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Newly isolated strains of potentially microcystin-producing cyanobacteria in potable water: case study of Mawoni village, South Africa

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Toxic cyanobacterial species occur in aquatic ecosystems when favourable environmental conditions prevail. These bacteria can produce natural hepatotoxic metabolites called microcystins that can affect the quality of water. Human exposure to microcystins results from ingesting contaminated drinking water and therefore cyanobacterial species producing these toxins should be monitored in these waters. The present study aimed to trace and identify cyanobacterial strains that potentially produce microcystins in drinking water. To achieve this objective, advanced digital flow cytometry and polymerized chain reaction were used for the detection and identification of cyanobacterial strains in water samples collected from water storage containers in Mawoni village. Full-length 16S rRNA genes from cultured cyanobacteria were amplified and sequenced using the 16S primers. Three novel strains of *Chroococcus* sp. (m64187e-7881, m64187e-2143, and m64187e-0930) and two strains of *Microcystis aeruginosa* (m64187e-6729 and m64187e-1069) were detected and identified in drinking water samples. The presence of these strains could indicate the potential of microcystins occurrence in drinking water, which therefore, could present potential human health risk due to exposure to such cyanotoxins.

KEYWORDS

cyanotoxins, drinking water, human health, molecular identification, microcystins

1 Introduction

The cyanobacteria phylum of the photosynthetic prokaryotes has been in existence for over 3.5 billion years and can be found in various habitats including freshwater bodies (Chia et al., 2022; Wang et al., 2024). They are considered the oldest organisms in the evolutionary process which are capable of performing oxygenic photosynthesis and are known to oxygenate the atmosphere of the earth (Balasooriya, 2019). Favourable physicochemical

conditions support the growth of these phyla (Chia et al., 2022). According to Chia et al. (2022); Melaram et al. (2022), factors influenced by global warming, such as eutrophication, high temperatures and increased solar irradiation can influence the excessive growth of harmful cyanobacteria in aquatic systems. Amongst the cyanobacteria species are those that produce and release a copious number of metabolites called cyanotoxin-microcystin. Currently, cyanobacteria have more than 60 species that are known as microcystin producers worldwide (Usman et al., 2022), and some of these species include *Microcystis* and *Chroococcus* sp. (Magonono et al., 2018; Chen et al., 2021; Mirasbekoc et al., 2021; Valadez-Cano et al., 2022; Thawabteh et al., 2023). Apart from being the water quality problem such as affecting the aquatic ecosystem and aquatic animals, the occurrence of cyanobacterial species and their toxins can also pose severe health threats to humans and animals (Chen et al., 2021; Jia et al., 2024). These toxins have been reported to comprise potent neurotoxic, hepatotoxic, dermatoxic and cytotoxic agents that they produce (Nowruzi et al., 2023) as well as causing gastro-intestinal toxicity (Li et al., 2024). Many studies have been focused on microcystins globally, due to their extensive occurrence, steadiness and resilience to chemical and biological breakdown, as well as their ability to reach high concentrations in scums and blooms (Dai et al., 2016; Welten et al., 2020).

According to Carmichael et al. (2013), microcystins are clyclic heptapeptides tha consist five common amino acids and pairs of L-amino acids as variants; with the most known acids being methyl aspartic acid, N-methyldehydroalanine, alanine, glutamic acid, and Adda (3-amino-9-methoxy-2,6,8-trimethyl-10- phenyldeca-4,6 dienoic acid) (Pearson et al., 2010). It was further reported that microcystins fall within a group of small molecular weight causing human and animal poisoning that involves acute hepatotoxicosis (Sarma, 2013). Previous studies have reported that most human health problems are linked with continuous low microcystin concentrations exposure (Drobac et al., 2013), of which one of the common exposure routes is through drinking microcystins-contaminated water (Mutoti et al., 2022; Melaram et al., 2022). The health threats caused by microcystins have led the World Health Organization (WHO) to set the standards for drinking water quality for these toxins; for example, a guideline value for total MCs is 1 μg/L in drinking water (Farrer et al., 2015; WHO, 2017). A liver is one of the variety of organs that can be targeted by microcystins. Acute exposure to these toxins can result in headaches, eye pain, blurred vision, nausea, and vomiting (Lad et al., 2022; Lim et al., 2023). With many studies focused on the cyanotoxins impact on animal health, there is limited information on the human health impact of cyanotoxins. Therefore, the human health effects of microcystins are still not well understood in some cases, mostly chronic health impacts (O'Keeffe, 2019). Potential associations have been identified by various studies between liver disease and microcystin exposure. The tissue of dialysis patients in Brazil dialysis centre were found to be accumulated by microcystins, causing death to many patients due to liver failure (O'Keeffe, 2019). Similarly, correlation was observed between freshwater microcystins-producing cyanobacteria with incident of liver cancer and mortalities in central Serbia (Melaran et al., 2022).

Most drinking water authorities have been facing the challenges of treating water contaminated with cyanobacteria. Although a guideline document to assist portable water producers in dealing with toxic cyanobacteria in water sources has been developed (WHO, 2017), Mutoti et al. (2022) reported that there is still a lack of personnel who are skilled enough to develop protocols and programmes to monitor cyanobacteria toxins, consequently becoming the key challenge faced by water treatment works (DWTW) in Southern Africa.

