IN DEPTH CHARACTERIZATION OF MARINE CYANOBACTERIA COMMUNITY: TARGETING OF *PROCHLOROCOCCUS* ECOTYPES

I. Bosnjak [1]*, I. Petric [2], I. Cetinic [3], S. Bosak [1], M. Mejdandzic [1] and Z. Ljubesic [1]
[1] Department of Biology, Faculty of Science, Zagreb, Croatia - ivana.bosnjak@biol.pmf.hr
[2] Division for Marine and Environmental Research, Ruder Boskovic Institute, Zagreb, Croatia
[3] NASA Goddard Space Flight Space Center, MD USA / USRA, Columbia, Maryland, USA

Abstract

Molecular tools are used in order to investigate ubiquitous marine cyanobacteria *Prochlorococcus* ecotype diversity and distribution patterns and its ecology is extensively studied in different oceanic regions. Still, little is known about *Prochlorococcus* microdiversity in the oligotrophic Adriatic Sea. In this study, by using ITS (internal transcribed spacer) as a molecular marker, presence of 2 *Prochlorococcus* populations corresponding to HLI (*P. marinus* MED4) and LLI ecotype (*P. marinus* NATL1A), were confirmed, showing high patchiness throughout the water layer.

Keywords: Cyanobacteria, Genetics, South Adriatic Sea

Introduction: *Prochlorococcus* is the most ubiquitous and important picocyanobacteria and the smallest photosynthetic organism of oligotrophic marine environments. It has an important ecological function as major contributor to primary production and carbon cycle [1]. Two major groups of *Prochlorococcus,* also known as ecotypes, inhabit different layers of the marine photic zone: (i) high-light or HL (clades HLI – HLIV), adapted to higher light intensities and (ii) low-light or LL (clades LLI – LLIV), adapted to lower light intensities. HL- and LL ecotypes also differ by their temperature acquirements and nutrient acquisition, both correlated to specific genome size and presence/absence of genes [1]. Presence of the genus *Prochlorococcus* in the Adriatic Sea is well known, but certain environmental ecotypes, as well as their vertical and horizontal distribution, are still not determined. The ongoing identifications of novel clades across the oceans indicate that the whole diversity of *Prochlorococcus* has not been fully recovered and increase the chances of new discoveries, especially in under-sampled areas of Adriatic Sea. Thus, the main aim of this study was to use molecular tools based on the marker gene for 16S-23S rDNA ITS region [2] in order to analyze genetic diversity and distribution patterns of *Prochlorococcus* populations in the vertical profile of the northeastern peak of southern Adriatic Sea water column.

Material and Methods: In March 2015 a cruise was conducted in the Southern Adriatic Pit. For the specific molecular analyses water samples were collected by Niskin bottles at 3 different depths (20, 80, 140 m) at the station P150A (42.33°N, 17.58°S) and immediately filtered (filters stored at -20 °C). Total genomic DNA was extracted from filters by using phenol-chloroform protocol [2]. Clone libraries were generated by PCR amplification of the 16S-23S rRNA ITS using the 2F-ITS and 3R-ITS primers [2] and subsequent cloning into pGEM®-T vector. In total, 96 positive clones from all depths were sent for Sanger sequencing (Macrogen, Netherlands). Retrieved sequences were edited and checked manually and compared to the NCBI Genbank database using Blast tool. Multiple sequence alignments were performed with ClustalX 2.1 and BioEdit softwares, with default parameters. MEGA 6 software was used to perform phylogenetic analysis, both Neighbour Joining (NJ) and Maximum Likelihood (ML). Bootstrapping was based on 1000 replications. ML tree was chosen as best suited for phylogenetic display.

Results and Discussion: From the total number of sequences analyzed in the dataset 69% belonged to the HL I clade / eMED4 while 23% belonged to the LL I clade / eNATL2A (8% belonged to the uncultured Cyanobacteria; Fig. 1). To our knowledge this is the first record of *Prochlorococcus* clades diversity in the Adriatic Sea. Results are comparable to the total distribution of *Prochlorococcus* communities found across water column of the Red Sea (80% of analyzed sequences belonged to HL-adapted and 20% to LL-adapted clade) [2]. In vertical profile, HL I clade-affiliated sequences were found at all depths, including 140 m. This was expected as HL I clade / MED4 is known to dominate in the Mediterranean where it was found up to 100 m depth due to adaptation to low amounts of phosphorous in the oligotrophic water environment [3]. LL I clade-affiliated sequences (23% of total sequences) were only found at higher depths (80 and 140 m) that correspond to intermediate water layer [2] while were absent at 20 m (Fig. 1). All analyzed LL clade-affiliated sequences clustered only with LL I / NATL2A ecotype, suggesting it as the predominant LL *Prochlorococcus* clade in the Adriatic Sea. In conclusion, diversity study of *Prochlorococcus* in the South Adriatic suggested that certain *Prochlorococcus* ecotypes occupy specific water depth while for other overlapping light-intensity niches were found. HL I clade/ MED4 was shown to dominate *Prochlorococcus* community throughout the water column, similar as reported for the Mediterranean. In contrast to this, the diversity at higher depths (80 and 140 m) exhibited varied compositions of both HL I and LL I / NATL2A clades. Ongoing research will reveal exact diversity and the abundance of *Prochlorococcus* community in the Adriatic Sea.

Acknowledgments: This work has been supported by Croatian Science Foundation under the project BIOTA UIP-11-2013-6433.

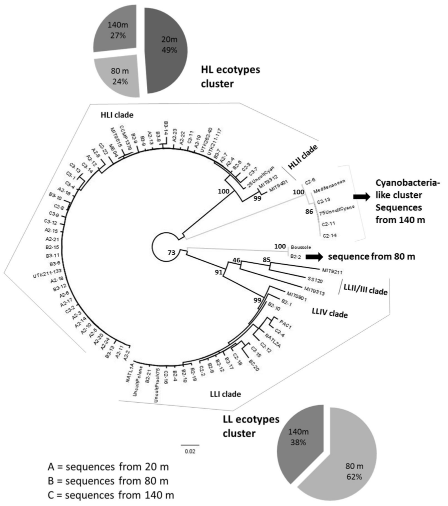

Fig. 1. ML phylogenetic tree showing the relationships of *Prochlorococcus* HL and LL ecotypes and clades, as well as the Cyanobacteria-like cluster. Branch support values of >75% are indicated.

References

1 - Biller et al. (2015) *Prochlorococcus*: the structure and function of collective diversity. *Nature Rev Microbiol* 13.1: 13-27.

2 - Shibl et al. (2014) Distribution and diversity of *Prochlorococcus* ecotypes in the Red Sea. *FEMS Microbiol Lett* 356: 118-126.

3 - Garczarek et al. (2007) High vertical and low horizontal diversity of *Prochlorococcus* ecotypes in the Mediterranean Sea in summer. *FEMS microbiology ecology* 60.2: 189-206.