First report of an *Anabaena* Bory strain containing microcystin-LR in a freshwater body in Africa

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In South Africa, little is known about the production of microcystin by the genus *Anabaena* Bory. In April 2012, during a cyanobacterial bloom event in Theewaterskloof Dam, Western Cape province, the plankton was sampled on 10 occasions. The dominant algae belonged to the genus *Anabaena*, a family of filamentous cyanobacteria known to produce cyanotoxins such as anatoxin-*a*, harmful to humans and the aquatic foodweb. The specimens isolated lacked the characteristic akinetes and/or heterocysts associated with this genus. Therefore the 16S rRNA gene was Sanger sequenced and a maximum parsimony tree was constructed, confirming its identity as *Anabaena ucrainica* (Schkorbatow) M. Watanabe. Enzyme-linked immunosorbent assay (ELISA) confirmed the presence of microcystin-LR in the isolated *A. ucrainica* field sample, while PCR analysis and sequencing further confirmed the presence of *mcy* genes in this species. It was speculated from the data that prevailing low water-column temperatures and strong gusty winds may have resulted in the lack of akinete or heterocyst production. The *Anabaena* strain isolated from Theewaterskloof Dam is the first report of a strain containing microcystin-LR belonging to this genus in a freshwater body in Africa.

Keywords: *Anabaena ucrainica*, heterocysts, microcystin-LR, remote sensing, temperature

Introduction

Blooms of *Anabaena* Bory are widespread in lakes and reservoirs throughout the world, especially during summer months (Laamanen and Kuosa 2005). These cyanobacterial cells can sometimes contain cyanotoxin and, in many cases, produce unpleasant odours (Oberholster et al. 2009). *Anabaena* is a filamentous, heterocystous cyanobacterium that forms akinetes through the transformation of vegetative cells to permit the organism to survive under adverse environmental conditions (Watanabe 1992).

In *Anabaena* spp., the morphology of the akinetes is a feature used during the taxonomic identification of a specific species, while the specific shape, size and position of akinetes relative to the heterocysts identify a specific species. Occasionally isolates are sampled in the field wherein the akinetes and heterocysts are absent, making classification on morphological characteristics at species level impossible. The literature indicates that these morphological characteristics may vary due to different environmental and/or growth conditions, or may even have been lost during cultivation (Li et al. 1997).

In Canada, Denmark, Finland, France, Norway and Egypt *Anabaena* produces a number of cyanotoxin variants such as anatoxin-*a*, anatoxin-*a*(S), microcystin-LR and cylindrospermopsin (Oberholster et al. 2004). However, the production of microcystin by African strains of *Anabaena* has remained a matter of speculation (Sivonen and Jones 1999). The latter authors reported that this genus is known to be one of the most important microcystin-producing cyanobacteria in temperate lakes. According to van Ginkel (2012), the genus *Anabaena*, although present in South African man-made lakes, is not associated with the presence of total microcystins. The knowledge gap about this genus in South Africa is partly due to stakeholders' perceptions that, here, *Microcystis* spp., not *Anabaena* spp., are the major producers of cyanotoxins during nuisance algae blooms in potable water supplies (Oberholster et al. 2009). Therefore, the development of a bloom of *Anabaena* sp. in an important drinking water supply reservoir for the metropolis of Cape Town requires special attention, due to the risk posed to human and animal health (Oberholster et al. 2009).

The aims of the present study were (i) to identify the *Anabaena* specimens isolated from a bloom in Theewaterskloof Dam, (ii) to determine if the species isolated contains genes of the *mcy* gene cluster and microcystin-LR equivalents, (iii) possibly to resolve environmental factors that may have dictated the loss of akinetes and heterocysts in the field strains isolated, and (iv) to determine if the *Anabaena* species isolated contains *nifJ* genes for nitrogen fixation.

Materials and methods

Study area

Theewaterskloof Dam (34°4′41″ S, 19°17′21″ E), completed in 1980 in the Riviersonderend valley near Villiersdorp (Figure 1), is the largest man-made lake in the Western Cape province and supplies potable water to Cape Town, a city of over one million inhabitants (River Health Programme 2005). Besides receiving runoff from the surrounding agricultural catchment, its inflow is supplemented by runoff from neighbouring catchment areas, transferred via a series of intake works, shafts and tunnels.

Physico-chemical analyses

Ten sampling trips to Theewaterskloof Dam were undertaken over a period of two weeks in April 2012 during the occurrence of a cyanobacterial bloom event. At each of five sampling sites (Figure 1), dissolved oxygen, water temperature, pH and electrical conductivity values were measured at the water surface, using a Hach sension™ 156 portable multiparameter probe (Loveland, USA). Surface water was sampled in triplicate, using a 1-litre scoop bottle sampler, for chemical and microcystin-LR equivalent analyses and phytoplankton identification. The data were pooled to give an average value over the 10 sampling dates. Water samples were filtered through 0.45 µm pore size Whatman GF/filters and stored in polyethylene bottles pre-rinsed with dilute sulphuric acid at pH 2.0 for analysis of dissolved nutrients. All chemical analyses were carried out according to standard methods (USEPA 1983; APHA et al. 1992). Concentrations of total nitrogen and total phosphorus were determined using the persulphate digestion technique. Nitrate concentrations were determined on an autoanalyser by the cadmium reduction method, while soluble reactive phosphorus concentrations were determined by the ascorbic acid method (APHA et al. 1992). Turbidity was measured *in situ* at the water surface using a Hach 2100P turbidimeter (Loveland, USA). The near-surface average nutrient concentration index (Forsberg and Ryding 1980) was used to classify the trophic state of the dam during the sampling period. Water transparency was measured with a 25 cm Secchi disc. The limit of the euphotic zone (Z_{eu}) was calculated as 2.7 times the Secchi depth (Cole 1994).

Sampling protocol and enumeration of phytoplankton species

Phytoplankton from 100 ml water samples were sedimented in a Sedgewick-Rafter counting chamber and analysed under an inverted microscope at 400× magnification using the strip-count method (APHA et al. 1992). All algae were identified using keys by Truter (1987), Wehr and Sheath (2001) and van Vuuren et al. (2006). A scanning electron microscope was used to identify doubtful taxa, mainly centric diatoms. The relative abundance of phytoplankton taxa at each sampling site was categorised according to Hörnström (1999): 1 = ≤250, 2 = 251–1 000, 3 = 1 001–5 000, 4 = ≥5 000 cells l^{-1}. Algal abundance in the epilithic zone was evaluated by counting the presence of each species as cells in a filament or equal number of individual cells. The Berger–Parker dominance index (BPDI) (Berger and Parker 1970) was used to measure

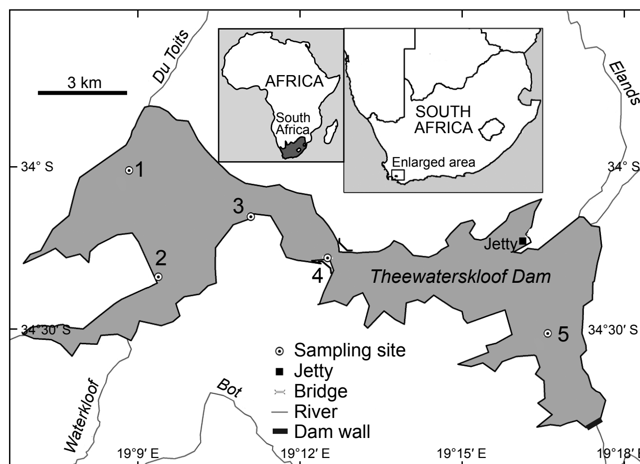


Figure 1: Map of Theewaterskloof Dam showing locations of the five sampling sites: 1 = North-west; 2 = South-west; 3 = Pump station; 4 = Bridge; 5 = South-east

the evenness or dominance of the algae at each sampling site. Net growth rate k (d^{-1}) was calculated for the sampling period at each site using the equation:

$$k = (\ln N_t - \ln N_0) / t \quad (1)$$

where t is time in days, and N_t and N_0 are population densities at times t and 0.

Equilibrial phytoplankton species (*sensu* Naselli-Flores et al. 2003) at the five sampling sites were determined over the two-week study period using the following criteria: (i) 1, 2 or 3 species of phytoplankton contribute more than 80% of the total biomass or bio-volume; (ii) their existence or coexistence persists for more than 1–2 weeks; and (iii) during this period, the total phytoplankton biomass did not increase significantly.