Currently, numerous analytical methods have been used for the assessment of microbiological characteristics of the water samples globally. A polyphasic approach has been utilized as a system of cyanobacterial species description that combines 16S rRNA, morphology, phycology, and ecology in trying to acknowledge the need to incorporate phylogenetics into cyanobacterial taxonomy (Willis and Woodhouse, 2020). This approach has been supported by taxonomists such as Komárek et al. (2014) and Komárek (2020) who pointed out that the polyphasic approach provides the best means of conducting taxonomic practice. Reviews have been conducted on DNA sequencing that is used for the phylogenetic and taxonomic analysis of cyanobacterial isolates (Mutoti et al., 2022). Many studies have utilized these approaches for the classification and identification of cyanobacteria species in drinking water (Mutoti et al., 2022; Valade-Cano et al., 2022). Moreover, FlowCAM has been used that capture images of cyanobacteria that are then identified based on their morphological parameters (area, length, width, equivalent spherical diameter, and area-based diameter) and shape; whereas, the PCR uses 16S rRNA gene sequencing to identify cyanobacterial to their species level (Wilson et al., 2000). Both FlowCAM and PCR techniques are taxonomic methods that enable the identification of cyanobacterial species in natural habitats (Lee et al., 2017) based on morphological characteristics and genomic information (Willis and Woodhouse, 2020). Komárek et al. (2014) reported that there is very limited data for most genera such as *Chroococcus* within the Chroococcaceae family; whereas, the well-known genus *Microcystis* is constantly monophyletic, but the species level classification within the genus can be controversial. This therefore triggers studies of this nature to try and fill up this vital data gap.

Containers (20–25 L) are used in rural areas such as Mawoni for collection and storing water for future use. The presence of pathogenic bacteria, including cyanobacteria in this water has been assumed. There has been some observation of cyanobacteria development inside these containers forming biofilm, which potentially pose threats to the households that use such water. Studies on the occurrence of cyanobacteria blooms have been seldom conducted in African aquatic resources (Chia et al., 2022). In Mawoni village and the surrounding areas located in the North part of South Africa in Limpopo, nothing or limited information has been reported regarding the underlying genetic characterization, morphology, and geographical origin among the strains of cyanobacteria. This gap in knowledge limits our understanding of cyanobacteria diversity, distribution, toxin production, and the nature of their congeners, highlighting the risks of exposure through potable water resource contamination. Therefore, to fill-up this gap, the present study is the first of its kind to assess the presence of cyanobacteria strains that potentially produce and release microcystins in drinking water collected from water storage containers in Mawoni village. This

information is fundamental to the water supply authorities that require critical control and management approaches to prevent health problems that are associated with these bioactive metabolites.

2 Materials and methods

2.1 Cyanobacteria culture and strains and microscopic analysis

In the present study, sixteen water samples (four batch of four samples: n = 16) were collected using Latex Free, Polypropylene Medline Basic Specimen containers with screw lids (manufactured by Medline, US). Samples were collected from water containers that contain green seeded biomass on their side walls and are used to store drinking water in Mawoni village. Sampling was conducted ones during summer season. During samples collection, physicochemical parameters were measured *insitu* using the Accsen PC 70 multimeter (manufactured by Accsen Instrumental in Italy) and the Turbicheck WL portable turbidity meter (TB 250 WL) (manufactured by Lovibond Water Testing, Germany) was used for turbidity measurements. Instruments were calibrated before use and all the physicochemical parameters were measured in triplicates from all the samples collected. Cyanobacterial strains from the collected water samples were cultured and grown in sterile shake flasks containing liquid medium (BG11). Before the examination, these cultures were incubated for 4 weeks at room temperature (25°C) and elevated temperature (40°C) under 24 h of white fluorescent lamp light (at 27 μmol m^{-2} s^{-1}) as described by Magonono et al. (2018); Mutoti et al. (2022). Since the taxonomic system of cyanobacteria according to Komárek et al. (2014) has radically changed due to the introduction of electron microscopy; and molecular and genetic methods for the characterization of cyanobacterial taxa; this study further utilizes a Leica DM3000 semi-automated laboratory microscope (Leica, Germany) to observe morphological characteristics of filaments and cells. Furthermore, the bench-top FlowCAM (8100-C, Fluid Imaging Technologies Inc., Scarborough., ME, United States) was used to generate images of cyanobacterial strains. Microscope and bench-top FlowCAM were adopted for preliminary analysis of water samples. The FlowCAM captures cyanobacterial cells as the water sample flows past the filter tube inside the FlowCAM device. The instrument was equipped with software (VisualSpreadsheet V3.2.2) used to view and select images of interest. During analysis, the instrument was on auto-image mode (capture rate of 20 frame/sec) and auto-focused for ×10 objective with the flow rate of 0.4 mL/min. Although the primary morphological characteristics that are diagnostic of the families were first given in Anagnostidis and Komárek (1990) and updated in Komárek (2013), the morphology of captured cyanobacterial strains in the present study was further identified according to van Vuuren et al. (2006); Balsooriya (2019) descriptions.

Furthermore, cyanobacterial cells harvested from cultures of four samples (each sample from each batch) by filtering were further used for molecular characterization and identification using PCR. The included analytical procedures were DNA extraction, amplification using PCR, sequencing, and phylogenetic analysis, and these procedures are described below.

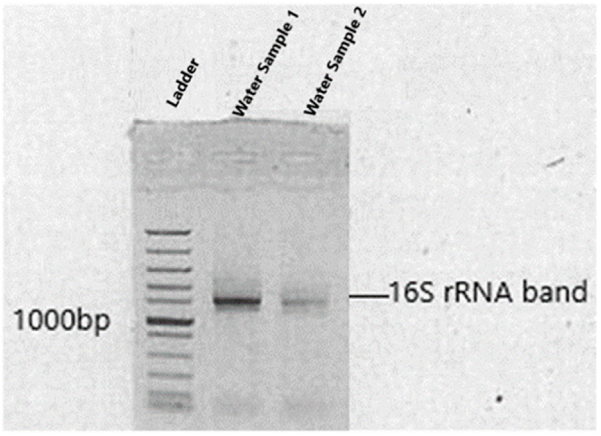

FIGURE 1
Amplified 16S rRNA of bacterial isolates shown on agarose gel electrophoresis. Levels of gene migration are expressed by lanes (water samples 1 and 2).

