

**Molecular approaches for monitoring potentially toxic marine
and freshwater phytoplankton species**

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Abstract

Harmful phytoplankton species are a growing problem in freshwater and marine ecosystems due to their ability to synthesize toxins that threaten both animal and human health. The monitoring of these microorganisms has been so far based on conventional methods, mainly involving the microscopic counting and identification of cells, and using analytical and bioanalytical methods to identify and quantify the toxins. However, the increasing number of microbial sequences in GeneBank database and the development of new tools in the last 15 years permit nowadays to propose molecular methods for the detection and quantification of harmful phytoplankton species and their toxins. These methods provide species-level identification of the microorganisms of interest, and their early detection in the environment by PCR techniques. Moreover, Real Time PCR can be used to quantify the cells of interest, and in some cases to evaluate the proportion of toxin producing and non-toxin producing genotypes in a population. Recently, microarray technologies have been also used to provide the simultaneous detection and semi-quantification of harmful species in environmental samples. These methods look very promising, but so far their use remained limited to research. The need of validation for routine use and the cost of these methods still hamper their use in monitoring programs.

Key words: Aquatic ecosystems, phytoplankton, phycotoxins, cyanotoxins, detection, molecular approaches

Introduction

Over the last ten years, increasing attention has been devoted to surveys of toxic photosynthetic microorganisms in marine and freshwater ecosystems due to the health risks associated with their presence in a growing number of these ecosystems, and also to their economic impact on tourism and aquaculture. Proliferations of these toxic microorganisms in aquatic ecosystems are stimulated by anthropogenic processes and, in particular, by high nutrient inputs (in particular of nitrogen and phosphorus) and, to a lesser extent, by climatic changes [1]. Surveys of these microorganisms are generally based on the microscopic identification and counting of the cells of interest. Similarly, the evaluation of the potential toxic risk of tested samples is based on the direct identification and quantification of the toxins by immunochemical or chemical methods or by using biological methods. These methods demonstrated their effectiveness in the context of monitoring programs, and will doubtless continue to be reference methods for many years to come, but they also have some limitations.

The difficulty to identify some of these microorganisms at the species or even genus level and the time-consuming process for microscopic examination and cell counting constitute the main restrictions on using these methods. In addition, identifying and quantifying the species present may not suffice to estimate the potential toxic risk, because all the strains of a given species may not produce toxins. Some strains of a same species can have the genes necessary to produce the toxins and some not. The cyanobacteria are a good example, as their ability to produce microcystins (hepatotoxins) can vary considerably within a given species. The main problems encountered in monitoring toxins result in the difficulty to quantify some of them by analytical approaches. Therefore, biological tests are still required to detect the presence of most of them such as the Paralytic Shellfish Poisoning (PSP) toxins in marine ecosystems.

Advances in the molecular methods for evaluating microbial diversity in natural environments means that new technical approaches such as real time-PCR (q-PCR) are now available to identify and quantify microorganisms in these complex matrices. Furthermore, the increasing number of complete microbial genomes available in databanks contributes to identify the genes involved in toxins biosynthesis. The aim of this review is to describe the current situation with regard to the development of the molecular tools for monitoring potentially toxic microorganisms in marine and freshwater ecosystems. We consider the molecular tools that allow the detection of potentially toxic species and the ones that separate toxic genotypes from non-toxic genotypes within a given population. Finally, we only examine the techniques based on the use of DNA/RNA molecules.

Molecular detection of potentially toxic phytoplankton in marine ecosystems

Most of the toxic phytoplankton species found in marine ecosystems are microalgae belonging to Dinophyceae, Bacillariophyceae, and Raphidophyceae. They are also few species of photosynthetic prokaryotes belonging to Cyanobacteria. The toxins produced by these marine microorganisms cause diseases including paralytic shellfish poisoning (PSP), azaspiracid poisoning (AZP), ciguatera fish poisoning (CFP), diarrhetic shellfish poisoning (DSP), and neurotoxic shellfish poisoning (NSP).

Detection of species of interest in marine ecosystems

Initially, several papers reported the interest of molecular probes for identifying and counting harmful algal species (e.g. [2]). Such probes, generally targeting ribosomal RNA, were used in the framework of the fluorescent *in situ* hybridization (FISH) approach coupled either with microscopic examination or with flow cytometry to count large numbers of cells quickly. After the early publications describing group- or strain-specific rRNA markers for harmful

algal species (e.g. [3]), numerous works tested their use in fluorescence *in situ* hybridization (FISH).

