Functional Screening of Microbial Resources for Healthy Food Fermentations through a Predictive Understanding of Genotype-Phenotype Relationships

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Increasing concerns about human and planetary health has raised the need for healthier and more sustainable food alternatives mainly obtained from plant sources. In this context, traditional fermentation offers a valuable biotechnological approach to maintain and/or improve plant-based foods nutritional, techno-functional and sensory properties. Accordingly, this Ph.D. research project aims to evaluate the suitability of different microbial resources to be employed for successful plant-based fermentation, by comparing their fermentative, phenotype, and metabolic capabilities when cultivated under optimal and/or food-like conditions. The most promising strains will be selected to produce three newly developed fermented plant-based food prototypes potentially having outstanding quality.

Screening funzionale di risorse microbiche per fermentazioni alimentari salutari attraverso un’analisi predittiva di relazioni genotipo-fenotipo

Le crescenti preoccupazioni per la salute umana e planetaria hanno evidenziato la necessità di alternative alimentari più sane e sostenibili, ricavabili principalmente da fonti vegetali. A questo proposito, la fermentazione offre un valido approccio biotecnologico per mantenere e/o migliorare proprietà nutrizionali, tecno-funzionali e sensoriali di alimenti vegetali. In tal senso, questo progetto di dottorato mira a valutare diverse risorse microbiche come potenziali starter per una fermentazione funzionale di substrati vegetali, confrontandone capacità fermentative, fenotipiche e metaboliche in condizioni di coltivazione ottimali e/o food-like. I ceppi più promettenti saranno impiegati nello sviluppo di prototipi di alimenti vegetali fermentati di migliorata qualità.

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

There is an increasing need in society, and in industry, for a revolutionary change in the food system to a more sustainable dietary pattern richer on plant-based foods (*Graça et al., 2019)*. Although a large portion of consumers has already started to follow this predominately plant-based lifestyle, due to healthy (i.e., lactose intolerance, diabetes, cardiovascular diseases and others), ethical (i.e., vegetarianism and/or veganism) or environmental reasons, still there is a part of the population that is resistant to undergo this transition process and overconsumes meat, dairy (and ultra-processed) foods and beverages (*Graça et al., 2019*). Interest in non-boring and tasteful alternative protein sources to replace the traditional animal-protein rich sources (i.e., dairy and meat) is thriving, leading to a continuous introduction of traditional and new sources of plant-based proteins, particularly from cereals, pseudo-cereals, and legumes (*Rizzello et al., 2010*). Indeed, their nutritional and functional properties make these sources particularly interesting alternative to the counterpart of animal origin (*Coda et al., 2017*). Despite being strongly recommended for human diet, plant-based protein sources have several limitations such as high anti-nutritional compounds content and lack of some essential amino acids (C*oda et al., 2017*). The fortification of cereal-based raw materials with legumes may represent a good strategy to complement the product nutritional quality (C*oda et al., 2017*). From a technological point of view, the interaction between the proteins and the carbohydrates present in grain- (and/or legume-) based raw materials may negatively interfere with foaming, gelling and other techno-functional properties. From a sensory point of view, bitter and beany (especially legumes) taste can form due to lipid oxidation causing bad taste and off-flavor (*Rizzello et al., 2010*). For these reasons, biotechnological approaches are required. Indeed, fermentation has been used for millennia for food preservation (and shelf-life increasing) and for enhancing food flavor. Recently, fermentation has become a simple and valuable biotechnology to keep and/or enhance the nutritional, textural, sensory properties of a raw material (*Di Cagno et al., 2013*). In this context, fermentation may offer a possible solution to overcome most of the challenges related to the application of high-protein plant-based raw materials in food production (*Coda et al., 2017*). From a nutritional point of view, fermentation may allow to improve protein digestibility, to reduce anti-nutritional factors and metabolize nutritional constituents (i.e., phenolic compounds) (*Coda et al., 2017*).

From a technological point of view, fermentation may improve foaming and emulsifying properties (*Coda et al., 2017*). Exopolysaccharides production by microorganisms can further enhance raw textural/structural properties (*Gobbetti et al., 2014*) while favoring the adhesion and the persistence of microbial cells, introduced in human body via consumption of probiotics and/or food containing living microorganisms (i.e., yogurt), at the intestinal epithelium level (*Gobbetti et al., 2014*). Finally, fermentation can be used to improve food flavor and sensory characteristics (*Gobbetti et al., 2014*). Facing this background, the following research project aims to explore how different microbiological resources (mainly lactic acid bacteria (LAB) including, fructophilic lactic acid bacteria (FLAB), and yeasts) can be fully exploited to improve traditional and/or develop innovative fermented plant-based (cereals and/or legume based) food, rich in proteins, with an enhanced nutritional, textural and sensory value, and with health-promoting potential.

# **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 1:

A1) **Literature Review Experimental and Plan Definition**

A2) **(*Existing*) microbial resource(s)** will be characterized for desired functional activities when individually cultivated in optimal and/or in food-like conditions (A2.1). Eventual differences in strains phenotypic profiling will be highlighted by OmniLog® Phenotype MicroArray (PM) platform (Biolog System) (A2.1). To the same purpose, autochthonous microbial resources will also be newly isolated and identified (A2.2).

A3) **Raw materials (legume- and/or grain- flours)** will beevaluated alone or in different combinations for their performances during prototypes production (A3.1). The best raw materials combination, identified during activity A3.1, will be used as a substrate for fermentation in activity A3.2.

A4) **Fermented prototype(s)** will be developed and characterized for their nutritional, techno-functional, and sensory properties, and for their possible effect on human gut (A4.1). The better-performing food prototype(s), from activity A4.1, will be selected and considered in the setting up of an optimized fermentation protocol to be applied at industrial level (A4.2).

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

***Table 1.***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) | ***Literature Review and Experimental Plan Definition*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | ***Microbial Resource(s)***  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Characterization of *existing* microbial resources  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Isolation and identification of *novel* microbial resources  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Raw Material(s)*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
|  | 1) Evaluation of raw materials (legume- and/or grain- flours) |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 2) Fermentation of raw materials (legume- and/or grain- flours)  |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
| A4) | ***Fermented Prototype(s)***  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) Development, characterization, and optimization of fermented prototype(s) |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
|  | 2) Upscaling of fermented prototype(s) production  |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| A5) | ***Thesis and Paper Preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

# **3. Selected References**

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