Phenotypic and genotypic diversity among potential next-generation probiotics

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The modulation of the gut microbiota is emerging as a promising target for the management or prevention of many diseases, especially by using traditional probiotics such as Lactobacilli and Bifidobacteria. This PhD thesis research project aims to isolate and investigate selected microbes and, by an in deep characterization, to discover potential next-generation probiotics (NGPs) that can exert health benefits and satisfied the World Health Organization (WHO) guidelines in terms of safety, functionality, and technological usability.

Diversità fenotipica e genotipica tra potenziali probiotici di nuova generazione

La modulazione del microbiota intestinale per la gestione e la prevenzione di molte patologie è un argomento di ricerca molto attuale. In questo contesto vengono spesso utilizzati i probiotici definiti tradizionali, principalmente Lattobacilli e Bifidobatteri. Questo progetto di tesi di dottorato mira a isolare e caratterizzare in modo specifico e mirato ceppi di microorganismi probiotici di nuova generazione, selezionati e identificati per la loro potenziale capacità di apportare benefici per la salute umana e soddisfare i criteri di sicurezza, funzionalità ed uso tecnologico stabiliti dall’Organizzazione Mondiale della Sanità.

# 1. State-of-the-Art

During the last years, the human gut microbiota has been appreciated as a pivotal reservoir of microorganisms present predominantly in the colon - bacteria, archaea, viruses, fungi, and others - that can influence health and disease. The gut microbiota possesses different functions: digestion and metabolism of dietary elements into bioactive food components, vitamin synthesis, protection from pathogen colonization by adhering to the mucosal surface, production of antimicrobial substances, and stimulation of the immune system.

Dysbiosis is the loss of the balance among microbes, their function, distribution, or metabolic activity. This phenomenon has been linked to the development of inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), atopic asthma, allergy, obesity, type 2 diabetes (T2D), cardiovascular and neurodegenerative diseases, behavioral disorders, autoimmunity, and cancer (Durack and Lynch, 2019).

Strategies to rebalance these harmful conditions are urgently needed, such as probiotic administration, largely used nowadays. Probiotics are live microorganisms that confer a health benefit when consumed in adequate amounts, as reported by the World Health Organization (WHO) in 2002 (Hill *et al*, 2014). However, the administration of traditional probiotics is not always sufficient in specific conditions.

*In silico* analyses has recently increased the knowledge on microorganisms with potential health benefit to develop probiotics addressing specific consumer needs and issues. These microorganisms are referred to as Next-Generation Probiotics (NGPs) and are considered as a health promoting strategy to re-establish an eubiosis condition (Langella *et al,* 2019). Contrary to traditional probiotics, they do not have a long history of use and their safety is thus not considered as proven. These NGPs are classified as novel foods, increasing the number of requirements to reach their commercialization as food ingredients. In addition, most NGPs are currently not commercially available, and more studies are needed to address their safety, efficacy, and technological robustness.

Candidates for NGPs are *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Bacteroides fragilis*, *Clostridium butyricum*, *Prevotella copri*, *Parabacteroides glodsteinii*, *Christensenella minuta*. Among these potential NGPs, *A. muciniphila* is one of the most promising microorganisms with probiotic properties (Derrien *et al*, 2004). *A. muciniphila* is a commensal bacterium that colonizes the intestinal mucosal layer, the only member of the phylum Verrucomicrobia present in the gut of healthy individuals. It is a Gram-negative, non-motile anaerobic microorganism that tolerates low oxygen levels, and produces no endospores. It degrades mucin and is also capable to induce its production by increasing the number and density of goblet cells. Its main positive functions for the host are the ability to strengthen the gut barrier, modulate insulin resistance, protect from metabolic inflammation, and exert anti-inflammatory effects (Si *et al*, 2022). Low levels of *A. muciniphila* has been associated with several diseases in both mouse models and in humans, such as IBD, UC, and Crohn’s disease. However, there are many controversial aspects that need to be clarified. For example, multiple sclerosis and Parkinson’s disease patients exhibited a higher abundance of *A. muciniphila* with respect to controls. The pasteurized *A. muciniphila* MucT has proven to be more efficient than the live microorganism (Cani *et al*, 2022) and at the moment the European Food Safety Authority (EFSA) has approved only pasteurized *A. muciniphila* ATCC BAA-835T strain as a novel food (<https://www.efsa.europa.eu/en/efsajournal/pub/6780>).

# 2. PhD Thesis Objectives and Milestones

The aim of this project is to isolate and characterize new potential NGPs investigating their crosstalk with the host, the cross-feeding processes among them, their function and mechanisms of action, their properties against intestinal pathogen colonization, their effectors responsible of the beneficial effects, also using omics and bioinformatic approaches. The results obtained with this research will deepen the knowledge about the molecular mechanisms of action of probiotics to achieve personalized treatments for the general population and for specific categories of patients.

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities (work packages, WPs) according to the Gantt diagram given in Table 1:

WP1) **Isolation of bacterial strains from fecal human samples**

Starting from stool samples from healthy donors (self-collected in sterile screw-cap specimen cups, transported refrigerated to the laboratory and processed in the same day), microorganisms of interest will be isolated (according to Filardi *et al*, 2022)

WP2) **Taxonomy identification of the isolates and genotypic characterization**

The isolates will be identified unambiguously at genus and species level by 16S rRNA gene sequencing analysis and whole genome sequencing.

WP3) **Phenotypic characterization of the new strains**

This WP3 will include different tasks (T). Indeed, the new potential NGPs will be evaluated for the macroscopic and microscopic morphological properties (T1), the tolerance to gastrointestinal conditions (T2), the ability to assimilate and/or ferment different carbon and nitrogen sources (T3), the capacity to hydrolyze compounds such as starch, lipids or proteins (T4), the production of extracellular compounds (T5), and the antibiotic susceptibility (T6).

WP4) ***In vitro* and/or *ex vivo* efficacy evaluation of the new strains**

This WP will include different tasks. Indeed, the most suitable *in vitro* and/or *ex vivo* 2D and 3D models will be used to determine the efficacy of the new potential NGPs in pathogen inhibition (T1), adhesion to eukaryotic cells (e.g., Caco-2) (T2), effect on intestinal permeability and tight junction integrity (T3), probiotic metabolite effect on different kind of cells (intestinal, endothelial and neuronal cells, adipocytes, or others) (T4).

**Table 1.***Gantt diagram for this PhD thesis project.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | *1st semester*  | *2nd semester* | *3rd semester* | *4th semester* | *5th semester* | *6th semester* |
| *WP1 Isolation of bacterial strains from human fecal samples* |  |  |  |  |  |  |
| *WP2 Taxonomy identification of the isolates and genotypic characterization* |  |  |  |  |  |  |
| *WP3 Phenotypic characterization of the new strains*  |  |  |  |  |  |  |
| *WP4 In vitro and/or ex vivo efficacy evaluation of the new strains* |  |  |  |  |  |  |
| *Manuscript(s) and PhD thesis preparation* |  |  |  |  |  |  |

# 3. Selected References

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