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Original Article

Effect of Actinobacteria as a single cell protein on growth performance of *Xiphophorus helleri*

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Abstract: The potential of Marine Actinobacteria particularly Streptomyces as a single cell protein (SCP) feed for the growth of ornamental fish, *Xiphophorus helleri* has been investigated. The Streptomyces strains used as SCP were isolated from the marine sponges, namely *Callyspongia diffusa*, *Mycale mytilorum*, *Tedania anhelans* and *Dysidea fragilis*. Six SCP feeds were prepared and their effects were compared with those of control diet. After 30 days of feeding trials, the growth parameters including absolute growth rate, specific growth rate and feed conversion efficiency were found to be significantly ($P<0.001$) higher in groups that received SCP feed than those of control one, whereas feed conversion ratio was lower. Thus it was found that in addition to being effective antibiotic agents against harmful pathogens, Streptomyces could also promote the growth of fish effectively. Marine Actinobacteria, particularly Streptomyces, could play an important role as a single cell protein (SCP) in aquaculture nutrition and is a promising microbe for the development of marine biotechnology.

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Introduction

Actinobacteria represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria, including five subclasses and 14 suborders (Stackebrandt, 2000). Among the five subclasses, actinobacteria belonging to the order Actinomycetales (commonly called actinomycetes) account for approximately 7,000 of the metabolites reported in the dictionary of natural products and the vast majority of these compounds are derived from the single genus *Streptomyces*. *Streptomyces* species are distributed widely in marine and terrestrial habitats (Pathomaree et al., 2006) and are of commercial interest due to their unique capacity to produce novel metabolites.

Marine *Streptomyces* are widely distributed in biological sources such as fishes, molluscs, sponges, seaweeds, mangroves, besides seawater and sediments (Dhevendaran and Anithakumari, 2002; Dhevendaran and Praseetha, 2004; Qianqun et al., 2004; Sivakumar et al., 2007; Das et al., 2008; El-Shatoury et al., 2009; Selvin et al., 2009). These organisms are gaining importance not only for their taxonomic and ecological perspectives, but also for their novel secondary metabolites production like antibiotics, enzymes, enzyme inhibitors, pigments and their biotechnological application such as probiotics and SCP (single cell protein) (Dharmaraj, 2010; Das et al., 2010).

Algae, fungi and bacteria are the chief of microbial protein (single cell protein-SCP) that has been utilized as a protein supplement (Anupama, 2000). Though marine Actinobacteria particularly *Streptomyces* are known to produce primary as well as secondary metabolites and also has advantages such as (1) the degradation of macromolecules, such as starch and protein in the culture pond water, (2)

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Table 1. Concentration of protein in the ingredients added to the formulated feeds.

S.	Ingredients	Protein (%)
1	Rice bran	10.522
2	Chickpea flour	35.540
3	Ground nut oil cake	40.010
4	Tapioca flour	2.520
5	Fish meal	55.004
6	*Streptomyces* sp.AQBCD03 (F1)	55.110
7	*Streptomyces* sp AQBCD11 (F2)	56.672
8	*Streptomyces* sp AQBMM35 (F3)	55.342
9	*Streptomyces* sp AQBMM49 (F4)	56.236
10	*Streptomyces* sp AQBTA66 (F5)	55.094
11	*Streptomyces* sp AQBDF81 (F6)	55.872

the production of antimicrobial agents and (3) the formation of heat and desiccation resistant spores. There are only few attempts on the usage of *Streptomyces* spp., as SCP in aquaculture. There has been report on *Streptomyces* incorporated feed as a SCP source, isolated from the fish gut of *Catla catla*. These diets exhibited a remarkable increase in growth pattern and better conversion efficiency compared to control feed (Suguna and Rajendran, 2012). Another report shows improved growth, feed conversion efficiency and higher protein content in juvenile prawns on 50 days of feeding trials of *Streptomyces* as SCP source (Manju and Dhevendaran, 1997). By considering the above facts, this study aimed to use *Streptomyces* as SCP feed for the ornamental fish *Xiphophorus helleri* and to evaluate its effects on the growth performance based on absolute growth rate (AGR), specific growth rate (SGR), feed conversion efficiency (FCE) and feed conversion ratio (FCR).

Materials and Methods

Experimental fish: Red swordtails (*X. helleri*, Cyprinidae) weighing about 1.5 g were stocked in seven 20-litre plastic troughs, each contains twenty fish. One trough fed with control feed, whereas the rest of the fish were supplemented with SCP feed. The experiment was conducted for 30 days and repeated in triplicate.

