

# Isolation and Effect of Isolated Gut Probiotics *Bacillus oleronius* on enzyme activities in *Koi carp*

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**Abstract**—*Koi carp* are colourful Ornamental fish. Probiotics are live microbes, utilized as a nourishment partner. The aim of the present study was to find out the effect of isolated gut probiotic bacteria on digestive enzyme activities in ornamental fish. *Bacillus oleronius* was isolated from *L.rohita* and identified as a probiotics based on the biochemical test and 16srRNA sequencing (TGTAACACCCGAAGTCGGTGAGGTAACCTTTGGAGCCAGCCGCCGAAGGTGGACCAGAT- Sequence ID :N R \_ 043325.1). *Koi carp* fingerlings in the control tank were fed only with supplementary fish feed and the fish in the experimental tank were fed with isolated gut probiotics along with supplementary fish feed. The digestive enzyme activities were analyzed on 0, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup> and 75<sup>th</sup> day of the experimental period. Increased amylase and lipases and decreased GOT and GPT activities were recorded in the experimental fish. Result reveals that isolated probiotics improved the growth and health status of ornamental fish.

**Keywords**— Isolation, probiotics, *Bacillus oleronius*, *Labeo rohita*, Blood, enzymes

## I. Introduction

Ornamental fish culture is one of the profitable area of fish farming activities and it plays an main role in the socio-economic upliftment of backward classes and women in our developing country. The prosperity of the ornamental fish industry has induced the indiscriminate use of antibiotics and chemotherapeutants for improved health and nutrition, which has led to the development of drug-resistant strains of pathogenic microorganisms [1]. There is a commercial growing interest in the ornamental fish trade in Asia and all over the world. Probiotics can be defined as live microbes that when administrated adequate amount confers the health benefits on the host [2]. Probiotics can produce vitamins and expel harmful substances in the diet, which causes nutritional improvement [3]. Probiotics are additionally considered as a naturally agreeable treatment technique. In aquaculture field, probiotics utilized as either as a nourishment partner or as an add on ingredient to water. *Bacillus* genes have been broadly accepted in the aquaculture sector and can improve survival, growth and development by fortifying the immune system [4]. *Bacillus* species are skilled to deliver amino acids, enzymes and antibiotics [5]. Probiotics reduces the cost of fish cultivation by lowering the cost of feed per unit growth rate of fish [6]. The use of probiotics in animal nutrition and human being is well documented and recently has been applied to fish culture [7]. The use of *Bacillus* species in aquaculture practices has also been reported previously [8]. The benefits of probiotics on koi carp health, growth, and intestinal bacteria are well documented [9] but there are few reports are available on the effect of isolated gut probiotic bacteria *B.oleronius* on digestive enzyme activities in koi carp.

## II. MATERIALS AND METHODS

### 2.1 Isolation of Gut Probiotic Bacteria and Experimental animal

Indian Major carp *Labeo rohita* were purchased from Sirago fish farm, Nerinjipet, Tamil Nadu, India. The body surface of fish was disinfected with 70 % alcohol, intestine were taken out and washed thrice with normal saline (NaCl 0.85% w/v) and cut into small pieces (1g) and homogenized [10]. Using serial dilution, 0.1ml of homogenized samples were spread on nutrient agar medium for 24 h incubation at 37°C to count total bacterial colony [11]. Single colony was isolated and purified on another nutrient agar medium. Isolated bacteria were identified based on the biochemical characteristics (Bergey's manual) and 16s rRNA sequencing. The healthy *Koi carp* (1.5 g weight) were purchased from Sirago fish farm, Nerinjipet, Tamil Nadu, India and acclimatized in nursery tank for a month. The fish were fed well with supplementary fish feed *ad libitum*. The selected supplementary fish feed (Hipro, Aptimum company, Thailand) contains ingredient such as crude protein (38 %), crude fat (4 %), crude fiber (3%), moisture content (12%) and crude ash content (12 %). The proximate composition of feed ingredients were done by the method of AOAC, (1999). Isolated probiotic *Bacillus oleronius* were identified based on its biochemical characteristics and (TGTAACACCCGAAGTCGGTGAGGTAACCTTTGGAGCCAGCCGCGAAGGTGGACCAGAT- Sequence ID :N R \_ 043325.1) 16s rRNA sequencing. Single colony was mass cultured and the colony forming units were counted.

