RESEARCH ARTICLE

Molecular detection of uncultured cyanobacteria and aminotransferase domains for cyanotoxin production in sediments of different Kenyan lakes

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Abstract

PCR-based denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA gene fragments was used to identify the cyanobacterial phylotypes in sediments and plankton of saline–alkaline and freshwater lakes of Kenya. The detection of the aminotransferase domain located on modules *mcyE* and *ndaF* using specific molecular markers confirmed the presence of potential toxin-producing cyanobacteria. The eight nucleotide sequences obtained from DGGE bands were placed in three divergent cyanobacterial clusters. Five nucleotide sequences were close to members of the genera *Anabaenopsis* and *Umezakia* (*Nostocales*), two sequences fell in the cluster with *Arthrospira* sp. (*Oscillatoriales*) and one sequence was related to *Chroococcidiopsis* sp. (*Pleurocapsales*). The presence of the latter taxon was demonstrated *de novo* in the investigated lakes. All nine attained nucleotide sequences of the aminotransferase region belonged to the *mcyE* module. Five sequences of the aminotransferase domain were included in the cluster having the nucleotide sequence of *Anabaena* sp. but showed a separate lineage. Other four aminotransferases were placed in the cluster represented by nucleotide sequence of *Microcystis aeruginosa*. To our knowledge, this is the first report on molecular detection of cyanobacterial phylotypes in sediments of African lakes and aminotransferase domains for cyanotoxin production from sediment samples in general.

Introduction

The Kenyan Rift Valley, which is a part of the Gregory Rift, has several lakes that are famous for their unique avifaunal and other wildlife diversity. Although most of the lakes are topographically endorheic, a few are freshwater (Baringo and Naivasha) while the rest are saline–alkaline (Bogoria, Elmenteita, Nakuru, Oloidien, and Turkana). The ecology and biology of most rift valley lakes have been extensively studied in the last few decades (Talling & Talling, 1965; Vareschi, 1978, 1982; Hopson, 1982; Kalff, 1986; Melack, 1988; Harper *et al.*, 2002, 2003; Ballot *et al.*, 2003, 2004a, 2005; Odada *et al.*, 2003; Oduor & Schagerl, 2007a; Schagerl & Oduor, 2008). These studies have paid a lot of attention to phytoplankton communities as key primary producers in the lakes. In Kenyan freshwater lakes, a highly diverse phytoplankton community composed by diatoms, green algae, euglenophytes, dinophytes, and cyanobacteria, has been reported. The saline–alkaline lakes are, however, less diverse and characterized exclusively by the presence and sometimes mass developments of cyanobacteria, namely *Arthrospira fusiformis* and *Anabaenopsis* sp. (Vareschi, 1982; Ballot *et al.*, 2004a, 2008; Krienitz *et al.*, 2005; Oduor & Schagerl, 2007b). Outside the Rift Valley, other lakes that have received limnological attention include Lake Victoria (Talling, 1966; Verschuren *et al.*, 2002) and the crater lake Simbi (Finlay *et al.*, 1987; Ballot *et al.*, 2005). Limited studies have also been carried out in a number of small water bodies found within the floodplains of Kenya's major rivers, notably the Tana river delta (Terer *et al.*, 2004).

The annual life cycle of cyanobacteria in water is characterized by different phenological stages with a benthic–pelagic coupling (Reynolds *et al.*, 1981). The presence of cyanobacteria, mostly *Microcystis* sp., in sediments even in deeper layers has been observed microscopically by several researchers (Takamura *et al.*, 1984; Boström *et al.*, 1989;

Tsujimura *et al*., 2000; Verspagen *et al*., 2005; Latour *et al*., 2007). Microscopic examinations of cyanobacteria are prone to erroneous identification of taxa, depending on the experience of the researcher. Establishment of DNA-extraction techniques from sediments facilitate the identification of bacteria in sediments of lakes (Duckworth *et al*., 1996; Rees *et al*., 2004). However, data on detection of cyanobacteria in sediments using molecular techniques are very rare (Innok *et al*., 2005). Molecular characterization of cyanobacterial communities using partial sequence of 16S rRNA gene is suitable to identify taxa that prevail in a particular habitat. Recently, cyanobacterial diversity in water and sediment samples from four representative sites of Salar de Huasco, a high-altitude saline wetland in northern Chile has been examined using denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene (Dorador *et al*., 2008). In spite of significant importance of cyanobacteria, especially in lake ecosystems, to our knowledge, no attempts have been made so far to apply molecular method (PCR-DGGE) to study the uncultured cyanobacterial population in sediments and planktons of African lakes.

A progressive increase in the production of cyanobacterial toxins in various habitats is hazardous to animals and amplifies problems of human health (Codd *et al*., 1999; Carmichael, 2001; Funari & Testai, 2008). Bloom and mat-forming cyanobacteria in freshwater, brackish, and saline–alkaline waters produce a wide variety of cyanotoxins worldwide. Among all cyanotoxins, microcystin and nodularin are the most common hepatotoxins produced by a wide range of cyanobacteria (Carmichael & Li, 2006; Rantala *et al*., 2008). Successive episodes of large-scale deaths of the Lesser Flamingo, *Phoeniconaias minor* Geoffrey, found in alkaline–saline lakes of Kenya, where the filamentous cyanobacterium *A. fusiformis* forms their main food resource have been reported. Recently, it was concluded that toxins produced by cyanobacteria could be one of the main causes of the mass die-offs of these birds (Codd *et al*., 2003; Harper *et al*., 2003; Krienitz *et al*., 2003; Kotut *et al*., 2006).

Numerous studies have been carried out on molecular characterization of toxic and nontoxic cyanobacteria based on the presence or absence of various gene loci with the potential of toxin production. Zhao *et al*. (2008) achieved phylogenetic analysis of type I polyketide synthase and nonribosomal peptide synthetase genes of bacteria and cyanobacteria in Antarctic sediment. Microcystin (*mcy*) and nodularin (*nda*) are cyclic heptapeptides, synthesized nonribosomally by synthetase gene clusters which encode peptide synthetase/polyketide synthase enzyme complexes that assemble microcystins and nodularins, respectively (Dittmann *et al*., 1997; Rantala *et al*., 2008). The aminotransferase domain in cyanobacteria is situated on the modules *mcyE* and *ndaF* of the microcystin and nodularin synthetase enzyme complexes, respectively, and plays an important role in the synthesis of all microcystins and nodularins. This domain has been applied for the detection of potential microcystin- and nodularin-producing cyanobacteria in various cyanobacterial blooms (Jungblut & Neilan, 2006).

The aim of this study was to identify uncultured cyanobacterial phylotypes in sediments and plankton of alkaline–saline and freshwater lakes of Kenya by PCR-DGGE method. The potential cyanotoxin-producing phylotypes were detected using molecular markers for the aminotransferase domain in sediment and water samples for early forecast of possible toxin occurrence in a particular water body.

