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Maximizing metabolome coverage and metabolism in *Microcystis* sp. : A comparative evaluation of monophasic and biphasic extraction-based workflow

Thomas Forjone^{1,2}, Melissa Eon^{1,2}, Charlotte Duval³, Sylvia Moreira¹, Benjamin Marie³, Binta Diémé⁴, Nicolas Creusot^{1,2}

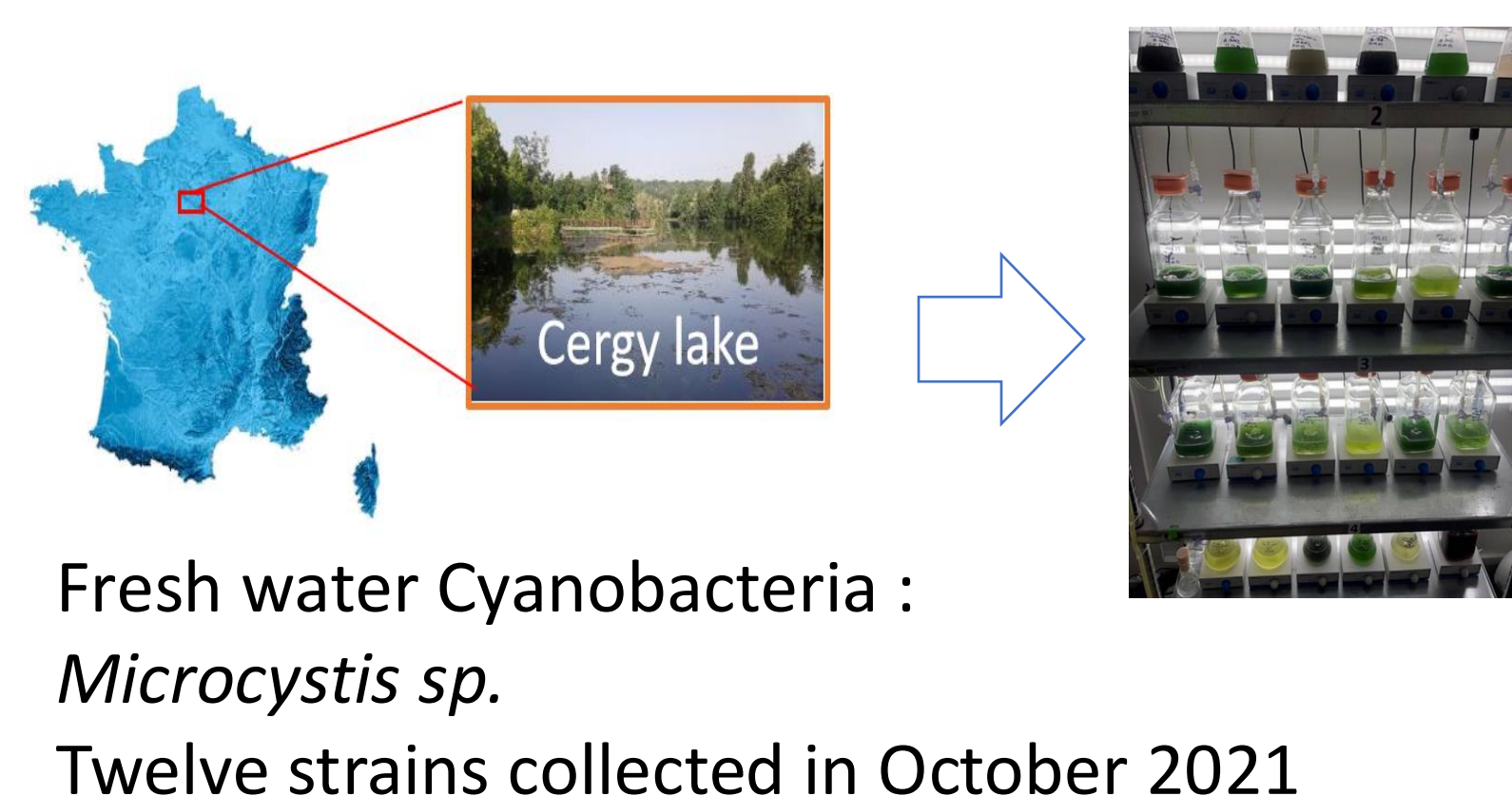
1 INRAE, UR1454 EABX, 2 Bordeaux Metabolome, MetaboHub, 3 Muséum National d'Histoire Naturelle, MCAM, Paris, France, 4 Université Clermont Auvergne