Detection of potentially toxic species using hybridization based methods

At the beginning, the hybridization of the probes was performed on whole cells, and the counting was done using epifluorescent microscopy or flow cytometry. Using this approach on seawater samples, Hosoi-Tanabe and Sako [4, 5] showed that a simple and rapid identification of two dinoflagellates species responsible for PSP, *Alexandrium tamarense* and *A. catanella*, can be obtained within 30 min. This PCR assay detected a single cell of *A. catanella* per reaction [4]. Similarly, Chen et al. [6] developed a whole-cell FISH protocol for the detection of *Heterosigma akashiwo*, a fish-killing Raphidophycean alga, in less than 1 hour. In all these papers, the detection and counting of the target species were performed by epifluorescent microscopic examination, which can also be time consuming. To reduce the time of these analyses, FISH protocols have been coupled with flow cytometry (e.g. [7]). In addition, Huang et al. [8] successfully applied this approach using a peptide nucleic acid (PNA) probe to detect of the toxic dinoflagellate *Takayama pulchella*. They also obtained a higher detection signal of the target species using the PNA probe instead of DNA probes.

Beside classical FISH hybridization assay on whole cells, the sandwich hybridization assay (SHA) developed by Scholin et al. [9, 10] on *Pseudo-nitzschia* was successfully tested for the detection of several other potentially toxic microalgae, such as *Alexandrium* spp. [11], *Heterosigma akashiwo* [12, 13], *Karenia brevis* and closely related species [14], or *Cochlodinium polykrikoides* [15]. This approach is based on the use of two probes, the first carries out the direct capture of the target rRNA sequence on a solid support (such as a 96-well plate) and the second detects the complex target rRNA-capture probe. The main advantage of the SHA over whole-cell FISH hybridization protocols is to allow the rapid

counting of potentially toxic microalgal species and the examination of a great number of environmental samples (about 50 samples per 8 h [11]) with a low analytical cost (around 5 USD/sample in triplicate [12]). In addition, the automation of the SHA protocol allowed the detection of *C. polykrikoides* at very low cell concentrations (1-3 cells/L) in field samples [15], demonstrating its efficiency for detecting the harmful species at pre-bloom concentrations. A variant of this method using a nuclease protection assay was also applied for the detection of *Prorocentrum* species with a limit of detection of 15 cells per mL of seawater [16]. The SHA approach was approved for commercial use in New Zealand in May 2004 [17] in the framework of the survey of toxic microalgae, in particular for the fragile microalgal cells difficult to identify and to count by microscopic examination. New methods based on the use of probes are still in development. For example, paramagnetic nanoparticles conjugated to a DNA-capture probe complementary to a specific 5.8S rRNA gene fragment of the genus *Alexandrium* has made possible to purify and concentrate *Alexandrium* DNA prior to its detection using a PCR assay [18]. In the same way, the coupling of different specific probes on polystyrene carboxylated beads combined with the use of PCR permitted to detect several harmful species simultaneously in marine water samples within less than 4 hours [19].

Detection of potentially toxic species by PCR based methods

In addition to hybridization-based methods, numerous papers described the use of PCR for monitoring Harmful Algal Bloom (HAB) species in marine environments. The target genes used to identify the species present were usually chosen within the rRNA operon. In a few studies, other genes were used such as the *rbcL* gene with a limit of detection of less than one cell per reaction [20] or the mitochondrial cytochrome b upstream region [21] with a detection limit of 0.1 *Pfiesteria piscidas* cell per mL of water sample using a Real Time PCR assay. This kind of approach was also successfully used to identify and detect several types of

potentially toxic microalgae: dinoflagellate cells in both the water column and the sediment [22], diatoms belonging to the *Pseudo-nitzschia* genus [23], and *Alexandrium* spp. in tissues of mussels [24]. Recently, the methodology of this PCR approach was enhanced to boost its sensitivity towards *Karenia mikimotoi* (Dinophyceae) 100 fold relative to regular PCR by using a loop-mediated isothermal amplification (the detection limit was around 6 pg of DNA) [25].

