Bacterioplankton community composition in 67 Finnish lakes differs according to trophic status

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ABSTRACT: The bacterioplankton composition of 67 Finnish lakes was characterised by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments, and subsequent sequence analyses of major fragments. The DGGE patterns grouped as a function of environmental characteristics describing the trophic status of the lakes, such as total phosphorus (TP), total nitrogen (TN), TN:TP, chlorophyll *a*, Secchi depth and water colour. Most sequences retrieved represented *Verrucomicrobia*, *Actinobacteria* and *Cyanobacteria*, while those representing *Alpha-* and *Gamma-proteobacteria*, *Chloroflexi* and *Acidobacteria* were less frequent. The presence of several sequences could be linked to the trophic status of the lakes, while that of others was more common and thus unrelated to the trophic status. These results suggest that individual responses towards environmental factors may occur among the bacterioplankton at the level of phyla as well as phylotypes.

KEY WORDS: Bacterioplankton community composition · Lake trophic status · DGGE · Canonical correspondence analysis · CCA

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INTRODUCTION

Bacterioplankton in freshwater ecosystems has a key role in nutrient cycling. Thus, detailed understanding of their distribution and the factors affecting bacterioplankton community composition are important. It has been suggested that relatively few groups of bacteria dominate freshwater systems and that these groups are widespread around the world (Zwart et al. 2002, Stepanauskas et al. 2003). According to previous studies, the most common freshwater bacteria belong to the divisions *Proteobacteria, Actinobacteria, Cyanobacteria* and *Verrucomicrobia* and to the *Cytophaga-Flavobacterium-Bacteroides* group (Zwart et al. 2002). In addition, it has been shown that lakes contain many bacterial groups that are distinct from those found in the oceans (Zwart et al. 2002).

The roles of the various factors governing bacterioplankton distribution are under debate. The influence of seasonality, such as water column mixing and thermal stratification, on bacterial community composition (BCC) is well established (Yannarell et al. 2003, Shade et al. 2007, 2008, Nelson 2009). Several studies have revealed a statistical relationship between BCC and temperature (Van der Gucht et al. 2001, Muylaert et al. 2002, Yannarell et al. 2003, Jardillier et al. 2004, Yannarell & Triplett, 2004, 2005, Lindström et al. 2005, Kritzberg et al. 2006, Shade et al. 2007, 2008). In addition, BCC has been shown to vary along gradients of pH (Lindström 2000, Lindström & Leskinen 2002, Stepanauskas et al. 2003, Lindström et al. 2005, Schauer et al. 2005, Yannarell & Triplett 2005, Haukka et al. 2006), humic acid content (Haukka et al. 2005) and lake water retention time (Lindström et

al. 2005). Muylaert et al. (2002) suggested that BCC was dependent on the dominant substrate source together with food web structure in 4 shallow eutrophic lakes. Grazing, biomass of microzooplankton and viral infections play an important role in controlling BCC (Lindström 2000, 2001, Hahn & Höfle 2001, Jürgens & Matz 2002, Kent et al. 2004, Van der Gucht et al. 2005). Jardillier et al. (2004) demonstrated that BCC depends on the combined effects of dominant substrate sources and selective predation by bacterial consumers. Nutrient availability, biomass of certain phytoplankton, lake productivity (concentration of chlorophyll *a*), water colour and transparency (Secchi depth) and dissolved organic carbon all appear to influence the BCC (Methé & Zehr 1999, Lindström 2000, Yannarell & Triplett 2004, 2005, Van der Gucht et al. 2005).

In the present study, we aimed to identify the features that best explained variation in BCC among 67 boreal lakes in Finland. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments and subsequent sequence analyses of specific fragments were used to characterise the bacterial community structure in water samples of these lakes. These data were then analysed by multivariate analysis in relation to physicochemical characteristics.

MATERIALS AND METHODS

Samples. Water samples included in this study originated from 67 lakes in southern and central Finland (see Table S1 in the supplement at www.int-res.com/articles/suppl/a062p241_supp.pdf). From each lake, a composite sample from a depth of 0 to 2 m was collected from an open lake area. Samples were collected at the beginning of July or the middle of August with 1 exception, which was collected on 16 September 2002 (Lake Vitträsk, no. 71). For DNA extraction, an aliquot of 75 to 1000 ml was filtered through a series of filters with pore sizes 10, 5, 1 and 0.2 µm as detailed by Rantala et al. (2006).

The physicochemical characteristics and cyanobacterial biomass of 55 and 50 lakes, respectively, have been analysed previously (Rantala et al. 2006), and these data were used in the statistical analyses. Cyanobacterial composition of the lakes was analysed by microscopy, and cell counts were converted to biovolumes using the cell volumes of the phytoplankton database of the Finnish Environment Institute (www.environment.fi) as described by Rantala et al. (2006). Nineteen of the lakes were oligotrophic (total phosphorus content, TP < 10 µg l^{-1}), 29 mesotrophic (TP: 10 to 34 µg l^{-1}), 15 eutrophic (TP: 35 to 100 µg l^{-1}) and 4 hypertrophic (TP > 100 µg l^{-1}).

