World J Microbiol Biotechnol (2010) 26:1473–1482
DOI 10.1007/s11274-010-0322-x

ORIGINAL PAPER

Molecular detection of potentially toxic cyanobacteria and their associated bacteria in lake water column and sediment

Konstantinos Ar. Kormas · Elisabeth Vardaka · Maria Moustaka-Gouni · Vasiliki Kontoyanni · Evi Petridou · Spyros Gkelis · Christos Neofitou

Received: 4 November 2009 / Accepted: 15 January 2010 / Published online: 30 January 2010
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Abstract We investigated the molecular diversity of cyanobacteria and bacteria during a water bloom in a lake with a long history of toxic cyanobacterial blooms (Lake Kastoria, Greece). We also tested the hypothesis whether bloom-forming cyanobacteria are preserved in the lake's sediment 2 years after the bloom. The dominant cyanobacteria during the bloom included the potentially toxin-producing *Microcystis aeruginosa* and several other Chroococcales forms closely related to the genus *Microcystis*. This suggests that the use of cyanobacterial-specific primers seems to be very informative in describing the cyanobacteria during the water blooms. The bacterial community showed high diversity, consisting mostly of singleton and doubleton phylotypes. The majority of the phylotypes were typical lake bacteria including some potential pathogens and toxin metabolising bacteria, suggesting that the dominant toxic cyanobacteria did not have any significant effect on the bacterial community structure. In the sediment, 2 years after the water bloom, no bloom-forming cyanobacteria were retrieved, suggesting that they cannot be preserved in the sediment. Similar to the water column, sediment bacterial diversity was also high, consisting mostly of yet-uncultured bacteria that are related to environments where organic matter degradation takes place.

Keywords Cyanobacteria · Bacteria · 16S rRNA · Water column · Sediment · Lake · Kastoria

Electronic supplementary material The online version of this article (doi:10.1007/s11274-010-0322-x) contains supplementary material, which is available to authorized users.

K. Ar. Kormas (✉) · V. Kontoyanni · E. Petridou · S. Gkelis · C. Neofitou
Department of Ichthyology and Aquatic Environment, University of Thessaly, 384 46 Nea Ionia, Greece
e-mail: kkormas@uth.gr

E. Vardaka
Department of Fisheries and Aquaculture Technology, Alexander Technological Educational Institute of Thessaloniki, Campus of New Moudania, P.O. Box 157, 632 00 New Moudania, Greece

M. Moustaka-Gouni · S. Gkelis
Department of Botany, Aristotle University of Thessaloniki, P.O. Box 109, 541 24 Thessaloniki, Greece

Introduction

Cyanobacterial blooms are recognized globally to cause detrimental ecological and economic effects. These include changes in food web structure that potentially reduce productivity at higher trophic levels, oxygen depletion and consequent death of aquatic animals, and reduced aesthetic value due to discoloration of water or foul odours and taste generated by the bacterial degradation of bloom forming biomass (Paerl and Fulton 2006). Of most concern, however, are the potent toxins that are produced by several cyanobacteria as secondary metabolites (Sivonen and Jones 1999). The presence of cyanobacterial toxins in drinking, recreational and irrigation water is known to pose a potential hazard to human health and to agricultural and aquaculture products destined for animal and human consumption (Falconer 1999; Falconer et al. 1999).

During a lake cyanobacterial bloom, a distinctive prokaryotic community may develop. However, little is known about these microbial communities and there is growing interest in the structure and possible ecophysiological role of these prokaryotes (Ye et al. 2009a; Zeng et al. 2009a). Some of these cyanobacterial bloom-associated heterotrophic bacteria have been found to inhibit and/or to enhance

cyanobacterial growth, and some others have been found to degrade cyanobacterial toxins. This suggests that there is the potential to use microbes to assess cyanobacterial blooms and their associated toxins (Berg et al. 2009). Some bloom-associated bacteria have been found to be potentially pathogenic. Together with the toxic effects of the bloom cyanobacteria, these may compound the negative health effects on humans and animals (Berg et al. 2009). In any case, such bacteria are difficult to isolate and identify with existing culture-based approaches.

In Greece, toxic cyanobacterial blooms occur frequently in most of the country's lakes (Cook et al. 2004; Vardaka et al. 2005) and the composition and biomass dynamics of these cyanobacteria are well known (e.g. Moustaka-Gouni 1993; Tryfon and Moustaka-Gouni 1997; Moustaka-Gouni et al. 2007). In addition, the effect of toxic blooms on plankton food web structure and particularly on the microbial loop has been identified in Lake Kastoria (Moustaka-Gouni et al. 2006).

The aims of this study were: (a) to characterize the cyanobacterial community during a water bloom in a Greek lake with a prolonged history of toxic water blooms (Lake Kastoria, Greece) and (b) to apply molecular approaches to describe the rest of the bacterial community accompanying the cyanobacterial bloom, in order to identify potential pathogens and cyanotoxin degraders. We also examined whether the sediment serves as an inoculum for future blooms by preserving a pool of viable cyanobacteria that have settled to the bottom of the lake after the bloom. We analyzed the small subunit ribosomal RNA (16S rRNA) gene diversity of Lake Kastoria, Greece using universal bacterial primers along with cyanobacteria-specific primers for 16S rRNA gene amplification.

Materials and methods

Approximately 500 ml of water were collected in sterile polyethylene bottles from a shallow area of the lake at 2 m depth during a water bloom on 13 August 2003. Upon return to the laboratory, 100 ml from each of the three carboys were filtered on a GF/C (Whatman, USA) filter under low vacuum ($\leq$150 mm Hg) and the filters were stored at −80°C until further analysis. Although we initially planned to use polycarbonate 0.2 μm isopore filters, their use was not feasible due to extreme clogging and breakage of filamentous cyanobacteria after the first 10–20 ml of water. In the summer of 2004, the lake's cyanobacterial bloom was of similar composition (Moustaka-Gouni and Katsiapi unpublished data), so we did not analyze this bloom with molecular tools. Since in the following summer, i.e. 2005, the lake's phytoplankton was dominated not by cyanobacteria but by chlorophytes, cryptophytes and diatoms (Moustaka-Gouni and Katsiapi unpublished data), we sampled the lake's sediment for the occurrence of dormant cyanobacterial cells (akinetes) or overwintering colonies/filaments.

Sediment samples were collected from three shallow areas on 16 October 2005, using a 20 ml syringe with a cut-off luer end. The top 1 cm of each of the three samples was pooled together in a 50 ml Falcon tube. The pooled sample was transferred to the laboratory immediately (<5 h) in darkness at 5°C, where it was stored at −80°C until further analysis.

DNA was extracted using the Ultra Clean Mega Soil DNA Isolation Kit (MoBio Laboratories Inc., USA) following the manufacturer's protocol and dissolved in 1 ml of PCR water (i.e. Milli-Q water autoclaved three times). For all PCR reactions we applied cycle optimization (i.e. to use the lowest number of PCR cycles giving a visible PCR product instead of the commonly used 30 cycles) in order to minimize the innate limitations of PCR (Spiegelman et al. 2005) and the differential representation of 16S rDNA genes with low and high copy numbers. The applied PCR primers and conditions are shown in Table 1.

The PCR products from both the Bacteria- and Cyanobacteria-specific amplifications were purified using the Montage purification kit (Millipore, USA), and the purified PCR products were cloned using the TOPO XL PCR cloning kit (Invitrogen, USA) using chemically competent cells according to the manufacturer's specifications. For each clone library a maximum of 96 clones were sequenced, each containing an insert of ca. 920 or 1,500 bp for the water column and sediment bacterial 16S rRNA, respectively, and ca. 680 bp for the water column and sediment cyanobacteria-specific amplification (CYA). These clones were grown in liquid LB medium with kanamycin and their plasmids were purified using the Nucleospin Plasmid QuickPure kit (Macherey–Nagel GmbH and Co. KG, Germany) for DNA sequencing.

