Team Name: LacPro

Engineering a Lactase-Producing Probiotic for Managing Lactose Intolerance

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ABSTRACT

Lactose intolerance affects approximately 65% of the global population due to a deficiency in lactase-phlorizin hydrolase (LPH), the enzyme responsible for hydrolyzing lactose into glucose and galactose. Current management strategies, including dietary restrictions and lactase supplements, address symptoms but fail to provide a sustainable solution. This proposal aims to develop a genetically engineered strain of *Streptococcus thermophilus* capable of producing lactase as a probiotic solution for lactose intolerance. By integrating the lacZ gene from *Escherichia coli* into *S. thermophilus*, the strain will continuously produce lactase in the gut, improving lactose digestion and reducing symptoms. The study will include genetic engineering, in vitro evaluation of enzyme activity and stability under gastrointestinal conditions, and in vivo testing in a lactose-intolerant animal model. This innovative approach seeks to offer a natural, cost-effective, and sustainable solution to lactose intolerance, enhancing dietary flexibility and quality of life for affected individuals.

INTRODUCTION

Lactose intolerance affects approximately 65% of the global population (Catanzaro et al., 2021) and is mainly caused due to a deficiency in lactase-phlorizin hydrolase (LPH), the enzyme that hydrolyzes lactose into glucose and galactose (Ugidos-Rodríguez et al., 2018). This deficiency leads to undigested lactose reaching the colon, where microbial fermentation produces gases and short-chain fatty acids, causing bloating, diarrhea, and abdominal discomfort (Misselwitz et al., 2019). Additionally, lactose intolerance often necessitates the avoidance of dairy products, potentially resulting in nutritional deficiencies due to reduced intake of calcium and other essential nutrients (Hodges et al., 2019).

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Lactose digestion in humans relies on lactase, an enzyme produced by intestinal enterocytes, to break lactose into glucose and galactose (ODELL, 2021). These monosaccharides are absorbed via SGLT1 (sodium-glucose co-transporter 1) transporters for energy production or storage (Gromova et al., 2021). In lactase-persistent individuals, this process remains efficient throughout life. However, in lactase non-persistent individuals, lactose fermentation in the colon leads to uncomfortable symptoms, highlighting the importance of maintaining lactase activity for gut health (Roice, 2021).

Current management strategies include dietary restrictions and the use of lactase supplements. While dietary restrictions prevent symptoms, they also limit nutritional intake and reduce dietary options (Shafi & Husain, 2022). Lactase supplements effectively aid lactose digestion but require consistent use with every lactose-containing meal, leading to inconvenience and recurring costs (Amin et al., 2023). Both strategies focus on symptom management rather than addressing the root cause of enzyme deficiency, making them less sustainable for long-term care (Fassio et al., 2018).

Synthetic biology and microbial engineering offer innovative solutions for lactose intolerance (Cruz et al., 2022). By genetically engineering probiotic bacteria like *Streptococcus thermophilus* to produce lactase, it is possible to create a sustainable and natural solution. Integrating the *lacZ* gene from *Escherichia coli* into *S. thermophilus* allows continuous lactase production in the gut, leveraging the probiotic's safety and colonizing properties. This approach promises improved lactose digestion, enhanced dietary flexibility, and reduced reliance on external supplements.

This study aims to develop a genetically engineered *S. thermophilus* strain capable of producing lactase as a probiotic for managing lactose intolerance. By addressing the limitations of current strategies, this innovative solution seeks to provide a natural, cost-effective, and sustainable enzyme source, ultimately improving dietary management and quality of life.

