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CHARACTERIZATION OF THE CYANOBACTERIAL COMMUNITY IN A LARGE SHALLOW LAKE IN CENTRAL ITALY BY FULL SHOTGUN METAGENOMICS

Nico Salmaso[1,2], *Leonardo Cerasino*[1], *Margherita Di Brizio*[3], *Massimo Pindo*[1], *Adriano Boscaini*[1]

[1] Research and Innovation Centre, Fondazione Edmund Mach, Via Edmund Mach 1,
38098 San Michele all'Adige, Italy
[2] NBFC, National Biodiversity Future Center, 90133 Palermo, Italy
[3] UOLM sito Perugia, ARPA Umbria, Via Pievaiola 207/B-3, 06132 Perugia, Italy

The polyphasic approach has important bottlenecks that limit the study to isolated and cultured specimens, single individuals or monospecific environmental samples. On the other hand, the low taxonomic resolution of short marker genes obtained by metabarcoding limits taxonomic classification to the genus level and hinders a thorough functional analysis. The increasing application of whole genome sequencing (WGS) methods in both genomic and metagenomic approaches, including short read assembly (Illumina) and long read assembly (PacBIO and Oxford Nanopore), has enabled the reconstruction of draft or circular cyanobacterial genomes in isolates or complex microbial communities, providing information on strain-level taxonomy and metabolic functions following genome annotation. In this paper, we report the results of a full shotgun metagenomic analysis performed on two environmental samples collected in the summer of 2023 in a large (124 km2 and 586×106 m3) and shallow (zmax 6 m) lake located at 258 m a.s.l. in central Italy (Lake Trasimeno). The taxonomic classification was based on the calculation of genome similarity metrics (ANI, average nucleotide identity and dDDH, digital DNA-DNA hybridization values) and phylogenomic approaches using reference databases (NCBI and GTDB). The metabolic characteristics of the species and the range of secondary metabolites (including toxins) were analyzed using selected functional annotation tools and databases (including KEGG). Lake Trasimeno is eutrophic and strongly dominated by cyanobacteria, mainly Raphidiopsis (Cylindrospermopsis) raciborskii, which was first formally documented in the lake in 1995. Besides Raphidiopsis, other less common cyanobacteria included species previously identified by microscopy belonging to Anabaenopsis and Pseudanabaena, as well as other cyanobacterial species never reported before (Prochlorothrix) or difficult to detect with conventional approaches (non-photosynthetic cyanobacteria, Vampirovibrionia strains). It is emphasized that genome-based approaches are the only ones that provide reliable information on cyanobacteria at the strain and functional level, delineating their evolutionary relationships and functional roles in ecosystems.