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Research Article

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Effect of isolated Probiotic bacteria on the histological changes of *Koi carp*.

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Keywords

Ornamental fish, probiotics, histology

Abstract

The ornamental fish culture is an economically important and profitable area of fish culture. Ornamental fish trade provides excellent opportunities as a non-food fishery activity for employment and income generation. Ornamental fish are peaceful, tiny with attractive colours and capable of living in confined spaces. Koi carp is high value of ornamental fish. The eco-friendly microbial feed supplements are used to improve the growth, immune responses of aquatic organism and play an important role in recycling nutrients, degrading organic matter and protecting fish against infectious diseases. The present study aims to find out the effect of isolated probiotic bacteria on the histological changes of K*oi carp.*

Introduction

Ornamental fish are often called as "living Jewell" due to their different colours, size, behaviour and its origin. They are peaceful, generally tiny and available in attractive colours and capable of living in confined spaces. There is a commercial growing interest in the ornamental fish trade in Asia and all over the world. Recently, several useful works have appeared, facilitating budding ornamental fish hobblers to maintain marine aquarium tank on scientific lines (Lamberton, 1996). The prosperity of the ornamental fish industry has induced the indiscriminate use of antibiotics and chemotherapeutants for improved health and nutrition, which leds to the development of drug-resistant strains of pathogenic microorganisms (Amabile - Cuevas *et al.*, 1995) . The occurrence of antimicrobial residues in the products of aquaculture is a main threat to human health (WHO, 2006).

Thus commercial application of probiotics in fish culture is not well developed compare to the culture of shrimp (Moriarty *et al*., 2005). The range of probiotics used in fish culture is quite broad and has encompassed both Gram-positive and Gram-negative bacteria as well as yeasts and

unicellular algae (Irianto and Austin 2002). The benefits of probiotics are also quite broad and inhibit the pathogenic microbes, improve the growth performance and increase the immune responses (Yanbo and Zirong, 2006). Lactic acid bacteria are also abundantly present in the intestine of healthy fish and therefore are commonly used probiotics in aquaculture(Gatesoupe 2008). Isolation and probiotic characterization of arsenic-resistant lactic acid bacteria for uptaking arsenic was observed by Bhakta *et al*., (2010). Harikrishnan *et al*., (2011a) isolated the probiotics and herbal mixtures enhance the nonspecific immune response in *Paralichthys olivaceus* against *Streptococcus parauberis*.

Histological methods remain the primary tools for the evaluation of pathological changes in tissues and are getting considerable attention while conducting sub-lethal exposure of different toxicant in aquatic organisms. The histological analysis of cells and tissues provide essential information on the pathological changes occurring in a variety of organelles, which can be related to both biochemical changes at cellular level and to tissue pathology. Light and electron microscopy demonstrated that pathogen-induced damage to the Atlantic salmon foregut could not be prevented or reversed, but could be marginally reduced in some cases. Dietary applications of *P. acidilactici* could significantly improve the length of microvilli in rainbow trout proximal intestine as compared to the control group (Merrifield *et al*., 2011).

Materials and Methods

Collection of Experimental Fish

The fish selected for the present study was Koi carp (*Cyprinus carpio).* Koi carp fingerlings with the average weight of 1.4 gm weight were purchased from Sirago fish farm, Nerinjipettai, Mettur Dam, Tamil Nadu, India and transported in double polyethylene bags filled with oxygenated water to an experimental tank which is located in Salem District and acclimatized for a month in separate nursery tank and fed with supplementary feed *ad libitum*.

Isolation and Identification And Selection Of Probiotics

Labeo rohita (20g) is Indian major carp were collected from Sirago fish farm located at Nerinjipett, Erode district, Tamil Nadu, India and brought alive to the laboratory. The surface of fish bodies were disinfected with 70% alcohol dissected the intestines and washed three times with normal saline (NaCl 0.85 % w/v). The intestines were then cut in small pieces (1 g) and homogenized (Rengpipat *et al*., 2008). 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 (Paludan-Muller *et al*., 1999). Single colony were isolated and purified on another Nutrient agar medium.

Identification was carried out based on biochemical tests (Holt *et al*., 1994) and 16s rRNA sequencing. Potential strains were identified up to species level based on Bergey's manual of systematic bacteriology (Whitman *et al*., 2009) 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 (Nguyen *et al*., 2007; Kopermsub and Yunchalard, 2010). The probiotic strain selected for the present study was *Bacillus oleronius.*

Supplementary Feed and Feed Coating

The supplementary fish feed Hipro was purchased from Aptimum company, Thailand. Supplementary fish 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 of

AQAC, (1995). Selected probiotic bacterial strain *Bacillus oleronius* was mass cultured and the concentration of colony forming units were determined by adjusting the culture to OD 1. Feed pellets were warmed to 60°C and blended with the molten agar containing fresh bacterial cells of *Bacillus oleronius*. 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 (2.9×10^8 CFU g^{-1})

