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Synthetic information

Application of molecular tools in Ecohydrology

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Abstract

This paper presents synthetic knowledge about the usefulness of molecular methods for implementation of the Ecohydrology principles as a framework for scientific investigation and problem solving associated with deteriorating quality of freshwater. Genetic markers are indispensable in the early monitoring of threats, precise analysis of the cause-effect relationships between biotic and abiotic components of environment and, consequently, in developing methods to improve water quality and enhancing ecosystem carrying capacity. All these elements provide the methodological background of Ecohydrology, and were described based on a study of the problem of water blooms dominated by toxic cyanobacteria.

Key words: gene, toxic genotype, cyanobacteria, microcystins, monitoring of threats, bacterial degradation.

1. Introduction

Ecohydrology (EH) is a subdiscipline of hydrology focused on the ecological aspect of hydrological cycle with special emphasis placed on using ecosystem processes as a management tool to enhance carrying capacity of a river basin (water, biodiversity, ecosystem services for society and resilience) (Zalewski 2011). According to the EH principles (hydrological, ecological and ecotechnological), quantification and monitoring of threats constitutes a fundamental step to solve the relevant problem. In this case, molecular analyses using knowledge of the structure of nucleic acids, the specificity of the genome and genes selected, represent some the most promising techniques for EH research. Molecular data are needed for a better understanding of the behavior of organisms and their dependence on the environment. Advantages offered by using genetic analysis include sensitivity, time saving and flexibility of the basic methodology (e.g. qualitative and quantitative PCR – polymerase chain reaction). The need for using molecular research in Ecohydrology was presented based on the example of harmful algal blooms (HAB). Toxic water blooms with a share of cyanobacteria (cyanobacterial blooms) represent a global problem (Chorus 2005), which is directly linked with an increased emission of biogenic substances to waters, and anthropogenic modification of biogeochemical cycles at the catchment scale (Zalewski 2000).

This paper presents a three step approach with the use of molecular methods, from monitoring of threats, through finding cause-effect relationships

determining the threat, to solving the problem. Each step was exemplified with research conducted by the author. Some general guidelines have also been included.

2. Harmful algal blooms

Harmful algal blooms containing cyanobacteria represent the key obstacles that are most difficult to control. According to Paerl and Paul (2012), climate warming can selectively promote the growth of cyanobacteria because as prokaryotes, their growth rate is optimized at relatively high temperatures (Robarts, Zohary 1986; Butterwick *et al.* 2005; Watkinson *et al.* 2005). Cyanobacteria in many aquatic ecosystems are an essential element in the trophic chain, which can affect the functioning of the whole ecosystem. Mass occurrences of HAB as a consequence of eutrophication of water bodies cause problems for production of drinking water, as well as recreational and agricultural use of water (Mankiewicz *et al.* 2003; van Apeldoorn *et al.* 2007; WHO 2003; 2011). Cyanotoxins can also be accumulated in various aquatic organisms, such as freshwater mussels, clams and fish, and after being transferred through the food chain, in terrestrial organisms (van Apeldoorn *et al.* 2007; Chen *et al.* 2009). Moreover, the presence of cyanotoxins, such as microcystins in water used for irrigation, may considerably affect growth and development of vegetables and crop plants (Mohamed, Shehri 2009).

Currently four main groups of toxins that can be produced by cyanobacteria in fresh waters, have been classified. They include: dermatotoxins, hepatotoxins (e.g. microcystins), neurotoxins (e.g. anatoxin-a) and cytotoxins (e.g. cylindrospermopsin) (Chorus, Bartram 1999; Mankiewicz *et al.* 2003; Codd *et al.* 2005). The above-mentioned groups of toxins can cause irritation to skin or mucous membranes, and allergy (dermatotoxins, hepatotoxins, cytotoxins), and in the later stage, skeletal muscle paralysis, impaired breathing (neurotoxin), diarrhea, acute gastroenteritis, and kidney and liver damage (hepato- and cytotoxins). The existing literature data indicate that microcystins classified as hepatotoxins are the most frequently reported cyanobacterial toxins in the world (Chorus 2005; van Apeldoorn *et al.* 2007).

