

MOLECULAR ANALYSIS OF THE PROKARYOTIC COMMUNITY IN THE SURFACE SEDIMENTS OF AN EASTERN MEDITERRANEAN DELTA FRONT AREA

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Abstract

The prokaryotic diversity in the muddy sediments of a Mediterranean delta front area (Maliakos Gulf, Greece) was examined by sequence analysis of 16S rDNA clone library. The analysis of 160 clones revealed 54 unique non-chimaeric sequences mainly belonging to the α , γ and δ subdivisions of *Proteobacteria* (5.5, 9.2 and 30%, respectively) and the divisions of *Acidobacteria* (5.5%), and *Chloroflexi*, *Verrucomicrobia*, *Planctomyces*, each accounting for 3.7% of the phylotypes found. High contribution of phylotypes closely related to bacteria involved in sulfur compounds and terrestrial organic matter cycling was observed. This indicates that in the submarine deltaic sediments adjacent to small rivers sulphur cycle and terrestrially derived organic matter may be important in diagenetic and biogeochemical processes.

Keywords : Aegean Sea, Bacteria, Coastal Systems, Deltas, Sediments.

Introduction

Large amounts of terrestrially derived organic material discharge through river runoff in coastal systems. Although much of this material is refractory and initially resistant to microbial degradation [1], in the submarine deltas of large tropical rivers, the remineralization of both terrestrial and marine organic carbon is comparably more efficient than in other marine environments [2]. Similarly, evidence of relatively high sediment community metabolic rates comes from the submarine depositions of smaller rivers in the Mediterranean and the Black Sea [3, 4]. However, little is known about the taxonomic and functional diversity of microorganisms involved in decomposition processes of terrestrially derived material in the suboxic deltaic sediments. In the present study, a culture-independent examination of microbial diversity in the muddy deltaic sediments of inner Maliakos Gulf (Aegean Sea, Greece) near Spercheios river mouth was carried out using sequence analysis of 16S rDNA clone library. This allows not only the assessment of prokaryotic diversity and community structure but also inferences to be made about the metabolic pathways used for the decomposition of organic material and the biogeochemical processes, in coastal systems influenced by riverine discharges.

Material and Methods

Sediment from the top 1 cm was collected under sterile conditions in May 2005 near Spercheios river mouth and remained for 2 months in -80°C. Total DNA was isolated using the Ultraclean Soil DNA isolation kit (MOBIO, USA). The PCR products, derived using special bacterial primers (8Forward and 1492Reverse), were cloned with the TOPO XL kit (Invitrogen USA). 160 clones were randomly selected from the library and the sequences retrieved (Macrogen) were aligned using CLUSTALX, and compared to the 16S GenBank database by the BLAST (<http://www.ncbi.nlm.nih.gov>) and FASTA programs. The phylogenetic tree was constructed using distance analysis with Jukes-Cantor corrections and maximum-likelihood from the PHYLIP package (<http://evolution.genetics.washington.edu/phylip.html>).

Results and Discussion

Of 160 clones randomly selected in the library, 65 contained non-chimaeric sequences. These belonged to 54 unique phylotypes, thus indicating high prokaryotic diversity in the delta front sediments of Maliakos Gulf. Bacterial sequences belonging to the following major divisions were identified: δ - *Proteobacteria* (30%), γ - *Proteobacteria* (9.2%), α - *Proteobacteria* (5.5%), *Acidobacteria* (5.5%), *Planctomyces* (3.7%), *Chloroflexi* (3.7%), *Verrucomicrobia* (3.7%). Phylotypes from the divisions *Flexibacteraceae*, *Bacteroidetes*, *Fusobacteria* and *Spirochaetes* contributed only up to 1.8% each while unidentified sequences accounted for 33% of the found unique sequences. Considerable similarity (ranging from 85 to 98%) was observed with phylotypes previously isolated from a variety of environments, including terrestrial wetlands and soils, as well as mud volcanoes and deep sea hydrothermal vents. The coexistence of phylotypes previously known from a variety of biotopes, both terrestrial and marine, may reflect not only material transportation and deposition processes through riverine and marine water circulation, but also high functional diversity of the deltaic prokaryotic community. Relatively high similarity (88-94%) of the present study sequences were observed

with bacteria characterized as sulphate reducers (i.e. *Desulfosarcina variabilis*) and species known to degrade hydroaromatic compounds, such as *Desulfobacterium aniline* and *Ilyobacter insuetus* sp. nov., as well as the sulphur oxidizer *Mesorhizobium thiogangeticum*, the nitrifier *Rhodopseudomonas julia* and the cellulolytic *Clostridium thermocellum*. This indicates that in the subaqueous deltaic sediments adjacent to small rivers sulphur cycle and terrestrially derived organic matter may be important in biogeochemical processes. The results of the present study are in accordance with findings in the submarine deltaic sediments deposited by large tropical rivers, in terms of bacterial diversity and community composition [5].

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