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Probiotic Lactic Acid Bacteria with Nitric Oxide Synthase Activity: A Promising Approach for Cardiovascular Health

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Abstract

Lactic acid bacteria (LAB) are widely recognized for their probiotic properties and potential health benefits. In humans cardiovascular diseases (CVDs) are a major cause of morbidity and mortality worldwide, necessitating the exploration of natural preventive strategies (World Health Organization, 2023). Recent investigations have highlighted the potential of probiotic LAB, particularly *L. plantarum* in promoting cardiovascular health through multiple mechanisms, including modulation of the gut microbiota, reduction of serum cholesterol levels, anti-inflammatory effects, antioxidant activity, antihypertensive action, glucose regulation, and nitric oxide (NO) production via nitric oxide synthase (NOS) activity. *L. plantarum* scavenges reactive oxygen species and enhances the activity of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), thereby mitigating oxidative stress and endothelial dysfunction. It also exerts lipid-lowering effects by producing bile salt hydrolase (BSH), modulating lipid metabolism via AMPK and PPARα pathways, and synthesizing conjugated linoleic acid (CLA). Furthermore, it contributes to blood pressure regulation through angiotensin-converting enzyme (ACE) inhibitory activity and γ-aminobutyric acid (GABA) production. The interaction of LAB with the gut microbiota enhances glucose uptake and reduces systemic inflammation—key factors in managing diabetes-related cardiovascular risks. Specific LAB strains, such as *L. plantarum*, *Lactobacillus fermentum*, and *Lactobacillus rhamnosus* have demonstrated resilience during gastrointestinal transit and the ability to exert beneficial immunomodulatory effects. In addition to their physiological benefits, LAB strains are also widely used in the fermentation of dairy, cereal, vegetable, and meat-based foods, enhancing their functional properties and serving as accessible dietary strategies to improve cardiovascular outcomes. This review explores the promising role of LAB as probiotics for cardiovascular health and outlines directions for future research

Keywords: Lactic Acid Bacteria (LAB), Cardiovascular Diseases (CVDs), Nitric Oxide Synthase (NOS), Probiotics, Functional Food, Gut Microbiota, Endothelial Function.

Introduction

Probiotics particularly Lactic acid bacteria (LAB) have earned significant attention for their beneficial effects on human health. Microorganisms such as *Lactobacillus planetarium*, *Lactobacillus fermentum* and *Lactobacillus rhamnosus*, found in fermented food having various therapeutic properties, including the enhancement of gut microbiota, boosting immune functions, and improving cardiovascular health (Ghosh and Chattopadhyay, 2013). Cardiovascular diseases (CVDs) represent a significant health burden globally, accounting for millions of deaths annually (Mensah et al., 2020). Probiotics, particularly LAB, have emerged as promising dietary interventions for cardiovascular disease management (Lordan et al., 2018). The ability of LAB to produce nitric oxide synthase, an enzyme play role in synthesis of Nitric Oxide, has opened up a promising avenue for their use as functional foods aimed at preventing and managing cardiovascular diseases. The primary objective of this review is to explore the isolation and characterization of LAB strains that exhibit nitric oxide synthase activity and their potential role in promoting cardiovascular health. We will also discuss the various methods used to assess the functional properties of LAB, including cell adhesion assays and cholesterol reduction assays, which contribute to their overall health benefits.

Methodology

This review employed a systematic and narrative approach to compile, synthesize, and evaluate the current literature on the cardioprotective mechanisms of *Lactiplantibacillus plantarum* and its application in fermented foods. The research focused on peer-reviewed articles, clinical studies, in vitro and in vivo experiments, and reviews published between 2000 and 2024. Relevant databases—including PubMed, Scopus, Web of Science, and Google Scholar—were searched using a combination of keywords such as “*Lactiplantibacillus plantarum*,” “cardiovascular health,” “antioxidant activity,” “cholesterol metabolism,”

“Blood pressure regulation,” “nitric oxide,” “GABA,” “fermented foods,” and “probiotics.” Boolean operators (AND, OR) were used to refine the search and capture comprehensive results. Inclusion criteria comprised studies focused on the probiotic activities of *L. plantarum* strains with relevance to cardiovascular parameters, such as oxidative stress markers, lipid profile modulation, ACE inhibition, and glucose metabolism. Studies detailing the use of *L. plantarum* in fermented food systems were also included (Marco et al., 2017; Cichońska et al., 2022). Clinical and preclinical studies demonstrating biochemical pathways, such as AMPK, PPAR α , and bile salt hydrolase-mediated cholesterol regulation, were prioritized (Jeong et al., 2018; Bermudez-Brito et al., 2012).

