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DETECTION OF MICROCYSTIN PRODUCING CYANOBACTERIA IN THE SVISLOCH RIVER, BELARUS

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Abstract

Comprehensive investigations of the Svisloch River phytoplankton were performed in 2006-2010. Samples were taken all-the-year-round at four sites: reservoirs Drozdy and Lake Komsomolskoye, sites along Vaneyev and Aranskaya streets (within the Minsk municipal section of the river). Species diversity of planktonic cyanobacteria and their contribution to phytoplankton abundance and biomass were determined. Potentially toxic cyanobacteria were detected based on the results of molecular-phylogenetic analysis. They contained a microcystin synthesis gene (*mcy*E). Obtained sequences belonged to the *Microcystis* and *Anabaena* genera, which were the most common in reservoirs worldwide and the most frequent agents of blooms. Several types of microcystins and a nontoxic peptide Oscillamide Y were detected in phytoplankton samples by means of LC/MS

Key words: Svisloch River, toxic cyanobacteria, *Microcystis*, *Anabaena*, microcystin, Oscillamide Y, *mcy*E gene, LC/MS

INTRODUCTION

The Svisloch River like many other water streams of Europe is subject to anthropogenic pollution and changes of the river course caused by construction of artificial reservoirs. This trend in the flow regulation caused eutrophication and deterioration of water quality. Decreased rate of water exchange in regulated rivers and increase of the surface area exposed to sunlight generate the growth of cyanobacteria (blue-

green algae) and water-bloom formation (Chorus and Bartram 1999). Cyanobacteria synthesize a large number of biologically active metabolites, including toxins. One of the most well-known and common cyanotoxins in freshwater ecosystems is microcystin (MC). Microcystins are cyclic heptapeptides which are synthesized nonribosomally by a large multienzyme complex (Nishizawa et al. 1999). At present, over 90 variants of MCs are known (Welker et al. 2004). Highly toxic MC-LR is the most common in which variable L-amino acids are represented by leucine and arginine. Microcystins possess a wide spectrum of biological activity: hepatotoxicity, inhibition of protein phosphatases, destruction of cytoplasmic membranes, and carcinogenicity (Honkanen et al. 1990, MacKintosh et al. 1990). Monitoring of the MC concentration in the water is performed in many countries. According to recommendations of the World Health Organization (WHO), such methods as high performance liquid chromatography (HPLC), immunoassays, protein phosphatase inhibition assays, mouse bioassays, and others can be used for monitoring. Liquid chromatography-mass spectrometry (LC/MS) is considered to be the most precise and sensitive method for microcystin determination (Chorus and Bartram 1999). According to the standards specified by WHO, concentration of MC-LR in drinking water should not exceed 1 μg/L, the abundance of cyanobacteria 20×10^6 cells/L and concentration of microcystins 2-4 μg/L in recreational waters are considered dangerous (Guidelines for safe... 2003, Guidelines for drinking-water... 2004).

Discovery of a gene locus responsible for MC synthesis in *Microcystis aeruginosa* (*mcy*A–J-genes) allowed the development of genus- and species-specific markers which are widely used for detection of toxigenic cyanobacteria (Nishizawa et al. 1999). In this work, a domain of aminotransferase (AMT) was chosen as a marker which plays a key role in biosynthesis of microcystins transferring an aminogroup to amino acid Adda (Moffitt and Neilan 2004). Aminotransferase is included in microcystin synthetases and nondularin synthetase (*nda*) coding synthesis of hepatotoxin – nodularin, produced by the species of the genus *Nodularia* (Moffitt and Neilan 2004). Genes of AMT synthesis were found in *Microcystis*, *Anabaena*, *Nodularia*, *Nostoc*, *Planktothrix*, and *Phormidium* from different water bodies. Their presence positively correlated with the production of microcystins and nodularins (Rantala et al. 2004, Jungblut and Neilan 2006). In phylogenetic analysis, sequences of *mcy*E and *nda*F genes group according to the taxonomy of cyanobacteria and are not affected by lateral gene transfer (Rantala et al. 2004). Thus, the use of primers targeted the AMT domain in the *mcy*E/*nda*F gene makes it easier to identify both microcystin- and nodularin-producing cyanobacteria in the natural samples.

Cases of toxic cyanobacterial blooms have been registered in many European countries (Codd et al. 2005). In Belarus water bodies, however, toxic cyanobacteria have not been recorded before.

This work was aimed at studying the species composition of cyanobacteria, their growth and contribution to total abundance and biomass of phytoplankton from 2006 to 2010 and at detecting microcystin synthesis genes and toxins in the Svisloch River within Minsk.

