



NMR Metabolite Screening of a Human Faecal Metagenomic Library for Identification of Polyphenol Bioconversion Activity.

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BACKGROUND



EU-project: Transfer of Knowledge GUT-system

Why:

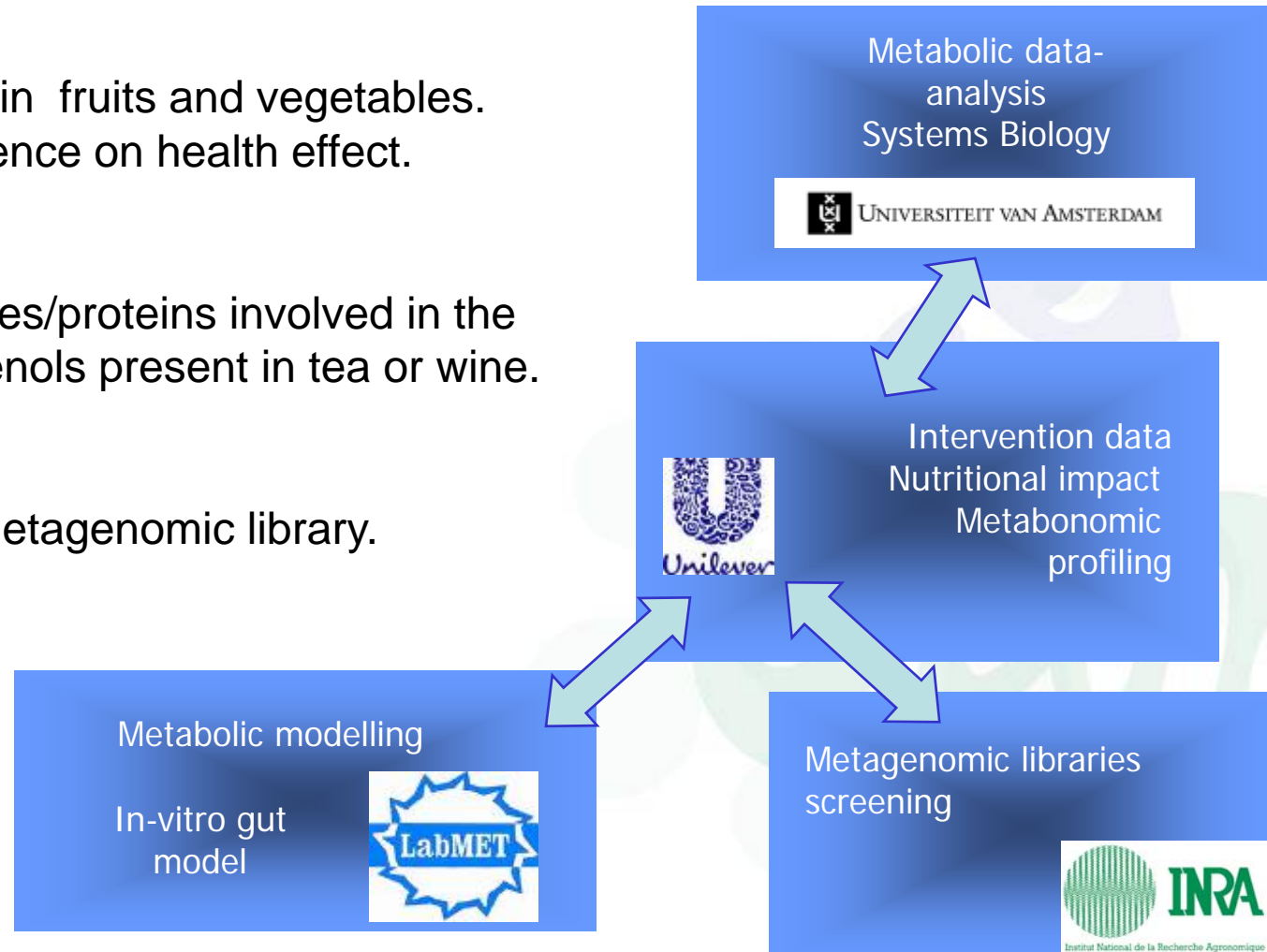
- Polyphenols present in fruits and vegetables.
- epidemiological evidence on health effect.