Reference management and duplicate removal were performed using Zotero and EndNote. Articles were critically analyzed for relevance, scientific rigor, and contribution to understanding the cardioprotective potential of *L. plantarum*. This integrative methodology ensures a comprehensive overview of the strain’s mechanisms and supports evidence-based conclusions regarding its role in cardiovascular disease prevention and functional food development.

Probiotic LAB-mediated Modulation of Nitric Oxide Pathways and Cardiovascular Benefits:

Nitric oxide (NO) plays an indispensable role in cardiovascular physiology by regulating vasodilation, inhibiting platelet aggregation, and preventing smooth muscle proliferation. Dysregulation of NO production is a hallmark of several cardiovascular pathologies, including hypertension and atherosclerosis. Emerging evidence suggests that probiotic LAB strains can influence NO metabolism either through direct nitric oxide synthase (NOS)-like activity or by modulating host endothelial NOS (eNOS) expression.

Certain LAB strains, notably *Lactobacillus plantarum* and *Lactobacillus fermentum*, have demonstrated the ability to produce NO under specific environmental conditions, such as in the presence of nitrate and nitrite. Additionally, LAB can release bioactive peptides during fermentation that stimulate endothelial NO production, contributing to improved vascular function. This dual mechanism—direct microbial NO production and host modulation—positions LAB as a valuable adjunct in cardiovascular therapy.

Furthermore, the anti-inflammatory properties of probiotic LAB reduce oxidative stress and systemic inflammation, indirectly preserving NO bioavailability. The strengthening of intestinal barrier function also minimizes endotoxemia, a critical factor implicated in endothelial dysfunction. Collectively, these probiotic-mediated effects converge to promote cardiovascular resilience. Probiotic Studies on animals and humans evidences that they have a crucial role in modulation of immune and inflammatory pathways. The benefits of probiotics decided by other parameters like Diet, age of person, BMI i.e. body mass index and medicines (Cristofori et al. 2021). Incorporating LAB strains with proven NOS activity into functional foods or supplements may offer a safe, accessible, and sustainable strategy to combat the rising prevalence of cardiovascular diseases. Use of Kefir in diet containing *Lactobacillus plantarum* shown significant decrease in systolic BP in heavy smokers. In another study consumption of probiotic soy milk enriched with *Lactobacillus plantarum* by a person with clinical type II diabetes mellitus, reported that significantly decrease in systolic/diastolic BP. Spontaneously hypertensive rats (SHR) is genetically hypertensive animal is widely used in understanding of cardiovascular abnormalities. Kefir has been feed to SHR to understand role in protection of vascular endothelial dysfunction and correction of impaired autonomic cardiovascular function, its inhibitory effects on angiotensin-converting enzyme. In another study of Minas Frescal probiotic cheese given to SHR showed a drastically lowered BP, improvement in cardiovascular health parameters such as triglycerides and cholesterol levels in blood as compared to control (Vasquez et al. 2019). Future investigations should aim at strain-specific characterization, dosage optimization, and elucidation of the molecular mechanisms underpinning LAB-induced NO production in vivo.

Role of Nitric Oxide in Cardiovascular Health

Nitric oxide acts as a critical regulator of vascular function by promoting vasodilation, inhibiting platelet aggregation, and preventing leukocyte adhesion (Stuehr, 1999). Insufficient NO levels are linked to the progression of hypertension, atherosclerosis, and endothelial dysfunction (Mensah et al., 2020). Enhancing NO bioavailability through probiotic interventions represents an innovative therapeutic approach.