Context

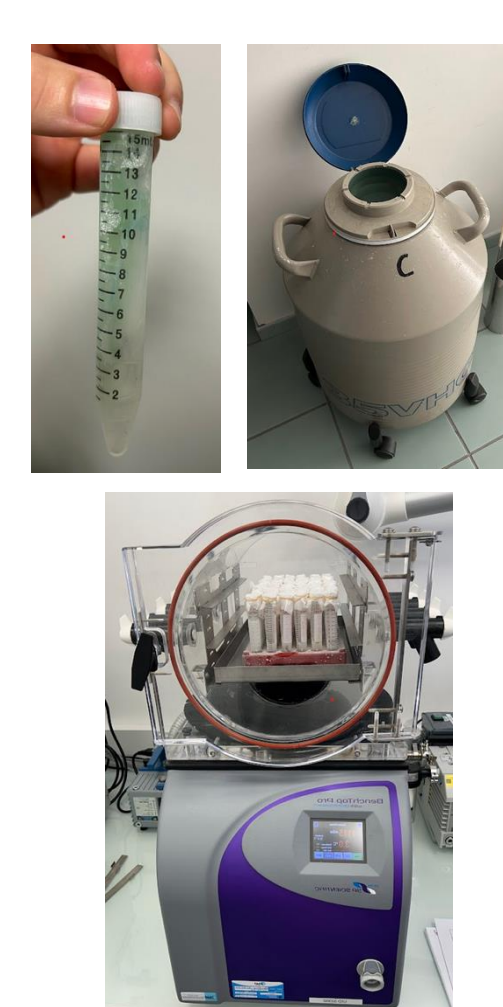
- Cyanobacterial blooms are a phenomenon of growing international concern, due to their potential ecological, human and economic impacts.
- Recently, some studies have highlighted the potential role of metabolic interactions between heterotrophic bacteria and cyanobacteria in the phycosphere in promoting cyanobacterial blooms.
- In this context, there is a growing need to comprehensively depict metabolome and metabolism of cyanobacteria and heterotrophic bacteria consortium.
- This study aims to explore various metabolomic workflows in order to provide a comprehensive picture of the chemical landscape and so the metabolism for twelve strains of *Microcystis* sp. To such an end, biphasic (MTBE/MeOH/H₂O leading to a hydrophilic and lipophilic extracts) and monophasic extraction (MeOH/H₂O leading to a single extract) coupled to UPLC-HRMS/MS analyses were compared**

