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First report of potentially microcystin-producing Microcystis in the Dominican Republic

Alfaniris Vargas Fernandez [a,1,*], Patri Ramírez Ramirez [a,1], Iris García Cruz [b], Claudia Reyes Gutierrez [c], Adrián Gutiérrez Cepeda [a], Alexander Valdez Disla [b], Roel Alejandro Chávez Luzania [d], Sergio de los Santos Villalobos [d,1,**], Pedro Leao [e], Vitor Vasconcelos [e,f]

[a] Research Group on Natural and Marine Products. Faculty of Sciences and Faculty of Health Sciences. Autonómous University of Santo Domingo (UASD), Dominican Republic
[b] Research Group on Natural and Marine Products. Faculty of Health Sciences. Autonomous University of Santo Domingo (UASD), Dominican Republic
[c] Faculty of Health Sciences. Technological University of Santiago (UTESA). Santo Domingo (UASD), Dominican Republic
[d] Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Col. Centro, Ciudad Obregón, Sonora, Mexico
[e] Interdisciplinary Center for Marine and Environmental Research. (CIIMAR/CIMAR-LA), University of Porto, Portugal
[f] Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007, Porto, Portugal

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SUMMARY

When the amount of nutrients in water bodies increases, cyanobacteria tend to proliferate rapidly in freshwater lakes and reservoirs, which can trigger cyanobacterial blooms. This increases the risk of cyanotoxin generation in water sources intended for human consumption, crop irrigation, and livestock. This study focused on identifying the presence of cyanobacteria and cyanotoxins in the Valdesia reservoir, which supplies drinking water to approximately 4 million people in Santo Domingo, Azua, San Cristóbal, San José de Ocoa, and Peravia in the Dominican Republic. Morphological observation suggested the presence of the genus *Microcystis*, which was confirmed by amplification and sequencing of two fragments of the 16S rRNA gene, as well as a fragment of the *mcyA* gene involved in encoding microcystins. This is the first report to highlight the urgent need to establish continuous monitoring of potentially microcystins-producing *Microcystis* sp. in this important reservoir, to implement appropriate water management measures to prevent their negative impact on public health and the environment.

Introduction

When there is an increase in nutrients (eutrophication), cyanobacteria develop and spread rapidly in freshwater lakes and reservoirs, which can lead to cyanobacterial blooms, commonly known as blue-green algae blooms (Rodriguez et al., 2024). If a bloom becomes too dense, causing various water quality problems, such as releasing toxins, reducing the amount of oxygen in the water, or releasing toxic gases, it is hazardous because it degrades water quality and poses risks to human and environmental health. Cyanotoxins can be classified as neurotoxins (affect the nervous system), hepatotoxins (affect the liver), cytotoxins (affect diverse organs), and dermatotoxins (affect the skin) (Corbet et al., 2014) and when consumed by animals or humans, they can result in the death or severe disease (Wood, 2016; Ruiba-Contil, 2019).

Thus, cyanobacterial blooms need to be monitored to control toxin-producing cyanobacteria, as well as to address cases of poisoning caused by their toxins. (Huisman et al., 2018; Rocha et al., 2024). It is crucial to measure and manage toxins produced by cyanobacteria, as they remain the main source of natural toxins present in surface water supplies. Acute hepatotoxicity, associated with hepatotoxins, is the most common form of cyanobacteria-related poisoning, with microcystins and nodularins being the primary toxins produced by various strains and species

* Corresponding authors at: Instituto de Investigaciones en Salud (INSIS), Facultad de Ciencias de la Salud, Universidad Autónoma de Santo Domingo (UASD), Santo Domingo. República Dominicana, 7mo. Piso, ala sur, Edificio Administrativo, Zona Universitaria.
** Corresponding authors at: Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Col. Centro, Ciudad Obregón, Sonora, México.
E-mail addresses: alfaniris.vargas@gmail.com (A. Vargas Fernandez), sergio.delossantos@itson.edu.mx (S. de los Santos Villalobos).
[1] These authors have contributed equally to this work.

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of genera such as *Microcystis, Dolichospermum, Hapalosiphon, Nodularia, Nostoc,* and *Oscillatoria* (Lalić, 2024). In this sense, identifying potentially toxin-producing cyanobacteria is crucial to designing mitigation strategies through fast, easy, and economical approaches such as amplification and sequencing of specific DNA fragments related to their taxonomical identification and potential toxicity. In the case of the Dominican Republic, although there are isolated species *(Limnothrix* sp., *Symploca* sp., *Leptolyngbya* sp., *Nostoc* sp., *Scytonema* sp., *Calothrix* sp., *Phormidium* sp., *Nodosilinea* sp. and *Hapalosiphon* sp.) (Vargas et al., 2021), their potential toxicity has not been reported (Vargas et al., 2023).