Materials and methods

Site description

The lakes studied are located in various parts of Kenya (Fig. 1). Along the Eastern Gregory Rift Valley, the lakes studied included lakes Turkana, Baringo, Bogoria, Nakuru, and Oloidien. In the eastern and coastal part of the country, those studied within the River Tana delta included the oxbow-lakes Moa and Kenyatta, and Buffalo pond, which was formed by road building excavation works. From the western region, samples were collected from Lake Victoria (Nyanza Gulf) and the crater lake Simbi. Some of the main geomorphological and physicochemical features of the lakes are summarized (Table 1). Overall, the waters studied ranged from freshwater to saline–alkaline. Freshwater lakes comprised of lakes Baringo and Victoria, and water bodies of the Tana Delta (Moa and Kenyatta). All the other lakes were saline–alkaline.

Sample collection and measurements

All sediment samples were taken by hand using a plastic liner during dry season (in January/February 2004, 2005, and 2006). Sediment samples were collected at a distance of between 1 and 50 m from the shoreline, depending on the water depth. At each selected site, the tube was forced into the sediment, stoppered at the top and, while still under water, a piston was pushed in from the bottom. On land, the piston was pressed toward the top, hence pushing the sediment core into a smaller tube held by hand to the top of the sediment sampler. The sediment core was sliced into 2.5-cm-thick portions, which were air-dried on site within 24 h under dry tropical conditions. In addition to sediment samples, for plankton sampling, a known volume of water was filtered onto Whatman glass fiber filters (GF/C, Whatman International Ltd, Maidstone, UK) in the field and air-dried on the site (Schober & Kurmayer, 2006; Haverkamp *et al*., 2008). Sample identification numbers and their collection sites are presented (Table 2). Conductivity, pH, and salinity were measured directly in the water bodies

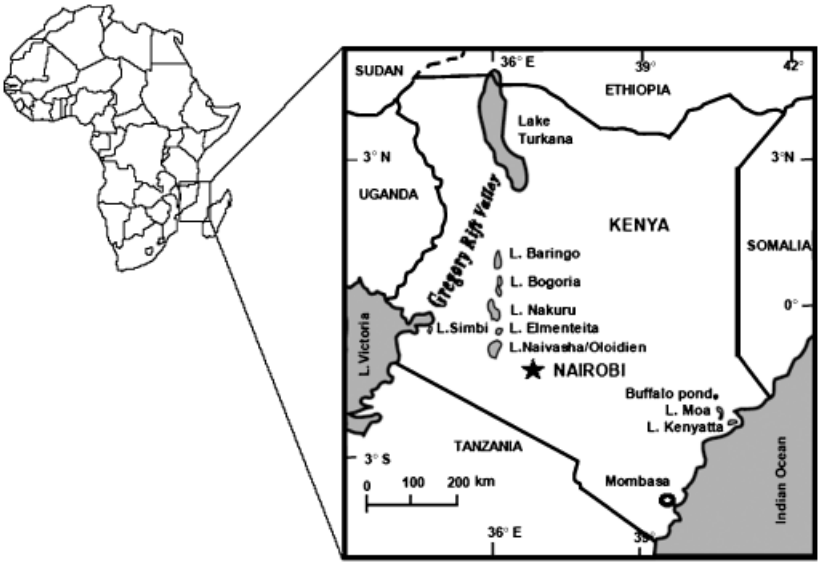

Fig. 1. Geographical location of Kenya in Africa and the Kenyan water bodies from which samples were used in this study.

Table 1. Geographic location and physicochemical characteristics of the studied lakes

	Baringo	Bogoria	Nakuru	Elmenteita	Oloidien	Simbi	Turkana	Victoria	Moa	Kenyatta	Buffalo
Position	00°36′N 36°01′E	00°15′N 36°05′E	00°20′S 36°05′E	00°27′S 36°15′E	00°48′S 36°16′E	00°22′S 34°38′E	04°00′N 36°00′E	00°05′S 34°42′E	02°21′S 40°19′E	02°24′S 40°41′E	02°23′S 40°23′E
Altitude	998	963	1759	1782	1995	1142	362	1135	7	8	12
Surface area (km^2)	160	34	40	c. 20	6.5	0.29	6500	68 800	2	2	0.01
$Depth_{max}$ (m)	8	10	0.2–4.5	0.3–3.1	8	23	73	84	NA	NA	NA
pH*	8.56	9.83	10.13	9.76	9.70	9.89	9.31	7.61	7.86	8.41	10.01
Conductivity* ($\mu S\,cm^{-1}$)	1195	78 100	58 800	50 100	6500	17 800	3630	181	250	1066	2880

*Measured at sampling date.
NA, not applicable.

Table 2. Location of the water bodies studied and details on sample type, source, and sampling period for all samples

Sample ID	Location	Region	Sediment depth (cm)	Year/month
2004/01	Lake Moa[F]	Tana-delta	0–2.5	2004/02
2004/02	Lake Moa[F]	Tana-delta	2.5–5.0	2004/02
2004/03	Lake Kenyatta[F]	Tana-delta	0–2.5	2004/02
2004/05	Buffalo pond[Sa]	Tana-delta	Mix (0–10)	2004/02
2004/06	Lake Victoria[F], near Dunga	Western	0–2.5	2004/02
2004/07	Lake Victoria[F], near Dunga	Western	2.5–5.0	2004/02
2005/01	Lake Oloidien[Sa]	Rift Valley	0–2.5	2005/02
2005/23	Lake Baringo[F]	Rift Valley	0–2.5	2005/02
2005/54	Lake Turkana[Sa]	Northern	0–2.5	2005/01
2006/38	Lake Baringo[F]	Rift Valley	0–2.5	2006/02
2006/P02	Lake Nakuru[Sa]	Rift Valley	Planktonic	2006/02
2006/P05	Lake Oloidien[Sa]	Rift Valley	Planktonic	2006/02
2006/P13	Lake Bogoria[Sa]	Rift Valley	Planktonic	2006/02
2006/06	Lake Nakuru[Sa]	Rift Valley	2.5–5.0	2006/02
2006/28	Lake Simbi[Sa]	Western	2.5–5.0	2006/02
2006/P07	Lake Victoria[F]	Western	Planktonic	2006/02
2006/P18	Lake Elmenteita[Sa]	Rift Valley	Planktonic	2006/02

Sa, saline–alkaline; F, freshwater; ID, identification numbers.

with a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten, Weilheim, Germany) at the time of sampling.