MATERIALS AND METHODS

Site description

The River Svisloch, a tributary of the Berezina River, belongs to the basin of the Dnieper River. It is 297 km long with a catchment area of 5,160 km^2. The river has experienced major changes in the last four decades, including the construction of multipurpose dams, water transfer from the Vileiskoye Reservoir, and increased inputs of untreated sewage as the 42 km section of the river passes through the capital of Belarus – Minsk. The flow of sewage from the city territory is the main source of the river pollution by organic substances, oil products, ammonium and nitrite nitrogen, and mineral and organic compounds of phosphorus and heavy metals. Daily average discharge of poorly treated sewage is 85,000 m^3. In 2000-2006, dredging and cleaning of the river bed from bottom sediments were carried out to improve the flowage and water quality of the river. Moreover, the bank was protected and the embankment was improved. The Svisloch River is the main element in the urban environment, it provides important aesthetic and recreational benefits of the city, and is the source of technical water supply. The research was carried out in that section of the Svisloch River which passes through Minsk including both river sites and its reservoirs.

Phytoplankton analysis

In 2006-2010, samples were collected every month at four sites, including regulated reservoirs Drozdy and Lake Komsomolskoye, and at the river sites along Vaneyev and Aranskaya streets. Water samples (0.5 l) were fixed with Lugol's solution and concentrated by a sedimentation method. Quantitative measurements of phytoplankton were performed according to T. Mikheyeva (1989). Species were identified under light microscopes Zeiss "Axiolab" and "Axiostar". Phytoplankton samples collected with an Apstein net in July-September of 2008-2010 were fixed in 80% ethanol for molecular-biological analysis. Phytoplankton samples were also concentrated on a fiberglass filter for the analysis of microcystins, dried at 60°C and kept at -20°C.

Amplification, cloning, sequencing

Universal primers HepF and HepR (Jungblut and Neilan 2006) were used to reveal genes involved in the synthesis of microcystins and nodularins. Isolation of DNA, PCR, cloning and sequencing were performed as described earlier (Belykh et al. 2011). All sequences decoded in this work have been deposited in the GenBank under accession numbers JF441235-JF441242.

Phylogenetic analysis

Newly decoded *mcy*E gene sequences were compared with the sequences from the GenBank using the BLASTn programme (www.ncbi.nlm.nih.gov/BLAST) to find close relatives. The most similar reference sequences, preferably of cultivated

strains, were downloaded into the alignment. The alignment of sequences and evolutionary and phylogenetic analyses were preformed using the MEGA software, version 5.0 (Tamura et al. 2011). Phylogenetic reconstructions were carried out by neighbour-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) methods. Evolutionary distances for the NJ and MP trees were calculated by the Kimura-2 parameter model. An optimal model of nucleotide substitutions (T92+I) was calculated using MEGA software (Tamura et al. 2011). The tree topology was evaluated by bootstrap support (1,000 and 500 replicons for NJ-MP and ML analyses, respectively).

Microcystin analysis

The dried phytoplankton biomass was diluted with distilled water and frozen and thawed 5 times to lyse cells. Further treatment and analysis (LC/MS) of samples were performed according to the published techniques (Belykh et al. 2011) at the temperature of chromatographic separation 60°C.

RESULTS

Species composition of phytoplankton. Contribution of cyanobacteria to abundance and biomass

The species composition of phytoplankton at the sites of the reservoirs Drozdy and Lake Komsomolskoye and at the river sites within the urban section along Vaneyev and Aranskaya streets was represented by 28, 29, 26, and 20 species of cyanobacteria, respectively. The phytoplankton communities of the studied ecosystems included 142, 148, 125, and 114 members of different divisions. The composition of dominating species complexes was polydominant throughout the whole period of observations but changeable in different years. Cyanobacteria were dominated by 1-5 species, more often by 2-3 species. In August, when we collected samples to reveal toxigenic species, the following species prevailed in biomass: *Aphanothece clathrata* W. et G. S. West (10-66%), *Microcystis aeruginosa* (Kütz.) Kütz. (8-57%), *Microcystis flos-aquae* (Wittr.) Kirchn. (8-79%), *M. wesenbergii* (Kom.) Kom. in Kondrat. (5-18%), *M. viridis* (A. Br.) Lemm. (8-32%), *Synechococcus aeruginosus* Näg. (25-50%), *Synechocystis aquatilis* Sauv. (6-30%), and *Aphanizomenon flos-aquae* (L.) Ralfs (10-21%). The most numerous among 9 species of the genus *Anabaena* were *A. flos-aquae* (Lyngb.) Bréb. and *A. lemmermanni* P. Richt, and *Planktothrix agardhii* Gom. among other genera.