2.1. Monitoring of toxic genotype – early identification of potential threat

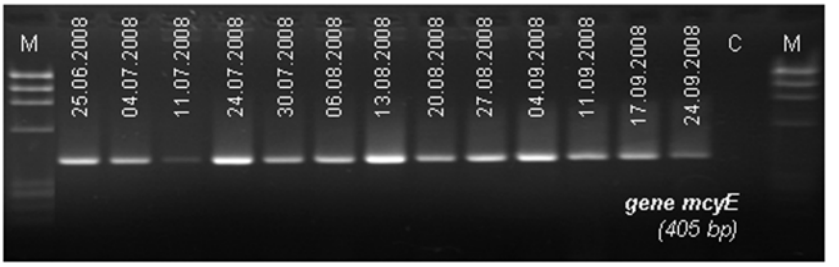
In terms of evolution, cyanobacteria are Gram-negative bacteria, and therefore they belong to a group which includes many well-known pathogenic and potentially harmful organisms. However, only toxic genotypes (toxigenic strains) among cyanobacterial species are able to produce cyanotoxins. The inability to differentiate between toxic and nontoxic strains of cyanobacteria by microscopic analysis has led to applying a sensitive molecular method, i.e. PCR amplification, which enables to identify the toxin-producing genotype (Diettmann *et al.* 1996; Neilan *et al.* 1999). It is possible to identify the total cyanobacterial genotype by amplification of the 16S rRNA gene (Wilmotte 1994). In order, to distinguish toxic and nontoxic strains, the *mcy* genes in the microcystin biosynthesis pathway have been applied (Tillett *et al.* 2000). Microcystins are synthesized nonribosomally by the *mcyABCDEFGHIJ* gene cluster (according to *Microcystis* PCC 7806 genome), including peptide synthetase, polyketide synthase and modifying enzymes. The total size of this region is 55 kb of DNA. Similarly, the *cyrABCDEFGHIJKLMNO* gene cluster (according to *Cylindrospermopsis raciborski* AWT205 genome) responsible for the synthesis of cylindrospermopsin has been described. The total size of this region is 43 kb of DNA (Mihali *et al.* 2008).

We have shown that it is possible to obtain routinely PCR products from surface water samples and then determine the occurrence of toxic genotypes responsible for synthesis of microcystins (freshwater hepatotoxins) or cylindrospermopsin in different water bodies (Fig. 1), regardless of the sampling point (Mankiewicz-Boczek *et al.* 2006a; Gagała *et al.* 2011). Few genes of the *mcy* cluster: *mcyA*, *mcyB*, *mcyD* and *mcyE* have been tested for the detection of microcystins-producing cyanobacteria (Mankiewicz-Boczek *et al.* 2006ab). The molecular analysis indicated that the *mcyE* gene which encodes the glutamate-activating adenylation domain (ADDA), responsible for toxic properties to the microcystin molecule, is very sensitive molecular marker for the determination of potential hepatotoxicity of cyanobacteria in different environmental samples, even if cyanobacterial biomass was below 0.1 mg/l (Mankiewicz-Boczek *et al.* 2011). The *mcyA* gene encoding a protein synthetase is the other equally sensitive molecular marker used in the regular monitoring of microcystins-producing cyanobacteria (Gagala *et al.* 2012). In turn, the *cyrJ* gene encoding the sulfotransferases seems to be an appropriate genetic marker for detection of cyanobacteria capable to cylindrospermopsin production (Mankiewicz-Boczek *et al.* 2012). Summing up, it has been observed that the toxic potential (i.e. toxigenicity) of cyanobacteria can be detected at the beginning of bloom expansion, and monitored throughout the vegetation season to determine occurrence of the toxic genotypes (Fig. 1). Therefore the molecular monitoring acts as an effective alert to possible health threat.

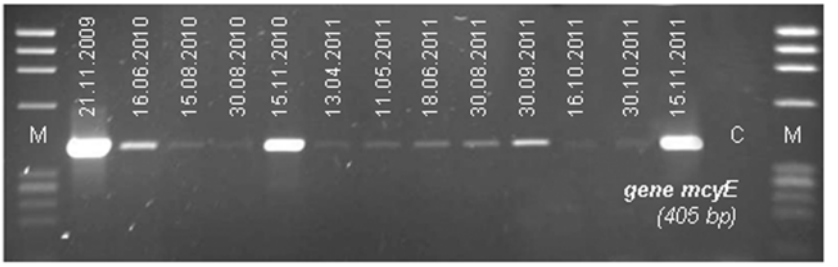
2.2. Interaction between cyanobacteria and environmental parameters – assessment of the cause-effect relationships

The next step in the molecular study according to the methodological background of EH includes the monitoring of dynamics of growth of toxins-producing cyanobacteria in relation to the total population of HAB (quantitative study based of real-time PCR) with regard to changes in environmental conditions. The quantitative RT-PCR is increasingly used in the monitoring of cyanobacteria (Kurmayer *et al.* 2002; Rinta-Kanto *et al.* 2005; Pearson, Neilan 2008; Ha *et al.* 2009; Martins *et al.* 2011). Quantitative molecular data on the number of cyanobacteria containing toxic genotype are necessary to understand how environmental conditions, including biotic and abiotic factors (physicochemical and hydrological parameters of water), can contribute to formation of the bloom in the study water body, and what the role of toxic genotypes in the bloom functioning is (Yoshida *et al.* 2007; Kardinaal *et al.* 2007; Davis *et al.* 2009).

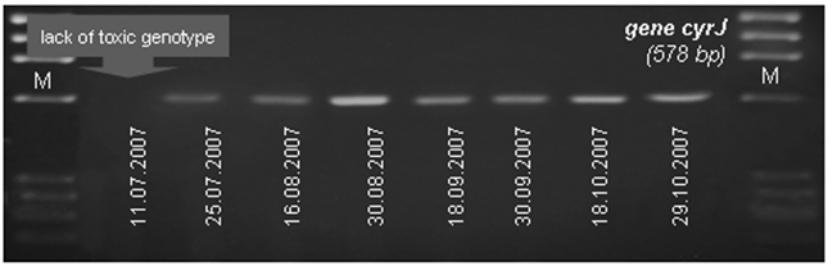
In our last study comprising application of quantitative RT-PCR (based on 16S rRNA gene copies) the key factor for the development of cyanobacteria in lowland dam reservoir was the water retention time (Gagała *et al.* in review). Shortening the retention time from 55 to 18 days resulted in a decrease in the average number of cyanobacteria, despite the favourable physicochemical conditions for the eutrophication (Fig. 2). It was observed that due to the poor hydrological conditions, the amount of toxic *Microcystis* genotypes (based on *mcyA* gene copies) increased to approx. 70% of the total amount of cyanobacteria (Fig. 2). It should also be noted that there were no significant differences in the mean concentrations of microcystins (1 μg/L



Jeziorsko – lowland reservoir in Central Poland.



Tana – the bigest Ethiopian lake.



Bnińskie – eutrophic lake in the Western Poland.

Fig. 1. The PCR analyses of water samples for the presence of genes involved in the synthesis of: microcystins – *mcyE* gene (405 bp) or cylindrospermopsin – *cyrJ* gene (578 bp); samples taken from different water bodies; C – negative control without DNA; M – marker ΦX174 DNA-*HaeIII* Digest.

of MCs) in both summer seasons, despite the large differences in the amount of cyanobacteria, including toxic genotypes (Fig. 2).

The cited results demonstrate how important role the toxic genotype performs in the functioning of cyanobacterial bloom. Active toxic genotype seems to be an important factor in keeping the concentration of cyanotoxins at the level that ensures the presence of cyanobacteria in the studied ecosystem.

2.3. Biodegradation of toxins – elaboration of methods

Knowledge of the interactions between organisms at the molecular level is indispensable in the development of methods to improve water quality, through the use of natural ecosystem properties. According to the review papers, microorganisms play an important role in the degradation of cyanobacterial hepatotoxins (Manage *et al.* 2010; Gągała, Mankiewicz-Boczek 2012). Bacteria can utilize microcystin molecule as a source of carbon and nitrogen for their growth (Jones *et al.* 1994). Based on the molecular knowledge it is possible to select these bacteria that are involved in the biodegradation of cyanotoxins. The first pathway of microcystins degradation by bacteria *Sphingomonas* sp. (MJ-PV) was described by Bourne *et al.* (1996, 2001). The four intracellular hydrolytic enzymes which are encoded by *mlrABCD* gene cluster (5.8 kb of DNA) are involved in this process.