Sequence data were obtained by capillary electrophoresis (Macrogen Inc., Seoul, Korea) using the BigDye Terminator kit (Applied Biosystems Inc., USA) with the primer M13F (5′-GTAAAACGACGGCCAG-3′) and M3R (5′-CAGGAAACAGCTATGAC-3′). Each sequence read was approximately 850 bp. For each individual clone, forward and reverse reads were assembled for the water column and sediment 16S rRNA sequences, and then the assembled sequences were checked for chimeras using the CHIMERA-CHECK function of the Ribosomal Database Project II (Maidak et al. 2001). All sequences were compared with the BLAST function (http://www.ncbi.nlm.nih.gov/BLAST/) for the detection of closest relatives. Sequence data were compiled using the MEGA4 software (Tamura et al. 2007) and aligned with sequences obtained from the GenBank (www.ncbi.nlm.nih.gov) database,

Table 1 Sequences of oligonucleotide primers used for PCR amplifications

PCR	Primer sequence (5′–3′)	Optimum PCR cycle number	PCR conditions (reaction volume)
Water column Bacteria	BAC-8f: AGAGTTTGATCCTGGCTCAG[a]	28	Pre-PCR: 95°C, 9 min
	BAC-907r: CCCGTCAATTCCTTTGAGTTT[a]		Cycle: 95°C, 45 s; 52.5°C, 45 s; 72°C, 2 min
			Post-PCR: 72°C, 10 min (50 µl)
Water column Cyanobacteria	CYA106f: CGGACGGGTGAGTAACGCGTGA[b]	8	Pre-PCR: 94°C, 5 min
	CYA781r(a): GACTACTGGGGTATCTAATCCCATT[b]		Cycle: 94°C, 30 s; 57°C, 30 s; 72°C, 30 min
	CYA781r(b): GACTACAGGGGTATCTAATCCCTTT[b]		Post-PCR: 72°C, 5 min (25 µl)
Sediment Bacteria	BAC-8f: AGAGTTTGATCCTGGCTCAG[a]	14	Pre-PCR: 95°C, 9 min
	BAC-1492r: CGGCTACCTTGTTACGACTT[a]		Cycle: 95°C, 45 s; 52.5°C, 45 s; 72°C, 2 min
			Post-PCR: 72°C, 10 min (50 µl)
Sediment Cyanobacteria	CYA106f: CGGACGGGTGAGTAACGCGTGA[a]	25	Pre-PCR: 94°C, 5 min
	CYA781r(a): GACTACTGGGGTATCTAATCCCATT[b]		Cycle: 94°C, 30 s; 57°C, 30 s; 72°C, 30 min
	CYA781r(b): GACTACAGGGGTATCTAATCCCTTT[b]		Post-PCR: 72°C, 5 min (25 µl)

In all cases, 10–20 ng DNA was used as template. *f* forward, *r* reverse primer

[a] Lane (1991)

[b] Nübel et al. (1997)

using the ClustalX aligning utility. Phylogenetic analyses were performed using minimum evolution and parsimony methods implemented in MEGA4 (Tamura et al. 2007). Heuristic searches under minimum evolution criteria used 1,000 random-addition replicates per data set, each followed by tree bisection-reconnection topological rearrangements. The topology of the tree was based on neighbour-joining according to Jukes-Cantor. Bootstrapping under parsimony criteria was performed with 1,000 replicates. Sequences of unique phylotypes found in this study have GenBank accession numbers EU376160–EU376620 (water column, bacterial 16S rRNA), FJ204870–FJ204887 (water column, cyanobacterial-specific 16S rRNA), EF203172–EF203209 (sediment, bacterial 16S rRNA) and GQ240853–GQ240877 (sediment, cyanobacterial-specific 16S rRNA).

Library clone coverage was calculated by the formula of the Good's C estimator $[1 - (n_1/N)]$ (Good 1953), where n_1 is the number of phylotypes represented by only one clone and N is the total number of clones examined in each library. The number of predicted phylotypes for each clone library was estimated after the abundance-based richness formula S_{Chao1} (Chao 1984, 1987):

$$S_{\text{Chao1}} = S_{\text{obs}} + \left[F_1^2/2(F_2+1)\right] - \left[(F_1F_2)/2(F_2+1)^2\right]$$

where S_{obs} is the number of phylotypes observed in the library, and F_1 and F_2 are the number of phylotypes occurring either one or two times. This estimator is particularly appropriate for data sets in which most phylotypes are relatively rare (Chao 1987).

Results

In the water column, a total of 82 bacterial and 49 cyanobacterial-specific clones were analyzed, corresponding to 61 and 18 unique phylotypes, respectively (Tables S1, S2). Clone coverage analysis (Fig. 1) based on Good's C estimator (Good 1953; Kemp and Aller 2004), suggests that retrieval of the full extent of extant diversity of Bacteria in this lake was not achieved, and that only 44.2% of the predicted phylotypes based on the S_{Chao1} index was revealed. On the other hand, the coverage of the cyanobacterial-specific library was satisfactory, with a S_{Chao1} index of 76.6% and a curvilinear mode around 0.9.

The PCR amplification with the cyanobacterial-specific primers in the water bloom, resulted in cyanobacterial (33/49 phylotypes) and non-cyanobacterial phylotypes (16/49 phylotypes). The cyanobacterial phylotypes recovered by this study affiliated with the Chroococcales, Nostocales and Oscillatoriales. Three phylotypes, were affiliated to "plastids" (Table S1; Fig. 2). In total, true cyanobacterial and plastid-related phylotypes represented 75.5% of all the recovered phylotypes. The majority of the non-cyanobacterial phylotypes belonged to the Verrucomicrobia (4/49 phylotypes, 18.4% relative abundance), and two singleton phylotypes were related to the Actinobacteria and to the candidate division OP11 (Table S1; Fig. 2).

Regarding the bacterial community, the dominant phylum, in terms of unique phylotypes (17/61) and clones (32.9%) was the Proteobacteria, and in particular the subphyla of α-, β- and γ-Proteobacteria. The next most

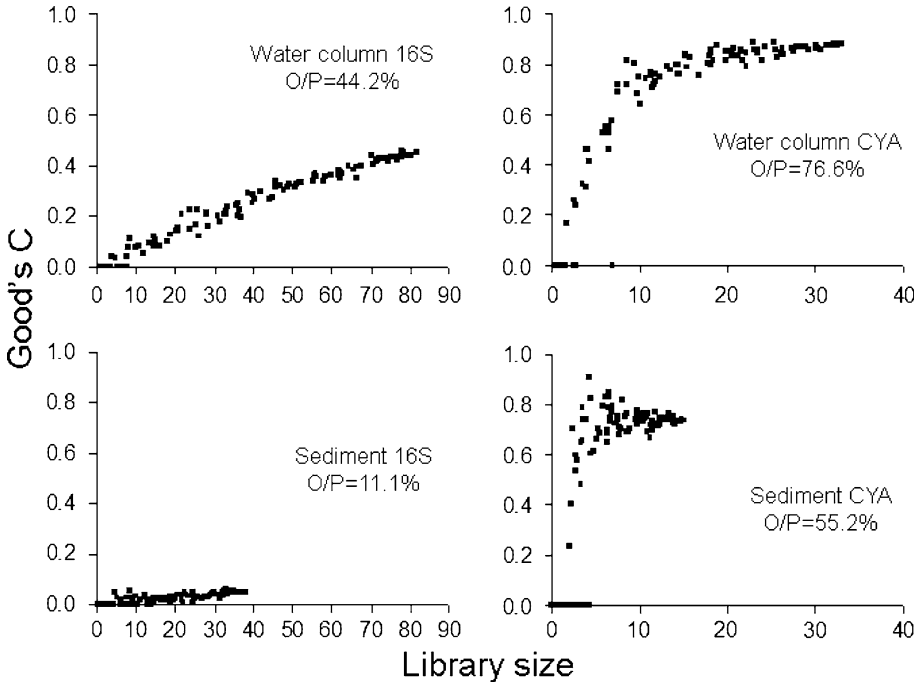


Fig. 1 Clone library coverage based on Good's C estimator of the bacterial and cyanobacterial clone libraries from the water column and sediment of Lake Kastoria, Greece. *O* observed number of phylotypes, *P* predicted number of phylotypes based on the S_{Chao1} index

dominant phylum was the Actinobacteria (11/61 phylotypes, 20.7% relative abundance). The rest of the occurring phylotypes belonged to the phyla of Verrucomicrobia, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Fusobacteria, Planctomycetes. In addition, seven phylotypes were not affiliated with any of the described bacterial phyla, and were most closely related to uncultivated microorganisms retrieved from natural habitats and bioremediation processes (Table S2; Fig. 3).

In the sediment, a total of 38 cyanobacteria-specific and 38 bacterial clones were analyzed, corresponding to 25 and 37 unique phylotypes, respectively (Tables S3, S4). Clone coverage analysis (Fig. 1) based on Good's C estimator (Good 1953; Kemp and Aller 2004), suggests that the full extent of the existing diversity of Bacteria was not recovered, and that only 11.2% of the predicted phylotypes based on the S_{Chao1} index was revealed. Clone coverage of the cyanobacteria-specific library was more satisfactory, with the S_{Chao1} index at 55.2% and a curvilinear mode around 0.8.

The sediment cyanobacteria-specific library (Table S3; Fig. 4) was dominated by the Verrucomicrobia (14/38 phylotypes, 44.7% relative abundance) followed by cyanobacteria (5/38 phylotypes, 39.5%). The most dominant (11/38) phylotype belonged to the cyanobacteria. The rest of the phylotypes were affiliated to the Chloroflexi, Acidobacteria and Firmicutes.

