



PROBIOTICS IN AQUACULTURE FOR PROMOTING FISH HEALTH

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ABSTRACT

The objective of the present study is to understand the importance and use of Probiotics in Aquaculture. The research on the use of probiotics in aquaculture is huge and extensively used. The *Lactobacilli* was isolated from fish gut. For experimental treatment, the fish were divided into two groups; one with Probiotics & commercial food, other group was supplemented only with commercial food. For feeding probiotics to fish the microencapsulation method was used which is employed by extrusion technique. Weight taken after every 5 days during 30 days experiment. At the end fish growth parameters was determined by the fish body length, weight, survival ratio and then compared between probiotic and non-probiotic group. Result showed that the increasing body length, weight and survival ratio is significantly higher than the control group. According to this data, the use of probiotics in aquaculture has an important nutritional feed to promote fish healthy gut which affects their immune system, growth performance and survival ratio.

Keywords: Probiotics, Microencapsulation; Growth Evolution

INTRODUCTION

The Aquaculture is fastest growing food production region in world. The Microorganisms have been involved due to

bacterial diseases, infections and its control in aquaculture is a big challenge [1]. A probiotics is expected to have a few certain

properties as a beneficial effect in aquaculture industry [2]. Probiotics such as *Lactobacillus spp.* is set out to have an inhibitory activity against common human pathogens.

The probiotics are health boost functional and useful food, as well as healing, health giving and growth supplementation in animal production. Probiotics includes harmless i.e. non-toxic bacteria and they help to protect of the host animal who contribute directly or indirectly against the harmful bacterial pathogens. For probiotics to be profitably, a strain should be able to colonize the gastrointestinal tract and aid host health through its metabolic activities. Probiotics have positive effects to enhance immune system, growth performance and prevention from pathogens of the host animal to increase their effectiveness [1].

The probiotic has of combination which contains yeast and lactic acid bacteria which helps in increasing the intestinal micro flora resulting in increased body weight and survival ratio. The effect of probiotic on fish and shrimp culture was explore and studied in some papers. There are many research papers which is been look out in the field of aquaculture nowadays [6, 7] with the mechanism of competitive exclusion of effective probiotics on the rainbow trout

(cold-water tributaries) [8, 9]. Probiotics has also been said to provoke the immune response in rainbow trout which is been studied by several researchers [9-11, 20].

Therefore, this experiment was carried out to study the effects of probiotics such as lactic acid bacteria on the growth rate and survival ratio in zebra fish (*Danio rerio*).

MATERIALS AND METHODS

SAMPLE COLLECTION

Fish samples were collected in sterile plastic bag with ice. The samples were stored in cold condition with ice and it transported to laboratory [5].

ISOLATION AND IDENTIFICATION OF ISOLATED FISH GUT FROM PROBIOTICS

ISOLATION OF BACTERIA

The collected fish samples were washed with sterile distilled water to remove the unwanted particles. Then the fish were dissected to remove the digestive tracts under the sterilization condition. The digestive tracts were homogenized in the phosphate buffer saline for centrifugation [12]. After centrifugation the supernatant was taken and serially diluted in sterile distilled water in test tubes. dilution were made up to 10^6 dilution and one hundred microliter of culture were spread on different selective media such as MRS agar, bifidobacterium agar, LB agar

plate and incubated anaerobically for 24 to 48 hrs. at room temperature. After Incubation, growth was observed on agar plate [4, 11].

IDENTIFICATION OF BACTERIA

Bacterial isolates were identified by using direct sequencing of the 16s rRNA Gene and BLAST analysis.

PROTEOLYTIC ACTIVITY

PREPARATION OF SKIM MILK AGAR FOR PROTEOLYTIC ACTIVITY

Proteolytic activity was followed qualitatively by using the skim-milk agar and made which as follows; -25gm of non fat dry milk (skim milk) was reconstitute with 250ml of distilled water. The mixture was stirred thoroughly and autoclaved at 121°C for 15min. Likewise, 500ml of 2.5% agar solution was made and sterilized. For plating skim milk and agar solution were held in a water bath at 50°C. Then the skim milk was poured into the agar bottle and mixed thoroughly. The skim milk agar was poured quickly into the plates. Cultures was serially diluted with sterilized 0.1% peptone water to 10^{-7} culture was spread on to the plates. Plates were incubated at 30° C anaerobically for 24 to 48 hrs. [15].

MICROENCAPSULATION OF PROBIOTIC CELLS

PREPARATION OF CELL SUSPENSION

The isolated cultures was inoculated in MRS, Bifidobacterium & LB broth for 24 h under aerobic and anaerobic conditions at 30°C for 24 to 48 hrs, then biomasses were separated i.e. harvested by centrifuging at 4000 rpm for 10 min at 4 °C. The cultures then washed twice by sterile saline solution (0.9%) and cultures were used for the microencapsulation process [14].

MICROENCAPSULATION

PROCEDURE

All glassware and solutions that is been used in the methods (protocols) were sterilized at 121 °C for 15 min. Hydrocolloid solution (sodium alginate) was prepared, transferred to carrier solution of probiotic culture of each bacteria with stirring under sterile condition to protect from contamination and ensure uniform distribution of the cells. Cell suspension were passed through syringe needle to form a droplets, containing a hardening solutions that holds cations which was directly dripped in to calcium chloride 0.1M. When the droplets come in contact with hardening solution, alginate polymers were surrounded with the core to form a three-dimensional lattice structure by cross-linking calcium ions which is used for further use [14].

FEEDING AND PROBIOTIC SUPPLEMENTS PREPARATION

The experiment was conducted for 30 days with *Zebra Fish*. The probiotic supplement with appropriate amount was given in fish tank. The commercial food was set for non-probiotics group was also added in the same way. The experiment was divided into two experimental groups, with probiotic and without Probiotics.

DETERMINATION OF NUTRITIONAL EFFECT AND SURVIVAL

Weekly the fish were taken for wet weight measurement. The number of mortality was also recorded. Electronic weighing scales was used for wet weight analysis which was calibrated at 0.00 g. Marks i.e. indicators of growth includes: body weight increase (BWI), specific growth rates (SGR), feed conversion ratio (FCR) and survival rate (SR) were as follows:

$$BWI = W_T - W_0;$$

$$SGR = 100 * \ln(W_2) - \ln(W_1) / T_2 - T_1$$

$$FCR = \text{Dry weight of food eaten by fish} / \text{Wet weight of fish (g)}$$

$$SR = \text{Final no. of fish (g)} / \text{Initial no. of fish (g)} * 100$$

Where, t- is the time period (culture period) in a days, food eaten by fish

$\ln W_1$ 0 - natural logarithm of the wet weight of the fish at beginning of experiment and $\ln W_t$ - natural logarithm of wet weight of fish at day t. (W_0 and W_t are in gram) [20].

STATISTICAL ANALYSIS:

The data was collected and subjected to one way ANNOVA, significant difference was determined by Duncan's test. The data presented as treatment mean \pm Standard deviation. The values of P \leq 0.05 were determined and showed a considered significantly difference.

RESULT-

ISOLATION AND IDENTIFICATION OF BACTERIA

The collected fish samples were dissected and gut region removed under aseptic condition. Four different bacterial strains were isolated by using selective medium from the fish gut (**Figure 1**).

Further Identification of fish gut isolates was done by using 16SrRNA sequencing till genus to species level [13]. The phylogenetic position based on 16SrRNA gene sequencing analysis of fish gut isolates as shown in **Figure 2-4**.

PROTEOLYTIC ACTIVITY-

The skim milk agar is an effective medium to detect the extracellular cell bound proteases, also to determine a clear zone surrounding by the growth of bacteria. The clear zone on skim milk agar were produced by different isolated LAB by showing their proteolytic activity. All isolated protease producing LAB produce different sized clear zones. Among

all isolated LAB shows differences in clear zones, out of these *Bifidobacterium* showed largest size clear zone than other isolated LAB [16] (Figure 5).

MICROENCAPSULATION OF PROBIOTIC CELLS

The number of significant differences between coated and uncoated beads in microencapsulation yields. Microencapsulation process resulting by showing small formulated micro capsules of four different fish gut LAB isolates as shown in below figure. Microencapsulation with *Hydrocolloid* is one of the most useful and effective method for probiotic survival [14] (Figure 6).

