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ORIGINAL PAPER

Diversity of *Microcystis aeruginosa* isolates (Chroococcales, Cyanobacteria) from East-African water bodies

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Abstract With exception of South Africa, very little is known about the presence and abundance of toxic cyanobacteria and cyanobacterial blooms on the African continent. The close proximity between society and nature, and the use of the sparse water resources as drinking water in large parts of Africa, lead to the recognition that more knowledge on toxic cyanobacterial blooms is of major importance. The bloom forming cyanobacterium *Microcystis aeruginosa* is known to produce cyclic heptatoxins (microcystins) which can be toxic to humans. In this study the morphological, genetic, and chemical characters of 24 strains of *M. aeruginosa* from several water bodies in Kenya and Uganda, some of them used as drinking water sources, were examined. The *M. aeruginosa* strains possessed different levels of diversity depending on characterisation method. Four morphotypes were identified based on the traditional morphological approach, 10 genotypes by DNA sequence comparison of the PC-IGS and ITS1 rDNA regions, and 10 chemotypes based on MALDI-TOF-MS oligopeptide analysis. Only 4 of the 24 isolated strains from East Africa were found to produce microcystins, while oligopeptides belonging to the aeruginosin and cyanopeptolin class were detected in most strains.

Keywords *Microcystis aeruginosa* · Microcystin · Morphology · ITS1-rDNA · PC-IGS · Phylogeny · Matrix-assisted laser desorption/ionization time-of-flight MALDI-TOF mass spectrometry · ELISA

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Introduction

Cyanobacteria of the genus *Microcystis* are among the most commonly reported bloom-forming species in lakes and reservoirs worldwide (e.g. Charmichael 1996; Codd et al. 1999). *Microcystis aeruginosa* is known to produce potent hepatotoxic metabolites, called microcystins, which can cause considerable hazards to aquatic ecosystems, domestic as well as wild animals, and human health (Christoffersen 1996; Krienitz et al. 2003; Bell and Codd, 1994; Kuiper-Goodman et al. 1999). Microcystins are a group of closely related cyclic heptapeptides sharing a common structure and varying by the degree of methylation, amino acid sequence, and toxicity (Carmichael et al. 1988). Microcystins inhibit protein phosphatases (Kuiper-Goodman et al. 1999) and have been linked to liver cancer in humans (Humpage and Falconer 1999). More than 70 structural

variants of microcystins are known to date. Due to the toxic properties of microcystins, the World Health Organization has provided a guideline value for the maximum allowable concentration in drinking water of 1 μg l^{-1} (WHO 1998). In addition to the microcystins, various other linear and cyclic oligopeptides are found within *M. aeruginosa* and they show a variety of bioactivities (Namikoshi and Rinehart 1996).

Microcystis aeruginosa has a cosmopolitan distribution (Komárek and Anagnostidis 1999) and is one of the most studied genera of cyanobacteria, and broad attention has been given to the advances in knowledge of its identification, toxicity, and diversity. However, no consistent relationship has been found between morphology, genetic characterisation, and geographical origin (Bittencourt-Oliveira 2001, Tillett et al. 2001, Janse et al. 2004, Wilson et al. 2005). Very little is known on the occurrence of *M. aeruginosa* and microcystins in African waters. A recent UNESCO survey concludes that the overall knowledge on the occurrence of cyanobacteria and cyanobacterial blooms in the vast continent of Africa, with exception of Southern Africa, Kenya, Ethiopia and Morocco, is poor (Codd et al. 2005). Several important water bodies are situated in East Africa, including Lake Victoria and several larger and smaller lakes in the Great Rift Valley. These lakes, which include some of the oldest, largest, and deepest lakes in the world, form a freshwater eco-region of great biodiversity and play an important role in the livelihood of the local people (Bootsma and Hecky 2003). Many questions regarding blooms of *Microcystis* in these African waters are still to be answered, including which species of *Microcystis* are present and to which extent they produce microcystins.

Traditionally, *M. aeruginosa* have been classified on the basis of morphological characteristics such as cell size and shape, colony shape and structure, and the presence and thickness of mucilage (Komárek and Anagnostidis 1999). However, the differentiation within the species is difficult as a large variety of morphotypes occurs (Otsuka et al. 2000). Problems in the application of morphological criteria for classification of *M. aeruginosa* in field samples arise from variation in response to environmental changes (Dor and Hornoff 1985). Further, strains of *M. aeruginosa* tend to change their morphological characteristics when isolated and subjected to different culture conditions (e.g. Krüger et al. 1981; Komárek 1991; Otsuka et al. 2000). Molecular genetic approaches have been applied in order to resolve relationships between cyanobacteria. Variable non-coding regions like the internal transcribed spacer region (ITS) of the rDNA or the intergenic spacer (IGS) of the phycocyanin (PC) operon between the bilin subunit genes *cpc*B and *cpc*A have been introduced for the purpose of delineating inter- and intraspecific variations within *M. aeruginosa* (Neilan et al. 1995; Boyer et al. 2001). The diversity of *M. aeruginosa* can also be related to the production of bioactive peptides. The majority of peptides found to be produced by *M. aeruginosa* can be grouped into six classes (Welker et al. 2004). However, the total diversity of peptide compounds occurring in *M. aeruginosa* is probably not recognised by the number of already known peptides. The peptide diversity can be quantitatively detected by using the technique of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and chemotypes of *M. aeruginosa* can be defined (Fastner et al. 2001; Welker et al. 2004).

