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Research Article

ISOLATION OF ENZYME PRODUCING BACTERIA FROM GUT OF CHANNA STRIATUS FED ON DIFFERENT HERBS AND PROBIOTICS DIET

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ABSTRACT

Objective: Isolation and enumeration of heterotrophic bacteria from the gastrointestinal tract of murrel (*Channa striatus)* fingerlings (2.2±0.43g) fed with probiotics, herbals and chicken intestine diet. The Cellulolytic, Amylolytic and Proteolytic activity of bacteria were detected in the fish gut. Methods: The six groups were treated with herbals and probiotics diet of concentration 5g in each treatment respectively. The seventh group were treated with fresh chicken intestine and eighth group served as the control. Results: At the end of culture the maximum average growth of fish treated with group IV was (3.51±0.20) but in control (2.01±0.21). At the end of experimental period the survival in group was (98%) but in control (90%). The celluloytic activity was higher in fish fed with group IV (93.2±0.81 Umg^{-1}) but in control group (5.9±0.84 Umg^{-1}). The amylolytic activity was higher in group III (3.3±0.53Umg^{-1}) but in control group (0.1±0.06 Umg^{-1}).The proteolytic activity was higher in group III (53.4±0.31Umg^{-1}) but in control group (3.5±0.42 Umg^{-1}).All these results were statistically found significantly (P<0.05).Conclusion: The result present study shows the chicken intestine and *Bacillus subtilis* has better impact on murrel culture.

Keywords: Herbs, Probiotics, *Channa striatus*, Gastrointestional, Chicken intestine.

INTRODUCTION

Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Beings rich in nutrient, the environment of the digestive tract of fish confers a favorable culture environment for the microorganisms. Importance of intestinal bacteria in the nutrition and well-being of their hosts has been established for homeothermic species. The murrels, commonly called snakeheads belonging to the family *channidae* constitue of the most common and dominant group of air breathing freshwater fishes. It is high taste and nutritional contents. It has long been commercially cultured in Thailand, Taiwan, Indian and etc. The aquaculture diets are conventionally based on expensive feed stuffs such as fish and fish meal. Development of aquaculture will be greatly enhanced by finding alternative and less expensive ingredients. The culture of *Channa striatus* is a very promising industry in Asian countries like India but the most serious constraints are availability of seeds and lack of knowledge of feeding murrel farming is socially more acceptable and technically and economically more viable and sustainable. It culture is a profitable enterprise and even small farmers of Nigeria can afford to culture tilapia to augment their income. It is consumed by poor people as it is relatively low priced commodity techniques.

The probiotics are live microbial cells that are administered to the gastrointestinal tract of the host as a feed supplement and Improving the intestinal microbial balance and health [8].The most important microbial strains such as *Lactobacillus sp, Pseudomonas fluorescens*, *sacharomycis cervisiae*are used as biological control agents in aquaculture. They are non pathogenic bacteria can survive in the gut and remain stable and viable for long periods. The importance of intestinal bacteria in the nutrition and well being of the hosts. It has been established for homoeothermic species, such as birds and mammals [7].Many herbs have been used in growth and disease prevention by incorporation into fish feeds; in China about 10 herbs are commonly used to treat diseases like enteritis, gill rot, white head and white mouth disease [19]. The endogenous digestive enzymes in fish have been studied substance that stimulate the growth of other microorganisms[6-14].The functional additive like probiotics is a new concept on aquaculture ,where show a positive effect on growth caused by the best use of nutritional effect .In the present study was to identify the dietary supplementation of probiotics, herbs and chicken intestine on growth and relative amount of proteolytic, amylolytic and cellulolytic activity producing bacteria in the gastrointestinal tract of *channa striatus.*

MATERIALS METHODS

Collection and preparation of feed additives

The healthy plants, *Murraya koenigii, Sesbania grandiflora*, *Amaranthus thandu* were collected from Tirunelveli local market, Tamilnadu, India. It was washed twice using fresh water to remove debris and other extraneous malter from the plants, and they were shade dried, well ground to make a fine powder .The powered probiotics of *Bacillus coagulants*, *Bacillus subtilis*, and mixed probiotics were obtained from Centre for Aquaculture research and Extension (CARE),St.Xaviers college,Palayamkotai, India. The chicken intestine was collected from a slaughter house in a local market in Tirunelveli.