2.2 Extraction of genomic DNA

Standard procedures for extraction of total gDNA were performed according to the instructions supplied by the manufacturer. Quick-DNA/RNA™ Miniprep Plus Kit (supplied by Inqaba Biotech Laboratories, South Africa) was used for the isolation of DNA. The first step of extraction began with cyanobacteria cell lysis, where an equal mixture of 400 μL of 2X concentrate with 400 μL of nuclease-free water was homogenized in a Lysis Tubes to get 1X DNA/RNA Shield™, followed by 10 min of centrifugation at 1600 × g and transfer of supernatant into an RNase-free tube. An equal volume of DNA/RNA Lysis Buffer was added, mixed well and then transferred into a Spin-Away™ Filter in collection tubes and centrifuged for 1 min. The Spin-Away™ Filter was again transferred into a new collection tube, 400 μL DNA/RNA Prep Buffer was added to the column and centrifuged for 30 s and the flow-through was discarded. 700 μL of DNA/RNA Wash Buffer was then added and centrifuged for 30 s and again the flow-through was discarded. 400 μL of DNA/RNA Wash Buffer was added and centrifuged for 2 min to ensure complete removal of wash buffer then the column was carefully transferred into clean microcentrifuge tubes. A volume of 100 μL of DNase/RNase-Free Water was directly added to the column matrix, let stand for 5 min, and then centrifuged to elute DNA from the respective column. The eluted DNA was stored at −70°C and then sent to Inqaba Biotech Laboratories for further analysis.

2.3 Molecular characterization of strains

The basic molecular characterization of strains were performed after 16S rRNA gene fragments amplification with PCR using the primers: 27F (/5AmMC6/gcagtcgaacatgtagctgactcaggtcac-AGAGTTTGATCCTGGCTCAG) and 1492R (/5AmMC6/tggatcacttgtgcaagcatcacatcgtag-TACGGYTACCTTGTTACGACTT), which were adopted from Meirkhanova et al. (2023). The methods for the multi-gene analysis and sequencing were carried out as per Mutoti

TABLE 1 Physicochemical parameters (mean and STDEV) status of water determined during the collection of water sample (n = 16).

TDS (mg/L)	EC (mS/m)	Turbidity (NTU)	DO (mg/L)	pH	Salinity (ppm)	Temp (°C)
37.8 ± 1.1	52.6 ± 0.4	2.2 ± 0.2	7.7 ± 0.2	7.8 ± 0.4	23.8 ± 0.4	20.1 ± 0.2
38.2 ± 0.7	52.8 ± 0.6	1.2 ± 0.2	7.8 ± 0.2	7.3 ± 0.2	25.1 ± 0.2	21.1 ± 0.1
38.4 ± 0.2	56.3 ± 0.4	22.3 ± 0.4	7.5 ± 0.2	6.8 ± 0.4	25.5 ± 1.4	19.5 ± 0.7
36.6 ± 1.1	50.8 ± 2.2	17.9 ± 0.7	13.0 ± 0.5	6.9 ± 0.2	27.3 ± 0.4	21.6 ± 0.7
37.3 ± 1.3	52.6 ± 0.4	2.2 ± 0.1	7.5 ± 0.2	7.5 ± 0.3	24.5 ± 0.5	18.8 ± 0.3
37.7 ± 0.5	53.2 ± 1.4	1.2 ± 0.1	7.5 ± 0.2	7.5 ± 0.1	25.1 ± 0.2	20.6 ± 0.4
38.4 ± 0.2	55.4 ± 0.4	24.2 ± 0.9	7.3 ± 0.2	6.5 ± 0.3	24.2 ± 0.2	19.9 ± 0.6
36.2 ± 1.2	50.0 ± 2.3	17.4 ± 0.6	12.7 ± 0.4	6.7 ± 0.2	28.0 ± 0.2	21.3 ± 0.9
38.0 ± 0.4	55.4 ± 0.1	3.1 ± 0.2	8.7 ± 0.3	7.2 ± 0.0	25.3 ± 0.3	19.4 ± 0.1
36.6 ± 0.4	56.5 ± 0.4	2.5 ± 0.1	8.7 ± 0.3	7.2 ± 0.1	24.7 ± 0.3	20.1 ± 0.2
36.1 ± 0.7	48.9 ± 0.3	38.7 ± 0.3	8.6 ± 0.3	6.3 ± 0.2	25.2 ± 0.3	20.1 ± 0.4
38.1 ± 0.2	48.7 ± 0.4	18.7 ± 0.3	11.4 ± 0.3	6.4 ± 0.0	27.7 ± 0.4	22.6 ± 0.4
37.1 ± 0.1	49.5 ± 0.4	2.5 ± 0.2	9.3 ± 0.1	7.1 ± 0.0	26.2 ± 0.2	20.2 ± 0.2
36.9 ± 0.5	58.5 ± 1.1	1.7 ± 1.0	7.8 ± 0.0	7.4 ± 0.1	26.0 ± 0.2	20.9 ± 0.2
37.7 ± 0.8	49.0 ± 0.2	38.9 ± 0.1	8.5 ± 0.0	6.8 ± 0.2	24.7 ± 0.1	21.7 ± 0.3
36.0 ± 0.4	49.1 ± 0.2	19.0 ± 0.3	12.2 ± 0.1	6.4 ± 0.1	27.2 ± 0.4	22.3 ± 0.6

