

MOLECULAR AND EVOLUTIONARY INVESTIGATIONS INTO THE CYANOTOXIN CYLINDROSPERMOPSIN

PhD Thesis Abstract – Rebecca Jane CAMPBELL

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Cyanobacteria are ubiquitous throughout global water bodies. They have the proclivity to produce a number of potent secondary metabolites that can sully potable waters' with potentially serious implications in respect to public health. Furthermore, these compounds can impact on aquaculture, agriculture and recreational activities. It is therefore imperative that water quality managers are able to rapidly differentiate between problematic and nonproblematic cyanobacterial populations, and thus ensure appropriate measures are initiated early to guarantee public health. Molecular based approaches are proving attractive alternatives to current detection strategies, where molecular probes can be designed to be specific, sensitive and lend themselves to greater portable and rapid technology platforms. However, there is a paucity of molecular data available for the biosynthetic mechanisms involved for many of these cyanobacterial secondary metabolites.

Cylindrospermopsin (CYN) is a potent cyanotoxin that inhibits mammalian protein synthesis, leading to toxicity in a range of different organs. CYN is a mutagen and preliminary evidence suggests that long term exposure may have carcinogenic consequences. Identification of CYN in global water bodies is becoming more frequent, perhaps reflective of improved detection strategies in addition to the proliferation of cyanobacteria promoted by eutrophication and global warming. Historically, CYN has had serious repercussions in regard to Australian water quality, and has been implicated as the causative agent for an acute gastrohepatic illness that affected a community on Palm Island, QLD. The cyanotoxin has also been attributed to livestock deaths.

To date there have been three candidate genes, correlated with CYN toxicity including an amidinotransferase, a polyketide synthase (PKS) and a peptide synthetase (PS); where the PKS and PS have already been applied for molecular detection strategies. However, without full consideration of the molecular architecture involved in CYN biosynthesis, it is impossible to conclude whether the most explicit molecular target has been selected to unequivocally detect CYN-producing cyanobacteria. Further to this, acquiring the molecular information in respect to the biosynthesis of CYN will facilitate future investigations into the regulation and ecological role of this metabolite.

This thesis describes the detection, sequencing and characterisation of a putative 43KB biosynthetic gene cluster isolated from CYN-producing *Cylindrospermopsis raciborskii* AWT205. A suite of molecular methods were employed to acquire the gene sequence. Through rigorous PCR screening of over 40 CYN- and non-CYN producing cyanobacterial species and strains, the genes involvement in CYN production was implicitly defined. Additionally, the screening permitted identification of suitable candidate genes allowing for unambiguous detection of CYN-producing cyanobacteria in future applications.

The acquired molecular knowledge was also practically applied to track a CYN-toxic cyanobacterial bloom using real-time PCR. The outcomes provided novel insights into the molecular mechanics of bloom-forming *Cylindrospermopsis raciborskii* and the production of CYN in the bloom environment. This knowledge may prove beneficial in guiding water quality managers to target algicide treatments at optimal time points.

Phylogenetic analyses of 21 CYN-producing cyanobacteria, from three distinct genera, was also undertaken to investigate the evolutionary history of the CYN biosynthetic cluster. In addition to multiple alignments, three tree construction methods and codon usage analyses were employed, to investigate the involvement of horizontal gene transfer in disseminating the cluster throughout cyanobacterial genomes.