All of the recovered phylotypes in the sediment bacterial 16S rRNA gene library, were singletons except one, which was a doubleton (Table S4; Fig. 5). The Proteobacteria dominated this library (14/37 phylotypes, 39.5% relative abundance), with the β- and γ-Proteobacteria equally dominating (13.2% each), followed by the α- and δ-Proteobacteria. The next most dominant phyla were the Bacteroidetes (8/37 phylotypes, 21.1% relative abundance) and the Verrucomicrobia (7/37 phylotypes, 18.4% relative abundance). The rest of the recovered phyla included the Gemmatimonadetes, Acidobacteria, Fibrobacteres, Firmicutes and Spirochaetes. Finally, two phylotypes were not affiliated to any of the known bacterial phyla (Table S4; Fig. 5).

Discussion

We investigated the water column and sediment molecular diversity of Cyanobacteria and Bacteria in Lake Kastoria, Greece, known for many years to host toxic cyanobacterial water blooms (Lanaras et al. 1989; Cook et al. 2004; Moustaka-Gouni et al. 2006; Gkelis et al. 2005). Our universal bacterial 16S clone library coverage revealed that in both the water column and in the sediment, the species richness of Bacteria is immense, and is dominated by "rare" phylotypes (i.e. singletons and doubletons) (Tables S1, S3; Fig. 1). In contrast, the cyanobacteria-specific clone libraries for both the water column and sediment showed much higher ratios of observed to predicted phylotypes (76.6 and 55.2% for the water column and sediment, respectively). This implies that the cyanobacteria-specific primers used here (Nübel et al. 1997) are more appropriate for monitoring cyanobacterial diversity, especially during water blooms, than universal bacterial primers, although further testing with cultured strains is needed along with in

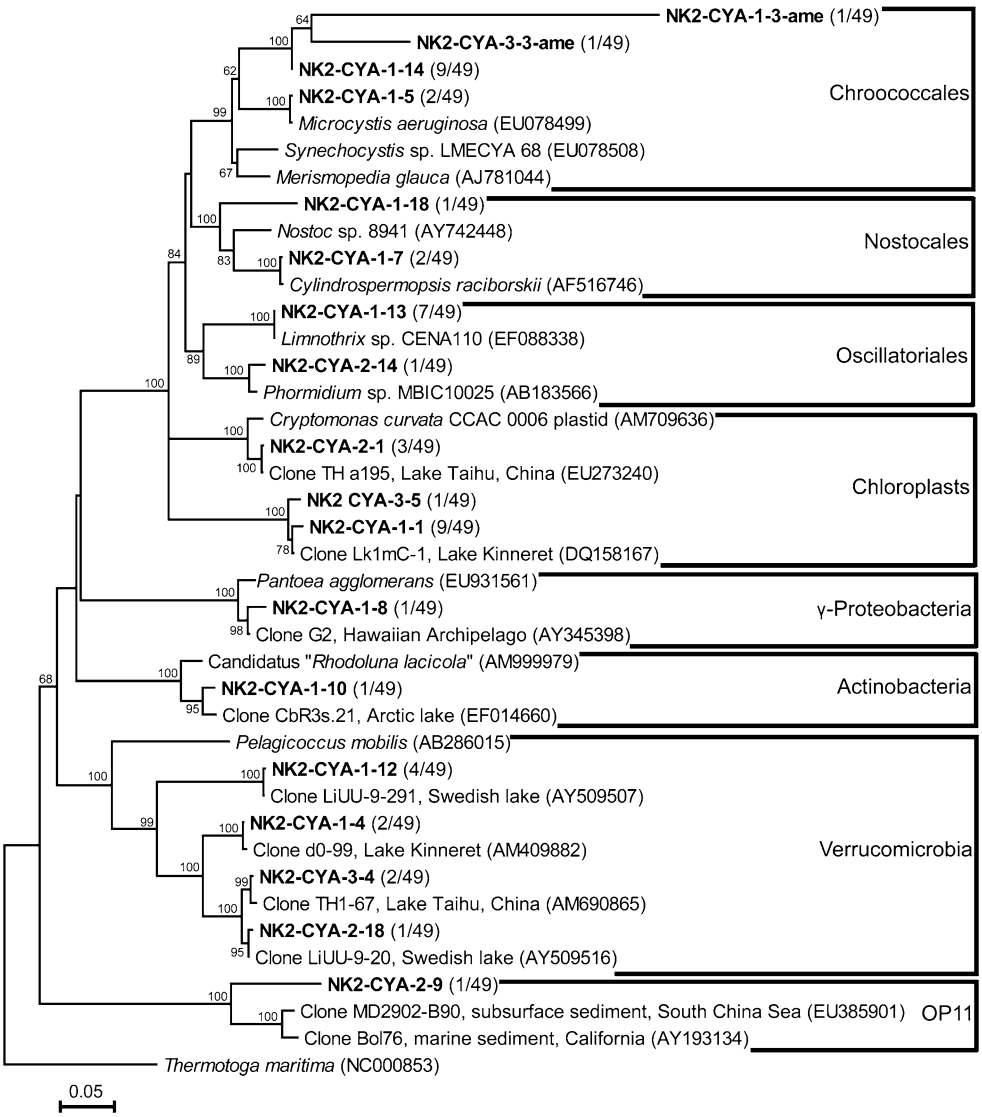
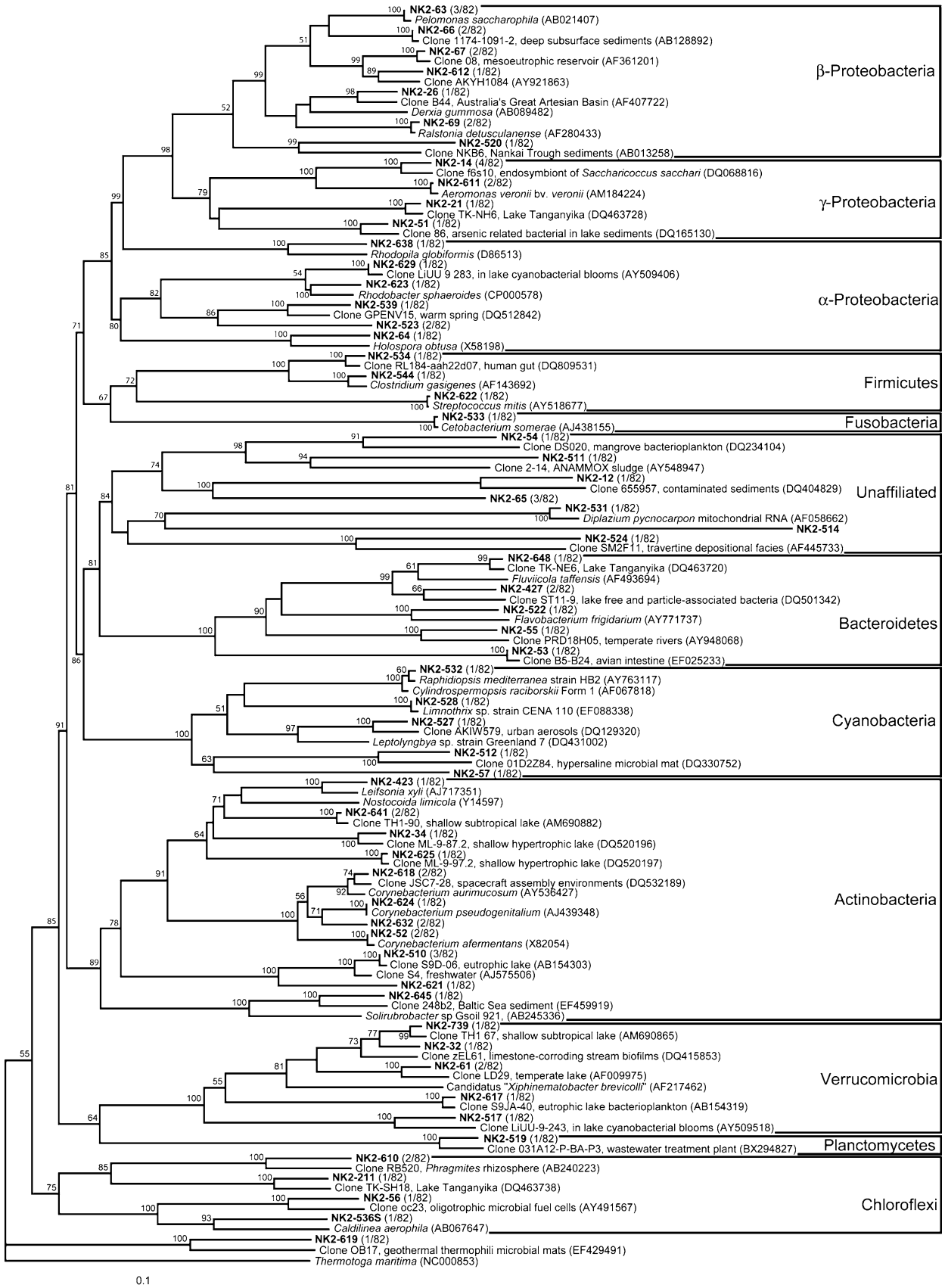


Fig. 2 Phylogenetic tree of relationships of 16S rDNA (ca. 680 bp) of the unique cyanobacterial phylotypes found in the water column of Lake Kastoria, based on the neighbour-joining method as determined by distance Jukes–Cantor analysis. The found phylotypes are in *bold*, with numbers of identical (≥98%) phylotypes in *parentheses*. One thousand bootstrap analyses (distance) were conducted, and percentages greater than 50% are indicated at the *nodes*. The numbers in *brackets* are GenBank accession numbers. *Scale bar* represents 5% estimated distance

silico analysis. However, these primers seem to capture not only the sequences of Cyanobacteria but also sequences from other phyla such as the Verrucomicrobia (Figs. 1, 3). The phylum Verrucomicrobia seems to be associated with cyanobacterial blooms not only in our study, but also has been found in other eutrophic lakes (Wu et al. 2007; Ye et al. 2009a; Kormas et al. submitted).

