ENVironmental Control of cyAnoToxins production (ENVICAT)
FP7-PEOPLE-2009-IEF-255054

General context and project relevance

Harmful cyanobacterial blooms (cHABs) have significant impacts that jeopardize the ecosystem goods and services provided by freshwaters. There is growing evidence that the spatial and temporal occurrence of cHABs is increasing due to anthropogenic pressures on freshwater ecosystems. In the context of the Water Framework Directive (WFD), which aims to improve the ecological status of freshwaters throughout Europe, it is of paramount importance to understand better the physiology and ecology of bloom-forming toxic cyanobacteria in order to (i) implement management procedures to improve the quality of freshwater ecosystems and ensure their safe use and (ii) forecast responses to future environmental change.

Cyanobacteria produce a wide range of bioactive compounds (cyanotoxins) that have a deleterious impact on human health and ecosystem sustainability of which the microcystins (MCs) are the most widely encountered. In addition, β-*N*-methylamino-L-alanine (BMAA), a non-protein amino acid considered to be a potent glutamate agonist, is suspected to be produced by most cyanobacteria and has been linked to serious neurodegenerative illnesses (ALS/PDC syndrome). This toxin, about which very little is known, is suspected to be present in most British waterbodies impacted by cHABs.

One of the main questions remaining to be answered concerning cyanotoxins is why they are produced and what controls their production. Despite great advances in the understanding of the metabolic pathways involved in cyanotoxins production, it is still impossible to predict the concentration of toxins found in the environment. Cyanobacterial biomass is indeed considered as a poor correlate of *in situ* cyanotoxins concentration and the environmental factors controlling the abundance of toxin-producing sub-population (the strains that possess the genes involved in toxin synthesis) and the toxin production itself need to be better understood.

In this context, the ENVICAT project (07/2011 – 06/2013) project aimed to provide a clearer understanding of the control of cHABs toxicity related to MCs and BMAA in response to environmental conditions. The plan was to achieve this via three interlinked tasks.

Note: Due to the appointment of the researcher to a permanent position, the project was terminated after 18 months, in January 2013.

Scientific objectives and project results

Task1 - Impact of growth conditions (light, temperature N, P and C) and their interaction on MCs and BMAA production by M. aeruginosa

The first objective of the project was to carry out a series of growth experiments in order to assess the impact of the main limiting environmental factors on the growth and cyanotoxins (MCs and BMAA) production of cyanobacteria. A factorial experimental design (Central Composite Design) was used to define experimental

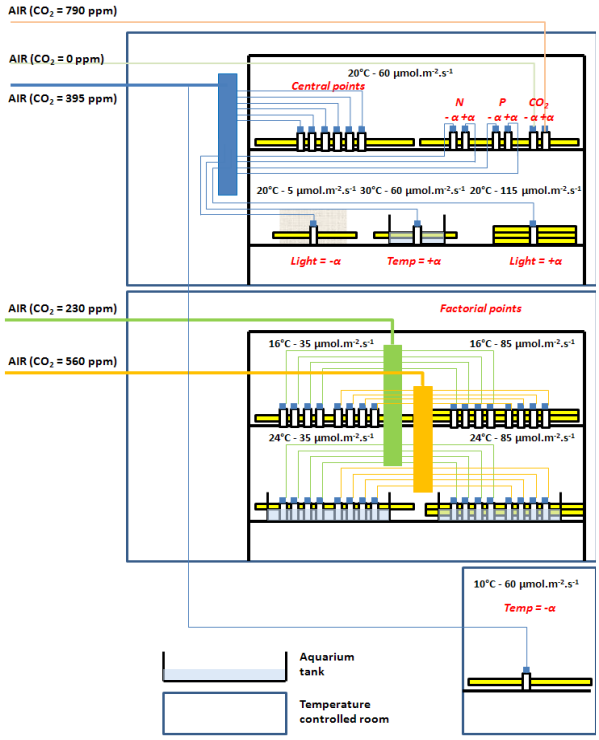

Fig. 1. Schematic diagram of the experimental setup.

conditions. This allowed the quantitative impact of each factor and their interactions on toxin production to be assessed and a predictive statistical model (GAM-based model) to be derived from the experimental data. The factorial design required an experimental setup to be built that allowed 48 different batch cultures to be controlled simultaneously (Figure 1). Due to the large number of experimental conditions, the three replicate experiments had to be run successively (i.e. true replicates). A recently isolated *Microcystis aeruginosa* strain (PMC 729.11) was used in order to prevent selective pressure or genetic drift that may occur during long-term artificial maintenance of microalgae. Cell growth was measured daily by optical density. Samples were collected at two growth phases (exponential and stationary) in order to measure cell density, cell volume, C and N, total protein, microcystins and BMAA content. Due to methodological uncertainties related to BMAA analysis and arising from a recent debate on its true occurrence in freshwater planktonic cyanobacteria, the analysis of BMAA samples is still under way. Regarding MCs production, a GAM model was selected based on GCV scores. The best model (88% of explained variance) showed that the gravimetric MC content (G_{MC}) is positively correlated to growth rate (μ), when μ is positive. The model also illustrates that reduced nitrogen availability leads to a reduction in G_{MC}. The level

