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"ISOLATION AND CHARACTERISATION OF BACTERIA ASSOCIATED WITH GUT MICROFLORA OF MULLET (*MUGIL CEPHALUS*)"

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Abstract
The gray mullet, Mugil cephalus, commonly referred to as the striped, gray, or black mullet is a species that inhabits tropical and subtropical coastal regions of the world between 42°N and 42°S .M. cephalus is an important commercial marine fish species for aquaculture . They have great importance in aquaculture and fishery trade of many countries as well as protein source. The gut microbiota is associated with many key functions of the host, such as resistance to infectious diseases and the decomposition of nutrients, and it provides the host with physiologically active materials, such as enzymes, amino acids and vitamins.

Microbial consortium that is present in fish gut systems works together to achieve unknown specific roles. In the present study, populations of Mugil cephalus (Mullet) from four geographical locations were analysed for their gut microflora, namely Thiruvananthapuram, Kollam, Alappuzha and Ernakulam samples. Bacterial isolates from each specimen were obtained, by removing the gut from the fishes through serial dilution and plating into Luria Agar plates. Grown colonies from each samples were cultured on liquid medium and isolated total bacterial genomic DNA using standard protocols. Isolated DNA were PCR amplified using 16S rRNA gene specific primers and the amplicons were given for sequencing for identifying the bacterial species associated with each samples.

The sequence analysis data shows the diversity of bacteria present among different samples used for the study. From the conserverd domain sequence analysis of bacterial colonies isolated from different mullet (Mugil cephalus) population used for the present study, it was understood that the gut microflora of mullet is rich in the presence of diverse bacterial species wherein Bacillus species is the most common among them. Certain other species like Ralstonia were also detected from the gut of mullet species. This is the first report of identification of gut microfloral diversity from mullet species in Kerala.

TOTAL GENOMIC DNA ISOLATION FROM BACTERIA ISOLATED FROM GUT MICRLORA OF *MUGIL CEPHALUS*

In order to characterize the bacterial diversity in the gut microflora of *Mugil cephalus,* sequencing should be done with the selected colonies isolated from the gut of each samples. For this purpose, DNA isolation from selected bacterial colonies is a prerequisite wherein the isolated DNA can be PCR amplified using conserved region specific primers and the products can be sequenced to know the bacterial species present in each sample used for the study.

PCR was carried out in Eppendorf Nexus Master cycler. PCR programme was set with initial denaturation at 94^{o}C for 2 min followed by 30 cycles of denaturation at 95^{o}C for 30 sec, annealing at 55.5^{o}C for 30 sec and extension at 72^{o}C for 1 min. Final extension was done at 72^{o}C for 8 min. Control reactions were carried out to distinguish the target products from non-target products and primer dimer. The amplified products (1500bp) along with PCR Marker (1 kb plus) from 'Thermo Scientific' were loaded on agarose gel (1%). The gel was run at 80 V for 45 min. The gel was then visualized under UV transilluminator for analyzing the results.

