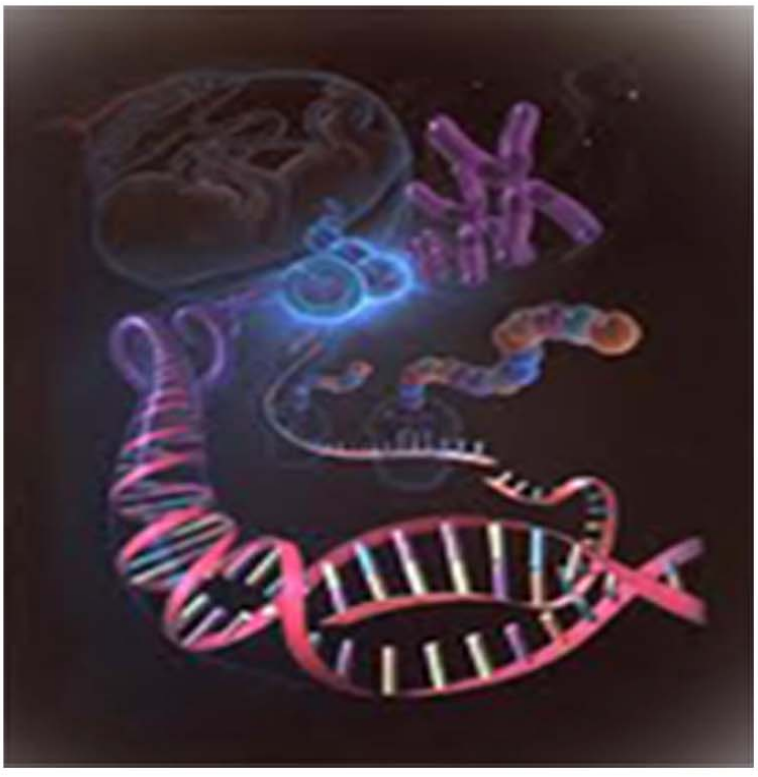
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 3, July 2013

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 3, July 2013

Research Paper

INFLUENCE OF PROBIOTIC ADMINISTRATION, DOMINANT GUT BACTERIAL STRAIN *BACILLUS SUBTILIS* ON GROWTH PERFORMANCE OF *LABEO ROHITA* (HAMILTON) FINGERLINGS

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The growth of aquaculture as an industry has accelerated over the past decades; this has resulted in environmental damages and low productivity of various crops. The need for increased disease resistance, growth of aquatic organisms, and feed efficiency has brought about the use of probiotics in aquaculture practices. The objective of present study was to evaluate the effect of dietary administration of *Bacillus subtilis*, the dominant bacteria in the gut of fish on weight gain, growth performance and survival of *Labeo rohita* fingerlings. Fingerlings of average weight 3-4 g were divided into four experimental groups each with three replicates. Tanks T1, T2, T3 were fed with prepared diets (30±0.4% crude protein) incorporated with isolated intestinal bacteria *Bacillus subtilis* at three different levels ($1x10^5$, $1x10^8$ and $1 x 10^{10}$cells g^{-1} respectively). T0 was control group. The experiment was conducted for 90 days. The results demonstrated that fish group (T2) supplemented with *Bacillus subtilis* at the level of $1 x 10^8$ cells g^{-1} showed significant improvement in growth parameters (Body weight gain, Specific Growth Rate, Condition Factor and RNA DNA ratio, food conversion efficiency) and survival in comparison to other fish groups. Hence, *Bacillus subtilis* can be used as growth promoting probiotic in the diet of *Labeo rohita* fingerlings. Better results for weight gain, growth performance, survival and feed utilization can be achieved by administering *Bacillus subtilis* at the level of $1 x 10^8$ cells g^{-1}.

Keywords: *Bacillus subtilis*, *Labeo rohita,* Fingerlings, Growth

INTRODUCTION

Aquaculture has made significant advances in recent years in the production of a wide range of aquatic organisms, both for human consumption and as ornamental species. As a negative impact to the success of aquaculture, increased intensification has led to higher disease outbreaks. Owing to the high mortality and contagious nature of these diseases, large amounts of antibiotics are often used for disease prevention and control.