DNA recovery from samples

Cyanobacterial cell lysis and DNA extraction from sediment samples were performed using the Ultraclean Soil DNA Isolation Kit (MoBio Inc., Solana Beach, CA) following the manufacturer's instructions. Briefly, dried sediment samples were ground thoroughly and 0.5 g was transferred to 2-mL bead solution tubes. Extraction buffer and inhibitor removal solution were then added, and the tubes vortexed horizontally by a vortexer equipped with a shaking plate (Vortex Genie® 2, Scientific Industries, Bohemia, NY) at maximum speed for 10 min. The tubes were then centrifuged at 10 000 ***g*** for 1 min, and the DNA in the supernatants was purified using the provided spin filters. The extracted DNA was visualized on a 1% agarose gel using ethidium bromide staining and stored at $-20\,^{\circ}C$.

Phytoplankton samples were scrubbed from the dried filter with sterile scalpel, and genomic DNA was extracted using Dynabeads DNA Direct System I (Invitrogen/Dynal Biotech, Oslo, Norway).

PCR amplification of DNA and purification

PCR amplifications were conducted using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) in a Peltier Thermal Cycler PTC 200 (MJ Research Inc., San Francisco, CA). All amplified products were purified through Qiaquick PCR purification columns (Qiagen GmbH).

16S rRNA gene amplification for DGGE

A nested PCR approach was used for the amplification of 16S rRNA gene fragments from cyanobacterial genomic DNA present in samples, as this technique has greater specificity than regular PCR (Zwart *et al.*, 2005). For the first round, CYA106F (Nübel *et al.*, 1997; Li *et al.*, 2001) and R4R (Li *et al.*, 2001) primers were used to selectively amplify long fragments of cyanobacterial 16S rRNA genes from the samples as described previously (Ballot *et al.*, 2004b). For the second round, the forward primer CYA359F containing a GC clamp and reverse primers 781Ra and 781Rb in equimolar concentrations were used (Nübel *et al.*, 1997). The reaction mixture contained 0.1 μL Taq DNA polymerase (concentration 5 U μL^{-1}), 0.6 μL dNTP mix (10 mM), 2 μL buffer [10 × concentrated, containing Tris Cl, KCl, $(NH_4)_2SO_4$, and 15 mM $MgCl_2$], 1 μL forward and reverse primers (10 μM), and 1 μL genomic DNA in a total volume of 20 μL. The thermal cycling conditions for reamplification of DNA fragments of the amplified 16S rRNA gene included an initial denaturation at 94 °C for 3 min, followed by 30 cycles at 94 °C for 1 min, 55 °C for 50 s, and 72 °C for 1 min, followed by a final extension of 5 min at 72 °C.

DGGE profiling

The products of the second PCR reaction were analyzed by DGGE according to Muyzer *et al.* (1993) with slight modification. An equal volume of all amplified products was loaded onto gel. DGGE was performed using INGENYphorU-2 System (Ingeny International BV, GP Goes, The Netherlands) with 7% acrylamide gel and denaturing gradient from 40% to 70%. Electrophoresis was performed at 60 °C, 80 V for 15 min, followed by 200 V for 5 h. The gel was stained with SyberGold solution (Biozym, Hessisch Oldendorf, Germany). Bands were visualized under UV light and gel image was captured and digitized by Alpha-Imager 2200 (Alpha Innotech, San Leandro, CA) for further analysis. It has been recognized that DGGE bands occurring at the same position in the gel throughout in different lanes corroborate the same phylotypes (Wichels *et al.*, 2004). Therefore, bands detected at different positions were excised from DGGE gels by puncturing the gel using sterile P10 pipette tips and incubated in 30 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH 8) at 4 °C for 24 h. Eluates from each band were used as template DNA for reamplification, using the thermal cycling conditions as described above except that the forward primer CYA359F did not contain a 5′ GC clamp. The banding profiles were analyzed using the GELQUEST software v2.2.3 (Hepperle, 2008). Cluster analysis of DGGE bands was conducted in order to find out similarities in cyanobacterial composition between samples and site of origin. A dendrogram was constructed by the unweighted pair group method with arithmetic mean (UPGMA) method, calculating the Pearson's correlation coefficient by CLUSTERVIZ v1.2.1 software (Hepperle, 2008) to determine the relationship of DGGE fingerprintings of 16S rRNA genes.

Amplification of the aminotransferase domain

The PCR mixture concentrations and volume were similar to that used for amplification of 16S rRNA gene, except in the choice of primers. The amplification of the aminotransferase domain from bulk genomic DNA of different sediments and planktons was carried out with HEP primers (HEPF and HEPR for the detection of microcystin and nodularin producing cyanobacteria) (Jungblut & Neilan, 2006). The PCR amplification was performed as follows: initial denaturation at 94 °C for 3 min, then 35 cycles at 92 °C for 20 s, 52 °C for 30 s, and 72 °C for 60 s, followed by elongation at 72 °C for 5 min. The amplified PCR products

were detected on 1% (w/v) agarose gel with ethidium bromide staining.

Sequencing of DGGE bands, aminotransferase domains, and phylogenetic analysis

The purified PCR products of the aminotransferase domain and reamplified amplicons of the 16S rRNA gene from DGGE bands were sequenced for both strands on an ABI 3100 Avant Genetic Analyzer using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) according to the manufacturer's manual. The nucleotide sequences obtained were checked for sequence similarity on-line with the BLAST search program at the National Center for Biotechnology Information website (NCBI, http://www.ncbi.nlm.nih.gov/), and sequences found to share a high level of similarity were used for phylogenetic studies.

The sequences were aligned using manual sequence alignment editor Align v05/2008 (Hepperle, 2008) and the sequence alignments were corrected manually. Phylogenetic analyses of partial sequences of 16S rRNA genes and aminotransferase domains of cyanobacteria were performed by MEGA software v 4.0 with the model of Tamura–Nei for nucleotide (Tamura *et al.*, 2007). Phylogenetic trees were constructed using a neighbor-joining (NJ) algorithm and nodes of the tree were tested by 1000-fold bootstrap analysis.

The nucleotide sequences reported in this study have been deposited in the NCBI database under the GenBank accession numbers EF568406–EF568413 (16S rRNA gene from DGGE bands) and EF579692–EF579700 (aminotransferase domains).

