Preliminary metabolomic approach on cyanobacterial co-cultures: Chemically mediated interactions between Microcystis and Planktothrix

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General context

Freshwater cyanobacteria, are well known for their ability to produce a wide variety of bioactive compounds, some of which have been described as allelochemicals. There is growing evidence that these secondary metabolites play an important role in shaping community composition through biotic interactions; however, for the most part, **the biological role and mode of regulation of the production of these bioactive compounds are poorly understood.** In temperate eutrophic freshwaters, *Microcystis* and *Planktothrix* often co-occur, with *Planktothrix* being an early colonizer and *Microcystis* appearing subsequently. **We tested if the production of a range of peptides by co-existing species could be regulated through interspecific interactions.**

Growth and morphological alterations of the Planktothrix cells in co-culture

Figure 1

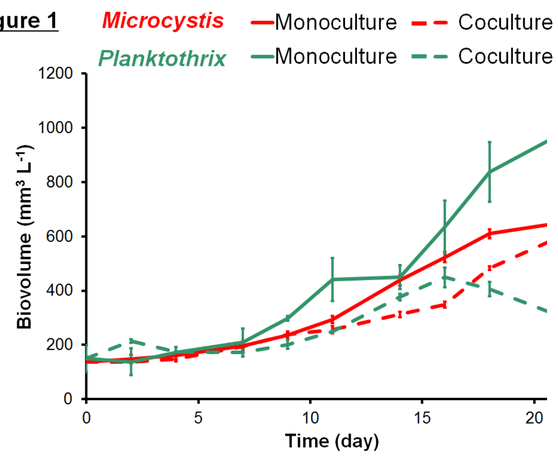
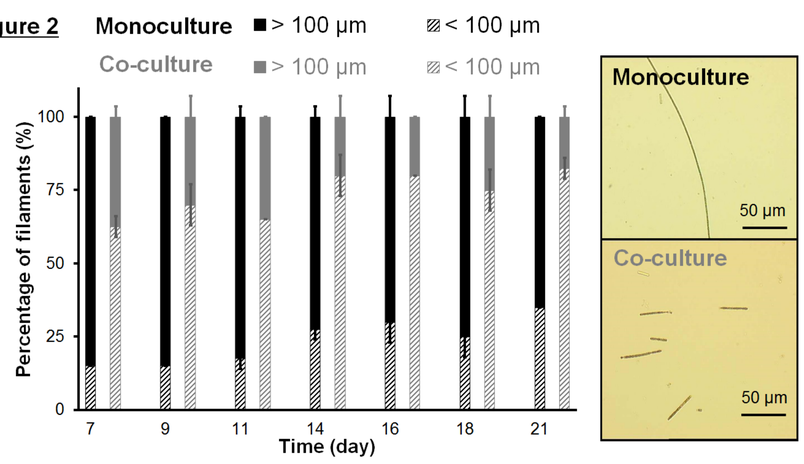
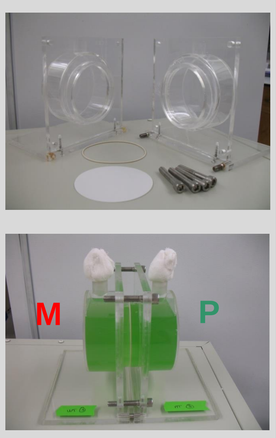


Figure 2



➢ **Culturing *Microcystis* cells with *Planktothrix* resulted in a reduction of the growth of *Planktothrix* (Fig. 1) together with a decrease of its filament size and alterations in the morphology of its cells (Fig. 2).**

Our 4 Steps Metabolomics Workflow



1- Design of the co-culture chamber

A purpose-built growth incubator was constructed, in which two cultures were physically separated by a 0.45 µm membrane filter that enabled passage of fluids and dissolved substances, but not cells.

Two strains:
Microcystis aeruginosa PCC 7806 (**M**)
Planktothrix agardhii PCC 7805 (**P**)

The **growth**, **morphology** and **metabolites production and release** of the strains were compared (i) in mono- and (ii) in co-culture in the growth chamber.