The composition of dominant cyanobacteria by biomass and cell abundance in July-September of 2008 and 2010 and their relative percentage of total phytoplankton are presented in Table 1. The complexes of dominant species demonstrate their diverse composition at various habitats and within one water body in different months. This is attributed not only to oscillations of climatic factors but also to the intense anthropogenic impact. It should be noted that throughout the whole period of observations potentially toxic cyanobacteria were recorded as dominant species: in August-

Table 1

Quantitative development of dominant cyanobacterial species in 2008 and 2010 and their relative percentage of total phytoplankton at regulated and river sites of the Svisloch River

Month	Dominants by organism abundance	%	Dominants by cell abundance	%	Dominants by biomass	%
1	2	3	4	5	6	7
2008	**Drozdy Reservoir**					
Jul.	*Synechococcus* sp.	76.1	*Aphanothece clathrata* *Synechococcus* sp.	51.6 9.7	None	
Aug.	*Aphanothece clathrata*	47.4	*Aphanothece clathrata*	94.6	*Aphanothece clathrata* *Microcystis flos-aquae* *Coelosphaerium dubium* *	66.5 7.9 5.4
Sept.	*Synechocistis aquatilis* *Aphanothece clathrata*	41.4 13.5	*Aphanothece clathrata* *Coelosphaerium dubium*	61.5 33.7	*Coelosphaerium dubium* *Aphanothece clathrata*	13.2 12.0
	Lake Komsomolskoye					
Jul.	*Synechococcus* sp.	54.3	*Aphanothece clathrata* *Merismopedia minima* *Synechococcus* sp. *Coelosphaerium dubium*	41.2 26.4 13.0 5.1	*Synechococcus* sp.	5.3
Aug.	*Aphanothece clathrata* *Merismopedia tennuissima*	35.1 7.6	*Aphanothece clathrata*	94.9	*Aphanothece clathrata*	63.4
Sept.	*Aphanothece clathrata* *Synechocistis aquatilis*	21.8 6.9	*Aphanothece clathrata* *Coelosphaerium dubium*	79.2 9.1	*Aphanothece clathrata* *Microcystis aeruginosa* *Oscillatoria agardhii*	16.1 12.9 10.6
	Transect at Aranskaya site					
Jul.	*Synechococcus* sp. *Aphanothece clathrata*	34.4 5.7	*Aphanothece clathrata* *Coelosphaerium dubium*	68.4 24.1	*Aphanothece clathrata* *Coelosphaerium dubium* *Microcystis aeruginosa* *Gloeocapsa minuta*	22.9 16.2 11.3 11.2

1	2	3	4	5	6	7
Aug.	*Aphanothece clathrata* *Gloeocapsa minor*	23.3 12.8	*Aphanothece clathrata* *Coelosphaerium dubium*	90.7 6.1	*Aphanothece clathrata* *Microcystis flos-aquae* *Coelosphaerium dubium*	57.4 13.4 7.7
Sept.	*Synechocistis aquatilis* *Aphanothece clathrata*	11.7 8.2	*Aphanothece clathrata* *Coelosphaerium dubium*	67.3 29.4	*Aphanothece clathrata* *Coelosphaerium dubium*	20.3 17.7
	Transect at Vaneyev site – *No sampling*					
2010	**Drozdy Reservoir**					
Jul.	*Synechocystis aquatilis*	89.8	*Microcystis aeruginosa* *Synechocystis aquatilis*	69.2 24.3	*Microcystis aeruginosa* *Synechocystis aquatilis*	54.8 19.2
Aug.	*Synechocystis aquatilis* *Anabaena flos-aquae* f. *aptecariana*	62.0 24.3	*Microcystis aeruginosa* *Anabaena flos-aquae* f. *aptecariana* *Microcystis wesenbergii* *Microcystis viridis*	52.8 22.3 14.1 5.9	*Anabaena flos-aquae* f. *aptecariana* *Microcystis aeruginosa*	46.5 34.9
Sept.	*Synechocystis aquatilis* *Microcystis wesenbergii* *Microcystis aeruginosa*	59.1 13.3 5.4	*Microcystis wesenbergii* *Microcystis aeruginosa* *Microcystis viridis*	58.2 35.4 5.3	*Microcystis aeruginosa* *Microcystis wesenbergii* *Microcystis viridis*	69.2 16.3 7.4
	Lake Komsomolskoye					
Jul.	*Synechocystis aquatilis*	79.9	*Synechocystis aquatilis* *Microcystis aeruginosa*	52.6 27.8	*Synechocystis aquatilis* *Microcystis aeruginosa*	23.7 12.6
Aug.	*Anabaena flos-aquae* f. *aptecariana* *Phormidium mucicola*	63.6 14.0	*Microcystis viridis* *Anabaena flos-aquae* f. *aptecariana* *Microcystis wesenbergii* *Microcystis aeruginosa*	52.4 19.1 17.5 9.2	*Anabaena flos-aquae* f. *aptecariana* *Microcystis viridis* *Microcystis aeruginosa*	51.3 32.0 7.8
Sept.	*Synechocystis aquatilis* *Microcystis wesenbergii* *Microcystis aeruginosa*	54.8 9.4 5.8	*Microcystis aeruginosa* *Microcystis wesenbergii*	55.2 41.1	*Microcystis aeruginosa* *Microcystis wesenbergii*	76.2 8.1