Results

DGGE of 16S rRNA gene

DGGE fingerprinting of PCR-amplified 16S rRNA genes of cyanobacterial communities from sediments and surface waters of different lakes comprised between five and 11 bands (Fig. 2). Seven conspicuous bands were identified in most lanes of DGGE analysis. The DGGE banding pattern obtained was similar among replicate samples. The UPGMA dendrogram cluster analysis of DGGE band patterns exhibited four major clusters (Fig. 3). Sediment sample (lane 14) and planktonic sample (lane 11) from the saline–alkaline Lake Nakuru formed a distinct cluster-II. Cluster-III was incorporated by eight DGGE banding patterns from samples of saline–alkaline lakes: Bogoria (lane 13), Oloidien (lanes 7, 12), and freshwater lakes Baringo (lanes 8, 10), Victoria (lanes 5, 6), as well as freshwater Buffalo pond (lane 4). Freshwater lakes Moa (lanes 1, 2), Kenyatta (lane 3), and the moderately saline Lake Turkana (lane 9) were included in the cluster-IV. DGGE fingerprints of the planktonic sample from Lake Oloidien did show high similarity to the freshwater Lake Baringo sediment sample (lanes 12), while the lineage of sediment DGGE profile from Lake Oloidien fitted into another subcluster. The banding pattern of the surface sediment sample (0–2.5 cm) of Lake Victoria was similar to that of the Buffalo pond, while deeper sediment samples (2.5–5.0 cm) from the same lake fitted into a cluster with a different lineage. Of all the samples investigated, the DGGE banding pattern from the sediment sample from Lake Simbi (lane 15) was quite distinct, suggesting an absence of a close relationship with samples from other lakes.

We were able to sequence eight bands out of 11 major bands detected from DGGE profiles. The other observed bands could not be successfully reamplified and sequenced despite the many attempts. A phylogenetic tree was constructed using the NJ method for partial sequences of 16S rRNA genes (307 bp) from each sequenced band and closely

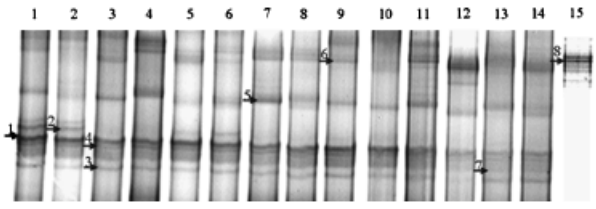

Fig. 2. DGGE banding patterns of 16S rRNA gene-defined diversity among sediment and planktonic samples from Kenyan Lakes. Lanes: 1, 2004/01 (Moa); 2, 2004/02 (Moa); 3, 2004/03 (Kenyatta); 4, 2004/05 (Buffalo); 5, 2004/06 (Victoria); 6, 2004/07 (Victoria); 7, 2005/01 (Oloidien); 8, 2006/23 (Baringo); 9, 2005/54 (Turkana); 10, 2006/38 (Baringo); 11, 2006/P02 (Nakuru); 12, 2006/P05 (Oloidien); 13, 2006/P13 (Bogoria); 14, 2006/06 (Nakuru); 15, 2006/28 (Simbi). Arrows and numbers denote bands that were successfully sequenced.

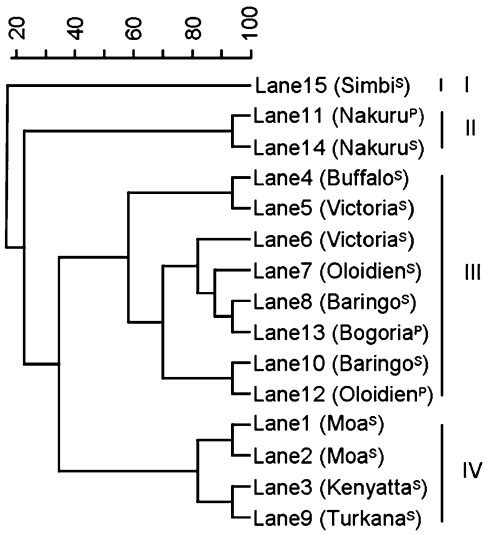

Fig. 3. A dendrogram generated by cluster analysis (UPGMA – Pearson's correlation index) showing the relationship of DGGE fingerprinting of 16S rRNA genes obtained from sediment and planktonic samples from Kenyan lakes. S, sediment samples; P, planktonic samples.

related sequences retrieved after a BLAST similarity search (Fig. 4). The percentage similarity between the sequences attained from DGGE bands and the most-matched sequence available in GenBank was ascertained to determine phylotypes (Table 3). The eight phylotypes fell within three distinct cyanobacterial clusters. The phylotypes of DGGE bands sb1, sb2, sb3, sb4, and sb7 (cluster I) belong to nostocalean cyanobacteria (*Anabaenopsis* sp. and *Umezakia* sp.). The phylotypes identified from nucleotide sequence of bands sb6 and sb8 belonged to the cluster including the oscillatorian member *Arthrospira* sp. with 100% similarity. The phylotype belonging to band sb5 is comprised within the cluster of the *Pleurocapsales*.

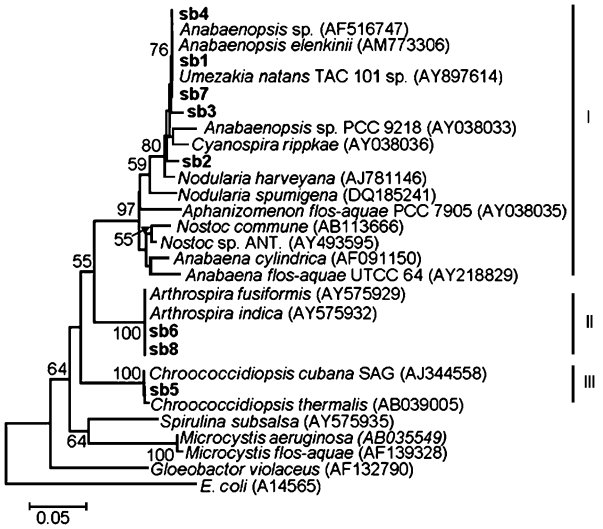

Fig. 4. NJ tree based on partial 16S rRNA gene sequences showing the phylogenetic relationship among sequences from excised bands from DGGE of sediment and planktonic samples from Kenyan lakes (bold), with reference sequences obtained through BLAST analysis. Bootstrap values > 50 were included.

Aminotransferase domain

PCR-amplification of the aminotransferase domain was successfully attained from bulk genomic DNA, using HEP primers from nine samples from both sediments and planktonic habitats, out of 15 samples investigated from different Kenyan lakes (Table 4). We got only one band in all PCR products (data not shown).

