenic bacterial load.³

Research over the past few decades has shown that the nutrients and minerals in fish, and particularly the omega 3 fatty acids found in fishes, are useful in brain development and reproduction.⁴ This has highlighted the role of fish in the functionality of the human body.

Owing to above maintained beneficial property of LAB. Researchers are in a constant hunt of for probiotic with improved properties with this in mind. The present study was undertaken to investigate the micro flora of intestinal gut in two native varieties of fishes and also characterize their probiotic properties.

MATERIALS AND METHODS

Sample collection

Water samples were collected in a sterilized polyethylene container from Periyar Lake Kerala. One set of sample is

used for spot analysis. Another set of samples were collected in sterilized polyethylene containers and transferred to the laboratory using an ice box container for further analysis. Live Fish samples were procured from catchers collected from Periyar Lake, Kerala. The live fish samples were collected from the (Mannarkudi tribal fisher man) catchers. The fish samples were selected at random and collected in pre-sterilized polyethylene bags containing the habitat water and transported to the laboratory. The fishes were identified using standard reference manuals (Journal Bombay natural hist society. vol. 93 (1996).

Analysis of physico-chemical parameters

The collected water samples were analyzed for various physico-chemical parameters, such as pH, Temperature, Electrical Conductivity, Total Dissolved Solids, Silica, Sodium, Potassium and Alkalinity. All the parameters were analyzed as per International Standard methods (IS:3025) of sampling and tests (physical and chemical) for water and wastewater as per APHA 1998. The analysis of water samples was carried out within 48 h after the sampling to avoid changes in the water quality.

Isolation of LAB from fish intestine gut

The Tilapia (*Oreochromis mossambica*) and Channa (*Channa striata*) fishes were washed with sterile distilled water to remove any undesired dust particles. Then the fishes were dissected to remove the gastrointestinal tract under sterilized conditions. The gastrointestinal tract was homogenized using sterile distilled water and centrifuged at 13000 rpm for 10 min. After centrifugation, the supernatant was serially diluted and pour plated on nutrient agar plates. The plates were incubated at 37°C for 24 h. Individual colonies of bacterial culture were inoculated in nutrient broth for further studies.



Identification of LAB

The isolated colonies of LAB were identified based on their morphological, cultural, physiological and biochemical characteristics which include Grams staining, Motility, Glucose, Lactose, Galactose, Mannitol, Esculine, Cellobiose, Raffinose and Catalase activities.

Antibiotic Susceptibility Test (AST)

AST was performed for isolated LAB against three antibiotics such as Penicillin G - HiMedia -india, Amoxicillin - HiMedia - india and Cephalothin - HiMedia - india. The single isolated bacterial culture grows in broth culture which was adopted for AST. Kirby Baur Disc Diffusion method was performed by using a cotton swab in which the bacterial suspension was spread on Mueller Hinton agar (MHA - HiMedia - M173) plate. Antibiotic discs were prepared at a concentration of 25 µg/ml, placed on the prepared MHA medium plate and incubated at 37°C for 24 h. The zone of inhibition of each disc was measured (ZOI) in values (mm)

Antimicrobial test

The antagonistic property of selected lactobacilli was performed according to Kirby bauer method briefly the pathogenic strains were swabbed in to nutrient agar medium and discs which were coated with lactobacillus strain was placed over. The plates were incubated at 37°C and examined for clearance around the disc. A clear zone around the disc suggest for the antimicrobial activity of the isolate.

Auto aggregation test

The assay was performed according to the method of.⁷ The auto aggregation percentage is expressed as bacteria which were grown at 37°C for 24 h in nutrient broth. The cells were harvested by centrifugation and suspended in PBS to 0.5 optical density (O.D.) units at 600 nm. After incubation at 37°C for 1 h, 1ml of the upper suspension was transferred to another tube and the O.D. value was measured. Aggregation was expressed as

$$1 - (A_t/A_0) \times 100$$

Where, A_t = Absorbance at t time (1 h) and

A_0 = Absorbance at 0 times (0 h)

Hemolytic Assay

The hemolytic assay was performed as per the procedure described.⁸ Isolates were screened on blood agar plates containing 5% sheep blood and incubated at 37°C for 48 hours. Hemolytic activity was scored as the presence of a clear zone around bacterial colonies.

RESULTS

The physico-chemical characteristics sub surface of water Periyar lake such as pH, Temperature, Turbidity, Conductivity, TDS, Phosphate, Silicate, Potassium, Alkalinity and Sodium were tested and the results are given in Table 1.

Table 1: The physico-chemical characteristics of water in the Periyar lake, Kerala

Parameters (surface water samples)	Values
pH	7.1
Temperature	26°C
Turbidity	Clear
Conductivity	74.0 µs/cm
TDS	48.0 ppm
Phosphate	0.1 mg/l
Silicate	1.9 mg/l
Potassium	1.3 mg/l
Alkalinity	28.0 mg/l
Sodium	3.2 mg/l

The isolated bacteria selected from gut region of fishes were identified subjected to the biochemical test such as Catalase, Glucose, Lactose Mannitol, Esculin Cellobiose, Galactose, Raffinose, were performed and the results are shown in table 2. The biochemical tests suggest that the isolate could belong to genus lactobacillus.