1	2	3	4	5	6	7
	Transect at Aranskaya site					
Jul.	*Synechocystis aquatilis*	97.3	*Microcystis aeruginosa* *Synechocystis aquatilis* *Microcystis wesenbergii* *Microcystis viridis*	64.7 17.3 10.8 5.4	*Microcystis aeruginosa* *Synechocystis aquatilis*	61.1 16.3
Aug.	*Anabaena flos-aquae* f. *aptecariana* *Synechocystis aquatilis*	44.6 22.2	*Anabaena flos-aquae* f. *aptecariana* *Microcystis pulverea* f. *incerta* *Microcystis viridis*	42.4 29.9 22.5	*Anabaena flos-aquae* f. *aptecariana* *Microcystis viridis*	64.5 7.8
Sept.	*Microcystis wesenbergii* *Microcystis aeruginosa* *Gloeocapsa minor*	9.0 6.0 6.0	*Microcystis aeruginosa* *Microcystis wesenbergii*	63.0 30.6	*Microcystis aeruginosa* *Microcystis wesenbergii*	81.5 5.7
	Transect at Vaneyev site					
Jul.	*Synechocystis aquatilis*	93.9	*Microcystis viridis* *Synechocystis aquatilis* *Aphanothece clathrata*	51.5 22.9 20.6	*Microcystis viridis* *Synechocystis aquatilis*	48.2 30.1
Aug.	*Synechocystis aquatilis*	95.9	*Synechocystis aquatilis* *Microcystis viridis* *Microcystis aeruginosa* *Anabaena flos-aquae* f. *aptecariana*	39.2 22.5 20.3 16.2	*Anabaena flos-aquae* f. *aptecariana* *Synechocystis aquatilis* *Microcystis aeruginosa* *Microcystis viridis*	38.3 29.5 15.2 12.1
Sept.	*Microcystis wesenbergii* *Microcystis aeruginosa*	26.5 11.4	*Microcystis wesenbergii* *Microcystis aeruginosa* *Microcystis viridis*	66.6 20.4 12.3	*Microcystis aeruginosa* *Microcystis wesenbergii* *Microcystis viridis*	49.4 23.0 21.2

