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RESEARCH ARTICLE

ENZYMATIC AND ANTIBACTERIAL ACTIVITY OF INTESTINAL BACTERIA OF ZEBRA FISH (*DANIO RERIO*)

***Suganya, D., Rajan, M. R., Sivakumar, P. and Anisha Banu, S.**

Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram-624 302, Dindigul Dist, Tamil Nadu, India

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ABSTRACT

Aim: The objective of this study was to explore isolation, identification, enzymatic and antibacterial activity of intestinal bacteria of Zebra fish (*Danio rerio*).

Methods and Results: The serially diluted (10^{-6}) sample was plated in sterilized nutrient agar medium and incubated at 37°C for 24 hrs. After incubation the pure colonies were characterized for their morphological and various biochemical characteristics using the Berge's Manual of Determinative Bacteriology. The isolated and identified bacteria was *Aeromonas* sp., *Pseudomonas* sp., *Micrococcus* sp., *Escherichia* sp., and *Bacillus* sp., The digestive enzyme productivity of isolated bacteria were identified from the culture plate using selective media. The isolated bacteria were subjected for its efficacy to produce enzymes like amylase, cellulase, lipase and protease. The antibacterial activity of isolated bacteria was tested with five fish pathogenic bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*). Based on the results the selected bacteria was *Pseudomonas* sp., produced higher enzyme productivity of amylase, cellulose and lipase and also higher zone of inhibition in antibacterial activity test.

Conclusion: Based on the above characteristics the selected intestinal bacteria was multiplied and recommended to the preparation of probiotic feed.

Significance and Impact of Study: Zebra fish have near by 75% of genome is same in human genome. In future isolated Zebra fish intestinal bacteria has been formulated as probiotic feed preparation and it can be further characteristic and also will produce large scale for enhance the growth.

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INTRODUCTION

Aquaculture and ornamental fish culture in India has shown a rapid progress during past few years but some major problems are hindering the progress path and disease being one of them. Because water sources make a suitable media for the high level microbial proliferation and its easily cause several bacterial diseases like dropsy, kidney disease, gill disease, pop eye, vibriosis, epizootic ulcerative syndrome, ulcerative disease, fin and tail rot (Gahlawat *et al.,* 2006). Bacterial pathogen cause heavy mortality in both cultured and wild fishes/shell fish species all over the world (Ram C. Sihag and Parvati Sharma, 2012).

Corresponding author: Suganya, D.
Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram-624 302, Dindigul Dist, Tamil Nadu, India.

For successful ornamental fish culture quality feed, good environment and disease free seeds were among the different techniques. Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria.

The study of digestive enzymes is an essential step towards understanding the mechanism of digestion and the organism adapts to changes in the nutrition environment. Few reports concerning microbial enzymatic production in the gastrointestinal tract of fish are available (Stickney and Shumway 1974; Prejs and Blaszcyk 1977). The study related to the isolation, identification enzymatic and antibacterial activity of intestinal bacteria of ornamental zebra fish is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

Collection of sample and Isolation of bacteria

Zebra fish (*Danio rerio*) was collected from Angel Aquarium, Dindigul, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. The intestinal sample was collected by dissecting the abdomen of the fish. The sample collected from the intestine of the fish was serially diluted and the appropriate dilutions (10^{-6}) were selected for the isolation of bacteria. The serially diluted sample was plated over sterilized Nutrient agar plates and incubated at 37^{o}C for 24hrs. After incubation the colonies were enumerated and the predominant colonies were selected. Morphological, physiological characteristics was carried out by biochemical tests (Bergey's manual of Determinative Bacteriology, 1994).

Antibacterial Activity of Isolated bacteria

Isolated bacteria were tested for antibacterial activity by using well diffusion method. 24 hrs bacterial broth culture was centrifuged at 6,000 rpm for 5 minute and supernatant was used for well diffusion method. Five fish pathogenic bacteria (*Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*) were used for the determination of antibacterial effect of the isolated strains and also used commercial antibiotic for purpose of comparison study.

RESULTS

The biochemical characteristics of intestinal bacteria of Zebra fish is given in table 1. Five distinct colonies were isolated from the intestinal content of Zebra fish. The isolated colonies were *Aeromonas* sp., (ZB1), *Pseudomonas* sp.,(ZB2), *Micrococcus* sp.,(ZB3), *Escherichia* sp., (ZB4) and *Bacillus* sp.,(ZB5) from the intestinal content of Zebra fish. The enzymatic productivity of intestinal bacteria of Zebra fish is given in Table 2. The enzyme productivity of intestinal bacteria of *Pseudomonas* sp., (ZB2) was higher in amylase, cellulose, protease and lower in other bacteria. The antibacterial activity of intestinal bacteria of zebra fish was given in table 3. Antibacterial activity of intestinal bacteria *Pseudomonas* sp., (ZB2) was higher zone of inhibition of intestinal bacteria of Zebra fish and compared with commercial antibiotic of *Streptomycin*.

