Study of Enzymatic Complexes in Termites and their Common Symbionts: GC×GC-TOFMS Analysis of Metabolites Produced by Termites on Different Diets

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What Are Termites?

Efficient System from Termites? Symbionts? → SYNERGY?

Termitofuel Project

PARTNERS :

- Bio-Industries Unit (BIO) → MICROBIOLOGY
- Microbiology and Genomics Unit (BAM) → GENOMIC
- Functional and Evolutionary Unit (EFE) → PROTEOMIC
- ✓ Mass Spectrometry Unit (LSM) → METABOLOMIC

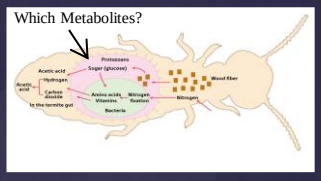
→ INTEGRATION OF COMPLEMENTARY APPROACHES

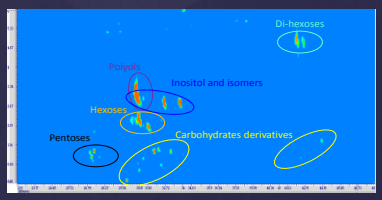
Sample Preparation:

- *Reticulitermes santonensis* de Feytaud (Lower termite), Oleron Island, France
- Laboratory cultured 27°C, 70% Humidity
- Adult workers collected, Total gut extracted (Fore-Mid-Hindgut) : **Gut volume < 1 µL !!**
- Pool of 1-10 guts, Piston pellet, MeOH/H_2O/$CHCl_3$ extraction, Evaporation to dryness
- Methoximation/silylation (60°C – 1h), Derivatization (MSTFA + 1% TMCS) (30°C – 2h)

Analytical Approach: GC×GC-TOFMS

- **DANI Master TOF GCxGC MLP v 01.20.59 (**ZX1-LN_2 cooled loop Modulation, ZOEX**)**
- P_M 4s-6s ; 100 scans/s ; 35-600 *m/z*
- **Reverse columns set** :
  - [1]D SLB IL-61 30 m x 0.25 mm 0.20 µm
  - [2]D Rtx-1 1 m x 0.10 mm 0.10 µm





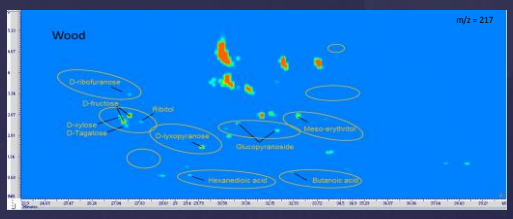
GC×GC-TOFMS carbohydrates analysis of 5 guts extract

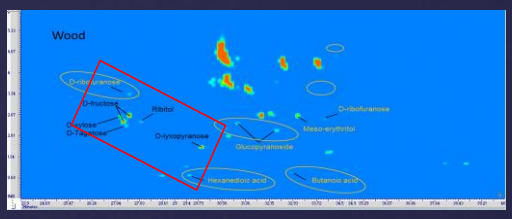
Application : Diet Influence

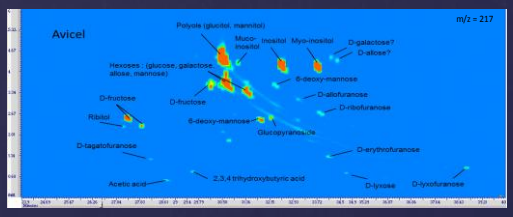
Wood

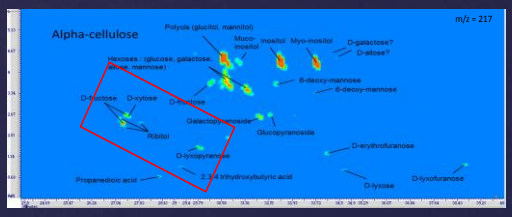
Cellulose Powder

Avicel®, alpha-cellulose
4 months diet



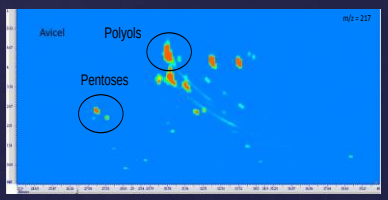






- Wood = lignine + hemicellulose + cellulose
  - [Phenols]$_n$ / [Hexoses + pentoses]$_n$ / [Glucose]$_n$
- Avicel = cellulose ⟶ [Glucose]$_n$
- Alpha-cellulose = cellulose + % xylan
  - [Glucose]$_n$ / [Xylose]$_n$

Polyols and pentoses are interesting compounds of specific metabolic pathways. They are detected even if termites were fed with polymer of glucose during 4 months



Conclusion:

- ✓ **Method suitable for metabolites detection in termite gut**
- ✓ **Sensitivity on 1 single gut**
- ✓ **Diet influence specificity**
- ✓ **Perspectives for analysis of fraction specificity**

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