

Elucidation of spatiotemporal variations in functional genes involved in the nitrogen cycle along the west coast of India

Coastal upwelling systems is considered to be the most productive areas in the world's oceans. The west coast of India is located at the Eastern Arabian Sea upwelling system receiving upwelled nutrient inputs are hotspots of mineralization. In such ecosystems, the organic matter input undergoes intense biogeochemical processing mainly by heterotrophic microbial assemblages. In seasonally upwelling regions such as the west coast of India, the intense biological productivity lead to oxygen deficient conditions with dissolved oxygen concentrations reaching $<1.42 \text{ ml L}^{-1}$ termed as hypoxia. Depletion of oxygen demands bacteria to utilize nitrate via the denitrification process. Thus, such ecosystems are hotspots for oxygen-sensitive nitrogen transformations, where nitrate serves as the main terminal electron acceptor in the process of organic matter oxidation and would harbor unique, diverse communities of heterotrophic bacteria. Implicitly, these processes ought to bring alterations in microbial communities.

In order to study spatiotemporal variations in phylogenetic composition of bacterial community, seawater samples were collected from the surface, mid and near bottom depths, from three coastal locations along the west coast of India (off Goa, off Mangalore and off Kochi) during the fall intermonsoon (FIM, October 2013), summer monsoon (SuM, July 2014) and spring intermonsoon (SIM, March 2015). This region is well known to experience intense oxygen deficiency in particular during summer monsoon (June-August) and fall intermonsoon (September-October). 16S rRNA gene sequencing analyses by culture dependent method resulted in 24 different bacterial genera belonging to five phyla such as Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, Bacteroidetes and Firmicutes. Of the 900 cultures obtained from the study area, 673 cultures were nitrate reducers accounting for 75% of the total bacterial population in this study, which grouped into 42 representative species. Nitrate Reduction Rate (NRR) ranged from $0.04\text{--}0.27 \mu\text{Md}^{-1}$. Nitrous oxide (N_2O) production was found to be in the range of $7.00\text{--}119.32 \text{ nMd}^{-1}$. Culture-independent bacterial diversity was investigated based on 16S rDNA clone library approach and the clones were affiliated with Alpha Proteobacteria, Gamma Proteobacteria, Beta Proteobacteria and Delta Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Cyanobacteria, Marinimicrobia, Verrucomicrobia, Chloroflexi, Planctomycetes and Omnitrophica bacterium.

The copy numbers of nitrous oxide reductase (*nosZ*), nitrite reductase (*nirS*) and nitrate reductase (*narG*) genes from water samples were quantified using qPCR. Functional gene sequencing of bacterial communities were diverse. Bacteria possessing *narG*, *nirS*, and *nosZ* indicated that complete denitrification occurred in the samples collected close to the bottom. *narG* ranged highest among the functional genes ($1.9 - 7.9 \times 10^7 \text{ copies L}^{-1}$), while *nirS* ranged from as low as $0.001 - 0.30 \times 10^6 \text{ copies L}^{-1}$, whereas *nosZ* was observed in the range of $0.3 - 2.9 \times 10^6 \text{ Copies L}^{-1}$ across the sampling sites. Functional gene sequencing of bacterial communities possessing *narG*, *nirS* and *nosZ* showed spatiotemporal differences and, dominated by members of Gamma-, Beta- and Alpha - proteobacteri