

1. INTRODUCTION

"It is completely unclear why certain strains of Microcystis aeruginosa are able to produce toxins, whereas others are not".

Meissner (1996)*

The worldwide occurrence of toxic cyanobacterial blooms in fresh and brackish eutrophic waters creates a problem for all life forms. Most water-based poisonings by cyanobacteria occur when heavy surface growths or scums accumulate near shorelines of lakes, ponds and reservoirs where animals have free access to high concentrations of these toxic cells. Deaths attributed to cyanobacterial toxins have been reported for man, animals, birds and fish.

Cyanobacterial blooms are ubiquitous, often associated with eutrophication and appear to be on the increase, also in South Africa. N, P and C are important nutrients for high growth rates and the ratios in the supply concentrations are often decisive in selecting for cyanobacterial dominance. These organisms are capable of scavenging their environments for resources and excessive or "luxury" uptake of nutrients allow them to survive extreme nutrient deficient conditions. Cyanobacteria flourish at high temperatures, neutral to alkaline conditions, high nutrient concentrations especially where the ratios of N:P are low and an adequate supply of iron is present. The buoyancy of certain species, due to especially the production of gas-vacuoles gives them a competitive advantage over other phytoplankton. All blooms of *Microcystis*, one of the most ubiquitous species, should be considered to be toxic and toxins are easily leached from the cells. They persist for long periods and are not easily degraded.

Microcystis aeruginosa is distributed worldwide that often form seasonal blooms, as is common in South Africa. This organism produces a vast number of peptides (microcystins), some of which are highly toxic. Microcystins consist of a seven-

* MEISSNER KE, DITTMANN E & BÖRNER T (1996) Toxic and non-toxic strains of the cyanobacterium *Microcystis aeruginosa* contain sequences homologous to peptide synthetase genes. FEMS Microbiology Letters **135**: 295-303.

membered peptide ring made up of five non-protein amino acids and two protein amino acids. More than 50 microcystins have been identified to date, most representing minor components of the total toxin complement of the cyanobacteria from which they were isolated. The most commonly occurring toxin is microcystin-LR, a cyclic heptapeptide hepatotoxin, where symptoms of exposure too, includes skin irritation, possible liver cancer as a result of cronic exposure, and even death. These toxins are inhibitors of serine/threonine protein phosphatase enzymes and are among the most potent tumour-promoting compounds. The mechanism of toxicity is exerted by the general inhibition of dephosphorylation of protein phosphatases 1 and 2A, leading to hyperphosphorylation in the cytosol.

The genus *Microcystis* contains non-toxic and toxic strains and the toxicity is affected by various environmental factors such as water temperature, pH, intensity of solar radiation, dissolved oxygen and CO₂ availability. It has been reported that microcystin concentrations are usually higher under conditions, when the organisms are stressed by any of the above factors.

The molecular basis of toxin-production in *M. aeruginosa* was partially elucidated elsewhere and it was found that both toxin-producing and non toxin-producing strains of *M. aeruginosa* contained sequences that revealed a high degree of homology with several well-characterised peptide synthetases. In blotting experiments, a PCR fragment based on a portion of one of these peptide synthetases hybridised exclusively to restricted genomic DNA from toxin-producing strains indicating that this peptide synthetase was involved in toxin production.

2. AIMS OF THE PROJECT

The aims of the project were to:

- To acquire and maintain viable toxic and non-toxic cyanobacteria, specifically *Microcystis aeruginosa* strains.
- Determination of optimal growth conditions as well as those affecting maximal toxin production.
- Mass culturing of toxic cyanobacteria for use in tests as required.

- Elucidation of the genetic control of microcystin synthesis.
- Determination of possible correlations between the occurrence of *Microcystis* blooms and environmental conditions in natural systems.
- To attempt to develop a molecular screening tool for naturally occurring blooms of *M. aeruginosa* based on the presence or absence of the gene *mcyB*, which has previously been implicated in toxin production in *M. aeruginosa*.

3. METHODOLOGY, RESULTS AND DISCUSSION

3.1 Test material

Unrelated strains of *M. aeruginosa* were obtained from the Pasteur Institute, France; the National Institute for Environmental Studies, Japan; the Institute of Freshwater Ecology, UK; Universities of Pretoria and the Free State culture collections (Table 1). Based on conserved regions present in known sequences of the *mcyB* gene, four primer pairs were designed. The strains were maintained under standard conditions and the total genomic DNA was extracted from toxin-producing strains PCC 7813, UV 027 and non toxin-producing strain CCAP 1450/1.

3.2 DNA and HPLC analyses

PCR reactions were performed and the fragments generated with the various primer pairs were compared with expected fragment sizes. PCR products of the expected size were amplified in both toxin-producing strains with all four-primer pairs, signifying that these toxin-producing strains possessed a copy of the *mcyB* gene. It was also possible to generate PCR fragments with three primer pairs from the non toxin-producing strain CCAP 1450/1. These results indicated that this strain contained at least partial elements of *mcyB*.

