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Influences of Isolated probiotic Bacterial strain *Bacillus oleronius* on Physico Chemical parameters of *Koi carp* rearing water

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Abstract: **The present investigation aims to findout the influences of isolated gut probiotic bacterial strain *Bacillus oleronius* on Physico Chemical parameters of Koi carp rearing water. 100 Koi carp was introduced into control tank and Experimental tank. Fish in the control tank were fed only with supplementary fish feed without probiotics and the fish in the experimental tank were fed with isolated probiotic bacterial strain *Bacillus oleronius* mixed with supplementary fish feed. The experiment was carried out for 75 days and the water was analyzed for its physico chemical parameters. Results of the present study reveal that the probiotics bacteria improved the water quality in fish rearing tank.**

Keywords: **Isolated gut probiotic, *Bacillus oleronius*, fish feed, physico chemical parameters, Koi carp.**

1. INTRODUCTION

Aquaculture is an important food producing sector for ever increasing human population [1] Probiotic feed provides protein rich substances. [2,3] led many investigations to assess the nutritional value. Probiotics is a live microbial feed supplement which affects the host organisms by improving its intestinal microbial balance"[4]. In aquaculture field, probiotics utilized either as a nourishment partner or as an add on ingredient to water [5]. Genus *Bacillus* is gaining momentum because of their ability to resist and survive under harsh conditions. *Bacillus* is a heterogeneous group of Gram-positive, facultative anaerobic, endospore-forming bacteria. They are characterized by their rod-shaped morphology, catalase production and their ubiquitous distribution, even though soil is generally accepted as its natural reservoir. The ability to produce endospores allows *Bacillus* to withstand extreme environmental conditions.

Nowadays, the use of probiotics in fish culture is widely accepted with an increasing demand for environment friendly fish culture [6-9]. Application of probiotics in fish culture is a technology and research on probiotics as bioremediation and biocontrol agents in increasing with the demand for eco- friendly fish culture [10-13].The ornamental fish culture is economically important and much profitable area of fish culture. The ornamental fish industry has induced the indiscriminate use of antibiotics and chemotherapeutants for improving the health and nutrition, which has led to the development of drug-resistant strains of pathogenic microbes [14]. Koi carp is a high-value ornamental fish species. The benefits of probiotics on koi carp health, growth, and intestinal bacteria are well documented [15-17] but there are few reports investigating the variation intestinal microbiota community of koi carp treated with *B. oleronius.* Hence, present study aims to find out the influences of isolated gut probiotic bacterial strain *B. oleronius* on water quality of Koi carp rearing water.

II. MATERIALS AND METHODS

A. Isolation of Probiotic Strain

Koi carp is the experimental fish selected for the present study. Koi is an colored variants of *C.carpio*.it needs good water quality to survive. Koi carp fingerlings with the average weight of **1.6 gm and 2.5cm** length were purchased from Sirago fish farm, Nerinjipettai, Mettur Dam, Tamil Nadu. Indian major carp *Labeo rohita* (21g) were purchased from Sirago fish

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farm located at Nerinjipett, Erode district, Tamil Nadu, India. The fish body surface was disinfected with alcohol (70%), dissected out and cut the intestine into small pieces (1 g) washed with normal saline (NaCl 0.85 % w/v) and homogenized [18]. Using serial dilution (up to 10^{-6} CFU/ml, NS), 0.1 ml of homogenized intestine samples were spread on Nutrient agar medium followed by 24 h incubation at 37°C to count total colony of bacteria[19]. Single colony were isolated and purified on another Nutrient agar medium. Identification was carried out based on biochemical tests and 16s rRNA profile. The purified isolates were tested for gram staining, arabinose, catalase activity, oxidase activity, methyl red test, voges proskauer test, indole production, citrate utilization, glucose test, lactose test, mannitol, maltose reduction, nylose test, salicin production, skim milk production and carbohydrate fermentation and also morphology using phase contrast microscope [20,21]. The selected probiotic strain is *Bacillus oleronius* Gram positive, aerobic bacteria.

B. Feed coating and feeding

Selected strain of probiotics *Bacillus oleronius* was mass cultured and the concentration of colony forming units were determined. Feed pellets were warmed to 60°C and blended with the molten agar containing fresh *B. oleronius* bacterial cells. The mixture was stirred well with sterile glass rods to have a uniform coating of the bacteria over the feed pellets. Food pellets was produced for *B. oleronius* live cells (3.0×10^8 CFU g^{-1}) at 3 % w/w of fish. The selected supplementary fish feed Hipro (Aptimum Company, Thailand. Supplementary feed) contains crude protein 38%, crude fat 4 %, crude fibre 3%, moisture content 12% and crude ash content 12 %. The proximate composition of organic feed ingredients was observed by the method [22].

