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ORIGINAL RESEARCH ARTICLE

Production of Probiotics from *Streptomyces* sp. Associated with Fresh water Fish and its Growth Evaluation on *Xiphorous helleri*

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ABSTRACT

In aquaculture, supplementary feeding is commonly used with a mixture of rice bran, oil cake and fishmeal in proportion. Proteins are the most expensive of all parts of feed. Fish meal forms the principal source of protein in complete commercial fish feed. The supply of fish meal has become increasingly uncertain ant the price has raised rapidly. As the expense of ingredients increase, the need for cheaper alternate protein sources increase. So for the replacement of fishmeal, microbial source has been chosen as an alternative. Four different strains of *Streptomyces* spp. isolated from fresh water fish gut of *Catla catla.* These diets exhibited a remarkable increase in growth and better conversion efficiency as compared to control feed. Hence it is clear that microbial SCP can serve to supply essential proteins.

Key words: *Streptomyces*, fish meal and *Catla catla*

1. INTRODUCTION

Fish plays a key role to serve as food as well as pet to the human beings. The fish enjoys a very special consideration and place in human civilization from times immemorial. Aquaculture can make a significant contribution to produce food stuffs for domestic consumption and even for export markets [1]. Fish meal is being used as feed major source of protein in pelleted feed the world over. However, fish meals expensive besides being increasingly scarce. Therefore it is an urgent need to develop alternative low cost and nutritionally balanced diets. Preferentially, the microbial single cell protein (SCP) for the smooth and the successful aquaculture operation can be utilized. The microorganisms present in the gut of many aquatic organisms act as secondary source of nutrition [2, 3].

Manju and Dhevendaran [4] conducted the experiments on the use of bacteria and Actionmycetes SCP as a feed ingredient for freshwater prawn, *Macrobrachium idella* and studied their food conversion efficiency. Waksman [5] worked on the occurrence of asparaginase in actionmycetes. The salt tolerance of soil *Streptomyces* has been studied by Klevanskya [6] and found that limiting salt concentration for growth of number of strains of *Streptomyces* varied from NaCl between 1.5% - 7%.

The single cell protein such as nutritional grouping bacteria and *Streptomyces* helped to replace fish meal to a certain extract in the diet of *Puntious vittatos* [7] in which the conversion efficiency was found to be maximum in fish fed with *Streptomycetes* incorporated diet. In this present investigation to evaluate the nutritional value of *Streptomyces* associated with fresh water fish and growth evaluation in *Xiphporous helleri*

2. MATERIALS AND METHODS

2.1. Isolation of *Streptomyces*

Fish sample, *Catla catla* was collected using cast net from the local fish pounds of Thanjavur, Tamil Nadu, India. The sample was kept in sterile polyethylene bag and transported to the laboratory under ice for microbiological analysis. The body surface was wiped with 70% ethanol by using sterile cotton and fish skin was peeled with sterilized forceps and the gill was dissected using scissors [8]. Abdoman was opened aseptically and then alimentary tract was carefully taken out. The contents of alimentary tract were squeezed out with forceps add squeezed out gut (1gm) was taken and homogenized separately in a sterile mortar and pestle. Then it was serially diluted with filtered and sterilized 50% sea water upto 10^{-}

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^{2} dilution. Serially diluted sample (1ml) was plated in petriplates containing Glycerol Aspargine Agar (GGA) medium in triplicates, which was incubated at 35^{0}C for 10 days. The leathery colonies of *Streptomyces* that appeared on the petriplates were counted from the 5th day onwards . All the colonies that grew on the petriplates were separately streaked for sub culture so as to ensure axenicity and were maintained in slants.

2.2. Mass culture

The strains are mass cultured in *A*ctinomycetes broth for 7-10 days. After incubation the cells mat are collect and dried for feed preparation.

2.3. Feed preparation:

The ingredients used for feed preparation consisted of fish meal, rice bran, tapioca powder, ground nut oil cake, Bengal gram and *Streptomyces* spp. Feed was formulated using square method [9].

The ingredients fish meal; Rice bran, Tapioca powder, Ground nut oil cake and Bengal gram were grind to fine powder. Weighed quantities of ingredients were mixed thoroughly with sufficient water to obtain smooth dough. The dough thus prepared was steam cooked for 30 minutes and then was allowed to cool. Then it was extruded through a pelletizer. The pellets were dried overnight in a hot air oven at 60^{0}C and then stores in dry air tight containers.

