

**THE ANTI-*Escherichia coli* EFFECT OF *Lactobacillus rhamnosus* IN THE PRESENCE OF POLYETHYLENE GLYCOL, GLYCERO-GELATIN AND THEOBROMA**

**BY**



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**NOVEMBER, 2025.**

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**A RESEARCH WORK SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF  
PHARMACY DEGREE BY THE FACULTY OF PHARMACY  
UNIVERSITY OF BENIN**

**NOVEMBER, 2025.**

## DECLARATION

I, **OTARU TESTIMONY AFIGIE** with matriculation number **PHA1908594** hereby declare that the project work entitled **THE ANTI-*Escherichia coli* EFFECT OF *Lactobacillus rhamnosus* IN THE PRESENCE OF POLYETHYLENE GLYCOL, GLYCERO-GELATIN AND THEOBROMA** is the original work carried out by me under the supervision of Dr E. Oloton in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin, Benin-city, Nigeria.

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**OTARU TESTIMONY AFIGIE**

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**DATE**

## **CERTIFICATION**

This is to certify that this is an original research work carried out by **OTARU TESTIMONY AFIGIE** in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin, in partial fulfillment of the requirements for the award of the Doctor of Pharmacy (PharmD) degree.

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**DR E. OLOTON**  
**(PROJECT SUPERVISOR)**

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**DATE**

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**DR (MRS) UPE BABAIWA**  
**(HEAD OF DEPARTMENT)**

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**DATE**

## **DEDICATION**

This project work is dedicated to God for his grace and strength throughout this project work and to all the healthcare professionals and healthcare professionals in training who given up part of their lives so that they can save the lives of others.

## **ACKNOWLEDGEMENT**

I express my gratitude to God and to everyone who contributed to the success of this project. I appreciate the guidance and support of my supervisor, Dr. E. Oloton, whose expertise and encouragement were invaluable.

My sincere gratitude also goes to my parents, Mr. and Mrs. Otaru for their prayers, advice and financial support during the course of this program.

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## ABSTRACT

This study examined the modulation of *Escherichia coli* growth by *Lactobacillus rhamnosus* in the presence of polyethylene glycol (PEG), glycerol-gelatin, and Theobroma. Growth kinetics were analysed using regression models of colony-forming unit counts over time. In monoculture, *E. coli* exhibited exponential growth ( $y = 6.6 + 0.21x$ ,  $R^2 = 0.874$ ). Co-culture with *L. rhamnosus* significantly suppressed *E. coli* proliferation ( $y = 5.97 + 0.02x$ ,  $R^2 = 0.324$ ;  $p < 0.001$ ). The inclusion of glycerol-gelatin moderately stabilized this inhibition ( $y = 5.93 + 0.03x$ ,  $R^2 = 0.462$ ;  $p < 0.001$ ), while PEG produced a synergistic effect, completely halting *E. coli* growth ( $y = 4.46 - 0.003x$ ,  $R^2 = 0.005$ ;  $p < 0.001$ ). In contrast, Theobroma reversed inhibition ( $y = 8.13 + 0.17x$ ,  $R^2 = 0.679$ ;  $p = 0.436$ ). These results indicate that physicochemical conditions strongly influence probiotic-pathogen interaction, with PEG amplifying inhibition, glycerol-gelatin enhancing stability, and Theobroma antagonizing probiotic action against *Escherichia coli*.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background of the study

Aerobic vaginitis is an inflammatory condition of the vagina associated with a change in the normal microbial flora and the proliferation of aerobic microorganisms. It is primarily linked to bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Streptococcus viridans*, and *Staphylococcus epidermidis*. The condition represents a disruption of the protective *Lactobacillus*-dominated flora, resulting in the dominance of aerobic commensal or pathogenic bacteria that trigger mucosal irritation and inflammation. Clinically, aerobic vaginitis presents with characteristic symptoms that include yellowish vaginal discharge, erythema of the vaginal mucosa, itching, burning sensations, and an elevated vaginal pH, typically around 5. Inflammatory responses are evident through leukocytic infiltration and the presence of sticky secretions. The condition occurs most frequently among women of reproductive age, particularly within the 15–45-year range (Achdiat *et al.*, 2025).

The disorder has been identified as a notable cause of vaginal discharge and is associated with adverse reproductive outcomes, including premature rupture of membranes, spontaneous abortion, preterm delivery, and stillbirth (Ma *et al.*, 2022). These complications are attributed to the inflammatory environment and microbial imbalance that compromise the integrity of the vaginal and cervical mucosa.

Probiotics are often used in the treatment of Aerobic Vaginitis since they directly antagonize the causative organisms.

## **1.2 Aim of study**

The aim of the study is to determine the anti-*Escherichia coli* effect of *Lactobacillus rhamnosus* in the presence of Polyethylene glycol, Glycerogelatin and Theobroma.

## **1.3 Objectives of the Study**

1. To determine the growth characteristics of *Escherichia coli*.
2. To investigate the growth characteristics of *Escherichia coli* and *Lactobacillus rhamnosus*.
3. To investigate the growth characteristics *Escherichia coli* in the presence of *Lactobacillus rhamnosus*, Glycerogelatin, Polyethylene Glycol and Theobroma respectively.
4. To determine which of the bases would best for the formulation of *Lactobacillus rhamnosus* pessaries.

