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# Microbial community structure of surface sediments from a tropical estuarine environment using next generation sequencing

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ABSTRACT

Microbial community structure was analyzed from tropical monsoon influenced Mandovi-Zuari (Ma-Zu) estuarine sediment by means of Next Gen Sequencing (NGS) approach using Ion Torrent PGM™. The sequencing generated 80,282 raw sequence reads. Barcoding with Ion Tags allowed multiplex analysis of microbial community and helped in identifying shifts in microbial community structure. Analysis of sequence data revealed that sediment at both the stations in the Mandovi estuary was dominated by Archaeal group, *Euryarchaeota* (53.1% and 64.01%). Among *Euryarchaeota, Methanomicrobia* was dominant. *Methanococci* was present only at the mouth and *Methanopyri* was detected at the mid-estuarine station. Whereas, both the stations of Zuari estuary were dominated by Bacteria, *Proteobacteria*, mainly *Gammaproteobacteria* (97.67% and 54.41%). A clear influence of mangrove ecosystem on the bacterial diversity was evident in the Zuari estuary. These results suggest that the two estuaries have a very distinct microbial community structure. Characterization of microbial communities in this study area using NGS for the first time points out that even within geographically close habitats, the microbial population structure is significantly influenced by localized interactions. The signatures obtained from sediments can thus be used to reconstruct habitat characteristics and serve as biomarkers. Future studies should focus on the functional gene profiling of different microbial communities and the influence of seasons and tide in such monsoon influenced estuaries.



## 1. Introduction

Estuarine systems have dynamic and diverse bacterial communities owing to the mixing of fresh water and sea water and are altered by autochthonous biological activity (Crump et al., 2003). The Mandovi and Zuari estuaries are tropical, tide dominated, monsoon influenced estuaries located in the south-eastern Arabian Sea. Although both these estuaries are adjacent to each other, they are influenced by different factors. Mandovi estuary receives greater run-off than Zuari owing to many tributaries feeding terrestrial inputs into this estuary (Qasim, 2003), and is also narrower and experiences more frequent navigational activities when compared to Zuari. Mandovi estuary receives an influx of nutrients, especially nitrates (Sardessai and Sundar, 2007), the sources of which can be attributed to terrestrial inputs, the mangrove swamps (Qasim and Gupta, 1981) and from the discharge of iron ore extraction-plants at the upstream end (De Souza et al., 2003). Due to an increased mining activity in both the estuaries, high concentrations of iron and manganese have been reported (Kessarkar et al., 2015). They also receive heavy sewage outfalls adding both organic and inorganic inputs.

Although few studies report the distribution of various pathogenic, indicator as well as limno-tolerant and halo-tolerant bacteria in the water column (Divya et al., 2009; Nagvenkar and Ramaiah, 2009; Rodrigues et al., 2011; Khandeparker et al., 2015), the work on the microbial community in the sediments is sparse. Sediments act as a repository of the events occurring in the pelagic environment and the processes occurring and the communities present in the surface sediments have a profound effect on the local and global cycling of elements (Arrigo, 2005). Recently, Singh et al. (2010) reported the Archaeal community distribution through phylogenetic analyses using 16s rRNA gene with an emphasis on the *Aamo*A gene in the sediments of these estuaries. Despite their ubiquity, relatively less is known about micro-organisms, largely because more than 99% of prokaryotes in the environment cannot be cultured in the laboratory and show resistance to culture under standard laboratory conditions, a phenomenon that limits the understanding of microbial physiology, genetics and community ecology (Woese et al., 1990). Among the different methods used to characterize and unravel the genetics of uncultured microor-

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ganisms, the genomic analysis of a population of microorganisms (metagenomics), has emerged as a powerful tool which involves direct isolation of genomic DNA from an environment (Schloss and Handelsman, 2005; Tringe and Rubin, 2005). Both the Polymerase Chain Reaction (PCR) based analysis of 16S rRNA and shotgun metagenomic studies have been used to characterize soils (Fierer et al., 2011), oceans (Caporaso et al., 2011), and the atmosphere (Bowers et al., 2011).

Ion Torrent PGM™, a newly introduced next-generation benchtop sequencing platform uses sensor array chips that can monitor millions, and potentially billions, of simultaneous sequencing reactions (Merriman et al., 2012). Recently, Whiteley et al. (2012) assessed the bacterial and archaeal dynamics within covered anaerobic digesters used to treat piggery wastes using Ion Torrent PGM™. It is clear from their study that the PGM platform provides a low cost, scalable and high throughput solution for both Tag sequencing and metagenomic analyses (Whiteley et al., 2012). Yergeau et al. (2012) also applied the Ion Torrent technology to 16S rRNA-based profiling of complex bacterial communities of the Athabasca river.

In the present study, the microbial community in the sediments of two different tropical estuaries was evaluated using the Ion Torrent Personal Genome Machine (PGM) as microbial community characterization using culturable methods and phylogenetic analyses using 16s rRNA gene provide partial information, whereas metagenomics is comprehensive and holistic. For this study, two stations from Mandovi estuary viz., Campal and Panjim and two stations from Zuari estuary viz., Chicalim and Siridao were selected. It was hypothesized that the diversity in the physico-chemical conditions and the dissimilarities in the degree of anthropogenic influence at these two different estuarine ecosystems would reflect on the microbial communities prevailing in their respective environments. To the best of our knowledge this is the first study in this region.