### 2.2 .Experimental Design

The two cement tanks (4 ft, 3 ft) were chosen for the present study and 100 healthy *Koi carp* (1.5 g) were introduced in to each control tank and experimental tank. The fish in the control tank were fed only with supplementary fish feed (without probiotics) and the fish in the experimental tank were fed with supplementary fish feed blended with the isolated probiotic bacteria *Bacillus oleronius*. Feed was given twice a day regularly as per body weight of fish (3% body wt of fish per day). The experiment was carried out for 75 days and digestive enzyme such as amylase, GOT and GPT activities were analyzed on 0, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup> and 75<sup>th</sup> day of the experimental period.

### 2.3 Assay Of Enzyme Activities in Koi carp

The intestinal tissues were removed, thoroughly washed with chilled distilled water to remove blood and mesenteries, collected in ice-cooled petri dishes, weighed and cut into small pieces. 50% of homogenate was prepared with an ice-cooled 0.1M phosphate buffer (pH 7.0) and centrifuged in a refrigerated centrifuge at 2,500 rpm for 15 min. The supernatant was used for enzyme assay. Amylase activity was assayed by the Dinitrosalicylic acid (DNSA) method based on the estimation of reducing sugars at 540 nm using maltose as the standard [12]. GOT and GPT activities were determined by using colorimetric method suggested by [13]

## 2.4 Statistical Analysis

The results are presented as Mean + SD, differences were analyzed by One way analysis of variance (ANOVA) and statistical analysis was carried out by using SPSS software (16 versions).

## III.RESULTS AND DISCUSSION

*Bacillus oleronius*(JP1) *Bacillus subtilise* (JP2) and *Bacillus licheniformi* (JP3) were isolated from the Indian major carp *Labeo rohita* and among these three bacterial strains, *B.oleronius* (JP1) were identified as a probiotic based on its biochemical characteristics and 16s rRNA sequencing. *B. oleronius* was selected as a probiotic bacterial strain(TGTAACACCCGAAGTCGGTGAGGTAACCTTTGGAGCCAGCCGCCGAAGGTGGACCAGAT- Sequence ID :N R \_ 043325.1)(Plate;1).



Plate:1. Isolation of Bacterial colony from the gut of *L.rohita*

Probiotics are eco friendly, can be acquainted into the cultured condition to promote the growth and development of the cultured organism [14]. The strains of *Bacillus* sp. used as probiotics for terrestrial livestock have telluric origins. They are not autochthonous in the gastrointestinal tract but may be active at the time of intestinal transit [15].

Insignificantly increased digestive enzyme amylase activity ( $220.7 \pm 0.15$  (U/L) was recorded in the fish fed with isolated prbiotics over the control fish at the end of the experimental period ( Table 1 & Figure 1). Digestive enzymes could be increased by adding probiotics to the diets [16] . Studies shows that digestive enzyme activities are affected by amount of feed, chemical composition of feed, and the nutritional requirements of the fish. Elevated digestive enzyme activities in the present study may be due to the fact that higher ability of probiotics to secrete wide range of exo enzymes or enhance the activities of digestive enzymes paves the way for increased food absorption results in better growth performance in Koi carp [17]. Present results are supported by [18] who observed the increased digestive enzyme activities in *C.carpio* fed with *Lactobacillus* sp.

EXPERIMENTAL PERIOD(DAYS)	AMYLASE (U/L)	
	CONTROL	EXPERIMENT
0	47±0.09	47.2±0.09
15	48.2±0.09	56.1±0.15
30	50.3±0.09	58.29±0.01
45	70.2±0.1	150.2±0.1
60	110.2±0.1	170.4±0.09
75	199.4±0.15	220.7±0.15

Values are in Mean ± SD, n=3; Significant level at P < 0.05 (One way ANOVA)

Table. 1. Changes in the Amylase activity of *Koi carp* fed with isolated gut probiotics *B.oleronius*.