C. Experimental Design

The experiment was conducted in two rectangular cement tanks (4.5 ft L, 3 ft W, 2 ft Depth) (Control tank and Experimental tank) situated in Salem district (12.33°N 78.36°E) Tamil Nadu, India. The study was carried out for 75 days with the fingerlings of *koi carp* with average size of **1.6 g** were stocked in the rate of 100/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 mixed with isoloated gut probiotic bacteria *Bacillus oleronius.* Feed was given twice a day early morning (7.00 am-8 a.m) as per body weight of fish (3% body wt of fish per day) and evening (5.00 pm- 6 p.m) regularly. The experiment was carried out for 75 days and the samples were taken from control group and experimental group on 15^{th}, 30^{th}, $45^{th,}$ 60^{th} and 75^{th} day of the experimental period for further analysis by using standard methods**.** The physico chemical parameters such as such as pH, temperature,total alkalinity, dissolved oxygen and ammonia were estimated by standard methods [23]. 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

The experiment was carried out for the period of seventy five days and physico chemical parameters of Koi carp rearing water was analyzed and results were presented in the tables and figures. The water pH was ranged from 7.8 ± 0.009 to 7.6± 0.01 in the control tank and ranged from 8.0±0.1 to 8.1±0.009 in the experimental tank water (Table 1 and Figure 1). Gradually decreased pH was recorded in the control tank water whereas the pH was maintained with slight fluctuations in the experimental tank water.

TABLE 1: Influences of isolated probiotics *B. oleronius* on water pH of Koi carp rearing water.

Experimental period(Days)	p^H	
	Control	Experiment
15	7.8±0.009	8.0±0.1
30	7.8±0.1	8.1±0.45
45	7.7±0.009	8.1±0.009
60	7.6±0.009	8.1±0.45
75	7.6±0.01	8.1±0.009

Significant level at $P < 0.05$ (one way ANOVA)

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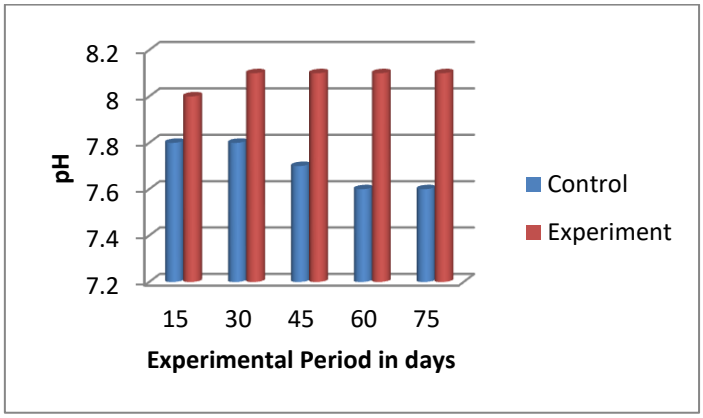

Fig 1: Influences of isolated probiotics *B. oleronius* on water pH.

The water temperature was ranged from 28±1 ^{o}C to 31.2±0.1^{o}C in the control tank and ranged from 28±1 ^{o}C to 34.3±0.15 ^{o}C in the experimental tank water (Table 2 and Figure 2). Significantly ($P < 0.05$) increased temperature was recorded in the experimental tank water (probiotic fed water).

TABLE 2: Influences of isolated probiotics *B. oleronius* on water temperature of Koi carp rearing water.

Experimental period(Days)	Temperature(^{0}C)	
	Control	Experiment
15	28±1	28±1
30	29±1	28.3±0.1
45	27.8±0.1	27±1
60	30.7±0.1	30.4±0.1
75	31.2±0.1	34.3±0.15

Significant level at $P < 0.05$ (one way ANOVA)