Nucleic acid extraction and PCR amplification. DNA was extracted from the cells on 10, 5, 1 and 0.2 µm filters using a modified hot phenol method and purified by Nucleotrap PCR purification (BD Biosciences) and QuickStep PCR Purification (Edge BIOSystems) kits as detailed previously by Rantala et al. (2006). Primers F-968-GC and R-1401 targeting eubacterial 16S rRNA genes (Nübel et al. 1996) were used to amplify PCR products for DGGE. PCR reactions were performed in a volume of 16 µl containing 1 µl of template DNA, 200 nM of primers F-968-GC and R-1401, 200 µM dNTP solution, 1× PCR buffer, 1 M betaine (Sigma), 0.3 U DyNAzyme^{TM} II DNA polymerase (Finnzymes) and sterile water up to 16 µl. The PCR programme consisted of a denaturing step at 94°C for 3 min, 35 cycles at 94°C for 1 min, 51°C for 1 min and 72°C for 2 min, and a final extension step at 72°C for 10 min. The size and purity of the amplification products were checked on a 1.5% agarose gel. Two parallel PCR reactions from each sample were prepared and combined after amplification. The combined PCR products (approx. 80% of the reaction) were loaded into the DGGE gel.

DGGE. DGGE was performed on a Dcode^{TM} Universal Mutation Detection System (Bio-Rad) using 6% polyacrylamide and 35 to 55% formamide-urea gradients as previously described by Kolmonen et al. (2004). The samples were run in 4 gels and each contained 3 standard lines (see Fig. S1 in the supplement at www.int-res.com/articles/suppl/a062p241_supp.pdf). Standards contained reamplified 16S rRNA gene fragments from a lake sample selected to cover the migration range of the bands. The standards with 6 band positions were applied for normalisation using Bionumerics software (Applied Maths BVBA, v 4.0). DNA bands were excised from the DGGE gels with a sterile scalpel and eluted from the gel slices using sterile water at 4°C overnight. These eluted products were reamplified and the PCR products were separated again in DGGE. In some cases, the band separation step was performed twice to ensure proper band separation. Several bands from different gels but the same position were sequenced as well as run again to ensure that each band position represented only 1 phylotype. From 46 band positions with sequenced bands, only 11 contained sequences from different phyla. Separated products from DGGE gels were reamplified with primers F-968 (without the GC-clamp) and R-1401. PCR products were purified using Microcon PCR purification tubes (Millipore) and sequenced using the Big Dye cycle sequencing ready reaction kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing reactions were analysed with the Applied Biosystems 310 genetic analyser.

Sequence analysis. The partial 16S rRNA gene sequences derived from DGGE gels were analysed using NCBI-BLAST (Altschul et al. 1997) in order to identify the most similar sequences in the GenBank database (April 2009). Taxonomical classification of the bacterial sequences was confirmed with the Classifier analysis tool (Ribosomal Database Project II, Wang et al. 2007) (see Table 1). All the sequences were deposited in GenBank under accession numbers EU861397–EU861477, FJ970038.

Statistical data analysis. DGGE images were transferred to the BioNumerics software (Applied Maths BVBA, v 4.0) for normalisations of the electrophoresis patterns between the gels. The normalisation was based on standard samples run alongside the lake samples. The alignment was corrected by means of visual inspection of the gels. Automatic band search (minimum profiling 5%) and band matching (position tolerance 1%) were performed as implemented in Bionumerics. The band matching was corrected manually by means of visual inspection of the gels and comparison with sequencing data. The data matrix based on the presence or absence of DNA bands was used for statistical analyses.

Principal component analysis (PCA) was used to investigate the variation (presence/absence of the bands) in the DGGE profiles, by clustering of samples and taxa (bands) along axes on the basis of the banding patterns alone. PCA was performed with the CANOCO 4.52 software package (ter Braak & Smilauer 2002).

In order to reveal relationships between the presence of bacterial groups and environmental factors, canonical correspondence analysis (CCA) in the CANOCO 4.52 software package (ter Braak & Smilauer 2002) was used. Environmental variables, except pH and temperature, were log $(x + 1)$ transformed. The environmental variables used were: water temperature and colour, pH, TP, total nitrogen (TN), nitrate and nitrite nitrogen ($N(NO_3\text{-}NO_2)$), transparency as determined by Secchi depth, chlorophyll a (chl a), ratio of dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN:DIP), TN:TP ratio, and latitude and longitude of the lake location.

CCA was applied to determine which environmental variables best explained patterns of similarity in DGGE profiles among bacterial communities in the lakes studied. Those band positions that occurred in fewer than 5 lakes were excluded from the analyses to avoid their too pronounced effect on the analysis. The environmental variables that contributed to the model significantly ($p < 0.05$) were included in the further analysis, using the manual selection of variables provided by CANOCO. This way the importance of individual variables can be determined (ter Braak & Smilauer 2002).

RESULTS

Lake characteristics

The lakes sampled are situated in southern and central Finland between northern latitudes 60° 10′ and 62° 50′ and eastern longitudes 22° 12′ and 28° 11′. The size of the lakes varied from 0.02 to 863.3 km². According to the TP content, the studied lakes ranged from oligotrophic to hypertrophic. pH values ranged from 5.8 to 10. The pH of most of the lakes was neutral or alkaline, and 11 of the lakes were acidic. The lake temperature ranged from 15.4 to 22.8°C, and the water colour from 8 to 160 mg platinum (Pt) l⁻¹. Of the lakes studied, 35 were clearwater lakes or the humus content was low (<40 mg Pt l⁻¹), and 32 were humic (humus content >40 mg Pt l⁻¹). The chl a concentration varied from 1.2 to 130 mg l⁻¹. In 6 lakes, cyanobacterial biomass ranged from 1 to 5.5 mg l⁻¹, and in all the other lakes cyanobacterial biomass was <1 mg l⁻¹. A higher biomass of cyanobacteria was detected in eutrophic and hypertrophic lakes than in oligo- and mesotrophic lakes. *Microcystis*, *Anabaena* and *Aphanizomenon* spp. were the most common cyanobacteria detected in this study by means of microscopic counting. Detailed lake characteristics are provided as supplementary material (Table S1 in the supplement).

