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VARIABILITY OF MICROCYSTINS AND ITS SYNTHETASE GENE CLUSTER IN *MICROCYSTIS* AND *PLANKTOTHRIX* WATERBLOOMS IN SHALLOW LAKES OF HUNGARY

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Waterbloom samples *of Microcystis aeruginosa* and *Planktothrix agardhii* were collected from a variety of ponds, lakes and reservoirs in Hungary. Samples were tested with matrix-assisted laser desorption/ionization – time-of-flight mass spectrometry (MALDI-TOF MS) to identify the microcystin forms. The concentration of the microcystins was measured with capillary electrophoresis and the toxicity was tested by sinapis test. DNA was extracted from the samples and tested using a range of primers linked to the biosynthesis of microcystin. All of the fourteen collected samples gave positive results for the presence of the *mcy* genes with PCR products with sizes between of 425 and 955 bp, respectively, indicating the presence of the genes implicated in the production of microcystins. The results showed that a wide range of microcystin (MC) forms were detected in the *Microcystis* containing samples, among which MC-LR, -RR, and -YR were the most common. The highest MC concentration was 15,701 mg g^{-1}, which was detected in an angling pond. The samples containing *Planktothrix agardhii* were less toxic, and the most common form in this species was the Asp3-MC-LR.

Keywords: Microcystis – *Planktothrix* – waterbloom – microcystins – MALDI-TOF

INTRODUCTION

Waterbloom-forming cyanobacteria are known risk factors for humans and livestock due to the production of toxic metabolites, most prominently the cyclic heptapeptide microcystins. These cyanotoxins have been implicated in the death of humans and various incidents of animal intoxication, and have been linked to liver cancer in humans. Microcystins were associated with liver failure and the death of fifty-two patients in a hemodialysis center in Brazil [4]. Their toxicity is believed to be the consequence of their inhibitory effect on eukaryotic protein phosphatases (PP) 1 and 2A [25]. The World Health Organization (1998) has set an advisory level for MCs in drinking water of 1.0 μg L^{-1} MC-LR [4].

Microcystins have a common structure containing three D-amino acids (alanine, b-linked erythro-b-methylaspartic acid and a-linked glutamic acid), two variable L-amino acids, R1 and R2, and two unusual amino acids, N-methyldehydroalanine

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(Mdha) and 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyldeca-4,6-dienoic acid (Adda) [26].

To date, over 60 chemical forms of microcystin have been identified, with molecular weights ranging from 800 to 1,100 Da. These compounds are produced by species belonging to several different genera of cyanobacteria including *Microcystis, Planktothrix, Anabaena, Nostoc*. In addition to the microcystins, many strains from these genera have been shown to produce other bioactive peptides like aeruginosins, anabaenopeptins and oscillamides [28].

Planktonic cyanobacteria, including unicellular colony forming *Microcystis* and the filamentous, nonheterocystous *Planktothrix,* commonly form toxic blooms in highly productive freshwater ecosystems worldwide [15].

The enzymes responsible for the biosynthesis of MC are encoded by the nonribosomal peptide synthesis NRPS pathway [17]. The interspecific and intraspecific variability in the production of bioactive nonribosomal peptides is known to be high in strains or colonies of cyanobacteria such as species of *Microcystis* or *Planktothrix* isolated from water samples [5, 28].

Laboratories worldwide and in several European countries studied the microcystin pattern of some species of bloom forming cyanobacteria, and the most potent species seems to be from the *Microcystis* and *Planktothrix* genera [8].

Previously, we have reported on the occurrence of cyanobacterial toxins and toxic waterblooms with interesting conditions and consequences in Hungary in several cases [1, 19, 20, 22].

In this study, *Microcystis aeruginosa* or *Planktothrix agardhii* containing cyanobacterial mass samples were collected and investigated from a variety of lakes, ponds and reservoirs in Hungary. The toxicity of the waterbloom-samples and the MC's concentration were also measured. The presence of genes linked to the biosynthesis of microcystins was analyzed using several primer pairs and the diversity of cyclic heptapeptides was also analyzed by MALDI-TOF MS to investigate patterns of production.

