

Metabolomics (general profiling)

Extraction of metabolites from bacteria and host

Background:

- Successful metabolomic research requires effective metabolite extraction. For non-targeted metabolomics, extraction methods need to capture a broad range of cellular and biofluid metabolites, while excluding components such as proteins that are not intended for analysis. Extraction is made more challenging by the physico-chemical diversity of metabolites and by metabolite abundances that can vary by many orders of magnitude.
- Biphasic, liquid-liquid extraction is often used to extract metabolites. The nature of the organic and aqueous solvents, their volumes, solvent ratios, and aqueous solvent pH, however, must be considered carefully. They can significantly affect the total number of metabolites extracted and experimental reproducibility.
- We use here a solvent mixture of very polar (water and methanol) and non-polar solvents (acetonitrile) to extract many different metabolites of our symbiotic system

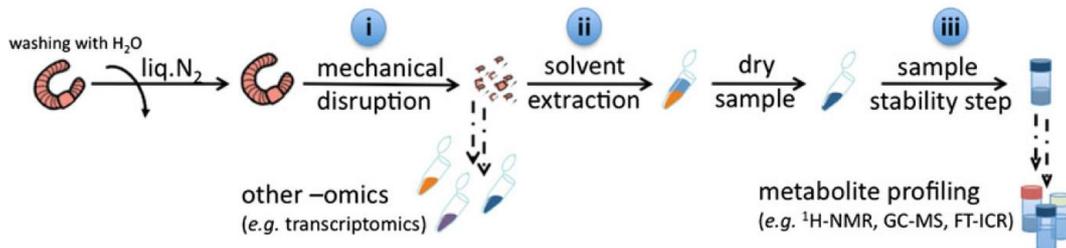


Fig.1 general extraction and sample preparation scheme

To be prepared:

- Ice bucket
- Solvent mixture (acetonitrile/methanol/water-solution mix (2:2:1)) **AMW** (all MS grade)
- Thaw internal standard solution (ribitol) beforehand, keep on ice

1. For cell culture

- a. spin cells down to get a pellet
- b. decant supernatant (keep SN, store SN in freezer)
- c. proceed with 3.)

2. For tissue

- a. If possible measure weight of tissue used for metabolomics sample

- b. Transfer piece of tissue into pre-chilled solvent mixture (in field)
 - c. Transfer piece of tissue in pre-chilled beadbeating tube (in lab)
3. add ice-cold 750µl AMW solution
 4. vortex and transfer everything into BeadBeating tube for cell disruption
 5. Wash the sample tubes with 750 µl AMW, to get remaining material transferred into BeadBeater vial
 6. For generating a blank add 750 µl AMW into an empty sample tube or a tube with the solvent mixture used in 2a, vortex, and transfer into one BeadBeater vial + 750 µl AMW
 7. Add 40 µl of ribitol standard per sample, close vials tight!
 8. Treat samples with bead beating for 40 sec., 4 m/s to disrupt tissue/cells. **Crucial:** *The tissue/cells should be completely dissolved/disrupted.*
 9. Centrifuge at 13.000 rpm for 1 min
 10. Transfer supernatant to new 2 ml eppendorf tubes
 11. Add 1 ml of AMW to the ,old' BeadBeater vials to repeat extraction.
 12. Vortex for 20sec
 13. Centrifuge for 1 min
 14. Combine each sample's supernatants in eppendorf tubes
 15. Centrifugal evaporation using speed-vac, 30 °C for 4 h. **Crucial:** *Open lids when the eppendorf tubes were located in the machine. Then turn the speed-vac on.*
 16. Store dried samples in freezer

Sample preparation for GC-MS measurements

Background:

- Involves a sample being vaporized to a gas and injected into a column
- Sample is transported through the column by an inert gas mobile phase
- Column has a liquid or polymer stationary phase that is adsorbed to the surface of a polymer tube
- Columns are 20-30 m in length and couple of µm in internal diameter
- Samples are usually derivatized with Trimethylsilane (TMS) to make them volatile

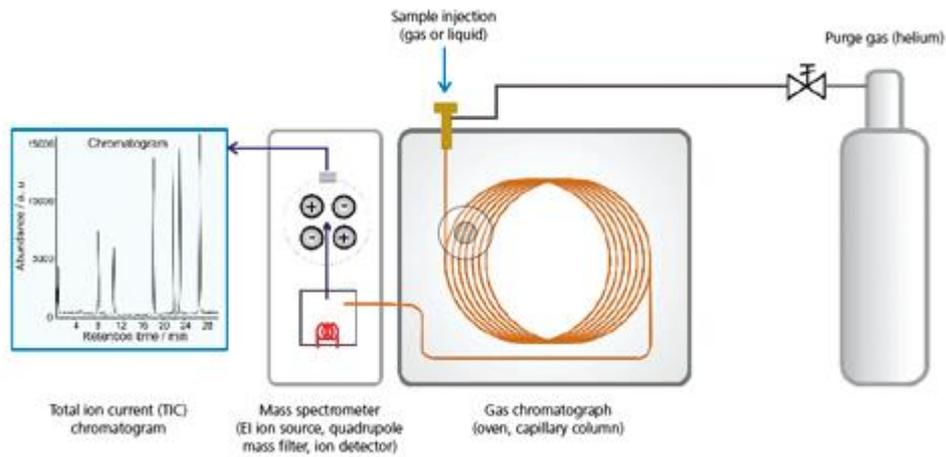


Fig.2 Scheme of a GC-MS setup

- Samples are usually derivatized with Trimethylsilane (TMS) to make them volatile
- Metabolites are detected as there TMS forms in the mass spectrometer

