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Original Article

Effect of Probiotic Bacteria on the Growth rate of Fresh Water Fish, *Catla catla*

M.M. Abdul Kader Mohideen[a], T. Selva Mohan[b], S. Peer Mohamed[a], and M.I. Zahir Hussain[c]
[a]Department of Microbiology, Sadakathullah Appa College, Tirunelveli-627011, India
[b] Department of Microbiology, Sivanthi Aditanar College, Nagercoil-629501, India
[c]Department of Advanced Zoology and Biotechnology, Sadakathullah Appa College, Tirunelveli-627011,Tamil Nadu, India
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Abstract

Isolation and screening of microbes from fish gill for the production of antimicrobial activity against *Vibrio harveyi*. A total of nine bacterial isolates were obtained from the rhizosphere soil of a medicinal plant. Characterization of selected microbes limits the growth of *Vibrio harveyi*.. Out of nine strains, only one was showed antagonistic activity and it was identified as *Bacillus* sp. Identified strain was examined for its effect on fish growth in the form of probiotics. Both water and feed application of *Bacillus* sp was studied and found that application of the *Bacillus* culture through water improved not only improved water quality parameters but also significantly enhanced the length and weight of *Catla catla*.

***Key words*:** *Vibrio harveyi*, Probiotic, *Catla catla*

Introduction

Fish diseases are one of the major problems in the fish farm industry. Even though vaccines are being developed and marketed, they cannot be used as a universal disease control measure in aquaculture. The use of antibiotics to cure bacterial infection and prevent fish mortality in aquaculture is becoming limited as pathogens develop resistance to the drugs (Gonzalez *et al*., 2000; Gomez-Gil *et al*., 2000). Further, beneficial bacterial flora are killed or inhibited by orally administered antibiotics, leading to efforts to find alternative disease prevention methods such as the use of non-pathogenic bacteria as probiotic biocontrol agents. Fuller (1987) defined probiotics as "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment". The use of commercial probiotics in fish is relatively ineffective as most commercial preparations are based on strains isolated from non-fish sources that are unable to survive or remain viable at high cell density in the intestinal environment of fish during the active growth phase of the fish (Gram *et al*., 2001). Hence, there is elegant logic in isolating putative probiotics from the host in which the probioticis intended for use. Such strains should perform better because they have already adhered to the gut wall of the fish and, thus, are well-adapted to compete with pathogens for nutrients. Presumably, strains that develop dominant colonies in the fish intestine are good candidates for preventing the adhesion of pathogens on the gut wall. The present study was undertaken to isolate probiotic bacterial strains from fish intestines and screen them by *in vitro* testing of their antagonism to pathogens and attachment to substrates.

Materials and Methods

Sample Collection

Soil samples were collected from the rhizosphere soil of medicinal plants such as *Azadiracta indica*, *Acalypha indica* brought to the laboratory and analysed immediately for microbial population.

Isolation of bacteria

For isolation of probiotic bacteria, the Pikovskaya's medium (Gullian *et al*., 2004) was prepared. The composition of the medium is Tri-calcium phosphate - 5.0g, Glucose - 10.0g, Ammonium sulphate - 0.5 g, Sodium chloride - 0.2g, Magnesium sulphate - 0.2g, Yeast extract - 0.5g, Manganese sulphate – Trace, Ferrous sulphate – Trace, Distilled water - 1000ml, Agar - 15.0 g, adjusted pH - 7.5

All the inoculated plates were incubated in an inverted position at 28±1^{o}C. Probiotic

bacteria were identified by clear solubilizing zones that were formed around their colonies at the end of the third day of incubation. The population is expressed as colony forming unit (CFU) per gram of soil sample. The probiotic bacterial colonies were picked up from the Petri dishes and re-streaked in appropriate nutrient agar plates. Then the pure cultures were maintained agar slants.

Seed germination assay

The bacterial strains were tested for their ability to promote/inhibit seedling growth by using the method as described by Fuller, 1987. Certified seeds of rice were purchased from the Agriculture Department. Then the seeds were kept in Petri dishes containing the microbial cultures of probiotic bacterial strains separately at 10^8cells ml^{-1}. The seeds were thus inoculated for 1 h in the Petri dishes. The seeds were taken out and spreaded onto the Petri plates having filter paper moistened with 10 ml of sterilized distilled water. Five replicates of 100 seeds of rice were maintained for each bacterial strain and incubated at 30ºC. In control plates, seeds were treated with sterilized distilled water alone. After 24hrs days, seed germination (%) was recorded. Plant growth promoting properties were determined based on the vigour index.

Vigour index (VI) was calculated as follows

VI= root length+ shoot length x germination

$$\textbf{Increased VI (\%)} = \frac{\text{VI of the isolates x VI of the Control}}{\text{VI of the Control}} \textbf{ x100}$$

Antagonistic Assay

Twenty four hours old broth culture of *Vibrio harveyi* were prepared. The isolated strains were tested for antibacterial activity against *V. harveii* by using cross-streak method. After 24h of incubation, zone of inhibition was recorded.

