PhD DISSERTATION PROJECTS

**Genomic characterization of Lactic Acid Bacteria strains for novel pro- and post-biotics development**

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This PhD thesis research project is aimed to exploit the autochthonous microbial communities of typical Italian fermented products to design new microbial consortia that have pro- and postbiotic properties. A plant-based psychobiotic yogurt will be developed to deliver neurotransmitter-producing strains and/or their psychobiotic metabolites into the gut and tested in a gut ecosystem simulator (SHIME), monitoring changes in the microbiome and metabolome. The expected results will open new avenues towards modulating the microbiome for mental health, linking health promotion with a sustainable food product.

Caratterizzazione genomica di ceppi di batteri lattici per lo sviluppo di nuovi pro e post-biotici

Questo progetto di tesi di dottorato ha lo scopo di sfruttare le comunità microbiche autoctone dei prodotti fermentati tipici italiani per progettare nuovi consorzi microbici che abbiano proprietà psicobiotiche. Uno yogurt psicobiotico a base vegetale sarà sviluppato per trasferire ceppi produttori di neurotrasmettitori all'intestino umano e testato in un simulatore dell'ecosistema intestinale (SHIME), monitorando i cambiamenti nel microbioma e nel metaboloma. I risultati attesi apriranno nuove strade verso la modulazione del microbioma per la salute mentale, collegando la promozione della salute all’utilizzo di un prodotto sostenibile.

# **1. State-of-the-Art**

Improving human health through modulation of microbial interactions during all phases of life is an evolving concept that is increasingly important for consumers, food manufacturers, health-care professionals and regulators. Postbiotics is a research area of great relevance within the field of functional foods. The term ‘postbiotics’ is increasingly found in the scientific literature, and it means a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” (Salminen *et al*., 2021). Recent studies suggest the viability of bacteria may not be necessary to achieve health-promoting effects with undoubted advantage to circumvent the problem of acquisition of antibiotic resistance genes and virulence factors. The use of postbiotics for human health is still at a preliminary stage. The growing volume of genomic information may facilitate systematic efforts to determine the metabolic pathways that may lead to obtain the desired postbiotic metabolites. Of notable interest in the field of postbiotics is the production of molecules with a beneficial effect on the nervous system. Lactic acid bacteria (LAB) have been reported to produce neuroactive molecules, capable of modulating mood and cognition in humans (psychobiotics). Fermented foods (FFs) may be considered as a still underexplored reservoir of microbial resources of beneficial microbes, that may positively affect human mental health, releasing molecules with the potential to modulate pathways of the microbiome–gut–brain axis. Indeed, it was recently suggested that a diet rich in fermented food may positively impact on stress (Berding *et al*., 2023). Therefore, In Table 1, the main neuroactive molecules produced by bacteria within the human gut are reported, including their precursors, and their regulatory functions (Casertano *et al*., 2022).

***Table 1***Representative list of neurotransmitters produced from bacteria within the human gut, precursors, and their regulatory functions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Neuroactive compound** | **Precursors** | **Genus** | **Regulatory function** |
| Gamma-aminobutyric acid (GABA) | Glutamate | *Lactobacillus, Bifidobacterium, Bacteroides* | Stress responsiveness, anxiety |
| Acetylcholine | Choline | *Lactobacillus, Bacillus* | Encoding of new memories |
| Dopamine | Tyrosine | *Lactococcus, Lactobacillus, Streptococcus* | Motivational decision-making |
| Serotonin  | Tryptophan | *Lactococcus, Lactobacillus, Streptococcus* | Modulation of intestinal secretion and motility; Brain development  |

Based on this background, the thesis project aims to exploit the autochthonous microbial communities of typical Italian fermented foods to design new microbial consortia capable of modulating the gut-brain axis through the direct production of neuroactive molecules during fermentation and/or through the modulation of intestinal microbiome activity in simulated digestion. A plant-based postbiotic yoghurt will be developed to deliver neurotransmitter-producing strains into the gut and tested in a gut ecosystem simulator, monitoring changes in the microbiome and metabolome. The expected results will open new avenues towards modulating the microbiome for mental health, linking health promotion with sustainability benefits.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Developing a collection of Lactic Acid Bacteria from typical Italian FFs:** LAB will be isolated and selected from traditional Italian FFs, including cheeses, sourdoughs used for the manufacturing of traditional breads and fermented olives (A1.1); Metagenome of FFs will be screened for the presence of microbial genes/pathways related to the production of neurotransmitters and neuroactive molecules (A1.2);

A2) **Developing three microbial consortia with postbiotic activities:** Strain testing for postbiotic and pro-technological activities in vitro. The production of neurotransmitters will be tested using broth containing monosodium glutamate, tyrosine, choline or tryptophan, precursors for GABA, catecholamine, acetylcholine and serotonin production. Supernatants will be analysed by LC-MS/MS. Strains will be also tested for pro-technological activities, considering their ability to ferment different plant-based matrices (rice, legumes, cereals) (A2.1); Strains showing the best pro-technological performances and being able to release significant concentration of neuroactive molecules during fermentation will be considered suitable for fermentation of a yogurt-like (YL) product and so used to prepare the microbial consortia (A2.2)

A3) **Developing a plant-based, yogurt-like product with psychobiotic properties:** The microbialconsortia will be tested for their ability to ferment different plant-based matrices like gelatinized mixture of rice and non-conventional flours (e.g., from legumes, pseudo- or minor-cereals) (A3.1); their growth and acidification ability will be monitored, as well as the ability to release neurotransmitters in the matrices (A3.2);

A4) **Testing the product in the SHIME model:** the best performing YL product will be tested in m-SHIME. During each run, a daily consumption of one portion (125g) of the YL product for 2 weeks will be tested (A4.1); all samples will be analyzed before microbiome stabilization, after stabilization, at different time-points during fermentation and at the end of the treatment. Microbiome will be analysed by shotgun metagenomics and neurotransmitters will be detected in the different SHIME compartments (A4.2);

A5)  **Writing and Editing** of the PhD thesis, scientific papers and oral and poster communications.

***Table 2***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Psychobiotic activities in Italian FFs*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) FFs collection and strain isolation |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Metagenomic characterization |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***Developing microbial consortia***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Strain testing  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Consortia development  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A3) | ***Developing a YL product*** |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Formulation  |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
|  | 2) Monitoring of acidification ability and psychobiotic properties |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| A4) | ***Testing in the SHIME model*** |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 1) SHIME model tuning |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 2) Processing of collected data |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A5) | ***Thesis and Papers Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# **3. Selected References**

Berding K, Bastiaanssen TFS, Moloney GM, Boscaini S, Strain CR, Anesi A, Long-Smith C, Mattivi F, Stanton C, Clarke G, Dinan TG, Cryan JF, (2023), Feed your microbes to deal with stress: a psychobiotic diet impacts microbial stability and perceived stress in a healthy adult population*, Mol Psychiatry* **28**: 601–610.

Casertano M, Fogliano V, Ercolini D, (2022), Psychobiotics, gut microbiota and fermented foods can help preserving mental health, *Food Research International,***152**.

Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, Sanders ME, Shamir R, Swann JR, Szajewska H, Vinderola G, (2021), The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, *Nat Rev Gastroenterol Hepatol* **18**: 649–667.