The Nizao basin has four dams (Jiguey, Aguacate, Valdesia, and the Las Barías reservoir). The Valdesia dam is one of the sources of drinking water supply for the country, representing approximately 40 % of the needs of the metropolitan aqueduct of Santo Domingo (6.25 m3 /s) and supplies 50 % of potable water to Santo Domingo, Azua, San Cristóbal, San José de Ocoa y Peravia, and also supplies agricultural water to approximately 30,000 hectares in Baní, San Cristóbal, and Western Cibao (Ramírez and Farias, 2003; Rubio and Ortega, 2017). It is estimated that around 4 million people receive this resource (CAASD, 2017). This hydroelectric generator has a tremendous impact on the Dominican Republic because it produces energy, serves as irrigation for agriculture, and is used for fishing, and food consumption. Recently, in the Dominican Republic, multiple cases of mass fish deaths have been reported in several locations in the country, including Puerto Plata, Azua, and in particular, in the Hatillo and Valdesia reservoirs (MMARN-Rep. Dom., 2024). However, the species of cyanobacteria present in these water bodies and their toxicity potential have not been determined, due to inadequate surveillance and low detectability. This has resulted in a lack of effort in monitoring cyanotoxin poisoning. Therefore, adequate reporting would allow a full understanding of the mechanisms that cause cyanobacterial poisoning incidents in this and other reservoirs, allowing the development of effective surveillance systems (De Jesús, 2022). This study aimed to identify the presence of potentially toxin-producing cyanobacteria in the Valdesia reservoir in the Dominican Republic, by amplifying and sequencing specific DNA fragments for their identification, taxonomy, and potential toxicity.

Material and methods

Sample collection

On March 8, 2024, two samples were collected from water bodies with previous reports of algal blooms in the Valdesia reservoir (Fig. 1). 1200 ml of water were collected from each sampling point (Point 1: Floating platform, side of the dam where the boat is taken_18.393804, −70.282033) (Point 2: Next to the dam's compounds, opposite to point 1_18.392684, −70.278607), and filtered using 0.45 μm filters. Then, a homogenized aliquot of samples was fixed using Phenol to prevent alterations in the natural composition of cyanobacterial communities. These fixed samples were used for microscopy visualization and high-quality DNA extraction.

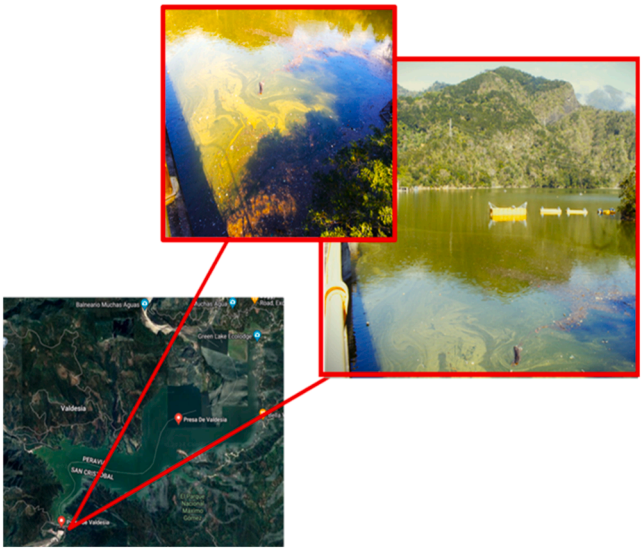

Fig. 1. Cyanobacteria blooms in the Valdesia reservoir, Dominican Republic.

Morphological identification

An inverted trinocular microscope (MOTIC AE 2000) equipped with an image acquisition system (MOTICAMProS5 Plus) was used to visualize cyanobacteria in the study site, using 50x and 1000x magnification. Identification followed the key for the morphological characterization of *Microcystis* species developed by Komárek and Anagnostidis (1998, 2005), Anagnostidis and Komárek (1985, 1988), and Komárek and Komárková-Legnerová (2002, 2007). Subsequently, *Microcystis* colony counts were performed using the Neubauer chamber, and the results were expressed in colonies/mL.