The aim of this study was to investigate the morphological, genetic, and chemical diversity among strains of *M. aeruginosa* isolated from different water bodies in the East-African countries Uganda and Kenya. Twenty-four strains of *M. aeruginosa* were isolated and morphologically examined. Further, the strains were genetically characterised using the PC-IGS nucleotide sequence and flanking regions of the genes *cpc*B and *cpc*A and the internal transcribed spacer region (ITS1) of the rDNA. These sequences were compared to sequences of *Microcystis* sp. from all continents obtained from DNA databases, in order to study the overall genetic variation and reveal possible geographical differences. The presence of microcystins and other oligopeptides in the African strains was determined by using MALDI-TOF MS. The strains were also analysed for microcystin content by ELISA. To our knowledge, this is the first study of this kind including strains of *M. aeruginosa* from East Africa.

Materials and methods

Strain isolation and culture conditions

The *M. aeruginosa* strains were isolated from water samples from eight different Ugandan and Kenyan water bodies (Table 1). The cyanobacterial strains were isolated either by capillary isolation, agar plate spreading or by serial dilution, and placed in capped tubes containing 10 ml culture medium. The growth medium used for isolation and cultivation of all Ugandan *M. aeruginosa* strains was the Z8 medium (Kotai 1972). The Kenyan *M. aeruginosa* stains were isolated and grown in BG-11 medium (Rippka et al. 1979). All strains included in this study were maintained at 18°C and illuminated under a 12 h:12 h light–dark cycle with an average photon flux density of 20 μmol photons m^{-2} s^{-1}. The strains were not axenic.

Table 1 *Microcystis aeruginosa* strains from water bodies in Uganda and Kenya used in this study

Strain	Geographic origin	Year of isolation
NIVA-CYA 431	Murchison Bay, Uganda[a]	2000
NIVA-CYA 432	Murchison Bay, Uganda[a]	2000
NIVA-CYA 433	Murchison Bay, Uganda[a]	2000
NIVA-CYA 463	Murchison Bay, Uganda[a]	2002
NIVA-CYA 464	Murchison Bay, Uganda[a]	2002
NIVA-CYA 465	Murchison Bay, Uganda[a]	2002
NIVA-CYA 475	Murchison Bay, Uganda[a]	2003
NIVA-CYA 476	Murchison Bay, Uganda[a]	2004
NIVA-CYA 477	Murchison Bay, Uganda[a]	2003
NIVA-CYA 478	Murchison Bay, Uganda[a]	2004
NIVA-CYA 482	Lake Mburo, Uganda[b]	2004
NIVA-CYA 495	Kazinga Channel, Uganda[c]	2004
NIVA-CYA 496	Kazinga Channel, Uganda[c]	2004
NIVA-CYA 497	Kazinga Channel, Uganda[c]	2004
NIVA-CYA 502	Murchison Bay, Uganda[a]	2003
NIVA-CYA 503	Murchison Bay, Uganda[a]	2003
NIVA-CYA 522	Murchison Bay, Uganda[a]	2004
AB2002/21	Nakuru final sewage pond, Kenya[d]	2002
AB2002/22	Kenyatta University sewage pond, Kenya[e]	2002
AB2002/23	Lake Baringo, Kenya[f]	2002
AB2002/24	Pilsner Pond, Kenya[g]	2002
AB2002/40	Lake Naivasha, Kenya[h]	2002
AB2002/52	Lake Baringo, Kenya[f]	2002
AB2002/55	Kenyatta University sewage pond, Kenya[e]	2002

[a] 00°14,48′N 32°38,59′E
[b] 00°39,36′S 30°56,03′E
[c] 00°10,16′S 29°59,23′E
[d] 00°18,21′S 36°05,72′E
[e] 01°11,64′S 36°57,86′E
[f] 00°36,79′N 36°01,40′E
[g] 00°19,02′S 36°08,32′E
[h] 00°48,92′S 36°18,90′E

Morphological characteristics

All strains were examined using a Nikon Optiphot 2 light microscope (Nikon, Tokyo, Japan) and classified to species level according to Komárek and Anagnostidis (1999). Mean cellular diameter was determined ($n = 30$ cells) for each strain, measuring to the nearest 0.5 μm at ×1,000 magnification. Photomicrographs of cells and colonies were taken with a Kodak DC 290 Digital Camera (Kodak Company, Rochester, NY, USA) attached to the microscope.

Oligopeptide chemotyping with MALDI-TOF MS

Oligopeptide chemotyping is a powerful and well-established tool for studies into cyanobacterial diversity (Fastner et al. 2001; Welker et al. 2002, 2004). Like other freshwater cyanobacteria, *M. aeruginosa* produces a vast number of oligopeptides and peptide-like compounds. The cellular oligopeptide composition is strain-specific, genetically determined and not a function of extracellular stimuli (Welker and von Döhren 2006). In the present study, cellular oligopeptide patterns were therefore used as chemical fingerprints to group *M. aeruginosa* strains.

Around 10 mg of lyophilised strain material was extracted with 100 μl of 50% methanol with 0.01% trifluoroacetic acid. This mixture was sonicated for 10 min and left for 1 h. One microlitre of the extract was directly prepared onto a stainless steel template and immediately 1 μl of matrix (10 mg of 2.5-dihydoxybenzoic acid per ml in water, ethanol and acetonitrile [1:1:1] with 0.03% trifluoroacetic acid) was added.