All PCR-amplified products obtained from various lakes were sequenced. Nine sequences (410 bp) representing the aminotransferase domain showed 75–100% similarity to each other. Percent similarity between attained sequences and closely related GenBank sequences was determined (Table 4). To explore the homology and the uniqueness of the sequences, a phylogenetic tree was constructed using the nucleotide sequences of the aminotransferase domain obtained, and sequences with high similarity in the BLAST searches (Fig. 5). The sequences were grouped in two major clusters. Five nucleotide sequences of the aminotransferase

Table 3. Identity of partial 16S rRNA gene sequences obtained from DGGE of community DNA of cyanobacteria from different Kenyan lakes

Band number	Accession number	Nearest phylogenetic neighbors	GenBank accession number	Percent similarity
sb1	EF568406	*Anabaenopsiselenkinii*	AM773306	100
sb2	EF568407	*Anabaenopsiselenkinii*	AM773306	98
sb3	EF568408	*Anabaenopsis* sp.	AF516747	99
sb4	EF568409	*Anabaenopsiselenkinii*	AM773306	100
sb5	EF568410	*Chroococcidiopsis cubana*	AJ344558	100
sb6	EF568411	*Arthrospira fusiformis*	AY575929	100
sb7	EF568412	*Umezakia natans*	AY897614	100
sb8	EF568413	*Arthrospira fusiformis*	AY575929	100

Table 4. Nucleotide sequence similarity and source of aminotransferase domain for *mcyE* gene detected in different Kenyan lakes

Sample ID	Accession number	Source	Nearest phylogenetically related cyanobacterial aminotransferase domain	GenBank accession number	Percent similarity
2004/01	EF579692	Lake Moa[S] (0–2.5 cm)	*Anabaena* sp.	AY817157	95
2004/02	EF579693	Lake Moa[S] (2.5–5.0 cm)	*Anabaena* sp.	AY817157	96
2004/03	EF579694	Lake Kenyatta[S]	*Anabaena* sp.	AY817157	95
2004/05	EF579695	Buffalo Pond[S]	*Microcystis aeruginosa*	AY817161	100
2005/01	EF579696	Lake Oloidien[S]	*Anabaena* sp.	AY817157	95
2006/38F	EF579697	Lake Baringo[S]	*Microcystis aeruginosa*	AY817161	99
2006/P02	EF579698	Lake Nakuru[P]	*Anabaena* sp.	AY817157	95
2006/P13	EF579699	Lake Bogoria[P]	*Microcystis aeruginosa*	AY817161	99
2006/18	EF579700	Lake Elmenteita[P]	*Microcystis aeruginosa*	AY817161	99

S, sediment; P, planktonic; ID, identification numbers.

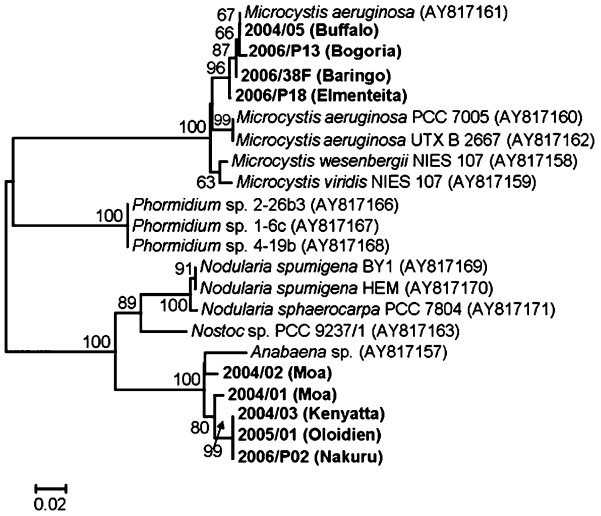

Fig. 5. NJ tree based on partial nucleotide sequences of the aminotransferase domain showing the phylogenetic relationship among sequences from sediment and planktonic samples of Kenyan lakes (bold), with reference sequences obtained through BLAST analysis. Bootstrap values > 50 were included.

domain included a cluster having one sequence of *Anabaena* sp., while four others fell in a separate cluster represented by *Microcystis* sp. (Fig. 5). Nucleotide sequences obtained from sediment samples of lake Moa (2004/02) showed proximity to the aminotransferase domain of the genus *Anabaena*, whereas other sequences depicted separate lineages in the same cluster. The four sequences attained in this study belonging to the aminotransferase domain of *Microcystis* sp. showed 99% and 100% similarity to GenBank sequence AY817161 (Table 4), but $< 97\%$ sequence similarity to other sequences of this region available in GenBank.

Discussion

Molecular study of uncultured cyanobacteria

The studies on cyanobacterial diversity of Kenyan saline–alkaline and freshwater lakes have until very recently been limited to conventional taxonomic procedure. The presence of various planktonic cyanobacterial genera has been demonstrated during the traditional exploration of biodiversity of saline–alkaline lakes Bogoria, Nakuru, Elmenteita (Vareschi, 1982; Hindák, 1985; Melack, 1988; Ballot *et al.*, 2004a), crater lakes Sonachi and Simbi (Njuguna, 1988; Verschuren *et al.*, 1999; Ballot *et al.*, 2005), Lake Baringo (Beadle, 1932; Patterson & Wilson, 1995; Ballot *et al.*, 2003), and Lake Victoria (Talling, 1987; Lung'Ayia *et al.*, 2000; Krienitz *et al.*, 2002). No comparable molecular data on PCR-based DGGE of cyanobacterial community found in waters and sediments of Kenyan lakes are available so far. In this study, for the first time, a molecular approach has been used to detect cyanobacterial phylotypes in sediments and plankton of different water bodies of Kenya.

The UPGMA dendrogram of DGGE band patterns demonstrated divergence among (1) freshwater lakes and saline–alkaline lakes as well as (2) sediment and planktonic samples of different lakes. However, in some cases, a clear relationship was not recorded between sample type and site. Dorador *et al.* (2008) revealed the same controversy when studying cyanobacterial diversity of a high-altitude saline wetland in northern Chile using DGGE of 16S rRNA genes. According to DGGE major bands, five to 11 phylotypes were present in the Kenyan lakes studied. In view of the DGGE band numbers, we did not observe apparent difference between the number of phylotypes of saline–alkaline and freshwater lakes. This finding differs from earlier conventional studies in which higher cyanobacterial diversity was recorded in saline–alkaline lakes than freshwater lakes (Ballot *et al.*, 2003, 2004a, 2005).

On the basis of the nucleotide sequence percent similarity between obtained sequences and the sequences available in the GenBank database, we identified eight cyanobacterial phylotypes belonging to three cyanobacterial genera. All sequences showed 98–100% sequence homology to their representative taxa. According to bacteriological approach, threshold values of 97.5% have often been used to distinguish between cyanobacterial species (Suda *et al.*, 2002; Taton *et al.*, 2003, 2006). Sequence similarities more than the threshold were considered the same phylotype. Cyanobacterial phylotypes belong to genera *Anabaenopsis*, *Umezakia* (cluster I), and *Arthrospira* (cluster II); *Chroococcidiopsis* (cluster III) are documented for the first time by a molecular method in sediments of African lakes. According to conventional characterization, the genus *Anabaenopsis* is frequently found and has wide distribution in saline–alkaline and freshwater habitats of tropical and subtropical regions (Komárek, 2005). The *Arthrospira* taxon commonly thrives in planktonic state. In the present investigation, we were able to detect phylotypes of this genus even in sediment samples. This cyanobacterium has great significance with its high protein content, and is the most favored food of Lesser Flamingo, which are predominantly found around saline–alkaline lakes of East Africa (Krienitz *et al.*, 2003; Schagerl & Oduor, 2008).

