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ASSESMENT OF MICROBIAL DIVERSITY IN GASTRO INTESTINAL TRACT OF ESTUARINE FISHES

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ABSTRACT

Bacterial strains were isolated from the gastrointestinal tract of seven fishes collected from Thengapattinam estuary of Kannyakumari District, Tamil Nadu. The fishes were identified by standard keys. An intestinal contents were removed aseptically from the fishes, from that bacterial flora was isolated by serial dilution-agar platting method. In this present study a total of 12 bacterial gut flora was identified by studied their cultural, morphological and biochemical studies viz. *Escherichia coli, Streptococcus sp, Staphylococcus sp, Micrococcci sp, Pseudomonas aeroginosa, Salmonella sp, Serratia sp, Shigella sp, Vibrio alginolytic, Vibrio parahaemolytics, Corynebacterium sp* and *Proteus sp.* Among these, *E. coli., Staphylococcus* and *P. auroginosa* were found as dominant gut flora.

KEYWORDS: Estuary fish, gastrointestinal tract, bacterial gut flora, & microbial diversity

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INTRODUCTION

The microflora of fish is a reflection of the aquatic environment in which it lives and colonized by pathogenic and non pathogenic bacteria in body surface, gills, and intestine[1]. The indigenous microflora of fish in aquaculture has been studied for many purposes. This includes, description of microbial spoilage[2], the relation between environment and fish micro flora[3], monitoring changes in fish form[4], microbial flora as food of fish[5], microbial flora help in production of enzymes[6] and antibiotic resistance profile of indigenous flora[7]. The micro flora of rearing fish hhave also been studied as a source of protection against diseases[8]. For all these reasons, study of bacterial flora of the gut is important[9]. Also, it is generally accepted that identification of the gastrointestinal tract microbiota is undoubtedly important for understanding the functional mechanisms between the microbes and the host [10]. The diversity of the gastrointestinal tract microbiota of fish is influenced by environmental factors such as ingested food and habitat[11]. However, the correlation between gut microbiota and its corresponding environmental microbiota is per not fully understood[12]. The objective of the study was to isolate and to analyze the diversity of bacteria, gut flora of estuarine fishes collected from Kanyakumari District.

MATERIALS AND METHODS

Sample Collection

In the present study fishes were collected from Thengapattinam estuary of Kannyakumari District, Tamil Nadu, using nets with the help of local fishermen and fishes were stored in an ice container. The collected fishes were identified by using standard keys described in FAO fish identification catalogue. Among the collected fishes, seven species were chosen for this study, because of their abundant distribution in this estuary. They were *Mugil cephalus* (Mullet), *Tilapia mossambicus* (Tilapia), *Etroplus suratensis* (Perl Spot), *Mystus gulio* (Catfish), *Caranx ignobilis* (Carangids), *Lenthrinces sp.* and *Gerus sp.*

Isolation and identification bacterial gut flora

The fishes were dissected out and the gastrointestinal tract was immediately removed under sterile condition. After measuring the length and weight of the gastrointestinal tract was opened aseptically and grounded well using 1% phosphate saline buffer. This was used as a stock solution for enumerating microbes. One ml of the stock solution was serially diluted upto 10^{-6} dilutions. Then 1 ml of the aliquot from each dilution was seeded in the nutrient agar by pour plate method. The inoculated plates were incubated at 35-37^{0}C for 48 hours and the bacterial growth was observed. The total number and morphologically different colonies were enumerated. After enumeration they were identified based on colony morphology, physiological and biochemical tests.

RESULTS

In the present study morphometric and gravimetric measurement of the gastrointestinal tract of the estuarine fishes was studies as the first part. The data on the morphometric and gravimetric measurements of the gastrointestinal tract (GT) of the selected estuarine fishes are mentioned in table 1. The total length of the fish varied from 8.70cm to 24.6cm being the maximum for *Mystus gulio*. The total weight of the fish was also varied much and it ranged between 20.0g to 153.0g. With respect to these variations the weight of the gut also varied and it ranged between 1.40 to 3.68g.