Identification of Microorganism

The isolated bacterial strain was identified by following the standard morphological and biochemical tests (Ahilan *et al*., 2004, Thapa *et al*., 2006, DeSch and Ollevier, 2000) .

Collection and acclimatization of Test Animals

The fish *Catla catla* weighing about an average of 1.42 ± 0.5g animals were collected from Surya Fish Farm in Madurai.

Water Quality parameters

Temperature, pH, Dissolved oxygen, Nitrate–Nitrogen, Nitrite–Nitrogen and Ammonia from the fish tank water samples were analysed once in a week.

Analysis of fish growth

Total length was measured using steel graduated scale. Fresh weight was taken by weighing the animal in live condition in an electronic balance once in a week. From the data collected, food consumed, mean weight, production, growth rate, average growth, relative growth were calculated.

Results

Seed germination assay

A total of nine strains were isolated from the rhizosphere soil of medicinal plants. These strains were tested for their efficiency in seed germination as well as their growth of root and shoot growth. Seed germination of rice was 72% in control. The seeds inoculated with bacteria exhibited enhanced germination. The germination varied from 76 to 94%.

Root length in rice was 6.5 ± 0.5mm in control. The seeds inoculated with bacterial strains exhibited enhanced growth from 6.7mm to 16.9mm. Shoot length of rice was 13.3± 0.5mm in the control. The seeds inoculated with bacterial strains varied from 14.2 to 23.1mm. Number of Root hairs of rice seedlings was 2±1 in the control. The seeds inoculated with bacterial strains were exhibited increased number of root hairs (Table-1).

Antagonistic Activity of Probiotic bacteria

Of nine isolates, seven isolates showed inhibition against V*ibrio harvei*. The inhibition zone was measured and tabulated (Table-1).

Identification of Probiotic Bacteria

The bacterial strain was characterized as *Bacillus* sp after According to Gunasekaran, (1994) method using morphological identification and biochemical properties (Table- 3).

Table- 1: Paddy seed germination, shoot and root growth and antagonistic activity as influenced by the bacterial strains.

Strain	Seed Germination (%)	Root Length (mm)	Shoot Length (mm)	No. of Root Hairs	VI	IVI (%)	Zone of Inhibition (mm)
Control	72	6.5	14.2	2	1028.9	0	-
TA1	80	16.4	13.3	4	1080.4	5.0	3(+)
TA2	76	8.5	15.6	4	1194.1	16.1	5 (+)
TA3	78	12.2	21.3	3	1673.6	62.7	7 (++)
TA4	80	16.7	16.7	3	1352.7	31.5	7 (++)
TA5	76	11.9	21.4	8	1638.3	59.2	6 (++)
TA6	94	16.9	23.1	8	2188.3	112.7	11 (+++)
TA7	80	11.7	18.9	7	1523.7	48.1	6 (++)
TA8	86	15.0	17.4	5	1511.4	46.9	-
TA9	76	16.7	16.6	2	1278.3	24.2	-

VI – Vigour Index; IVI – Increased vigour Index
Numbers inside the parentheses indicate the diameter of zone of inhibition in mm;
'-', No inhibition; '+', low (1- 5); '++', medium (6 - 10); '+++', High (11- 15).

Table- 2: Water quality parameters in culture tanks treated with microbial stains

Variables	Average Value			
	CF	CF+TA3	CF + TA5	CF + TA6
pH	7.8	7.9	7.8	7.8
Ammonia (mg/l)	137	75	100	50
Nitrite (µg/l)	11.99	11.76	11.80	11.58
Nitrate(µg/l)	32.29	31.2	31.6	29.2
Dissolved oxygen ((mg/l)	4.3	4.7	4.3	4.7

Table- 3: Cultural and biochemical characteristic features of the selected bacterial strain

Characters	Results	Characters	Results
Gram Staining	Gram Positive	Methyl Red Reaction	+
Bacterial Shape	Rods	Voges Proskauer	-
Motility	Motile	Citrate Utilization	+
Glucose	+	Urease activity	-
Fructose	+	Catalase activity	+
Maltose	+	Oxidase activity	+
Mannitol	+	Starch hydrolysis	+
H_2S production	-	Lipid Hydrolysis	+
Nitrate Reduction	+	Casein Hydrolysis	+
Indole Production	-	Gelatin Hydrolysis	+

Water Quality paprameters

Water quality parameters such as pH, temperature, Nitrate, nitrite and ammonia were recorded from water collected from the fish tanks treated with feed probiotics and water probiotics (Table- 2).

Fish Growth analysis

In general fish growth was more in experimental animals than in control. There was no significant difference between control and treated tanks. However the linear Growth of fish was higher in the probiotic treated than control tank. Growth of fish (in terms of wet weight gain) was significantly higher in treated than the control tanks (Table 4).

