Influence of bioactive compounds of milk on intestinal cell cultures

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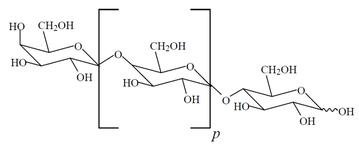
Introduction

- Milk- and milk dairy products are part of our daily life
- Milk- and galacto-oligosachharides **(MOS/GOS)** are natural compounds in mammalian milks
- Bio-functional properties
  - Prebiotic
  - Immune modulating
  - Bifidogenic
  - Anti-inflammatory

➡ Mechanism behind the effects is still unknown



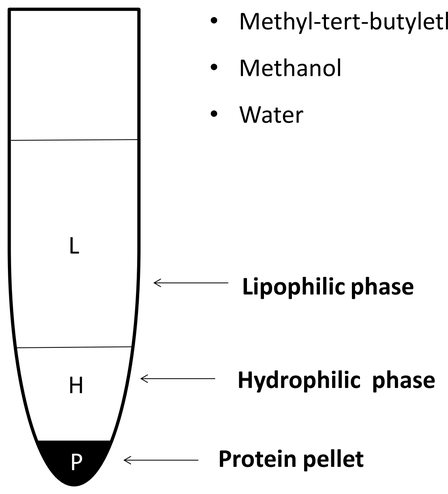
Basic structure galacto-oligosachharides (GOS)

Aim of the project

The aim of this study is to analyze the effects of MOS/GOS of different origins (cow/ goat/ *K.lactis*) and in this context of different concentrations on the lipidome and metabolome of tumorous intestinal cells.
In addition, specific metabolic patterns and corresponding pathways should be identified.

Extraction

- Methyl-tert-butylether
- Methanol
- Water



Disposition of the phases in the SIMPLEX approach

Measurements

- Method hydrophilic small molecules (75-2000 Da)
- Method lipophilic molecules (150-3000 Da)

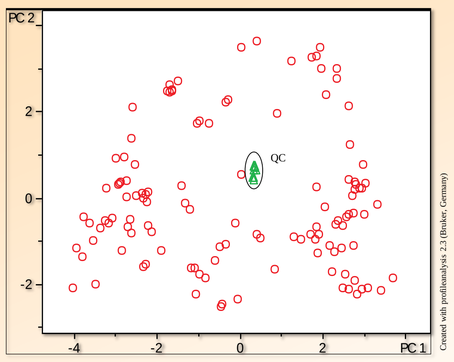
Solarix FTICR-MS; 7T (Bruker , Germany)

Methods

- **Cell cultivation**
  Treatment with MOS/GOS for 24h
- **Cell harvesting**
  Acidified water and physical force
- **Cell disruption**
  Ultrasonic on ice
- **Extraction** (liquid-liquid)
  SIMPLEX-approach
- **Measurements**
  FTICR-MS ESI (flow injection) pos./neg.
- **Data analysis**
  Targeted / non targeted

Results

- **Stability of measurements**



PCA model of the quality control (QC), method hydrophilic metabolites pos.

The quality control approach

- Based on Demetrowitsch et al. (2015)
- The QC was measured each 20 samples
- Information about
  - Repeatability
  - Stability
  - Precision

The principal component analysis (PCA)

- Reduction of data
- Identification of clusters
- Identification of characteristic metabolites

Results

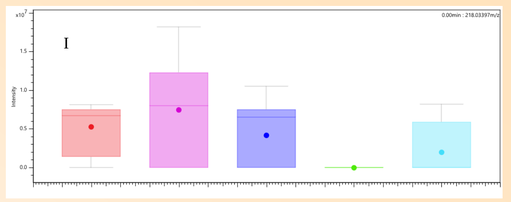
- **The PCA model B**

Hydrophilic compounds negative

The PCA model shows a tight cluster only for the treatment 2

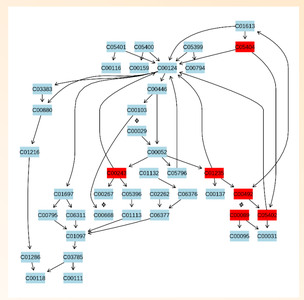
32 of 7122 metabolites were identified to be characteristic for the arrangement in the model B

The boxplot shows an example that was detected in the neg. mode with a regulatory effect by treatment 2



Boxplot of the metabolite with the mass 218,03397 predicted formula C8H11O5P
Possible inhibiting effect of GOS (not identified yet)

Pathway analysis



Pathway of galactose metabolism
All identified metabolites were alignt with MetaboAnalyst 3.0
It was possible to detect six (red) degradation products of galactose

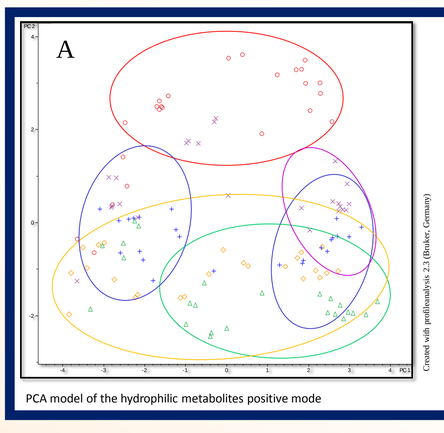
- **The PCA model A**

Hydrophilic compounds positive

The PCA model shows clusters for each treatment, all replicates of cultivation and extraction are close together