Genetic identification

High-quality DNA extraction from fresh environmental samples was carried out according to the protocol described by Raeder and Broda in 1985. The integrity and quality of the extracted DNA were assessed using 1 % agarose gel electrophoresis at 100 V for 60 min and a NanoDrop 2000C (Thermo Fisher Scientific), respectively. Then, the extracted DNA was used as a template in PCR reactions, for identifying the presence of cyanobacteria by using i) primers to amplify the 16S rRNA gene from cyanobacteria [CYAN738F: ATACCCCWGTAGTCCTAGC and CYAN1281R: GCAATTACTAGCGATTCCTCC, Ta; 57° C (Valério et al., 2009)], and ii) primers to amplify the 16S rRNA gene from *Microcystis* [MICR184F; GCCGCRAGGTGAAAMCTAA and MICR431R: AATCCAAARACCTTCCTCCC, Ta; 54° C (Martins and Vasconcelos, 2011)], as well as primers to amplify the *mcyA* associated with microcystin biosynthesis [mcyA-Cd-1F: AAAATTAAAAGCCGTATCAAA and mcyA-Cd-1R; AAAAGTGTTTTATTAGCGGCTCAT, Ta; 57° C (Sabart et al., 2015)] (Chávez-Luzanía et al., 2024).

The PCR reaction was carried out using the PCR Master Mix 2X PCR kit (Promega, M7502), using a total volume of 25 μL, which included the following components: 12.5 μL of PCR Master Mix 2X, 9.5 μL of nuclease-free water, 1 μL of forward primer and 1 μL of reverse primer at a concentration of 10 mM, and 1 μL of template DNA at a concentration of 30 ng/μL. The reactions were carried out in a T100™ Thermal Cycler, BIO-RAD under the following conditions: the process was started with an initial denaturation at 94 °C for 180 s; subsequently, 30 cycles were carried out, which included a denaturation at 94 °C for 30 s, an annealing phase with temperatures adjusted according to the optimization described previously, for 40 s, and an elongation at 72 °C for 30 s. After 30 cycles, the process was completed with a final elongation at 72 °C for 300 s.

Visualization of PCR products was performed using 1.5 % agarose gel electrophoresis at 100 V for 60 min. The amplicons obtained were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega) and subsequently sequenced by Sanger technology. The raw sequences were visualized using FinchTV V1.4.0 to remove low-quality regions based on chromatogram inspection. High-quality sequences were aligned in CLC Sequence Viewer 8 to construct a phylogenetic tree using the Neighbor-Joining construction method with a Jukes-Cantor nucleotide distance model with 1000 bootstrap replicates and *Escherichia coli* AN-ASH-9 as an outgroup. Taxonomic affiliation was performed using the high-quality sequences in the NCBI BLAST® tool (http://blast.ncbi.nlm.nih.gov/), considering query cover and similarity as selection criteria.

Results

Morphological identification

Based on morphology, the observed cyanobacteria showed colonies clathrate or not, and mucilage broad; in addition, cells concentrated in the center of the colonies, which are traits associated with *Microcystis aeruginosa* (Fig. 2). Besides, irregular slime colonies of spherical cells were observed, as well as cells irregularly distributed within the colony and colorless and fine mucilage, which is delimiting and not very dense. Cells are 4–7 μm in diameter (McGregor et al., 2007). Different cell abundances were found in the two samples from this bloom event. *Microcystis* colony counts at sampling point 1 were 3.15×10^5 colonies/mL, and at sampling point 2, the count was 1.20×10^5 colonies/mL. This genus *Microcystis,* is a cyanobacterium found in eutrophic freshwater bodies, and dominates phytoplankton due to its buoyancy regulation ability. Detecting this genus and its toxin, microcystin, is crucial in the study region, as drinking water reservoirs nearby present a persistent risk due to the toxin's stability even after conventional treatments (Esqueda et al., 2016). This allows us to assess ecological and human health risks associated with the presence of the toxin in treated drinking water. Global reports of algal blooms have been recorded across several continental regions (Vasconcelos, 2006; Harke et al., 2016; Sakamoto et al., 2021; Aguilera et al., 2023). Additionally, neighboring countries in different regions, such as Cuba (Tito et al., 2022; Valle et al., 2021), Venezuela (González et al., 2012; Marín et al., 2023), and Mexico (Esqueda et al., 2016).

Identification of the species detected in the Valdesia reservoir, Dominican Republic

Genetic identification

High-quality DNA was extracted as indicated by the electrophoresis (a band of intact genomic DNA), obtaining a concentration of 43.9 ng/μL and quality ratios of 1.61 (260/280) and 1.95 (260/230). These results indicate a suitable DNA concentration and purity for subsequent PCR reactions (Kadri, 2020; Lucena-Aguilar et al., 2016). The obtained high-quality DNA was used for the amplification of cyanobacteria-specific and *Microcystis*-specific 16S rRNA regions, as well as the *mcyA* gene (Fig. 3), where amplicons at the expected band sizes ~550 bp (Valério et al., 2009), ~220 bp (Martins and Vasconcelos, 2011), and ~290 bp (Sabart et al., 2015) were observed, respectively (Chávez-Luzanía, et al., 2024).

