World Journal of Fish and Marine Sciences 5 (6): 582-586, 2013
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2013.05.06.74154

Application of *Streptomyces* as a Single Cell Protein to the Juvenile Fish *Xiphophorus maculatus*

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Abstract: The potential of *Streptomyces* as a single cell protein (SCP) feed for the growth of ornamental fish, *Xiphophorus maculatus* has been investigated. Sediment samples from Akkulam Lake harbored a diversified microbial population particularly *Streptomyces* species. Four SCP feeds were prepared and their effects were compared with those of control diet. After 15 days of feeding trials, the growth parameters namely food conversion ratio and food conversion efficiency were assessed. The food conversion efficiency were found to be significantly ($p<0.05$) higher in groups that received SCP feed than in the control, whereas food conversion ratio was lower. Thus it was found that in addition of being effective antibiotic agents, *Streptomyces* could also promote the growth of the fish effectively and thus it play an important role as a single cell protein (SCP) in aquaculture nutrition.

Key words: Single Cell Protein (SCP) · *Streptomyces* · Ornamental Fish · *Xiphophorus maculates*

INTRODUCTION

Single cell protein broadly refers to the microbial biomass or protein extract used as food or feed additive. The high requirement for dietary protein in fish has been subject to much critical analysis in recent years. Proteins are complex organic compounds composed of a number of amino acids linked together through peptide bonds. The ingested proteins of food are split into simpler substances either by pepsin secreted in the stomach or trypsin of the pancreas. These small substances (peptides) are further, broken down by hydrolyzing enzymes, viz, peptidases into amino acids which in turn get absorbed in the blood stream through the gastro-intestinal tract. Subsequently, they are resynthesized into new tissue proteins or are catabolized for energy or broken down for further metabolism [1].

In aquaculture, supplementary feeding is commonly used with a mixture of rice bran and oil cake in equal proportion, but Varghese *et al*. [2] have reported that this conventional feed is nutritionally imbalanced to achieve fast growth of fish. 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 and the price has risen rapidly. As the expense of ingredients increase, the need for cheaper alternate protein sources increase. Weeds like *Nymphoides* and *Spirodella* [3], *Colocassia escIulenta* [4] and *Eichornia* [5] were used to replace the major ingredients partially in supplementary feeds.

Sound nutrition promotes reproductive success and healthy larval development and is essential in the production of high quality aquaculture products. To reduce dependence on live-food, countermeasures such as shortening of the preweaning period [6] and development of complete artificial diets for precocious cofeeding [7] were undertaken with some success. However, the exclusive use of artificial dry diets as first feed was deemed unsuitable until in developing a complete feed containing yeast and fish protein hydrolysate for the sea bass larvae, totally replacing live-prey [8]. Nevertheless, providing an acceptable first food with reliable availability, proper size and nutrient composition, remains a major challenge in aquaculture

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science. Single-cell proteins (SCPs) are playing a greater role in the evolution of aquaculture diets. With excellent nutrient profiles and capacity to be mass produced economically, SCPs have been added to aquaculture diets as partial replacement for fishmeal [9-11] and for Highly unsaturated Fatty acids (HUFA) -fortification of rotifer and *Artemia* [12].

In aquaculture operations, essential and expensive components of the feed are proteins, especially in the fishmeal. Since the supply of fishmeal has become uncertain, it is of great importance to replace the fishmeal to a minimum possible extent in fish rations. Among unconventional protein sources, single cell protein (SCP) of microbial origin appear to be a promising substitute for fishmeal, which can replace up to 25-50% fishmeal. Marine streptomyces incorporated into the artificial diet on growth, can enhance food conversion efficiency and protein increment of the juvenile prawn, Macrobrachium idella and fish, Oreochromis mossombicus [13].

Considering the above facts an attempt made on isolation of *Streptomyces* from sediment samples collected from Akkulam Lake, Kerala, India, preparation and of incorporation of *Streptomyces* in formulated feeds and supplementation of formulated feeds to the *Xiphophorus maculates*.

MATERIALS AND METHODS

In the present study sediment samples were colleted from polluted Akkulam Lake, Kerala, India. The Akkulam Lake is the smallest of the lakes confined to the southern most part of the Kerala State, situated 5 km north west of the Thiruvananthapuram, the capital city of Kerala to 8°28' N latitude and 76°57' E the longitude. The lake is about 4 km long and 3 km broad. The lake is shallow; the average depth of 1.5-2.5m. The samples were collected using polythene bags. The samples were transported to the laboratory within minimum possible time to avoid external microbial contamination and excessive proliferation.

Isolation and Maintenance of *Streptomyces* Culture: The *Streptomyces* colonies were cultured by poured plate method in which sediment sample were homogenized and added to selective media like Glycerol asparagine agar. The *Streptomyces* colonies appeared in the media were isolated at random and sub cultured on Glycerol Asparagine Agar. The media were amended with rifampicin (2.5µg/ml) and amphotericin B (75µg/ml) to inhibit bacterial and fungal contamination [14]. The isolated culture was maintained as slant culture at 28±2ºC. The strain was characterized by acid-fast staining and Gram staining techniques. The isolate was also studied by employing various parameters detailed below. The pigmentation of aerial and substrate mycelia was noted. Isolation and mass culture of the selected strains were carried out in Glycerol Asparagine broth. The pure culture of *Streptomyces* spp. was inoculated in Glycerol Asparagine broth medium and incubated for 14 days. The *Streptomyces* grew as a mat on the surface of the broth (non-motile form). The mat was harvested and the cell mass was dried and mixed with the formulated feed ingredients.

