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Annual dynamics of bacterioplankton assemblages in the Gulf of Trieste (Northern Adriatic Sea) ☆

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Abstract

Bacterioplankton community diversity was investigated monthly in coastal waters of the Gulf of Trieste (NE Adriatic Sea) throughout 2003. Superficial bacterial assemblages of two differently freshwater influenced stations were studied using PCR-DGGE fingerprinting techniques. Bacterial genetic diversity of the sampled area, as estimates of the number of DGGE bands was high (36–64) compared to that reported in other studies employing this fingerprint technique. The similarity index (Sorensen Index) between assemblages showed a defined operational taxonomic units (OTUs) succession pattern in the more typically marine station with stable winter communities and quickly changing summer ones. On the contrary in the station affected by riverine inputs no clear pattern was detected. In both sites, according to cluster analyses performed on the DGGE banding pattern, three seasonal assemblages were identified: winter-spring, summer and fall.

Sequence analysis of fifty-six among the brightest gel bands led to the observation of bacteria affiliated to Gram positive, Cyanobacteria, Cytophaga–Flavobacteria–Bacteroides (CFB) lineages and the alpha-, gamma- and delta- subdivisions of the Proteobacteria. Gamma-Proteobacteria constituted the main fraction (60%) of sequences in the more typically marine station, whereas the river-influenced station was characterised by more heterogeneous assemblages (39% alpha-Proteobacteria, 32% Flavobacteria).

Keywords: Bacteria; PCR-DGGE; Cluster analysis; Principal component analysis; 16S rDNA sequences

1. Introduction

The application of molecular biology techniques to microbial ecology, including those that use the gene sequences of the small subunit ribosomal RNA as a molecular marker for identification of microorganisms, has changed considerably our view of aquatic bacterial communities. The genes encoding small subunit ribosomal RNAs reflect the evolutionary relationship of microorganisms (Woese, 1987) and the sequences of these genes allow one to group and identify microorganisms. Denaturing gradient gel electrophoresis (DGGE) is a cultivation-independent technique that can separate different PCR-amplified gene fragments in a gradient of DNA according to differences in sequence (Muyzer, 2000). Diversity profiles from different microbial communities can be compared according to their gel patterns and the sequences of representative bands. Therefore PCR-DGGE can be used to investigate the diversity of microbial communities, to determine the spatial and temporal variability of bacterial populations, and to monitor community behaviour after natural or induced environmental perturbations.

Bacteria are a major biological force in the oceanic carbon cycle and ecosystem structure (Azam and Worden, 2004). The taxonomic composition of bacterial assemblages and their temporal and spatial dynamics in the oceans are likely to be of major importance in determining the role of bacteria in marine biogeochemistry (Castle and Kirchman, 2004). Seasonal

Abbreviations: BLAST; basic local alignment search tool; CFB; Cytophaga-Flavobacteria-Bacteroides; Chl *a*; chlorophyll *a*; DGGE; denaturing gradient gel electrophoresis; DO; dissolved oxygen; EDTA; ethylene diamine tetraacetic acid; MMO; Isonzo River monthly mean outflows; OTU; operational taxonomic unit; PCR; polymerase chain reaction; PC; principal component; PCA; principal component analysis; TAE; Tris-acetate EDTA.

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variations of bacterial-community structures have been observed in marine, estuarine and freshwater ecosystems (Höfle et al., 1999; Pinhassi and Hagström, 2000; Selje and Simon, 2003). Some authors have reported that bacteria are likely influenced by abiotic characteristics and microbial foodweb structures of aquatic ecosystems (Yannarell and Triplett, 2005). Previous studies have shown that bacterial community structure is correlated with salinity (Bouvier and del Giorgio, 2002), nutrients (Biddanda et al., 2001), pH, water clarity (Yannarell and Triplett, 2005), substrates resource (Crump et al., 2003), phytoplankton, chlorophyll *a* concentration (Murray et al., 1998; Pinhassi et al., 2004), grazing (Höfle et al., 1999) and viral lysis (Fuhrman and Suttle, 1993; Suttle, 1994). However, the environmental variables that affect the bacterial communities' composition may also vary by site and time. Furthermore, temporal patterns in marine bacterial community composition have recently been defined as "predictable" by Fuhrman et al. (2006) and Kan et al. (2006) who found a seasonal repeatability of assemblages off the southern California coast and in Chesapeake Bay respectively. In the Gulf of Trieste (Northern Adriatic Sea) some authors have already described bacterial annual dynamics (Turk et al., 2001; Paoli et al., 2006), the phylogenetic diversity of bacteria associated with marine snow (Rath et al., 1998) and bacterial community structure in mucilaginous aggregates (Cataletto, 2004), but there is no evidence of spatial and temporal variability within the basin. On the contrary, the larger-sized plankton fractions have been often studied both in terms of seasonal variations and in relation to freshwater inputs and/or to the Gulf's main circulation (Malej et al., 1995; Mozetič et al., 1998; Fonda Umani et al., 2007).