Goal:

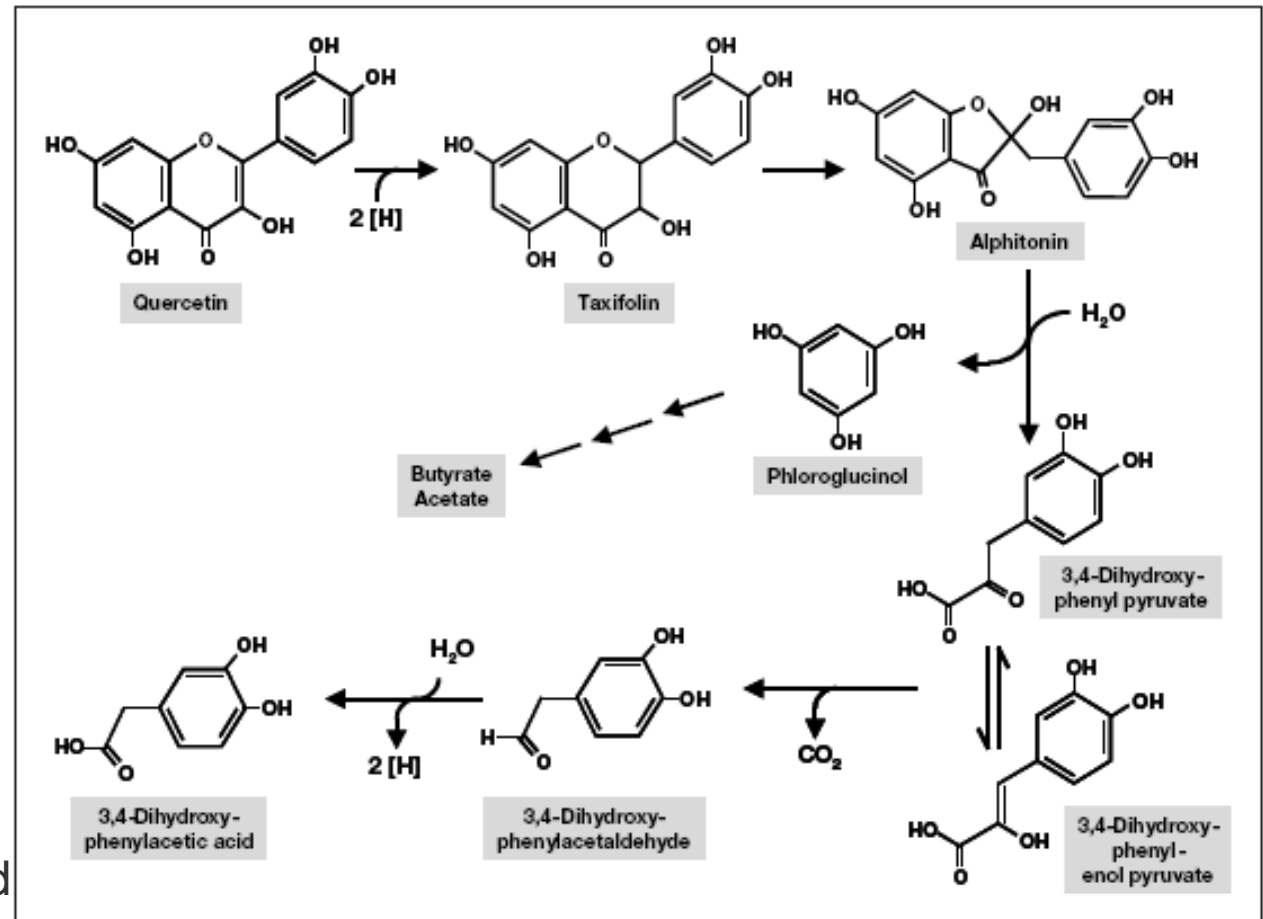
- Identify for novel genes/proteins involved in the breakdown of polyphenols present in tea or wine.

How:

- Screening a faecal metagenomic library.



