Changes in bacterial community structure associated with phytoplankton succession in outdoor experimental ponds

YUKI KOBAYASHI^{1, 5, *}, YOSHIKUNI HODOKI², KAKO OHBAYASHI³, NOBORU OKUDA^{4, 6} & SHIN-ICHI NAKANO⁴

¹ Research Center for Environmental Changes, Academia Sinica Academia Sinica Road, Sect 2, NanKang, 11529 Taipei, Taiwan ROC

² Department of Biology, Keio University, 4–1–1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223–8521, Japan

³ Department of General Systems Studies, Graduate School of Arts and Science, The University of Tokyo, 3–8–1 Komaba, Meguro-Ku, Tokyo 153–8902, Japan

⁴ Center for Ecological Research, Kyoto University, 2–509–3 Hirano, Otsu, Shiga 520–2113, Japan

⁵ National Research Institute of Fisheries and Environment of Inland Sea, 2–17–5 Maruishi, Hatsukaichi, Hiroshima 739–0452, Japan

⁶ Research Institute for Humanity & Nature 457–4 Motoyama, Kamigamo, Kyoto P.O. 603–8047, Japan

Received 21 December 2013; Accepted 26 December 2014

Abstract: Changes in bacterial community structure were followed in two outdoor experimental ponds. To compare the changes in bacterial community, *Microcystis* was inoculated into one of the ponds but not to the other one. In both ponds, Chlorophyceae algae from the genera *Cosmarium* and *Scenedesmus* were dominant in the first and last months of the study. During the middle period of the study, cyanobacteria were dominant. *Microcystis* and *Aphanizomenon* dominated in one pond, and *Planktothrix* dominated in the other. To investigate bacterial phylogenetic abundance and these compositions, we used catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) and denaturing gradient gel electrophoresis (DGGE). A significant relationship was observed between the number of α -proteobacterial operational taxonomic units (OTUs) and the abundance of Chlorophyceae algae ($p < 0.001$), although no significant relationship was identified between the abundances of the two groups. The sequencing analysis of DGGE bands detected microcystin-degrading bacteria belonging to the α -proteobacteria as one of the dominant bacterial phylogenetic groups when *Microcystis* was the dominant phytoplankton. To our knowledge, this is the first report demonstrating changes in the abundance and composition of bacterial groups during the wax and wane of dominant phytoplankton taxa, using two different molecular methods.

Key words: *Microcystis aeruginosa*, Catalyzed reporter deposition fluorescence *in situ* hybridization, Denaturing gradient gel electrophoresis, Cyanophyta, Chlorophyta

Introduction

In the pelagic areas of lakes, bacterioplankton play important roles in material cycling in planktonic food webs (Corner & Biddanda 2002, Grossart et al. 2005, Rooney-Varga et al. 2005, Šimek et al. 2008). Extracellular dissolved organic carbon derived from phytoplankton is a major source of organic matter for bacterioplankton (van Hannen et al. 1999, Kirkwood et al. 2006). Many researchers have investigated the relationships between the domi-

nant phytoplankton and different bacterial phylogenetic groups, and demonstrated that *Flavobacteria* (Pinhassi et al. 2004, Niu et al. 2011), α -proteobacteria, and the *Cytophaga-Flavobacterium* (CF) cluster (Riemann et al. 2000) were abundant during diatom blooms. Šimek et al. (2008) showed that β -proteobacteria dominated during a Cryptophyceae algal bloom, and Hube et al. (2009) revealed that α -proteobacteria and *Bacteroidetes* were highly abundant in laboratory cultures of the genus *Planktothrix* (Cyanophyceae). Most of these studies were carried out by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (Muyzer et al. 1995). The advantage of this method is that bacterial taxa can be identified by se-

*Corresponding author: Yuki Kobayashi; E-mail, kobadon0718@gmail.com

quencing the bands derived from PCR-DGGE. Unfortunately, PCR-DGGE is subject to some of the biases that result from DNA extraction methods and PCR, and the results derived from this technique must be viewed with caution (Castle & Kirchman 2004).

Another method for the examination of bacterial phylogenetic groups is catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) (Schönhuber et al. 1997, Pernthaler et al. 2002). This method does not require PCR, and the abundance of each phylogenetic group can be quantitatively estimated. However, using this method, bacterial taxa cannot be identified by sequencing.

By using both the DGGE and FISH methods, we can quantitatively determine bacterial community structure and identify the dominant bacterial species. However, we still have only limited information on bacterial community structure and/or dominant bacterial species in relation to phytoplankton succession.

In the present study, our aim was to examine the changes in bacterial community structure, as well as the dominant bacterial taxa, in relation to succession of dominant phytoplankton taxa. We used CARD-FISH to examine bacterial community structure and DGGE to identify dominant bacterial taxa.

Materials and Methods

The present study was conducted in two mesoscale experimental ponds (area, 10 m²; maximum depth, 1.7 m; water volume, ca. 70 m³) located in the Center for Ecological Research, Kyoto University, Japan (34°58'2.24"N, 135°57'38.93"E). We introduced *Microcystis* collected from Lake Biwa to the ponds as a seed population. In August 2009, 3.0 m³ lake water containing a *Microcystis* bloom was collected, concentrated to volume with a 5- μ m-mesh size plankton net (ca. 1.8×10^6 cells mL⁻¹), and added to Pond A (Hodoki et al. 2011). Pond B was used as control, without addition of the lake phytoplankton. At the beginning of the experiment, we added MA medium (Ichimura 1979) with modification (final concentration, 118.6 μ M for dissolved inorganic nitrogen, DIN; 6.5 μ M for dissolved inorganic phosphorus, DIP). Water samples were collected from the surface of each pond with a 5-L plastic bucket and poured into sterilized polycarbonate bottles. Samples were taken once or twice weekly from 20 August to 19 November in 2009. At times of sampling, water temperature and pH were measured with a bar thermometer and pH meter (FE20/EL20; Mettler Toledo, Columbus, OH, USA), respectively.

