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Degradation of microcystins by water and bottom sediment bacterial communities from a eutrophic freshwater lake

Aleksandra Bukowska*, Tomasz Kaliński, Ryszard J. Chróst

Department of Microbial Ecology & Environmental Biotechnology, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089, Warszawa, Poland

ABSTRACT: Biodegradation is the most effective way to transform hepatotoxic microcystins produced by cyanobacteria in natural environments. An increasing number of new bacterial strains capable of microcystin degradation are being discovered, and there is still only one fully described metabolic pathway responsible for degradation, encoded by the *mlr* gene cluster. We aimed to identify microcystin-degrading bacteria in assemblages from water and bottom sediment of the eutrophic freshwater Lake Mikołajskie, Poland. Bacteria from water and sediment differed in taxonomic composition, and only in the community from sediment was the *mlr*A gene detected. In the presence of microcystins, bacteria from the sediment showed higher proteolytic activity, while in the water community, there was no change in the activity of proteases. Bacterial assemblages from both environments also showed different time profiles of microcystin utilization. All these results indicate that other metabolic pathways may be involved in the degradation of microcystins by bacteria living in the water and bottom sediment. Identified microcystin-degrading and potentially microcystin-degrading bacteria belong to *Betaproteobacteria* (genera: *Hydrogenophaga*, *Comamonas*, *Rhodoferax*) and *Gammaproteobacteria* (genera: *Acinetobacter*, *Citrobacter*, *Rahnella*, *Serratia*, *Pseudomonas*, *Stenotrophomonas*), according to denaturing gradient gel electrophoresis analysis, as well as isolation and testing of the properties of individual strains. No bacteria belonging to family *Sphingomonadaceae* (*Alphaproteobacteria*) were identified, which, until recently, were considered to be the main microcystin degraders. Several bacterial taxa detected in this study have never been previously described as potential microcystin degraders: *Comamonas* sp., *Hydrogenophaga* sp., *Rhodoferax* sp., *Citrobacter* sp., and *Serratia* sp.

KEY WORDS: Microcystins · Biodegradation · *Betaproteobacteria* · *Gammaproteobacteria* · Degradation pathway · *mlr*A gene

INTRODUCTION

In recent decades, toxic cyanobacterial blooms have become an increasingly important global environmental problem (Paerl & Otten 2013). Microcystins (MCs), belonging to hepatotoxins, are the most common cyanobacterial toxins in freshwater ecosystems and are produced by a wide spectrum of cyanobacteria taxa (Christiansen et al. 2003, Metcalf & Codd 2004). MCs are cyclic heptapeptides, containing 5 constant non-protein amino acids and 2 variable protein amino acids in their structure (Christiansen et al. 2003). Over 90 different variants of MCs have been identified (Pearson et al. 2010). Due to their cyclic structure and possession of a few very rare amino acids, MCs are extremely resistant to degradation (Manage et al. 2010). In the natural environment, MCs are degraded in 2 ways: through

*Corresponding author: a.bukowska@biol.uw.edu.pl

UV photolysis and microbial decomposition. However, solar radiation is largely ineffective because MCs are degraded mainly under UV-C radiation, which is very limited in the sunlight spectrum (Tsuji et al. 1995). The most important way to remove MCs from natural ecosystems is biodegradation (Christoffersen et al. 2002, Ho et al. 2007a, Chen et al. 2008). Bacteria capable of MC degradation were found in both lake water and bottom sediments (Li et al. 1017). There are studies showing that this process is faster in the sediments (Rapala et al. 1994, Christoffersen et al. 2002, Holst et al. 2003). The first bacterium with a proven capability to decompose MCs was a *Sphingomonas* sp. ACM-3962 strain (Bourne et al. 1996, 2001). Thereafter, other bacteria from the *Sphingomonadaceae* family (*Alphaproteobacteria*), also possessing degradative activity, were described (Park et al. 2001, Ishii et al. 2004, Ho et al. 2007b, Okano et al. 2009, Jiang et al. 2011). These bacteria degrade MCs via the pathway described by Bourne et al. (2001). Detailed descriptions of the degradation products and the characteristics of the enzymes involved were reported later (Hashimoto et al. 2009, Dziga et al. 2012, 2016). To date, this is the only fully described pathway for degradation of MCs. Genetically, a cluster of 4 *mlr* (*mlr*ABCD) genes is responsible for the degradation process. Numerous studies indicate the existence of microorganisms other than the *Sphingomonadaceae* family that can degrade MCs, belonging to *Alphaproteobacteria* (Mu et al. 2009, Jing et al. 2014, Zhu et al. 2016), *Betaproteobacteria* (Yan et al. 2004, Rapala et al. 2005, Lemes et al. 2008), *Gammaproteobacteria* (Takenaka & Watanabe 1997, Chen et al. 2010a, Li & Pan 2014, Mankiewicz-Boczek et al. 2015, Yang et al. 2018), *Actinobacteria* (Manage et al. 2009, Eleuterio & Batista 2010, Ramani et al. 2012) and *Bacilli* (Nybom et al. 2007, Alamri 2010). New metagenomic studies indicate that degradation activity is much more widespread among bacteria than was previously thought. Mou et al. (2013) found that in one lake ecosystem, MC decomposition ability can be exhibited by as many as 89 different bacterial orders, belonging to *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria* and *Verrucomicrobia*, of which the *Methylophilales* and *Burkholderiales* orders may be the most important MC degraders. Most of the newly described MC-degrading bacteria species do not have the *mlr* genes, so they carry out the degradation via different metabolic pathways. One alternative pathway may involve xenobiotic metabolism because, in this process, related genes were over-represented in MC-degrading metagenomes (Mou et al. 2013). However, there may also be other, currently unrecognized pathways of MC degradation.

Environmental bacteria capable of MC degradation are intensively studied because of their high biotechnological potential for the purification of water bodies contaminated with cyanotoxins, and drinking water resources are of utmost importance. The present study concerns the abundance, as well as the metabolic and taxonomic characteristics, of MC-degrading bacteria in water and sediments of Lake Mikołajskie, which is part of the Great Mazurian Lakes system in northeastern Poland, where toxic cyanobacteria and MCs have been previously reported (Bukowska et al. 2014, 2017).

MATERIALS AND METHODS

Sample collection

Water and sediment samples were collected in September 2015 from Lake Mikołajskie, one of the Great Mazurian Lakes located in northeastern Poland. It is a eutrophic lake and is characterized by a variety of cyanobacteria species, some of which, such as *Planktothrix* spp. and *Microcystis* spp., can produce MCs (Bukowska et al. 2014, 2017). Water samples were taken at 0.5 m depth intervals from the epilimnion water column and integrated in 1 container. Sediment samples were taken from the surface layer of sediment (0 to 5 cm) at a depth of 22 m.

Microcosm incubation

Lake water was gravity filtered through 3.0 µm pore-size polycarbonate filters (Nuclepore Whatman) to exclude bacterivores. To concentrate the number of bacteria, 4 l of filtrate was filtered again through 0.2 µm pore-size polycarbonate filters (Nuclepore Whatman), and bacteria deposited on the filters were resuspended in 1 l of water previously filtered through 0.2 µm pore-size filters (Nuclepore Whatman).

A total of 80 ml of sediment was resuspended in 1 l of lake water filtered through 0.2 µm pore-size polycarbonate filters.

Experimental variants were amended with a mixture of inorganic nitrogen and phosphorus (5 mM NH_4Cl, 5 mM $NaNO_3$, and 1 mM NaH_2PO_4, final concentrations) and preincubated for 4 d to allow the

bacteria to consume labile dissolved carbon compounds, according to the procedure described by Mou et al. (2013).

After preincubation, final experimental microcosms were set up. From the preincubated lake water, there were 2 microcosms: Water+MC and Water Control. From the preincubated sediment, there were also 2 microcosms: Sediment+MC and Sediment Control. Additionally, a control NoBacteria+MC microcosm was designed. This was lake water mixed with sediment and autoclaved to kill all living bacteria. All +MC microcosms were amended with a mixture of 10 µg l^{-1} MC-LR and 10 µg l^{-1} MC-RR (Microcystin-LR ALX-350-012-C100 and Microcystis-RR ALX-350-043-C100, Enzo Life Sciences) (final concentrations). The final volume of each microcosm was 500 ml. Microcosms were incubated for 72 h in the dark at the temperature of the epilimnion water (17°C). They were agitated by shaking by hand every 12 h.

MC concentration

MC concentrations were measured at the beginning (0 h) and after 72 h of incubation. Samples were taken from Water+MC, Sediment+MC and NoBacteria+MC microcosms. Collected samples were filtered through 0.2 µm pore-size filters, and the MC concentration was measured using a Microcystins ADDA specific ELISA kit (Enzo Life Sciences), according to the manufacturer's instructions. Absorbance on test plates was read using Synergy H1 microplate reader with Gen5 software (BioTek Instruments). All samples were measured in duplicate.

Total bacterial number

The number of bacteria was counted after 0, 24, 48 and 72 h by the DAPI method (Porter & Feig 1980) in the Water+MC, Water Control, Sediment+MC and Sediment Control microcosms. Aliquots of 0.1 to 1 ml of sample were incubated with DAPI stain (final concentration: 1 µg ml^{-1}) in the dark for 15 min and then settled on 0.2 µm pore-size black polycarbonate filters (Isopore Membrane Filter GTBP02500, Merck Millipore). Bacteria were counted in 10 randomly selected fields per filter using a Nikon Eclipse E400 epifluorescent microscope at 1000× magnification and NIS-Elements microscope imaging software (Nikon Instruments). All samples were analyzed in triplicate.