Table 1
Morphometric and gravimetric measurements of GI of the fishes

Sample code	Fishes	Total length of Fish(cm)	Total weight of Fish(g)	Total gut weight(g)
01	*Mugil cephalus*	20.6	132.0	3.68
02	*Tilapia mossambicus*	17.8	153.0	3.10
03	*Etroplus suratensis*	14.8	73.0	1.70
04	*Mystus gulio*	24.6	91.2	2.90
05	*Caranyx ignobilis*	10.9	37.0	1.60
06	*Lenthrinces sp.*	8.7	20.0	1.40
07	*Gerus sp.*	11.2	21.0	1.70

Table 2 provides the data on Total Viable Count (TVC) of aerobic heterotrophic bacterial population in the gastrointestinal tract of the selected estuarine fishes. Among the selected fishes the maximum average CFU ml-1 was recorded in *M. cephalus* (2.02 CFU ml-1×10^6), *T.mossambicus* (2.37 CFU ml-1×10^6), M.gulio (2.26 CFU ml-1×10^6), and C.ignobilis (2.24 CFU ml-1×10^6). On the other hand the minimum number of viable count (0.94 CFU ml-1×10^6), was recorded for the *Gerus* species. In *E.suratensis* and *Lenthrinces* sp. The average viable count recorded were 1.89 and 1.49 CFU ml-1×10^6, respectively. This results indicated that the maximum average viable colonies per gram gut was recorded in *E.suratensis* (1.10 CFU g-1 gut×10^6), *C.ignobilis* (1.40 CFU g-1 gut×10^6), and *Lenthrinces* sp (1.06 CFU g-1 gut×106). In all other species studied more or less uniform values were recorded.

Table 2
Total viable count of bacteria

Fishes	Dilutions					Average CFU $ml^{-1}×10^6$
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
Mugil cephalus	132	86	30	16	8	2.02
Tilapia mossambicus	TNTC	76	19	12	8	2.37
Etroplus suratensis	221	168	25	10	8	1.89
Mystus gulio	TNTC	42	19	8	8	2.26
Caranyx ignobilis	TNTC	44	20	7	8	2.24
Lenthrinces sp.	TNTC	31	14	8	5	1.49
Gerus sp.	TNTC	23	11	6	3	0.94

The distribution of the aerobic heterotropic bacterial population recorded in the gastrointestinal tract of the selected estuarine fishes are shown in table 3. The results clearly indicated that the distribution and generic composition of the aerobic heterotrophic bacterial population varied between fish species studied. For instance, in *M. cephalus* a total of 12 bacterial genera were identified. Among the identified genera *E.coli* (15.38%), *Staphyloccus* (15.38%) and *Pseudomonas aeroginosa* (15.38%) were the dominant microbes. The other microbial genera identified were *Micrococcus, Salmonella, Vibrio alginolyticus, Corynebacterium* and *Proteus* sp. and these genera were represented uniformly with 7.69% occurrence. In *T.mossambicus* eleven bacterial genera were identified. In this fish the bacterial genera like *Staphylococcus* (12.5%) and *P.aeroginosa* (18.75%) were dominated. In addition to this *V.alginolyticus* (12.50%) and *V.parahaemolyticus* (12.50%) were also represented (6.25%) bacterial genera were *Streptococcus, Micrococci* sp., *Salmonella* sp., *Serratia sp.,* and *Proteus sp.*

Table 3
Distribution of bacterial species

Si. No.	Bacterial genera	Fish species code						
		01	02	03	04	05	06	07
1	*Escherichia coli*	2(15.38%)	1(6.25%)	2(10.0%)	2(10.53%)	3(15.80%)	1(12.50%)	1(8.33%)
2	*Streptococcus sp*	1(7.69%)	1(6.25%)	2(10.0%)	2(10.53%)	1(5.26%)	1(12.50%)	1(8.33%)
3	*Staphylococcus sp*	2(15.38%)	2(12.5%)	3(15.0%)	3(15.80%)	1(5.26%)	1(12.50%)	1(8.33%)
4	*Micrococcci sp*	1(7.69%)	1(6.25%)	-	-	1(5.26%)	-	1(8.33%)
5	*Pseudomonas aeroginosa*	2(15.38%)	3(18.75%)	3(15.0%)	3(15.80%)	3(15.80%)	2(25.0%)	3(25.0%)
6	*Salmonella sp*	1(7.69%)	1(6.25%)	2(10.0%)	1(5.26%)	2(10.53%)	1(12.50%)	1(8.33%)
7	*Serratia sp*	1(7.69%)	1(6.25%)	1(5.0%)	2(10.53%)	2(10.53%)	-	1(8.33%)
8	*Shigella sp*	1(7.69%)	1(6.25%)	2(10.0%)	1(5.26%)	1(5.26%)	1(12.50%)	1(8.33%)
9	*Vibrio alginolytic*	1(7.69%)	2(12.50%)	3(15.0%)	3(15.80%)	3(15.26%)	-	-
10	*Vibrio parahaemolytics*	1(7.69%)	2(12.50%)	-	1(5.26%)	-	-	-
11	*Corynebacterium sp*	1(7.69%)	-	1(5.0%)	-	1(5.26%)	-	1(8.33%)
12	*Proteus sp*	1(7.69%)	1(6.25%)	1(5.0%)	1(5.26%)	1(5.26%)	1(12.50%)	1(8.33%)