MALDI-TOF MS analyses were performed on a Voyager-DE PRO Biospectrometry workstation (Applied Biosystems) equipped with a reflectron, post-source decay (PSD) and collision-induced dissociation (CID) options as described previously (Fastner et al. 2001; Welker et al. 2002). Positive ion mass spectra were recorded, and mass signals were compared with a database of known cyanobacterial metabolites. High-intensity peaks of known or unknown masses were further analysed by recording post-source decay (PSD) fragment spectra. Peptides were identified by the presence of immonium ions of amino acids in the fragment spectra. Assignment to known classes of cyanobacterial oligopeptides was done on the basis of class-specific fragments such as the typical Adda-fragment for microcystins (m/z 135). Further information on the identity of oligopeptides was gained from the comparison with published fragmentation data from pure substances and from a fragment database. Each *M. aeruginosa* strain was analysed three times to ensure reproducibility of the results.

ELISA microcystin analysis

The cyanobacterial strains were analysed for microcystins by Abraxis Microcystin ELISA kit (Abraxis LLC, Warminister, PA, USA) following the instructions of the manufacturer. The test is an indirect competitive ELISA and allows a congener-independent detection of all microcystins based on recognition by specific antibodies. Before the analysis, 10 ml of dense culture material from each cyanobacterial strain was frozen and thawed three times to extract the toxins. The colour reaction of the ELISA test was evaluated by a microtiterplate reader (Thermo Max, Molecular Devices, Sunnyvale, CA, USA) at 450 nm wavelength.

DNA extraction, PCR amplification, and sequencing

Fifty millilitres of culture material were centrifuged, and total genomic DNA was extracted and purified using Dynabeads DNA DIRECT System I (Deutsche Dynal GmbH, Hamburg, Germany). The DNA was loaded onto an agarose gel (0.8 %) to check the quality.

PCR was performed in a PTC-200 Peletier Thermal Cycler (MJ Research, Watertown, USA) using primers PCβ-F and PCα-R for amplification of PC-IGS (Neilan et al. 1995) and primers 322 and 340 for ITS rDNA (Iteman et al. 2000). The PCR amplifications for the PC-IGS were carried out in a final volume of 50 µl containing 5 µl of 10× PCR reaction buffer, 5 µl of 25 mM $MgCl_2$ (Qiagen, Hilden, Germany), 5 µl of 2 mM dNTP, 1 µl of 10 µM of each primer, 0.2 µl of 5 U *Taq* DNA polymerase (Qiagen) and 1 µl DNA and 31.8 µl water. The amplification was performed with an initial denaturation step of 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, followed by a final extension step at 72°C for 10 min. ITS1 amplification was performed as for PC-IGS, but with an initial denaturation step of 97°C for 5 min, followed by 30 cycles of 97°C for 15 s, 48°C for 30 s and 72°C for 1 min, followed by a final extension step at 72°C for 5 min. PCR products were visualised by 1.5% gel electrophoresis in TAE buffer according to standard protocols (Sambrook et al. 1989). Gels were visualised and photographed in an AlphaImager 2200 MultiImage Light Cabinet (Biozyme Diagnostik GmbH, Oldendorf, Germany).

PCR products for sequencing were purified through Qiaquick PCR purification columns (Qiagen), and DNA was redissolved in elution buffer according to the manufacturer's protocol. Four microlitres of purified DNA were sequenced with Big Dye Terminator Cycle Sequencing Ready Reaction Kit 3.1 (Applied Biosystems, Darmstadt, Germany) as described in the user guide of the kit. Both strands were sequenced with an ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems, Darmstadt, Germany), and the primers used for sequencing were the same as used for the amplification.

Sequence alignment and phylogenetic analysis

The DNA sequences were edited in BioEdit sequence alignment Editor Version 5.0.6 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Each sequence was compared with EMBL entries using Basic Local Alignment Tool (BLAST) in order to select reference sequences and to obtain a preliminary affiliation of the sequences. Sequences of *Microcystis* were aligned by using the Clustal W option in BioEdit and the sequence alignment was corrected manually. Gaps and highly variable and ambiguous positions where a proper alignment was impossible were excluded from the alignments. MrModeltest (Nylander 2002) based on Modeltest (Posada and Crandall 1998) was used to find the best substitution model for each alignment.

Phylogenetic trees were constructed by using the maximum likelihood method in PHYML (Guindon 2003), by the neighbour-joining method on Jukes and Cantor pairwise distances using PAUP* 4.0 (Swofford 1998) and by MrBayes v3.0 (Huelsenbeck and Ronquist 2001). In the PHYML-analyses, both proportion of invariable sites and the gamma distribution parameter were estimated. The number of categories was set to 4. In the MrBayes analysis the G distribution of site rates was approximated using four rate categories with equal probability. Other prior settings were set to default values. The Markov chain Monte-Carlo (MCMC) chains lasted for 10,000,000 generations, and trees were saved each 100 generations. After burn-in, which was based on visual inspection of the stationary phase of the MCMC chains, the remaining trees were used for calculating the consensus tree and posterior probability values. In the case of PHYML and MrBayes, the parameter values for the best substitution model were imported from Mr. Modeltest. The trees were statistically evaluated with non-parametric bootstrap-analysis (number of replicates = 1,000), and bootstrap values for each phylogenetic method were given at the nodes. Sequences of *Microcystis wesenbergii* NIES 111 for PC-IGS (AF385390) and ITS1 (AB015388) were used as outgroups in the PC-IGS trees and the ITS1 tree, respectively. The sequence data were submitted to the EMBL database and the accession numbers are listed in Table 2.

