Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(6): 509-511

Received: 19-09-2019
Accepted: 23-10-2019

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Isolation of lactobacillus species from gastrointestinal tract of gift tilapa (*Oreochromis nloticus*) as potential probiotics

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Abstract
Genetically improved farmed tilapia (*Oreochromis niloticus*) is the food fish of 21st century; it can grow in marine as well as freshwater environments and omnivorous fish. The culture practice of tilapia is providing nutritional and income security to small scale farmers all over the world. Current study was designed to isolate lactobacillus species from gastrointestinal tract of GIFT tilapia (*O. niloticus*) as potential probiotic. The whole gut of GIFT tilapia fingerlings was taken for the study on MRS agar. Three colonies were isolated on the basis of colony morphology similar to lactobacillus which was further conformed by biochemical test like catalase negative and endospore forming bacteria were identified as lactobacillus species. For probiotic potential enzymatic activity index was measured for two selected colonies C: 2 showed the lipase activity 7mm; while C: 5.6mm. For protease activity C: 2 and C: 3 showed 8 and 7.5mm respectively. Both the colonies were amylase negative. The findings of the study indicate that the gastrointestinal tract of GIFT tilapia (*O. niloticus*) contains lactobacillus species, which helps in utilization of feed protein and lipids.

Keywords: GIFT tilapia, protease, lactobacillus, biochemical test, catalase

Introduction
Aquaculture industry represents rapidly growing animal protein producing sector in the world and the sector contributes 80 million tons of food fish and 30.1 million tons of aquatic plants to the world fish production of 179.9 million tons [1]. The demand of fish production from this sector is estimated to double in 2030 so the researchers are focusing to make sustainable production from this sector in terms of disease resistance, improved immunity, faster growth rate and adaptibility to the changing climate. GIFT tilapia is the selective breeding strain of tilapia (*Oreochromis niloticus*) which shows 60% faster growth and 50% better survival at harvest than most commonly farmed strain of tilapia [2, 3]. Tilapia is called as food fish of 21st century as well as aquatic chicken and second most farmed fish in the world, providing nutrition and livelihood security to small scale farmers. Tilapia is omnivorous fish can be cultured in both freshwater and marine waters due to wide tolerance of salinity. The gut of *Oreochromis niloticus* was also evaluated for presence of extracellular enzymes producing microbes as potential probiotics by researchers [4] as the gut microbiota study of marine and fresh water fishes is centre of attraction for scientific researchers as the findings of the several authors indicated towards the symbiotic role of gut microflora with their host [5]. Various authors attempted to use beneficial gut bacilli isolated from the gut of rohu fish as probiotics for fishes [6, 7]. The gastrointestinal tract of *Oreochromis* species have been evaluated for microbial colonization, extracellular enzyme production and isolation of pathogenic bacteria previously [8-9]. Gastrointestinal tract of two tilapia species viz. *Oreochromis mossumbicus* and *O. niloticus* revealed heterotrophic beneficial bacterial populations in both the species [10]. The gastrointestinal microbes of fishes are supposed to improve the immunity and feed utilization in the fishes and ultimately enhancing the growth. Hence the current research work was designed to isolate and identify lactobacillus species from gastrointestinal tract of GIFT tilapia (*Oreochromis niloticus*).

Materials and Methods
1. Sample collection
Fingerlings of *Oreochromis niloticus* (GIFT tilapia) were procured from Manikonda tilapia

Hatchery, near Vijaywada, Andhra Pradesh. Fingerlings of GIFT tilapia (*O. niloticus*) were randomly selected for isolation of symbiotic bacterial flora from gastrointestinal tracts [11]. The gastrointestinal tract was emptied and thoroughly rinsed in sterile 0.9% saline in order to remove non-adherent bacteria. Then it was separately homogenized with 10 parts of chilled 0.89% sodium chloride solution for sample preparation [12].

2. Preparation of MRS media (De man Rogosa and Sharpe) and inoculation of bacterial samples

MRS media was prepared by adding 1% of sterilized potassium sorbate in the media before pouring. Then 250µl of respective dilutions (10^{-3}, 10^{-4} and 10^{-5}) were used for spreading in duplicate to avoid any contamination. The plates were incubated inside the anaerobic jar at room temperature for 48 hrs at 37˚C.