Analysis of DGGE fingerprint patterns

DGGE analysis retrieved highly variable patterns with 2 to 26 (average 17, SD 3.9; 1 lake with only 2 bands) fragments depending on the sample. Bands were detected in 61 different positions in the DGGE gels. The 16S rRNA gene fragment sequences were obtained from 82/267 (30.7%) DGGE bands, representing 46 band positions (75%). The closest relatives (by BLAST analysis) for these sequences are shown in Table 1. The majority (80/82) of the sequences represented 6 bacterial phyla: *Verrucomicrobia* (22 sequences), *Actinobacteria* (19), *Cyanobacteria* (17), *Proteobacteria* (15), *Chloroflexi* (6), and *Acidobacteria* (1). Two sequences were of eukaryotic (chloroplast) origin.

Despite the variation in location, trophic status or water chemistry of the lakes, certain dominant band positions were common in all DGGE profiles. The most common (represented by FL22; Table 1) was observed in 62 of the total 67 lake samples analysed. The sequence of the band FL22 was similar to that of an uncultured alphaproteobacterium (clone PIB-1), previously detected in the Piburger See in Austria (Table 1). The second most common band position was observed in 54 of the 67 lakes and all the sequences (FL8, FL165, FL267; Table 1) derived from that band position were

Table 1. Sequence similarities of the excised DGGE bands from 67 Finnish lakes, their taxonomic classification in the Ribosomal Database using the Classifier analysis tool and closest relatives in the GenBank database determined by BLAST (NCBI). BD: band designation; BD in databank: number of the band position found in all DGGE profiles is given in brackets

