«*In vitro* fermentation of *Pleurotus ostreatus* and *Ganoderma lucidum* by human gut microbiota: metabolomic analysis of the products»

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INTRODUCTION

Gut microbiota is the most complex microbial community in the body, associated with various health-promoting activities. β-(1→3,1→6)-D-glucans, abundant in the cell wall of fungi, are non-starch polysaccharides with differences in their chemical structure and bioactivity. Dietary interventions for modulation of gut microbiota by fungi rich in β-glucans, have not been widely conducted; nevertheless, increasing experimental evidence supports their potential prebiotic action and their beneficial health effects, anti-cancer and immuno-modulating effects included. The aim of this project is to investigate the impact of edible Basidiomycetes, of high β-glucan content, on the gut microbiota using an *in vitro* batch culture fermentation system inoculated with fecal samples from healthy volunteers. Hence, two types of edible Basidiomycetes, *Pleurotus ostreatus* and *Ganoderma lucidum,* are being tested.

MATERIALS AND METHODS

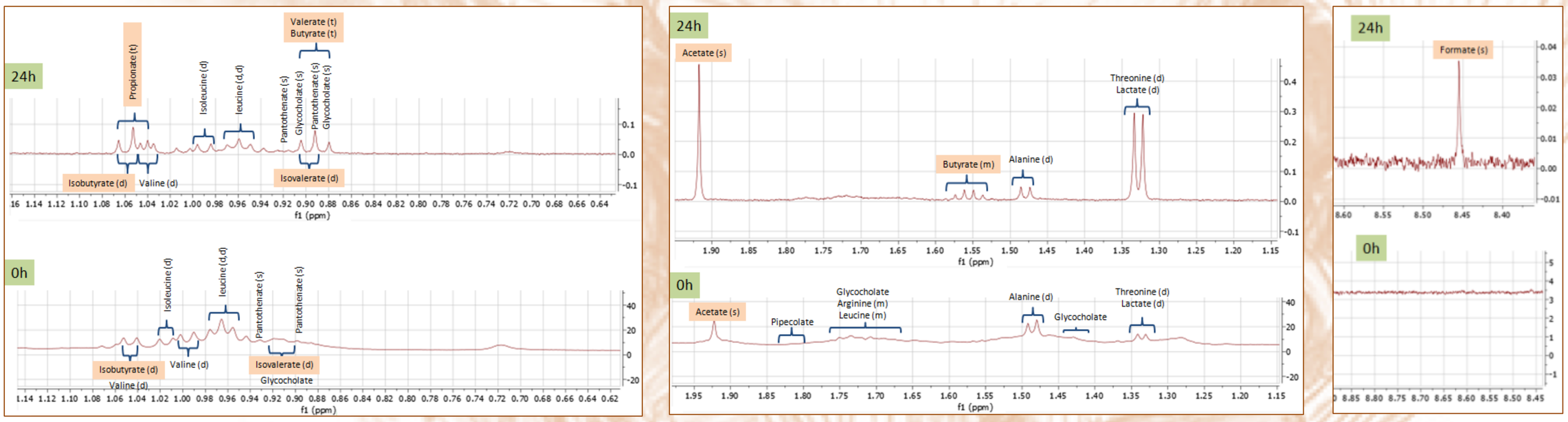
The global metabolic profiling of fermented products is assessed by the use of ^{1}H NMR spectroscopy, following *in vitro* fermentation of the whole fungus as well as β-glucan enriched extracts by fecal slurry of healthy volunteers. Lyophilized fungal substrates and inulin, an established prebiotic, at appropriate concentrations, are *in vitro* fermented for 24 hours. *In vitro* fermentation without any additional carbon source is in parallel carried out as reference.

In vitro fermentation: basal medium + fecal slurry of healthy volunteers + carbon source → Sampling at 0h and 24h → Lyophilization of samples → Mixture with: -540μL of acid phosphate buffered (400 mM pH 7.2) in D_2O -60μL TSP 5 mM → Insertion of final solution in NMR tubes, 5 mm in diameter → Receiving ^{1}H spectra in a Varian 600MHz spectrometer (599.9 MHz resonance frequency) → Apply the NOESY-presat 1D pulse sequence and quench the resonance peak of H20 → Usage Mnova program (Mestrelab Research) : -phase and base-line correction of ^{1}H NMR spectra -normalization based on the TSP resonance peak at 0 ppm → Usage of Chenomx NMR Suite 6.0 software and literature data to identification of spectrum resonance peaks → Placement of the identified metabolites on the spectra peaks and evaluation of the presence of single / double / triple / multiple peaks for each metabolite

RESULTS

Preliminary analysis of the *in vitro* fermentation products when inulin is used as the carbon source, revealed variations in the metabolic profile of fermented products as follows:

- Production of acid metabolites – Short Chain Fatty Acids (SCFAs) 24 hours post fermentation (propionate, butyrate, isobutyrate, valerate, isovalerate, formate, acetate).
- Significant variations in other metabolites such as amino acids, sugars, vitamins, phenolic compounds.



Indicative spectra, where differences in metabolites within 24 hours are evident (semi-quantitative assessment).

DISCUSSION

The next step is to calculate the longitudinal quench time of the nuclei by the T1 inversion recovery method and the results will show the appropriate application of the quench time to take the ^{1}H spectra with the NOESY-presat sequence to allow further quantification of the results. The same analysis will be applied for the fermentation products of *Pleurotus ostreatus* and *Ganoderma lucidum*. A comparative survey between all the above substrates, using chemometrics in combination with 2D NMR spectroscopy will be further applied, in order to identify biomarkers associated with the health promoting effects and the biological activities of *Pleurotus ostreatus* and *Ganoderma lucidum.*

REFERENCES: Anthony C. Dona, Michael Kyriakides, Flora Scott, Elizabeth A. Shephard, Dorsa Varshavi, Kirill Veselkov, Jeremy R. Everett (2016). A guide to the identification of metabolites in NMR-based metabonomics / metabolomics experiments. *Computational and Structural Biotechnology Journal 14 (2016) 135–153*

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