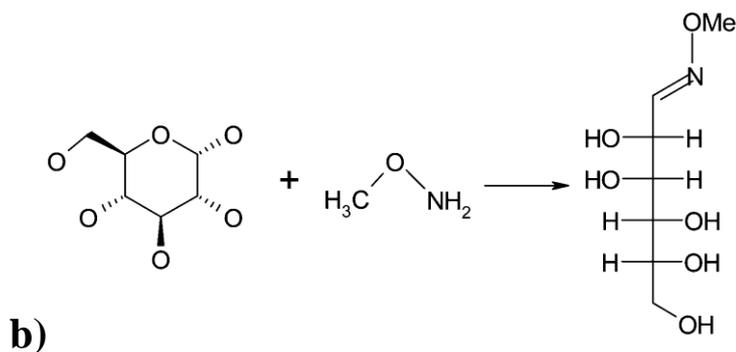
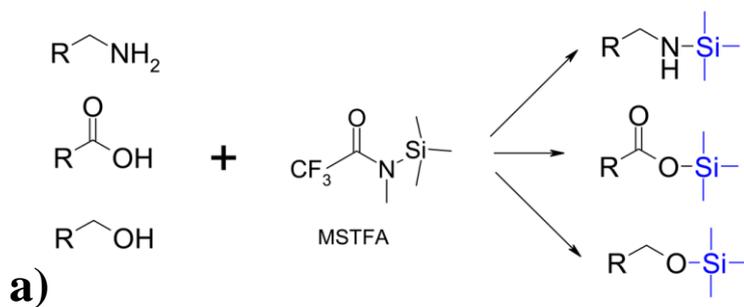


Fig.3 Derivatization reaction: a) general trimethylsilylation, b) methoxymation plus trimethylsilylation

To be prepared:

- Prepare derivatization reagent I (methoxyamin (20 mg/ml pyridine))
- Get reagent II (*N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA))) from fridge

17. Add 40 μ l reagent I to the pellets
18. Mix on thermomixer for 90 min at 37 $^{\circ}$ C, 1250 rpm.
19. Short centrifugation (30 sec)
20. Add 40 μ l reagent II
21. Mix on thermomixer for further 30 min at 37 $^{\circ}$ C
22. Short centrifugation (30 sec)
23. Prepare GC vials
24. Transfer 80 μ l of the liquid (\pm) into the GC vials.
25. Acquire data

Background data analysis:

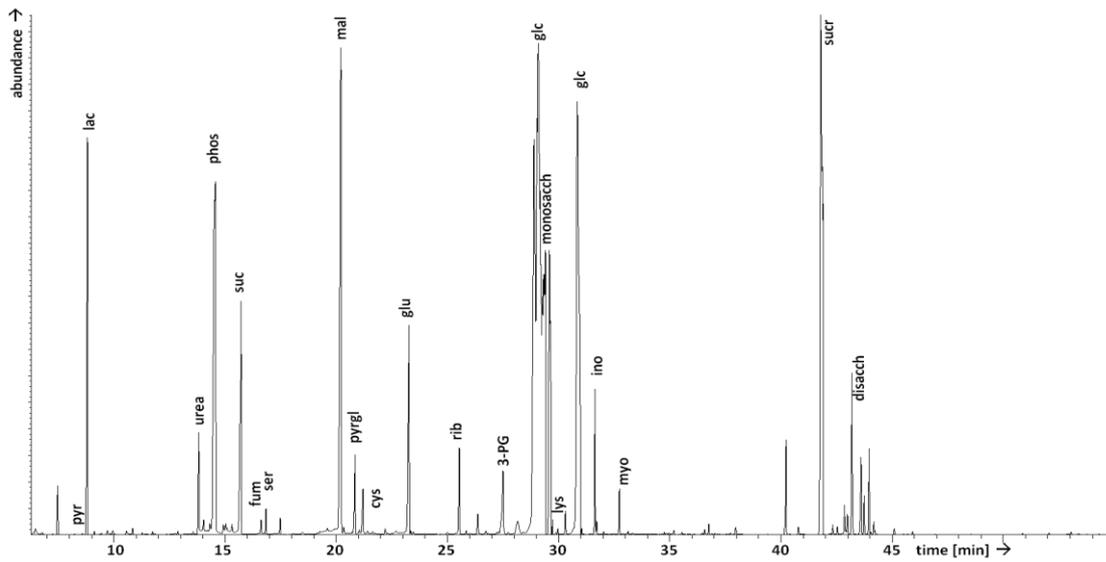


Fig.4 GC-MS chromatogram, identification of compounds done by mass spectral comparison with database

Notes: