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Transdisciplinary approaches for the study of cyanobacteria and cyanotoxins

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ABSTRACT

Cyanobacteria, ancient aerobic and photoautotrophic prokaryotes, thrive in diverse ecosystems due to their extensive morphological and physiological adaptations. They play crucial roles in aquatic ecosystems as primary producers and resource providers but also pose significant ecological and health risks through blooms that produce harmful toxins, called cyanotoxins. The taxonomic affiliation of cyanobacteria has evolved from morphology-based methods to genomic analysis, which offers detailed structural and physiological insights that are essential for accurate taxonomic affiliation and monitoring. However, challenges posed by uncultured species have been extrapolated to the detection and quantification of cyanotoxins. Current advances in molecular biology and informatics improve the precision of monitoring and allow the analysis of groups of genes related to toxin production, providing crucial information for environmental biosafety and public health. Unfortunately, public genomic databases heavily underrepresent cyanobacteria, which limits the understanding of their diversity and metabolic capabilities. Despite the increasing availability of cyanobacterial genome sequences, research is still largely focused on a few model strains, narrowing the scope of genetic and metabolic studies. The challenges posed by cyanobacterial blooms and cyanotoxins necessitate improved molecular, cultivation, and polyphasic techniques for comprehensive classification and quantification, highlighting the need for advanced genomic approaches to better understand and manage cyanobacteria and toxins. This review explores the application of transdisciplinary approaches for the study of cyanobacteria and cyanotoxins focused on diversity analysis, population quantification, and cyanotoxin monitoring, emphasizing their genomic resources and their potential in the genomic mining of toxin-related genes.

1. Introduction

Cyanobacteria are among the most primitive organisms found on earth and have an estimated origin of approximately 2700 million years (Shestakov & Karbysheva, 2017). Their considerable morphological and physiological adaptations as aerobic and photoautotrophic prokaryotic microorganisms have allowed them to colonize both terrestrial and aquatic ecosystems, as well as diverse extreme environments (Salomón et al., 2020). Thus, cyanobacteria have been able to colonize a large number of ecological niches, since they have been reported from the poles to the tropical regions of the planet, in both aquatic and terrestrial systems according to the Global Biodiversity Information Facility (www.gbif.org). In addition, cyanobacteria can tolerate extreme environments since their growth capacity is highly favored in alkaline or neutral water environments with pH ranging from 6 to 9, and temperatures between 15 and 30 °C with high concentrations of nutrients or chemical residuals (nitrogen and phosphorus), even with low concentrations of carbon dioxide (Beasley, 2020; Glibert et al., 2018; Pal et al., 2020).

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These bacteria have been studied with two opposing sides; (i) they are crucial primary producers for aquatic trophic networks, as well as great sources of resources that have allowed their use as the provision of vitamins, proteins, biofertilizers, bioremediating agents, and for the production of biofuel and pollution control agents; on the other hand, (ii) as a significant economic, ecological, and hygienic problem of water management and aquatic ecosystems (Cobo, 2015), due to their ability to suddenly increase their growth rate caused by a complex interaction of high nutrient concentrations, sunlight, warm temperature, turbidity, pH, conductivity, salinity, carbon availability and slow-flowing or stagnant water (Beasley, 2020; Codd et al., 2020). This phenomenon is known as bloom, which modifies the physical and chemical characteristics of water, such as the presence of foam and unpleasant odors, decreased transparency, and disturbances in color and taste; in addition, some taxa can produce cyanotoxins (Salomón et al., 2020), significantly reducing the survival rates of various aquatic species, leading to the death of a wide range of organisms, including humans (Bonilla et al., 2015; Cobo, 2015).

Thus, the negative effect of cyanobacteria is due to increased biosynthesis of a wide range of unwanted metabolites, such as cyanotoxins, which are biotoxins responsible for acute and (sub)chronic poisonings of wildlife/domestic animals and humans (Sini et al., 2021). Cyanotoxins can follow multiple routes: (i) orally, via drinking water, or via consumption of health food tablets or other organisms that have accumulated these metabolites along the food chains; (ii) in labor or recreational water environments dermally; or (iii) by inhalation exposure (Sini et al., 2021). Their natural state can also be influenced by a set of geological, environmental, and anthropogenic factors. Most of the attention given to the adverse effects of cyanobacteria on human health has focused on acute poisoning (Codd et al., 2017). Cyanotoxins, which are secondary metabolites, are more numerically diverse than the cyanobacteria that produce them (Bashir et al., 2023). According to the CyanoMetDB database, over 2000 cyanobacterial metabolites have been identified, including, among others, microcystins, cyanopeptolins, other depsipeptides, anabaenopeptins, microginins, aeruginosins, cyclamides, cryptophycins, saxitoxins, spumigins, microviridins, and anatoxins (Jones et al., 2021). Most cyanotoxins are released into the water column only after cyanobacterial death, although some can also be actively released by the intact cells. These toxins exhibit diverse mechanisms of action, including cyto-, neuro-, hepato-, and neurotoxicity. The toxicity of many cyanobacterial metabolites still needs to be studied further (Falfushynska et al., 2023). Even so, the currently known cyanotoxins are only a small fraction of (bio)chemically similar metabolites produced by cyanobacteria, and their function for the producing cells is yet unclear (Janssen, 2019).

Cyanotoxins are spread worldwide, both in aquatic and terrestrial habitats, constituting an indispensable part of global biodiversity, food chains, and biogeochemical cycles. Usually, the formation of these blooms requires increased temperature, high availability of photosynthetically active radiation, windless weather, and pH in a 6–9 range (Falfushynska et al., 2023). According to the recorded climate conditions in places where acute poisonings and mass mortalities of wild animals occurred showed that dry and hot weather stimulates the proliferation of cyanotoxins, which considerably increases the risk of poisoning (H. Wang et al., 2021). Cyanobacterial blooms continue to increase in response to eutrophication where agriculture is intensified or urban settlements expand without sufficient wastewater treatment (Chorus et al., 2021).

The rise in cyanobacterial blooms has increased the demand for integrative evaluations of these microorganisms, such as the use of genomic approaches (Alvarenga et al., 2017). Identifying and quantifying gene clusters responsible for toxin biosynthesis is crucial for assessing the potential toxigenicity of blooms to ensure environmental and public health safety (Casero et al., 2019). Molecular biological methods offer rapid and sensitive detection of toxic cyanobacteria and are valuable for ecological studies. Nucleic acid-based methods are favored for their specificity, sensitivity, and speed in targeting toxin biosynthesis genes (Ouellette & Wilhelm, 2003). Techniques such as end-point PCR, qPCR, microarrays, and next-generation sequencing are useful for cyanobacterial monitoring, though standardized protocols for detecting harmful algal blooms or cyanotoxins are still needed (Saleem et al., 2023).

Thus, at least two strategies have been studied: (i) the taxonomic affiliation of cyanobacteria and (ii) the analysis of the cyanotoxins produced under specific culture conditions. Initially, the taxonomy of cyanobacteria was based on morphology, using pre-existing rules, but as more was learned about their diversity, it became clear that more information needed to be considered when taxonomically affiliating them, where genomic analysis became a necessary consideration (Dextro et al., 2021). However, it is important to note that many species have not been able to be cultured, which limits taxonomic analyses, being a challenge to unraveling their diversity and functional roles, which could be overcome with omics approaches and the continued development of innovative culturing methods (Dextro et al., 2021). For example, although the 16S rRNA gene effectively identifies some cyanotoxigenic cyanobacteria, it is not specific enough for all cyanobacteria and may result in overestimates or false positives (Lu et al., 2020). Thus, attempts have been made to counteract non-specificity through the use of cyanobacterial barcoding (integrating PCR amplification and next-generation DNA sequencing), which may allow the use of downstream bioinformatics to obtain a higher level of confidence in cyanobacterial identification (Saleem et al., 2023), where nucleic acid-based microbial population and species diversity quantification techniques can provide the information needed to make decisions about the risk posed by a bloom (Alvarenga et al., 2017, 2018).

On the other hand, it has been reported that eutrophication events generate blooms of cyanobacteria that in turn secrete into the environment concentrations of toxins that affect human health and the fauna of the affected environmental matrix (Ballot, Fastner, & Wiedner, 2010; Gao et al., 2015; Savela et al., 2015; Wiltsie et al., 2018). Thus, direct and indirect methods are available to estimate the concentration of intra- or extracellular cyanotoxins, such as biological tests and chromatographic technologies to determine cyanotoxin concentration (Hiskia et al., 2020; Sanseverino et al., 2017). Recent advances in molecular biology and informatics have enabled accelerated improvements in PCR, DNA sequencing, RNA analysis, microfluidics, and machine learning methods to probe and monitor cyanobacterial density and cyanotoxin production (Saleem et al., 2023). Nucleic acids-based techniques can determine the number of transcripts of those genes or gene clusters related to toxin production; however, the correlation with the cyanotoxin biosynthesis and accumulation in the environment is still a challenge (Moreira et al., 2020; Radkova et al., 2020; Sanseverino et al., 2017; Wiltsie et al., 2018).

This review presents a critical analysis of the application of techniques based on cyanobacterial nucleic acids for the assessment of their population, diversity, taxonomic affiliation, and quantification of cyanotoxin, for integrative and comprehensive studies of cyanobacteria.

2. Taxonomic affiliation

Classically, the taxonomic affiliation of cyanobacteria has been based on their morphological characteristics (Komárek et al., 2014; Rippka et al., 1979) (Fig. 1). However, with the advancement of new technologies based on DNA, the use of molecular methods, and ecological analyses, a new panorama has been reached for the polyphasic taxonomy of cyanobacteria (Komárek, 2016a).

2.1. Morphological analysis and other techniques

For a long time, the taxonomy of cyanobacteria has generated debate among researchers. Many of these taxa have arisen and/or have been lost several times during evolution. The works of Anagnostidis and

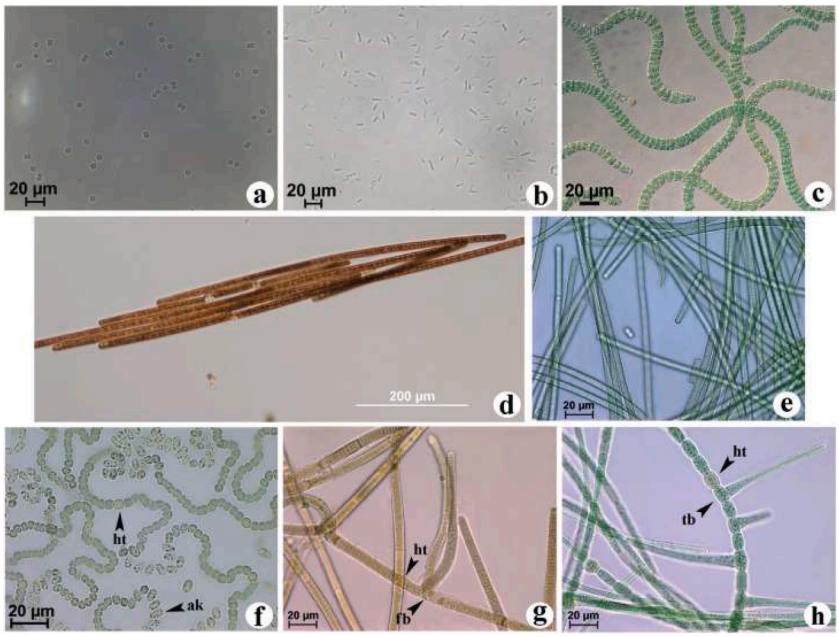

Fig. 1. Morphological diversity of different cyanobacterial genera where (a) belongs to *Synechocystis* sp. PCC6803, (b) *Synechococcus elongatus* PCC4972, (c) Arthrospira maxima, (d) *Trichodesmium* sp. CCM-UFV034, (f) *Nostoc* sp. CCM-UFV028, (g) *Brasilonema octagenarum* CCM-UFVE1 and (h) *Stigonema* sp. CCM-UFV036. Arrows with abbreviations correspond to (ak) akinetes; (fb) false branching; heterocytes (ht) and (tb) true branching. From (Esteves-Ferreira et al., 2017).

Komárek are among the most consulted for microscopic work. The characteristics used for their classification are the shape and size of the cells, the cellular organization by forming colonies of mucilaginous sheaths or trichomes, the methods of reproduction and specialized structures as akinetes, heterocysts, or gas vesicles, as well as the distribution of the thylakoids and pigments present in the cell. (Komarék, 2013; Komarék & Anagnostidis, 1999, 2005). In this sense, Bergey's Manual of Systematic Bacteriology compiles five orders, such as *Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales*, and *Stigonematales*. In which they are described based on their morphological, ecological, biochemical, physiological, and ultrastructural criteria (Komárek, 2016b; Morales-Sandoval et al., 2021) (Fig. 2).

A more recent compilation shows an increase from five to eight orders, with the addition of *Gloeobacterales, Chroococcidiopsidales,* and *Spirulinales*, due to new parameters generated by the use of a polyphasic approach. This is based on genetic studies, such as 16S rRNA gene

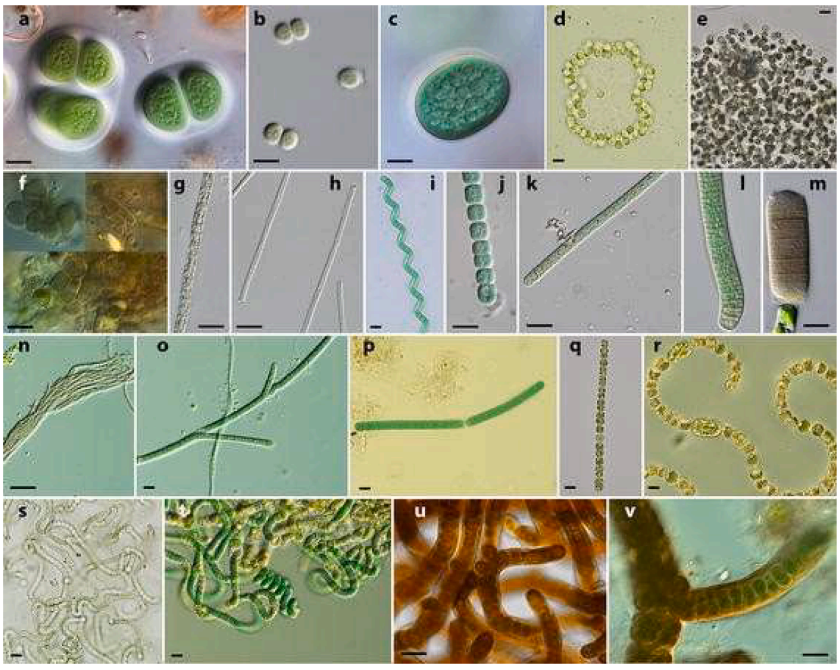

Fig. 2. Morphological classification by optical microscopy of the different cyanobacteria. Order *Chroococcales*: (a) *Chroococcus subnudus*, (b) *Ch. limneticus*, (c) *Cyanothece aeruginosa*, (d) *Snowella litoralis*, (e) *Microcystis aeruginosa*. Order *Pleurocapsales*: (f) *Pleurocapsa minor*. Order *Oscillatoriales*: (g) *Planktothrix agardhii*, (h) *Limnothrix redekei*, (i) *Arthrospira jenneri*, (j) *Johanseninema constricum*, (k) *Phormidium* sp., (l) and (m) *Oscillatoria* sp., (n) *Schizothrix* sp., (o) *Tolypothrix* sp., (p) *Katagnymene accurata.*, Order *Nostocales*: (q) *Dolichospermum planctonicum*, (r) *Dolichospermum* sp., (s) *Nostoc* sp., (t) *Nodularia moravica*. Order *Stigonematales*: (u) and (v) *Stigonema* sp. From (Dvořák et al., 2015).

sequences, accompanied by morphological, ecophysiological (N_2-fixation, light, temperature, and salinity optima) and ecological (geography, type of habitat, and trophic status) analysis criteria, essential for the construction of monophyletic genera (Komárek, 2016a; Komárek et al., 2014). There is also an online database of cyanobacteria based on the polyphasic approach known as CyanoDB2.0 (as described in Section 4). The platform offers a large amount of literature and molecular data on the main cyanobacterial taxa reported, being a tool for taxonomic assignment. It currently has 2175 taxa, with 451 genera and 1724 species (Hauer & Komárek, 2022).

Microscopic techniques are a fundamental tool in the identification of cyanobacteria and provide valuable information for their taxonomic affiliation; however, traditional methods are time-consuming and require skilled professionals (Zhang et al., 2022). It is necessary to use optical microscopes with 10–15 magnification eyepieces, along with a counting reticle and ruler for colonies and large filaments. In addition, a set of 100× objectives to increase resolution when searching for cyanobacteria <10 μm wide, and when searching for other morphological details (Bonilla, 2009; Cronberg & Annadotter, 2006; Wilmotte et al., 2017) (Fig. 3). This identification is based on the morphological shape and size of the cyanobacteria, in some cases the use of staining favors their observation, such as Lugol, methylene blue, or nigrosine, where the sheaths, membranes, and filamentous transversal walls are highlighted (Bonilla, 2009; Wilmotte et al., 2017).

Electron microscopy is an alternative to light microscopy, which has a higher resolution that helps to understand the structural organization and intracellular space of cyanobacteria (Mondal et al., 2024). These techniques included: (i) scanning electron microscopy (SEM), used primarily for surface topology and prepared with chemical or freeze fixation; (ii) transmission electron microscopy (TEM) which provided information on the size, shape, and arrangement of organelles from specific fixation and staining reagents for each part of the cell; and (iii) confocal laser scanning microscopy (CLSM) where fluorescent staining is used to obtain a 3D image (Figs. 4 and 5) (Hernández Mariné et al., 2004; Winey et al., 2014). The methods complemented each other to provide a physical and physiological description of cyanobacteria but had some disadvantages, such as dehydration and sample collapse (Hernández Mariné et al., 2004). As time progresses, alternatives to the toxic staining agents used in TEM are being sought. With the elimination of staining, a low voltage system of 15 and 25 kV is used, which coupled with a scanning transmission electron microscope (STEM) allows a sharper image and reduces chromatic aberration (Mrazova et al., 2023). The equipment used must have qualified personnel for its operation. In the case of SEM, it usually has large and expensive equipment, which is a drawback *in situ* monitoring (Vaughan et al., 2022).