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However, the use of antibiotics as a preventive measure has been questioned because they can alter the gut microbiota and induce resistant bacterial populations, with unpredictable long-term effects on public health from a scientific point of view, the use of probiotics has been suggested to be an alternative method for the prevention and control of various diseases in aquaculture.

Selection of probiotics is very critical because inappropriate microorganisms can lead to undesirable effects in host. An ideal probiotic strain, irrespective of its source should be able to colonize, establish and multiply in the host gut Therefore, there is a general consensus that probiotics from autochthonous source have a greater chance of competing with resident microbes and of becoming predominant within a short period of intake, which can assist in returning a disturbed microbiota to its normal beneficial composition and therefore enhanced the disease resistance of host.

The bacteria present in the aquatic environment influence the composition of the gut biota and vice versa as the host and micro-organisms share the eco system (Verschuere *et al.,* 2000). So it is preferable to give probiotics to the fish in larval stage, because the larval forms of most fish and shellfish are released in the external environment at an early ontogenetic stage, these larvae are highly exposed to gastro intestinal-associated disorders, because they start feeding even though the digestive tract is not yet fully developed and the immune system is still incomplete (Balcazar *et al.,* 2006; Kesarcodi-Watson *et al.,* 2008). The word "probiotic" was introduced by Parker (1974). Fuller (1989) revised the definition as 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'.

In present study an attempt has been made to investigate the probiotic effect of *Bacillus subtilis* at different standardized rates through supplementary diet on the growth performance of *Labeo rohita* fingerlings.

MATERIALS AND METHODS

Bacterial Strains-Probiotic

Intestinal bacterial flora was isolated from intestine of ten healthy fingerlings of rohu, *Labeo rohita* (average weight 10 g) of the earthen pond. These fish were starved for 24 h and the intestine from all the fish were dissected out aseptically and homogenized with 0.8% NaCl solution (10:1) (Das and Tripathi, 1991). Fish intestine was homogenized in the homognizer with 10 ml of sterilized water and dilution of 10^{-6} was made by carrying serial dilution stepwise through additional dilution tubes. One ml volume of the dilution was placed in a sterilized petri-plate and about 15 ml of liquefied agar medium (43-45^{0}C) was added in the petri-dish. Then the sample was thoroughly mixed and allowed to solidify. The solidified plates were then kept in an incubator at 30±2^{0}C for 24-48 h (Figure 1). Bacteria that were morphologically different and well isolated on plates were transferred to slants and reincubated at 30±2^{0}C for 24-48 h. All the isolates were rechecked for purity by streaking them on a second fresh Nutrient agar plate and then transferred on slants and maintained at 4^{o}C temperature. Isolated colonies were characterized and identified following Bergey's Mannual of Determinative Bacteriology (Breed *et al.,* 1957) for their colony and cell morphology, staining, biochemical and physiological tests (Ghosh *et al.,* 2002). However biochemical and physiological tests are essential

tools for identification of bacterial genera and species. Various isolates were tested for 10-12 biochemical and physiological tests following Microbes in Action Harry and Paul (1962). Among them, *Bacillus subtilis* was selected for incorporation into diet as it is more prominent on both agar plates and supposed to be suitable bacteria with excellent protease and moderate cellulase producing capacity.

Diet Preparation

Four basal (approximately 31% crude protein) experimental diets were prepared in the form of dry pellets. Diets (D1-D3) were supplemented with the isolated bacterial strain of *Bacillus subtilis* at three different levels ($1x10^5$, $1x10^8$ and $1 x 10^{10}$ cells g^{-1} respectively) and fed to the experimental group in tanks, T1-T3. The control diet (D0) was not supplemented with bacterial cells and fed to control group in tank T0.

Experimental Design

The experiment was carried out in College of Fisheries, Pantnagar. The feeding trial was conducted in four cemented rectangular tanks with flow rate of 25 L per min. Fingerlings were obtained from the instructional fish farm and well acclimatized for four weeks. Each tank was stocked with 55 fingerlings of *Labeo rohita* of average weight 3.0 to 4.0 g.

