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Influences of Isolated Gut Probiotic *Bacillus subtilis* on Total Growth Parameters of Freshwater fish *Labeo rohita* (Hamilton, 1822)

S. Jayaprakash[1*], Dr. K. Parvathi[1]

[1]Assistant Professor of Zoology, Erode Arts and Science College (Autonomous), Erode, Tamil Nadu, India.
[*1]Research Scholar in Zoology, Erode Arts and Science College (Autonomous), Erode, Tamil Nadu, India.
***Corresponding Author**: jprksh297@gmail.com

Abstract: **To find out the effect of isolated gut probiotics on the total growth parameters of freshwater fish fingerlings *Labeo rohita.* The isolated gut probiotic *Bacillus subtilis* (SUB3845847 SeqJP2 MH128358) on Length, Weight, Weight gain percentage and Specific growth rate (SGR) of freshwater fish *Labeo rohita* for the period of 60 days. The two tubs such as Control tub (fed without probiotics) and Experimental tub (feed fed with *Bacillus subtilis*) were maintained and 30 fingerlings were introduced into each tub and continuous aeration was given. Each treatment had triplicates. The feed was given twice a day of 6 am and 6 pm regularly at the rate of 3% body weight and water changed every day with the water pump. At the end of the evening 15 days, fish Weight and Length were measured up to 60 days. Results indicate that Weight and Length were significantly increased in the experimental tub than the control tub. Isolated gut probiotic diet improved gastrointestinal activity and stimulate the better growth parameters of freshwater fish *Labeo rohita*.**

Keywords: **Gut probiotic *Bacillus subtilis,* Growth parameters, *Labeo rohita* fingerlings.**

I. INTRODUCTION

The human population is estimated to reach 9.3 - 9.6 billion by 2050 [1 and 2]. This increase, often fastest in developing countries, inevitably leads to an increase in demand for high-quality sources of protein foodstuffs. Presently India is the second largest fish producing and second largest aquaculture nation in the world. India is also a major producer of fish through aquaculture and ranks second in the world. Freshwater aquaculture accounts for nearly 55% of the total fish production in India [3]. Aquaculture resources in India include 2.36 million hectares of ponds and tanks. Ponds and tanks are the prime resources for freshwater aquaculture in India. However, less than 10 percent of India's natural potential is used for aquaculture currently [4]. The three Indian major carps, namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute the bulk of production to the extent of 70 to 75 percent of the total freshwater fish production. Parker [5] modified the definition to "organisms and substances which contribute to intestinal balance". The use of probiotics in aquaculture is now widely accepted with an increasing demand for environment friendly aquaculture [6]. Fuller [7] 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". When probiotics like *Bacillus subtilis* and *B. circulans* were supplemented in the diets of Rohu (*Labeo rohita*) fingerlings, the final body weight and SGR significantly increased than those fed only formulated diets [8]. The experimental tub (feed fed with *Bacillus subtilis* SUB3845847 SeqJP2 MH128358) results indicate that weight, length, weight gain percentage, and length gain percentage were significantly increased in the experimental tub than the control tub for the experimental period of 15th and 30th days. Isolated probiotic diet improved digestive activity and stimulate the growth of freshwater fish *Labeo rohita* [9]. The objective of the current study was to evaluate the effects of probiotics the bacteria *Bacillus subtilis* total growth parameters of freshwater fish *Labeo rohita* for with the following objectives.

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II. MATERIALS AND METHODS

A. Collection of Experimental Animals

Healthy, freshwater fish *Labeo rohita* (5.00 gm weight and 4.5 cm length) were bought from Government fish farm, Bhavanisagar, Erode District, Tamil Nadu and acclimatized in research laboratory conditions in a plastic tub (25^0 C/77^0 F, pH 7.0) with continuous aeration for three weeks prior to the commencement of the experiment. Stocked fish were fed with supplementary diet *ad libitum* [10].