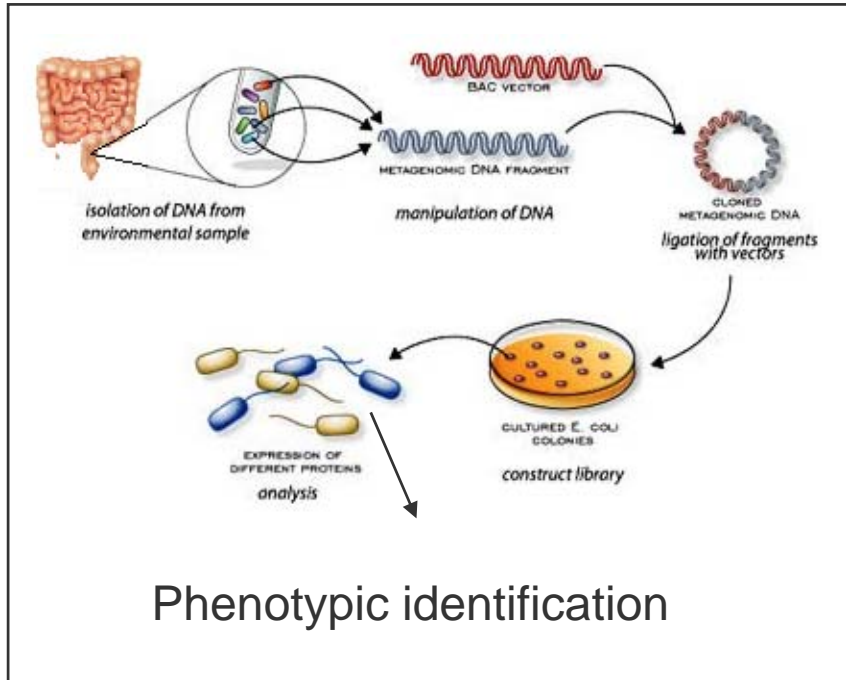
polyphenolic conversion pathway



Pathway of quercetin degradation as catalyzed by *E. ramulus*.

M. Blaut, Int. J. Vitam. Nutr. Res., 73 (2), 2003

Metagenomic library screening



Human genome Human metagenome

The comparison shows the **Human genome** as a set of 22 pairs of chromosomes, a Vitruvian Man figure, and a mouse. The **Human metagenome** is represented by a large, complex circular map, a microscopic view of purple bacteria, and an elephant. A large **X 100** indicates the scale difference between the two.

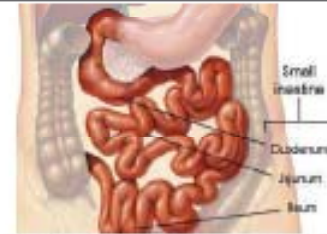
- **Metagenomics potential**
 - **Novel biocatalysts**
 - **Detailed knowledge of enzymatic pathways**
 - **Novel therapeutic compounds**



Banques métagénomiques et séquences-UEPSD



2 Banques fécales



Banque mucosale-Iléon

Individus	6 Sains	6 Malades Crohn	1
Echantillons	Feces		Pièce opératoire
Nombre de clones	25 000	25 000	20 000
Séquençage	1300 genes ADNr16S 10 010 extrêmités		96 inserts de 40kb 96 inserts of 40kb
Volume de séquences	7 Mb		4 Mb annotés

