Pyrosequencing reveals benthic bacteria changes responding to heavy deposition of *Microcystis* scum in lab — searching bacteria for bloom control

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Abstract. Bacteria capable of degrading cyanobacteria *Microcystis* are crucial for determining the ecological consequences of *Microcystis* blooms in freshwater lakes. Scum derived from *Microcystis* blooms tends to accumulate in bays of large lakes and then sink to the sediments where it is finally consumed by benthic bacteria. Understanding the response of benthic bacterial communities to massive *Microcystis* deposition events may help identify the bacteria best suited to *Microcystis* hydrolyzation and even bloom control. For that purpose, an experimental system was set up in which intact sediment cores were incubated in the laboratory with normal and heavy deposits of *Microcystis* detritus. Pyrosequencing was performed in order to describe a phylogenetic inventory of bacterial communities in samples taken at 0–1, 1–2 and 2–3 cm depths in incubated sediments and in original untreated sediment. A hierarchical cluster tree was constructed expose differences between sediments. Similarity percentage calculations were also performed to identify the bacterial species contributing to variation. The results of this study suggest that: (1) deposition of *Microcystis* scums exerts a strong effect on the bacterial community composition in the surface (0–1 cm) and sub-surface (1–2 cm) sediment layers; (2) bacterial community responses to *Microcystis* detritus deposition vary across vertical gradients. A list of bacteria with potential roles in *Microcystis* degradation was compiled. These findings may inform the development of future measures for *Microcystis* bloom control in lakes.

1. Introduction

*Microcystis* blooms are very commonplace in eutrophic freshwater lakes, ponds and rivers, where their effects include increased turbidity, smothering of macrophyte growth and negative impacts on important habitats for invertebrates and fish [1]. Some *Microcystis* species produce toxins like microcystin, which is potentially lethal to diverse organisms including humans [2]. In some large lakes, *Microcystis* blooms drift and accumulate in nearshore areas as dense scum layers up to 30 cm thick [3-4]. In such dense accumulations, only a small proportion of *Microcystis* cells are decomposed or consumed in the water. The majority sink into the sediments as detritus [5-6], where they are ultimately consumed by benthic bacteria causing oxygen depletion, endotoxin release and an unpleasant smell [7-8]. Elucidating the
structure of bacterial communities in these sediments and observing their response to massive depositions of detritus may improve understanding of the effects of Microcystis blooms. Furthermore, identifying bacteria capable of hydrolyzing Microcystis may inform future efforts to manage and control bloom events.

Scientists have been actively seeking bacteria capable of killing phytoplankton or degrading phytoplankton-derived detritus efficiently. These bacteria sometimes increase in abundance as algal blooms decline, and may play a significant role in bloom dynamics [9]. Previous studies have identified a number of pelagic bacteria important in Microcystis degradation [4, 10-13], including several species with proven algicidal properties and applicability in the management of blooms to avoid bloom causing nuisance issues [14-16].

In sediment, as in most ecosystems, in situ organic resources are generally exploited by relatively few species of abundant microbes; the remaining species lie dormant until roused by favourable environmental conditions [17-19]. It was hypothesized that massive inputs of fresh organic matter in the form of Microcystis detritus may trigger an explosion of activity and growth in otherwise cryptic sediment microbes, some of which may be Microcystis hydrolyzation specialists.

An experimental system was devised in which intact sediment cores were incubated the laboratory with differing loads of Microcystis detritus (normal and heavy). Pyrosequencing was applied to bacterial communities sampled from these incubated sediments and also from untreated sediment in order to reveal the benthic bacteria able to take advantage of Microcystis deposition. Pyrosequencing is a DNA sequencing technique based on the detection of pyrophosphates released during DNA synthesis [20] and can offer a hundred times more phylogenetic information than previous PCR-DGGE (Polymerase Chain Reaction and Denatured Gradient Gel Electrophoresis) methods [21]. The twin objectives of this bacterial inventory were to determine how sediment bacterial community structure changes in response to the deposition of cyanobacterial scum across vertical gradients and to identify bacteria potentially specialised in Microcystis degradation.