In complement to these “classical” PCR approaches, the q-PCR approach can also be used to perform in less than 4 h a semi-quantification or a quantification of the cells in addition to identifying them. This kind of assay was successfully tested for dinoflagellate species such as *Pfiesteria* [21, 26], *Cryptoperidiniopsis brodyi* [27], *Cochlodinium polykrikoides* [28], *Alexandrium minutum* [29] and *Karlodinium veneficum* [30], and for raphidophyte species such as *Chattonella cf verruculosa*, *C. subsalsa* or *Heterosigma akashiwo* [31, 32]. In the paper of Handy et al. [32], it was shown that multiplexing (using several species-specific sets of primers and probes) was more efficient than multiprobing (using the same set of primers with species-specific probes) for the simultaneous detection of several species. The use of the nucleic acid sequence-based amplification (NASBA) was also tested for the quantification of *K. brevis* (Dinophyceae) [33] and that of *K. mikimotoi* [34] with a detection limit of one cell per reaction. This approach is based on a real-time PCR amplification coupled with molecular beacon technology performed on the mRNA of the *rbcL* gene. Recently, Casper et al. [33] developed a handheld version of the whole system, which provided a portable assay kit to detect *K. brevis* in 20 minutes. Comparing the different methods available (whole-cell FISH, real-time PCR and morphotaxonomy analyses) for counting *A. minutum* (Dinophyceae) in seawater samples, Touzet et al. [35] showed that the three approaches provided congruent cell quantification data. Q-PCR was also successfully tested for the quantification of *A. tamarensis* and *A. catenella* (Dinophyceae) cysts in marine sediments [36].

Detection of potentially toxic species by microarray based methods

The recent advances in microarray technologies provided new tools for the monitoring of toxic microorganisms in marine environments opening a new approach to research. The pioneering paper of Ki and Han [37] demonstrated the potential interest of using low-density oligonucleotide arrays for the simultaneous detection of several HAB species in natural samples. In the framework of the “Fish and Chips” European Project [38], a phytoplankton DNA chip for all microalgal classes, including many toxic species was developed, and the ALEX-CHIP [39] was evaluated in the frame of the identification and the monitoring of *Alexandrium* with the aim of proposing a sensitive tool for the high-throughput survey of this genus.

Detection of toxic genotypes in marine ecosystems

Until now, the use of molecular methods for the detection of marine strains containing the genes involved in the toxins biosynthesis remains impossible. The main reason is that characterization of these genes is still elusive despite an important research effort [40-42] (Table 1). This is partly due to the complexity of the algae genomes and to the frequent bacterial-algal symbiosis. Despite the efforts of the marine research community to retrieve the origin of the most potent algal toxin of toxic Dinoflagellates, the genes for the biosynthesis of saxitoxins was recently retrieved from freshwater cyanobacterial species [41, 43]. However, this is not yet sufficient to define a molecular detection tool for the monitoring of saxitoxins producers even in freshwater systems (see below).

The only genes involved in toxin production that have been described in marine microorganisms (Table 1) are those allowing the cyanobacterium *Lyngbya majuscula* strain JBH collected from Hector Bay in Jamaica to produce jamaicamides toxins [44]. The

jamaicamides A to C are neurotoxic compounds produced by a 58-kb gene cluster named *Jam*, which is very similar to other cyanobacterial and bacterial PKS and NRPS genes clusters. Concomitant to the discovery of the jamaicamides pathway, Gerwick's team (Institution of Oceanography, University of California) also investigated curacin A, an antitubulin product and potent anticancer agent produced by another *L. majuscula* strain L19 isolated in Curucaó [45]. The biosynthetic pathway of curacin A and the associated NRPS/PKS *Cur* gene cluster were recently described [46, 47]. The comparison of the *Cur* and *Jam* genes revealed their high similarity at the nucleotide and amino acid levels [48, 49]. This exemplifies, once more, that the genes involved in the jamaicamides pathway are not all equivalent in terms of monitoring, as some of them can lead both to the target toxins and to a potent anticancer compound. Their detection could lead to a false positive in the context of jamaicamides detection. Therefore, the choice of the appropriate genes to target appears essential. Moreover, the diversity of these genes remains to be identified, as they were so far retrieved from only one strain. However, the *Jam* cluster discovery is a first step in the development of molecular monitoring tools for field surveys of jamaicamides-producing cyanobacteria.