MATERIALS AND METHODS

Sources of samples

The samples were collected during 2005–2010 in ponds and lakes situated in the Charpathian basin in Hungary (Fig. 1). Information regarding the source of the samples is provided in Table 2. All the sampling sites are used for recreation and/or drinking water and operated as fishing ponds. All the samples were collected from visible floating mass of algae caused by *Microcystis aeruginosa* or *Planktothrix agardhii*. Bloom material was collected by 50 μm mesh size plankton net. Samples were disrupted by freezing and thawing several times (at least 5 times) and than lyophilised and the materials kept at –20 °C until extractions were made. *M. aeruginosa* and *P. agardhii* were identified according to the morphological criteria described in Komarek [6].

Fig. 1. Map of Hungary with the sampling sites. Symbols ● indicate localities where samples of *M. aeruginosa* were collected [1. Velencei, lake; 2. Bárdos, pond; 3. Nagycsécs, pond; 4. Kis-Balaton(3T), reservoir; 5. Ónod, pond; 6. Békés, pond; 7. Balaton, lake; 8. Gyula, ornamental lake; 9. Doboz, pond; 10. Kis-Balaton(2T), reservoir]. Symbols ○ indicate the locality where samples of *P. agardhii* were collected [11. Várpalota, pond; 12. Gyula, pond; 13. Hajdúhadház, pond; 14. Kis-Balaton(4T), reservoir]

Toxicity of the samples

The Blue-Green Sinapis Test (mustard test, BGST) was performed as described earlier by our laboratory with minor modifications [21]. This test is a well-known biotest for MC detection because it is a simple, efficient and selective method as described by Marsalek and Blaha [10]. Briefly, white mustard seeds were sterilized with 5% H_2O_2 under laminar hood and imbibed overnight in the dark (16 h). The imbibed seeds were placed on the surface of a 1% plant nutrient supplemented agar (Bacto) layer (100 ml) and complemented with cyanotoxin-containing samples in the wells of microtiter plates in parallel (one seed per well). The waterbloom samples were mixed with water (10 mg in 500 µl water) and this solution was diluted into wells of microtiter plates before adding the plant nutrient-containing agar solutions to the wells. The seeds on the microtiter plates (without lid) were grown in a sterile metal box in the dark at 28 °C. The hypocotyl length of etiolated seedlings (practically plant length) was measured after 3 days of growth and the mean length and SD of hypocotyls were calculated. Fifty percent inhibition of plant growth (IC_{50}) was calculated when necessary. Throughout the experiments, white mustard seeds (*Sinapis alba* L. convar. Budakalászi sárga) were used.

Quantification of the MCs

The toxin concentration of the extracts was determined by micellar electrokinetic chromatography (Fig. 2) as we published earlier [19] (MEKC) (Prince CEC-770 instrument; polyimide coated fused silica capillary [Supelco, 60 cm 9 50 µm id., effective length: 52 cm]; hydrodynamic injection 100 m bar 9 s^{-1}; applied voltage:

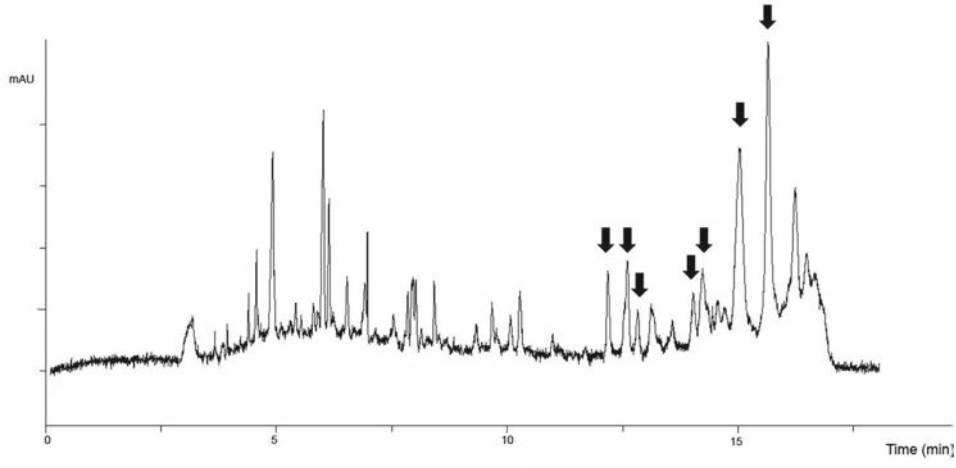

Fig. 2. The result of the applied MEKC method: the cyanotoxin pattern of the collected Microcystis cell from Kis-Balaton(3T) reservoir. The arrows show the microcystin peaks on the electropherogram

