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Genetic diversity in the production of small bioactive peptides in cyanobacteria

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Cyanobacteria are an important group of freshwater algae favoured by eutrophication processes and dominate aquatic systems frequently. Many cyanobacteria species are distributed worldwide, however a few species can be found in specific climatic regions only. Cyanobacteria do show an impressive diversity in the production of small bioactive peptides (700 – 1400 Da), i. e. microcystins, anabaenopeptins, aeruginosins, etc. Typically isolates differing in production of those small peptides can not be discriminated either in the microscope nor by traditional molecular based taxonomic approaches. Those peptides are formed by modular peptide synthetases and polyketide synthases which belong to the largest enzymes inside the cell. The ecological mechanisms and processes favouring this diversity in the synthesis of those secondary metabolites are not understood.

The filamentous cyanobacterium *Planktothrix* spp. occurs in the temperate region of the Northern hemisphere, the green pigmented species *P. aghardii* in shallow and eutrophic lakes and the red pigmented species *P. rubescens* in deep, physically stratified and less eutrophic systems. We have analysed 70 isolates from lakes in Europe (i) for the genes of the microcystin-, aeruginosin- and anabaenopeptin synthetase by molecular techniques (ii) for the ability to produce those peptides using mass spectrometry and liquid chromatography. The distribution of genes encoding the synthesis of microcystin clearly depends on the ecosystem, i. e. microcystin genotypes do occur among isolates of *P. rubescens* with a 100% frequency. While the gene linked to

anabaenopeptin is found in nearly all strains, the gene encoding aeruginosin is more coupled to the presence of microcystin. In addition a number of isolates have been found inactive, i. e. although containing the gene(s) for specific peptides no peptide could be detected. In a number of inactive isolates such inactivity could be explained by natural mutations possibly caused by transposable elements.

In order to quantify genotypes differing in production of specific peptides directly in nature, a number of gene probes have been designed and are used in single filament analyses and quantitative real time PCR techniques. First results obtained by quantitative PCR methods show that the proportion of microcystin genotypes differs between ecological habitats, however in most cases within habitats the proportion of microcystin genotypes has been found stable over the season. In addition inactive genotypes can be frequently detected under natural conditions, and make up a relatively high proportion of the total population. Inactive genotypes also persist in the population at least over several years. The genetic diversity in peptide production is an excellent opportunity to study biogeographic patterns and local adaptation processes.