## 2. Materials and methods

### 2.1. Site description and sampling

Mandovi and Zuari estuaries have been described as the lifelines of Goa, originate in the Western Ghats and flow through a narrow coastal plain and along the west coast of India and are extensively used in transportation, fisheries and recreational activities. They differ in their geomorphology, rainfall pattern and complex estuarine ecosystem. Both estuaries are of nearly identical length (~50 km each), are highly productive and dynamic systems and have wide mouth regions and longer flushing periods. In this study, Chicalim (15°24′10.92″N, 73°51′8.55″E) and Siridao (15°25′41.89″N, 73°52′38.84″E) were sampled in the Zuari estuary (Fig. 1). Chicalim (Zu-Ch), is highly influenced by anthropogenic activity and shipping industry when compared to Siridao which receives inputs from the mangrove area and is relatively pristine (Zu-Si). High numbers of pathogenic bacteria have been previously reported in this area (Nagvenkar and Ramaiah, 2009; Rodrigues et al., 2011; Khandeparker et al., 2015). Campal (15°29′36.26″N, 73°48′42.08″E) and Panjim (15°30′9.76″N, 73°50′10.24″E) in Mandovi estuary were selected as sampling stations (Fig. 1). Panjim is a mid-estuarine station (Ma-Pa), receives inputs from different anthropogenic activities as it is close to the city centre and large number of fishing trawlers, whereas, Campal is situated at the mouth of the estuary (Ma-Ca), receives lesser inputs other than the influx carried towards this location through currents and tides. All the sediment samples were collected using van Veen grab during pre-monsoon season (Zuari – April 2013; Mandovi – March 2014) in sterile 50 ml falcon tubes and transported to the laboratory on ice for DNA extraction and stored at −20 °C until further processing.

### 2.2. Sample processing and DNA isolation

Sediment DNA extraction was performed using Ultraclean Soil DNA Kit, (MoBio lab. Geneworks, Australia). The DNA was extracted using bead beating and column purification which was performed according to the manufacturer's guidelines. The metagenomic DNA was quantified by Eppendorf-Biospectrometer, and run on 0.8% agarose gel. The gel was viewed using Bio-Rad Gel Doc™ EZ Gel documentation system after staining with Ethidium Bromide (EtBr). Metagenomic DNA was stored at −80 °C until further downstream processing.

### 2.3. PCR based analysis using ion tags

All chemicals for PCR viz., DNA polymerase, deoxynucleotide triphosphates (dNTPs), Taq DNA polymerase, nuclease free water, 10× reaction buffer, $MgCl_2$ were purchased as PCR Core Kit with Taq DNA Polymerase from Sigma Aldrich, USA.

V6 hyper variable region of bacterial 16S rRNA gene was amplified by PCR as described in Sogin et al. (2006) using A-967F (5′- CAACGCGAAGAACCTTACC-3′) and B-1046R (5′-CGACAGCCATGCANCACCT-3′) primers. PCR amplification mix contained 5 units of Taq DNA polymerase, 1× reaction buffer, 200 μM dNTPs and 0.2 μM concentration of each primer in a volume of 100 μl. Genomic DNA (3–10 ng) was added to two separate 50-μl amplification mixes. Cycling conditions included an initial denaturation step at 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 57 °C for 45 s, and 72 °C for 1 min; and a final 3 min extension step at 72 °C. PCR amplification of the PCR products were processed in duplicates, were checked for size and specificity by electrophoresis on 2% w/v agarose gel.

DNA concentration was assessed on Qubit high sensitivity assay kit (Invitrogen, Life Technologies). PCR amplicons, which were amplified in duplicates, were pooled in an equimolar concentration. Prior to sequencing, library preparation, end repair, adaptor ligation was done according to the protocol specified by the manufacturer. PCR purification (Agencourt *AMPure* XP beads, Beckman Coulter) and quantification (Qubit 2.0 Flourometer) was performed in between each step. Subsequently, the samples were adjusted to a final concentration of 25–30pM and pooled in an equimolar concentration. The pooled amplicons were attached to the surface of Ion Sphere Particles (ISPs) using an Ion Xpress Template 100 bp (base pairs) kit (Life Technologies, USA). Enrichment of total ISPs resulted in >80% templated-ISPs which was sequenced on the 314′ (10 Mega bases) micro-chips on the Ion Torrent Personal Genome Machine (Life Technologies, USA) for 65 cycles (260 flows) using Ion Express Template 100 bp chemistry resulting in an expected average read length of >100 bp. The Ion Torrent PGM has an inbuilt software plugin capability to filter low quality reads, polyclonal sequences and automatic trimming of the sequences matching the PGM 3′ adapter (adapter trimming).

Filtered PGM data was exported as Standard Flowgram Format (SFF) files and uploaded on the Ribosomal Database Pipeline (RDP), a pyrosequencing pipeline (https://pyro.cme.msu.edu). Before trimming, both reverse and forward primers in the sequences were retained. The RDP initially sorts low quality sequences, trimming off the key tags, primers and minimum length sequences ≤50 bp) (Cole et al., 2013). Trimmed "Fastq" files containing quality reads were converted to fasta (sequence), qual (quality score), flow (flowgram) files using Mothur version v.1.36.1 (Schloss et al., 2009) by command line "fastq.info (fastq = sample.fastq)". All quality sequences "fasta" files were checked for chimeric sequences by UCHIME reference algorithm (Edgar et al., 2011) where reference sequence