+25 kV; 25 mM sodium-tetraborate–100 mM SDS buffer, pH: 9.3; detection by diode-array detector at 239 nm). Dax 3D 8.1 software was used for the evaluation of the electropherograms. MC-LR was used as a standard purified in our laboratory, purity approx. 97% (HPLC-DAD).

MALDI-TOF MS analysis

Lyophilized waterbloom samples were screened for microcystins by MALDI-TOF MS. Five mg of lyophilized sample was mixed with 200 µl of 50% aqueous methanol, sonicated for 5 min, and allowed to stand for 1 h. The samples were examined in positive-ion mode using a Bruker Biflex MALDI-TOF mass spectrometer equipped with delayed-ion extraction. A 337-nm nitrogen laser was used for desorption/ionization of the sample molecules. Spectra from multiple (at least 100) laser shots were summarized using 19-kV accelerating and 20-kV reflectron voltage. External calibration was applied using the $[M+Na]^{+1}$ peaks of malto-oligosaccharides dp 3–7, m/z values 527.15, 689.21, 851.26, 1013.31, and 1175.36, respectively. The measurement was performed in 2,5-dihydroxybenzoic acid (DHB) matrix, by mixing 0.5 ml of matrix solution with 0.5 ml of sample on the sample target and allowing it to dry at room temperature. DHB matrix solution was prepared by dissolving DHB (10 mg) in a mixture (0.5 ml) of ethanol and water (1:1, v:v). The compounds were identified on the basis of the mass of $[M+H]^+$ peak. After determination of mass values, post-source decay (PSD) measurements were performed directly from the same sample on the template and microcystins and other peptides identified by PSD fragment structure analysis.

DNA extraction and PCR analyses

Total genomic DNA extraction was achieved by the phenol–chloroform method [12]. PCR was used to analyze the presence or absence of those gene regions (Table 1), which are important parts of the gene cluster responsible for microcystin biosynthesis [13, 17]. PCR amplification was accomplished as described [12]. PCR products were separated by 1.5% agarose gel in 1× TAE buffer. The gels were stained with ethidium bromide and photographed under UV transillumination. The molecular mass marker was GeneRuler TM 1 kb DNA Ladder (Fermentas), and the photo preparation and the analysis of results were achieved using a Cleaver GelDoc system.

RESULTS

Ten *Microcystis aeruginosa* and four *Planktothrix* waterbloom samples were collected in the Charpathian basin region in Hungary. Ten samples from a variety of water bodies conformed to the taxonomic description of *M. aeruginosa* and four to *P. agardhii*. Visible algal mass and floating colonies occurred in the sampling site and more than 90% was the frequency of the dominant species. The results presented in Table 2 show that *M. aeruginosa* is more toxic bloomforming cyanobacteria than *P. agardhii* in Hungary. The calculated IC_{50} was 245 μg in the most toxic sample collected at Bardos pond. In four cases, the IC_{50} was undetectable: the *P. agardhii* containing samples from Kis-Balaton reservoir, Hajdúhadház and Gyula; and the *M. aeruginosa* containing sample from Ónod pond. The IC_{50} of the samples correlated with the amount of microcystin content measured by capillary electrophoresis calculated for MC-LR equivalent. The highest MC content was measured in the Bardos pond sample, which concentration suits for another phenomenon published by our laboratory when a Microcystis bloom occurred. In the four cases, where the samples were not toxic, we detected the lowest MC concentrations. Twelve different chemical forms of microcystin were detected in the fourteen samples. Of those strains that produced at least one form of microcystin, 7 (70%) contained MC-LR in *M. aeruginosa,* and all the three samples of *P. agardhii* contained Asp3MC-LR. The most common microcystins in terms of occurrence were in MC-LR 7, MC-RR 6, MC-YR 4, MC-WR 4, Dha7-RR 4, and Asp3MC-LR 3 cases. MC LL, LF, Dha7-FR were relatively uncommon (Table 2). Among the strains that contained microcystins, between one and six chemical forms were detected in the collected samples (Table 2). All of the fourteen collected samples gave positive results for the presence of one of the *mcy* genes with PCR products with sizes between 425 and 955 bp, respectively, indicating the presence of the genes implicated in the production of microcystins. These results are in good agreement with capillary electrophoresis and MALDI-TOF MS analysis, as shown in Table 2, since the samples containing these genes contained detectable amounts of microcystin in the CE assay and at least one chemotype of this toxin in the MALDI-TOF MS analysis. The only exceptions were the Kis-Balaton(4T) reservoir sample of *P. agardhii*. This sample was found to contain