The water column cyanobacterial bloom in Lake Kastoria was dominated by phylotypes related to *Microcystis aeruginosa*. The toxin producing *M. aeruginosa* is known to be one of the dominant bloom cyanobacteria in this lake (Cook et al. 2004; Gkelis et al. 2005; Vardaka et al. 2005), while it has been shown to be a major cause of hepatotoxic blooms worldwide (Sivonen and Jones 1999). The use of the cyanobacteria-specific primers revealed the occurrence of three more Chroococcales phylotypes. According to our microscopic observations (Moustaka-Gouni unpublished data) we observed several morphospecies of *Microcystis* (*M. aeruginosa*, *M. flos-aquae*, *M. ichthyoblabe*, *M. novacekii*, *M. wesenbergii*), *Aphanocapsa*-like and *Aphanothece*-like colonies. However, none of the recovered Chroococcales phylotypes were closely related to the genera *Aphanocapsa* and *Aphanothece*, suggesting that these are probably novel phylotypes of the *Microcystis* group. *Microcystis* morphospecies are the dominant cyanobacteria late in succession of phytoplankton in L. Kastoria (Moustaka-Gouni et al. 2007). The rest of the

Fig. 3 Phylogenetic tree of relationships of 16S rDNA (ca. 920 bp) ▶ of the unique bacterial phylotypes found in the water column of Lake Kastoria, based on the neighbour-joining method as determined by distance Jukes–Cantor analysis. The found phylotypes are in *bold*, with numbers of identical (≥98%) phylotypes in *parentheses*. One thousand bootstrap analyses (distance) were conducted, and percentages greater than 50% are indicated at the *nodes*. The numbers in *brackets* are GenBank accession numbers. *Scale bar* represents 10% estimated distance


NK2-63 (3/82)
Pelomonas saccharophila (AB021407)
NK2-66 (2/82)
Clone 1174-1091-2, deep subsurface sediments (AB128892)
NK2-67 (2/82)
Clone 08, mesoeutrophic reservoir (AF361201)
NK2-612 (1/82)
Clone AKYH1084 (AY921863)
NK2-26 (1/82)
Clone B44, Australia's Great Artesian Basin (AF407722)
Derxia gummosa (AB089482)
NK2-69 (2/82)
Ralstonia detusculanense (AF280433)
NK2-520 (1/82)
Clone NKB6, Nankai Trough sediments (AB013258)
β-Proteobacteria
NK2-14 (4/82)
Clone f6s10, endosymbiont of Saccharicoccus sacchari (DQ068816)
NK2-611 (2/82)
Aeromonas veronii bv. veronii (AM184224)
NK2-21 (1/82)
Clone TK-NH6, Lake Tanganyika (DQ463728)
NK2-51 (1/82)
Clone 86, arsenic related bacterial in lake sediments (DQ165130)
γ-Proteobacteria
NK2-638 (1/82)
Rhodopila globiformis (D86513)
NK2-629 (1/82)
Clone LiUU 9 283, in lake cyanobacterial blooms (AY509406)
NK2-623 (1/82)
Rhodobacter sphaeroides (CP000578)
NK2-539 (1/82)
Clone GPENV15, warm spring (DQ512842)
NK2-523 (2/82)
NK2-64 (1/82)
Holospora obtusa (X58198)
α-Proteobacteria
NK2-534 (1/82)
Clone RL184-aah22d07, human gut (DQ809531)
NK2-544 (1/82)
Clostridium gasigenes (AF143692)
NK2-622 (1/82)
Streptococcus mitis (AY518677)
Firmicutes
NK2-533 (1/82)
Cetobacterium somerae (AJ438155)
Fusobacteria
NK2-54 (1/82)
Clone DS020, mangrove bacterioplankton (DQ234104)
NK2-511 (1/82)
Clone 2-14, ANAMMOX sludge (AY548947)
NK2-12 (1/82)
Clone 655957, contaminated sediments (DQ404829)
NK2-65 (3/82)
NK2-531 (1/82)
Diplazium pycnocarpon mitochondrial RNA (AF058662)
NK2-514
NK2-524 (1/82)
Clone SM2F11, travertine depositional facies (AF445733)
Unaffiliated
NK2-648 (1/82)
Clone TK-NE6, Lake Tanganyika (DQ463720)
Fluviicola taffensis (AF493694)
NK2-427 (2/82)
Clone ST11-9, lake free and particle-associated bacteria (DQ501342)
NK2-522 (1/82)
Flavobacterium frigidarium (AY771737)
NK2-55 (1/82)
Clone PRD18H05, temperate rivers (AY948068)
NK2-53 (1/82)
Clone B5-B24, avian intestine (EF025233)
Bacteroidetes
NK2-532 (1/82)
Raphidiopsis mediterranea strain HB2 (AY763117)
Cylindrospermopsis raciborskii Form 1 (AF067818)
NK2-528 (1/82)
Limnothrix sp. strain CENA 110 (EF088338)
NK2-527 (1/82)
Clone AKIW579, urban aerosols (DQ129320)
Leptolyngbya sp. strain Greenland 7 (DQ431002)
NK2-512 (1/82)
Clone 01D2Z84, hypersaline microbial mat (DQ330752)
NK2-57 (1/82)
Cyanobacteria
NK2-423 (1/82)
Leifsonia xyli (AJ717351)
Nostocoida limicola (Y14597)
NK2-641 (2/82)
Clone TH1-90, shallow subtropical lake (AM690882)
NK2-34 (1/82)
Clone ML-9-87.2, shallow hypertrophic lake (DQ520196)
NK2-625 (1/82)
Clone ML-9-97.2, shallow hypertrophic lake (DQ520197)
NK2-618 (2/82)
Clone JSC7-28, spacecraft assembly environments (DQ532189)
Corynebacterium aurimucosum (AY536427)
NK2-624 (1/82)
Corynebacterium pseudogenitalium (AJ439348)
NK2-632 (2/82)
NK2-52 (2/82)
Corynebacterium afermentans (X82054)
NK2-510 (3/82)
Clone S9D-06, eutrophic lake (AB154303)
Clone S4, freshwater (AJ575506)
NK2-621 (1/82)
NK2-645 (1/82)
Clone 248b2, Baltic Sea sediment (EF459919)
Solirubrobacter sp Gsoil 921, (AB245336)
Actinobacteria
NK2-739 (1/82)
Clone TH1 67, shallow subtropical lake (AM690865)
NK2-32 (1/82)
Clone zEL61, limestone-corroding stream biofilms (DQ415853)
NK2-61 (2/82)
Clone LD29, temperate lake (AF009975)
Candidatus "Xiphinematobacter brevicolli" (AF217462)
NK2-617 (1/82)
Clone S9JA-40, eutrophic lake bacterioplankton (AB154319)
NK2-517 (1/82)
Clone LiUU-9-243, in lake cyanobacterial blooms (AY509518)
Verrucomicrobia
NK2-519 (1/82)
Clone 031A12-P-BA-P3, wastewater treatment plant (BX294827)
Planctomycetes
NK2-610 (2/82)
Clone RB520, Phragmites rhizosphere (AB240223)
NK2-211 (1/82)
Clone TK-SH18, Lake Tanganyika (DQ463738)
NK2-56 (1/82)
Clone oc23, oligotrophic microbial fuel cells (AY491567)
NK2-536S (1/82)
Caldilinea aerophila (AB067647)
Chloroflexi
NK2-619 (1/82)
Clone OB17, geothermal thermophili microbial mats (EF429491)
Thermotoga maritima (NC000853)
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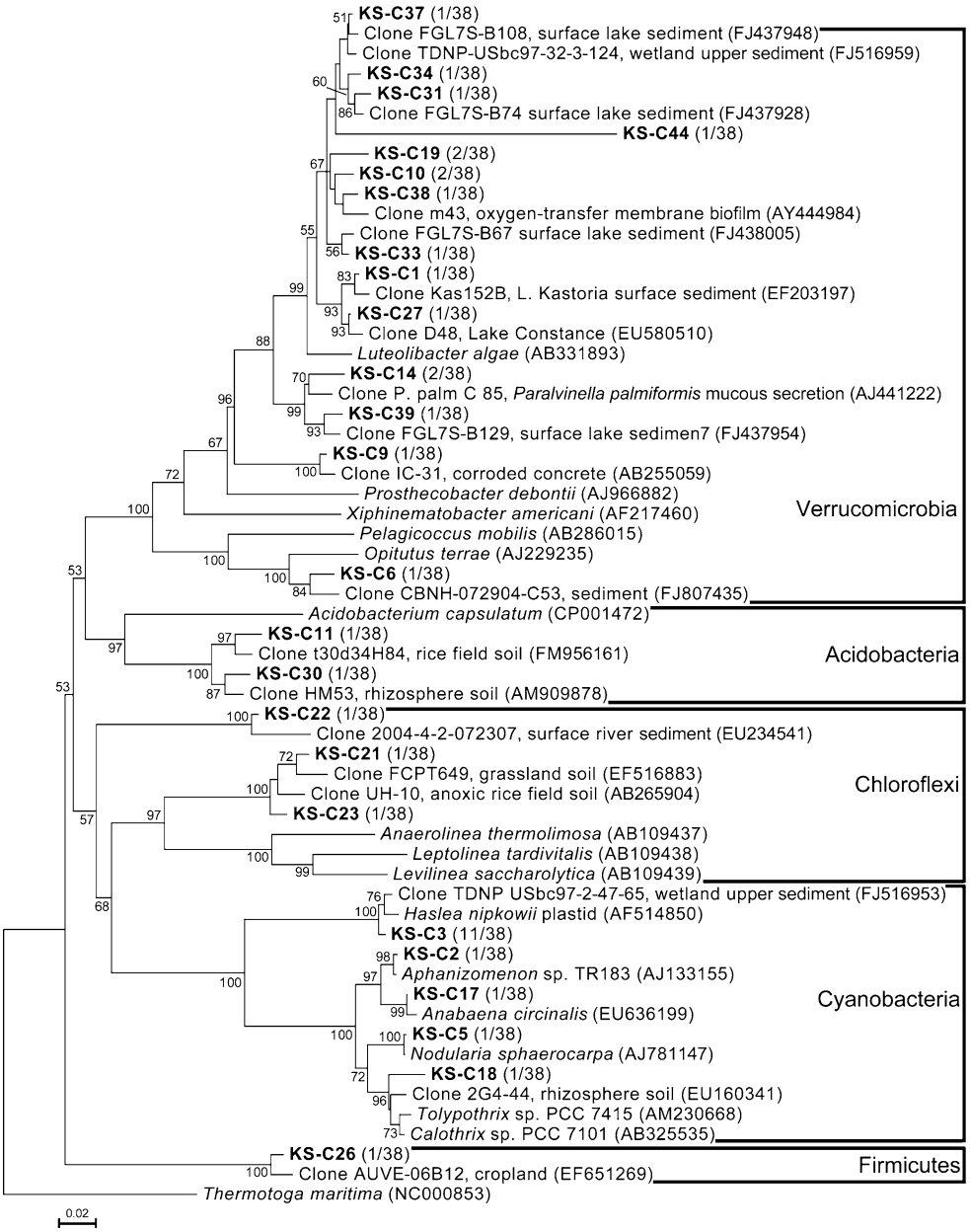

