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MICROBIAL DIVERSITY AND SCREENING CHARACTERIZATION OF ETROPLUS SURATENSIS

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ABSTRACT

Isolation and enumeration of microbial diversity from the gastro intestinal tract (foregut, midgut and hindgut) of Etroplus suratensis have been carried out to find out their importance in the nutrition of the host fish. Proteolytic, amylolytic, lipolytic and cellulolytic bacteria were detected in the fish gut. Among specific enzyme producing bacteria, proteolytic and cellulolytic bacteria were present at higher number within the foregut, midgut and hindgut of E. suratensis respectively. Altogether twenty bacterial genera were identified by biochemical approaches. In general Bacillus sp., Pseudomonas sp. and Micrococcus sp. were the dominant groups, which were followed by Enterobacter, Klebsiella, Staphylococcus, Flavobacterium and Chromobacterium.

KEYWORDS: Etroplus suratensis, gastrointestinal tract, enzyme producing bacteria.

INTRODUCTION

Gut microbes are known to play crucial roles in maintaining gut integrity, in strengthening immunity and disease resistance and in contributing to digestion in higher animals (Cahill, 1990). The variation in the microbial flora in different fish species depending of nutrition, intestinal micro environmental age, geographical location, environment factors, stress and etc., (Yang et al., 2007). The bacterial flora of the gastrointestinal tract in general represents a very important and diversified enzymatic potential and it seems logical to think that the enzymatic mass lodged in the digestive tract might interfere in a considerable way with a major part of the metabolisms of the host animal (Bairagi et al., 2002).

MATERIALS AND METHODS

Fish collection

Healthy fishes were collected from the Rajakkamangalam estuary. Fishes were collected using cast nets and were transported to the laboratory in plastic containers immediately.

Collection of Gastrointestinal tract

The collected fishes were starved for 24h in order to make their intestinal tract clear and also to eliminate the bacteria that were transit in nature. After starvation period, the fishes were sacrificed and gastrointestinal tract was removed aseptically.

Sample preparation

A homogenate solution was made by grinding GI tract with 0.89% sodium chloride solution (10: 1 volume/weight) (Das and Tripathi, 1991). Serial dilutions were made by mixing homogenate solution with sterilized distilled water using vortex mixer to use as inoculums.

Screening of isolates for extracellular production

(a). Protease activity

The culture were streaked as single streak on skim milk agar plates and incubated at 37^{o}C for 24-48h. Presence of zone of clearance surrounding the culture streak was taken as a measure of protease production.

(b). Amylase activity

Cultures were streaked on starch agar plates and incubated at 37^{o}C for 24-48h. The culture plates were then flooded with 1% lugol, iodine solution to identify amylase activity by formation of transparent zone arrounding the colony.

(c). Cellulase activity

The strain was streaked single line on the Carboxy Methyl Cellulose (CMC) plates incubated at 37^{o}C for 48h. After incubation the plates were flooded with congo

red dye and then NaCl solution. Appearance of clear zone due to hydrolysis CMC around the bacterial colony indicates cellulose production on the medium.

(d). Lipase activity

The bacterial isolates were streaked on sprit blue agar plates and sterilized by autoclaving at 121°C for 20min. Formation of zone around the colony indicates the lipid synthesis.

Characterization and identification of the bacterial isolate

The characterization experiments and identification were performed as per the 8th edition of the Bergey's Manual of Determinative Bacteriology.

RESULTS

Total Bacterial Count

Total bacterial count (CFU/ml) recorded in the gastro intestinal tract of the experimental fish is presented in the Table 1. The maximum number of bacterial count (15 CFU/ml) was recorded in hindgut in 10^{-4} dilution when compared with foregut and midgut.

Table 1: Bacterial count (CFU/ml) in the foregut, midgut and hindgut of E.suratensis.

Sl. No.	Experimantal tissues	Dilution factor	Average (CFU/ml)
1	Foregut	10^{-1}	180
		10^{-2}	80
		10^{-3}	43
		10^{-4}	8
2	Midgut	10^{-1}	195
		10^{-2}	93
		10^{-3}	40
		10^{-4}	12
3	Hindgut	10^{-1}	198
		10^{-2}	97
		10^{-3}	52
		10^{-4}	15

Screening of proteolytic bacteria

Out of seven foregut bacterial isolates, five showed protease enzyme production. Likewise among eight midgut isolates five showed positive results. In the hindgut region, out of eight isolates six showed protease activity.

Screening of amylolytic bacteria

In the for gut, out of seven bacterial isolates four showed amylase enzyme production. In the midgut, four showed positive results, out of eight isolates. Likewise, four among eight isolates from hindgut showed amylase activity.

Screening of lipolytic bacteria

Out of seven foregut bacterial isolates, two showed lipase enzyme production. But in the midgut only one showed positive result out of eight isolates. Likewise, three among eight were showed lipase activity in the hindgut.

Screening of cellulolytic bacteria

In the foregut seven bacterial isolates were screened and among them four showed cellulose enzyme production. Among eight midgut bacterial isolates, five showed positive results. Likewise, five among eight isolates from hindgut showed cellulose activity.