Table 1
PCR primers used in this study

Gene	Primer pair	Sequence (5'-3')	$T_{annealing}$ (°C)	Size (bp)	Reference
mcyB	2156-F	ATCACTTCAATCTAACGACT	52	955	Mikalsen et al. [13]
	3111-R	AGTTGCTGCTGTAAGAAA			
mcyC	PSCF1	GCAACATCCCAAGAGCAAAG	52	674	Ouahid et al. [14]
	PSCR1	CCGACAACATCACAAAGGC			
mcyD1	PKDF1	GACGCTCAAATGATGAAAC	52	647	Ouahid et al. [14]
	PKDR1	GCAACCGATAAAAACTCCC			
mcyD2	PKDF2	AGTTATTCTCCTCAAGCC	52	859	Ouahid et al. [14]
	PKDR2	CATTCGTTCCACTAAATCC			
mcyE	PKEF1	CGCAAACCCGATTTACAG	52	755	Ouahid et al. [14]
	PKER1	CCCCTACCATCTTCATCTTC			
mcyG	PKGF1	ACTCTCAAGTTATCCTCCCTC	52	425	Ouahid et al. [14]
	PKGR1	AATCGCTAAAACGCCACC			
mcyB	mcyB.fw	ATTACAGCAGAGAAAATCCAAGCA	61	555	Mbedi et al. [12]
	mcyB.rew	TCGCAATAGCGGGGATCA			

Table 1 (cont.)

Gene	Primer pair	Sequence (5'-3')	$T_{annealing}$ (°C)	Size (bp)	Reference
mcyCJ	mcyCJ.fw	TTGGATACAAGCGACAAAAGG	59	524	Mbedi et al. [12]
	mcyCJ.rew	TCTCCAGCTTGAAGTTCTGC			
mcyE	mcyE.fw	TTACCTAATTATCCCTTTCAAAG	50	589	Mbedi et al. [12]
	mcyE.rew	CAATGGGTAAGGTTTGCTT			
mcyEG	mcyEG.fw	GAATTCATTTTTGTTGAGGAAGG	61	775	Mbedi et al. [12]
	mcyEG.rew	AGAAAACAAGCCCAGAGTGC			
mcyHA	mcyHA.fw	TTAGATGAAGCCACCAGTGC	59	540	Mbedi et al. [12]
	mcyHA.rew	GATTAAAAATTGAATAGCTGCTAGG			
mcyT	mcyT.fw	CCCAATCTAACCCCAACTGC	57	747	Mbedi et al. [12]
	mcyT.rew	CAATAGCGATTTTCCCAAGC			
mcyTD	mcyTD.fw	ATCCGCCCATACTGTGACC	61	763	Mbedi et al. [12]
	mcyTD.rew	GATTTTGCCCGGTTTACTCC			

Table 2
Place of sampling, dominant species, microcystin content (MC-LR equivalent), toxicity (IC_{50}), identified MC variants and the detected *mcy* genes in Hungarian freshwaters

