**PhD DISSERTATION PROJECTS**

Exploiting fermented foods microbiome to improve food quality and human health

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This PhD research project is aimed at investigating the microbial biodiversity in fermented foods and its potential implications for human health. The complex microbial communities of fermented foods will be investigated using a shotgun metagenomic approach, focusing on the microbiome functional potential, and highlighting the metabolic pathways involved in health-promoting activities. Finally, a long-term human intervention trial with milk kefir will be carried out to test *in vivo* the effects on gut microbiome and human health.

Utilizzo del microbioma degli alimenti fermentati per migliorare la qualità del cibo e la salute umana

Questo progetto di ricerca di dottorato mira a studiare la biodiversità microbica negli alimenti fermentati e le sue potenziali implicazioni per la salute umana. Le complesse comunità microbiche degli alimenti fermentati saranno studiate utilizzando un approccio di metagenomica shotgun, focalizzandosi sul potenziale funzionale del microbioma ed evidenziando i percorsi metabolici coinvolti nelle attività di promozione della salute. Infine, sarà condotto uno studio di intervento a lungo termine sull'uomo con kefir di latte per verificarne *in vivo* gli effetti sul microbioma intestinale e sulla salute umana.

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

Fermented foods (FFs) have always been part of human cultures, including culinary traditions and health practices. These foods undergo microbial fermentation, a process in which complex microbial communities transform the raw ingredients into flavourful, preserved, and nutritionally rich products. Beyond these attributes, FFs have been associated with potential health benefits, ranging from improved digestion and nutrient absorption to enhanced immune function (Marco et al., 2017). The health benefits attributed to FFs stem from various factors. Microorganisms produce an array of bioactive compounds during fermentation, including organic acids, antimicrobial peptides, and vitamins, many of which have physiological effects on the human body. Furthermore, fermentation increases the bioavailability of nutrients, making them easier to be absorbed (Cani, 2018; Sharma et al., 2020). Numerous FFs have been tested on humans, investigating their potential effects and benefits. In Table 1 main results from intervention studies are summarised. Understanding the intricate relationship between FFs and human health has been greatly advanced by the application of shotgun metagenomic sequencing, which allows for a comprehensive analysis of the genetic material present in complex microbial communities, enabling the identification of microbial strains and their associated functional genes (Quince et al., 2017). Through bioinformatic analysis, reconstruction of metagenome-assembled genomes can assess metabolic capabilities of FFs microbiome, unravelling the microbial diversity and functional dynamics, offering a deeper understanding of the potential health-promoting properties, including immune-modulating functions, metabolic processes, and mental wellness (De Filippis et al., 2020).

***Table 1***Main FFs used for intervention studies exploring effects in human health.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diet or fermented food matrix** | **Study design** | **Variations in the gut microbiome** | **Health outcome targeted** | **Health outcome achieved** |
| High-FFs diet | RCT | ↑ *Ruminococcaceae* and *Streptococcaceae*↓ *Lachnospira* | Inflammation | Yes |
| Grana Padano | Double blind placebo-controlled | Not detected | Hypertension | Yes |
| Kefir | RCT | ↑ *Lactobacillus* and *Bifidobacterium* spp. | Metabolic syndrome | Yes |
| Fermented milk | Cross-over trial | ↑ *Bacteroides ovatus, Lachnospira and Ruminococcus* | IBS and obesity | No |
| Kimchi | RCT | ↑ *Prevotella* and *Bacteroides*↓ *Blautia* | Metabolic syndrome | Yes |
| Yogurt | Before/after trial | ↑ LAB and *Clostridium perfringens* ↓ *Bacteroides* | None | None |

FFs: fermented foods; RCT: randomised control trial; IBS: irritable bowel syndrome: LAB: lactic acid bacteria.

While shotgun metagenomic sequencing has improved our understanding of FFs and their impact on human health, challenges remain. Large-scale studies and well-designed intervention trials are needed to establish causal relationships between FFs consumption, gut microbial community changes, and health outcomes (Johnson et al., 2020). This PhD project develops within the framework of the EU project DOMINO (GA 101060218). Within this project, a database of FFs metagenomes will be developed, aiming to map the complex microbial communities present in different types of FFs. The database will be screened to identify microbial markers (species, strains and/or genes) potentially associated with positive health outcomes. Finally, a randomised controlled trial in subjects with metabolic syndrome will be developed to highlight the effect of FFs on human health and the FFs-gut microbiome axis.

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

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

A1) **Sampling of FFs and beverages** (e.g., cheeses and dairy products, kombucha, fermented vegetables), in order to expand the knowledge on FFs microbiome (A1.1). The analysis of molecular compounds (Volatile Organic Compounds, antioxidants) will be also performed (A1.2).

A2) **Shotgun metagenomic sequencing** will be carried out to investigate microbial communities in different FFs. Thus, microbial DNA will be extracted, and metagenomic libraries prepared and sequenced (A2.1). Then, metagenome bioinformatic analysis will reveal FFs microbial strains and predict metabolic pathways related to them (A2.2).

A3) **A database of FFs microbiome** will be obtained through the post-processing of metagenomic data (A3.1) and a large-data statistical analysis (A3.2). Microbial genes related to potential health effects will be identified.

A4) **FFs human trial** to validate the health effect of FFs, as well as the effect on the gut microbiome and the potential transfer of microbial strains.

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

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activity** | **Months** | **11** | **02** | **03** | **04** | **05** | **06** | **07** | **08** | **09** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Sampling of FFs and beverages*** |  |   |  |  |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) FFs sampling |  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Metabolomics |  |  |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***Shotgun metagenomic sequencing*** |  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) DNA extraction and sequencing  |  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Metagenome bioinformatic analysis |  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A3) | ***FFs database construction***  |  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Post-processing of metagenomic data |  |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
|  | 2) Big-data statistical analysis |  |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| A4) | ***Trial/intervention with FFs*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Thesis and paper preparation*** |  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

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