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Analysis of gut microflora of fish, *Ophiocephalus striatus* in the selected ponds at Kanyakumari District, Tamilnadu

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The gut microflora of the murrel *Ophiocephalus striatus* in Kanyakumari district, Tamilnadu was estimated quantitatively and qualitatively, and the isolates were identified at species level. Total viable microbial count measured a highest range of $9.49 \pm 0.25 \times 10^6$ CFUg^{-1} and a lowest range of $6.12 \pm 0.09 \times 10^6$ CFU g^{-1} in the digestive tract of *O. striatus* collected from pond C and A respectively. Six bacterial genus were isolated and identified in the digestive tract of the fish, viz., *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas fluorescens*, *Micrococcus* sp., *Bacillus* sp., and *Proteus rettgeri*. The total viable microbial count and bacterial species were also isolated and identified from the water samples and bottom sediments of the selected ponds.

Gut Microflora /Microbiology

The digestive tract of fish consists of aerobic, facultative anaerobic and obligate anaerobic bacteria (Gomez and Balcazar, 2008), but the composition has been suggested to change with host age, nutritional status, environmental conditions and the complexity of the fish digestive system (Eddy and Jones, 2002; Verner-Jeffreys et al. 2003). In general, the gut microflora has been suggested to hinder the colonization of pathogenic bacteria (Spanggaard et al.2001), stimulate immune response (Olafsen, 2001) or produce some beneficial bioactive substances such as essential fatty acids (Ringo et al. 1992), vitamins (Sugita et al.1991), digestive enzymes (Skrodenyt - Arba_iauskien, 2007) and antibacterial substances (Sugita et al. 2002). Therefore, it is generally accepted that there is a possible symbiotic relationship between fish and gut microflora (Verschuere et al. 2000). During the life cycle, the growth rate of fish varies greatly and can be affected by a variety of factors such as temperature, water quality available nutrition (Baltz et al.1998). In addition, the gut microflora plays a very important role in the health and growth of the host (Vine et al. 2006; Comstock, 2007; Mazmanian et al. 2008). Evidence indicates that the bacteria found in the digestive tract of fish was highly variable and were a reflection of their aqueous environment, especially the food choice of the individual fish (Nieto et al. 1984). Reports indicate that bacteria such as *Pseudomonas fluorescens*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Vibrio* sp. and *Myxobacteria* are ubiquitous in the aquatic environment (Allen et al. 1983). Certain bacteria such as *Salmonella* sp. or *Escherichia coli* could cause human diseases (Fernandes et al.1997).Contamination of these edible portions could originate from digestive tract of the fish. By monitoring the bacterial contents of fish digestive tract, the quality of fish can be measured. The purpose of this study is to isolate and identify the gut microbial diversity of *O. striatus*. They were collected in and around Nagercoil region during January to June, 2010.

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Materials and Methods

Fish examined

Carnivore, bottom feeder *Ophiocephalus striatus* (murrel) were sampled by gill net from the local ponds for the present study. Fishes were collected from three ponds Nullikulam, Thathayarkulam and Thoppukulam designated as pond A, B and C. During the sampling periods, the water temperature varied between 25°C to 28°C, pH 6.79 to 7.1 and dissolved oxygen 2.5 ml/l to 3.7ml/l. The average weight, total length (L_T) and gut length (L_G) of the fish were measured. Relative gut length was reported as the ratio of the gut length to the total length (L_G/L_T).

Post mortem examination

To isolate stable aerobic heterotrophic bacterial population from the digestive tracts, three fishes from each pond were starved for 24 hours in order to make their intestinal tract clear and also to eliminate the bacteria that were transit in nature. After starvation period, the digestive tract was removed from the fishes. A homogenate was made by adding the digestive tract with 0.89% sodium chloride (NaCl) solution (10: 1; volume: weight) (Das and Tripathi, 1991). Serial dilutions were made by mixing this homogenate solution with sterilized distilled water using vortex mixer to use as inoculums.

