ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 11, pp.6468-6477, November, 2017

RESEARCH ARTICLE

CHRACTERIZATION, DIGESTIVE ENZYME PRODUCTIVITY AND ANTI BACTERIAL ACTIVITY OF INTESTINAL BACTERIA OF ROHU *LABEO ROHITA*

***Sivakumar, P., Suganya, D. and Rajan, M.R.**

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

ARTICLE INFO

Article History:
Received 17th August, 2017
Received in revised form
24th September, 2017
Accepted 06th October, 2017
Published online 30th November, 2017

Key words:

Characterization,
Enzymatic Productivity,
Antibacterial activity,
Intestinal
Bacteria and *Labeo rohita*.

ABSTRACT

The present study deals with the characterization, digestive enzymatic productivity and antibacterial activity of intestinal bacteria of Rohu *Labeo rohita*. Pure colonies were isolated from the intestinal content of Rohu was characterized morphologically and biochemically using the Berge's manual of Determinative bacteriology. The isolated and identified intestinal bacteria was *Aeromonas* sp., (R1), *Pseudomonas* sp., (R2), *Bacillus* sp., (R3), *Enterobacter* sp., (R4) and *E.coli* sp., (R5). The isolated intestinal bacteria were subjected for its efficacy to produce digestive enzymes like amylase, cellulase, lipase, and protease were studied using selective media. The intestinal bacteria of *Enterobacter* sp., (R4) was higher enzymatic productivity and other was lower productivity. The antibacterial activity of intestinal bacteria was higher zone of inhibition was *Enterobacter* sp., (R4) with compare to the commercial antibiotics of Gentamycin. Based on the results the *Enterobacter* sp., (R4) was higher enzymatic productivity and higher inhibition of antibacterial activity of intestinal bacteria in Rohu. The presence of this organism in the intestinal flora of the fish enhance the probiotic nature and helps in the nutritional benefits of fish.

INTRODUCTION

Aquaculture has become an important economic activity in many countries. It's tremendous and judicious exploitation of the resource contributes a sizable production of aquaculture. It is the most appropriate tool for poverty reduction, food security and overall rural development (Subasinghe, 2005). Fish receive bacteria in the digestive tract from aquatic environment through water and food that are populated with bacteria. Being rich in nutrient the environment of digestive tract of fish confers a favorable culture environment for the microorganisms but some major problems are hindering the progress path and disease being one of them. Hence for successful aquaculture we must ensure quality feed, good environment and disease free seeds. The enzymes producing intestinal bacteria, their source and significant in fish is scarce. The relative amount of amylase, cellulose, lipase and protease producing bacteria in the intestinal tract of fishes. Intestinal isolates were evaluated for extra cellular enzyme producing capacities (Nibeditakar and Koushik Gosh, 2008).

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

The microorganisms used as probiotics, including yeasts, lactic acid bacteria, *Pseudomonas* sp., have been originally incorporated into feed to increase the animals growth and improve its health by increasing its resistance to disease (Samsh H. Saged *et al.,* 2011). The study related to the isolation, enzymatic characterization and anti bacterial activity of intestinal bacteria of Rohu *Labeo rohita* is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

Collection of sample and isolation of bacteria

The experimental fish Rohu *Labeo rohita* (4.25±0.023) was collected from SGC Fish form Periyakulam, Theni District, 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 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°C for 24hrs.

Identification of bacteria

After incubation the colonies were enumerated and the predominant colony were selected and identified based on the morphological, microscopic and biochemical characteristics like Gram staining, Indole test, Methyl red test, Voges-proskauer test, Citrate, Starch test, Gelatin hydrolysis test, using Bergey's Manual of Determinative Bacteriology, (1994).

RESULT AND DISCUSSION

The biochemical characterization of intestinal bacteria of Rohu is presented Table 1.

Nibedita Kar and Koushik Ghosh (2008) reported higher enzymatic productivity of intestinal bacteria of *Pseudomonas* sp., in *Labeo rohita*. Abhinanda Bairagi *et al.,* (2002) reported the distinct microbial source of the digestive enzyme Amylase, Cellulase, lipase and Protease apart from the endogenous source in fish gut. Ananth *et al.,* (2015) also reported the enzymatic productivity of *Bacillus* sp., from the intestine of Koi carp *Cyprinus carpio var koi*. The antibacterial activity of intestinal bacteria of Rogu was given table 3. The intestinal bacteria of *Enterobacter* sp., (R4) was higher zone of inhibition and other was lower zone formation using fish pathogens of *Staphylococcus aereus* (P1), *Shigella sonnei* (P2), *Enterococcus faecalis* (P3), *Pseudomonas aeruginosa* (P4) *and Klebsilla pneumonia* (P5) compare with Commercial Antibiotics of Gentamycin.