Results

Morphological characterisation

Twenty-four strains of *Microcystis* sp. were isolated from different water bodies in Kenya and Uganda. All strains were confirmed to the taxonomic descriptions of *Microcystis aeruginosa* (Kützing) Kützing. The colonial characteristics are shown in Fig. 1, and the morphological characteristics of the cyanobacterial strains in culture are listed in Table 2. All strains presented spherical cells with gas vesicles, and the cell diameter ranged from 3.7 to 6.0 µm. Over time, some strains lost the ability to form colonies in culture. We defined four

Table 2 Morphological, chemical and genetic properties of *Microcystis aeruginosa* strains from waterbodies in Uganda and Kenya

Strain	Cell diameter (μm), mean (SD)	Morpho type[a]	Chemo type	Geno type[b]	Accession number	
					ITS1	PC-IGS
NIVA-CYA 431	5.2 (0.3)	M3+	C1	G1	AM421549	AM048614
NIVA-CYA 432	5.2 (0.4)	M3+	C1	G1	AM421550	AM421573
NIVA-CYA 433	5.1 (0.3)	M3+	C2	G1	AM421551	AM421574
NIVA-CYA 463	4.1 (0.2)	M2+	C3	G2	AM421552	AM421575
NIVA-CYA 464	4.1 (0.2)	M2+	C3	G2	AM421553	AM421576
NIVA-CYA 465	4.4 (0.3)	M2−	C3	G2	AM421554	AM048615
NIVA-CYA 475	4.1 (0.2)	M2−	C4	G2	AM421555	AM048616
NIVA-CYA 476	4.5 (0.4)	M2+	C5	G3	AM421556	AM048620
NIVA-CYA 477	3.7 (0.3)	M1+	C5	G4	AM421557	AM048621
NIVA-CYA 478	4.2 (0.2)	M2−	C6	G2	AM421558	AM048617
NIVA-CYA 482	4.2 (0.3)	M2+	C1	G5	AM421559	AM421577
NIVA-CYA 495	4.2 (0.3)	M2+	C7	G6	AM421560	AM421578
NIVA-CYA 496	4.2 (0.3)	M2+	C7	G6	AM421561	AM421579
NIVA-CYA 497	4.5 (0.3)	M2+	C1	G7	AM421562	AM421580
NIVA-CYA 502	4.1 (0.2)	M2+	C8	G2	AM421563	AM048618
NIVA-CYA 503	4.2 (0.3)	M2+	C8	G2	AM421564	AM048619
NIVA-CYA 522	4.2 (0.3)	M2+	C1	G2	AM421565	AM421581
AB2002/21	4.0 (0.2)	M2+	C4	G8	AM421566	AM421582
AB2002/22	4.1 (0.2)	M2+	C4	G8	AM421567	AM421583
AB2002/23	4.2 (0.3)	M2+	C1	G2	AM421568	AM421584
AB2002/24	6.0 (0.4)	M4+	C9	G9	AM421569	AM421585
AB2002/40	4.4 (0.4)	M2+	C10	G10	AM421570	AM421586
AB2002/52	4.3 (0.3)	M2+	C1	G2	AM421571	AM421587
AB2002/55	4.1 (0.2)	M2+	C4	G8	AM421572	AM421588

[a] + Colony forming in culture, − not producing colonies in culture

[b] PC-IGS and ITS1

different morphotypes based on (1) the cell size, (2) the arrangement of the cells in colony and (3) the colony shape (Table 2); M1: <4 μm (densely and regularly packed, irregular colony shape), M2: 4.0–4.9 μm (densely and irregularly packed, holes within colony, irregular colony shape), M3: 5.0–5.9 μm (loosely and irregularly packed, irregular colony shape) and M4: >5.9 μm (densely and regularly packed, irregular colony shape).

Chemical characterisation

In the MALDI-TOF MS analysis of the 24 *M. aeruginosa* strains more than 30 mass signals could be detected over the mass range from *m/z* 545 to 1,825. Among the compounds that could be fully elucidated were microcystins and an aeruginosin called microcin SF 608 (Table 3). Some additional oligopeptides of the cyanopeptolin and aeruginosin class were found as well, but in too small amounts to allow a complete structural elucidation. The strains NIVA-CYA 495, NIVA-CYA 496, AB2002/24, and AB2002/40 contained microcystins. Microcystin desmethyl-YR was found to be produced by all four strains. Microcystin-YR was found in NIVA-CYA 495, NIVA-CYA 496, and AB2002/24, whereas only AB2002/24 contained microcystin-LR. Based on the peptide patterns shown in Table 3, the 24 *M. aeruginosa* strains of the present study could be divided into 10 chemotypes (Table 2). Differences in the microcystin profile among the strains measured by MALDI-TOF MS were in congruence with results from the ELISA test (Table 3).

Genetic characterisation

Amplification of ITS rDNA with primers 322 and 340 gave single amplification products of ca. 470 bp. Without the flanking 16S and 23S rDNA regions, the ITS1 sequence lengths varied from 356 to 361 bp. The ITS1 alignment used in the phylogenetic analysis contained 360 positions, and one gap (4 bp) was excluded from the alignment. The conserved domains (D1, D1', D2, D3, D4, D5 and Box A) and one tRNA gene, $tRNA^{Ile}$, described by Iteman (2000), were found in all 24 sequences. No polymorphism was found in the conserved domains. Neighbour-joining analysis and the MrBayes inference gave trees congruent with the maximum likelihood tree. The maximum likelihood analysis produced a tree consisting of four major clusters with moderate to high bootstrap support (Fig. 2a). Cluster I consisted of strains from two waterbodies in Uganda and from one lake and two ponds in Kenya. Cluster II consisted of two toxin producing Ugandan strains. Cluster III contained two toxin producing strains from Kenya, in addition to one strain from Uganda. Finally, Cluster IV contained three strains