Microbial culture

Microbial culture of the homogenized digestive tracts of fish from each pond was carried out separately for isolation of bacteria. Diluted samples (0.3 ml) were poured aseptically within a laminar airflow on sterilized tryptone Soya Agar (TSA) to determine, the total heterotrphic bacterial population. Microbial culture of water samples and bottom sediments from each pond was carried out separately for isolation of bacteria.The genus and species of selected bacterial isolates were identified by various biochemical tests using the criteria provided in Bergey's manual of systemic bacteriological classification (Holt et al. 1994).

Results

Relative gut length

The digestive tract is relatively longer in *O. striatus* fish (8.4 ± 0.29 cm) in pond A and shorter (6.8 ± 0.37 cm) in pond B. Mean ± SD of relative gut length (LG/LT) of *O. striatus* was significantly shorter in pond A than the pond B (Table- 1).

Table - 1: Average weight, total length, weight of the gut, gut length and relative gut length of the *O. striatus*

Pond	Average weight (g)	Total length (cm) (L_T)	Weight of the gut (g)	Gut length (cm) (L_G)	Relative gut length (L_G/L_T)
A	51.60 ± 0.27	8.4 ± 0.29	4.13 ± 0.51	8.9 ± 0.28	1.06 ± 0.25
B	38.00 ± 0.75	6.3 ± 0.15	3.02 ± 0.40	6.8 ± 0.37	1.08 ± 0.31
C	46.33 ± 1.13	7.6 ± 0.71	3.71 ± 0.76	8.3 ± 0.65	1.09 ± 0.72

Results are mean ± SD of six determinants.

Bacteria in fish digestive tract

Analysis of bacterial flora in the digestive tract of the *O. striatus* fish examined showed higher bacterial population in the pond C (9.49 ± 0.25 x 10^6 CFU g^{-1}) and lower in pond A (6.12 ± 0.09 x 10^6 CFU g^{-1}) (Table 2).

Table - 2: Heterotrophic bacterial population in the digestive tract of *O. striatus*

Pond	Bacterial population in the digestive tract (10^6 CFUg^{-1})
A	6.12 ± 0.09
B	7.31 ± 0.18
C	9.49 ± 0.25

Results are mean ± SD of the six determinants

Bacteria in pond water

The total bacteria count recorded in the water samples of the three ponds A, B and C were given in the Table-3. The bacterial count recorded in the pond A, B and C showed a minimum of 19.42 ± 0.88 x 10^6 CFU ml^{-1}, 12.81 ± 0.039 x 10^6 CFU $ml^{-1}$and 20.41 x 10^6 CFU ml^{-1} respectively in station I and the maximum of 26.75 ± 0.34 x 10^6 CFU ml^{-1}, 30.25 ± 0.38 x 10^6 CFU $ml^{-1}$and 32.40 ± 0.59 x 10^6 CFU ml^{-1}respectively in station III (Table-3).

Table -3: Heterotrophic bacterial population of experimental animal *O. striatus*

Pond	Heterotrophic bacterial population					
	Pond water (10^6 CFU ml^{-1})			Bottom sediment (10^6 CFU g^{-1})		
	Stations			Stations		
	I	II	III	I	II	III
A	19.42 ±0.88	24.31±0.17	26.75±0.34	17.33±0.16	9.79±1.13	21.41±0.32
B	12.81±0.39	27.00±0.72	30.25±0.38	26.81±0.92	18.33±0.26	39.29±0.47
C	20.41±0.24	28.79±0.85	32.40±0.59	29.64±0.79	25.38±0.11	18.49±1.07

Results are the mean ± SD of six determinants

Table - 4: Identification of gut micro flora isolated from *O. striatus*

Pond	Microorganisms	Incidence (%)
A	*Bacillus* sp. (n = 27)	43.54 ± 1.19
	*Escherichia*s *coli* (n = 33)	53.22 ± 2.67
	Micrococcus sp. (n = 2)	3.22 ± 0.11
B	*Staphylococcus* sp. (n = 19)	26.38 ± 1.27
	Micrococcus sp. (n = 5)	6.94 ± 0.33
	Escherichia coli (n = 38)	52.77 ± 1.88
	Pseudomonas fluorescens (n =4)	5.55 ± 0.05
	Proteus rettgeri (n = 6)	8.33 ± 0.12
C	*Bacillus* sp. (n = 21)	30.88 ± 0.91
	Escherichia coli (n = 34)	50.00 ± 2.63
	Pseudomonas fluorescens (n=11)	16.17 ± 0.27
	Staphylococcus sp. (n = 2)	2.94 ± 0.27

