International Journal of Pure and Applied Zoology
Volume 3, Issue 2, pp: 148-153, 2015
http://www.ijpaz.com

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
Rishan Publications

Research Article

CHARACTERIZATION AND EVALUATION OF PROBIOTIC FISH FEED

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Article History: Received 1st October 2014; Accepted 21st April 2015; Published 12th May 2015

ABSTRACT

Probiotic bacteria confer beneficial effect to the host animal. Probiotic diets are being incorporated together with prebiotics in recent years. Probiotics with synbiotics elicit synergistic and more favorable actions. Administration of probiotic feed like *Bacillus subtilis, Lactococcus lactis, Saccharomyces cerevisiae* in the diets kept the histo-architectural structure intact and promoted regeneration in the intestine of fish to increase the functionality of fish. This increased advantage of formulated probiotic and its benefits in maintaining good water quality and rapid increase in growth of *L. rohita* were evaluated in this study. Feeds with commericial probiotic feed obtained were evaluated with the isolated probiotic feed. The adherence of isolated probiotic feed in the intestine of *Labeo rohita*, alter the enzymes, microbial metabolism and improve the weight gain and survival rate.

Keywords: Probiotic, *Labeo rohita*, Fish feed, Aquaculture, Water quality.

INTRODUCTION

Probiotics are traditionally defined as "a live microbial feed supplement which beneficially affects the host animals by improving microbial balance". This definition of Probiotics has been modified through the years particularly on its applicability in aquaculture. Lazaro simplified the definition of probiotics which states, "live or dead, or even a component of the bacteria that act under different modes of action in conferring beneficial effects to the host or to its environment". Probiotics, the beneficial microbial population, are considered to enhance the immunity of fish under stressful environmental conditions, by modulating the gut colonization of the probiotic bacterial strains, and production of antibodies, acid phosphatase, lysozyme and anti-microbial peptides (Mohapathra *et al.*, 2012; Panigrahi, 2007; Taoka *et al.*, 2006; Salminen *et al.*, 1999).

The research on the live cell preparations in aquatic organisms is being increased to sustain the aquaculture industry. The *Lactobacillus* spp., *Bacillus* spp., *Saccharomyces cerevisiae*, and *Lactococcus* spp. are the commonly used probiotics in carps. The quality and cost effectiveness of commercial feeds are a primary concern for the feed manufacturer. In order to produce a quality feed one has to understand variations in ingredient cost, quality and availability. Additionally, the effects of the ingredient on processing, nutrient content of the ingredient and the response of the animal must be understood to make a high quality diet that consistently meets the nutrient requirements of the culture species. Such diets are typically a mixture of a few primary ingredients that produce the desired results. To reduce culture costs, considerable work has been put towards reducing the cost of fish feed formulations by replacing relatively expensive ingredients with more economical alternatives. Traditionally, the development of commercial aquatic feeds has been dependent on fish meal (FM) as the main protein source because of its balanced essential amino acid (EAA) profile, high protein content,

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and excellent source of essential fatty acids (EFAs), digestible energy, vitamins and minerals. However, because of high cost and limited availability, feed formulations have shifted to increasing levels of alternative protein sources using limited amounts of fish meal or animal proteins. The use of probiotics addresses the concerns on administration strategies, multifaceted benefits and bacterial viability. To keep up with the rapid growth of aquafarming, which grows annually at 11% increase the price of feed. Ingredients together with their limited supply play a tremendous role to meet the demand. While remaining cost effective and competitive, the formulation of alternative feed ingredients generally meets the nutritional requirements of the target species. Recent studies (Lazado *et al.,* 2014) show that incorporation of probiotics stimulates the hemopoesis and also induces the non specific immunity in fish (Marzouk *et al.,* 2008).

Fish which are poikilothermic vertebrates inhabit aquatic ecosystem and are most susceptible to seasonal and diurnal variations in water temperature. Temperature regulated the growth and other physiological and biochemical functions of fish (Swain *et al.,* 2007; Smith, 1989). Alarming rise in temperature caused by global warming is thought to negatively affect the wild capture fisheries as well as the world aquaculture production (Ficke *et al.,* 2007). Among alternative feed, probiotic feed provide protein source with high digestible protein and energy contents and good amino acid profile. Several studies have been conducted to evaluate the nutritional value (Nayak, 2010; Gomes *et al.,* 2009). Present study was undertaken to evaluate the fish growing water and the benefits of improving the water quality in which fish grows.

MATERIALS AND METHODS

Preparation of probiotic

Isolation of the organism: The *L. rohita* fishes were dissected to remove the intestine, after washing the ventral surface area of the fish with sterile distilled water. The entire intestine was homogenized in 9 ml of PO_4 buffer saline and mixed with a vortex mixer by 10:1 dilution. The sample was serially diluted and plated on the nutrient agar plates, by spread plate technique. The plates were incubated at 37^{o}C for 24 hours.

Identifications of the organisms

The isolated probiotic bacterium was identified by following morphological and biochemical characterization methods.