The spatial distribution of the cyanobacterial bloom was recorded using satellite imagery from the European Space Agency's medium resolution imaging spectrometer (MERIS) and newly-developed techniques by Matthews et al. (2012). Three images from dates nearest to the sampling period were acquired and processed following Matthews et al. (2012) to derive the concentration of chlorophyll *a* (Chl *a*) in the range 0.1–500 mg m^{-3}, as well as the occurrence of cyanobacteria.

Separation, isolation and culturing of Anabaena *sp. strain and DNA extraction*

Floating *Anabaena* filaments from the water column at the sampling sites were separated from non-buoyant phytoplankton species according to Oberholster et al. (2006). The cells were then washed three times with phosphate buffer saline (PBS)-Tween 20 (pH 7.4) to remove any residue. The PBS buffer consists of the following: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4. *Anabaena* filaments were isolated and purified according to the method of Rippka (1988). A single axenic filament was cut out under sterile conditions from the agar plate containing MA medium. The latter was done by using

a micro-spade under a dissecting microscope. The single axenic filament was transferred and cultured in liquid MA medium (Ichimura 1978) at 25 °C under continuous illumination of 25 µmol photons m^{-2} s^{-1}. At 21-days growth, 2 ml of the axenic culture was transferred to a serum vial and freeze-dried for 48 h. The axenic culture was then stored under vacuum until the DNA was extracted The DNA was extracted using the DNAzol®-Genomic DNA isolation reagent, following the manufacturer's recommended protocol (Molecular Research Centre, Inc., Cincinnati, Ohio).

PCR amplification of 16S rRNA, **mcy** *and* **nifJ** *genes*

The PCR analysis was performed using gene specific primers (Appendix 1) by employing a GeneAmp PCR system 9700 thermocycler (Applied Biosystems). The thermal cycling protocol included an initial denaturation at 94 °C for 3 min, followed by 35 cycles. Each cycle started with a 10 s interval at 93 °C, followed by 20 s at the annealing temperature (T_m °C) for the specific primer pairs (Appendix 1), and ended with 1 min at 72 °C. A final step of 15 min at 72 °C concluded the thermal cycling. The amplification reactions contained a 10× amplification buffer with 1.5 mmol l^{-1} $MgCl_2$, 0.2 mmol l^{-1} dNTPs, 20 pmol of each primer and 1 U Taq DNA polymerase, and 3–5 ng extracted DNA in a final volume of 40 µl. PCR assay results were visualised on 1.5% agarose-TAE gels.

Phylogenetic analysis of 16S rRNA and **mcy** *genes*

The InsTAclone™ PCR cloning kit cloning procedure recommended by the manufacturer was followed for the ligation and transformation of amplified 16S rRNA and *mcy* genes (Fermentas, Thermo Fisher Scientific). Single colonies were inoculated into 50 µl of distilled water, and 13 µl of each was used as template for colony-PCR amplification using M13 primers (Appendix 1). Multiple copies of these 16S rRNA and *mcy* gene containing colony-PCR fragments, identified on agarose gels, were selected for subsequent identification by sequencing (Central Analytical Facilities, Stellenbosch University, RSA). The obtained sequenced data were used to conduct homology searches on GenBank using BLASTn (Altschul et al. 1997, http://blast.ncbi.nlm.nih.gov/blast.cgi), and for further bioinformatic analyses to perform phylogenetic analysis using CLC sequence viewer 6 (CLC bio, Cambridge, Massachusetts).

For phylogenetic analyses, representative sequences were downloaded from National Center for Biotechnology Information (NCBI; Altschul et al. 1997) (Appendix 2). Sequences were exported to and analysed with the MEGA 5.05 package containing ClustalW files (Tamura et al. 2007) to construct maximum parsimony (MP) phylogenetic trees. Heuristic tree searches were carried out under equal and unordered weights criterion with 1 000 random sequence additions and the tree bisection-reconnection (TBR) branch swapping algorithm, permitting 10 trees to be held at each step. The option to collapse branches at zero length was selected. The Kimura two-parameter nucleotide substitutions model was used for the distance analysis following the guidelines of Nei and Kumar (2000). Datasets including and excluding the gap-scoring were analysed. Results from the distance analysis are only given and discussed where different from those of the parsimony analysis. One thousand bootstrap replications (Felsenstein 1985) were conducted as an indication of the relative support for each node. To evaluate whether datasets can be combined for parsimony analysis, partition homogeneity tests were conducted on all possible pairwise comparisons (Farris et al. 1994; Cunningham 1997). The amount of phylogenetic information in the parsimony analysis was estimated using the consistency index (Kluge and Farris 1969), the retention index (Farris 1989), and the g1 statistic (Hillis and Huelsenbeck 1992). To achieve the g1 statistic the tree length distribution of 100 000 random parsimony trees was calculated and used to assess the amount of non-random structure in the data.

Determining microcystin-LR toxicity using ELISA

Microcystin-LR equivalents of the bloom were determined by using the sampling method of Boyer et al. (2004) and Oberholster et al. (2009). Samples collected from all five sites were poured gently through 934-AH glass fibre filters in the field, frozen on dry ice, and returned to the laboratory in a cooler box. Filters for toxin analysis were extracted by grinding with 10 ml of 50% methanol containing 1% acetic acid and clarified by centrifugation. Microcystin-LR equivalents were also determined in the isolated axenic *Anabaena* strain. The cyanobacterial extract of both the field samples and the isolated axenic laboratory *Anabaena* sample were analysed for microcystin-LR equivalents using an enzyme-linked immunosorbent assay (ELISA) assay. The ELISA assay was conducted by employing the Quanti™ kit for microcystins (EnviroLogix, USA) following the manufacturer's protocol.

Data analysis

In this study, results were recorded on standard Microsoft Excel 2010 spreadsheets for data processing and statistical analysis. Statistical differences were analysed by calculating the Pearson correlation and a *t*-test using Sigma Plot 10 (Jandel Scientific). Values of $p \leq 0.05$ were regarded as significant. Correlations of *r* near zero were regarded as unrelated (Systat 7.0 [1997]).

Results

Physico-chemical analyses and phytoplankton assemblage

Surface water electrical conductivity values varied between 881 and 1 222.5 µS cm^{-1} throughout the study ($n = 10$) at all five sampling sites. The average surface water temperature measured during the 10 sampling trips was 14.5 °C, while turbidity measurements fluctuated between 24.5 and 76 NTU. The average pH at the five sampling sites was 7.18, while total nitrogen (TN) was 1 mg l^{-1} and total phosphorous (TP) 279.8 mg l^{-1} (Table 1). Large differences in the silica concentrations between the different sampling sites were observed. Site 4 with the lowest numerical cell numbers of *Anabaena* sp. also contained the lowest average silica concentration of 0.4 mg l^{-1}, while Site 2 had the highest concentration of 3.8 mg l^{-1} silica (Table 1). The phytoplankton assemblage is shown in Table 2. The dominant cyanobacteria present at all sampling sites was *Anabaena* sp., with an average cell number of

Table 1: Physico-chemical variable values (n = 10) at five sampling sites in Theewaterskloof Dam in April 2012

Site no.	Total silica (mg l⁻¹)	Total nitrogen (mg l⁻¹)	Total phosphorous (mg l⁻¹)	Conductivity (µs cm⁻¹)	pH	Temperature (°C)	Turbidity (NTU)
1	0.7	1.0	290.0	881.0	7.3	15.0	38.1
2	3.8	0.1	276.0	1 222.5	7.1	14.0	66.5
3	1.3	2.0	369.0	898.0	7.1	15.0	76.0
4	0.4	1.0	291.5	925.0	7.3	14.3	24.5
5	2.0	1.0	173.0	1198.0	7.2	14.5	61.5

Table 2: Phytoplankton community composition at five sampling sites in Theewaterkloof Dam in April 2012. + = rare, ++ = scarce, +++ = common, ++++ = abundant, +++++ = predominant. Relative abundance (cells l^{-1}) of each phytoplankton taxon was grouped into: 1 = ≤50 (rare); 2 = 51–250 (scarce); 3 = 251–1 000 (common); 4 = 1 001–5 000 (abundant); 5 = ≥5 000 (predominant)