Despite having microscopy as a basis for morphological identification, time and experience are still an obstacle, and many references come from obsolete drawings or illustrations (Li et al., 2019). Deep learning has recently been used to employ image pattern recognition in

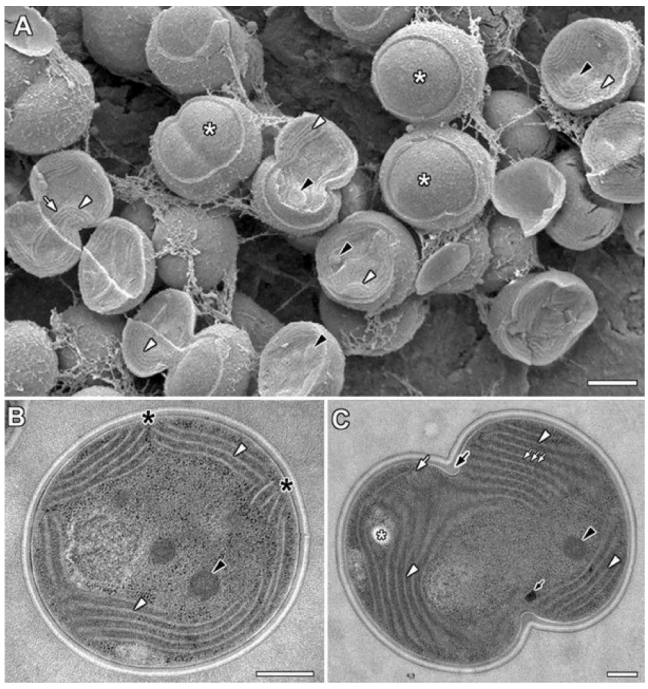

Fig. 4. Images obtained by cryo-scanning electron microscopy (A) and transmission electron microscopy (B and C) of *Synechocystis* sp. PCC 6803 cells. From (Van De Meene et al., 2006).

the identification and counting of cyanobacterial cells. Five species of microalgae were analyzed within which two cyanobacteria (*Chroococcus dispersus* and *Aphanocapsa elachista*) were included, both images obtained by light microscopy (LM) and SEM) together with 5 convolutional neural network (CNN) models. The VGG16 and EfficientNetV2 models achieved the highest success compared to the other networks. Both, a 99 % success rate was obtained in both optical microscopy and scanning electron microscopy classification, presenting an alternative for the classification of different cyanobacteria based on their morphology (Sonmez et al., 2023).

In terms of observation and population counting, flow cytometry is a technique that offers a smaller amount of work, in less time and with greater precision than conventional microscopy techniques (Vaughan et al., 2022). Coupling this technique with a camera creates a flow cytometry image, providing structural information as well as spectral properties of the cells with high image resolution on morphology and internal structure. Additionally, the use of artificial intelligence and machine learning has been suggested, facilitating the analysis itself without the supervision of a technician (Dimitriadis et al., 2024). A method was developed for the analysis of water from the FlowCam

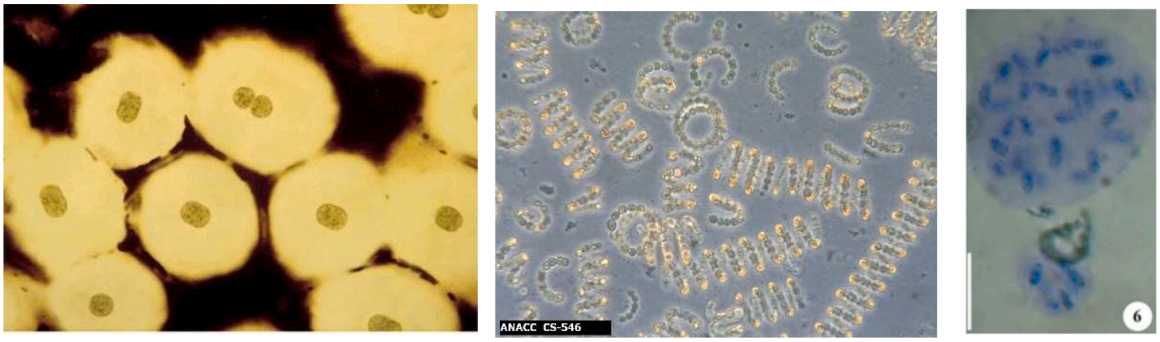

Fig. 3. Left: Photomicrograph of *Cyanothece* sp. CE 4 capsule in India ink/nigrosin stain (775×; brightfield) From (De Philippis & Vincenzini, 1998), center: lugol stain of *Dolichospermum spiroides* (Australian National Algae Culture Collection's - ANACC), right: methylene blue stain of *Lemmermanniella terrestris* colony. From (Gama et al., 2012).

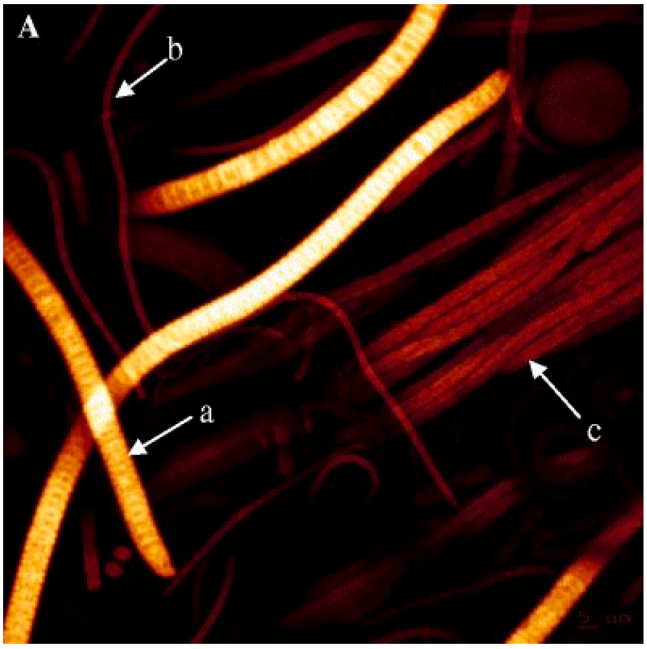

Fig. 5. Confocal laser scanning microscopy (CLSM) image showing different cyanobacteria: *Lyngbya* (a); *Pseudanabaena* (b); and *Microcoleus* spp. (c). From (Solé et al., 2009).

system for taxonomic identification of cyanobacteria, using Visual-Spreadsheet software for automatic counting. Despite generating higher taxonomic data compared to light microscopy, challenges were encountered due to cell size and lack of resolving power for accurate species-level identification (Fig. 6) (Graham et al., 2018).

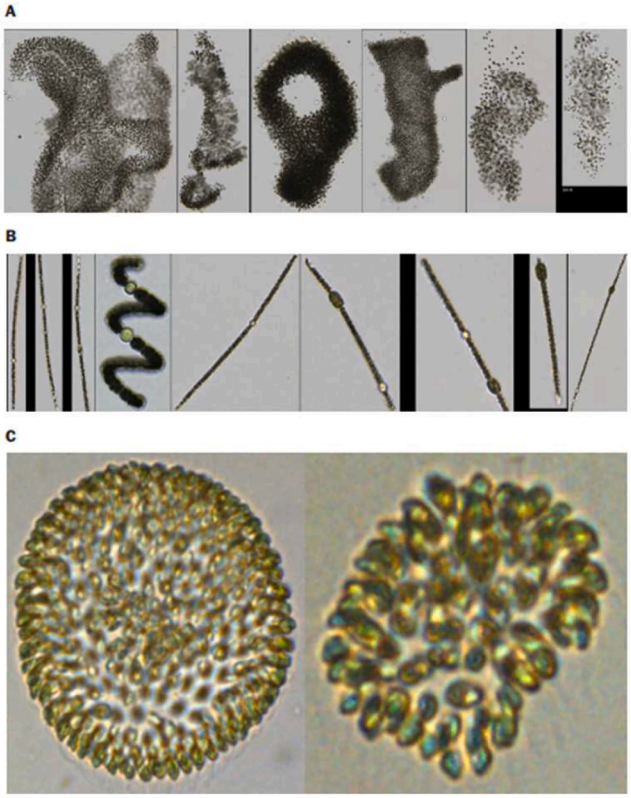

Fig. 6. Images obtained by FlowCam of cyanobacteria. (A) *Microcystis,* B) *Dolichospermum* (formerly *Anabaena*), and (C): *Gomphosphaeria.* From (Barrowman et al., 2024).

Cyanobacteria contain phycobilisomes, protein complexes that capture light energy during photosynthesis. These are classified into three types: phycoerythrins (λ_{max} ~565 nm), phycocyanins (λ_{max} ~620 nm), and allophycocyanins (λ_{max} ~650 nm) (Chakdar & Pabbi, 2015). Native mass spectrophotometry emerged as a rapid alternative for the detection of cyanobacterial since small volumes can be identified before the formation of the algal bloom. By tracking phycobiliproteins, spectral "fingerprints" were created; however, further development and generation of a database of native mass spectra is needed. Six species of cyanobacteria were taken for data analysis: *Spirulina subsalsa, Spirulina major, Nodularia harveyana, Oscillatoria nigroviridis, Gloeocapsopsis crepidinum*, and *Coccochloris elabens*; the result was a unique fingerprint for each species. One advantage that this method could offer is the identification of Multiple species in a water sample and the possible automation of the data. Overall, the data are very promising for the simultaneous detection and identification of coexisting cyanobacteria *in situ* (Sound et al., 2021).

In addition, based on the fluorescence of cyanobacterial pigments (*Microcystis aeruginosa* and *Anabaena flos-aquae*), an automated imaging technique was developed for their quantification and identification. Fluorescent light was used to excite the natural photosynthetic pigments of the cells to increase contrast and various morphological attributes were evaluated for differentiation, such as eccentricity, compactness, convex area, solidity, extent, and perimeter. The results demonstrated a decrease in the time and resources required to analyze water samples, increased sensitivity, and the ability to differentiate between the two species of cyanobacteria and an alga of similar morphology in mixed samples (*Ankistrodesmus*) (Jin et al., 2018) (Fig. 7).

Although the techniques mentioned above are valuable, phenotypic traits are not perceptible even when the genomic background is different; thus, the genomic approach becomes necessary and a promising complementary strategy to distinguish cyanobacteria taxa (Alvarenga et al., 2017; Komárek, 2016a).

2.2. Molecular techniques

The innovation and cost reduction of next-generation sequencing technologies facilitate the development of new techniques for the molecular identification of cyanobacteria by sequencing genomes, RNA, or amplicons (Oliveira et al., 2018). With this approach, two groups of techniques based on nucleic acid extraction and sequencing can be distinguished, those that rely on an axenic culture and those that do not (Alvarenga et al., 2017). These aspects are explored below and how the

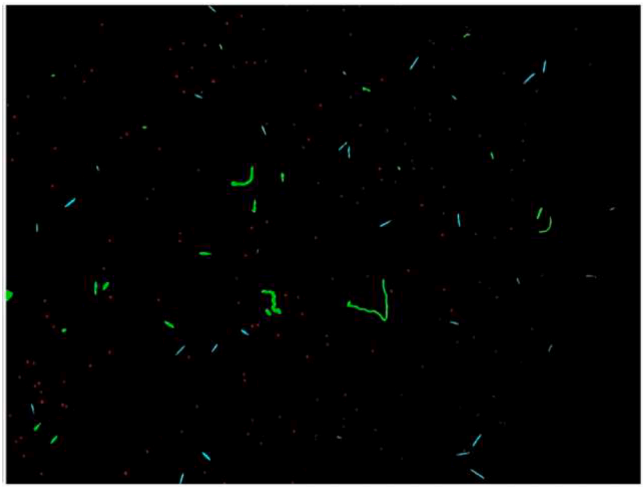

Fig. 7. Representative monofluorescent processed images of a mixed suspension of *Microcystis* (blue), *Anabaena* (red), and *Ankistrodesmus* (green). From (Jin et al., 2018).

difficulty of microbiological work with cyanobacteria influences the technology to be used (Cornet et al., 2018).

2.2.1. Axenic cultures

The establishment of an axenic culture is essential to obtain a complete taxonomic characterization of an organism, identify compounds of interest or interactions with other species and is a prerequisite for the success of molecular techniques, such as those shown below (Šulčius et al., 2017). Complications arise when isolating cyanobacterial cells, as they are in contact with environmental pollutants and contaminating microorganisms that adhere to their cell wall, some of these forming symbiotic associations (Shiraishi, 2015; Šulčius et al., 2017). It should be noted that each strain requires specific approaches for their isolation, being a complex and time-consuming process (Alvarenga et al., 2017; Vu et al., 2018).

2.2.1.1. Genomics. Due to the wide variety of characteristics of cyanobacteria and their coexisting organisms, it is impossible to provide a universal protocol for establishing axenic cultures. Vu and co-workers provide a review of different microalgae isolation techniques to establish an axenic culture, providing a tool to select the most suitable method to achieve the researcher's goals (Vu et al., 2018) (Fig. 8). With the application of axenic cultures in genomic sequencing, the amount of noise and contaminating sequences is reduced.

Genomic sequencing shows advantages in the characterization of conserved or specialized cyanobacterial genes, comparing data on a well-resolved cyanobacterial phylogeny, and providing reliable information on association with specific taxa (Chen et al., 2021). The analysis of genes of environmental and health interest, such as those related to the production of cyanotoxins, can be identified by holistic genome analysis. Using this approach a taxonomic analysis was carried out on *Nostoc azollae* which is a cyanobacterium with symbiosis to a fern. Sequencing of 10 strains from Zimmerman and Meeks' collection was carried out to differentiate the isolates; all these 10 strains were divided into three distinct groups: six strains were nearly identical to the non-symbiotic strain, *Nostoc (Anabaena) variabilis* ATCC 29413; three were similar to the symbiotic strain, *Nostoc punctiforme*, and one, *Nostoc* sp. 2RC was more similar to the non-symbiotic strains of *Nostoc linckia* (Pratte & Thiel, 2021).

Fig. 8. Overall scheme for obtaining axenic cultures from natural field samples (Vu et al., 2018).

Affiliation by molecular methods alone, such as marker genes, creates a vast array of invalid genus and species data (Komárek, 2020). Hirose et al. proposed the establishment of cryopreservation protocols based on the assembly of genomes of 28 heterozygous and 3 non-heterozygous strains of cyanobacteria from the microbial culture collection of the National Institute of Environmental Sciences, Japan Whole genome sequencing, assembly, and gap closure were performed using the MiSeq platform (Illumina) and bioinformatics processing. Phylogenetic relationships between genomes and taxonomic differences in mapping were identified, suggesting that taxonomic inconsistencies in cyanobacteria are due to morphological plasticity (Hirose et al., 2021).

2.2.1.2. Amplicon sequencing. Compared to the genomic approach, the presence of repeated sequences in cyanobacteria allows their use in the differentiation of species or strains, providing a faster and cheaper alternative to DNA sequencing. Valerio in 2009 conducted research where molecular techniques were applied to 118 isolates of three orders of cyanobacteria: *Chroococcales, Oscillatoriales*, and *Nostocales*. In which a relationship was observed between STTR and LTRR-based clustering showing specificity for cyanobacteria (Fig. 9), mainly 16S rRNA and rpoC1 gene phylogeny, and taxonomic affiliation, indicating the usefulness of these PCR footprint profiles for cyanobacterial identification (Valério et al., 2009).

The 16S rRNA gene sequences are not always a criterion for species differentiation in cyanobacteria, molecular markers such as nifH genes and ITS regions were used together to identify them as highly conserved regions and their relationship with the nitrogenase enzyme present in cyanobacteria. These parameters were used in the study of two seawater samples in China, these were purified to obtain axenic cultures, and a polyphasic approach was applied for their characterization. This led to the description of two new species along with two new genera, *Marileptolyngbya sina* SCSIO T-2 and *Alileptolyngbya diazotrophicum* SCSIO 43686 (Zhou et al., 2018). Along the same line, the use of genes involved in cyanotoxin synthesis can help in the identification of potentially toxic cyanobacteria, the HEPF and HEPR primer pair in particular, can effectively serve in water monitoring and identification of cyanobacteria. In the case of mycE PCR fragments, research is still needed due to insufficient data; however, there are studies on the amplification of genes related to toxin production, as will be discussed below (Radkova et al., 2020).

The study of cyanobacterial strains from remote locations increases the possibility of expanding cyanobacterial diversity at taxonomic, genomic, and metabolic levels. Thus, based on the isolation of 81 strains from diverse habitats in Europe, the 16S rRNA gene, the ITS 16S-23S rRNA, and the cpcBA-IGS operon were used to describe five new genera (*Speleotes, Haliplanktos, Olisthonema, Speos,* and *Iphianassa*) and their type species (*S. anchialus, H. antonyquinny, O. eestii, S. fyssassi, I. zackieohae*), four new species were also described (*Komarekiella chia, Stenomitos pantisii, Cyanocohniella. hyphalmyra, Nodularia mediterannea*). Showing a great diversity ignored by classical morphology, hydrogen cyanide production was also evaluated (Panou & Gkelis, 2022).

The development of specific primers could support the identification of cyanobacteria present in low concentrations that are not detectable by microscopy. Primers were developed for the marker genes rbcLX, rpoB, rpoC1, and cpcBA, to compensate for the limitations of 16S rRNA, specifically for the cyanobacteria *Nostocalaes* of the genera *Chrysosporum, Cuspidothrix, Cylindrospermopsis, Raphidiopsis,* and *Sphaerospermopsis*. It has become useful in molecular monitoring for morphologically and genetically similar genera, without neglecting light microscopy,

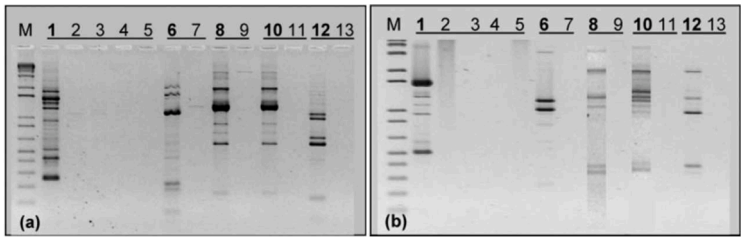

Fig. 9. Fingerprinting profiles of cyanobacteria and cultivable bacteria from non-axenic cultures with different methods to be tested (a) STRR PCR fingerprinting and (b) LTRR PCR fingerprinting. From (Valério et al., 2009).

however, there is currently insufficient data for identification at the species level (Kim et al., 2021),

2.2.2. *Non-axenic cultures*

It is known that complications in the isolation of cyanobacteria can limit the information that can be obtained with a genomic approach (Davison et al., 2014). Non-axenic cultures in cyanobacteria are commonly composed of a consortium of microorganisms that are sometimes considered impossible to isolate (Vázquez-Martínez et al., 2004). Therefore, non-axenic culture-dependent techniques play an important role in understanding the diversity of cyanobacteria and allowing the analysis of their toxic potential.

