Seasonal and spatial variability of free-living bacterial community composition along an estuarine gradient (Ria de Aveiro, Portugal)

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Abstract

We examined the dynamics of the free-living bacterial community in the Ria de Aveiro estuary (Portugal) using 16S rDNA PCR–denaturing gradient gel electrophoresis (DGGE). Samples were collected along the salinity gradient in April, July and October 2003 and January 2004. Analysis of DGGE profiles indicated that the compositional shifts within this community occurred between the brackish and freshwater sections. Those shifts were particularly evident in samples collected in April, October and January. Less pronounced changes were also detected along the salinity gradient in the marine and brackish sections. Seasonally driven changes in microbial community in this estuary also occur. Canonical correspondence analysis (CCA) revealed that salinity and temperature accounted for a significant amount of the variability in the bacterioplankton community composition (26%). Dominant phylotypes were identified either by direct sequencing or by cloning and sequencing DGGE bands. Analysis of DNA sequences revealed that the dominant bacterial groups changed from Bacteroidetes, Alpha-proteobacteria and Gamma-proteobacteria in the marine-brackish section to Bacteroidetes, Beta-proteobacteria, Delta-proteobacteria and Epsilon-proteobacteria in the freshwater section of the estuary.
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1. Introduction

Estuaries are usually characterised by strong environmental gradients resulting from the mixing of fresh and marine waters. Salinity, nutrient concentration and organic matter composition gradients are known to strongly influence the composition and structure of the prokaryotic assemblages (Ducklow and Carlson, 1992; Bouvier and del Giorgio, 2002). Those gradients, as well as the mixing of freshwater and seawater communities and the probable existence of a typical prokaryotic estuarine community, are known to create a high degree of bacterial diversity and considerable spatial and temporal variability (del Giorgio and Bouvier, 2002; Crump et al., 2004).

Culture-dependent methods are known to be inadequate for analysis of microbial community because only a small fraction of bacteria in environmental samples are culturable (Ward et al., 1990). Molecular methods based on 16S rRNA genes are now widely used for providing new insights into microbial diversity and into structure and dynamics of microbial communities. Particularly, fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA fragments have been found suitable to analyse multiple samples simultaneously and to access temporal and spatial dynamics of microbial community (Muyzer et al., 1993). DGGE analysis has been used to characterise bacterioplankton community succession along several geographically diverse estuaries, such as the Rhone River estuary (Thousselier

et al., 2002), the Weser estuary (Selje and Simon, 2003), the Plum Island Sound estuarine system (Crump et al., 2004) and the Delaware estuary (Castle and Kirchman, 2004). Those studies gave important insights into the composition and variability of bacterioplankton community along the estuarine gradients, namely the confirmation that salinity plays a key role in determining the bacterioplankton distribution.

Ria de Aveiro is a mesotrophic estuarine system, located on the northwest coast of Portugal, with a complex network of inner channels into which industrial and domestic effluents have been discharged for many years. Despite the higher pollution levels Ria de Aveiro has a great economic importance: professional fishing in the lagoon is a traditional activity and recreational activities and aquaculture have been developed with greater intensity in recent years. Moreover, the estuary offers good conditions for agricultural development along its borders and for the setting up of a large number of small and medium industries.

Previously, the estuary has been extensively characterised in what concerns physical and chemical parameters like temperature, salinity, available nutrients and dissolved oxygen (Cunha et al., 2000; Almeida et al., 2002; Lopes et al., 2005). Along the salinity gradient in Ria de Aveiro, bacterioplankton populations displaying different metabolic capabilities have been reported. However, whether these differences are due to metabolic plasticity or to genetically diverse microbial assemblages was not elucidated, since data on their phylogenetic composition was not available (Cunha et al., 2000, 2001; Almeida et al., 2002).