Abbreviations: TDS: total dissolves solids, EC: electrical conductivity, DO: dissolved oxygen, Temp: temperature.

et al. (2022). The Barcoded Universal Primer approach was used for multiplexing 16S amplicons using two rounds of PCR workflow (Supplementary Table S1). The first round used the universal primer-tailed 16S primers (targeting the bacterial 16S rRNA genes) and the PacBio Barcoded Universal Primers were used in the second round (Gueidan et al., 2019). DNA bands of amplified PCR products were clearly shown on agarose gel sizing at base pairs of ≥1100 (Figure 1). A PacBio (www.pacb.com) sequel system was used for sample sequencing. Version 9.0 Single Molecular Real Time (SMRT) link was used for processing raw subreads to produce highly accurate reads. The V-search (https://github.com/torognes/vsearch) was then used to process these highly accurate reads and based on QIMME2 the taxonomic information was determined.

2.4 Accession numbers of nucleotide sequence and phylogenetic sequence analysis

GenBank was used to deposit sequences of novel strains found in this study under accession numbers OP323090, OP323094, and OP323097 for *Chroococcus* sp. and accession numbers OP323093 and OP323094 for *Microcystis aeruginosa*. The alignment of sequences obtained was performed using a program (BioEdit software) for DNA sequencing and a ClustalW (MEGA-X Version 4.0). To validate the origin of cyanobacteria of sequenced samples, NCBI/BLAST (obtainable from http://www.ncbi.nlm.nih.gov/BLAST/) was used for comparison of 16S rRNA gene sequences compiled from available databases with sequences obtained in this study; therefore, these sequences were used to construct phylogenetic trees. The p-distance method (Nei and Kumar, 2000) and the neighbour-joining method (Saitou and Nei, 1987) were used to calculate and construct evolutionary distances and phylogenetic trees, respectively, using 1000 replicates for bootstrap analysis and only bootstrap above 50%. *Bacillus subtilis* strain was used as an out-group taxon when contracting the phylogenetic tree.

2.5 Data analysis

Pearson's correlation coefficient analysis was utilized in the present study to determine the aspects of a linear relationship between various physical and chemical parameters observed during water sample collection.

3 Results

3.1 Physicochemical parameters

The physicochemical parameters that were determined in this study indicated values that met World Health Organization recommended standards for domestic requirements (WHO, 2022). The mean and standard deviation (STDEV) for seven important physicochemical parameters were determined and shown in Table 1. Furthermore, Pearson's correlation analysis was conducted in the present study (Table 2); and this analysis revealed a strong correlation between parameters with values highlighted in blue and further indicated moderate positive

TABLE 2 Relationship between physicochemical parameters determined by the Pearson's correlation analysis (n = 16).

	TDS	EC	Turbidity	DO	pH	Salinity	Temp
TDS	1						
EC	0.339*	1					
Turbidity	−0.134	−0.470	1				
DO	−0.572	−0.571	0.225	1			
pH	0.242	0.481*	−0.794	−0.517	1		
Salinity	−0.452	−0.445	0.137	0.886**	−0.517	1	
Temp	−0.260	−0.550	0.303*	0.678**	−0.470	0.662**	1

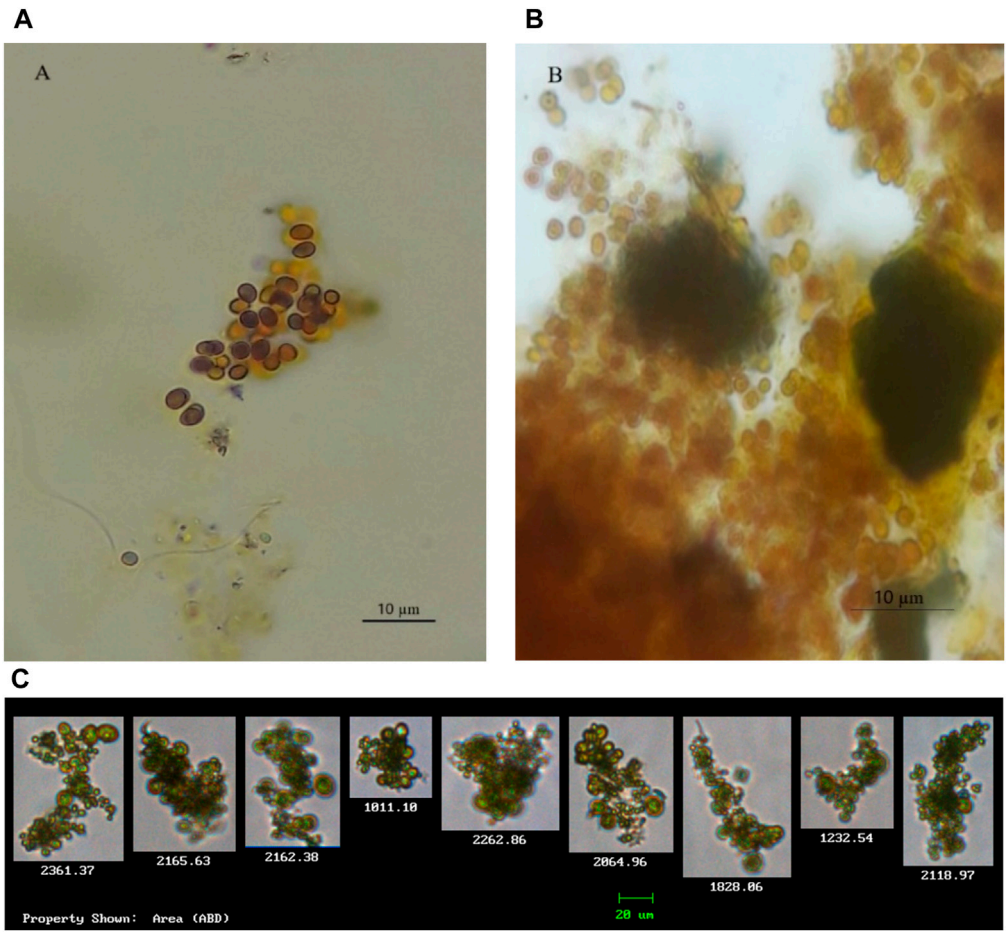

FIGURE 2
Isolated cyanobacterial species and their morphological features. **(A,B)** *Chroococcus* strains (observed with a microscope); **(C)** *Chroococcus* sp. (observed and captured with FlowCAM).

correlations between parameters with values highlighted in orange at a 0.01 level of significance.