## **1.4 Justification of the Study**

In recent years, probiotics have gained increasing scientific and clinical attention due to their various health-promoting properties, particularly their role in maintaining intestinal microbial balance, modulating immune responses, and preventing or mitigating pathogenic infections. As antimicrobial resistance continues to escalate globally, the search for alternative or complementary therapeutic approaches has become a pressing public health priority. Probiotics

when administered in the right concentration at the right site of action, present a promising avenue for addressing this challenge. However, the efficacy and stability of probiotic action are strongly influenced by environmental factors and formulation components. Understanding how excipients and carriers modulate the antimicrobial efficacy of probiotics is therefore essential for optimizing their therapeutic potential.

Polyethylene glycol (PEG), Theobroma, and Glycerogelatin are widely used excipients in pharmaceutical and food formulations, yet their interactions with probiotics remain insufficiently characterized.

Examining the effects of these substances on probiotic antimicrobial activity is of critical importance, as formulation components may significantly alter probiotic performance in vivo. Understanding these interactions could guide the rational design of probiotic-based therapeutic systems and functional foods with improved stability, bioactivity, and clinical efficacy. This study therefore seeks to evaluate the influence of polyethylene glycol, Theobroma, and Glycerogelatin on the antimicrobial activity of *Lactobacillus rhamnosus*, a known probiotic. The findings are expected to contribute to the broader understanding of excipient–microbe interactions, providing valuable information for developing optimized probiotic formulations and new antimicrobial strategies.

## **1.5 What are Probiotics?**

Probiotics are defined as live microorganisms which, when administered in adequate quantities, confer health benefits upon the host. This definition is widely accepted and endorsed by both the Food and Agriculture Organization of the United Nations (FAO) and the World Health

Organization (WHO) and demonstrates the importance of viable microbial populations in promoting host well-being (Bodke & Jogdand, 2022; Gan *et al.*, 2021).

Commercially available probiotic formulations consist primarily of strains belonging to the *Bifidobacteriaceae* and *Lactobacillaceae* families. These organisms are commonly incorporated into fermented food products and nutraceutical preparations due to their established safety profile and historical usage (Zaccaria *et al.*, 2023). Beyond their conventional role as dietary supplements, probiotics have also been investigated for their potential prophylactic and therapeutic applications across both gastrointestinal, vaginal and systemic disease conditions. Such conditions include, but are not limited to, obesity, type 2 diabetes mellitus, aerobic vaginitis, inflammatory disorders, colorectal cancer, and various forms of infectious diarrhoea (Asgari *et al.*, 2020).

### **1.5.1. Organisms Known to be Probiotics**

Probiotic microorganisms may be categorized based on their taxonomic hierarchy; specifically genus, species, and strain, with distinct groups demonstrating well-characterized health-promoting effects. The main groups recognized for probiotic application include the following:

#### **1. *Lactobacillus***

Members of the genus *Lactobacillus* are among the most extensively studied and commercially utilized probiotic bacteria. Characterized by their lactic acid production, these organisms contribute to maintaining an acidic gut environment, thereby inhibiting the proliferation of pathogenic microorganisms (Sadaqat., 2024). Common probiotic species within this genus include:

- *Lactobacillus acidophilus*

In 1900, Moro first isolated the bacterium from infant feces, initially naming it *Bacillus acidophilus*. However, in 1970, Hansen and Møcquot revised its classification, renaming it *Lactobacillus acidophilus* (Anupama, 2021). Since its initial discovery, extensive investigations have elucidated various biological characteristics and functional attributes of this species. *L. acidophilus* comprises numerous strains, such as LA-1, LA-5, NCFM, ATCC 4356, and DDS-1 each exhibiting distinct probiotic capacities and physiological functions (Goa *et al.*, 2022).

*Lactobacillus acidophilus*, is a Gram-positive, non-spore-forming bacillus. Morphologically, the organism appears as slender rods with rounded termini, typically ranging from 2 to 10 µm in length (Goa *et al.*, 2022). Most strains of *L. acidophilus* exhibit microaerophilic growth preferences, displaying enhanced growth under anaerobic conditions or in atmospheres containing 5–10% CO<sub>2</sub>, in contrast to standard aerobic environments.

The optimal cultivation temperature for *L. acidophilus* generally lies between 35°C and 38°C, while temperatures below 20°C are largely non-conducive to growth (Gao *et al.*, 2022). The species demonstrates limited thermal tolerance and exhibits optimal growth in mildly acidic conditions, with a preferred pH range of 5.5 to 6.0. Although slight variations in growth parameters exist among different strains, *L. acidophilus* is broadly characterized by its acidophilic nature and its capacity to tolerate both acidic pH and bile salts. These traits enable it to colonize ecological niches where other lactic acid bacteria (LAB) may be unable to survive. It is metabolically versatile, capable of utilizing glucose, fructose, lactose, and sucrose through homolactic fermentation pathways, and it predominantly produces DL-lactic acid as a terminal metabolite (Tang *et al.*, 2023).

- ***Lactobacillus delbrueckii***

*Lactobacillus delbrueckii* is composed of six subspecies, *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis*, *L. delbrueckii* subsp. *jakobsenii*, *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *sunkii*, and *L. delbrueckii* subsp. *Indicus* (Rizzello & De Angelis, 2022).

