PhD DISSERTATION PROJECTS

Identification of Microbial Functions Interfering with Host-Cell Physiology: a Quick View into *Streptococcus thermophilus* Metabolic Potential

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This PhD thesis research project is aimed at demonstrating how significant interactions between specific microbial activities and host can play a potentially crucial role in upgrading the health status in fragile consumers categories. Particularly, the study focuses on the modulation of Streptococcus thermophilus urease and β-galactosidase activities through a metabolic boost, required for the development of new functional products able to affect gastro-intestinal diseases patients’ symptoms as a completely new way of treatment, i.e. from traditional probiotics to “precision probiotics”.

**Identificazione di funzioni microbiche in grado di interferire con le interazioni fisiologiche ospite-cellula: uno sguardo al potenziale metabolico di *Streptococcus thermophilus*.**

Questo progetto di tesi di dottorato si pone come obbiettivo di dimostrare come alcune interazioni significative tra specifiche attività microbiche e ospite possano avere un ruolo cruciale nel *miglioramento* delle condizioni di salute nelle categorie fragili di consumatori. In particolare, lo studio si incentra sulla possibile modulazione dell’attività ureasica e β*-*galattosidasica di *Streptococcus thermophilus* tramite specifiche azioni di *potenziamento* metabolico. Queste attività sono funzionali allo sviluppo di nuovi prodotti in grado di ridurre i sintomi in pazienti affetti da specifiche malattie legate al tratto gastro-intestinale e rappresentano un primo passo nella transizione dai prodotti probiotici tradizionali ai “probiotici di precisione”.

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

*Streptococcus thermophilus*, which is employed in dairy and probiotic industry, has an efficient lactose catabolism managed first by LacS, a permease system, and then hydrolyzed by β-galactosidase to yield glucose and galactose (Arioli et al., 2022), but it also owner of an urease positive activity trait that showed a significant positive correlation with β-galactosidase, increasing lactose consumption rate and acidification potential (Mora et al., 2014). Increasing β-galactosidase activity in *S. thermophilus* could be resolutive in the development of a new product able to reduce lactose intolerance symptoms in those subjects having a limited expression or activity of lactase in the small intestine, thus suffering of discomfort and pain after dairy consumption. Lactose intolerant subjects often react by excluding milk and dairy from their diet thereby leading to essential nutrient deficiency, such as vitamin D and calcium increasing the risk of osteoporosis (Ratajczak et al., 2020). In this context a food supplement based on *S. thermophilus* metabolically activated for lactose consumption will also be inline with the claim of “lactose digestion” by yogurt cultures recognized by EFSA (EFSA Journal 2010; 8(10):1763) and with the more recent World Gastroenterology Organisation Global Guidelines – Probiotics and prebiotics (2023).

More recently it was reported that a *S. thermophilus* strain releasing β-galactosidase was able to significantly reduce tumorigenesis of colorectal cancer in mice (Li et al., 2021) by modulating the metabolism of cancer cells, *i.e.* reducing the aerobic glycolysis and activating the oxidative phosphorylation. Therefore, the possibility to prepare specific *S. thermophilus* strains to be prone in releasing β-galactosidase *in vivo* will be an issue of this PhD project.

IBD (Inflammatory Bowel Disease) is a term used to describe different disorders that involve chronic inflammation of the digestive tract. IBD can manifest by various gastrointestinal expressions that share a common genesis in an initial state of dysbiosis (Strober et al., 2007) which is often related to high level of pathogenic urease activity in colon. Ammonia released by urease activity of harmful gut bacteria increases the inflammatory status in these patients. We recently observed that administration of urease-positive *S. thermophilus* cells in healthy subject determined a decrease in urease content in fecal samples (Martinović et al.*,* 2023). Therefore, the chance to prepare *S. thermophilus* with high content of urease activity and to test them *in vivo* will also be a target of this PhD project.

Based on these, the aim of the study is to prove that an efficient modulation of *S. thermophilus* metabolic activities and an adequate administration of freeze-dried cells could be useful to host treatment by:

* Improving lactose intestinal absorption better than commercial lactase would do.
* Modulating cancer cells metabolism and decreasing tumorigenesis of colorectal cancer in mice.
* Providing a correction of dysbiosis state with a re-population by harmless bacteria and a decrease of pathogenic urease activity in the gut.

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

Within the overall objective mentioned above, this PhD thesis project would be performed subdivided into the following activities (A) according to the Gantt diagram given in **Table 1**:

A1) **Screening and selection** of *S. thermophilus* strainsbased on the level of their β-galactosidase and urease activities.

A2) **Metabolic improvement** of *S. thermophilus* strain selected in A1 to increase β-galactosidase and urease activities.

A3) **Scale-up** **of the metabolic improvement** from a laboratory-scale to an industrial-pilot-scale.

A4) **Optimization of the freeze-drying process** to maintain the highest level of β-galactosidase and urease activities in *S. thermophilus* cells.

A5) **Proof-of concept *in vivo* study in lactose malabsorber human subjects** to verify the efficacy of metabolically activated *S. thermophilus* freeze-dried biomasses in reducing the lactose-intolerance symptoms.

A6) **Proof-of concept *in vivo* study on C57BL/6J-ApcMin/J mice**, which harbor a germline mutation in the tumor suppressor gene Apc and develop intestinal polyps spontaneously, to verify the role of *S. thermophilus* releasing and not releasing β-galactosidase on tumorigenesis.

A7) **Proof-of concept *in vivo* study on mice** to evaluate the decrease of fecal urease activity by administering *S. thermophilus* prepared to be urease-positive or urease-negative.

A8) **Dissemination, preparation and publication** of the results of the project

A9) **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) | *Selection of S. thermophilus strain* |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A2) | *Metabolic improvement of S. thermophilus selected strain*  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |   |   |  |  |  |  |
| A3) | *Scale-up process from a laboratory scale to an industrial/pilot scale* |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
| A4) | *Optimization of the freeze-drying process*  |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
| A5) | *Proof-of concept in-vivo study in lactose malabsorber human subjects* |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |
| A6) | *Proof-of concept in-vivo study on C57BL/6J-ApcMin/J mice to verify the role of S. thermophilus releasing and not releasing β-galactosidase on tumorigenesis* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A7) | *Proof-of concept in-vivo study on mice with administration of urease positive or urease negative S. thermophilus cells* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A8) | *Project’s results dissemination, preparation and publication* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A9) | *PhD thesis writing and editing* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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