# DEVELOPMENT OF ORIGINAL ANALYTICAL TOOLS FOR THE CHEMICAL PROFILING OF MICROALGAE

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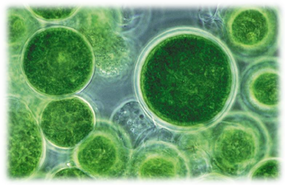
## Context

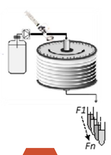
Research efforts are necessary to sustain the development of blue biotechnology as an emerging industrial field by diversifying industrial microalgae applications in high value added sectors such as materials, nutraceuticals, cosmetics or drugs. Access to these new products has to be done through the development of disruptive and safer processes with low environmental impacts as well as to adapt the micro-algae biorefinery according to chemodiversity potential, process routes, and market needs. Such an approach necessarily implies **an increased knowledge of the chemical diversity of the "microalgae" raw material**.

## Introduction

An original and efficient analytical tool was developed for the chemical profiling of microalgae extracts.[1,2] This dereplication strategy combines extract fractionation by **centrifugal partition chromatography** and **$^{13}$C NMR spectroscopy combined with unsupervised classification** for the compound-by-compound grouping of experimental chemical shift values according to their chromatographic emergence profile. The grouping proceeds through the **hierarchical clustering analysis performed on $^{13}$C chemical shifts**. The chemical shift clusters are then used as search targets in **a database enriched with data from literature and predicted chemical shifts**.

## General workflow[1]

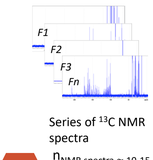





Series of chemicaly simplified CPC fractions

$n_{NMR\ spectra} \simeq 10\text{-}15$

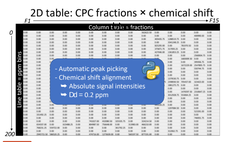
1 Fractionation by centrifugal partition chromatography



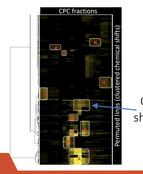

Series of $^{13}$C NMR spectra

$n_{NMR\ spectra} \simeq 10\text{-}15$

2 $^{13}$C NMR analysis of the chemically simplified CPC fractions



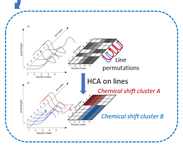

3 $^{13}$C NMR data alignment & binning




HCA ( Euclidean distance, Ward's agglomeration method)

$$D_W(C_i, C_j) = \sum_{x \in C_i} (x - r_i)^2 + \sum_{x \in C_j} (x - r_j)^2 - \sum_{x \in C_{ij}} (x - r_{ij})^2$$

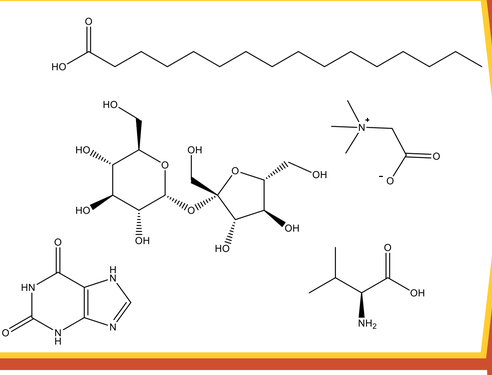
4 $^{13}$C NMR Unsupervised clustering of chemical shift values according to fractionation profile similarities: Hierarchical clustering values (**HCA**)




5 $^{13}$C NMR data Chemical shift clusters as targets for structure search in database

Database containing NP chemical structures and corresponding calculated $^{13}$C NMR spectra by ACD/Lab CNMR Predictor

