UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.10

Proponent: Prof. Patrizia Di Gennaro

Project Title: Design and characterization of an *in vitro* reconstructed human gut microbiota to model microbe-microbe and host-microbe reciprocal interactions

Scientific background and 'open issues'

It is known that human health can be influenced by the presence of different bacteria, known as microbiota, which inhabit our body districts. Among all compartments, the intestinal one is the most densely populated by a highly diversified microbial community represented by more than 1,000 prevalent species (Elzinga et al. 2019). The outstanding properties of these bacteria are functionality, redundancy, and resilience (Swanson et al. 2020) having a key role in the complex interplay between microorganisms and the host. However, the overall effects depend on the mechanisms underlying the networking between bacteria. Thus, to simplify and try to establish a cause-and-effect connection, the search for bacteria representative in a reliable way the gut microbiota and new *in vitro* systems mimicking the intestinal compartment have raised attention. The intrinsic challenge is the cultivation of the most representative human gut microbiota under regulated environmental conditions and the study of the complex interspecies interactions of the microbial community established within it. Currently, *in vitro* models permit the exploration of the influence of different factors such as dietary compounds, microbial pathogens, and bioactive molecules in a controlled manner, helping in the identification of keystone species, redundant functions, beneficial molecules, and conditions that contribute to the stability of the community. (Nissen et al. 2020; Gutierrez et al. 2019; Arnold et al. 2016). Various strategies have been introduced to modulate the composition and function of the microbiota, such as the use of different carbon sources and the administration of beneficial probiotic strains (Swanson et al. 2020).

Indeed, it is widely known that diet-driven interspecies relationships within the intestinal tract can have a crucial impact on host health. Dietary fibres that reach the colon undigested are converted to beneficial metabolites like short-chain fatty acids (SCFAs) (Shetty et al., 2022). Additionally, some bacteria, including probiotics, are capable of producing antimicrobial molecules, among which bacteriocins are of particular interest for their several applications as novel therapeutic agents (Peng et al., 2023; Martin et al., 2023). However, the intricate interplay between the microbial community of the gut microbiota and complexity of this micro-environment is not yet completely understood.

Objectives

The project aims to define a reconstructed human gut microbiota *in vitro* to model microbe-microbe and host-microbiota reciprocal interactions in presence of different carbon sources

and probiotic bacteria to better understand the underlying complex network, including the possible effects on the host.
To achieve this goal, a model of human gut microbiota will be reconstructed *in vitro* by assembling representative species of the intestinal bacterial community and implemented with probiotic strains previously characterized by the proponent research group. This *in vitro* model will be employed to study the defined gut microbiota modulation in presence of different carbon sources and to evaluate the microbial reciprocal interactions and the production of selected secondary metabolites (principally SCFAs and antimicrobial compounds).
The experimental conditions that will most improve the composition of the defined gut microbiota, favoring the beneficial microorganisms, will be used to test the response of the host, employing *in vitro* models of intestinal cell lines.

Methodologies
The present Project will be developed through the following project's design:
1. **Design of a new reconstructed human gut microbiota *in vitro***: bacterial species from human gut community will be selected and assembled *in vitro* to reconstruct a model representative of the human gut microbiota. This model will be tested in a batch fermentation system in presence of different carbon sources and different probiotic bacteria characterized by the proponent research group (Presti et al. 2015).
2. **Analysis of microbe-microbe interactions in the *in vitro* gut microbiota**: the microbe-microbe interactions will be determined by the growth of the cultures and the analysis of the microbial metabolites at different time points. Abundance modulation of the strains included in the *in vitro* model will be evaluated through qPCR assays and using next-generation sequencing (Illumina technology). The metabolic network that is established between the different bacteria of the model will be analyzed following the expression of genes of interest via qPCR assays. In addition, the production of bacterial molecules like antimicrobial peptides will be investigated through different antagonism assays.
3. **Effects on the host:** the effects of the bacterial secondary metabolites will be evaluated on human colorectal epithelial or immune system cells, using different *in vitro* approaches (monolayer cultures, or transwell models) thanks to the collaboration with industrial and university partners. The viability of the cells and the possible responses activated will be assessed through ELISA tests, qPCR analyses, and suitable methods.

Collaboration / Co-tutoring opportunities
1. Collaboration with Laboratory of Prof. Paola Fusi (UNIMIB-BTBS) who leads a biochemistry group working on human colon cell systems and adequate competencies to execute antioxidant analyses
2. Collaboration with Laboratory of Dr. Federica Facciotti (UNIMIB-BTBS) who leads an immunology group working on all the responses connected to the intestinal mucosal immunological environment
3. Collaboration with the Sequencing Laboratory of the DISAT Department for the bacterial community's analyses

4. Collaboration with Amita HC and Flanat Research and Roelmi HPC industrial partners specialized in health care, nutraceutical, and food. They have adequate competence in natural extracts preparation.
5. Collaboration with Prof. Tom Van de Wiele (Gent, Belgium, Center for Microbial Ecology and Technology at Ghent University) and Prof. Massimo Marzorati (Gent, Belgium, Center for Microbial Ecology and Technology at Gent University) to execute experiments on human gut microbiota thanks to the high technological model SHIIME.

Project's Sustainability & Mobility

Competences of the laboratory:

The laboratory proposing the project has strong expertise in the field of general microbiology. Prof. Di Gennaro has great expertise in the selection of new microbial strains and in their cultivation in both aerobiosis and anaerobic environments. In addition, the laboratory deals with molecular investigations carried out with *-omic* approaches (i.e., whole genome sequencing, microbial community sequencing, RNAseq) and more traditional techniques (PCR, qPCR). With the latest projects, the experience is also pushing towards the development of *in vitro* microbial communities and the evaluation of effects on human cell lines, thanks to the collaboration with university and industrial partnerships.

Pertinent Research Articles:

- De Giani A., Oldani M., Forcella M., Lasagni M., Fusi P., Di Gennaro P. Synergistic antioxidant effect of prebiotic Ginseng berries extract and probiotic strains on healthy and tumoral colorectal cell lines. Int J Mol Sci. (2022); 24(1): 373. doi: 10.3390/ijms24010373.
- De Giani A., Sandionigi A., Zampolli J., Michelotti A., Tursi F., Labra M., Di Gennaro P. Effects of inulin-based prebiotics alone or in combination with probiotics on human gut microbiota and markers of immune system: a randomized, double-blind, placebo-controlled study in healthy subjects. Microorganisms. (2022); 10(6): 1256. doi: 10.3390/microorganisms10061256
- Sandionigi A. & De Giani A., Tursi F., Michelotti A., Cestone E., Giardina S., Zampolli J., Di Gennaro P. Effectiveness of multistrain probiotic formulation on common infectious disease symptoms and gut microbiota modulation in flu-vaccinated healthy elderly subjects. Biomed Res Int. (2022); 2022:3860896. doi: 10.1155/2022/3860896
- De Giani A., Perillo F., Baeri A., Finazzi M., Facciotti F., Di Gennaro P. Positive modulation of a new reconstructed human gut microbiota by Maitake extract helpfully boosts the intestinal environment in vitro. Currently under revision on PlosOne (submission number PONE-D-23-23807 – *under revision*)

Putative Foreign Institutions for mobility:

Prof. Tom Van de Wiele (Gent, Belgium, Center for Microbial Ecology and Technology at Ghent University) and Prof. Massimo Marzorati (Gent, Belgium, Center for Microbial Ecology and Technology at Gent University)

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