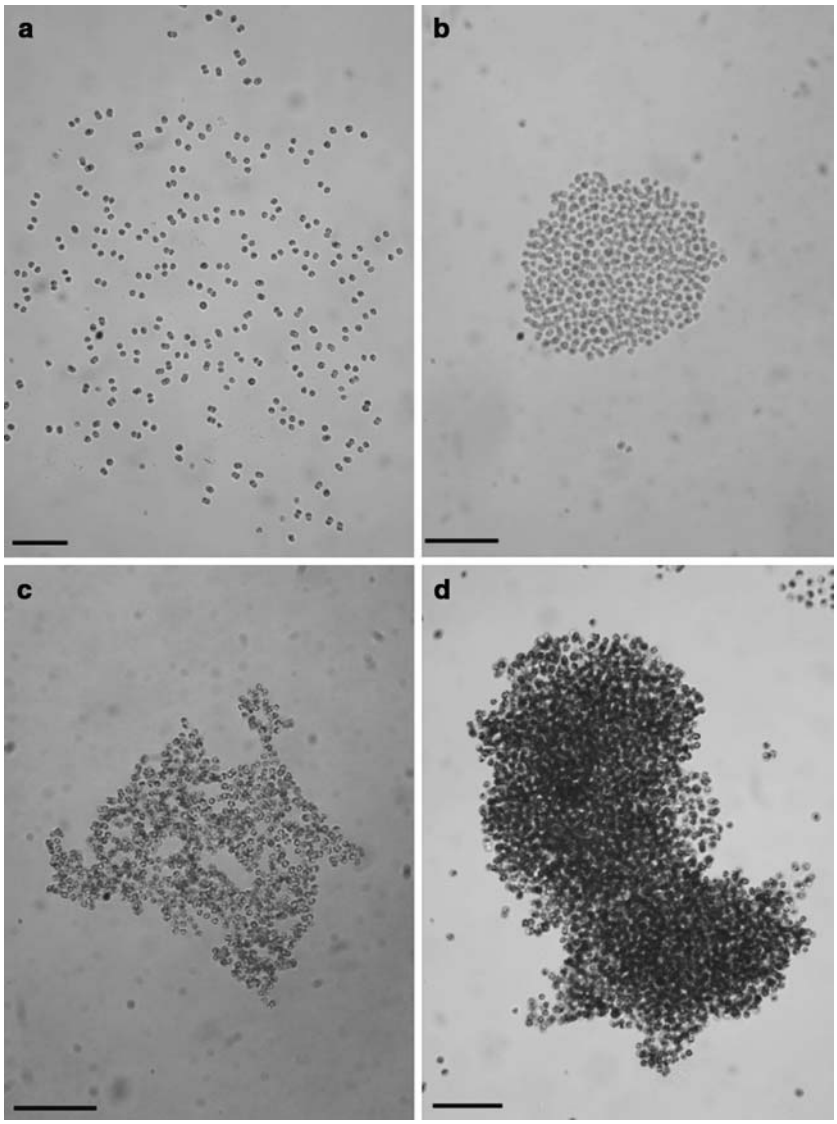

Fig. 1 Morphological characteristics of *Microcystis aeruginosa* **a** M3 *M. aeruginosa*, CYA 432, **b** M1 *M. aeruginosa*, CYA 477, **c** M2 *M. aeruginosa*, CYA 503 and **d** M4 *M. aeruginosa* AB2002/24. The *scale bars* indicate 50 μm

with identical sequences from Uganda. The branching order for the strain NIVA-CYA 477 from Uganda is uncertain.

The primers PCβ-F and PCα-R gave single amplification products of ca. 610 bp from the 24 strains of *M. aeruginosa*. A 583-bp fragment of the PC-IGS sequences could be clearly resolved and unambiguously aligned for phylogenetic analysis. No insertions or deletions were noted in the coding or IGS region among the sequences examined. The three different phylogenetic approaches produced similar tree topology. The maximum likelihood tree consisted of two major clusters and two branches separated with moderate to high bootstrap support (69/75/97 for NJ/ML/MB bootstrap analysis; Fig. 2b). Cluster I consisted of ten identical sequences from strains isolated from Murchison Bay in Uganda and from Lake Baringo in Kenya, three identical sequences of Kenyan strains isolated from two ponds and two sequences from Ugandan strains isolated from Lake Mburo and Kazinga Channel. Cluster II consisted of only sequences from strains isolated from Murchison Bay and Kazinga Channel in Uganda, two of them toxin producing strains NIVA-CYA 495 and NIVA-CYA 496. The toxin producing strain AB2002/40 isolated from Lake Naivasha formed a separate Branch III. Branch IV consisted of the toxin producing strain AB2002/24 isolated from Pilsner Pond in Kenya. The branching order of the Ugandan strain NIVA-CYA 477 was again uncertain.