Recently we used 16S rDNA clone libraries to examine the free-living bacterial community structure within the middle and outer sections of Ria de Aveiro (Henriques et al., 2004). Results obtained revealed a prokaryotic diversity comparable to other coastal and estuarine environments previously studied. On the other hand, some differences were observed in the types of sequences recovered from the two sections of the estuary. However, the above mentioned study included only two sampling sites and didn't assess the temporal variability.

The aim of this study was to examine the spatial and temporal distribution of the *Bacteria* phylotypes present in the free-living bacterial community in the estuarine system Ria de Aveiro. For that we used DGGE analysis of PCR amplified fragments of the 16S rDNA. The contribution of salinity and temperature on bacterioplankton community distribution was assessed using CCA (Canonical Correspondence Analysis). The identity of the predominant community members was assessed by analysis of sequences retrieved from DGGE-separated DNA fragments and comparison with sequences stored in databases.

2. Materials and methods

2.1. Site description and sampling

Ria de Aveiro is a shallow coastal lagoon situated on the northwest coast of Portugal, separated from the sea by a sand barrier. The estuary has an irregular and complex geometry, where four main channels can be identified. It has been observed that the differences between surface and bottom salinity and temperature values are very low, indicating that Ria de Aveiro is a well mixed estuary (Lopes et al., 2005). Studies were conducted along a longitudinal profile, extending across the inner estuary down to the outer segment of the lagoon (Canal de Ílhavo). This channel has 15 km in length, contains a water volume of $2.8 \times 10^6\,\text{m}^3$ at low tide and $9.3 \times 10^6\,\text{m}^3$ at high tide, and it connects to a permanent freshwater stream (Rio Boco) at its upper end, which creates a distinct salinity gradient.

Samples were collected in 2-L autoclaved dark bottles always during daytime, at low tide, approximately 0.2 m below the surface, in April (spring), July (summer), and October (autumn) 2003 and in January (winter) 2004. Seven sampling sites spaced regularly at 3 km were defined. From North to South, stations were designated N-1 (in the transition to the coastal zone), I-2, I-4, I-6, I-8 (middle-estuary) and I-10 and RB (defined as the mixing zone between the fresh and marine waters) (Fig. 1). To simplify the presentation of results, subsequently station N-1 will be referred as the marine section of
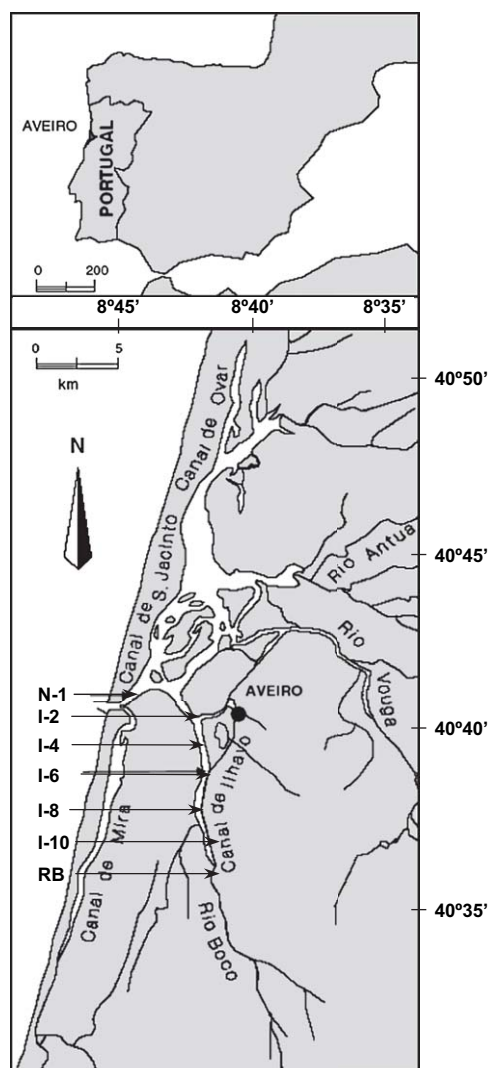

Fig. 1. Ria de Aveiro lagoon with sampling stations indicated by arrows.