B. Isolation and Identification of Probiotic Bacteria in fish Intestine

The freshwater fish *Labeo rohita* were washed with sterile distilled water and dissected to remove the fish intestine by the sterilization condition. The fish intestine was homogenized in the same sterile distilled water and centrifugation. After centrifugation, the supernatant was taken and serially diluted in sterile distilled water in the test tubes to 10^{-5} and 10^{-6} dilution and was pour plated on a nutrient agar plate and incubated for 24 h at room temperature [11]. Selective colonies were characterized and identified following Bergey's Manual of Systematic Bacteriology [11] for their colony and cell morphology, gram staining, biochemical and physiological tests [12] and 16S rRNA sequencing [13] identification of bacterial genera and species. Pure culture was maintained in MRS broth at -30^{o}C with 10% (v/v) glycerol [14]. Finally, all the sequences of *Bacillus subtilis* strain were deposited in NCBI Genbank.

C. Feed preparation

To prepare the diet, the following ingredients such as (Rice bran, Groundnut oil cake, Dry fish meal, Soya meal, Maize and vitamin & mineral mix) were purchased from local Erode market, Tamil Nadu, India. All the ingredients were mixed and powdered by a machine and were made into dough with the help of distilled water [15]. Feed ingredients and experimental pellet diet were analysed for the proximate composition according to [10] procedure as follows; Carbohydrate, Protein, Lipid, Moisture, and Ash content was a determination for [10] protocols (Table 1)

Table 1: Proximate Composition (% dry matter basis) analysis of experimental diets

Ingredients	**Control (%)**	**Experiment (%)**
Rice bran	40	40
Groundnut	20	20
Fish meal	15	15
Soya Meal	15	15
Maize	9	8
Vitamin & Mineral mix	1	1
Bacillus subtilis	-	1
Proximate Composition (%)		
Dry matter	99.90	99.80
Carbohydrate	20.72	21.23
Protein	35.65	35.53
Lipid	9.16	9.14
Moister	9.34	9.40
Ash	13.14	14.10
Digestible energy (k.cal/kg)	3294.27	3258.45

(-) The absence of *Bacillus subtilis*

D. Experimental Design

Experimental fish were divided into two groups such as control and experimental tub. Fish in the control tub were fed only with formulated feed (without any probiotic), fish in the experimental tub is fed with formulated feed mixed with isolated probiotic bacteria *Bacillus subtilis*. Each treatment had triplicates. The feed was given twice a day of 6 am and 6 pm regularly at the rate of 3% body weight and water changed every day with the water pump [16]. The experiment was conducted up to 60 days [10]. The experiment was conducted up to 60 days. At the end of 15 days, the weight and length of fish were noted.

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Control - Formulated diet only

Experiment - Formulated diet + Probiotics bacteria (*Bacillus subtilis*)

E. Growth Parameters

The total growth parameters of the freshwater fish *Labeo rohita* fingerlings were measured by taking their body weight and length was measured at the end of 15 days up to 60 days. The total growth parameters were calculated using the following formulas:

$$\text{Length (cm)} = \text{Final length (cm)} - \text{Initial length (cm)}$$

$$\text{Length gain percentage (\%)} = \frac{\text{Final length(cm)} - \text{Initial length (cm)}}{\text{Initial length (cm)}} \times 100$$

$$\text{Live Weight (gm)} = \text{Final weight (gm)} - \text{Initial weight (gm)}$$

$$\text{Weight gain percentage (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight(g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific Growth Rate (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Days of experiment}} \times 100$$

F. Statistical Analysis

Means ± SD with the different superscripts within a column is significantly ($P<0.05$) One-way analysis of variance ANOVA by using SPSS 16.0 software