Molecular detection of potentially toxic phytoplankton in freshwater ecosystems

In freshwater ecosystems, the main toxic species in the phytoplankton communities are cyanobacteria. Several cyanobacterial genera are able to produce hepatotoxins, comprising the small peptides microcystins (MCs, produced by several genera including *Microcystis*, *Anabaena*, *Planktothrix*, *Anabaenopsis*, *Hapalosiphon*, review in [50]) and nodularins (NODs, only found so far in *Nodularia spumigena* and *N. harveyana* PCC 7804 [51, 52]), and the alkaloid cylindrospermopsin (CYN, mainly produced by *Cylindrospermopsis raciborskii*,

Anabaena ovalisporum and *Umezakia natans*). Moreover, since the 1970s the ability of cyanobacteria to synthesize neurotoxins has been demonstrated [53]. Until now, three kinds of these toxins were evidenced in freshwater cyanobacteria: anatoxins (anatoxin-a and homoanatoxin-a), anatoxin-a(s) and saxitoxins.

Detection of toxic cyanobacteria in freshwater ecosystems

There are far fewer papers dealing with the use of molecular methods for the detection of cyanobacteria in freshwater ecosystems (Table 2) than in marine environments. This is in part attributable to the fact that the health authorities have only been taking cyanobacteria into account in monitoring programs for the past ten years, whereas harmful algae have been monitored in marine environments for much longer. A second reason could be that toxic cyanobacteria are easier to detect with classical methods in freshwater ecosystems than microalgae in marine ecosystems, due to the fact that they generally form very considerable biomasses.

Detection of cyanobacteria species using PCR based methods

Since the initial papers by Rudi et al. [54, 55], numerous publications have dealt with the evaluation of molecular methods for monitoring toxic cyanobacteria in freshwater ecosystems. The intergenic spacer region in the phycocyanin operon was used by Baker et al. [56, 57] to identify several potentially-toxic cyanobacterial species in environmental samples. In the same way, the *rpoCI* gene was successfully tested for the specific detection of *Cylindrospermopsis raciborskii* [58], and a fragment of the 16S rRNA gene was used for the early detection of *Microcystis* spp. [59]. A combination of a PCR on the rRNA 16S-23S ITS fragment with a digestion of the amplified fragments by restriction enzymes (amplified rDNA restriction analysis: ARDRA) was also applied successfully by Masseret and Sukenik [60] to monitor toxic cyanobacteria in the catchment area of Lake Kinneret. While all these studies

dealt with environmental samples, a multiplex PCR was also tested on dietary supplements produced from the nontoxic cyanobacterium *Aphanizomenon flos-aquae* to find out whether any toxigenic cyanobacteria are present [61]. This PCR targeting both a 220-bp fragment of the 16S rRNA gene and a 300-bp fragment of the *mcyA* gene (involved in the microcystin biosynthesis), demonstrated the presence of toxic *Microcystis* cells in these dietary supplements.

Detection of cyanobacteria species using q-PCR and Reverse Transcriptase-PCR

A few papers reported the use of q-PCR for identifying and counting cyanobacteria in aquatic ecosystems [62, 63], but it was mainly used for the specific evaluation of microcystin-producing cyanobacteria, as described below. Using q-PCR approach, Rueckert et al. [63] were able to detect at least five *Anabaena planktonica* cells per sample and Rinta-Kanto et al. [62], 5000 gene copies per liter of lake water. A Reverse Transcriptase-PCR assay on the *rnpB* gene was also evaluated for the detection of *Anabaena variabilis* for the purpose of its application in biosensors [64], but there was no further development since this first publication.

Detection of cyanobacteria species using DNA array

The use of DNA array for the field detection of cyanobacterial species was also examined. Rudi et al. [65] established ten specific 16S rDNA oligonucleotide probes, which were spotted onto a solid support. This array was tested on water samples from eight lakes. Good results were obtained for the qualitative estimation of the presence or absence of the various cyanobacterial genera. However this array did not provide quantification. After this first method, an universal microarray was developed for cyanobacteria in the framework of the European project MIDI-CHIP [66]. This new array was constructed using 16S rRNA gene

probes targeting 19 cyanobacterial groups and was evaluated on environmental samples. Only 1 fmol of PCR product (corresponding to 1 ng of amplified DNA) was necessary to obtain a positive signal. The comparison of the microarray data with those obtained by microscopic examination of the same samples leads to consistent results demonstrating the potential interest of this approach for monitoring cyanobacteria.