Species	Site 1	Site 2	Site 3	Site 4	Site 5
Bacillariophyceae					
Asterionella formosa	++++	++++	+++	++++	++++
Surirella angusta					+++
Aulacoseira ambigua	++++	++++	++++	++++	++++
Navicula capitatoradiata	+++	++			+++
Chlorophyceae					
Staurastrum paradoxum	+++	+++	+++	+++	+++
Chlorogonium euchlorum	+++				
Monoraphidium sp.		+++	+++		+++
Pandorina morum		++++			++++
Scenedesmus acutiformis	++++	+++	+++	++++	+++
Cyanophyceae					
Anabaena ucrainica	+++++	+++++	+++++	+++++	+++++
Chroococcus limneticus			++		++
Microcystis aeruginosa		+++++			+++++
Dinophyceae					
Sphaerodinium fimbriatum	++++	++++	++++	++++	++++
Euglenophyceae					
Trachelomonas intermedia		+++			

≥5 000 cells l^{-1}, which correlated positively (r = 0.9104, $p \leq 0.05$) with the low average TN of 1 mg l^{-1} at these sites. During the first week of sampling in April 2012 the *Anabaena* sp. bloom of 2.3×10^6 cells l^{-1} and 9.7 $mm^3\ l^{-1}$ of the total biovolume occurred at Site 5 in the main dam basin. However, due to wind action, this bloom was transported passively from the south-eastern basin of the dam and spread out throughout the dam, to Sites 1 and 3 in the south-western basin of the dam, reaching a cell count of 1.2×10^5 cells l^{-1} and 7.7 $mm^3\ l^{-1}$ of the total biovolume.

During the second week of sampling, a moderate wind of 1–3.5 m s^{-1} was measured, with periodic overcast conditions. The average Secchi disc readings were 52.2 cm at Site 1, 54.3 cm at Site 2, 65.1 cm at Site 3, 82.5 cm at Site 4, and 86.7 cm Site 5, during the two-week sampling period and could be related to the remote sensing time-series showing the passive movement of the cyanobacterial bloom from the eastern to the western dam basin. The lowest average euphotic zone (Z_{eu}) limit of 141 cm in the north-western basin was measured at Site 1 at the end of the second week. This was in association with the increase in cell numbers of 1.2×10^5 cells l^{-1} and 7.7 $mm^3\ l^{-1}$ of the total biovolume at Site 1 in the western basin, and which coincided with the high turbidity of 56.1 NTU measured at Site 1.

Remote sensing

Figure 2 shows the spatial distribution of Chl *a* and the cyanobacterial bloom in March–April 2012. Imagery coinciding with the dates of sampling was not available, as the Envisat mission ended unexpectedly on 8 April 2012. Nevertheless, imagery data before 8 April 2012 showed a high concentration of Chl *a* of <300 mg m^{-3} in the western basin and in the clearer water of >10 mg m^{-3} towards the eastern basin, as was observed *in situ*. This indicates that the western basin generally had a higher phytoplankton biomass than the eastern basin by the end of the sampling period, with a mixed phytoplankton assemblage composed of Baccillariophyceae, Chlorophyceae, Dinophyceae and Cyanobacteria species (Table 2). Water identified by the algorithm as dominated by cyanobacteria was visible in patchy formation in both the western and eastern basins, as indicated by shaded, faceted pixels. The bloom dominated by *A. ucrainica* appeared persistent in the north-west corner of the dam, as well as in the central regions, while the eastern basin appeared to be clear on 5 April 2012 due to the degradation or movement of the bloom.

Microscope analyses of ≥300 cells of *Anabaena* sp. sampled over the period of two weeks revealed the total absence of akinetes and heterocystes (Figure 3). The microscopic analyses of ≥300 cells of the *Anabaena*

2012-03-25

Warning: Cyanobacteria detected (shaded).

2012-04-05

Warning: Cyanobacteria detected (shaded).

1 5 10 20 30 50 100 300 500

2012-04-02

Warning: Cyanobacteria detected (shaded).

Figure 2: Chlorophyll *a* (mg m^{-3}) derived using the MPH algorithm (Matthews et al. 2012) from MERIS Full Resolution imagery acquired on 3 days in March/April 2012. White pixels indicate presence of clouds. Shaded and faceted pixels indicate presence of water dominated by cyanobacteria

sp. specimens collected at the sampling sites revealed the following morphological variations: cell width 5.0 µm (SD 0.3) and cell length 6.1 µm (SD 1.1). Furthermore, microscopic analyses of all the *Anabaena* sp. trichomes collected in the field showed no curled or spiral forms, which were also distinct from the isolated *Anabaena* cultured strain after 21 days.

High numbers of diatoms, between 1 001–5 000 cells l^{-1}, were observed throughout the study at all five sampling sites, with *Aulacoseira ambigua* (Grunow) Simonsen as the dominant diatom species (BPDI, 0.591). *Asterionella formosa* (Hassall) was abundant at all sampling sites, reaching a maximum cell count of 2 200 cells l^{-1} at Site 1 during the first week of sampling. The average lower biovolume of 5.1 and 4.3 mm^{3} l^{-1} of the different species of diatoms sampled at Sites 1 and 4 correlated negatively (r = −0.8816, $p \leq 0.05$; 0.8475, $p \leq 0.05$) with the low silica concentrations of 0.4 and 0.3 mg l^{-1} measured at these sites. The cyanobacterium *Anabaena* sp. accounted for

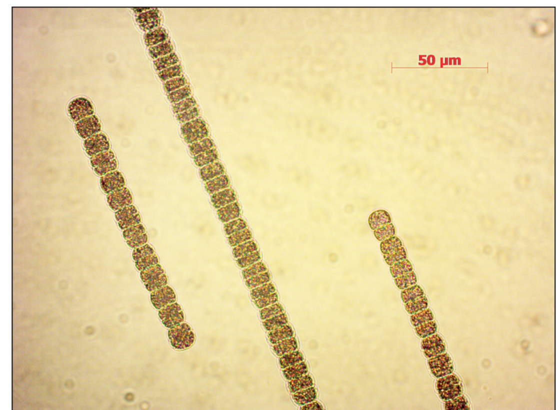
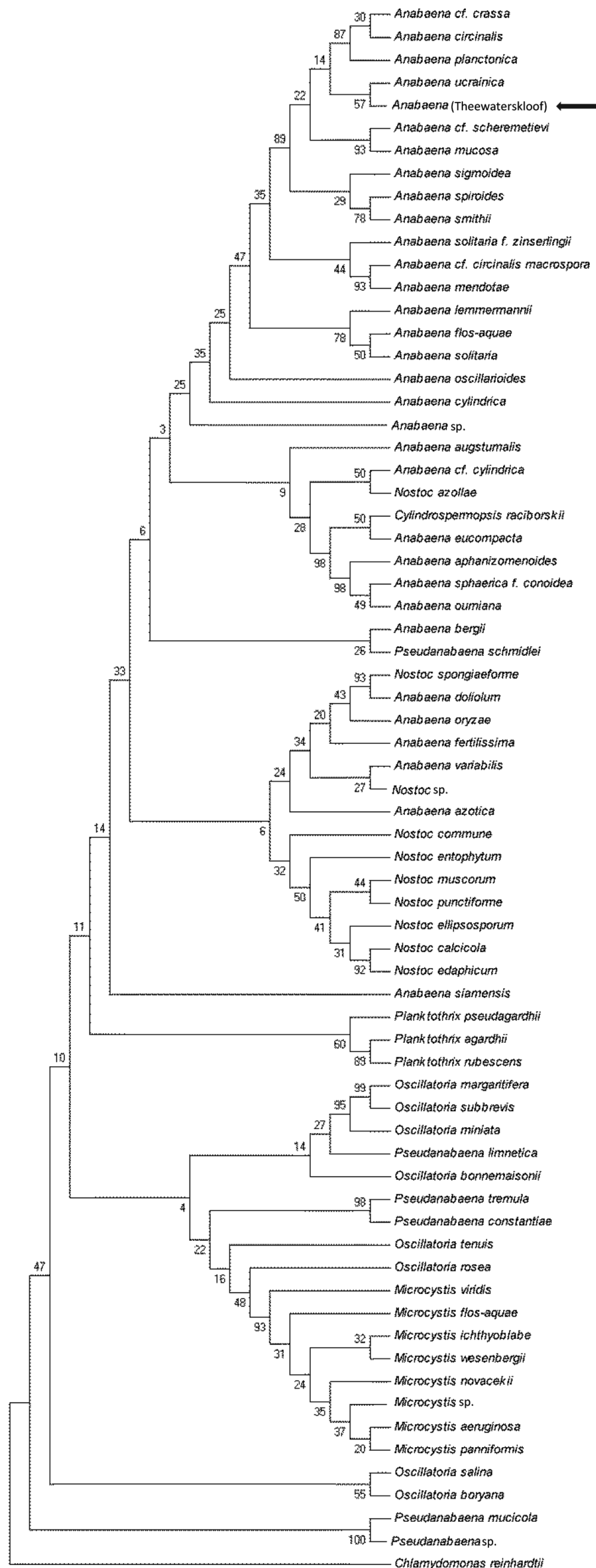