The aim of this work was to compare the bacterial composition of surface coastal assemblages in two stations characterized by different hydrological conditions. These two sites were sampled monthly throughout 2003 and by use of a distance matrix constructed from DGGE band profiles (absence and presence of the bands) we obtained several bacterial seasonal patterns. Then, the environmental variables that may explain the bacterial seasonal patterns were determined by application of Principal Component Analysis (PCA) and finally we examined the composition of bacterial communities by sequencing selected bands from DGGE.

2. Materials and methods

2.1. Study area and sampling

The Gulf of Trieste (Fig. 1) is the northernmost part of the Adriatic Sea with a surface area of about 600 km^2 and maximum depth of 26 m (Olivotti et al., 1986). The main freshwater input comes from the Isonzo River. Rivers from the south-eastern coast are of a torrential nature. Freshwater inputs show a high interannual variability (Malej et al., 1995), which affects salinity, whose values range from 32 to 38 at the surface (Fonda Umani, 1991; Celio et al., 2002). Water temperatures show a regular annual pattern from winter minima, as low as 6 °C in February, to summer maxima > 25 °C (Cardin and Celio, 1997). A high variability of the water column profile, due to riverine outflows and temperature variations, is enhanced by an alternance of cold winds from the east-north east (i. e. Bora) and mild winds from the south (i. e. Scirocco). Two sites were

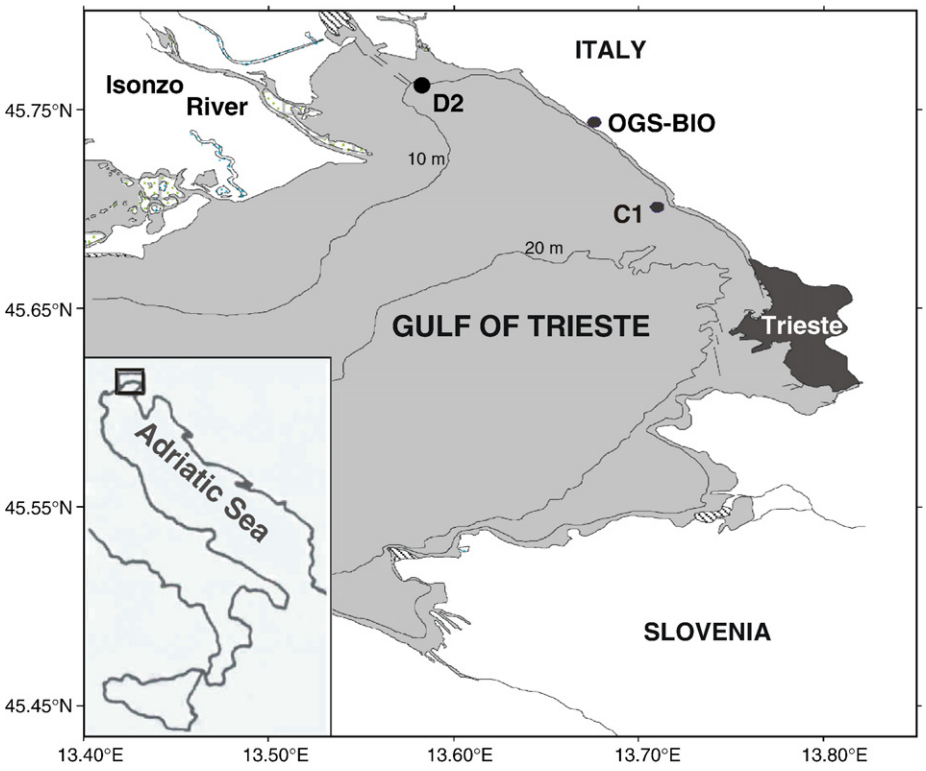

Fig. 1. Location of the C1 and D2 sampling stations in the Gulf of Trieste (Northern Adriatic Sea).