Fig. 4 Phylogenetic tree of relationships of 16S rDNA (ca. 680 bp) of the unique cyanobacterial phylotypes found in the top 1 cm sediment of Lake Kastoria, based on the neighbour-joining method as determined by distance Jukes–Cantor analysis. The found phylotypes are in *bold*, with numbers of identical (≥98%) phylotypes in *parentheses*. One thousand bootstrap analyses (distance) were conducted, and percentages greater than 50% are indicated at the *nodes*. The numbers in *brackets* are GenBank accession numbers. *Scale bar* represents 2% estimated distance

Nostocales and Oscillatoriales phylotypes that were found frequently in the study by Moustaka-Gouni et al. (2007), particularly the bloom forming *Cylindrospermopsis raciborskii*, are potentially toxic.

In contrast to the clearly structured cyanobacterial community, the bloom-associated bacterial community showed immense species richness with no defined dominance of any phylotype. This might imply that the cyanobacterial bloom did not impose any constraints on bacterial species richness. The high diversity at the phylotype level, with most phylotypes belonging to the Actinobacteria and to the Proteobacteria (mostly β- and α-), seems to be typical for lakes (Spring et al. 2000), as this has been confirmed from culture dependant (Berg et al. 2009), culture-independent (e.g. Wu et al. 2007, Nelson 2009, Ye et al. 2009a) and metagenomic approaches (e.g. Debroas et al. 2009). According to Debroas et al. (2009), several of these bacteria are related to those involved in the degradation of xenobiotics (especially the α-Proteobacteria). This capability could extend to the degradation of cyanotoxins produced by the bloom forming cyanobacteria. Since L. Kastoria has a long history of toxic cyanobacterial blooms (Lanaras et al. 1989; Cook et al. 2004; Moustaka-Gouni et al. 2006; Gkelis et al. 2005), it is possible that some of the α-Proteobacteria phylotypes recovered here represent toxin degraders. Based on current knowledge (Berg et al. 2009), none of the phylotypes we recovered are known to have such activity, although phylotype NK2-522

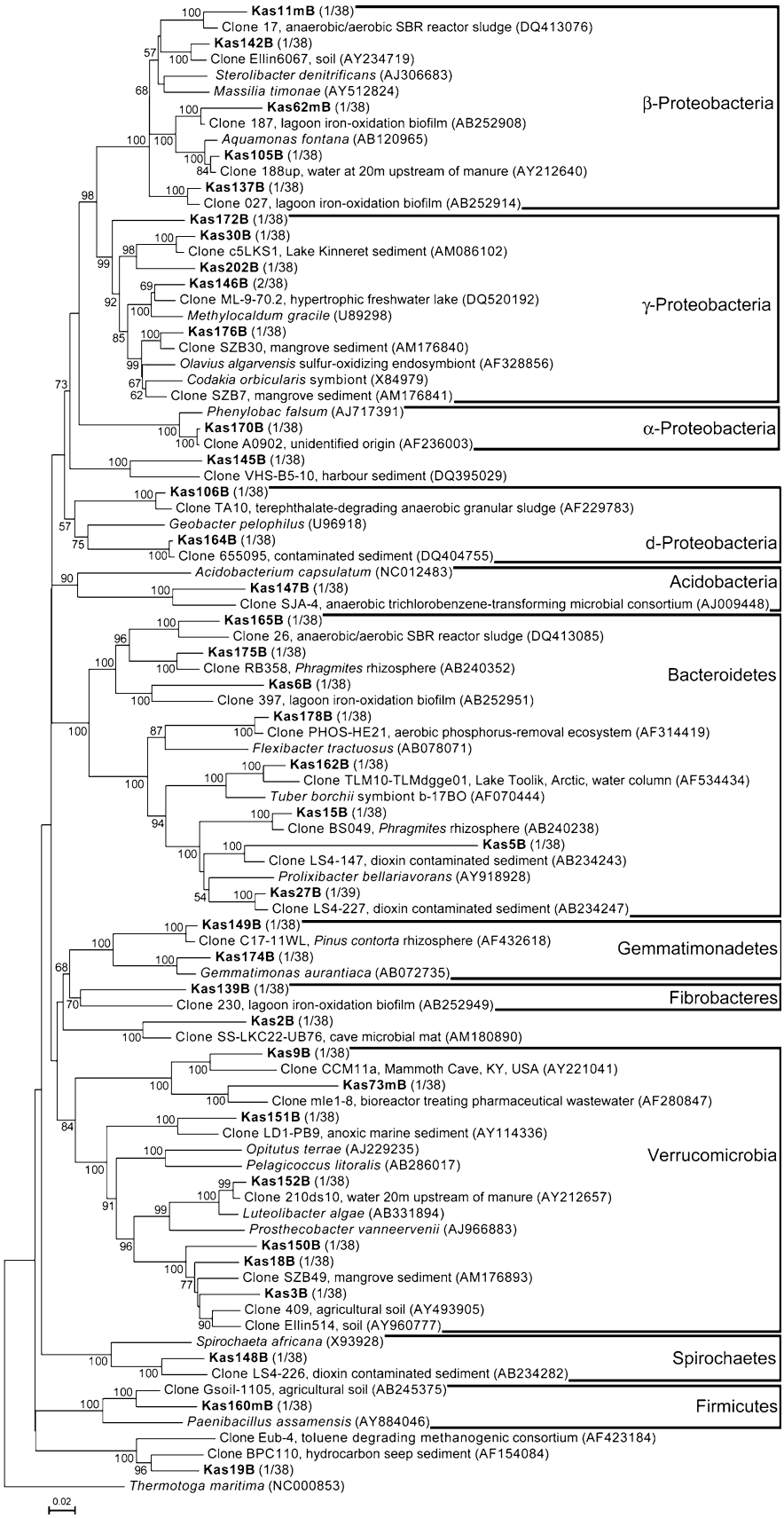

Fig. 5 Phylogenetic tree of relationships of 16S rDNA (ca. 1,500 bp) of the unique bacterial phylotypes found in the top 1 cm sediment of Lake Kastoria, based on the neighbour-joining method as determined by distance Jukes–Cantor analysis. The found phylotypes are in *bold*, with numbers of identical (≥98%) phylotypes in *parentheses*. One thousand bootstrap analyses (distance) were conducted, and percentages greater than 50% are indicated at the *nodes*. The numbers in *brackets* are GenBank accession numbers. *Scale bar* represents 2% estimated distance