Growth Parameters

Fish were batch weighed from each tank fortnightly. Growth parameters were assessed in terms of Weight Gain (WG), Specific Growth Rate (SGR), Condition Factor (CF), RNA DNA ratio, Food Conversion Ratio (FCR), biochemical analysis of fish and survival. Final sampling was done after 90 days by weighing all the surviving fish from raceways. Average live weight gain (%) and specific growth rate were calculated using standard methods outlined by Steffens (1989). DNA and RNA contents were estimated following the methods of Munro and Fleck (1956), Burton (1966) and Marham (1955), respectively. The ratio of RNA to DNA was used as an index to growth. Proximate composition of the diets and biochemical analysis of fish was estimated according to the AOAC procedures (1998). The water quality parameters of water, viz., temperature, transparency, pH, dissolved oxygen, free carbon di oxide, total alkalinity were analyzed weekly till the end of the experiment following the methods outlined by APHA (2005)

RESULTS AND DISCUSSION

Fish cultures are increasing to compensate for the shortage of animal protein all over the world. The parallel use of biological products namely the probiotic either alone or in combination with prebiotics is recently the goal of the disease biocontrol strategy in aquaculture as they improve the fish health and modify the fish associated microbial community (Gibson and Roberfroid, 1995).The advantages of using probiotics in fish aquaculture were recently reviewed by Nayak (2010) and Qi *et al.* (2009).

The main strategy of using probiotics is to isolate intestinal bacteria with favorable properties from mature fish and mix it in the feed of immature fish of the same species (Gildberg *et al*., 1997). Intestinal bacterial flora was isolated from intestine of earthen pond raised adult rohu and *Bacillus subtilis* was selected for incorporation into diet as it is more prominent in agar plates and supposed to be suitable bacteria with excellent protease and moderate cellulase producing capacity. Ghosh *et al*. (2002) also reported the

significance of Bacilli in digestion of this fish species.

This study was planned to evoke the differential aspects of using dominant bacteria in the gut of fast growing fish by administrating it at different standardized rates. Concerning the growth performance of the experimental groups, the results revealed that probiotic-supplemented diets showed higher growth rate than those kept on a basal diet, suggesting that the addition of probiotics enhanced the growth performance and feed utilization (Table 1).

The statistical analysis of different growth parameters of *Labeo rohita* fingerlings at the end of experimental period indicated significant increase in the body weight between the four groups. Fingerlings of experimental tank T2 kept on diet D2 were the fast grower followed by fingerlings of tank T3, T1 and least in control group. The specific growth rate and condition factor take almost the same pattern of weight gain in which tank T2 had highest SGR and CF followed by tank T3, T1 and T0. Only the FCR of *Labeo rohita* fingerlings kept on a basal diet (control) was higher than other three groups receiving the diets supplemented with probiotics which in turn represented a positive aspect of probiotic supplemented diets. The best FCR values observed with probiotic-supplemented diets suggested that, the addition of probiotics improved feed utilization, in practical terms this means that probiotic used can decrease the amount of feed necessary for animal growth which could result in production cost reduction. Similar results have been reported by Lara-flores *et al.* (2003).

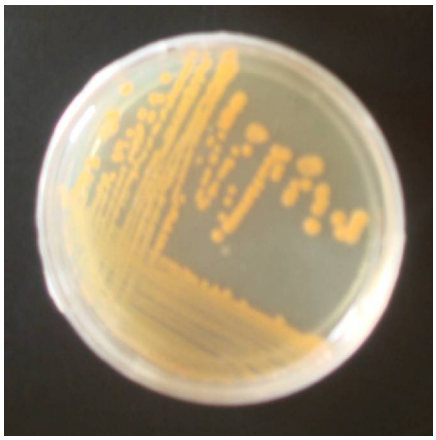
RNA-DNA contents in the test specimen were measured after the termination of the experiment and increased RNA-DNA ratio in the treated group indicates better growth increment and higher protein synthesis which could be attributed to probiotics bacterium used. On biochemical analysis of specimen at the end of the experiment, it has been found that probiotics supplemented diet fed group of fish contain higher protein percent in body muscles (18.95-20.68%) over the control group (17.21%).

