**Engineering of microorganisms for the production of metabolites of interest and the transformation of molecules during fermentation processes**

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The aim of this PhD project is to study and optimize lactic acid bacteria (LAB) fermentation processes capable to metabolize substrates to produce or degrade molecules of interest for the food sector. At first, the ability to degrade molecules, with focus on allergens, will be investigated. At the beginning, the research will be aimed to explore the diversity between the strains, investigating their proteolytic activity (*in silico*) and optimizing the fermentation parameters (*in vitro*). Analyzes will follow to verify its effect on allergens. The last phase will be directed to engineering the most promising LAB for optimizing the degradation process.

Ingegnerizzazione di microrganismi per la produzione di metaboliti di interesse e la trasformazione di molecole durante processi di fermentazione

Lo scopo di questo progetto di dottorato è quello di studiare e ottimizzare processi di fermentazione con batteri lattici (LAB) in grado di metabolizzare substrati per produrre o degradare molecole di interesse per il settore alimentare. Inizialmente, verrà valutata la capacità di degradare molecole proteiche, con focus sugli allergeni. La prima parte della ricerca sarà pertanto rivolta ad esplorare la diversità tra i ceppi, studiandone l’attività proteolitica (*in silico*) e ottimizzando i parametri di fermentazione (*in vitro*). Seguiranno delle analisi per verificarne l’effetto sugli allergeni. L’ultima fase sarà indirizzata all’ingegnerizzazione dei LAB più promettenti per ottimizzare il processo di degradazione.

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

Climate change and population growth are driving the search for more sustainable feed and alternative foods to conventional sources. In this context, new protein sources could be introduced into the food chain, raising many health problems, especially in the field of allergies. Overall, more than 170 foods can cause allergic reactions in humans, but 90% of these allergies are induced by allergens from 8 major foods, including shellfish, soy, peanut, milk, tree nut, egg, wheat and fish. Food allergy corresponds to a type I hypersensitivity (mediated by immunoglobulin E, IgE) to otherwise harmless food proteins of animal or plant origin (allergens), affecting 5-10% of children and 1- 5% of adults. These allergens bind to IgE, on the surface of basophils or mast cells, and cause the release of pro-inflammatory mediators, such as histamine, and induce the symptomatic phase of allergy, causing urticaria, rhinitis, swelling, anaphylactic shock, death, etc. (El Mecherfi *et al.*, 2020; Pi *et al.*, 2021). Therefore, a significant challenge for global food safety and health concerns the study of new strategies to reduce food allergenicity.

Among the various strategies being studied at the moment, fermentation with LAB, in addition to improving the physical-chemical properties and nutritional values of foods, can represent an excellent solution as it allows to modify the protein structure and consequently reduce the sensitivity of the human body to food allergens. In fact, LAB play an essential role in food fermentation processes. LAB are auxotrophic and depend on their proteolytic system to meet their nutritional needs for amino acids in food matrices deficient in nitrogen sources necessary for growth. (El Mecherfi *et al.*, 2020). Thanks to this proteolytic system composed of cell envelope proteinases (CEP) the LAB can deactivate IgE epitopes by degrading the proteins into oligopeptides which are subsequently taken up by the cells via specific peptide transport systems for further degradation into oligopeptides and amino acids by means of a concerted action of various intracellular peptidases (Guo *et al.*, 2016; Pescuma *et al.*, 2015). Through a comparative genomic analysis, it was observed that the number of CEP genes can vary from one to four in a specific strain and that the simultaneous presence of two or more CEPs can improve the efficiency of breakdown of the proteins of interest (Guo *et al.*, 2016).

In this context, this PhD project will be aimed at investigating the proteolytic activity of LAB strains, selected among those belonging to the University of Parma Culture Collection (UPCC), aimed at the degradation of allergens. The characteristics of the different strains and allergens of interest will then be explored, verifying the presence of CEP proteases and applying different growth (time, temperature, inoculum concentration, etc.) in the presence of the allergens. We want to conduct a study not only on different types of LAB strains, but also on allergens from different food proteins, in particular insects, soy and gluten.

# **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) ***In silico* analysis of LAB genomes to study the distribution of CEP proteases.**

A2) **Test the proteolytic activity *in vitro*** by fermentation processes applying different growth conditions (time, temperature, inoculum concentration, etc.) in the presence of allergens, by using LAB extracts or enzymes purified from different LAB strains.

A3) **Identify the metabolites produced during fermentation and verify the degradation of the allergens** using different techniques.

A4) **Optimization of the process through the engineering of the strains.**

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) | ***In silico* analysis** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | **Test the proteolytic activity *in vitro*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | **Identification of metabolites** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | **Strain engineering** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | **Thesis and Paper Preparation** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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