2- Extraction Process

- **Intracellular:** Supelclean™ LC-18 (Supelco Analytical)

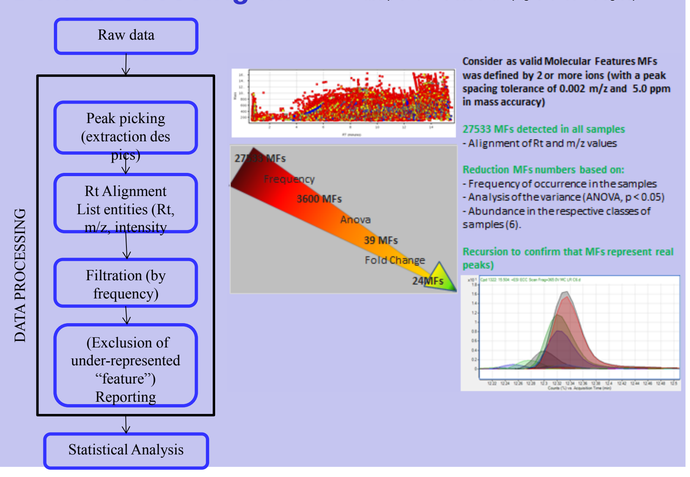
- **Extracellular:** Discovery® DSC-18, 1000 or 5000 mg (Supelco Analytical)

3- Chemical & Data Analysis

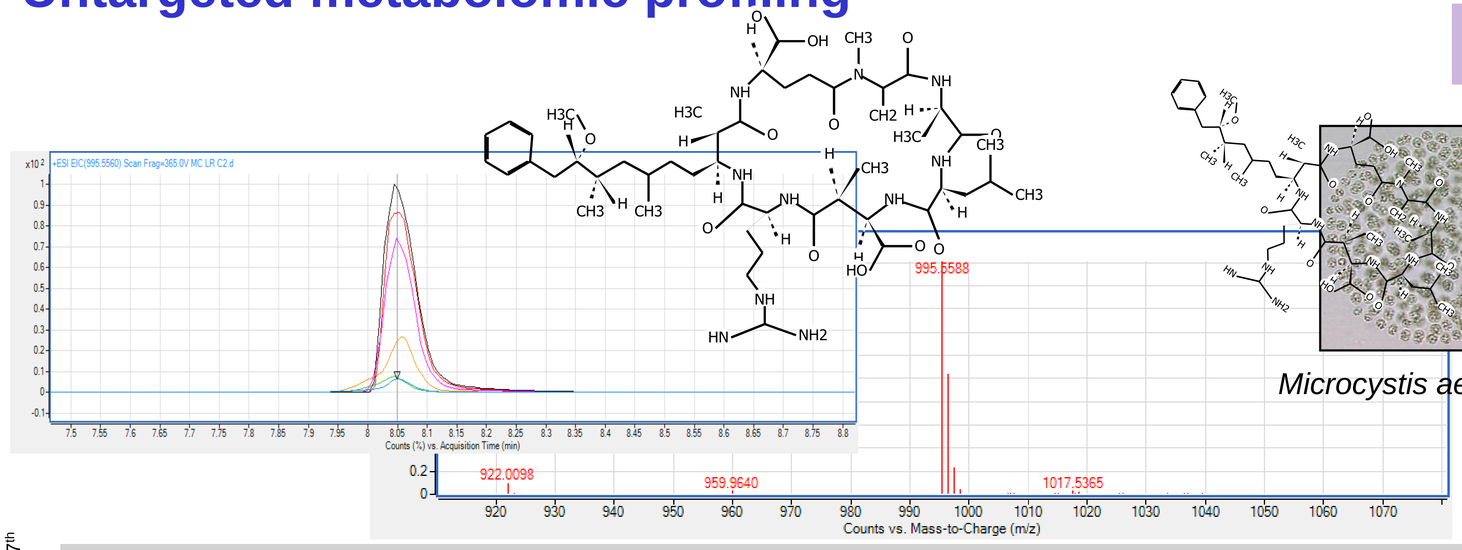
1290 Infinity II UHPLC / Jetstream ESI 6550 ifunnel QTOF MS (Agilent Technologies)

4- Data Processing MPP*

Mass profiler Professional *(Agilent Technologies)