BD in databank	BD in PCA	Taxonomic description (confidence threshold)	Closest relative in GenBank (accession number)	Identities (query coverage)	Source
FL62 (8)	Act1	*Actinobacteria* (100%)	Uncultured actinobacterium clone IW1H7 (EU117747)	376/377 (99%)	Ike Walton Lake, WI, USA
FL48, FL100 (17)	Act2	*Actinobacteria* (99%)	Uncultured actinobacterium clone IW1H7 (EU117747)	377/377 (97%), 381/381 (100%)	Ike Walton Lake, WI, USA
FL252 (8)	Act3	*Actinobacteria* (100%)	Uncultured bacterium clone ML-9-97.2 (DQ520197)	377/377 (98%)	Lake Taihu, China
FL63, FL101 (10)	Act4	*Actinobacteria* (99%), *Actinobacteria* (97%)	Uncultured actinobacterium clone CB7D9 (EU117633)	365/368 (99%), 375/377 (99%)	Crystal Bog Lake, WI, USA
FL219 (20)	Act5	*Actinobacteria* (100%)	Uncultured actinobacterium clone TW1G8 (EU117987)	377/377 (97%)	Twin Valley Lake, WI, USA
FL53 (46)	Act6	*Actinobacteria* (100%)	Uncultured actinobacterium clone IW1A8 (EU117729)	377/377 (100%)	Ike Walton Lake, WI, USA
FL8; FL165, FL267 (54)	Act7	*Actinobacteria* (100%)	Uncultured actinobacterium clone TW1G8 (EU117987); Uncultured actinobacterium clone SA1E1 (EU117920)	376/377 (100%); 369/372 (100%), 362/363 (97%)	Twin Valley Lake, WI, USA; Salmo Pond, WI, USA
FL9, FL133; FL166 (44)	Act8	*Actinobacteria* (100%)	Uncultured actinobacterium clone TR1H5 (EU117967); Uncultured actinobacterium clone SA1E1 (EU117920)	376/377 (97%), 373/373 (97%); 370/373 (100%)	Trout Lake, WI, USA; Salmo Pond, WI, USA
FL26, FL125 (37)	Act9	*Actinobacteria* (98%), *Actinobacteria* (99%)	Uncultured actinobacterium clone Jo153-01 (AJ620849)	379/380 (99%), 375/376 (99%)	Lake Joutikas, Finland
FL11 (38)	Act10	*Actinobacteria* (100%)	*Arthrobacter ramosus* strain IMER-B4-15 (FJ796455)	388/388 (99%)	Unpubl.
FL12 (3)	Act11	*Actinobacteria* (100%)	*Arthrobacter* sp. PR35-2 (EU057864)	385/385 (99%)	*Hemidactylium scutatum* skin, George Washington National Forest, VA, USA
FL27 (8)	Act12	*Actinobacteria* (99%)	Uncultured actinobacterium clone Jo153-01 (AJ620849)	379/380 (99%)	Lake Joutikas, Finland
FL198 (15)	Chlo1	*Chloroflexi* (60%)	Uncultured bacterium clone 5C231719 (AM888159)	366/367 (100%)	Lake Gatun, Panama
FL207, FL223 (38)	Chlo2	*Chloroflexi* (64%), *Chloroflexi* (61%)	Uncultured *Chloroflexi* bacterium clone PIB-212 (AM888159)	370/370 (98%), 370/370 (99%)	Piburger See, Austria
FL13, FL204; FL71 (47)	Chlo3	*Chloroflexi* (55%), *Chloroflexi* (67%); *Chloroflexi* (64%)	Uncultured *Chloroflexi* bacterium clone PIB-212 (AM888159); Uncultured bacterium clone CR99-35-31 (AF428989)	372/372 (100%), 367/367 (100%); 372/372 (100%)	Piburger See, Austria; Changjiang River, China
FL163 (16)	Ver1	*Verrucomicrobia* (100%)	Uncultured verrucomicrobium sp. clone VE34-98 (AJ507504)	379/379 (100%)	Lake Vesijärvi, Finland
FL49 (18)	Ver2	*Verrucomicrobia* (100%)	Uncultured gammaproteobacterium clone ADK-LNe02-02(EF520568)	364/374 (98%)	Adirondack lake, NY, USA
FL6, FL42; FL130 (36)	Ver3	*Verrucomicrobia* (60%), *Verrucomicrobia* (49%); *Verrucomicrobia* (49%)	Uncultured bacterium clone AS 34 (AJ550448); Uncultured verrucomicrobium sp. clone VE51-98 (AJ507510)	385/385 (99%), 384/385 (99%); 375/376 (98%)	Enclosure experiment Archipelagic Sea, Finland; Lake Vesijärvi, Finland
FL7, FL121, FL243; FL131, FL150 (30)	Ver4	*Verrucomicrobia* (56%), *Verrucomicrobia* (46%), *Verrucomicrobia* (50%); *Verrucomicrobia* (40%), *Verrucomicrobia* (49%)	Uncultured *Verrucomicrobia* clone Jo119-01 (AJ620838); Uncultured Crater Lake bacterium CL120-10 (AF316720)	381/385 (100%), 375/383 (100%), 386/387 (100%); 370/376 (98%), 370/376 (100%)	Lake Joutikas, Finland; Crater Lake, OR, USA
FL122, FL244; FL139 (27)	Ver5	*Verrucomicrobia* (46%), *Verrucomicrobia* (59%); *Verrucomicrobia* (63%)	Uncultured *Verrucomicrobia* clone Jo119-01 (AJ620838); Uncultured Crater Lake bacterium CL120-10 (AF316720)	384/384 (100%), 386/386 (100%); 372/377 (99%)	Lake Joutikas, Finland; Crater Lake, OR, USA
FL54 (21)	Ver6	*Verrucomicrobia* (100%)	Uncultured *Verrucomicrobia* bacterium, clone MAH16 (AJ629853)	373/373 (97%)	Mesocosm sample, Lake Ahvenlammi, Finland
FL55, FL56 (1)	Ver7	*Verrucomicrobia* (100%)	Uncultured *Verrucomicrobia* clone NE09 (AJ575732)	371/373 (96%), 376/379 (99%)	Lake Grosse Fuchskuhle, Germany
FL28 (15) Finland	Ver8	*Verrucomicrobia* (100%)	*Verrucomicrobia* bacterium clone AH18 (AJ629852)	383/385 (100%)	Lake Ahvenlamm
FL29, FL227 (25)	Ver9	*Verrucomicrobia* (86%)	Uncultured *Verrucomicrobia* bacterium clone Jo27-02 (AJ620839)	379/380 (98%), 380/380 (98%)	Lake Joutikas, Finland
FL192 (11)	Ver10	*Verrucomicrobia* (84%)	Uncultured *Verrucomicrobia* clone Jo27-02 (AJ620839)	374/374 (100%)	Lake Joutikas, Finland
FL33; FL234 (12)	Ver11	*Verrucomicrobia* (80%); *Verrucomicrobia* (100%)	Uncultured *Verrucomicrobia* clone Jo27-02 (AJ620839); Uncultured bacterium clone L73_ML_194 (FJ354097)	377/380 (98%); 381/383 (99%)	Lake Joutikas, Finland; 17th Street Canal, New Orleans, LA, USA
FL47, FL96 (8)	Alfa-P1	α-*proteobacteria* (84%), α-*proteobacteria* (80%)	Uncultured bacterium clone 5C231136 (EU803548)	375/391 (99%), 377/391 (99%)	Lake Gatun, Panama
FL34, FL97, FL193 (18)	Alfa-P2	α-*proteobacteria* (86%), α-*proteobacteria* (89%), α-*proteobacteria* (84%)	Uncultured bacterium clone 5C231661 (EU803984)	380/382 (98%), 381/382 (100%), 378/380 (100%)	Lake Gatun, Panama

Table 1 (continued)