2. Materials and Methods

2.1. Study area and experimental design
When Lake Taihu (30°55′–31°32′N, 119°52′–126°36′E) is the third largest lake in China, with a surface area of ~2338 km² and an average depth of 1.9 m. The sampling site lies in Meiliang Bay in the northern part of the lake (31°28′39″, 120°12′35″) in Wuxi, an area characterised by high densities of Microcystis species [3]. The water temperature at the time of study was 28.5 °C, and the illumination intensity at the bottom of the lake was 0.

On August 22, 2012, three intact sediment cores (inner diameter 15 cm) were sampled to a depth of 20 cm depth with 20cm overlying water and transported to the laboratory. After a 24 h acclimation and sediment stabilisation period, one core was sampled to represent the initial, untreated condition of all sediment communities (group O). The next core (group C) was supplied with 24.3 μg Microcystis detritus, representing the natural conditions of detritus deposition in Meiliang Bay. The third core (group H) was treated with 242.5 μg of Microcystis detritus to simulate heavy deposition comparable to that previously recorded after a severe bloom event in Lake Taihu with thick accumulations of scum [11]. All three chambers were incubated in the dark at 28.5 °C for 5 days. The cores were subsampled at depths of 0–1, 1–2 and 2–3 cm, pH tested and then freeze-dried and stored at -80 °C for later analysis of DNA.

2.2. DNA extraction, 16Sr RNA gene construction and pyrosequencing
Total microbial community DNA was extracted from approximately 1 g of material per subsample using the MoBio UltraClean Soil DNA isolation kit (MoBio Laboratories, USA) and following the manufacturer’s protocol. DNA was used as templates for library construction. DNA concentrations were quantified using a NanoDrop spectrophotometer (NanoDrop Technologies Inc., DE). V3–V5 regions of the 16S rRNA gene (Escherichia coli positions 357 to 926) are targeted using barcoded primers.
Sequences of the partial 16S rRNA genes were determined using a GS-FLX 454 sequencer (Roche). Raw sequence reads were retrieved from all sediment samples and processed with the PyroNoise algorithm to remove 454 sequencing errors. Sequences were analyzed for the presence of chimeras using the UChime algorithm on the Mothur platform. Pairwise distances between aligned sequences were calculated at a 97% similarity (or 0.03 distance) cutoff and were then clustered into unique operational taxonomic units (OTUs) in Mothur. Taxonomic assignment was performed by the RDP classifier, and Fastq files containing sequences were submitted to the NCBI Sequence Read Archive (SRX 1462880). A Bray–Curtis dissimilarity matrix was calculated using PRIMER v5.0 software, and a hierarchical cluster tree was constructed at phylum level based on the resulting matrix. PRIMER v5.0 was also used to perform similarity percentage calculations (SIMPER).

3. Results and Discussion

The pyrosequencing analysis of 16S rRNA gene amplicons from nine sediment samples produced 204,034 sequence reads, with an effective read number of 124,701 after noise removal. The average sequence length was 435 nucleotides, excluding the adaptor and barcode primer sequences. A total of 8606 operational OTUs were obtained at the 97% sequence identity threshold. The number of OTUs is more than an order of magnitude greater than previously reported for benthic bacterial communities in Lake Taihu [22-23] and for other sediment bacterial groups associated with phytodetritus deposition [24], providing a much more detailed bacterial profile.

OTUs from all samples were assigned to 23 categories at phylum level, 71 at class level, 117 at order level, 217 at family level and 437 at the level of genus. At phylum level, Proteobacteria made the largest contribution to all samples, ranging from 25.96% to 45.43% of identified OTUs, with the smallest relative abundances observed in the 1–2 cm sediment layer of the high biomass group (H2). Other phyla contributing more than 1% of recorded OTUs were Bacteroidetes (average proportion of 5.58%), Actinobacteria (5.22%), Chloroflexi (2.98%), Firmicutes (1.08%) and Acidobacteria (1.04%). Proteobacteria dominated in all sediment samples and have previously been reported as the dominant group in freshwater, intertidal wetland and marine sediments [25]. Bacteroidetes, Actinobacteria, Chloroflexi, Firmicutes and Acidobacteria are also common phyla in eutrophic lake sediments [22, 26].

