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Inferences of gut bacterial diversity from next-generation sequencing of 16S rDNA in deep sea blind ray - *Benthobatis moresbyi*

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ABSTRACT

The complex polymicrobial physiology of the fish gut, 'worlds within worlds' are governed by various factors. Taxonomic profiling of fish gut microflora through metagenomics approach has not received much attention to date. The present study explored the gut microbiota composition of *Benthobatis moresbyi*, sampled from the Arabian Sea and compared it to the existing metagenomic datasets. V3 region of 16S rRNA gene was amplified from metagenomic DNA and sequenced on Illumina MiSeq system. Taxonomic classification was done at various levels. *Actinobacteria, Proteobacteria* and *Acidobacteria* were the predominant phyla, in stark contrast to human and other gut microbiomes. *Alphaproteobacteria* and *Actinomycetales* were the predominant classes. *Acidobacteriales* and *Solibacterales* were dominant in the phylum *Acidobacteria.* Comparison of the taxonomic distribution of *Benthobatis moresbyi* gut microflora to other metagenomic datasets in MG-RAST metagenomics analysis server revealed more similarity to the microbiota of various ocean depths rather than guts of other terrestrial animals.

1. Introduction

Fish gut is a highly complex ecosystem representing an interface between the external environment and the body. The diverse, polymicrobial ecology of fishes are governed by factors such as fish type, developmental stage, diet, conditions of the surrounding environment, climate and other stress factors [1–6]. Gut microbiota of fish are involved in nutrient metabolism, homeostasis, xenobiotic metabolism, development of immune system, renewal of epithelial cells and several physiological processes of the host system [7–9]. They encompass both beneficial bacteria and pathogens, which is in accordance with the overall health status of fishes.

The NIH-sponsored human microbiome project and the valuable insights offered therein has accelerated interest in the taxonomic profiling of the highly selective gut environment of various vertebrates. However fish gut microbiome has not received much attention. Most of the earlier studies on fish gut microbes were based on culture dependent approaches. But, only 0.001–0.1% of microbes in seawater are cultivable [10]. Thus, metagenomic or the culture independent approach remains the most viable option to catalogue the microbial diversity of such 'worlds within worlds'. The emergence of various next generation sequencing (NGS) platforms has further revolutionized the analysis of the composition and biodiversity of such metagenomic datasets.

Benthobatis moresbyi, locally named as dark blindray is a bathydemersal fish, found at depths ranging from 787 to 1071 m and little is known about its biology yet [11]. This study uses universal phylogenetic marker, 16S rDNA to characterise the microbial gut composition of this fish. Out of the nine hypervariable regions found in 16S rDNA, V3 and/or V6 regions have been used extensively for metagenomic studies [12,18], as they can provide sufficient information regarding the bacteria present in samples. Roche 454 and Illumina are the preferred NGS platforms used in metagenomic analyses of environmental samples. To date, few studies have used Illumina tags for the purpose [13–16]. Though Illumina reads are short, they can provide accurate taxonomic information comparable to the use of full length 16S rDNA sequence [17,18].

In this context, the present study uses Illumina MiSeq platform to sequence V3 region of 16S rDNA to analyse the diversity of gut microbiota of *B. moresbyi*. The study also seeks to compare the gut bacterial diversity of this deep sea ray fish to metagenomic datasets of gut microbiota of other organisms and that of marine environments.

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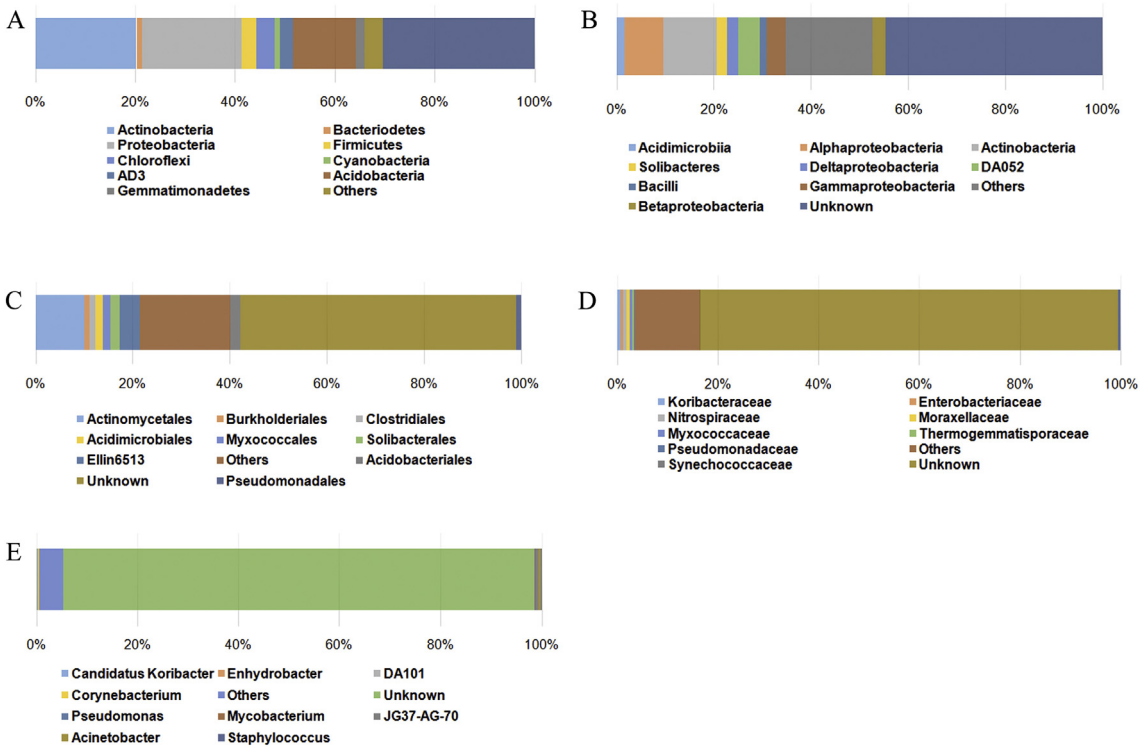