Morphological characterization

Studies on Nutrient Agar: Morphological and cultural characteristics such as abundance of growth pigmentation, optical characteristics, size, form, margin and elevation were studied on nutrient agar plates.

Simple Staining: For simple staining, the bacterial smears were treated with crystal violet (60 seconds) and rinsed with distilled water. Then smears were air dried and observed under microscope.

Gram Staining: A thin smear of the isolate was made on a clean glass slide and heat fixed. Then the smear was stained with crystal violet for 1 minute and then washed with water, gram's iodine was added for 1 minute and decolorized with alcohol. After decolourization the smear was counter stained with saffranin for 1 minute. Finally the smear was washed with water and air-dried. Then the slide was observed under the microscope.

Biochemical Characteristics: The following biochemical tests were carried out according to the method described by Cappuccino and Sherman (1996).

Catalase Test: A clean glass slide was taken and a drop of culture suspension was placed on the glide. To the culture few drops of hydrogen peroxide was added. A positive reaction indicates the release of air bubbles from the suspension.

Starch Hydrolysis Test: Starch agar medium was prepared and transferred aseptically into sterile petridish. The isolated colonies were streaked on starch agar plates and incubated at 37^{o}C for 48 hours. The plates were flooded with Gram's Iodine. Amylase production was indicated by colorless zone surrounded by bacteria and rest of the plate appeared purple.

Casein Hydrolysis: Skim milk agar medium was prepared and transferred aseptically into sterile Petri plates. The medium was allowed to set and the isolated colonies were streaked on skim milk agar plate and incubated at 37^{o}C for 48 hours. The opaque zones surrounding the microbial growth consist of casein milk powder, indicating protease activity.

Lipid Hydrolysis: Spirit blue agar medium with lipid was prepared and transferred aseptically into sterile Petriplates. The isolated colonies were streaked on the plate, and incubated at 37^{o}C for 48 hours. The zone surrounding the microbial growth indicates the lipolytic activity.

Indole Production Test: One percentage peptone broth was prepared, sterilized and incubated with the isolated colonies and incubated at 37 ^{o}C for 48 hours. After incubation 1 ml of Kovac's regent was added and gently shaken. The results were observed after allowing the tubes to stand. A cherry red ring indicates the positive reaction.

Methyl Red Test: MR-VP broth was prepared, sterilized and incubated with the isolates, 5 drops of methyl red indicator was added and the tubes were observed for a color to red that indicates a positive reaction.

Voges

Nitrate Reduction Test: Nitrate broth was prepared, sterilized and **Proskauer Test:** MR-VP broth was prepared sterilized and incubated with the isolated, incubated at 37^{o}C for 48 hours. After, incubation few drops of Baritt's regent B and A were added and the results noted. Development of crimson to pink color indicates a positive reaction.incubated with the isolates, incubated at 37^{o}C for 24-48 hours. Presence of nitrate was tested by adding a few drops of sulfanilic acid and napthalamine regent to each of the tubes. Results were observed without shaking the tubes. A distinct red colour that may turn brown indicates reduction of nitrate.

Citrate Utilization Test: Simmon's citrate agar medium was prepared, sterilized and transferred aseptically to the test tubes and slant was prepared. The isolated colonies were streaked on the surface of the slant and incubated at 37^{o}C for 24 hours. A change in green colour to Prussian blue indicates the positive results.

Probiotics like *Bacillus subtilis, Lactococcus lactis* and *Saccharomyces cerevisiae* were identified. The Luria broth was prepared and supplemented with various nutrient supplement such as Vitamin B, Methionine, Cystine and serine, spirullina, oilcake and wheat bran. The isolated colonies *Bacillus subtilis, Lactococcus lactis, Saccharomyces cerevisiae* were incubated in the respective medium and incubated at 37^{o}C for 48 hours.

Determination of the growth

The minimal broth was prepared in the fish growing water and supplemented with various nutrient supplements such as oil cake, Black gram, Green gram and wheat bran (Sample A: Fish feed with Commericial probiotic feed, Sample B: Fish feed with Formulated feed, Sample C: Fish feed (Control). The length and weight of the control and experimental fishes were measured using scale and weighing machine.

Histological assessment of intestine

To determine the effect of probiotics diets on intestine, *L. rohita* fry in each treatment were sampled for histological sections at the end of experimental period. Fish samples were stored in formalin solution (4%) and then eviscerated to remove their digestive tract. Paraffined blocks of fish intestine were prepared and then sliced by microtom to give sections of 4 to 5 µ. Sections were stained by the coloration methods of hematoxilin and eosin and then studied under microscope (Mumford, 2004).

RESULTS AND DISCUSSION

Determination of length and weight of fish: Control and experimental fishes were exposed to various nutrient supplements such as oil cake, Black gram, Green gram and wheat bran along with supplemented probiotics (*Bacillus subtilis, Lactococcus lactis, Saccharomyces cerevisiae*). Increased length and weight were observed at the end of 10, 20 and 30 days from various sampling groups than control (Table 1) as reported by Safoura Sedaghat, *et al.* (2014) and Madhavi Rane and Aishwarya Markad (2015).