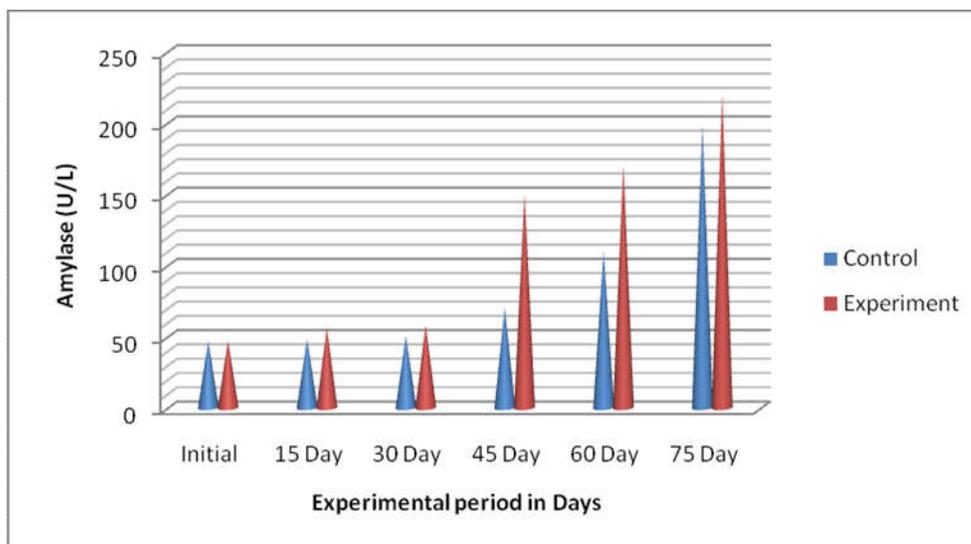


Fig. 1. Changes in the Amylase of the *Koi carp* fed with isolated gut probiotics *B.oleronius*

Table. 2 & Figure.2 depicts the activity of SGOT in the control fish and fish fed with isolated probiotic *B.oleronius*. The SGOT activity in the control fish were determined as 50 ± 0.1 U/L on 0 day, 51.6 ± 0.1 U/L on 15<sup>th</sup> day, 55.1 ± 0.1 U/L on 30<sup>th</sup> day, 69.8 ± 0.1 U/L on 45<sup>th</sup> day, 128 ± 1 U/L on 60<sup>th</sup> day and 144 ± 1 U/L on 75<sup>th</sup> day of the experimental period. Whereas, the SGOT activity in the probiotic fed fish were determined as 50.2 ± 0.1 U/L on 0 day, 50.6 ± 0.1 U/L on 15<sup>th</sup> day, 52.7 ± 0.09 U/L on 30<sup>th</sup> day, 70.8 ± 0.1U/L on 45<sup>th</sup> day, 74.1 ± 0.1 U/L on 60<sup>th</sup> day and 76.3 ± 0.15 U/L on 75<sup>th</sup> day of the experimental period. Significantly (P<0.05) decreased SGOT activity was recorded in the probiotic fed fish than the control fish.

EXPERIMENTAL PERIOD(DAYS)	SGOT (U/L)	
	CONTROL	EXPERIMENT
0	50 ±0.1	50.2±0.1
15	51.6±0.1	50.6±0.1
30	55.1±0.1	52.7±0.09
45	69.8±0.1	70.8±0.1
60	128±1	74.1±0.1
75	144±1	76.3±0.15

Values are in Mean ± SD, n=3; Significant level at P < 0.05 (One way ANOVA)

Table. 2. Changes in the Serum Glutamic-oxaloacetic transaminase activity of *Koi carp* fed with isolated probiotics *B.oleronius*.

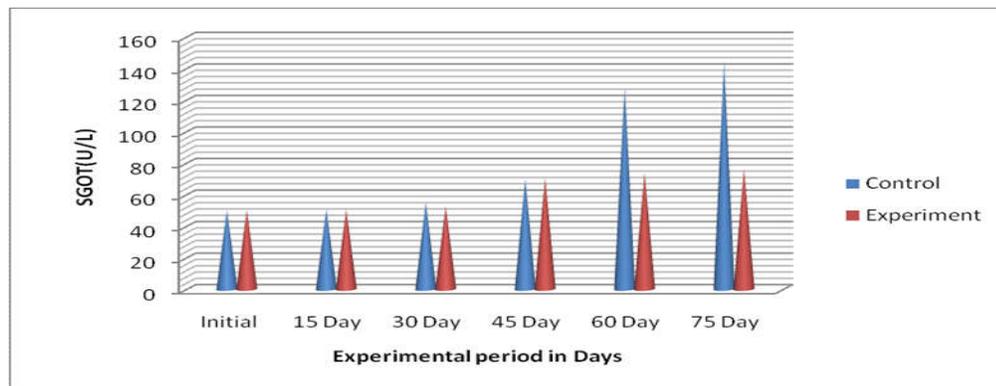


Fig. 2. Changes in the Serum Glutamic-oxaloacetic transaminase activity of the *Koi carp* fed with isolated probiotics *B.oleronius*.