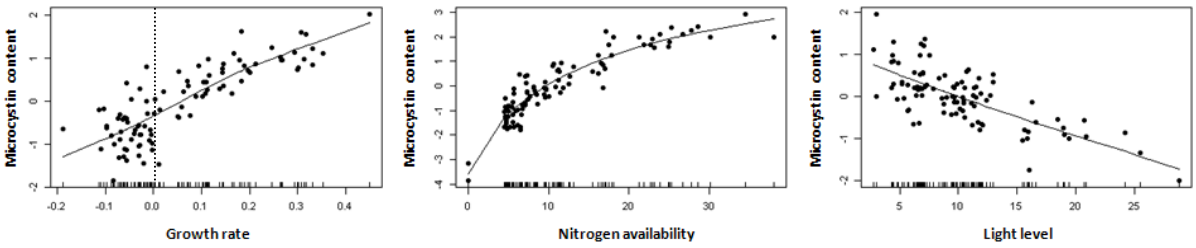

Fig. 2. Fitted model plots for growth rate (d^{-1}), nitrogen availability ($pM.\mu m^{-3}$) and light level ($aM.\mu m^{-3}.s^{-1}$) corresponding to the reduced GAM for microcystin gravimetric content ($mg.g^{-1}$).

light received by *M. aeruginosa* cells (expressed as the photon flux divided by the biomass level) showed a negative correlation with G_{MC}, which is consistent with a down-regulation of the *mcyD* gene involved in MC synthesis and a reduced MC:protein ratio recently described by Deblois & Juneau (*Harmful Algae* (2010) 9:18-24) and Sevilla et al. (*Ecotoxicology* (2012) 21:811-819) and suggests a role of microcystins in photoacclimatation processes. The data and some results from the GAM model are illustrated in (Figure 2). A full analysis of the results for publication is underway.

Task 2 - Relative fitness of MCs producing versus non-MCs producing strains.

The aim of this task was to assess if the conditions that maximize/minimize MCs production (identified in Task 1) are related to different competitive ability of MCs-producing (MC+) or MCs-non-producing (MC-) genotypes. Due to well-known phenotypic differences between strains, we selected 3 *M. aeruginosa* MC+ and 3 MC- strains. The objective was to define an experimental cyanobacteria community and to study the evolution of MC+ and MC- genotypes relative abundance in response to growth conditions. Before carrying-out the experiment, a method to quantify the sub-populations (n=6) was required. We first planned to use ITS sequence variability to define molecular probes that could be use for qPCR or RING-FISH analysis. However, after sequencing the ITS of a collection of 20 *M. aeruginosa* strains, insufficient sequence variability was found. We then used sequences published in GenBank, for microcystin (*mcy*) genes and housekeeping genes from *M. aeruginosa* to identify candidate gene sequences. Two candidate genes (*mcyB*, a nonribosomal peptide synthetase gene of the *mcy* operon, and *tpi*, a gene coding for a triosephosphate isomerase) showing sufficient sequence variability were selected for further study. However, early termination of the project did not allow the method development for this task to be completed.

Task 3.1. Analysis of in situ *distribution of MCs producing genotypes*

A large set of phytoplankton samples was collected from three geographic areas: the English Lake District (UK, n = 20 lakes, sampled once), the Paris area (France, n = 50 lakes, sampled 6 times) and the French Mediterranean region (France, n = 48 lakes, sampled twice). The aim was to quantify the % of MC+ genotypes in a large array of natural samples and identify the environmental conditions that may explain the observed % of MC+ genotypes. A qPCR method was selected using a recently published primer pair DQmcyF/R (Al-Trebineh et al, *Toxicon* (2011) 57:546-554) shown to amplify a gene from the *mcy* gene (*mcyE*) cluster in all MCs producing species tested. A reference primer pair (PCβF/PCαR) targeting the phycocyanin gene (*cpc*) was selected for quantifying total cyanobacteria populations. At the time the project ended, the analysis of samples had started. This work will be carried on at the researcher's new institute (MNHN) and will help to develop collaborations between the two institutes.

Task 3.2. Large-scale assessment of BMAA distribution

Based on the same set of samples used for Task 3.1., the aim of Task 3.2. was to provide the first large-scale assessment of the distribution of BMAA in lakes and reservoirs. Following, the debate regarding the occurrence of BMAA in cyanobacteria, the analysis of theses samples aimed to clarify the extent of lakes and reservoirs contamination by this cyanotoxin. At the time the project ended, the analysis of BMAA samples were still under way. This work will be carried on through collaboration with the project supervisor and analytical chemists from CEH (Dr Dos Santos Pereira and Dr Llewellyn).

Potential socio-economic impact

The results obtained from this research are a first step to provide the data necessary to complement the PROTECH model developed at CEH Lancaster, one of the most advanced deterministic models aiming at simulating phytoplankton dynamics in lakes, in order to produce a module to predict *in situ* toxins concentration (MCs and BMAA). The implementation of a module allowing predicting *in situ* cyanotoxins concentration would be a significant breakthrough for managing health related issues associated to cHABs. We hope to develop this module as a collaborative project even though this fellowship has now finished.

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