Non-ribosomal Biosynthesis of the Cyanobacterial Toxin Nodularin

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ABSTRACT

The complete gene cluster encoding the enzymatic machinery required for nodularin synthesis in the toxic *Nodularia spumigena* strain NSORl0 was sequenced and characterised. The 48 kb gene cluster consists of nine open reading frames (ORF), *ndaA-I,* transcribed on two polycistronic mRNA from a bi-directional regulatory promoter region. Upstream of the promoter region, *ndaC-I* encodes non-ribosomal peptide synthetase (NRPS) modules, polyketide synthase (PKS) modules and tailoring enzymes that catalyse the synthesis of the C_{20} amino acid, 3-amino-9-methoxy-2,6,8,-trimethyl-10-phenyl-4,6-decadienoic acid (Adda) and its subsequent condensation with D-glutamic acid. Downstream of the promoter, *ndaAB* encodes three NRPS modules that catalyse the condensation of the substrate amino acids onto the peptide chain, and the cyclisation and release of nodularin.

The ORF flanking the *ndaS* gene cluster in the genome of *N. spumigena* strain NSORl0 were identified and found to encode proteins that have homology to previously-characterised transposases, high light and high temperature stress associated proteins and hypothetical proteins. A putative transposase has also been associated with the microcystin synthetase *(mcyS)* gene clusters that have been characterised to date. This relationship indicates a possible mechanism for the distribution of these biosynthetic gene clusters between various cyanobacterial genera. Furthermore, there is evidence that indicates that the duplication of the NdaA C-domain was likely to have occurred during a deletion event, resulting in the loss of two modules from a putative microcystin synthetase progenitor, resulting in nodularin synthetase. Further phylogenetic analysis indicated that nodularin synthetase diverged from the microcystin synthetase lineage well before transfer of the *mcyS* gene cluster between *Microcystis* and *Planktothrix.* In order to further characterise the evolution of non-ribosomal hepatotoxin synthesis in cyanobacteria, the highly-conserved type I ketosynthase (KS) domain was used as a marker. The nodularin synthetase KS domains were found to cluster with a group of type I KS domains isolated from cyanobacteria and myxobacteria, however these were distinct from KS domains isolated from actinobacteria. The KS domain of NdaC appeared to be structurally distinct from other KS domains of nodularin synthetase and was found to cluster with a group of KS domains that are required for the condensation of a starter unit activated by an NRPS module with a PKS extender unit. Such KS domains are not restricted to the hepatotoxic cyanobacteria.

In order to propose associated physiological functions of nodularin, the growth of *N. spumigena* NSORIO, the accumulation of *ndaS* transcripts and the intracellular concentration of nodularin in response to environmental factors were investigated. Increased levels of *ndaS* transcripts were identified as a result of specific growth temperature (20°C), light intensity (30 µmol photons.$m^{-2}.s^{-1}$) and salinity (0% and 3% NaCl), however transcription appeared to be cell density dependent. The concentration of nitrate was shown to have no apparent effect on the growth of *N. spumigena* NSORl0, and neither the transcription of *ndaS* nor the synthesis of nodularin.

A global comparative analysis of gene expression in a toxic and a non-toxic mutant strain of *N. spumigena* was performed using micro array hybridisations. Results indicated that the expression of putative iron(III)-starvation regulated genes was increased in the non-toxic mutant strain, thereby suggesting that this may be associated with the absence of nodularin synthesis in this strain. The results presented in this dissertation may therefore help provide a greater understanding of the complex factors associated with the evolution, genetics and physiology of nodularin synthesis in the cyanobacterium *Nodularia spumigena*. In addition, an appreciation of the mechanisms involved in non-ribosomal synthesis and its evolution will be vital for future combinatorial engineering and rational design of novel metabolites and pharmaceuticals.