Figure 3: Unstained, bright-field microscopy image (400×) of *Anabaena ucrainica* (Skorbatov) M. Watanabe sampled from Theewaterskloof Dam, showing total absence of akinetes and heterocysts. Scale bar = 50 µm. Total number of cells analysed $n \geq 300$



more than 80% of the biovolume of the phytoplankton community at Sites 1, 2, 3 and 5. From the latter observation it was evident that this species was the ‘equilibrial species’ in the dam (*sensu* Naselli-Flores et al. 2003). The population growth net rate of *Anabaena* sp. at the start of the study was estimated at 0.17 d^{-1} but declined to 0.12 d^{-1} at the end of the sampling period.

Phylogenetic analysis and microcystin-LR toxicity

Since microscope analyses of the *Anabaena* sp. revealed the total absence of akinetes and heterocysts (Figure 3), PCR analyses were conducted to assess the presence of a gene required for nitrogen fixation. Separation of the *nifJ* gene amplicons from the isolated axenic *Anabaena* strain gave two fragments of approximately 155 and 316 bp, respectively, after agarose electrophoresis (not shown), indicating that the cells contained the *nifJ* genes for nitrogen fixation. To identify further the species of *Anabaena* collected from Theewaterskloof Dam, samples of the axenic *Anabaena* strain were sequenced with primers specific to 16S rRNA, *mcyA*, *mcyB* and *mcyD* (Appendix 2). The sequences obtained were analysed and submitted to GenBank (http://blast.ncbi.nlm.nih.gov/blast.cgi) and GenBank accession numbers were obtained as follows KJ578658 (16S rRNA), KJ578661 (*mcyA*), KJ578659 (*mcyB*) and KJ578660 (*mcyD*).

Homology searches on GenBank using BLASTn (Altschul et al. 1997, http://blast.ncbi.nlm.nih.gov/blast.cgi), of the 16S rRNA sequence obtained from the Theewaterskloof strain revealed a 99% similarity (E-value = 0.0; query cover 99%; bit score 573) to *Anabaena ucrainica* (Skorbatov) M. Watanabe (GU197638.1). By using the 16S rRNA sequences, the studied *Anabaena* sp. strain clustered with *Anabaena ucrainica* in the phylogenetic tree (Figure 4) and in a different cluster from other genera such as *Cylindrospermopsis* spp., *Microcystis* spp., *Nostoc* spp., *Oscillatoria* spp., *Pseudanabaena* spp. and *Planktotrix* spp. Homology searches on GenBank using BLASTn (Altschul et al. 1997, http://blast.ncbi.nlm.nih.gov/blast.cgi) of the *mcyA* (KJ578661), *mcyB* (KJ578659) and *mcyD* gene

Figure 4: Maximum parsimony analysis tree for cyanobacterial 16S rRNA gene sequence showing *Anabaena* sp., *Cylindrospermopsis* sp., *Microcystis* sp, *Nostoc* sp., *Oscillatoria* sp., *Planktothrix* sp. and *Pseudanabaena* sp. The outgroup was the green alga *Chlamydomonas reinhardtii*. Arrow shows position of the sampled Theewaterskoof Dam axenic strain. Evolutionary history was inferred using the maximum parsimony tree. The best of 24 most-parsimonious trees (length = 2 863) is shown. The consistency index is 0.431648, the retention index is 0.724216, and the composite index is 0.416804 (0.312606) for all sites and for parsimony-informative sites (in parentheses). Percentage of replicated trees in which the associated taxa clustered together in the bootstrap test (10 000 replicates) is shown next to the branches. The MP tree was obtained using the close-neighbour-interchange algorithm with search level 1, in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 69 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1 605 positions in the final dataset. Evolutionary analyses were conducted in MEGA5

(KJ578660) sequences obtained from the Theewaterskloof strain revealed respective similarities of 91% (E-value = 0.0; query cover 45%) to the *mcyA* from *Microcystis aeruginosa* UWOCC 019 (AP1383321); 90% (E-value = 0.0; query cover 84%) to the *mcyB* gene from *Microcystis panniformis* BCCUSP 019 (HQ852442.1), and 96% (E-value = 3e−138; query cover 74%,) to *Microcystis aeruginosa* AICB8689 polyketide synthase (*mcyD*) gene, partial cds. To support this observation further, phylogenetic trees were constructed using the sequenced *mcyB* and *mcyD* genes of the sampled *A. ucrainica*. These genes were used since their presence was previously shown to be a determining factor in the production of microcystins in South African impoundments (Botha and Oberholster 2008). After construction of the phylogenetic trees it was found that the gene sequences obtained from the Theewaterskloof Dam strain clustered with *Microcystis* sp. and *Planktothrix* sp. (Figures 5 and 6).

To characterise the studied axenic *Anabaena* strain further, sample primers were designed for the non-ribosomal microcystin synthetase genes *mcyA*, *mcyB* and polyketide synthetase gene *mcyD* (Botha and Oberholster 2008) to identify any toxigenic potential of this strain (Figure 7). The amplicons obtained from the *Anabaena* strain revealed the presence of *mcy* genes, which are normally associated with toxin production (Figure 7). These PCR products, after amplification with the MSF/MSR primer, produced a band size of <1.5 kb, while the MSI/MSR primer produced a band size of <300 bp. The first fragment was larger than the expected size of ~1.3 kb, while the latter fragment was significantly smaller than the expected size of ~1.3 kb as reported by Tillett et al. (2001), and may point towards a nodularin type cluster, or even a new type of microcystin-related peptide as indicated by the low sequence query cover 45% score. The Tox2+/Tox2− primer set produced a band size of ~400 bp, and the FAA/RAA primer set a band size ~900 bp, while the McyDF2/McyDR2 primer set produced a fragment of ~290 bp. To confirm whether the Theewaterskloof Dam *Anabaena* strain produced microcystin-LR, an ELISA assay was conducted on both the axenic laboratory strain and the field samples. They contained an average of 2.07 mg of protein and 8.75 µg g^{-1} dry weight biomass of microcystin-LR equivalents, while the dry weight biomass of microcystin-LR equivalents measured in the field samples was 11.7 µg g^{-1}.