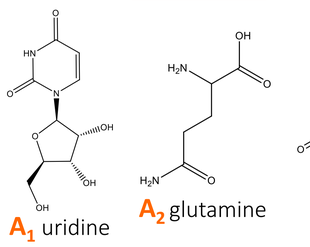
ACD/Labs



*Nannochloropsis gaditana*

Intact cells — Milled cells

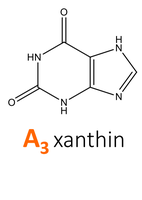
Comparison of MeOH/$H_2O$ extract



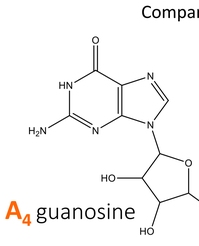
A $A_1$ uridine



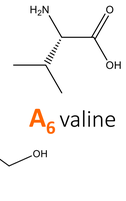
$A_2$ glutamine



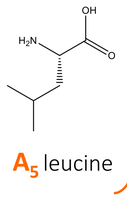
$A_3$ xanthin



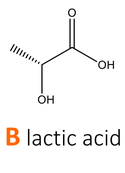
$A_4$ guanosine



$A_6$ valine



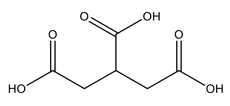
$A_5$ leucine



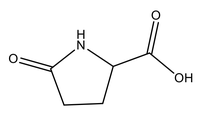
B lactic acid

Compounds only present in the milled extract :