Detection of toxic genotypes in freshwater ecosystems

Several studies of toxic cyanobacterial populations reported the presence of both toxin-producer and non-toxin producer genotypes (e.g. [67-69]), which cannot be distinguished by using the methods listed above. As demonstrated by chemical and genetic studies on the biosynthetic pathways, cyanobacterial hepatotoxins result from a non-ribosomal biosynthesis involving a particular cluster of genes. In the last five years, characterization of these clusters permitted the development of molecular methods for the detection and monitoring hepatotoxic cyanobacterial genotypes. On the contrary, several reviews on cyanobacterial neurotoxins and their toxicological effects [51, 70, 71] were reported, but the biosynthetic pathways and the coding genes clusters were far less known until recently. Thus, the development of tools for the detection of toxic cyanobacteria genotypes in freshwater ecosystems has mainly concerned hepatotoxin producers, especially MC-producers.

Detection of hepatotoxic genotypes using PCR based methods

Initially, Otsuka et al. [72] investigating the phylogenetic relationships between toxic and non-toxic strains of *Microcystis* revealed that these strains could be distinguished to some extent by their 16S-23S rRNA Internal Transcribed Spacer amplified by PCR. They constructed a phylogenetic tree in which *Microcystis* strains were divided into three clusters. The first cluster included both toxic and non-toxic strains, the second only toxic strains, and

the third only non-toxic strains. Later, microcystin-producing and non-microcystin producing cyanobacteria of natural populations were distinguished by PCR on the rRNA ITS coupled with denaturing gradient gel electrophoresis typing (rRNA ITS-DGGE) [73-75] .

As a result of the discovery of the MC genes cluster, PCR reactions directly targeted those genes involved in toxin production (Table 2). The *mcy* gene cluster responsible for the production of MCs was first described for *Microcystis* [76, 77] and subsequently in *Planktothrix* [78] and *Anabaena* [79] (Fig 1). The genes *mcyA*, *mcyB* and *mcyE* are predominantly used for the detection of MC-producing genotypes (Table 2). They can be used separately in simple PCR [57, 80, 81] or in combination in multiplex PCR [82]. The genes cluster involved in the NODs biosynthesis presents a high degree of sequence homology with the *mcy* cluster (Fig 2) [83]. Using a set of primers (the HEP primer set) targeting a conserved aminotransferase domain of the *mcyE* and *ndaF* genes, Jungblut and Neilan [84] proposed a PCR test for the simultaneous detection of all MC- and NOD-producing cyanobacteria (*Microcystis*, *Planktothrix*, *Anabaena*, *Nostoc*, *Nodularia*).

The main limitation of the use of PCR results in a possible overestimation of the potential toxicity. Indeed, a positive detection of the toxic genotypes does not reveal mutations, insertions or deletions that can inactivate genes within the cluster [68, 85, 86] and hamper the MC production. However, the recent paper of Christiansen et al. [87] demonstrated that, in general, less than 10% of the *mcy* genes cluster persist in non-toxic *Planktothrix* strains, and that the remaining elements were usually located in the flanking regions (*mcyT* or *mcyJ*), which are never targeted for PCR.

Detection and quantification of hepatotoxic genotypes using q-PCR

The data provided by the use of the PCR indicate the presence/absence of potentially toxic genotypes, but not their proportions in a given population. However, these proportions can

vary during the course of the bloom and also differ from one bloom to another. In order to estimate the abundance of a cyanobacterial population and the proportion of toxic genotypes within this population, several q-PCR assays were successfully tested on different genera: *Microcystis* [88-91] and *Planktothrix* [92, 93] (Table 2). In the paper of Kurmayer [88], the detection limit was only of one target cell per PCR reaction. These assays were also useful to estimate the spatiotemporal changes in the proportions of MC-producer genotypes within these cyanobacterial populations. Recently applied for CYN producers, Mihali et al. [94] characterized the cluster of genes involved in the CYN biosynthesis (Fig 3). They suggested that *cyrJ* could be used to detect CYN-producing cyanobacteria, because this gene encodes for a sulfotransferase, and is only present in CYN-producing cyanobacteria. At the same time, Rasmussen et al. [95] developed two q-PCR tests to detect and quantify CYN producers with a limit of detection of 100 copies per reaction or 1,000 cells ml⁻¹. The first test detects all CYN producers, whereas the second only recognizes toxic *C. raciborskii* genotypes. The successful use of a portable q-PCR device in this study suggested the possibility to use such molecular methods in the field for the rapid screening of water samples. However, as already reported for the *mcy* gene cluster, Rasmussen et al. [95] demonstrated that some mutations found in the *cyr* genes cluster can inactivate CYN production. In order to limit false positive results, they proposed to target preferentially *aoaA*, *aoaB* and *aoaC* (homologs of the *cyrA*, *cyrB* and *cyrC* genes respectively described by Mihali et al. [94]). The *aoaA* gene was previously proposed as a potentially good target gene [96, 97].