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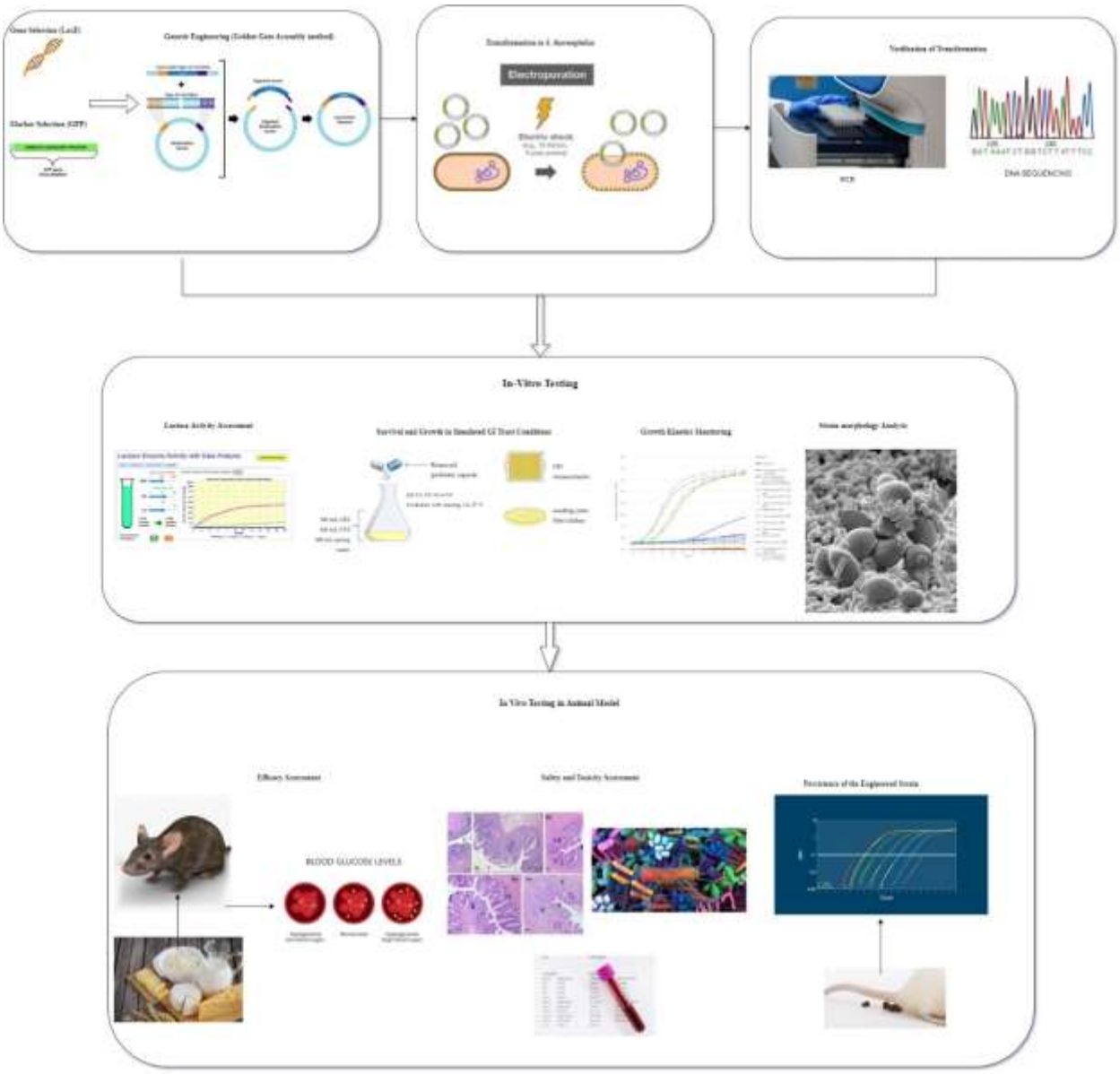
MATERIALS AND METHODS



Workflow

Gene Selection

The *lacZ* gene from *Escherichia coli*, encoding β-galactosidase (lactase), will be selected for its high enzymatic activity (Tomizawa et al., 2016). This gene is well-characterized and known to produce an efficient enzyme capable of breaking down lactose into glucose and galactose, making it an ideal candidate for this study.

Genetic Engineering

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Construction of Recombinant Vector

The genetic engineering process will begin with the construction of a recombinant vector using the Golden Gate Assembly method (Sorida & Bonasio, 2023). This method employs Type IIS restriction enzymes, such as BsaI, to create compatible overhangs, enabling the precise and seamless assembly of multiple DNA fragments into the pNZ8148 plasmid backbone (Sorida & Bonasio, 2023). The P23 promoter from *Lactococcus lactis* will be incorporated to drive strong, constitutive expression of the lacZ gene in *Streptococcus thermophiles* (Guan et al., 2016).