BD in databank	BD in PCA	Taxonomic description (confidence threshold)	Closest relative in GenBank (accession number)	Identities (query coverage)	Source
FL21 (41)	Alfa-P3	α-proteobacteria (91%),	Uncultured bacterium clone 2C229440 (EU801115)	382/382 (99%),	Delaware Bay, NJ, USA
FL22 (62)	Alfa-P4	α-proteobacteria (92%)	Uncultured alphaproteobacterium clone PIB-1 (AM887995)	386/386 (98%)	Piburger See, Austria
FL78 (41)	Alfa-P5	α-proteobacteria (100%)	Uncultured alphaproteobacterium clone ADK-WSe02-21 (EF520431)	388/390 (100%)	Adirondack lake, NY, USA
FL10, FL68 (12)	Alfa-P6	α-proteobacteria (100%)	Methylobacterium sp. strain B25a (EU240206)	388/389 (100%), 377/377 (100%)	Unpubl., Mexico
FL164 (11) Finland	Gamma-P1	γ-proteobacteria (95%)	Uncultured proteobacterium clone AH14 (AJ629859)	374/374 (100%)	Lake Ahvenlamm
FL197 (6)	Gamma-P2	γ-proteobacteria (82%)	Uncultured bacterium clone OTU074 (DQ684068)	368/374 (99%)	Rhizosphere soil sample, Canada
FL20, FL183 (21)	Gamma-P3	γ-proteobacteria (100%), Bacteria (100%)	Pseudomonas sp. G1125 (DQ910470), Pseudomonas sp. T14 (EU111695)	388/388 (99%), 297/317 (100%)	Unpubl., unflooded rice paddy soil; Unpubl.
FL60 (2)	Gamma-P4	γ-proteobacteria (97%)	Uncultured bacterium clone OTU535 (DQ684529)	375/387 (100%)	Rhizosphere soil sample, Canada
FL179 (7)	Acid-P	Acidobacteria (86%)	Uncultured Acidobacteria bacterium clone (DQ501294)	383/386 (100%)	Lake Breiter Luzin, Germany
FL92, FL94; FL264 (9)	Cya-An1	Cyanobacteria, family I (100%)	Anabaena affinis strain NIES40 (AY701541); Aphanizomenon sp. 16D11 (FJ234882)	381/381 (100%), 378/379 (100%); 381/381 (96%)	Japan; Scharmuetzelsee, Germany
FL110	1)[a]	Cyanobacteria, family I (100%)	Aphanizomenon flos-aquae var. Klebahnii, strain 218 (AJ293123)	383/383 (100%)	Lake Birkerød, Denmark
FL93, FL253 (2)	Cya-An2	Cyanobacteria, family I (100%)	Aphanizomenon flos-aquae AFA-3 (EU157999), Aphanizomenon issatschenkoi strain TAC419 (AY196087)	378/378 (99%), 377/378 (100%)	China, unknown
FL231	1)	Cyanobacteria, family I (100%)	Gloeotrichia echinulata, environmental colony URA3 (AM230705)	385/385 (99%)	Lake Urajärvi, Finland
FL2, FL151 (25)	Cya-Sy1	Cyanobacteria, family II (100%); Cyanobacteria, family II (99%)	Uncultured bacterium clone ML-5-119 (DQ235028)	372/372 (98%), 365/372 (98%)	Lake Taihu, China
FL3, FL202 (24)	Cya-Sy2	Cyanobacteria, family II (100%)	Uncultured cyanobacterium clone TH1-92 (AM690887), Uncultured bacterium, clone: S9JA-38 (AB154318)	379/379 (100%), 383/383 (100%)	Lake Taihu, China; Lake Kasumigaura, Japan
FL1, FL120	1)	Cyanobacteria, family II (100%)	Synechococcus sp. MH301 (AY224199)	372/378 (100%), 379/379 (100%)	Lake Mondsee, Austria
FL233	1)	Cyanobacteria, family II (100%)	Cyanobium sp., strain JJR2A5 (AM710381)	379/379 (97%)	Rimov reservoir, Czech Republic
FL188, FL257 (13)	Cya-Mi1	Cyanobacteria, family XI (100%)	Microcystis aeruginosa strain 039 (DQ363255)	379/379 (100%), 343/347 (100%)	Unpubl.
FL187 (2)	1)	Cyanobacteria, family XI (100%)	Microcystis aeruginosa strain (AB271211)	378/378 (100%)	Lake Biwa, Japan
FL211 (20)	Euc1	Chloroplast (70%)	Uncultured cryptophyte chloroplast, clone Jo117-01 (AJ620853)	337/350 (96%)	Lake Joutikas, Finland
FL212 (18)	Euc2	Cyanobacteria, family IV (9%)	Uncultured phototrophic eukaryote clone PRD18E11; chloroplast (AY948042)	292/314 (88%)	Parker River, MA, USA

[a]Band was excluded from the PCA

similar to uncultured actinobacterium sp. (clones TW1G8 and SA1E1) found in lakes in the USA. Sequences (FL13, FL71, FL204; Table 1) from the third most common band position observed in 47 lakes in Finland were very similar to *Chloroflexi* sequences obtained from the Piburger See in Austria and the Changjiang River in China. Four other bands were observed in DGGE analyses of samples from more than 40 lakes. Sequences (FL53, FL9, FL133, FL166; Table 1) derived from 2 of these bands were related to those of *Actinobacteria*; sequences (FL21, FL78; Table 1) from the other 2 were related to those of *Alphaproteobacteria* and the closest relatives were found in each case in freshwater environments.

Differences in bacterioplankton community composition in PCA

In PCA, DGGE banding patterns clustered mainly according to the trophic status (TP concentration) of the lakes (Fig. 1). The first principal component of PCA explained 42% and the second axis 33% of the variation. Sequenced DGGE bands, which are indicated with arrows in the PCA plot (Fig. 1), showed that certain bacterial phylotypes were typical of a specific trophic level. Most of the oligotrophic and mesotrophic lakes are placed on the right side and bottom of the plot (Fig. 1). A few of the oligo- and mesotrophic lakes are placed at top of the plot
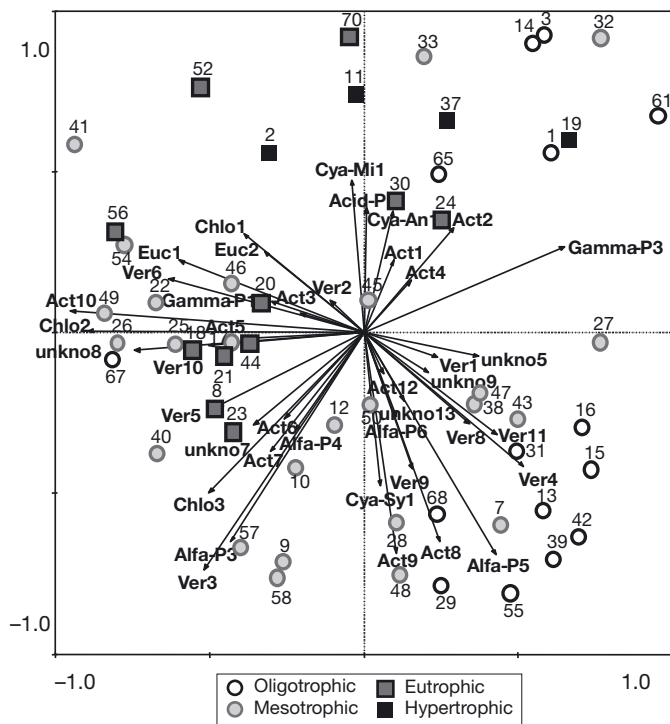
Fig. 1. Principal component analysis (PCA) plot showing clustering of the DGGE fingerprint patterns obtained from 55 Finnish lakes. Arrows indicate DGGE bands. Abbreviation 'unkno' indicates those DGGE bands that could not be sequenced; other abbreviations are as in Table 1. Total phosphorus concentration in oligotrophic lakes was <10 mg l^{-1} (○); in mesotrophic lakes, 10 to 34 mg l^{-1} (●); in eutrophic lakes, 35 to 100 mg l^{-1} (■); and in hypertrophic lakes, >100 mg l^{-1} (■). Numbers refer to the lakes (see Table S1 in the supplement)

