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Detection and identification of potentially toxic cyanobacteria in Polish water bodies

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The main goal of this study was to determine the distribution of potentially toxic cyanobacteria in 39 selected Polish water bodies. From the water bodies with blooms and also from those in which blooms were not visible 87 samples were investigated. For the first time samples from ponds localized in villages with high agricultural activities were included. Lakes for which microcystin concentrations had been determined before were included as a reference for the research. The detection of cyanobacteria was conducted by microscopic observation as well as by PCR amplification of the *rpoC1* gene fragment. Cyanobacteria were present in 75 out of 87 samples. The presence of potentially toxic cyanobacteria was detected by amplification of the *mcyB* and *mcyE* genes, which are involved in the biosynthesis of microcystins. Both genes were detected in 7 out of 9 blooms investigated. In the case of samples collected from water bodies in which blooms were not observed, the *mcyB* and *mcyE* genes were detected in 20 out of 36. In order to identify the cyanobacteria occurring in selected reservoirs, 16S plus ITS clone libraries were constructed. The method allowed distinguishing 18 different genotypes. After sequence analysis, cyanobacteria belonging to genera *Microcystis, Planktothrix, Anabaena, Pseudanabaena, Synechocystis, Synechococcus* and *Woronichinia* were identified. Results confirmed the usefulness of the *rpoC1* and *mcy* genes for monitoring water bodies and detection of potentially toxic cyanobacteria. Application of molecular markers allowed detecting potentially toxic cyanobacteria before the bloom was visible. This is the first comprehensive study concerning cyanobacteria present in different types of Polish water bodies performed using molecular markers.

Keywords: *Cyanobacteria*, toxins, microcystins, *rpoC1*, *mcyE*, *mcyB*, 16S rRNA

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INTRODUCTION

Cyanobacteria (blue-green algae) are one of the largest group of Gram-negative, chlorophyll-*a* containing, photosynthetic prokaryotes. They are morphologically diverse and widespread in both marine and terrestrial habitats. Cyanobacteria have been traditionally distinguished on the basis of phenotypic (morphological and physiological) properties. Because of limitations of this approach, it is commonly accepted that the modern cyanobacterial taxonomy should be supplemented by molecular methods. Molecular techniques have facilitated culture-independent studies and identification of microorganisms collected from environment and difficult to culture. In addition, molecular methods do not require axenic cultures. The importance of the polyphasic approach, combining morphological observations with molecular data in improving the taxonomy of cyanobacteria is unquestionable.

The *rpoC1* gene, coding for the γ subunit of RNA polymerases, is recommended as a molecular marker for the detection and identification of cyanobacteria (Jameson *et al.*, 2008; Yoshida *et al.*, 2008; Valerio *et al.*, 2009). The DNA-dependent RNA polymerase of cyanobacteria contains a unique core subunit γ, which is not present in the RNA polymerases of other eubacteria (Bergsland & Haselkorn, 1991). This subunit is a common and unique feature of the core RNA polymerase of cyanobacteria and plastids, so the *rpoC1* gene coding for this subunit is a good molecular marker for detection and identification of these microorganisms.

Certain species of cyanobacteria are able to produce cyanotoxins, which can be hazardous for humans and animals. Cyanotoxins are arranged in four classes according to their specific activities: the neurotoxins (anatoxin, anatoxin-a(s), saxitoxin), hepatotoxins (microcystins, nudularins, cylindrospermopsins), dermatotoxins and cytotoxins (lyngbyatoxin-a, aplysiatoxins), endotoxins (LPS, lipopolysaccharides) (Codd *et al.*, 2005).

In Polish water reservoirs, the most commonly detected cyanotoxins are microcystins, which can be produced by strains of the distantly related cyanobacterial genera: *Microcystis*, *Anabaena*, *Planktothrix,* and more rarely *Anabaenopsis*, *Hapalosiphon* and *Nostoc* (Jurczak *et al.,* 2004; Izydorczyk *et al.,* 2005; Mankiewicz *et al.,* 2005; Palus *et al.*, 2007; Mazur-Marzec, 2008, Mankiewicz-Boczek *et al.*, 2009; Gągała *et al.*, 2010, WIOŚ Gdańsk, 2010).

Microcystins can be accumulated in organs of plants and animals (fish, water birds, turtles) and create a potential risk to human health in case of their consumption (Chen *et al.*, 2009). Microcystins are potent inhibitors of eukaryotic protein phosphatases types 1 and 2A and act as tumor promoters (Codd *et al.*, 2005). It has been reported that 50–75% of cyanobacterial blooms release

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Abbreviations: Adda, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid; BLAST, Basic local alignment search tool; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization/time of fligh; MS, mass spectrometry; PPIA, protein phosphatase inhibition assay; WIOŚ Regional Inspectorate for Environment Protection.

the toxins in the water, and even up to 95% of blooms formed by *Microcystis* are toxic (Sivonen, 1996).

Presently the main efforts are concentrated on the monitoring and prediction of the appearance of cyanobacterial toxic water blooms. Because cyanobacteria with the potential to produce toxins have no characteristic morphological traits, their distinction from those that are nontoxic is not possible by microscopic observations. Routinely the detection of microcystins is carried out by enzyme-linked immunosorbent assay (ELISA), phosphatase inhibition assays (PPIA) as well as high performance liquid chromatography (HPLC) or mass spectrometry (MS), matrix-assisted laser desorption/ionization/time of fligh (MALDI-TOF). However, such analyses do not indicate the potential of the appearance of toxins and do not allow the identification of the cyanobacteria producing toxins.

Polymerase chain reaction (PCR)-based detection of genes involved in the synthesis of microcystins (*mcyB* and *mcyE*) is rapid and cost-effective. Assays such as real-time PCR, multiplex-PCR, semi-nested-PCR and microarray DNA chip technology have increased the sensitivity and specificity of the PCR. Recently, molecular methods are more often used for the detection of microcystin-encoding genes (Ouellette *et al.*, 2005; Sivonen, 2008; Ha *et al.*, 2009).

Molecular techniques allow identification of cyanobacteria, including the microcystin-producing cyanobacteria and also provide information about the presence of genes coding for the toxins in water bodies before populations of cyanobacteria develop fully (Codd *et al.*, 2005). This is essential for health protection of water users and for the conservation of water resources and habitats.

Microcystins are cyclic heptapeptides, which are synthesized nonribosomally by multifunctional enzymes that include polypeptide synthetase and polyketide synthase modules. This group of toxins is encoded by microcystin synthetase (mcy) gene cluster, which contains 55 kb of DNA (Tillett *et al.*, 2000; Rouhiainen *et al.*, 2004). More than 70 microcystin structural variants are known (Codd *et al.*, 2005).