Experimental setup

C.striatus fingerlings (2.2+0.43g) were collected from the Kumar private fish form in Tirunelveli, India, and transported to the center for Aquaculture Research and Extension (CARE) in Palayamkotai, India. They were stocked in (12×10×3m) plastic tank and fed with commercial pellet feed for 7 days of acclimatization.

Experimental group

After the acclimation period the fishes were divided in to eight groups and stocked in the 50 l tank at a stocking density 25 fishes per tank.Filled with fresh water at a rate of 20 fingerlings per through the water control 26 to 27^0c, pH 6.5to7.0, maintained for each treatments. The Six groups were treated with herbal and probiotic diet concentration of 5g in each treatment. The seventh group was treated with chicken intestine and eighth group served as the control without herbal diet. The triplicate tanks were maintained for each group this experiment was conducted for a period of 60 days. The details are given below.

Group 1= basal diet +5g of *Murraya koenijii*

Group 2= basal diet +5g of *Sesbania grandiflora*

Group 3= basal diet +5g of *Amaranthus thandu*

Group 4= basal diet +5g of *Bacillus coagulants*

Group 5= basal diet +5g of *Bacillus subtilis*

Group 6= basal diet +5g of Mixed probiotics

Group 7= chicken intestine

Group 8= basal diet only (Control)

Experimental diet

The experimental diets were formulated to contain protein level of 40% in each experimental diets Table 1.Fishmeal, ground oil cake, wheat flour, tapioca flour, fish oil were homogenized thoroughly in a food mixer. After adding water to the mixer ingredients, a paste was made by hand mixing. The paste were sealed in a plastic bag and stored at -20^{0}c until use. The prepared feed ratio was divided in two times a day 10.00 am and 4.00 pm respectively.

Enzyme producing bacteria in the gut

At the end of the experimental period each group of fishes were harvested, and gut was separated.The separated gut samples were rinsed with cold distilled water. Total intestinal content was homogenized in phosphate buffer (p^H 7.5; PBS) using a hand held glass homogenizer at 4^{0}c. The homogenate was centrifuged at 4 ^{0}C at 15000×g for 15 min. The supernatant was collected and serially diluted. The 1ml of each dilution was spread on bacterial plates of Starch agar, CMC agar and skim milk agar plates respectively [2]. The culture plates were incubated at 37^{0}C overnight and examined for development of bacterial colonies after the inoculation period. It was assumed that the microflora, which had formed colonies on the SA paltes.CMC plates and Skim milk plates had amyoloytic, celluloytic and proteolytic activites respectively.

Statistical analysis

The data are reported as Mean +SD. One way analysis of variance was used to determine significant variation between the treatments the difference between means were determined and compared by Duncan multiple rang test (version 17.0) were performed to find significant level of $p<0.05$

RESULT

Survival

The survival rate of all experimental groups applied with herbs, probiotics and chicken intestine treatment were observed to be higher than the control group. At the end of culture the maximum survival rate was observed in group IV was (98%) followed by group III (92%), group VII (92%),group VI (94%) group V (94%),group II (92%),group I (90%) respectively. The survival was found to be very low (86%) in the as control group.

Table 1: Composition of basal diet

Ingredients	Group1	Group2	Group3	Group4	Group5	Goup6	Group7	Group8
Fish meal	35	35	35	35	35	35	-	36
[1]GOC	30	30	30	30	30	30	-	30
Soy flour	14	14	14	14	14	14	-	15
Wheat flour	8	8	8	8	8	8	-	10
Tapioca flour	6	6	6	6	6	6	-	9
Fish oil	1	1	1	1	1	1	-	1
[2]Vitamine and Mineral premix	1	1	1	1	1	1	-	1
Additives	5	5	5	5	5	5	Chicken intestine	-

[1]GOC-Ground nut oil cake

[2]Vitamin and mineral premix, each kg of premix contained, vitamin A (4,000,000 IU), vitamin D(666,666.7 IU), vitamin H (3,333.3 mg,), vitamin K3 (333.3 mg), vitamin B1 (333.3 mg), vitamin B2 (1,666.7 mg), vitamin B6 (500 mg), vitamin B1 (3.33 mg), pantothenic acid (3,333.3 mg), folic acid (333.3 mg), biotin (16.7 mg) niacin (10,000 mg), iron (10,000 mg), manganese (20,000 mg), copper (1,333.3 mg), zinc (166,666.7 mg), iodine (100 mg), cobalt (33.3 mg) and selenium (33.3 mg).