A hierarchical cluster tree was constructed from the phylum profiles of all samples (Figure 1). Groups O and C were clustered together by similarities in bacterial community composition at phylum level for each depth (O1 and C1, O2 and C2 and O3 and C3). The 2–3 cm subsample of the heavy deposition group (H3) also exhibited similarities with groups O and C, with H3, O3 and C3 forming a distinct cluster. The upper layers of group H (H1 and H2), however, occupy two different branches, indicating changes in the structure of the bacterial community consistent with the differences in community composition observed at phylum level.
Figure 1. Visual representation of the similarity between samples based on bacterial community composition at phylum level, including a cluster tree calculated using Bray–Curtis dissimilarity and a bar chart displaying the community composition of each sample, giving the 22 recognised taxa in all samples, with unidentified taxa included as ‘Other’. Note: O1, O2 and O3 represent samples taken at 0–1, 1–2 and 2–3 cm from the untreated sediment core; C1, C2 and C3 represent samples from 0–1, 1–2 and 2–3 cm in the control treatment; H1, H2 and H3 represent samples at 0–1, 1–2 and 2–3 cm in the treatment subject to heavy *Microcystis* deposition.

Fewer than 1% of OTUs recorded in this study contributed more than 50% of bacterial abundance in each sample. Data from H3, O3 and C3 are not shown because of their lack of variability.

The ten most dominant OTUs in group O and their variations in the treated samples are shown in Table 1. SIMPER analysis was conducted to compare species variation in sediments with heavy *Microcystis* deposition with the untreated samples and the control treatment. The OTUs that contributed most significantly to differences between bacterial communities are shown in Table 2.
phytodetritus, which constitutes an effectively inexhaustible sup
C) and heavy deposition groups (group H) at 0–1 cm and 1–2 cm. Taxa are given at the level to which they could be identified.

| Depth | Dominant OTU | Taxa | Initial Abundance | Control Abundance | Bloom Abundance | Contribution % |
|-------|--------------|------|-------------------|-------------------|-----------------|----------------|
| OTU0000 | Unidentified | 0   | 0                 | 853               | 5.01%           | 6.01%          |
| OTU0000 | Steroidobacter | 8   | 84                | 863               | 5.75%           |                |
| OTU0000 | Desulfo bacteraceae | 7   | 6                 | 559               | 3.89%           |                |
| OTU0002 | Betaproteobacteri a | 24  | 116              | 586               | 3.63%           |                |
| OTU0000 | Acidobacteria Gp17 | 3   | 4                 | 325               | 2.26%           |                |
| OTU0000 | Unidentified | 14  | 8                 | 277               | 1.87%           |                |
| OTU0006 | Comamonadaceae | 6   | 6                 | 258               | 1.80%           |                |
| OTU0010 | Clostridium III | 3   | 4                 | 213               | 1.48%           |                |
| OTU0002 | Acidobacteria Gp6 | 7   | 4                 | 1284              | 8.14%           |                |
| OTU0004 | Bradyrhizobium | 3   | 0                 | 961               | 2.36%           |                |
| OTU0005 | Unidentified | 1   | 0                 | 957               | 2.35%           |                |
| OTU0004 | Acidobacteria Gp6 | 5   | 2                 | 926               | 2.27%           |                |
| OTU0005 | Micromonosporaceae | 0   | 0                | 919               | 2.26%           |                |

The dominant OTUs from the untreated lake sediment remained active in incubated samples regardless of the level of Microcystis detritus added. This group is accustomed to daily deposition of phytodetritus, which constitutes an effectively inexhaustible supply of organic material allowing the
bacteria to remain continuously active. These components of the bacterial communities share similar traits to soil local bacteria as depicted by Fontaine et al. [27], in that they grow slowly and maintain continuous activity regardless of level of fresh organic matter input.

