Heterologous Expression of Microviridins

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Microviridins are a unique tricyclic class of ribosomally synthesized and post-translationally modified peptides produced by cyanobacteria. They are synthesised through biosynthetic gene clusters with a precursor peptide and two ATP-grasp ligases responsible for a lactam and two lactone bonds. The biosynthetic gene clusters typically further contain a GNAT-type *N*-acetyltransferase and an ABC transporter likely functioning as scaffolding protein. Occasionally, the clusters contain multiple precursor peptides.

In this work, heterologous expression platforms were developed to investigate the substrate tolerance and precursor peptide promiscuity of microviridin biosynthetic machinery. Direct Pathway Cloning was used to capture microviridin biosynthetic gene clusters from a *Nostoc* sp. TH1SO1 strain containing four distinct microviridin clusters. Region 108 was selected for further study because it represents a complete biosynthetic gene cluster. In parallel, a previously obtained microviridin biosynthetic gene cluster from *Microcystis aeruginosa* LEGE 91341 was used as a second expression platform.

Native precursor peptide genes were replaced with precursor peptide genes originating from three *Nostoc* biosynthetic gene clusters, and all constructs were heterologously expressed in *Escherichia coli*. The Mae platform retained the native *Microcystis* precursor peptide gene MdnA2, which was consistently expressed in all constructs, while additional *Nostoc* precursor peptide genes showed variable production. Interestingly, expression of three adjacent precursor peptide genes from region 108 resulted predominantly in production of the middle precursor peptide, *Nostoc* MdnA2. Similar behaviour was observed in the native region 108 platform. Replacement experiments further demonstrated differences in precursor peptide compatibility, as the precursor peptide gene from region 99 did not produce detectable products in Nos platform, whereas the precursor peptide gene from region 215 yielded multiple peptides in both heterologous platforms. The precursor peptide gene from region 215 in Mae platform was selected for large-scale expression, which is currently undergoing.

These results demonstrate substantial but selective substrate flexibility of microviridin biosynthetic enzymes and establish heterologous platforms suitable for future engineering of microviridin diversity.