The aims of this study was to check usefulness of the application of molecular methods for detection of cyanobacteria by PCR amplification of the *rpoC1* gene fragment and monitoring of the presence of toxic cyanobacteria by PCR amplification of genes coding for microcystin synthetase in 36 selected Polish water bodies.

MATERIALS AND METHODS

Samples collected from water bodies. Sampling were conducted from nine water bodies Bnińskie Lake, Bytyńskie Lake, Klasztorne Duże Lake, Trzesiecko Lake, Tuchom Lake, pond in Drzewce, pond in Gumnisko, pond in Mniewo and Sulejów Reservoir during bloom of cyanobacteria and also, when mass occurrence of cyanobacteria was not observed (Table 1). Samples gathered when bloom was not visible were from 20 lakes: Bielsko, Chodzieskie, Ciemino, Dobre, Dolsko, Jeleń, Jelonek, Karlikowskie, Kamienickie, Kielno, Klasztorne Duże, Krąg, Mausz, Ostrzyckie, Raduńskie Dolne, Straszyn, Trzesiecko, Tuchom, Wysoka and Żur; five reservoirs: Dojlidy, Sulejów, Zemborzyce, Nadarzyce and Lagoon of Wistula; three ponds in Gumnisko, Kowalewo, and Mniewo; six rivers: Gwda, Łeba, Noteć, Słupia, Tralalka and Radunia; Gulf of Gdańsk (Orłowo, Puck, Gdynia and Osłonino); Baltic Sea (Orzechowo and Rowy) (Table 1).

Cultures of cyanobacteria. Strains *Microcystis* sp. PCC 7806, *Microcystis* sp. PCC 7941, *Microcystis* sp. PCC 7005, *Planktothrix* sp. PCC 7821, *Planktothrix* sp. PCC 7805, *Cyanobium* sp. PCC 6307, *Gloeothece* sp. PCC 6501 and *Dermocarpella* sp. PCC 7326 were obtained from the Pasteur Culture Collection, Paris, France. These strains of cyanobacteria were grown in BG11, BG112N10 and Z8 medium (Rippka, 1988) at 20 °C and under photoperiod conditions (18 h day: 6 h night).

Sampling and DNA extraction. To examine the community of cyanobacterial populations in blooming water, samples were collected early morning from the surface and a depth of 0.5 m in the offshore zone. Nine water samples were collected during blooms of cyanobacteria (Table 1). The dominant cyanobacteria were identified directly in samples using a light microscope (Nicon, Eclipse E600). For DNA preparation, cells were harvested by centrifugation and suspended in TE buffer (50 mM Tris/HCl, 40 mM EDTA, pH 8.0). Cyanobacterial DNA was extracted using SDS/proteinase K.

Seventy-eight water samples were collected from 36 water reservoirs in which cyanobacterial blooms were not observed. For microscopic identification 1–2 ml samples of water were centrifuged, pellet was resuspended in 100 μl volume of water and subsamples placed into a nanoplankton counting chamber filamentous, colonial and single-celled algal forms were identified to the generic level according to taxonomic keys based on cell or colony morphology (Komarek & Anagnostidis, 2000; 2005).

For DNA analysis 500 ml of each sample was filtered by using two kinds of filters (Φ 8 μm and 0.2 μm). This procedure allowed separation of colony-forming and filamentous cyanobacteria fraction from single-cell forms. Filters with collected cyanobacterial cells were frozen at –20 °C. Cyanobacterial DNA was extracted from cells using the protocol described by Waleron *et al.* (2007).

DNA amplification. The detection of cyanobacteria in water samples was conducted by amplification of the *rpoC1* gene fragment. PCR products were amplified using primers rpoC3 (5'-CCCGCNAARGAYTGGGAATG-3') and rpoC4 (5'-GCTTCYTGCARCATCCGYTTYTC-3') to produce a 729-base pair (bp) product. The amplification reaction was performed with the Biometra Thermocycler, as follows: initial denaturation step (94 °C, 5 min), followed by 36 cycles of denaturation (94 °C, 50 s), annealing (53 °C, 1 min) and extension (72 °C, 2 min), with a final extension step (72 °C, 7 min). Each reaction was carried out in a volume of 50 μl containing: 5 μl of 10x reaction buffer (Fermentas), 2 mM Mg^{2+}, 0.25 mM of each of the four deoxynucleoside triphosphates (dNTPs), 5 μl of BSA, 10 mg/ml, 20 pmol of each primer, 50–100 ng of DNA and 1 U of recombinant *Taq* DNA polymerase (MBI Fermentas). The obtained products were electrophoretically separated in 1.2% agarose gel at 7 V/cm for 2 h in TAE buffer and subsequently stained with ethidium bromide (0.5 μg/ml) and visualized under UV. In order to detect potentially toxic cyanobacteria, *mcyB* and *mcyE* gene fragments were amplified. For each sample, two separate PCRs were conducted. The 320 bp fragment of the microcystin peptide synthetase B gene (*mcyB*) was obtained with Mcy F1 and Mcy R1 primers (Nonneman & Zimba, 2002). Fragment of

mcyE gene (809–812 bp) was amplified using primers mcyE-F2 and mcyE-R4 (Vaitomaa *et al.*, 2003). Mcy F1 and Mcy R1 are specific primers to detect potentially microcystin-producing *Microcystis*, whereas mcyE-F2 and mcyE-R4 are general primers to detect different potentially toxic strains of cyanobacteria. Both PCR reactions were conducted according to the protocols previously described by Nonneman and Zimba (2002) and Vaitomaa *et al.* (2003). The obtained products were electrophoretically separated under the same conditions as described for the *rpoC1* gene.

Amplification of 16S rRNA + ITS + 5' end of 23S rRNA gene was carried out as the first step to generate clone libraries. Primers CYA359F, 23S30R and PCR parameters were as described by Nübel *et al.* (1997) and Taton *et al.* (2003). Products were electrophoretically separated as above.

Clone library generation and analysis. For each library, three separate PCR reactions were carried out. The aim of this step was to minimize the bias caused by the PCR reaction. Obtained products were mixed and purified with a Clean-Up system (A&A Biotechnology), according to Vendor's instructions. Clone libraries were created using pGEM-T® Easy Vector (Promega) and Strata Clone™ PCR Cloning Vector pSC-A cloning kit. White and light-blue transformants were purified by streaking and screened for the presence of insert DNA by performing colony PCR with primer pair CYA359F and 23S30R for amplification of 16S rRNA + ITS + 5' end of 23S rRNA gene. The amplification conditions were as described above.

Restriction fragment length polymorphism analysis (RFLP) of cloned PCR product of *rrn* fragment (16S rRNA + ITS + 5'end of 23S rRNA).

