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Bacterioplankton diversity and community composition in the Southern Lagoon of Venice

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ABSTRACT

The Lagoon of Venice is a large water basin that exchanges water with the Northern Adriatic Sea through three large inlets. In this study, the 16S rRNA approach was used to investigate the bacterial diversity and community composition within the southern basin of the Lagoon of Venice and at one inlet in October 2007 and June 2008. Comparative sequence analysis of 645 mostly partial 16S rRNA gene sequences indicated high diversity and dominance of *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* at the lagoon as well as at the inlet station, therefore pointing to significant mixing. Many of these sequences were close to the 16S rRNA of marine, often coastal, bacterioplankton, such as the *Roseobacter* clade, the family *Vibrionaceae*, and class *Flavobacteria*. Sequences of *Actinobacteria* were indicators of a freshwater input. The composition of the bacterioplankton was quantified by catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) with a set of rRNA-targeted oligonucleotide probes. CARD-FISH counts corroborated the dominance of members of the phyla *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes*. When assessed by a probe set for the quantification of selected clades within *Alphaproteobacteria* and *Gammaproteobacteria*, bacterioplankton composition differed between October 2007 and June 2008, and also between the inlet and the lagoon. In particular, members of the readily culturable copiotrophic gammaproteobacterial genera *Vibrio*, *Alteromonas* and *Pseudoalteromonas* were enriched in the southern basin of the Lagoon of Venice. Interestingly, the alphaproteobacterial SAR11 clade and related clusters were also present in high abundances at the inlet and within the lagoon, which was indicative of inflow of water from the open sea.

Introduction

Littoral ecosystems such as lagoons, estuaries, and salt marshes are among the most productive natural ecosystems on Earth [1,14]. The ecology of coastal lagoons is strongly influenced by the physical and chemical environment, such as the fluxes caused by currents and tides, the import of marine sediment and water, and terrestrial inputs by rivers and groundwater. The sporadic mixing of fresh and marine waters results in a high physico-chemical variability both in space and time [50]. The relatively closed lagoon ecosystems are strongly exposed to nutrient input from the surrounding area, including anthropogenic pollution [9]. Coastal lagoons are usually surrounded by extensive areas of freshwater and salt marshes which are important sources of dissolved organic matter (DOM) [50]. The Venice lagoon is a shallow water basin located on the coast of the Northern Adriatic Sea, just north of the River Po delta. The lagoon is connected to the sea by three 400–900 m wide and 15–50 m deep inlets which divide it into three hydrological basins [58] called the Northern Lagoon or Basin of Lido, the Central Lagoon or Basin of Malamocco, and the Southern Lagoon or Basin of Chioggia. The high water exchange with the sea, which mainly depends on a tidal regime, is one of the most important factors for the quality of waters, sediments and biota. This exchange with the sea also determines the dispersion and reduction of pollutant levels [65]. In the last decade, the Lagoon of Venice has been declared UNESCO patrimony, and as a consequence several studies have focused on the pollution in this ecosystem [7]. The current situation of the lagoon is alarming in view of its apparent degradation, with signs of anoxia in bottom layers in summer, a decline of zooplankton, and a build-up of sulphide in the upper layers of bottom sediments accompanied by a shift in bottom flora composition [59]. Several studies have proved the presence of a wide range of contaminants in its different basins [39] [10] but little is known about the microbial community. Only recent studies have focused on the seasonal and spatial dynamics of the

Abbreviations: CARD, Catalyzed reporter composition; FISH, Fluorescence *in situ* hybridization; FA, formamide.

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microbial community of the Lagoon of Venice, and they have highlighted that there is a strong seasonality of the planktonic bacterial assemblage [16], as well as spatial differences in community composition between sub-basins [12].

The aim of this study was to examine the bacterial diversity and community composition in surface waters of the southern basin of the Lagoon of Venice near the city of Chiogga. Here, we present data obtained by the rRNA approach to microbial ecology [6] at the Chioggia inlet and within the lagoon in two different seasons. Insights into bacterioplankton diversity were obtained by comparative sequence analysis of several 16S rRNA gene clone libraries. Furthermore, the quantitative bacterial community composition was investigated by catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) with rRNA-targeted oligonucleotide probes. We hypothesized that the high availability of inorganic and organic nutrients in the lagoon should result in high cell numbers, and shift the taxonomic composition of the bacterial community towards copiotrophic taxa. A particular focus of the study was on members of the class *Flavobacteria* of the phylum *Bacteroidetes* for which we hypothesized high diversity and abundance in this nutrient-rich coastal setting of the Basin of Chioggia based on recent reports [4].

Materials and methods

Study site and sampling procedure

Surface water samples were taken around 9 am near the city of Chioggia, Italy, on October 17, 2007 and June 10, 2008 at two stations (Fig. 1): (i) within the lagoon [45°13′24″ N, 12°17′5″E] (in front of the Hydrobiological Station of Chioggia), and (ii) in the Southern Chioggia inlet [45°13′58″N, 12°17′30″E]. Water depth at the inlet station was around 20 m, whereas the lagoon station only had a depth of 4 m. On October 17, 2007 the general direction of water flow at the inlet was into the lagoon, while on June 10, 2008 the flow was directed outwards. Water samples were collected in replicates directly in acid-rinsed plastic bottles, and immediately transported to the laboratory. For DNA extraction, 1 L aliquots were directly filtered onto 0.2 μm pore size polycarbonate membrane filters (GTTP, 47 mm diameter, Millipore, Eschborn, Germany). Filters were immediately frozen, and kept at −20 °C until processed for nucleic acid extraction.