Sampling area	Species	MC conc. (mg/g)	IC_{50} µg	MC forms	MC genes
Velencei, lake	*Microcystis aeruginosa*	3.342	1134	LR, YR, WR	*mcyB, mcyC, mcyD1, mcyD2, mcyE, mcyG*
Bárdos, pond	*Microcystis aeruginosa*	15.701	245	Dha7-RR, RR, WR	*mcyB, mcyC, mcyD1, mcyD2, mcyE, mcyG*
Nagycsécs, pond	*Microcystis aeruginosa*	0.801	1522	LL, LR, RR	*mcyB, mcyD1, mcyE, mcyG*
Kis-Balaton(3T), reservoir	*Microcystis aeruginosa*	3.911	1044	[Asp3]Mcyst-LR, LR, Dha7-RR, RR, YR	*mcyB, mcyD1, mcyE, mcyG*
Ónod, pond	*Microcystis aeruginosa*	0.050	>3000	Dha7-FR	*mcyB, mcyE, mcyG*
Békés, pond	*Microcystis aeruginosa*	3.724	654	WR	*mcyB, mcyC, mcyD1, mcyD2, mcyE, mcyG*
Balaton, lake	*Microcystis aeruginosa*	2.843	934	LR, RR	*mcyB, mcyG*
Gyula, ornamental lake	*Microcystis aeruginosa*	2.111	1100	LR, Dha7-RR, RR, YR, WR	*mcyB, mcyD1, mcyE*
Doboz, pond	*Microcystis aeruginosa*	0.902	1454	LF, LR	*mcyC, mcyD1, mcyE, mcyG*
Kis-Balaton(2T), reservoir	*Microcystis aeruginosa*	4.238	839	LR, Dha7-RR, RR, YR	*mcyB, mcyC, mcyD1, mcyD2, mcyE, mcyG*
Várpalota, pond	*Planktothrix agardhii*	3.227	916	[Asp3]Mcyst-LR, Asp3, Dha7-RR, Dha7-RR	*mcyA, mcyB, mcyCJ, mcyE, mcyEG, mcyHA, mcyT, mcyTD*
Gyula, pond	*Planktothrix agardhii*	0.020	>3000	[Asp3]Mcyst-LR	*mcyB, mcyE, mcyEG, mcyHA, mcyT, mcyTD*
Hajdúhadház, pond	*Planktothrix agardhii*	0.012	>3000	[Asp3]Mcyst-LR	*mcyB, mcyEG, mcyT, mcyTD*
Kis-Balaton(4T), reservoir	*Planktothrix agardhii*	0.010	>3000	–	*mcyT*

microcystin synthetase gene *(mcyT)* but microcystins could not be detected by MALDI-TOF MS. Several samples lacking some of the investigated *mcy* genes were found to contain microcystin(s) (Table 2).

DISCUSSION

Microcystins and their production have been studied intensively during the last two decades, because of the potential and real threats of these cyanobacterial toxins. Many regions and countries monitor the presence of the potent toxinproducing cyanobacterial strains in waters and investigate their hazard and risk for human population [2, 3, 9, 11]. In our study we analysed fourteen waterblooms occurring in shallow lakes in Hungary. The aim was to obtain more information on real bloom material caused by two well-known bloom-forming cyanobacterial species in Hungary. Two of the sampling sites were the two largest recreational shallow lakes in Hungary. Lake Balaton is a freshwater lake in the Transdanubian region of Hungary and it is the largest lake in Central Europe. Although the water is primarily characterized by the presence of nitrogen fixing cyanobacteria [24], we observed a local *Microcystis* bloom in 2010. The collected sample was positive for microcystin and 2.843 mg g^{-1} microcystin content was detected. Lake Velencei is the third largest lake in Hungary and it is a popular holiday destination. The first observation of hepatotoxic *Microcystis* strain from Hungary was reported from this shallow lake by Kós et al. [7]. The *Microcystis aeruginosa* BGSD 243 was isolated from that bloom and two main microcystin the –LR and –YR was identified form the strain. Nearly 20 years later we collected a waterbloom material from that water and the identified forms were the LR, YR and WR. The Kis-Balaton reservoir system, a natural reserve area consisting of the upper and lower reservoirs, is located near the mouth of the Zala River. One of the main tasks of the reservoir is to protect the Lake Balaton from the higher nutrient load (to reduce phosphorus loading), consequently the waterblooms of several cyanobacterial species is common [16]. In two cases, we observed blooms caused by *M. aeruginosa* and at the sampling site (4T) where we collected *P. agardhii* samples. There are plenty of angling waters in Hungary, the two big rivers of the Pannonian basin, the Danube and the Tisza cross the country with their backwaters and several mine-lakes, many smaller natural and artificial shallow lakes are famous in Hungary. In this study we found 6 cases of *M. aeruginosa* blooms and 3 cases of *P. agardhii* blooms in this type of water. The highest MC concentration was detected in an artificial angling pond in Gyula where the MC level is similar to another bloom phenomenon in an artificial garden pond reported by us [1]. Total microcystin concentrations varied between 0.010 and 15.701 mg microcystin-LR equivalents g^{-1} DW (Table 2). A few studies reported total concentrations of microcystins over 10 mg g^{-1} DW, in the Bautzen reservoir (Germany) with microcystin equivalents up to 14.7 mg g^{-1} DW and in waters of northeastern Wisconsin with microcystin equivalents up to 12.8 mg g^{-1} DW [3] and in Lake Baringo in Kenya [2].

