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Phylogenetic diversity of archaeal 16S rRNA and ammonia monooxygenase genes from tropical estuarine sediments on the central west coast of India

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Abstract

Phylogenetic diversity analyses of archaeal 16S rRNA and ammonia monooxygenase subunit *A* (A*amoA*) genes were carried out on sediment samples from the Mandovi and Zuari estuaries on the central west coast of India. The 16S rRNA gene libraries revealed quite high diversity of archaea in these sediments compared to previous reports from tropical and temperate estuarine sediments. Uncultured members of Crenarchaeota accounted for ~78% of 433 archaeal 16S rRNA gene clones from both of the estuaries. We detected archaeal 16S and *amoA* gene-related organisms capable of ammonia oxidation. Among Crenarchaeota, marine group I (MG I) was the most predominant. Clones matching the uncultured methanobacteria were predominant among the ribogroups of Euryarchaeota. Our results indicate that archaeal diversity in tropical estuarine sediments is influenced by the mangrove vegetation bordering the lower stretches of both estuaries. Higher diversity may be related to elevated land drainage during the monsoon, particularly in the Mandovi estuary sediments. Also, the diversity of A*amoA* sequences was higher in Mandovi sediments than those from Zuari and other tropical and/or temperate estuaries studied previously.

Keywords: Tropical estuaries; Sediment; Phylogeny; Archaea; Marine group I; DGGE; 16S rRNA; OTU; *amoA*; Ammonia oxidation

1. Introduction

Application of molecular methods has changed our perception of the diversity, distribution and function of archaea in natural marine ecosystems. Analyses of ribosomal RNA gene sequences from environmental samples have revealed that archaea are ubiquitous (Stein and Simon, 1996) and far more abundant (Karner et al., 2001) than previously assumed. 16S rRNA gene-based archaeal diversity analyses reveal the existence of archaea in the open ocean, marine sediments, soils and freshwater lake sediments (Schleper et al., 2005). Mesophilic archaea in the subkingdoms, Crenarchaeota and Euryarchaeota, are now recognized to be widespread in the oceans and are reported to contribute significantly to cycling of carbon and nitrogen, the two biologically most essential elements (e.g., Francis et al., 2007; Ingalls et al., 2006).

Biological fixation and microbial denitrification losses of nitrogen are ultimately connected by nitrification, which is an important process throughout the oceans and coastal systems (Ward, 2005). While decomposition of NO_3^- to N_2 gas leads to N loss, the conversion of ammonia (NH_4^+) to nitrite (NO_2^-) has been shown to be predominantly carried out by archaea, in particular in soils and sediments (Ando et al., 2008; Leininger et al., 2006). Recent discoveries from metagenomic sequences and pure cultures also suggest that the archaea are capable of performing chemoautotrophic nitrification (Könneke et al., 2005; Venter et al., 2004). Many studies have shown the presence, diversity and distribution of archaeal *amoA* (A*amoA*) genes in different ecosystems and geographic locations (Beman and Francis, 2006; Beman et al., 2008; Bernhard et al., 2005; Bothe et al., 2000; Dang et al., 2008). The

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amoA-encoding archaea are increasingly recognized as important because of their potential for ammonia oxidization in terrestrial and marine ecosystems (Dang et al., 2008; Leininger et al., 2006; Wuchter et al., 2006). However, the extent of occurrence and composition of ammonia-oxidizing archaea (AOA) in the tropical marine systems and their relative importance in the N cycle will require further investigation.

Phylogenetic studies using the 16S rRNA gene, the traditional marker, suggest a certain level of differentiation of bacterial and archaeal operation of taxonomical units (OTUs) between different ecosystems (Nicol and Schleper, 2006). Since two monophyletic lineages are reported for the 16S rRNA gene of ammonia-oxidizing bacteria (AOB), it is used for describing AOB phylogeny as well (Bothe et al., 2000). Recently, a substantial congruence of crenarchaeal 16S rRNA and A*amoA* genes has also been shown (Prosser and Nicol, 2008). However, 16S rRNA gene diversity within Crenarchaeota is far greater than that of AOA (Schleper et al., 2005).

