**Production of γ-aminobutyric acid by *Levilactobacillus brevis*: basic aspects and applications in foods**

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The PhD thesis project is focused on the exploitation of lactic acid bacteria, as bio-factories for the production of bioactive compounds useful for the development of functional foods. Specifically, the production of γ-aminobutyric acid (GABA) by Levilactobacillus brevis will be optimised, and the metabolite will be purified and supplemented in several foods. Moreover, the in-situ production of GABA will be also evaluated by using a selected Lvb. brevis strain as adjunct culture during food fermentations.

Produzione di acido γ-aminobutirrico da *Levilactobacillus brevis*: aspetti basilari e applicazioni nell’industria alimentare

Il progetto di tesi di Dottorato è focalizzato sullo sfruttamento dei batteri lattici, come bio-fabbriche per la produzione di composti bioattivi utili allo sviluppo di alimenti funzionali. In particolare, la produzione di acido γ-aminobutirrico (GABA) da parte di *Levilactobacillus brevis* sarà ottimizzata e il metabolita sarà purificato e aggiunto in diverse matrici alimentari. Inoltre, la produzione *in-situ* di GABA sarà valutata anche utilizzando, come coltura aggiuntiva durante le fermentazioni, un ceppodi *Lvb. brevis* opportunamente selezionato.

**1. State of the Art**

Microorganisms are a potential source of bioactive compounds (e.g. organic acids, biopeptides, short chain fatty acids, exopolysaccharides, antioxidants, prebiotics) and may be used as cell factories to produce functional foods through the *in-situ* production of bioactive metabolites (directly in food matrices) or by food supplementation with their postbiotics (Nataraj et al., 2020). Among the beneficial microbial metabolites, γ-aminobutyric acid (GABA) is receiving great attention in food, pharmaceutical and cosmetic industries. GABA is a non-proteinogenic aminoacid that may act several physiological functions (e.g. brain development, regulation of neurological disorders, hypotensive, analgesic, antianxiety, antidiabetic effects) that result in proven benefits to human health (Dhakal et al., 2012; Diana et al., 2014; Xu et al., 2017). GABA may be produced via chemical synthesis or through the decarboxylation of glutamate by pyridoxal 5’-phosphate (PLP)-dependent microbial glutamate decarboxylases (Xu et al., 2017). Microbial bioconversion is preferred to the traditional chemical methods because provides food-grade and eco-friendly product that can be used as supplement in fortified and functional foods, as well as in drugs and cosmetics. The global GABA market is expected to grow in the next years and the production by microbial fermentation is expected to exceed that by chemical synthesis. Several microorganisms have been recognised as potential GABA-producers (Dhakal et al., 2012; Diana et al., 2014); among them, lactic acid bacteria (LAB; especially those belonging to *Levilactobacillus brevis* and *Lentilactobacillus buchneri* species) are considered the most promising GABA-producing group (Li and Cao, 2010; Dhakal et al., 2012). The genetic equipment of LAB strains is crucial for the bioconversion of glutamate to GABA, even if other factors, such as pH values, temperatures, time of fermentation, composition of growth substrate and cell density, may affect the functionality of GABA production system. Often, the downstream processes needed for separation and purification of GABA from culture broth are expensive and may impair the large-scale production and the marketability of GABA. Therefore, the optimisation of fermentative processes to ensure high-yield GABA production and the development of low-cost downstream protocols may be of practical relevance. The natural content of GABA in foods (Diana et al., 2014) is low and strategies to increase its concentration are recently gaining interest. However, the production of GABA-enhanced foods is still at experimental levels, and different approaches to incorporate the GABA-producing strains or the purified biocompound in food matrices are critical to ensure the appropriate content and functionality of GABA in supplemented foods. Several authors investigated the use of resting cells (non-growing but metabolically active) for bioconversion processes as they have several advantages compared to growing cells (costs of fermentation process) or purified enzymes (limitations of separation techniques). On the other hand, other studies, have addressed on the use of strategies for the protection of microbial cells and GABA (e.g. immobilization, microencapsulation) to improve their stability in food matrices (Thangrongthong et al., 2020; Ozer et al., 2022). However, these data, although promising, are still preliminary and further studies are needed for the development of GABA-functionalized foods, able to meet the needs of the market and consumers.

Based on the above considerations, the aim of PhD project will be the optimisation of GABA production (by testing and selecting appropriate conditions in bioreactor cultivation), as well as of extraction and purification methods. GABA-producing strains or the purified biocompound will be also use to develop potential functional foods and/or beverages, with enhanced GABA content.

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

The PhD project will be divided in several activities, as described below and in the Gantt diagram (Table 1):

A1) **Optimization of biomass and GABA production**: GABA production by *Lvb. brevis* strains will be optimised in controlled conditions using cultivations in bioreactor (A1.1). Biomass will be used for GABA production in different buffer systems and glutamate/GABA content will be measured in both low-cost media and buffers. Chromatographic techniques will be used for detecting glutamate consumption and GABA production (A1.2). Gene expression (RT-qPCR) will be carried-out to elucidate the bioconversion mechanisms and regulation pathways.

A2) **Separation and purification of GABA from synthetic media and buffer systems**: Several strategies (e.g. membrane filtration, precipitation, solvent extraction, desalting, size exclusion chromatography) will be combined and optimised to remove residual components from culture supernatants of both synthetic media and buffer system. GABA in cleaned supernatants will be separated by using chromatography approaches, combining different resins and elution parameters (A2.1).

A3) **Microencapsulation of GABA-producer strain and purified GABA**: Different food-grade biopolymers/hydrocolloids, lipids and/or proteins will be combined and used as coating agents to produce spry-dried microencapsulated GABA-producing cells or purified GABA (A3.1). Encapsulation efficiency will be evaluated; a cost analysis will be carried out to identify the best protocol of microencapsulation (A3.2).

A4) **Food functionalization with GABA-producer strain or purified GABA**: Fermented milks and/or fruit juices will be used as model foods, and will be functionalized through an *in-situ* bioconversion using the microencapsulated producer strain (A4.1) or purified GABA. The chemical-physical and sensory properties of GABA-enhanced foods will be evaluated over-time to verify the effect of GABA supplementation (A4.2).

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

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Year (1)** | | | | | | **Year (2)** | | | | | | **Year (3)** | | | | | |
| **Activity Months** | | **2** | **4** | **6** | **8** | **10** | **12** | **14** | **16** | **18** | **20** | **22** | **24** | **26** | **28** | **30** | **32** | **34** | **36** |
| A1 | ***Optimization of biomass and GABA production*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Cultivations in bioreactor |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2) Analyses of biomass and GABA |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2 | ***Separation and purification of GABA*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Chromatography techniques |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3 | ***Microencapsulation (ME) of GABA-producer strain and purified*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Optimisation of ME protocol |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2) Evaluation of ME efficiency |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4 | ***Food functionalization with GABA*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Development of GABA-enhanced foods and beverages |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2) Chemical-physical and sensory analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5 | ***Bibliographic research, thesis and paper preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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