Discussion

Anabaena ucrainica *bloom and environmental variables*

The low silica concentrations, rather than turbidity observed at Sites 1–5, may have suppressed the growth and dominance of the diatom *Aulacoseira ambigua*. Under these silica stress conditions, it appears that *Anabaena ucrainica* outcompeted *Aulacoseira ambigua*. According to Reynolds et al. (1994), algae such as *Aulacoseira* sp. that succeed in rivers and lakes with high turbidity are considered to have a higher capacity to capture light. These algae can adapt to fast changes in light conditions and also to their intermittent passage through the euphotic zone as

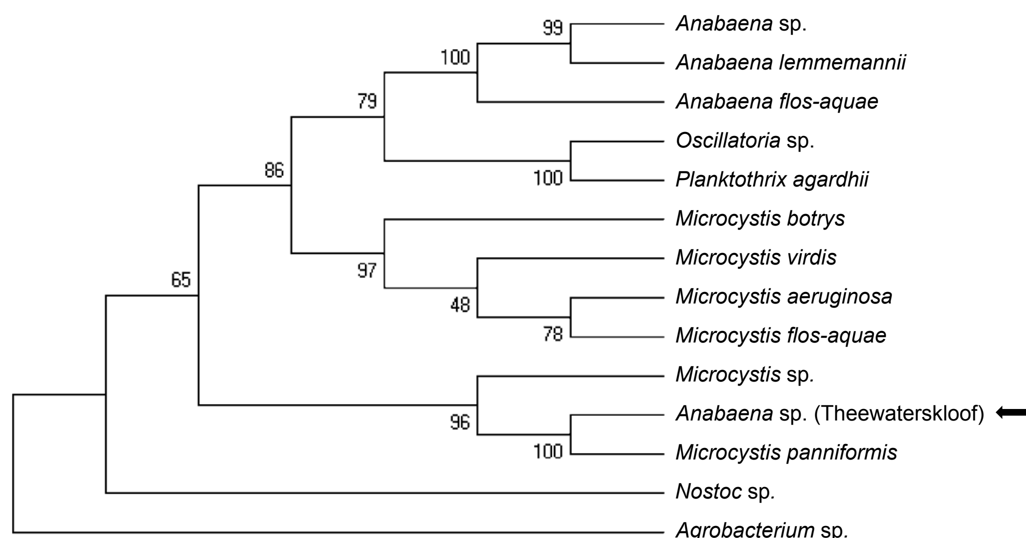


Figure 5: Maximum parsimony analysis tree for cyanobacterial *mcyB* gene sequence showing *Anabaena* sp., *Microcystis* sp., *Nostoc* sp., *Oscillatoria* sp. and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek. The outgroup was *Agrobacterium* sp. Arrow indicates position of the sampled Theewaterskloof strain. The evolutionary history was inferred using the maximum parsimony method. The bootstrap consensus tree inferred from 10 000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10 000 replicates) is shown next to the branches. The MP tree was obtained using the close-neighbour-interchange algorithm with search level 1, in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 14 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 3 551 positions in the final dataset. Evolutionary analyses were conducted in MEGA5

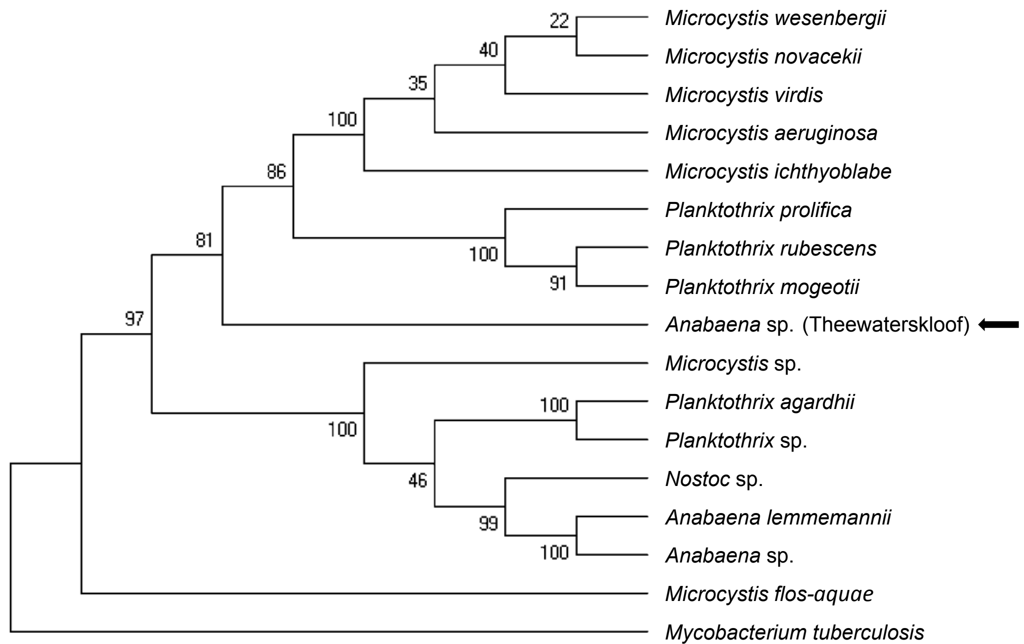


Figure 6: Maximum parsimony analysis tree for cyanobacterial *mcyD* gene sequence showing *Anabaena* sp., *Microcystis* sp., *Nostoc* sp. and *Planktothrix* sp. The outgroup was *Mycobacterium tuberculosis.* Arrow indicates position of the sampled Theewaterskloof strain. The bootstrap consensus tree inferred from 10 000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10 000 replicates) is shown next to the branches. The MP tree was obtained using the close-neighbour-interchange algorithm with search level 1, in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 17 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 5 497 positions in the final dataset. Evolutionary analyses were conducted in MEGA5

they move continuously through distinctive light gradients. *Anabaena* sp. and *Aulacoseira* sp. also occupied opposite ends of the light/nutrient spectrum, as indicated by Lewis (1978). However, turbulent mixing caused by the moderate (>3.5 m s^{-1}) to strong (>6 m s^{-1}) wind regimes during our sampling period may have been one reason for the decline in the net growth of *A. ucrainica.* According to Tsujimura and Okubo (2003), the growth rate of *A. ucrainica* might decrease temporarily under conditions of turbulent mixing, possibly induced by wind. This is supported by a previous study by Reynolds et al. (1983) which showed a reduction in growth of *Anabaena* sp. under a non-stratified regime in enclosure experiments with artificial mixing.

Although we cannot readily explain why the sampled *Anabaena* sp. strains revealed a total absence of akinetes and heterocysts, previous studies have shown that environmental factors such as nutrients, light and temperature affect the formation of akinetes (Herdman 1987; Li et al. 1997). However, turbulence and water-column mixing, which are related to temperature and stratification, have been suggested as playing a major role in the dominance of different cyanobacterial species (Steinberg and Hartmann 1988). According to Oliver and Ganf (2000), stratification leads to decreased nutrient availability in the surface water column, favouring species able to migrate vertically, such as cyanobacteria. Nevertheless, it is known that cyanobacteria are sensitive to turbulence, which leads to the breakage of filaments and decreases in growth, nitrogen fixation and photosynthesis (Moisander and McClinton 2002). According to Reynolds (1984), water-column mixing suppressed net growth of *Anabaena* spp., but the stock persists in the water column, as in the case of our study, when high cell numbers and Chl *a* concentrations were still observed during wind mixing. Furthermore, it is also known that wind-driven hydrodynamics can change the morphology of the filament of *Anabaena* (Becker et al. 2004). Sullivan et al. (2003) reported that filament lengths of *A. circinalis* were related to wind direction, and thus the effect of turbulence may differ among species, and can be greater in larger species. In a laboratory study, Li et al. (1997) showed that temperatures of between 10 and 15 °C induced akinete formation at high frequencies in five cultured planktonic species of *Anabaena*, including *A. ucrainica* strain TAC449. However, in our field study, no akinete or heterocyst formation was observed in *A. ucrainica* strains sampled from the water column with an average temperature of 14.5 °C, suggesting that wind mixing may have been the main reason contributing to the absence of akinete or heterocyst formation, since the presence of the *nifJ* genes for nitrogen fixation was confirmed in the isolated axenic strain.