DNA extraction from microcosms

Samples of 50 ml were collected from the Water+MC, Water Control, Sediment+MC and Sediment Control microcosms every 24 h (0, 24, 48 and 72 h). Samples were filtered onto 47 mm diameter, 0.2 µm pore-size membrane filters (Nuclepore). Single filters were placed in 1.5 ml Eppendorf-type sterile tubes and frozen at –30°C until further analyses. Then, DNA was extracted from filters using a GeneMATRIX Soil DNA Purification Kit (EURx), according to the manufacturer's instructions.

DGGE-16S rRNA gene analysis

Denaturing gradient gel electrophoresis (DGGE) analysis was performed to monitor changes in the structure of the bacterial community in microcosms amended with MC. Samples from Water+MC and Sediment+MC were collected every 24 h (0, 24, 48, and 72 h). A primer pair (GC)Eub388F 5′-AC TCC TAC GGG AGG CAG CAG-3′ and 907R 5′-CCG TCA ATT CMT TTG AGT TT-3′ (Lane 1991, Konopka et al. 1999), targeting the 16S rRNA gene fragment was used. The specificity of both primers for the *Bacteria* domain was confirmed by the Ribosomal Database Project's Probe Match tool (http://rdp.cme.msu.edu/probematch/search.jsp). A 40 nucleotide GC clamp 5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C-3′), which prevents the complete separation of 2 DNA strands in DGGE, was added to the 5′ end of the forward primer. PCR reactions were performed in a Mastercycler Epgradient S thermocycler (Eppendorf), using the Taq PCR Core Kit (Qiagen) in a volume of 25 µl. The reaction mix (25 µl) contained 1 µl (~20 ng) of template DNA, 0.4 mM of each primer, 0.5 U Taq polymerase, 0.2 mM dNTPs, 4 mM $MgCl_2$, 1× reaction buffer, 1× Q solution and deionized water. PCR program (30 cycles) was as follows: denaturation for 30 s at 95°C, annealing for 45 s at 53°C, and 1 min elongation at 72°C. An initial step of denaturation at 95°C for 5 min and a final step of 10 min extension at 72°C were also included.

DGGE was performed on 1 mm thick vertical 7% (w/v) polyacrylamide (acrylamide/bisacrylamide, ratio 37.5:1.0) gels, with the linear gradient of denaturants in the gel increasing from 20% to 60% (100% denaturant defined as 7 M urea and 40% [v/v] formamide). Electrophoresis was carried out in a DCode™ Universal Mutation Detection System (BioRad) in a TAE buffer at a constant temperature of 60°C initially at 200 V for 5 min, followed by 16 h at 50 V. Gels

were stained with a mixture of 14 ml of 10 000 × SYBR Green (S9430, Sigma-Aldrich) and 200 ml of deionized water for 15 min, viewed under UV light, and photographed. Selected bands were excised from the gel with a sterile scalpel, placed in 40 µl of sterile deionized water, and incubated at 4°C for 24 h. The eluent was used as a template for reamplification with the same primer pair and PCR program. Then, the reamplified samples were Sanger-sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions.

Amplification of *mlr*A gene

The presence of *mlr*A gene, which encodes the first step of the only completely described MC degradation pathway, was analyzed by PCR, at the beginning and at the end of microcosm incubation, using primer pair: MF 5′-GAC CCG ATG TTC AAG ATA CT-3′ and MR 5′-CT CCT CCC ACA AAT CAG GAC-3′ (Saito et al. 2003, Chen et al. 2010b). Amplification was run in a Mastercycler Epgradient S thermocycler (Eppendorf), using the Taq PCR Core Kit (Qiagen). The reaction mix (25 µl) contained 1 µl (~20 ng) of template DNA, 0.4 mM of each primer, 0.5 U Taq polymerase, 0.2 mM dNTPs, 4 mM $MgCl_2$, 1× reaction buffer, 1× Q solution and deionized water. The PCR program included 35 cycles of denaturation at 95°C for 20 s, annealing at 50°C for 10 s, and elongation at 72°C for 30 s. An initial step of denaturation at 95°C for 5 min and a final step of 10 min extension at 72°C were also included.

Community-level physiological profiling

The capability of microbial communities in microcosms to utilize a variety of carbon sources was analyzed using the EcoPlates™ method (Biolog). Each plate contained 31 different carbon sources, all in triplicate (Preston-Mafham et al. 2002). The EcoPlates™ method allows comparison of the functional diversity of microbial communities in environmental samples (Gryta et al. 2014). Originally, there were also 3 blank wells, containing only a water sample, which were replaced by the addition of MC-LR and MC-RR in a final concentration of 20 µg l^{-1} in this analysis. Each well also contained tetrazolium redox dye as an indicator of substrate oxidation. Samples were collected from the Water and Sediment variants after the 4 d preincubation period and were inoculated on test plates (150 µl in each well). Absorbance was measured every ~9 h, for 54 h in total, at a wavelength of 590 nm using a Synergy H1 microplate reader with Gen5 software (BioTek Instruments).

The 31 carbon sources on the Biolog EcoPlates were assigned to 6 categories (carbohydrates, amino acids, carboxylic acids, phenolic compounds, polymers, and amines, according to Feigl et al. 2017); additionally, MCs were treated as a seventh category. The carbon source with the smallest absorbance increase on the EcoPlate was treated as a negative control. Average well color development (AWCD) was calculated by subtracting the initial absorbance from the absorbance value after 40 h of incubation for each well and then averaged for the whole plate or for each carbon source category (Garland & Mills 1991). The Shannon index (H) was calculated according to Gryta et al. (2014): $H = -\Sigma Pi$ (ln Pi), where Pi is the proportional color development of the well over the total color development of all wells of a plate. The substrate richness (R) means the number of utilized substrates and was calculated as the sum of wells on the EcoPlate where the absorbance increase was >0.1 after 40 h of incubation. The lag time period for the carbon source means the time after which the absorbance started to increase and was calculated in the Gen5 software of the Synergy H1 microplate reader.

Proteolytic activity

To detect protease activity, we used the EnzChek Protease Assay Kit (Thermo Fisher Scientific), applying the procedure proposed by the manufacturer. Incubations were performed at room temperature (22°C) for 17 h on black 96 well plates (30296, SPL Life Sciences). Fluorescence (excitation 485 nm/emission 528 nm) was read using a Synergy H1 microplate reader. Samples from all 5 microcosms were collected after 24 h of incubation. Additionally, distilled water was used as a negative control. All samples were analyzed in triplicate. Protease activity was expressed as fluorescence change per unit sample because samples could contain unknown proteases, and therefore, it was not possible to prepare the relevant standard curve.

Isolation of MC-degrading bacterial strains

To isolate bacterial strains capable of MC degradation, samples were collected from microcosms

showing degradation activity after the 72 h incubation period. Isolation was carried out in 2 ways. The first method allowed the identification of potentially MC-degrading strains. To begin, 100 µl of the samples was transferred onto Petri dishes with M9 minimal salts medium (6.78 g l^{-1} Na_2HPO_4, 3 g l^{-1} KH_2PO_4, 1 g l^{-1} NH_4Cl, and 0.5 g l^{-1} NaCl) with 1.5% agar (w/v), amended with 20 µg l^{-1} (final concentration) of MCs (MC-LR and MC-RR) as a carbon source. Plates were incubated for 7 d at 22°C in the dark. Single colonies were individually transferred into liquid LB medium (L3022, Sigma-Aldrich) and incubated for 2 d at 22°C in the dark. Then, 2 ml of the bacterial liquid culture was centrifuged (5000 rpm [2800 × *g*] for 3 min), and the pellets were used for DNA isolation.

In the second method of strain isolation, 100 µl of the samples from the microcosms was transferred onto Petri dishes with LB agar medium (L2897, Sigma-Aldrich) to obtain a pool of cultivable bacteria. Plates were incubated for 2 d at 22°C in the dark. To obtain pure bacterial strains, colonies were streaked onto other LB agar plates and incubated again for 2 d at 22°C in the dark. Single colonies were individually transferred into liquid LB medium (L3022, Sigma-Aldrich) amended with 20 µg l^{-1} (final concentration) of MCs and into the lake water autoclaved and filtered through 0.2 µm pore-size polycarbonate filters (Nuclepore Whatman) and also amended with 20 µg l^{-1} (final concentration) of MCs. Bacteria were incubated for 8 d at 22°C in the dark, and the MC concentration was measured at the beginning and at the end of the experiment using a Microcystins ADDA specific ELISA kit (Enzo Life Sciences). Negative controls composed only of LB medium or sterile 0.2 µm pore-size filtered lake water, both amended with MCs, were also included. All strains as well as controls were incubated in triplicate. Strains showing MC degradation activity were selected, 2 ml of their cultures was centrifuged at 5000 rpm (2800 × *g*) for 3 min, and the pellets were used for DNA isolation.

DNA from bacterial cultures were isolated using GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit (EURx). Amplification of 16S rRNA gene fragment was performed using the same primer pair, but without the GC clamp in the case of forward primer, the same cycling conditions, and the same reaction mix as in the DGGE analysis. The amplification products were Sanger-sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions.