III. RESULT

The total growth parameters in the *Labeo rohita* were fed with probiotic and the length, length gain percentage, weight gain, and weight gain percentage and specific growth rate of the fish were measured at the end of 15 days and 60 days of an experimental period. The live weight of the fish was increased as (13.5±0.46) gm in experiment fish than the (7.8 ±0.83) gm control fish at the end of 60 days of an experimental period (Table 2 & Figure 1). Whereas at the end of 60 days, the total length of fish was gradually increased as (10.5 ±0.70) cm in experimental fish than the control fish (8.4 ±0.71) cm (Table 3 & Figure 2). The weight gain percentage was found to be maximum increased as (73.07 ±0.72) in the experimental group than the control group (56 ±0.70) at the end of 60 days of an experimental period (Table 4 & Figure 3). The length gain was found to be increased as (25.0 ±0.70) in the experimental group than the control group (15.06 ±0.56) at the end of 60 days (Table 5 & Figure 4). The specific growth rate was found to be increased as (12.50 ±0.70) in the experimental group than the control group (4.66 ±0.55) at the end of 60 days (Table 6 and Fig.5). Therefore suggesting that the high concentrations of isolated gut probiotic diet promote the total growth parameters of *Labeo rohita* in this research study. This present study supported the result report of [12 and 9] the use of better concentration of the isolated gut probiotic always leads to better growth performances of the *Labeo rohita*. The results of the total growth parameters of *Labeo rohita* fingerlings fed with isolated gut probiotics *Bacillus subtilis* were improved the total growth parameters of *Labeo rohita.* The support the resulting study with the findings of [22 and 23] reported that species of *Bacillus* showed inhibitory activity against various fish pathogens. Probiotic bacteria produce digestive enzymes and required growth parameters and improving the feed absorption resulting in a better growth rate in host animals.

Table. No.2. Changes in the live weight on the freshwater fish *Labeo rohita* of a different exposure period.

Exposure Period in Days	Live Weight (gm)	
	Control	Experiment
15	6.0 ±0.35	7.9 ±0.65
30	6.4 ±0.65	8.7 ±0.76
45	7.0 ±0.61	10.6 ±0.65
60	7.8 ±0.83	13.5 ±0.46

Values are in mean ±SD

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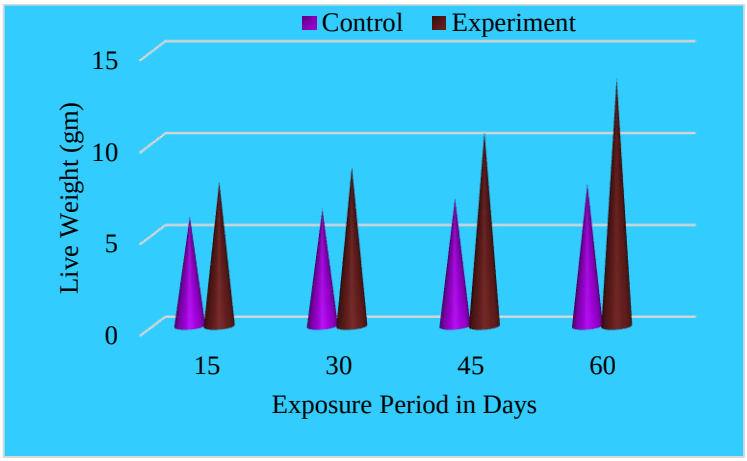

Fig.1. Changes in the live weight on *Labeo rohita* fed with isolated probiotic *Bacillus subtilis* of a different exposure period.

Table. No.3. Changes in the total length on the freshwater fish *Labeo rohita* of a different exposure period.

Exposure Period in Days	Total Length (cm)	
	Control	Experiment
15	7.3 ±0.45	8.1 ±0.40
30	7.4 ±0.64	8.5 ±0.71
45	8.0 ±0.60	9.3 ±0.56
60	8.4 ±0.71	10.5 ±0.70