Our preliminary study, based on the detection of the *mlrA* gene fragment (Saito *et al.* 2003), indicates a possibility of degradation of microcystins by the genus *Sphingopyxis* in lowland reservoir in the Central Poland (unpublished data). Ho *et al.* (2010) described that the LH21 isolate belonging to the genus *Sphingopyxis* could be very effective in degradation of microcystins in biological sand filtration.

Conclusion

The molecular analyses, such as quantitative PCR and qualitative RT-PCR using knowledge of the structure of nucleic acids and function of genes, are effective and indispensable techniques for methodological background of Ecohydrology. Advantages offered by using genetic analysis include sensitivity, time saving and flexibility of the methodology.

Based on our experience, it has been shown that the use of genetic markers allowed to determine the potential threat caused by the cyanobacteria

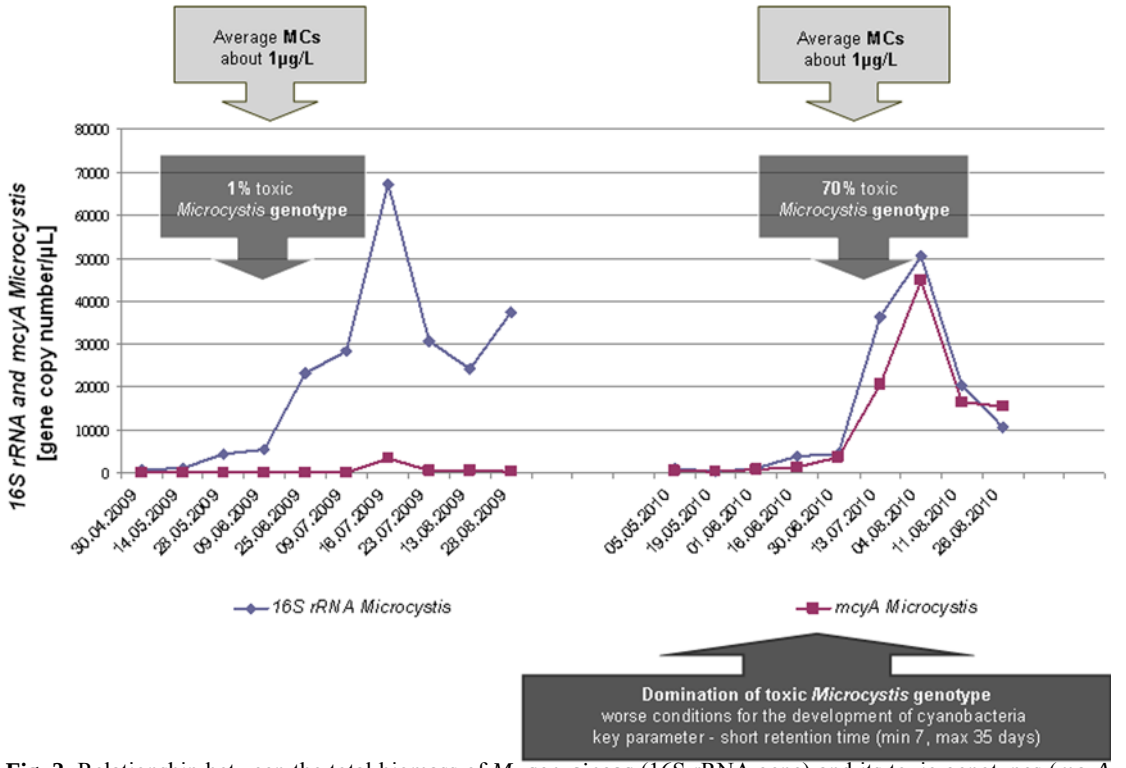


Fig. 2. Relationship between the total biomass of *M. aeruginosa* (16S rRNA gene) and its toxic genotypes (*mcyA* gene), in Sulejów Reservoir, in the context of different hydrological conditions; MCs – microcystins.

(representing harmful algal blooms) in the water bodies based on detection of toxic genotypes at the beginning of the bloom formation. Then analysis of the relationship between environmental parameters and the presence of cyanobacteria, including toxic genotypes, and microcystins (cyanotoxins), helped to identify the retention time as the key factor determining the cyanobacterial threat in the study lowland reservoir. Finally, the amplification of the bacterial gene involved in the synthesis of microcystin-degrading enzymes allowed to undertake further research to develop methods to reduce cyanobacterial toxins in different sources of freshwater.

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