The bacteria dominating in group H samples (heavy deposition) contributed most to community structure variation at the sediment surface (0–1 cm layer) (Table 1, 2), with most presenting in only very small numbers (close to the detection limit) in the untreated (O) and control (C) sediments. This pattern is consistent with a microbial dormancy strategy as described by Jones and Lennon [17, 18]. The sudden superabundance of Microcystis detritus biomass allows enhanced activity and growth of these previously starving microbial populations, which expand rapidly to exploit the new substrate and result in remarkable changes in the structure of the microbial community [28-29]. In the present context, group H conditions might be expected to promote the growth of bacteria specialising in the decomposition of Microcystis, and indeed, most of the bacteria dominating this group are known to be associated with the pelagic Microcystis degradation. For example, members of the phylum Bacteroidetes can degrade biopolymers, in particular all kinds of polysaccharides [30]. The increased abundance and concentration of the unidentified bacteroidete OTU00043 may be indicative of a role in the degradation of Microcystis sp. mucilage, which consists mainly of polysaccharides [31]. The bacteria Hydrogenophaga, Acidovorax, Dechloromonas, Comamonadaceae and Clostridium have previously been reported with algicidal properties [4, 12-13, 16]. The current study is the first to indicate a role in phytodetritus degradation for the genus Hophaga.

The shifts in dominant bacteria in the subsurface (1–2 cm) sediment layer with heavy deposition were totally different to those at the surface (Table 2). Remarkable increases in abundance were observed among members of the Acidobacteria GP6 subgroup. Acidobacteria GP6 are considered to be ubiquitous and abundant in soil but are rarely cultured and thus remain poorly studied [32]. Naether et al. [33] reported that members of the Acidobacteria GP6 subgroup exhibit their highest relative abundances in soils with high nutrient levels. This being the case, the increases observed in heavily deposited sub-surface sediments may be a response to nutrient released from Microcystis detritus during degradation by surface sediment bacteria. Members of the order Rhizobiales including Bradyrhizobium, can serve as denitrifiers [34-35], and are likely to flourish in response to the release of nitrates from deeply degraded cyanobacteria detritus. The genus Microcystis comprises a number of highly competitive polyphosphate-accumulating bacteria [36] and is likely to serve in phosphate removal. Members of the Actinomycetales such as the Micromonosporaceae and Pseudonocardia sp are potentially involved in the mineralisation of refractory organic compounds [37]. Thus members of the Micromonosporaceae and Pseudonocardia sp. may benefit from the availability of some recalcitrant forms of Microcystis carbon that persist after surface sediment bacteria have assimilated the more accessible forms.

In general, the bacterial communities recorded in our sediment samples can be divided into three functional groups: local dominant bacteria, bacteria activated by massive deposition of Microcystis detritus, and dormant bacteria. Totally different groups of bacteria were activated by phytodetritus deposition at the sediment surface (0–1 cm) and sub-surface (1–2 cm) layers, resulting in increased differentiation. We suggest that the bacteria dominating in different sediment layers may reflect different processes of Microcystis degradation. Furthermore, we suspect that some of the bacteria thriving in the 1–2 cm sediment layer were not involved directly with Microcystis detritus degradation but responded to the nutrients released by other bacteria. The low levels of species variation observed in the 2–3 cm layer between treatments may indicate the exhaustion of the organic resources.

4. Conclusion
In conclusion, by setting up an experimental system with intact sediment cores incubated with different levels of Microcystis detritus and then pyrosequencing the resulting bacterial communities, we found that the deposition of Microcystis scums exerted a strong influence on community composition in the surface (0–1 cm) and sub-surface (1–2 cm) sediments. These microbial community compositions reflect the activation of bacteria adapted to exploit the organic resource of Microcystis detritus. The bacterial
community response to heavy deposition of detritus varied across vertical gradients, possibly reflecting different degradation processes. We conclude that the unidentified bacteroidete bacteria OTU00043, the *Hoplogha* sp OTU00146, *Hydrogenophaga* sp OTU00009, *Acidovorax* sp OTU00075, *Dechloromonas* sp OTU000145, *Clostridium* sp OTU00158 and the comamonadacean OTU00069 are potentially involved in the early stage of *Microcystis* degradation, and may be crucial in *Microcystis* bloom termination. Pure cultures of these bacterial strains will be isolated in order to testify their ability to hydrolyze *Microcystis* cells.