***** Until 2010, *M. wesenbergii* and *Coelosphaerium dubium* were not considered as separate species

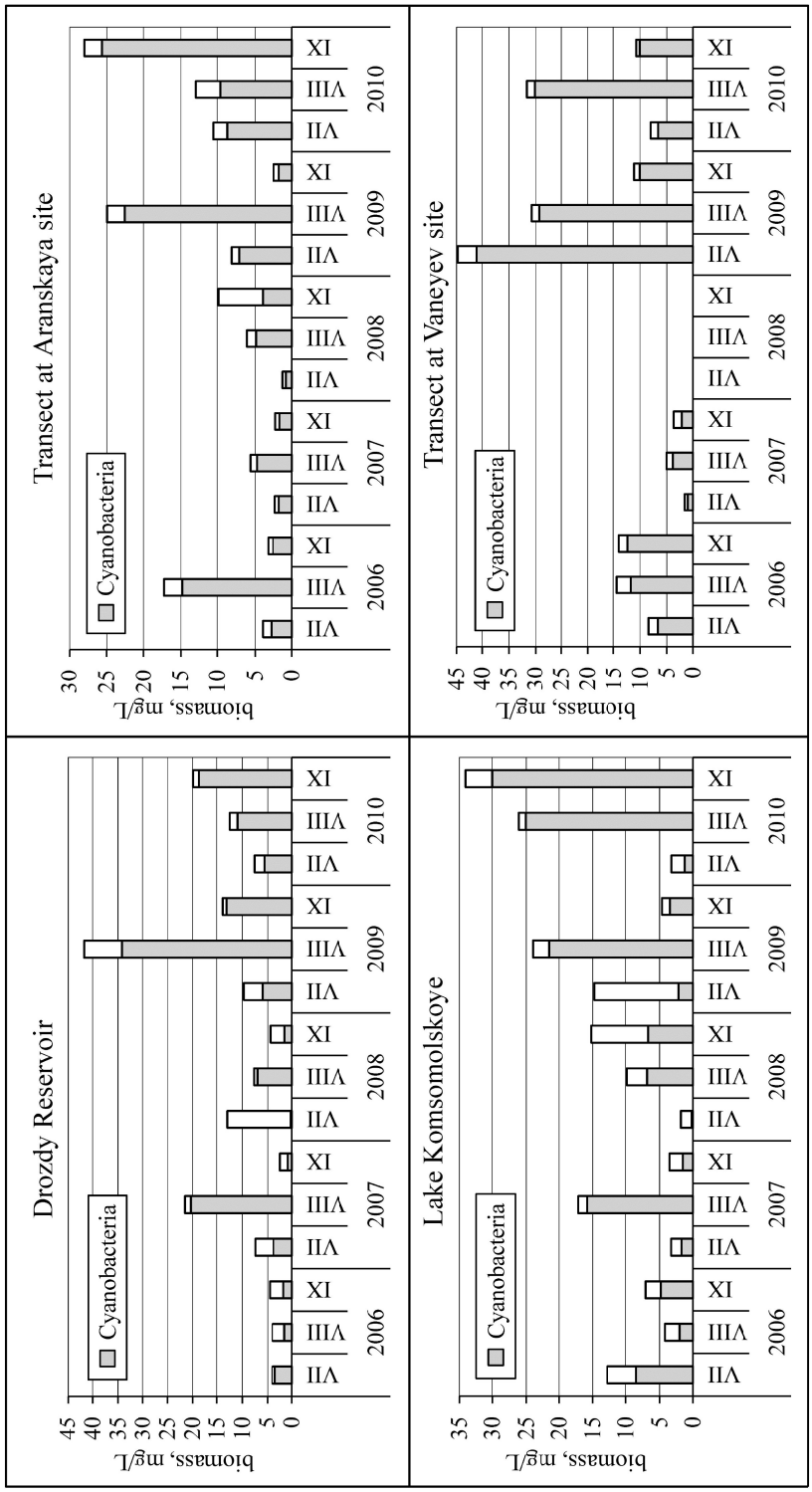

Fig. 1. Total phytoplankton and cyanobacteria biomass in different years at the regulated and river sites of the Svisloch River

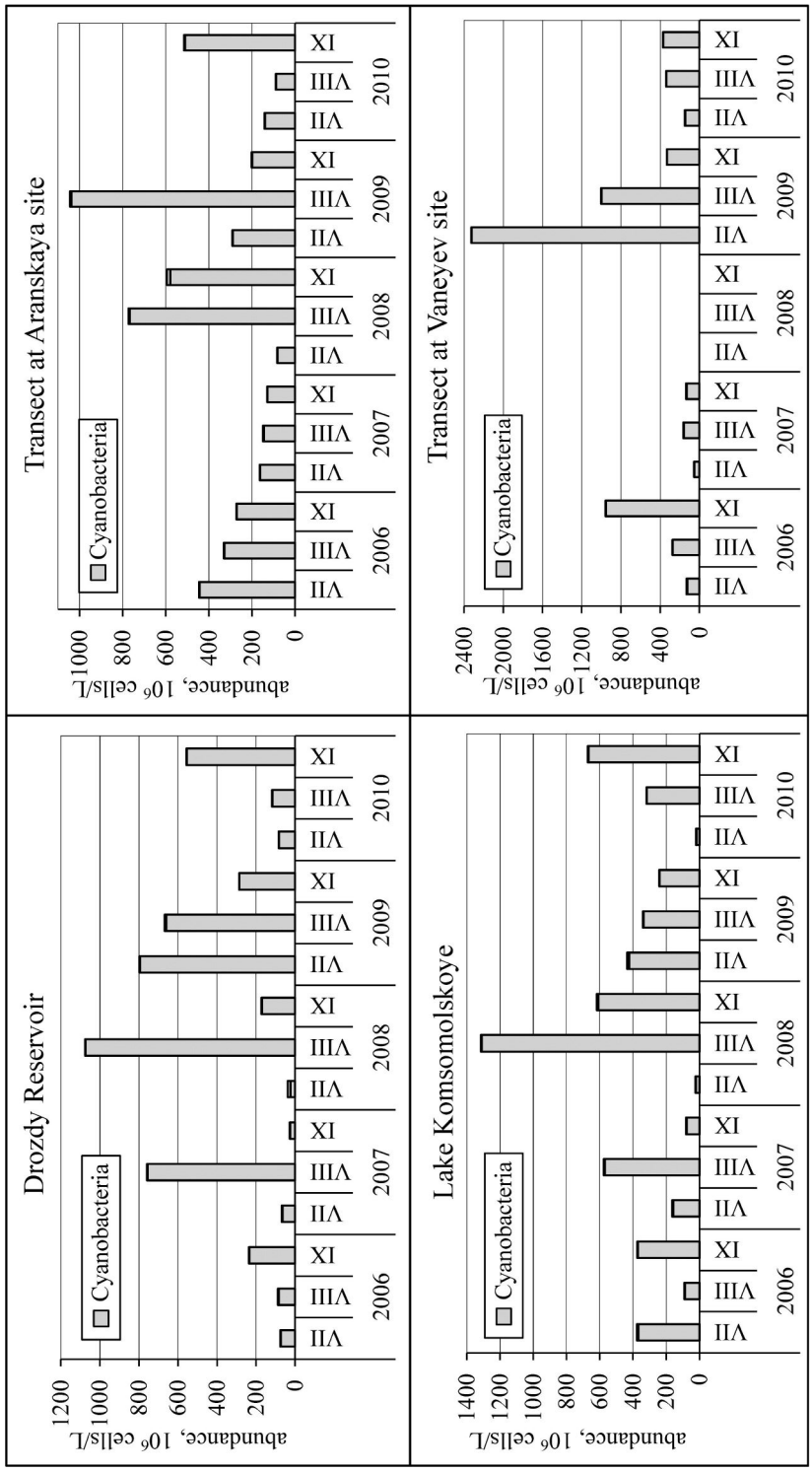

Fig. 2. Total phytoplankton and cyanobacteria cell abundance in different years at the regulated and river sites of the Svisloch River

September of 2010, their mean contribution to total phytoplankton biomass was 76% (SD=25, n=20).