EXPERIMENTAL PERIOD(DAYS)	SGPT(U/L)	
	CONTROL	EXPERIMENT
0	66±1	66.2±1
15	68±1	68±1
30	62.3±0.09	70.1±0.01
45	96.3±0.2	80.1±0.1
60	158.9±0.15	91.2±0.1
75	168.03±0.02	98.2±0.1

Values are in Mean ± SD, n=3; Significant level at P < 0.05 (One way ANOVA)

Table. 3.Changes in the Serum glutamate-pyruvate transaminase (GPT) of *Koi carp* fed with isolated probiotics *B.oleronius*.

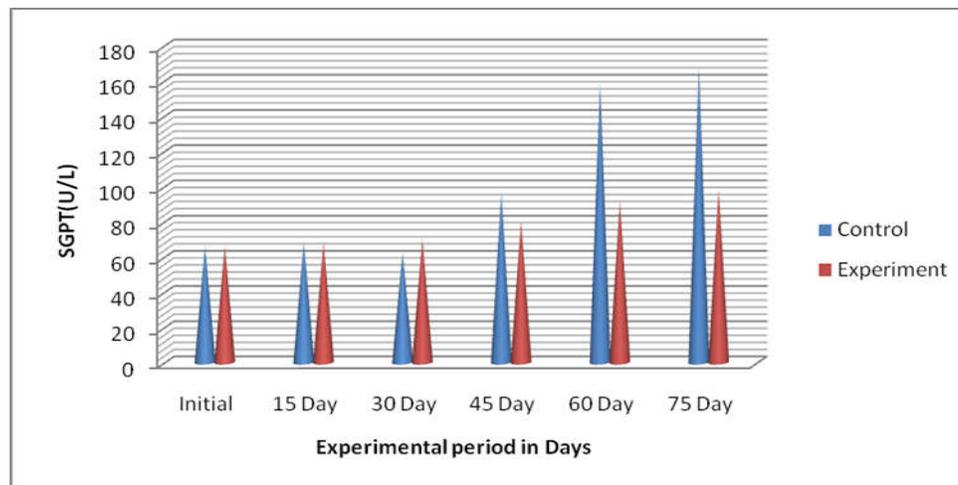


Fig. 3. Changes in the Serum glutamate-pyruvate transaminase (GPT) of the Koi carp fed with isolated gut probiotics *B.oleronius*.

The SGPT activity in the control *Koi carp* was determined as  $66 \pm 1$  U/L on 0 day,  $62.3 \pm 0.09$  U/L on 15<sup>th</sup> day,  $62.3 \pm 0.09$  U/L on 30<sup>th</sup> day,  $96.3 \pm 0.2$  U/L on 45<sup>th</sup> day,  $108.9 \pm 0.15$  U/L on 60<sup>th</sup> day and  $128.03 \pm 0.02$  U/L on 75<sup>th</sup> day of the experimental period. Whereas, the SGPT activity in the probiotic fed fish were determined as  $66.2 \pm 1$  U/L on 0 day,  $68 \pm 1$  U/L on 15<sup>th</sup> day,  $70.1 \pm 0.01$  U/L on 30<sup>th</sup> day,  $80.1 \pm 0.1$  U/L on 45<sup>th</sup> day,  $91.2 \pm 0.1$  U/L on 60<sup>th</sup> day and  $98.2 \pm 0.1$  U/L on 75<sup>th</sup> day of the experimental period. Significantly ( $P < 0.05$ ) decreased SGPT activity was recorded in the probiotic fed fish than the control fish (Table.3 & Figure.3). This decrease was more prominent in the control fish over the probiotic fed fish. GPT (ALT) and GOT (AST) enzymes are important liver enzymes and it is a valuable tool in diagnosis of liver damages and diseases. When the tissues gets damaged, extra GOT and GPT enzymes are released into the blood stream causing the increased enzymes level [19]. They are indicators for liver health and function. [20] noted the elevation of liver transaminases of *O.niloticus* intoxicated with AFB1. Similarly, the decreased metabolic enzyme activities was recorded in the probiotic fed *Koi carp* may be due to the fact that isolated probiotics might protect the liver cells and maintains the health status of liver without any damages. Normal size of hepatocytes without inflammation was recorded in the histology of liver in the present study.

## CONCLUSION

From the present study, it is concluded that isolated bacteria *B.oleronius* served as potent probiotic and enhanced the digestive amylase activity and thus improved better digestion, absorption and decreased SGOT, SGPT proves good condition of liver cells in *Koi carp*.

## ACKNOWLEDGMENT

The author is very grateful to my Research Supervisor Dr.K.Parvathi, Assistant Professor of Zoology, Erode Arts and Science College, Erode for her support to complete our Ph.D research work on time.

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