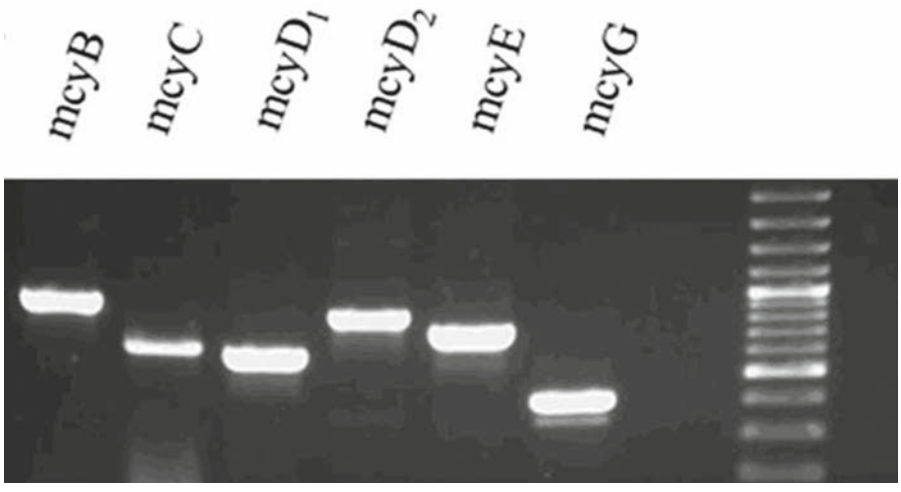

Fig. 3. PCR amplification of the selected gene from the *mcy* gene cluster in the *Microcystis* cell mass collected from Bárdos, pond (Molecular marker: GeneRuler TM 1kb DNA Ladder; Fermentas)

Applying matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a great variety of microcystins can be detected with accuracy, resulting in bloom-specific MC pattern [27]. Since we were especially interested in the MC diversity of the Hungarian blooms of *M. aeruginosa* and *P. agardhii* we focused on the cyclic heptapeptid content of these samples. Our investigation revealed that our *M. aeruginosa* samples are producers of microcystins. Microcystin-LR, microcystin-RR and microcystin-YR were found as a frequent form in this species. These types of microcystins are the dominant microcystins in blooms of *Microcystis* spp. [4]. Interestingly, where the highest MC content was measured by CE (supported by Sinapis Test) the main MC forms were the Dha7-RR, -RR and -WR. Worldwide, more than 60 structural variants of microcystins have been identified and in 50–75% of the isolated cyanobacterial blooms, toxins can be found. In several investigations from many countries (Germany, Czech Republic, Korea, Finland) 80 to 90% of the samples containing *Microcystis* also contained microcystins [23]. From all the *Microcystis* bloom samples we collected in Hungary more recently and in the last decades, we could detect some form of MC. The composition of MCs in Hungarian waters were similar to those in field samples and *Microcystis* strains from Japan (variants of MC-LR, -RR, and -YR mainly dominated) and MC-RR was also more abundant in the majority of *Microcystis* strains from Japanese lakes [29]. In general, MC-LR was proposed to be the major toxin in bloom samples and strains from temperate waters of Europe and Canada [4], and we can confirm this fact by our results.

