Secondary metabolites of Microcystis aeruginosa (Cyanobacteria): study of their production and role by a metabolomic approach

Final Report Summary - CYANOMIC (Secondary metabolites of Microcystis aeruginosa (Cyanobacteria): study of their production and role by a metabolomic approach)

Cyanobacteria produce a wide variety of toxins that cause animal poisonings, human health risk and that have deleterious impact on ecosystem sustainability. They also produce a large number of bioactive compounds that are a valuable and inspirational source for the biotechnological and biomedical industries. In the last decade, many efforts have been done to solve the biosynthesis of cyanobacterial toxins and bioactive compounds. A major part of cyanobacterial secondary metabolites are peptides or possess peptidic substructures, originating mainly from non-ribosomal peptide synthetase (NRPS) or mixed...
polyketide synthase (PKS)-NRPS biosynthesis. Despite great advances in the understanding of the metabolic pathways involved in their production, the natural functions and the ecological role played by these metabolites are still not well understood. It is assumed that those compounds are secondary metabolites and by definition are not essential for the growth but should play an important role to increase the competitive ability of the producer in the environment and in shaping of the community composition through inter- and intraspecies interactions. Hence, the overall scientific aim of CYANOMIC project was to better understand the effect of inter- and intraspecies interactions on the production of cyanobacterial secondary metabolites. In particular, to achieve a step-change in the understanding of (1) the impact of surrounding biota (between metabolite producing strains and non-producing strains and between producing species and target microorganisms) and (2) the role of associated heterotrophic bacteria on the production of cyanobacterial secondary metabolites. To attempt to respond to these questions, we focus our research on one model organism, the freshwater cyanobacteria, Microcystis aeruginosa. This ubiquitous species is occurring worldwide and is implicated in numerous intoxications because of its ability to produce harmful toxins, microcystins (MC), which inhibit protein phosphatase 1 and 2A. Besides microcystins, Microcystis is a rich source of biologically active compounds mainly protease inhibitors.

The project has four linked objectives organized within four Workpackages (WP):

Objective 1: To determine and compare the secondary metabolic profiles of MC- and non-MC-producing Microcystis aeruginosa strains when they are cultivated in mono- and co-culture conditions (WP1).

Objective 2: To determine the impact of the presence of bacteria in the culture of MC- and non-MC-producing Microcystis aeruginosa strains on the production and the fate of cyanobacterial secondary metabolites (WP2).

Objective 3: To determine the impact of interspecies interaction on the production of secondary metabolites by Microcystis strains (WP3).

Objective 4: To develop a model of the relative dynamics of these cyanobacterial metabolites and of their impact on surrounding biota in presence of associated heterotrophic bacteria (WP4).

Workpackage 1 (Led by W. Gerwick): Comparison of secondary metabolic profiles of MC- and non-MC-producing Microcystis aeruginosa strains

MC- and non-MC-producing cyanobacterial strains co-exist in different proportions during a natural bloom. In our study we investigated whether co-culturing cyanobacterial strains with the potential to produce different secondary metabolites could affect their metabolic profiles and the production of those compounds. We addressed the following questions: (1) Is co-culturing a MC- and a non-MC-producing strains affect their growth, the metabolic profile and the production of bioactive compounds? (2) Are MCs involved in these differences? (3) Are these differences depending on other secondary metabolites? An innovative co-culturing/molecular networking approach allowed us to monitor the growth and to compare the secondary metabolic profiles of MC- and non-MC-producing Microcystis strains. Despite no influence of the intraspecies interaction on their individual growth, LC-MS measurements revealed a pronounced up-regulation of cyanopeptolins, cyanobactins, aeruginosins and anabaenopeptins for non-MC-producing strains. Of interest is the observed increase in MC concentration of the toxin producer in co-culture condition. This work provides novel insights into the question of the production of MC and other secondary metabolites by cyanobacteria and that these compounds may have interchangeable or complementary functions. Moreover, using recent advanced mass spectrometry techniques (MS/MS networking) we may identify new analogues of known class of peptides as well as unknown compounds.