(Fig. S2) seems to be distantly related to *Flavobacterium frigidarium*. Strains of *Flavobacterium* might be involved in the degradation of cyanobacterial hepatotoxins or other recalcitrant, complex organic compounds (Berg et al. 2009).

A few phylotypes were closely related to (16S rRNA gene similarity > 98%) known pathogenic bacteria such as *Aeromonas veronii* bv. *veronii* (NK2-611), *Corynebacterium afermentans* (NK2-52), *Corynebacterium aurimucosum* (NK2-618), *Corynebacterium pseudogenitalium* (NK2-624) and *Streptococcus mitis* (NK2-622), suggesting a potential risk for public health. However, genera of pathogenic bacteria have also been found in other cyanobacterial blooms (Berg et al. 2009).

The sediment cyanobacterial community consisted of benthic and planktic representatives. However, the dominant phylotype KS-C3 (Table S4; Fig. 5) is not a true cyanobacterium but rather is related to the plastid of the epipelic diatom *Haslea nipkowii* (Ohtsuka 2005). It is most likely that this phylotype belongs to a pennate diatom similar to *H. nipkowii.* This diatom was observed microscopically in sediment samples of L. Kastoria. Two other cyanobacterial phylotypes were related to the benthic genera of *Tolypothrix/Calothrix* and to the mat forming, potentially toxin producing *Nodularia sphaerocarpa* (Komárek et al. 1993).

Only two phylotypes were related to the planktic and toxic forms of the nostocalean genera of *Anabaena* and *Aphanizomenon* that have been found in the overlying water column of L. Kastoria (Moustaka-Gouni et al. 2007). The fact that no *Cylindrospermopsis/Raphidiopsis* phylotypes were retrieved, might be due to the lack of these organisms in the lake water in 2005 and also suggests that their dormant cells/filaments were not detectable a year after their bloom. In 2004, the bloom species in this lake seem to have rarely produced akinetes (Moustaka-Gouni et al. 2009) and this may have resulted in a small sedimentary inoculum for the next year's growth. Furthermore, the akinete distribution in the sediment may be patchy since they can be accumulated in deposition zones (Padisak 2003). The presence and size of the sedimentary pool of cyanobacteria is an important issue in water quality management that has been largely overlooked.

The bacterial species richness in the sediment was high, as was the case for the water column and other lake sediment habitats (Tamaki et al. 2005; Schwarz et al. 2007; Zhao et al. 2008, Ye et al. 2009b, Zeng et al. 2009b). The sediment bacterial community showed the expected profile with dominance of the β-, γ-, α- and δ-Proteobacteria along with Verrucomicrobia and Bacteroidetes (Wobus et al. 2003, Ye et al. 2009a, Zeng et al. 2009b). The majority of the retrieved phylotypes were related phylotypes of uncultured Bacteria from similar lake and non-freshwater sediments, rendering, thus, their inferred ecophysiological role rather dubious. However, such bacterial communities have been connected to the degradation processes of organic matter in sediments (Wobus et al. 2003; Ye et al. 2009b). In contrast to the water column, no pathogenic or toxic bacterial phylotypes could be recognised in the sediment based on phylogeny.

In conclusion, we revealed the bacterial and cyanobacterial diversity during a eutrophic lake water bloom using universal and cyanobacterial PCR primers. The water column cyanobacterial diversity corroborated well with morphological observations but also revealed new phylotypes with unknown morphology. The dominant toxic cyanobacteria did not appear to impose any shaping pattern on the bacterial community structure, since it was composed of typical freshwater phylotypes. However, 16S rRNA gene sequences of some potential pathogens and toxin-metabolising bacteria were recovered. When we investigated the cyanobacterial molecular diversity in the sediment 2 years after the water bloom, no bloom forming cyanobacteria were retrieved, suggesting that either they cannot be preserved in the sediment or are in cellular forms that cannot be detected by the methods applied. The sediment bacterial diversity was also high, like the water column, consisting mostly of yet-uncultured bacteria related to Bacteria found in environments where high rates of organic matter degradation take place.

Acknowledgments This work was partially funded by the Research Committee of Alexander Technological Educational Institute of Thessaloniki. The authors thank the two anonymous reviewers and Virginia P. Edgcomb whose comments improved the manuscript.

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SUPPLEMENTARY MATERIAL: Four (4) tables

Kormas et al., submitted to "World J. Microbiol. Biotechnol."

Table S1. Cyanobacterial-specific 16S rRNA retrieved phylotypes from the water column of Lake Kastoria, Greece.

Clone	***# of similar clones (≥98%)***	***Putative affiliation***	***Closest phylotype (%) [GenBank #]***	***Description***	***Closest microorganism (% [GenBank #]***
NK2-CYA-1-1	9	Cyanobacteria	Clone Lk1mC-1 (99) [DQ158167]	Lake Kinneret (Israel)	-
NK2-CYA-1-14	9	Cyanobacteria	Strain LMECYA 68 (93) [EU078508]	wastewater pond Marrakesh, Morocco	*Synechocystis* sp. (93) [EU078508]
NK2-CYA-1-13	7	Cyanobacteria	Strain CENA 110 (99) [EF088338]	waste pond, Brazilia	*Limnothrix* sp. (99) [EF088338]
NK2-CYA-1-12	4	Verrucomicrobia	Clone LiUU-9-291 (98) [AY509507]	Swedish lake	-
NK2-CYA-2-1	3	Chloroplast	Clone TH_a195 (99) [EU273240]	Lake Taihu, China	-
NK2-CYA-1-4	2	Verrucomicrobia	MEsu06b11G10 (99) [FJ828495]	eutrophic lake	-
NK2-CYA-1-5	2	Cyanobacteria	Isolate cln_0TU23C141 (99%) [AM259247]	Shallow, eutrophic lake	*Microcystis aeruginosa* (99) [AB271211]
NK2-CYA-1-7	2	Cyanobacteria	Strain Florida I (99)	St Johns River, Florida, USA	*Cylindrospermopsis*

			[AF516746]		*raciborskii* (99) [AF516746]
NK2-CYA-3-4	2	Verrucomicrobia	Clone TH1-67 (97) [AM690865]	Lake Taihu, China	-
NK2-CYA-1-3-ame	1	Cyanobacteria	Clone 5b/562g (77) [EF160024]		-
NK2-CYA-1-8	1	γ-Proteobacteria	Bacterium G2 (98) [AY345398]	Hawaiian Archipelago	-
NK2-CYA-1-10	1	Actinobacteria	clone CbR3s.21 (97) [EF014660]	stamukhi lake, Arctic	-
NK2-CYA-1-18	1	Cyanobacteria	Strain CCG2 (90) [DQ235802]	geothermal region in New Zealand	*Mastigocladopsis* sp. (90) [DQ235802]
NK2-CYA-2-9	1	OP11	Clone MD2902-B90 (87) [EU385901]	subseafloor sediments, South China Sea	-
NK2-CYA-2-14	1	Cyanobacteria	clone Erie 21 (99) [AY858013]	Lake Erie, USA	*Phormidium* sp. (97) [AB183566]
NK2-CYA-2-18	1	Verrucomicrobia	Clone LiUU-9-20 (99) [AY509516]	Swedish lake	-
NK2-CYA-3-3_ame	1	Cyanobacteria	Clone 0BB39S01 (82) [AJ781044]		-
NK2-CYA-3-5	1	Chloroplast	Clone Lk1mC-1 (98) [DQ158167]	Lake Kinneret (Israel)	-

Table S2. Bacterial 16S rRNA retrieved phylotypes from the water column of Lake Kastoria, Greece.