Table 2: Average growth performance and survival of *Channa striatus (*Mean ±SD).

Feeding habits	Average weight (g)	Average length (cm)	Survival (%)
Mixed Probiotics	2.21±0.24^a	6.4±0.24^b	90
B.coagulants	2.61±0.54^a	6.7±0.51^a	92
B.subtilis	3.24±0.96^a	7.1±0.64^a	96
Chicken intestine	3.51±0.20^a	6.8±0.22^a	98
Murraya koenijii	3.13±0.47^b	6.7±0.31^b	94
Sesbania grandiflora	3.11±0.78^a	6.4±0.74^a	94
Amaranthus thandu	2.41±0.92^a	6.1±0.25^a	92
Control feed (Semi moist)	2.01±0.21^a	6.5±0.20^a	86

[a,b]Means in the same row with different superscript are significantly different ($p<0.05$)

(g)-gram,cm-Centimeter

Table 3: Bacterial Count in gut of *Channa striatus* (Mean ± SD)

Feeds	CFU/mg gut in bacterial count		
	Amylolytic Bacetria(×10^5)	Cellilolytic Bacteria(×10^5)	Proteolytic bacteria(×10^5)
Mixed Probiotics	--	22.2±0.83^a	40.2±0.15^a
B.coagulants	--	24.71±0.46^b	52.3±0.42^c
B.subtilis	3.3±0.53^a	53.6±0.28^b	53.4±0.31^a
Chicken intestine	--	93.2±0.81^a	22.3±0.53^a
Murrayakoenijii	0.2±0.04^c	--	49.6±0.41^a
Sesbaniagrandiflora	0.06±0.74^a	--	9.0±0.23^c
Amaranthusthandu	--	--	3.9±0.38^a
Control feed (Semi moist)	0.1±0.06^a	5.9±0.84^a	3.5±0.42^a

[a,b,c]Means in the same row with different superscript are significantly different ($P<0.05$)