For nutrient measurements, water samples were filtered through precombusted, acid-washed glass fiber filters (GF/F; Whatman, Maidstone, Kent, UK) for 3 h at 420°C. Filtrates were poured into acid-washed polystyrene bottles and stored at -30°C until analysis. The concentrations of nitrate, nitrite, and DIP were measured spectrophotometrically using an autoanalyzer (AACS II; Bran+Luebbe, Nor-

derstedt, Germany). Ammonium concentration was determined fluorometrically with a TD-700 fluorometer (Turner Designs, Sunnyvale, CA, USA) according to the method of Holmes et al. (1999), and DIN (=nitrite+nitrate+ammonium) was calculated.

For chlorophyll *a* (Chl *a*) concentration, a 20-mL volume of each water sample was filtered through a GF/F to retain the seston, and the filter containing the retained seston was kept at -20°C until analysis. To extract Chl *a*, the filter was placed in a glass test tube and 10 mL of *N,N*-dimethylformamide (DMF) was added. The Chl *a* quantity was then determined by the fluorometric method using a spectrofluorophotometer (RF-5300; Shimadzu, Kyoto, Japan) (Welschmeyer 1994).

To determine bacterial abundance, a 10-mL volume of each water sample was fixed with paraformaldehyde (2% final concentration) and 1–2 mL of fixed sample was filtered through a black-stained 0.2- μ m polycarbonate membrane filter (Whatman). Bacterial cells were stained with 2 μ g mL⁻¹ of 4',6-diamino-2-phenylindole (DAPI) for 10 min and counted by epifluorescence microscopy (BX60; Olympus, Tokyo, Japan) at 1000 $\times$ magnification. A 100-mL volume of each water sample was fixed with glutaraldehyde (1% final concentration), and *Microcystis* cells were enumerated with a Fuchs–Rosenthal type hemocytometer (Hirschmann, Eberstadt, Germany) under a microscope at 200 $\times$ or 400 $\times$ magnification (Hodoki et al. 2011). For other phytoplankton, 500 mL of each water sample was fixed with acid Lugol's solution (1% final concentration) and phytoplankton cells were concentrated by natural sedimentation. Phytoplankton cells were enumerated with a Sedgwick–Rafter chamber under a microscope at 200 $\times$ or 400 $\times$ magnification.

CARD-FISH was performed according to the method of Okazaki et al. (2013) with modifications. Briefly, water samples were fixed with formaldehyde (2% final concentration) and stored at 4°C for 16–18 h. The probes used in this study are listed in Table 1. For hybridization, 3 mL of each water sample was filtered onto a 0.2- μ m pore-diameter membrane filter (polycarbonate membrane, 25 mm diameter; Whatman). The collected cells were coated with 0.1% (w/v) low-melting-point agarose and permeabilized with lysozyme solution [0.05 M EDTA, 0.1 M Tris-HCl, 10 mg mL⁻¹ lysozyme (MP Biomedicals, Santa Ana, CA)] for 1 hour at 37°C. All probes were horseradish peroxidase (HRP) labeled oligonucleotide (Thermo Fisher Scientific, MA) as shown in Table 1. The filters were cut into six pieces and soaked with hybridization buffer [900 mM NaCl, 20 mM Tris-HCl, 10% (w/v) dextran sulfate, 0.05% TritonX100, 20% (v/v) formamide, 35%] containing 0.5 mg mL⁻¹ of each probe. The hybridization reaction was performed overnight at 37°C with mild agitation. After hybridization, the DAPI-positive cells and hybridized cells were counted manually under an epifluorescence microscope (Olympus BX60).

DNA extraction was performed according to the method

Table 1. Oligonucleotide sequences of probes used for CARD-FISH and PCR primers used for DGGE fingerprinting. * denotes PCR primers.

Phylogenetic group	Probe or Primer	Oligonucleotide sequence (5'-3')	Reference
<i>Eubacteria</i>	Eub338-I	GCTGCCTCCCGTAGGAGT	Amann et al. 1995
	Eub338-II	GCAGCCACCCGTAGGTGT	Daims et al. 1999
	Eub338-III	GCTGCCACCCGTAGGTGT	Daims et al. 1999
<i>Alphaproteobacteria</i>	Alfa968	GGTAAGGTTCTGCGCGTT	Glöckner et al. 1999
<i>Betaproteobacteria</i>	Beta42	GCCTTCCCACTTCGTTT	Manz et al. 1992
<i>Gammaproteobacteria</i>	Gam42a	GCCTTCCCAACATCGTTT	Manz et al. 1992
<i>Cytophaga-Flavobacterium</i>	CF319	TGGTCCGTGTCTCAGTAC	Manz et al. 1996
<i>Actinobacteria</i>	HGC69a	TATAGTTACCACCGCCGT	Glöckner et al. 2000
<i>Verrucomicrobia</i>	VER139	CGAGCTATTCCCCTCTTG	Dedysh et al. 2006
	VER1463	CCATCCATACCTTCGGCA	Dedysh et al. 2006
* <i>Eubacteria</i>	GC-341F	CGCCCGCCGCGCCCGCGCCCGGCCCGCCG CCCCCGCCCCCTCCTACGGGAGGCAGCAG	Muyzer et al. 1995
	907R	CCGTCAATTCTTTGAGTTT	Muyzer et al. 1995
* <i>Alphaproteobacteria</i>	GC-517F	CGCCCGCCGCGCCCGCGCCCGGCCCGCCG CCCCGCCCGTGCAGCAGCCGCGG	Lane 1991
	968R	GGTAAGGTTCTGCGCGTT	Neef 1997
* <i>Cytophaga-Flavobacterium</i>	GC319F	CGCCCGCCGCGCCCGCGCCCGGCCCGCC GCCCCGCCCGTACTGAGACACGGACCA	Manz et al. 1996