Untargeted metabolomic profiling



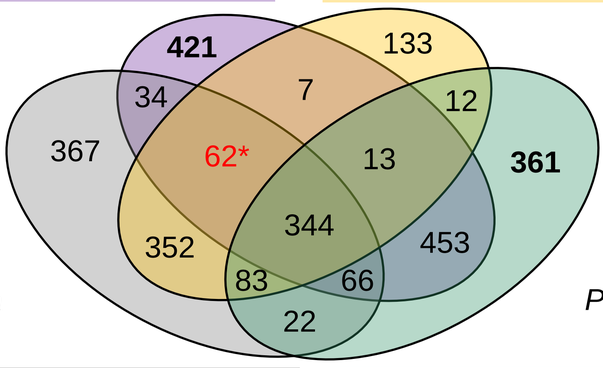
Microcystis aeruginosa

Planktothrix agardhii

Figure 3

1400 entities *Microcystis*: Intra Mono- and Co-culture

1006 entities *Planktothrix*: Extra Mono- and Co-culture



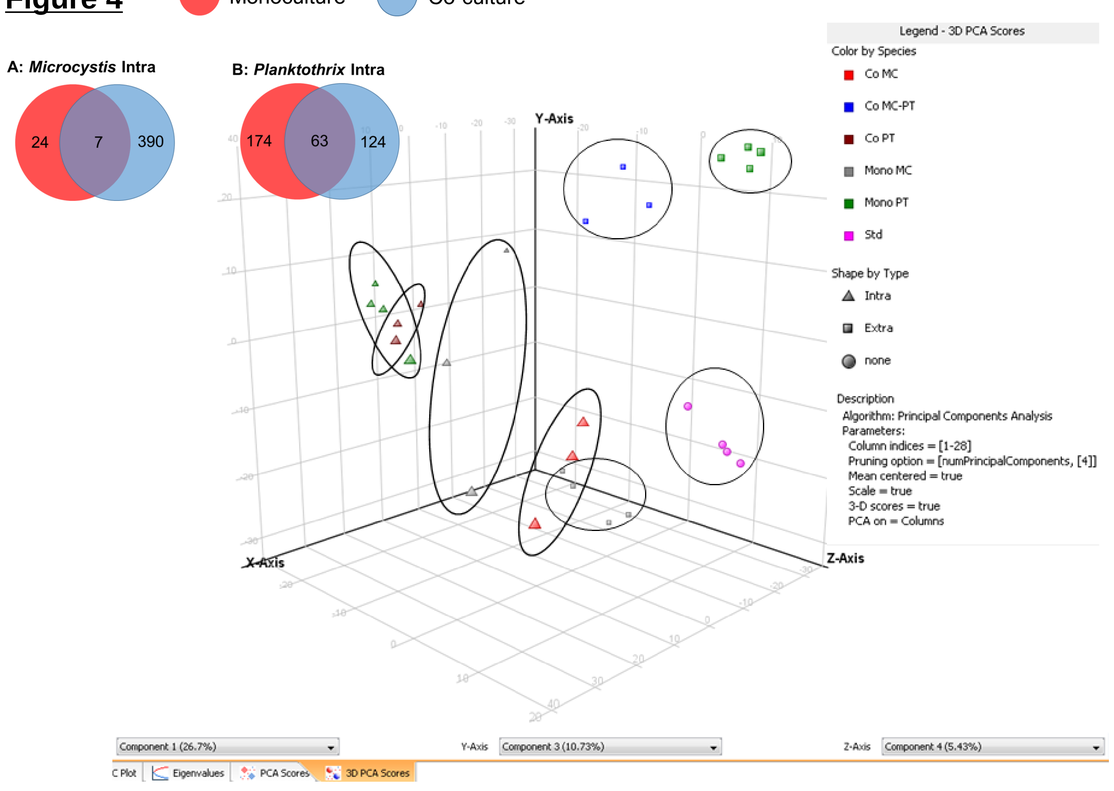
1330 entities *Microcystis*: Extra Mono- and Co-culture

1354 entities *Planktothrix*: Intra Mono- and Co-culture

Microcystin -LR: $C_{49}H_{74}N_{10}O_{12}$ – M+H⁺ = 995.556 – A toxic metabolite synthesized by Microcystis

➢ **Out of the 2730 compounds, 822 (421+34+367) were specific to *Microcystis*, 506 (133+12+361) were only found in *Planktothrix*, whereas 1402 compounds were shared by both strains independently of the culture condition and the fraction (Fig. 3).**

Figure 4



➢ **Out of the 421 compounds specific to the intracellular fraction for *Microcystis,* 390 were produced under co-culture (Fig. 4A).**

➢ **Out of the 361 compounds specific to the intracellular fraction for *Planktothrix*, 124 were produced under co-culture (Fig. 4B).**

➢ ***Microcystis* produced a lower number of intracellular compounds under monoculture than *Planktothrix*.**

➢ **Inversely, *Microcystis* produced a higher number of compounds under co-culture condition than *Planktothrix.***

Conclusion & Perspectives

❖ **The presence of *Microcystis* altered the growth and the morphology of the *Planktothrix* cells.**

❖ **However, the production of specific intracellular compounds by *Planktothrix* was not different between mono and co-culture conditions. In general, *Microcystis* produced a lower number of intracellular compounds under monoculture than *Planktothrix*, and a higher number of compounds than *Planktothrix* under co-culture condition.**

❖ **Our investigation did not allow us to identify specifically the compounds involved in the observed physiological and morphological changes of *Planktothrix* cells, but suggests that specific compounds produced by *Microcystis* in the presence of *Planktothrix* have been specifically produced as potential allelochemicals.**

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