Results are mean ± SD of the six determinants

Bacteria in pond sediments

The total bacterial count recorded in the bottom sediment of the three pond A, B and C were given in the table 3. In the pond A, the bacterial count recorded a minimum of 9.79 ± 1.13 x 10^6 CFU g^{-1} in station I and the maximum of 21.41 ± 0.32 x 10^6 CFU g^{-1} in station III. In the pond B, the bacterial count recorded a minimum of18.33 ± 0.26 x 10^6 CFU g^{-1} in station II and the maximum of 39.29 ± 0.47 x 10^6 CFU g^{-1} in station III. In the pond C, the bacterial count recorded a minimum of 18.49 ± 1.07 x 10^6 CFU g^{-1} in station III and the maximum of 29.64 ± 0.79 x 10^6 CFU g^{-1} in station I (Table- 3).

Identification of bacteria in the gut of the fish

The gut microbial species recorded in the experimental fish *O. striatus* from the pond A, B and C were given in the Table 4. The dominant group of gut microbial genera, *Escherichia coli* (53.22 ± 2.67%, 52.77 ± 1.88 and 50.00 ± 2.63% respectively) were recorded in the pond A, B and C and the least group of gut bacterial genera recorded were *Micrococcus* sp. (3.22 ± 0.11%) in pond A, *Pseudomonas fluorescens* (5.55 ± 0.05%) in pond B and *Staphylococcus* sp.(2.94 ± 0.27%) in pond C (Table- 4).

Identification of bacteria in the pond and bottom sediments

The microbial species recorded in the pond water and sediments of experimental pond A, B and C were given in the Table 5. The dominant group of microbial species *Escherichia coli* were recorded in the water samples of Pond A, B and C (43.67 ± 1.16%, 37.27 ± 0.96% and 37.50 ± 1.33% respectively) and the least group of microbial species *Klebsiella* (6.89 ± 0.93%) in pond A, *Pseudomonas fluorescens* (1.81 ± 0.01%) in pond B and *Proteus* (4.16 ± 0.17%) in Pond C. The dominant group of microbial genera *Escherichia* were recorded in the sediment samples of pond A, B and C (51.11 ± 2.41%, 36.44 ± 2.13% and 45.55 ± 3.12% respectively) and the least species of *Proteus rettgeri* in pond A and B (2.22 ± 0.07% and 1.86 ± 0.09% respectively) and *Klebsiella* (4.44 ± 0.28%) in pond C (Table -5).