For the East-African strains of *M. aeruginosa*, we had a set of ITS1 sequences and a set of PC-IGS sequences. Based on sequence similarity, identical sequences of ITS1 and PC-IGS respectively, grouped together in congruent sequence genotypes, designated genotypes G1 to G10 (Table 2). The same strains grouped together in a genotype using the two DNA

Table 3 Results from MALDI-TOF MS analysis for the presence of peptides in strains of *Microcystis aeruginosa* isolated from water bodies in Kenya and Uganda. Results from the ELISA test are also included

	Microcin SF 608	Unknown aeruginosin	Unknown aeruginosin	Unknown aeruginosin	Unknown cyanopeptolin	Unknown cyanopeptolin	MC-LR	Desmeth-MC-YR	MC-YR	ELISA
Masses (m/z)	609	617	651	695	943	957	995	1031	1045	
NIVA-CYA 431										
NIVA-CYA 432										
NIVA-CYA 433	■									
NIVA-CYA 463	■					■				
NIVA-CYA 464	■					■				
NIVA-CYA 465	■					■				
NIVA-CYA 475				■						
NIVA-CYA 476					■					
NIVA-CYA 477					■					
NIVA-CYA 478		■								
NIVA-CYA 482										
NIVA-CYA 495			■					■	■	■
NIVA-CYA 496			■					■	■	■
NIVA-CYA 497										
NIVA-CYA 502	■		■			■				
NIVA-CYA 503	■		■			■				
NIVA-CYA 522										
AB2002/21				■						
AB2002/22				■						
AB2002/23										
AB2002/24	■						■	■	■	■
AB2002/40								■		■
AB2002/52										
AB2002/55				■						

regions. Identical genotypes were found in different water bodies in Uganda and Kenya. G2 consisted of 10 identical sequences, 8 strains from Murchison Bay, Uganda, and 2 strains from Lake Baringo, Kenya. Genotype G8 consisted of identical sequences of *M. aeruginosa* from two different ponds in Kenya.

Figure 3 presents a maximum likelihood tree based on PC-IGS sequences from the 24 strains of *M. aeruginosa* from East Africa and additionally 42 sequences of *Microcystis* spp., with origin from different parts of the world, obtained from the EMBL database. The neighbour-joining analysis and the MrBayes inference gave trees congruent with the maximum likelihood tree. Three major Clusters were produced. The strains in this study mainly fell in Cluster I together with strains of *M. aeruginosa* from Brazil and Australia and one strain of *Microcystis flos-aquae* from USA. The strains NIVA-CYA 477 and AB2002/24 were found in Cluster II together with strains of mainly *M. aeruginosa* and some *M. flos-aquae* from all continents.

Discussion

The results from this study demonstrated that the 24 strains of *M. aeruginosa* isolated from water bodies in Uganda and Kenya possessed a large degree of morphological, genetic, and chemical diversity. The isolated strains showed a considerable morphological plasticity; hence showing that populations of *M. aeruginosa* can comprise a large variety of morphotypes. Some strains lost their ability to form colonies when cultured, and all strains were expected to have undergone morphological changes in culture. We found some correspondence between morphotypes and genotypes (Table 2). Various genotypes in previous studies have similarly shown uniform morphological characteristics (Kato et al. 1991; Nishihara et al. 1997; Otsuka et al. 1999; Bittencourt-Oliveira et al. 2001). Other studies, however have shown that the morphology may correlate poorly to the ITS and PC-IGS genotypes in *M. aeruginosa*, and *Microcystis* sp. in general (Otsuka et al. 1999, 2000; Tillett et al. 2001; Janse et al. 2004; Yoshida et al. 2005).

Our study revealed a presence of genetic diversity within *M. aeruginosa* isolates in East-African water bodies. The majority of the strains were isolated from Murchison Bay in Lake Victoria, Uganda (Table 1), and both DNA regions analysed indicate that at least four different genotypes of *M. aeruginosa* are present. However, it is likely that the diversity is higher due to the possibility that some colonies of *M. aeruginosa* are less suited to grow in artificial medium than others. Also, data from regular sampling in Murchison Bay in 2002–2004 have shown that the microcystin concentration in the water at times was 0.5–1 μg l^{-1}, and that the most abundant genus of potentially toxic cyanobacteria was *M. aeruginosa* (Haande et al., unpublished), indicating a presumable presence of toxin producing strains of *M. aeruginosa*. Previous studies have found two to six *Microcystis* sp. genotypes in a single water body (Kato et al. 1991; Bolch et al. 1997; Bittencourt-Oliveira et al. 2001). Janse et al. (2004) studied the genetic diversity of 107 *Microcystis* sp. colonies (7 morphospecies) from 15 European and Moroccan lakes and characterised 59 distinct classes, showing a considerable genetic variation within and across habitats. There was no indication of a geographical restriction of the *Microcystis* sp. strains. Recently, Wilson et al. (2005) surveyed the genetic diversity within *M. aeruginosa* within and among geographically closely