Selection Marker and Reporter Gene

To facilitate the selection of successfully transformed cells, a fluorescence-based reporter gene, Green Fluorescent Protein (GFP), will be included. GFP provides a non-antibiotic marker that enables easy confirmation of successful transformation and gene expression by emitting fluorescence under UV light (Nienhaus & Nienhaus, 2022). The lacZ gene will be flanked by specific recognition sites to ensure correct insertion into the vector.

Transformation of S. thermophilus

Transformation of the recombinant plasmid into *S. thermophilus* will be performed using electroporation (Kong et al., 2021). This technique involves applying an electrical pulse to bacterial cells to increase their membrane permeability, allowing the uptake of the plasmid.

Verification of Transformation

Following transformation, verification steps will be undertaken to confirm the successful integration and expression of the lacZ gene. PCR and sequencing will be used to verify the presence of the gene, while Western blotting will detect the β-galactosidase protein. Additionally, enzyme activity assays will be performed to confirm the functionality of the β-galactosidase enzyme, ensuring the engineered strain's ability to hydrolyze lactose effectively.

In Vitro Testing

Lactase Activity Assessment

In vitro experiments will be conducted to assess the activity of the engineered *Streptococcus thermophilus* strain in hydrolyzing lactose. Lactase activity will be measured using an enzyme

assay with the o-nitrophenyl-β-D-galactopyranoside (ONPG) substrate. The ONPG assay will allow for the quantification of lactase activity by measuring the release of o-nitrophenol, which can be detected spectrophotometrically. This assay will evaluate the strain's efficiency in breaking down lactose into its monosaccharide components (Amin et al., 2023).

Survival and Growth in Simulated GI Tract Conditions

The engineered strain's survival and growth will be tested in a simulated gastrointestinal (GI) tract model. The model will expose the strain to acidic conditions (pH 2-3) to mimic the stomach environment, followed by exposure to bile salts and digestive enzymes to replicate conditions in the small intestine. The strain's survival rate will be determined by plating and counting colony-forming units (CFUs) at various time points. This will help assess the strain's ability to withstand the harsh conditions of the human GI tract and its potential for successful colonization and activity in the gut (Cheng et al., 2021).

Long-Term Stability Evaluation

The long-term stability of the engineered strain will be evaluated by storing it under gut-like conditions for extended periods. After cryopreservation and recovery, the strain's ability to retain lactase activity and viability will be assessed. This will involve measuring lactase activity and CFUs after storage to determine the strain's ability to remain functional over time, ensuring its viability for use in long-term treatments for lactose intolerance.

Growth Kinetics Monitoring

Growth kinetics of the engineered strain will be monitored through optical density (OD) measurements at various time intervals. This will track the strain's growth rate and overall health under simulated gut conditions, providing insights into its potential for sustained growth and activity in the human gut.

Strain morphology Analysis

Scanning electron microscopy (SEM) will be used to examine the strain's morphology and surface properties after exposure to gut-like conditions. SEM imaging will provide detailed information about the strain's structural integrity, surface characteristics, and potential for adhesion to the intestinal mucosa, which is crucial for its effectiveness in the gut environment. These analyses will

help determine the strain's ability to maintain its functional properties and structural stability under conditions that simulate the human gut (Kiepś et al., 2023).

In Vivo Testing in Animal Model

Animal Model and Experimental Groups

Preliminary in vivo testing will be conducted using lactose-intolerant mice, with lactose intolerance induced through genetic predisposition. The animals will be divided into two groups: a control group, which will receive a placebo (saline solution), and a treatment group, which will be administered the engineered *Streptococcus thermophilus* strain. The strain will be delivered orally, either as a suspension, with the dosage and frequency determined based on prior in vitro studies.