Streptomyces as single cell protein (SCP): Single colonies of *Streptomyces* strains were isolated from marine sponges namely *Callyspongia diffusa*, *Mycale mytilorum*, *Tedania anhelans* and *Dysidea fragilis* collected at a depth of 5 to 10 m from Vizhinjam port, situated about 16 km to the south of Trivandrum at 8°22'30" N latitude and 76°59'16" E longitude on the South-West coast of India. Among 94 cultures isolated, only six *Streptomyces* strains namely AQBCD03, AQBCD11, AQBMM35, AQBMM49, AQBTA66 and AQBDF81 were used as SCP source and the characterization of the isolates has been detailed in Dharmaraj and Sumantha (2009) and Dharmaraj (2011). The strains were inoculated in 500 mL of starch casein broth in a 1 liter Erlenmeyer flask and incubated at room temperature for seven 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 (Das et al., 2006).

Feed preparation: Fish were fed with seven different feeds including the SCP feed and control feed for 30 days. Formulated diets were prepared according to the least square method (Hardy, 1980). The protein content in the ingredients was estimated by Kjeldahl method (Belcher and Godbert, 1954). The compositions of the ingredients used for preparing formulated diets and their protein mass fractions are shown in Tables 1 and 2.

Control feed: The ingredients of control feed consist

Table 2. Composition of ingredients in formulated feeds.

Ingredients	Formulated feeds (g %)	
	Control feed	SCP feed
Rice bran	16.50	16.50
Chickpea flour	18.50	18.50
Groundnut oil cake	23.50	23.50
Tapioca flour	16.00	16.00
Fish meal	25.50	15.50
Streptomyces dried cell mass	-	10.00

Table 3. Proximate composition of different feeds.

Formulated feeds	Proximate composition (%)					
	Moisture	Crude protein	Crude fat	Ash	Crude fibre	Nitrogen-free extract (NFE)
Control	10.87	35.04	12.02	13.80	2.45	25.82
F1	11.90	45. 20	14.20	13.34	2.85	12.51
F2	11.51	43.25	13.90	13.50	2.62	15.22
F3	11.91	44.22	13.21	13.66	2.78	14.22
F4	11.95	45.15	13.31	12.33	2.94	14.32
F5	11.48	44.08	13.28	13.58	2.70	14.88
F6	11.56	44.56	13.26	13.28	2.81	14.53

of fish meal, rice bran, groundnut oil cake and chickpea flour with tapioca flour as binder. The ingredients were ground to a fine powder and mixed thoroughly with sufficient water to obtain smooth dough. The dough thus prepared was steam cooked for 30 min and allowed to cool. This was extruded through a pelletiser. The pellets were dried and then stored in dry airtight containers at 28°C.

Single cell protein (SCP) feed: The steam cooked dough was prepared and cooled as given above. To this dough, a known quantity dried cell mass of *Streptomyces*, was added and mixed. This was extruded through a pelletiser, the pellets were dried and then stored in dry airtight containers at 28°C.

Determination of growth performance: The fish were fed with prepared feeds at the rate of 5% body mass once a day. The unconsumed feed was siphoned out 6 hours after feeding. The following morning, the faecal matter was collected from each trough. The unconsumed feed and faecal matter were dried in an oven at 60°C and their mass was recorded. About 75% of water of each trough was changed every day with minimum disturbances to the fish. The initial mass was measured before the experiment and the final mass on the 30th day after the feed supplementation. The growth parameter like absolute growth rate (AGR), specific growth rate (SGR), feed conversion efficiency (FCE) and feed conversion ratio (FCR) were calculated by the method described by Halver (1972). The formulae for calculation are given by the Eqs. 1-4, respectively:

AGR = (final mean mass–initial mean mass)/g [1]

SGR = [(ln final mass–ln initial mass)/ rearing period] ×100 [2]

FCE = [(final mass–initial mass)/ (feed given–unconsumed feed)] ×100 [3]

FCR = [(feed given–unconsumed feed)/ (final mass–initial mass)] [4]

Chemical analysis: The proximate composition of feed and tissue of the fish were analysed for moisture, crude protein, crude fat, crude fiber and ash and nitrogen-free extract according to the standard methods of AOAC (2000). The proximate

Table 4. Proximate composition of Red Swordtail fish fed on different diets.

Formulated feeds	Proximate composition (%)					
	Moisture	Crude protein	Crude fat	Ash	Crude fibre	Nitrogen-free extract (NFE)
Control	77.00	15.00	3.00	3.20	1.00	0.80
F1	78.00	15.25	3.15	2.65	0.75	0.20
F2	77.65	15.14	3.09	2.90	0.85	0.37
F3	78.04	15.02	3.10	3.00	0.59	0.25
F4	77.97	15.31	3.02	2.67	0.75	0.28
F5	78.01	15.06	3.04	3.12	0.46	0.31
F6	78.05	15.00	3.02	3.10	0.48	0.35

Table 5. AGR, FCE, FCR and SGR in *Xiphophorus helleri* fed with control feed and SCP feeds.