2.2.2.1. Metagenomics. Metagenomics is a broader perspective to obtain genomic information from non-axenic samples. This has the advantage of avoiding the isolation and culture of microorganisms and in addition, obtaining individual genomes from bacterial consortia, accelerating the research work in obtaining results. Recent advances have provided new possibilities for *in vitro* studies, renewing the value of microbial consortia as a resource for the rapid assessment of cyanobacteria (Alvarenga et al., 2017).

Such is the case of a study in which new genomic sequences were obtained for polar cyanobacterial strains, were obtained medium-quality genomes with a high level of completeness (high complexity), and produced a very low level of contaminating sequences (high specificity), which could be very useful for phylogenomic analyses (Cornet et al., 2018). Genome-resolved metagenomics provides a reliable and simple method to obtain new cyanobacterial genomes. From a metagenomic approach applied to 17 polar cyanobacterial species, 37 assembled metagenome genomes (MAG) were obtained, that were affiliated to different taxa such as *Pseudanabaena, Leptolyngbya, Microcoleus/Tychonema and Phormidium*, and to a lesser extent *Crinalium and Chamaesiphon*. Discovering a cyanobacterium without thylakoids from cold environments of the genus *Gloeobacterales*, being designated as *Candidatus Sivonenia alaskensis* (Fig. 10) (Pessi et al., 2023).

Also, an application of this approach has been carried out in research conducted on sludge and water samples from a water treatment plant, *Synechococcus, Microcystis,* and *Dolichospermum* were identified as the predominant genera from shotgun metagenomics. However, differences emerged with the organisms detected in the taxonomic counts (Jalili et al., 2021). Similarly, Linz et al., (2023) carried out metagenomic mapping to identify the main cyanotoxin producers in 12 large rivers, the results showed a considerable presence of the genera *Planktothrix, Microcystis, Dolichospermum, Nodularia*, and others, these could be inducers of toxin-producing blooms, with a greater presence of *Microcystis* and *Planktothrix* spp. Sequencing of the *mcyE* gene was also carried out to determine genotypic diversity between the genera (Linz et al., 2023).

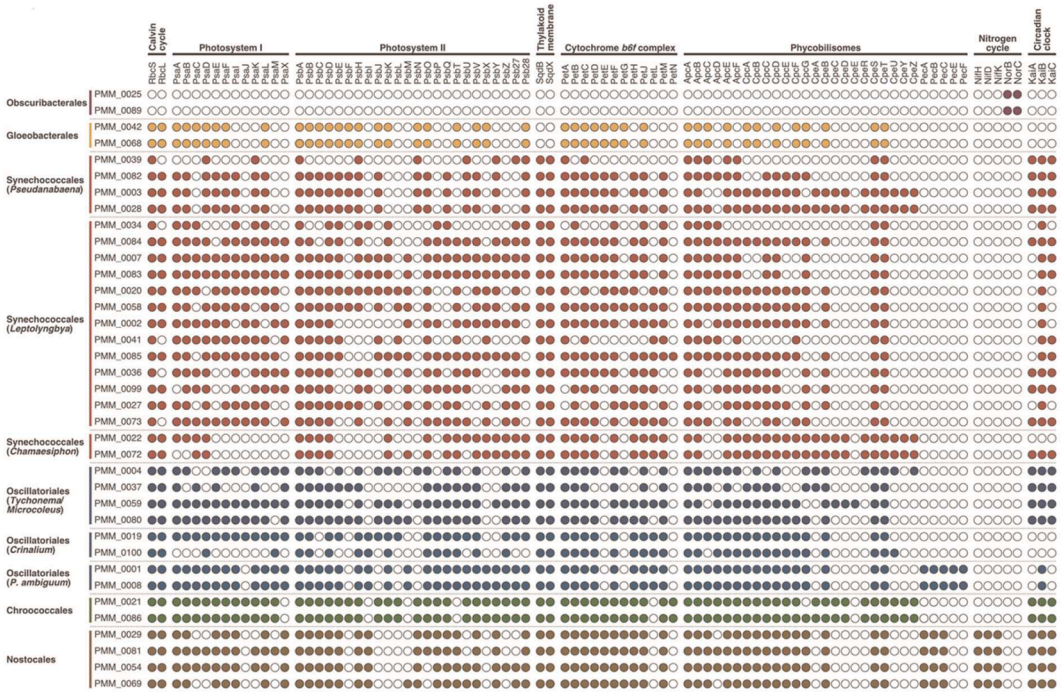

Fig. 10. Presence of genes involved in carbon fixation, photosynthesis, nitrogen cycle, and circadian clock in 37 metagenome-assembled genomes of cyanobacteria and melainabacteria. From (Pessi et al., 2023).

2.2.2.2. Metataxonomic. Metataxonomy, in contrast with the use of complete/draft genomes in metagenomics, allows the construction of taxonomic profiles from the sequencing of marker genes, commonly for ribosomal RNA regions, the 16S RNA gene one of its main targets (Aguiar-Pulido et al., 2016). The main difference with other techniques is the sequencing of a single gene to identify a wide variety of microorganisms (Breitwieser et al., 2019). Li and coworkers investigated the diversity of cyanobacteria using 16S rRNA sequence amplicons with more than 97 % similarity, using monophyletic species instead of OTU to estimate cyanobacterial diversity. The combination of these two methods allowed them to reveal cryptic genera and species and to better distinguish between freshwater and seasonal blue-green algal communities. Cryptic genera and species were found that provide a much clearer separation of the cyanobacterial community (Li et al., 2020).

Amplicon sequence variant (ASV) analysis studies, together with high-throughput sequencing, provide information on the population dynamics of cyanobacteria at high taxonomic resolution. The specific sequence of the 16S rRNA-ITS region of approximately 350 bp was used. The elimination of variant sequence extensions by negative controls (ASV) was performed, obtaining a total of 201 ASVs where six were assigned to family-level *Leptolyngbyaceae, Coleofasciculaceae, Microcystaceae, Cyanobiaceae,* and 193 were assigned to genus-level *Planktothrix, Cyanobium, Synechococcus, Snowella, Aphanizomenon, Microcystis, Pseudanabaena* and *Gloeocapsa* the most abundant (Fig. 11) (Nwosu et al., 2021).

Another type of marker used for phylogeny and classification of cyanobacteria is the structural nitrogenase genes (*nifH, nifK*, and *nifD*). Recently, two new markers were developed from regulatory genes in nitrogenase production from the taxon *Nostocales*, being designed for the nifE and nifN genes. The results showed that the markers can distinguish between *Nostocales* strains with common morphological and physiological characteristics (Giannakopoulos et al., 2024).

2.2.2.3. Others strategies. In another research, morphological identification was compared with next-generation sequencing, including metabarcoding. From bacterial communities of 7 water bodies, saturated sequences were obtained and compared with morphological identification. Ribosomal data classification was achieved through the Silva database and blast analysis at NCBI to identify cyanobacterial OTUs and assign them to taxa. The result yielded higher species detection than classical morphology, metabarcoding may be more sensitive to cyanobacteria, as evidenced by detecting certain genera in a greater number of samples than morphological identification (Fig. 12) (Li et al., 2019).

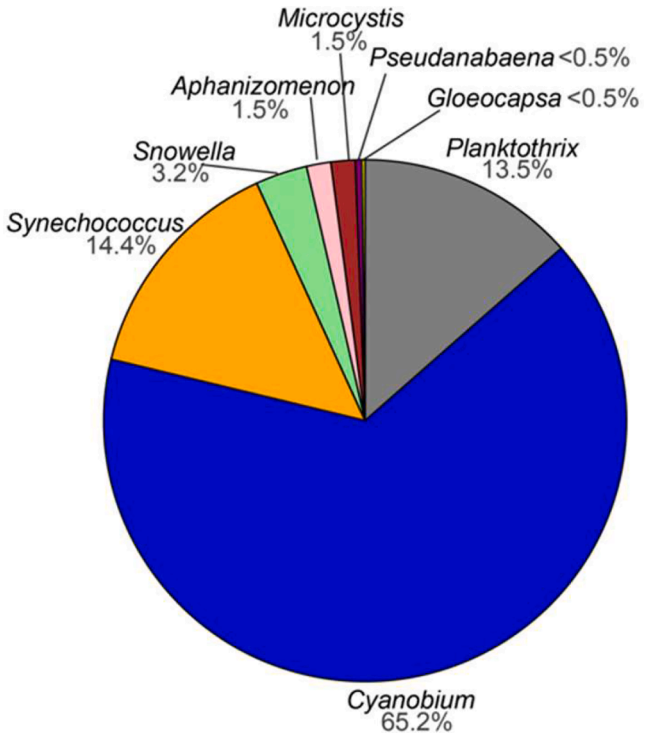

Fig. 11. Pie chart showing the percentage of amplicon sequence variants (ASVs) assigned to cyanobacteria from Lake Tiefer, Klocksin, Germany). From (Nwosu et al., 2021).

3. Cyanotoxin production

As mentioned, cyanobacterial species produce biologically active compounds (cyanotoxins) that can be harmful to both wildlife and humans, generating problems of public health and economic losses for millions of US dollars (Agha et al., 2022; Glibert et al., 2018). Over 40 cyanobacteria genera are recognized as toxin producers, in addition to an unspecified count of species deemed "potentially" toxic (Zhang et al., 2023). The main toxin-producing genera include *Anabaena, Aphanizomenon, Cylindrospermopsis, Gloeotrichia, Hapalosiphon, Lyngbya, Microcystis, Nodularia, Nostoc, Oscillatoria, Schizothrix, Spirulina*, and *Synechocystis* (Hambright et al., 2014). The risk of increased cyanotoxins varies depending on the levels of harmfulness, which can have an impact on all water uses, including recreational activities, agricultural irrigation, and, most notably, the safety of the drinking water supply (Munoz et al., 2021). Cyanotoxins are classified in two ways, by their: (i) chemical structures as alkaloids, non-ribosomal peptides, polypeptides, non-protein amino acids, indole alkaloids, organophosphates, lipopeptides and lipoglycans (Ricciardelli et al., 2023); and (ii) by the target organ that they affect, the liver being hepatotoxins (microcystin, nodularin, cylindrospermopsin), neurotoxins acting on the nervous system (saxitoxins, anatoxin-a, anatoxin-a(s), homoanatoxin-a), cytotoxins (aplysiatoxin, debromoaplysiatoxin, lingbyatoxin, lipopolysaharide endotoxin) and skin and gastrointestinal irritants (Table 1) (Codd et al., 2020).

Microcystin (MCs) and nodularin (NODLN) are extensively studied cyanotoxins, which are pretty similar in their structural chemical (Liu et al., 2021). Microcystins, a cyclic heptapeptide, received their name from the cyanobacterium *Microcystis aeruginosa*, from which they were initially identified (Botes et al., 1984). To date, 280 microcystin variants have been fully characterized, with molecular weights in the range of 800–1100 Da (Bouaïcha et al., 2019). These toxins are found in several

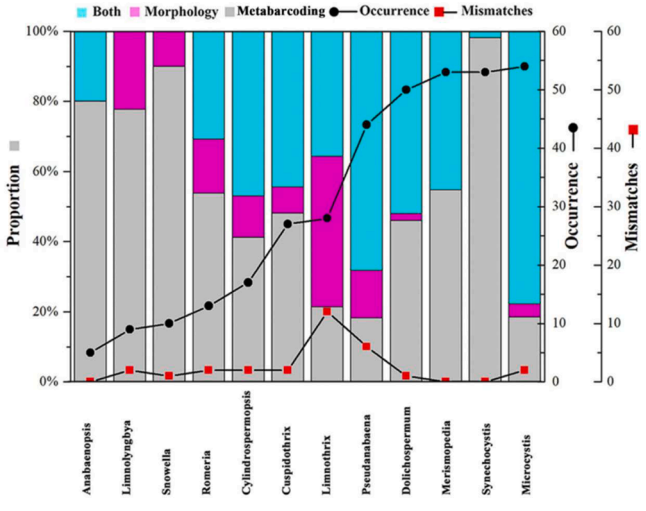

Fig. 12. Frequency of occurrences for 12 genera detected in metabarcoding and morphological datasets, and the proportion of occurrences in each dataset and both datasets. Mismatches indicate taxa detected only by morphology. From (Li et al., 2019a).

Table 1
Biosynthetic gene clusters by cyanotoxins and their effect on health.

Organ	Chemical classification	Cyanotoxin	Abv	Biosynthetic cluster	Reference
Hepatotoxic	Non-ribosomal peptide	Microcystin	MCs	mcyA-J	(Tillett et al., 2000)
Hepatotoxic	Non-ribosomal peptide	Nodularin	NODLNs	ndaA-I	(Moffitt & Neilan, 2004)
Neurotoxic	Alkaloid	Anatoxin-a	ATXs	anaA-H	
Neurotoxic	Alkaloid	Saxitoxin	STXs	sxtA-Z	(Méjean et al., 2009)
Neurotoxic	Non-protein amino acid	β-N-methylamino-L-alanine	BMAAs	Unknown	(Hackett et al., 2013)
Neurotoxic	Lipoglycans	Lipopolysaccharides	LPs	Unknown	
Neurotoxic	Lipopeptide	Antillatoxin	ANTXs	Unknown	
Cytotoxic	Lipopeptide	Anabaenolysin	ABLs	ablA-F	
Cytotoxic	Alkaloid	Cylindrospermopsin	CYNs	cyrA-O aoaA	(Shishido et al., 2015)
Dermatoxic	Polyketide	Aplysiatoxin	APTXs	Unknown	(Dittmann et al., 2013)

higher taxa of cyanobacteria, including the orders *Chroococcales, Oscillatoriales, Nostocales*, and *Stigonematales*, although data on Pleurocapsales are limited (Jungblut & Neilan, 2006). The distribution of microcystins at the genus or species level is inconsistent (Chorus & Welker, 2021). On the other hand, nodularins have been found predominantly in the genus Nodularia, especially in *Nodularia spumigena* (Melaram et al., 2024).

Cylindrospermopsin (CYN) is a cyclic sulfated guanidine alkaloid that causes general cytotoxic, hepatotoxic, and neurotoxic effects (Chorus & Welker, 2021). While most cyanotoxins exist primarily intracellularly, up to 100 % of total CYN can be released into water bodies, likely increasing absorption by aquatic organisms (Yang et al., 2021). Poisoning incidents involving CYN date back to 1979 (Byth, 1980), but its chemical structure was not identified until 1992 (Ohtani et al., 1992). CYN is a 415 Da alkaloid with a molecular formula of $C_{15}H_{21}N_5O_7S$, consisting of a tricyclic guanidine moiety combined with hydroxymethyluracil and a sulfonic acid group (Du et al., 2019). To date, CYN-producing cyanobacteria have been recorded in the orders Nostocales and Oscillatoriales worldwide (Nowruzi & Porzani, 2021).

Anatoxin-a (ATX) is another toxin that although less reported than microcystins (MCs) or cylindrospermopsin (CYN) (Plata-Calzado et al., 2024) has been reported to be responsible for the death of dogs, livestock, and wildlife due to ATX poisoning in France, Ireland, the Netherlands, New Zealand, Scotland and the United States (WHO, 2020). This bicyclic amine alkaloid, with the molecular formula C10H15NO, was first found in strains of *Anabaena* (formerly *Dolichospermum*) *flosaquae* originating from Canada (Carmichael et al., 1975). Anatoxins (ATX) are produced mainly by strains of *Anabaena* although they have been described in other cyanobacterial genera such as *Aphanizomenon, Planktothrix, Cylindrospermum, Arthrospira, Cuspidothrix, Microcystis, Oscillatoria* and *Phormidium* (Chorus & Welker, 2021).

Not only anatoxin-a has been linked to acute animal neurotoxic poison, but also saxitoxin, which is a highly known and toxic neurotoxin commonly found in shellfish, produced by dinoflagellate and cyanobacterial blooms in seawater or fresh water (Ishak et al., 2023) and is the most researched paralytic shellfish toxin (Christensen & Khan, 2020). This cyanotoxin was first described in Alaska butter clams (*Saxidomus giganteus*) in 1957 (Schantz et al., 1957) but in 1995 the cyanobacterium *Anabaena circinalis* was reported to also produce this toxin (Podduturi et al., 2021). Saxitoxins are produced by a range of species and strains belonging to the Nostocales, that is, *Dolichospermum* (*Anabaena*), *Aphanizomenon,* and *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* mainly in Brazil, Scytonema and Oscillatoriales such as *Planktothrix* and *Microseira* (*Lyngbya*) *wollei* (Chorus & Welker, 2021).

These cyanotoxins are part of a complex process by some cyanobacteria which regulate this production by their genetic and phenotypic differences between species and even between strains, as well as environmental and temporal conditions (Bashir et al., 2023). Due to this, it is impossible to differentiate morphologically between species and strains that produce cyanotoxins and those that do not. Therefore, identification and quantification techniques as well as molecular analyzes of gene clusters responsible for the biosynthesis of these molecules represent a strategy to assess the potential toxigenic capacity of cyanobacterial blooms to ensure environmental biosafety as well as the public health of society (Casero et al., 2019).

3.1. Classic cyanotoxin identification techniques

The development of diverse techniques and methods of detecting cyanotoxins are prerequisites for the accurate identification of cyanobacterial toxins in the environment including bioassays, immunoassays, and molecular methods (Du et al., 2019). These methods are described below, where it is possible to see how the detection of a toxin produced provides accurate information on the presence and quantity of the molecule in the environment under a given condition, on the other hand, the genetic approach provides information on the total toxic potential of an organism, being able to determine whether it possesses genes or clusters of integrated genes that would allow the synthesis of toxins under different environmental conditions (Bashir et al., 2023; Sanseverino et al., 2017; Sini et al., 2021).