3.2 Morphological characterization

Morphological characteristics were used for the identification of two cyanobacteria species *Microcystis* and *Chroococcus*. The isolated *Chroococcus* sp. (Figure 2) was observed as a group of two and/or four cells enclosed with clear and an amorphous mucilage sheath, with each cell having an individual envelope. *Chroococcus* sp. are prokaryotic organisms that have chlorophyll pigment, are unicellular, have a coccus cell shape, and form a mucous membrane (Goshtasbi et al., 2022; Fendiyanto et al., 2023). *Chroococcus* are egg to rod-shaped unicellular algae with a diameter of 0.4–50 µm. It is an autotrophic organism capable of

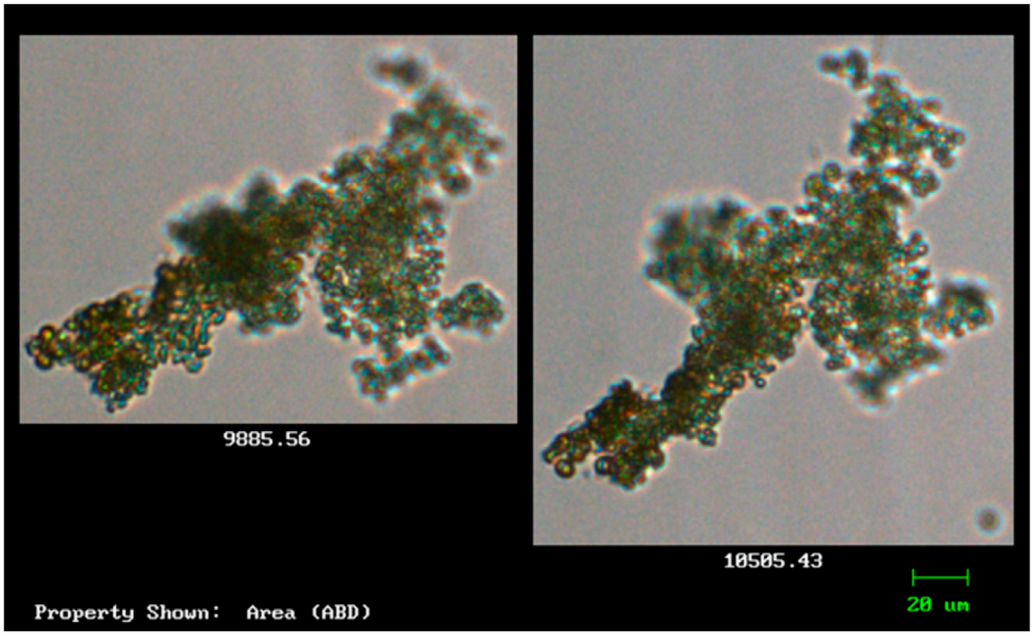

FIGURE 3
The morphological features of the isolated cyanobacteria species *Microcystis* (observed and captured with FlowCAM).

surviving without oxygen. The mucus layers of different colonies stick together so that they can form a biofilm visible to the naked eye. *Chroococcus* sp. is classified as division cyanophyta, class cyanophyceae, order chroococcales, and genus *Chroococcus*. In the new classification system, this species, based on microalgae phylogeny studies by Shayler and Siver (2006), is classified closer to bacteria than other eukaryotic algae organisms (Fendiyanto et al., 2023).

Species with cells that are green in colour and spherical were observed and captured using the FlowCAM. Characteristics of such species resemble that of *Microcystis* (Figure 3). According to Balsooriya (2019), Mutoti et al. (2022), and Thawabteh et al. (2023), cells of this species are spherical and green in colour and strains are classified as large since their diameter varies from 0.5 to 9 µm. They further reported that the cells of *Microcystis* are densely or sparsely, and irregularly arranged with fine and colourless mucilage that sometimes forms a wide margin around the cells. Cells of *Microcystis* are pale-blue–green and are spherical or hemispherical after division, but they appear brownish due to aerotopes that mask the blue–green colour of the protoplast. Due to vacuoles that are found within the cells, when they are viewed through a light microscope, they often have a black appearance (Mutoti et al., 2022).

3.3 Molecular identification of cyanobacteria strains

Sequences of cyanobacteria were targeted by primers set that were adopted in this study to target the 16S rDNA genes. Amongst these, were the sequences that resemble that of *M. aeruginosa* and *Chroococcus* sp. from the order chroococcales with sequences of *Chroococcus* sp. dominating the water samples. Table 3 shows strains of cyanobacteria that could not be identified using morphological characters, instead, were identified using molecular characters performed after culturing and isolation of samples. Sequence homology search performed by use of the BLAST program shows strains of cyanobacteria (Table 3) aligning most confidently to their closest related sequences from the NCBI database. Table 3 shows that strains m64187e-7881, m64187e-2143, and m64187e-0930 were closely related to *Chroococcus* sp. JJCM with percentage similarities of 95%, 96%, and 94%, respectively. On the other hand, cyanobacterial species *M. aeruginosa* NIES-1062 and strain m64187e-6729 had a similarity of 93%. Strain m64187e-1069 and species *M. aeruginosa* NIES-933 were found with a similarity of 93%.