Phylogenetic analysis

Homology searches for 16S rRNA gene sequences obtained in DGGE analysis and from isolated MC-degrading and potentially MC-degrading bacteria strains were performed using BLASTN network service (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignment of the sequences was carried out using CLUSTAL W (Thompson et al. 1994) implemented in MEGA6 software (Tamura et al. 2013). The phylogenetic trees were constructed with MEGA6 software (Tamura et al. 2013) using the Maximum Likelihood method and GTR model parameters with bootstrap analysis of 1000 replicates. The obtained 16S rRNA gene sequences were deposited in GenBank under the following accession numbers: MG586876 to MG586884 and MG586901 to MG586903.

Statistical analysis

The statistical analyses were performed using Microsoft Office Excel 2011, OriginLab Origin 6.1, PAST: Paleontological Statistics software package for education 3.18 and StatSoft Statistica 12 software packages. The Student's *t*-test and 1-way ANOVA were performed to determine whether there were any statistically significant relationships between the parameters of bacteria number, proteolytic activity, AWCD values in Biolog EcoPlate tests, Shannon diversity (*H*) and substrate richness (*R*). The level of significance was $\alpha < 0.05$.

RESULTS

Response of bacterial communities in microcosms to MCs

During 72 h of incubation in both the Water and Sediment microcosms with the addition of MC-LR and MC-RR, almost all MCs (94 to 96%) were degraded, while in the NoBacteria+MC microcosm, on average <10% of MCs disappeared (Fig. 1).

DGGE-16S rRNA gene profiling showed a substantial change in the structure of the bacterial community in the Water+MC microcosm during the 3 d incubation period (Fig. 2a). At the beginning, immediately after MC addition, there were 18 different bands on the denaturing gel, indicating 18 different bacterial operational taxonomic units (OTUs). The number of OTUs decreased each day: 16 after 24 h, 12 after 48 h and 10 after 72 h. There was also a

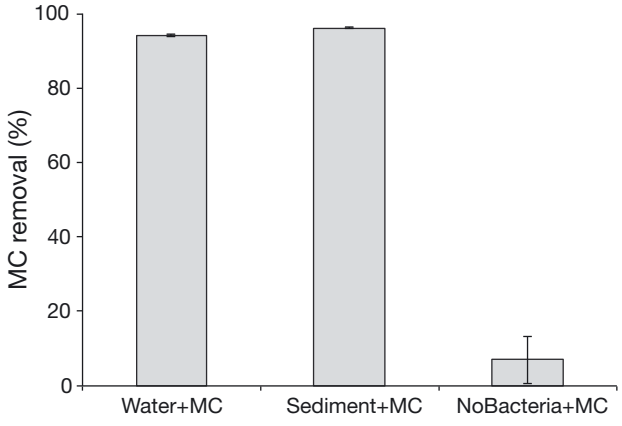

Fig. 1. Percentage removal of microcystins (MCs) in a concentration of 20 µg l^{-1} in microcosms of Water+MC, Sediment+MC and NoBacteria+MC, during 72 h of incubation. Error bars indicate standard deviation calculated from 2 measurements of each sample

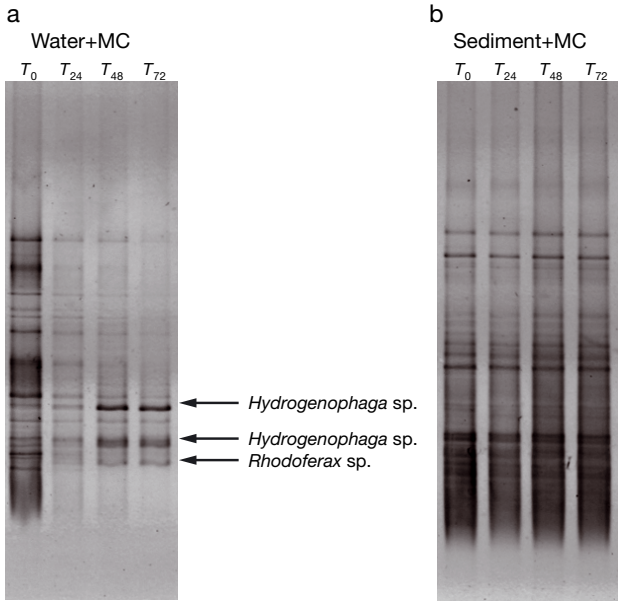

Fig. 2. Results of DGGE-16S rRNA gene profiling of bacterial communities from (a) Water+MC and (b) Sediment+MC microcosms at the beginning of incubation (T_0) and after every 24 h (T_{24}, T_{48}, and T_{72}). Arrows indicate the OTUs dominating at the end of incubation

clearly visible change in the dominating taxa during incubation with MCs. After 3 d, there were 3 dominating OTUs that were previously present, but their bands were much weaker (Fig. 2a). Sequences from dominating OTUs were assigned to *Betaproteobacteria*. W01DGGE and W02DGGE sequences showed the closest homology to genus *Hydrogenophaga* and the W03DGGE sequence to genus *Rhodoferax*. The position of these OTUs is shown on the phylogenetic tree (Fig. 3). Details of the taxonomic lineage and the GenBank accession numbers are given in Table 1.

In the case of the Sediment+MC microcosm, there was no visible change in the bacterial community structure during the 72 h of incubation. The same 19 OTUs were observed in gel profiles at 24 h intervals (Fig. 2b).

The number of bacteria during incubation was compared in microcosms with and without supplementation with MCs. In Water microcosms, the density of bacteria at the beginning of the experiment was ~8×10^6 cells ml^{-1}. There was an initial decrease in bacteria number in both variants, which continued in the microcosm without MCs to the end of incubation (2.6-fold decrease after 72 h). The number of bacteria in the Water+MC microcosm started to increase after 48 h (3.2-fold decrease at 48 h and 0.8-fold increase during the last 24 h) (Fig. 4a). The bacteria number in Sediment microcosms was initially almost 100-fold higher than that in Water microcosms. In both the Sediment+MC and Sediment Control, the bacterial density decreased throughout the whole incubation period (~3.4- to 3.8-fold decrease during 72 h) (Fig. 4b).

Presence of *mlr*A gene

The *mlr*A gene fragment was amplified in the microcosms containing sediment. The presence of this gene was confirmed in sediment after 4 d of preincubation prior to addition of MCs and after the 72 h incubation in both the Sediment+MC and Sediment Control microcosms. In contrast, in microcosms containing bacteria from lake water, no *mlr*A gene was detected at the beginning of or after the incubation period.

Community-level substrate utilization analysis

Biolog EcoPlates were used to indicate the microbial activity in the Water and Sediment experimental variants after preincubation in carbon-limited conditions. The functional diversity measured as the Shannon index (H) and utilized substrate richness (R) were not significantly different in both variants (1-way ANOVA: $F_{1,4} = 2.247$, $p > 0.05$ for H; $F_{1,4} = 1.471$, $p > 0.05$ for R) (Table 2). The mean AWCD values after 40 h of incubation were, on average, 40% higher in the Sediment variant. In both the Water and Sediment variants, the EcoPlate carbon source utilization