The most productive years in the investigated ecosystems were 2009 and 2010: the maximal phytoplankton biomass reached 45 mg/L (Fig. 1). The maximal abundance of phytoplankton (2.8×10^8 cell/L) was recorded in 2009 (Fig. 2). The highest discrepancies in cell abundance and biomass were observed at the Vaneyev site, whereas the lowest ones – at the Aranskaya site. Due to the presence of multicellular non-heterocystous colonial cyanobacteria at all sites, they did make up the total abundance of phytoplankton (Fig. 2). Cyanobacteria amounted to 60-70% and 5-8% in total cell abundance of phytoplankton during the vegetation (May-October) and winter periods, diatoms – 13-15% and 36-50% and cryptophytes 13-16% and 7-13%, respectively. The percentage of cyanobacteria, diatoms and cryptophytes in the total abundance of organisms was approximately similar – about 30%. In the summer, the contribution of cyanobacteria was on average 84% (SD=22), whereas that to the total phytoplankton abundance and biomass could reach 99.9% and 96%, respectively (Figs. 1, 2).

PCR-analysis. Comparative and phylogenetic analyses

The PCR-analysis of the total DNA in all studied reservoirs and at river sites gave positive results for the presence of microcystin synthetase gene in August and September of 2008-2010. Eleven incomplete sequences of *mcy*E gene of 442 b.p. were obtained from the phytoplankton samples of the Svisloch River (the Vaneyev site) in August and September of 2010. Three identical sequences were grouped and registered as JF441242. Comparative analysis of sequences from the Svisloch River with those from the GenBank revealed high similarity (98-100%) in seven amplicons with *mcy*E genes of *Microcystis* spp. and one with *A. lemmermannii*. Sequences with high homology to nodularin synthetase gene have not been found. Microscopic analysis has not revealed species of the genus *Nodularia* in the phytoplankton either.

As the phylogeny of *mcy*E gene sequences inferred by different methods (NJ, MP, ML) displayed similar topologies of the trees, the NJ tree only is shown in Fig. 3. Seven sequences of *mcy*E-gene from the Svisloch River (JF441236-JF441242) belong to a stable clade of the genus *Microcystis* grouping with the strains of three different species. Sequence JF441238 is in the group formed by *M. aeruginosa* NIES-843 (Japan) and uncultured *Microcystis* sp. from the water bodies of Russia. Sequence JF441237 and strain *M. viridis* NIES-102 form a stable clade within this group. Four sequences are clustered into a monophyletic group with *M. wesenbergii* NIES-107 having high levels of bootstrap support (89-97%). The position of JF441241 is unstable on the phylogenetic tree, and the sequence has not been included in one of the clusters formed by closely related species. The only sequence JF441235 belongs to the *Anabaena* genus clade representing a monophyletic group with strains of *A. lemmermannii* from the Scandinavian lakes and with uncultured *Anabaena* sp. from Lake Kotokel, Russia.