because of their divergent DGGE profiles. The majority of the DGGE profiles from the eutrophic as well as mesotrophic lakes with near eutrophic phosphorus levels are placed on the left side and in the middle of the plot (Fig. 1). All 4 hypertrophic lakes are placed at the top of the PCA plot (Fig. 1) due to their similar DGGE profiles.

Canonical correspondence analysis

In the CCA plot, lakes grouped according to the trophic status based on TP (Fig. 2). Of the environmental variables used, TP and chl a autocorrelated strongly (r = 0.86; see Table 3). In the CCA model, only 2 variables were statistically significantly (p < 0.05) related to the bacterioplankton community composition at a time: one of

the autocorrelating variables (chl a or TP) and the DIN:DIP ratio (Fig. 2). For the final CCA model, chl a was chosen as a more general biological variable that includes the effect of TP. In the CCA clustering of the lake samples, chl a has the most pronounced effect; samples from the oligotrophic and mesotrophic lakes are placed to one side, and the samples from the eutrophic and hypertrophic lakes are placed on the other side together with the chl a vector (Fig. 2A). The same clustering was found when TP was used instead of chl a in the model (Fig. 2B). According to the CCA, species and significant environmental variables based on the 2 ordination axes explained 19% of the taxonomic variation of the bacterioplankton in the 55 samples included in the analysis (Table 2).

Chl a, the factor that was related to bacterial community composition, had a statistically significant positive correlation to TP, TN and water colour, and negative correlation to Secchi depth and TN:TP ratio (Table 3). Thus, higher TP and TN, darker water colour, lower transparency and lower TN:TP ratio associated with bacterial communities of eutrophic and hypertrophic lakes. The DIN:DIP ratio was significantly and positively correlated to N(NO$_3$-NO$_2$) and the TN:TP ratio (Table 3).

DISCUSSION

Results of the CCA of bacterioplankton community DGGE profiles and environmental parameters as explanatory factors indicated that phytoplankton biomass, as detected by chl a or TP, and the DIN:DIP ratio were significantly related to the bacterial community composition. The importance of phytoplankton to the BCC has been shown earlier in many other studies (e.g. Horner-Devine et al. 2003, Jardillier et al. 2004, Yannarell & Triplett 2004, Kritzberg et al. 2006), whereas the DIN:DIP ratio has been found to be more

Table 2. Summary of canonical correspondence analysis (CCA) of the bacterioplankton community composition data obtained from 55 lake samples

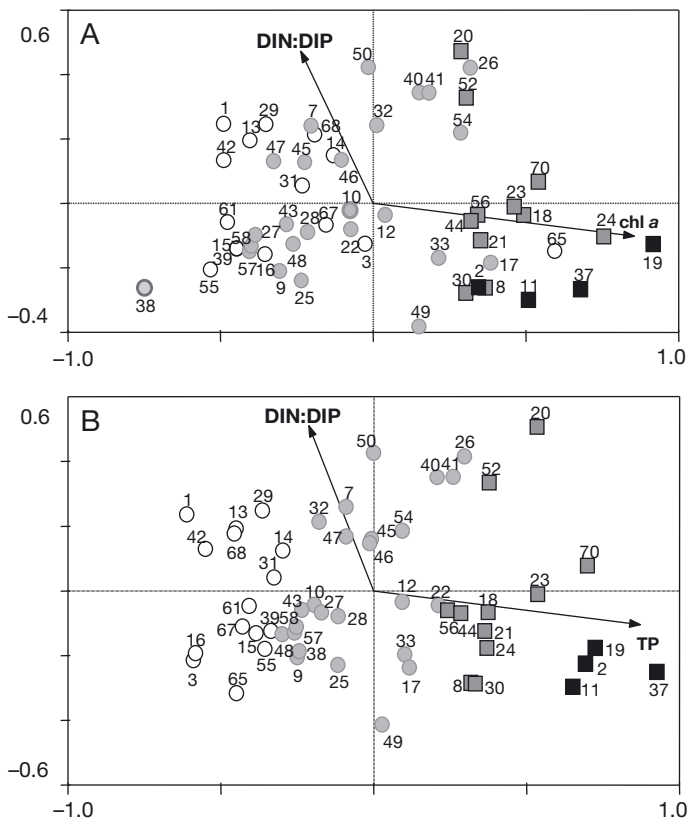| Parameter	Axis 1 (chl a)	Axis 2 (DIN:DIP)
Number of samples (lakes)	55	
Number of species (DGGE band positions)[a]	49	
Eigenvalues	0.144	0.046
Species-environment correlations	0.863	0.725
Cumulative percentage variance of species data	7.6	10.0
Cumulative percentage variance of species-environment relation	76.0	100.0
Sum of all canonical eigenvalues	0.190	
[a]Rare bands (12 out of 61) were excluded from the CCA		