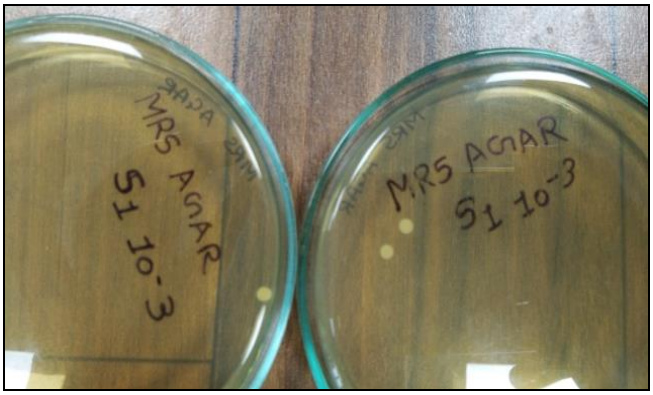

Fig 1: Lactobacillus colonies on MR Sagar

Estimation of probiotic potential of isolated colonies

The probiotic potential was estimated by measuring enzymatic activity index value for protease, amylase and lipase enzyme production on milk agar, starch agar and tributrin agar.

$$\text{Enzymatic activity index (mm)} = \frac{\text{Colony diameter + Holo diameter}}{\text{Colony diameter}}$$

Results and Discussion

After incubation, the three different colonies observed and further proceded for colony morphology study (Table 1), gram's staining and biochemical analysis (Table 2). The results of the biochemical test indicated that colony 2 (C:2) and colony 3 (C:3) showed similarity with lactobacillus species as lactobacillus species are endospore forming catalase negative rod shaped gram's positive rods [13]. The results were also supported by Dhanasekaran *et al.*, (2010) [14] according to them the isolated microbes, which are catalse negative, oxidase negative, VP negative, citrate negative and endospore positive might confirm the *Lactobacillus* sp. Colonies C:2 and C:3 were further analyzed for production of digestive enzymes as potential probiotics. The results of enzymatic activity index indicated that these colonies produced considerable amount of protease, lipase and did not produce amylase. The findings of current study were supported by the findings of Ghosh *et al.* (2017) [15]. They investigated ten out of 97 isolated bacteria as the efficient exo-enzyme producing strains from the gut of nile tilapia (*O. niloticus)* as novel probiotics, based on extracellular digestive (amylase, protease and lipase) and degradation (cellulase, phytase, and xylanase) enzymes-producing ability, pathogen inhibition and bio-safety. They identified best amylase producing bacteria as *Bacillus licheniformis* (KT362744), through 16S /18S rRNA gene fragment analysis. The findings of their study was also supported by Alonso *et al.* (2019) [16] as they isolated 45 gram-positive strains with antimicrobial activity from the gut microbiota of 13 marine fishes and out of which, nine were identified as lactic acid bacteria by 16S rRNA gene sequencing. These isolates were five *Lactococcus lactis* subsp. *lactis*, two *Enterococcus* sp., one *Lactobacillus plantarum*, and one *Leuconostoc mesenteroides* subsp. *mesenteroides*. The findings of the current study were also supported by Ray *et al.* (2012) [17]. They reviewed enzyme producing bacteria isolated from fish gut and suggested that fish gut microbiota might have positive effects to the digestive processes of fish. The findings of the current study indicated that gastrointestinal tract of GIFT tilapia contains potential probiotic lactobacillus species, which helps in improving feed utilization and improving immunity in GIFT tilapia (*O. niloticus*) under cultured condition.

Table 1: Colony morphology study of isolated colonies

S. No	Size	Colour	Shape	Elevation	Margin	Surface	Gram's staining
1	Medium	Creamy white	Circular	Raised	Entire	Moist	Bacilli (+)
2	Medium	Creamy white	Circular	Raised	Entire	Moist	Bacilli (+)
3	Small	Creamy white	Circular	Raised	Entire	Moist	Bacilli (+)

Table 2: Biochemical identification of colonies

Biochemical parameters	Colonies		
	C:1	C:2	C:3
a. Gram's staining	+	+	+
b. Endospore staining	+	+	+
c. Catalase test	+	–	–
d. Cellulase prduction	+	+	+
e. Citrate utilizaion	+	–	–
f. Lipase production	+	+	+
g. Amyase production	+	+	+
h. Protease production	+	+	+
i. DNAse reduction	+	+	+

Table 3: Enzymatic activity index of isolated colonies

Enzymes	Colonies	Colony diameter (mm)	Holo diameter (mm)	Enzyamtic activity index (EAI)
Amylase				
	C:2	2	0.0	0.0
	C:3	2	0.0	0.0
Lipase				
	C:2	2	12	7
	C:3	3	14	5.6
Protease				
	C:2	2	14	8
	C:3	2	13	7.5

Acknowledgement
The authoress is highly thankful to ICAR for financial assistance in the form of ICAR SRF and ICAR- CIFT, Veraval for guidance and laboratory facilities. She is also thankful to College of Fisheries Science, Junagadh Agricultural University for providing all the facilities for research.

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