PhD DISSERTATION PROJECTS

**Exploitation of functional potential of autochthonous microorganisms from fermented foods**

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This PhD thesis research aims to isolate new candidate probiotic strains possessing traits potentially useful to hinder NCDs, to introduce them in traditional and new functional foods.

**Valorizzazione del potenziale funzionale di microrganismi autoctoni da alimenti a fermentazione naturale**

Questo progetto di tesi di dottorato mira ad isolare nuovi ceppi probiotici in grado di ostacolare le malattie non trasmissibili, allo scopo di introdurli in alimenti funzionali tradizionali e non tradizionali.

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

Nowadays consumer’s demand for safe, high-quality, and health-promoting foods is growing more and more. Research has been demonstrating that nutrition can prevent several diseases, by reducing risk factors and increasing certain physiological functions. There are several studies that highlight the link between diet and a specific class of diseases, called non communicable diseases (NCDs). NCDs are slow-developing and long-lasting diseases, such as cancer, diabetes, cardiovascular, respiratory, and neuro-degenerative diseases, which are the cause of more than 71% of annual deaths worldwide. In recent years, scientific evidence that emphasizes the correlation between the development of NCDs and gut microbiota has emerged: microbiota dysbiosis is a common trait in people affected by these pathologies. Also, gut microbiota is closely related to nutrition. Among the microbiota species, it is particularly important to monitor the so-called next generation probiotics (NGPs): *Faecalibacterium pausnitzii*, *Akkermansia muciniphila* and *Eubacterium hallii* are the most promising. NGPs recently gain attention for their role in prevention and treatment of many chronic diseases. For example, *Akkermansia muciniphila* helps to prevent type 2 diabetes and to maintain glucose homeostasis (De Filippis et al., 2022).

From this perspective, functional foods can play a central role. Functional foods are defined as those foods which, besides providing nutrients and energy, are also able to enhance health or reduce the risk of disease. One of the main classes of functional foods are fermented foods. The main agents of fermentation are lactic acid bacteria (LAB), which play a key role in guaranteeing safety, enhancing shelf-life, and developing typical sensory properties of the product. Moreover, during fermentation, vitamins, minerals, biologically active peptides, bacteriocins, antioxidants are produced, and some anti-nutritional factors are removed. As a result, these products provide many health benefits. In traditional fermented foods fermentation occurs spontaneously, thanks to autochthonous microorganisms that are naturally present in the raw food and can be promising probiotic candidates (Grujović et al., 2021). There are several studies regarding the indigenous microflora, but studies focusing on their addition in functional foods with the aim to hinder NCDs are still lacking.

The potential of indigenous LAB from fermented foods is huge, but the selection of the best probiotic candidates to use in the development of a functional food is challenging. It is indeed necessary to consider functional, safety, technological and physiological aspects. As for the functional aspects, numerous studies have confirmed antioxidant, anti-inflammatory, immunomodulatory, anti-carcinogenic, anti-cholesterol, antidiabetic, and anti-obesity effects of natural microflora and their metabolites. LAB not only show several metabolic pathways useful for health, but they may also modulate gut microbiota by producing active metabolites (e.g., short chain fatty acids (SCFAs) and antimicrobial substances) in the gut, which contribute to prevent microbiota dysbiosis (Grujović et al., 2021). In this regard, in vitro models that simulate large intestine fermentations are crucial to assess the effect of probiotics on gut microbiota and its metabolites. As for safety aspects, probiotic candidates must obviously be safe for human consumption, so they must be non-pathogenic strains with no toxic properties. Most LAB are included in the list of species presumed to be safe, in accordance with the QPS (Qualified Presumption of Safety) concept introduced in 2007 by the European Food Safety Authority (EFSA). From a technological point of view, probiotics must be able to survive and maintain a high vitality in the food matrix during all processing steps and shelf-life, since the physiological effect is guaranteed only in the presence of high concentrations of viable cells (at least 106-107 CFU/g). Furthermore, in the case of fermented foods, they must not interfere with the metabolic activities of the indigenous microflora of the product, nor negatively influence the physico-chemical, structural, and sensory characteristics of the product. Lastly, to exert the health effects they are selected for, physiological features are relevant. Probiotics must survive the gastrointestinal transit (GIT), which depends first on their tolerance to gastric acidity, antimicrobial activity of pepsin, bile salts and pancreatin (Marino et al., 2021).

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

Within the overall objective mentioned above, this project can be divided into the following activities according to the Gantt diagram (Table 1):

A1) **Literature review** in order to select the most suitable food products for strains’ isolation and a definitive list of functional activities to look for.

A2) **Isolation of autochthonous LAB strains from different food matrices and evaluation of their probiotic potential** in order to collect safe bacterial strains able to reach and interact with the target site.(A2.1) Safety assessment (absence of biogenic amines production, absence of horizontal transfer of antibiotic-resistance genes). (A2.2) Probiotic assessment: survival ability to GIT stressors (pH, gastric juices, and bile salts) and intestinal colonization ability (adherence to enterocytes, auto-aggregation, cell surface hydrophobicity, co-aggregation). (A2.3) Species assignment through 16S rDNA sequencing, and molecular fingerprinting.

A3)***In vitro* evaluation of functional properties** (anti-cholesterol activity, ACE-inhibitory activity, immunomodulatory and anti-inflammatory activity, pro-Next Generation Probiotics activity, alfa-amylase inhibition, GABA production, folates production, EPS production, …) in order to collect the most promising bacterial cultures.

A4) **Development of new functional foods**. (A4.1) Selection of possible food targets (e.g., dairy products, plant-based products, …). (A4.2) Development of functional foods and assessment of the microbiological, physico-chemical, and sensory characteristics. (A4.3) Assessment of strains’ viability during production process, shelf-life, and *in vitro* digestion. (A4.4) Assessment of the impact on gut microbiota and its metabolites.

A5) **Writing and editing of scientific papers and PhD thesis**.

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



# **3. Selected 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.

Grujović M, Mladenović KG, Nikodijević DD, Čomić LR (2019) Autochthonous lactic acid bacteria - presentation of potential probiotics application. *Biotechnology Letters* **41**: 1319-1331.

Marino M, Innocente N, Melchior S, Calligaris S, Maifreni M (2021) Main technological challenges associated with the incorporation of probiotic cultures into foods. *Advances in Probiotics* 479–495.