2.4. Determination of food conservation ratio, food conservation efficiency by using microbial incorporated feed to the fish *Xiphorus helleri*:

The experiment to study the effect of *Streptomyces* on growth was conducted for 15 days. The experiment was carried out in five plastic troughs of 10 litre capacity (Figure - 4). Five fishes were stocked in each trough. The total weight of fish in troughs was ascertaining fishes were given one week for acclimatization to the experimental diet for 1 week and starved for 24 hours prior to the initiation of the experiment.

The fishes were fed at the rate of 15% body weight once daily. The unconsumed feed was siphoned out six hours after feeding. The next day morning, the faecal matter was collected from each trough. The unconsumed feed and faecal matter were dried in an oven at 60^{0}C and weights are recorded. About 75% if weights water from each trough was changed daily with minimum disturbances to the fish. The final weights were taken on the 15thday after the feed supplementation and the initial weight before the experiment is given.

Food conversion efficiency was estimated by following the method of Halver [10]. Food conversion efficiency was calculated by

$$\text{FCE} = \frac{\text{Wf-Wi X 100}}{\text{Total food consumed}}$$

Food conversion ratio was calculated by

$$\text{FCR} = \frac{\text{Total food consumed (g)}}{\text{Wf-Wi (g)}}$$

Where Wf and Wi are mean final and initial weights respecting, FCR and FCE are calculated.

3. RESULTS AND DISCUSSION

The present investigation was an attempt to understand the distribution pattern of *Streptomyces* spp in the micro environment of gut regions of fishes of fresh water. The primary isolation of *Streptomyces* was carried out under selective media like glycerol aspargine agar [11]. This occurrences of *Streptomyces* colonies inhibited the growth of bacteria because it has already been proved that fresh water *Streptomyces* synthesized antibiotics, anti cancer agents, L-asparginase enzyme as reported earlier [10, 11]. (**Table 1**) showed the formulation of feed preparation with *Streptomyces* cells. The different ingredients were added in different ratios for the preparation of the formulated feed.

The results showed (**Table 2**) that FCE values were increased when compared to control and FCR values were less then compared to control. The strains of *Streptomyces* spp that incorporated may be rich in protein and they may synthesize the lower weight precursors like macromolecules and vitamins. This may be the reason for the better growth rate. The results collaborated with the finding of Manju and Dherendaran [12], Anitha Kumary and Dherendaran [13], in which the SCP fed fish, provided better growth and conversion efficiencies. From the present investigation, it has been well established that *Streptromyces* culture has been fully utilized as single cell protein (SCP) for fish growth and a new venture in applied biological sciences

4. CONCLUSION

As far as the microbial feed supplementation to the ornamental fish Xhelleri showed good results for the growth of the fish, which were increased and the fish remained healthy. The feed prepared with the strains isolated from gut of fresh water fish *Catla catla* samples clearly proved that these strains can be used as single cell protein (SCP) source and has vast biotechnological application. The supply of fish meal has become increasingly uncertain and the price has been raised rapidly. Thus increase in the cost is necessitated to look for cheaper alternate source with efficient growth

promoters. It is better and cost efficient to supplement microbial incorporated feed as reported.

Table 1: ingredients composition of formulated diets (25%) expressed in grams

Ingredients	Control	Feed 1	Feed2	Feed 3	Feed 4
Rice bran	20	20	20	20	20
Groundnut oil cake	35	35	35	35	35
Bengal gram	15	15	15	15	15
Fish meal	15	15	15	15	15
Tropioca powder	15	12	12	12	12
Streptomyces spp	-	3 (FWS1)	3 (FWS2)	3 (FWS3)	3 (FWS4)

Control – RB+GNOC+BG+FM+TP
Feed 1 - RB+GNOC+BG+FM+TP+FWS1
Feed 2- RB+GNO+BG+FM+TP+FWS2
Feed 3- RB+GNO+BG+FM+TP+FWS3
Feed 4- RB+GNO+BG+FM+TP+FWS4
RB - Rice bran; GNO- Ground nut oil cake; BG - Bengal gram; FM - Fish meal; FWS- Fresh water strain

Table 2: Food Conservation ratio and conservation efficiency of X.Helleris with formulated diets

PARAMETERS	DIETS				
	Control	Feed1	Feed2	Feed3	Feed4
Weight of feed given	0.2	0.2	0.2	0.2	0.2
Weight of unfeed	0.071	0.067	0.069	0.085	0.071
Weight of feed intake	0.129	0.133	0.14	0.115	0.079
Weight of excreta	0.049	0.042	0.030	0.042	0.047
Initial weight of fish	0.4	0.4	0.4	0.4	0.3
Final weight of fish	0.44	0.5	0.47	0.45	0.35
Food conservation ratio (FCR)	3.22	1.33	2	2.3	1.58
Food conservation Efficiency (FCE)	31.0	75.1	50	43.4	63.3

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