3.4 Phylogenetic analysis

In Figures 4, 5, the neighbour-joining method (Saitou and Nei, 1986) was used to construct the topology of sequences isolated from the present study and their closely related sequences from the NCBI database. The percentage of replicates for the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). Five base substitutions for nucleotide positions are represented by the scale bar. An outgroup taxon is shown by red dot.

4 Discussion

This study aimed at detecting and identifying the presence of cyanobacteria strains producing microcystin in drinking water.

TABLE 3 Cyanobacterial strains and their closest species from blast search.

Strain	Closest species	Accession number	Similarity (%)
m64187e-7881	*Chroococcus* sp. JJCM	AM710384.1	95
m64187e-2143	*Chroococcus* sp. JJCM	AM710384.1	96
m64187e-0930	*Chroococcus* sp. JJCM	AM710384.1	94
m64187e-6729	*Microcystis aeruginosa* NIES-1062	KX014841.1	93
m64187e-1069	*Microcystis aeruginosa* NIES-933	LC557455.1	93

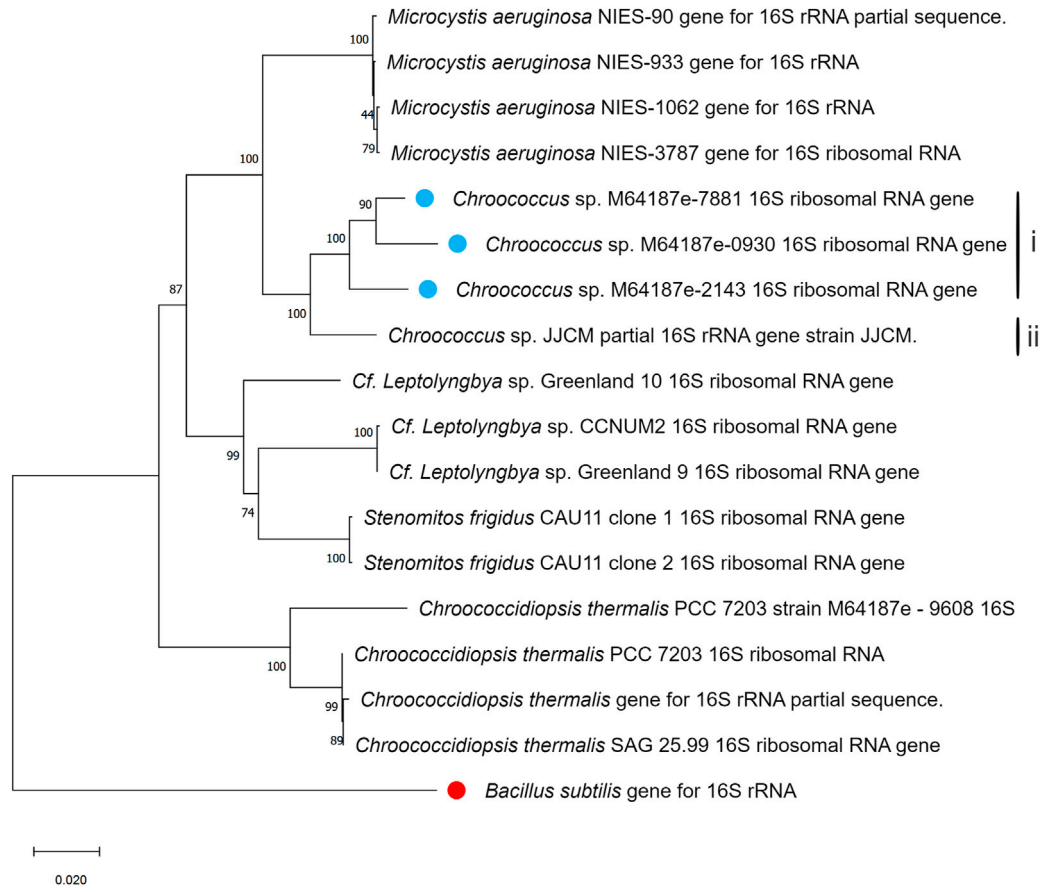

FIGURE 4
Phylogenetic tree of isolated *Chroococcus* sp. based on sequences isolated from the present study and sequences of its neighbouring species from the NCBI database. The tree was calculated using a maximum-likelihood algorithm with the bootstrap values given at the nodes. The cyanobacterial strains from the current study are indicated by a blue dot.

Leica DM3000 Semi-Automated Laboratory Microscope and FlowCAM were used for morphological characterization of cyanobacteria species detected in the present study. Captured images of cyanobacterial species were identified through comparison based on available literature. Cyanobacteria species (*Microcystis* and *Chroococcus*) from order Chroococcales were detected and identified, showing diverse morphological characteristics and phylogenetic relationships. Their morphological characteristics resemble that of Balsooriya (2019); Wang et al. (2021); Fendiyanto et al. (2023). Moreover, further morphological description of *Chroococcus* sp. in the present study was also consistent with previous descriptions by Balasooriya (2019); Goshtasbi et al. (2022). In a study conducted by Fendiyanto et al. (2023) in Dramaga Bogor, water samples were collected from freshwater found in high and low light-intensity environments. In their study, they aimed to identify the morphological diversity level of microalgae-based on their environment (high light and low light environment). Their results indicated that cyanobacterial species *Chroococcus* and *Microcystis* from order chroococcales were found among the most dominating species in freshwater samples collected from high-light intensity environment. They concluded that light was the main environmental factor influencing the growth and production