Clone	*No. of similar (≥98) clones*	*Putative affiliation*	*Closest sequence (% similarity) [GenBank accession No.]*	*Description*	*Closest organism (similarity) [GenBank accession No.]*
NK2-14	4	γ-Proteobacteria	Clone O16 (99) [AY376693]	Endosymbiont of the pink sugarcane mealybug, *Saccharicoccus sacchari*	*Enterobacter aerogenes* (99) [AF395913]
NK2-65	3	WS3	Clone 072DZ78 (82) [DQ397490]	Hypersaline microbial mat	
NK2-63	3	β-Proteobacteria	Clone SM1G08 (99) [AF445700]	Travertine depositional facies	*Pelomonas saccharophila* (99) [AB021407]
NK2-510	3	Actinobacteria	Clone S9D-06 (99) [AB154303]	Eutrophic lake bacterial communities	-
NK2-52	2	Actinobacteria	isolate BF0002D108 (99) [AM697597]	Indoor dust	*Corynebacterium afermentans* (99) [X82054]
NK2-61	2	Verrucomicrobia	Clone LD29 (97) [AF009975]	Temperate freshwater lake	-
NK2-66	2	β-Proteobacteria	Clone 1174-1091-2 (99) [AB128892]	Deep subsurface sediments	-
NK2-67	2	β-Proteobacteria	Clone 08 (98) [AF361201]	Mesoeutrophic reservoir	-
NK2-69	2	β-Proteobacteria	Clone TIIF1 (99) [DQ297956]	Hydrocarbon contaminated soil	*Ralstonia detusculanense* (99) [AF280433]
NK2-427	2	Bacteroidetes	Clone ST11-9 (94) [DQ501342]	Lake free- and particle-associated bacteria	*Fluviicola taffensis* (91) [AF493694]
NK2-523	2	α-Proteobacteria	*Bradyrhizobium* clone YJQ-17 (98) [AY569292]	Hot spring microbial mat	*Bradyrhizobium* sp. ORS278 (98) [CU234118]
NK2-610	2	Chloroflexi	Clone RB520 (93)	Rhizosphere of *Phragmites*	-

			[AB240223]		
NK2-611	2	γ-Proteobacteria	Clone aab00h06 (99) [DQ816884]	Zebrafish and mice gut bacteria	*Aeromonas veronii* bv. veronii (99) [AM184224]
NK2-618	2	Actinobacteria	Clone JSC7-28 (98) [DQ532189]	Spacecraft assembly environments	*Corynebacterium aurimucosum* (98) [AY536427]
NK2-632	2	Actinobacteria	*Corynebacterium accolens* (96) [AJ439346]		
NK2-641	2	Actinobacteria	Clone TH1-90 (99) [AM690882]	Shallow subtropical lake	*Nostocoida limicola* (91) [Y14597]
NK2-12	1	Unidentified	clone 655957 (93) [DQ404829]	Contaminated sediments	-
NK2-21	1	γ-Proteobacteria	clone TK-NH6 (98) [DQ463728]	Lake Tanganyika	-
NK2-26	1	β-Proteobacteria	Clone B44 (97) [AF407722]	Australia's Great Artesian Basin	*Derxia gummosa* (93) [AB089482]
NK2-32	1	Verrucomicrobia	Clone zEL61 (96) [DQ415853]	Limestone-corroding stream biofilms	-
NK2-34	1	Actinobacteria	Clone ML-9-87.2 (97) [DQ520196]	Shallow hypertrophic lake	-
NK2-51	1	γ-Proteobacteria	Clone 86 (97) [DQ165130]	Arsenic related bacterial in lake sediments	-
NK2-53	1	Bacteriodetes	Clone B5_B24 (99) [EF025233]	Avian intestine	-
NK2-54	1	Unidentified	Clone DS020 (88) [DQ234104]	Mangrove bacterioplankton	-
NK2-55	1	Bacteriodetes	Clone PRD18H05 (93) [AY948068]	Temperate rivers	-
NK2-56	1	Chloroflexi	Clone oc23 (93)	Oligotrophic microbial fuel	-

			[AY491567]	cells	
NK2-57	1	Cyanobacteria	*Scenedesmus obliquus* plastid (91) [DQ396875]		-
NK2-64	1	α-Proteobacteria	*Holospora obtusa* (93) [X58198]	Uncultured bacterial endosymbionts	
NK2-211	1	Chloroflexi	Clone TK-SH18 (95) [DQ463738]	Lake Tanganyika	
NK2-423	1	Actinobacteria	*Leifsonia xyli* (96) [AJ717351]	Non-saline alkaline environment	-
NK2-511	1	Unidentified	Clone 2-14 (85) [AY548947]	Microbial communities of ANAMMOX sludge	-
NK2-512	1	Cyanobacteria	Clone 01D2Z84 (92) [DQ330752]	Hypersaline microbial mat	-
NK2-514	1	Unidentified	Clone d064 (77) [AF422655]	Trichloroethene-contaminated site	-
NK2-517	1	Verrucomicrobia	Clone LiUU-9-243 (94) [AY509518]	Lake bacteria associated with cyanobacterial blooms	-
NK2-519	1	Planctomycetes	Clone 031A12_P_BA_P3 (96) [BX294827]	Municipal wastewater treatment plant	-
NK2-520	1	β-Proteobacteria	Clone NKB6 (89) [AB013258]	Nankai Trough sediments	-
NK2-522	1	Bacteroidetes	*Flavobacterium frigidarium* isolate (93) [AY771737]	Diversity of Arctic bacteria	-
NK2-524	1	Unidentified	Clone SM2F11 (82) [AF445733]	Travertine depositional facies	-
NK2-527	1	Cyanobacteria	Clone AKIW579 (96) [DQ129320]	Urban aerosols	*Leptolyngbya* sp. Greenland 7 (92) [DQ431002]

NK2-528	1	Cyanobacteria	*Limnothrix* sp. CENA 110 (99) [EF088338]	Facultative waste stabilization pond	-
NK2-531	1	Unidentified	*Diplazium pycnocarpon* mitochondria (98) [AF058662]		
NK2-532	1	Cyanobacteria	*Raphidiopsis mediterranea* HB2 (99) [AY763117]		
NK2-533	1	Fusobacteria	*Cetobacterium somerae* (99) [AJ438155]		
NK2-534	1	Firmicutes	Clone RL184_aah22d07 (98) [DQ809531]	Human gut bacteria	-
NK2-536S	1	Chloroflexi	*Caldilinea aerophila* (91) [AB067647]		
NK2-539	1	α-Proteobacteria	Clone GPENV15 (96) [DQ512842]	Warm spring	-
NK2-539S	1	Verrucomicrobia	Clone d0-99 (98) [AM409882]	Lake Kinneret	-
NK2-544	1	Firmicutes	clone 288c2 (99) [EF460016]	Baltic Sea sediment	*Clostridium gasigenes* (98) [AF143692]
NK2-612	1	β-Proteobacteria	Clone AKYH1084 (96) [AY921863]	Comparative metagenomics of microbial communities	-
NK2-617	1	Verrucomicrobia	Clone S9JA-40 (98) [AB154319]	Eutrophic lake bacterioplankton	-
NK2-619	1	Chloroflexi	Clone OB17 (90) [EF429491]	Tropical geothermal thermophilic microbial mats	-
NK2-621	1	Actinobacteria	Clone S4 (99) [AJ575506]	Actinobacteria from freshwater habitats	-

NK2-622	1	Firmicutes	*Streptococcus* sp. clone 2.5 (100) [DQ346438]	Oral microflora	*Streptococcus_mitis* (99) [AY518677]
NK2-623	1	α-Proteobacteria	*Rhodobacter sphaeroides* (96) [CP000578]		
NK2-624	1	Actinobacteria	*Corynebacterium pseudogenitalium* (100) [AJ439348]		
NK2-625	1	Actinobacteria	Clone ML-9-97.2 (99) [DQ520197]	Shallow hypertrophic lake	
NK2-629	1	α-Proteobacteria	Clone LiUU-9-283 (99) [AY509406]	Lake bacteria associated with cyanobacterial blooms	*Rhodobacter sphaeroides* (95) [CP000578]
NK2-638	1	α-Proteobacteria	*Rhodopila globiformis* (94) [D86513]		
NK2-645	1	Actinobacteria	Clone 248b2 (95) [EF459919]	Baltic Sea sediment	*Solirubrobacter* sp. Gsoil 921 (91) [AB245336]
NK2-648	1	Bacteroidetes	Clone TK-NE6 (98) [DQ463720]	Lake Tanganyika	-
NK2-739	1	Verrucomicrobia	Clone TH1-67 (99) [AM690865]	Shallow subtropical lake	Candidatus *Xiphinematobacter brevicolli* (98) [AF217462]

Table S3. Cyanobacterial-specific 16S rRNA retrieved phylotypes from the sediment of Lake Kastoria, Greece.