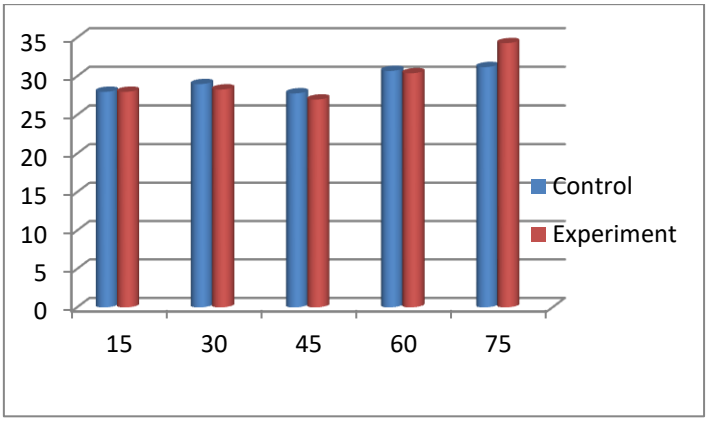

Fig 2: Influences of isolated probiotics *B. oleronius* on water temperature.

The total alkalinity was observed from 330 ± 1 to 310 ± 1 mg/l in the control water. The total alkalinity was recorded from 350.00 ± 0.15 mg/l to 350.0±1 mg/l in the experimental water. Significantly ($P < 0.05$) increased total alkalinity was found in experimental period. (Table 3 and Figure 3).

TABLE 3: Influences of isolated probiotics *B. oleronius* on Total Alkalinity of Koi carp rearing water.

Experimental period(Days)	Total alkalinity (mg/l)	
	Control	Experiment
15	330±1	350.00±0.15
30	332±1	360.00±1.17

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45	330±1	375.10±0.01
60	320±1	380±1
75	310±1	350.0±1

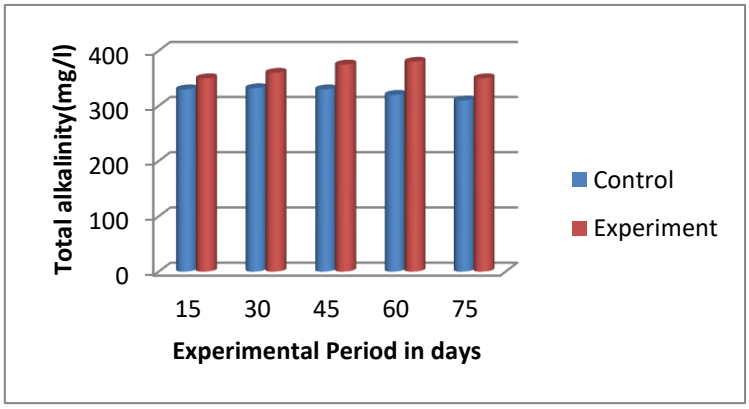

Fig 3: Influences of isolated probiotics *B. oleronius* on Total Alkalinity.

Dissolved oxygen level was ranged between 0.43±0.01 mg/l to 0.78±0.01 mg/l in the control tank water and the Do level was ranged between 1.20±0.01 mg/l to 1.64±0.01 mg/l in the experimental tank water. Significantly ($P < 0.05$) increased dissolved oxygen level was calculated in the experimental tank water. (Table 4 and Figure 4).

TABLE 4: Influences of isolated probiotics *B. oleronius* on Dissolved oxygen level in the Koi carp rearing water.

Experimental period(Days)	Dissolved oxygen(mg/l)	
	Control	Experiment
15	0.43±0.01	1.20±0.01
30	0.32±0.01	1.28±0.01
45	0.41±0.01	1.36±0.01
60	0.51±0.01	1.40±0.01
75	0.78±0.01	1.64±0.01

Significant level at $P < 0.05$ (one way ANOVA)

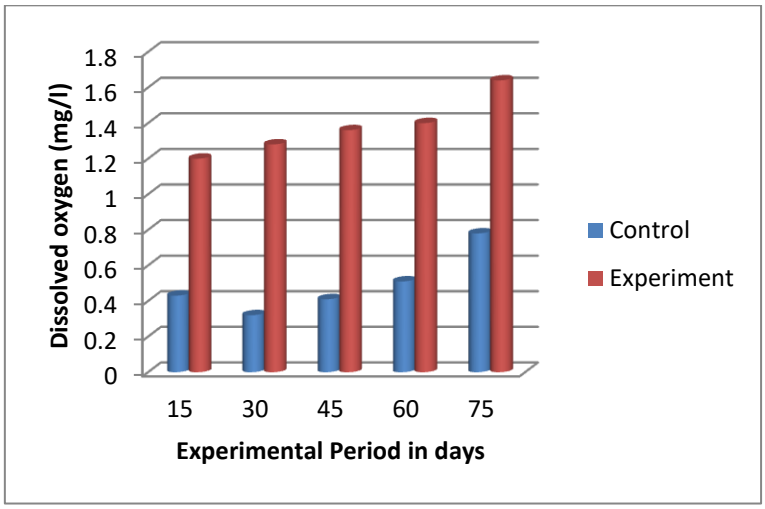

Fig 4: Influences of isolated probiotics *B. oleronius* on Dissolved oxygen level.