Table- 4: Average Length and wet weight gain of *Catla catla* against *Bacillus* sp. during 35 days of tank culture. Values are average of 24 fish with SD.

Treatment		Average Value					
		Initial	7th day	14th day	21st day	28th day	35th day
Control	Length (cm)	2.40±0.44	2.80±0.37	3.20±0.87	3.50±0.59	3.90±0.68	4.10±0.84
	Weight (g)	14.10±0.68	14.80±0.88	15.30±0.96	15.90±0.73	16.80±0.88	17.50±0.56
CF	Length (cm)	2.30±0.88	2.90±0.47	3.20±0.78	3.90±0.58	4.30±0.56	4.60±0.87
	Weight (g)	14.00±0.75	14.60±0.55	15.80±0.66	17.70±0.47	19.60±0.88	22.30±0.66
CF + TA3	Length (cm)	2.30±0.64	3.00±0.39	3.40±0.58	4.10±0.59	4.40±0.56	4.80±0.39
	Weight (g)	14.30±0.82	15.20±0.58	16.30±0.97	18.60±0.55	21.00±0.75	23.50±0.59
CF + TA5	Length (cm)	2.50±0.94	2.90±0.68	3.30±0.87	3.80±0.68	4.10±0.84	4.40±0.67
	Weight (g)	13.90±0.83	14.70±0.77	16.70±0.55	17.90±0.67	19.30±0.94	21.00±0.84
CF + TA6	Length (cm)	2.40±0.54	3.10±0.94	3.60±0.48	3.90±0.98	4.20±0.57	4.60±0.88
	Weight (g)	15.00±0.69	16.10±0.62	17.30±0.87	18.50±0.58	19.60±0.59	21.80±0.94

Discussion

In recent years there has been considerable increase in the probiotic in aquaculture. The probiotics were defined as live microbial feed supplement that improve the health of man and terrestrial livestock (Ghosh *et al*.,2002). In order to overcome fish diseases, scientists have selected certain beneficial microbes, to be used as feed additives. Hjelm *et al*., 2004 coined the term probiotics and defined the term as "organisms and substances which contribute to intestinal microbial balance". Probiotics can also be considered as microbes to improve the nutritive value of an animal feed (Ibrahim *et al*., 2004). Until recently, one of the most frequent procedures used to avoid the establishment of undesirable bacteria in a target organism was the administration of antibiotics in the water.

In the present study, bacteria isolated from medicinal plants showed higher seed germination efficiency and also had antagonistic activity against fish pathogenic bacteria. Most efficient bacteria was identified as *Bacillus* sp. Bacillus is known to improve seed germination and plant growth in several crop plants (Irianto, and Austin, 2003). In addition, *Bacillus* sp are also producing siderosphores, growth hormones, enzymes and organic acids that enhance the growth of agricultural crop plants.

The selection of probiotic bacteria was usually based on their antagonistic activity against pathogens. Our potential bacterial stain was antagonistic to *Vibrio harvei* that was isolated from fish intestine (Jack *et al*., 1994). In present study, a total of nine isolates were tested against *Vibrio herveyi*, among these only one strain inhibited the growth of pathogen at higher level then other strains. Jones *et al*., 2002 and her co-workers found that *Bacillus* produces bacteriocins, siderophores, lysozymes, proteases, hydrogen perioxides that are inhibiting the pathogenic microbes.

In aquaculture practice, water quality deteriorates mainly due to accumulation of metabolic wastes. In this work *Bacillus* sp improved the water quality parameters and also reduced pathogenic bacterial load at significant level than the commercial strain *Lactobacillus* sp. This could be due to the degradation of organic matter facilitates nutrients recycling and competes with other pathogenic bacteria (Sanders *et al*., 2003). This is true in our study also. But, compared to Bacillus, *Lactobacillus* improved the health of fishes to some extent but water quality parameters were much improved than control.

In present study the water quality reduces higher in water probiotic then feed probiotic. Because water probiotic are directly applied the tank culture, that reducing the

organic matter load. *Bacillus* sp of bacteria are reported to more efficiently improve water quality. *Bacillus* also reduced the quantity of ammonia, nitrite in the water. (Skjermo, and Vadstein, 1999).

In the present study, application of water probiotic significantly reduced the levels of ammonia, nitrite, nitrate than feed probiotic application. Bacterial culture incorporated into pellet feed gave good result but not comparatively better than water probiotic. Because, probiotic bacteria such as Lactic acid bacteria, *Streptococcus*, *Saccharomyces* showed enhanced survival, growth and immunity of fish (Sugita *et al*., 1996).

In the present study, *Bacillus* sp improved water quality parameters as well as fish growth by reducing bacterial pathogens, which is comparable to commercial strain of Lactobacillus and control. In addition, application of water probiotics gave good result than feed probiotics.

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