Marine Genomics 17 (2014) 9–15

Contents lists available at ScienceDirect

Marine Genomics

CIESM 2013

Diversity of rare and abundant bacteria in surface waters of the Southern Adriatic Sea

Grazia Marina Quero, Gian Marco Luna *

Institute of Marine Sciences (CNR-ISMAR), National Research Council, Venezia, Italy

ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form 26 March 2014
Accepted 1 April 2014
Available online 13 April 2014

Keywords:
Bacterioplankton
ARISA
Pyrosequencing
Rare biosphere
Adriatic Sea

ABSTRACT

Bacteria are fundamental players in the functioning of the ocean, yet relatively little is known about the diversity of bacterioplankton assemblages and the factors shaping their spatial distribution. We investigated the diversity and community composition of bacterioplankton in surface waters of the Southern Adriatic sub-basin (SAd) in the Mediterranean Sea, across an environmental gradient from coastal to offshore stations. Bacterioplankton diversity was investigated using a whole-assemblage genetic fingerprinting technique (Automated Ribosomal Intergenic Spacer Analysis, ARISA) coupled with 16S rDNA amplicon pyrosequencing. The main physico-chemical variables showed clear differences between coastal and offshore stations, with the latter displaying generally higher temperature, salinity and oxygen content. Bacterioplankton richness was higher in coastal than offshore waters. Bacterial community composition (BCC) differed significantly between coastal and offshore waters, and appeared to be influenced by temperature (explaining up to 30% of variance) and by the trophic state. Pyrosequencing evidenced dominance of *Alphaproteobacteria* (SAR11 cluster), uncultured *Gammaproteobacteria* (*Rhodobacteraceae*) and *Cyanobacteria* (*Synechococcus*). Members of the *Bacteroidetes* phylum were also abundant, and accounted for 25% in the station characterized by the higher organic carbon availability. Bacterioplankton assemblages included a few dominant taxa and a very large proportion (85%) of rare (<0.1%) bacteria, the vast majority of which was unique to each sampling station. The first detailed census of bacterioplankton taxa in the SAd sub-basin, performed using next generation sequencing, indicates that assemblages are highly heterogeneous, spatially structured according to the environmental conditions, and comprise a large number of rare taxa. The high turnover diversity, particularly evident at the level of the rare taxa, suggests to direct future investigations toward larger spatial or temporal scales, to better understand the role of bacterioplankton in the ecosystem functioning and the biogeochemistry of the basin.

1. Introduction

Marine microorganisms are crucial players in the ecosystem functioning of the global ocean, due to their role in the cycling of organic matter and the major elements, and the existence of the "microbial loop" (Azam et al., 1983; Cho and Azam, 1988; Karl, 2007). Planktonic Bacteria and Archaea dominate biomass and are centrally involved in the fluxes of energy and matter in the sea (DeLong et al., 2006). Heterotrophic bacteria process about half of the primary production and play a key role in the microbial carbon pump, by transforming labile organic matter into refractory forms that persist in the ocean (Benner and Herndl, 2011).

For decades, the study of bacterial diversity in aquatic ecosystems has been hampered by the inability to cultivate and further identify them using conventional microbiological methods. With the advent of molecular biology techniques, pioneered by early cloning and sequencing studies (Giovannoni et al., 1990) and later improved by the application of high-throughput massive sequencing of ribosomal genes (Sogin et al., 2006), it has become possible to study the richness and community composition of bacterioplankton under unprecedented detail. These studies have led to important discoveries about the taxonomic identity of bacterioplankton communities, and are shedding light on the patterns and drivers of diversity in surface and deep waters. Morris et al. (2002) discovered that the SAR11 clade (*Alphaproteobacteria*) accounts for up to a third of the cells in surface waters. Several other studies have documented the spatial variability in aquatic microbial communities over different spatial scales (e.g. Hewson et al., 2006; Pommier et al., 2007; Fuhrman et al., 2008) and investigated the relationships between diversity and the environmental variables, such as temperature, salinity or the availability of resources (Lozupone and Knight, 2007; Fuhrman et al., 2008; Galand et al., 2010; Fortunato et al., 2012). The application of high-throughput sequencing has also led to the discovery of the "rare" prokaryotic biosphere (Sogin et al., 2006), which is believed to consist of dormant microorganisms that can be resuscitated under

* Corresponding author at: National Research Council, Institute of Marine Sciences (CNR-ISMAR), Castello 2737/f, Arsenale Tesa 104, 30122 Venezia, Italy. Tel.: +39 041 2407993.
E-mail address: gianmarco.luna@ve.ismar.cnr.it (G.M. Luna).

http://dx.doi.org/10.1016/j.margen.2014.04.002
1874-7787/

different environmental conditions (Galand et al., 2009; Campbell et al., 2011). Studies on the diversity of bacterioplankton have been also carried out in the Mediterranean Sea (Ghiglione et al., 2005; Alonso–Saez et al., 2007; Pommier et al., 2010). However, more studies are needed to better understand the spatial patterns of rare and abundant bacterioplankton species in the Mediterranean Sea and to predict their potential response to natural or anthropogenic changes.