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**References**

[1] H.W. Paerl, J. Huisman, Bloom likes it hot, Science 320 (2008) 57-58.
[2] D.R. de Figueiredo, U.M. Azeteiro, S.M. Esteves, F. Goncalves, M.J. Pereira, Microcystin-producing blooms—a serious global public health issue, Ecotox. Environ. Safe. 59 (2004) 151-163.
[3] B.Q. Qin, P. Xu, Q. Wu, L. Luo, Y. Zhang, Environmental issues of Lake Taihu, China Hydrobiologia 581 (2007) 3-14.
[4] P. Xing, L. Guo, W. Tian, Q.L. Wu, Novel Clostridiun populations involved in the anaerobic degradation of Microcystis blooms, ISME. J. 5 (2011) 792-800.
[5] R.D. Fallo, T.D. Brock, Planktonic blue-green algae: production, sedimentation, and decomposition in Lake Mendota, Wisconsin, Limnol. Oceanogr. 25 (1980) 72-88.
[6] N. Takamura, M. Yasuno, Sedimentation of phytoplankton populations dominated by *Microcystis* in a shallow lake, J. Plankton. Res. 10 (1988) 283-299.
[7] L.A. Meyer-Reil. Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight, Mar. Biol. 77 (1983) 247-256.
[8] B.C. Sander, J. Klaff 1993 Factors controlling bacterial production in marine and freshwater sediments, Microb. Ecol. 26 (1993) 79-99.
[9] X. Mayali, F. Azam, Algicidal bacteria in the sea and their impact on algal blooms, J. Eukaryot. Microbiol. 51 (2004) 139-144.
[10] K. Christoffersen, S. Lyck, A. Winding Microbial activity and bacterial community structure during degradation of microcysts, Aquat. Microb. Ecol. 27 (2002) 125-136.
[11] H. Li, P. Xing, M. Chen, Y. Bian, Q.L. Wu, Short-term bacterial community composition dynamics in response to accumulation and breakdown of Microcystis blooms, Water. Res. 45 (2011) 1702-1710.
[12] H. Li, P. Xing, Q.L. Wu Characterization of the bacterial community composition in a hypoxic zone induced by Microcystis blooms in Lake Taihu, China, FEMS. Microbiol. Ecol. 79 (2012) 773-778.
[13] H. Cai, H. Jiang, L.R. Krumholz, Z. Yang, Bacterial community composition of size-fractioned aggregates within the phylosphere of cyanobacterial blooms in a eutrophic freshwater lake Plos One 9 (2014) e102879.
[14] P.M. Manage, Z. Kawabata, S.I. Nakano, Algicidal effect of the bacterium Alcaligenes denitrificans on *Microcystis* spp, Aquat. Microb. Ecol. 22 (2000) 111-117.
[15] K.K. Rashidian, D.F. Bird. Role of predatory bacteria in the termination of a cyanobacterial bloom, Microbiol. Ecol. 41(2001) 97–105.
[16] Y.-K. Kang, S.-Y. Cho, Y.-H. Kang, T. Katano, E.-S. Jin, D.-S. Kong, Isolation, identification and characterization of algicidal bacteria against *Stephanodiscus* hantzschii and *Peridinium* bipes fot the control of freshwater winter algal blooms, J. Appl. Phycol. 20 (2008) 375-386.
[17] S.E. Jones, J.T. Lennon. Dormancy contributes to the maintenance of microbial diversity, Proc. Natl. Acad. Sci. USA. 107 (2010) 5881-5886.
[18] J.T. Lennon, S.E. Jones, Microbial seed banks: the ecological and evolutionary implications of dormancy, Nat. Rev. Microbiol. 9 (2013) 119-130.

[19] M. de Nobili, M. Contin, C. Mondini, P.C. Brookes, Soil microbial biomass is triggered into activity by trace amounts of substrate, Soil. Biol. Biochem. 33 (2001) 1163-1170.

[20] M. Ronaghi, Pyrosequencing sheds light on DNA sequencing, Genome Res. 11 (2001) 3-11.