For RFLP analysis of the *rrn* amplicon, restriction endonuclease *Msp*I (Fermentas) was applied. Digestion was carried out 4 h at 37°C. Each reaction was carried out in a volume of 30 µl containing: 0.25 µl *Msp*I (10 U/µl), 3 µl of 10×buffer B (Promega) and sterile water (Fluka). Fragments were separated in 2% agarose gel electrophoresis and visualized as described above. The genotypes with similar RFLP patterns were grouped and one clone of each genotype (each RFLP pattern) was sequenced.

Plasmid isolation and sequencing. Plasmids containing *rrn* fragments differentiated by RFLP were isolated with Plasmid Mini (A&A Biotechnology) miniprep system. Before sequencing, the concentration of plasmid DNA was measured with NanoDrop (NanoDrop Technologies, Inc.). The DNA was further diluted with sterile water to give a concentration of 70–80 ng/µl. Sequencing of samples that gave different RFLP patterns was conducted using the CYA359F primer (Nübel *et al.*, 1997). Sequencing was carried out in the Pomeranian Science and Technology Park in Gdynia, Poland.

Analysis of sequence data. Obtained sequences were manually edited with BioEdit software (http://www.mbio.ncsu. edu/BioEdit/bioedit.html), which was incorporated for sequence alignment based on automated ClustalW alignment. Basic local alignment search tool (BLAST) algorithm was conducted through the NCBI Web site (http://www.ncbi.nih.gov). For each cloned sequence, the BLAST result with the highest sequence similarity was chosen (Table 2). The attribution of detected cyanobacterial sequences to particular genus was conducted according to definition of bacterial "species" and "genus" proposed by Stackebrandt and Goebel (1994). Therefore, the similarity value for the 16S rRNA gene sequence higher than 97.5% indicated that two cyanobacterial strains belong to the same genus.

RESULTS

Microscopic observation and identification of cyanobacteria

On the basis of morphological observation of bloom samples, two genera of cyanobacteria were identified. In lakes Bnińskie, Bytyńskie, Klasztorne Duże and in the pond of Gumnisko thin, filamentous, nonheterocystous cyanobacteria belonging to genus *Planktothrix* were observed. In the remaining water bodies, we identified colonies of *Microcystis* (Table 1).

The microscopic analysis of samples collected from water bodies in which a mass occurrence of cyanobacteria was not observed, indicated the presence of cyanobacteria in 54 samples (Table 1). Potentially toxic cyanobacteria were identified in 19 water bodies. *Microcystis, Planktothrix* and *Anabaena* were identified, respectively, in 12, 7 and 13 water bodies (Table 1).

Detection of cyanobacteria using molecular markers

The presence of cyanobacteria in water bodies was analyzed using PCR amplification of the *rpoC1* gene fragment. The occurrence of cyanobacteria was revealed in 75 samples (86.2%) collected from 39 different water bodies including all nine blooming water bodies (100%) and 34 non-blooming water bodies (Table 1).

To determine the presence of potentially toxic (toxigenic) cyanobacteria in water bodies with blooms the amplification of *mcyB* and *mcyE* genes required for synthesis of microcystins was applied (Table 1). These genes were also amplified from cultures of *Microcystis* sp. PCC 7806, *Microcystis* sp. PCC 7941, *Planktothrix* sp. PCC 7821, PCC 7805, whose ability to produce microcystins is well known (Table 1). As negative controls, DNA extracted from nontoxic strains: *Microcystis* sp. PCC 7005, *Cyanobium* sp. PCC 6307, *Gloeothece* sp. PCC 6501 and *Dermocarpella* sp. PCC 7326 was used as a template (Table 1).

The presence of *mcyB* was confirmed in 7 out of 9 water bodies and of *mcyE* in all water bodies in which cyanobacterial blooms were observed (Table 1). Among the 36 non-blooming water bodies the presence of rpoC1 PCR product was confirmed in 34 (84.6%). Cyanobacteria that have the potential for the production of toxins were identified by detection of *mcyE* and *mcyB* genes in 41 samples collected from 20 water bodies (Table 1). It is worth underlining that the *mcyB* or/and *mcyE* PCR products were not detected in samples in which the presence of cyanobacteria was not confirmed by amplification of the *rpoC1* gene fragment and microscopic observation (Table 1).

The analysis of one sample from Mausz Lake (July 2004) indicated the presence of potentially toxic cyanobacteria other than *Microcystis,* because only the *mcyE* gene was amplified (Table 1). Both toxicity markers, *mcyE* and *mcyB,* were detected in only one out of five running waters, the Notec.

Monitoring of water bodies showed that in Klasztorne Duże, Ostrzyckie, Kielno and Lagoon of Wistula, the presence of toxic cyanobacteria was observed in samples collected between July and October, in Trzesiecko lake between June and August, in Tuchom

Table 1. Detection and identification of cyanobacteria and potentially toxic cyanobacteria

No.	Name and nature of water bodies	Date of sampling	Presence of cyanobacteria					Microcystin's concentration Sampling Date (References)
			Microscopic observation	PCR *rpoC1*	PCR *mcyE*	PCR *mcyB*	Identification of potentially toxic cyanobacteria	
Reference strains								
			Microcystis sp. PCC 7806	+	+	+	*mcyB, mcyE* — positive	
			Microcystis sp. PCC 7941	+	+	+	*mcyB, mcyE* — positive	
			Planktothrix sp. PCC 7821	+	+	–	*mcyE* — positive	
			Planktothrix sp. PCC 7805	+	+	–	*mcyE* — positive	
			Microcystis sp. PCC 7005	+	–	–	*mcy* — negative	
			Cyanobium sp. PCC 6307	+	–	–	*mcy* — negative	
			Gloeothece sp. PCC 6501	+	–	–	*mcy* — negative	
			Dermocarpella sp. PCC 7326	+	–	–	*mcy* — negative	
Samples collected during bloom:								
1	**Bnińskie Lake** agricultural and forestry land	20.09.2004	*Planktothrix*	+	+	–	*Planktothrix*	10.47 µg/L 20.09.2004 (Mankiewicz-Boczek *et al*., 2006)
2	**Bytyńskie Lake** agricultural and forestry land	20.09.2004	*Planktothrix*	+	+	–	*Planktothrix*	23.8, 34.6 µg/L 06.2007/09.2007 (Mankiewicz-Boczek *et al*., 2009)
3	**Klasztorne Duże Lake** urban land	11.08.2004	*Planktothrix Microcystis*	+	+	+	*Planktothrix Microcystis*	
4	**Trzesiecko Lake** agricultural and urban land	20.08.2004	+	+	+	+	*Microcystis*	
5	**Tuchom Lake** agricultural land	30.08.2003	*Microcystis Anabaena*	+	+	+	*Microcystis*	>5 µg/L 23.06.2003 (Mazur-Marzec, 2006)
6	**Pond in Drzewce** agricultural land	15.09.2004	*Microcystis Anabaena*	+	+	+	*Microcystis*	
7	**Pond in Gumnisko**	02.09.2004	*Planktothrix*	+	+	+	*Planktothrix*	
8	**Pond in Mniewo** agricultural land	10.08.2003	*Microcystis Anabaena*	+	+	+	*Microcystis*	
9	**Sulejów Reservoir** agricultural and forestry land	20.09.2004	*Microcystis*	+	+	+	*Microcystis*	4.67 µg/L 22.09.2004 (Mankiewicz-Boczek *et al*., 2006)
Samples collected from reservoirs in which bloom was not visible:								
1	**Bielsko Lake** agricultural and forestry land	20.04.2004	*Merismopedia Synechococcus*	+	–	–	n.i.	
2	**Chodzieskie Lake** urban land	17.06.2004	*Microcystis Anabaena Leptolyngbya*	+	+	+	*Microcystis*	
		01.07.2004	+	+	+	+	*Microcystis*	
3	**Ciemino Lake** forestry land	09.08.2002	*Synechococcus Phormidium*	+	–	–		
4	**Dobre Lake** forestry land	20.08.2004	*Merismopedia Leptolyngbya*	+	–	–		