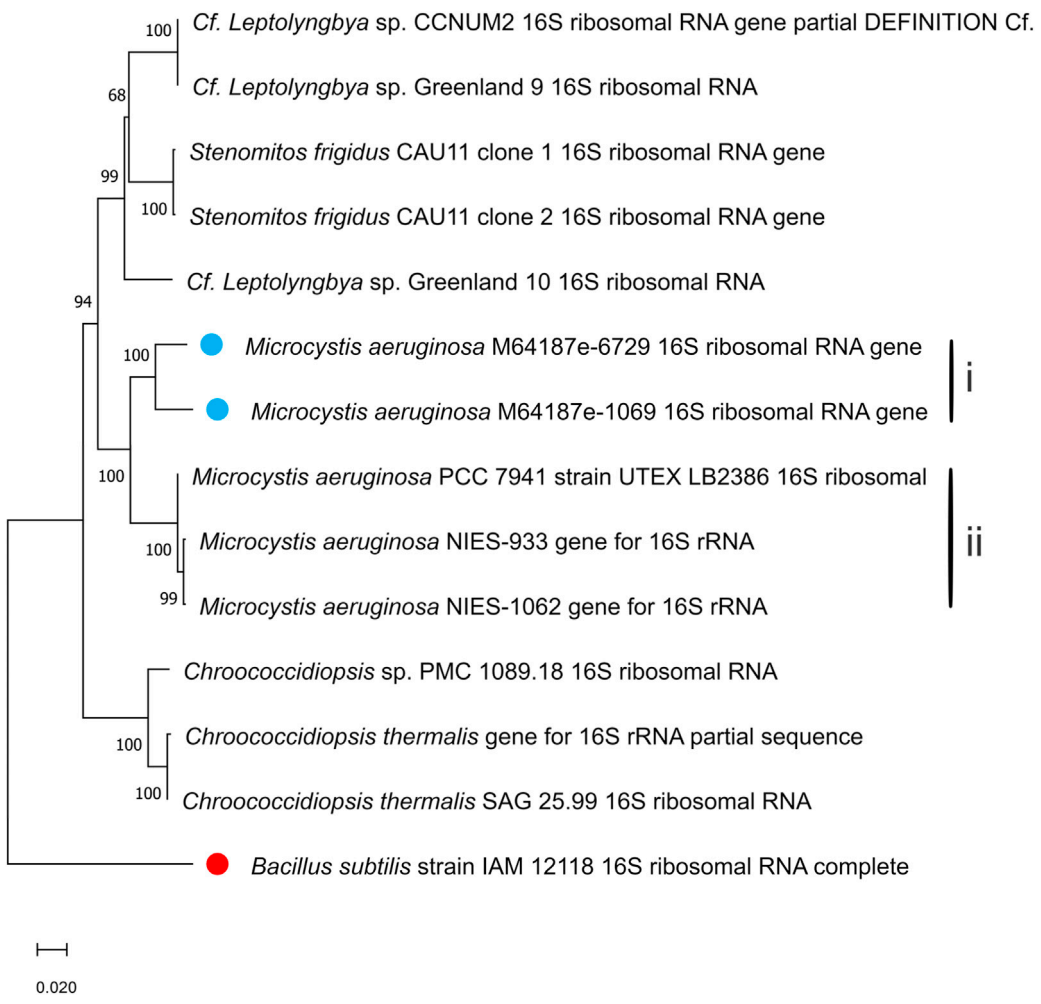

FIGURE 5
Phylogenetic tree of isolated *Microcystis aeruginosa* based on sequences isolated from the present study and sequences of its neighbouring species from the NCBI database. The tree was calculated using a maximum-likelihood algorithm with the bootstrap values given at the nodes. The cyanobacterial strains from the current study are indicated by a blue dot.

of these microalgae in freshwater, posing a threat to humans and the aquatic environment.

In addition, the BLAST program (NCBI) was further utilized in the present study to search for strains of cyanobacteria, which are closely related to the strains of cyanobacteria found in this study. The BLAST search confirmed that the stains of cyanobacteria found in this study were closely related to species of cyanobacteria that have the potential to produce microcystins, such as *Microcystis* and *Chroococcus* (Mirazbekov et al., 2021). Borowitzka (2018) reported that the genus *Microcystis* contains several toxins-producing species, with *M. aeruginosa* being the best-known and globally distributed. They further highlighted that microcystins produced by *Microcystis* are a family of monocyclic heptapeptides with a characteristic feature, the unusual β-amino acid, Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4E,6E-dienoic acid). Numerous research studies that have been conducted previously have reported on microcystins released by *Microcystis* and *Chroococcus* species in freshwater bodies. A review study by Chia et al. (2022) reports on the presence of potentially toxic *Microcystis* strains in natural lakes found in the Middle Atlas Mountains in Morocco. In their study, they detected microcystin synthetase *mcy* genes using PCR. They further reported a study that characterized 27 potentially toxic cyanobacterial strains including *Microcystis* from seven reservoirs in Tunisia, which also detected microcystin synthetase *mcy* cluster (*mcy*A, -B, -C, -D, -E, and -G). Apart from these findings, there are numerous studies that have been conducted previously across the world and have reported on microcystins-producing cyanobacteria in drinking water (Table 4).

In humans, exposure to low doses of microcystin may lead to liver and kidney failure (Mutoti et al., 2022; Chia et al., 2022); on the other hand, exposure to high doses may lead to immediate liver necrosis and intrahepatic hemorrhage (Chia et al., 2022). Therefore, understanding the potential health effects of microcystins exposure underscore the importance of implementing comprehensive measures to prevent and minimize the presence of these toxins in drinking water. Hence, standards that serve as benchmarks for water quality management have been established by regulatory agencies and health organizations such as WHO and DWS

TABLE 4 Cyanobacterial species responsible for the production of microcystins in water.