*Lactobacillus delbrueckii* was originally described by Leichmann in 1896 under the designation *Bacillus delbrueckii*. It was subsequently reclassified by Beijerinck in 1901 as *Lactobacillus fermentum* var. *delbrueckii*. Later, the Judicial Commission of the International Committee on Systematics of Prokaryotes (ICSP) ruled that the name *Lactobacillus delbrueckii* Beijerinck 1901 should be published as a distinct species (Baek *et al.*, 2023).

The 16S rRNA gene sequence similarity among the six recognized subspecies of *L. delbrueckii* ranges from 99.21% to 99.54%, reflecting their close phylogenetic relatedness (Al-Mujahidy 2024). However, the majority of industrial applications focus on two subspecies in particular: *L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *lactis*.

*L. delbrueckii* subsp. *bulgaricus* is widely used in combination with *Streptococcus thermophilus* for the commercial production of yogurt and various cheeses (Ge *et al.*, 2024). In contrast, *L. delbrueckii* subsp. *lactis* is primarily employed in the manufacture of hard cheeses. Due to their significance in food biotechnology, the genetic bases underlying the metabolic distinctions between these two subspecies have been elucidated through comparative genomic analyses.

A primary differentiating feature lies in the genes associated with carbohydrate metabolism. *L. delbrueckii* subsp. *bulgaricus* exhibits a comparatively narrow metabolic profile, predominantly fermenting lactose along with a limited range of other carbohydrates. Conversely, *L. delbrueckii*

subsp. *lactis* displays broader fermentative capabilities, including the utilization of various plant-derived sugars such as maltose, mannose, sucrose (saccharose), and trehalose (Baek *et al.*, 2023).

- ***Lactobacillus plantarum***

*Lactobacillus plantarum* is characterized by its nonmotile and non-spore-forming nature. While species within the genus *Lactobacillus* are generally classified as catalase-negative, certain strains of *L. plantarum* have demonstrated true catalase activity as well as manganese-dependent pseudocatalase activity under specific conditions (Garcia-Gonzalez *et al.*, 2021). These enzymatic features, although atypical for the genus, have been documented in various strains that continue to be classified within the *L. plantarum* species, despite exhibiting traits such as nitrate reduction and pseudocatalase activity that deviate from the expected genus-level characteristics.

Morphologically, *L. plantarum* cells are rod-shaped with rounded ends, measuring approximately 0.9–1.2 µm in width and 3.0–8.0 µm in length (Sahu, 2025). These cells may appear singly, in pairs, or form short chains. The composition of the cell wall includes teichoic acids based on either ribitol or glycerol, and the peptidoglycan structure incorporates diaminopimelic acid (DAP), which is notable in distinguishing its taxonomic position (Martínez *et al.*, 2020). The organism requires specific nutritional supplements for optimal growth, including amino acids, vitamins, metal ions, buffers, and other compounds (Zhang *et al.*, 2021). *L. plantarum* has an optimal growth at 37 °C and also low pH tolerance (2.0–3.5) (Katiku *et al.*, 2022).

Phenotypic heterogeneity is a well-documented characteristic of *L. plantarum*, contributing to taxonomic ambiguity, particularly in distinguishing it from the closely related species *Lactobacillus pentosus*. To address this issue, molecular typing techniques, most notably

randomly amplified polymorphic DNA PCR, (RAPD-PCR), have been employed for both species' differentiation and strain-level identification within *L. plantarum* (Tsai & Lai, 2024).

Ecologically, *L. plantarum* is primarily associated with plant-derived substrates and is frequently isolated from fermented plant products such as silage, sauerkraut, pickled vegetables, sourdough and Nigerian ogi. However, its occurrence is not limited to plant materials; it has also been isolated from animal-derived sources and diverse environments including cow dung, dairy products, meat and fish products, as well as various human-associated niches such as the oral cavity, vaginal microbiota, gastrointestinal tract, feces, and sewage (Yilmaz *et al.*, 2022). Although generally regarded as a commensal or beneficial microorganism, certain strains of *L. plantarum* have been implicated in opportunistic infections, including subacute bacterial endocarditis, systemic septicemia, and localized abscess formation (Ioannou *et al.*, 2024; Rossi *et al.*, 2024).

- ***Lactobacillus crispatus***

The species name *Lactobacillus crispatus* is derived from the Latin term *crispatus*, meaning "curled," in reference to the bacterium's morphology (Zheng *et al.*, 2020). *L. crispatus* is a lactic acid-producing bacterium and a common inhabitant of the lower female reproductive tract, where it contributes significantly to the maintenance of vaginal health (Zheng *et al.*, 2021). Through the production of lactic acid, it reduces the local pH, thereby creating a hostile environment for pathogenic microorganisms. This acidification mechanism is a key factor in its ability to prevent infections.

In addition to its role in female reproductive health, *L. crispatus* has been identified as a prevalent microorganism in semen, where it is believed to influence sperm quality positively.

Specifically, its presence has been associated with improvements in sperm concentration and morphology (Jayamurali *et al.*, 2023). It also contributes to the maintenance of a stable microbial ecosystem within the semen, a factor that may support overall male fertility. Consequently, probiotic strains such as *L. crispatus* and *Lactobacillus rhamnosus* have been employed to enhance semen quality, particularly in cases of infertility.