UEPSD 16 11 06

Proof of principle

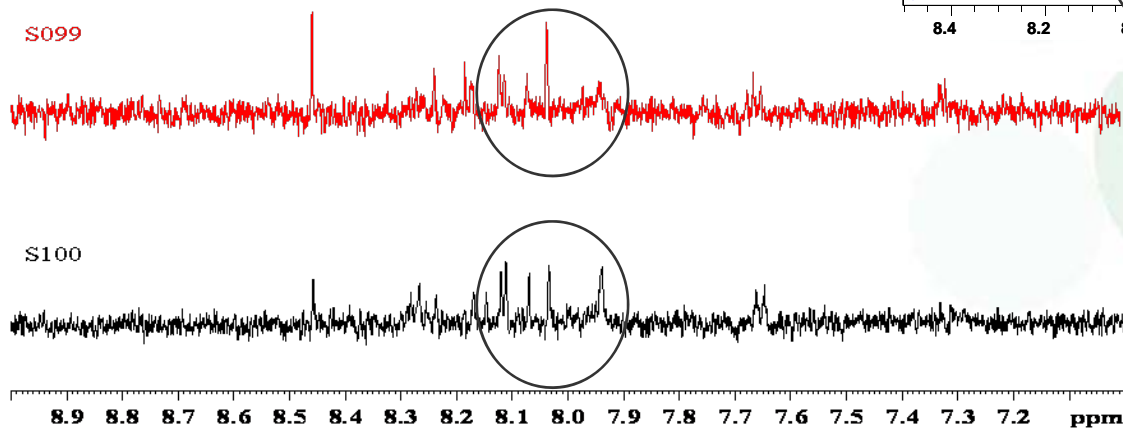
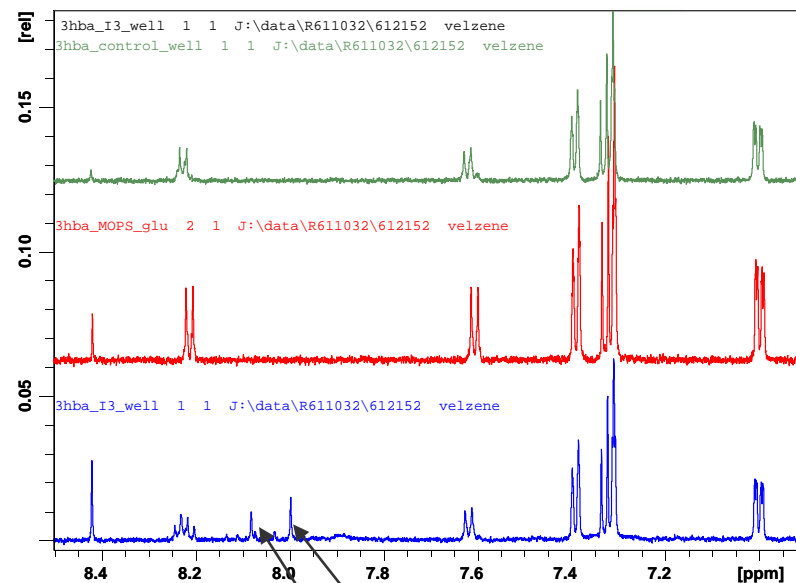


• Production of a riboflavin like compound shown by NMR.

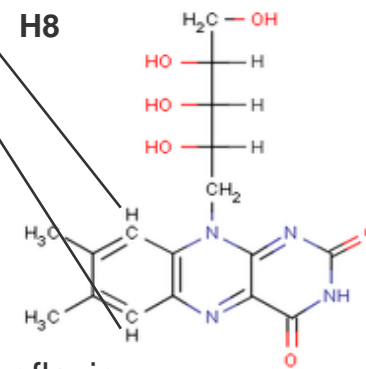
(Leclerc et al. in prep.)



- 1) XDAB0029C D12 (S099)
- 2) XDAB0119C H2 (S100)
- 3) XDAB0256C G4
- 4) *E. coli* DH10b (empty vector)

