INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: *Lactobacillus delbrueckii* subsp. *bulgaricus* as a Microbial Chassis for D-Lactic Acid Biosynthesis: Strain Improvement, Metabolic Engineering, and Development of Molecular Tools

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SHORT ABSTRACT

D-lactic acid (DLA) serves as a vital precursor for producing biodegradable polylactic acid (PLA), offering a sustainable alternative to conventional plastics. This thesis presents a comprehensive metabolic engineering strategy to enhance the biosynthesis of optically pure DLA in Lactobacillus delbrueckii. Through systematic strain screening, strategic pathway optimization, dynamic metabolic regulation, and random mutagenesis, this study maximized DLA production while maintaining robust strain performance. Among the screened strains, Lactobacillus delbrueckii subsp. bulgaricus VI104 emerged as a superior candidate due to its efficient lactose utilization, high DLA production, and excellent electroporation efficiency. A multifaceted engineering approach was applied, including the reconstruction of the Leloir pathway for enhanced galactose metabolism, ATP balance optimization, and targeted overexpression of key glycolytic enzymes such as D-lactate dehydrogenase (dldh), phosphofructokinase (pfk), and phosphoglycerate kinase (pgk). These synergistic interventions resulted in a remarkable 240% increase in DLA titres and a 273% improvement in acid tolerance. To alleviate the metabolic burden associated with constitutive gene expression, a DLA-inducible promoter-repressor system derived from Pseudomonas species was engineered. This dynamic regulatory system enabled precise modulation of dldh expression in response to DLA accumulation, autonomously transitioning between growth and production phases, resulting in a 63% increase in DLA titres during bioreactor-scale fermentation. Additionally, strain screening identified L. bulgaricus ATCC 11842 as a high DLA producer with exceptional optical purity. However, its low electroporation efficiency limited genetic modifications. To address this, random mutagenesis using UV irradiation and chemical mutagenesis was applied, leading to a mutant strain with a 97% increase in DLA production and a 37% enhancement in glucose uptake rates. Further optimization of fermentation parameters using One-Factor-At-a-Time (OFAT) and Response Surface Methodology (RSM) achieved a 300% improvement in DLA titres compared to the wild-type strain. Fermentation using whey permeate as

an economical carbon source yielded DLA with 99.09% optical purity. The purified DLA was characterized using Fourier Transform Infrared (FTIR) and proton Nuclear Magnetic Resonance (NMR) spectroscopy, confirming its chemical equivalence to commercial standards. Beyond DLA biosynthesis, the metabolic engineering strategies and molecular tools developed in this study provide a robust framework for further exploration of L. bulgaricus as a versatile host for therapeutic and probiotic applications. This work advances the industrial-scale production of DLA, contributing to sustainable and innovative biotechnological solutions.