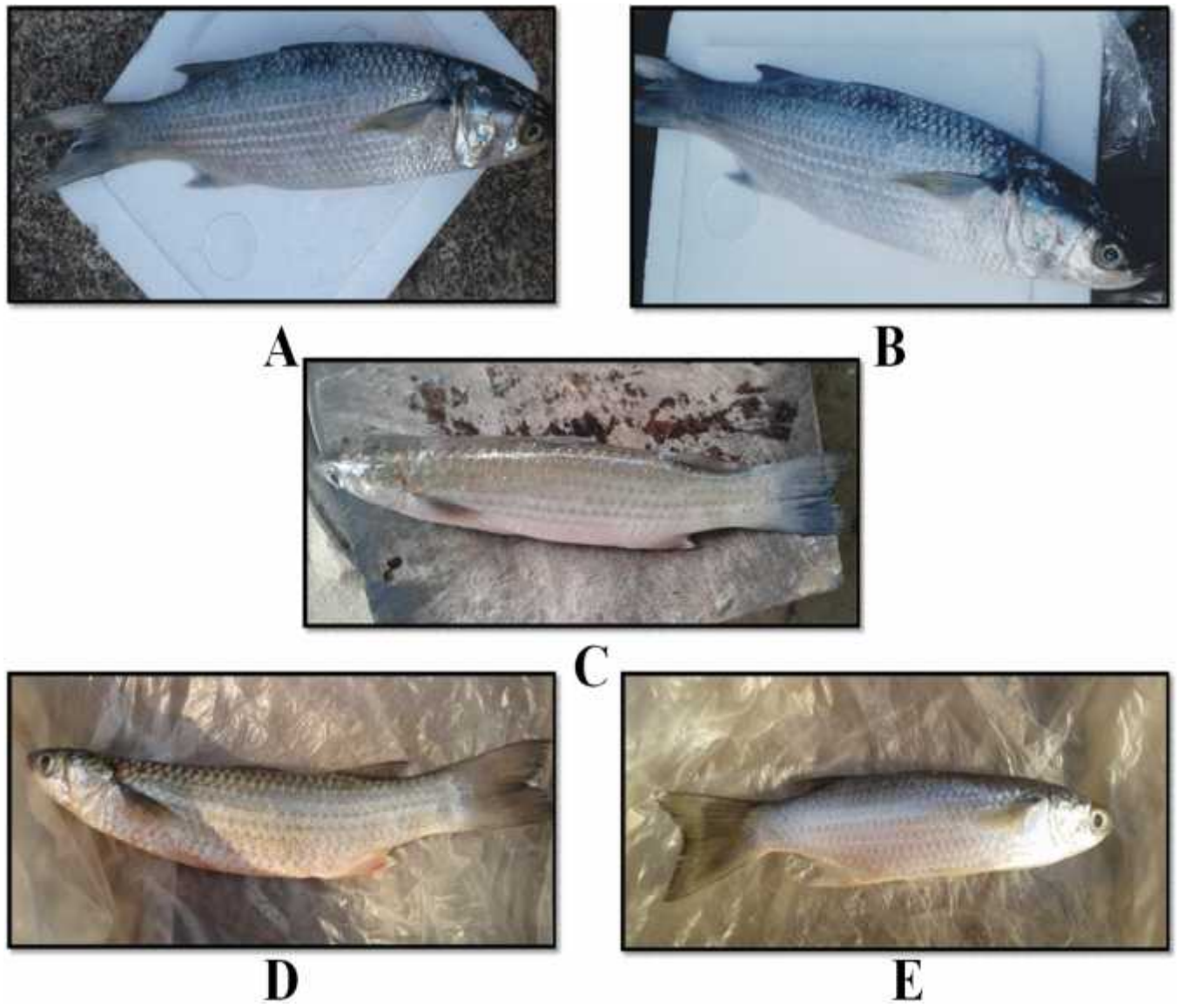
DNA purification (Gel elution of the PCR product)
The 16S rRNA gene PCR product (1500bp) was eluted from the gel using QI Aquick Gel extraction kit (QIAGEN) using the following protocol.

Sequencing and Bacterial species identification

The eluted samples (PCR products) (1500bp) were quantified using nano spectrophotometer and diluted to 50ng/ml and given for sequencing (dideoxy method of sequencing) using 16SrRNA forward primer to Agrigenome labs (Cochin). NCBI nucleotide blast (www. ncbi.nlm.nih.gov) was used to compare the sequences, obtained as database sequences, and each sequence was then assigned to the closest match in the database from an identified species .

Results

Sample Collection



ISOLATION OF BACTERIA FROM MULLET GUT

Figure no. 1: Mullet samples collected from different locations of Kerala for the study. A- Thiruvananthapuram sample, B- Kollam sample, C- Alappuzha sample (Freshwater), D-Alappuzha sample (Marine), E- Ernakulam sample

NUCLEIC ACID EXTRACTION FROM MULLET

DNA isolation

DNA was extracted from overnight culture of single colonies isolated from serially diluted homogenate from mullet gut and the quality and quantity were analyzed using agarose gel electrophoresis.