Probiotic LAB: Mechanisms of Cardiovascular Protection

LAB exhibits multiple mechanisms beneficial for cardiovascular health. LAB assist in lowering serum cholesterol by assimilating cholesterol during growth (Gilliland et al., 1984), deconjugating bile salts (Moser & Savage, 2001), and converting cholesterol into coprostanol. LAB strains with NOS activity contribute to NO production and blood pressure regulation (Stuehr, 1999; Qi et al., 2021). LAB exert antioxidant and anti-inflammatory effects that protect vascular integrity (Gunathilake et al., 2018). LAB promote beneficial microbiota composition, strengthen the gut barrier, and reduce systemic inflammation (Ouweland & Salminen, 2003).

Natural Sources of LAB with Cardiovascular Benefits

Beetroot provides a nitrate-rich environment that enhances NO production. LAB strains isolated from beetroot may offer synergistic cardiovascular benefits (Chen et al., 2021). Milk and curd serve as traditional sources of probiotic LAB with cholesterol-lowering and antioxidant properties (Ghosh & Chattopadhyay, 2013). Idli batter contains diverse LAB strains beneficial for cardiovascular health (Ghosh & Chattopadhyay, 2013).

Functional Evaluation of LAB Strains

Key functional assessments included safety evaluations through hemolysis and antibiotic susceptibility profiling (Abdel Tawab et al., 2022), acid, bile, and NaCl tolerance tests (Gilliland et al., 1984), cell surface hydrophobicity, autoaggregation, and coaggregation (Polak-Berecka et al., 2014), survivability in simulated gastric

and intestinal conditions (Botta et al., 2014), adhesion capacity to epithelial cells (Ouwehand & Salminen, 2003), and antimicrobial activity evaluation (Mounyr et al., 2016).

NOS Activity Assessment in LAB

Quantification of NO production was performed via the Griess assay (Griess, 1879). It is simple and widely used for analysis of NO production in biological samples like CSF, plasma (serum), urine, saliva and in cell cultures. The principle of test is based on reaction when nitrite is treated with a sulfanilamide (a diazotizing reagent) in acidic media to form a transient diazonium salt, it is an intermediate compound is allowed to react with N-naphthyl-ethylenediamine (a coupling reagent) to form purple colored azo compound. The absorbance of this azo product is measured at 540 nm is directly proportional to the nitrite concentration in the test sample. The nitrite assay is highly sensitive, measure nitrite concentration as low as ~0.5 µM level. The increased assay demand for the nitrate and nitrite analysis in biological samples, number of companies such as Cayman Chemical, Oxford Biomed. Research, Inc., Roche Diagnostics GmbH, Phoenix Pharmaceutical and many more made. Griess Reaction assay kit available to researchers in recent years (Sun, J et al. 2003).

Endothelial cell-based evaluations (Stuehr, 1999). Strains with significant NOS activity were identified through 16S rRNA sequencing (Lane, 1991). In silico analyses for pathogenicity and genome stability were conducted using PHASTER and CRISPR Cas Finder tools (Arndt et al., 2016; Couvin et al., 2018, Yin, Y et al., 2019)

Nutraceutical Properties of LAB

LAB strains demonstrated multiple nutraceutical properties including antioxidant activities (Kim et al., 2022), anti-inflammatory effects (Gunathilake et al., 2018), antidiabetic activities (Kazeem et al., 2013), gut health promotion (Ouwehand & Salminen, 2003), anti-obesity and anti-aging potential (Álvarez-Arraño & Martín-Peláez, 2021), and anti-infection properties (Ouwehand & Salminen, 2003).

Conclusion and Future Perspectives:

The reviewed studies suggest that probiotic LAB strains with NOS activity hold immense promise for cardiovascular disease prevention. Utilizing traditional fermented foods as LAB reservoirs offers an accessible and culturally relevant intervention strategy. Future research should prioritize clinical validations, the exploration of synergistic interactions with nitrate-rich foods, and the development of LAB-enriched functional foods.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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