The microbial composition of the vagina is sensitive to a variety of physiological and external factors, including hormonal changes, immune modulation, and antimicrobial usage. Among the vaginal lactobacilli, *L. crispatus* is notable for its production of robust amounts hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a compound that further contributes to its antimicrobial defense mechanisms (Miko & Barakonyi, 2023).

Taking advantage of its protective properties, a live biotherapeutic product containing *L. crispatus*, known as LACTIN-V (also referred to as CTV-05 or *Lactobacillus crispatus* CTV-05), has been developed for potential clinical application. Administered intravaginally, LACTIN-V is under investigation for the prevention of bacterial vaginosis and urinary tract infections (UTIs) (Armstrong *et al.*, 2022). It is described as the first live biotherapeutic product designed specifically for the restoration and stabilization of the vaginal microbiome.

The depletion of H<sub>2</sub>O<sub>2</sub>-producing *Lactobacillus* species, including *L. crispatus*, has been strongly associated with an increased risk of bacterial vaginosis and UTIs. However, most commercially available probiotic formulations do not contain vaginal strains and therefore may not effectively improve genitourinary health outcomes. In contrast, LACTIN-V has demonstrated promising results in clinical studies. According to the 2021 Sexually Transmitted Infections Treatment Guidelines issued by the United States Centers for Disease Control and Prevention (CDC),

intravaginal administration of LACTIN-V significantly reduced the recurrence of bacterial vaginosis following antibiotic therapy with metronidazole.

As of July 2025, LACTIN-V is undergoing clinical trials for the treatment of UTIs and bacterial vaginosis and is not yet commercially available (Hemmerling *et al.*, 2025). The product was originally developed by GyneLogix and is currently under further development by Osel Inc., in collaboration with the National Institute of Allergy and Infectious Diseases and other research institutions.

- ***Lactobacillus gasseri***

*Lactobacillus gasseri* was first identified in 1980 by François Gasser and colleagues (LPSN, 2025). It is considered a normal commensal organism in the human gastrointestinal tract and the lower reproductive tract of healthy women. Along with *Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Lactobacillus iners*, *L. gasseri* constitutes one of the four predominant species that are naturally found in a healthy vagina.

Functionally, *L. gasseri* exhibits a range of probiotic activities that have been proven by both genomic and clinical studies. These include the production of antimicrobial peptides such as gassericin A, a bacteriocin with inhibitory effects on pathogenic microorganisms, and lactocillin, a ribosomally synthesized antimicrobial compound (Taghizad *et al.*, 2021). These bacteriocins contribute to *L. gasseri*'s ability to maintain microbial homeostasis within host environments.

The probiotic potential of *L. gasseri* results from several niche-specific adaptations that enhance its colonization and persistence in human mucosal surfaces. These include acid tolerance, resistance to bile salts, and a strong adhesion capacity to epithelial cells. Such adaptations not only facilitate its survival in the gastrointestinal tract but also enhance its therapeutic utility.

Clinically, *L. gasseri* has demonstrated several health-promoting effects. These include the regulation of gastrointestinal function, promotion of vaginal health, immunomodulation of both innate and adaptive immune responses, and antioxidative activity. Additionally, *L. gasseri* has been observed to be involved in metabolic regulation, including the mitigation of obesity and related metabolic disorders.

One well-characterized strain, *L. gasseri* BNR17, was originally isolated from human breast milk. Preclinical studies using animal models have indicated that BNR17 may exert anti-obesity and anti-diabetic effects (Lee *et al.*, 2022). These findings support its investigation as a metabolic probiotic, though further clinical research is necessary to validate efficacy in humans.

The therapeutic potential of *L. gasseri* has also been explored in the context of liver disease. Fatty liver, a prevalent hepatic condition globally, has been associated with dietary and metabolic factors, including high cholesterol intake. Using a zebrafish larvae model, a recent study investigated the effects of *L. gasseri* on high-cholesterol diet (HCD)-induced hepatic injury (He *et al.*, 2023). The study assessed a range of pathological indicators, including liver morphology, lipid accumulation, oxidative stress, and inflammatory responses. Notably, administration of *L. gasseri* was found to reduce morphological and histological damage, reduce lipid deposition, suppress oxidative stress, and limit macrophage aggregation in hepatic tissue.

- ***Lactobacillus jensenii***

*Lactobacillus jensenii* was first identified in 1969 by F. Gasser, M. Mandel, and M. Rogosa. Although it shares several phenotypic characteristics with *Lactobacillus leichmannii*, the two species were distinguished through gel electrophoretic analysis of their lactic dehydrogenase

enzymes. The species was named in honor of Sigurd Orla-Jensen, a prominent Danish microbiologist and pioneer in the field of biotechnology (Zheng *et al.*, 2020).

In terms of ecological distribution, *L. jensenii* is a regular constituent of the female urinary microbiota and has been isolated from women both with and without urinary tract symptoms. Its presence in the urinary tract highlights its potential role in maintaining urogenital health.

*L. jensenii*, plays a role in inhibiting the growth of uropathogens. In particular, *L. jensenii* has demonstrated the capacity to suppress the proliferation of *Escherichia coli* strains implicated in urinary tract infections (UTIs) (Hudson *et al.*, 2020). This antagonistic activity is indicative of the protective function these lactobacilli perform within the urinary tract microenvironment.