After Sanger sequencing of the fragment amplified with cyanobacteria-specific primers (accession number: PQ475782), the obtained nucleotide sequences were used to construct a phylogenetic tree shown in Fig. 4, and BLAST® searches were carried out. The phylogenetic tree grouped the studied sample in the same clade of *Microcystis aeruginosa*, which was confirmed with the BLAST results indicating that the obtained sequences had a query cover of 98 %, and a similarity of 99.23 % to *Microcystis aeruginosa* NIES-3807 (accession number: LC455674.1). The taxonomic affiliation of these microorganisms can be achieved by analyzing the variability of the 16S rRNA regions, which have conserved and hypervariable regions where there are regions that are conserved among groups of organisms by which phylogenetic closeness can be differentiated and a taxon can be assigned (Shahi et al., 2017; Valério et al., 2009). However, a polyphasic approach is necessary to perform this task accurately (Morales-Sandoval et al., 2021).

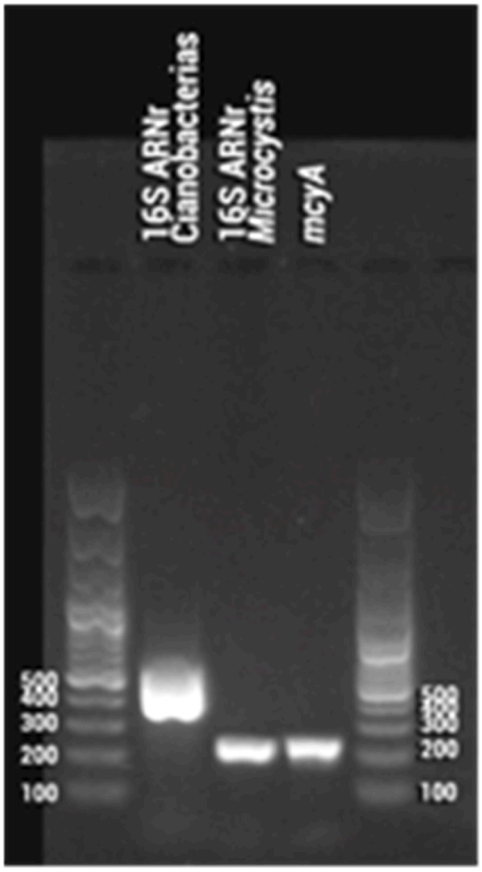

Fig. 3. Amplification of cyanobacteria-specific and *Microcystis*-specific 16S rRNA regions, as well as the *mcyA* gene from the studied sample.

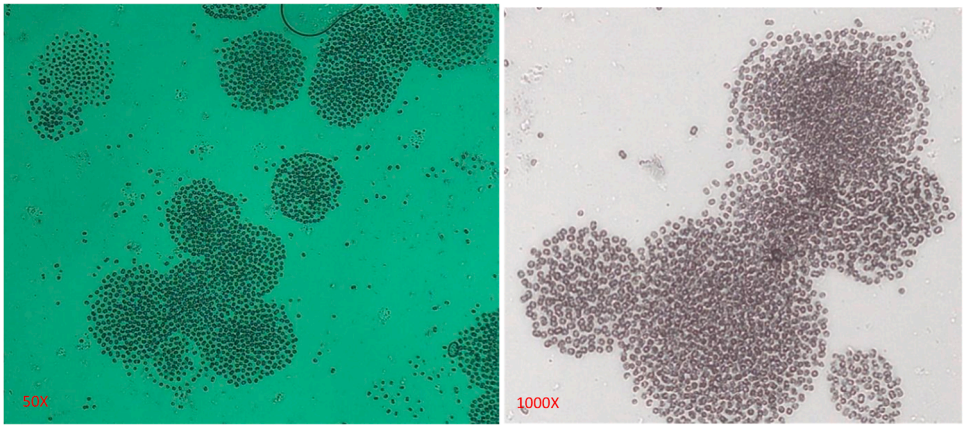

Fig. 2. Photomicrographs of the cyanobacteria detected from the Valdesia reservoir, Dominican Republic.