Histology of intestine: In the present study, no difference among various treatments was observed in histological assessment of intestine (Figure 1-3). Similar findings reported by Pooramini *et al.* (2014).

Probiotic feed obtained commercially were feeded with normal fish feed and compared with the formulated isolated feed. The isolated probiotic, strains are more efficient in converting organic matter, large polymer into smaller units and adhere to the intestine of *L. rohita.*

Water quality: As a result of probiotic activity, water quality improved and reduced the organic matter load. Nitrifying bacteria also reduced, which lead to good water quality (Table 2). The probiotic bacteria adhesion increased and altered certain enzymes, microbial metabolisms which are beneficial.

Table 1. Length and weight of fishes exposed to various nutrient supplement groups.

Feed	Parameter	0th Day	10th Day	20th Day	30th Day
A	Length (cm)	11.3±0.4	12.1± 0.2	12.6 ± 0.2	13.1 ± 0.2
	Weight (gm)	23.1±0.2	24.9± 0.4	25.5 ± 0.4	27.3 ± 0.6
B	Length (cm)	09.6±0.5	10.8± 0.3	11.7 ± 0.2	12.8 ± 0.4
	Weight (gm)	18.9±0.2	19.7± 0.3	21.2 ± 0.5	23.6 ± 0.4
C	Length (cm)	10.8±0.6	11.2± 0.2	11.4 ± 0.1	11.5 ± 0.4
	Weight (gm)	17.6±0.3	17.7 ±0.4	17.9 ± 0.8	18.14±0.51

A. Fish feed with Commericial probiotic feed.
B. Fish feed with Formulated feed.
C. Fish feed (Control).

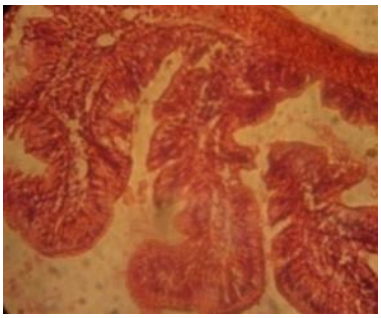

Figure 1. Histology of intestine microvilli in control fish, *Labeo rohita*.

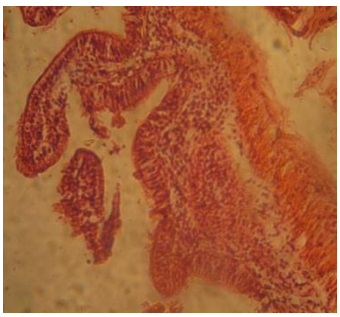

Figure 2. Histology of intestine microvilli of commercial probiotic fed fish.

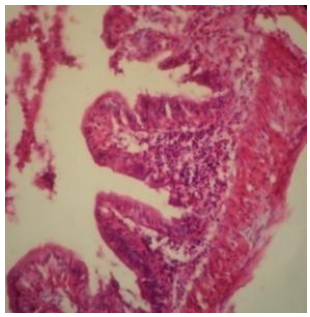

Figure 3. Histology of intestine microvilli of formulated probiotic feed fish.

Table 2. Physico-chemical properties of water.

Sl. No	Parameters	Sample-A	Sample-B	Sample-C
1	pH	7.15	7.12	7.13
2	EC µmho/cm	0.74	0.75	0.75
3	Turbidity, NTU	0.48	0.36	0.38
4	TDS mg/L	1300	1200	1400
5	TSS mg/L	1200	1000	1600
6	Total Hardness mg/L	280	260	290
7	Calcium mg/L	46.09	42.08	40.08
8	Magnesium mg/L	40.09	37.66	41.17
9	Alkalinity mg/L	70.4	47.5	57.5
10	Fluoride mg/L	1.24	1.22	1.20
11	Sulphate mg/L	1.52	1.42	1.08
12	Nitrate mg/L	0.45	0.13	0.17
13	Sodium mg/L	42.9	43.3	43.3
14	Potassium mg/L	4.1	3.9	4.4
15	Dissolved oxygen mg/L	8.42	8.24	7.16
16	Biological Oxygen Demand mg/L	4.18	4.04	3.95
17	Chemical Oxygen Demand mg/L	32.0	28.0	20.0
18	Chloride mg/L	124.3	110.2	98.4

CONCLUSION

A mixture of isolated bacterial strains positively influenced growth and survival of *L. rohita*. This evaluation study showed the nutritional value of probiotic feed and its utilization. The appropriate feed selected for feeding fish *L. rohita* 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 *L. rohita* fish culture. The selection of probiotics for aquaculture for growth, attachment to the intestinal mucus and production of beneficial compounds can be considered for improving *L. rohita* fish growth.

ACKNOWLEDGMENT

Authors like to thank the Principal and Head of the Department of for providing the facilities to carry out this research work.

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