* Forward (F) and reverse (R) primers

of Murray and Thompson (1980) with some modification. Fifty milliliters of each water sample was filtered onto a 47-mm, 0.2- μ m pore-diameter polycarbonate membrane filter (Millipore, Eshborn, Germany); filters were kept at -30°C until analysis. DNA was extracted from the filters using cetyltrimethylammonium bromide (CTAB) and chloroform/isoamyl alcohol (CIAA; 24:1), and phenol/chloroform/isoamyl alcohol (PCA; 25:24:1). Fragments containing the V3 region of the bacterial 16S rRNA gene were PCR-amplified using primers for eubacteria (GC-341F–907R) (Muyzer et al. 1995), α -proteobacteria (GC517F–968R) (Gich et al. 2005), and the CF-cluster (GC319F–907R) (Gich et al. 2005) (Table 1). Eubacterial sequences were amplified with a Blend Taq PCR Kit (Toyobo, Tokyo, Japan) and a touchdown program was performed on a thermal cycler (iCycler; Bio-Rad, Hercules, CA, USA) as follows: initial denaturation at 94°C for 5 min; 30 cycles at 94°C for 1 min, annealing at 64 – 55°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 7 min. The annealing temperature was decreased from 64°C to 55°C by 1°C every second cycle. For amplification of α -proteobacteria and the CF-cluster, step-down PCRs were performed as described by Gich et al. (2005). PCR products were resuspended in $40\ \mu\text{L}$ TE buffer and quantified with a UV spectrophotometer (model 1000; NanoDrop Technologies, Wilmington, DE, USA).

DGGE was performed using the D-Code Universal Mutation Detection System (Bio-Rad; Muyzer et al. 2004). PCR products (250 ng per sample) mixed with loading buffer (Bio-Rad), and a DGGE marker (Nippon Gene, Tokyo, Japan) for migration comparisons were run on a gel with a 20–60% denaturing gradient (100% denaturant was 7 M

urea and 40% deionized formamide). Electrophoresis was performed at 50 V for 16 h at 60°C . The gels were stained with 1 $\times$ SYBR Gold (Molecular Probes, Eugene, OR, USA) and photographed with a Gel DOCTM 2000 Gel Documentation System (Bio-Rad). The gel pictures were analyzed to measure the migration distances using the Quantity One software package (Bio-Rad). Prominent DGGE bands were excised and DNA was extracted from the excised bands. Theoretically, each DGGE corresponds to a single operational taxonomic unit (OTU), where the total banding pattern is reflective of a community's species richness and diversity (Muyzer et al. 1993). Sequencing analysis was performed on the excised DGGE bands. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions and sequenced using a BigDyeTM Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI3100-Avant Capillary Auto Sequencer (Applied Biosystems). Sequences were aligned using the BioEdit program (Hall, 2004) and sequence results were checked using the BLAST search program against nucleotide sequences (Altschul et al. 1990). Sequences were deposited at the DNA Data Bank of Japan under the accession nos. AB787222–AB787264. Correlation analyses between bacterial and phytoplankton communities were performed using SigmaPlot 5.0 (SPSS, Chicago, IL, USA).

Results and Discussion

In both ponds, the water temperature gradually decreased from 31.0°C to 10.8°C during the study period

(Table 2). While the pH value, DIP concentration, and DIN concentration fluctuated during the study period (pH 6.9–10.9, DIP: 0.2–40.3 $\mu\text{mol L}^{-1}$, and DIN: 0.8–1580 $\mu\text{mol L}^{-1}$), we could detect no seasonal patterns (Table 2). Although we added lake phytoplankton to Pond A, Chl *a* concentrations were relatively stable in both ponds after the first 2 weeks (Fig. 1A, B). The phytoplankton dynamics have already been reported in Hodoki et al. (2011), but are described briefly below. In Pond A, cyanobacterial

Table 2. Summary of the environmental variables for both experimental ponds. *M. aeruginosa* was added to pond A (A) but not to pond B (B).

Variables	Pond	Mean $\pm$ SD	Range
Temperature ($^{\circ}\text{C}$)	A	20.3 $\pm$ 4.7	11.0–27.8
	B	20.3 $\pm$ 4.9	10.8–31.0
pH	A	9.4 $\pm$ 0.7	7.8–10.9
	B	8.3 $\pm$ 0.7	6.9–9.9
DIN ($\mu\text{mol L}^{-1}$)	A	126.3 $\pm$ 328.7	0.8–1576.9
	B	57.2 $\pm$ 119.5	1.1–380.5
DIP ($\mu\text{mol L}^{-1}$)	A	4.2 $\pm$ 7.3	0.8–40.3
	B	2.2 $\pm$ 1.9	0.2–6.6
Chl.a ($\mu\text{g l}^{-1}$)	A	80.2 $\pm$ 30.7	6.5–150.6
	B	73.7 $\pm$ 36.9	27.0–180.1

abundance increased beginning on day 21 and reached 5.0×10^4 cells mL^{-1} on day 63, corresponding to 67% of the total phytoplankton abundance (Fig. 1A). In this pond, *Microcystis aeruginosa* dominated, followed by *Aphanizomenon issatschenkoi*. *M. aeruginosa* abundance increased

Fig. 1. Changes in the Chlorophyll *a* concentration and the relative abundances of phytoplankton. (A) Pond A and (B) Pond B. (●) Chlorophyll *a* concentration.

Fig. 2. Time-course variations in the absolute and relative abundances of different bacterial phylogenetic groups in the experimental ponds. Absolute bacterial abundance in (A) Pond A and (B) Pond B. Relative bacterial abundance in (C) Pond A and (D) Pond B. ALP: α -proteobacteria, BET: β -proteobacteria, GAM: γ -proteobacteria, CF: *Cytophaga-Flavobacteria* cluster, HGC: high G+C, and VER: *Verrucomicrobia*.

markedly, attained a peak concentration of 4.7×10^4 cells mL^{-1} on day 42, and then significantly decreased afterward. The decrease in the cyanobacterial contribution to total phytoplankton abundance (Fig. 1A) was due to the decrease in *M. aeruginosa* abundance. In contrast to Pond A, only the genus *Planktothrix* (Cyanophyta) dominated in Pond B (Fig. 1B); its trichome density increased, peaking at 16.8×10^4 cells mL^{-1} on day 63, which was 75% of the total phytoplankton abundance.