Efficacy Assessment

To assess the strain's efficacy, lactose intolerance symptoms, including diarrhea, bloating, abdominal cramps, and flatulence, will be monitored through behavioral observations and clinical scoring systems. Blood glucose levels will be measured during lactose tolerance tests to evaluate the efficiency of lactose digestion and absorption. A significant increase in blood glucose levels after lactose ingestion will indicate improved lactose hydrolysis due to the engineered strain. Additionally, gut microbiota composition will be analyzed using 16S rRNA sequencing or metagenomic analysis to evaluate any changes in the microbial community caused by the engineered strain (Cheng et al., 2021).

Safety and Toxicity Assessment

Safety assessments will be conducted through histopathological examination of tissue samples from the intestines, liver, and kidneys to ensure that the engineered strain does not cause adverse effects or toxicity. Hematology and serum biochemistry tests will be performed to monitor the overall health of the animals (L.M et al., 2021).

Persistence of the Engineered Strain

Fecal samples will be collected periodically to assess the persistence of the engineered strain in the gut, with the presence of the strain confirmed through quantitative PCR (qPCR). These in vivo

animal studies will provide essential data on the safety, efficacy, and persistence of the engineered *S. thermophilus* strain, which will inform future clinical trials.

RESULTS AND DISCUSSION

Genetic Engineering

The recombinant plasmid contain LacZ gene and GFP gene, constructed using the Golden Gate Assembly method, is expected to demonstrate correct assembly, as verified by gel electrophoresis and sequencing data. Successful transformation of the recombinant vector to *Streptococcus thermophilus* is predicted to be indicated by GFP fluorescence (green color), confirming the uptake and expression of the plasmid. Western blot analysis is anticipated to detect the expression of β-galactosidase, while enzyme activity assays are expected to confirm the functional activity of the enzyme in hydrolyzing lactose.

In Vitro Testing

Lactase Activity Assessment

The engineered *S. thermophilus* strain is expected to exhibit significant lactase activity, as determined by ONPG assays. The release of o-nitrophenol, quantified spectrophotometrically, should confirm the strain's ability to hydrolyze lactose efficiently.

Survival and Growth in Simulated GI Tract Conditions

The strain is expected to maintain viability under simulated gastrointestinal conditions, with CFU counts indicating survival in acidic environments (pH 2–3) and in the presence of bile salts and digestive enzymes. Growth kinetics data are anticipated to show a stable growth rate, further confirming the strain's resilience.

Long-Term Stability

The engineered strain is expected to retain lactase activity and viability after storage under gut-like conditions. CFU counts and enzyme activity assays are anticipated to show minimal loss of functionality over time, demonstrating the strain's potential for long-term therapeutic applications.

Strain Morphology Analysis

Scanning electron microscopy (SEM) is expected to reveal intact cellular morphology and surface properties conducive to adhesion to the intestinal mucosa, which are critical for effective colonization and activity in the gut.

In Vivo Testing

Efficacy Assessment

In lactose-intolerant mice, the treatment group is expected to exhibit reduced symptoms of lactose intolerance, such as diarrhea, bloating, and flatulence, compared to the control group. Blood glucose levels during lactose tolerance tests are anticipated to show a significant increase in the treatment group, indicating improved lactose digestion. Gut microbiota analysis is expected to reveal favorable changes in microbial diversity, suggesting a positive impact of the engineered strain on the gut ecosystem.

Safety and Toxicity Assessment

Histopathological examination of tissue samples from the intestines, liver, and kidneys is expected to show no adverse effects or signs of toxicity. Hematological and biochemical parameters are anticipated to remain within normal ranges, confirming the safety of the engineered strain.

Persistence of the Engineered Strain

Quantitative PCR analysis of fecal samples is expected to confirm the persistence of the engineered strain in the gut over the study period, indicating its potential for sustained therapeutic activity.

CONCLUSION

This proposal outlines the development of a genetically engineered *Streptococcus thermophilus* strain to address the root cause of lactose intolerance by enabling continuous lactase production in the gut. By leveraging synthetic biology and microbial engineering, this solution is expected to offer improved lactose digestion, reduced symptoms, and enhanced dietary flexibility. The anticipated outcomes of this research could pave the way for a sustainable and cost-effective alternative to current management strategies, significantly improving the quality of life for individuals with lactose intolerance.

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