Classification of the Theewaterskloof* Anabaena *sp. strain

A species is generally defined as a group of strains if they share approximately 70% or more DNA-DNA relatedness

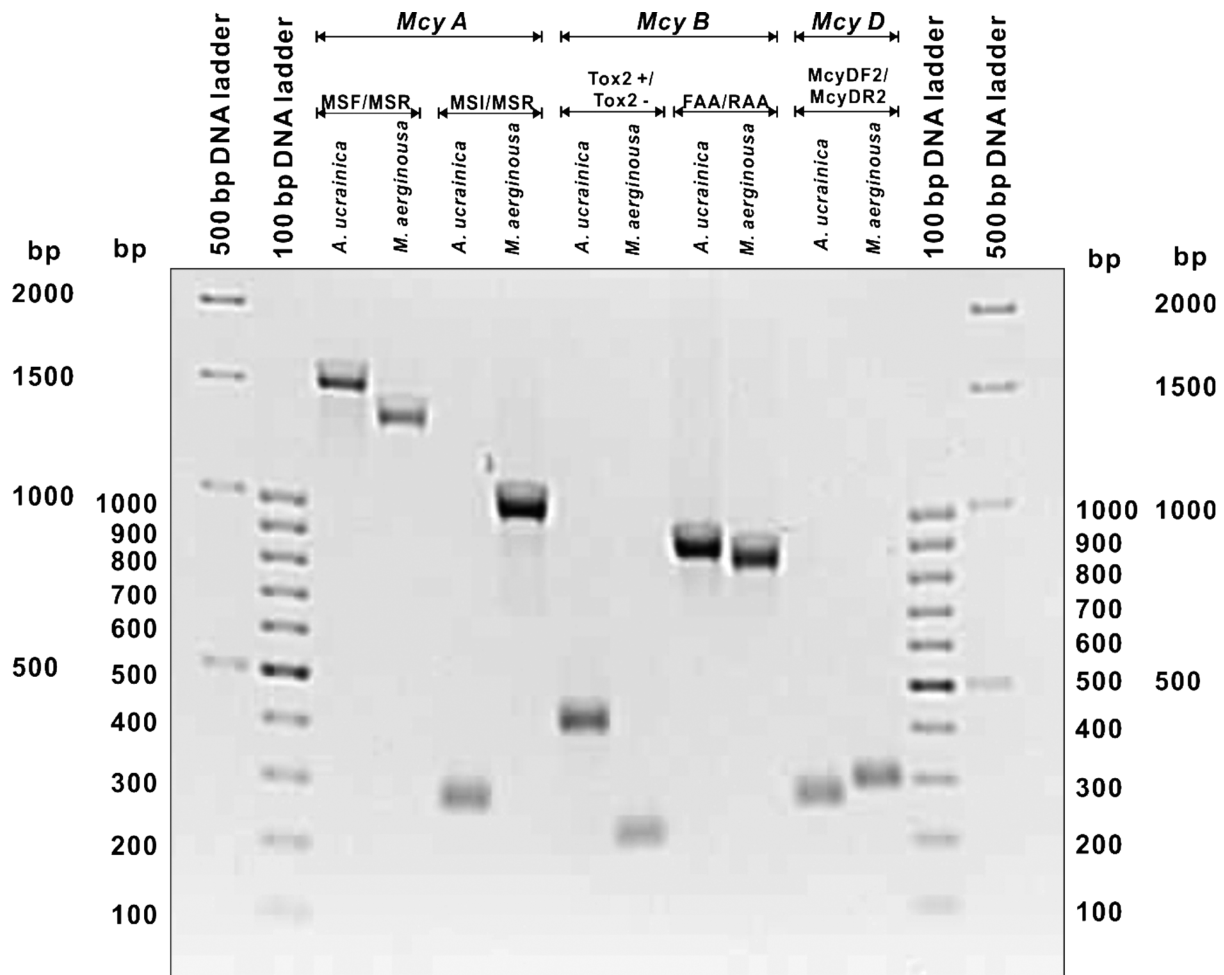


Figure 7: PCR amplicons obtained after amplification of DNA from *Anabaena ucrainica* (Theewaterskloof axenic strain) and *Microcystis aeruginosa.* DNA fragments were electrophoresed on 1% agarose. Lane 1: 500 bp DNA ladder; Lane 2: 100 bp DNA ladder; Lane 3: MSF/MSR primers on *A. ucrainica*; Lane 4: MSF/MSR primers on *M. aeruginosa* control; Lane 5: MSI/MSR primers on *A. ucrainica*; Lane 6: MSI/MSR primers on *M. aeruginosa* control; Lane 7: Tox2+/Tox2– primers on *A. ucrainica*; Lane 8: Tox2+/Tox2– primers on *M. aeruginosa* control; Lane 9: FAA/RAA primers on *A. ucrainica*; Lane 10: FAA/RAA primers on *M. aeruginosa* control; Lane 11: McyDF2/McyDR2 primers on *A. ucrainica*; Lane 12: McyDF2/McyDR2 primers on *M. aeruginosa* control; Lane 13: 100 bp DNA ladder; Lane 14: 500 bp DNA ladder

and 5 °C ΔT_m, and if their phenotypic characteristics agree with this definition (Wayne et al. 1987). The planktonic *Anabaena* sp. isolated from Theewaterskloof Dam shared a sequence identity of more than 96.6% with the *A. ucrainica* sequence deposited in GenBank, suggesting that the *Anabaena* sp. isolated from Theewaterskloof Dam is *A. ucrainica*.

Toxicity of the axenic **Anabaena ucrainica** *strain*

Microcystis spp., *Planktothrix* spp. and *Anabaena* spp. consist of hepatotoxic and non-hepatotoxic strains (Carmichael and Gorham 1981). Dittmann et al. (1999) found that hepatotoxic and non-toxic strains differ in their content of microcystin synthetase (*mcy*) genes that encode specific peptide synthetases, which have been shown to be involved in the production of the toxic heptapeptide microcystin. This 55 kb gene cluster consists of six open reading frames (ORFs) with a mixed nonribosomal peptide synthetase/polyketide synthase nature (*mcyA* to *mcyE* and *mcyG*), and four smaller ORFs, *mcyF* and *mcyH* to *mcyJ*, with putative precursor and tailoring functions (Tillett et al. 2000). It could be demonstrated that the occurrence of *mcy* genes in cells correlates with their ability to synthesise microcystin and, *vice versa*, that microcystin-free cells usually do not contain *mcy* genes (Kurmayer et al. 2002). The data generated in the present study, using an ELISA assay and the PCR amplification of the *mcy* genes, showed that non-ribosomal microcystin synthetase gene *mcyA*, *mcyB* and polyketide synthetase gene *mcyD* were present in the axenic *A. ucrainica* strain isolated from Theewaterskloof Dam, and also confirmed that this strain has the potential to be toxic.

The *A. ucrainica* strain isolated from Theewaterskloof Dam is the first *Anabaena* strain containing microcystin-LR equivalents to be reported from fresh water in Africa. It was also evident that wind mixing may have been the main contributor to the absence of akinete or heterocyst formation in this strain.

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Appendix 1: Oligonucleotides used for PCR analysis

Gene region	Primer	Sequence	T_m (°C)	Fragment size	Source
16S rRNA	16SF	5′-GCGTTATCCGGAATTATTGG-3′	51.4	~600 bp	
	16SR	5′-CCACTAAGAACGAGGGTTGC-3′	55.7		
M13	M13F	5′-GTAAAACGACGGCCAGT-3′	53		
	M13R	5′-CAGGAAACAGCTATGAC-3′	55		
mcyA	MSF	5′-ATCCAGCAGTTGAGCAAGC-3′	59	~1.3 kbp	Tillett et al. (2001)
	MSR	5′-TGCAGATAACTCCGCAGTTG-3′	60		
	MSI	5′-GAGAATTAGGGACACCTAT-3′	48	~1.3 kbp	
	MSR	5′-TGCAGATAACTCCGCAGTTG-3′	60		
mcyB	TOX 2+	5′-AGGAACAAGTTGCACAGAATCCGCA-3′	50	~200 bp	Kaebernick et al. (2002)
	TOX 2−	5′-ACTAATCCCTATCTAAACACAGTAACTCA-3′	50		
	FAA	5′-CTATGTTATTTATACATCAGG-3′	40	~580 bp	Neilan et al. (1999)
	RAA	5′-CTCAGCTTAACTTGATTATC-3′	43		
mcyD	McyDF2	5′-GGTTCGCCTGGTCAAAGTAA-3′	50	~297 bp	Oberholster et al. (2006)
	McyDR2	5′-CCTCGCTAAAGAAGGGTTGA-3′	50		
nifJ-1	NifJF-1F	5′-CGGAATGGTCAGATGCTTGG-3′	55	~155 bp	
	NifJ-1R	5′-CTGGGATGCGGTAAATGTCT-3′	55		
nifJ-2	NifJ-2F	5′-GCGTTGCAAACAGGGTCATT-3′	59	~316 bp	
	NifJ2-R	5′-CGTGGGAGGTACGAAAACCA3′	59		

Appendix 2: Origins and accession numbers of 16S rRNA sequences from phytoplankton used in this study (the accession numbers refer to the assigned National Center for Biotechnology Information [NCBI; Bethesda, Maryland] sequence numbers)