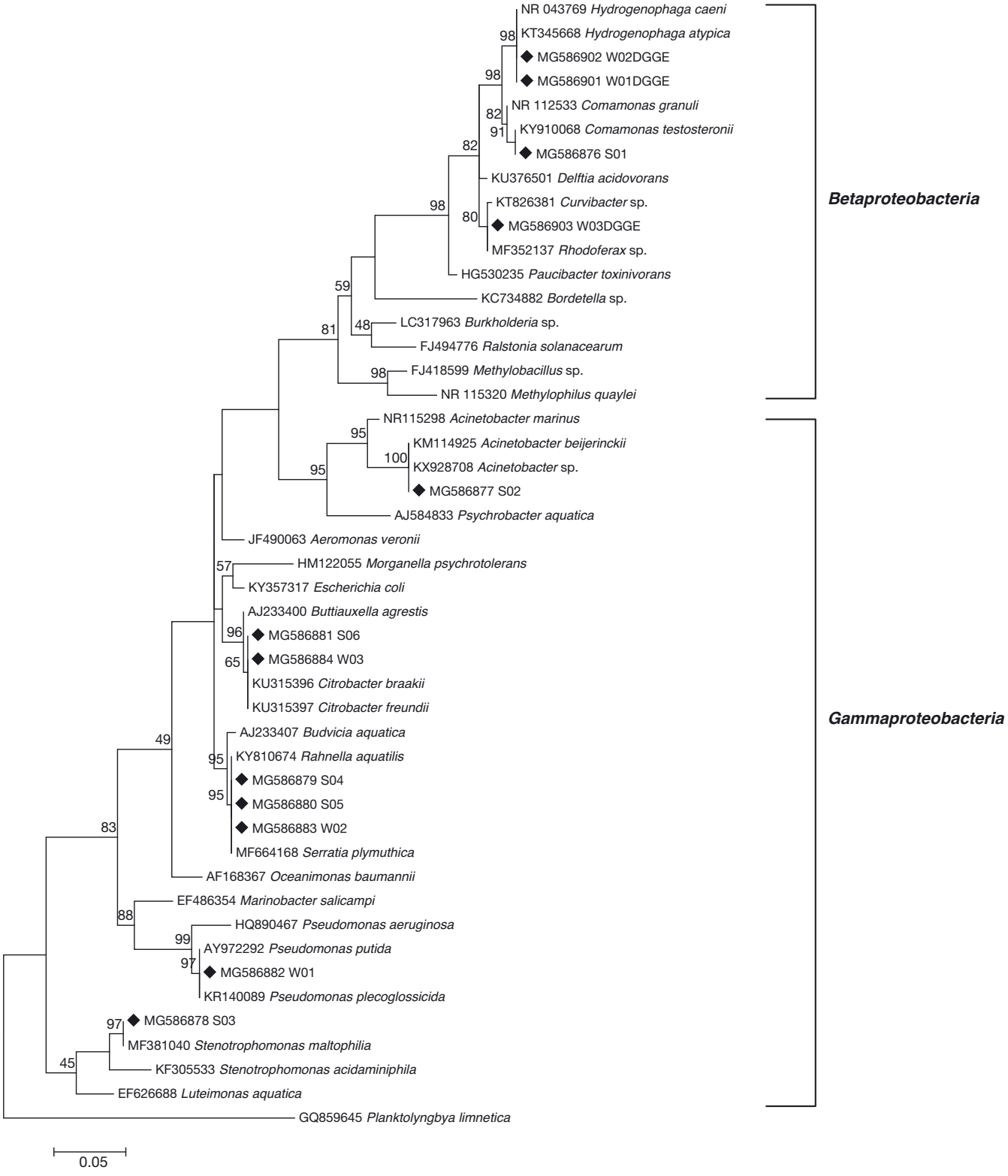

Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences inferred by using the maximum likelihood method and general time-reversible model. Numbers near the nodes indicate bootstrap values ≥40 as a percentage of 1000 replicates resulting from the analysis. The tree with the highest log likelihood (–4098.5915) is drawn to scale, with branch lengths measured in the number of substitutions per site. ◆: sequences of MC-degrading and potentially MC-degrading bacteria obtained in this study from isolated strains or from DGGE bands (see Table 1). Other sequences are derived from the GenBank database

Table 1. Characteristics and taxonomic lineage based on 16S rRNA gene sequences of microcystin (MC)-degrading bacteria and potentially MC-degrading bacteria identified in this study

Strain/ sequence name	GenBank accession number	Source	Identification method	Taxonomic lineage class-order-family-genus
Potentially MC-degrading bacteria				
S01	MG586876	Sediment	Isolation M9 medium	*Betaproteobacteria-Burkholderiales-Comamonadaceae-Comamonas*
S02	MG586877	Sediment	Isolation M9 medium	*Gammaproteobacteria-Pseudomonadales-Moraxellaceae-Acinetobacter*
S03	MG586878	Sediment	Isolation M9 medium	*Gammaproteobacteria-Xanthomonadales-Xanthomonadaceae-Stenotrophomonas*
S04	MG586879	Sediment	Isolation M9 medium	*Gammaproteobacteria-Enterobacterales-Yersiniaceae-Rahnella*
W01	MG586882	Water	Isolation M9 medium	*Gammaproteobacteria-Pseudomonadales-Pseudomonadaceae-Pseudomonas*
W02	MG586883	Water	Isolation M9 medium	*Gammaproteobacteria-Enterobacterales-Yersiniaceae-Rahnella*
W01DGGE	MG586901	Water	DGGE band	*Betaproteobacteria-Burkholderiales-Comamonadaceae-Hydrogenophaga*
W02DGGE	MG586902	Water	DGGE band	*Betaproteobacteria-Burkholderiales-Comamonadaceae-Hydrogenophaga*
W03DGGE	MG586903	Water	DGGE band	*Betaproteobacteria-Burkholderiales-Comamonadaceae-Rhodoferax*
MC-degrading bacteria				
S05	MG586880	Sediment	Isolation LB medium	*Gammaproteobacteria-Enterobacterales-Yersiniaceae-Serratia*
S06	MG586881	Sediment	Isolation LB medium	*Gammaproteobacteria-Enterobacterales-Enterobacteriaceae-Citrobacter*
W03	MG586884	Water	Isolation LB medium	*Gammaproteobacteria-Enterobacterales-Enterobacteriaceae-Citrobacter*

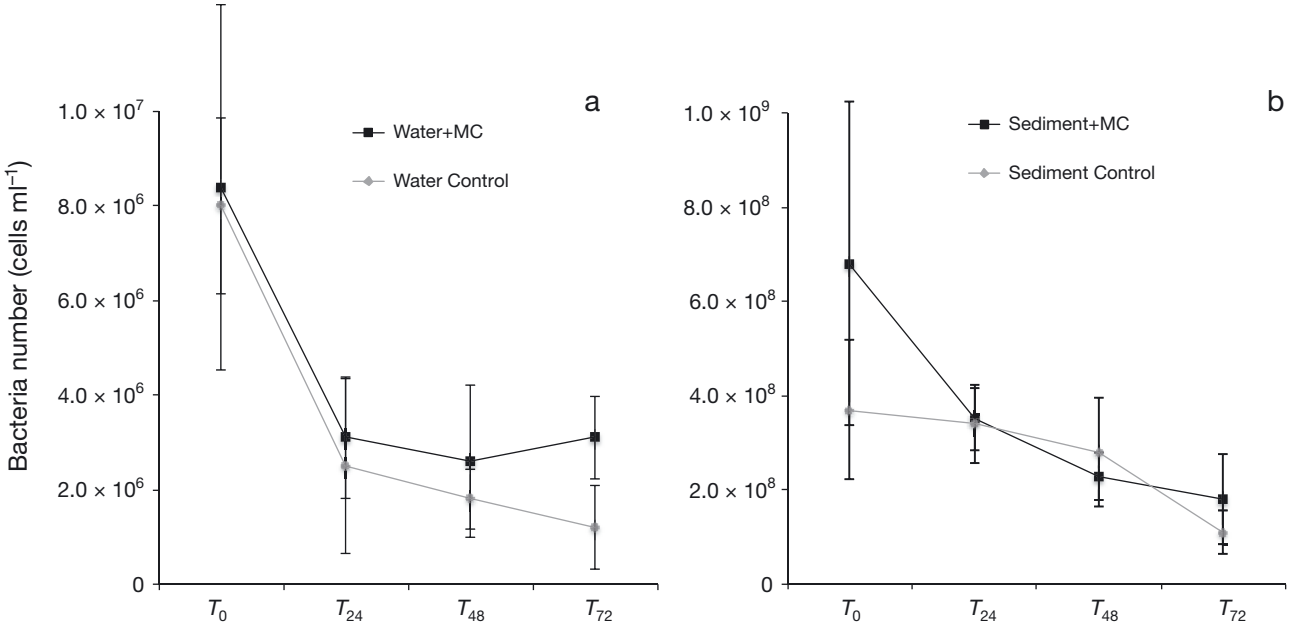

Fig. 4. Bacteria number calculated by the DAPI method in (a) Water+MC and Water Control microcosms and (b) Sediment+MC and Sediment Control microcosms, at the beginning of incubation (T_0) and after every 24 h (T_{24}, T_{48}, and T_{72}). Error bars indicate standard deviation calculated from triplicate counts of bacteria numbers

Table 2. Shannon diversity index (H) and substrate richness (R) in water and sediment experimental variants after preincubation in carbon-limited conditions, calculated after 40 h of incubation on Biolog EcoPlates. SD: standard deviation

	Water	SD	Sediment	SD
Shannon index (H)	2.96	0.05	3.02	0.05
Richness (R)	18	2.1	19	1.2

patterns were similar. The highest AWCD values were observed for polymers, amino acids, and carbohydrates, while the lowest AWCD values were noted for carboxylic acids (Fig. 5). Utilization of MCs was at the same level in experimental variants with lake water and sediment ($F_{1,4}$ = 4.341, p > 0.05). The AWCD level for MCs in the water variant was significantly (34%) lower than that in the polymer group ($F_{1,4}$ = 362.7, p < 0.05) but higher (37–69%) than those in other carbon source categories (Fig. 5). Utilization of MCs in the sediment variant was on a significantly lower level than that of polymers ($F_{1,4}$ = 27.69, p < 0.05) (57% lower AWCD) and significantly higher than that of phenolic compounds and carboxylic acids ($F_{1,4}$ = 5.675, p < 0.05; $F_{1,4}$ = 23.91, p < 0.05) (29 and 35%, respectively) (Fig. 5).

The AWCD profiles in the wells containing MCs showed the utilization patterns of these compounds in water and sediment bacterial communities from the experimental variants (Fig. 6). In the Water variant, there was a shorter lag time period (17 ± 1 h) than in Sediment (27 ± 0 h). The AWCD values increased until 40 h of incubation in both variants (Fig. 6).