For CARD-FISH, 10 mL aliquots were collected and fixed with paraformaldehyde (EM Grade, Hatfield, PA) for 1–24 h at 4 °C (1% final concentration). The fixed samples were subsequently filtered onto 0.2 μm pore size polycarbonate filters (GTTP, 47 mm diameter, Millipore) and stored at −20 °C.

Determination of chlorophyll a and b

For determination of chlorophyll concentrations, cells were collected on glass fibre filters (nominal pore size 0.7 μm; Whatman, Dassel, Germany). Samples were immediately frozen at −20 °C. Frozen samples were placed in 15 mL centrifuge tubes, 5 mL of acetone were added, and the tubes were sonicated on ice for 10 × 1 min. Pigments were extracted from the mixture at −20 °C, overnight. Samples were then filtered (Acrodisc CR syringe filter, 0.45 μm pore size, PALL, Gellman Laboratory; Dreieich, Germany), and the filtrate was diluted with water to a final concentration of 70% acetone. Photo-pigments were separated on an HPLC 2695 separation module (Waters, Eschborn, Germany), equipped with a Reprosil-Pur-120 C18, 5 μm 250 × 4.6 mm (Trentec Analysentechnik, Gerlingen, Germany), according to the method given in [63]. Absorption spectra were measured on a Waters 996 photo diode array detector, and the pigments were quantified and identified by comparison to pigment standards (DHI Waters and Environment, Horsholm, Denmark).

CARD-FISH and cell counting

CARD-FISH was performed according to Pernthaler et al. [51] with slight modifications. Briefly, cells were immobilized on GTTP filters by embedding in 0.2% agarose (Metaphor, Cambrex Bio Science, Rockland, USA), and they were subsequently permeabilized by treatment with 10 mg mL^{-1} lysozyme in 50 mM EDTA, 100 mM Tris/HCl for 1 h at 37 °C. Filter sections were cut and hybridized with horseradish peroxidase (HRP)-labelled oligonucleotide probes (Biomers, Ulm, Germany) for 2.5 h at 46 °C. Probes targeted members of the *Bacteria* (EUB338 I-III, 35% FA, [24]), the alphaproteobacterial SAR11 clade (SAR11-441, 25% FA, [45]) and the *Roseobacter* clade (Ros537, 35% FA, [26]), *Alphaproteobacteria* general probe (ALF968, 35% FA, [47]), the gammaproteobacteria affiliated to the family *Vibrionaceae* (GV, 30% FA, [30]), the genus *Pseudoalteromonas* (PSA184, 30% FA, [26]), the NOR5/OM60 clade (mix of probes NOR5-730, [27] and NOR5-1238, 50% FA, [64]), the *Alteromonas/Glaciecola* clade (ALT1413, 40% FA, [26]), *Alteromonas macleodii* (Amac83, 35% FA, [2]), the *Gammaproteobacteria* general probe (GAM42at 35% FA, with competitors according to Manz et al., [43]), the *Betaproteobacteria* (BET42a 35% FA, with competitors according to Manz et al., [43]), the *Cytophaga/Flavobacterium* cluster of *Bacteroidetes* (CF319a, 35% FA, [42]), and the *Planctomycetes* (PLA46, 30% FA, [48]). The hybridized and washed filter sections were subsequently embedded in a mounting mixture containing 1 μg mL^{-1} DAPI [51]. The fraction of DAPI-stained objects detected by a particular probe was determined by epifluorescence microscopy (AXIOPLAN 2, Zeiss, Jena, Germany). Controls with the antisense probe NON338 [60] were always negative. At least 1000 DAPI-stained cells were manually counted.

DNA extraction and PCR amplification

Total nucleic acids were extracted from the 0.2 μm pore size GTTP filters, as described previously [66]. Nearly complete 16S rRNA genes were amplified by PCR using the general bacterial primers GM3 and GM4 [46]. The 50 μL PCR reactions contained 0.3 mg mL^{-1} BSA, 2.5 mM total dNTPs, 1 × PCR buffer including 1.5 mM of Mg chloride, 0.5 mM of each primer, and 1 U of Master Taq Polymerase (Eppendorf, Hamburg, Germany). The amplification was carried out with a Thermocycler Mastercycler (Eppendorf) as follows: an initial denaturation step at 95 °C for 3 min, followed by 25 cycles of 1 min denaturation at 95 °C, 2 min annealing at 44 °C, and 2 min elongation at 72 °C. There was a final extension step of 15 min at 72 °C.

Clone library generation, sequencing and phylogenetic analysis

In order to avoid cloning of small and non-specific PCR products, fragments of the correct size of ∼1.5 kb were excised from 1.5% agarose gel, and subsequently extracted and purified using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). Cleaned PCR products were cloned either into the pGEM-T Easy vector (Promega, Madison, WI) or the pCR4 TOPO vector (Invitrogen, Groningen, the Netherlands), according to the manufacturers' instructions. The recombinant vectors were transformed into chemically competent *E. coli* cells, DH10B strain (Invitrogen, Groningen, the Netherlands), plated, and 192 clones from each library were picked. Clone inserts were amplified by PCR using vector primers M13F (5′-GTAAAACGACGGCCAG-3′) and