The Adriatic Sea is an elongated basin located in the central Mediterranean Sea, between the Italian peninsula and the Balkans (Artegiani et al., 1997). It can be divided into three sections: the northern one is very shallow and gently sloping (average bottom depth of about 35 m), the middle one is on average 140-m deep (with two depressions reaching 260 m) and the southern section is characterized by a wide depression more than 1200-m deep. A large number of rivers discharge into the basin, with the Po River in the northern basin being the most considerable (Manini et al., 2004). Bacterioplankton communities in the Adriatic Sea have been the subject of many studies. However, they have mostly addressed the spatio-temporal patterns of bacterioplankton abundance and metabolism (Puddu et al., 1997; La Ferla et al., 2005; Fonda Umani et al., 2007; Del Negro et al., 2008) and the biotic and abiotic factors influencing them (Solic and Krstulovic, 1994; Weinbauer and Peduzzi, 1995). Other studies have been carried out to study the bacterial role in the formation of large organic matter aggregates (Herndl and Peduzzi, 1988; Degobbis et al., 1999; Danovaro et al., 2005). Conversely, only two studies have focused on bacterioplankton diversity and have addressed the temporal patterns of diversity in the northern Adriatic by using the low-resolution, DGGE fingerprinting technique (Celussi et al., 2011; Šilović et al., 2012). Consequently, the Southern Adriatic sub-basin (SAd) has so far remained completely unexplored in terms of bacterioplankton diversity and BCC.

In this study, we used a whole-assemblage genetic fingerprinting technique (ARISA; Fisher and Triplett, 1999; Danovaro et al., 2006) and 16S rDNA amplicon pyrosequencing to describe, for the first time so far, the diversity and community composition of bacterioplankton assemblages in surface waters of the SAd sub-basin. We also assessed their spatial distribution, the role played by the main environmental variables in shaping diversity, and the contribution of rare versus dominant taxa to the assemblage diversity.

2. Methods

2.1. Sampling activities and sample processing

Seawater samples were collected in the South Adriatic Sea onboard the italian R/V Minerva Uno during the "ODW2012" cruise carried out from 23 March to 02 April 2012 (Fig. 1). Surface water was collected from 7 stations using a CTD–rosette system consisting of a CTD SBE 911 plus, and a General Oceanics rosette with 12 Niskin Bottles (having the capacity of 12 l). The sampling strategy was designed to cover stations located close to the coast (<100 m bottom depth, stations 44, 46 and 60) and offshore stations (>100 m bottom depth, stations 29, 39B, 42, 43B). These stations differed in the main physical–chemical variables (see the Results section). At all sampling stations, temperature, salinity, dissolved oxygen concentration and fluorescence (as a proxy for chlorophyll-a concentrations) were measured. Temperature measurements were performed with two SBE-3/F thermometers (resolution of 10^{-3} °C), and conductivity measurements were performed with two SBE-4 sensors (resolution of 3×10^{-4} S/m). Dissolved oxygen was measured with a SBE-43 sensor (resolution 4.3 μM), and data were checked against Winkler titration. The CTD probe has been calibrated before the cruise. In addition, surface water samples were collected for the determination of total suspended matter (TSM), which were carried out by a standard filtration (onto GF/F filters) and weighting method.

For analyses of bacterial diversity, seawater samples were filtered (2 l for each sample, two duplicate filters from each station) through a sterile 200-μm mesh net (to remove larger eukaryotic organisms containing plastids, which may interfere with the molecular analyses; Fuhrman et al., 2006) before concentration onto 0.22 μm Cellulose Nitrate membrane filters (Sartorius). Samples were processed onboard immediately after collection. Each filter was put into a sterile Petri dish using sterile forceps and stored at −20 °C until return to the laboratory.

2.2. DNA extraction

DNA was extracted from each filter using the PowerWater® DNA Isolation Kit (MoBio Laboratories Inc., California), according to the manufacturer's instructions with some slight modifications to increase the DNA yield and quality. These modifications included two additional

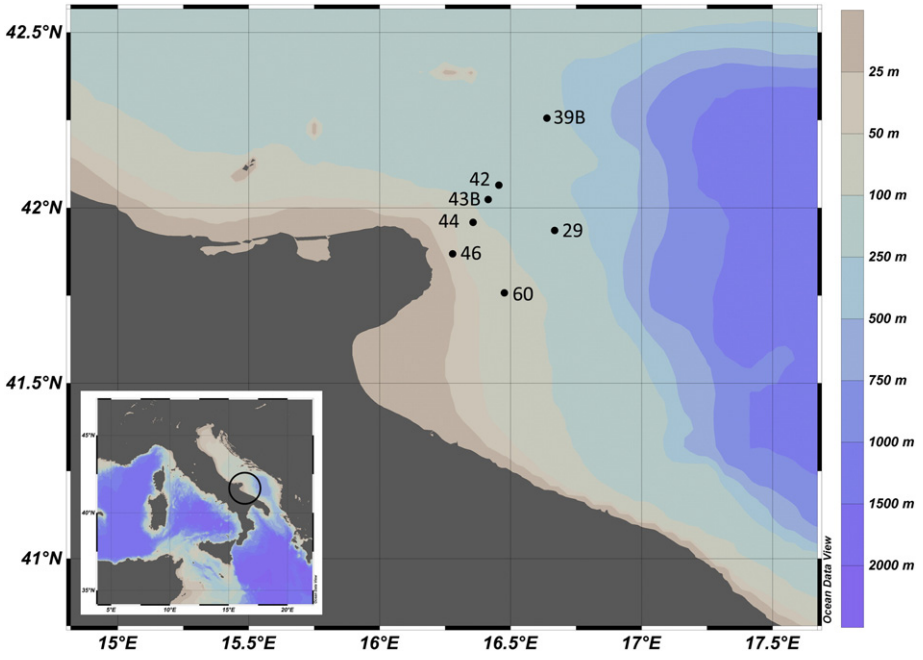

Fig. 1. Map of the investigated area with the location of the sampling stations. The map has been created using the Ocean Data View software.