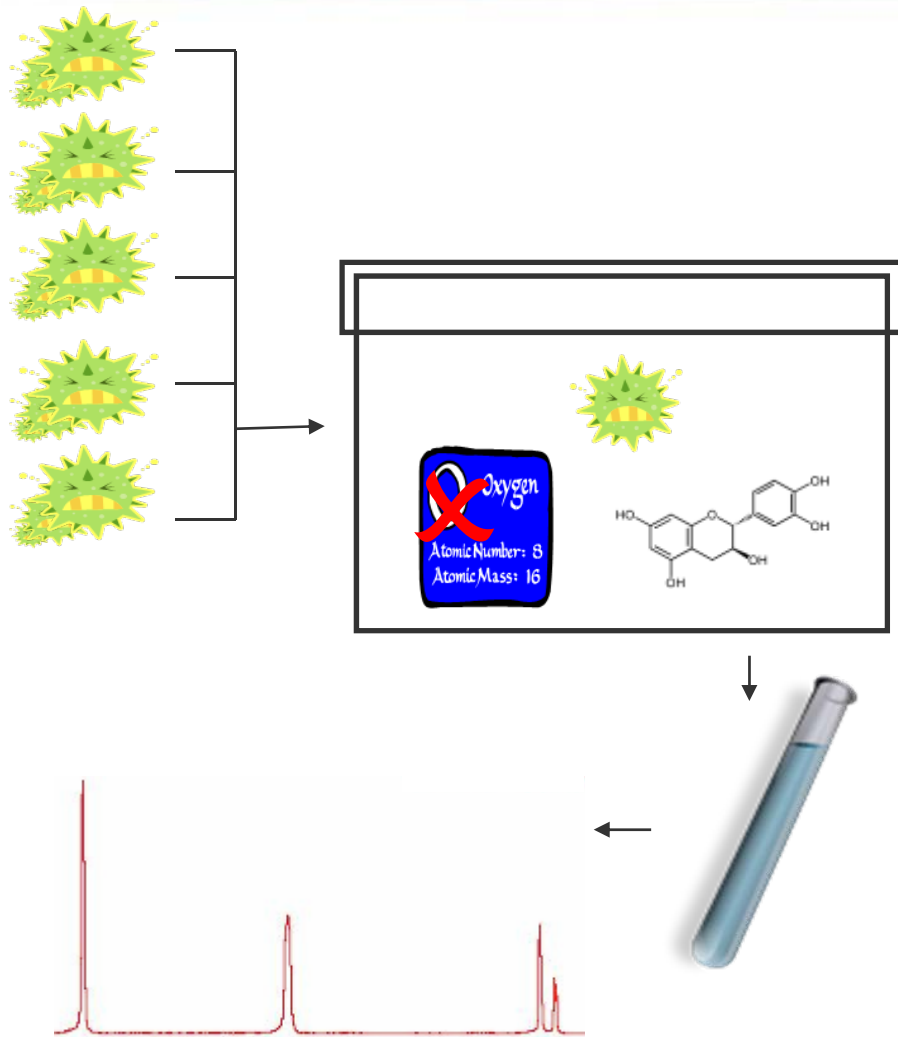


H8
H5



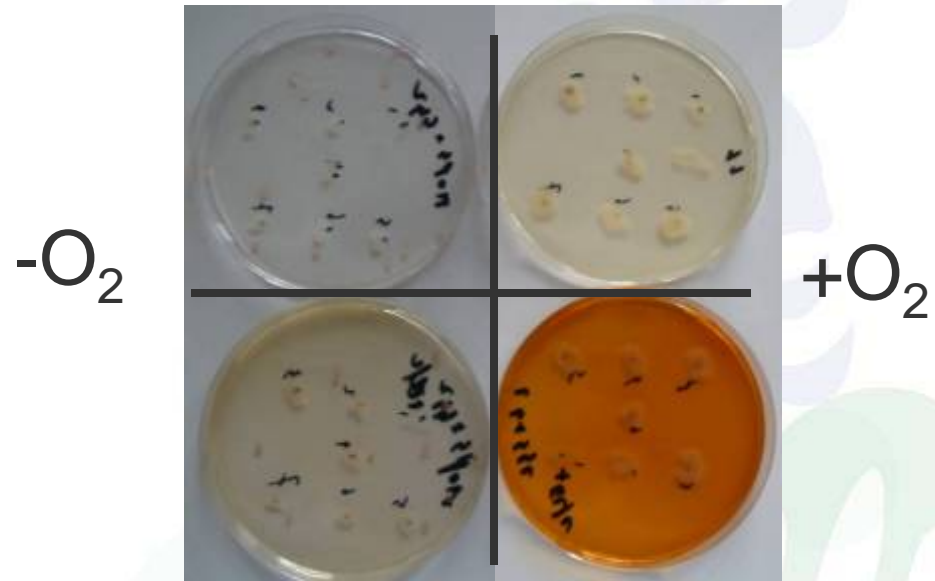
Riboflavin