Proteolytic activity

Protease activity varied significantly between the Water and Sediment microcosms ($F_{1,4}$ = 1088, p < 0.05 for microcosms without MCs; $F_{1,4}$ = 1664, p < 0.05 for microcosms with amendment of MCs) (Fig. 7). The fluorescence change during 17 h of incubation in the protease activity assay was 3.8-fold (±0.4) higher in the Sediment Control in comparison to the Water Control microcosm. The addition of MCs caused a significant change in the proteolytic activity in sediment ($F_{1,4}$ = 64.11, p < 0.05): the fluorescence change was 1.2-fold (±0.1) higher in the Sediment+MC than in the Sediment Control microcosm. In contrast, in

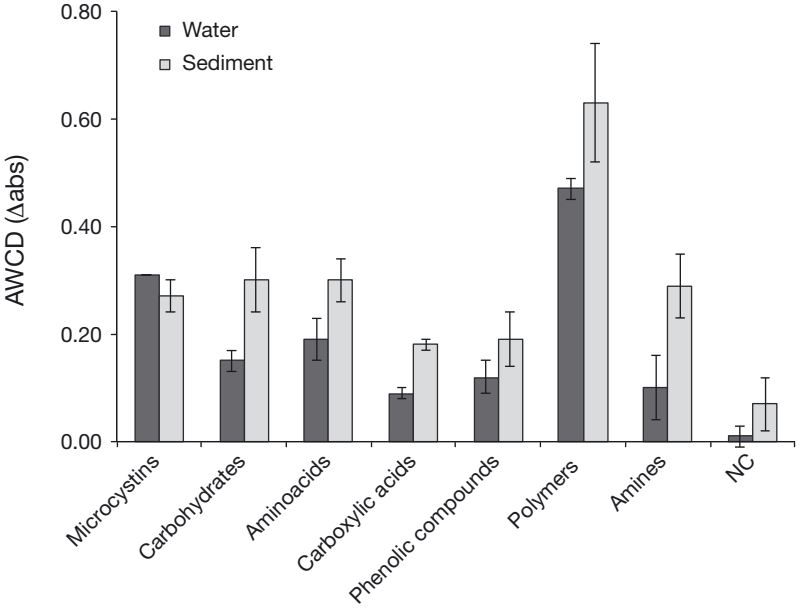

Fig. 5. Average well color development (AWCD, as change in absorbance) for different carbon source categories in Water and Sediment experimental variants after preincubation in carbon-limited conditions, calculated after 40 h of incubation on Biolog EcoPlates. Negative control (NC) means the carbon source with the lowest absorbance (abs) increase on the EcoPlate. Error bars indicate standard deviation calculated from triplicate incubations with each carbon source on Biolog EcoPlates

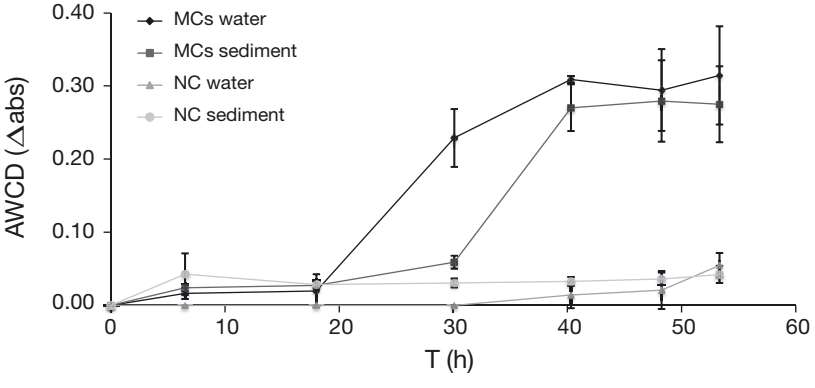

Fig. 6. Average well color development (AWCD) time profiles in the wells containing MCs (MCs water and MCs sediment) and in negative control wells (NC water and NC sediment) on Biolog EcoPlates incubated with Water and Sediment experimental variants after preincubation in carbon-limited conditions. Error bars indicate standard deviation calculated from triplicate incubations on Biolog EcoPlates

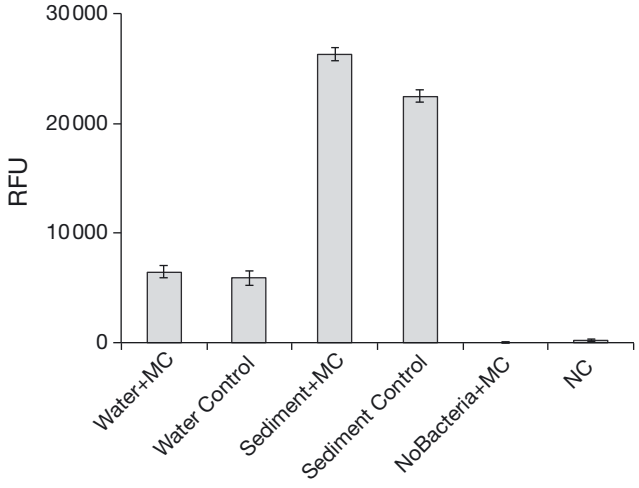

Fig. 7. Proteolytic activity in microcosms expressed as the fluorescence change (RFU) per unit sample measured after 24 h of incubation. Distilled water was used as a negative control (NC). Error bars indicate standard deviation calculated from triplicate incubations

microcosms with lake water, no significant change in proteolytic activity was observed after MC addition ($F_{1,4}$ = 1.227, p > 0.05) (Fig. 7).

Isolation and phylogenetic characteristics of MC-degrading and potentially MC-degrading bacterial strains

In the first method using M9 minimal salts medium amended with MC, 11 single bacterial colonies were isolated. After the elimination of repetitive strains based on 16S rRNA gene sequence analysis, there were finally 6 different strains capable of growing on MCs: 2 isolated from the Water+MC microcosm and 4 isolated from the Sediment+MC microcosm. BLAST searches and phylogenetic analyses showed that all strains belonged to *Proteobacteria*. Strain S01 isolated from sediment belongs to *Betaproteobacteria* and shows the closest homology with the *Comamonas* genus from the *Comamonadaceae* family (Fig. 3). The 5 other strains isolated on M9 medium belong to *Gammaproteobacteria* and can be assigned to the genera *Acinetobacter* (S02), *Stenotrophomonas* (S03), *Rahnella* (S04, W02), and *Pseudomonas* (W01) (Fig. 3). Table 1 presents the taxonomic lineage and accession numbers of the 16S rRNA gene sequences in the GenBank database.

In the second method of MC-degrading strain isolation using LB medium, 8 pure bacterial colonies were collected from Petri dishes: 3 from the Water+MC microcosm and 5 from the Sediment+MC microcosm. After 8 d of incubation in liquid LB medium and sterile filtered lake water, both with addition of MCs, the disappearance of MCs was measured (Fig. 8). A bacterial strain was considered as MC-degrading when the degradation rates during incubation in at least 1 of the experimental variants (LB medium or lake water) was >20%. Three strains, one from the Water+MC microcosm (W03) and 2 from the Sediment+MC microcosm (S05 and S06), were selected for further analyses as MC degraders. Strains W03 and S06 showed higher degradation rates in lake water (27.9 ± 15.3% SD and 42.0 ± 4.5%, respectively), but they also had the ability of MC degradation in the LB medium (21.1 ± 0.7% and 19.1 ± 3.5%, respectively) (Fig. 8). BLAST searches and phylogenetic analyses allowed us to assign these strains to the genus *Citrobacter*, *Gammaproteobacteria* (Fig. 3). In contrast, strain S05 only showed the ability MCs to degrade in the LB medium (20.9 ± 3.1% after 8 d) (Fig. 8). This strain was assigned to the genus *Serratia*, *Gammaproteobacteria* (Fig. 3). Details of the taxonomic classification and GenBank accession numbers are given in Table 1.

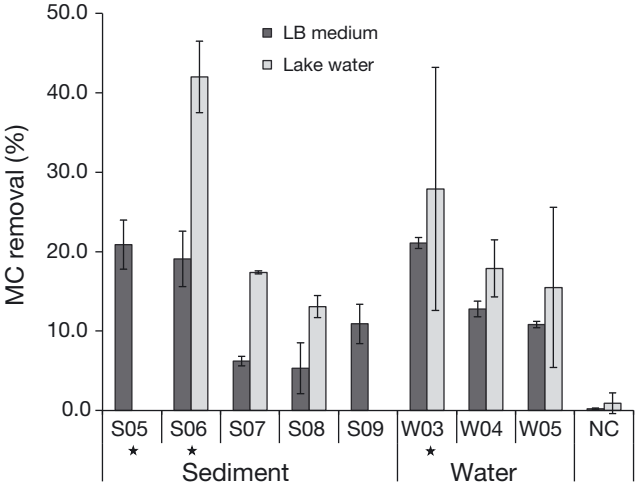

Fig. 8. Removal of MCs by bacterial strains isolated on LB agar medium amended with MCs from the Water+MC and Sediment+MC microcosms. The graph shows the percentage degradation of MCs after 8 d of incubation in liquid LB medium and sterile filtered lake water, both amended with MCs at a concentration of 20 µg l^{-1}. Negative control (NC) means liquid LB medium and sterile filtered lake water, both amended with MCs and without addition of bacteria. Error bars indicate standard deviation calculated from 3 independent incubations of each sample. Stars indicate strains considered as MC-degrading, which show percentage removal of MCs >20% in at least 1 medium

DISCUSSION

The aim of the present study was to identify MC-degrading bacteria in lake water and lake sediment and to characterize the degradation processes occurring in both environments. Two variants of MCs were used for the experiments: MC-LR is one of the most common types of this toxin (Christiansen et al. 2003, Metcalf & Codd 2004), and MC-RR, also commonly present in aquatic environments (Christiansen et al. 2003), was previously detected in Lake Mikołajskie (Bukowska et al. 2017). The *Planktothrix* genus, which was found to be the main producer of MCs in the studied lake (Bukowska et al. 2017), produces mainly demethylated variants of MC-RR (Fastner et al. 1999, Christiansen et al. 2003, Grabowska et al. 2014).