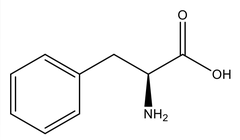
M not yet identified



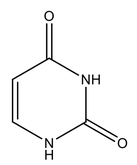
N citric acid



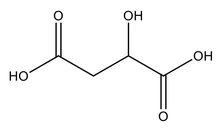
O pyroglutamic acid



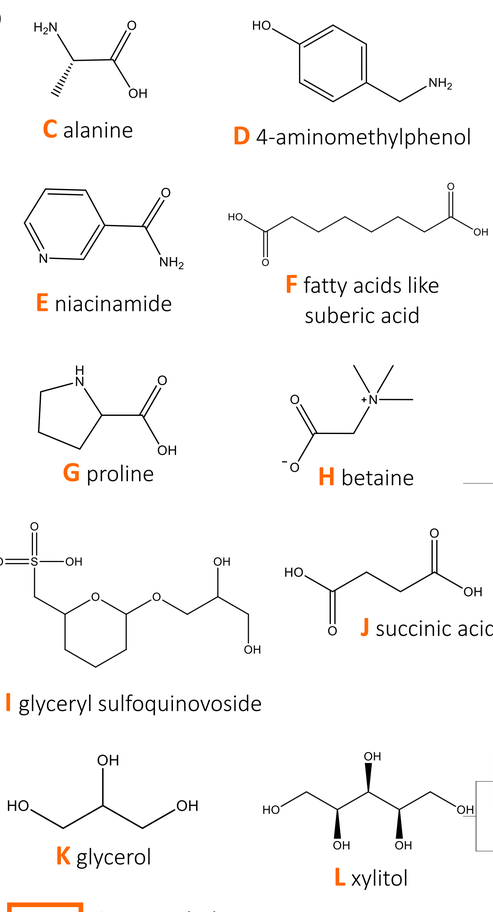
P phenylalanine



Q uracile



R malic acid

C alanine

D 4-aminomethylphenol

E niacinamide

F fatty acids like suberic acid

G proline

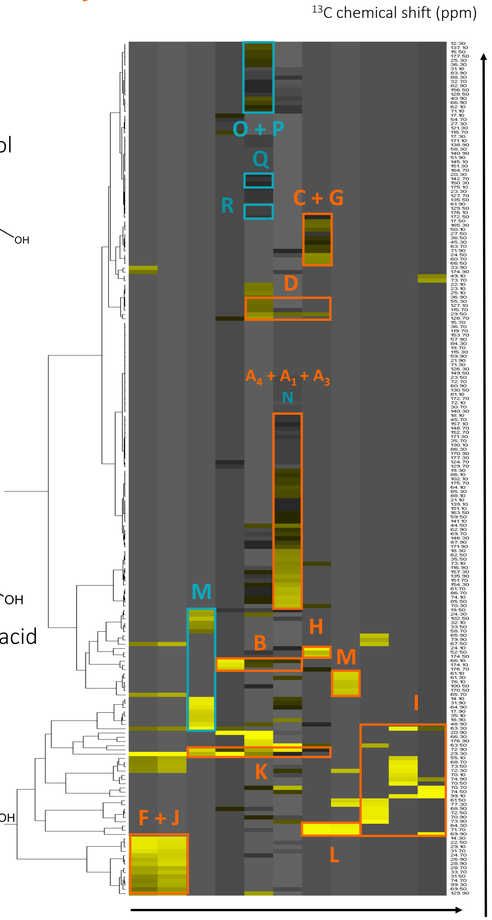
H betaine

I glyceryl sulfoquinovoside

J succinic acid

K glycerol

L xylitol



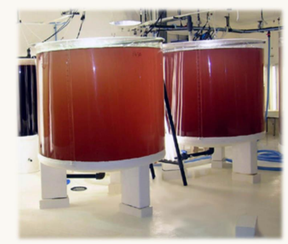

Common to both extracts

Compounds only present in the milled extract

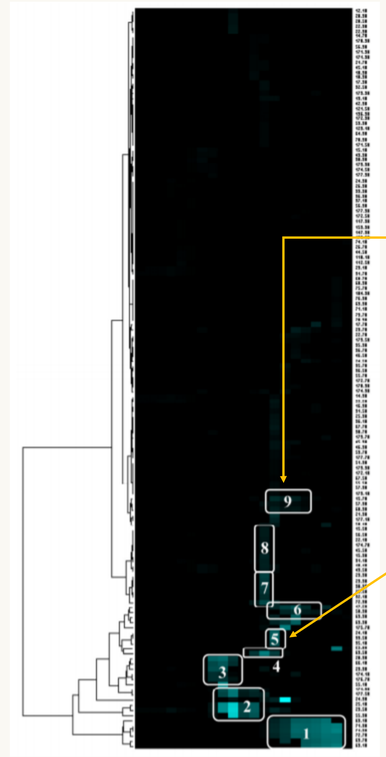
### Example[3]

Mariliance

Investigation of the neuro-soothing activity of a water-soluble hydrolysate obtained from the red microalgae *Rhodosorus marinus* Geitler (Stylonemataceae).



*Production plant (Givaudan, Britany)*




HCA heat map
Ward's agglomeration method

8: α-methyl-2-oxo-1-pyrrolidineacetic acid

9: GABA-alanine

6: alanine

7: 2-oxopyrrolidine

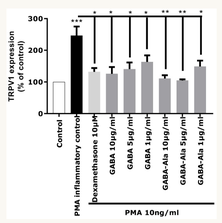
4: glycerol

5: γ-aminobutyric acid

3: lactic acid + succinc acid

1: sorbitol

2: pyroglutamic acid




*Biological evaluation of pure γ-aminobutyric acid (GABA) and GABA-Alanine on TRPV1 expression in inflammatory conditions. Statistical analyses were performed using Student's t-test with $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.*

The chemical profiling of the extract revealed the presence of two active molecules, GABA and its structural derivative GABA-alanine. This *Rhodosorus marinus* extract called Mariliance provides an efficient solution to treat skin disorders such as sensitive skin, atopia, dermatitis or psoriasis.

## Conclusion & Perspectives

The combination of an efficient multigram-scale fractionation method with $^{13}$C NMR analysis and HCA for pattern recognition of $^{13}$C signals across spectra of the fraction series enabled the direct identification of the main metabolites present in a crude natural extract (*i.e.* from microalgae) without compound-by-compound tedious, time and solvent consuming purification steps.[4] It also allows the study of the **impact of the fractionation and production process on the chemical profile of the microalgae extracts**.

[1]Hubert, J.; Nuzillard, J.-M.; Purson, S.; Hamzaoui, M.; Borie, N.; Reynaud, R.; Renault, J.-H. *Anal. Chem.* **2014**, *86* (6), 2955–2962.

[2]Bakiri, A.; Plainchont, B.; Emerenciano, V. de P.; Reynaud, R.; Hubert, J.; Renault, J.-H.; Nuzillard, J.-M. *Mol. Inform.* **2017**, *36* (10), 1700027.

[3]Scandolera, A.; Hubert, J.; Humeau, A.; Lambert, C.; De Bizemont, A.; Winkel, C.; Kaouas, A.; Renault, J.-H.; Nuzillard, J.-M.; Reynaud, R. *Mar. Drugs* **2018**, *16* (3), 96.

[4]Wolfender, J.-L.; Nuzillard, J.-M.; van der Hooft, J. J. J.; Renault, J.-H.; Bertrand, S. *Anal. Chem.* **2019**, *91* (1), 704–742.