Methodology

Sampling and cultivation



Sampling, quenching, freeze drying



Monophasic extraction

MeOH/H₂O (1:5)
→ Ultrasonication

Biphasic extraction

Lipophilic phase
MTBE/MeOH (3:1)
Hydrophilic phase
H₂O/MeOH (3:1)
→ Grinding

Metabolomic workflow

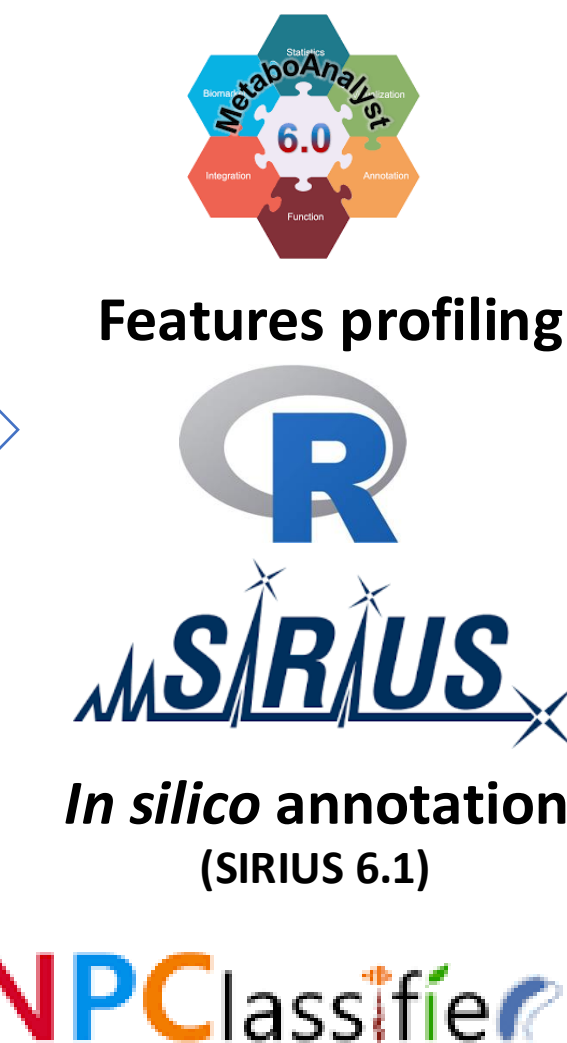
UPLC-HRMS/MS analysis



Data processing



Data analysis & annotation

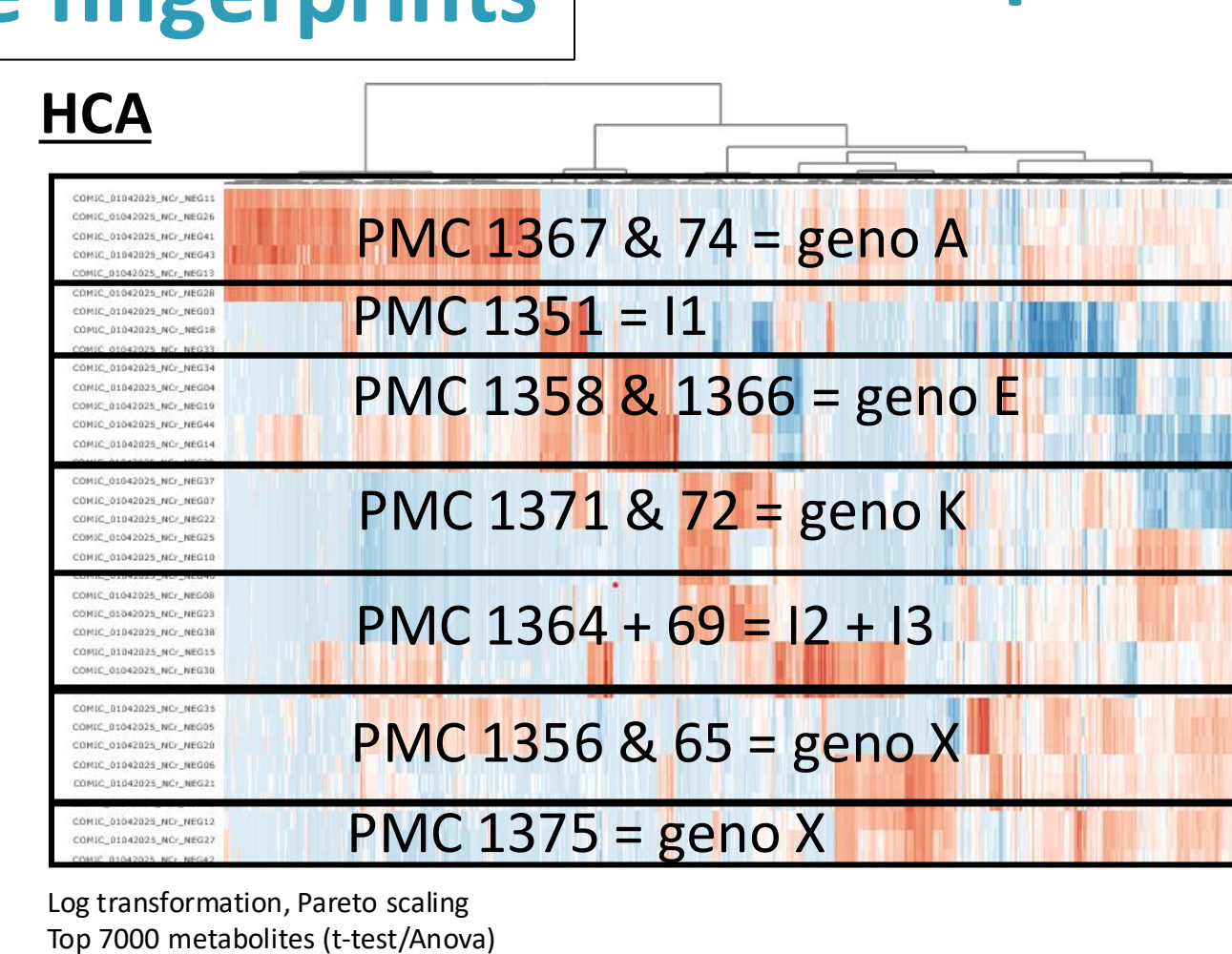
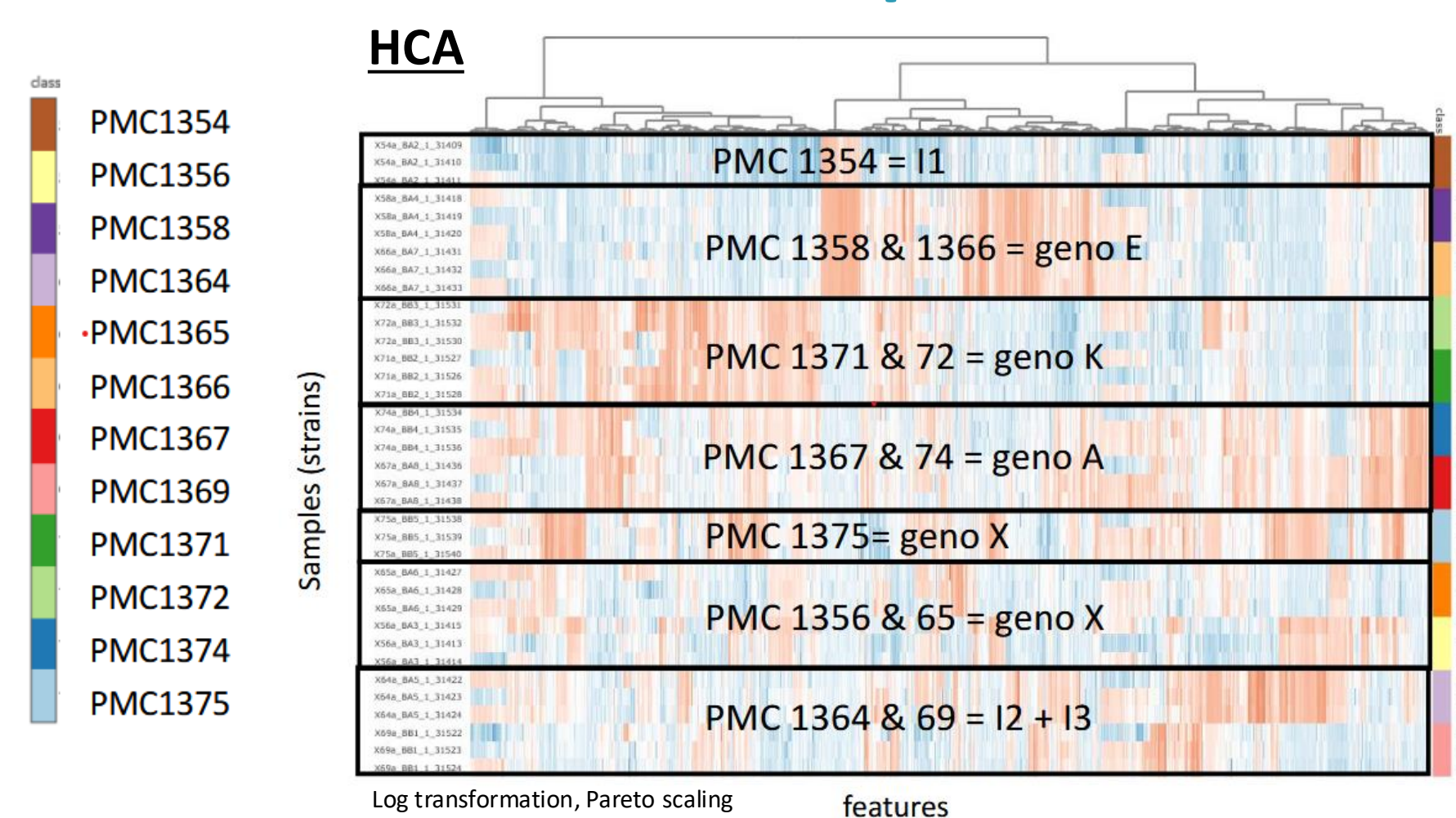