Ammonia level was ranged from 0.31±0.01 mg/l to 0.32±0.01 mg/l in the control tank water and the ammonia level in the experimental tank water was gradually decreased from 0.30±0.01mg/l to 0.27±0.01 mg/l on 60th day and gradually increased to 0.30±0.01 mg/l at the end of the experimental period (Table 5 and Figure 5).

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TABLE 5: Influences of isolated probiotics *B. oleronius* on Ammonia level in the Koi carp rearing water.

Experimental period(Days)	Ammonia (mg/l)	
	Control	Experiment
15	0.31±0.01	0.30±0.01
30	0.33±0.01	0.30±0.01
45	0.32±0.01	0.31±0.01
60	0.31±0.01	0.27±0.01
75	0.32±0.01	0.30±0.01

Significant level at $P < 0.05$

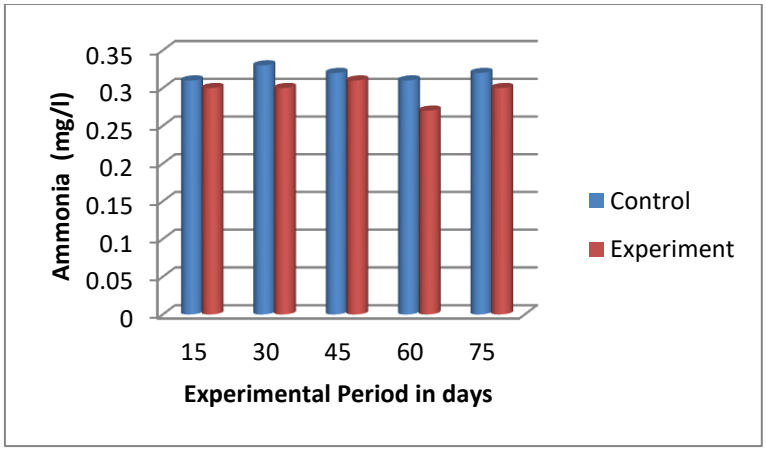

Fig 5: Influences of isolated probiotics *B. oleronius* on ammonia level.

IV. DISCUSSION

Probiotic bacteria known to improve the water quality. A rise in temperature of water accelerates chemical reactions, reduces solubility of gases and elevates metabolic activity of organisms. Application of probiotics in fish culture is a method for controlling illness, upgrading immune response, giving nutritional and enzymatic contributions to the digestion of the host, and enhancing water quality [24] . Heterotrophic bacteria necessitating various organic materials in addition with inorganic materials for their growth which has significant role in the decomposition of organic materials and production of food particulates from the dissolved materials [25]. Intense photosynthetic activity of green algae, the free carbon di oxide or bicarbonates drawn out by algae which may results in decreased free Co2 and increased carbonates [26]. Increased dissolved oxygen content in the *Bacillus pumilus* treated water may be due to the photosynthetic activity of green plants and algae. [27] reported that the total hardness is usually related to the total alkalinity as the cations of hardness and anions of alkalinity are derived from the carbonate mineral solution. [28] noted high positive correlation exists between total alkalinity and total hardness of the pond waters. Critical water quality parameters must be maintained at optimum level in fish ponds and tanks because in fish ponds mineralization of chemical fertilizers , excess feed and waste often increases the ammonia level in the fish ponds which is very harmful to fish above 0,1mg/l. Heterotropic bacteria in the aquatic medium are known to utilize nitrogen rich materials and release more ammonia and its salts [29].Consumption of organic materials as their sources of carbon, converts the ammonia into nitrites and to nitrates and hence low level of ammonia were recorded in the probiotic bacteria treated water. By using probiotic bacteria improves the water quality and prevents the pathogenic bacteria in fish ponds and tanks.

V. CONCLUSION

From the present study it is concluded that the probiotic bacteria improves the water quality by increasing dissolved oxygen content by enhanced photosynthetic activity of algae and conversion of ammonia as a source of nutrition by bacteria.

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