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SEGREGATION AND RECOGNITION OF EFFICACY OF PROBIOTIC BACTERIA ISOLATED FROM THE INTESTINE OF FISH *TILAPIA MOSSAMBICA*

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ABSTRACT

In aquaculture, probiotics have been tested for enhancing the immune system and promoting growth and survival rate of many marine species like shrimps. The gastrointestinal tract of fish is a complex ecosystem possessing a specific micro-biota consisting of aerobic, facultative anaerobic and obligate anaerobic bacteria. In order to isolate bacteria with a high probiotic potential for marine shellfish aquaculture, homogenates of the gastro intestinal tract from adult fish were isolated. Live Fish *Tilapia mossambica* was bought from Penna River with the help of fisherman. The animals were brought to the laboratory in live condition and homogenates were prepared aseptically in 1% peptone broth. Sample was serially diluted and plated on to MRS (De man, Rogosa and Sharpe) agar plates. two unknown bacterial isolates were isolated and morphologically studied by gram staining and biochemical tests and observed as gram positive Bacilli and Lactobacillus. Antibacterial activity of unknown samples was studied by antibacterial activity test against to *Vibrio harveyi* pathogen by well diffusion method. After incubation period, isolate –I shows 8mm of zone of inhibition, isolate –II shows 9mm of zone of inhibition. The immune response of isolates against the pathogen results that they have the potential use as probiotics in aquaculture culture.

Keywords: Fish gut, Probiotics, *Vibrio harveyi*, immunity, *Tilapia mossambica*.

Introduction

Aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants (FAO, 2000) under controlled conditions. Andhra Pradesh ranks first in coastal aquaculture and fresh water aquaculture. It ranks second in fresh water fish production and overall value of fish production. Aquaculture is one of the fastest growing industries in the food sector worldwide. However, this rapid increase in growth has been marred by the outbreak of numerous diseases, mainly bacterial, viral and leading to very high stock mortality.

Prevention and control of diseases have led to a substantial increase in the use of veterinary medicines in recent years. The utility of antimicrobial agents and antibiotics as remedial measure has been questioned. These huge amounts of antibiotics have exerted a very strong pressure on the resistance among bacteria. Hence, Aquaculture industry is focusing on the alternate intervention in terms of bioremediation, vaccine, immune stimulants and use of probiotics for fish and shrimp culture. Since, the efficacy of these commercial probiotics is uncertain in the gastrointestinal tract of the aquatic animals.

Tilapia, because of their enormous adaptability to a wide range of physical and environmental conditions, ability to reproduce in captivity, relative resistance to handling stress and disease-causing agents compared to other cultured finfish species, good flesh quality, feed on a low trophic level and excellent growth rate on a wide variety of natural and artificial diets, are the most abundantly cultured species worldwide.

The gastrointestinal tract of fish is a complex ecosystem possessing a specific micro-biota consisting of aerobic, facultative anaerobic and obligate anaerobic bacteria (Gomez and Balcazar, 2008). The predominant bacterial species isolated from most of the fish digestive tracts have been reported to be aerobes or facultative anaerobes (Bairagi *et al.*, 2002; Saha *et al.*, 2006). The isolated many lactic acid bacteria are proved to function as probiotics, which are benefit to host health, when ingested in sufficient quantities. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion and by the production of organic acid and antimicrobial compounds.

Hence, most attempts have been aimed at seeking autochthonous strains with probiotic properties. In aquaculture, probiotics have been tested for enhancing the immune system and promoting growth and survival rate of many marine species like shrimps. In order to isolate bacteria with a high probiotic potential for marine shellfish aquaculture, homogenates of the gastro intestinal tract from adult fish were isolated. Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Beings rich in nutrients, the environment of digestive tract of fish confers a favorable culture environment for microorganisms. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion, production of organic acid and antimicrobial compounds.

Materials and Methods

Collection and processing of fish samples

A total of live *Tilapia* fish was collected from Local River with the help of fisherman. The animals were brought to the laboratory in live condition. The surface of fish bodies were disinfected by alcohol (70%) and dissected under antiseptic conditions, intestines taken out and washed three times with normal saline solution (NaCl 0.85%). The intestines were cut open and the dietary materials were pooled out. Then the gut samples were homogenized in 10ml of sterile saline solution (NaCl0.85%) with a mechanic tissue homogenizer (Rengpipat *et al.*, 2008) for centrifugation. After centrifugation the supernatant was taken and serially diluted in sterile distilled water in the test tubes to 10^{-2}, 10^{-3} ,10^{-4} ,10^{-5} ,10^{-6} and10^{-7} dilution and were pour plated on Nutrient agar and MRS agar plates and incubated for 24 h in a incubator.