Results

Monophasic extraction

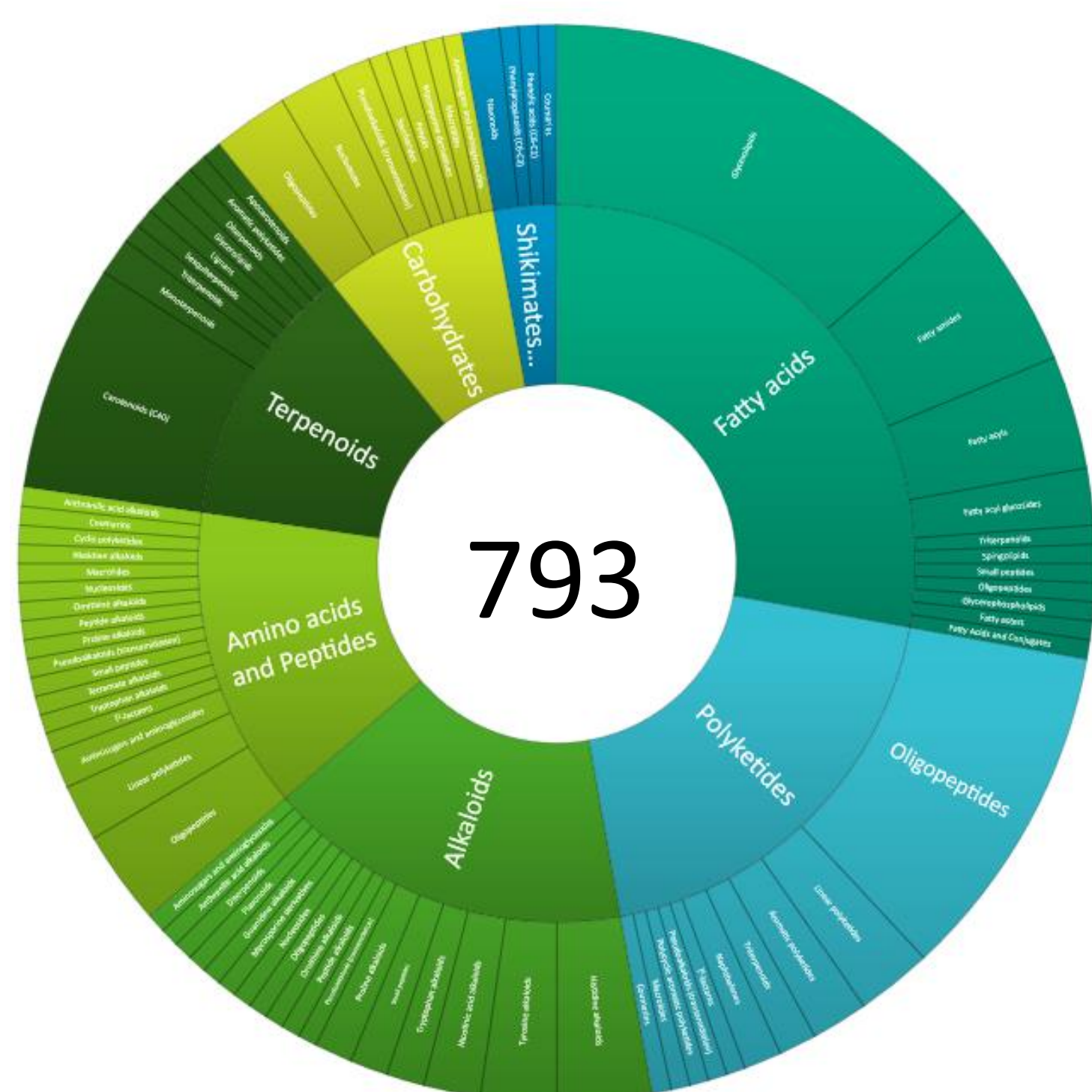
1. Whole metabolome fingerprints

Biphasic extraction



These results suggest that neither the extraction method nor the type of mass analyzer has a major impact on the clustering of metabolic profiles across *Microcystis* strains.