Screening procedure



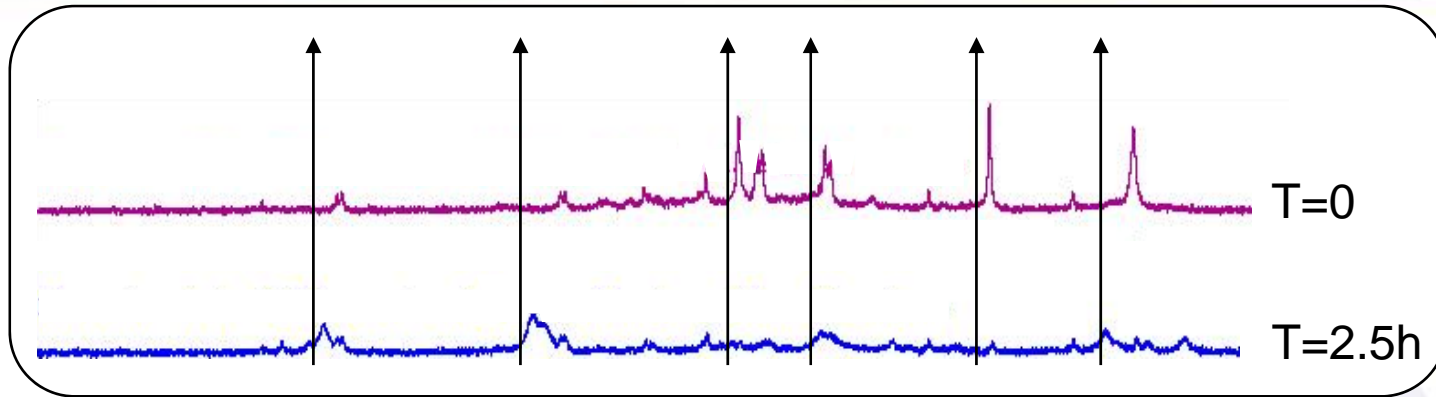
- Aerobic incubation causes polyphenolic conversion