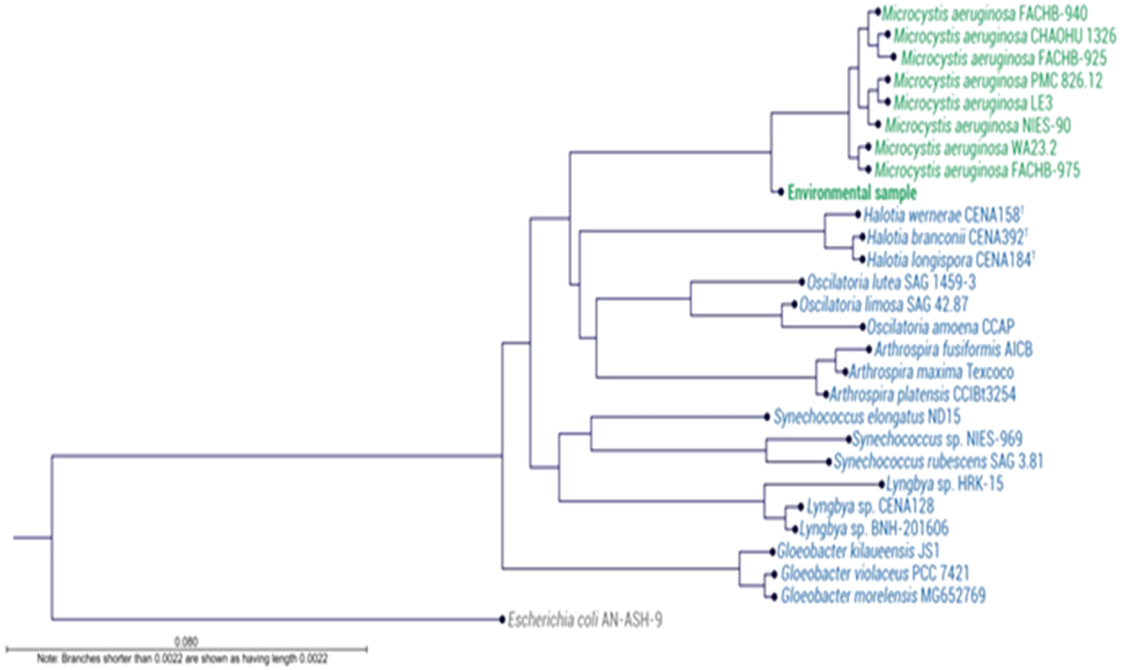

Fig. 4. The relationship of the cyanobacteria detected in the environmental sample using the sequence of the amplicon produced by the 16S rRNA cyanobacteria-specific primers, based on the 16S rRNA genes of the most related cyanobacterial species. The presence of the m*cyA* gene indicates the potential for the production of the toxin microcystin, but confirmation of its expression is needed using HPLC or ELISA.

Thus, the morphological assessment, 16S rRNA amplification using cyanobacteria-specific primers, phylogenetic tree, and Sanger sequencing suggest that *Microcystis aeruginosa* is one of the most abundant species colonizing the Valdesia reservoir in the Dominican Republic. This was further strengthened by sequencing of the amplicon produced by the 16S rRNA *Microcystis*-specific primers, indicating 99 % coverage and 100 % similarity to *Microcystis aeruginosa* LMECYA 59 (accession number: EU078492.1), due to the conserved sequences in the V2-V3 hypervariable regions of 16S rRNA conserved among the genus *Microcystis*, allowing its specific amplification (Henderson et al., 2019; Neilan et al., 1997; Shahi et al., 2017).

In this sense, the potential of this species to biosynthesize microcystin was determined by using DNA amplification and Sanger sequencing of the *mcyA* gene. The results indicated the presence of the *mcyA* gene in the environmental sample, whose sequence showed 100 % coverage and 99.61 % similarity with a microcystin synthetase from *Microcystis aeruginosa* FACHB-1174 (accession number: OQ291090.1). The detection of the *mcyA* gene suggests a potential risk of microcystin production, as described in previous studies where a positive correlation is demonstrated between the detection of the mcyA gene with the concentration of microcystins in environmental water samples associated with the presence of *Microcystis*, which was evaluated by the study of the 16S rRNA gene (Singh et al., 2015; Dong et al., 2016; Hu et al., 2016), therefore, microcystin production could exist in the blooms in the Valdesia reservoir.

Conclusions

Based on morphology and genetic analysis, we can conclude that *Microcystis aeruginosa* is one of the most dominant cyanobacterial species present in the Valdesia reservoir in the Dominican Republic, which has the potential to biosynthesize microcystin. This indicates that there is a preponderant need to detect and quantify microcystins in the reservoir in the future and that it is of vital importance to establish a monitoring plan.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Given his/her/their role as Editor, Sergio de los Santos Villalobos had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to David Ojcius.

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Data availability

Data will be made available on request.

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