Fig. 2. Canonical correspondence analysis (CCA) biplot showing bacterial community composition in relation to the 2 strongest environmental variables: ratio of dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN:DIP), and (A) chlorophyll a (chl *a*) or (B) total phosphorus (TP). TP concentration in oligotrophic lakes was <10 mg l^{-1} (O); in mesotrophic lakes, 10 to 34 mg l^{-1} (⊙); in eutrophic lakes, 35 to 100 mg l^{-1} (▨); and in hypertrophic lakes, >100 mg l^{-1} (■). Numbers refer to the lakes (see Table S1 in the supplement)

relevant in determining the cyanobacterial community (Hyenstrand et al. 1998). In the CCA plot, the low DIN:DIP ratio correlated with a high chl *a* concentration and thus with high levels of phytoplankton. In addition, total concentrations of both nutrients (P and N) as well as water colour were positively correlated with chl *a* (Table 3). Thus, many environmental variables that are known to be related to trophic status were found to be associated with the BCC of the Finnish lakes studied.

Phosphorus and nitrogen often limit the growth of both bacterio- and phytoplankton (Wetzel 1983, Fisher et al. 2000, Vrede 2005), and enrichment of these nutrients in lakes can also influence BCC (Fisher et al. 2000, Haukka et al. 2006). High concentrations of TP have been shown to increase the cyanobacterial and phytoplankton biomass and thus also the concentration of chl *a* (Watson et al. 1997). Previously, in the study by

Rantala et al. (2006), it was found that higher pH and TN concentration as well as a larger surface area of the lake increased the occurrence probability of potentially hepatotoxic cyanobacteria in the lakes used in the present study. Analyses of large lake data sets from Europe found TN concentrations to be among the important factors contributing to overall BCC patterns together with other factors associated with densities/biomass of grazers, lake depth and pH as well as drainage area parameters (Van der Gucht et al. 2007). In our CCAs, 19% of the variation was explained by significant environmental parameters. This corresponds closely to the proportion of variation found to be explained by environmental factors in the BCC of other large data sets (Van der Gucht et al. 2007).

Similar to the present investigation, several studies have found the dominant forces structuring BCC to be primary productivity (measured as concentration of chl *a*) and dissolved organic carbon (DOC) (Crump et al. 2003, Horner-Devine et al. 2003, Stepanauskas et al. 2003, Jardillier et al. 2004, Yannarell & Triplett 2004, Kritzberg et al. 2006). It is well established that strong seasonal changes in BCC occur (e.g. Yannarell et al. 2003, Shade et al. 2007, 2008, Nelson 2009). In our study, the samples originated from 67 lakes, but were mainly taken during the middle of summer before the cyanobacterial bloom-forming season.

Sequence analysis of DGGE bands revealed the presence of *Verrucomicrobia*-, *Actinobacteria*-, *Cyanobacteria*-, *Alpha*- and *Gammaproteobacteria*-, *Chloroflexi*- and *Acidobacteria*-related phylotypes in the lakes studied. All these bacterial phyla are frequently found in freshwater environments (Table 1). Database analysis of 16S rRNA gene sequences from freshwater by Zwart et al. (2002) showed that a relatively small number of bacterial phyla dominates the freshwater plankton. Our results confirm that this is

Table 3. Correlation coefficients of environmental variables with variables used in the CCA model. Statistically significant correlations (p < 0.05) are marked in **bold**

Environmental variable	Chl *a*	DIN:DIP
Chl *a*	1.0	−0.466
Secchi depth	**−0.804**	0.322
Temperature	0.189	−0.274
Total phosphorus (TP)	**0.862**	−0.430
Total nitrogen (TN)	**0.792**	0.011
TN:TP	**−0.627**	**0.609**
pH	0.573	−0.317
Water colour	**0.631**	−0.056
N(NO$_3$-NO$_2$)	−0.095	**0.861**
DIN:DIP	−0.466	1.0
Latitude	−0.218	0.044
Longitude	−0.264	0.274

also the case in the Finnish boreal lakes studied. According to Zwart et al. (2002), the major bacterial divisions present in most freshwaters are *Alpha-* and *Betaproteobacteria*, *Cytophaga-Flavobacterium-Bacteroides* (CFB), *Actinobacteria* and *Verrucomicrobia*. Other studies have shown that *Actinobacteria, Betaproteobacteria* and CFB are the most numerous phylogenetic groups in freshwater clone libraries (Glöckner et al. 2000, Stepanauskas et al. 2003, Zwisler et al. 2003, Eiler & Bertilsson 2004). *Betaproteobacteria* or CFB were not detected in the present study. However, these groups might have been present, since we did not succeed in sequencing bands from every position in the DGGE gels. In addition, DGGE is known to detect the most abundant bacterial phylotypes (Ferris & Ward 1997). PCR amplification can distort the results, and the choice of the primer set can have an effect on the DGGE analysis. The F-968 primer used in the present study included an internal mismatch to many freshwater betaproteobacterial sequences, which could have lowered their detection frequency. However, in our earlier work (Kolmonen et al. 2004, Haukka et al. 2006), *Betaproteobacteria* and CFB were detected sporadically from the lake samples using DGGE with the same primer set.

