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The WRC operates in terms of the Water Research Act (Act 34 of 1971) and its mandate is to support water research and development as well as the building of a sustainable water research capacity in South Africa.

TECHNICAL BRIEF

Eutrophication

Cyanobacteria – detecting toxin-producing strains

A report discussing new methods to detect and identify toxic strains of cyanobacteria has been published by the WRC.

Cyanobacteria – Scope of the problem

Blooms of toxic cyanobacteria found in eutrophic municipal and domestic water supplies are an increasing environmental hazard in South Africa.

The most common of the toxic cyanobacteria species in South Africa is ***Microcystis aeruginosa***. It produces microcystins, the most abundant of the lethal cyanobacterial toxins, of which more than 65 have been isolated to date.

Death of livestock occurs through the drinking of water contaminated by these toxins. Hazards to human health may result from chronic exposure via contaminated water supplies. ***Microcystins*** are powerful tumour promoters and are suspected to be involved in the promotion of primary liver cancer in humans.

Monitoring the quality of water destined for potable water supply must of necessity include the detection of potentially toxic cyanobacteria and the determination of their population density. However, traditional diagnostic technology does not provide for ease of analysis.

Analyses that are specific have usually been laborious, requiring specialised, expensive equipment. Reasonably priced analyses have had the disadvantage of being non-specific.

This is especially true for the genus ***Microcystis*** because of the large variation in morphological features induced by varying environmental and growth conditions. Moreover, identification by microscopic morphology or molecular analysis does not indicate the potential for toxin production.

Different strains of one species can be morphologically identical but differ in genetics and the ability to produce toxins. ***Microcystis aeruginosa***, for example, has both toxigenic and non-toxigenic strains.

Origin of genetic diversity

Genetic diversification within the cyanobacteria is an ongoing process in which new types develop from continually modified cyanobacterial genotypes under different environmental conditions at different geographical locations. ***Microcystis aeruginosa***, for example, is characterised by the existence of a wide variety of genotypes that differ in their content of secondary metabolites and in toxicity among strains.

New methods for establishing genetic diversity

The development of molecular techniques has opened new avenues for distinguishing between different strains of cyanobacteria species such as ***Microcystis aeruginosa***. Many different types of DNA-based genetic markers, in particular, have become available for the analysis of genetic diversity.

Methods based solely on the detection of naturally-occurring DNA polymorphisms have, for instance, been developed through determining the degree of sequence conservation between bacterial genomes. These polymorphisms are a result of point mutations or rearrangements (i.e. insertions and deletions) in the DNA and can be detected by scoring the presence, verses absence, of bands in banding patterns that are generated by restriction-enzyme digestion and DNA amplification procedures.

Variations in banding patterns are a direct reflection of the genetic relationship between bacterial strains. Banding patterns can therefore be considered genomic fingerprints, allowing strains to be characterised and identified through numerical analysis.

Amplified fragment length polymorphism (AFLP) markers are among the recent innovations in genetic market technologies and have found application in scanning genome-wide variations of strains that would be impossible

to resolve with morphological features or other molecular systematic characters. With its broad taxonomic applicability, AFLP is therefore the method of choice in determining genetic diversity and population structure of cyanotoxins occurring in South Africa's dams. It is based on the selective amplification of genomic restriction fragments by polymerase chain reaction (PCR), here used to differentiate between geographically unrelated *Microcystis* strains. In total, 23 strains were subjected to AFLP fingerprinting.

Genetic diversity and population structure

Strains fell into four distinct clusters: Cluster 1 consisted mainly of strains that originated from Japan, while European strains grouped together in Cluster 2. South African strains that originated from the northern part of the country fell into Cluster 3, while the strains collected from the central and southern regions of South Africa, together with strains from the USA, group together in Cluster 4.

This exercise has not only provided evidence for the applicability of AFLP in cyanobacterial taxonomy, but has also demonstrated clearly the superior discriminative power of AFLP in differentiating geographically unrelated *Microcystis aeruginosa* strains that belong to the same species.

Detecting toxin-producing cyanobacteria

Although the above molecular techniques have improved the accuracy of strain identification, they have not been able to distinguish toxigenic from non-toxigenic strains of the same species. The recent identification of the role of *mcy* genes in the production of microcystin synthetase provides a new avenue for studying microcystin production at a genetic level.

The occurrence of *mcy* genes in cells is correlated with their ability to synthesise microcystin, and, conversely, microcystin-free cells usually do not contain *mcy* genes. This approach, which is PCR-based, therefore has appeal as an early warning diagnostic and is also very sensitive due to the amplification achieved through PCR.

It is therefore logical to determine the potential of using the *mcy* gene sequences as a diagnostic tool in raw water to detect the presence of toxin-producing cyanobacterial spp. in South African reservoirs. To achieve this objective, two main strategies have been followed. Firstly, insertions and deletions (indels) present in the *mcyB* gene sequence have been used as a diagnostic tool for establishing the presence of 'known' toxic strains in water reservoirs; secondly, PCR-based technologies have been used for detecting toxic cyanobacteria and the expression of the *mcy* genes as representatives of the microcystin peptide synthetase genes.

The PCR-based methods are found to be highly suitable as a method for early detection of putative toxigenic strains, even at very low cell counts. This has been established by correlating the presence of PCR and/or quantitative real-time (qRT) PCR products with 'known' levels o toxicity, as measured independently using ELISA (enzyme-like immunosorbent assay) and PPIA (protein phophatase inhibition assay). Unlike the PCR-based methods and ELISA, PPIA occasionally gave 'false' positives, indicating sensitivity to other compounds besides microcystins.

Medium to high levels of toxicity were measured during a survey of reservoirs in South Africa. The *Microcystis aeruginosa* strain with the highest measured toxicity was collected from Krugersdrift Dam during December 2004, while other strains with high levels of toxicity were collected at various times from Hartbeespoort Dam, Roodeplaat Dam, and Bon Accord Dam.

In summary, the population structure of *Microcystis aeruginosa* strains from South Africa proves to be very diverse, and different from strains from other geographic regions (i.e. North America, Europe and Asia). PCR-based RFLPs appear to be a cheap, effective alternative for species and/or strain identification, provided that an array of enzymes are used to ensure proper identification.

The use of the *mcyB* gene in PCR assays, applied directly to the environmental samples provides a useful indicator of putative toxicity, since the genetic potential of a strain to produce microcystin is measured. These assays detect toxigenic cells rather than toxins and require little sample preparation and modest capital costs. Thus, they make water monitoring more feasible and allow for the early application of corrective action before cyanobacterial blooms start to decompose or disintegrate.

Further reading:
To obtain the *PCR-based Markers for Detection and Identification of Toxic Cyanobacteria* **(Report No: 1502/07)** contact Publications at Tel: (012) 330-0340; Fax: (012) 331-2565; E-mail: orders@wrc.org.za; or Visit: www.wrc.org.za