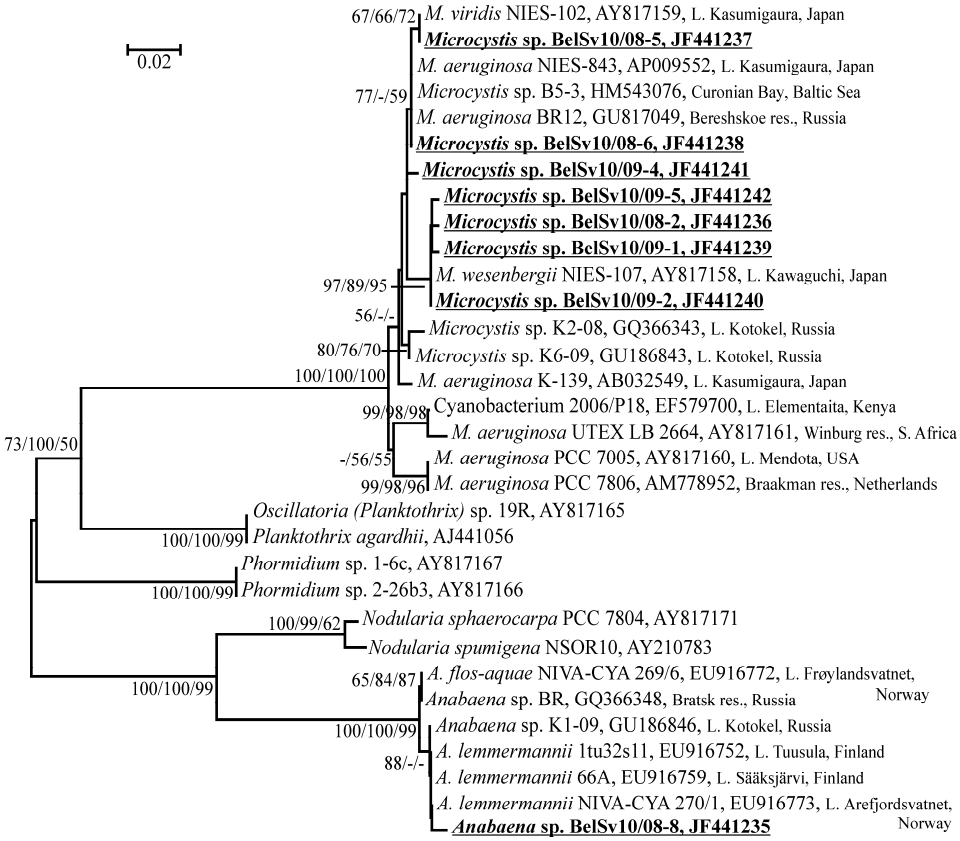

Fig. 3. Unrooted phylogenetic tree of cyanobacteria based on sequencing results of microcystin and nodularin synthetase genes (*mcy*E, *nda*F) inferred by neighbour-joining method. Numbers show the results of bootstrap analysis (NJ/MP/ML). Sequences obtained in this work are underlined

Intracellular microcystin and related peptide concentration and variety

The extracts from phytoplankton samples analysed by HPLC exhibited four major peaks with the following retention times (T_R): 17.4, 19.5, 21.9, and 24.6 min; the UV spectra were typical of microcystins. LC/MS analysis revealed the presence of two MC variants with characteristic m/z values [D-Asp^3,Dha^7]MC-LR (m/z=967.4918 $[M+H]^+$) and MC-VF (m/z=972.4467 $[M+H]^+$), eluting as one peak with T_R 17.4 min. Characteristic m/z value 984.4181 $[M+H]^+$ corresponded to two types of microcystins: [Dha^7]MC-EE(OMe) or [D-Asp^3,Dha^7]MC-E(OMe)E(OMe). Characteristic m/z value 995.5569 $[M+H]^+$ is usually referred to MC-LR, although at present there are two more variants of MC with this mass: [D-Asp^3,D-Glu$(OCH_3)^6$]MC-LR and [(6Z)-Adda^5]MC-LR. Further investigations are needed for exact identification of microcystin type. The total content of MCs was 2.4 μg/g of dry weight (DW). Dominant types were [D-Asp^3,Dha^7]MC-LR and MC-VF making up 64%.

One of the peaks corresponded to the mass of peptide Oscillamide Y, closely related to microcystins (m/z=858.4369 $[M+H]^+$) the content of which was low – 0.36 μg/g DW.

DISCUSSION

Based on the studies carried out in 1973, species diversity of phytoplankton in the Svisloch River is represented by 210 species and intraspecific taxa from 77 genera (Mikheyeva and Ganchenkova 1979). Year-round monthly investigations of 1995-1996 revealed only 102 species (Mikheyeva et al. 1998). In 2006-2010, from 114 to 118 species were recorded at these sites, and this agrees with the values of species richness registered in the river earlier.

Studies of the processes of biological self-purification and production-destruction characteristics of the river plankton (Mikheyeva et al. 1998, Ostapenya et al. 1999) allowed the conclusion that the eutrophication of the Svisloch River waters is very high upstream of the city, and trophic indices increase within the urban section of the river. For example, in 1995, the chlorophyll *a* concentration in July-August was 40.1 µg/L above the city, and at the exit from the city – 150.5 µg/L, whereas the phytoplankton biomass – 6.35 and 33.5 mg/L, respectively (Mikheyeva et al. 1998). The tendency of biomass increase from site to site along the river flow was disturbed in the period of our investigations. The analysis of inter-annual dynamics of phytoplankton abundance and biomass shows a positive effect from the river improving activities (bottom cleaning and bank protection) performed in 2000-2006. The most significant decrease of phytoplankton biomass was recorded in 2006 in the reservoirs Drozdy and Lake Komsomolskoye, and in 2007 – in the range of the river sites. In 2008, positive changes were retained. However, in the next years the trophic indices of the river flow increased again. In 2009-2011, the phytoplankton biomass reached its maximal values at all sites. This was attributed mainly to climatic specific features of the vegetation season: plenty of hot days with storm rainfall.

In many countries, recreational use of water bodies is restricted when the concentration of cyanobacteria is over 20×10^6 cell/L (Chorus and Bartram 1999, Guidelines for safe... 2003). The abundance of cyanobacteria at the river sites recorded in the summer every year was 1-2 orders higher compared to that recommended by WHO for recreational water bodies, and in 2006-2010, its mean value amounted to 20×10^8 cell/L.