Primer set	**sequence (5'-3')**
16SrRNA forward primer	GAGTTTGATCCTGGCTCAG
16SrRNA reverse primer	ACGGCTACCTTGTTACGACTT

Table no 2: details of 16SrRNA primers used for the study

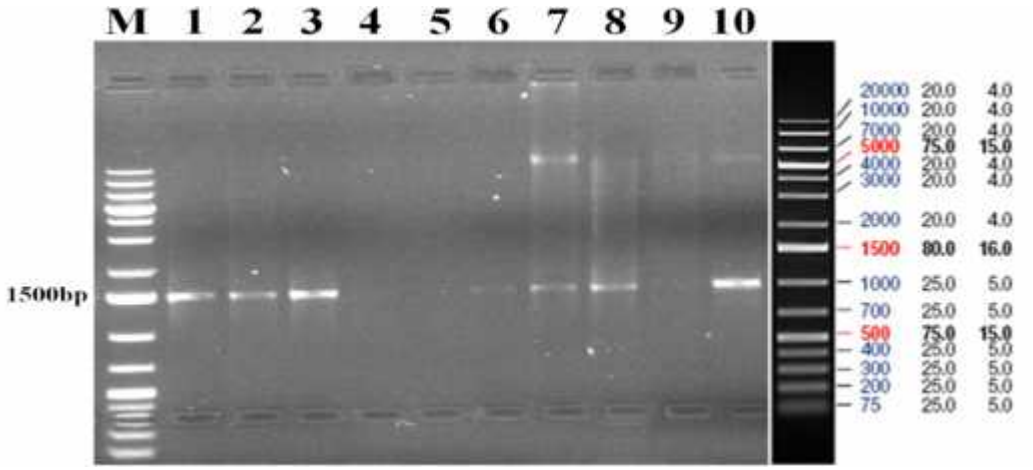


Figure no. 7: PCR product (1500bp) of genomic DNA, isolated from single bacterial colonies obtained through serial dilution of mullet gut homogenate, using 16SrRNA primer set. 1 and 2 - colonies from Thiruvananthapuram, 3 and 4- colonies from Kollam, 5 and 6- colonies from Alappuzha (Freshwater), 7 and 8- colonies from Alappuzha (Marine), 9 and 10- colonies from Ernakulam, M- 1kb plus DNA marker.

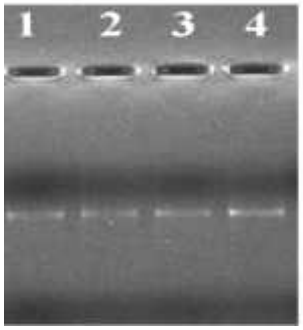


Figure no. 8: PCR products purified from gel using QIAquick Gel extraction kit (QIAGEN) (used for sequencing). 1- colony from Thiruvananthapuram, 2- colony from Alppuzha (Marine), 3- colony from Alppuzha (freshwater), 4- Colony from Ernakulam

Conclusion

From the conserved domain sequence analysis of bacterial colonies isolated from different mullet (*Mugil cephalus)* population used for the present study, it was understood that the gut microflora of mullet is rich in the presence of diverse bacterial species wherein *Bacillus* species is the most common among them. Certain other species like *Ralstonia* were also detected from the gut of mullet species. This is the first report of identification of gut microfloral diversity from mullet species in Kerala. Further studies need to be carried out in the wide range of mullet populations in other geographical locations of Kerala for better assessment of gut bacterial diversity in these species since bacteria found in the digestive tract of these fishes are highly variable and are a reflection of their aqueous environment, especially the food choice of the individual fish thereby have a great impact on aquaculture.

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