Microcystin-producing species	References
Eucapsis sp.	Mioni et al. (2011)
Oscillatoria sp.	Christoffersen and Kaas (2010); Mohamed (2016)
Leptolyngbya sp.	Frazão et al. (2010); Somdee et al. (2013)
Pseudanabaena sp.	Borges et al. (2015); Wiltsie et al. (2018); Graham et al. (2020)
Nostoc sp.	Graham et al. (2020); Ivanov et al. (2021)
Phormidium sp.	Graham et al. (2020); Wood et al. (2017)

(Mutoti et al., 2022; Mutoti et al., 2022). Maximum permissible levels of microcystins in portable water have been established to protect consumers from the potential health risks associated with these toxins (WHO, 2017). They safe guide public health and ensure the quality of water supplies. Moreover, these standards guide the implementation of monitoring and treatment measures to mitigate microcystin contamination.

Furthermore, sequences of different nucleotides together with those observed in the present study were utilized for the construction of the phylogenetic trees, where *B. subtilis* strain was included as an external group. The phylogenetic trees generated based on 16S rDNA displayed the phylogenetic position of the strains among their closely related cyanobacteria (Figures 4, 5). According to the phylogenetic tree in Figure 4, the maximum likelihood tree of the 16S rDNA sequences showed a tight cluster of *Chroococcus* sp. strains separated from all the other strains of the order chroococcales included in the present study. The whole cluster was supported by a bootstrap value of 100%. Two separate sub-clusters within the *Chroococcus* sp. cluster could be distinguished in the phylogenetic tree (Figure 4). The first cluster (I) comprised the strains m64187e-7881, m64187e-2143, and m64187e-0930, and the second cluster (II), which was separated from cluster I comprised *Chroococcus* sp. JJCM. Cluster I was supported by a bootstrap value of 100%. All the strains within cluster I, that is, m64187e-7881 (now referred to as *Chroococcus* sp. M64187e-7881 16S ribosomal RNA gene), m64187e-2143 (now referred to as *Chroococcus* sp. M64187e-2143 16S ribosomal RNA gene), and m64187e-0930 (now referred to as *Chroococcus* sp. M64187e-0930 16S ribosomal RNA gene) showed identical 16S rDNA sequences similarities of 94%–95% with strain *Chroococcus* sp. JJCM (with accession number AM710384) in cluster II isolated from the freshwater reservoir in South Bohemia (Jezberova, 2006).

Another tight cluster of *M. aeruginosa* is shown by the maximum likelihood three of the 16S rDNA sequences in Figure 5, supported by a bootstrap of 100%. Figure 5 also distinguished two separated clusters within the *Microcystis* cluster, with cluster I comprising strains m64187e-6729 and m64187e-1069, and cluster II comprising three different strains of *M. aeruginosa*; that is, *M. aeruginosa* PCC 7941, *M. aeruginosa* NIES-933, and *M. aeruginosa* NIES-1062. Both cluster I and II were supported by 100% bootstrap. Strain m64187e-6729 (thereafter called *M. aeruginosa* M64187e-6729 16S ribosomal RNA gene) had identical 16S rDNA sequences similarities of 93% with strain *M. aeruginosa* NIES-1062 gene (KX014841) isolated from farm ponds in USA by Yuan et al. (2020). Moreover, another identical 16S rDNA sequence similarity of 93% was observed between strain m64187e-1069 (thereafter called *M. aeruginosa* M64187e-1069 16S ribosomal RNA gene) and strain *M. aeruginosa* NIES-933 gene (LC557455) isolated from Japan by Suzuki et al. (2022).

5 Conclusion

In conclusion, this study has successfully identified and characterized two microcystin-producing cyanobacteria, *M. aeruginosa* and *Chroococcus* sp., isolated from drinking water samples in Mawoni village, South Africa. Utilizing a polyphasic approach combining morphological and molecular techniques, we employed FlowCAM imaging and PCR analysis to confirm the presence of these cyanobacteria species, which gave positive results for the identification and classification of members of the genus from the order Chroococcales, supported by BLAST analysis and phylogenetic tree construction. Notably, this study reports for the first time the presence of these biofilm-producing strains in drinking water, highlighting potential health risks associated with stored water. The findings of this study suggest that the potential for treated water stored in storage containers to support a range of toxic cyanobacteria may currently be underestimated, and therefore, isolation and screening of more cultured strains from treated water is required to further investigate the diversity of toxin-producing cyanobacteria. As such, future research should prioritize the analysis of microcystin synthetase genes and quantification of microcystins in potable water to assess health risks accurately. This approach could lead to improved monitoring and management strategies for cyanobacterial toxins in drinking water supplies. By focusing on these advancements, researchers and public health authorities can better safeguard water quality and protect community health. Furthermore, future research should also be focused on conducting cytotoxicity studies in these microcystin-producing species and more experimental studies to understand the detailed change in microbial diversity considering the variation in temperature and pH range in freshwater.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, OP323090, OP323093, OP323097, OP323100, and OP323094.

Author contributions

MM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Validation, Writing–original draft, Writing–review and editing. JG: Conceptualization,

Methodology, Project administration, Resources, Supervision, Validation, Writing–review and editing. AK: Data curation, Formal Analysis, Methodology, Validation, Writing–review and editing. AJ: Conceptualization, Formal Analysis, Supervision, Validation, Writing–review and editing.

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Conflict of 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2024.1423339/full#supplementary-material

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