(CFU)-Colony forming unit,(mg)-Milligram,(--)-Not detected

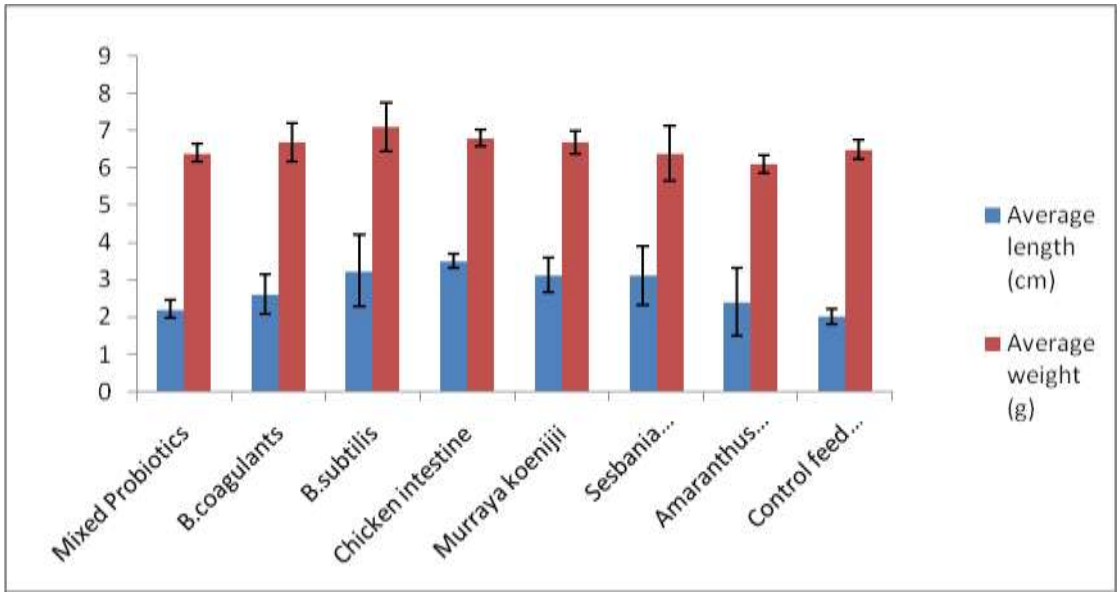

Fig. 1: Average length and weight relationship of *C.striatus.*

Growth

The maximum growth was observed in the group IV. The result showed significantly ($p<0.05$) growth rate in all the treated groups than that of the control group. At the end of the experimental period the maximum growth rate was observed in group IV (3.51±0.20) and followed by group III (3.24±0.96), group VII (2.41±0.92), group VI (3.11±0.78), group V (3.13±0.47), group II (2.61±0.54), group I (2.21±0.24) and control group (2.01±0.21) respectively (Table.2).

DISSCUSSION

The use of herbs and probiotics is widely expected to become an alternative therapy in aquaculture as a prophylactic and to control fish diseases. These studies concentrating the isolation and identification of enzymes in gut with different diets. The role of plant in growth rates was confirmed with production of the digestive enzymes like protease ,amylases and cellulose [15].According to FCR of *O. niloticus* on basal diet (control) was higher (5.8) than diet supplemented with probiotics, *S. cerevisiae* and *B.subtilis* (4.7) due to probiotic supplementation by improved feed utilization. The bacteria present in the aquatic environment may influence the composition of the gut micro biota in fish [3].The result of the present study showed variation in the relative abundance of the enzyme producing bacteria in *C.striatus* with different herbal, probiotic and chicken intestine diet .This may be due to varied bacterial load of diets (Table.3).The possible correlation between the intestional microbiota of fish and bacterial content of the water has been demonstrated [12].In the present study was three herbal plants such as Murraya *koenigii ,Sesbania grandiflora, Amaranthus thandu* and three probiotics such as *Bacilus coagulants, Bacilus subtilis,* Mixed Probiotics and control diet were tested with *C.striatus.* The probiotics promoted colonization of bacteria in the fish gut for a prolonged period and had capacity to adhere and grow well *in vitro* in the intestinal mucus from turbot [16]. Observed a constant increase in Probiont (*Carno bacterium sp.*) population in the gut of rainbow trout and Atlantic salmon fingerlings fed with probiotic diet [18]. Rainbow trout (*Oncorhynchus mykiss*) was fed with the diet supplemented with probiotic, *Bacillus spp*. The count of bacteria was higher (3.39 ± 2.06 ×107 CFU g-1) than the control (12.5 ± 1.08 ×107 CFU g^{-1} [9]. Similarly the present study the maximum cellulolytic bacteria were observed in chicken intestine (93.2±0.81Umg^{-1}) and *B.subtilis* (53.6 ± 0.28Umg^{-1}).The enhanced growth performance compare to the herbal diets. Several herbal principles have been tested for their growth-promoting activity in aquatic animals [4,13] .The use of probiotics, herbals, and their active compounds in aquaculture is comparatively new, but they are becoming recognized as being important for disease control. The result of the study probiotics supplementation of the experimental diets resulted in higher growth and feed utilization as compared with herbal and control diet [12]. The increase in growth of *C.striatus* s by inclusion of *B. subtilis* may be due to that most of *Bacillus spp* can produce secondary metabolites which have been used industrially for production of antibiotics ,bioinsecticides, fine chemicals and enzymes that readily hydrolyze carbohydrates, lipids and proteins in to sugars, fatty acids, peptides and amino acids [9,17,20].

CONCLUSION

The aim of the present study was to determine the effect of growth and isolation of proteolytic amylolytic and cellulolytic activity from the gasterointestinal tract of *C. striatus* with probiotics and herbals diet. The maximum growth as well as enzyme producing bacteria was determine in *C.striatus* fed with chicken intestine and *B.subtilis*

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