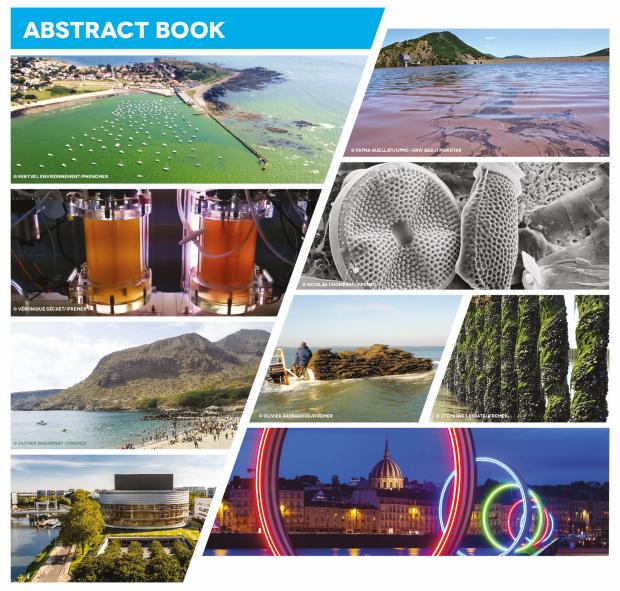
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Taxonomy

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The use of High-Throughput Sequencing in the study of the diversity of toxigenic cyanobacteria

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Abstract: The study of cyanobacterial diversity in aquatic environments has been traditionally carried out through the microscopic examination of samples and the use of molecular techniques based on culture-dependent approaches. A correct identification of cyanobacteria is of the utmost importance, due to the ability of these organisms to produce a wide variety of toxins. Nonetheless, in environmental studies the traditional approaches have many limitations, because they underestimate the number of cyanobacterial taxa, whereas identifications might be affected by taxonomic ambiguities. This is especially true in the detection of rarest or smaller individuals. This issue in only partially softened by the use of traditional and expensive and time-consuming cultivation-independent approaches, such as gel electrophoresis based methods, CARD-FISH, and clone and sequencing. This situation has radically changed with the advent of new cultivation-independent high-throughput sequencing (HTS) technologies, which opened new perspectives in the evaluation of both biological and functional diversity. In this contribution, the application of marker gene amplification metagenomics in the study of cyanobacterial diversity will be evaluated with practical examples. A recent study carried out in Lake Garda highlighted pros and cons of this approach based on the amplification of 16S rRNA genes. Results obtained from two years investigations allowed discovering a wide cyanobacterial diversity. The most abundant OTUs identified coincided with the most abundant and larger taxa quantified using traditional microscopic and molecular approaches (e.g. the toxic Tychonema bourrellyi and Planktothrix rubescens, and non-toxic Dolichospermum lemmermannii), as well as metabolomic profiling of cyanotoxins. In addition, HTS identified many other abundant but smaller Synechococcales and Chroococcales, along with other rare Nostocales and the new nonphotosynthetic cyanobacterial groups ML635J-21 and Melainabacteria, never identified so far in the large lakes south of the Alps. The potential of these techniques to unravel the bacterial and metabolomic diversity in non axenic cultures will be further evaluated considering a few approaches used to study algal inocula for photo-bioreactors (project H2020-MSCA-RISE AlgaeCeuticals). The application of marker gene amplification is not free of complications, e.g. due to the short length of the most used 16S rRNA gene sequences obtained with Illumina MiSeq technologies, the scarsity of information available in reference databases for other molecular markers, and limits inherent to the most common bioinformatic pipelines. The use of less widespread approaches, such as full shotgun sequencing, represents powerful complementary tools to disentangle biodiversity and functions of cyanobacteria and plankton.

Disclosure of Interest: None Declared