We were probably unable to detect phylotypes of the order *Chroococcales* in DGGE analysis due to the little input of these taxa into the sediments investigated. Most notably, the detection of the phylotype *Chroococcidiopsis* sp. (*Pleurocapsales*) is documented for the first time from sediment samples using DGGE of 16S rRNA gene. The DGGE band patterns showed that the phylotype of *Chroococcidiopsis* sp. was present in most of the samples except L. Simbi. The occurrence of investigated phylotype of *Chroococcidiopsis* in alkaline lakes of Kenya corresponded to *Chroococcidiopsis*

cubana in 16S rRNA gene sequence. The finding of *Chroococcidiopsis cubana* in alkaline waters (Komárek & Anagnostidis, 1999) demonstrated an ecological similarity between these two taxa. Previous microscopic investigations of Kenyan lakes have not recorded the presence of this genus. The *Chroococcidiopsis* sp. has been characterized as a slow-growing unicellular cyanobacterium, existing in a wide range of extreme environments, as they have been reported on rocks from Antarctic valleys and hot deserts worldwide (Friedmann & Ocampo, 1976; Büdel & Wessels, 1991; Büdel, 1999).

PCR-based detection of the aminotransferase domain

Cyanotoxins can have significant ecological impacts on aquatic food webs in lakes and cause serious health problems (Carmichael, 1997; Codd *et al*., 1999; Funari & Testai, 2008). We confirm from our study the existence of potential toxic cyanobacterial strains in sediment as well as in planktonic state, using molecular markers for the aminotransferase region. The aminotransferase domain has been successfully used in the detection of microcystin- and nodularin-producing synthetase genes in hepatotoxic cyanobacterial isolates and blue-green algal bloom (Jungblut & Neilan, 2006). It is evident from this study that the aminotransferase domain for *mcyE* and *ndaF* could be considered a good molecular tool for the detection of potential toxic phylotypes not only in planktonic samples but also in sediments.

All nine nucleotide sequences of the aminotransferase region obtained from different samples in the present study appeared to be phylogenetically related to the aminotransferase domain only for *mcyE* gene but never for the *ndaF* gene, and they were included in the clusters of microcystin-producing cyanobacteria. It is very likely that the four aminotransferase sequences obtained from sediment and water samples of different Kenyan lakes are associated with *Synechococcus* sp. or *Synechocystis* sp. Based on microscopic analyses, the presence of *Microcystis* sp. has been reported only in freshwater lakes and not in saline–alkaline lakes of Kenya, while other chroococcalean members (*Synechococcus* sp. and *Synechocystis* sp.) have been described in both categories of lakes (Ballot *et al*., 2003, 2005). The capability of microcystin production by *Synechocystis* sp. has been recorded for drinking water reservoirs and ponds in Morocco (Oudra *et al*., 2002) and isolated strains of *Synechocystis* sp. from marine habitats (Martins *et al*., 2005). The potential to produce this cyanotoxin by *Synechococcus* sp. was also elucidated in Salton Sea (Carmichael & Li, 2006) and in a cultured sample from Portugal (Martins *et al*., 2005). Probably, the aminotransferase domain of *Synechococcus* sp. and *Synechocystis* sp. is phylogenetically very close to *Microcystis* sp. Unfortunately, a sequence of the aminotransferase region from *Synechococcus* sp. and *Synechocystis* sp. is not available so far in the GenBank database for comparison with *Microcystis* sp. At least in one of the studied lakes (L. Bogoria), hepatotoxins microcystin-LR, -RR, -LF and -YR and the neurotoxin anatoxin-a were detected analytically from cyanobacterial mats dominated by *Synechococcus bigranulata* and were related to the mysterious mass deaths of Lesser Flamingos (Krienitz *et al*., 2003).

The significant sequence divergence ($\geq$5%) and non-availability of adequate sequences (one only) of the aminotransferase region in public database indicates that our five sequences are presumably related to the taxon *Anabaenopsis* or another species of *Anabaena*. Our deduction is supported from earlier microscopical findings, in which nostocalean taxa *Anabaenopsis* and *Anabaena* have been reported from Kenyan saline–alkaline and freshwater lakes (Krienitz *et al*., 2002; Ballot *et al*., 2003, 2004a, 2005; Kotut *et al*., 2006). The notion regarding the relationship of the obtained aminotransferase sequences in this study to taxa of *Anabaenopsis* are favored by from findings in which the potential of microcystin production by *Anabaenopsis* sp. has been demonstrated in extracts of a natural bloom of cyanobacteria predominantly composed of *Anabaenopsis milleri* in Lake Porto Lagos, Greece (Lanaras & Cook, 1994). Phylogenetic analysis of amino acid sequences of the aminotransferase region showed that the aminotransferase domain of nodularin synthetase had the closest relationship to the aminotransferase from heterocyst-forming microcystin producers (Jungblut & Neilan, 2006). The *mcyE* and *ndaF* gene regions have been considered highly reliable genetic markers for potential microcystin/nodularin production (Rantala *et al*., 2008). The microcystin and anatoxin-a produced by the phytoplanktonic cyanobacterial community in L. Bogoria and L. Nakuru may have an impact on the flamingo populations at both lakes (Ballot *et al*., 2004b). Our results on the detection of the aminotransferase domain for potential cyanotoxin production support our earlier findings that cyanotoxins contribute to the mysterious death of flamingos in Kenyan saline–alkaline lakes (Krienitz *et al*., 2003), which are important conservation reserves and tourist attractions (Schagerl & Oduor, 2008). However, the aminotransferase domain located on module *mcyE* was detected in nine samples out of 15 investigated. It is therefore obvious that not all strains possess the aminotransferase domain for microcystin production. The presence of DNA fragments of the aminotransferase domain in several sediments as well in the water samples studied demonstrates the prevalence of potential toxin-producing cyanobacterial phylotypes in these lakes. Our results have revealed that a variety of aminotransferase domains are widely distributed in the Kenyan water bodies. We can, therefore, conclude that molecular tools such as the PCR-based aminotransferase domain detection in sediments could be successfully used in

routine monitoring to provide an early warning on possible toxin production in a particular water body.

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References

Ballot A, Pflugmacher S, Wiegand C, Kotut K & Krienitz L (2003) Cyanobacterial toxins in Lake Baringo, Kenya. *Limnologica* **33**: 2–9.

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd GA & Pflugmacher S (2004a) Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya – Lakes Bogoria, Nakuru and Elmenteita. *J Plankton Res* **26**: 925–935.

Ballot A, Dadheech PK & Krienitz L (2004b) Phylogenetic relationship of *Arthrospira*, *Phormidium* and *Spirulina* strains from Kenyan and Indian waterbodies. *Arch Hydrobiol/Algol Stud* **113**: 37–56.