No.	Name and nature of water bodies	Date of sampling	Presence of cyanobacteria				Microcystin's concentration Sampling Date (References)
			Microscopic observation	PCR *rpoC1*	PCR *mcyE*	PCR *mcyB*	Identification of potentially toxic cyanobacteria	
5	**Dolsko Lake** forestry and agricultural land	09.08.2002	*Synechocystis Cyanobium Phormidium*	+	–	–		
6	**Jeleń Lake** agricultural land	09.08.2002	*Oscillatoria Anabaena*	+	–	–		
7	**Jelonek Lake** agricultural land	28.07.2003	–	–	–	–		
		11.08.2003	+	+	–	–		
		10.10.2003	–	–	–	–		
		10.07.2004	*Microcystis Synechococcus Nostoc*	+	+	+	n.i.	
8	**Karlikowskie Lake** agricultural and urban land	10.07.2004	*Microcystis, Anabaena*	+	+	+	n.i.	1–5 µg/L 08/27.08.2003 (Mazur-Marzec, 2006)
		17.07.2004	+	+	+	+	n.i.	
9	**Kamienickie Lake** agricultural and forestry land	25.07.2004	*Microcystis*	+	–	–	*Microcystis*	
10	**Kielno Lake** agricultural land	20.07.2003	+	+	+	+	n.i.	<1 µg/L 29.08.2003 (Mazur-Marzec, 2006)
		10.08.2003	+	+	+	+	n.i.	
		10.10.2003	+	+	+	+	*Microcystis*	
11	**Klasztorne Duże Lake** urban land	10.07.2003	*Microcystis, Planktothrix Pseudanabaena*	+	+	+	*Microcystis, Planktothrix*	1–5 µg/L 27.08.2003, 4.09.2003 (Mazur-Marzec, 2006)
		19.09.2003	+	+	+	+	*Planktothrix*	
		09.10.2003	+	+	+	+	*Microcystis*	
12	**Krąg Lake** agricultural land	11.08.2003	*Microcystis, Anabaena*	+	+	+	*Microcystis*	
		29.08.2004	+	+	+	+	n.i.	
13	**Mausz Lake** agricultural and forestry land	06.07.2004	*Microcystis Anabaena Leptolyngbya*	+	+	–		
14	**Ostrzyckie Lake** agricultural and forestry land	31.07.2003	*Planktothrix Microcystis Anabaena*	+	+	+	n.i.	
		11.08.2003	+	+	+	+	*Planktothrix*	
		09.09.2003	+	+	+	+	*Planktothrix*	
		09.10.2003	+	+	+	+	n.i.	
15	**Raduńskie Dolne Lake** agricultural and forestry land	31.07.2003	*Synechococcus Pseudanabaena Anabaena Microcystis*	+	+	+	n.i.	
		11.08.2003	+	+	+	+	*Planktothrix Anabaena*	
		09.09.2003	+	+	–	+	n.i.	
		09.10.2003	+	–	–	–		
		17.07.2004	+	+	–	+	n.i.	
16	**Straszyn Lake** agricultural and urban land	31.07.2003	+	+	+	+	n.i.	
		11.08.2003	–	–	–	–		
		09.09.2003	–	+	–	–		
		09.10.2003	–	+	–	–		

No.	Name and nature of water bodies	Date of sampling	Presence of cyanobacteria					Microcystin's concentration Sampling Date (References)
			Microscopic observation	PCR *rpoC1*	PCR *mcyE*	PCR *mcyB*	Identification of potentially toxic cyanobacteria	
17	**Trzesiecko Lake** agricultural and urban land	14.06.2003	*Microcystis*	+	+	+	n.i.	1–5 µg/L 23.07.2003 (Mazur-Marzec, 2006) 6.29 µg/L 27.08.2002 (Mankiewicz *et al*., 2005)
		30.06.2003	+	+	+	+	n.i.	
		10.07.2003	+	+	+	+	*Microcystis*	
		20.07.2003	+	+	+	+	*Microcystis*	
18	**Tuchom Lake** agricultural land	09.07.2004	*Microcystis Anabaena*	+	+	+	*Microcystis*	
		17.07.2004	+	+	+	+	*Microcystis*	
		12.08.2004	+	+	+	+	n.i.	
19	**Wysoka Lake** forestry and agricultural land	10.09.2003	*Microcystis Synechococcus Nostoc*	+	+	+	n.i.	
		10.10.2003	*Microcystis Synechococcus Pseudanabaena*	+	+	+	n.i.	
20	**Żur Lake** forestry and agricultural land	16.08.2004	*Microcystis Leptolyngbya*	+	–	–		
21	**Pond in Gumnisko** agricultural land	05.10. 2004	*Planktothrix Anabaena Cyanobium*	+	+	+	*Planktothrix*	
22	**Pond in Kowalewo** agricultural land	11.06.2003	–	+	–	–		
		31.07.2003	–	+	–	–		
		11.08.2003	–	–	–	–		
		10.09.2003	*Microcystis Anabaena*	+	+	+	n.i.	
		10.10.2003	+	+	+	+	n.i.	
23	**Pond in Mniewo** agricultural land	10.10.2003	*Microcystis Anabaena Pseudanabaena*	+	+	+	n.i.	
24	**Sulejów Reservoir** agricultural and forestry land	21.05.2005	*Microcystis*	+	+	+	*Microcystis*	
25	**Zemborzyce Reservoir** forest and agricultural land	30.08.2004	–	+	–	–		
26	**Dojlidy Reservoir** agricultural and urban land	17.08.2004	–	+	+	+	n.i.	
27	**Nadarzyce Reservoir** forest land	09.07.2002	*Phormidium Leptolyngbya Merismopedia*	+	+	+	*Microcystis*	
28	**Lagoon of Wistula** agricultural, urban and forestry land	31.07.2003	*Microcystis Anabaena Phormidium Lyngbya*	+	+	+	n.i.	
		11.08.2003	+	+	+	+	n.i.	
		09.09.2003	+	+	+	+	n.i.	
		09.10.2003	+	+	+	+	n.i.	
29	**Gwda River**	09.07.2002	–	–	–	–		
		10.08.2002	–	+	–	–		
30	**Łeba River** — Chocielewko	14.08.2002	–	+	–	–		
31	**Noteć River** — Milcz	18.07.2004	–	+	+	+	n.i.	