Experimental Design

Experiment were carried out in two rectangular cement tanks (4.5 ft L, 3 ft W, 2 ft Depth) which is in situated in Salem district (12.33°N 78.36°E) Tamil Nadu, India. Each tank was provided with well organized inflow and outflow system to maintain the required water level. The healthy fingerlings of Koi carp was purchased from Sirago fish farm, Nerinjipet, Erode district, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fingerlings of *Koi carp* were weighed accurately by using digital electronic balance before starting up to the experiment. 100 animals were introduced into each control tank and experimental tank. The fish in the control tank were fed only with supplementary fish feed without probiotics and the fingerlings in the experimental tank were fed with supplementary fish feed mixed with isolated gut probiotic bacteria *Bacillus oleronius.* Feed was given twice a day early morning (7.00 am-8 am) and evening (5.00 pm- 6 pm) regularly as per body weight of fish (3% body wt of fish per day). The experiment was carried out for 75 days and the samples were taken from control group and experimental group on 0 and 75th day of the experimental period for further histological analysis**.**

Results and Discussion

Histological observations are an important tools in finding out the changes in the cellular levels that may occur in target organs (Dutta, 1996). Digestion and immunity are complicated physiological processes that have coevolved (Van Loo, 2007). Hence, the histological changes in the intestine and liver of *C.carpio var.koi* was observed on initial day and 75th day of the experimental period and results were presented in Plates.

Plate 1.i-ii.Section shows the Intestine of *C. carpio var.koi*

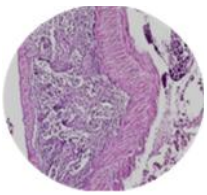

Plate 1.i.Intestine of Control fish

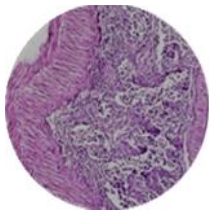

Plate 1.ii.Intestine of Experimental fish (75th day)

Plate. 2. Section shows the liver of *C. carpio var.koi*

Plate.2 i. Liver of Control fish

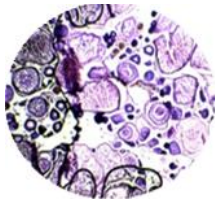

Plate 2. ii. Liver of Experimental fish (75th day)

Normal number of goblet cells and epithelial layer of villi were noticed in the intestine of control fish. Increased epithelial layer of the villi and more goblet cells were noticed in the intestine of probiotic fed fish than the intestine of control fish (1.i & ii). . No evidences of abnormalities noticed in the intestine of both the control and experimental fish. Increased epithelial villous gap is to provide spaces for food particles to be absorbed by the villi. Epithelial layer in intestine is a single layer form the type of absorptive columnar cells which plays an important role in protecting the fish against the harmful agents in lumen. Goblet cells reside throughout the length of intestines which is responsible for the production and maintenance of the protective mucus blankets by secreting high molecular weight glycoprotein's called mucins. In the present investigation, increased epithelial layer of the villi and more goblet cells were noticed in the intestine of probiotic bacteria fed Koi carp fish than the intestine of control fish. No evidences of any abnormalities were noticed in the intestine of the control and experimental fish. Increased length of epithelial villi provides more spaces for food particles to be absorbed by the villi. Goblet cells reside throughout the length of intestines is responsible for the production of the protective mucus blankets in the gut against infections. Similarly, improved intestinal histological changes without any damages in tilapia fed supplemented diet with probiotic and enzymes (Ayodeji *et al*., 2016). Similar to the present findings, Reda & Selim (2015) also recorded more villi and goblet cell number in fish fed supplemented with *B. amyloliquefaciens* in Nile tilapia.

Cytoplasmic alterations in fish hepatocytes are a very early and unspecific signal of disturbance of hepatocellular homeostasis being difficult to establish a threshold for what should be considered a fish farm healthy liver (Braunbeck, 1998). Hypertrophy, vacuolar degeneration and increase of lipid droplets in hepatocytes of fish are some of parameters normally analyzed to predict liver health and integrity. In farmed fish it is known that commercial feed causes accumulation of lipid droplet, hepatic cell membrane degeneration, and hepatocyte vacuolization and can cause circulatory disturbances (Bilen and Bilen, 2013). In the present research, more number of normal hepatic cells with prominent nuclei is noticed in the experimental fish. No abnormalities like necrosis, structural damage of liver and liver cells in the liver of experimental fish (2.i &ii). No damages in the liver cells were recorded in the presence study is confirmed with decreased level of GST GPT and AP enzymes. From the study, it is concluded that, more number of normal hepatic cells with prominent nuclei are noticed in the experimental fish. No abnormalities like necrosis, structural damage in the liver cells were observed in the liver of experimental fish.

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