Taxonomic assignment	Strain	Geographical origin	Accession numbers for 16S rRNA
		Cyanobacteria	
Anabaena aphanizomenoides	1LT27S09	Lake Trasimeno, Italy	FM177473
A. augstumalis	SCMIDKE JAHNKE/4a	Rostock, Germany	AJ630458
A. azotica	LCRSM-1	India	JN705716
A. bergii	AB2008/43	Lake Kinneret, Israel	FR822615
A. bergii	AB2008/44	Lake Kinneret, Israel	FR822616
A. bergii	AB2008/45	Lake Kinneret, Israel	FR822617
A. bergii	AB2010/02	Lake Kinneret, Israel	FR822618
A. bergii	AB2010/03	Lake Kinneret, Israel	FR822619
A. bergii	AB2010/07	Lake Kinneret, Israel	FR822622
A. bergii	AB2010/08	Lake Kinneret, Israel	FR822623
A. bergii		Australia	AF160256
A. cf. *circinalis macrospora*	0tu25s6	Lake Tuusulanjarvi, Finland	AJ630412
A. cf. *circinalis macrospora*	1tu23s3	Lake Tuusulanjarvi, Finland	AJ630408
A. cf. *circinalis macrospora*	1tu26s10	Lake Tuusulanjarvi, Finland	AJ630409
A. cf. *circinalis macrospora*	1tu27s5	Lake Tuusulanjarvi, Finland	AJ630410
A. cf. *circinalis macrospora*	1tu28s13	Lake Tuusulanjarvi, Finland	AJ630411
A. cf. *crassa*	1tu27s7	Lake Tuusulanjarvi, Finland	AJ630413
A. cf. *cylindrica*	133	France	AJ293110
A. cf. *scheremetievi*	PMC9701	France	AJ293117
A. circinalis	1tu30s11	Lake Tuusulanjarvi, Finland	AJ630416
A. circinalis	1tu33s12	Lake Tuusulanjarvi, Finland	AJ630417
A. circinalis	1tu34s5	Lake Tuusulanjarvi, Finland	AJ630415
A. circinalis	123	France	AJ293103
A. compacta	ANACOM-KOR	Hnivkovice-Kooensko, Czech Republic	AJ630418
A. compacta	189	France	AJ293111
A. cylindrica	XP6B	Gulf of Finland, Baltic Sea, Finland	AJ630414
A. cylindrica		Australia	AF091150
A. doliolum	Ind2	Varanasi, Uttar Pradesh, India	GU563894
A. eucompacta	CHAB464	China	GU197627
A. eucompacta	CHAB915	China	GU197626
A. eucompacta	CHAB923	China	GU197628
A. fertilissima	LCRSM-NDa	Varanasi, Uttar Pradesh, India	JN705712
A. flos-aquae	0tu33s2a	Lake Tuusulanjarvi, Finland	AJ630421
A. flos-aquae	0tu33s15	Lake Tuusulanjarvi, Finland	AJ630420
A. flos-aquae	1tu30s4	Lake Tuusulanjarvi, Finland	AJ630422
A. flos-aquae	1tu31s11	Lake Tuusulanjarvi, Finland	AJ630419
A. flos-aquae	1tu35s12	Lake Tuusulanjarvi, Finland	AJ630423
A. flos-aquae	PCC 9302	France	AY038032
A. lemmermannii	1tu32s11	Lake Tuusulanjarvi, Finland	AJ630424
A. mendotae	57	France	AJ293107
A. mucosa	1tu35s5	Lake Tuusulanjarvi, Finland	AJ630425
A. oryzae	Ind 4	Varanasi, Uttar Pradesh, India	JN197410
A. oscillarioides	BECID22	Gulf of Finland, Baltic Sea, Finland	AJ630426
A. oscillarioides	BECID32	Gulf of Finland, Baltic Sea, Finland	AJ630427
A. oscillarioides	BO HINDAK 1984/43	Canada	AJ630428
A. oumiana	CHAB502	China	GU197612
A. oumiana	CHAB508	China	GU197614
A. oumiana	CHAB1036	China	GU197635
A. planctonica	1tu28s8	Lake Tuusulanjarvi, Finland	AJ630430
A. planctonica	1tu30s13	Lake Tuusulanjarvi, Finland	AJ630431
A. planctonica	1tu33s8	Lake Tuusulanjarvi, Finland	AJ630432
A. planctonica	1tu33s10	Lake Tuusulanjarvi, Finland	AJ630429
A. planctonica	1tu36s8	Lake Tuusulanjarvi, Finland	AJ630433
A. planctonica	2LT27S01	Lake Tuusulanjarvi, Finland	FM177474
A. siamensis	TISTR 8012	Thailand	JQ657825
A. sigmoidea	0tu36s7	Lake Tuusulanjarvi, Finland	AJ630434
A. sigmoidea	0tu38s4	Lake Tuusulanjarvi, Finland	AJ630435
A. smithii	1tu39s8	Lake Tuusulanjarvi, Finland	AJ630436
A. solitaria f. *zinserlingii*	2LT27S03	Lake Trasimeno, Umbria, Italy	FM177475
A. solitaria f. *zinserlingii*	2LT27S04	Lake Trasimeno, Umbria, Italy	FM177476
A. solitaria	82	France	AJ293105

Appendix 2: (cont.)

Taxonomic assignment	Strain	Geographical origin	Accession numbers for 16S rRNA
Anabaena sp.	0tu37s9	Lake Tuusulanjarvi, Finland	AJ630438
Anabaena sp.	0tu39s7	Lake Tuusulanjarvi, Finland	AJ630439
Anabaena sp.	1LT27S02	Lake Trasimeno, Umbria, Italy	FM177477
Anabaena sp.	1LT27S03	Lake Trasimeno, Umbria, Italy	FM177478
Anabaena sp.	1tu34s7	Lake Tuusulanjarvi, Finland	AJ630437
Anabaena sp.	14	Finland	AJ133152
Anabaena sp.	66A	Finland	AJ133157
Anabaena sp.	90	Finland	AJ133156
Anabaena sp.	299	France	AJ293106
Anabaena sp.	A202A1	Finland	AJ133159
Anabaena sp.	A277	Finland	AJ133160
Anabaena sp.	Baldwin Park Type 2	Australia	JQ811777
Anabaena sp.	Baldwin Park Type 3	Australia	JQ811778
Anabaena sp.	Buayanup Drain Type 2	Australia	JQ811781
Anabaena sp.	Chelodina Reserve Type 1	Australia	JQ811782
Anabaena sp.	GS-4	Australia	JQ811786
Anabaena sp.	GS-6	Australia	JQ811789
Anabaena sp.	GS-7	Australia	JQ811790
Anabaena sp.	Ind7	Varanasi, Uttar Pradesh, India	JN197411
Anabaena sp.	DB14a	Noonmati Refinery, Guwahati, India	JQ894522
Anabaena sp.	DB24a	Noonmati Refinery, Guwahati, India	JQ894528
Anabaena sp.	KUAC 3903d	Khaldiyah, Kuwait	HQ533199
Anabaena sp.	LCRSM-243	India	JN705709
Anabaena sp.	PCC 7108	India	AJ133162
Anabaena sp.	Vasse River Type 1B	Australia	JQ811796
Anabaena sp.	Vasse River Type 2	Australia	JQ811797
A. sphaerica f. *conoidea*	1LT27S01	Lake Trasimeno, Umbria, Italy	FM177480
A. sphaerica f. *conoidea*	2LT27S09	Lake Trasimeno, Umbria, Italy	FM177479
A. spiroides	1tu39s17	Lake Tuusulanjarvi, Finland	AJ630440
A. spiroides	PMC970	France	AJ293118
A. ucrainica	CHAB1431	China	GU197643
A. ucrainica	CHAB1432	China	GU197649
A. ucrainica	CHAB1433	China	GU197640
A. ucrainica	CHAB1434	China	GU197644
A. ucrainica	CHAB2152	China	GU197651
A. ucrainica	CHAB2154	China	GU197645
A. ucrainica	CHAB2161	China	GU197647
A. ucrainica		Theewaterskloof Dam, South Africa	
A. variabilis	a	USA	CP000117
A. variabilis	b	USA	CP000117
A. variabilis	c	USA	CP000117
A. variabilis	d	USA	CP000117
Cylindrospermopsis raciborskii	1LT32S01	Lake Trasimeno, Umbria, Italy	FM177492
C. raciborskii	05E	Australia	AF516732
C. raciborskii	Brazil 1	Brazil	AF516733
C. raciborskii	Florida 1	Florida, USA	AF516746
C. raciborskii	T3	Norway	EU439566
Microcystis aeruginosa	2LT25S03	Lake Trasimeno, Umbria, Italy	FM177496
M. aeruginosa	2LT27S08	Lake Trasimeno, Umbria, Italy	FM177497
M. aeruginosa	rRNA-a NIES-843	Japan	NC_010296
M. aeruginosa	rRNA-b NIES-843	Japan	NC_010296
M. aeruginosa	MCYS-LB01	Lebna Dam, Tunisia	EU541970
M. aeruginosa	MCYS-LB02	Lebna Dam, Tunisia	EU541971
M. aeruginosa	PCC7806	Australia	AF139299
M. aeruginosa	PCC7820	Australia	AF139300
M. aeruginosa	UWOCC 001	Australia	AF139292
M. aeruginosa	UWOCC 006	Australia	AF139293
M. aeruginosa	UWOCC 017	Australia	AF139294
M. aeruginosa	UWOCC 019	Australia	AF139295
M. aeruginosa	UWOCC 023	Australia	AF139296
M. aeruginosa	UWOCC 024	Australia	AF139297
M. aeruginosa	UWOCC 026	Australia	AF139298
M. aeruginosa	UWOCC 84/1	Australia	AF139301