Table 2: Morphological and biochemical test for the conformation of LAB

Tests	LAB 1.	LAB 2.
Gram staining	+	+
Motility	-	-
Catalase	-	-
Glucose	A	A
Lactose	A	A
Mannitol	A	NA
Esculin	A	A
Cellobiose	A	A
Galactose	A	A
Raffinose	A	NA

Note: + (Positive), - (Negative), A (Acid), NA (No Acid).

Table 3: Antibiotic sensitivity test

Organisms	Penicillin G (mm)	Amoxicillin (mm)	Cephalothin (mm)
LAB 1.	35 ± 2	30 ± 2	25 ± 2
LAB 2	30 ± 1	37 ± 1	31 ± 1

The isolates were tested for their susceptibility against three different antibiotics such as Penicillin G, Amoxicillin, Cephalothin table (3).The isolate LAB1 showed a zone of clearance of Penicillin G (35mm), Amoxicillin (30mm),and Cephalothin (25mm) and LAB 2 with clearance zone of Penicillin G (30mm), Amoxicillin (37mm) and Cephalothin (31mm) respectively.

The isolates LAB 1 and LAB 2 was studied for their antimicrobial activity against human pathogen are shown in table 4 LAB1 showed in clearance zone of (12mm),



klebsiella (14mm), E.coli (13mm) for staphylococcus and (13mm), bacillus respectively. LAB 2 should zone of clearance (10mm), klebsiella (15mm), E.coli (20mm), staphylococcus and (8mm) for bacillus suggesting their efficient antibacterial activity.

Table 4: Antimicrobial test against *E. coli*, *Klebsiella*, *Staphylococcus*, *Bacillus*.

Bacterial strains	<i>Klebsiella</i> (mm)	<i>E. coli</i> (mm)	<i>Staphylococcus</i> (mm)	<i>Bacillus</i> (mm)
LAB 1.	12 ± 2	14 ± 2	13 ± 2	13 ± 2
LAB 2	10 ± 1	15 ± 1	20 ± 1	8 ± 1

Table 5: Results of auto aggregation test

Isolated strains	Auto aggregation (Absorbance at 600 nm)
LAB 1.	58.30%
LAB 2	49.93%

The aggregation ability of the isolates LAB1 and LAB 2 was performed and results are shown in table 5. Both the isolates should significant aggregation property with LAB 1 (58.30%) and LAB 2 (49.93%) respectively.

Table 6: Results of hemolytic assay

Organisms	Hemolytic test at 5% blood
LAB 1.	No lysis (-)
LAB 2	No lysis (-)

Hemolytic activity of LAB1 and LAB2 was performed as blood agar base medium with 5% (table 6). Both in strain showed no hemolytic activity confirming that they are non pathogenic and could be used as probiotic.

DISCUSSION

Tilapia (*Oreochromis mossambica*) and Channa (*Channa striata*) were collected from Periyar lake, Kerala. The gut was suspected isolated and cultured for lactic acid producing bacteria. We obtained so different colonies from the two species of fishes on the basis of morphology and biochemical tests. The isolates were identified as *lactobacillus*. The *lactobacillus* species that was isolated in the present study were relatively similar to the species described by Ringo et al.⁹ The biochemical characterization used for identification of *lactobacillus sp* may suggest some clear in relation to occurrence of the strain in nature. Fish at all life stages may expose to the bacteria found on the environment. Current methods for control of pathogens in the fish farms should be improved by studying the beneficial bacteria. As *lactobacillus* has many documented health effects,¹⁰ and are naturally present in the gastrointestinal tract of man and fish,¹¹ knowledge on the presence of *lactobacillus* as a natural flora in fish may lead to further application to improve fish health.

Two strain of *lactobacillus* strains are identified in present study showed promising results and could be developed as a potential probiotic. The strain LAB 1 showed highest

activity against E.coli and it was least active against klebsiella sp. The strain LAB 2 was highly active against bacillus and was modernly active against E.coli both the strain established good rate of autoaggregation of 58.30% and 49.93% suggesting their ability to adhere to intestinal epithelial cells of fish gut.¹²⁻¹⁴ The non pathogenic property of two strains was confirmed by hemolytic activity on blood agar plate which showed a negative result. The two strains isolated in the present study could be employed as feed supplement for fishes as it process probiotic property.

CONCLUSION

Lactobacillus culture was isolated from the gut region of fish. This lactobacillus culture helps the fish in many ways like boosting the immune system, prevents the diarrhea from various causes it is also used to inhibit the growth of pathogenic organisms.

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