B139 PCR based screening approach for the assessment of secondary metabolism in cyanobacteria

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Cyanobacteria are a rich source of secondary metabolites, in particular non-ribosomal peptides and polyketides (1). Cyanobacterial metabolites of this type not only exhibit a fascinating range of structural diversity, but also represent natural products with many interesting bioactivities; e.g. antibacterial, antifungal, antiviral and cytotoxic. Polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), which are responsible for the biosynthesis of these polyketides and peptides, form large multi-enzyme complexes with a modular structure.
Fifteen cyanobacterial strains from the genera *Synechocystis*, *Nostoc*, *Scytonema*, *Tolypothrix*, *Oscillatoria*, *Plectonema* and *Fischerella* were screened in an extended PCR study. Degenerate PCR primers were designed on the basis of conserved sequence motifs of PKS and NRPS (2). While the NRPS derived primers yielded gene ampilcons of the expected size from only one *Fischerella* strain, PKS fragments could, however, be amplified and sequenced from several cyanobacterial strains. Positive results from the PCR screening indicate the presence of the corresponding biosynthetic gene clusters in these cyanobacterial strains and provide valuable information about their metabolic potential.
In addition to this screening approach, the filamentous cyanobacterium *Plectonema* sp., which was found to be PKS-positive, was further investigated chemically. NMR spectroscopic measurements made with HPLC fractions of a methanol extract of this cyanobacterium indicated some fractions to have a high polypeptide content. From the NMR and MALDI-TOF data of these fractions it was possible to speculate that the peptides are composed of 15 to 20 amino acids.

References: 1. Dittmann, E. et al. (2001) Appl. Microbiol. Biotechnol. 57: 467-473. **2.** Beyer, S. et al. (1999) Biochim. Biophys. Acta 1445 : 185-195.

B140 Secondary metabolites of the North Sea bryozoan *Flustra foliacea* and their biological significance

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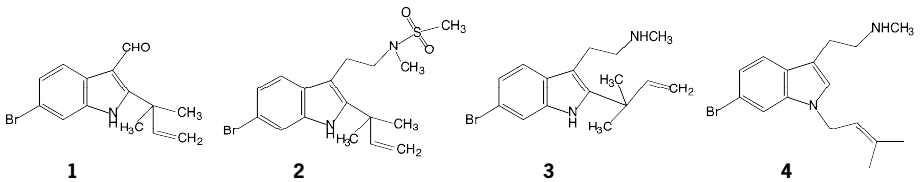
The marine bryozoan *Flustra foliacea* is abundant in various parts of the North Sea and contains biologically active brominated alkaloids and terpenoid metabolites. HPLC separation of the lipophilic extract of *F. foliacea* yielded twelve brominated indole alkaloids, four of which had unusual and new structures (**1 – 4**). GC-MS analysis of various *F. foliacea* extracts allowed to monitor the variation of the secondary metabolite content. It could be shown that samples of *F. foliacea* from different collection sites vary to a big extent, whereas the time of collection has no big influence. All compounds were tested for their antibacterial activity in agar diffusion assays towards bacteria isolated from *F. foliacea* itself and terrestrial bacteria. Significant activities were only found towards marine derived bacteria. Quorum sensing seems to be influcened by one of the metabolites. Pharmacological investigations show flustramin A to have an unspecific blocking activity on voltage activated Kv1.4 potassium channels.



1 **2** **3** **4**