Most studies on MC-degrading bacteria focus only on the isolation of individual strains (Park et al. 2001, Rapala et al. 2005, Amé et al. 2006, Ho et al. 2007b, Manage et al. 2009, Choi et al. 2010, Yang et al. 2014b, Lemes et al. 2015, Mankiewicz-Boczek et al. 2015). It is also important to know how the degradation processes occur in conditions similar to natural environments. Such research may show whether the degradation in bacterial consortia is more effective than degradation carried out by individual bacterial strains. This type of study was previously undertaken by Mou et al. (2013). Microcosms are widely used in ecological research, but they cannot fully reproduce natural environmental conditions. We tried to make them as similar to nature as possible: bacteria were suspended in water and sediment from a lake from which they originally derived, and they were incubated at the lake water temperature. However, the 'bottle effect' cannot be excluded. This phenomenon concerning aquatic bacteria transferred from the natural environment to a smaller volume container can cause replacement of natural community by opportunistic bacteria and increase the participation of heterotrophs (Baltar et al. 2012, Ionescu et al. 2015). It can result in the loss of some of the microorganism diversity that occurs in the environment (Elliott et al. 1986). Only aerobic bacteria were considered in this study. We were not able to maintain stable anaerobic conditions in this type of microcosm. It is now known that the bacterial degradation of MCs can also be anaerobic, although to date, these processes have been less frequently investigated than aerobic degradation (Holst et al. 2003, Chen et al. 2010b, Bao & Wu 2016).

It was confirmed in this study that in both water and sediments from eutrophic Lake Mikołajskie, MC-degrading bacteria occurred that can degrade both MC-LR and MC-RR at concentrations of 10 µg l^{-1} over 72 h. The declining numbers of bacteria in microcosms during a 3 d incubation could be due to the bottle effect and the preincubation effect (Fig. 4). In the Water+MC microcosm, the number of bacteria began to increase after 48 h of incubation with MCs — this is probably due to the effect of using MCs as a carbon source. In this microcosm, a bacterial consortium capable of degrading MCs has already developed. This can also be seen in the DGGE results, where after 48 h, there are clearly visible dominant OTUs, which are most likely responsible for the cleavage of toxins (Fig. 2a). In the case of the Sediment+MC microcosm, the number of bacteria was initially ~2 orders of magnitude higher than that in Water+MC (Fig. 4). Bacteria effectively degraded MCs, but for the 3 d period of the experiment, there was no change in the community structure in DGGE analysis (Fig. 2b). Most likely, only some of the bacterial taxa present in the experiment with sediment can degrade MCs, but during the 3 d incubation period, these bacteria did not reach a predominance over other bacteria. The MC concentration could also be too low to cause a visible change in the taxonomic structure. Perhaps the more detailed changes in the community structure and bacterial taxa potentially responsible for the degradation of toxins can be noticed using other methods such as next-generation sequencing.

The presence of the *mlr*A gene from the *mlr* cluster, encoding the toxin-cleavage pathway in some MC-degrading bacteria, was investigated. The *mlr*A product is the most important enzyme in this pathway because it catalyzes the opening of the ring and the linearization of MC molecules (Bourne et al. 1996, 2001, Hashimoto et al. 2009). The *mlr*A gene was found in the sediment but was not found in lake water, indicating that the MC-degrading bacterial communities in both environments are different. The results do not exclude the possibility that homologues of the *mlr*A gene are present in bacteria from the Water+MC microcosm or in bacteria from the Sediment+MC microcosm. The exact sequences of these homologues are unknown, and they cannot be detected by the primers used (Hu et al. 2009, Mou et al. 2013). To date, the *mlr* genes have been mainly detected in the family *Sphingomonadaceae* (*Alphaproteobacteria*) (Jones et al. 1994, Maruyama et al. 2006, Okano et al. 2009, Jiang et al. 2011) and in several other species from other taxonomic groups (Alamri 2010, Chen et al. 2010a, Yang et al. 2014a, Zhu et al. 2016). It is probable, however, that the MC

degradation in our experiments is not only related to the *mlr*-dependent pathway but also being carried out via alternative degradation pathways. An alternative pathway based on xenobiotic metabolism-related genes was postulated by Mou et al. (2013), while the existence of a specialized MC-degradative protease was found by Takenaka & Watanabe (1997).

The proteolytic activity in the experimental variants was tested to determine whether degradation by bacteria originating from water and sediment occurred in the same manner. The protease activity increased in the Sediment microcosm with MC addition in relation to the Sediment Control microcosm without MC amendment (Fig. 7). Such a change was not observed in the microcosms with water bacteria, although the degradation of MCs in the water was as effective as in the sediment (Fig. 7). The higher proteolytic activity in the Sediment+MC microcosm indicates the presence of a degradation pathway involving the protease enzymes. Most likely, it is an *mlr* pathway, where the enzyme encoded by *mlr*A, as well as some other enzymes, is a protease (Bourne et al. 2001, Dziga et al. 2012). However, they can also be other unknown proteases that are capable of MC degradation. For example, Takenaka & Watanabe (1997) described an alkaline protease from a *Pseudomonas aeruginosa* bacterium capable of decomposing MCs. In the experimental variant with Water+MC, neither *mlr* genes nor increased proteolytic activity were observed. This indicates that these bacteria may degrade MCs via another pathway. Perhaps it is the potential pathway described by Mou et al. (2013), similar to the xenobiotic metabolism pathway.

Community-level physiological profiling did not show differences in preferences for the utilization of various substrate groups by lake and bottom sediment bacteria (Fig. 5). Metabolic activity in the Sediment+MC microcosm was higher, but this is probably the result of a higher number of bacteria. However, the substrate preferences in both environments were very similar. Therefore, it is not possible to conclude that bacterial communities displayed different metabolic pathways on the basis of community-level metabolic profiling.

MCs on the Biolog EcoPlates were effectively used in both bacterial communities, and in the case of water bacteria, MCs were the second most effectively used substrate group. The time profile of MC utilization showed that bacteria from lake water reacted faster to the presence of MCs: the lag phase was ~10 h shorter than for sediment bacteria (Fig. 6). Perhaps these bacteria already have active metabolic pathways that may be responsible for toxin degradation, but in the case of sediment bacteria, these pathways have to be activated. In both cases, the maximum absorbance was reached after 40 h of incubation, which means that after this time, all the MCs were likely utilized. In the sediment variant, the degradation rate was higher, and the entire substrate at the concentration of 20 µg l^{-1} was used in ~13 h, whereas in the water bacteria community, 23 h was required for complete degradation. The metabolic time profile of MC utilization shows that there are differences in the kinetics of degradation reactions between these communities, which may be the result of using different metabolic pathways. In the literature, degradation rate analyses have been primarily based on the measurement of MC loss over time (Li et al. 2011, Mankiewicz-Boczek et al. 2015, Lezcano et al. 2016, Dai et al. 2017, Morón-López et al. 2017). To date, only Manage et al. (2009) and Giaramida et al. (2013) used Biolog MT2 plates to measure the metabolic activity of bacterial strains during MC degradation. The Biolog EcoPlate analysis allows us to trace the actual activity of the entire bacterial community, which gives new insights into the dynamics of degradation processes.

To identify the taxonomy of MC-degrading and potentially MC-degrading bacteria in water and bottom sediment of Lake Mikołajskie, pure bacterial strains were isolated. Bacteria isolated by the first method — on plates containing M9 medium and MCs as the only carbon source — were considered to be potentially MC-degrading. Although they were able to grow on MCs, it was not possible to determine the degradation rate because these strains could not be effectively grown in liquid media. Strains isolated by the second method, whose degradation efficiency of MCs in liquid media was >20% during 8 d of incubation, were considered MC-degraders. In the case of bacteria from water, sequences from dominating bands in DGGE after 3 d of incubation with MCs were also identified as belonging to potentially MC-degrading bacteria. All identified taxa were *Proteobacteria*, both beta and gamma classes (Table 1). Both of these classes were identified in water and sediment, but only the strain of *Betaproteobacteria* was isolated from the sediment. The DGGE analysis indicated a predominant role of *Betaproteobacteria* in the degradation of MCs in the Water+MC microcosm, but only strains of *Gammaproteobacteria* were isolated from this microcosm. This points to the differences in the ability to cultivate individual taxa and indicates that research based only on cultured bacteria can be very incomplete (Cho & Giovannoni 2004).

Sphingomonadales from the class *Alphaproteobacteria* were considered to have a predominant role in MC degradation for a long time (Jones et al. 1994, Maruyama et al. 2003, Saitou et al. 2003, Amé et al. 2006, Jimbo et al. 2010, Zhang et al. 2010). In the present study, no strain nor any 16S rRNA gene sequences belonging to these taxa was isolated. Although the *mlr*A gene in the bottom sediment indicates that these bacteria were present, they do not appear to play a dominant role in toxin degradation in Lake Mikołajskie. Further published studies expanded the MC-degraders group, initially with *Actinomycetales*, *Bacillales*, *Burkholderiales* and *Methylophilales* (Yan et al. 2004, Lemes et al. 2008, Hu et al. 2009, Manage et al. 2009, Alamri 2010), while recent reports indicate that MC-responsive bacteria even belong to 89 orders within the phyla of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria* of the alpha, beta, gamma and delta/epsilon subdivisions, and *Verrucomicrobia* (Mou et al. 2013). Lezcano et al. (2017) indicates that the bacteria highly responsive to the release of MCs to the environment belong to phyla *Proteobacteria*, *Bacteroidetes*, *Acidobacteria* and *Gemmatimonadetes* and that they are bacteria known from their ability to degrade complex and persistent organic compounds.