Systemic Study of *Streptomyces* spp: The International *Streptomyces* Project (ISP) was initiated in 1964 to overcome the problem in *Streptomyces* taxonomy. The important characters which are taken into consideration for the classification and identification are color of spores, spore morphology, spore surface ornamentation [15]. For spore morphology**,** the culture was grown on a Petri dish containing casein-starch-peptone-yeast extract (CSPY) agar medium, with a cover slip inserted at an angle of 45º. The cover slip was removed after seven days of incubation, air dried and observed under the scanning electron microscope.

Feed Preparation: Feed was formulated using square method [16]. 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 in a domestic pressure cooker for 30 minutes and then was allowed to cool. The steam cooked dough was cooled and weighed quantity of mass cultured *Streptomyces* spp. was added after homogenization and mixed well. Then it was palletized, dried and packed in dry airtight containers. The protein content for *Streptomyces* was 55% of crude protein per gram.

Determination of Food Conversion Ratio, Food Conversion Efficiency by Using Microbial Incorporated Feed to the Fish *Xiphophorus maculates*: The experiment to study the effect of *Streptomyces* spp. on growth was conducted for 15 days. The experiment was carried out in five plastic troughs of 10 L capacity. Fishes of same brood having approximately 0.4 g weight were selected. Ten fishes were stocked in each trough. The total weight

of fish in troughs was ascertained. Fishes were given one week for acclimatization to the experimental diet and starved for 24 hours prior to the initiation of the experiment.

The fishes were fed at the rate of 5% body weight once daily. The unconsumed feed was siphoned out six hours after feeding. The next day morning, the fecal matter was collected from each trough. The unconsumed feed and fecal matter were dried in an oven at 60°C and weights were recorded. About 75% if water from each trough was changed daily with minimum disturbances to the fish. The final weights were taken on the 15th day after the feed supplementation and the initial weight before the experiment was carried out.

The food conversion efficiency and food conversion efficiency were calculated by the method described earlier by Halver [17]. The formulae for calculation of FCE and FCR are given below:

$$FCE = [(\text{final weight} - \text{initial weight}) / (\text{feed given} - \text{unconsumed feed})] \times 100$$

$$FCR = [(\text{feed given} - \text{unconsumed feed}) / (\text{final weight} - \text{initial weight})]$$

Statistical Analysis: One way analysis of variance (ANOVA; Sigma Stat v. 3.5, Systat Software Inc, San Jose, CA, USA) was used to determine whether significant variation between the treatments existed. Difference between means was determined and compared by Duncan's new multiple range test [18]. All tests used a significance level of $p<0.05$. Data are reported as mean values ± standard errors.

RESULTS AND DISCUSSION

In the present investigation, the distribution pattern of *Streptomyces* in the sediments collected from Akkulam Lake was assessed. The isolation of *Streptomyces* was carried out using the selective media, glycerol asparagine agar. Four isolates of *Streptomyces* were obtained. The isolation of *Streptomyces* was carried out using the selective media, glycerol asparagine agar. The total *Streptomyces* population was shown in Table 1. Nearly 13 strains of Streptomyces were isolated. Among them four isolates were chosen for the experiment. Mycelial color study is one of the important characteristics of *Streptomyces*. The mycelial color characteristics of selected strains of *Streptomyces* spp. was seen using Glycerol asparagine media which were shown in Table 2. The aerial mycelial color expressed was white, creamy white and grey. The substrate mycelial color shows brown, orange and yellow. These showed that different coloration may be due to medium provided with different macro and micro nutrients, which leads to more secondary metabolite production and expression. The strain was acid-fast negative and was found to be Gram-positive. The scanning electron microscope results showed the spore morphology as having a smooth surface and rectiflexibiles (RF) hyphae. The isolated colonies showing typical morphology of *Streptomyces* as described earlier [19-21]. All the isolates fitted the genus as reported by several investigators [15].