DETERMINATION OF FISH GROWTH AND SURVIVAL

In this research there is a significant differences ($P < 0.05$) is initial mean weight (0.42 ± 0.13 a g) among the treatments (Table 1). Survival ratio in the probiotic group was higher than the control group i.e. Non-probiotic group. However, average fish weight was conducted and recorded to see the effect of fish fed with probiotic supplementation. In the probiotic group average body weight increased was seen (BWI), and was significantly ($P < 0.05$). In probiotic group the food provided conversion ratio was higher than the non-probiotic groups i.e. control group and the specific growth rate (SGR) between the treated fish and control fish showed different characters.

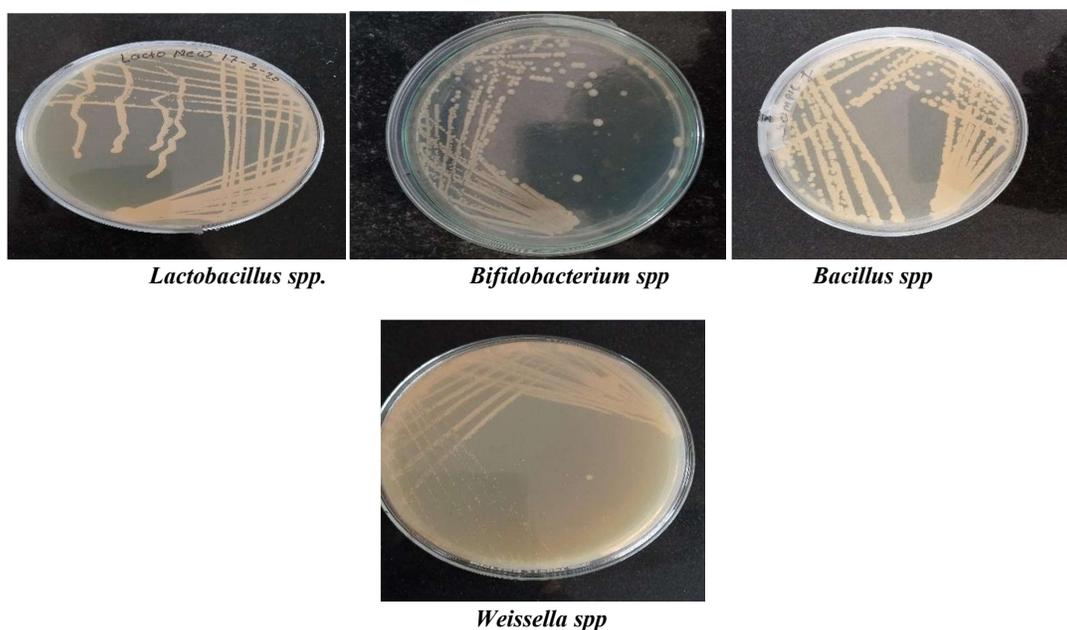


Figure 1: Different isolates from Fish gut

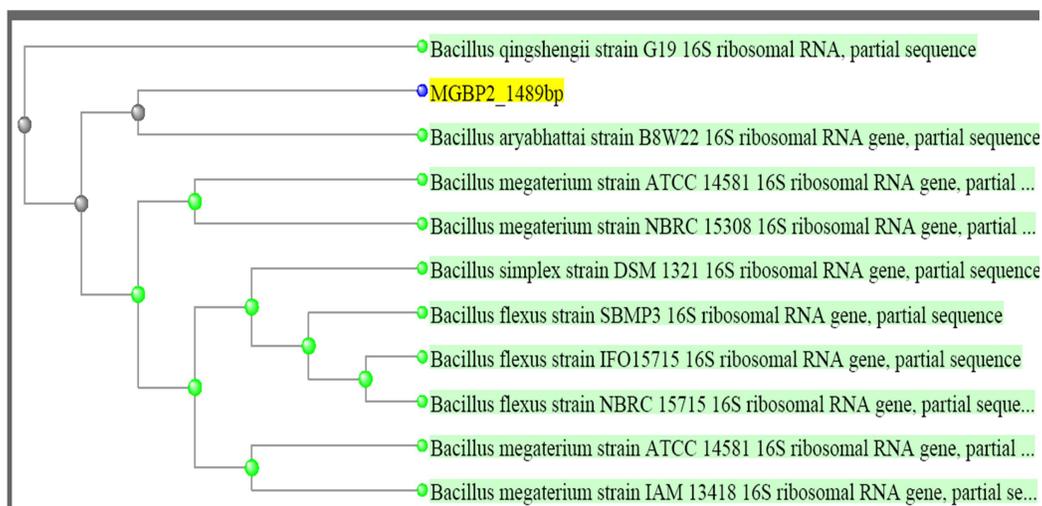


Figure 2: Phylogenetic position based on 16SrRNA gene sequence analysis bacteria *Bacillus* spp

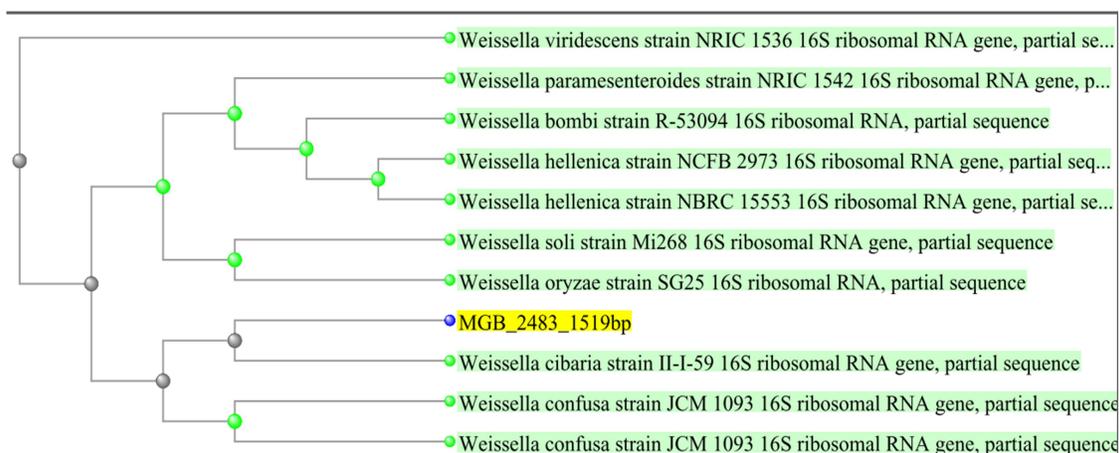


Figure 3: Phylogenetic position based on 16SrRNA gene sequencing analysis of *Weissella* species

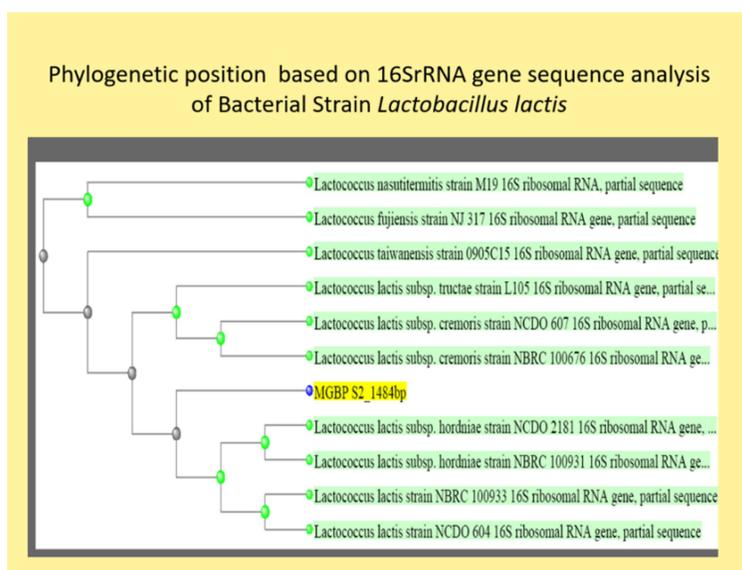


Figure 4: Phylogenetic position based on 16SrRNA gene sequencing analysis of *Lactobacillus lactis*