No.	Name and nature of water bodies	Date of sampling	Presence of cyanobacteria				Microcystin's concentration Sampling Date (References)
			Microscopic observation	PCR *rpoC1*	PCR *mcyE*	PCR *mcyB*	Identification of potentially toxic cyanobacteria
32	**Radunia River** — Somonino	31.07.2003	–	+	–	–	
		11.08.2003	–	–	–	–	
		09.09.2003	–	–	–	–	
		09.10.2003	–	–	–	–	
33	**Słupia River** — Soszyca	20.09.2004	–	–	–	–	
34	**Tralalka River**	01.08.2002	–	–	–	–	
35	**Gulf of Gdańsk** — Orłowo	10.09.2003	*Anabaena*	+	–	–	
		10.10. 2003	–	+	–	–	
	Gulf of Gdańsk — Puck	04.08.2004	+	+	–	–	
	Gulf of Gdańsk — Gdynia	10.08. 2002	–	+	–	–	
	Gulf of Gdańsk — Osłonino	16.08.2004	*Merismopedia Aphanothece*	+	–	–	
36	**Baltic Sea** — Orzechowo	31.07.2003	–	+	–	–	
	Baltic Sea — Rowy	31.07.2003	–	–	–	–	

lake the presence was between July and August, in Raduńskie Dolne lake between July and September (Table 1, Fig. 1). In the pond in Kowalewo, both genes were detected in October and September (Table 1, Fig. 1).

However, in samples isolated from eight water bodies (Bielsko, Ciemino, Dobre, Dolsko, Jeleń, Kamienickie, Żur, reservoir in Zemborzyce) and from four rivers (Gwda, Łeba, Słupia, Tralalka) as well as from the Gulf of Gdańsk, in which *rpoC1* gene was detected, the presence of genes involved in biosynthesis of microcystin was not observed (Table 1).

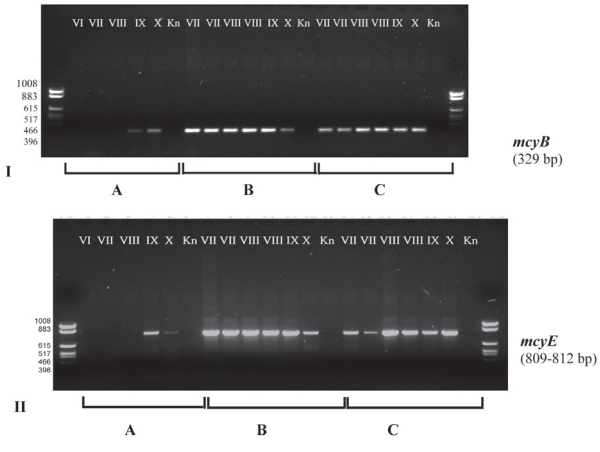

Figure 1. Detection of potentially toxic cyanobacteria in water samples collected periodically from three water bodies.
(A) pond in Kowalewo, **(B)** Klasztorne Duze Lake, **(C)** Lagoon of Wistula. Electrophoresis was performed in 1.2% agarose gel. **I,** detection of *mcyB* gene fragment. **II,** detection of *mcyE* gene fragment. Roman figures represent the month, in which samples were collected. Kn, negative control. M, marker: pKO3/*Hinf*I.

Identification of cyanobacteria using clone library analysis

To determine the composition of cyanobacteria communities in selected water bodies in which blooms appeared and to compare them with those in which mass occurrence of cyanobacteria was not observed, clone libraries were generated for seven water samples (Table 2).

The analysis of the clone libraries of the seven selected reservoirs indicated the presence of cyanobacteria belonging to genera *Microcytis, Planktothrix, Synechococcus, Cyanobium, Anabaena* and *Woronichinia* (Table 2). Strains from the genus *Microcystis* were the most prominent (186 clones out of 528) in the clone libraries. *Microcystis*-related genotypes, 4M and 5M, constituted 35% of all tested clones (Fig. 2, Table 2). The genotype 5M was dominant and the most widespread and was observed in 6 out of 7 analyzed water bodies, besides the pond in Gumnisko in which three different genotypes of *Planktothrix* were accrued (Table 2).

One-hundred and forty-six out of 528 (27.6%) clone sequences exhibited the highest level of similarity with 16S rDNA sequences of *Planktothrix*. For this genus of cyanobacteria 4 different genotypes were discriminated, 12M, 13M, 14M and 16M (Fig. 2, Table 2). The genus *Planktothrix* was abundant in two lakes, Klasztorne Duże and Ostrzyckie, and one pond in Gumnisko (Table 2). Cyanobacteria from the genus *Planktothrix* strongly dominated the cyanobacterial community in two water bodies, the Gumnisko pond and Ostrzyckie Lake, in which

Table 2. Identification of cyanobacteria on the basis of sequences obtained from the rrn clone library

Reservoirs	Number of clones	Genotype on the basis of 16S rDNA	Blast Relatives	
			Name of closest relatives	Max_{id}
Pond in Drzewce* (15.09.2004)	46	5M	*Microcystis sp.*	99 %
	14	10M	*Anabaena sp.*	91 %
	7	9M	*Anabaena sp.*	97 %
	5	4M	*Microcystis sp.*	96 %
Pond in Gumnisko* (02.09.2004)	29	14M	*Planktothrix sp.*	98 %
	23	12M	*Planktothrix sp.*	98 %
	5	15M	*Cyanobium sp.*	99 %
	4	16M	*Planktothrix sp.*	99 %
Klasztorne Duże Lake** (09.10.2003)	63	5M	*Microcystis sp.*	99 %
	10	16M	*Planktothrix sp.*	99 %
	5	12M	*Planktothrix sp.*	98 %
Ostrzyckie Lake*** (11.08.2003)	44	12M	*Planktothrix sp.*	98 %
	23	13M	*Planktothrix sp.*	98 %
	3	5M	*Microcystis sp.*	99 %
	6	11M	*Woronichinia sp.*	99 %
	5	14M	*Planktothrix sp.*	98 %
	2	10M	*Anabaena sp.*	91 %
Raduńskie Dolne*** Lake (11.08.2003)	44	2M	*Synechococcus sp.*	98 %
	9	1M	*Synechococcus sp.*	93 %
	8	9M	*Anabaena sp.*	97 %
	7	5M	*Microcystis sp.*	99 %
Wysoka Lake*** (10.09.2003)	61	11M	*Woronichinia sp.*	99 %
	14	5M	*Microcystis sp.*	99 %
	7	6M	*Synechococcus sp.*	99 %
	5	1M	*Synechococcus sp.*	93 %
	2	7M	*Synechocystis sp.*	99 %
	2	8M	*Synechococcus sp.*	92 %
Jelonek Lake*** (11.08.2003)	45	5M	*Microcystis sp.*	99 %
	9	6M	*Synechococcus sp.*	99 %
	6	15M	*Cyanobium sp.*	99 %
	3	17M	*n.i.*	No data
	3	18M	*Synechococcus sp.*	99 %
	3	3M	*Synechococcus sp.*	90 %
	3	4M	*Microcystis sp.*	96 %
	3	16M	*Planktothrix sp.*	99 %

