Thesis Abstract: Unnatural Production of Natural Products: Heterologous Expression and Combinatorial Biosynthesis of Cyanobacterial-Derived Compounds

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Cyanobacteria produce a myriad of structurally unique secondary metabolites with useful bioactive properties. Heterologous expression of a variety of microbial natural compounds has been used to harness their diversity and facilitate their combinatorial biosynthesis. However, these genetic techniques have not been developed for secondary metabolite-producing cyanobacteria. Therefore the genetically manipulable *Escherichia coli* and *Synechocystis* sp. PCC6803 were engineered in order to develop effective heterologous hosts and promoters for the expression of cyanobacterial-derived compounds.

The phosphopanthetheinyl transferase (PPT), Sppt, from *Synechocystis* sp. PCC6803 was characterised to determine its ability to activate carrier proteins from secondary metabolite pathways. Despite *in silico* evidence which suggested Sppt was able to activate a wide range of carrier proteins, biochemical analysis revealed that it is dedicated for fatty acid synthesis. Consequently, *E. coli* and *Synechocystis* sp. PCC6803 were engineered to encode a broad-range PPT, from the filamentous cyanobacteria *Nodularia spumigena* NSOR10, for the activation of carrier proteins from nonribosomal peptide synthesis.

Cyanobacterial natural product engineering was also explored with the characterisation of two relaxed specificity adenylation domains (A-domains) from the biosynthetic pathway of the toxin microcystin. The wide variety of microcystin compounds produced by cyanobacterial species suggests that multiple amino acids can be activated by the same A-domain. This was supported by preliminary ATP-[^{32}P]PP$_i$ exchange assays and was subsequently harnessed in the production of a variety of dipeptides using two reconstituted modules *in vitro*.

Transposition was investigated as a potential mechanism for the transfer of nonribosomal peptide synthetase gene clusters to heterologous hosts. This was performed via the characterisation of the putative transposase, Mat, physically linked with the microcystin synthetase gene cluster (*mcyS*). PCR screening, *in silico* analysis and nitrocellulose filter binding assays indicated that this transposase may have mediated *mcyS* gene cluster rearrangements but not entire gene cluster mobilisation between species. The potential role of transposases in the natural combinatorial biosynthesis of microcystin has evolutionary implications for the dynamic nature of cyanobacterial genomes and applications for use in the engineering of novel bioactive compounds. Therefore, the results from this study may provide a biotechnological platform for the transfer, expression and combinatorial biosynthesis of novel cyanobacterial-derived natural products.

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