Appendix 2: (cont.)

Taxonomic assignment	Strain	Geographical origin	Accession numbers for 16S rRNA
M. aeruginosa	UWOCC AK(GV-)	Australia	AF139302
M. aeruginosa	UWOCC AK-1	Australia	AF139303
M. aeruginosa	UWOCC AubB1	Australia	AF139304
M. aeruginosa	UWOCC Bauld5A	Australia	AF139305
M. aeruginosa	UWOCC C1	Australia	AF139315
M. aeruginosa	UWOCC C4	Australia	AF139316
M. aeruginosa	UWOCC C5	Australia	AF139319
M. aeruginosa	UWOCC E7	Australia	AF139326
M. aeruginosa	UWOCC M4	Australia	AF139318
M. aeruginosa	UWOCC MR-A	Australia	AF139320
M. aeruginosa	UWOCC MR-B	Australia	AF139321
M. aeruginosa	UWOCC MR-C	Australia	AF139323
M. aeruginosa	UWOCC MR-D	Australia	AF139324
M. aeruginosa	UWOCC MSU28-2	Australia	AF139313
M. aeruginosa	UWOCC P3	Australia	AF139314
M. aeruginosa	UWOCC RID-1	Australia	AF139317
M. aeruginosa	UWOCC S-15-b	Australia	F139325
M. aeruginosa	UADFM1	Vela Lake, Western Central Portugal	HM854730
M. aeruginosa	UADFM2	Vela Lake, Western Central Portugal	HM854731
M. aeruginosa	UADFM3	Vela Lake, Western Central Portugal	HM854732
M. aeruginosa	UADFM7	Vela Lake, Western Central Portugal	HM854736
M. flos-aquae	UWOCC C2	Australia	AF139328
M. flos-aquae	UWOCC C3	Australia	AF139329
M. flos-aquae	UWOCC N	Australia	AF139327
M. ichthyoblabe	2LT25S02	Lake Trasimeno, Umbria, Italy	FM177498
M. ichthyoblabe	2LT25S04	Lake Trasimeno, Umbria, Italy	FM177499
M. novacekii	MCYS-CH01	Algeria	EU541973
M. panniformis	MCYS-OB06	Algeria	EU541974
Microcystis sp.	1tu31s06	Lake Tuusulanjarvi, Finland	AM259270
Microcystis sp.	UADFM4	Vela Lake, Western Central Portugal	HM854733
Microcystis sp.	UADFM8	Vela Lake, Western Central Portugal	HM854737
Microcystis sp.	UADFM10	Vela Lake, Western Central Portugal	HM854738
Microcystis sp.	UWOCC BauldB	Australia	AF139306
Microcystis sp.	UWOCC BauldE	Australia	AF139312
Microcystis sp.	UWOCC CBS	Australia	AF139307
Microcystis sp.	UWOCC F	Australia	AF139308
Microcystis sp.	UWOCC K	Australia	AF139309
Microcystis sp.	UWOCC MSU28-1	Australia	AF139310
Microcystis sp.	UWOCC Q	Australia	AF139311
M. viridis	UADFM5	Vela Lake, Western Central Portugal	HM854734
M. viridis	UADFM6	Vela Lake, Western Central Portugal	HM854735
M. wesenbergii	MCYS-LB03	Tunisia	EU541972
Nostoc azollae	0708a		NC_014248
N. azollae	0708b		NC_014248
N. azollae	0708c		NC_014248
N. azollae	0708d		NC_014248
N. calcicola	III	Ceske Budejovice, Czech Republic	AJ630447
N. calcicola	VI	Dobre Pole, Czech Republic	AJ630448
N. commune	KUAC 3906d	Khaldiyah, Kuwait	HQ533202
N. edaphicum	X	Chelcice, Czech Republic	AJ630449
N. ellipsosporum	V	Nezamyslice, Czech Republic	AJ630450
N. entophytum	LCRSM-4	India	JN705719
N. muscorum	I	Dlouha Ves, Czech Republic	AJ630451
N. muscorum	II	Jevany, Czech Republic	AJ630452
N. muscorum	Ind34	Varanasi, Uttar Pradesh, India	JN197415
Nostoc sp.	1tu14s8	Lake Tuusulanjarvi, Finland	AJ630453
Nostoc sp.	2LT05S03	Lake Trasimeno, Umbria, Italy	FM177500
Nostoc sp.	HKAR-2	Bihar, Rajgir, India	FJ939126
Nostoc sp.	PCC 7120a	Japan	BA000019
Nostoc sp.	PCC 7120b	Japan	BA000019
Nostoc sp.	PCC 7120c	Japan	BA000019
Nostoc sp.	PCC 7120d	Japan	BA000019

Appendix 2: (cont.)

Taxonomic assignment	Strain	Geographical origin	Accession numbers for 16S rRNA
N. spongiaeforme	Ind41	India	GU563895
Oscillatoria bonnemaisonii	LC4_3	La Saline, La Réunion Island (south-western Indian Ocean), France	GQ251524
O. boryana	NTDM11	India	JQ867393
O. margaritifera	NAC8-54	USA	GU724207
O. margaritifera	NAC8-55	USA	GU724208
O. miniata	NAC8-50	USA	GU724203
O. rosea	ZDa	Urla-Gulbahce, Turkey	HQ916863
O. salina	simbu3	India	JQ258938
O. subbrevis	SGBRA04	India	JQ083642
O. tenuis	CCAP 1459/4PCC 9107	Italy	FN813345
Planktothrix agardhii	No260	Austria	EU266178
P. agardhii	No277	Austria	EU266176
P. pseudagardhii	2LT34S02	Lake Trasimeno, Umbria, Italy	FM177501
P. rubescens	No161	Austria	EU266168
P. rubescens	No169	Austria	EU266170
P. rubescens	No178	Austria	EU266172
Pseudanabaena constantiae		Germany	DQ393595
Pseu. limnetica	0LI36S3	Luxembourg	DQ264245
Pseu. mucicola	PMC268.06	France	GQ859642
Pseu. mucicola	PMC269.06	France	GQ859643
Pseu. mucicola	PMC279.0	France	GQ859644
Pseu. schmidlei	BDU 20761	India	EF544989
Pseu. sp.	0tu30s18	Lake Tuusulanjarvi, Finland	AM259268
Pseu. sp.	1tu24s9	Lake Tuusulanjarvi, Finland	AM259269
Pseu. tremula	UTCC 471	USA	AF218371
		Green algae	
Chlamydomonas reinhardtii	CC-1342 mt-	Canada	FJ458259
C. reinhardtii	CC-1952 S1 C5 mt-	Canada	FJ458258
C. reinhardtii	CC-2343 mt+	Canada	FJ458260
C. reinhardtii	CC-2344 mt+	Canada	FJ458261
C. reinhardtii	CC-2931 mt-	Canada	FJ458262
C. reinhardtii	CC-503 cw92 mt+	Canada	FJ458256