The majority of the DGGE band-derived sequences were related to *Verrucomicrobia.* The study also indicated that there were verrucomicrobial phylotypes that preferred oligo-mesotrophic conditions, whereas the other phylotypes were frequent in eutrophic lakes (Fig. 1). In our earlier study of the bacterioplankton community in lake mesocosms (Haukka et al. 2006), *Verrucomicrobia* correlated with more eutrophic conditions and *Actinobacteria* with less eutrophic conditions. In addition, our previous studies on BCC, conducted in just a few lakes, indicated that *Verrucomicrobia* are more prevalent in eutrophic lakes than in oligo- or mesotrophic lakes (Kolmonen et al. 2004, Haukka et al. 2005). The present extensive analysis of 67 lakes indicates that studies of individual lakes may not reveal general trends in how environmental factors affect BCC. Our results are in agreement with the mesocosm study by Sipura et al. (2005) in the Baltic Sea Archipelago, where *Verrucomicrobia* were most frequent in control mesocosms, whereas nutrient additions decreased the DGGE band intensities.

Actinobacteria-related sequences were frequently retrieved from the lake samples. Previous studies have shown *Actinobacteria* to be a common and important component of the freshwater bacterioplankton (Glöckner et al. 2000, Urbach et al. 2001, Warnecke et al. 2005). Recently, strains of aquatic *Actinobacteria* have been isolated from oligotrophic to hypereutrophic lakes (Hahn et al. 2003). It has been suggested that some *Actinobacteria* are possibly

common in the bacterioplankton because they are very small in size and resist predation by protists (Pernthaler et al. 2001, Hahn et al. 2003, Warnecke et al. 2004). In the present study, some *Actinobacteria* (e.g. Act 1, 2, 4; Fig. 1) were associated with eutrophic lakes, indicating that certain phylotypes of this group may be important degraders of phytoplankton biomass. On the other hand, other *Actinobacteria*-related phylotypes (Act 6 to 8) were found associated with less eutrophic lakes, suggesting an important role for this phylum in mid-summer bacterioplankton communities in Finnish lakes. Previously, Lindström & Leskinen (2002) found that lakes in Swedish Lapland (bog lakes) commonly contain *Actinobacteria,* whereas our samples all came from lakes in southern and central Finland.

Cyanobacteria occurred most commonly in hypereutrophic lakes, according to the DGGE results. In the oligo- and mesotrophic lakes, the picocyanobacterial genera were detected, while the bloom-forming genera, *Anabaena* and *Microcystis*, were more often present in the eutrophic and hypertrophic lakes. In previous studies using the same environmental data set, potential cyanobacterial hepatotoxin (microcystin) producers, *Anabaena*, *Microcystis* and *Planktothrix*, were detected in approximately 90% of the lakes by PCR (Rantala et al. 2006) and the cyanobacterial genera *Snowella* and *Woronichinia* were commonly found through microscopy in the oligo- and mesotrophic lakes (Rajaniemi-Wacklin et al. 2006). In the present study, cyanobacterial genera were detected less frequently using DGGE with the eubacterial primer set. The primer R-1401 had a mismatch for most of the cyanobacterial sequences in the RDP Database, most probably causing the lower detection frequency. Our earlier study demonstrated that it is preferable to use cyanobacteria-specific primers in DGGE and cloning when targeting the cyanobacterial community (Rajaniemi-Wacklin et al. 2008).

In the present study, *Alphaproteobacteria* sequences were encountered more frequently than *Gammaproteobacteria*. An *Alphaproteobacteria*-related sequence was retrieved from a band (FL22) located in the most prominent band position that was present in 62 of the 67 lakes investigated (Table 1), suggesting that certain members of this phylum are common in Finnish lakes. *Alphaproteobacteria* were found dominating in the mesotrophic Biwa Lake throughout the season (Nishimura & Nagata 2007). In the northern boreal lakes, *Alpha-* and *Betaproteobacteria* were dominant classes in the clone libraries of 3 eutrophic Swedish lakes during the cyanobacterial bloom (Eiler & Bertilsson 2004). In Swedish lakes, *Alphaproteobacteria* appeared to be more common in lakes with neutral to alkaline pH, in contrast to more acidic waters (Lind-

ström & Leskinen 2002). Similar to the present study, Lindström et al. (2005) also found *Gammaproteobacteria* to occur only rarely in 15 studied lakes in northern Europe. *Betaproteobacteria* are common in freshwater lakes (e.g. Zwart et al. 2002) and are probably capable of degrading complex organic macromolecules (Kirchman 2002), but this group and *Deltaproteobacteria* were not detected in our study.

Through DGGE analysis, we found that certain band positions, some with sequences related to *Alphaproteobacteria* and *Actinobacteria,* were common in all lakes despite differences in the trophic status or water chemistry of the lake. Similarly, many previous studies have found certain strains or bacterioplankton phylotypes to be common regardless of the various environmental gradients or regions (Hahn et al. 2003, Lindström et al. 2005, Van der Gucht et al. 2007). Similar phylotypes (based on the 16S rRNA gene sequence) may, however, represent various ecotypes differing in their environmental requirements, as was recently shown with isolates of *Actinobacteria* (Hahn & Pöckl 2005). Genome projects of various strains of the same organisms have shown that ribosomal genes belong to the core genes of the organisms and may not reflect the enormous niche adaptation gene pool contained in the individual strains (Medini et al. 2005). Our extensive analysis of the BCC in Finnish lakes showed that trophic status is an important factor in shaping the bacterioplankton communities, reflected in changes at the phyla level as well as in individual phylotypes. In future, next generation sequencing technologies are likely to reveal detailed information about bacterioplankton community composition as well as niche adaptation of individual organisms.

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