- ***Lactobacillus iners***

*Lactobacillus iners*, initially characterized in 1999, constitutes a common member of the vaginal microbiota (Zheng *et al.*, 2021). Unlike other dominant *Lactobacillus* species, *L. iners* demonstrates a complex ecological behavior and an atypical role in vaginal health. One of the most striking features of *L. iners* is its dual presence across both healthy and dysbiotic vaginal states. It frequently dominates in Community State Type (CST) III, which is generally considered a *Lactobacillus*-dominated, low-diversity state (Dong *et al.*, 2024). However, it is also commonly observed in CST IV, characterized by a diverse microbial composition often associated with bacterial vaginosis and elevated vaginal pH. Unlike *L. crispatus*, *L. jensenii*, and *L. gasseri*, which are strongly negatively correlated with bacterial vaginosis, *L. iners* can coexist with bacterial vaginosis-associated organisms and is frequently detected during episodes of dysbiosis.

Evidence suggests that *L. iners* may not confer the same degree of protection against vaginal pathogens as other *Lactobacillus* species (Nilsen *et al.*, 2020). Instead, it appears to function as a

transitional species, often emerging as the dominant microorganism following antibiotic treatment for bacterial vaginosis particularly with metronidazole. This temporal dominance may reflect an opportunistic strategy in which *L. iners* colonizes the disturbed vaginal environment but does not necessarily reestablish a protective microbial community.

Further complicating its characterization, *L. iners* has the smallest genome among known *Lactobacillus* species and displays limited metabolic capabilities. Its growth characteristics also distinguish it from other *Lactobacilli*. It does not readily grow on de Man-Rogosa-Sharpe (MRS) agar, a standard medium for *Lactobacillus* culture, but can grow under anaerobic conditions on blood agar (Zheng *et al.*, 2021). As a result, *L. iners* only received significant attention with the advent of molecular methods such as 16S rRNA gene sequencing.

Although *L. iners* possesses some probiotic features, its presence has also been associated with adverse reproductive outcomes, including increased susceptibility to sexually transmitted infections and complications during pregnancy.

- ***Lactobacillus rhamnosus***

*Lactobacillus rhamnosus* also known as *Lacticaseibacillus rhamnosus*, is one of the most extensively studied probiotic strains in the context of women's health. Its beneficial roles in maintaining urogenital health are well recognized, particularly in reducing the recurrence of bacterial vaginosis and urinary tract infections. Clinical use among HIV-positive individuals has also demonstrated its safety, with some reports noting reduced incidences of diarrhea and improvements in CD4 cell counts (Petrova *et al.*, 2021). Unlike many other probiotic strains applied for urogenital purposes, *L. rhamnosus* has been thoroughly investigated not only for its functional properties but also for its genomic composition, metabolic profile, and surface characteristics, all of which contribute to its adaptability and efficacy within the host environment.

*Lactobacillus rhamnosus* is regarded as nomadic microorganism, exhibiting a broad ecological distribution. Isolates have been recovered from a range of habitats including the human gastrointestinal and vaginal tracts. The capacity of individual strains to express specific genes enables adaptation to diverse environmental conditions.

## **2. *Bifidobacterium***

*Bifidobacterium* is a genus of gram-positive, nonmotile, and typically branched anaerobic bacteria. These microorganisms are predominantly found in the gastrointestinal tract (GIT) of mammals. In addition to their presence in the gut, certain strains have also been isolated from other mucosal surfaces, such as the vaginal environment and the oral cavity, for example, *Bifidobacterium dentium* has been identified in the latter.

*Bifidobacterium* species are notable for their health-promoting properties, which have led to their inclusion in many probiotic formulations (Cizeikiene & Jagelaviciute, 2021). Evidence now suggests that many of their beneficial effects are mediated through modulation of the host immune system and attenuation of inflammatory processes. *Bifidobacterium* has been shown to enhance the population of regulatory T cells and to strengthen the integrity of the intestinal epithelial barrier (Gavzy *et al.*, 2023).

Metabolically, *Bifidobacterium* contributes to the gut microbial ecosystem by producing essential metabolites, which are utilized by other commensal bacteria. Through the fermentation of carbohydrates, they generate short-chain fatty acids (SCFAs), primarily acetate and butyrate. These SCFAs play a protective role in host physiology and are involved in the prevention and mitigation of various disease processes (Xiong *et al.*, 2022).

Bifidobacteria exhibit strict nutritional requirements and are highly selective with respect to the composition of their growth media. Optimal cultivation demands the presence of readily assimilable nitrogen sources, a broad spectrum of amino acids, fermentable carbohydrates, unsaturated fatty acids, essential vitamins, and various mineral elements (You *et al.*, 2023).

Species of Bifidobacterium include:

- *Bifidobacterium animalis* subsp. *Lactis*
- *Bifidobacterium longum*
- *Bifidobacterium adolescentis* which is a known producer of nicotinic acid (Zhang *et al.*, 2025)
- *Bifidobacterium breve*

### **3. *Streptococcus* species**

Certain species within the *Streptococcus* genus, particularly *Streptococcus thermophilus* and *Streptococcus salivarius*, are commonly employed in probiotic formulations, especially in the context of fermented dairy products. These species have gained attention for their potential to support gastrointestinal health and influence host immune function, especially since *Streptococcus salivarius* is known to produce antimicrobial peptides.

