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Modulatory effects of calcium-alginate encapsulated *Akkermansia muciniphila* in healthy and pathogen-infected faecal models

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Introduction

Gut microbiota are the collection of bacteria, archaea and eukarya that colonise the gastrointestinal tract of animals and humans [1].

Prebiotics, **probiotics** and postbiotics have a positive effect on improving intestinal homeostasis by **modulating both the microbiota profile and metabolic functions**. This modulation may lead to beneficial changes in the production of specific **organic acids** and the consumption of **extracellular ammonia**. [2]. To study the effects of macro- or micronutrients, bioactive compounds and/or pre- or probiotics on the gut microbiota, in vitro digestion and **colonic fermentation models** have been developed. These models are a powerful tool to determine the effect of prebiotics or probiotics on the **microbiota profile** and/or the effect of these on **metabolite production** [4].

Objectives

The main goal of the study was to evaluate the influence of a pre-digested **encapsulated** next-generation probiotic, ***Akkermansia muciniphila***, on gut microbiota during **dysbiosis**, caused by the oro-gastrointestinal pathogen *Escherichia coli* O157:H8. The specific aim is to determine whether the encapsulated probiotic has a therapeutic effect or primarily acts as a preventive measure. The analysis is focused on ammonia and short chain fatty acids (SCFAs) to understand the effect of the encapsulated ***A. muciniphila*** on **gut microbiota metabolism.** Furthermore, this work focused on **evaluating the modulation of the gut microbiota** in terms of **microbial diversity**.

Methods

A. muciniphila loaded capsules production

Encapsulation procedure via extrusion

Strain: *Akkermansia muciniphila* DSM 22959

Matrix: Sodium alginate and Ca^{2+}

Exposure to simulated gastrointestinal tract conditions

1. Exposure to gastric and intestinal conditions*
2. Lyophilization of the digestion products
3. Dialysis of the lyophilized digestion products

*Protocol according to INFOGEST assay (2019) [5]

Faecal Fermentation

Experiment included four conditions: (A) control;
(B) infection with *E. coli* O157:H7;
(C) addition of encapsulated *A. muciniphila*;
(D) treatment (infection with *E. coli* O157:H7 and addition of encapsulated *A. muciniphila*).

Protocol according to Carvalho et al. (2022) [6]

Total Ammonia Nitrogen and SCFAs determination

Total Ammonia Nitrogen Determination:
Ammonium concentration was measured using a 9663 ion-selective electrode, at constant temperature (room temperature, 20 °C) and pH (6.7-6.9).

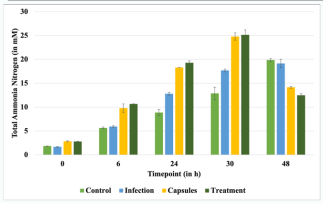
SCFAs determination:
An Agilent 1260 II series HPLC instrument with a refractive index detector and a diode array detector at 220 nm and an Aminex HPX-87H ion-exclusion column operated at 50 °C was used to monitor SCFA produced.

DNA extraction and samples sequencing

DNA extraction was prepared according to the instructions of the NZY Tissue gDNA Isolation kit
Samples were prepared according to Novogene's Instructions and sent for sequencing.
The DNA samples (25 µL) underwent Illumina metagenomic sequencing analysis.
Quality control was performed at every step for better accuracy and reliability of sequencing data
Analysis was performed afterwards for the abundance in at the genus and species levels, to compare the richness and uniformity information of the samples

Main Findings

1) Determination of Total Ammonia Nitrogen throughout 48-hour faecal fermentation



- ✓ Steady increase in total ammonia nitrogen until the 30-hour mark across all tested conditions
- ✓ After 48 hours, the two conditions, which included encapsulated *A. muciniphila*, showed a **significant decrease** of approximately 50% in total ammonia nitrogen.
- ✓ Nitrogen was possibly **assimilated** by the metabolic pathways of *A. muciniphila* or by other species stimulated by *A. muciniphila* loaded capsules.

Figure 1. Evolution of Total Ammonia Nitrogen (in mM) throughout the 48-hour faecal fermentation process in control (■); infection with *E. coli* O157:H7 (■); addition of encapsulated *A. muciniphila* (■); and infection with *E. coli* O157:H7 and addition of encapsulated *A. muciniphila* (■) treatments.

