Non-ribosomal biosynthesis of peptides in planktonic cyanobacteria
Leo Rouhiainen, David P. Fewer, Jouni Jokela and Kaarina Sivonen

Department of Applied Chemistry and Microbiology, P.O. Box 56, Viikki Biocenter, Viikinkatu 9, FIN-00014, University of Helsinki, Finland

Background
Cyanobacteria produce a large variety of peptides, many made by non-ribosomal peptide synthetases (NRPSs) and occasionally in combination with polyketide synthases (PKS). Non-ribosomal cyanobacterial peptides include protein phosphatase inhibitors, protease inhibitors and cytotoxic compounds. The NRPSs are large modular enzymes catalyzing the activation, modification and incorporation of amino acids or sometimes carboxylic acids into the peptide chain in a stepwise process. We have characterized the NRPSs of Anabaena, and Nostoc genetically and biochemically. The gene clusters coding for the biosyntheses of protease inhibitors anabaenopeptins from Anabaena strain 90, and mildly cytotoxic nostophycin from Nostoc strain 152 have been studied in the Finnish Program for The Centers of Excellence in Research started in 2008.

Alternative starter modules are employed by Anabaena to create structural variation of anabaenopeptins

Anabaenopeptin biosynthesis in Anabaena strain 90.
The genes aptA1-aptD code for NRPSs, aptE putatively the change of peptide bond to ureido bond and aptF ABC transporter. Anabaenopeptin synthetase of Anabaena spp. makes use of alternative starter modules with high substrate specificity to assemble a group of peptides with limited structural variance. Anabaenopeptin synthesis constitutes a novel exception to the coremodulearity rule of non-ribosomal peptide production. Abbreviations of the domains: A, adenylation; PCP, peptidyl carrier; C, condensation; E, epimerase domain; M, N-methyltransferase; Te, thioesterase.

NRPSs and NRPS/PKSs characterized from Nostoc 152

Structure and genetic organization of the mixed PKS-NRPS, nostophycin synthetase, from Nostoc strain 152.
The genes npnx and npnB encode mixed NRPS/PKS and npnC NRPS. Abbreviations of the domains: A, adenylation; KR, ketoreductase; ACP, acyl carrier; KS, ketosynthase; AT, acyltransfrase; DH, dehydratase; ER, enoylreductase; AMT, aminotransferase; MonoOx, mono-oxidase; T, peptidyl carrier; E, epimerase; C, condensation; Te, thioesterase.

Biochemical characterization of anabaenopeptin synthetase by ATR/PPi exchange assay.
Anabaena sp. strain 90 is recalcitrant to genetic manipulation so the substrate specificities of expressed adenylation domains were studied. Six of the eight adenylation domains were overexpressed in E. coli BL21 Star (DE3) and their substrate specificities determined in an ATP-PPi exchange assay.

Peptides produced by Nostoc 152

Nostophycin produced by Nostoc strain 152.
The mildly cytotoxic nostophycin contains the novel β-amino acid moiety, Ahoa, 3-amino-2,5-dihydroxy-8-phenyloctanoic acid, and seven amino acid ring with two proline residues.

Microcystins produced by Nostoc strain 152.
The protein phosphatase inhibiting hepatic toxic microcystins are characterized by the rare amino acid Adda, 5-amino-9-methoxy-2,6,8-trimethyl-deca-4,6-dienoic acid, and seven amino acid ring with N-methyldehydroalanine.

Future work
We need a method to manipulate genetically Anabaena 90 and anatoxin producing Anabaena 37, and we are working to develop conjugation system. The electroporation method used has shown to be ineffective. The biochemical characterization of the peptide synthetases will be continued.

Publications in 2008 - 2010