Values are in mean ±SD

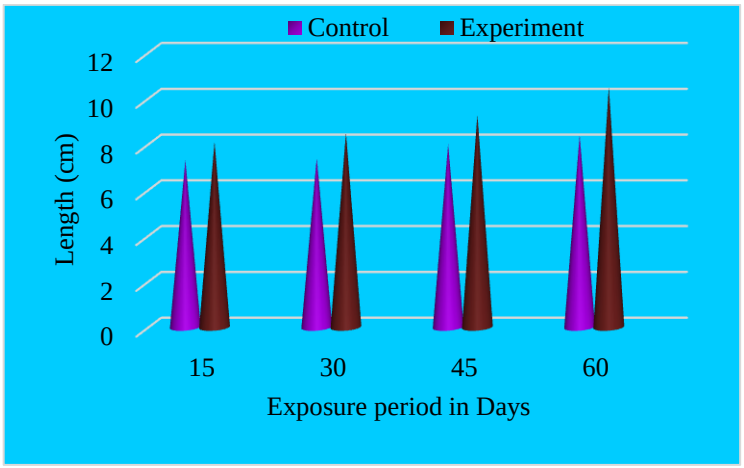

Fig.2. Changes in the total length on *Labeo rohita* fed with isolated probiotic *Bacillus subtilis* of a different exposure period.

Table. No.4. Changes in the weight gain percentage on the freshwater fish *Labeo rohita* of a different exposure period.

Exposure Period in Days	Weight Gain Percentage (%)	
	Control	Experiment
15	20 ±0.61	31.66 ±0.54
30	28 ±0.86	35.93 ±0.59
45	40 ±0.61	51.42 ±0.85
60	56 ±0.70	73.07 ±0.72

Values are in mean ±SD

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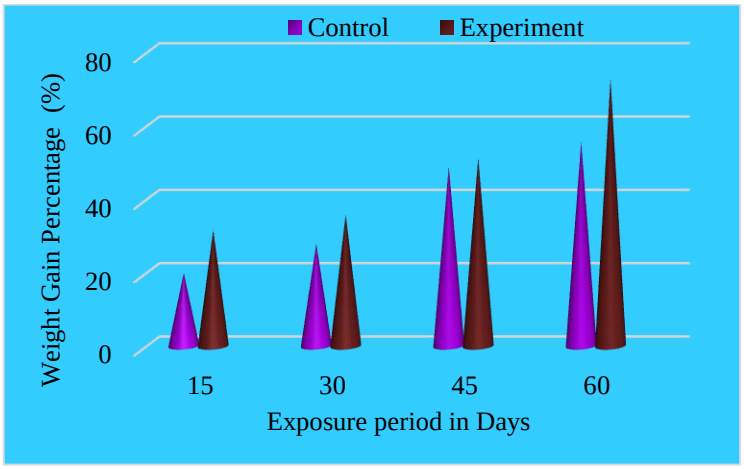

Fig.3. Changes in the weight gain percentage on *Labeo rohita* fed with isolated probiotic *Bacillus subtilis* of a different exposure period.

Table. No.5. Changes in the length gain on the freshwater fish *Labeo rohita* of a different exposure period.

Exposure Period in Days	Length Gain (%)	
	Control	Experiment
15	4.28 ±0.56	10.95 ±0.71
30	5.71 ±0.44	14.86 ±0.57
45	14.28 ±0.43	16.25 ±0.42
60	15.06 ±0.56	25.0 ±0.70

Values are in mean ±SD

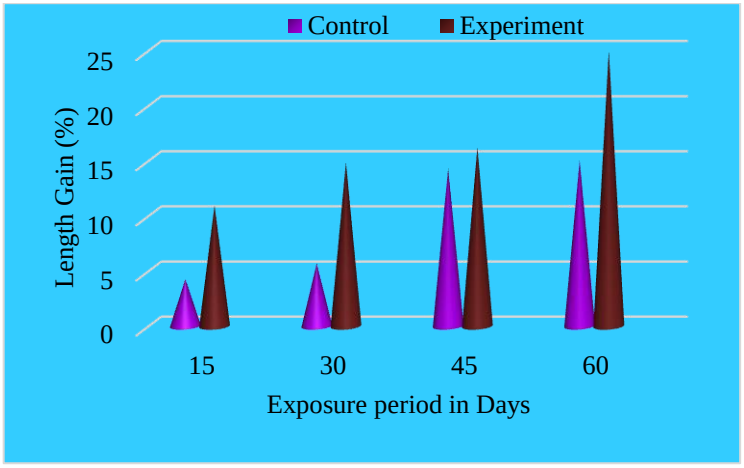

Fig.4. Changes in the length gain on *Labeo rohita* fed with isolated probiotic *Bacillus subtilis* of a different exposure period.