*Reservoirs with bloom of cyanobacteria; **Samples were collected 2 months after bloom; ***Reservoirs, in which bloom was not observed during specimen collection

they constituted about 86.7% and 91.8%, respectively (Table 2).

DISCUSSION

In this study, the presence and identification of cyanobacteria in Polish waterbodies was verified using microscopic observation, standard PCR methods and clone libraries constructed for environmental samples. Most of the studied waterbodies are under an excessive nutrient loading from agricultural, industrial and urban runoff, in effect many of them are eutrophicated (Table 3).

Microscopic observation allowed detecting of cyanobacteria in 63 (72.4%) samples, while amplification of *rpoC1* gene specific for cyanobacteria allowed for detection of this group of bacteria in 75 (86.2%) samples (Table 1). These results indicate the high value of *rpoC1* PCR method for detection of cyanobacteria in environmental samples. The usefulness of the *rpoC1* gene fragment as a molecular marker for detection, identification

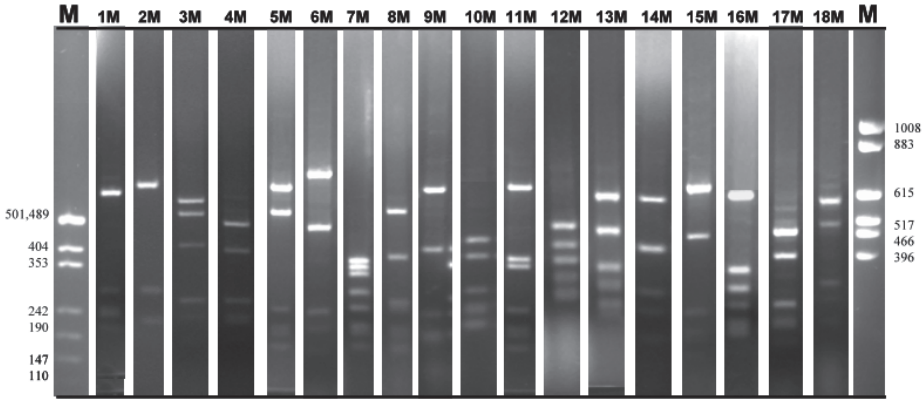

Figure 2. RFLP genotypes discriminated on the basis of MspI restriction endonuclease digestion of amplified *rrn* operon fragments of 533 clones
Electrophoresis was performed in 2% agarose gel in TEA buffer. Molecular marker (M_{left}) MspI/pUC18DNA, (M_{right}) pKO3/*Hinf*I. Figure was created using BioNumerics program.

and differentiation of cyanobacteria has previously been described (Toledo & Palenik, 1997; Jameson *et al.*, 2008; Yoshida *et al.*, 2008; Valério *et al.*, 2009; Han *et al.*, 2009).

The results of microscopic observation were in general agreement with the molecular data. However, in the case of samples collected from Raduńskie Dolne Lake (October 2003), cyanobacteria were observed under the microscope, but the *rpoC1* PCR product was not detected. In contrast, in two samples from Straszyn Lake (September 2003; October 2003), we did not see any cells of cyanobacteria during microscopic observation, but we got *rpoC1* PCR products (Table 1). The procedure of sampling (filtration of water samples using filters) allowed checking the presence of cyanobacteria cells in a large volume of water sample and thus facilitated the generation of the specific PCR product.

Because microcystins are most widespread cyanotoxins, we focused on the detection of cyanobacteria that have the genetic potential to produce these toxins. In all water blooms investigated in this study, PCR amplification of *mcyB* and *mcyE* genes confirmed the presence of these genes in the environment (Table 1). According to these results, we concluded that 7 analyzed blooms were potentially toxic and contained *Microcystis* and *Planktothrix* cells. Only *mcyE* gene was detected in Bnińskie and Bytyńskie Lake. This indicates that potentially toxic *Planktothrix* strains were present in these lakes (Table 1).

It is worth to emphasize that the presence of microcystins-related genes (*mcyB* and *mcyE*) is not a proof of the occurrence of the toxins, but only shows, that potentially toxic cyanobacteria are present in the tested water bodies. Some strains may have only fragments of the *mcy* genes cluster or mutations within these genes, and in spite of being a source of DNA for successful amplification with primers specific for *mcy* genes, they are not toxin-producers (Vaitomaa *et al.*, 2003). Earlier studies (Rantala *et al.*, 2004; Mankiewicz-Boczek *et al.*, 2006) have indicated that the *mcyE* gene is the best molecular marker for the determination of potentially toxic cyanobacteria in environmental samples. The detection of a *mcyE* PCR product means that potentially toxic cyanobacteria are present in the sample, because this gene encodes modules responsible for incorporation of 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid, (Adda) and D-glutamate into the microcystin molecules. These two amino acids are crucial for toxicity, and moreover they vary less than the other amino acids comprising the many toxin isoforms (Sivonen & Jones, 1999). Mankiewicz-Boczek *et al.* (2006) tested primers for amplification of *mcyA, mcyB, mcyD* and *mcyE*, and showed that the *mcyE* gene fragment was amplified in all water samples containing different genera of microcystins-producing cyanobacteria. The next most frequently amplified gene was *mcyB* (in 96% of tested water samples). However, in the cited publications another *mcyB* gene fragment was applied and other primers were used.

An overwhelming majority of the selected water bodies were examined for the first time, however, seven of them had previously been examined by others by biochemical, physicochemical (Table 3) and partly molecular methods and can serve as a control for the results obtained by the applied molecular analysis.

The combination of microscopic and molecular analyses of the sample collected from Bnińskie Lake indicated that bloom (observed in September 2004) was caused by cyanobacteria belonging to genus *Planktothrix* (Table 1). This result is in accordance with previous investigations of this lake indicating toxic cyanobacteria dominated by *Planktothrix agardhii* (Mankiewicz-Boczek *et al.*, 2006; 2009). Mankiewicz-Boczek (2006) determined the toxicity of the water samples collected from Bnińskie Lake in September 2004, using protein phosphatase inhibition assay (PPIA) and high performance liquid chromatography (HPLC). Microcystin concentration estimated by HPLC was 10.47 μg/L. A compilation of literature and our data confirms that cyanobacteria present in Bninskie Lake were not only potentially toxic, but also efficiently produced toxins.