[21] P.N. Polymenakou, C.A. Christakis, M. Mandalakis, A. Oulas, Pyrosequencing analysis of microbial communities reveals dominant cosmopolitan phylotypes in deep-sea sediments of the eastern Mediterranean sea, Res. Microbiol. 166 (2015) 448-457.

[22] K. Shao, G. Gao, B. Qin, X. Tang, Y. Wang, K. Chi, J. Dai. Comparing sediment bacterial communities in the macrophyte-dominated and algae-dominated areas of eutrophic Lake Taihu, China, Can. J. Microbiol. 57 (2011) 263-272.

[23] K. Shao, G. Gao, Y. Wang, X. Tang, B. Qin, Vertical diversity of sediment bacterial communities in two different trophic states of the eutrophic Lake Taihu, China, J. Environ. Sci. 25 (2013) 1186-1194.

[24] M.A. Franco, I. de Mesel, M.D. Demba, K. van der Gucht, D. van Gansbeke, P. van Rijswijk, M.J. Costa, M. Vincx, J. Vanaverbeke, Effect of phytoplankton bloom deposition on benthic bacterial communities in two contrasting sediments in the southern North Sea, Aquat. Microb. Ecol. 48 (2007) 241-254.

[25] Y. Wang, H.F. Sheng, Y. He, J.Y. Wu, Y.X. Jiang, N.F. Tam, H.W. Zhou. Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags, Appl. Environ. Microbiol. 78 (2012) 8264-8271.

[26] H. Tamaki, Y. Sekiguchi, S. Hanada, K. Nakamura, N. Nomura, M. Matsumura, Y. Kamagata, Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques, Appl. Environ. Microbiol. 71 (2005) 2162-2169.

[27] S. Fontaine, A. Mariotti, L. Abbadie, The priming effect of organic matter: a question of microbial competition? Soil. Biol. Biochem. 35 (2003) 837-843.

[28] B. Behera, G.H. Wagner, Microbial growth rate in glucose-amended soil, Soil. Sci. Soc. Am. J. 38 (1974) 591-594.

[29] B.S. Griffiths, K. Ritz, N. Ebblewhite, G. Dobson, Soil microbial community structure: effects of substrate loading rates, Soil. Biol. Biochem. 31(1998) 145-153.

[30] J.I.Schwarz, W. Eckert, R. Conrad. Response of the methanogenic microbial community of a profundal lake sediment (Lake Kinneret, Israel) to algal deposition, Limnol. Oceanogr. 53(2008) 113-121.

[31] Y. Amemiya, K. Kato, O. Nakayama, Changes in the chemical composition of carbohydrates and proteins in surface water during a bloom of Microcystis in Lake Suwa. Ecol. Res. 5 (1990) 153-162.

[32] W.J. Sul, S. Asuming-Brempong, Q. Wang, D.M. Tourlousses, C.R. Penton, Y. Deng, J.L.M. Rodrigues, S.G.K. Adiku. Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon, Soil. Biol. Biochem. 65 (2013) 33-38.

[33] A. Naether, B.U. Foesel, V. Naegele, P.R. Wü st, J. Weinert, M. Bonkowski, F. Alt, Y. Oelmann, A. Polle, Environmental factors affect acidobacterial communities below the subgroup level in grassland and forest soils, Appl. Environ. Microbiol. 78 (2012) 7398-7406.

[34] A.H. Treusch, S. Leininger, A. Kletzin, S.C. Schuster, H.P. Klenk, C. Schleper, Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling, Environ. Microbiol. 7 (2005) 1985-1995.

[35] C.M. Jones, B. Stres, M. Rosenquist, S. Hallin. Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification, Mol. Biol. Evol. 625 (2008) 1955-1966.

[36] K. Nakamura, A. Hiraishi, Y. Yoshimi, M. Kawaharasaki, Y. Kamagata, Microlunatus phosphovorus gen. nov. sp. nov. a new gram-positive polyphosphate-accumulating bacterium
isolated from activated sludge, Int. J. Syst. Evol. Microbiol. 45 (1995) 17-22.

[37] M.D. Chengalroyen, E.R. Dabbs, Characterization of rubber degrading isolates, J. Microbiol. 2 (2012) 872-885.