Many different approaches and methods have been explored around cyanotoxin detection from environmental samples, most of the research has been focused on microcystin (they are more widespread in water supplies), but there is also important research in nodularin, cylindrospermopsin, anatoxin-a (this was the first toxin from cyanobacteria that was chemically and functionally defined), saxitoxins and neosaxitoxins, other types like aplysiatoxins and debromoaplysiatoxin are relevant because they cause diseases in humans, but are less researched than the previous ones mentioned. The classic identification techniques, also known as biological or biochemical screening assays, provide simple and rapid screening of samples based on the biological and overall toxic effects (Bashir et al., 2023; Kaushik & Balasubramanian, 2013).

One of the most used classical techniques in the research is the enzyme-linked immunoassay also known as ELISA, appearing in 1971 to detect antigens in human blood serum, revolutionizing the clinical investigation. Extrapolating it to the cyanobacteria research, we can deduce that the cyanotoxins in waters are like the antigens in serum. As mentioned above, these biologically active compounds are of interest because they can be harmful to the life forms in an ecosystem. The ELISA method is used to detect and quantify a specific substance, usually an antigen. The antigen is immobilized in a microplate well, either directly or by a specific antibody known as a “capture antibody”, and a “primary detection antibody” is added, forming an antigen-antibody complex (De La Rica & Stevens, 2012). ELISA kits are commercially available for the detection of microcystins in water, depending upon the antibody and the procedure employed, these kits can achieve a detection limit as low as 4 ng/L, with an upper quantitation limit of 5 μg/L. These kits have also been made commercially available for the detection of anatoxin-a, cylindrospermopsin, and saxitoxin, being the most significant advantage that these methods do not require expensive and high-upkeep analytical instrumentation to be maintained (Sundaravadivelu et al., 2022). Enzyme-linked immunosorbent assays are used to analyze

cyanotoxins in water, the assays can be configured as qualitative (positive or negative) or semiquantitative, also, they're easy to perform and are relatively inexpensive. They can detect toxins produced by inactive and active microcystin genotypes of cyanobacteria. Despite the availability of commercial ELISA's for cyanotoxins, a reliable supply of antibodies is limited (Vogiazi et al., 2019).

A study conducted in La Plata River, Argentina, revealed the presence of cyanobacteria species, including *Chroococcus* sp. and *Microcystis* sp., with *Chroococcus* sp. showing significant growth in the area. To determinate the total microcystins, they used the ELISA assay, revealing concentrations that varied from low levels that couldn't be detected (<0.16 μg/L) and extremely high levels (>2500 μg/L) (Ruibal et al., 2005). The Interdisciplinary Centre of Marine and Environmental Research (CIMAR) from the University of Porto, conducted a study in the North and Center Regions of Portugal, using samples of microcystins, cylindrospermopsins, anatoxin-a and saxitoxins collected from bloom and non-bloom events and compared with ELISA results that allow to infer the toxicity level of a given sample. The evaluation consisted of checking the ELISA proportion values obtained in the samples, in comparison with the proposed guideline value for each cyanotoxin tested, showing that results for microcystins, anatoxin-a, and saxitoxin from samples from Northern and Central Regions of Portugal exceeded the guideline values (Moreira et al., 2020). Some of the most recent cyanobacterial research used ELISA assays to quantify cyanotoxins in surface water, specifically self-produced broad-spectrum ELISA kit under the indirect competitive principle, showing satisfactory detection levels with a limit of 0.15 μg/l for Microcystin-LR standard in various water environments (Liu et al., 2023).

In contrast with these recent studies, we can mention that in the '90s, studies made with colorimetrical methods found that microcystin and nodularin were potent inhibitors of protein phosphatase type 1 (PP1) and 2A, and even suspected that these toxins were involved with the promotion of primary liver cancer in humans exposed to long-term low doses of these cyanotoxins. The PP1 assay made with five microcystins and two nodularins was developed, and the activity of PP1 was determined by measuring the rate of color production from the liberation of p-nitrophenol from p-nitrophenol phosphate using the microtiter plate reader. This colorimetric method was found to be very sensitive and able to detect the bioactive microcystins used in the study, even not using radioactive marks (An & Carbachael, 1994).

Today we have a wide range of colorimetric or fluorometric biosensors as alternative candidates to the ELISA for monitoring water-contaminating toxins, like the ones produced by cyanobacteria. Chemical array sensing has proved to be an effective technology for detecting single analytes or distinguishing multiple components in a sample, in addition, there are technologies adapted to smartphones, facilitating the data analysis of images obtained by specific detectors (Li et al., 2019). Paper-based colorimetrical assays have versatile applications varying from environmental monitoring, food safety, and clinical diagnostics, this assay, in integration with biorecognition materials (named paper-based colorimetric biochip) is less sensitive than ELISA but is a base for the nanozyme enhanced paper-based biochip developed for the rapid detection of cyanotoxins in water with low cost and high sensitivity, to detect microcystins and nodularins in water samples (Liu et al., 2022).

Colorimetrical assays are versatile for the investigations, but there are also other kinds of assays, the ones using biological organisms. The bioassays could be another alternative for toxin identification, consisting of experimentation, using mice and marine toxins that cause human poisoning. The experiments involve an initial extraction of the toxins, then are diluted, and centrifuged to obtain a clear liquid that is injected into the mice, and then the effects are observed (Pierce & Kirkpatrick, 2001). In studies from the 80s, rat hepatocytes were isolated and prepared, then were added *Microcystis aeuroginosa* and *Oscillatoria agardhii* extracts. Morphological cell damage was observed microscopically in small samples, and classified in a semiquantitative way, with the finality of observing cytotoxic effects (Aune & Berg, 1986).

At present, there are numerous natural approaches (vertebrates, invertebrates, and mammalian cell lines) established to spot toxins generated by cyanobacteria based on their interactions, immunological response, and several enzymatic actions. As seen, "lab rats" are still a good option in research, nowadays, mouse bioassay is the most preferred alternative for testing microcystins (Ramya et al., 2020). The detection of cyanotoxins in aquatic environments is substantial to maintain a healthy ecosystem, and the biological assay method can be used as a detection method for these harmful toxins. The bioassay is responsible for measuring the effect of cyanotoxins qualitatively and quantitatively on biological tissues including vertebrate animals (as seen on mice) based on clinical, biochemical, behavioral, and histopathological changes in tissue (Dilrukshi et al., 2023). Also, some investigations involve plants like *Sinapis alba, Lepidium sativum,* or *Nicotiana tabacum* and their interaction with cyanobacteria species that produce toxins (Mishra & Gupta, 2023).

Some methods, like the analytical ones, make use of the physicochemical properties of cyanobacterial toxins and the reactivities they exhibit with different chemicals for their detection. Examples include UV chromophores, capillary electrophoresis, nuclear magnetic resonance, mass spectrometry coupled with liquid or gas chromatography, and matrix-assisted laser desorption time-of-flight mass spectrometry, also known as MALDI-TOF MS (Kaushik & Balasubramanian, 2013). Radioimmunoassay is another immunochemical method, like ELISA, this was the primary immunoassay technique that was developed and applied towards the biorecognition of insulin in human blood, this technique can perform multiple analyses with high sensitivity and specificity but suffers disadvantages like requiring pure state antigen and use radioactive isotopes that could be a potent health hazard, being limitations to the common use of this assay and the cause of often being replaced with ELISA (Gupta et al., 2021).

3.2. Molecular cyanotoxin identification techniques

Molecular techniques are not only useful for the taxonomic affiliation of cyanobacteria but they can also be used to observe the expression of genes implicated in the production of toxins, such as cyanotoxins. These genes could also help us consider their pathogenic potential for their ecosystem based on the similarity between toxin-producing genes, as will be seen below.

These methods are recently developed compared to the classic techniques. The DNA-based detection techniques have become popular for their specificity, sensitivity, and speed rate. These methods can identify cyanobacteria and detect their toxic potential by analyzing their genomic content, detecting specific genes, or studying the gene expression of clusters related to toxin production, rather than the cyanotoxins directly (Fig. 13) (Kaushik & Balasubramanian, 2013).

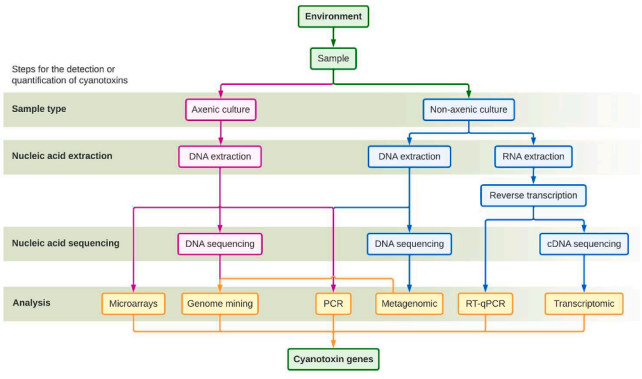

Fig. 13. Steps for the detection or quantification of cyanotoxins using nucleic acid-based techniques.

3.2.1. PCR of genes related to the production of cyanotoxins

As seen above, cyanobacteria have specific genes that codify the synthesis of toxins, these genes could be amplified by PCR using specific primers (Table 2). The use of nucleic acid-dependent methods for the affiliation of cyanobacteria and the detection of genes related to the biosynthesis of cyanotoxins has gained momentum in recent decades, allowing the prediction of the toxic potential of cyanobacteria present in sites of environmental or health interest (Alvarenga et al., 2017; Bashir et al., 2023). Toxin production can be predicted by gene detection using DNA and techniques such as conventional PCR, or RNA allowing the expression of these genes to be quantified by RT-qPCR (Sanseverino et al., 2017). This is crucial to assess the risks associated with these cyanobacterial blooms and provide information to decision-makers for taking preventive or corrective measures. Therefore, a review of the most reported genes in the synthesis of cyanotoxins and the primers designed for their detection and quantification of expression is provided.

Using this approach, studies made in 2012, with Qatar cyanotoxin samples, based on the results observed in classical methods of toxin identification, observed the presence of microcystins observed in HPLC-DA and confirmed by ELISA. The classic methods were supported by the detection of the gene *mycD* DNA by PCR, indicating a genetic origin in the analyzed species (Metcalf et al., 2012).

On the other hand, Li et al. 2011 worked with various axenic strains of cyanobacteria (*M. aeruginosa, A, flos-aquae*), obtained from the Culture Collections of the Freshwater Algae of the Institute of Hydrobiology in Wuhan, China, to compare them with environmental water samples from the Yellow River in eastern China. A PCR was conducted to observe genes like *phy, mcyA, mcyE, mcyAcd, mcyAB*, and *mcyB*. Subsequently, RT-qPCR was performed to quantify the template cell numbers in unknown samples, the standard curves were generated by correlating the number of cells and the threshold cycle values. The results of this study demonstrated that PCR amplifications of individual 16S rRNA, in the previously mentioned genes, were conducted across three strains: *M. aeruginosa* FACHB975 (used as the toxic target) and an *Anabaena* strain (used as a non-toxic cyanobacterial control). The cyanobacterial-specific 16S rRNA PCR resulted in a 782 bp partial fragment for all the strains.

Quantitative real-time PCR molecular techniques have been successfully developed to detect toxigenic genes. While most qPCR for monitor cyanotoxins are uniplex, targeting only a single genetic marker, multiplex-qPCR can amplify multiple targets in a single reaction. Experiments made in Taiwan demonstrated that amplification curves for the 16s rRNA, *mycB* gene, *rpoC1*, and *pks* genes are valuable for detecting potential toxin production (Chiu et al., 2017). Results from another study showed that the microcystin gene mcyE was amplified in all samples investigated, which included two samples from dry periods and two from rainy periods. The study also used four sets of primers to asses cylindrospermopsin genes, finding that only the *cyrC* gene was amplified from the DNA samples collected during the first dry period. Additionally, saxitoxin-related genes, particularly *sxtB* and *sxtI*, were predominantly present in samples from the dry periods. Controls for the study included *M. aeruginosa*, and two strains of *R. raciborskii*, all of which are known producers of microcystin, saxitoxin, and cylindrospermopsin (Ribeiro et al., 2020).

The qPCR results frequently uncover new information that cannot be obtained through other methods. One notable finding is the potential for cylindrospermopsin and saxitoxin production in biofilms within certain water bodies. In Slovenia, cylindrospermopsin was detected in low amounts in a planktonic sample for the first time, while saxitoxins have never been detected. Despite this, the potential for their production is significant and relevant for future monitoring and research efforts (Zupančič et al., 2021).

3.2.2. Microarrays

DNA microarray or DNA chips is a modern biological research technology for analyzing gene expression, offering the possibility to measure expression levels of thousands of genes during important biological processes (Dabba et al., 2021). Microarrays offer new insights into cyanobacterial populations and their potential cyanotoxin production in natural environmental settings. The monitoring of cyanobacteria is based on microscopic identification and cell counting, being a long and tedious process (Sivonen, 2008).

A reverse transcriptase microarray was developed to detect toxin expression, probes immobilized on the microarray slide captured the mRNA and were extended directly on the microarray. The reverse transcription extension incorporated fluorescently labeled oligonucleotides to ensure a high signal detected by the microarray scanner. Strong signals were obtained for some toxin pathways, emphasizing the toxic potential of the cyanobacteria sampled (Medlin, 2018).

Recently, a novel multiplexed electrochemical platform for the detection of multiple cyanotoxins in freshwater was developed. Based on high affinity for targeted marine toxins, the platform utilizes five specific aptamers individually immobilized on a microarray composed of eight carbon electrodes modified with gold nanoparticles. The detection mechanism relies on changes in electron transfer resulting from conformational changes in the DNA. Initial results demonstrated high sensitivity for five toxins (microcystin-LR, cylindrospermopsin, anatoxin-a, and saxitoxin), making this method an excellent alternative to single sensors by reducing detection and monitoring time in contaminated waters (Rhouati & Zourob, 2024).

3.2.3. Sequencing methods

Sequencing methods are also involved in determining toxin production. The toxicological potential of cyanobacteria colonies could be determined by isolating the total DNA found in environmental samples and applying PCR to amplify crucial genes implied in toxin biosynthesis (Petcheneshky, 2017). Sequencing of cyanobacterial genomes led to the description of gene clusters responsible for cyanotoxin production, paving the way for targeting these genes in PCR and qPCR applications (Alvarenga et al., 2017; Sanseverino et al., 2017). Nowadays, the next generation sequencing has boosted research on cyanobacterial metagenomics in freshwaters around the world, offering the possibility of researching several cyanotoxin genes (Casero et al., 2019).

Using genomic DNA sequencing, genome mining enables the identification and characterization of natural product gene clusters. Although the number of cyanobacterial genomes remains limited compared to other phyla, genome mining has identified gene clusters that correlate with known metabolites and has also revealed a significant number of clusters with unknown products. This highlights the need for further research to enhance our understanding of cyanobacteria (Micallef et al., 2015). Additionally, comparative metagenomics allows a more comprehensive examination of microbial functional genes in various natural environments, enabling the study of toxin-producing organisms as well as their associated heterotrophic communities and their potential role in driving the blooms (Steffen et al., 2012).

A cyanobacterial genomic DNA library for shotgun metagenome analysis was constructed in 2018. Metagenome pipeline analysis of gene fragments was conducted following steps like quality control, assembly, gene prediction, alignment, and taxonomic annotation. For example, in the saxitoxin-producing potentials of the cyanobacterial community, the *sxtA* and *sxtG* genes were determined by PCR using DNA obtained from water and sediment samples. After amplification, the determined sequences of saxitoxin-producing genes were applied to a BLAST search against the GenBank database of NCBI (Kim et al., 2018).

3.2.4. Genome mining

In addition to the above, and due to the lack of available information related to cyanobacterial genomics, it is essential to develop strategies that allow the correct identification and characterization of secondary metabolites gene clusters produced by cyanobacteria, specifically cyanotoxins that have a wide range of chemical structures and bioactivities (Micallef et al., 2015) of which it is necessary to understand their

Table 2
Reports of primer pairs for amplification of genes related to cyanotoxin production.

