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IN SILICO CHARACTERIZATION OF MICROBIAL DIVERSITY IN FRESHWATER BODIES AND DETECTION OF CYANOTOXIN BIOSYNTHETIC GENE CLUSTERS TO EVALUATE THEIR EXPRESSION DURING BLOOMS

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Cyanobacteria are photosynthetic microorganisms that live in almost all water bodies and have an important ecological role. Nutrient excess and rising water temperatures can alter the equilibrium of the water bodies resulting in "blooms", defined as a rapid and large rise in cyanobacteria number. Cyanobacteria may produce hazardous secondary metabolites known as cyanotoxins during certain blooms. These are referred to as "harmful blooms" and are of great public health importance because of their potential threat to animal and human health. Nowadays, microcystin is the most commonly monitored cyanotoxin, however, there are several other cyanotoxins potentially present in the freshwater bodies. The essential genes for cyanotoxin synthesis are located in biosynthetic gene clusters (BGCs). Their genetic sequences are useful targets for finding toxin-producing bacteria. Our hypothesis states that it is possible to detect BGCs by means of bioinformatic analysis of the water bodies metagenomes, and this information could be useful for designing proper monitoring tools. Our research involves long-read sequencing of the metagenomes of several water bodies in Argentina to determine the structure and composition of microbial communities and the presence of cyanobacteria and BGCs. The cyanotoxin biosynthesis genes in the samples were identified by similarity with a manually created database derived from genomic sequences accessible through NCBI Nucleotide (<http://www.ncbi.nlm.nih.gov/nucleotide>). So far, we have studied three water bodies in Argentina, a lagoon in Buenos Aires Province (PBA) and two water reservoirs in Córdoba (COR) and Salta (SLA). For the lagoon in PBA the abundance of the 16S molecular marker showed a higher proportion of the phyla Cyanobacteria, Proteobacteria and Bacteroidetes. The two most abundant cyanobacterial species were classified within picocyanobacteria. The alignment of cyanotoxin biosynthetic gene sequences to the metagenome revealed the

presence of genes responsible for microcystin, nodularin, saxitoxin, anatoxin and cylindrospermopsin production. In the SLA Reservoir, the most abundant phyla according to 16S analysis were Proteobacteria, Bacteroidetes and Cyanobacteria. *Raphidiopsis* CRJ1 and *Microcystis* PCC 7914 were the most prevalent cyanobacteria. Biosynthetic genes for nodularin, microcystin, and saxitoxin were mapped and the presence of microcystin BGC was confirmed by PCR of the gene *mycB*. The molecular marker analysis in COR lake resulted in a higher dominance of the phyla Dinoflagellata, Proteobacteria and Actinobacteria in decreasing order of abundance. The 16S sequence of only one *Nostocaceae* cyanobacteria was detected and microcystin and anatoxin biosynthetic genes were mapped. It remains to confirm by qPCR and RT-qPCR the abundance of genes and their transcripts, however, the observation of BGCs in addition to microcystin biosynthetic genes highlights the importance of the monitoring of other cyanotoxins.

Palabras clave: cyanotoxin - cyanobacteria - environmental ecology - water resources