Table 1. Biochemical characterization of intestinal bacteria of Rohu *Labeo rohita*

Tests	R1	R 2	R 3	R 4	R 5
Simple staining	Rods	Rods	Cocci	Rods	Rods
Gram's staining	Negative	Negative	Positive	Positive	Negative
Motility	Motile	Motile	Motile	Non-Motile	Motile
Indole	Positive	Negative	Positive	Positive	Positive
Methyl Red	Positive	Negative	Negative	Positive	Positive
Voges Prokauser	Positive	Negative	Negative	Positive	Positive
Citrate	Positive	Positive	Positive	Negative	Not performed
Catalase	Positive	Positive	Positive	Positive	Positive
Starch	Positive	Positive	Negative	Negative	Negative
Gelatin hydrolysis	Positive	Positive	Positive	Negative	Negative
Lipid	Not Performed	Positive	Positive	Negative	Positive
Identification result	*Aeromonas sp.,*	*Pseudomonas sp.,*	*Bacillus* sp.,	*Enterobacter* sp.	*E.coli. sp.,*

Table 2. Enzymatic productivity of intestinal bacteria of Rohu *Labeo rohita*

Fish species	Bacterial strains	Amylase	Cellulase	Lipase	Protease
Rohu *(Labeo rohita)*	*Aeromonus* sp.,(R 1)	++	+++	++	++
	Pseudomonas sp., (R 2)	++	+	++	++
	Bacillus sp., (R 3)	++	++	+	++
	Enterobacter sp. (R 4)	+++	++	+++	+++
	Escherichia sp., (R 5)	++	+	++	++

Table 3. Antibacterial activity of intestinal bacteria of Rohu *Labeo rohita*

S.No	Intestinal Bacteria	Zone of Inhibition in mm									
		P1	CA	P2	CA	P3	CA	P4	CA	P5	CA
1	*Aeromonus* sp.,(R 1)	08	04	07	04	09	05	08	05	06	04
2	*Pseudomonas* sp., (R 2)	06	04	08	05	08	06	07	04	05	04
3	*Bacillus* sp., (R 3)	07	05	06	04	07	07	06	03	04	06
4	*Enterobacter* sp. (R 4)	10	06	09	06	10	06	09	04	08	04
5	*Escherichia* sp., (R 5)	07	04	07	05	07	05	05	03	07	06

CA – Commercial Antibiotic (Zendamycin), P1-*Staphylococcus aureus*, P2-*Shigella sonnei,* P3-*Enterococcus faecalis*, P4-*Pseudomonas aeruginosa,* P5-*Klebsilla pneumoni.*

From present study, the isolated and identified bacteria from intestine of Rohu *Labeo rohita* was *Aeromonas* sp., (R1), *Pseudomonas* sp., (R2), *Bacillus* sp., (R3), *Enterobacter* sp., (R4) and *Escherichia* sp., (R5). The bacteria isolated from the intestinal tract of fresh water fishes are generally colonized by *Aeromonus* sp., *Entereobacter* sp., and *Micrococcus* sp., (Horshey *et al.,* 1997). Shubhadeep Ghosh *et al.,* (2007) reported the isolation and identification of intestinal bacteria such as *Pseudomonas* sp, *Aeromonus* sp, and *Bacillus* sp., from Indian major carps. Sivakumar *et al.,* (2014) also reported the isolation and identification of intestinal bacteria from Common carp *Cyprinus carpio.* The enzymatic productivity of intestinal bacteria of Rohu was given table 2. The higher enzymatic productivity of intestinal bacteria was *Enterobacter* sp., (R4) and other bacteria the productivity is lower.