Cyanotoxin	Gen	F Name	F Sequence	R Name	R Sequence	Reference
Anatoxin	*anaC*	anaC-gen-F2	TCTGGTATTCAGTMCCCTCYAT	anaC-gen-R2	CCCAATARCCTGTCATCAA	(Sabart et al., 2015)
	anaC	anxgenF	ATGGTCAGAGGTTTTACAAG	anxgenR	CGACTCTTAATCATGCGATC	(Rantala-Ylinen et al., 2011)
	anaC	anxCF	TGAGGGAACAAGTGAGTT	anxCR	ATCATCTCCGATCCCAATCC	(Rantala-Ylinen et al., 2011)
	anaC	anxgen F	ATGGTCAGAGGTTTTACAAG	anxgen R	CGACTCTTAATCATGCGATC	(S. Wang et al., 2015)
	anaC	anaC-gen F	TCTGGTATTCAGTCCCCTCTAT	anaC-gen R	CCCAATAGCCTGTCATCAA	(S. Wang et al., 2015)
	anaC	anaC-gen-F2	TCTGGTATTCAGTMCCCTCYAT	anaC-gen-R2	CCCAATARCCTGTCATCAA	(Sabart et al., 2015)
	ATX	atoaf	TCGGAAGCGCGATCGCAAATCG	atxar	GCTTCCTGAGAAGGTCCGCTAG	(Hodoki et al., 2013)
	pks	atxoa	TCGGAAGCGCGATCGCAAATCG	atxar	GCTTCCTGAGAAGGTCCGCTAG	(Ballot, Fastner, Lentz, et al., 2010)
Cylindrospermopsins	*cyrA*	CatF1	AGATGGTGCTTATTTTGAAC	CatR1	TCTTCACAGATGACCTTCTT	(Ribeiro et al., 2020)
	cyrA	CYLATF	ATTGTAAATAGCTGGAATGAGTGG	CYLATR	TTAGGGAAGTAATCTTCACAG	(Lei et al., 2019)
	cyrA	cyrF	GTCTGCCCACGTGATGTTATGAT	cyrR	CGTGACCGCCGTGACA	(Al-Tebrineh et al., 2012)
	cyrB	CPS-F	AGTATATGTTGCGGGACTCG	CPS-R	CCCGCCAAGACAGAGGGTAG	(Ribeiro et al., 2020)
	cyrB	CPSF	AGTATATGTTGCGGGACTCG	CPSR	CCCGCCAAGACAGAGGGTAG	(Lei et al., 2019)
	cyrC	CkcF3	AATGATCGAAAACAGCAGTCGG	CkcR3	TAGAACAATCATCCCACAACCT	(Ribeiro et al., 2020)
	cyrC	A205PKF	AATGACAGAGACTTGTGCGGGG	A205PKR	TTATCGGTATTGGTGGTAGCAACT	(Lei et al., 2019)
	cyrJ	cynsulfF	ACTTCTCTCCTTTCCCTATC	cylnamR	GAGTGAAAATGCGTAGAACTTG	(Lei et al., 2019)
	cyrJ	cyrJ –F1	TGATTCGCCAACCCAAAGAA	cyrJ –R1	GATCGTTCAGCAAGTCGTGT	(Lei et al., 2019)
	cyrJ	cyrJ207-F	CCCCTACAACCTGACAAAGCTT	cyrJ207-R	CCCGCCTGTCATAGATGCA	(Zupančič et al., 2021)
	pks	pksK18	CCTCGCACATAGCCATTTGC	pksM4	GAAGCTCTGGAATCCGGTAA	(Gkelis & Zaoutsos, 2014)
	pks	K18	GAAGCTCTGGAATCCGGTAA	M4	AATCCTTACGGGATCCGGTGC	(Lei et al., 2019)
	pks	cyl2	GGCATTCCTAGTTATATTGCCATACTA	cyl4	GCCCGTTTTTGTCCCTTTCGTGC	(Zhang et al., 2014)
	pks	k18	CCTCGCACATAGCCATTTGC	m4	GAAGCTCTGGAATCCGGTAA	(Rasmussen et al., 2008)
	pks	M4	GAAGCTCTGGAATCCGGTAA	M5	AATCCTTACGGGATCCGGTGC	(Schembri et al., 2001)
	ps	psM13	GGCAAATTGTGATAGCCACGAGC	psM14	GATGGAACATCGCTCACTGGTG	(Gkelis & Zaoutsos, 2014)
	ps	M13	GGCAAATTGTGATAGCCACGAGC	M14	GATGGAACATCGCTCACTGGTG	(Lei et al., 2019)
	pks	m4	GAAGCTCTGGAATCCGGTAA	k18	CCTCGCACATAGCCATTTGC	(Chiu et al., 2017)
Microcystin	*mcyE*	PKEF1	CGCAAACCCGATTTACAG	PKER1	CCCCTACCATCTTCATCTTC	(Gkelis & Zaoutsos, 2014)
	mcyA	MISYf	CGACCGAGGAATTTCAAGCT	MISYr	AGTATCCGACCAAGTTACCCAAAC	(Foulds et al., 2002)
	mcyA	MSF	ATCCAGCAGTTGAGCAAGC	MSR-2	TGCAGATAACTCCGCAGTTG	(Furukawa et al., 2006)
	mcyA	mcyA-Cd1F	AAAATTAAAAGCCGTATCAAA	mcyA-Cd1R	AAAAGTGTTTTATTAGCGGCTCAT	(Gkelis & Zaoutsos, 2014)
	mcyA	mcyA-Cd-1F	AAAATTAAAAGCCGTATCAAA	mcyA-Cd-1R	AAAAGTGTTTTATTAGCGGCTCAT	(Sabart et al., 2015)
	mcyA	MSF	ATCCAGCAGTTGAGCAAGC	MSR	TGCAGATAACTCCGCAGTTG	(Tillett et al., 2001)
	mcyA-C	M13	GGCAAATTGTGATAGCCACGAGC	M14	GATGGAACATCGCTCACTGGTG	(Schembri et al., 2001)
	mcyB	MIf	GCAGCGAACTCTTGAAGGGTTTATG	MIr	GCGGATTCTGTGCAGCTTGTTCTTC	(Foulds et al., 2002)
	mcyB	30F	CCTACCGAGCGCTTGGG	108R	GAAAATCCCCTAAAGATTCCTGAGT	(Kurmayer & Kutzenberger, 2003)
	mcyB	mcyB2959F	TGGGAAGATGTTCTTCAGGTATCCAA	mcyB3278R	AGAGTGGAAACAATATGATAAGCTAC	(Gkelis & Zaoutsos, 2014)
	mcyB	mcyB#04F	TGTGGAGTCTATTTATCCTCTTTCC	mcyB#04R	GAGTTTGACTACAATAAATCCCTGAAT	(Chiu et al., 2017)
	mcyE	mcyE-F2	GAAATTTGTGTAGAAGGTGC	MicmcyE-R8	CAATGGGAGCATAACGAG	(Vaitomaa et al., 2003)
	mcyE	mcyE-F2	GAAATTTGTGTAGAAGGTGC	AnamcyE-12R	CAATCTCGGTATAGCGGC	(Vaitomaa et al., 2003)
	mcyE	mcyE-F2	GAAATTTGTGTAGAAGGTGC	mcyE-R4	AATTCTAAAGCCCAAAGACG	(Ribeiro et al., 2020)
	mcyE/ ndaF	HEPF	TTTGGGGTTAACTTTTTTGGGCATAGTC	HEPR	AATTCTTGAGGCTGTAAATCGGGTTT	(Gkelis & Zaoutsos, 2014)
	mycB	FAA	CTATGTTATTTATACATCAGG	RAA	CTCAGCTTAACTTGATTATC	(Neilan et al., 1999)
Nodularin	*nda*	NPSF3	CTTATCGAGGAGGTCGTGAAG	HLIPR	CAGAAAGTCAGTATTAGG	(Moffitt & Neilan, 2004)
	ndaF	DQmcyF	TTTAGAACSGGVGATTTAGG	DQmcyR	CGRBTVADTTGRTATTCAATTTCT	(Al-Tebrineh et al., 2011)
	ndaF	mcyF	TTTAGAACSGGVGATTTAGG	mcyR	CGRBTVADTTGRTATTCAATTTCT	(Al-Tebrineh et al., 2012)
	ndaF	DQmcyF	TTTAGAACSGGVGATTTAGG	DQmcyR	CGRBTVADTTGRTATTCAATTTCT	(Al-Tebrineh et al., 2011)
	ndaF	NPF	TATTTTGTGGTGGAGAAGCACTA	NPR	GGAACTATCTGATAATTAGAC	(Moffitt & Neilan, 2001)
	ndaF	ndaF8452	GTGATTGAATTTCTTGGTCG	ndaF8640	GGAAATTTCTATGTCTGACTCAG	(Koskenniemi et al., 2007)

(*continued on next page*)

Table 2 (*continued*)

Cyanotoxin	Gen	F Name	F Sequence	R Name	R Sequence	Reference
	NRPS	NPF	TATTTTGTGGTGGAGAAGCACTA	NPR	GGAACTATCTGATAATTAGAC	(Moffitt & Neilan, 2004)
	pks	MNKF	GTTCYTCYTCAYTRGTRGCG	MNKR	CCYAAGAACAACWAYTCCACA	(Moffitt & Neilan, 2004)
Saxitoxin	*sxtA*	sxtA-F	GATGACGGAGTATTTGAAGC	sxtA-R	CTGCATCTTCTGGACGGTAA	(Ribeiro et al., 2020)
	sxtA	sxtA-F	GATGACGGAGTATTTGAAGC	sxtA-R	CTGCATCTTCTGGACGGTAA	(Al-Tebrineh et al., 2010)
	sxtA	sxtF	GGAGTGGATTTCAACACCAGAA	sxtR	GTTTCCCAGACTCGTTTCAGG	(Al-Tebrineh et al., 2012)
	sxtA	sxtA-F	GCGTACATCCAAGCTGGACTCG	sxtA-R	GTAGTCCAGCTAAGGCACTTGC	(Ballot, Fastner, & Wiedner, 2010)
	sxtA4	sxtA4-F	CTGAGCAAGGCGTTCAATTC	sxtA4-R	TACAGATMGGCCCTGTGARC	(Gao et al., 2015)
	sxtB	sxtB_F2	TGTTGTGCTTGCTGCTCTATCAG	sxtB_R2	CAGCGTTTTCAGCGTAYCGAC	(Savela et al., 2015)
	sxtB	SXTB-F	TTTGTAGGRCAGGCACTT	SXTB-R	ATCATCGGTATCATCGGTA	(Ribeiro et al., 2020)
	sxtI	SxtI-F	GCTTACTACCACGATAGTGCTGCCG	SxtI-R	GGTTCGCCGCGGACATTAAA	(Gkelis & Zaoutsos, 2014)
	sxtI	OCT-F	TGCCGTTTTGTGCTTAGATG	OCT-R	GGACGGAAGGACTCACGATA	(Ribeiro et al., 2020)

evolution, organization, and distribution of the genes involved in their biosynthesis (Alvarenga et al., 2017), since, as mentioned above, they represent a risk to human, animal (Méjean & Ploux, 2013), and vegetation health (Weralupitiya, Wanigatunge, Gunawardana, Vithanage, & Magana-Arachchi, 2022).

Although genome mining aims to assess the genetic potential of strains to produce secondary metabolites by analyzing DNA sequences, matching biosynthetic gene clusters (BGCs) to their corresponding secondary metabolites remains a challenge (May et al., 2020). This hinders our ability to map the distribution of biosynthetic pathways and to better understand the physiological function of cyanotoxins (Micallef et al., 2015). There are different bioinformatics platforms dedicated to the prediction of BGCs, the most used is the web server Antibiotics & Secondary Metabolite Analysis Shell (AntiSMASH, https://antismash.secondarymetabolites.org/), which can permit the rapid identification, annotation, and analysis of gene clusters associated with the biosynthesis of secondary metabolites, non-ribosomal peptide synthetases, polyketide synthases, type I and II polyketide synthases, lasso peptides, antibiotic oligosaccharides, and others (Blin et al., 2021). Recently, studies focused on the identification of secondary metabolites in cyanobacteria have used the software AntiSMASH to perform genome mining, one of these is the one carried out by (Hernandez et al., 2021), where secondary metabolites were identified within discrete BGCs and some of them were known toxins, such as microcystin, nodularin, cylindrospermopsin, and saxitoxin. Similarly, (Pérez-Carrascal et al., 2019) performed a population genomic analysis of some strains of the *Microcystis* genus, responsible for harmful blooms in ecosystems worldwide, and used AntiSMASH to identify 34 groups of known BGC, among these, microcystin (ecotoxic), cyanopeptolin (hepatotoxic), microcyclamide and Puwainaphycins (both cytotoxic). In addition, a study developed by (Yamaguchi et al., 2019) characterized the genome of *Microcystis aeruginosa* NIES-102 and identified 11 BGC, including microcystin, microviridin B, aeruginosin, and micropeptin. Another study carried out by (Soares et al., 2021) identified gene clusters associated with the synthesis of proteusin, terpenes, arylpolyene, and other secondary metabolites using AntiSMASH, nevertheless, due to the importance of cyanotoxins, the presence of genes related to their biosynthesis was carried out using a manually-curated dataset of genes sequences from the NCBI database, then processed with additional methods. Furthermore, the information available online is scarce, according to the in-depth literature review focused on genomic mining tools for the rapid identification of BGCs related to cyanotoxins using bioinformatics tools.

In addition to referring to the use of AntiSMASH, an alternative of performing "manual" genomic mining is shown, using a command line software tool in conjunction with literature regarding the genes associated with the biosynthesis of cyanotoxins. The use of bioinformatics tools for the manual detection of genes associated with the biosynthesis of cyanotoxins constitutes a work path with a greater investment of time but with more specific inquiries, being able to include in databases the most recent reports on the subject. In this manner, it is suggested the use of Prokka, a software tool that is capable of accomplishing a rapid annotation of prokaryotic genomes, identifying all the pertinent features on a sequence, and coordinating a suite of existing software tools to achieve a well-founded annotation of genomic bacterial sequences (Seemann, 2014). The app Annotate Assembly and Re-annotate Genomes with Prokka - v1.14.5, is available in the open-source software and data platform, Systems Biology Knowledgebase (KBase, http://kbase.us), which facilitates data sharing, integration, and other analysis (Arkin et al., 2018). Specifically, this tool allows for the annotation of the cyanobacterial genomes, providing access to different features, such as gene, coding sequences, mRNA, product function, contig length and location, protein length and translation, and DNA length and sequence, among other details. Thus, once the coding sequences associated with the biosynthesis of cyanotoxins have been identified, their future comparison with databases specifically created and focused on the description of their possible functions is viable, opening the way to carrying out genomic mining manually.

Genome mining advances as a viable approach in evaluating the potential of a microorganism to produce secondary metabolites. Nevertheless, different obstacles persist, such as the lack of taxonomic diversity available in cyanobacterial genome databases, which provides an incomplete picture of this phylum and influences the lack of efficient bioinformatic tools, the optimization of the growth conditions provided to cyanobacteria to express their BGCs and, to be able to identify the predicted secondary metabolites (Alvarenga et al., 2017; May et al., 2020). An alternative is to combine genome mining approaches with analytical methods such as mass spectrometry, and demonstrate the basis of a robust dereplication strategy for efficient discovery of natural products (Kalaitzis et al., 2016).

4. Public genetic resources

Although the genomic approach is fundamental for the understanding of the functions and the participation of an organism in the environment, some organisms are less studied than others. Cyanobacteria are a clear example of this, being underrepresented in public genomic databases, making up 0.6 % of the total genomes of prokaryotes (Alvarenga et al., 2017), or 1.3 % of the total taxonomic units in NCBI, and more than 70 % of the cyanobacteria reported are affiliated at order level or higher taxonomic levels (Dextro et al., 2021). This generates a biased view of the phylum limiting the finesse with which the species diversity of metabolic capabilities is analyzed.

In 1995, the first articles reporting the complete sequence of

bacterial genomes (*Hemophilus influenzae* and *Mycoplasma genitalium*) were (Fleischmann et al., 1995; Fraser et al., 1995). Soon after, the sequence of the 3573,470 bp chromosome of the well-studied unicellular cyanobacterium *Synechocystis* PCC 6803 was reported (Kaneko et al., 1996). Currently, about 2000 complete or draft sequences of cyanobacterial genomes are accessible in public databases such as the DOE Joint Genome Institute (genome.jgi.doe.gov/portal/) and the Microbial Genome Database for Comparative Analysis (mbgd.nibb.ac.jp/). This number is steadily increasing in the frame of metagenomic analyses (Cassier-Chauvat et al., 2021). There are databases dedicated to cyanobacteria with information on (i) strain collections, (ii) regulatory interaction, (iii) taxonomy, (iv) literature, (v) proteins, and (vi) genomics (Kumar & Arya, 2020). The following are public genetic information databases containing information on cyanobacteria (Table 3).

The growing number of fully sequenced cyanobacterial genomes offers significant opportunities to explore their metabolic organization in diverse environments (Peter et al., 2015). However, genetic manipulation has been limited to a few strains, narrowing the scope of research (Baunach et al., 2024). Research has mainly focused on models like *Synechocystis* PCC 6803, *Synechococcus* PCC 7942, and *Synechococcus* PCC 7002, which are easy to culture and modify but represent only a small fraction of cyanobacterial diversity. This narrow focus may hinder both fundamental discoveries and the commercialization of broader applications (Cassier-Chauvat et al., 2021).

Cyanobacteria are highly versatile microorganisms that can adapt to extreme conditions by differentiating specialized cells and producing diverse metabolites, such as lipids, polysaccharides, and pigments (Cassier-Chauvat et al., 2021). Their wide distribution and metabolic diversity make them ideal for studying environmental impacts and exploring potential industrial and biotechnological applications. Collecting and analyzing these organisms is essential for discovering novel species with valuable properties (Cui et al., 2023). The field of cyanobacterial genomics holds an interesting example of a research subject that has been moving forward at a pace that is relatively slower than currently observed for some of the other bacterial phyla (Alvarenga et al., 2017). To fully understand and utilize cyanobacterial biodiversity, we need to focus on large-scale analyses of gene and sRNA functions in model organisms. Fields like comparative genomics will reveal the pangenome, encompassing all genes across analyzed cyanobacteria. Additionally, predicting which metabolic pathways to engineer increases the production of biotechnologically valuable chemicals (Cassier-Chauvat et al., 2021).

5. Conclusion

Cyanobacterial blooms and the presence of cyanotoxins in various ecological niches pose significant environmental, economic, and health challenges. Despite the availability of molecular approaches and NGS technologies for quantifying these toxins, these methods can be inefficient and complicate comprehensive taxonomic classification. Current cultivation techniques also struggle with isolating different cyanobacterial strains, highlighting the need for more effective polyphasic approaches that combine morphological, biochemical, and genetic analyses. Therefore, it is essential to continue research along robust omics approaches that guide the development and future application of state-of-the-art tools that contribute to a thorough understanding of the quantification of cyanotoxin production, the cyanobacterial population, their diversity, and the respective functional roles of cyanobacteria and cyanotoxin production.

Author contributions

Sergio de los Santos-Villalobos: The conceptualization of the review, formal analysis, and revisions of the manuscript. The rest of the authors: writing of the original draft.

Table 3
Public genetic databases with information on cyanobacteria.

Database	Site
BacDive	https://bacdive.dsmz.de/
cTFbase	http://cegwz.com/
Culture Collection of Algae and Protozoa	http://www.ccap.ac.uk
Cyano DB	http://www.cyanodb.cz/
CyanoBase	http://genome.microbedb.jp/cyanobase
CyanoClust	http://gclust.jp/CyanoClust/index.html
CyanoCyc	https://cyanocyc.org/
CyanoEXpress	http://cyanoexpress.sysbiolab.eu/
CyanoLyase	http://cyanolyase.genouest.org/
CyanoOmicsDB	http://www.cyanoomics.cn/
Cyanorak	http://cyanorak.sb-roscoff.fr/cyanorak
Cyanosite	https://www-cyanosite.bio.purdue.edu/
CYANOTYPE	https://lege.ciimar.up.pt/cyanotype/
DDBJ	https://www.ddbj.nig.ac.jp/
LEGE	http://lege.ciimar.up.pt/cyanotype
NCBI	https://www.nih.gov/
µgreen-db	http://microgreen-23sdatabase.ea.inra.fr/

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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Data availability

No data was used for the research described in the article.

References

Agha, R., Gerphagnon, M., Schampera, C., Rohrlack, T., Fastner, J., Wolinska, J., 2022. Fate of hepatotoxin microcystin during infection of cyanobacteria by fungal chytrid parasites. Harmful Algae 118, 102288. https://doi.org/10.1016/j.hal.2022.102288.

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., Narasimhan, G., 2016. Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis. Evol. Bioinform. 12, 5–16. https://doi.org/10.4137/EBO.S36436/ASSET/IMAGES/LARGE/10.4137_EBO.S36436-FIG3.JPEG.