Detection of hepatotoxic genotypes using DNA microarray

For the detection of hepatotoxic cyanobacterial genotypes, the genes involved in the biosynthesis of MC or NOD were also used in the context of the development of a DNA microarray coupled to a PCR reaction, [98] (Table 2). Genus-specific probes (*Anabaena*, *Microcystis*, *Planktothrix*, *Nostoc* and *Nodularia*) were designed for the detection of both

mcyE and *ndaF* genes. As already observed by Castiglioni et al. [66], these probes have high specificity and sensitivity allowing to lower the detection limit to 1 to 5 fmol of the PCR product. These features suggested the possibility to develop this technology for routine use in monitoring and screening environmental samples.

Toward a future detection of neurotoxic cyanobacteria genotypes in freshwater ecosystems using molecular tools?

Recent studies from Neilan's Laboratory (Sydney, AUS) permitted to identify a putative biosynthesis pathway for STX [41, 43, 99, 100]. The genes cluster responsible for STX production is modular as demonstrated by its recent characterization in different cyanobacterial strains [43, 94, 101, 102]. The mosaic structure and the absence of synteny of the *sxt* genes cluster from one organism to another is important to keep in mind if the aim is to provide early warnings using PCR-based systems. It will be necessary to characterize the genes involved in the biosynthesis of STX in a wider range of toxin-producing organisms displaying different toxin profiles before a molecular detection tool can be proposed to monitor saxitoxin producers.

The anatoxins (anatoxin-a and homoanatoxin-a) were first isolated from an *Anabaena flos-aquae* strain [53], and were subsequently retrieved from diverse cyanobacterial genera. Two decades later, the first biosynthesis pathway common to both anatoxins was proposed [103]. The genes involved in the putative biosynthetic pathway were recently proposed and a PCR-based methodology was tested on isolated strains for the purpose of detecting the presence of anatoxin-producing *Oscillatoria* [104, 105]. However anatoxins can also be produced by cyanobacteria other than *Oscillatoria*. As for saxitoxin-producers, it will be necessary to identify these genes in other anatoxin-producing genera prior to monitor these toxic microorganisms using molecular detection tool.

Conclusions

This review reveals that the use of molecular tools for field monitoring of potentially toxic phytoplankton species is still in its infancy. The main drawback of these methods for detecting and quantifying toxic organisms is that they provide information about the potential toxicity of the organisms present, but not real estimation of the associated risk. Their ability to do so is hampered by the variable capacity of toxin-producing cells within the same species, and also by changes in the toxin production of a given toxic cell over time. Another important issue is the high cost of some of these methods in terms of both time and money, which is not compatible with their routine use in protocols for analyzing large numbers of samples. Despite these issues, molecular methods are undoubtedly useful for the early detection of potentially toxic organisms, as they are effective even at low presence of the target. The automation of these methods will be the key to real-time monitoring of aquatic ecosystems and, in particular, for early stages in the development of potentially toxic microorganisms. This is already an undergoing research area with the development of integrated Real Time PCR tool to quantify in less than 30 min the proportion of potentially toxic genotypes in water samples containing cyanobacteria. Finally, in regard to the recent development of high throughput technologies of DNA sequencing and to the decrease of the costs of DNA sequencing, new approaches based on the use of these technologies will emerge in the next years, allowing to evaluate the potential toxicity due to phytoplankton species in natural water samples.

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Figure legends:

Fig. 1. Structural organization of the microcystin gene cluster from *Microcystis aeruginosa*, *Planktothrix agardhii* and *Anabaena* sp. (from [106]).

Fig. 2. Structural organization of the nodularin gene cluster from *Nodularia spumigena* (from [106]).

Fig. 3. Structural organization of the cylindrospermopsin gene cluster from *Cylindrospermopsis racibordkii* (from [94]).