The isolated strain showed maximum inhibition against the pathogenic strain and selected for further study. Ana Zapata (2013) reported that the antibacterial activity of lactic acid bacteria showed high ability to inhibit growth of fresh water fish pathogens particularly *Vibrio* sp., and *Mycobacterium* sp. and lactic acid bacteria have high potential probionts to use in Nile tilapia. Antibacterial ability and growth promoting effects of feed supplemented with probiotic bacterium isolated from gut micro flora of *Cirrinus mrigala* (Anita Bhat Nagar and Ritu Lamba 2014). Parthasarathi and Ravi (2011) also reported the antibacterial effect of *Bacillus* sp., and *Aeromonus* sp., in *Catla catla* combination with two probiotic bacteria. So the present study, based on the biochemical tests, enzymatic productivity and antibacterial activity, the selected bacteria was *Enterobacter* sp., (R4).

Conclusion

The present study concludes that the isolated and identified intestinal bacteria was *Aeromonas* sp., (R1), *Pseudomonas* sp., (R2), *Bacillus* sp., (R3), *Enterobacter* sp., (R4) and *Eschershia* sp., (R5) the selected bacteria was indicates there is a distinct microbial source of digestive enzymes (Amylase, cellulase, lipase, and protease) apart from the endogenous sources in fish gastrointestinal tracts and the higher inhibitory zone of antibacterial activity was *Enterobacter* sp., (R4). So the presence of this organism in the intestinal flora of the fish enhance the probiotic nature and helps in the nutritional benefits to the fish.

REFERENCES

Abhinanda Bairagi, Keka Sarkar Ghosh, Sukantakumar Sen, and Arunkumar Rai 2002. Enzyme producing bacterial flora isolated from fish digestive tract. Aquaculture International, 10: 109 - 112.

Ana, A., Zapata and Maurilio Lara-Flores 2013. Antimicrobial activity of Lactic acid bacteria strains isolated from Nile Tilapia Intestine (*Oreochromis niloticus*). *Journal of Biology and Life Science*, (1): 164 - 171.

Ananth, A., Rajan, M.R and Sivakumar, P 2015. Isolation, identification, enzymatic productivity, antibacterial activity and molecular characterization of intestinal bacteria of Koi carp (*Cyprinus carpio var. koi*) and its role on growth. *International Journal of Scientific Research,* 4 (6) : 582 - 585.

Anita Bhatnagar and Ritu Lamba 2014. Antimicrobial ability and growth promoting effect of feed supplemented probiotic bacterium isolated from gut microflora of Cirrhinus mrigala. *Journal of Integrative Agriculture Advance Online Publication,* 3119 (14): 1 - 18.

Bergey's Manual of Determinative Bacteriology 1994. Williams and Wilkins, 428 East Presto street, Baltimore, Maryland. 21202, USA.

Horseley R.W. 1997. A review of the bacterial flora of teleosts and elasmobranches, including methods for analysis. *Journal of Fish Biology,* 10: 529 - 553.

Nibedita Kar and Koushik Ghosh 2008. Enzyme producing bacteria in the gastrointestinal tracts of *Labeo rohita* (Hamilton) and *Channa puntatus* (Bloch). *Turkish Journal of Fisheries and Aquatic Science,* 8 : 115 - 120.

Parthasarathi, R and Ravi, D. 2011. Probiotic bacteria as growth promoter and biocontrol agent against *Aeromonas hydrophila* in *Catla catla* (Hamilton, 1822), *Indian Journal of Fisheries,* 58 (3) : 87 – 93.

Sameh H. Sayed, Zakaria, A., Mohamed, G.A and Mohamed, K.K 2011. Use of probiotics as growth promoter, anti-bacterial and their effects on the physiological parameters and immune response of *Oreochromis Niloticus* Lin. Fingerlings. *Journal of the Arabian Aquaculture Society*, 6 (2) : 201 - 221.

Shubhadeep Ghosh., Archana Sinha and Chittaranjan Sahu 2007. Isolation of pupative probionts from the intestine of Indian major carps. *The Israeli Journal of Aquaculture - Bamidgeh,* 59 (3): 127 - 132.

Sivakumar, P., Rajan, M. R., and Ramachandran, P. 2014 Effect of probiotics on growth performance of Common carp *(Cyprinus carpio var communis) International Journal of Pharma and Bio Sciences.* 5 (1) : 835 - 839.

Subasinghe, R.P 2005. Epidemiological approach to aquatic animal health management : opportunities and challenges for developing countries to increase aquatic production through aquaculture, *Prev. Vertinary Medicine*, 67: 117 - 124.

****