Al-Tebrineh, J., Gehringer, M.M., Akcaalan, R., Neilan, B.A., 2011. A new quantitative PCR assay for the detection of hepatotoxigenic cyanobacteria. Toxicon. 57 (4), 546–554. https://doi.org/10.1016/j.toxicon.2010.12.018.

Al-Tebrineh, J., Mihali, T.K., Pomati, F., Neilan, B.A., 2010. Detection of saxitoxin-producing cyanobacteria and anabaena circinalis in environmental water blooms by quantitative PCR. Appl. Environ. Microbiol. 76 (23), 7836–7842. https://doi.org/10.1128/AEM.00174-10.

Al-Tebrineh, J., Pearson, L.A., Yasar, S.A., Neilan, B.A., 2012. A multiplex qPCR targeting hepato- and neurotoxigenic cyanobacteria of global significance. Harmful Algae 15, 19–25. https://doi.org/10.1016/j.hal.2011.11.001.

Alvarenga, D.O., Fiore, M.F., Varani, A.M., 2017. A metagenomic approach to cyanobacterial genomics. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00809 (MAY).

Alvarenga, D.O., Fiore, M.F., Varani, A.M., 2018. Overview of next-generation sequencing technologies. Curr. Protoc. Mol. Biol. 122 (1). https://doi.org/10.1002/cpmb.59.

An, J., & Carbachael, W.W. (1994). Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins (Vol. 32, Issue 12).

Arkin, A.P., Cottingham, R.W., Henry, C.S., Harris, N.L., Stevens, R.L., Maslov, S., Dehal, P., Ware, D., Perez, F., Canon, S., Sneddon, M.W., Henderson, M.L., Riehl, W. J., Murphy-Olson, D., Chan, S.Y., Kamimura, R.T., Kumari, S., Drake, M.M.,

Brettin, T.S., Yu, D., 2018. KBase: The United States department of energy systems biology knowledgebase. Nat. Biotechnol. 36 (7), 566–569. https://doi.org/10.1038/nbt.4163.

Aune, T., Berg, K., 1986. Use of freshly prepared rat hepatocytes to study toxicity of blooms of the blue-green algae microcystis aeruginosa and oscillatoria agardhii. J. Toxicol. Environ. Health 19 (3), 325–336. https://doi.org/10.1080/15287398609530931.

Ballot, A., Fastner, J., Lentz, M., Wiedner, C., 2010a. First report of anatoxin-a-producing cyanobacterium Aphanizomenon issatschenkoi in northeastern Germany. Toxicon. 56 (6), 964–971. https://doi.org/10.1016/j.toxicon.2010.06.021.

Ballot, A., Fastner, J., Wiedner, C., 2010b. Paralytic Shellfish poisoning toxin-producing cyanobacterium aphanizomenon gracile in northeast Germany. Appl. Environ. Microbiol. 76 (4), 1173–1180. https://doi.org/10.1128/AEM.02285-09.

Barrowman, P., Adams, H., Southard, M., Hoppe-Jones, C., Weinrich, L., McNaught, K.J., Vogel, S., Klein, M., Clay, K., 2024. Flow imaging microscopy for harmful algal bloom monitoring. J. Am. Water. Works. Assoc. 116 (3), 36–48. https://doi.org/10.1002/awwa.2247.

Bashir, F., Bashir, A., Bouaïcha, N., Chen, L., Codd, G.A., Neilan, B., Xu, W.-L., Ziko, L., Rajput, V.D., Minkina, T., Arruda, R.S., Ganai, B.A., 2023. Cyanotoxins, biosynthetic gene clusters, and factors modulating cyanotoxin biosynthesis. World J. Microbiol. Biotechnol. 39 (9), 241. https://doi.org/10.1007/s11274-023-03652-x.

Baunach, M., Guljamow, A., Miguel-Gordo, M., Dittmann, E., 2024. Harnessing the potential: advances in cyanobacterial natural product research and biotechnology. Nat. Prod. Rep. 41 (3), 347–369. https://doi.org/10.1039/D3NP00045A.

Beasley, V.R. (2020). Harmful algal blooms (phycotoxins). In ***Reference Module in Earth Systems and Environmental Sciences***. Elsevier. 10.1016/B978-0-12-409548-9.11275-8.

Blin, K., Shaw, S., Kloosterman, A.M., Charlop-Powers, Z., Van Wezel, G.P., Medema, M. H., Weber, T., 2021. AntiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic. Acids. Res. 49 (W1), W29–W35. https://doi.org/10.1093/nar/gkab335.

Bonilla, S. (2009). *Programa Hidrológico Internacional 16 PHI-VII /Documento Técnico N °Cianobacterias Planctónicas del Uruguay Manual para la identificación y medidas de gestión.* http://www.unesco.org.uy/phi.

Bonilla, S., Haakonsson, S., Somma, A., Gravier, A., Britos, A., Vidal, L., De León, L., Brena, B., Pírez, M., Piccini, C., Martínez, G., 2015. Cyanobacteria and cyanotoxins in freshwaters of Uruguay. Revista Del Laboratorio Tecnológico de Uruguay 10, 9–22.

Botes, D.P., Tuinman, A.A., Wessels, P.L., Viljoen, C.C., Kruger, H., Williams, D.H., Santikarn, S., Smith, R.J., Hammond, S.J., 1984. The structure of cyanoginosin-LA, a cyclic heptapeptide toxin from the cyanobacterium Microcystis aeruginosa. J. Chem. Soc., Perkin Trans. 1, 2311. https://doi.org/10.1039/p19840002311.

Bouaïcha, N., Miles, C., Beach, D., Labidi, Z., Djabri, A., Benayache, N., Nguyen-Quang, T., 2019. Structural diversity, characterization and toxicology of microcystins. Toxins. (Basel) 11 (12), 714. https://doi.org/10.3390/toxins11120714.

Breitwieser, F.P., Lu, J., Salzberg, S.L., 2019. A review of methods and databases for metagenomic classification and assembly. Brief. Bioinform. 20 (4), 1125–1136. https://doi.org/10.1093/BIB/BBX120.

Carmichael, W.W., Biggs, D.F., Gorham, P.R., 1975. Toxicology and pharmacological action of anabaena flos-aquae toxin. Science 187 (4176), 542–544. https://doi.org/10.1126/SCIENCE.803708.

Casero, M.C., Velázquez, D., Medina-Cobo, M., Quesada, A., Cirés, S., 2019. Unmasking the identity of toxigenic cyanobacteria driving a multi-toxin bloom by high-throughput sequencing of cyanotoxins genes and 16S rRNA metabarcoding. Sci. Total Environ. 665, 367–378. https://doi.org/10.1016/j.scitotenv.2019.02.083.

Cassier-Chauvat, C., Blanc-Garin, V., Chauvat, F., 2021. Genetic, genomics, and responses to stresses in cyanobacteria: biotechnological implications. Genes. (Basel) 12 (4), 500. https://doi.org/10.3390/genes12040500.

Chakdar, H., Pabbi, S., 2015. Cyanobacterial phycobilins: production, purification, and regulation. Front. Discoveries and Innovations in Interdisciplinary Microbiol. 45–69. https://doi.org/10.1007/978-81-322-2610-9_4/TABLES/1.

Chen, M.Y., Teng, W.K., Zhao, L., Hu, C.X., Zhou, Y.K., Han, B.P., Song, L.R., Shu, W.S., 2021. Comparative genomics reveals insights into cyanobacterial evolution and habitat adaptation. ISME J. 15 (1), 211–227. https://doi.org/10.1038/s41396-020-00775-z.

Chiu, Y.T., Chen, Y.H., Wang, T.S., Yen, H.K., Lin, T.F., 2017. A qPCR-based tool to diagnose the presence of harmful cyanobacteria and cyanotoxins in drinking water sources. Int. J. Environ. Res. Public Health (5), 14. https://doi.org/10.3390/ijerph14050547.

Chorus, I., Fastner, J., & Welker, M. (2021). Cyanobacteria and cyanotoxins in a changing environment: concepts, controversies, challenges. *Water 2021, Vol. 13, Page 2463*, 13(18), 2463. 10.3390/W13182463.

Chorus, I., & Welker, M. (2021). Toxic cyanobacteria in water. Toxic Cyanobacteria in Water. 10.1201/9781003081449.

Christensen, V.G., Khan, E., 2020. Freshwater neurotoxins and concerns for human, animal, and ecosystem health: a review of anatoxin-a and saxitoxin. Sci. Total Environ. 736, 139515. https://doi.org/10.1016/J.SCITOTENV.2020.139515.

Cobo, F., 2015. Métodos de control de las floraciones de cianobacterias en aguas continentales. Limnetica 34, 247–268. https://doi.org/10.23818/limn.34.20.

Codd, G.A., Meriluoto, J., & Metcalf, J.S. (2017). Introduction: cyanobacteria, cyanotoxins, their human impact, and risk management. In ***Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*** (pp. 1–8). John Wiley & Sons, Ltd. 10.1002/9781119068761.ch1.

Codd, G.A., Testai, E., Funari, E., & Svirčev, Z. (2020). Cyanobacteria, cyanotoxins, and human health. In ***Water Treatment for Purification from Cyanobacteria and Cyanotoxins*** (pp. 37–68). Wiley. 10.1002/9781118928677.ch2.

Cornet, L., Bertrand, A.R., Hanikenne, M., Javaux, E.J., Wilmotte, A., Baurain, D., 2018. Metagenomic assembly of new (Sub)polar cyanobacteria and their associated microbiome from non-axenic cultures. Microb. Genom. (9), 4. https://doi.org/10.1099/mgen.0.000212.

Cronberg, G. (Gertrud), & Annadotter, H. (2006). *Manual on aquatic cyanobacteria : a photo guide and a synopsis of their toxicology*. Intergovernmental Oceanographic Commission, Unesco.

Cui, J., Sun, H., Chen, R., Sun, J., Mo, G., Luan, G., Lu, X., 2023. Multiple routes toward engineering efficient cyanobacterial photosynthetic biomanufacturing technologies. Green Carbon 1 (2), 210–226. https://doi.org/10.1016/j.greenca.2023.11.004.

Dabba, A., Tari, A., Meftali, S., Mokhtari, R., 2021. Gene selection and classification of microarray data method based on mutual information and moth flame algorithm. Expert. Syst. Appl. 166. https://doi.org/10.1016/j.eswa.2020.114012.

Davison, M., Hall, E., Zare, R., Bhaya, D., 2014. Challenges of metagenomics and single-cell genomics approaches for exploring cyanobacterial diversity. Photosynth. Res. 126 (1), 135–146. https://doi.org/10.1007/S11120-014-0066-9. *2014 126:1*.

De La Rica, R., Stevens, M.M., 2012. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nat. Nanotechnol. 7 (12), 821–824. https://doi.org/10.1038/nnano.2012.186.

De Philippis, R., Vincenzini, M., 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol. Rev. 22 (3), 151–175. https://doi.org/10.1111/J.1574-6976.1998.TB00365.X.

Dextro, R.B., Delbaje, E., Cotta, S.R., Zehr, J.P., Fiore, M.F., 2021. Trends in free-access genomic data accelerate advances in cyanobacteria taxonomy. J. Phycol. 57 (5), 1392–1402. https://doi.org/10.1111/jpy.13200.

Dilrukshi, K.T., Abeysiri, H.A.S.N., Imanthi, K.P.A., Wijerathna, P.A.K.C., Manage, P.M., & Kulasooriya, S.A. (2023). Detection of cyanotoxins using vertebrate bioassays in mice. In ***Protocols for Cyanobacteria Sampling and Detection of Cyanotoxin*** (pp. 197–203). Springer Nature Singapore. 10.1007/978-981-99-4514-6_28.

Dimitriadis, S., Dova, L., Kotsianidis, I., Hatzimichael, E., Kapsali, E., Markopoulos, G.S., 2024. Imaging flow cytometry: development, present applications, and future challenges. ***Methods Protoc.*** 7 (2). https://doi.org/10.3390/MPS7020028.

Dittmann, E., Fewer, D.P., Neilan, B.A., 2013. Cyanobacterial toxins: biosynthetic routes and evolutionary roots. FEMS Microbiol. Rev. 37 (1), 23–43. https://doi.org/10.1111/J.1574-6976.2012.12000.X.

Du, X., Liu, H., Yuan, L., Wang, Y., Ma, Y., Wang, R., Chen, X., Losiewicz, M.D., Guo, H., Zhang, H., 2019. The diversity of cyanobacterial toxins on structural characterization, distribution and identification: a systematic review. Toxins. (Basel) (9), 11. https://doi.org/10.3390/TOXINS11090530.

Dvořák, P., Poulíčková, A., Hašler, P., Belli, M., Casamatta, D.A., Papini, A., 2015. Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification. Biodivers. Conserv. 24 (4), 739–757. https://doi.org/10.1007/S10531-015-0888-6/FIGURES/3.

Esteves-Ferreira, A.A., Cavalcanti, J.H.F., Vaz, M.G.M.V., Alvarenga, L.V., Nunes-Nesi, A., Araújo, W.L., 2017. Cyanobacterial nitrogenases: phylogenetic diversity, regulation and functional predictions. Genet. Mol. Biol. 40 (1), 261. https://doi.org/10.1590/1678-4685-GMB-2016-0050, 1 Suppl.

Falfushynska, H., Kasianchuk, N., Siemens, E., Henao, E., Rzymski, P., 2023. A review of common cyanotoxins and their effects on fish. Toxics. 11 (2), 118. https://doi.org/10.3390/toxics11020118.

Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F., Kerlavage, A. R., Bult, C.J., Tomb, J.-F., Dougherty, B.A., Merrick, J.M., McKenney, K., Sutton, G., FitzHugh, Will, Fields, C., Gocayne, J.D., Scott, J., Shirley, R., Liu, L., Glodek, A., Venter, J.C, 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science (1979) 269 (5223), 496–512. https://doi.org/10.1126/science.7542800.

Foulds, I.V., Granacki, A., Xiao, C., Krull, U.J., Castle, A., Horgen, P.A., 2002. Quantification of microcystin-producing cyanobacteria and E. coli in water by 5'-nuclease PCR. J. Appl. Microbiol. 93 (5), 825–834. https://doi.org/10.1046/j.1365-2672.2002.01772.x.

Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., Fritchman, J.L., Weidman, J.F., Small, K.V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T.R., Saudek, D.M., Phillips, C.A., Venter, J.C., 1995. The minimal gene complement of *Mycoplasma genitalium*. Science (1979) 270 (5235), 397–404. https://doi.org/10.1126/science.270.5235.397.

Furukawa, K., Noda, N., Tsuneda, S., Saito, T., Itayama, T., Inamori, Y., 2006. Highly sensitive real-time PCR assay for quantification of toxic cyanobacteria based on microcystin synthetase a gene. J. Biosci. Bioeng. 102 (2), 90–96. https://doi.org/10.1263/jbb.102.90.

Gama, W.A., De Paiva Azevedo, M.T., Komárková-Legnerová, J., Sant'anna, C.L., 2012. A new species of Lemmermanniella (Cyanobacteria) from the Atlantic rainforest, Brazil. Braz. J. Bot. 35 (4), 319–324. https://doi.org/10.1590/S0100-84042012000400005.

Gao, Y., Yu, R.-C., Murray, S.A., Chen, J.-H., Kang, Z.-J., Zhang, Q.-C., Kong, F.-Z., Zhou, M.-J., 2015. High Specificity of a quantitative PCR assay targeting a saxitoxin gene for monitoring toxic algae associated with paralytic shellfish toxins in the yellow sea. Appl. Environ. Microbiol. 81 (20), 6973–6981. https://doi.org/10.1128/AEM.00417-15.

Giannakopoulos, C., Panou, M., Gkelis, S., 2024. Phylogenetic analysis of Nostocales (Cyanobacteria) based on two novel molecular markers, implicated in the nitrogenase biosynthesis. FEMS Microbiol. Lett. 371. https://doi.org/10.1093/FEMSLE/FNAD136.

Gkelis, S., Zaoutsos, N., 2014. Cyanotoxin occurrence and potentially toxin producing cyanobacteria in freshwaters of Greece: a multi-disciplinary approach. Toxicon. 78, 1–9. https://doi.org/10.1016/j.toxicon.2013.11.010.

Glibert, P.M., Gross, E., Cullen, J., Fukuyo, Y., Gentien, P., Anderson, D.M., Blackburn, S., Elbrächter, M., Enevoldsen, H., Estrada, M., Fennel, W., Kononen, K., Lagos, N., Osborn, T., Pitcher, G., Sierra-Beltrán, A.P., Thorpe, S., Urban, E.R., Zhang, J., & Zingone, A. (2018). ***Global Ecology and Oceanography of Harmful Algal Blooms*** (P. M. Glibert, E. Berdalet, M. A. Burford, G. C. Pitcher, & M. Zhou, Eds.; Vol. 232). Springer International Publishing. 10.1007/978-3-319-70069-4.

Graham, M.D., Cook, J., Graydon, J., Kinniburgh, D., Nelson, H., Pilieci, S., Vinebrooke, R.D., 2018. High-resolution imaging particle analysis of freshwater cyanobacterial blooms. Limnol. Oceanogr.: Methods 16 (10), 669–679. https://doi.org/10.1002/lom3.10274.

Gupta, R., Raza, N., Bhardwaj, S.K., Vikrant, K., Kim, K.H., Bhardwaj, N., 2021. Advances in nanomaterial-based electrochemical biosensors for the detection of microbial toxins, pathogenic bacteria in food matrices. J. Hazard. Mater. 401. https://doi.org/10.1016/j.jhazmat.2020.123379.

Hackett, J.D., Wisecaver, J.H., Brosnahan, M.L., Kulis, D.M., Anderson, D.M., Bhattacharya, D., Gerald Plumley, F., Erdner, D.L., 2013. Evolution of saxitoxin synthesis in cyanobacteria and dinoflagellates. Mol. Biol. Evol. 30 (1), 70–78. https://doi.org/10.1093/MOLBEV/MSS142.

Hambright, K.D., Zamor, R.M., Easton, J.D., Allison, B., 2014. Algae. Encyclopedia of Toxicology. Elsevier, pp. 130–141. https://doi.org/10.1016/B978-0-12-386454-3.00983-0. Third.

Hauer Tomas, & Komárek Jin. (2022). *CyanoDB 2*. http://www.cyanodb.cz/.