Among the MC-degrading and potentially MC-degrading bacteria found in Lake Mikołajskie (Table 1), there are taxa that have already been described as MC degraders: *Acinetobacter* sp. (Li & Pan 2014, Yi et al. 2015, Li et al. 2016), *Stenotrophomonas* sp. (Chen et al. 2010a, Yang et al. 2014b, 2018, Zhou et al. 2014) and *Pseudomonas* sp. (Takenaka & Watanabe 1997, Li & Pan 2014, Lemes et al. 2015). In the case of the genus *Rahnella,* there is only one mention that it could degrade MCs under anaerobic conditions (Li et al. 2017). However, in our study, *Rahnella* sp. S04 and W02 strains also degraded MCs in aerobic conditions.

In the present study, potentially MC-degrading bacteria, which have not been previously reported in the literature, were found. The interesting group is the family *Comamonadaceae*, representatives of which were found in the MC-degrading bacterial community in the Water+MC microcosm. So far, only one bacterial species from this family, *Delftia acidovorans*, was confirmed to have the ability of MC degradation (Zhou et al. 2006). In this study, we confirmed the presence of 3 other potentially MC-degrading *Comamonadaceae* genera: *Comamonas* sp., *Hydrogenophaga* sp. and *Rhodoferax* sp. (Table 1). These bacteria are also closely related to several other *Betaproteobacteria* taxa that were previously described as MC degraders: *Ralstonia solanacearum*, *Paucibacter toxinivorans*, *Burkholderia* sp., *Methylobacillus* sp., and *Bordetella* sp. (Yan et al. 2004, Rapala et al. 2005, Lemes et a. 2008, Hu et al. 2009, Zhang et al. 2011, Yang et al. 2014a).

Two *Gammaproteobacteria* taxa isolated in this study — *Citrobacter* sp. and *Serratia* sp. — were also not previously described as MCs degraders. An interesting observation is that these bacterial strains degrade MCs with different efficiencies depending on the medium in which they are cultivated (Fig. 8). The *Serratia* sp. S05 strain did not decompose MCs in lake water with a lower nutrient content but did show degradation activity in the nutrient-rich LB medium. Li et al. (2014) and Lezcano et al. (2016) have already noted that the MC degradation rate may largely depend on total organic carbon, total nitrogen or other compound concentrations, but further studies are needed to better understand the mechanisms of these processes. Our research also shows that the degradation efficiency by individual bacteria strains is much lower than that by the bacterial consortia. The nearly complete decomposition of 20 µg l^{-1} MCs in microcosms with bacterial communities from water and sediment took <3 d, while the highest MC degradation rate by the *Citrobacter* sp. S06 strain was 42% after 8 d. Further studies are required to determine whether different species in bacterial communities can 'cooperate' by performing the various steps of the degradation processes. Perhaps the metabolic pathways of degradation should be considered as a process carried out by a multi-species bacterial consortium. This study provides a clear indication that the degradation of MCs is widespread among bacteria, although more research is needed for understanding the different mechanisms of this process.

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LITERATURE CITED

Alamri SA (2010) Biodegradation of microcystin by a new *Bacillus* sp. isolated from Saudi freshwater lake. Afr J Biotechnol 9:6552–6559

Amé VM, Ricardo EJ, Stephan P, Alberto WD (2006) Degradation of microcystin-RR by *Sphingomonas* sp. CBA4 isolated from San Roque reservoir (Córdoba — Argentina). Biodegradation 17:447–455

Baltar F, Lindh MV, Parparov A, Berman T, Pinhassi J (2012) Prokaryotic community structure and respiration during long-term incubations. MicrobiologyOpen 1:214–224

Bao ZY, Wu Y (2016) Biodegradation of microcystin-LR by an amino acid-degrading anaerobic bacterium. Desalination Water Treat 57:870–880

Bourne DG, Jones GJ, Blakeley RL, Jones A, Negri AP, Riddles P (1996) Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR. Appl Environ Microbiol 62:4086–4094

Bourne DG, Riddles P, Jones GJ, Smith W, Blakeley RL (2001) Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environ Toxicol 16:523–534

Bukowska A, Bielczyńska A, Karnkowska-Ishikawa A, Chróst RJ, Jasser I (2014) Molecular (PCR-DGGE) versus morphological approach: analysis of taxonomic composition of potentially toxic cyanobacteria in freshwater lakes. Aquat Biosyst 10:2–11

Bukowska A, Kaliński T, Koper M, Kostrzewska-Szlakowska I, Kwiatowski J, Mazur-Marzec H, Jasser I (2017) Predicting blooms of toxic cyanobacteria in eutrophic lakes with diverse cyanobacterial communities. Sci Rep 7:8342

Chen W, Song L, Peng L, Wan N, Zhang X, Gan N (2008) Reduction in microcystin concentrations in large and shallow lakes: water and sediment-interface contributions. Water Res 42:763–773

Chen J, Hu LB, Zhou W, Yan SH, Yang JD, Xue YF, Shi ZQ (2010a) Degradation of microcystin-LR and RR by a *Stenotrophomonas* sp. strain EMS isolated from Lake Taihu. Int J Mol Sci 11:896–911

Chen X, Yang X, Yang L, Xiao B, Wu X, Wang J, Wan H (2010b) An effective pathway for the removal of microcystin LR via anoxic biodegradation in lake sediment. Water Res 44:1884–1892

Cho JC, Giovannoni SJ (2004) Cultivation and growth characteristics of a diverse group of oligotrophic marine Gammaproteobacteria. Appl Environ Microbiol 70:432–440

Choi JH, Kim MS, Jung MJ, Roh SW, Shin KS, Bae JW (2010) *Sphingopyxis soli* sp. nov., isolated from landfill soil. Int J Syst Evol Microbiol 60:1682–1686

Christiansen G, Fastner J, Erhard M, Borner T, Dittmann E (2003) Microcystin biosynthesis in Planktothrix: genes, evolution, and manipulation. J Bacteriol 185:564–572

Christoffersen K, Lyck S, Winding A (2002) Microbial activity and bacterial community structure during degradation of microcystins. Aquat Microb Ecol 27:125–136

Dai G, Peng N, Zhong J, Yang P and others (2017) Effect of metals on microcystin abundance and environmental fate. Environ Pollut 226:154–162

Dziga D, Władyka B, Zielińska G, Meriluoto J, Wasylewski M (2012) Heterologous expression and characterisation of microcystinase. Toxicon 59:578–586

Dziga D, Zielińska G, Władyka B, Bocheńska O, Maksylewicz A, Strzałka W, Meriluoto J (2016) Characterization of enzymatic activity of MlrB and MlrC proteins involved in bacterial degradation of cyanotoxins microcystins. Toxins (Basel) 8:76

Eleuterio L, Batista JR (2010) Biodegradation studies and sequencing of microcystin-LR degrading bacteria isolated from a drinking water biofilter and a fresh water lake. Toxicon 55:1434–1442

Elliott ET, Hunt HW, Walter DE, Moore JC (1986) Microcosms, mesocosms and ecosystems: linking the laboratory to the field. In: Megusar F, Gantar M (eds) Perspectives in microbial ecology. Slovene Society for Microbiology, Lubljana, p 472–480

Fastner J, Erhard M, Carmichael WW, Sun F, Rinehart KL, Ronicke H, Chorus I (1999) Characterization and diversity of microcystins in natural blooms and strains of the genera *Microcystis* and *Planktothrix* from German freshwaters. Arch Hydrobiol 145:147–163

Feigl V, Ujaczki É, Vaszita E, Molnár M (2017) Influence of red mud on soil microbial communities: application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies. Sci Total Environ 595:903–911

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57:2351–2359

Giaramida L, Manage PM, Edwards C, Singh BK, Lawton LA (2013) Bacterial communities' response to microcystins exposure and nutrient availability: linking degradation capacity to community structure. Int Biodeterior Biodegradation 84:111–117

Grabowska M, Kobos J, Toruńska-Sitarz A, Mazur-Marzec H (2014) Non-ribosomal peptides produced by *Planktothrix agardhii* from Siemianówka Dam Reservoir SDR (northeast Poland). Arch Microbiol 196:697–707

Gryta A, Frąc M, Oszust K (2014) The application of the Biolog EcoPlate approach in ecotoxicological evaluation of dairy sewage sludge. Appl Biochem Biotechnol 174: 1434–1443

Hashimoto EH, Kato H, Kawasaki Y, Nozawa Y, Tsuji K, Hirooka EY, Harada K (2009) Further investigation of microbial degradation of microcystin using the advanced Marfey method. Chem Res Toxicol 22:391–398

Ho L, Gaudieux AL, Fanok S, Newcombe G, Humpage AR (2007a) Bacterial degradation of microcystin toxins in drinking water eliminates their toxicity. Toxicon 50: 438–441

Ho L, Hoefel D, Saint CP, Newcombe G (2007b) Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. Water Res 41: 4685–4695

Holst T, Jørgensen NOG, Jørgensen C, Johansen A (2003) Degradation of microcystin in sediments at oxic and anoxic, denitrifying conditions. Water Res 37:4748–4760

Hu LB, Yang JD, Zhou W, Yin YF, Chen J, Shi ZQ (2009) Isolation of a *Methylobacillus* sp. that degrades microcystin toxins associated with cyanobacteria. N Biotechnol 26: 205–211

Ionescu D, Bizic-Ionescu M, Khalili A, Malekmohammadi R, Morad MR, de Beer D, Grossart HP (2015) A new tool for long-term studies of POM–bacteria interactions: overcoming the century-old Bottle Effect. Sci Rep 5:14706

Ishii H, Nishijima M, Abe T (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Res 38: 2667–2676

Jiang Y, Shao J, Wu X, Xu Y, Li R (2011) Active and silent members in the mlr gene cluster of a microcystin-degrading bacterium isolated from Lake Taihu, China. FEMS Microbiol Lett 322:108–114