### **4. *Bacillus* species**

Species within the genus *Bacillus* are increasingly recognized for their probiotic potential, particularly in aquaculture and human health applications. These bacteria are known for their ability to produce a variety of extracellular enzymes and exhibit resilience under extreme environmental conditions, including high temperatures and dehydration. When administered at optimal concentrations in aquatic environments, *Bacillus* strains have been associated with

improvements in the physiological, morphological, hematological, and immunological parameters of aquatic organisms (Monier *et al.*, 2023).

*Bacillus* species are Gram-positive, rod-shaped bacteria, many of which possess the ability to form endospores under adverse conditions. This sporulation capacity, however, varies among strains. While *Bacillus* organisms are predominantly soil-dwelling, they are also frequently found in water, air, and food matrices. Among them, *Bacillus subtilis* and *Bacillus coagulans* are particularly noteworthy for their established probiotic properties. These strains contribute significantly to gastrointestinal health by supporting the native gut microbiota.

A distinguishing feature of *Bacillus subtilis* is its ability to endure transit through the gastrointestinal tract. This is primarily due to its capacity to form spores, which are resistant to extreme temperatures, pH fluctuations, and gastric acidity (Zhang *et al.*, 2020). Upon reaching the intestine, these spores germinate, allowing the bacterium to exert its probiotic effects. Furthermore, *Bacillus subtilis* demonstrates adaptability to both aerobic and anaerobic environments, enhancing its functionality as a probiotic under diverse conditions.

A notable characteristic of many *Bacillus* species is their ability to synthesize antimicrobial peptides, especially bacteriocins (Bahaddad *et al.*, 2023). Bacteriocins produced by *Bacillus* species typically target Gram-positive bacteria by binding to their outer membranes and disrupting membrane biosynthesis. In this context, bacteriocins may also function as colonizing agents, facilitating the establishment of probiotic *Bacillus* strains on the intestinal epithelium, even in the presence of an existing microbial community. These antimicrobial peptides are valued not only for their broad-spectrum antibacterial activity but also for their stability under extreme temperature and pH conditions.

Despite their many beneficial attributes, the safety of *Bacillus* probiotics remains an important concern. Some isolates have been found to harbor antibiotic resistance genes, which are frequently located on plasmids or mobile genetic elements such as transposons and insertion sequences. This raises the possibility of horizontal gene transfer, with implications for both human and animal health (Jin *et al.*, 2025).

Both vegetative cells and spores of *Bacillus* species are used in probiotic formulations. Their inherent resistance to heat, gastric conditions, and moisture contributes to their stability and viability in various commercial applications. Commonly used commercial probiotic strains include *Bacillus cereus*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus polyfermenticus*, *Bacillus pumilus*, and *Bacillus subtilis* (Luise *et al.*, 2022; Payne, *et al.*, 2024). These strains are attributed with diverse health-promoting properties, including antimicrobial, anticancer, antioxidant, and vitamin-synthesizing activities.

Nonetheless, it is important to note that certain *Bacillus* strains may produce toxins and biogenic amines, or carry transferable antibiotic resistance genes. These issues necessitate thorough safety assessments prior to their use in probiotic products. Research exploring the interaction between *Bacillus* probiotics and the human microbiome remains limited. Most existing studies have focused on animal models such as chickens, mice, and pigs. Additionally, several *Bacillus* strains are employed as fermentation starters in plant-based and soybean-derived products, and as carriers in probiotic-enriched baked goods.

## **5. *Pediococcus* species**

Members of the genus *Pediococcus*, classified as lactic acid bacteria (LAB), have gained increasing attention for their probiotic potential across human and animal health sectors.

Traditionally known for their roles in the fermentation of foods, pediococci have been used for centuries in the production of fermented products, contributing to preservation, safety, and organoleptic properties. In modern food processing, these bacteria are employed as starter cultures due to their fermentative capabilities, allowing for the generation of distinct textures and flavors in a variety of food matrices.

Beyond their historical and industrial relevance, pediococci have been proposed as promising probiotic candidates due to their ability to influence host physiology and immune response (Todorov *et al.*, 2022). Their beneficial effects span a range of applications, including immunomodulation, pathogen inhibition, enhancement of health status during and after antibiotic therapy, and improved recovery following viral infections such as COVID-19 (Todorov *et al.*, 2022). These attributes have been documented in both human and veterinary contexts, where strains of *Pediococcus* have demonstrated positive impacts on gastrointestinal health, reduction in respiratory tract infection incidence, and mitigation of lactose intolerance symptoms. In livestock production, pediococci supplementation has also been associated with growth enhancement and improved feed utilization.

A defining probiotic trait of many *Pediococcus* species is their capacity to produce antimicrobial metabolites, notably bacteriocins such as pediocins. These compounds inhibit a range of Gram-positive pathogens, including *Listeria monocytogenes*, and are now being explored as natural bio-preservatives (Khorshidian *et al.*, 2021; Darbandi *et al.*, 2022). Their broad-spectrum antimicrobial activity and stability under food processing conditions make them valuable not only for food safety but also for potential applications in veterinary and human medicine.