As they are under the influence of both oceans and rivers, the estuaries are reported to harbor strong microbial diversity. Recent phylogenetic analyses of archaea from temperate (Abreu et al., 2001) and tropical (Vieira et al., 2007; Zeng et al., 2007) estuaries indicate remarkable diversity, perhaps as a consequence of the presence of estuarine, riverine and coastal ocean assemblages. Though some tropical regions are believed to be hot-spots of biodiversity, our knowledge of archaeal diversity from these ecosystems is limited mainly due to the lack of use of molecular phylogenetic investigations (Vieira et al., 2007; Zeng et al., 2007). In view of the dearth of such information, we sought to examine archaeal assemblages and compare their phylogenetic relationships in two tropical and ecologically similar estuaries through culture-independent 16S rRNA and A*amoA* gene analyses. These moderately to highly productive estuaries are fringed by mangroves in their lower stretches and receive substantial land runoff during the monsoon months of June to September (Shetye et al., 2007). We aimed to elucidate the differences in archaeal community structure in the sediments of these estuaries and to detect the archaeal *amoA* (A*amoA*) gene which is functionally important in ammonia oxidation, one of the very crucial steps in marine nitrogen cycle.

2. Methods

2.1. Description of sampling sites

Mandovi and Zuari are the two major rivers of Goa on the central west coast of India. Before draining into the Arabian Sea, they form a confluent estuarine system between 15°09′ and 15°33′ N and between 73°45′ and 74°14′ E (Fig. 1). The Mandovi river has many tributaries and receives a greater runoff than the Zuari (Shetye et al., 2007). Both experience semi-diurnal tides and higher freshwater influxes during the monsoon months. A hilly terrain with dense vegetation girdles the lower stretches of both estuaries, and thus there is rich input of humus. Sediments in the lower stretches of these estuaries are reported to be mostly sandy (Mandovi: sand: 65–70 > clay 15–20 > silt:10–15%; Zuari: sand 60–65 > clay 25–30 > silt 5–10%). The annual variations in salinity range from close to freshwater ones during monsoon months (June–September) to 34 during pre-monsoon months (March–May). Annual fluctuation of water temperature is between 20 and 29 °C (Shetye et al., 2007). The high nutrient content, along with low wave action and mild currents near the banks, also lead to a favorable environment for the growth of mangroves. During the post-monsoon (October–February) months, total organic carbon is reported to be in the range of 0.8–1.2% in Zuari (Dessai et al., 2009) and 1.6–3% in Mandovi (Nasnolkar et al., 1996) sediments. In these estuaries, the average total bacterial counts are $\sim 6.31 \times 10^6$ ml^{-1} water and 3.02×10^9 g^{-1} dried sediment (Ramaiah et al., 2002).

2.2. Sampling

Sediment samples were collected during January 2005 from the locations shown in Fig. 1. They were collected using van Veen grab from three sites each in the estuarine mouths of both Mandovi (15°29′ N′; 73°48 E; water depth 5 m; salinity in overlying water ~32 psu and temperature 24 °C) and Zuari (15°25′; 73°51′ E; depth, 5 m; salinity of overlying water ~34 psu and temperature 25 °C). The top 2.5 cm sediment layer were aseptically removed and stored separately in sterile Falcon tubes. These sediment samples were transported to the laboratory on ice for DNA extraction and stored at −70 °C until taken up for further processing.

2.3. DNA extraction

Two replicates of ~0.5–1 g subsamples of all six samples were subjected to DNA extraction. The MOBIO soil DNA extraction kit (MOBIO, USA) was used for extraction as per the protocol provided by the manufacturer. DNA extracts from each subsample were pooled and stored at −20 °C.

2.4. Denaturing gradient gel electrophoresis (DGGE) analysis

DGGE-PCR was carried out using primers, PARCH 340f: 5′-CCCTACGGGG(C/T)GCA(G/C)CAG with GC clamp: 5′-TCGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGG and PARCH 934R: 5′-GTGCTCCCCCGCCAATTCCT as described by Ovreas et al. (1997). The sediment DNA extracts were amplified in triplicate to maximize diversity. Five hundred nanogram-pooled and purified PCR products were loaded on 6% (w/v) polyacrylamide gels containing a 40–60% denaturing gradient of urea and formamide. The gel was run in a D-Code universal mutation detection system (Bio-Rad, USA) for 16 h at 60 °C at 60 V. The gel was stained in SYBR green. Intra- and interestuarine variations were examined by running all 6 DNA samples on a single gel. Bands were defined manually as well as by Quantity-one software (Version 4.66, Bio-Rad, USA).