Jimbo Y, Okano K, Shimizu K, Maseda H, Fujimoto N, Motoo U, Sugiura N (2010) Quantification of microcystin-degrading bacteria in a biofilm from a practical biological treatment facility by real-time PCR. J Water Environ Technol 8:193–201

Jing W, Sui G, Liu S (2014) Characteristics of a microcystin-LR biodegrading bacterial isolate: *Ochrobactrum* sp. FDT5. Bull Environ Contam Toxicol 92:119–122

Jones GJ, Bourne DG, Blakeley RL, Doelle H (1994) Degradation of the cyanobacterial hepatotoxin microcystin by aquatic bacteria. Nat Toxins 2:228–235

Konopka A, Bercot T, Nakatsu C (1999) Bacerioplankton community diversity in a series of thermally stratified lake. Microb Ecol 38:126–135

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York, NY, p 115–175

Lemes GAF, Kersanach R, Pinto Lda S, Dellagostin OA, Yunes JS, Matthiensen A (2008) Biodegradation of microcystins by aquatic *Burkholderia* sp. from a South Brazilian coastal lagoon. Ecotoxicol Environ Saf 69:358–365

Lemes GAF, Kist LW, Bogo MR, Yunes JS (2015) Biodegradation of [D-Leu1] microcystin-LR by a bacterium isolated from sediment of Patos Lagoon estuary. J Venom Anim Toxins Incl Trop Dis 21:4

Lezcano MÁ, Morón-López J, Agha R, López-Heras I, Nozal L, Quesada A, El-Shehawy R (2016) Presence or absence of mlr genes and nutrient concentrations co-determine the microcystin biodegradation efficiency of a natural bacterial community. Toxins (Basel) 8:318

Lezcano MÁ, Velázquez D, Quesada A, El-Shehawy R (2017) Diversity and temporal shifts of the bacterial community associated with a toxic cyanobacterial bloom: an interplay between microcystin producers and degraders. Water Res 125:52–61

Li H, Pan G (2014) Enhanced and continued degradation of microcystins using microorganisms obtained through natural media. J Microbiol Methods 96:73–80

Li JM, Shimizu K, Sakharkar MK, Utsumi M, Zhang ZY, Sugiura N (2011) Comparative study for the effects of variable nutrient conditions on the biodegradation of microcystin-LR and concurrent dynamics in microcystin-degrading gene abundance. Bioresour Technol 102: 9509–9517

Li JM, Peng LJ, Li J, Qiao YH (2014) Divergent responses of functional gene expression to various nutrient conditions during microcystin-LR biodegradation by *Novosphingobium* sp. THN1 strain. Bioresour Technol 156:335–341

Li H, Ai HN, Kang L, Sun XF, He Q (2016) Simultaneous *Microcystis* algicidal and microcystin degrading capability by a single *Acinetobacter* bacterial strain. Environ Sci Technol 50:11903–11911

Li J, Li R, Li J (2017) Current research scenario for microcystins biodegradation — a review on fundamental knowledge, application prospects and challenges. Sci Total Environ 595:615–632

Manage PM, Edwards C, Singh BK, Lawton LA (2009) Isolation and identification of novel microcystin-degrading bacteria. Appl Environ Microbiol 75:6924–6928

Manage PM, Edwards C, Lawton LA (2010) Bacterial degradation of microcystin. In: Hamamura N, Suzuki S, Mendo S, Barroso CM, Iwata H, Tanabe S (eds) Interdisciplinary studies on environmental chemistry-biological responses to contaminants. TERRAPUB, Tokyo, p 97–104

Mankiewicz-Boczek J, Gągała I, Jurczak T, Jaskulska A, Pawełczyk J, Dziadek J (2015) Bacteria homologus to *Aeromonas* capable of microcystin degradation. Open Life Sci 10:119–129

Maruyama T, Kato K, Yokoyama A, Tanaka T, Hiraishi A, Park HD (2003) Dynamics of microcystin-degrading bacteria in mucilage of *Microcystis*. Microb Ecol 46:279–288

Maruyama T, Park HD, Ozawa K, Tanaka Y and others (2006) *Sphingosinicella microcystinivorans gen*. nov., sp. nov., a microcystin degrading bacterium. Int J Syst Evol Microbiol 56:85–89

Metcalf JS, Codd GA (2004) Cyanobacterial toxins in water. Foundation for Water Research, Marlow

Morón-López J, Nieto-Reyes L, El-Shehawy R (2017) Assessment of the influence of key abiotic factors on the alternative microcystin degradation pathway(s) (mlr-): a detailed comparison with the mlr route (mlr+). Sci Total Environ 599–600:1945–1953

Mou X, Lu X, Jacob J, Sun S, Heath R (2013) Metagenomic identification of bacterioplankton taxa and pathways involved in microcystin degradation in Lake Erie. PLOS ONE 8:e61890

Mu RM, He YJ, Liu SX, Wang XR, Fan ZQ (2009) The algicidal characteristics of one algae-lysing FDT5 bacterium on *Microcystis aeruginosa*. Geomicrobiol J 26:516–521

Nybom SM, Salminen SJ, Meriluoto JA (2007) Removal of microcystin-LR by strains of metabolically active probiotic bacteria. FEMS Microbiol Lett 270:27–33

Okano K, Shimizu K, Kawauchi Y, Maseda H and others (2009) Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. J Toxicol 2009:954291

Paerl HW, Otten TG (2013) Blooms bite the hand that feeds them. Science 342:433–434

Park HD, Sasaki Y, Maruyama T, Yanagisawa E, Hiraishi A, Kato K (2001) Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environ Toxicol 16:337–343

Pearson L, Mihali T, Moffitt M, Kellmann R, Neilan B (2010) On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Mar Drugs 8:1650–1680

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25: 943–948

Preston-Mafham J, Boddy L, Randerson PF (2002) Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles — a critique. FEMS Microbiol Ecol 42:1–14

Ramani A, Rein K, Shetty KG, Jayachandran K (2012) Microbial degradation of microcystin in Florida's freshwaters. Biodegradation 23:35–45

Rapala J, Lahti K, Sivonen K, Niemelä SI (1994) Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. Lett Appl Microbiol 19: 423–428

Rapala J, Berg KA, Lyra C, Niemi RM and others (2005) *Paucibacter toxinivorans* gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. Int J Syst Evol Microbiol 55: 1563–1568

Saito T, Okano K, Park HD, Itayama T and others (2003) Detection and sequencing of the microcystin LR-degrading gene, mlrA, from new bacteria isolated from Japanese lakes. FEMS Microbiol Lett 229:271–276

Saitou T, Sugiura N, Itayama T, Inamori Y, Matsumura M (2003) Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. J Water Supply 52:13–18

Takenaka S, Watanabe MF (1997) Microcystin LR degradation by *Pseudomonas aeruginosa* alkaline protease. Chemosphere 34:749–757

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30:2725–2729

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tsuji K, Watanuki T, Kondo F, Watanabe MF and others (1995) Stability of microcystins from cyanobacteria. II. Effect of UV light on decomposition and isomerization. Toxicon 33:1619–1631

Yan H, Pan G, Zou H, Li X, Chen H (2004) Effective removal of microcystins using carbon nanotubes embedded with bacteria. Chin Sci Bull 49:1694–1698

Yang F, Zhou YL, Song RL, Wei HY, Li YH, Yin LH, Pu YP (2014a) Biodegradation of microcystin-LR and -RR by a novel microcystin-degrading bacterium isolated from Lake Taihu. Biodegradation 25:447–457

Yang F, Zhou YL, Yin LH, Zhu GC, Liang GY, Pu YP (2014b) Microcystin degrading activity of an indigenous bacterial strain *Stenotrophomonas acidaminiphila* MC-LTH2 isolated from Lake Taihu. PLOS ONE 9:e86216

Yang F, Massey IY, Guo J, Yang S, Pu Y, Zeng W, Tan H (2018) Microcystin-LR degradation utilizing a novel effective indigenous bacterial community YFMCD1 from Lake Taihu. J Toxicol Environ Health A 81:184–193

Yi YL, Yu XB, Zhang C, Wang GX (2015) Growth inhibition and microcystin degradation effects of *Acinetobacter guillouiae* A2 on *Microcystis aeruginosa*. Res Microbiol 166:93–101

Zhang X, Hu HY, Men YJ, Christoffersen KS (2010) The effect of *Poterioochromonas* abundance on production of intra- and extracellular microcystin-LR concentration. Hydrobiologia 652:237–246

Zhang ML, Yan H, Pan G (2011) Microbial degradation of microcystin-LR by *Ralstonia solanacearum*. Environ Technol 32:1779–1787

Zhou J, Yan H, He HS, Zhong GR, Zhang C, Lin H (2006) Activity of *Delftic acidovorans* for the biodegradation of microcystins. Kexue Jishu Yu Gongcheng 6:166–170

Zhou YL, Yang F, Liang GY, Yin LH, Pu YP (2014) Biodegradation of microcystin-RR and -LR by an indigenous bacterial strain MC-LTH11 isolated from Lake Taihu. J Southeast Univ 30:68–71 (English edn)

Zhu XY, Shen YT, Chen XG, Hu YOO, Xiang HY, Tao J, Ling Y (2016) Biodegradation mechanism of microcystin-LR by a novel isolate of *Rhizobium* sp. TH and the evolutionary origin of the mlrA gene. Int Biodeterior Biodegradation 115:17–25

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