Table. No.6. Changes in the specific growth rate on the freshwater fish *Labeo rohita* of a different exposure period.

Exposure Period in Days	Specific Growth Rate (%)	
	Control	Experiment
15	6.66 ±0.42	10.00 ±0.61
30	4.66 ±0.55	9.00 ±0.61
45	4.44 ±0.51	10.22 ±0.75
60	4.66 ±0.55	12.50 ±0.70

Values are in mean ±SD

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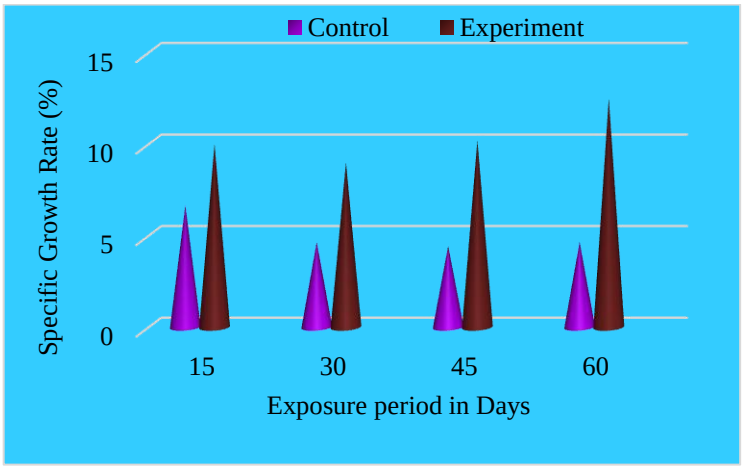

Fig.5. Changes in the specific growth rate on *Labeo rohita* fed with isolated probiotic *Bacillus subtilis* of a different exposure period.

IV. DISCUSSION

Probiotics are live microbes, nowadays probiotics *Bacillus subtilis* are widely used in aquaculture, its beneficial effect on host animals improving digestive activity and improve growth performance of aquatic animals. Probiotics are the useful microorganisms promoting growth performance and protecting the host against pathogens. The support research study was isolated probiotic *Bacillus subtilis* on improving growth of fingerlings freshwater fish *Labeo rohita.* The experimental tub feed fed with *Bacillus subtilis* results indicates that improved the weight, length, weight gain percentage, and length gain percentage were significantly increased in the experimental tub than the control tub. Isolated probiotic diet improved digestive activity and stimulate the growth and better health condition of freshwater fish *Labeo rohita* [9]. The effects of probiotic diet have been studied in the many aquatic animals. The aquatic animal growth improving has been reported by feeding of *Bacillus sp*. in the *Labeo rohita* [17]. In the present study, the growth of *Labeo rohita* was significantly increased by probiotic diet. Such increase in the growth of aquatic animals fed with probiotic diets may be improved digestive activity by improving the synthesis of vitamins, enzymatic activity, with a consequent improvement of the digestibility and weight gain [18]. Probiotics have been suggested as an option to improve the health and well-being of aquatic animal fish culture [19]. Results of this study substantiate the fact that probiotics have direct growth-promoting effects on *Labeo rohita* which in accordance with the reports [20]. As supplementary components in aquaculture feed, probiotics have strong adhesive and growth abilities [21].

V. CONCLUSION

Probiotics are the useful microbes stimulating growth parameters and protecting the host against fish pathogens. My research work isolated probiotic *Bacillus subtilis* on improving growth of fingerlings freshwater fish *Labeo rohita*. The experimental tub (feed fed with *Bacillus subtilis* SUB3845847 SeqJP2 MH128358) results indicate that weight, length, weight gain percentage, length gain percentage and specific growth rate were significantly increased in the experimental tub than the control tub. Isolated gut probiotic *Bacillus subtilis* with formulated diet improved digestive activity, better health and stimulate the total growth parameters of fingerling freshwater fish *Labeo rohita* in same species diet.

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