Among pediococci, *Pediococcus pentosaceus* has emerged as a particularly promising probiotic strain. This homofermentative, Gram-positive bacterium, taxonomically associated with the

phylum *Firmicutes* and class *Bacilli*, is frequently isolated from fermented foods, animal sources, plants, and fecal matter. *P. pentosaceus* exhibits multiple functional properties, including anti-inflammatory, anticancer, antioxidant, detoxifying, and lipid-lowering effects. In food systems, it enhances flavor and acts as a preservative, while simultaneously preventing colonization by foodborne pathogens such as *Salmonella spp.*, *Escherichia coli*, and *Listeria spp.*

In addition to its antibacterial properties, *P. pentosaceus* also demonstrates antifungal activity, further reinforcing its utility in food preservation and health promotion (de Souza de Azevedo *et al.*, 2020). Its ability to inhibit fungal contaminants places it among the most effective LAB with antifungal capabilities, offering a natural solution to fungal spoilage in food products.

Despite its growing use and recognition as a generally safe organism for consumption, *P. pentosaceus* requires continued evaluation concerning its safety profile. While its bacteriocins and other metabolic products are generally regarded as safe, rigorous assessments, including cytotoxicity assays, are necessary to ensure the absence of antimicrobial resistance genes and other toxicological risks, particularly when considered for therapeutic or clinical applications.

## **6. *Leuconostoc* species**

The genus *Leuconostoc*, comprising heterofermentative cocci primarily associated with plants and, to a lesser degree, with meat, milk, and dairy products, includes approximately eleven recognized species. Among these, *Leuconostoc mesenteroides* with its three subspecies (*mesenteroides*, *cremoris*, and *dextranicum*) and *Leuconostoc lactis* are the most frequently encountered. Taxonomic revisions continue to modify this relatively small genus, particularly as several species currently classified within *Leuconostoc* exhibit limited genotypic homology both with the common species and among themselves.

Recent investigations into the probiotic and antimicrobial properties of *Leuconostoc* strains have highlighted their promising potential in diverse biotechnological and health-related applications. Two strains, *Leuconostoc citreum* KCC-57 and KCC-58, demonstrated notable probiotic capabilities, including strong antibacterial activity against a range of pathogenic microorganisms (Muthusamy *et al.*, 2023). These findings suggest that such strains may serve as viable alternatives to traditional antibiotics for managing infectious agents, thus contributing to the reduction of antibiotic resistance development.

Another strain, *Leuconostoc lactis*, isolated from fish, exhibited several adaptive traits conducive to survivorship and colonization within the gastrointestinal environment. These traits include robust aggregation ability and tolerance to harsh conditions such as acidic pH, bile salts, trypsin, and pepsin exposure, which mimic the gastrointestinal milieu (Gumustop & Ortakci 2022). The strain also demonstrated inhibitory effects against pathogenic bacteria, low susceptibility to antibiotics, and effective coaggregation with pathogens, indicating its probiotic potential. These characteristics underscore the suitability of *Leuconostoc lactis* as a candidate probiotic strain for marine aquaculture, where effective pathogen control and gut health maintenance are critical.

Advancing the exploration of novel lactic acid bacteria (LAB), a strain identified as *Leuconostoc mesenteroides* I1/53 was isolated from sugarcane juice and characterized using 16S rRNA gene sequencing. Comprehensive safety assessments revealed that this strain lacks antibiotic resistance genes and virulence factors, supporting its safety profile for probiotic use. Genomic analysis further identified numerous genes associated with adaptation and stress tolerance to the human gastrointestinal environment, alongside genes encoding antioxidant and antibacterial activities. Notably, the presence of a secondary metabolite gene cluster suggested the capacity to synthesize bacteriocin lactococcin G via the expression of the *lagD* gene (Su *et al.*, 2024).

Experimental validation confirmed this strain's broad-spectrum antibacterial activity against multiple foodborne pathogens.

During fermentation processes, *L. mesenteroides* I1/53 influenced the chemical composition of sugarcane juice by increasing concentrations of organic acids, phenols, flavonoids, alkaloids, terpenoids, lignans, and coumarins, while reducing aromatic compounds. These modifications enhance the nutritional and functional properties of the fermented product, suggesting valuable applications in food fermentation. Collectively, these findings establish *L. mesenteroides* I1/53 as a safe and effective probiotic strain with notable fermentation capabilities, offering a strong theoretical foundation for its incorporation into food production systems.

Further research into the antagonistic effects of LAB against oral pathogens has identified *Leuconostoc mesenteroides* MJM60376 as a potent inhibitor of *Streptococcus mutans*, a primary etiological agent of dental caries. Isolated from fermented foods, this strain exhibited the highest antagonistic activity against *S. mutans* strains tested (Gu *et al.*, 2023). It also demonstrated key oral probiotic properties such as weak acid production, tolerance to lysozyme, adhesion to oral epithelial cells, susceptibility to antibiotics, and strong coaggregation ability with *S. mutans*. Co-culture experiments revealed a significant reduction in *S. mutans* biofilm formation, which was corroborated by scanning electron microscopy showing diminished bacterial attachment and disruption of biofilm matrix structures.