Before and after cyanobacterial dominance, Chlorophyta algae dominated in both ponds (Fig. 1). The dominant genera were *Cosmarium* and *Scenedesmus*, and their contributions were 79.1% and 22.5% of the total Chlorophyta abundance at maximum, respectively. In both ponds, α -proteobacterial abundance increased, reaching concentrations of 5.5×10^6 and 3.8×10^6 cells mL^{-1} in Ponds A and B, respectively (Fig. 2A, B). In Pond A, β -proteobacteria and the CF-cluster dominated at the beginning, followed by the dominance of α -proteobacteria (31.0%) on day 76 (Fig. 2C). In Pond B, α -proteobacteria and the CF-cluster dominated throughout the study period

(Fig. 2B); however, in the middle of the experiment, counts of β -proteobacteria in CARD-FISH were high (4.6×10^6 cells mL^{-1} on day 18 and 3.4×10^6 cells mL^{-1} on day 46; Fig. 2B). In Pond A, the *Verrucomicrobia* represented 1–5% of the bacterial abundance from days 14 to 76 (Fig. 2A). We did not detect any *Verrucomicrobia* in Pond B (Fig. 2D).

Using Spearman's rank correlation analysis, we examined the relationships between the abundance of each phytoplankton taxon and each bacterial phylogenetic group. However, we found no significant relationships between phytoplankton and each phylogenetic bacterial abundance. The only significant relationship that we detected was between the abundance of Chlorophyceae algae and the number of α -proteobacteria OTUs ($p < 0.001$).

DGGE profiles of PCR-amplified bacterial 16S rRNA gene fragments are shown in Fig. 3A–F. In Pond A, the number of DGGE bands ranged from 13 to 27, 9 to 21, and 4 to 13 on the DGGE gels for eubacteria (EUB-DGGE), α -proteobacteria (ALP-DGGE), and the CF-cluster (CF-DGGE), respectively. In pond B, 12–28, 5–14, and 6–14

Fig. 3. DGGE images of eubacteria, α -proteobacteria, and CF-cluster 16S rRNA gene fragments. M1 and M2 denote the marker and *M. aeruginosa* strain (NIES 843), respectively. The number on each lane indicates the sampling day. Numbers on the gels in white boxes indicate DNA bands that were excised and sequenced. EUB-DGGE for (A) Pond A and (B) Pond B; ALP-DGGE for (C) Pond A and (D) Pond B; CF-DGGE for (E) Pond A and (F) Pond B.

Table 3. Sequenced DGGE bands. EUB, ALP, and CF indicate bands from the EUB-DGGE, ALP-DGGE, and CF-DGGE gels, respectively. The letters a and b indicate sequences from Ponds A and B, respectively. The numbers after the hyphen correspond to the band numbers in white boxes on the DGGE gel images in Fig. 3.

Name	Band		Closest relative				Source
	Accession no.	Similarity (%)	Accession no.	Phylogenetic affiliation	Organism	References	
EUBa-1	AB787222	98	HQ659580	<i>Actinobacteria</i>	<i>Rhodococcus</i> sp. SDB1	Ali et al. (2011)	Sawdust (Kuwait)
EUBa-2	AB787223	94	FM208180	<i>β-Proteobacteria</i>	<i>Polynucleobacter acidiphobus</i>	Hahn et al. (2011)	Rock pool from a streambed of mountain brook in Corsica (France)
EUBa-3	AB787224	93	AJ289885	<i>β-Proteobacteria</i>	<i>Limnobacter thiooxidans</i> , strain CS-K2.	Spring et al. (2001)	Sediment of a freshwater lake Chiemsee in Bavaria (Germany)
EUBa-4	AB787225	93	AY234491	<i>β-Proteobacteria</i>	<i>Bacterium</i> Ellin5074	Joseph et al. (2003)	Soil under the pasture in Ellinbank (Australia)
EUBa-5	AB787226	98	AB308368	Bacteria	<i>Bacterium</i> TG133	Tamaki et al. (2009)	Sediment from Lake Kasumigaura (Japan)
EUBa-6	AB787227	98	FJ377413	<i>α-Proteobacteria</i>	<i>Sphingobacteriales</i> bacterium Ana3	Shi et al. (2009)	
EUBa-7	AB787228	95	DQ522217	<i>Spirochaetes</i>	<i>Leptospira interrogans</i> clone PAD39J_1	Ganoza et al. (2006)	Water samples from puddles and gutters in Belen (Peru)
EUBa-8	AB787229	97	EF636196	<i>Bacteroidetes</i>	<i>Sphingobacteriales</i> bacterium TP91	Bollmann et al. (2007)	
EUBa-9	AB787230	90	JF728926	<i>Bacteroidetes</i>	<i>Chryseobacterium</i> sp. DAB_4Ecl	—	
EUBa-10	AB787231	99	AP009552	<i>Cyanobacteria</i>	<i>Microcystis aeruginosa</i> NIES-843	Kaneko et al. (2007)	
EUBa-11	AB787232	93	GQ369058	<i>Actinobacteria</i>	<i>Iamia</i> sp. T2-YC6790	—	
EUBa-12	AB787233	98	FJ763789	<i>Cyanobacteria</i>	<i>Synechococcus</i> sp. BE08071	Jasser et al. (2011)	Water from Mazurian lake (Poland)
EUBb-1	AB787234	96	AB369258	<i>α-Proteobacteria</i>	<i>Roseomonas stagni</i>	Furuhata et al. (2008)	Pond water (Japan)
EUBb-2	AB787235	98	CP002859	<i>Bacteroidetes</i>	<i>Runella slithyformis</i> DSM 19594	—	
EUBb-3	AB787236	99	EU770258	<i>α-Proteobacteria</i>	<i>Alpha</i> proteobacterium A4	—	
EUBb-4	AB787237	99	GQ921957	<i>α-Proteobacteria</i>	<i>Ancylobacter</i> polymorphus strain T10AII	Arbeli and Fuentes (2010)	Soil with atrazine histroy (Colombia)
EUBb-5	AB787238	97	AJ876403	<i>β-Proteobacteria</i>	<i>Polynucleobacter acidiphobus</i>	Wu et al. (2006)	Surface water from Taihu Lake (China)
EUBb-6	AB787239	94	AY151251	<i>Cyanobacteria</i>	<i>Synechococcus</i> sp. MW97C4	Crosbie et al. (2003)	Water from central European subalpine lakes
ALPa-1	AB787240	99	AB098002	<i>α-Proteobacteria</i>	<i>Alpha</i> proteobacterium ANRB21-3 gene	Yamamoto et al. (2005)	
ALPa-2	AB787241	93	AY960770	<i>Verrucomicrobia</i>	<i>Bacterium</i> Ellin507	Sangwan et al. (2005)	Source
ALPa-3	AB787242	93	AB371592	<i>α-Proteobacteria</i>	<i>Methylocystis</i> sp. SD3-5	—	
ALPa-4	AB787243	91	AF150790	<i>α-Proteobacteria</i>	<i>Methylocystis</i> sp. LW5	Costello & Lidstrom (1999)	
ALPa-5	AB787244	95	FM886866	<i>α-Proteobacteria</i>	<i>Sphingomonas</i> sp. OTSz_A_294	—	Biofilm on the surface of the reed in Lake Biwa (Japan)
ALPa-6	AB787245	96	EU770264	<i>α-Proteobacteria</i>	<i>Alpha</i> proteobacterium A10	—	Soil from pasture in Ellinbank (Australia)
ALPa-7	AB787246	92	EU685337	<i>α-Proteobacteria</i>	<i>Rhodobacter</i> sp. CC-SAL-30	—	
ALPa-8	AB787247	97	EU180516	<i>α-Proteobacteria</i>	<i>Alpha</i> proteobacterium BAC153	Magic-Knezev et al. (2009)	Sediment in lake Washington in Seattle (USA)
ALPa-9	AB787248	98	AB297501	<i>α-Proteobacteria</i>	<i>Roseomonas</i> sp. K-20	Furuhata et al. (2007)	
ALPa-10	AB787249	98	HM124367	<i>α-Proteobacteria</i>	<i>Hyphomicrobium</i> sp. 16-60	—	