Clone	*No. of similar (≥98) clones*	*Putative affiliation*	*Closest sequence (similarity) [GenBank accession No.]*	*Description*	*Closest organism (similarity) [GenBank accession No.]*
KS-C3	11	Cyanobacteria	Clone TDNP_USbc97_2_47_65 (98) [FJ516953]	Wetland upper sediment	-
KS-C10	2	Verrucomicrobia	Clone FGL7S_B67 (96) [FJ437926]	Lake surface sediments	-
KS-C14	2	Verrucomicrobia	Clone P. palm C 85 (95) [AJ441222]	*Paralvinella palmiformis* mucus secretions	-
KS-C19	2	Verrucomicrobia	Clone TDNP_USbc97_32_3_124 (95) [FJ516959]	Wetland upper sediment	-
KS-C1	1	Verrucomicrobia	Clone Kas152B (98) [EF203172]	L. Kastoria sediment	-
KS-C2	1	Cyanobacteria	*Aphanizomenon gracile* UADFA16 (99) [FJ895128]		-
KS-C5	1	Cyanobacteria	*Nodularia sphaerocarpa* BECID36 (99) [AJ781147]		-
KS-C6	1	Verrucomicrobia	Clone CBNH_072904_C53 (96) [FJ807402]	Sediment	-
KS-C9	1	Verrucomicrobia	Clone IC-31 (97) [AB255059]	Corroded concrete	-
KS-C11	1	Acidobacteria	Clone t30d34H84 (97) [FM956161]	Rice field soil	-

KS-C17	1	Cyanobacteria	Anabaena circinalis ACMB13 (99) [EU636199]		-
KS-C18	1	Cyanobacteria	Clone 2G4-44 (96) [EU160341]	Rhizosphere soil	*Tolypothrix* sp. PCC 7415 (96) [AM230668]
KS-C21	1	Chloroflexi	Clone FCPT649 (97) [EF515877]	Grassland soil	-
KS-C22	1	Chloroflexi	Clone 2004-4-2-072307 (95) [EU234541]	Surface river sediment	-
KS-C23	1	Chloroflexi	Clone UH-10 (98) [AB265904]	Anoxic rice field soil	-
KS-C26	1	Firmicutes	Clone AUVE_06B12 (97) [EF651269]	Cropland	-
KS-C27	1	Verrucomicrobia	Clone D48 (98) [EU580470]	Lake Constance	-
KS-C30	1	Acidobacteria	Clone HM53 (96) [AM909878]	Rhizosphere soil from rice field	-
KS-C31	1	Verrucomicrobia	Clone FGL7S_B74 (97) [FJ437926]	Lake surface sediments	-
KS-C33	1	Verrucomicrobia	Clone FGL7S_B5 (98) [FJ437926]	Lake surface sediments	-
KS-C34	1	Verrucomicrobia	Clone TDNP_USbc97_32_3_124 (97) [FJ516959]	Wetland upper sediment	-
KS-C37	1	Verrucomicrobia	Clone FGL7S_B108 (98) [FJ437926]	Lake surface sediments	-
KS-C38	1	Verrucomicrobia	Clone m43 (97) [AY444967]	Biofilm on oxygen-transfer membrane	-

KS-C39	1	Verrucomicrobia	Clone FGL7S_B129 (97) [FJ437926]	Lake surface sediments	-
KS-C44	1	Verrucomicrobia	Clone TDNP_Bbc97_32_3_94 (86) [FJ516798]	Wetland upper sediment	-

Table S4. Bacterial 16S rRNA retrieved phylotypes from the sediment of Lake Kastoria, Greece.

Clone	*No. of similar (≥98) clones*	*Putative affiliation*	*Closest sequence (similarity) [GenBank accession No.]*	*Description*	*Closest organism (similarity) [GenBank accession No.]*
Kas146B	2	γ-Proteobacteria	Clone SS_LKC22_UB76 (90) [AM180890]	Cave microbial mat	-
Kas2B	1	Unidentified	Clone 409 (94) [AY493905]	Agricultural soil	-
Kas3B	1	Verrucomicrobia	Clone LS4-147 (96) [AB234243]	Dioxin contaminated sediment	-
Kas5B	1	Bacteroidetes	Clone C06 (93) [EF589963]	Nitrobenzene-contaminated river sediment	-
Kas6B	1	Bacteroidetes	Clone CCM11a (91) (AY221041)	Mammoth Cave, KY, USA	-
Kas9B	1	Verrucomicrobia	Clone 17 (97)	Anaerobic/aerobic SBR	*Strerolibacterium denitrificans* (92)

			[DQ413076]	reactor sludge	[AJ306683]
Kas11mB	1	β-Proteobacteria	Clone BS049 (96) [AB240238]	*Phragmites* rhizosphere	*Prolixibacter bellariavorans* (88) [AY918928]
Kas15B	1	Bacteroidetes	Clone SZB49 (93) [AM176893]	Mangrove sediment	-
Kas18B	1	Verrucomicrobia	Clone BPC110 (91) [AF154084]	Hydrocarbon seep sediment	-
Kas19B	1	Unidentified	Clone LS4-227 (97) [AB234247]	Dioxin contaminated sediment	-
Kas27B	1	Bacteroidetes	Clone cSLKS 1 (96) [AM086102]	L. Kinneret sediment	Candidatus *Competibacter phosphatis* (93) [AY962318]
Kas30B	1	γ-Proteobacteria	Clone 187 (99) [AB252908]	Lagoon iron-oxidation biofilm	-
Kas62mB	1	β-Proteobacteria	Clone mle1-8 (91) [AF280847]	Bioreactor treating pharmaceutical wastewater	-
Kas73mB	1	Verrucomicrobia	Clone 188up (98) [AY212640]	Water 20 m upstream of manure	*Aquomonas fontana* (96) [AB120965]
Kas105B	1	β-Proteobacteria	Clone TA10 (96) [AF229783]	Terephthalate-degrading anaerobic granular sludge	-
Kas106B	1	δ-Proteobacteria	Clone 027 (96) [AB252914]	Lagoon iron-oxidation biofilm	-
Kas137B	1	β-Proteobacteria	Clone 230 (88) [AB252949]	Lagoon iron-oxidation biofilm	-
Kas139B	1	Fibrobacteres	Clone Ellin6067 (96) [AY234719]	Soil	-
Kas142B	1	β-Proteobacteria	Clone VHS-B5-10 (89) [DQ395029]	Harbor sediment	-

Kas145B	1	Proteobacteria	Clone ML-9-70.2 (94) [DQ520192]	Hypertrophic freshwater lake	-
Kas147B	1	Acidobacteria	Clone SJA-4 (88) [AJ009448]	Anaerobic trichlorobenzene-transforming microbial consortium	-
Kas148B	1	Spirochaetes	Clone LS4-226 (91) [AB234282]	Dioxin contaminated sediment	*Spirochaeta africana* (92) [X93928]
Kas149B	1	Gemmatimonadetes	Clone C17.11WL (98) [AF432618]	*Pinus contorta* rhizosphere	-
Kas150B	1	Verrucomicrobia	Clone *Ellin514* (90) [AY960777]	Soil	-
Kas151B	1	Verrucomicrobia	Clone LD1_PB9 (90) [AY114336]	Anoxic marine sediment	-
Kas152B	1	Verrucomicrobia	Clone 210ds10 (97) [AY212657]	Water 20 m upstream of manure	-
Kas160mB	1	Firmicutes	Clone m Gsoil 1105 (95) [AB245375]	Agricultural soil	*Paenibacillus assamensis* (92) [AY884046]
Kas162B	1	Bacteroidetes	Clone TLM10/TLMdgge01 (94) [AF534434]	L. Toolik, Arctic, water column	-
Kas164B	1	δ-Proteobacteria	Clone 655095 (98) [DQ404755]	Contaminated sediment	-
Kas165	1	Bacteroidetes	Clone 26 (92) [DQ413085]	Anaerobic/aerobic SBR reactor sludge	-
Kas170B	1	α-Proteobacteria	Clone AO902 (98) [AF236003]	Undefined origin	*Phenylobacterium falsum* (97) [AJ717391]
Kas172B	1	γ-Proteobacteria	Clone S1-4-CL1 (98) [AY725260]	Decayed velvetleaf seed	-

Kas174 B	1	Gemmatimonadetes	*Gemmatimonas aurantiaca* (95) [AB072735]	-	-
Kas175B	1	Bacteroidetes	Clone RB358 (94) [AB240352]	*Phragmites* rhizosphere	-
Kas176B	1	γ-Proteobacteria	Clone SZB30 (96) [AM176840]	Mangrove sediment	-
Kas178 B	1	Bacteroidetes	Clone PHOS-HE21 (96) [AF314419]	Aerobic phosphorus-removal ecosystem	*Flexibacter tractuosus* (87) [AB078071]
Kas202B	1	γ-Proteobacteria	Clone SZB7 (89) [AM176841]	Mangrove sediment	-

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