Fragments amplified by PCR from toxin-producing strains were cloned into pGemT®-Easy (Promega) and sequenced. Basepair and translated amino acid alignment of

the assembled fragments showed a high degree of homology with previously deposited sequences of *mcyB* in the Genbank database.

A fragment amplified by PCR from strain PCC 7813 with primer pair Tox 7P/3M was randomly labelled and used as a probe to screen other strains of *M. aeruginosa* for the presence of *mcyB*. This probe hybridised to a fragment of the expected size in all toxin-producing strains as well as the non toxin-producing strain confirming PCR results that all strains contained this particular portion of *mcyB*.

Table 1 Different *Microcystis* strains used, their culture collection codes, collection organisation, nutrient medium, and known toxicity.

Culture ID	Culture collection	Nutrient Medium	Toxicity
PCC7806	Pasteur, France	BG-11	?
PCC7813	Pasteur, France	BG-11	?
PCC7820	Pasteur, France	BG-11	?
SAG14	Göttingen, Germany	BG-11	?
UV027	UFS, South Africa	BG-11	Toxic
CCAP1450/1	CCAP, UK	BG-11	Non-toxic
N88	NIES, Japan	BG-11	?
N89	NIES, Japan	MA	?
N90	NIES, Japan	MA	?
N91	NIES, Japan	MA	?
N99	NIES, Japan	MA	?
N101	NIES, Japan	MA	?
N299	NIES, Japan	MA	?
UP01	UP, South Africa	BG-11	?

A second probe generated from strain PCC 7813 with primer pair Tox 1P/1M representing the fragment of *mcyB* not amplified by PCR in strain CCAP 1450/1 was

synthesised. This probe hybridised to a fragment of the expected size in all toxin-producing strains and the non toxin-producing strain. Hybridisation of this probe to *Pvu*II digested DNA from CCAP 1450/1 indicated that there was enough target DNA in the CCAP 1450/1 genome for the Tox 1P/1M/PCC 7813 probe to hybridise to, hinting at the possibility that this strain also possessed a complete copy of the gene.

Amplified fragment length polymorphism (AFLP) was used to determine the genetic relationships of the geographically unrelated strains. A total of 909 bands were amplified from the eight primer combinations, of which 665 was informative, 207 non-informative and 37 monomorphic, with an average of 83.12 polymorphic bands per primer combination. Definite groupings were obtained, that confirmed the value of AFLP analyses for the identification of genetic diversity and population structures of *M. aeruginosa*.

Based on conserved motifs present in known sequences of *mcyB* four primer pairs were designed and used to identify strains with toxicity or not. Analysing the strains and using the insertions/deletions (indels) to discriminate between *M. aeruginosa* and *M. wesenbergii* in raw water samples it confirmed the value of PCR assays as an indicator of toxicity and taxonomical characteristics.

Crude aqueous cell extracts made from all strains, were investigated and analysed by HPLC for the presence of microcystin-LR. Microcystin-LR was detected in all toxin-producing strains as well as the 'non toxin-producing' strain CCAP 1450/1.

3.3 Photosynthetic characteristics

The thirteen different *Microcystis* strains varied more than 3-fold in their photosynthetic characteristics. Large variations were also seen in the microcystin YR and LR contents. The relatively low I_k values (onset of light saturated photosynthesis) and high α^B rates (photosynthetic efficiencies) indicated highly efficient usage of low light intensities, making them suitable organisms for turbid environments. UV027, a known highly toxic *Microcystis* strain did not contain the highest total microcystin content. The highest toxin contents (YR + LR) were found in

the strains SAG14, N 88, 89, 91, 99 and 101. Neither the photosynthetic potential, nor a measure such as the Chl a content appeared to give an indication of the potential toxicity of a particular strain.

Microcystis is essentially a photoautotrophic organism, but may also be mixotrophic. This implies that production of the secondary metabolites, such as microcystin has to be derived from primary photochemistry. Using chlorophyll fluorescence it was found that the different strains showed marked variations in both the primary photochemical characteristics, such as photon absorption per reaction centre and the electron transport per reaction centre, as well as oxygen liberation at different light intensities. The results also demonstrated acclimation to differing conditions, indicating the adaptability of *Microcystis*. There was some indication that photosynthetic performance may imply higher toxicity and vice versa.

Many factors and combinations of factors influence bloom formation of cyanobacteria and the only conclusions that can be made from this study are that there is a high probability that cyanobacteria may form blooms, when eutrophic conditions are present, water temperature is high and water pH's are alkaline. Water temperature appeared to be the most important factor influencing bloom development in a eutrophic pond and little growth was seen at temperatures below 18 °C. Once blooms develop toxin measurements are the only means of determining the presence or not of these secondary metabolites and PCR assays should be used.