Table 1. The biochemical characteristics of Intestinal bacteria in Zebrafish

Test	Bacterial Result				
Simple staining	Rods	Cocci	Rods	Rods	Rods
Gram's staining	Gram positive	Gram positive	Gram positive	Gram Negative	Gram Negative
Indole test	+ve	-ve	+ve	+ve	-ve
Methyl Red	+ve	+ve	+ve	+ve	+ve
Voges Prokauser	+ve	-ve	+ve	-ve	-ve
Citrate test	-ve	-ve	+ve	+ve	-ve
Catalase test	+ve	+ve	+ve	+ve	+ve
Starch test	+ve	-ve	+ve	+ve	+ve
Gelatin hydrolysis	+ve	- ve	+ve	NP	+ve
Identification result	*Aeromonas* sp.,	*Pseudomonas* sp.,	*Micrococcus* sp.,	*Escherichia coli* sp.,	*Bacillus Sp.,*

Table 2. Enzymatic productivity of intestinal bacteria in Zebrafish

S.No.	Bacteria strain		Amylase	Cellulase	Lipase	Protease
1.	*Aeromonas* sp.,	(ZB 1)	++	++	+++	++
2.	*Pseudomonas* sp.,	(ZB 2)	+++	+++	++	+++
3.	*Micrococcus* sp.,	(ZB 3)	+++	++	++	+
4.	*Escherichia* sp.,	(ZB 4)	NP	+	+++	++
5.	*Bacillus* sp.,	(ZB 5)	++	+	++	+

Table 3. Antibacterial activity (mm) of intestinal bacteria in Zebrafish

Isolated Bacteria		Zone of inhibition (mm)									
		P1	CA	P2	CA	P3	CA	P4	CA	*P5*	CA
Aeromonas sp.,	(ZB 1)	14	10	18	15	18	13	20	17	17	13
Pseudomonas sp.,	(ZB 2)	16	12	20	16	22	12	17	15	18	16
Micrococcus sp.,	(ZB 3)	16	12	20	13	19	17	18	11	18	20
Escherichia sp.,	(ZB 4)	12	10	20	14	16	14	15	13	17	18
Bacillus sp.,	(ZB 5)	18	16	17	11	18	16	14	11	16	13

P1- *Klebsiella pneumonia,* P2- *Pseudomonas aeruginosa,*P3- *Bacillus cereus*,
P4- *Staphylococcus aureus* and P5- *E. coli* CA- Commercial Antibiotic (Streptomycin)

DISCUSSION

Lewbert, 1998 were isolated intestinal bacteria such as *Aeromonas* sp., *Pseudomonas* sp., and *Enterococcus* sp., in five different ornamental fishes. Shubhadeep Ghosh *et al*., (2007) also isolated bacteria such as *Pseudomonas* sp., *Aeromonas* sp., and *Bacillus* sp., from the intestine of Indian major carps.

Hoshina *et al*. (1997) reported the higher enzyme productivity of protease in *Pseudomonas* sp., of marine fish Pacific cod. Sivakumar and Rajan (2015) also reported digestive enzyme productivity of (Amylase, Cellulose, Lipase and Protease of intestinal bacteria *Stenotrophomonas maltophilia* was higher in Yellow molly. Hidalgo *et al.* (1999) reported the enzymatic activity of protease and amylase in the intestinal bacteria of Gold fish(*Carraius auratus*). Prabu Narayanan *et al*. (2012) reported the antibacterial activities of *Vibrio* sp., and *Pseudomonas* sp., from ornamental fishes and evaluation of potential agent for its control. Sivakumar *et al*. (2014) also reported the antibacterial activity of intestinal bacterium *Escherchia fergusonii* of gold fish showed higher inhibition of pathogens which was noted by higher zone formation. Veerapan *et al*. (2009) reported the effect of probiotic bacteria *Lacto bacillus* on disease resistance of ornamental fish black molly against the pathogenic bacteria of *Vibrio alginoluticus*. Jawanar Abraham (2008) also reported the antibacterial activity of intestinal bacteria *Lactobaccilus* sp., from gold fish and the Zone of inhibition was higher against pathogens.

Conclusion

The present study demonstrate *Pseudomonas* sp., (ZB2) was isolated from the intestinal content of Zebra fish shows higher enzymatic productivity of amylase, cellulase and protease which present a scope for fish nutritionists to utilize the enzyme producing bacteria as a probiotic formulating cost effective fish diets. Also antibacterial activity of intestinal bacteria *Pseudomonas* sp., (ZB2) has higher zone of inhibition against *Bacillus cereus.* It can be recommended to the preparation of probiotic feed.

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