2. Chemical Landscape of putative annotated metabolites



87 alkaloids; 511 Amino acids and peptides; 67 carbohydrates; 118 Fatty acids; 47 Polyketides; 37 Terpenoids; 5 shikimates & Phenyls propanoids

793 putative annotated metabolites
78 in L2 (spectral DB) + 715 L3 (structural DB)

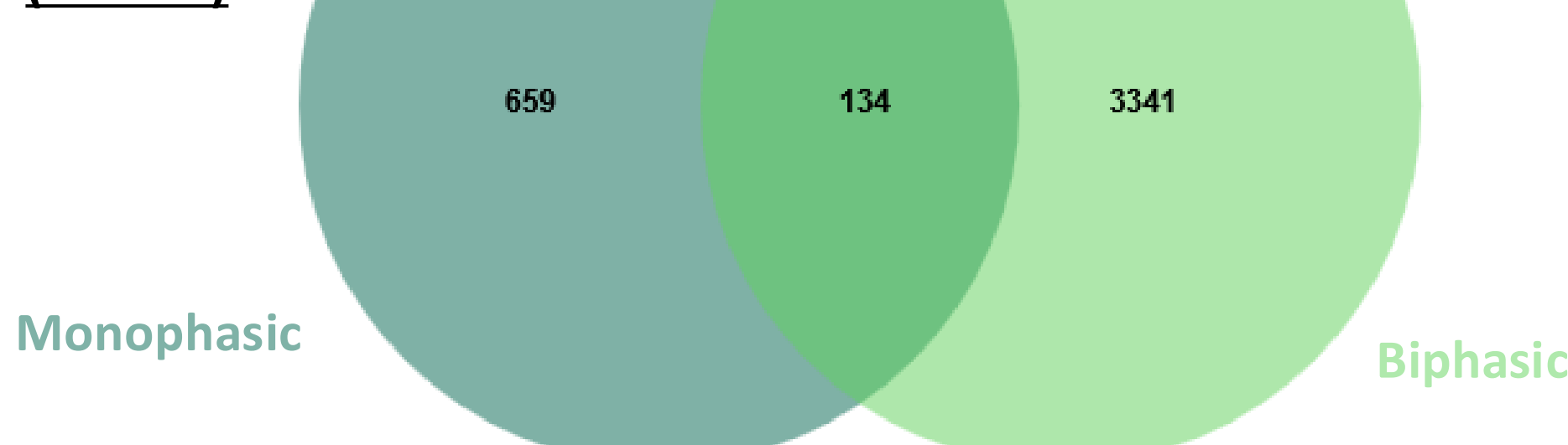


619 alkaloids; 2104 Amino acids and peptides; 259 carbohydrates; 660 Fatty acids; 161 Polyketides; 191 Terpenoids; 63 shikimates & Phenyls propanoids

3475 putative annotated metabolites
252 in L2 (spectral DB) + 3223 in L3 (structural DB)

Overall, the biphasic extraction led to a significantly higher number of putatively annotated metabolites (3475 vs 793), including a substantial increase across all major chemical classes, such as alkaloids, peptides, fatty acids, and terpenoids. Additionally, more high-confidence annotations (L2, spectral database) were obtained with the biphasic method (252 vs 78), highlighting its greater efficiency for metabolite detection and identification.

Venn diagram based on (SMILES)



- ✓ Only 134 annotated metabolites were shared between the two methods. Difference due to sonication (monophasic) vs grinding (biphasic extraction) ?
- Overall strong influence of both extraction and analysis methods on the metabolite profiles obtained
- Need for multiplexing to cover the broadest range of metabolites

Major outcomes

- The clustering results revealed that neither the extraction method or the mass analyzer had a major influence on the overall structure of metabolic profiles across *Microcystis* strains.
- However, annotation results showed a strong disparity in the number and diversity of metabolites detected, with the biphasic workflow yielding a much broader chemical coverage.
- Consistently, the Venn diagram showed a surprisingly low overlap between the two workflows, with only 134 shared features out of 793 (monophasic) and 3475 (biphasic) metabolites.
- These results demonstrate that capturing the full chemical landscape of a biological sample remains challenging, and highlight the value of combining complementary extraction and analytical approaches to achieve a more comprehensive view of the metabolome.**

References:

- [1] Igwaran et al. (2024). Cyanobacteria Harmful Algae Blooms : Causes, Impacts, and Risk Management. <https://doi.org/10.1007/s11270-023-06782-y>
[2] Louati, I. et al. (2023). The phyto-bacterioplankton couple in a shallow freshwater ecosystem : Who leads the dance ? Harmful Algae, 126, 102436. <https://doi.org/10.1016/j.hal.2023.102436>

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