Fig. 1. A. Bacterial diversity of *B. moresbyi* gut microbiome at phylum level. **B**. Bacterial diversity of *B. moresbyi* gut microbiome at class level. **C**. Bacterial diversity of *B. moresbyi* gut microbiome at order level. **D**. Bacterial diversity of *B. moresbyi* gut microbiome at family level. **E**. Bacterial diversity of *B. moresbyi* gut microbiome at genus level.

2. Materials and methods

2.1. Sampling and DNA barcoding

Deep sea ray fish was collected during cruise no. 321 of the FORV (Fishery and Oceanographic Research Vessel) *Sagar Sampada* of the Centre for Marine Living Resources & Ecology (CMLRE), under the Ministry of Earth Sciences, Government of India. The sample was collected along Arabian Sea region (8°11′N, 75°54′E) of southwest coast of India by High Speed Demersal Trawl (HSDT) net. Fish DNA was isolated using phenol-chloroform method and identification of fish was performed as described previously [19].

2.2. Metagenomic DNA isolation and sequencing

The gut contents of the fish were excised under aseptic conditions and metagenomic DNA was isolated from fish gut using QIAamp DNA stool minikit (Qiagen, India). The V3 region of 16S rDNA was amplified using primers 341F 5′CCTACGGGAGGCAGCAG 3′ and 518R 5′ATTAC CGCGGCTGCTGG 3’. The amplicons were sequenced on Illumina MiSeq system using 151 bp x 2 paired end reads.

2.3. Sequence assembly and taxonomic analysis

The amplicons reads were processed using QIIME pipeline (v 1.8) [20]. The raw fastq files obtained from the sequencer were subjected to analysis of base quality score distribution, average base content per read and GC distribution. V3 region was extracted from Illumina paired end sequences by trimming of spacer and conserved regions and a consensus V3 region was built from trimmed paired-end reads using ClustalW program [21]. Further, the reads were passed through filters for read quality and mismatch. All the candidate V3 sequences were then clustered into OTUs based on their sequence similarity using Uclust program (Similarity cut off = 0.97) [22]. Further, a representative sequence was constructed for each OTU and the sequence was aligned to Greengenes reference database [23] using PyNAST [24]. Taxonomic classification was performed using Ribosomal Database Project (RDP) classifier [25] against the Greengenes database.

2.4. Comparative analysis of microbiome diversity

The metagenomic dataset of the present study was compared to termite gut (4442701.3), canine gut (4444703.3), rabbit gut (4461372.3), pig gut (4461380.3), mouse gut (4529796.3), human gut (4461143.3), chicken gut (4440283.3), marine sponge (*Arenosclera brasiliensis*, 4461454.3), fresh water fish gut (common carp, 4449604.3), aquacultured fish (adult hybrid striped bass, 4440062.3), Indian Ocean (Global Ocean Sampling Expedition, 4441135.3) and microbial plankton communities at various depths (Project Microbial Community Genomics at the HOT/ALOHA, deep abyss: 4442503.3, below upper mesopelagic: 4442499.4, upper euphotic: 4442582.3) metagenomes within MG-RAST (the Metagenomics RAST) server [26].

3. Results

3.1. Identification of fish by DNA barcoding

Sequencing and BLAST analysis of amplicons from 5′ region of *cox1* gene confirmed the identity of the deep sea ray fish. The 681 bp sequence showed 94% identity to *Benthobatis moresbyi* with a query coverage of 95%. The sequence obtained was submitted to GenBank and accession number was obtained (KJ888144.1).

3.2. Sequence analysis and taxonomic distribution

The sequencing run produced 902,914 paired end reads of 151 bp length. The average Phred quality score was greater than Q30, implying a base call accuracy of 99.9%. More than 75% of the reads had GC