Microscopic analysis of water samples collected from Klasztorne Duże Lake indicated *Planktothrix* and *Microcystis* strains. The amplification of *mcyB* and *mcyE* genes confirmed the presence of toxic cyanobacteria (Table 1). The clone library generated for samples collected from this lake two months after bloom showed that strains of *Planktothrix* and *Microcystis* were still dominant in this reservoir. Mazur-Marzec (2008) also identified strains of *Planktothrix* and *Microcystis* in this lake and estimated microcystin concentration at 1–5 μg/L during summer 2003. In accord with the results obtained by Mankiewicz *et al.* (2005) and Mazur-Marzec (2008) for Trzesiecko Lake in 2002 and 2003, our data indicated the presence of potentially toxic strains of *Microcystis* also in 2004. This suggests that the population of toxic *Microcystis* in this lake is stable.

In this study, the presence of *Microcystis* strains was reported in Tuchom Lake in summer 2003 and 2004. The PCR products of *rpoC1*, *mcyB* and *mcyE* genes confirmed the occurrence of potentially toxic *Microcystis*. Mazur-Marzec (2008) monitored Tuchom Lake during summer in 2000, 2001, 2002 and 2003. They detected the genera *Microcystis* and *Anabaena*, and the concentration of micro-

Table 3. Physicochemical parameters of investigated lakes

Name of water bodies	Surface (km^2)	Mean depth (m)	TP (mg P/L)[a] Sampling date*	TN (mg N/L)[b] Sampling date*	Chl-a (µg/L)[c] Sampling date*
Bnińskie Lake	2.215	4.2	**0.2** 07–10.2004[1]	**2.3** 07–10.2004[1]	n.d
Bytyńskie lake	3.088	3.5	**0.092** 2000[2], 2009[3]	**2.95** 2000[2], 2009[3]	**47.3** 2000[2], 2009[3]
Chodzieskie Lake	1.15	3.1	**0.196** 2000[2]	**2.47** 2000[2]	**82.711** 2000[2]
Ciemino Lake	2.417	6.0	**0.1** 1998[4], 2008[5]	**1.4** 1998[4], 2008[5]	n.d.
Dolskie Lake	0.348	10	**0.019** 07.2003[6]	**2.26** 07.2003[6]	n.d.
Jeleń Lake	0.89	12.0	**0.07** 05-08.2006[7]	**1.35** 05-08.2006[7]	**2–5** 05–08.2006[7]
Karlikowskie Lake	0.31	5.0	**0.18** 1998[8]	**2.2** 1998[8]	n.d.
Kamienickie Lake	1.38	4.0	**0.50** 06-08.1995[9] **0.264** 2006[10, 11]	**2.3** 06-08.1995[9] **2.5** 2006[10, 11]	**78.2** 08.1995[9], **118.3** 2006[10]
Klasztorne Duże Lake	0.57	8.0	**0.26** 2007[12]	**2.50** 2007[12]	**108.9** 2007[12]
Krąg Lake	1.484	2.0	**0.6** 2002[11]	**2.1** 2002[11]	
Mausz Lake	3.84	12.8	**0.051** 2008[13]	**1.021** 2008[13]	**6.1** 2008[13]
Ostrzyckie Lake	3.08	6.7	**0.079** 2002[14] **0.060** 2005[10, 15]	**0.74** 2002[14] **0.90** 2005[10, 15]	**15.6** 2002[14] **27.5** 2005[10, 15]
Raduńskie Dolne Lake	11.2	7.372	**0.063** 2002[14] **0.104** 2006[10, 16]	**0.60** 2002[14] **1.150** 2006[10, 16]	**10.4** 2002[14] **72** 2006[10, 16]
Straszyn Lake	0.75	5	**<0.1** 2002 [14] **0.13** 2006 [11]	**<0.1** 2002[14] **1.29** 2006[11]	**15** 2002[14]
Trzesiecko Lake	2.95	5.4	**0.11** 08.2002[17]	**2.59** 08.2002[7]	
Wysoka (Wycztok) Lake	0.55	1.9	**0.050** 08.2001[18]	**1.11** 08.2001[18]	**57.7** 08.2001[18]
Żur Lake — Tleń	4.4	15	**>0.20** 2001[19] **0.26** 2005 [20]	**>1.5** 2001[19] **1.8** 2005[20]	**>15** 2001[19] **10.1–20** 06–08.2005[20]
Sulejów Reservoir	27	3.3	**0.2** 07–10.2004[1]	**1.0** 07–10.2004[1]	n.d.
Zemborzyce Reservoir	2.8	2.5	**0.36** 05–10.2003[21]	**0.075** $NaN0_3$ **0.33** $N\text{-}NH_4$ 05–10.2003[21]	**129** 05–10.2003[21]
Nadarzyce Reservoir	2.027	4	**0.05** 2004[4]	**<1.0** 2004[4]	**<8.0** 2004[4]
Lagoon of Wistula	838	2.4	**0.316** 07.2007[22] **0.263** 08.2008[22]	**4.8** 08.2007[22] **3.56** 07.2008[2]	**142.2** 06.2007[22] **145.9** 07.2008[22]
Łeba River — Lębork Mosty			**0.11** 2005[23]	**1.86** 2005[23]	**2.1** 2005[23]
Noteć River — Nakło			**0.24** 2004[20]	**1.96** 2004[20]	**25.3** 2004[20]
Radunia River — Somonino			**0.15** 2001[24] **0.1** 2004[25]	**1.5** 2001[24] **0.9** 2004[25]	 **7** 2004 (26)
Słupia River — Charnowo			**0.11** 2005[23]	**2.01** 2005[23]	**3.7** 2005[23]
Gulf of Gdańsk — Orłowo		6.5	 **0.05** 07.2009[26]	 **0.443** 07.2009[26]	**34.5** 07.2004[27] **14.4** 07.2005[27] **3.56** 07.2009[26]
Gulf of Gdańsk — Puck		1.5	**0.064** 07.2009[26]	**0.344** 07.2009[26]	**14.01** 07.2009[26]
Gulf of Gdańsk — Gdynia		10.0	**0.089** 07.2009[26]	**0.375** 07.2009[26]	**3.29** 07.2009[26]
Gulf of Gdańsk — Osłonino		2.5	**0.0509** 07.2009[26]	**0.349** 07.2009[26]	**8.42** 07.2009[26]
Baltic Sea — Rowy		6.5	**0.031** 06.2009[26]	**1.14** 06.2009[26]	**1** 06.2009[26]

