PhD DISSERTATION PROJECTS

Generating Novel Synbiotic Formulations with Multi-Functional Features: A Focus on Psychobiotic Effects

Amir Shazad (Amir.Shazad@student.unibz.it)

Food Engineering and Biotechnology, Faculty of Agricultural, Environmental and Food Sciences

Tutor: Prof. Raffaella Di Cagno

Internal Co-tutors: Dr. Ali Tlais Zein Alabiden and Dr. Claudia Cappello

External co-tutor: Dr. Daniela Pinto

The proposed PhD project aims to develop new synbiotic formulations combining probiotics and prebiotics to enhance gut health, immune function, and mental well-being. In vitro analyses will be conducted to evaluate the safety, stability, and efficacy of these formulations using experimental models that simulate gut-brain axis. Promising formulations will then undergo in vivo studies in animal models to assess their impact on mental health and cognitive function. This research has the potential to contribute to the creation of multi-functional synbiotics that can improve human mental health and cognitive performance, leading to development of new dietary supplements and functional foods.

**Generazione di nuove formulazioni simbiotiche con caratteristiche multifunzionali:**

**Un focus sugli effetti psicobiotici**

Il progetto di dottorato proposto mira a sviluppare nuove formulazioni simbiotiche che combinino probiotici e prebiotici per migliorare la salute dell'intestino, la funzione immunitaria e il benessere mentale. Saranno condotte analisi in vitro per valutare la sicurezza, la stabilità e l'efficacia di queste formulazioni utilizzando modelli sperimentali che simulano l'asse intestino-cervello. Le formulazioni promettenti saranno poi sottoposte a studi in vivo su modelli animali per valutare il loro impatto sulla salute mentale e sulla funzione cognitiva. Questa ricerca ha il potenziale per contribuire alla creazione di sinbiotici multifunzionali in grado di migliorare la salute mentale umana e le prestazioni cognitive, portando allo sviluppo di nuovi integratori alimentari e alimenti funzionali.

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

The human body is home to a diverse microbial ecology hosting about 90% microbial cells and 10 million microbial genes. The gastrointestinal tract, the most colonized portion of the human body, is the site of a complex and mutualistic relationship between the microbial habitat and the host, resulting in a health-promoting stable community (Kelly et al., 2016). Recent research has focused on the manipulation of the gut microbiota using various interventions, including probiotics, prebiotics, and their combination (synbiotics), to enhance health and treat various disorders (Allen et al., 2017). Synbiotics work synergistically to improve the microbial eubiosis, showing promising results in various disorders, including mental health disorders, such as depression and anxiety (Aggarwal et al., 2013).

Nowadays, research interest is gradually shifting to probiotics able to improve mental health thanks to their positive effect on the gut-brain axis. In the specific, several studies have shown that certain bacteria, including lactobacilli and bifidobacteria, can improve depressive symptoms in animal models and human clinical trials (Kelly et al., 2015). This subcategory of probiotics, defined as psychobiotics, confers a mental health benefit to the host showing a direct effect on brain function and behavior. Psychobiotics work through various mechanisms of regulation of the gut-brain axis, including regulating the production of neurotransmitters, reducing inflammation, and modulating the hypothalamic-pituitary-adrenal axis, which is a key regulator of the stress response and mood regulation (Dinan et al., 2013).

Moreover, recent research has focused on the development of novel synbiotic formulations with multi-functional features, with an emphasis on their psychobiotic effects. These formulations have shown promising results in various disorders, including mental health disorders and metabolic disorders (Schmidt et al., 2015). However, more studies are needed to optimize the formulation and dose of these synbiotics, as well as to discover the precise bacterial strains and prebiotic fibers that are most effective for their therapeutic effects and to develop individualized treatments for maximum efficacy.

# **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 and microbial strains selection**

Understanding the laboratory organization; reviewing the latest scientific literature related to my project and defining of the main research topics and selection of bacteria and yeasts strains from Micro4Food culture collection.

A2) **Screening of the strains’ performance and phenotypic characterization**

Screening of strains will be modulated by evaluating the performance of each strain through different activities like evaluating the gastrointestinal transit resistance, the enzymatic activities, the growth kinetics, and the acidification rates. The molecular characterization of glutamic acid decarboxylase (GAD) gene will be done as pre-selection for gamma-aminobutyric acid (GABA) producing strains. Microbial strains will also be characterized phenotypically to understand their carbon and nitrogen consumption profiles by using OmniLog Phenotype MicroArray (PM) Technology.

A3) ***In vitro* analyses**

Once the screening of the strains will be completed, the most promising probiotic strains will be selected using a statistical approach. Afterwards, the potential psychobiotic effect of the strains will be evaluated through the characterization of neurotransmitters and short chain fatty acids (SCFA) produced through fermentation Moreover, the anti-inflammatory response and the effect on the intestinal barrier permeability from the intervention using the candidate probiotic strains through in vitro analyses will be evaluated.

A4) **Hypothalamic Pituitary Adrenal (HPA)** **axis and serotonin production**

Evaluating the HPA axis in response to psychobiotics will help to determine if the microbial strains can affect stress hormone levels, regulate the stress response, and potentially alleviate stress-related conditions. To investigate this, adrenocorticotropic hormone (ACTH) stimulation test and gene expression analysis will be performed *in vivo.* The production of serotonin, a key neurotransmitter involved in mood regulation and mental well-being, will also be examined by using germ free mice.

A5) **Writing and Editing**

Participating to national and international scientific conference for oral and/or poster communications; writing and editing of the PhD thesis and scientific papers.

***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 strains selection*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Screening of the strains*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Strains performance |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Phenotypical characterization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***In vitro analyses*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***HPA axis and serotonin production*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Papers publication and thesis writing*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

Aggarwal, J., Swami, G. and Kumar, M., 2013. Probiotics and their effects on metabolic diseases: an update. Journal of clinical and diagnostic research: JCDR, 7(1), p.173.

Allen, A.P., Dinan, T.G., Clarke, G. and Cryan, J.F., 2017. A psychology of the human brain–gut–microbiome axis. Social and personality psychology compass, 11(4), p.e12309.

Dinan, T.G., Stanton, C. and Cryan, J.F., 2013. Psychobiotics: a novel class of psychotropic. Biological psychiatry, 74(10), pp.720-726.

Kelly, J.R., Clarke, G., Cryan, J.F. and Dinan, T.G., 2016. Brain-gut-microbiota axis: challenges for translation in psychiatry. Annals of epidemiology, 26(5), pp.366-372.

Kelly, J.R., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G. and Hyland, N.P., 2015. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Frontiers in cellular neuroscience, p.392.

Schmidt, K., Cowen, P.J., Harmer, C.J., Tzortzis, G., Errington, S. and Burnet, P.W., 2015. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology, 232, pp.1793-1801.