Table 3. Continued

Band			Closest relative			Source
Name	Accession no.	Similarity (%)	Accession no.	Phylogenetic affiliation	Organism	References
ALPb-1	AB787250	95	AF007948	<i>α-Proteobacteria</i>	<i>Rasbo bacterium</i>	Blomqvist et al. (1997)
ALPb-2	AB787251	95	DQ295210	<i>Cyanobacteria</i>	<i>Leptolyngbya</i> sp. (Leptolyngbya sp. CSC8)	Bruno et al. (2009)
ALPb-3	AB787252	100	AB074742	<i>α-Proteobacteria</i>	<i>Alpha proteobacterium</i> S-St (1)-8B	Miyake et al. (2003)
ALPb-4	AB787253	99	AB599867	<i>α-Proteobacteria</i>	<i>Alpha proteobacterium</i> HIN4	—
ALPb-5	AB787254	100	AB578881	<i>α-Proteobacteria</i>	<i>Alpha proteobacterium</i> S-18	—
CFa-1	AB787255	90	F1424814	<i>Bacteroidetes</i>	<i>Wandonia haliotis</i> strain Haldi-1	Lee et al. (2010)
CFa-2	AB787256	91	EU787448	<i>Bacteroidetes</i>	<i>Solitalea koreensis</i> strain R2A36-4	Weon et al. (2009)
CFa-3	AB787257	90	EU755021	<i>Bacteroidetes</i>	<i>Sejongia</i> sp. 5516J-09	—
CFa-4	AB787258	94	AB517714	<i>Bacteroidetes</i>	<i>Fluviicola</i> sp. NBRC 101268	—
CFa-5	AB787259	93	CP002542	<i>Bacteroidetes</i>	<i>Fluviicola taffensis</i> DSM 16823	—
CFa-6	AB787260	91	DQ660382	<i>Bacteroidetes</i>	<i>Brumimicrobium mesophilum</i> strain YH207	Yang et al. (2013)
CFb-1	AB787261	94	AB517714	<i>Bacteroidetes</i>	<i>Fluviicola</i> sp. NBRC 101268	—
CFb-2	AB787262	93	AJ229217	Unidentified	Unidentified eubacterium (clone BSV73)	Hengstmann et al. (1999)
CFb-3	AB787263	90	AY918928	<i>Bacteroidetes</i>	<i>Prolixibacter bellariivorans</i> strain F2	Holmes et al. (2007)
CFb-4	AB787264	91	EU787448	<i>Bacteroidetes</i>	<i>Solitalea koreensis</i> strain R2A36-4	Weon et al. (2009)

OTUs were detected in the EUB-DGGE, ALP-DGGE, and CF-DGGE, respectively. For the ALP-DGGE, the greatest number of bands in Pond A was found on day 42 when *M. aeruginosa* reached its peak abundance (Hodoki et al. 2011). The sequences of prominent DGGE bands were determined, and 43 bands detected by DGGE were identified (Table 3). They were mainly affiliated with α -, β -, and γ -proteobacteria, *Bacteroidetes*, and *Actinobacteria*. *Verrucomicrobia* were detected from the day 42 to day 49 (Fig. 3C, band 2). *Sphingomonadales* were detected from day 35 to day 91 in Pond A. Band 6 in the EUB-DGGE Pond A was identified as a *Sphingomonas* sp. belonging to the α -proteobacteria, and was detected from the day 35 to the day 91 on the gel (Fig. 3A, band 6), when abundance of *M. aeruginosa* was the highest (Hodoki et al. 2011). Similarly, in the ALP-DGGE Pond A, another *Sphingomonas* band was detected from day 42 to day 91 (Fig. 3C, band 5). From the CF-cluster, *Sphingobacteria* bands were detected in both the EUB-DGGE and the CF-DGGE gels (Fig. 3A, band 8 and Fig. 3E, band 2; Table 3).