At this stage this work gave rise to two articles in high impact international journals:
Workpackage 2 (Led by W. Gerwick): Role of associated heterotrophic bacteria on the production of Microcystis aeruginosa secondary metabolites

The freshwater cyanobacteria, Microcystis spp., commonly form large colonies with bacteria embedded in their mucilage. Positive and negative interactions between Microcystis species and their associated bacteria have been reported. However, the potential role of bacteria in the production and degradation of cyanobacterial secondary metabolites has not been investigated. Our main questions were: (1) Is there a difference in the secondary metabolic profile of Microcystis strains under axenic and non-axenic conditions? (2) Which heterotrophic bacteria are associated with Microcystis aeruginosa? (3) Are there specific interactions between the cyanobacterium and the associated bacteria? In this study, a Microcystis-associated bacterial community was isolated and added to the axenic M. aeruginosa PCC7806 liquid culture. After three years of co-cultivation, we studied the bacterial genetic diversity adapted to the PCC7806 strain and compared the intra- and extracellular metabolic profiles of the cyanobacterial strain under xenic and axenic conditions. Mass spectrometric analyses of cells and media extracts showed that the peptides measured were produced by the cyanobacterial strain independently of the presence of bacteria. These peptides were still detected in the axenic media but totally absent in the xenic media. This investigation revealed that the bacterial community, dominated by Alphaproteobacteria, was able to degrade the cyanobacterial peptides and utilize them as carbon and nutrient sources. In turn, this process may help sustain cyanobacterial growth through nutrient recycling. This putative mutualistic interaction may contribute to the ecological success of Microcystis.

At this stage this work gave rise to one article:

Workpackage 3 (Led by M. Bormans): Impact of cyanobacterial secondary metabolites on the physiology of cyanobacteria through interspecific interactions

To better simulate natural environmental conditions, where cyanobacterial genera may co-occurred in the same freshwater body, we tested whether cyanobacterial secondary metabolites may play an important role by shaping communities composition through interspecific interactions. Our main questions were: (1) What are the effects of extracellular compounds released by a cyanobacteria on the growth and the physiology of co-occurring cyanobacterial genera? and (2) How the production of cyanopeptides is affected by two cyanobacterial genera in co-culture? Co-culture experiments were performed between two co-occurring cyanobacterial genera: Microcystis aeruginosa and Planktothrix agardhii and between Microcystis aeruginosa and Anabaena cylindrica. We compared the growth, the photosynthetic activity and the production of cyanopeptides of each strain under mono- and co-culture conditions. We observed a negative impact of Microcystis strain on the growth and the physiology of Planktothrix cells resulting in a decrease of growth and photosynthetic activity together with an alteration and deformation in the normal morphology of the cells. Moreover, a slight impact of Anabaena on the growth of Microcystis was
observed. It is expectable that the observed changes were induced by the action of several, still unidentified allelopathic compounds. Secondary metabolites profiles of each strain under both conditions are still in progress.

At this stage this work gave rise to one master’s thesis:

Workpackage 4 (Led by M. Bormans): Modeling secondary metabolites dynamics and their impact on surrounding biota
The aim of this fourth workpackage was to develop a model that predicts the potential toxicity and of the interference ability of a Microcystis bloom on surrounding biota in presence of associated heterotrophic bacteria. This research objective is not yet completed as it is to some degree dependent on the analyses of secondary metabolites produced under interspecific interactions still in progress. However at the ecophysiological level we can already assert that interspecific interactions have an impact on the fitness of the species present, which will have implications on the community structure. A simple competition model is being developed, where the growth of each species is affected by both interspecific interactions and the presence of bacteria. Linking these fitness changes to secondary metabolites productions will allow better predictions of community evolution during cyanobacterial blooms.

Expected final results and their potential impact and use
The combination of the results from the different objectives have highlighted changes in the secondary metabolic profiles of MC and non-MC- producing Microcystis aeruginosa strains in response to intra- and interspecies interactions. This work has emphasized that a better understanding of the possible functions of bioactive compounds and in general of the cyanobacterial physiology and ecology requires studying bioactive peptides as a group rather than focusing on one of them in particular. Moreover, isolation and characterization of new analogues as well as unknown cyanobacterial bioactive compounds that have the potential to be used by biotechnological and biomedical industries, will contribute to European excellence and competitiveness. Issues associated with cyanotoxins in drinking water are a huge problem worldwide. Therefore the isolation of bacteria able to degrade those toxins will provide biological treatment option that can be implemented in treatment plants for optimum cyanobacterial metabolite removal.

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