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Metabolomic study of the red algae *Chondrus crispus*

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CONTEXT

The goal of this study was to establish the best protocol of extraction of the whole metabolome of algae.
Seaweeds contain a lot of polysaccharides. For instance, in red algae, carrageenans confer a gelling nature to tissue, resulting in difficulties to obtain a reproducible manual grinding. Thus, particular attention has to be paid to grinding and automated techniques to carry out such extraction steps, as cryo-grinding, should be preferred. To avoid metabolite degradation, freeze-drying the sample before grinding is important. Thus, we tested automated cryo-grinding for the metabolite extraction of a read algae, *Chondrus crispus*, submitted to elicitation by methyl jasmonate (MeJA).

MATERIAL

- **Precellys 24** homogenizer with **Cryolys** cooling device to have a constant temperature of – 20 ℃ within the homogenization chamber using liquid nitrogen.
- Precellys lysing kit : 03961-1-008 (MK28-R).
- Sample: 3x3 replicates of MeJA elicited & control *Chondrus crispus*.
- Extraction solvent: 1 ml ice-cold [MeOH/H2O (8:2)] for 200mg fresh tissue (added after homogenization).

PROTOCOL

- Each lysing tube was added of 150 mg Fontainebleau sand.
- Precellys 24: 1x30s 6800rpm - 1x50s 6000rpm - 1x30s 6800rpm - 1x90s 6200rpm (with 5min break between each run).
- Extraction was carried out at +4℃ during 1hour with constant agitation. Centrifugation 3000g, 15 min.
- Samples were injected in UHPLC/MS on Dionex U3000 RSLC coupled to a LTQ-Orbitrap Discovery (Thermo, Les Ullis). Data treatment with XCMS and Mev.

RESULTS

Our results clearly demonstrate the elicitation of the red algae *Chondrus crispus* by MeJA (results not shown). Morever, the 9 different replicates display very reproducible metabolomic profiles (Figure 1), thanks to simultaneous grinding with Precellys 24. During this study, the Cryolys guaranteed a constant temperature of -20°C within the homogenization chamber and thus avoided an uncontrolled defrosting of the frozen samples.


Chondrus crispus

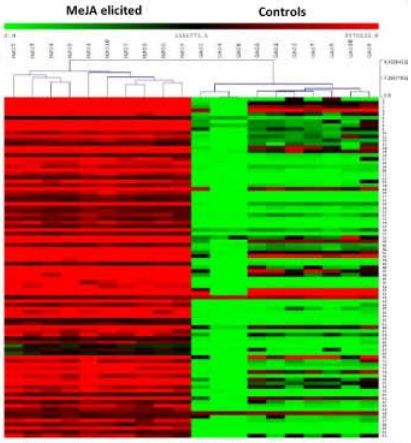

Figure 1: Hierarchical clustering of all detected ions from of control and MeJA elicited Chondrus crispus samples. Samples are in columns; each line represents a detected ion and its variations, from low amount (green) to higher amount (red). Here, only the 91 first ions are represented (2448 detected).

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METABOMER

CONCLUSION

Compare to manual grinding, simultaneous **cryo-grinding** avoided biological material degradation and allowed **higher reproducibility, faster grinding and better metabolite extraction.**

Cryo-grinding with the combo **Precellys 24 and Cryolys** is for our platform a daily necessary tool to perform **robust and high-throughput metabolomic analysis.**

For more details, please contact precellys@bertin.fr

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