2) Production of SCFAs throughout 48-hour faecal fermentation

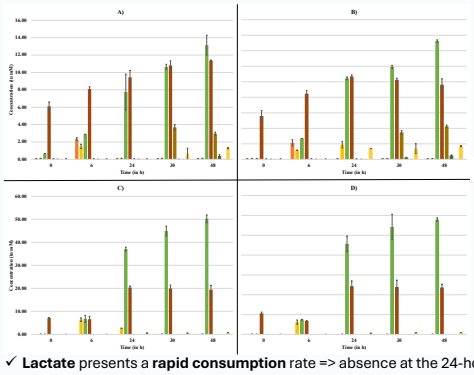


Fig. 2 Production of short chain fatty acids (SCFA) throughout faecal fermentation process of 48 h for (A) control; (B) infection with *E. coli* O157:H7; (C) addition of encapsulated *A. muciniphila*; and (D) treatment (infection with *E. coli* O157:H7 and addition of encapsulated *A. muciniphila*) conditions.

- ✓ **Lactate** presents a **rapid consumption** rate => absence at the 24-hour timepoint in all four conditions.
  - ✓ The **absence** of **lactate** in conditions C) and D) at all timepoints indicate a **beneficial shift** of the microbiota profile, with lactate being immediately consumed .
- ✓ **The production** of **acetate** and **propionate** is evident after 6 h , with its concentration increasing over the 48h fermentation process in all conditions.
- ✓ **Isobutyrate** and **butyrate** are notably **absent** after 24 h for conditions C) and D) involving encapsulated *A. muciniphila*. This indicates a **shift on microbiota** metabolism after *A. muciniphila* loaded capsules addition.

3) Modulation of gut microbiota throughout 48-hour faecal fermentation

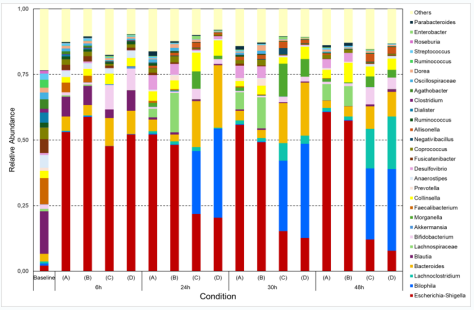


Fig. 3 - Microbial diversity by genus throughout the 48-hour faecal fermentation process.

- ✓ Observed **decline in *Faecalibacterium*** in conjunction with an **increase in *Escherichia-Shigella*** relative abundance serves to illustrate the **genus' sensitivity to modulation processes**
- ✓ **Increased presence** of three **butyrate-producing genera** such as *Bacteroidetes*, *Ruminococcus* and *Coprococcus* in conditions (C) and (D).
  - ✓ The **addition of encapsulated *Akkermansia*** stimulates the **growth of bifidobacteria** (the bifidogenic effect) and could promote the **presence of butyrate-producing colonic bacteria** (the butyrogenic effect) in the human colon during long-term interventions
- ✓ Administration of *A. muciniphila*-loaded capsules was associated with a **reduction in the prevalence of the pathogenic *E. coli* strain**, as evidenced by a decline in *Escherichia-Shigella* abundance in conditions (C) and (D)
- ✓ Overall, there was **greater diversity and a wider distribution of relative abundances** in conditions containing *A. muciniphila*, reversing the effect of the addition of *E. coli* O157:H7 over time.

References

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Conclusions

The incorporation of *A. muciniphila*, encapsulated in a calcium-alginate matrix, led to the **modulation** of both **healthy** and ***E. coli* O157:H7-infected microbiota**. This modulation resulted in an **increase in the microbial diversity** of the gut microbiota, a **decrease in extracellular ammonia**, and an **increase in the production of SCFA**, particularly acetate and propionate. In relation to total nitrogen ammonia, the results indicate that *A. muciniphila* takes in **ammonia intracellularly**, leading to a decrease in levels after 48 hours of faecal fermentation. The supplementation with calcium-alginate capsules containing *A. muciniphila* resulted in the modulation of microbiota diversity and metabolism, leading to **increased levels of propionate and acetate**, while **butyrate was absent**. This study found an **increase in beneficial microbial genera**, including *Morganella*, *Bifidobacterium*, and *Bacteroidetes*, indicating the **restoration of gut microbiota homeostasis** after a state of dysbiosis caused by infection with *E. coli* O157:H7.

Acknowledgements

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