Hernandez, B.Y., Zhu, X., Sotto, P., Paulino, Y., 2021. Oral exposure to environmental cyanobacteria toxins : Implications for cancer risk. Environ. Int. 148, 106381. https://doi.org/10.1016/j.envint.2021.106381.

Hernández Mariné, M., Clavero, E., Roldán, M., 2004. Microscopy methods applied to research on cyanobacteria. Limnetica 23 (1–2), 179–186. https://doi.org/10.23818/limn.23.16.

Hirose, Y., Ohtsubo, Y., Misawa, N., Yonekawa, C., Nagao, N., Shimura, Y., Fujisawa, T., Kanesaki, Y., Katoh, H., Katayama, M., Yamaguchi, H., Yoshikawa, H., Ikeuchi, M., Eki, T., Nakamura, Y., Kawachi, M., 2021. Genome sequencing of the NIES Cyanobacteria collection with a focus on the heterocyst-forming clade. DNA Res. (6), 28. https://doi.org/10.1093/dnares/dsab024.

Hiskia, A.E., Triantis, T.M., Antoniou, M.G., Kaloudis, T., & Dionysiou, D.D. (2020). *Water Treatment for Purification from Cyanobacteria and Cyanotoxins, First Edition. Edited*.

Hodoki, Y., Ohbayashi, K., Kobayashi, Y., Takasu, H., Okuda, N., Nakano, S., 2013. Anatoxin-a-producing Raphidiopsis mediterranea Skuja var. grandis Hill is one ecotype of non-heterocytous Cuspidothrix issatschenkoi (Usačev) Rajaniemi et al. in Japanese lakes. Harmful Algae 21–22, 44–53. https://doi.org/10.1016/j.hal.2012.11.007.

Ishak, S.M., Yahaya, N., Loh, S.H., Kamaruzaman, S., Zain, N.N.M., Waras, M.N., Abdullah, W.N.W., Miskam, M., Raoov, M., Aziz, N.A., Hassan, Y., 2023. Research progress on extraction and analytical methods for saxitoxin and its congeners. Chromatographia 86 (5), 349–373. https://doi.org/10.1007/S10337-023-04251-6. *2023 86:5*.

Jalili, F., Trigui, H., Guerra Maldonado, J.F., Dorner, S., Zamyadi, A., Shapiro, B.J., Terrat, Y., Fortin, N., Sauvé, S., Prévost, M., 2021. Can cyanobacterial diversity in the source predict the diversity in sludge and the risk of toxin release in a drinking water treatment plant? Toxins. (Basel) (1), 13. https://doi.org/10.3390/TOXINS13010025.

Janssen, E.M.L., 2019. Cyanobacterial peptides beyond microcystins – a review on co-occurrence, toxicity, and challenges for risk assessment. Water. Res. 151, 488–499. https://doi.org/10.1016/J.WATRES.2018.12.048.

Jin, C., Mesquita, M.M.F., Deglint, J.L., Emelko, M.B., Wong, A., 2018. Quantification of cyanobacterial cells via a novel imaging-driven technique with an integrated fluorescence signature. Sci. Rep. 8 (1). https://doi.org/10.1038/s41598-018-27406-0.

Jones, M.R., Pinto, E., Torres, M.A., Dörr, F., Mazur-Marzec, H., Szubert, K., Tartaglione, L., Dell'Aversano, C., Miles, C.O., Beach, D.G., McCarron, P., Sivonen, K., Fewer, D.P., Jokela, J., Janssen, E.M.L., 2021. CyanoMetDB, a comprehensive public database of secondary metabolites from cyanobacteria. Water. Res. 196, 117017. https://doi.org/10.1016/J.WATRES.2021.117017.

Jungblut, A.D., Neilan, B.A., 2006. Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. Arch. Microbiol. 185 (2), 107–114. https://doi.org/10.1007/S00203-005-0073-5/METRICS.

Kalaitzis, J.A., Ingrey, S.D., Chau, R., Simon, Y., Neilan, B.A., 2016. Genome-guided discovery of natural products and biosynthetic pathways from Australia's untapped microbial megadiversity. Aust. J. Chem. 69 (2), 129. https://doi.org/10.1071/CH15601.

Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirosawa, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Tabata, S., 1996. Sequence analysis of the genome of the unicellular cyanobacterium synechocystis sp. Strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res. 3 (3), 109–136. https://doi.org/10.1093/dnares/3.3.109.

Kaushik, R., Balasubramanian, R., 2013. Methods and approaches used for detection of cyanotoxins in environmental samples: a review. In: Critical Reviews in Environmental Science and Technology, 43, pp. 1349–1383. https://doi.org/10.1080/10643389.2011.644224.

Kim, I.S., Park, H.K., Kim, Y.J., 2021. Development of genus-specific PCR primers for molecular monitoring of invasive nostocalean cyanobacteria. Int. J. Environ. Res. Public Health 18 (11), 5703. https://doi.org/10.3390/IJERPH18115703/S1.

Kim, K.H., Yoon, Y., Hong, W.Y., Kim, J.B., Cho, Y.C., Hwang, S.J., 2018. Application of metagenome analysis to characterize the molecular diversity and saxitoxin-producing potentials of a cyanobacterial community: a case study in the North Han River. Appl. Biol. Chem. 61 (2), 153–161. https://doi.org/10.1007/s13765-017-0342-4.

Komarék, J. (2013). Cyanoprokaryota; Teil 3: heterocystous genera. In B. Budel, G. Gartner, L. Krienitz, & M. Schargel (Eds.), ***Süßwasserflora Von Mitteleuropa****: Vol.* 19/3 (p. 1131). Springer Spektrum Berlin, Heidelberg.

Komárek, J., 2016a. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. Eur. J. Phycol. 51 (3), 346–353. https://doi.org/10.1080/09670262.2016.1163738.

Komárek, J., 2016b. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. Eur. J. Phycol. 51 (3), 346–353. https://doi.org/10.1080/09670262.2016.1163738.

Komárek, J., 2020. Quo vadis, taxonomy of cyanobacteria (2019). Fottea 20 (1), 104–110. https://doi.org/10.5507/fot.2019.020.

Komarék, J., & Anagnostidis, K. (1999). Cyanoprokaryota 1 Teil: chroococcales. In H. Etts, G. Gartner, H. Heynig, & D. Mollenhauer (Eds.), ***Subwasserflora Von Mitteleuropa*** (p. 548).

Komarék, J., & Anagnostidis, K. (2005). Cyanoprokaryota 2 Teil/2nd Part: oscillatoriales. In B. Budel, L. Krienitz, G. Gartner, & M. Schargel (Eds.), ***Susswasserflora Von Mitteleuropa*** (p. 759). Spektrum Akademischer Verlag Heidelberg.

Komárek, J., Kaštovský, J., Mareš, J., & Johansen, J.R. (2014). Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. In *Preslia* (Vol. 86).

Koskenniemi, K., Lyra, C., Rajaniemi-Wacklin, P., Jokela, J., Sivonen, K., 2007. Quantitative real-time PCR detection of toxic nodularia cyanobacteria in the Baltic sea. Appl. Environ. Microbiol. 73 (7), 2173–2179. https://doi.org/10.1128/AEM.02746-06.

Kumar, A., & Arya, P.K. (2020). Database resources for cyanobacterial research. In ***Advances in Cyanobacterial Biology*** (pp. 47–54). Elsevier. 10.1016/B978-0-12-819311-2.00004-8.

Kurmayer, R., Kutzenberger, T., 2003. Application of real-time PCR for quantification of microcystin genotypes in a population of the toxic cyanobacterium microcystis sp. Appl. Environ. Microbiol. 69 (11), 6723–6730. https://doi.org/10.1128/AEM.69.11.6723-6730.2003.

Lei, L., Lei, M., Lu, Y., Peng, L., Han, B.-P., 2019. Development of real-time PCR for quantification of Cylindrospermopsis raciborskii cells and potential cylindrospermopsin-producing genotypes in subtropicalreservoirs of southern China. J. Appl. Phycol. 31 (6), 3749–3758. https://doi.org/10.1007/s10811-019-01898-3.

Li, L., Jia, R., Liu, Y., Zhang, H., 2011. Detection of microcystin-producing cyanobacteria in a reservoir by whole cell quantitative PCR. Procedia Environ. Sci. 10, 2272–2279. https://doi.org/10.1016/j.proenv.2011.09.355 (PART C).

Li, X.C., Huo, S., Zhang, J., Ma, C., Xiao, Z., Zhang, H., Xi, B., Xia, X., 2019a. Metabarcoding reveals a more complex cyanobacterial community than morphological identification. Ecol. Indic. 107, 105653. https://doi.org/10.1016/J.ECOLIND.2019.105653.

Li, X., Huo, S., Xi, B., 2020. Updating the resolution for 16S rRNA OTUs clustering reveals the cryptic cyanobacterial genus and species. Ecol. Indic. 117. https://doi.org/10.1016/j.ecolind.2020.106695.

Li, Z., Zhang, S., Yu, T., Dai, Z., Wei, Q., 2019b. Aptamer-based fluorescent sensor array for multiplexed detection of cyanotoxins on a smartphone. Anal. Chem. 91 (16), 10448–10457. https://doi.org/10.1021/acs.analchem.9b00750.

Linz, D.M., Sienkiewicz, N., Struewing, I., Stelzer, E.A., Graham, J.L., Lu, J., 2023. Metagenomic mapping of cyanobacteria and potential cyanotoxin producing taxa in large rivers of the United States. Sci. Rep. (1), 13. https://doi.org/10.1038/s41598-023-29037-6.

Liu, J., Xing, Y., Lin, Y., Xie, Y., Zhou, X., 2023. Effect of pretreatment approach on the ELISA-based detection of cyanotoxins in water: Analysis and application. Sci. Total Environ. 871, 161988. https://doi.org/10.1016/j.scitotenv.2023.161988.

Liu, J., Xing, Y., Xue, B., Zhou, X., 2022. Nanozyme enhanced paper-based biochip with a smartphone readout system for rapid detection of cyanotoxins in water. Biosens. Bioelectron. 205, 114099. https://doi.org/10.1016/j.bios.2022.114099.

Liu, J., Yang, Y., Zhu, Q., Wang, Z., Hu, G., Shi, H., Zhou, X., 2021. ELISA-based method for variant-independent detection of total microcystins and nodularinsviaa multi-immunogen approach. Environ. Sci. Technol. 55 (19), 12984–12993. https://doi.org/10.1021/ACS.EST.1C03330/SUPPL_FILE/ES1C03330_SI_001.PDF.

Lu, J., Struewing, I., Wymer, L., Tettenhorst, D.R., Shoemaker, J., Allen, J., 2020. Use of qPCR and RT-qPCR for monitoring variations of microcystin producers and as an early warning system to predict toxin production in an Ohio inland lake. Water. Res. 170, 115262. https://doi.org/10.1016/j.watres.2019.115262.

May, D.S., Crnkovic, C.M., Krunic, A., Wilson, T.A., Fuchs, J.R., Orjala, J.E., 2020. 15N stable isotope labeling and comparative metabolomics facilitates genome mining in cultured cyanobacteria. ACS. Chem. Biol. 15 (3), 758–765. https://doi.org/10.1021/acschembio.9b00993.

Medlin, L.K. (2018). CYANO RT-microarray: a novel tool to detect gene expression in cyanobacteria medlin LK. CYANO RT-microarray: a novel tool to detect gene expression in cyanobacteria. In *J. Environ. Microbiol.* (Vol. 1, Issue 1).

Méjean, A., Mann, S., Maldiney, T., Vassiliadis, G., Lequin, O., Ploux, O., 2009. Evidence that biosynthesis of the neurotoxic alkaloids anatoxin-a and homoanatoxin-a in the cyanobacterium Oscillatoria PCC 6506 occurs on a modular polyketide synthase