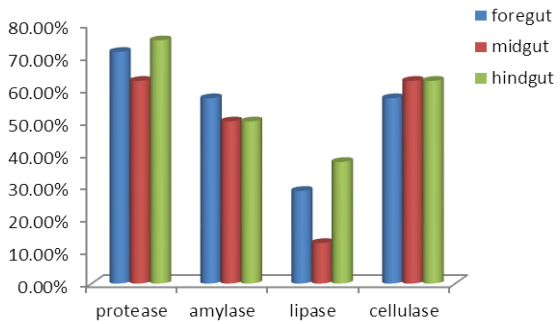

Fig. 1: Percentage occurrence of enzyme producing bacterial strains from various regions of E. suratensis.

Identification of bacterial strains

According to Bergey's Manual of Determinative Bacteriology, seven bacterial isolates from foregut, midgut and hindgut. Among the bacterial strains Bacillus sp., Pseudomonas sp. and Micrococcus sp. were the dominant groups which were followed by Enterobacter, Klebsiella, Staphylococcus, Flavobacterium and Chromobacterium.

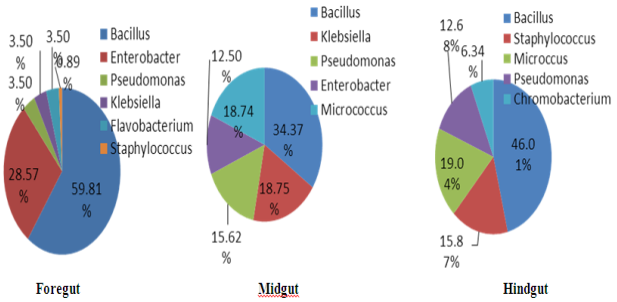

Fig. 2: Percentage Abundance of Bacterial Strains Isolated from Various Regions of E. Suratensis.

DISCUSSION

Fishes are continuously exposed to the microorganisms present in water and in sediment including the contaminants in sewage/faeces. They receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Majority of the organisms found in gut region were found to be Bacillus sp. and Pseudomonas sp. Both marine and freshwater fishes have been shown to have a specific indigenous microflora (Horsley, 1977; Ringo et al., 1995).

In order present study, the population density of the microflora in E.suratensis usually varies quantitatively and qualitatively in the gut regions. In the GI tract itself the bacterial diversity showed variation between foregut, midgut and hindgut regions. Venugopalan et al., 1985, suggested that the quantitative variation between the microbes was in the following order, foregut<midgut<hindgut in E.suratensis. The progressive increase of bacteria from midgut to hindgut might be due to the variation in concentration of nutrients and also the presence of faecal matter (Venugopalan et al., 1985). Lee and Lee (1995) noted that higher populations in the digestive tract of Dover Sole (Solea solea) with $5.2x10^5$, $8.0x10^5$ and $9.8x10^6$ CFU/g recovered from the stomach-foregut, midgut and hindgut- rectum respectively.

In the present study suggests that, on the basis of their enzyme production ability, amylase producing bacteria were found to be highly colonized in midgut and hindgut region rather than in the foregut. On the other hand cellulose and protease producing strains were highly colonized in the hindgut regionrather than in the foregut and midgut. Lipase producing bacteria were found to be highly colonized in hindgut region. In general the bacterial flora of the gastro intestinal tract represents a very important diversified enzymatic potential and it seems logical to think that the enzymatic mass lodged in the digestive tract might interferes in a considerable way with a major part of the metabolism of the host animal (Clements, 1997).

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REFERENCES

1. Bairagi, A., Ghosh, K., Sen, S. K. and Ray, A.K., Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 2002; 10: 109-121.
2. Cahill, M.M., Bacterial flora of fishes: a review. Microbial Ecology, 19: 21-41.
3. Clements, K. D., Fermentation and Gastrointestinal microorganisms in fishes, in gastrointestinal microbiology, New York: Chapman and Hall, 1997; 1: 156-198.
4. Horslay, R.W., A review of the bacterial flora of teleosts and elasmobranches, including methods for its analysis. Journal of Fish Biology, 1997; 10: 529-553.
5. Lee, S. and Lee, Y., Identification of intestinal microflora in rainbow trout. J. Microbiol., 1995; 33: 273-277.
6. Ringo, E., Strom, E. and Tabachek, J.A., Intestinal microflora of salmonids: a review. Aquaculture Research, 1995; 26: 773-789.
7. Venugopalan, V.K., Nandakumar, R. and Ramesh, A., Microflora of pond cultured milkfish. Chanos chanos (Forskal). In: Proceeding of the symposium on harvest and post-harvest technology. Ravindran et al., (Eds.), 1985; 680-688.
8. Yang, J., Nie, H., Chen, I., Zhang, X., Yang, F., Xu, X., Zhu, Y., Yu, J., Jin, Q., Revisiting the molecular evolutionary history of Shigella sp. Journal of Molecular Evolution., 2007; 64: 71-79.