The genus *Planktothrix (Oscillatoria)* [6] occurs frequently in lakes and reservoirs in the temperate zone of the Northern Hemisphere. The species *P. rubescens* and *P. agardhii* differ in pigmentation and planktonic life-form: *P. rubescens* is typically found in deep, stratified and oligo- to mesotrophic waters, while *P. agardhii* occurs in

shallow and more eutrophic waters [11]. As we observed, the *P. agardhii* is a common bloomforming species from this genus in Hungary. *P. rubescens* caused waterbloom with high MC content was seen and investigated by us in only one case (under publication). While MCs were detected in all the *M. aeruginosa* blooms, only one of the *P. agardhii* samples contained more than 1 mg g^{-1} MCs. Compared to the *M. aeruginosa*, this species less frequent and smaller concentrations of MC were detected. In *Planktothrix* blooms and strains, the dominant microcystin variants are [Asp3] variants of MC-LR, Mcyst-RR [8], as was the case in our toxin-producing samples.

Microcystin isoforms are an extremely large family of cyclic heptapeptid toxins, that are produced by a wide variety of cyanobacteria, including *Microcystis* and *Planktothrix* species [15, 28]. These secondary metabolites are synthesized non-ribosomally, similarly to linear, cyclic, and branched-cylcic peptides, including potent drugs, such as penicillin, cyclosporine and vancomycin [13].

They are synthetized by a giant enzyme complex containing non-ribosomal peptid synthetase (NRPS) and polyketide synthetase (PKS) modules, contain distinct activation domains (A-domains), thiolation domains (T-domains), condensation domains (C domains), metyltransferases (M-domains), epimerases (E-domains), heterocyclization domains, formyl-transferase domains, N-methylation domains, oxidation domains, reduction domains, phosphopantetheine-protein transferases, O-methyl transferases, dehydrogenases, thioesterase (TE domains), halogenases, and ABC transporters.

Functional units are encoded by the 55 kb *mcy* gene cluster, which is responsible for biosynthesis of microcystins. The difference between the *Mcy* cluster of *M. aeruginosa* and the *mcy* cluster of *P. agardhii* is *mcyT,* which can be found only in *Planktothrix* species. *Mcy* genes in *Microcystis* are organized in two operons, *mcyF* and *mcyI,* that are lacking in *Planktothrix* [26].

Although several chemo-, and genotypes of the same bloomforming species can be detected from a natural bloomsample the samples were tested using a range of primers linked to the biosynthesis of microcystin. All the primers gave positive result in the highest concentration of *myc* in *M. aeruginosa* and *P. agardhii* samples. Although all the *M. aeruginosa* sample MC positive, several genes could not be detected in these samples. This result could be related to the fact, that several primers are adequate only for unialgal laboratory samples and it's also important to note in real samples the toxin-producing genotypes could be present only in low frequency, as most cyanobacterial blooms are not formed by a single or a few geno- and chemotypes [25, 27].

On the other hand, microcystin production was higher in those samples, in which all genes in the *mcy* cluster were detectable (Fig. 3). If one of the genes was absent, the MC concentration was lower. As there are microcystin-producing and non-microcystin-producing strains, we can distinguish essential and non-essential genes for producing. The most essential genes for microcystin syntesis are *mcyABC* and *mcyE* as described earlier by Tillett et al. [18] and Mbedi et al. [12]. According to our data, *mcyT* was detected in producers and non-producers too, so *mcyT* and *mcyTD* regions are inadequate for the detection of microcystin-producing *Planktothrix* in field samples but can be applied to separate *Planktothrix* from *Microcystis* [12].

After some previous publications, this current result confirms that the *M. aeruginosa* and the MC containing *P. agardhii* represent a significant water quality problem in waters and in water bodies used for recreational purposes in Hungary.

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