initiated by L-proline. J. Am. Chem. Soc. 131 (22), 7512–7513. https://doi.org/10.1021/JA9024353/SUPPL_FILE/JA9024353_SI_001.PDF.
Méjean, A., & Ploux, O. (2013). Chapter six a genomic view of secondary metabolite production in cyanobacteria. In ***BS:ABR*** (Vol. 65). Elsevier. 10.1016/B978-0-12-394313-2.00006-8.
Melaram, R., Newton, A.R., Lee, A., Herber, S., El-Khouri, A., Chafin, J., 2024. A review of microcystin and nodularin toxins derived from freshwater cyanobacterial harmful algal blooms and their impact on human health. Toxicol. Environ. Health Sci. 1–9. https://doi.org/10.1007/S13530-024-00220-0/FIGURES/2.
Metcalf, J.S., Richer, R., Cox, P.A., Codd, G.A., 2012. Cyanotoxins in desert environments may present a risk to human health. Sci. Total Environ. 421–422, 118–123. https://doi.org/10.1016/j.scitotenv.2012.01.053.
Micallef, M.L., D'Agostino, P.M., Al-Sinawi, B., Neilan, B.A., & Moffitt, M. C. (2015). Exploring cyanobacterial genomes for natural product biosynthesis pathways. In *Marine Genomics* (Vol. 21, pp. 1–12). Elsevier B.V. 10.1016/j.margen.2014.11.009.
Mishra, N., & Gupta, S. (2023). Detection of cyanotoxins: bioassay using plants. In ***Protocols for Cyanobacteria Sampling and Detection of Cyanotoxin*** (pp. 233–241). Springer Nature Singapore. 10.1007/978-981-99-4514-6_33.
Moffitt, M.C., Neilan, B.A., 2001. On the presence of peptide synthetase and polyketide synthase genes in the cyanobacterial genus Nodularia. FEMS Microbiol. Lett. 196 (2), 207–214. https://doi.org/10.1111/j.1574-6968.2001.tb10566.x.
Moffitt, M.C., Neilan, B.A., 2004. Characterization of the nodularin synthetase gene cluster and proposed theory of the evolution of cyanobacterial hepatotoxins. Appl. Environ. Microbiol. 70 (11), 6353–6362. https://doi.org/10.1128/AEM.70.11.6353-6362.2004/ASSET/7A4C556D-AAF2-42F9-B19F-50D048207DB8/ASSETS/GRAPHIC/ZAM0110449150007.JPEG.
Mondal, S., Sharma, B., Majumdar, A., Tiwari, S., Maurya, P.K., Gupta, A., Singh, S.P., Singh, P., 2024. Sample preparation and visualization protocol for electron microscopy–based analysis of cyanobacteria. Methods in Cyanobacterial Res. 170–181. https://doi.org/10.1201/9781003398387-21.
Morales-Sandoval, P.H., Valenzuela-Ruiz, V., Ortega-Urquieta, M.E., Martínez-Vidales, A.D., Félix-Pablos, C.M., Chávez-Luzania, R.A., Parra-Cota, F.I., & de los Santos-Villalobos, S. (2021). ***Taxonomía Bacteriana Basada En índices Relacionados Al Genoma Completo***. *La Sociedad Académica*, 58, 39–51. https://www.ezbiocloud.
Moreira, C., Gomes, C., Vasconcelos, V., Antunes, A., 2020. Cyanotoxins occurrence in Portugal: a new report on their recent multiplication. Toxins. (Basel) (3), 12. https://doi.org/10.3390/toxins12030154.
Mrazova, K., Bacovsky, J., Sedrlova, Z., Slaninova, E., Obruca, S., Fritz, I., Krzyzanek, V., 2023. Urany-less low voltage transmission electron microscopy: a powerful tool for ultrastructural studying of cyanobacterial cells. Microorganisms (4), 11. https://doi.org/10.3390/microorganisms11040888.
Munoz, M., Cirés, S., de Pedro, Z.M., Colina, J.Á., Velásquez-Figueroa, Y., Carmona-Jiménez, J., Caro-Borrero, A., Salazar, A., Santa María Fuster, M.-C., Contreras, D., Perona, E., Quesada, A., Casas, J.A., 2021. Overview of toxic cyanobacteria and cyanotoxins in Ibero-American freshwaters: challenges for risk management and opportunities for removal by advanced technologies. Sci. Total Environ. 761, 143197. https://doi.org/10.1016/j.scitotenv.2020.143197.
Neilan, B.A., Dittmann, E., Rouhiainen, L., Bass, R.A., Schaub, V., Sivonen, K., Börner, T., 1999. Nonribosomal peptide synthesis and toxigenicity of cyanobacteria. J. Bacteriol. 181 (13), 4089–4097. https://doi.org/10.1128/JB.181.13.4089-4097.1999.
Nowruzi, B., Porzani, S.J., 2021. Toxic compounds produced by cyanobacteria belonging to several species of the order Nostocales: A review. J. Appl. Toxicol. 41 (4), 510–548. https://doi.org/10.1002/JAT.4088.
Nwosu, E.C., Roeser, P., Yang, S., Pinkerneil, S., Ganzert, L., Dittmann, E., Brauer, A., Wagner, D., Liebner, S., 2021. Species-level spatio-temporal dynamics of cyanobacteria in a hard-water temperate lake in the southern Baltics. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.761259.
Ohtani, I., Moore, R.E., Runnegar, M.T.C., 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green alga Cylindrospermopsis raciborskii. J. Am. Chem. Soc. 114 (20), 7941–7942. https://doi.org/10.1021/ja00046a067.
Oliveira, M.C., Repetti, S.I., Iha, C., Jackson, C.J., Díaz-Tapia, P., Lubiana, K.M.F., Cassano, V., Costa, J.F., Cremen, M.C.M., Marcelino, V.R., & Verbruggen, H. (2018). High-throughput sequencing for algal systematics. In *European Journal of Phycology* (Vol. 53, Issue 3, pp. 256–272). Taylor and Francis Ltd. 10.1080/09670262.2018.1441446.
Ouellette, A.J.A., Wilhelm, S.W., 2003. Toxic cyanobacteria: the evolving molecular toolbox. Front. Ecol. Environ. 1 (7), 359. https://doi.org/10.2307/3868188.
Pal, M., Yesankar, P.J., Dwivedi, A., Qureshi, A., 2020. Biotic control of harmful algal blooms (HABs): a brief review. J. Environ. Manage. 268, 110687. https://doi.org/10.1016/j.jenvman.2020.110687.
Panou, M., Gkelis, S., 2022. Unravelling unknown cyanobacteria diversity linked with HCN production. Mol. Phylogenet. Evol. 166. https://doi.org/10.1016/j.ympev.2021.107322.
Pérez-Carrascal, O.M., Terrat, Y., Giani, A., Fortin, N., Greer, C.W., Tromas, N., Shapiro, B.J., 2019. Coherence of microcystis species revealed through population genomics. ISME J. 13 (12), 2887–2900. https://doi.org/10.1038/s41396-019-0481-1.
Pessi, I.S., Popin, R.V., Durieu, B., Lara, Y., Tytgat, B., Savaglia, V., Roncero-Ramos, B., Hultman, J., Verleyen, E., Vyverman, W., Wilmotte, A., 2023. Novel diversity of polar Cyanobacteria revealed by genome-resolved metagenomics. Microb. Genom. (7), 9. https://doi.org/10.1099/mgen.0.001056.
Petcheneshky, T. (2017). *KOLMAN-2017-ciano.pdf*.
Peter, A.P., Lakshmanan, K., Mohandass, S., Varadharaj, S., Thilagar, S., Abdul Kareem, K.A., Dharmar, P., Gopalakrishnan, S., Lakshmanan, U., 2015. Cyanobacterial KnowledgeBase (CKB), a compendium of cyanobacterial genomes and proteomes. PLoS. One 10 (8), e0136262. https://doi.org/10.1371/journal.pone.0136262.
Pierce, R.H., & Kirkpatrick, G.J. (2001). Innovative techniques for harmful algal toxin analysis. In *Environmental Toxicology and Chemistry* (Vol. 20, Issue 1, pp. 107–114). SETAC Press. 10.1002/etc.5620200110.
Plata-Calzado, C., Prieto, A.I., Cameán, A.M., Jos, A., 2024. Analytical methods for anatoxin-a determination: a review. Toxins. (Basel) 16 (4), 198. https://doi.org/10.3390/TOXINS16040198/S1.
Podduturi, R., Schlüter, L., Liu, T., Osti, J.A.S., Moraes, M., de, A.B., Jørgensen, N.O.G., 2021. Monitoring of saxitoxin production in lakes in Denmark by molecular, chromatographic and microscopic approaches. Harmful Algae 101, 101966. https://doi.org/10.1016/J.HAL.2020.101966.
Pratte, B.S., Thiel, T., 2021. Comparative genomic insights into culturable symbiotic cyanobacteria from the water fern azolla. ***Microb. Genom.*** 7 (6). https://doi.org/10.1099/MGEN.0.000595.
Radkova, M., Stefanova, K., Uzunov, B., Gärtner, G., Stoyneva-Gärtner, M., 2020. Morphological and molecular identification of microcystin-producing cyanobacteria in nine shallow Bulgarian water bodies. Toxins. (Basel) 12 (1), 39. https://doi.org/10.3390/toxins12010039.
Ramya, M., Umamaheswari, A., Elumalai, S., 2020. Global health concern of cyanotoxins in surface water and its various detection methods. ***Current Botany*** 65–74. https://doi.org/10.25081/cb.2020.v11.6059.
Rantala-Ylinen, A., Känä, S., Wang, H., Rouhiainen, L., Wahlsten, M., Rizzi, E., Berg, K., Gugger, M., Sivonen, K., 2011. Anatoxin-a synthetase gene cluster of the cyanobacterium anabaena sp. strain 37 and molecular methods to detect potential producers. Appl. Environ. Microbiol. 77 (20), 7271–7278. https://doi.org/10.1128/AEM.06022-11.
Rasmussen, J.P., Giglio, S., Monis, P.T., Campbell, R.J., Saint, C.P., 2008. Development and field testing of a real-time PCR assay for cylindrospermopsin-producing cyanobacteria. J. Appl. Microbiol. 104 (5), 1503–1515. https://doi.org/10.1111/j.1365-2672.2007.03676.x.
Rhouati, A., Zourob, M., 2024. Development of a multiplexed electrochemical aptasensor for the detection of cyanotoxins. Biosensors 14 (6), 268. https://doi.org/10.3390/bios14060268.
Ribeiro, M.S.F., Tucci, A., Matarazzo, M.P., Viana-Niero, C., Nordi, C.S.F., 2020. Detection of cyanotoxin-producing genes in a eutrophic reservoir (billings reservoir, São Paulo, Brazil). Water. (Basel) 12 (3), 903. https://doi.org/10.3390/w12030903.
Ricciardelli, A., Pollio, A., Costantini, M., Zupo, V., 2023. Harmful and beneficial properties of cyanotoxins: two sides of the same coin. Biotechnol. Adv. 68, 108235. https://doi.org/10.1016/j.biotechadv.2023.108235.
Rippka, E., Deruelles, J., Waterbury, N.B., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. MicrobioZogy 111.
Ruibal, A.L., Rodríguez, M.I., & Angelaccio, C.M. (2005). *Ocurrencia de cyanobacterias y sus toxinas (microcistinas) en aguas del Río de la Plata: evaluación rápida usando el ensayo ELISA*.
Sabart, M., Crenn, K., Perrière, F., Abila, A., Leremboure, M., Colombet, J., Jousse, C., Latour, D., 2015. Co-occurrence of microcystin and anatoxin-a in the freshwater lake Aydat (France): analytical and molecular approaches during a three-year survey. Harmful Algae 48, 12–20. https://doi.org/10.1016/j.hal.2015.06.007.
Saleem, F., Jiang, J.L., Atrache, R., Paschos, A., Edge, T.A., Schellhorn, H.E., 2023. Cyanobacterial algal bloom monitoring: molecular methods and technologies for freshwater ecosystems. Microorganisms. 11 (4), 851. https://doi.org/10.3390/microorganisms11040851.
Salomón, S., Rivera-Rondón, C.A., Zapata, Á.M., 2020. Floraciones de cianobacterias en Colombia: estado del conocimiento y necesidades de investigación ante el cambio global. *Revista de La Academia Colombiana de Ciencias Exactas*. Físicas y Naturales 44 (171), 376–391. https://doi.org/10.18257/RACCEFYN.1050.
Sanseverino, I., Conduto António, D., Loos, R., & Lettieri, T. (2017). *Cyanotoxins: methods and approaches for their analysis and detection*. 10.2760/36186.
Savela, H., Spoof, L., Perälä, N., Preede, M., Lamminmäki, U., Nybom, S., Häggqvist, K., Meriluoto, J., Vehniäinen, M., 2015. Detection of cyanobacterial sxt genes and paralytic shellfish toxins in freshwater lakes and brackish waters on Åland Islands, Finland. Harmful Algae 46, 1–10. https://doi.org/10.1016/j.hal.2015.04.005.
Schantz, E.J., Mold, J.D., Stanger, D.W., Shavel, J., Riel, F.J., Bowden, J.P., Lynch, J.M., Wyler, R.S., Riegel, B., Sommer, H., 1957. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. J. Am. Chem. Soc. 79 (19), 5230–5235. https://doi.org/10.1021/JA01576A044.
Schembri, M.A., Neilan, B.A., Saint, C.P., 2001. Identification of genes implicated in toxin production in the cyanobacterium Cylindrospermopsis raciborskii. Environ. Toxicol. 16 (5), 413–421. https://doi.org/10.1002/tox.1051.
Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 30 (14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
Shestakov, S.V., Karbysheva, E.A., 2017. The origin and evolution of cyanobacteria. Biol. Bull. Rev. 7 (4), 259–272. https://doi.org/10.1134/s2079086417040090.
Shiraishi, H., 2015. Association of heterotrophic bacteria with aggregated Arthrospira platensis exopolysaccharides: implications in the induction of axenic cultures. Biosci. Biotechnol. Biochem. 79 (2), 331–341. https://doi.org/10.1080/09168451.2014.972333.
Shishido, T.K., Jokela, J., Kolehmainen, C.T., Fewer, D.P., Wahlsten, M., Wang, H., Rouhiainen, L., Rizzi, E., De Bellis, G., Permic, P., Sivonena, K., 2015. Antifungal activity improved by coproduction of cyclodextrins and anabaenolysins in Cyanobacteria. Proc. Natl. Acad. Sci. U. S. A. 112 (44), 13669–13674. https://doi.org/10.1073/PNAS.1510432112/SUPPL_FILE/PNAS.1510432112.SAPP.PDF.
Sini, P., Dang, T.B.C., Fais, M., Galioto, M., Padedda, B.M., Lugliè, A., Iaccarino, C., & Crosio, C. (2021). Cyanobacteria, cyanotoxins, and neurodegenerative diseases:

Dangerous liaisons. In *International Journal of Molecular Sciences* (Vol. 22, Issue 16). MDPI. 10.3390/ijms22168726.

Sivonen, K. (2008). Emerging high throughput analyses of cyanobacterial toxins and toxic cyanobacteria. In ***Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*** (pp. 539–557). Springer New York. 10.1007/978-0-387-75865-7_24.

Soares, D.M.M., Atum, S.V.F., Bechara, E.J.H., & Setubal, J.C. (2021). *Exploring the microbiota of the guarapiranga water reservoir with long-read sequencing technology*. 8 (December), 1–4. 10.3389/fmars.2021.791101.

Solé, A., Diestra, E., Esteve, I., 2009. Confocal laser scanning microscopy image analysis for cyanobacterial biomass determined at microscale level in different microbial mats. Microb. Ecol. 57 (4), 649–656. https://doi.org/10.1007/S00248-008-9463-Y/FIGURES/4.

Sonmez, M.E., Altinsoy, B., Ozturk, B.Y., Gumus, N.E., Eczacioglu, N., 2023. Deep learning-based classification of microalgae using light and scanning electron microscopy images. Micron. 172. https://doi.org/10.1016/j.micron.2023.103506.

Sound, J.K., Peters, A., Bellamy-Carter, J., Rad-Menéndez, C., MacKechnie, K., Green, D. H., Leney, A.C., 2021. Rapid cyanobacteria species identification with high sensitivity using native mass spectrometry. Anal. Chem. 93 (42), 14293–14299. https://doi.org/10.1021/acs.analchem.1c03412.

Steffen, M.M., Li, Z., Effler, T.C., Hauser, L.J., Boyer, G.L., Wilhelm, S.W., 2012. Comparative metagenomics of toxic freshwater cyanobacteria bloom communities on two continents. PLoS. One 7 (8). https://doi.org/10.1371/journal.pone.0044002.

Šulčius, S., Slavuckytė, K., Januškaitė, M., Paškauskas, R., 2017. Establishment of axenic cultures from cyanobacterium Aphanizomenon flos-aquae akinetes by micromanipulation and chemical treatment. Algal. Res. 23, 43–50. https://doi.org/10.1016/j.algal.2017.01.006.

Sundaravadivelu, D., Sanan, T.T., Venkatapathy, R., Mash, H., Tettenhorst, D., Danglada, L., Frey, S., Tatters, A.O., & Lazorchak, J. (2022). Determination of cyanotoxins and prymnesins in water, fish tissue, and other matrices: a review. In ***Toxins*** (Vol. 14, Issue 3). MDPI. 10.3390/toxins14030213.

Tillett, D., Dittmann, E., Erhard, M., Von Döhren, H., Börner, T., Neilan, B.A., 2000. Structural organization of microcystin biosynthesis in Microcystis aeruginosa PCC7806: an integrated peptide–polyketide synthetase system. Chem. Biol. 7 (10), 753–764. https://doi.org/10.1016/S1074-5521(00)00021-1.

Tillett, D., Parker, D.L., Neilan, B.A., 2001. Detection of toxigenicity by a probe for the microcystin synthetase a gene (mcyA) of the Cyanobacterial Genus Microcystis: Comparison of Toxicities with 16S rRNA and phycocyanin operon (phycocyanin intergenic spacer) phylogenies. Appl. Environ. Microbiol. 67 (6), 2810–2818. https://doi.org/10.1128/AEM.67.6.2810-2818.2001.

Vaitomaa, J., Rantala, A., Halinen, K., Rouhiainen, L., Tallberg, P., Mokelke, L., Sivonen, K., 2003. Quantitative real-time PCR for determination of microcystin synthetase E copy numbers for microcystis and anabaena in lakes. Appl. Environ. Microbiol. 69 (12), 7289–7297. https://doi.org/10.1128/AEM.69.12.7289-7297.2003.

Valério, E., Chambel, L., Paulino, S., Faria, N., Pereira, P., Tenreiro, R., 2009. Molecular identification, typing and traceability of cyanobacteria from freshwater reservoirs. Microbiology (N. Y) 155 (2), 642–656. https://doi.org/10.1099/mic.0.022848-0.

Van De Meene, A.M.L., Hohmann-Marriott, M.F., Vermaas, W.F.J., Roberson, R.W., 2006. The three-dimensional structure of the cyanobacterium Synechocystis sp. PCC 6803. Arch. Microbiol. 184 (5), 259–270. https://doi.org/10.1007/S00203-005-0027-Y/FIGURES/6.

Vaughan, L., Zamyadi, A., Ajjampur, S., Almutaram, H., Freguia, S., 2022. A review of microscopic cell imaging and neural network recognition for synergistic cyanobacteria identification and enumeration. Anal. Sci. 38 (2), 261–279. https://doi.org/10.1007/S44211-021-00013-2/TABLES/3.

Vázquez-Martínez, G., Rodriguez, M.H., Hernández-Hernández, F., Ibarra, J.E., 2004. Strategy to obtain axenic cultures from field-collected samples of the cyanobacterium Phormidium animalis. J. Microbiol. Methods 57 (1), 115–121. https://doi.org/10.1016/J.MIMET.2003.12.003.

Vogiazi, V., De La Cruz, A., Mishra, S., Shanov, V., Heineman, W.R., Dionysiou, D.D., 2019. A comprehensive review: development of electrochemical biosensors for detection of cyanotoxins in freshwater. ACS. Sens. 4 (5), 1151–1173. https://doi.org/10.1021/acssensors.9b00376.

Vu, C.H.T., Lee, H.G., Chang, Y.K., & Oh, H.M. (2018). Axenic cultures for microalgal biotechnology: establishment, assessment, maintenance, and applications. In *Biotechnology Advances* (Vol. 36, Issue 2, pp. 380–396). Elsevier Inc. 10.1016/j.biotechadv.2017.12.018.

Wang, H., Xu, C., Liu, Y., Jeppesen, E., Svenning, J.C., Wu, J., Zhang, W., Zhou, T., Wang, P., Nangombe, S., Ma, J., Duan, H., Fang, J., Xie, P., 2021. From unusual suspect to serial killer: cyanotoxins boosted by climate change may jeopardize megafauna. Innovation 2 (2). https://doi.org/10.1016/j.xinn.2021.100092.

Wang, S., Zhu, L., Li, Q., Li, G., Li, L., Song, L., Gan, N., 2015. Distribution and population dynamics of potential anatoxin-a-producing cyanobacteria in Lake Dianchi. China. Harmful Algae 48, 63–68. https://doi.org/10.1016/j.hal.2015.07.005.

Weralupitiya, C., Wanigatunge, R.P., Gunawardana, D., Vithanage, M., Magana-Arachchi, D., 2022. Cyanotoxins uptake and accumulation in crops: phytotoxicity and implications on human health. Toxicon. 211, 21–35.

WHO. (2020). *Cyanobacterial toxins: anatoxin-a and analogues background document for development of*. http://apps.who.int/bookorders.

Wilmotte, A., Dail Laughinghouse, H.I., Capelli, C., Rippka, R., & Salmaso, N. (2017). *Taxonomic identification of cyanobacteria by a polyphasic approach*.

Wiltsie, D., Schnetzer, A., Green, J., Borgh, M.Vander, Fensin, E, 2018. Algal blooms and cyanotoxins in Jordan Lake. Toxins. (Basel) (2), 10. https://doi.org/10.3390/toxins10020092.

Winey, M., Meehl, J.B., O'Toole, E.T., Giddings, T.H., 2014. Conventional transmission electron microscopy. Mol. Biol. Cell 25 (3), 319–323. https://doi.org/10.1091/MBC.E12-12-0863/ASSET/IMAGES/LARGE/319FIG2.JPEG.

Yamaguchi, H., Suzuki, S., Osana, Y., Kawachi, M., 2019. Genomic characteristics of the toxic bloom-forming cyanobacterium microcystis aeruginosa NIES-102. J. Genomics. 8, 1–6. https://doi.org/10.7150/jgen.40978.

Yang, Y., Yu, G., Chen, Y., Jia, N., Li, R., 2021. Four decades of progress in cylindrospermopsin research: the ins and outs of a potent cyanotoxin. J. Hazard. Mater. 406, 124653. https://doi.org/10.1016/J.JHAZMAT.2020.124653.

Zhang, J., Li, C., Rahaman, M.M., Yao, Y., Ma, P., Zhang, J., Zhao, X., Jiang, T., Grzegorzek, M., 2022. A comprehensive review of image analysis methods for microorganism counting: from classical image processing to deep learning approaches. Artif. Intell. Rev. 55 (4), 2875–2944. https://doi.org/10.1007/s10462-021-10082-4.

Zhang, W., Lou, I., Ung, W.K., Kong, Y., Mok, K.M., 2014. Analysis of cylindrospermopsin- and microcystin-producing genotypes and cyanotoxin concentrations in the Macau storage reservoir. Hydrobiologia 741 (1), 51–68. https://doi.org/10.1007/s10750-013-1776-2.

Zhang, Y., Whalen, J.K., Cai, C., Shan, K., Zhou, H., 2023. Harmful cyanobacteria-diatom/dinoflagellate blooms and their cyanotoxins in freshwaters: a nonnegligible chronic health and ecological hazard. Water. Res. 233, 119807. https://doi.org/10.1016/j.watres.2023.119807.

Zhou, W.G., Ding, D.W., Yang, Q.S., Ahmad, M., Zhang, Y.Z., Lin, X.C., Zhang, Y.Y., Ling, J., Dong, J.De, 2018. Marileptolyngbya sina gen. Nov., sp. nov. and Salileptolyngbya diazotrophicum gen. nov., sp. nov. (Synechococcales, Cyanobacteria), species of cyanobacteria isolated from a marine ecosystem. Phytotaxa 383 (1), 75–92. https://doi.org/10.11646/phytotaxa.383.1.4.

Zupančič, M., Kogovšek, P., Šter, T., Remec Rekar, Š., Cerasino, L., Baebler, Š., Krivograd Klemenčič, A., Eleršek, T., 2021. Potentially toxic planktic and benthic cyanobacteria in slovenian freshwater bodies: detection by quantitative PCR. Toxins. (Basel) (2), 13. https://doi.org/10.3390/TOXINS13020133.