



Multilayer approach for characterization of bacterial diversity in a marginal sea: From surface to seabed



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ARTICLE INFO

Keywords:

Bacterial diversity
High throughput sequencing
qPCR
Flow cytometry
Southern Adriatic Sea

ABSTRACT

Bacteria are the most important microorganisms in the world oceans, accounting for up to 75% of the total biomass. They are responsible for fundamental biogeochemical processes and therefore often used as ecological indicators. In this study, bacteria were quantified by flow cytometry and their diversity assessed by High Throughput Sequencing (HTS) in the southern Adriatic Sea. The most abundant bacterial groups were also quantified by qPCR. The samples were collected from the surface to the seabed over a total of 16 different depths at four stations during the late winter BIOTA (BIO-Tracing Adriatic water masses) cruise conducted in March 2016. The investigated area showed unusual water mass properties and was characterized by a shallow mixed layer, which differed from the usual winter convection conditions, typical of middle-altitude ecosystems and important for the seasonal picoplankton dynamics of this area. Heterotrophic bacteria were separated into HNA (relative High Nucleic Acid content) and LNA (Low Nucleic Acid content) subpopulations with abundances up to 1.8×10^5 and 8.8×10^5 cells mL⁻¹, respectively. HNA dominated at offshore stations reaching their maximum at depths below the euphotic zone. The bacterial community was dominated by *Alphaproteobacteria*, accounting for > 40% of the total sequence reads and were mainly represented by the SAR11 clade (90.84%), followed by *Marinimicrobia* (18% of the total sequence reads), mainly represented by clade SAR406 (8.44%). Distinctive bacterial groups were found in the euphotic layer (*Bacteroidetes* and *Actinobacteria*) and aphotic layer samples (*Deltaproteobacteria*, *Marinimicrobia*, *Chloroflexi*, *Acidobacteria* and *Planctomycetes*). Results of the qPCR analyses further confirmed HTS results with highest abundances obtained for *Alphaproteobacteria*, followed by *Gammaproteobacteria* and *Bacteroidetes*. The adopted multiple approach, combining different molecular tools, critically supported by optics and flow cytometry, reveal changes in the bacterial assemblages during the unusual thermohaline conditions observed in 2016 in the southern Adriatic Sea.

1. Introduction

Bacteria are important constituents of the picoplankton community, playing a critical role in biogeochemical cycling of key elements in marine ecosystems (Cole et al. 1988) and accounting for up to 75% of the total carbon biomass in surface and deep waters (Aristegui et al. 2009, Fuhrman et al. 1989). In spite of their widespread occurrence, relatively little is known about the factors driving their diversity, community composition and adaptation to the global ocean. Recent applications of new molecular techniques have revolutionized

microbial research and provided useful insights into complexity and the vast marine microbial diversity. High Throughput Sequencing (HTS) and bioinformatics analyses allow to detect both the most abundant community members and rare species which could not be retrieved by traditional cultivation, cloning and sequencing methods (Pedrós-Alió 2006), providing more robust and reliable information of the microbial community composition. These methods have been used during global research cruises, such as the Sorcerer II Global Ocean Sampling (GOS) expedition (Martiny et al. 2006, Rusch et al. 2007) or the Tara Oceans global expedition (Karsenti et al. 2011), aimed at better understanding

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