Screening & Enumeration of bacteria

Morphological identification

After 24 h individual colonies were taken with typical characteristics namely pure white, off white and small (2–3 mm diameter) with entire margins were picked from each plate, transferred to subculture in three times and quadrant streak for pure culture in single colony. Selective colonies were characterized and identified following Bergey's Manual of Systematic Bacteriology (Whitman et al., 2009) for their colony and cell morphology, gram staining, biochemical tests (Ghosh *et al*., 2002).

Gram staining

The most widely used staining procedure in microbiology is the Gram's staining, discovered by the Danish scientist and physician Hans Christian Joachim Gram in 1884. Gram staining is a differential staining technique that differentiates bacteria into two groups: Gram positive and Gram negative. The procedure is based on the ability of microorganisms to retain colour of the stains used during the gram stain reaction. Gram negative bacteria are decolourized by the alcohol, losing the colour of the primary stain, purple. Gram positive bacteria are not decolorized by alcohol and remain as purple. After decolorization step, a counterstain is used to impart pink colour to the decolorized gram-negative organisms.

Biochemical analysis

The biochemical tests were carried out according to the methods described by Cappuccino and Sherman (1991).

Antibacterial Activity Study of Isolates

Well Diffusion Method

The pathogenic bacteria *Vibrio harveyi* were cultured in TSB and incubated at 36° C for 24 hrs. There after, 10 µl of the cultures were spread on tryptone soya agar (TSA) by swabbing with cotton swabs. At the same time, the selected strain was cultured in TSB at 36° C for 24 hrs. The bacteria were harvested by centrifugation at 10,000 rpm for 2 min and the supernatants were used for antibacterial test using well diffusion methods (Balcázar *et al*., 2008; Allamesh *et al*., 2012).

Results and Discussion

Total count of bacteria in intestine

Total number of bacterial count/plates in fish intestine was determined on Nutrient agar and MRS agar medium.

Isolation, biochemical characterization and identification of probiotic bacteria

In the present study Tilapia fish samples were screened for isolation of probiotic bacterial species. Among different fish intestines, two isolates were isolated and identified as gram-positive bacillus and lactobacillus groups, Dhanasekaran *et al*. (2008, 2010). Isolates showed (Table.2) that their biochemical tests such as V.P, Citrate utilization, Catalase, Oxidase Nitrate reduction are positive and Gelatin hydrolysis negative. The results obtained from the pattern of carbohydrate fermentation all isolates are positive. However, in the present study different organisms were selected. This may be the result of different hosts, different habitats.

Antibacterial Activity Study of Isolates

Antibacterial activity of unknown samples was studied by antibacterial activity test against to *Vibrio harveyi* pathogen by well diffusion method (Table.2). After incubation period, isolate –I shows 8mm of zone of inhibition, isolate –II shows 9 mm of zone of inhibition **(**Fig.1).

Table.1 General and Biochemical characterization for selected 2 isolates

S.n.o	Characteristics	Isolate-1	Isolate-2
1	Colony Appearance	White	Yellow
2	Morphology	Unicellular rods	Long bent rods
3	Gram's Test	+	+
4	Ctalase Test	+	-
5	Oxidase Test	+	-
6	Indole Production	+	-
7	Methyl Red Test	+	+
8	Citrate Utilization	+	+
9	H_2S Production	+	-
10	Nitrate Reduction	+	-
11	Starch Hydrolysis	+	+
12	TSI Test	ALS/ACB	ACS/ACB
13	Possible Micro organisms	*Bacillus* spp.	*Lactobacillus* spp.

Table.2 Antibacterial activity test

S.no.	Name of the pathogen	Zone of inhibition (mm) by Isolates	
1	*Vibrio harveyi*	Isolate-1	Isolate-2
		8	9

Fig1 Antibacterial activity test Inhibition zone formed by isolated strain 1 and strain 2 against to *Vibrio harvey*

Conclusion

The present study concluded that *Bacillus spp.* and *Lactobacillus spp.* was normal in micro flora of *Tilapia* gut. In addition, these isolates showed high ability to inhibit growth of freshwater fish pathogens particularly *Vibrio harveyi.* Therefore, it seems that *Bacillus spp.* and *Lactobacillus spp.* has high potential probiotic needed in aquaculture systems for development of sustainable fish production.

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