In *E.suratensis* total 20 bacterial genera were screened. Among them *Staphylococcus, P. aeroginosa* and *V.alginolytic* were represented dominantly (15.0%). The next dominant (10%) genera were *E.coli*, *Streptococcus, Salmonella* sp., and *Shigella* sp. The less represented genera (50%) in this fish were *Serratia* sp., *Corynebacterium* and *Proteus* sp. In *M.gulio* 19 bacterial isolates were screened and 10 bacterial genera were identified. Among the identified genera *Staphylococcus, P.aeroginosa* and *V.alginolyticus* were the dominant (15.80%). The next dominant genera identified were (10.53%) *E.coli, Streptococcus* and *Serratia* sp. The less represented bacterial genera were (5.26%) *Salmonella* sp., *Shigella* sp., *V.parahaemolytics* and *Proteus* sp. In *C.ignobilis* 19 isolates were screened and 11 bacterial genera were identified. In this fish the dominant bacterial genera (15.80%) recorded were, *P.aeroginosa, V.alginolyticus* and *E.coli*. The next dominant genera were (10.53%), *Salmonella* sp. and *Serratia* sp. The less occurred bacterial genera in this were (5.26%) *Streptococcus, Staphylococcus, Micrococcus* sp., *Shigella* sp., *Corynebacterium* and *Proteus* sp. In *Lenthrinces* sp. 8 isolates were screened and seven bacterial genera were identified. In this fish the only dominant bacterial genera recorded was *P.aeroginosa*. The other less occurred (12.50%) bacterial genera were *E.coli, Streptococcus*, *Staphylococcus, Salmonella* sp, *Shigella* sp and *Proteus* sp. In *Gerus* sp. also 12 isolates were screened and ten bacterial gerera were identified. Among the identified genera, *P.aeroginosa* was the dominant one with 25% occurrence. The less dominant bacterial genera recorded were (8.33%). *E.coli, Streptococcus, Staphylococcus, Micrococcus, Salmonella sp., Serratia sp., Shigella sp., Corynebacteium and Proteus sp*.

DISCUSSION

In general the intestinal tract of fish is known to harbor indigenous facultative anaerobes as the predominant bacteria. If beneficial bacteria colonize more, the fish will grow at a faster rate in a healthy condition. On the other hand if pathogenic bacteria may colonize first in the gut, it will affect the host fish and eventually lead to death. Viewing the importance of fish microbes interaction, the present work was undertaken. Ecological studies on the intestinal and gill bacteria have been undertaken by several authors in several species[13]. Sugita *et al*.[14] studied the intestinal microflora of some coastal fish species in Japan, and they mentioned clearly about the common bacterial species and their population. Moreover the intestinal microflora of some selected carp species such as common carp *Cpprinus carpio, grass carp Ctenopharyngodom idella* and *Tilapia sarotherodon* (*Niloticus*) by Sugita *et al*.[15]. In the present study the distribution and

occurrence of aerobic heterotrophic bacterial population in the gastrointestinal tract of the chosen estuarine fish species clearly indicated the host species dependent variation. In *M.cephalus., E.coli., Staphylococcus* and *P.auroginosa* were dominant (15.38%) among the 12 identified genera. The other recorded bacterial genera were uniformly occurred (7.69%). In *T.mossambicus P.aeroginosa* were the dominant genera (18.75%) and the next dominant genera recorded were *Staphylococcus, V.alginolyticus* and *V.parahmolyticus* (12.50%). In this fish species the total 11 bacterial genera were recorded and others were uniformly distributed (6.25%). In *E.suratensis* three bacterial gerera such as *Staphylococcus, P.aeroginosa* and *V.alginolyticus* (15.0%) were dominated among the 10 identified isolates. A similar dominating trend was also noticed for *M.gulio* and here also 10 isolates were identified. In *C.ignobilis E.coli, P.aeroginosa, V.alginolyticus* (15.26%) was dominant among the 11 identified isolates. In *Lenthrinces* sp. and also in *Gerus* species *P.aeroginosa* was dominant among the 9 identified isolates. These results clearly indicated that *P.aeroginosa* was predominantly occurred in all the tested fish species and the occurrence of other species varied much. The variation in the number as well as percentage may be attributed to the prevalence of different environmental parameters in the gastrointestinal tract as well as different types of nutritional characteristics of the host species. This view has already been reported by the earlier workers like Campbell and Buswell[16], Sugita *et al*.[17] and Noguchi *et al*.[18, 19]. In conclusion the distribution of aerobic heterophic bacterial population and their composition varied much between fish species and were found to be host species dependent.

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