Table 1: Details of Growth Performance of *Labeo rohita* Fingerlings in Different Experimental Raceways

Details	T0	T1	T2	T3
No. of fish stocked	55	55	55	55
No. of fish harvested	52	54	55	55
Survival (%)	94	98	100	100
Total weight stocked (g)	180.0^{a}	179.10^{a}	178.75^{a}	180.7^{a}
Total weight harvested (g)	2154^{a}	3653^{b}	3892.5^{c}	3649.6^{d}
Net production in experimental tanks (g)	1974.0^{a}	3473.9^{b}	4071.2^{c}	3830.3^{d}
SGR	2.81^{a}	3.42^{b}	3.46^{c}	3.45^{d}
Condition Factor	1.66^{a}	2.1^{b}	2.7^{c}	2.3^{d}
FCR	5.86^{a}	4.7^{b}	4.3^{c}	4.5^{d}
RNA DNA Ratio	0.0185^{a}	0.0785^{b}	0.0988^{c}	0.0971^{d}

Note: *Values in the same row having same superscripts do not differ significantly ($P > 0.05$).

Figure 1: Tryptone Soya Agar Plates of Bacterial Colonies from the Intestine of Fingerlings of *Labeo rohita*



The differences in growth and survival of *Labeo rohita* fingerlings between control and treated group could be attributed to the quality of diets. Supplementation of intestinal microflora increased the nutritional efficiency of the formulated diet. The intestinal tract of fish fingerlings is much more simply organized and shorter than that of the adults (Stroband and D¹browski, 1979), which is associated to low production of digestive enzymes. The fingerlings therefore, simply do not have the necessary enzymes to digest the feed at the optimal level. Exogenous enzymes extracted from probiotic bacteria might have supplied digestive enzymes and certain essential nutrients to promote better growth and survival as indicated by Douillet and Langdon (1994), who observed faster growth and higher survival in Pacific oyster fed bacterium. The usefulness of RNA concentration as an indicator of growth has been proven by Neidhardt and Megasanik (1960) for bacteria and shown it to be equally applicable to fish. Rapidly growing organisms apparently synthesize and accumulate the RNA needed for protein synthesis. Consequently, RNA and the ratio of RNA to DNA are useful as an index of growth. In the present study, RNA-DNA ratios in *Labeo rohita* fingerlings were positively correlated to the trends in growth as has been indicated by several authors (Haines, 1980; Bulow *et al.,* 1981). Increased RNA-DNA ratios noticed in *Labeo rohita* fingerlings corresponding to growth increment are indicative of higher protein synthesis which could be attributed to probiotic effect of the bacterium used.

Water quality plays important role in growth and survival of aquatic organisms. It is determined by various physical, chemical and biological parameters of water body. Almost similar status of all physico chemical parameters (Temperature, 18-24ºC; pH, 7.0- 8.0; dissolved oxygen, 7.8-8.5 mg/l; free carbon dioxide, 0.2-0.8 mg/l and alkalinity, 180-225 mg/l) of the raceway water was observed. Only a sudden increase in pH value was observed in all raceways during 9th week of experiment which is due to the application of lime. Therefore, it is clear that addition of probiotic had no adverse effect on water quality.

CONCLUSION

In conclusion, the dominant gut bacteria *Bacillus subtilis* from the fish is significantly related to growth enhancement and survival of fish hence it would be an effective way to select candidate probiotics from fish gut itself. Administration of bacteria, *Bacillus subtilis* as probiotic must be practiced in diet formulation of fingerlings of *Labeo rohita* to obtain better growth, survival, feed utilization and it will produce more efficient results if it is used at the level of 1 x 10^8cells g^{-1}.

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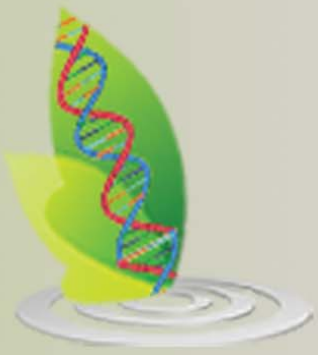

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ISSN 2250-3137