It was revealed that eutrophic and hypertrophic water bodies always contain potentially toxic cyanobacterial species – MC producers, and more often this is a combination of three genera: *Microcystis*, *Anabaena* and *Planktothrix* (Rantala et al. 2006). The most common potential MC producers among cyanobacteria detected in the Svisloch River plankton are dominant species of the phytoplankton community: *Microcystis aeruginosa*, *M. viridis*, *M. wesenbergii*, *Anabaena lemmermannii*, *A. flos-aquae*, *Aphanizomenon flos-aquae*, and *Planktothrix agardhii*. Members of the genus *Microcystis* are the most common toxigenic species in fresh waters. Microcystins were detected in over 80-90% of samples from the water bodies of Denmark, Germany, Czech Republic, and Korea where *Microcystis* spp. dominated (Sivonen and Jones 1999). The highest amount of toxic strains was recorded in *M. aeruginosa*, approximately half of them contained *mcy* genes and produced microcystins, whereas the MC concentration in strains of *M. aeruginosa* was higher compared to *M. viridis* and *M. wesenbergii* (Watanabe et al. 1988, Yasuno et al. 1998, Ozawa et al. 2005).

According to the data of molecular-phylogenetic analysis, three species of the genus

Microcystis and *Anabaena lemmermannii* containing AMT-domain of the microcystin synthetase gene were detected in the Svisloch River. The amount of clones belonging to the genus *Microcystis* was 10 times higher than that in *Anabaena*. Similar ratio of *mcy*E gene copies was recorded in the Finnish lakes and Lake Kotokel (Russia) where toxic blooms occurred and microcystins were found (Vaitomaa et al. 2003, Belykh et al. 2011).

Earlier works showed that cyanobacteria belonging to the genus *Microcystis* usually produce MC-LR, MC-RR and MC-YR as major microcystin variants. Their demethylated isoforms are detected more rarely (Welker et al. 2004). MC-LR, the most toxic of the main MC types, is likely to be in the samples from the Svisloch River. Its LD_{50} (mouse, i.p.) is 50 μg/kg (Chorus and Bartram 1999). It should be noted that in 1989 this type of microcystin together with MC-YR was detected in the Dnieper River (the Svisloch River belongs to the Dnieper basin) (Carmichael et al. 1993). Besides the common variant, a rare variant of microcystin (MC-VF) was recorded. It was for the first time found in the strain *Microcystis aeruginosa* PCC7820 (Bateman et al. 1995), whereas it was detected in Lake Ladoga during cyanobacterial bloom (Voloshko et al. 2008). Toxicity of MC-VF has not been revealed so far.

Demethylated microcystin variants detected in the Svisloch River phytoplankton are poorly studied and their toxicity is unknown. As it is shown that demethylated MC variants in some strains of cyanobacteria are dominant (Krüger et al. 2010) and can contribute considerably to the total content of microcystins in the water body, they should be taken into account and need further investigations.

Oscillamide Y, a nontoxic peptide related to microcystins, was detected in the Svisloch River. T. Sano and K. Kaya (1995) isolated for the first time it from a freshwater toxic cyanobacterium *Oscillatoria (Planktothrix) agardhii* and revealed its chymotrypsin inhibitory effect. Oscillamide Y was detected in the natural samples only in Lake Ladoga (Voloshko et al. 2008). We assume that it can be often recorded during cyanobacterial toxic blooms, but as it is nontoxic, it is not included in the ordinary monitoring.

CONCLUSION

Despite improving activities carried out on the Svisloch River in 2000-2006, annual cyanobacterial water blooms are observed in the urban site within Minsk which can be a threat to the population. Every year potentially toxic species are dominant in phytoplankton, and their contribution to the total biomass is over 90%. The abundance of cyanobacteria is considerably higher than the WHO standards recommended for recreational water bodies. The microcystin synthesis genes belonging to three species of the genus *Microcystis* and *Anabaena lemmermannii* were detected in the Svisloch River during molecular-biological studies. Microcystins and nontoxic peptide Oscillamide Y were revealed in the phytoplankton samples by means of LC/MC. The total content of MCs was low (2.4 μg/g DW). Prevailing MC variants were [D-Asp^3,Dha^7]MC-LR and MC-VF (64%). To protect public health from cyanotoxins, it is necessary to perform seasonal monitoring of cyanobacterial abundance and microcystin concentration in the Svisloch River.

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ВЫЯВЛЕНИЕ МИКРОЦИСТИН-ПРОДУЦИРУЮЩИХ ЦИАНОБАКТЕРИЙ В Р. СВИСЛОЧЬ, БЕЛАРУСЬ

Изложение

В течение 2006-2010 гг. проводилось комплексное исследование фитопланктона р. Свислочь. Пробы отбирались круглогодично на четырех станциях в черте г. Минска: в зарегулированных водохранилищах Дрозды и Комсомольское озеро и на речных участках в районе улиц Ванеева и Аранская. С помощью световой микроскопии определен видовой состав планктонных цианобактерий, оценен их вклад в численность и биомассу фитопланктона. В результате молекулярно-филогенетических исследований выявлены потенциально токсичные цианобактерии, содержащие ген синтеза микроцистина (*mcy*E). Полученные последовательности принадлежат представителям родов *Microcystis* и *Anabaena* (= subg. *Dolichospermum*), которые широко распространены в водоемах мира и являются частыми возбудителями токсичных цветений. Несколько типов микроцистинов и нетоксичный пептид осцилламид Y были детектированы в пробах фитопланктона с помощью ВЭЖХ-МС.