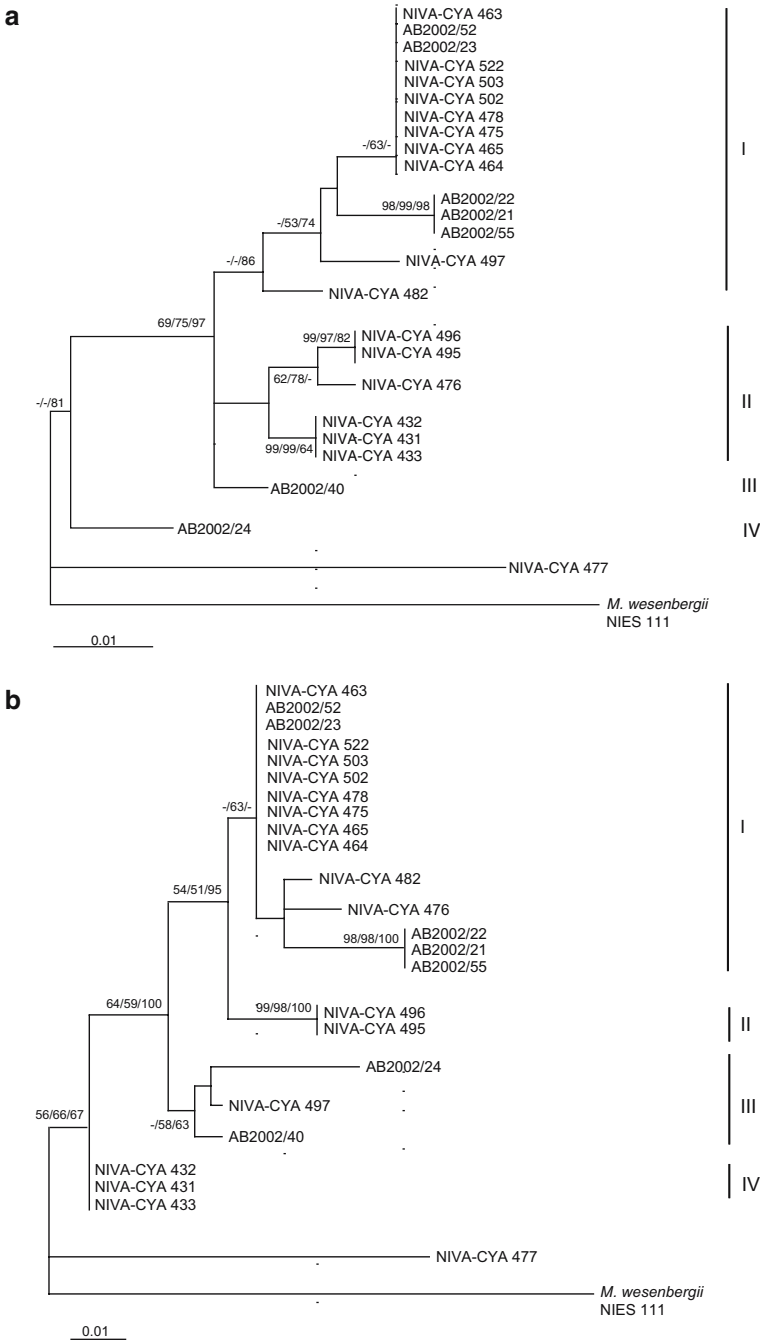

Fig. 2 **a** Maximum likelihood tree of *Microcystis aeruginosa* inferred from the ITS1 region. *Microcystis wesenbergii* NIES 111 was used as an outgroup. The ML tree was derived using the F81 model with parameter values according to Mr. Modeltest. The main clusters and branches are indicated to the *right*. Figures at the nodes are bootstrap support values (>50%): maximum likelihood/neighbour-joining/posterior probability. **b** Maximum likelihood tree of *Microcystis aeruginosa* inferred from the PC-IGS region within the phycocyanin operon. *Microcystis wesenbergii* NIES 111 was used as an outgroup. The ML tree was derived using the K2P model with parameter values according to Mr. Modeltest. The main clusters and branches are indicated to the *right*. Figures at the nodes are bootstrap support values (>50%): maximum likelihood/neighbour-joining/posterior probability

situated lakes in Southern Michigan, USA and found a substantial genetic diversity both in and among the lakes. For example, 53 of 67 isolates were shown to be genetically distinct and they found only one instance of the same genotype being present in two separate lakes.

The importance of comparing several regions of the genome in the study of species relationship has been stressed (Olsen and Woese 1993), yet most of the studies on genetic variation within the genus *Microcystis* only include one DNA region. In this study, we have included two DNA regions, PC-IGS and ITS1. The topology of the PC-IGS tree and the ITS1 tree differed slightly, but the sequence genotypes (G1–G10) were congruent for the two phylogenies. The obtained results from the two DNA regions were in good agreement and therefore the use of two DNA regions strengthened our genetic characterisation of the *M. aeruginosa* strains from East Africa. Recently, Sanchis et al. (2005) studied the genetic relationship (PC-IGS and ITS1) within strains of *Microcystis* sp. isolated from a Spanish water reservoir, and they found similar tree topology in the phylogenetic analysis of their

