Selection of Next-Generation probiotics from the gut microbiome of subjects with different dietary patterns

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The first activities of the PhD project are described. The aim of the project is to isolate and characterize novel microbial strains from the gut microbiome, for a potential use as Next Generation Probiotics (NGPs). Firstly, nine different culture media and enrichment procedures were tested for the isolation of microbial strains from the human gut, analyzing microbial cells collected from agar plates by 16S rRNA high-throughput amplicon sequencing. Secondly, the four best media were used to isolate microbial strains from the gut microbiome of vegans/vegetarians subjects.

**Selezione di probiotici di nuova generazione dal microbioma umano di individui con abitudini alimentari diverse**

Le prime attività del progetto di dottorato vengono di seguito descritte. Lo scopo del progetto è isolare e caratterizzare nuovi ceppi microbici dal microbioma intestinale, per un potenziale utilizzo come *Next Generation Probiotics* (NGPs). In primo luogo, sono stati testati nove diversi terreni di coltura e procedure di arricchimento per l’isolamento di ceppi microbici dall’intestino umano, analizzando le cellule microbiche raccolte direttamente dalle piastre mediante sequenziamento ad alto rendimento di ampliconi del gene codificante per l’rRNA 16S. In secondo luogo, sono stati utilizzati i quattro migliori terreni per isolare i ceppi microbici dal microbioma intestinale di soggetti vegani/vegetariani.

**Key words**: Gut microbiome, Next Generation Probiotics, probiotics, short-chain fatty acids, fibre fermentation

# **1. Introduction**

In accordance with the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

(A1) selection of suitable media that support the growth of the highest number of putative NGP species; to select strict anaerobes, we tested 9 different culture media and a pre-enrichment step of fecal samples in different broths for 48 h in anaerobic conditions;

(A2) use of the 4 best-performing media for the isolation of microbial strains from 9 vegetarian/vegan donors. Four-hundred twelve microbial isolates were screened and identified by 16S rRNA sequencing.

# **2. Materials and Methods**

We tested nine culture media with different formulations in terms of polysaccharidic source, vitamins, minerals and fatty acids to study the culturable fraction of the gut microbiome. In order to define the best-performing media (in terms of the highest number of putative NGP species growing on them), we collected bulk microbial colonies from agar plates of the different media and sequenced them by 16S rRNA high-throughput amplicon sequencing. The 4 best media were selected for further strain isolation from 9 vegetarian/vegan donors. Colonies were purified using repetitive streaking and the same colony was incubated both in aerobic and anaerobic conditions at 37°C to discard facultative anaerobes. Strains grown only in anaerobic condition were identified by 16S rRNA sequencing. To select strict anaerobes, we also tested a pre-enrichment step of fecal samples in different broths for 48 h in anaerobic conditions. Strains belonging to putative NGP species (as reported in literature, De Filippis et al., 2022) are characterized for the production of beneficial metabolites (e.g., short-chain fatty acids from fibre fermentation, urolithins from ellagic acid, equol from daidzein; Edwards et al., 2017). In addition, we are testing their ability to grow on different carbohydrate sources (e.g., pectin, cellulose, hemicellulose, resistant starch). Short-chain fatty acid will be detected in the growth supernatant using Gas Chromatography coupled to Mass Spectrometry, while urolithins and equol will be detected in supernatant using HPLC analysis.

Promising strains will be finally tested in SHIME (Simulator of Human Intestinal Microbial Ecosystem) to evaluate their effect on health and the ability to modulate the gut microbiome.

# **3. Results and Discussion**

## **3.1 Selection of suitable culture media**

We compared taxonomic composition of the donor fecal sample with that of bulk colonies collected from two plates (10-6 and 10-7 dilutions) of the 9 media tested, in order to define which media were able to support the growth of the highest diversity of taxa and to reproduce more reliably the microbiota composition of the fecal sample. Example of microbiota composition from one donor is reported in Figure 1. Results obtained allowed us to select four media that supported the growth of the highest number of putative NGP species, named: PYG; YCFAGSC; Rumen Bacteria medium, *Ruminococcus albus* medium; Chopped meat (Figure 1).



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**Figure 1** *Bar chart reporting microbiota composition of one donor and of bulk cells collected from two plates of 9 different media tested. The four media selected are circled. Ak = Akkermansia; R = Ruminococcus; F = Faecalibacterium; B = Bacteroides; Col = Collinsella; Cop = Coprococcus; D = Dorea; L = Lachnospiraceae*

## **3.2 NGP isolation from vegan/vegetarian’s gut microbiome**

We used the four best performing media (indicated with \* in Figure 1) for strain isolation from the fecal samples of 9 vegan/vegetarian donors. We isolated 412 colonies in total, that were purified and screened for growth in aerobic or anaerobic condition. Facultative anaerobes were discarded. After 16S rRNA sequencing and identification, we obtained 42 interesting isolates including the promising NGP candidates *Bacteroides uniformis* and *Bacteroides thetaiotaomicron*, *Collinsella aerofaciens* and *Dorea longicatena* (Table 1). Testing of these strains for the production of beneficial metabolites is on-going.

***Table 1*** *Putative NGP species identified and culture media where colonies were isolated from. t0, colony isolated from fresh fecal sample; t48, colony isolated from sample enriched in anaerobiosis for 48 hours.*

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| **Isolation media** | **Taxonomic identification** |
| CHOPPED MEAT t48; PYG + cellobiose + starch t0; RUMEN BACTERIA t48; YCFAGSC t48 | *Bacteroides uniformis* |
| RUMEN BACTERIA t48 | *Bacteroides thetaiotaomicron* |
| YCFAGSC t48 | *Bacteroides salyersiae* |
| PYG + HORSE SERUM t0 | *Bacteroides stercoris* |
| R. ALBUS t48; CHOPPED MEAT t48; YCFAGSC t48 | *Parabacteroides distasonis* |
| YCFAGSC t48; YCFAGSC t0; R. ALBUS t0; PYG + HORSE SERUM t48; R. ALBUS t0; R. ALBUS t48 | *Faecalicatena contorta* |
| PYG + HORSE SERUM t0; PYG + HORSE SERUM t48; YCFAGSC t0 | *Collinsella aerofaciens* |
| PYG + cellobiose + starch t0 | *Dorea longicatena* |
| YCFAGSC t0 | *Pseudoruminococcus massiliensis* |

# **4. References**

De Filippis F, Esposito A, Ercolini D (2022) Outlook on next‐generation probiotics from the human gut. *Cellular and Molecular Life Sciences***79**: 76.

Edwards CA, Havlik J, Cong W, Mullen W, Preston T, Morrison DJ, Combet E (2017) Polyphenols and health: Interactions between fibre, plant polyphenols and the gut microbiota. *Nutr Bull* **42**: 356-360.