Table - 5: Microflora isolated from the pond water and bottom sediments

Pond	Microorganisms	Incidence (%)
	In pond water	
A	*Bacillus* sp. (n = 31)	35.63 ± 1.43
	Escherichia coli (n = 38)	43.67 ± 1.16
	Proteus rettgeri (n = 8)	9.19 ± 0.14
	Klebsiella sp. (n = 6)	6.89 ± 0.93
	Micrococcus sp. (n = 4)	4.59 ± 0.07
B	*Bacillus* sp. (n = 27)	24.54 ± 1.03
	Escherichia coli (n = 41)	37.27 ± 0.96
	Staphylococcus sp. (n = 20)	18.18 ± 0.46
	Proteus rettgeri (n = 11)	10.00 ± 0.03
	Pseudomonas fluorescens (n =2)	1.81 ± 0.01
	Micrococcus sp. (n = 9)	8.18 ± 0.09
C	*Bacillus* sp. (n = 29)	30.20 ± 1.16
	Escherichia coli (n = 36)	37.50 ± 1.33
	Proteus rettgeri (n = 4)	4.16 ± 0.17
	Pseudomonas fluorescens (n= 7)	7.29 ± 0.25
	Staphylococcus sp. (n = 14)	14.58 ± 0.41
	Klebsiella sp. (n = 6)	6.25 ± 0.59
	In bottom sediments	
A	*Bacillus* sp. (n = 27)	30.00 ± 1.03
	Escherichia coli (n = 46)	51.11 ± 2.41
	Proteus sp. (n = 2)	2.22 ± 0.07
	Klebsiella sp. (n = 9)	10.00 ± 0.16
	Micrococcus sp. (n = 6)	6.66 ± 0.21
B	*Bacillus* sp. (n = 26)	24.92 ± 1.65
	Escherichia coli (n = 39)	36.44 ± 2.13
	Staphylococcus sp. (n = 18)	16.82 ± 1.01
	Proteus rettgeri (n = 2)	1.86 ± 0.09
	Pseudomonas fluorescens (n= 9)	8.41 ± 1.16
	Micrococcus sp. (n = 13)	12.41 ± 0.95
C	*Bacillus* sp. (n = 23)	25.55 ± 1.17
	Escherichia coli (n = 41)	45.55 ± 3.12
	Proteus rettgeri (n = 9)	10.00 ± 0.93
	Pseudomonas fluorescens (n= 5)	5.55 ± 0.06
	Staphylococcus sp. (n = 8)	8.88 ± 0.14
	Klebsiella sp. (n = 4)	4.44 ± 0.28

Results are mean ± SD of the six determinants.

Discussion

The results showed that bacterial diversity in the digestive tract of *O. striatus* varied in pond A, B and C. Generally, the bacterial diversity in the digestive tract of the fish vary due the hydrobiological fluctuations occurring in the natural systems (Rheinheimer,1985). Some workers suggested that the bacterial diversity in the digestive tract of the fish might be increased with the increase of water temperature (Hossain et al. 1999). In the present study total microbial density in the digestive tract of the *O. striatus* ranged from $6.12 \pm 0.09 \times 10^6$ g-1 to $9.49 \pm 0.25 \times 10^6$ g^{-1} in pond A,B and C. Our results are supported by Chowdhury et al. (1989). Sakata et al. (1984) and Sugita et al. (1985) who reported intestinal bacterial diversity of tilapia captured from various pond as 5.5×10^6 to 9.8×10^9 cfu g^{-1}. Ramakrishnan et al. (2008); Wache et al. (2006) reported that the increased bacterial count in *Cyprinus carpio* and *Oncorhynchus mykiss* due to the presence of probiotic diets. The predominant bacterial flora consisted of Gram-negative rods. The bacterial flora examined in the digestive tract of *O. striatus* in different ponds were *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas fluorescens*, *Micrococcus* sp., *Bacillus* sp. and *Proteus rettgeri*. These results are in partial agreement with those of Sakata *et al*. (1984), who observed that *Vibrio*, *Aeromonas* and *Pseudomonas* were the predominant bacterial genera in tilapia intestine. However, it differed with Dhanaraj et al. (2008) where *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Shigella* sp., *Aeromonas salmonicida*, and *Vibrio alginolyticus* were the dominant bacteria in the in the digestive tract of *Channa striatus*. The high prevalence of *Escherichia coli*, *Bacillus* sp., *Staphylococcus* sp., and *Pseudomonas fluorescens* throughout the study period suggests that these bacteria may be common bacterial commensals in the digestive tract of *O. striatus*. The dominance of the various species isolated in the present study, varied in different ponds and collectively it was observed that the presence of *Escherichia* coli, *Bacillus*, *Staphylococcus* sp. and *Pseudomonas fluorescens* were the dominant species in the water and sediment samples of the selected ponds. Similar findings were observed by Goni-Urriza et al. (2000); Sathyamurthy et al. (1991) and Simek et al. (2001) in water and sediments of different fresh water habitats. In present study, isolation of *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas fluorescens*, *Micrococcus* sp., *Bacillus* sp. and *Proteus rettgeri* in the digestive tract of *O. striatus* examined in different ponds shows that these pathogens are the important agents of food poisoning. The present results indicated that the commensal bacterial flora and bacterial pathogens which under stress could give rise to various diseases to fish. This information will help in controlling the storage quality of the fishery products.

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