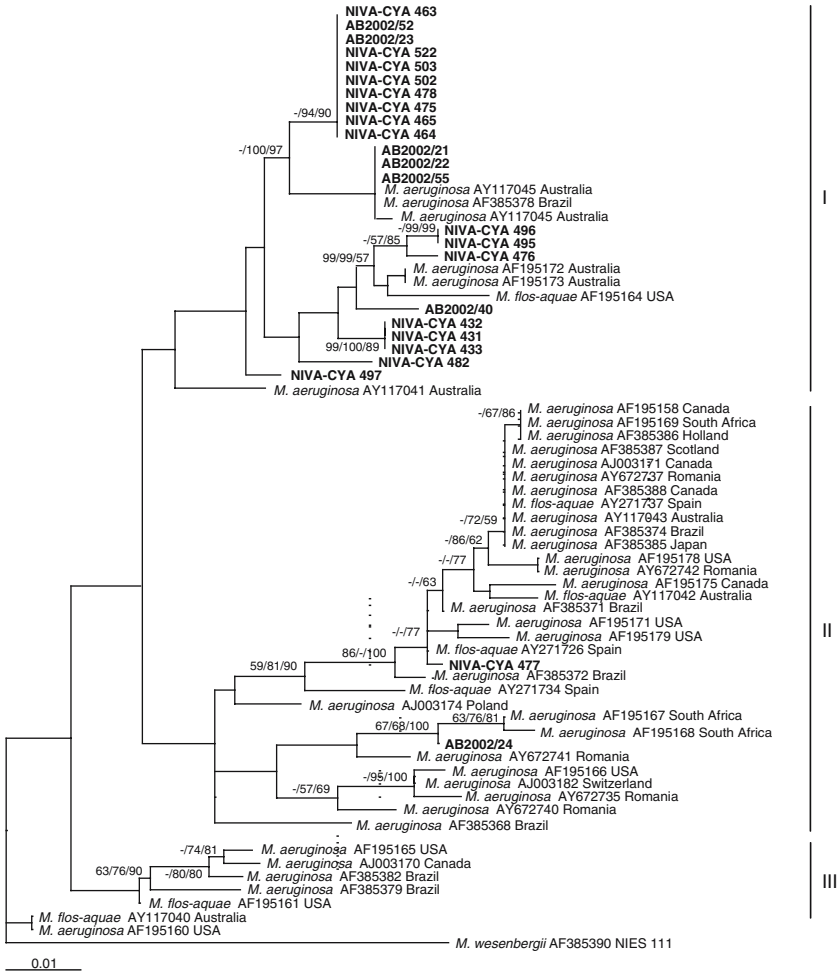

Fig. 3 Maximum likelihood tree of *Microcystis aeruginosa* inferred from the PC-IGS region within the phycocyanin operon. Strains from this study are marked in *bold*. *Microcystis wesenbergii* NIES 111 was used as an outgroup. The ML tree was derived using the GTR model with parameter values according to Mr. Modeltest. The main clusters and branches are indicated to the *right*. Figures at the nodes are bootstrap support values (>50%): maximum likelihood/neighbour-joining/posterior probability

sequences. The toxic strains are differently separated in the two trees. Both regions can be said to be equally valid to establish phylogenetic relationship among strains of the genus *M. aeruginosa*. The genetic variation was nearly the same within the two DNA regions, 4.6% for PC-IGS and 5.3% for the ITS1. This is in good agreement with studies of the PC-IGS region for Brazilian strains of *M. aeruginosa* (Bittencourt-Oliveira et al. 2001) and of the ITS1 region for several strains of *Microcystis* sp. from Japan (Otsuka et al. 1999).

The analysis of the PC-IGS region of the East-African *M. aeruginosa* strains and of the reference strains from different continents shows a separation in three Clusters. The phylogenetic tree in Fig. 3 showed that cluster I includes most of the strains from Uganda and Kenya, in addition to strains of *M. aeruginosa* from (sub)-tropical regions like Australia and Brazil, with exception of a strain of *M. flos-aquae* from USA. Cluster II, on the other hand, consists of strains of both *M. aeruginosa* and *M. flos-aquae* from all parts of the world. On the basis of the sequences included in this study, there seems to be one group (Cluster II) which includes the "cosmopolitan species" suggested for some temperate cyanobacterial species by Komàrek (1985). However, as noted by Bittencourt-Oliveira et al. (2001), the *Microcystis* sp. genotypes most likely represent a series of related populations sharing a common phylogenetic history.

The isolated *M. aeruginosa* strains from Uganda and Kenya were found to contain a mixture of microcystins and other oligopeptides. Only 4 of the 24 strains (17 %) were found to produce microcystins. In comparison, 62% of the 26 *M. aeruginosa* strains isolated from a Portuguese river were found to produce microcystins (Saker et al. 2005) whereas only 10% of the 20 isolated *M. aeruginosa* and *Microcystis* sp. strains from Lake Kasumigaura in Japan were microcystin producing (Ohtake et al. 1989). However, the bias introduced by strain isolation may be substantial (Wilson et al. 2005), and therefore analysis of freshly collected colonies of *M. aeruginosa* from environmental samples can be more appropriate for the comparison of the abundance of microcystin-producing strains in different geographical regions. Via-Ordorika et al. (2004) used this latter method and found that 72% of the colonies of

M. aeruginosa isolated from a range of European Lakes were microcystin producers.

The chemical forms of microcystin detected by MALDI-TOF MS analysis differed between the isolates. Microcystin desmethyl-YR was produced by all four strains and microcystin-YR by three strains, whereas microcystin-LR only was produced by one strain. An aeruginosin called Microcin SF608 was produced by six strains, none of them microcystin producers, all isolated from Murchison Bay in Lake Victoria, Uganda. This peptide is found to be a potent serine protease inhibitor (Banker and Carmeli 1999). Wiegand et al. (2002) studied the effects of Microcin SF608 on water moss (*Vesicularia dubyana*) and water flea (*Daphnia magna*) and found that the peptide adversely inhibited enzyme production (glutathione-S-transferase, serine protease, peroxidase) and caused oxidative stress. Traditionally, the production of the potent hepatotoxic microcystins has been the major concern related to waterblooms of cyanobacteria. However, less known biologically active compounds produced by cyanobacteria may also cause major harm to plant, animal, and human health. Interestingly, the 24 *M. aeruginosa* strains isolated from East Africa had a range of peptides of the aeruginosin and cyanopeptolin classes, both known as bioactive (Welker and von Döhren 2006). The production of particular peptides is specific for individual clones of *M. aeruginosa*, allowing a characterisation of chemotypes based on the peptide composition. The East-African strains could be divided into 10 chemotypes. This heterogeneity in oligopeptides is in good agreement with studies carried out in European lakes (Fastner et al. 2001; Welker et al. 2004). The results from the MALDI-TOF MS analysis on microcystins were in good agreement with the results from the ELISA analysis.

Taking into account the extreme variation and complexity and the worldwide distribution of *M. aeruginosa*, and also the implications of toxin production on ecological and public health, it is necessary to use morphological examination, chemical characterisation, and genetic analysis for precise species identification (polyphasic approach). *M. aeruginosa* represents a significant water quality problem in potable waters and in water bodies used for recreational purposes, and further knowledge on the occurrence of *M. aeruginosa* and cyanotoxins in African water bodies is of major importance for the livelihood of the local population.

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