- catechins

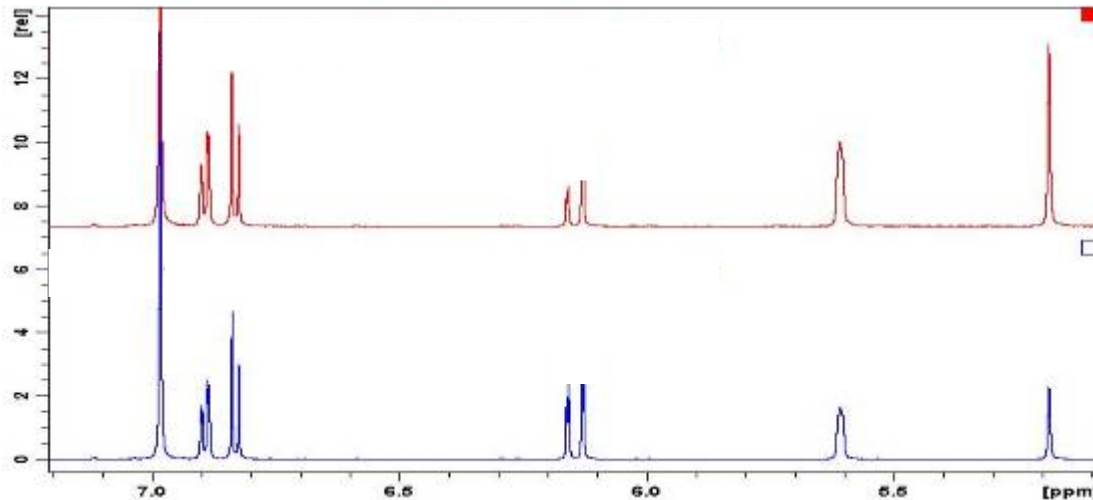


+ catechins

Polyphenol stability



Cyanidin 3-O-glucoside NMR profile over time



Epigallocatechin (EGC) NMR profile over time

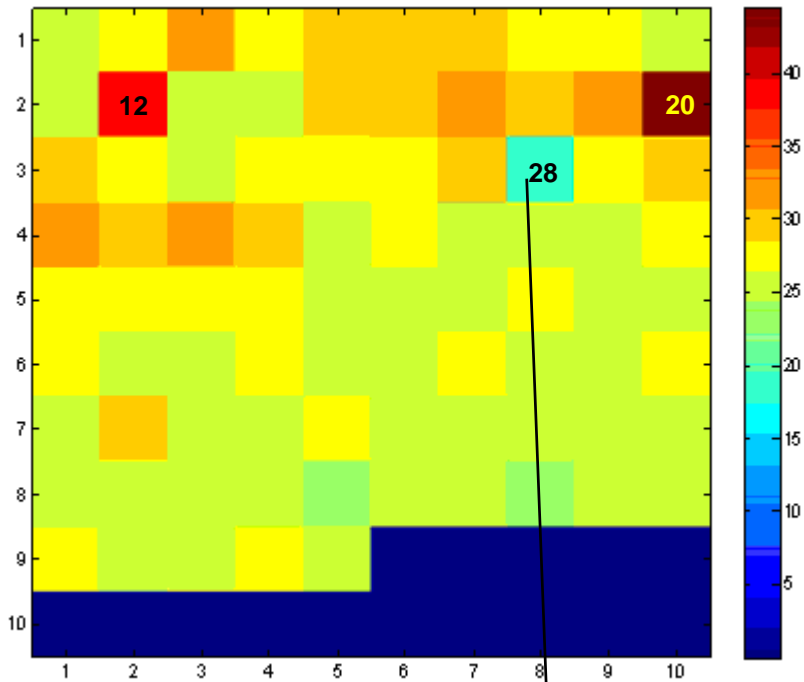
Conclusion: catechin & epicatechin stable under assay conditions.

Catechin bioconversion screen

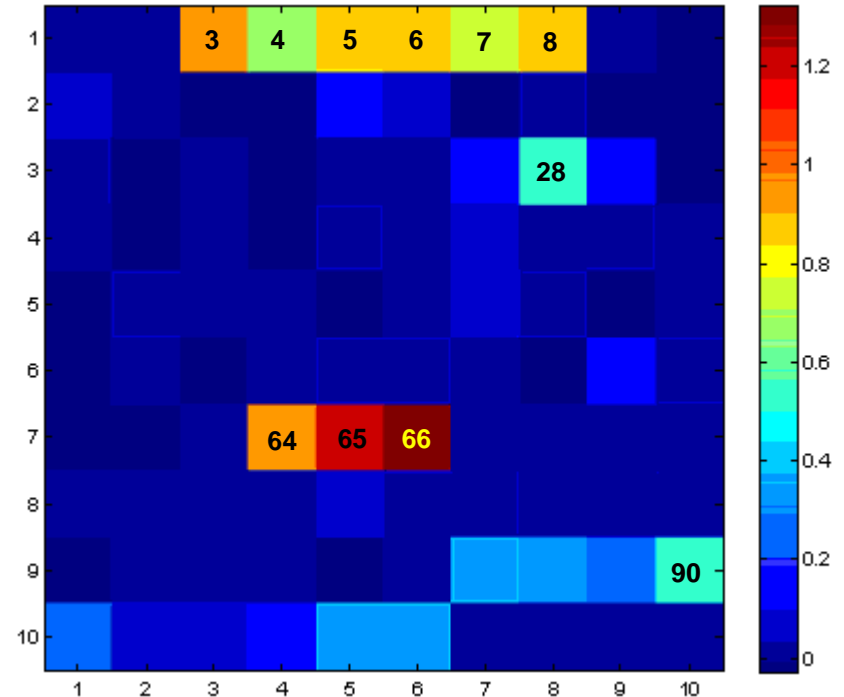


Catechin and formate signals in the supernatants of the pooled faecal metagenomic library clone(s) after incubation.

Catechin bioconversion



Formate (metabolic act.)



pool #28 shows growth & catechin degradation'



- Proof of principle shown
- Polyphenols show variable stability
- Anaerobic conditions required
- One clone pool identified that displays catechin breakdown.



A decorative graphic on the left side of the slide. It features a large teal letter 'M' at the bottom, a dark blue letter 'A' above it, and a white letter 'B' at the top. The letters are surrounded by various blue and teal shapes, including circles and abstract forms, creating a layered, artistic effect.

Thank you