Previous studies have revealed that succession of the phytoplankton community is one of the most important factors affecting changes in the phylogenetic composition of bacteria (Grossart et al. 2005, Šimek et al. 2008, Niu et al. 2011). We found no significant relationships between the abundances of dominant phytoplankton taxa and the abundances of bacterial phylogenetic groups. The only significant relationship we found was between the abundance of Chlorophyceae algae and the number of α -proteobacteria OTUs ($p < 0.001$). Several laboratory experiments have revealed interactions between Chlorophyceae algae and planktonic (Jasti et al. 2005), epiphytic (Tujula et al. 2010, Burke et al. 2011), and endosymbiotic α -proteobacteria (Meusnier et al. 2001). In the present study, the genus *Cosmarium* dominated the Chlorophyceae algae. *Cosmarium* species excrete extracellular polymeric substances (Kiemle et al. 2007), and organic matter derived from phytoplankton is an important food source for bacteria (van Hannen et al. 1999, Kirkwood et al. 2006). Organic matter released from *Cosmarium* was probably available for the bacteria in the present study. High biodiversity is known to be found in environments with high productivity, although controversy remains over the relationship between biodiversity and productivity in different ecosystems (Loreau et al. 2001). For freshwater bacteria, Logue et al. (2012) found a positive correlation between bacterial OTU richness and nutrient availability in 14 Swedish lakes. Thus, bacterial biodiversity likely becomes high when the supply of nutrients and/or organic matter is favorable to bacteria. Likewise, the significant relationship between the number of species (OTUs) of α -proteobacteria and *Cosmarium* in the present study might have been due to the growth of the bacteria on the organic matter derived from the algae. It is known that DGGE can not detect a population when its abundance is less than approximately 1% of the total abundance (Muyzer et al. 1993). Addition-

ally, in this study, we did not sequence all bands. Therefore, we need attention that we can not list accurately that all bacterial were present.

In this study, we assumed that all DGGE bands at the same vertical position in a gel had identical sequences (Riemann et al. 1999). As all sequences were compared with the GenBank database using BLAST, we assumed that the closest bacterial strain matches were positive identification. Notably, band 6 in the EUB-DGGE was detected from day 35 to day 66 in Pond A (Fig. 3A) during the wax and wane of *M. aeruginosa* (Fig. 1). The band was identified as a *Sphingomonas* sp. (Fig. 3A and Table 3), a member of the α -proteobacteria known to decompose microcystin (Shi et al. 2009). We also detected another sequences related to microcystin-decomposing bacteria during this period: ALPa-5 (Fig. 3A, Table 3). With regard to other bacterial groups, high abundances of *Verrucomicrobia* bacteria have been reported to be detected during *Microcystis* blooms (Riemann & Winding 2001, Eiler & Bertilsson 2004, Kolmonen et al. 2004). Its abundance increased during the *Microcystis* bloom in Pond A (Fig. 2A, C), although the abundance of this group was minor in the bacterial community in Pond B (Fig. 2B). Additionally, we identified *Verrucomicrobia* sequences (Fig. 3C, band 2 and Table 3) from day 18 to day 91 on DGGE gels when *M. aeruginosa* abundance increased, and this was also confirmed by CARD-FISH (Fig. 2A). From these changes in bacterial communities, we have demonstrated that succession of the phytoplankton community is one of the most important factors affecting changes in the phylogenetic composition of bacteria, as previously indicated by Grossart et al. 2005, Šimek et al. 2008, and Niu et al. 2011.

To our knowledge, the present study is the first to examine changes in bacterial abundance and composition using multiple molecular methods during the wax and wane of dominant phytoplankton groups. CARD-FISH and PCR-DGGE allowed us to collect information about the bacterial phylogenetic composition and dominant bacterial taxa, respectively. However, we still only have limited information about bacterial community structure in relation to phytoplankton blooms. Further studies are needed to collect information on bacterial community structures during the succession of dominant phytoplankton and to identify drivers affecting changes in dominant bacterial phylogenetic groups.

Acknowledgments

We sincerely thank Prof. Fereidoun Rassoulzadegan for kind perusal and constructive comments on our manuscript. This research was partly supported by the Environment Research and Technology Development Fund (D-0905) of the Ministry of the Environment, Japan, and the Japan Science and Technology Strategic International Research Cooperative Program (Japan–China) on Science and Technology for Environmental Conservation and Con-

struction of a Society with Less Environmental Burden "Fate of dissolved organic matter in lakes with special reference to loading and pollution" to S.N.

References

- Ali N, Eliyas M, Al-Sarawi H, Radwan SS (2011) Hydrocarbon-utilizing microorganisms naturally associated with sawdust. *Chemosphere* 83: 1268–1272.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215: 403–410.
- Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59: 143–169.
- Arbeli Z, Fuentes C (2010) Prevalence of the gene *trzN* and biogeographic patterns among atrazine-degrading bacteria isolated from 13 Colombian agricultural soils. *FEMS Microbiol Ecol* 73: 611–623.
- Blomqvist G, Wesslen L, Pahlson C, Hjelm E, Pettersson B, Nikkila T, Allard U, Svensson O, Uhlen M, Morein B, Friman G (1997) Phylogenetic placement and characterization of a new alpha-2 proteobacterium isolated from a patient with sepsis. *J Clin Microbiol* 35: 1988–1995.
- Bollmann A, Lewis K, Epstein SS (2007) Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates. *Appl Environ Microbiol* 73: 6386–6390.
- Bruno L, Billi D, Bellezza S, Albertano P (2009) Cytomorphological and genetic characterization of troglitic *Leptolyngbya* strains isolated from Roman hypogea. *Appl Environ Microbiol* 75: 608–617.
- Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S (2011) Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. *ISME J* 5: 590–600.
- Castle D, Kirchman DL (2004) Composition of estuarine bacterial communities assessed by denaturing gradient gel electrophoresis and fluorescence in situ hybridization. *Limnol Oceanogr* 2: 303–314.
- Corner JB, Biddanda BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. *Ecosyst* 5: 105–121.
- Costello AM, Lidstrom ME (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microbiol* 65: 5066–5074.
- Crosbie ND, Pockl M, Weisse T (2003) Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16S rRNA gene and *cpcBA*-intergenic spacer sequence analyses. *Appl Environ Microbiol* 69: 5716–5721.
- Daims H, Brühl A, Amann R, Schleifer KH, Wagner M (1999) The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. *Syst Appl Microbiol* 22: 434–444.
- Dedysh SN, Pankratov TA, Belova SE, Kulichevskaya IS, Liesack W (2006) Phylogenetic analysis and in situ identification of bacteria community composition in an acidic *Sphagnum* peat bog. *Appl Environ Microbiol* 72: 2110–2117.
- Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. *Environ Microbiol* 6: 1228–1243.
- Furuhata K, Goto K, Kato Y, Saitou K, Sugiyama J, Hara M, Yoshida S, Fukuyama M (2007) Characteristics of a pink-pigmented bacterium isolated from biofilm in a cooling tower in Tokyo, Japan. *Microbiol Immunol* 51: 637–641.
- Furuhata K, Miyamoto H, Goto K, Kato Y, Hara M, Fukuyama M (2008) *Roseomonas stagni* sp. nov., isolated from pond water in Japan. *J Gen Appl Microbiol* 54: 167–171.
- Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, Segura ER, Gilman RH, Gotuzzo E, Vinetz JM (2006) Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic *Leptospira*. *PLoS Med* 3: E308.
- Gich F, Schubert K, Bruns A, Hoffelner H, Overmann J (2005) Specific detection, isolation, and characterization of selected, previous uncultured members of the freshwater bacterioplankton community. *Appl Environ Microbiol* 71: 5908–5919.
- Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton composition of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl Environ Microbiol* 65: 3721–3726.
- Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. *Appl Environ Microbiol* 66: 5053–5065.
- Grossart HP, Levold F, Allgaier M, Simon M, Brinkhoff T (2005) Marine diatom species harbour distinct bacterial communities. *Environ Microbiol* 7: 860–873.
- Hahn MW, Lang E, Brandt U, Sproer C (2011) *Polynucleobacter acidiphobus* sp. nov., a representative of an abundant group of planktonic freshwater bacteria. *Int J Syst Evol Microbiol* 61: 788–794.
- Hall T (2004) BioEdit. Biological sequence alignment editor for win95/98/NT/2K/XP/7. Available at: <http://www.mbio.ncsu.edu/bioedit/bioedit.html> (accessed on 1 December 2014)
- Hengstmann U, Chin KJ, Janssen PH, Liesack W (1999) Comparative phylogenetic assignment of environmental sequences of genes encoding 16S rRNA and numerically abundant culturable bacteria from an anoxic rice paddy soil. *Appl Environ Microbiol* 65: 5050–5058.
- Hodoki Y, Ohbayashi K, Kobayashi Y, Okuda N and Nakano S (2011) Temporal variation in cyanobacteria species composition and photosynthetic activity in experimentally induced blooms. *J Plankton Res* 33: 1410–1416.
- Holmes RM, Aminot A, Kérouel R, Hooker BA, Peterson BJ (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can J Fish Aquat Sci* 56: 1801–1808.
- Holmes DE, Nevin KP, Woodard TL, Peacock AD, Lovley DR (2007) *Prolixibacter bellariivorans* gen. nov., sp. nov., a sugar-fermenting, psychrotolerant anaerobe of the phylum *Bacteroidetes*, isolated from a marine-sediment fuel cell. *Int J Syst Evol Microbiol* 57: 701–707.
- Hube AE, Heyduck-Söller B, Fischer U (2009) Phylogenetic classification of heterotrophic bacteria associated with filamentous marine cyanobacteria in culture. *Syst Appl Microbiol* 32: 256–

- 265.
- Jasser I, Krolicka A, Karnkowska-Ishikawa A (2011) A novel phylogenetic clade of picocyanobacteria from the Mazurian lakes (Poland) reflects the early ontogeny of glacial lakes. *FEMS Microbiol Ecol* 75: 89–98.
- Jasti S, Sieracki ME, Poulton NJ, Glewat MW, Rooney-Varga JN (2005) Phylogenetic diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. *Appl Environ Microbiol* 71: 3483–3494.
- Joseph SJ, Hugenholtz P, Sangwan P, Osborne CA, Janssen PH (2003) Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl Environ Microbiol* 69: 7210–7215.
- Kaneko T, Nakajima N, Okamoto S, Suzuki I, Tanabe Y, Tamaki M, Nakamura Y, Kasai F, Watanabe A, Kawashima K, et al. (2007) Complete Genomic Structure of the Bloom-forming Toxic Cyanobacterium *Microcystis aeruginosa* NIES-843. *DNA Res.* 14: 247–256.
- Kiemle SN, Domozych DS, Gretz MR (2007) The extracellular polymeric substances of desmids (Conjugatophyceae, Streptophyta): chemistry, structural analyses and implications in wetland biofilms. *Phycologia* 46: 617–627.
- Kirkwood AE, Nalewajko C, Fulthorpe RR (2006) The effects of cyanobacterial exudates on bacterial growth and biodegradation of organic contaminants. *Microb Ecol* 51: 4–12.
- Kolmonen E, Sivonen K, Rapala J, Haukka K (2004) Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas, Finland. *Aquat Microb Ecol* 36: 201–211.
- Lane DJ (1991) 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematics* (eds Stackebrandt E, Goodfellow M). John Wiley, Chichester, United Kingdom, pp. 115–175.
- Lee DH, Choi EK, Moon SR, Ahn S, Lee YS, Jung JS, Jeon CO, Whang KS, Kahng HY (2010) *Wandonia haliotis* gen. nov., sp. nov., a marine bacterium of the family Cryomorphaceae, phylum Bacteroidetes. *Int J Syst Evol Microbiol* 60: 510–514.
- Logue, JB, Langenheder S, Andersson AF, Bertilsson S, Drakare S, Lanzén A, Lindström ES (2012) Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species-area relationships. *ISME J* 6: 1127–1136.
- Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* 294: 804–808.
- Magic-Knezev A, Wullings B, Van der Kooij D (2009) *Polaromonas* and *Hydrogenophaga* species are the predominant bacteria cultured from granular activated carbon filters in water treatment. *J Appl Microbiol* 107: 1457–1467.
- Manz W, Amann R, Ludwig W, Wagner M, Schleifer KH (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: problems and solutions. *Syst Appl Microbiol* 15: 593–600.
- Manz W, Amann R, Ludwig W, Vancanneyt M, Schleifer KH (1996) Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* 142: 1097–1106.
- Meusnier I, Olsen JI, Stam WT, Destombe C, Valero M (2001) Phylogenetic analyses of *Caulerpa taxifolia* (Chlorophyta) and of its associated bacterial microflora provide clues to the origin of the Mediterranean introduction. *Mol Ecol* 10: 931–946.
- Miyake D, Kasahara Y, Morisaki H (2003) Distribution and characterization of antibiotic resistant bacteria in the sediment of southern basin of Lake Biwa. *Microbes Environ* 18: 24–31.
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8: 4321–4325.
- Muyzer G, De Waal ED, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified gene coding for 16S rRNA. *Appl Environ Microbiol* 59: 695–700.
- Muyzer G, Hottentrager S, Teske A, Waver C (1995) Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA—a new molecular approach to analyze the genetic diversity of mixed microbial communities. In: *Molecular Microbial Ecology Manual*, 2nd ed (eds Akkermans ADL, van Elsas JD, de Bruijn FJ). Kluwer, Dordrecht, pp. 3.4.4.1–3.4.4.22.
- Muyzer G, Brinkhoff T, Nübel U, Santegoeds C, Schäfer H, Waver C (2004) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In: *Molecular microbial ecology manual*, 2nd ed (eds Kowalchuk GA, de Bruijn FJ, Head IM, Akkermans ADL, van Elsas JD). Kluwer Academic Publishers, Dordrecht, pp. 743–770.
- Neef A (1997) Anwendung der *in situ*-Einzelzell-Identifizierung von Bakterien zur Populationsanalyse in komplexen mikrobiellen Biozönosen. Ph. D. thesis. Technical University, Munich, Germany.
- Niu Y, Shen H, Chen J, Xie P, Yang X, Tao M, Ma Z, Qi M (2011) Phytoplankton community succession shaping bacterial community composition in Lake Taihu, China. *Water Res* 45: 4168–4182.
- Okazaki Y, Hodoki Y, Nakano S (2013) Seasonal dominance of CL500–11 bacterioplankton (phylum *Chloroflexi*) in the oxygenated hypolimnion of Lake Biwa, Japan. *FEMS Microbiol Ecol* 83: 82–92.
- Pernthaler A, Pernthaler J, Amann R (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Appl Environ Microbiol* 68: 3094–3101.
- Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol Ò, Malits A, Marrasé C (2004) Changes in bacterioplankton composition under different phytoplankton regimens. *Appl Environ Microbiol* 70: 6753–6766.
- Riemann L, Steward GF, Fandino LB, Campbell L, Landry MR, Azam F (1999) Bacterial community composition during two consecutive NE monsoon periods in the Arabian sea studies by denaturing gradient gel electrophoresis (DGGE) of rRNA gene. *Deep Sea Res Pt II* 46: 1791–1811.
- Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* 66: 578–587.
- Riemann L, Winding A (2001) Community dynamics of free-living and particle-associated bacterial assemblages during a