Ballot A, Krienitz L, Kotut K, Wiegand C & Pflugmacher S (2005) Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful Algae* **4**: 139–150.

Ballot A, Dadheech PK, Haande S & Krienitz L (2008) Morphological and phylogenetic analysis of *Anabaenopsis abijatae* and *Anabaenopsis elenkinii* (Nostocales, Cyanobacteria) from tropical inland water bodies. *Microb Ecol* **55**: 608–618.

Beadle LC (1932) Scientific results of the Cambridge Expedition to the East African lakes, 1930–1931. 4. The waters of some East African Lakes in relation to their fauna and flora. *J Limn Soc Zool* **38**: 157–211.

Boström B, Pettersson AK & Ahlgren I (1989) Seasonal dynamics of a cyanobacteria-dominated microbial community in surface sediments of a shallow, eutrophic lake. *Aquat Sci* **51**: 153–178.

Büdel B (1999) Ecology and diversity of rock-inhabiting cyanobacteria in tropical regions. *Eur J Phycol* **34**: 361–370.

Büdel B & Wessels DCJ (1991) Rock inhabiting blue-green algae/cyanobacteria from hot arid regions. *Arch Hydrobiol/Algol Stud* **64**: 385–398.

Carmichael WW (1997) The cyanotoxins. *Adv Bot Res* **27**: 211–256.

Carmichael WW (2001) Health effects of toxin-producing cyanobacteria: "the cyanoHABs". *Hum Ecol Risk Assess* **7**: 1393–1407.

Carmichael WW & Li RH (2006) Cyanobacteria toxins in the Salton Sea. *Saline Systems* **2**: 1–5.

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA & Metcalf JS (1999) Cyanobacterial toxins, exposure routes and human healths. *Eur J Phycol* **34**: 405–415.

Codd GA, Metcalf JS, Morrison LF, Krienitz L, Ballot A, Pflugmacher S, Wiegand C & Kotut K (2003) Susceptibility of flamingos to cyanobacterial toxins via feeding. *Vet Rec* **152**: 722–723.

Dittmann E, Neilan BA, Erhard M, von Döhren H & Börner T (1997) Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. *Mol Microbiol* **26**: 779–787.

Dorador C, Vila I, Imhoff JF & Witzel KP (2008) Cyanobacterial diversity in Solar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion? *FEMS Microbiol Ecol* **64**: 419–432.

Duckworth AW, Grant WD, Jones BE & van Steenbergen R (1996) Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiol Ecol* **19**: 181–191.

Finlay BJ, Curds CR, Bamforth SS & Bafort JM (1987) Ciliated protozoa and other microorganisms from 2 African soda lakes (Lake Nakuru and Lake Simbi, Kenya). *Arch Protistenkd* **133**: 81–91.

Friedmann EI & Ocampo R (1976) Endolithic blue-green algae in dry valleys – primary producers in Antarctic desert ecosystem. *Science* **193**: 1247–1249.

Funari E & Testai E (2008) Human health risk assessment related to cyanotoxins exposure. *Crit Rev Toxicol* **38**: 97–125.

Harper DM, Boar RR, Everard M & Hickley P (2002) *Lake Naivasha, Kenya. Developments in Hydrobiology, Vol. 168*. Kluwer Academic Publishers, Dordrecht.

Harper DM, Childress RB, Harper MM *et al.* (2003) Aquatic biodiversity and saline lakes: Lake Bogoria, National Reserve Kenya. *Hydrobiologia* **500**: 259–276.

Haverkamp T, Acinas SG, Doeleman M, Stomp M, Huisman J & Stal LJ (2008) Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons. *Environ Microbiol* **10**: 174–188.

Hepperle D (2008) Align – multisequence alignment editor v05/2008, GelQuest – DNA fingerprint analysis software v2.3.3, ClusterVis – software for cluster analysis and visualization of fingerprint data v1.2.1. Available from SequentiX – digital DNA processing, Klein Raden, Germany, http://www.sequentix.de

Hindák F (1985) Morphology of trichomes in *Spirulina fusiformis* Voronichin from Lake Bogoria. *Arch Hydrobiol/Algol Stud* **71**: 201–218.

Hopson AJ (1982) Lake Turkana**:** A report on the findings of the Lake Turkana Project 1972–1975. Overseas Development Administration, London.

Innok S, Matsumura M, Boonkerd N & Teaumroong N (2005) Detection of *Microcystis* in lake sediment using molecular genetic techniques. *World J Microb Biot* **21**: 1559–1568.

Jungblut AD & Neilan BA (2006) Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and

nodularin, synthetase genes in three orders of cyanobacteria. *Arch Microbiol* **185**: 107–114.

Kalff J (1986) Phytoplankton and its dynamics in two tropical lakes – a tropical and temperate zone comparison. *Hydrobiologia* **138**: 161–176.

Komárek J (2005) Phenotypic diversity of the heterocystous cyanoprokaryotic genus *Anabaenopsis*. *Czech Phycol* **5**: 1–35.

Komárek J & Anagnostidis K (1999) Cyanoprokaryota, 1. Teil: chroococcales. *Süßwasserflora von Mitteleuropa, v. 19/1* (Ettl H, Gärtner G, Heynig H & Mollenhauer D, eds), pp. 1–548. Gustav Fischer Verlag, Jena.

Kotut K, Ballot A & Krienitz L (2006) Toxic cyanobacteria and its toxins in standing waters of Kenya: implications for water resource use. *J Water Health* **4**: 233–245.

Krienitz L, Ballot A, Wiegand C, Kotut K, Codd GA & Pflugmacher S (2002) Cyanotoxin-producing bloom of *Anabaena flos-aquae*, *Anabaena discoidea* and *Microcystis aeruginosa* (Cyanobacteria) in Nyanza Gulf of Lake Victoria, Kenya. *J Appl Bot* **76**: 179–183.

Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf JS, Codd GA & Pflugmacher S (2003) Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiol Ecol* **43**: 141–148.

Krienitz L, Ballot A, Casper P, Codd GA, Kotut K, Metcalf J, Morrison L, Pflugmacher S & Wiegand C (2005) Contribution of toxic cyanobacteria to massive deaths of Lesser Flamingos at saline–alkaline lakes of Kenya. *Verh Internat Verein Limnol* **29**: 783–786.

Lanaras T & Cook CM (1994) Toxin extraction from an *Anabaenopsis milleri*-dominated bloom. *Sci Total Environ* **142**: 163–169.

Latour D, Salençon MJ, Reyss JL & Giraudet H (2007) Sedimentary imprint of *Microcystis aeruginosa* (Cyanobacteria) blooms in Grangent reservoir (Loire, France). *J Phycol* **43**: 417–425.