n.d., not determined; *if months of sampling are not specified the year average is stated; [a]TP, total phosphorus; [b]TN, total nitrogen; [c]Chl-a, chlorophyll a; [1]Mankiewicz-Boczek *et al*., 2006 ; [2]WIOŚ Poznań, 2000; [3]WIOŚ Poznań, 2009; [4]ABRYS Technika 2004; [5]WIOŚ Szczecin, 2011; [6]D. Borowiak (pers. com.); [7]Witek & Jarosiewicz, 2010; [8]Gos & Bociąg, 1998; [9]Starostwo Powiatowe w Kartuzach, 2008; [10]Borowiak, 2007; [11]http://www.infoeko.pomorskie.pl/; [12]WIOŚ Gdańsk, 2008; [13]WIOŚ Gdańsk, 2009; [14]WIOŚ Gdańsk, 2003; [15]WIOŚ Gdańsk, 2006a; [16] WIOŚ Gdańsk, 2007; [17]Mankiewicz *et al*., 2005; [18]WIOŚ Gdańsk, 2002a; [19]WIOŚ Bydgoszcz, 2002; [20]WIOŚ Bydgoszcz, 2006; [21]Pawlik-Skowrońska *et al*., 2004; [22]WIOŚ Olsztyn, 2008; [23]WIOŚ Gdańsk, 2006b; [24]WIOŚ Gdańsk, 2002b; [25]WIOŚ Gdańsk, 2005; [26]WIOŚ Gdańsk, 2010; [27]Mazur-Marzec *et al.,* 2006.

cystins ranged from 1 to 5 μg/L (Table 1). These results indicate the long-term occurrence of *Microcystis* in Tuchom Lake.

Analysis of water samples collected from Sulejów Reservoir, which is an alternative source of drinking water for the city of Łódź, demonstrated the presence of toxic cyanobacteria from genus *Microcystis*. This result confirmed the previous data describing the appearance of toxigenic cyanobacteria and their toxins in this reservoir (Jurczak *et al*., 2004; Izydorczyk *et al*., 2005; Palus *et al*., 2007; Gągała *et al*., 2010) and data revealing the microcystin concentration in September 2004 (Mankiewicz *et al*., 2006).

The microscopic identification of cyanobacteria in blooming ponds in Drzewce, Mniewo and Gumnisko indicated the presence of *Microcystis* strains in the first two ponds and *Planktothrix* strains in the third one. The presence of cyanobacteria with the genetic ability to produce toxins was confirmed by PCR tests (Table 1).

The presented results are the first application of *rpoC1* gene for detection of cyanobacteria in samples from Polish water bodies. The data obtained through molecular analysis based on PCR amplifications of *rpoC1*, *mcyB* and *mcyE* genes turned out to be adequate for the detection of cyanobacteria including toxic strains and are in agreement with earlier studies obtained with other methods (Table1). A significant advantage of the application of molecular methods for the detection of cyanobacteria and their toxic strains is the fact that they allow prediction of the mass occurrence of potentially toxic cyanobacteria before the bloom appears. Janse *et al*. (2005) suggested that the strain that was present in very low abundance in wintertime was the same strain that would form a bloom in spring.

Some data indicate that toxic strains utilize more nutrients and grow better in eutrophic water bodies than nontoxic strains (Lee *et al*., 2000). For the first time, we studied water samples collected from ponds and small lakes in villages (ponds in Drzewce, Gumnisko, Mniewo, Kowalewo, and lakes Kielno and Krąg). We intended to verify the appearance of potentially toxic cyanobacteria in water bodies being under the influence of agriculture and to determine the potential risk of using these waters. The obtained results indicated a mass occurrence of potentially toxic cyanobacteria in the ponds in Drzewce, Gumnisko and Mniewo in which blooms were observed each year but no informations about the presence of toxic cyanobacteria were available (Table 1). Cyanobacteria occurring in these waters were assigned to *Microcystis* and *Planktothrix* on the basis of microscopic and molecular evaluations. *Microcystis* strains were also detected in Kielno Lake and Krąg Lake. In all these water bodies genes *mcyB* and *mcyE* were detected. On the basis of obtained results it can be concluded that all tested waterbodies, which were under the influence of agriculture, were contaminated by cyanobacteria able to produce microcystins (Table 1 and Table 3).

The investigation of water samples collected from reservoirs which are a source of drinking water for cities (Sulejów Reservoir and Straszyn Lake) also indicated the presence of potentially toxic cyanobacteria (Table 1). The obtained results support the earlier conclusions that this type of reservoirs should be carefully monitored for the presence of toxic cyanobacteria, especially when microcystin-related genes are present. The Sulejowski Reservoir is actually in constant monitoring for cyanobacteria, including physicochemical, genetic, biochemical and toxicological analyses (Jurczak *et al*., 2004; Izydorczyk *et al*., 2005; Mankiewicz-Boczek *et al*., 2006a; 2006b; Palus *et al*., 2007, Izydorczyk *et al*., 2008; Gągała *et al*., 2010).

The detection of cyanobacteria in running waters turned out to be difficult. We analyzed 10 samples collected from 6 rivers (Radunia, Gwda, Łeba, Noteć, Slupia, Tralalka). Positive results of the *rpoC1* PCR amplification were obtained for four samples (Table 1). The occurrence of potentially toxic cyanobacteria was revealed only in one sample from the Noteć. In that case the sampling point was located on a lowland area with a very slow water flow and the river was eutrophicated in this area (Table 3).

The identification of cyanobacteria was conducted using clone libraries constructed from environmental DNA samples from five selected lakes and two ponds (Table 2). In the Drzewce Pond, we detected cyanobacteria assigned to genera *Microcystis* and *Anabaena*. In Gumnisko Pond, *Planktothrix* and *Cyanobium* were identified (Table 2). Results demonstrated a lower diversity of cyanobacteria in reservoirs in which blooms were present. Lower biodiversity of cyanobacteria was also found in Klasztorne Duże Lake, which was investigated two months after a bloom. According to our knowledge, this is the first comprehensive study concerning cyanobacteria and their toxigenic strains present in different types of Polish water bodies (Table 1 and Table 3) performed using molecular markers.

CONCLUSIONS

The high effectiveness of the molecular marker *rpoC1* for the detection of cyanobacteria was illustrated. Applied molecular markers *mcyB* and *mcyE* allowed the detection of potentially toxic cyanobacteria in Polish water bodies. The present results indicate that potentially toxic cyanobacteria occur widely in reservoirs, lakes and ponds of Poland. Our results confirm the usefulness of the selected *cyanobacterial* genes for monitoring water bodies and rapid detection of potentially toxic cyanobacteria. Application of molecular methods allows one to choose a group of water bodies that have the potential to form toxic blooms and have to be monitored. The described procedure is applicable for the prediction of the occurrence of toxic blooms before they actually happen.

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