- freshwater phytoplankton bloom. *Microb Ecol* 42: 274–285.
- Rooney-Varga JN, Giewat MW, Savin MC, Sood S, LeGresley M, Martin JL (2005) Links between phytoplankton and bacterial community dynamics in a coastal marine environment. *Microb Ecol* 49: 163–175.
- Sangwan P, Kovac S, Davis KE, Sait M, Janssen PH (2005) Detection and cultivation of soil *Verrucomicrobia*. *Appl Environ Microbiol* 71: 8402–8410.
- Schönhuber W, Fuchs B, Juretschko S, Amann R (1997) Improved sensitivity of whole-cell hybridization by the combination of horseradish peroxidase-labeled oligonucleotides and tyramide signal amplification. *Appl Environ Microbiol* 63: 3268–3273.
- Shi L, Cai Y, Yang H, Xing P, Li P, Long L, Kong F (2009) Phylogenetic diversity and specificity of bacteria associated with *Microcystis aeruginosa* and other cyanobacteria. *J Environ Sci* 2: 1581–15.
- Šimek K, Horňák K, Jezbera J, Nedoma J, Zhachor P, Hejzlar J, Seďa J (2008) Spatio-temporal patterns of bacterioplankton production and community composition related to phytoplankton composition and protistan bacterivory in a dam reservoir. *Aquat Microb Ecol* 51: 249–262.
- Spring S, Kaempfer P and Schleifer KH (2001) *Limnobacter thiooxidans* gen. nov., sp. nov., a novel thiosulfate-oxidizing bacterium isolated from freshwater lake sediment. *Int J Syst Evol Microbiol* 51: 1463–1470.
- Tamaki H, Hanada S, Sekiguchi Y, Tanaka Y, Kamagata Y (2009) Effect of gelling agent on colony formation in solid cultivation of microbial community in lake sediment. *Environ Microbiol* 11: 1827–1834.
- Tujula NA, Crocetti GR, Burke C, Thomas T, Holmström C, Kjelleberg S (2010) Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. *The ISME J* 4: 301–311.
- van Hanne E, Mooij W, van Agterveld MP, Gons HJ, Laanbroek HJ (1999) Detritus- dependent development of the microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. *Appl Environ Microb* 65: 2478–2484.
- Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol Oceanogr* 39: 1985–1992.
- Weon HY, Kim BY, Lee CM, Hong SB, Jeon YA, Koo BS, Kwon SW (2009) *Solitalea koreensis* gen. nov., sp. nov. and the reclassification of [*Flexibacter*] *canadensis* as *Solitalea canadensis* comb. Nov. *Int J Syst Evol Microbiol* 59: 1969–1975.
- Wu QL, Hahn MW (2006) Differences in structure and dynamics of *Polynucleobacter* communities in a temperate and a subtropical lake, revealed at three phylogenetic levels. *FEMS Microbiol Ecol* 57: 67–79.
- Yamamoto M, Murai H, Takeda A, Okunishi S, Morisaki H (2005) Bacterial flora of the biofilm formed on the submerged surface of the reed *Phragmites australis*. *Microbes Environ* 20: 14–24.
- Yang SH, Seo HS, Oh HM, Kim SJ, Lee JH, Kwon KK. (2013) *Brumimicrobium mesophilum* sp. nov., isolated from a tidal flat sediment, and emended descriptions of the genus *Brumimicrobium* and *Brumimicrobium glaciale* *Int. J. Syst Evol Microbiol* 63: 1105–1110.