Formulated feeds	Initial weight (g)	Final weight (g)	AGR (g)	FCE (%)	FCR(g)	SGR (%)
C	1.530 ± 0.020	1.840 ± 0.030	0.310 ± 0.010^{a}	26.940 ± 1.875^{a}	3.724 ± 0.256^{g}	0.659 ± 0.012^{a}
F1	1.520 ± 0.010	2.050 ± 0.010	0.530 ± 0.010^{c}	46.167 ± 0.871^{c}	2.167 ± 0.041^{e}	1.068 ± 0.030^{c}
F2	1.530 ± 0.010	1.993 ± 0.015	0.463 ± 0.006^{b}	39.881 ± 0.947^{b}	2.508 ± 0.059^{f}	0.945 ± 0.006^{b}
F3	1.533 ± 0.025	2.540 ± 0.020	1.007 ± 0.006^{g}	78.732 ± 0.946^{g}	1.270 ± 0.015^{a}	1.803 ± 0.031^{g}
F4	1.520 ± 0.020	2.243 ± 0.020	0.723 ± 0.003^{e}	63.014 ± 0.812^{e}	1.587 ± 0.020^{c}	1.390 ± 0.016^{e}
F5	1.530 ± 0.010	2.35 ± 0.010	0.820 ± 0.005^{f}	67.326 ± 0.363^{f}	1.485 ± 0.008^{b}	1.532 ± 0.016^{f}
F6	1.535 ± 0.005	2.23 ± 0.020	0.695 ± 0.018^{d}	59.456 ± 0.685^{d}	1.682 ± 0.019^{d}	1.333 ± 0.028^{d}

Values in the same column sharing a common superscript are not significant (P<0.001)

Table 6. One way ANOVA for weight gain of *Xiphophorus helleri* fed with control feed and SCP feeds.

Source of Variation	Degrees of freedom	Sum of squares	Mean square	F
Between Groups	6	0.985	0.164	**1838.804*** **
Within Groups	14	0.00125	0.0000893	
Total	20	0.986		

*** Significant (P<0.001)

compositions of the formulated feeds are displayed in Table 3.

Statistical analysis: All experiments were performed in triplicate and the results are expressed as mean values with ± standard deviation (± SD). Data were subjected to one way analysis of variance (ANOVA; SigmaStat v. 3.5, Systat Software Inc, San Jose, CA, USA) and means were separated by Tukey test at P<0.001 significant level (Hayter,1986).

Results

Thirty days of feeding trials with single cell protein (SCP) result a marked increase in the growth performances of the fishes compared to control treatment. The proximate compositions of Red Swordtail fish fed on formulated diets are displayed in Table 4. When comparing the FCR, the control feed given fishes showing the higher value of 3.724 ± 0.256 than those of others. The FCE values of F3 (78.732 ± 0.946), F5 (67.326 ± 0.363) were higher than the control treatment (26.940 ± 1.875). There was significant increase in food conversion efficiency when fed with SCP feed and decreased values of food conversion ratio. The SCP feed was rich in protein and this may be the reason for the better growth rate. The SCP feeds given fishes were found to be significant than the control fishes with respect to AGR and SGR (P<0.001) (Table 5). The

weight gain showed significant differences at the 1% level by Tukey test, when comparing the control feed and SCP feeds given fishes (Tables 6).

Discussion

There are reports in support of the *Streptomyces* as SCP in aquaculture. The juvenile prawns *Macrobrachium idella* fed with *Streptomyces*-incorporated feed for 50 days, showed improved growth (140.54%) and feed conversion efficiency (45%), and higher protein content (54.72%) (Manju and Dhevendaran, 1997). Furthermore, preliminary report on the growth increment of tiger shrimp, *Penaeus monodon* on supplementation of *Streptomyces*-incorporated feed showed high growth in terms of length (15.79%) and weight (57.97%) compared with those of control treatment (length (4.08%) and weight (32.77%)) (Das et al., 2006). Also, *Oreochromus mossombicus* fed with single cell protein (SCP) from *Azolla* sp. showed better growth and conversion efficiencies (Manju and Dhevendaran, 2002). In the present work the SCP feed replaced nearly 30-40% of the fish meal incorporated in the feed. In aquaculture, essential and expensive components of the feed are proteins, especially the fish meal. Since the supply of fish meal has become uncertain and the prices have increased rapidly, the need for cheaper alternative protein sources has increased. Among unconventional protein sources, microbial origin appears to be a promising substitute for fish meal, replacing up to 25-50%. This research in line with above-mentioned works, indicates the importance of marine actinobacteria, particularly the application of Streptomyces in aquaculture.

Conclusions

Marine microorganisms synthesise macromolecules and vitamins which benefit animal nutrition. There are very limited reports on the application of Actinobacteria, particularly Streptomyces as SCP in aquaculture. This is the first report on the application of *Streptomyces* of marine origin act as SCP feed for the growth of ornamental fish and this study proved that there is a significant increment in the growth of the ornamental fish *X. helleri* fed with SCP feed. Therefore, in the near future the applications of *Streptomyces* as single cell protein (SCP) will play an important role in aquaculture nutrition and is a promising microbe for the development of marine pharmaceutical biotechnology.

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