Li RH, Debella HJ & Carmichael WW (2001) Isolates identifiable as *Arthrospira maxima* and *Arthrospira fusiformis* (Oscillatoriales, Cyanobacteria) appear identical on the basis of a morphological study in culture and 16S rRNA gene sequence. *Phycologia* **40**: 367–371.

Lung'Ayia HBO, M'Harzi A, Tackx M, Gichuki J & Symoens JJ (2000) Phytoplankton community structure and environment in the Kenyan waters of Lake Victoria. *Freshwater Biol* **43**: 529–543.

Martins R, Pereira P, Welker M, Fastner J & Vasconcelos VM (2005) Toxicity of culturable cyanobacteria strains isolated from the Portuguese coast. *Toxicon* **46**: 454–464.

Melack JM (1988) Primary producer dynamics associated with evaporative concentration in a shallow, equatorial soda lake (Lake Elmenteita, Kenya). *Hydrobiologia* **158**: 1–14.

Muyzer G, de Waal EC & Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microb* **59**: 695–700.

Nübel U, Garcia-Pichel F & Muyzer G (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl Environ Microb* **63**: 3327–3332.

Njuguna SG (1988) Nutrient–phytoplankton relationships in a tropical meromictic soda lake. *Hydrobiologia* **158**: 15–28.

Odada EO, Olago DO, Bugenyi F, Kulindwa K, Karimumuryango J, West K, Ntiba M, Wandiga S, Aloo-Obudho P & Achola P (2003) Environmental assessment of the East African Rift Valley lakes. *Aquat Sci* **65**: 254–271.

Oduor SO & Schagerl M (2007a) Temporal trends of ion contents and nutrients in three Kenyan Rift Valley saline–alkaline lakes and their influence on phytoplankton biomass. *Hydrobiologia* **584**: 59–68.

Oduor SO & Schagerl M (2007b) Phytoplankton primary productivity characteristics in response to photosynthetically active radiation in three Kenyan Rift Valley saline–alkaline lakes. *J Plankton Res* **29**: 1041–1050.

Oudra B, Loudiki M, Vasconcelos V, Sabour B, Sbiyyaa B, Oufdou K & Mezrioui N (2002) Detection and quantification of microcystins from cyanobacteria strains isolated from reservoirs and ponds in Morocco. *Environ Toxicol* **17**: 32–39.

Patterson G & Wilson KK (1995) The influence of the diel climatic cycle on the depth-time distribution of phytoplankton and photosynthesis in a shallow equatorial lake (Lake Baringo, Kenya). *Hydrobiologia* **304**: 1–8.

Rantala A, Rizzi E, Castiglioni B, de Bellis G & Sivonen K (2008) Identification of hepatotoxin-producing cyanobacteria by DNA-chip. *Environ Microbiol* **10**: 653–664.

Rees HC, Grant WD, Jones BE & Heaphy S (2004) Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. *Extremophiles* **8**: 63–71.

Reynolds CS, Jaworski GHM, Cmiech HA & Leedale GF (1981) On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kütz emend Elenkin. *Philos T Roy Soc B* **293**: 419–477.

Schagerl M & Oduor SO (2008) Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline–alkaline lakes. *Mar Freshwater Res* **59**: 125–136.

Schober E & Kurmayer R (2006) Evaluation of different DNA sampling techniques for the application of the real-time PCR method for the quantification of cyanobacteria in water. *Lett Appl Microbiol* **42**: 412–417.

Suda S, Watanabe M, Otsuka S, Mahakahant A, Yongmanitchai W, Nopartnaraporn N, Liu Y & Day JG (2002) Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. *Int J Syst Evol Micr* **52**: 1577–1595.

Takamura N, Yasuno M & Sugahara K (1984) Overwintering of *Microcystis aeruginosa* Kütz in a shallow lake. *J Plankton Res* **6**: 1019–1029.

Talling JF (1966) The annual cycle of stratification and phytoplankton growth in Lake Victoria (East Africa). *Int Rev Ges Hydrobio* **51**: 545–621.

Talling JF (1987) The phytoplankton of Lake Victoria (East Africa). *Arch Hydrobiol Beih Ergebn Limnol* **25**: 229–256.

Talling JF & Talling IB (1965) The chemical composition of African lake water. *Int Rev Ges Hydrobio* **51**: 421–463.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**: 1596–1599.

Taton A, Grubisic S, Brambilla E, de Wit R & Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo dry valleys, Antarctica): a morphological and molecular approach. *Appl Environ Microb* **69**: 5157–5169.

Taton A, Grubisic S, Ertz D *et al.* (2006) Polyphasic study of antarctic cyanobacterial strains. *J Phycol* **42**: 1257–1270.

Terer T, Ndiritu GG & Gichuki NN (2004) Socio-economic values and traditional strategies of managing wetland resources in Lower Tana River, Kenya. *Hydrobiologia* **527**: 3–14.

Tsujimura S, Tsukada H, Nakahara H, Nakajima T & Nishino M (2000) Seasonal variations of *Microcystis* populations in sediments of Lake Biwa, Japan. *Hydrobiologia* **434**: 183–192.

Vareschi E (1978) Ecology of Lake Nakuru (Kenya) I abundance and feeding of Lesser Flamingo. *Oecologia* **32**: 11–35.

Vareschi E (1982) The ecology of Lake Nakuru (Kenya) III abiotic factors and primary production. *Oecologia* **55**: 81–101.

Verschuren D, Cocquyt C, Tibby J, Roberts CN & Leavitt PR (1999) Long-term dynamics of algal and invertebrate communities in a small, fluctuating tropical soda lake. *Limnol Oceanogr* **44**: 1216–1231.

Verschuren D, Johnson TC, Kling HJ, Edgington DN, Leavitt PR, Brown ET, Talbot MR & Hecky RE (2002) History and timing of human impact on Lake Victoria, East Africa. *P Roy Soc Lond B Bio* **269**: 289–294.

Verspagen JMH, Snelder EOFM, Visser PM, Jöhnk KD, Ibelings BW, Mur LR & Huisman J (2005) Benthic–pelagic coupling in the population dynamics of the harmful cyanobacterium *Microcystis*. *Freshwater Biol* **50**: 854–867.

Wichels A, Hummert C, Elbrächter M, Luckas B, Schütt C & Gerdts G (2004) Bacterial diversity in toxic *Alexandrium tamarens*e blooms off the Orkney Isles and the Firth of Forth. *Helgoland Mar Res* **58**: 93–103.

Zhao J, Yang N & Zeng RY (2008) Phylogenetic analysis of type I polyketide synthase and nonribosomal peptide synthetase genes in Antarctic sediment. *Extremophiles* **12**: 97–105.

Zwart G, Agterveld MPK, Werff-Staverman I, Hagen F, Hoogveld HL & Gons HJ (2005) Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake. *Environ Microbiol* **7**: 365–377.