Based on the loading plots (not shown) of the statistical model it was possible to identify 23 of 3650 metabolites that are characteristic for the clustering

The following boxplots show some examples for characteristic metabolites



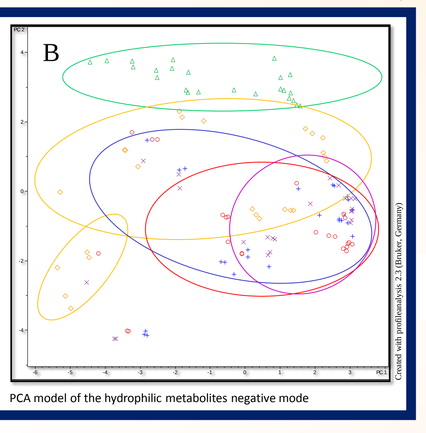
PCA model of the hydrophilic metabolites positive mode

Key of treatments PCA

- 1 GOS/MOS 12% origin cow
- 2 GOS 96.1% origin *K. lactis*
- 3 GOS/MOS 3,3% origin goat
- 4 Control sucrose
- 5 Control medium only

Replicates
8 for cultivation
3 for extraction

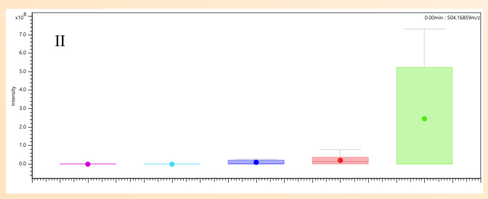
Conditions PCA model
Threshold 2.000.000
Occurrence in 2 of 3 replicates of extraction



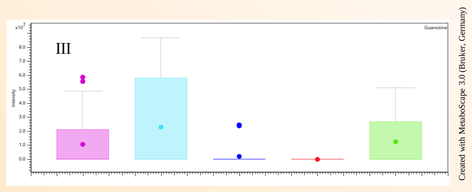
PCA model of the hydrophilic metabolites negative mode

Key of treatments

- 1 GOS/MOS 12% origin cow
- 2 GOS 96.1% origin *K. lactis*
- 3 GOS/MOS 3,3% origin goat
- 4 Control sucrose
- 5 Control medium only



Boxplot of the metabolite with the mass 504.16859, predicted formula $C_{18}H_{32}O_{16}$
Decreasing intensity with decreasing GOS amount in the treatment 2>1>3
Predicted as galactosyllactose a typical milk oligosaccharide

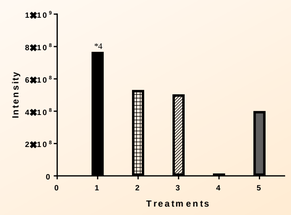


Boxplot of the metabolite with the mass 283.09167 identified as guanosine
Increased intensity with decreasing MOS amount

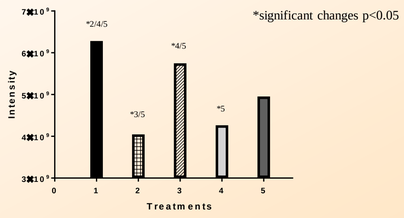
- **Lipophilic compounds**

Key of treatments

- 1 GOS/MOS 12% origin cow
- 2 GOS 96.1% origin *K. lactis*
- 3 GOS/MOS 3,3% origin goat
- 4 Control sucrose
- 5 Control medium only



Influence of the different treatments on PI (42:3) m/z 934.46372



Influence of the different treatments on PI (42:3) m/z 934.46372

It was possible to detect significant changes for six identified lipophilic metabolites
The graphs show the effects of the treatments for PI 40:3, PI 42:3
(PG 28:6, PG 12:5, PE-O 32:8 and PA 39:9 not shown)

Graphs created and calculated with Prism7.0

Results

Discussion

Hydrophilic compounds

A high intensity of a component in the treatment 2 suggests an effect of a high concentration of GOS (I)
A decreasing intensity of a component from treatment 2>1>3 suggests an effect of GOS (II)
An increasing intensity of a metabolite from treatment 1>3 suggest an effect of MOS (III)

Lipophilic compounds

It is known that phosphoinositoles (PI) have an anti-inflammatory effect by inhibiting the response of T-cells (van Dien et al. 2011)
As shown for PI (40:3) GOS/MOS could have an stimulating effect on PIs

Outlook

Repetition of the experiment

Treatment 1 and 3 are based on so-called nanofiltrated retentates so that an effect could also result from non-distant substances

The experiment will be repeated with pure substances as in the case of treatment 2

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References:

Federal Ministry of Education and Research
Inflammation at Interfaces
FoCus
MRI Max Rubner-Institut