Microbial incorporated feed was prepared and supplemented to fish *Xiphophorus maculates* which were shown in Table 3 and 4. The results showed that the FCE values were increased and FCR values were less, when

Table 1: *Streptomyces* population isolated from soil of Akkulam Lake

				CFU x 10 ^{4} g dry weight			
SL No	Medium used	Source	Stations	*Streptomyces*	Bacteria	Fungus	Selected strains
1	GAA	Soil	ST 1	10	-	-	2
2			ST 2	1	-	-	1
3			ST 3	2	-	-	1

GAA - Glycerol Asparagine Agar

Table 2: Mycelial color characteristics of selected strains (AQB-A $_{1,}$ AQB-A $_{2,}$ AQB-A $_{3,}$ AQB-A $_{4}$) of *Streptomyces*

		Glycerol Asparagine Agar medium	
SL No	Strains	Aerial mycelium	Substrate mycelium
1	AQB-A $_1$	White	Brown
2	AQB-A $_2$	Creamy white	Orange
3	AQB-A $_3$	Grey	Yellow
4	AQB-A $_4$	Grey	Yellow

Table 3: Ingredients composition of formulated diets (25%) expressed in grams

Ingredients	Control	Feed 1 AQB-A_1	Feed 2 AQB-A_2	Feed 3 AQB-A_3	Feed 4 AQB-A_4
Rice bran	20	20	20	20	20
Ground nut Oil cake (GOC)	35	35	35	35	35
Bengal gram	15	15	15	15	15
Fish meal	15	10	10	10	10
Tapioca	15	15	15	15	15
Streptomyces spp.	-	5	5	5	5

Table 4: Food conversion ratio and conversion efficiency of *X. maculates*

	Diets				
Parameters	Control	Feed 1	Feed 2	Feed 3	Feed 4
Weight of feed given	0.2	0.2	0.2	0.2	0.15
Weight of unfed	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 wt of fish	0.4	0.4	0.4	0.4	0.3
Final wt of fish	0.44	0.5	0.47	0.45	0.35
Weight gain	0.04	0.1	0.07	0.05	0.05
FCR	3.22	1.33	2	2.3	1.58
FCE	31.0	75.1	50	43.4	63.3

Food Conversion Ratio - FCR, Food conversion Efficiency -FCE; Values are significant when compared with control (p<0.05)

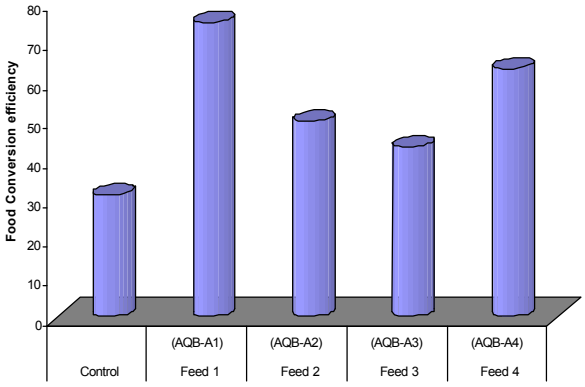

Fig. 1: Food conversion efficiency of *X. maculates* with formulated diets

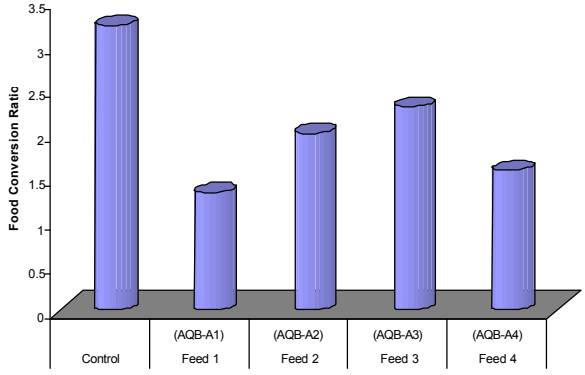

Fig. 2: Food conversion ratio of *X. maculates* with formulated diets

compared to control feed fed fishes (Figures 1 and 2). The strains of *Streptomyces* that incorporated may be rich in protein. The protein percent in each strain was assessed it was found to be AQB-A_1-55, AQB-A_2-47, AQB-A_3-51, AQB-A_4-45. This shows that protein content is rich in these strains and they may synthesize the lower weight precursors like macromolecules and vitamins. This may be the reason for the better growth rate. The results corroborated with the finding of Manju and Dhevendaran [13], in which the SCP fed fish provided better growth and conversion efficiencies. Supplementary feeding is a major expense in aquaculture operation. The commonly used conventional feed in India is a mixture of Rice bran and oil cake in equal proportions. But this feed is nutritionally imbalanced to achieve the fast growth of the fish [2]. The supply of fishmeal has become increasingly uncertain and the price has been raised rapidly. Thus the increase in the cost necessitated to look for cheaper alternate source with efficient growth promoters. It is better and cost efficient to supplement microbial incorporated feed. Statistical analysis by Duncan's new multiple range test showed significant differences of $p<0.05$ between the fish fed with SCP of *Streptomyces* and fish fed with control feed.

CONCLUSION

Single cell protein refers to the dried microbial cells or total protein extracted from pure microbial cell culture

which can be used as food supplement to animals as feed grade. Here in the experiment the growth of fishes was assessed by supplementing microbial incorporated feed. Since FCR values were less when compared to FCE values. The percentage of growth rate was high when compared with control. The high protein content of *Sterptomyces* may be one of the reasons which favor the growth of these ornamental fishes. Therefore, in the near future the applications of *Streptomyces* as probionts will play an important role in aquaculture nutrition.

ACKNOWLEDGEMENTS

The authors wish to thank Department of Aquatic Biology and Fisheries, University of Kerala, for providing necessary facilities.

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