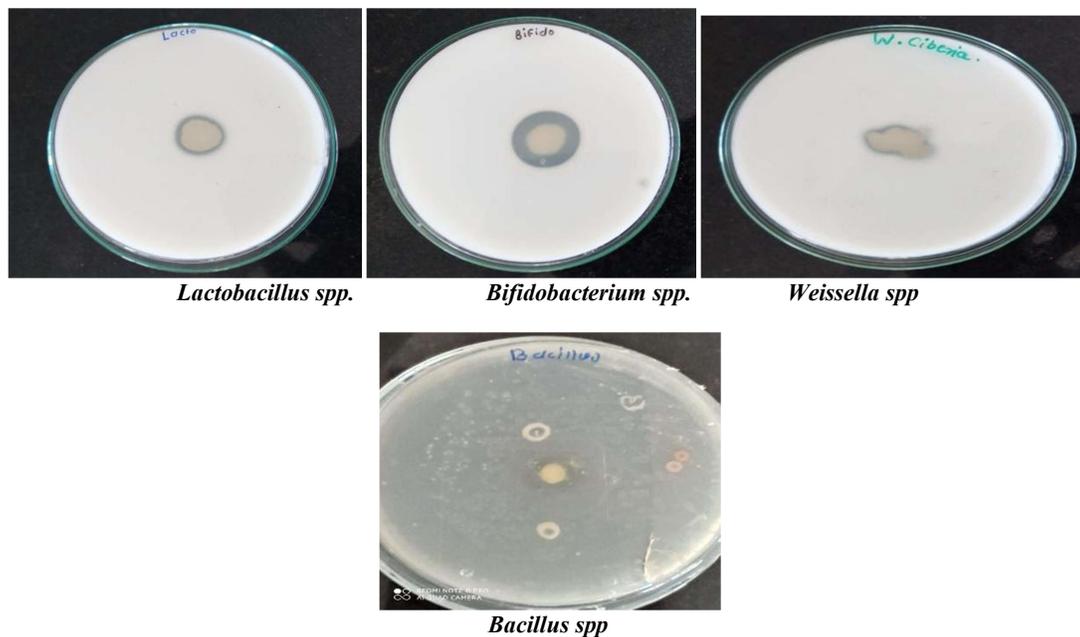


Figure 5: Proteolytic activity of protease producing on skim milk agar



Figure 6: Microencapsulation of probiotics cells

Table 1: Growth indices and survival (mean \pm S.E) of zebra fish

Treatment	BWI	SGR	FCR	SR
Probiotics	0.42 \pm 0.13 a	1.03	4.57	50
Non-probiotics	0.25 \pm 0.07 a	1.39	8	30

Letters with different superscripts at the same row differ significantly by $P < 0.05$ by means separation using the Duncan multiple ranges test

DISCUSSION

The growth parameters, such as survival, weight gain, specific growth rate and feed conversion efficiency rate were significantly higher in probiotics which was incorporated in fish fed by diet, followed by other

experimental groups when compared with control [19]. They have potential to improve the water quality, increases tolerance to stress, generate high quality livestock. Use of Probiotics had showed to have a beneficial effect on fish health, growth and hence

increasing economic production of fish farming.

Probiotics provide beneficial effects on fish growth and have become an important management tool in aquaculture industry. In this study, the effects of probiotics, the useful microorganisms on the growth, survival rate and nutritional status of fish was analyzed [19]. This present study shows that, best administration method, aquaculture systems and beneficial applications of probiotics. Prebiotics which holds oligosaccharides like β - glucane and mannose helps to an increases feeding efficiency conversion and reduced the mortality leads to enhance growth.

CONCLUSION

A mixture of isolated bacterial strains positively influenced growth and survival of fish. The present evaluation study showed the nutritional value of probiotic feed and its utilization. The appropriate feed selected for feeding fish regarded as a very promising feed and novel strategy to be used in aquaculture, from this research study. As a result formulated isolates were used in the fish culture. The choice of probiotics for aquaculture showed effects for growth, attachment to the intestinal mucus and production of beneficial compounds can be considered for improving fish growth rate.

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