Importantly, the cell-free supernatant derived from *L. mesenteroides* MJM60376 inhibited biofilm formation during the initial adherent stage and retained its activity following treatment with catalase, trypsin, or pH neutralization (Gu *et al.*, 2023). Gene expression analysis confirmed a marked downregulation of *S. mutans* genes involved in biofilm development when exposed to this supernatant. These various probiotic features position *L. mesenteroides* MJM60376 as a

promising candidate for development as a functional ingredient in oral healthcare products aimed at preventing dental caries.

## **7. *Enterococcus* species**

The genus *Enterococcus* comprises lactic acid bacteria that predominantly inhabit the gastrointestinal tracts of humans and animals. Among these, *Enterococcus faecium* and *Enterococcus faecalis* represent the most prevalent species within the human digestive system. Beyond their commensal presence, enterococci also contribute significantly to the fermentation of various traditional foods, including cheeses and sausages, thereby playing a role in food production and preservation.

Certain strains of *E. faecium* and *E. faecalis* have been developed as probiotics and are widely consumed, often in large quantities via pharmaceutical formulations. These probiotics are administered in clinical and veterinary contexts to address diverse health concerns. In humans, enterococcal probiotics are employed primarily to manage conditions such as diarrhoea, including antibiotic-associated diarrhoea, irritable bowel syndrome, and inflammatory bowel disease, as well as to promote immune system enhancement and cholesterol reduction. In veterinary medicine, they are utilized to prevent or treat diarrhoea, stimulate immune responses, and improve overall growth performance in animals (Krawczyk *et al.*, 2021).

While the beneficial applications of enterococcal probiotics are well recognized, their use raises significant safety considerations related to their intrinsic and acquired characteristics (Im *et al.*, 2023). Enterococci are known to possess virulence factors that can contribute to pathogenicity. These bacteria also possess mobile genetic elements such as plasmids and transposons, that facilitate the horizontal transfer of antibiotic resistance genes among strains and even across

different bacterial species. This process has led to the emergence of clinically relevant antibiotic-resistant enterococci, including vancomycin-resistant enterococci (VRE), which pose serious challenges in healthcare settings.

The commensal nature of enterococci, combined with their capacity for immune system modulation, underlies their probiotic potential. However, this same adaptability also contributes to their role as opportunistic pathogens, especially in hospital environments where outbreaks of enterococcal infections are frequently documented. Their ability to translocate from the gut to other tissues, coupled with virulence traits and antibiotic resistance makes their eradication difficult.

## **8. *Escherichia* species**

While *Escherichia coli* consists of a diverse range of strains, including many pathogenic variants, certain non-pathogenic strains have been incorporated into probiotic formulations due to their beneficial functions. These strains contribute to gut health by synthesizing essential vitamins and supporting the balance of the intestinal microbiota.

One notable example is *E. coli* strain Nissle 1917, which has been utilized as a probiotic and therapeutic agent for over a century. Experimental studies indicate that Nissle provides protective effects against infection by enterohemorrhagic *E. coli* (EHEC) O157:H7 strains. However, it is important to note that mice models exhibit limited susceptibility to O157:H7 infection, and therefore, do not fully replicate the human response to this pathogen. Furthermore, the close genetic relationship between strain Nissle and the uropathogenic *E. coli* (UPEC) strain CFT073 raises concerns regarding the potential pathogenicity of Nissle under certain circumstances (Falzone *et al.*, 2024).

The protective mechanisms attributed to Nissle can be broadly categorized into two main strategies. The first involves modulation of the host's immune and epithelial defenses. For example, the TcpC protein expressed by Nissle enhances epithelial barrier integrity through the activation of host signaling pathways, specifically protein kinase C and extracellular signal-regulated kinase (Helmy *et al.*, 2021). In experimental models of human intestinal organoids co-infected with pathogens, Nissle administration improved barrier function, as evidenced by increased retention of luminal markers and elevated expression of the adhesion molecule E-cadherin. Additionally, Nissle influences immune signaling by modulating the expression of both pro-inflammatory and anti-inflammatory pathways, with some observations suggesting a downregulation of inflammatory responses.

The second strategy involves direct competition with other bacterial strains within the intestinal environment (Pradhan & Weiss, 2020). Nissle exhibits a strong capacity for colonization, supported by multiple mechanisms facilitating adherence to the intestinal mucosa. Its competitiveness is further enhanced by several iron acquisition systems that enable efficient nutrient uptake in a resource-limited environment. Moreover, Nissle possesses genetic determinants encoding antibacterial compounds, including microcin H49, microcin M, and colibactin, which contribute to its ability to inhibit the growth of competing bacteria. For instance, the production of microcin M has been demonstrated to suppress *Salmonella enterica* proliferation in inflamed mouse intestines (Pradhan & Weiss 2020).

## **9. *Saccharomyces* species**

*Saccharomyces boulardii* (*Saccharomyces cerevisiae* var. *boulardii*) is a yeast widely recognized for its probiotic properties, particularly its antimicrobial activity and therapeutic potential in managing various gastrointestinal disorders. As one of the most extensively studied probiotic

strains, *S. boulardii* has been the subject of numerous clinical investigations over several decades, underscoring its significant role in gastrointestinal health (Abid *et al.*, 2022).

Between 1976 and 2015, approximately ninety randomized controlled trials were conducted to evaluate the efficacy of *S. boulardii* across fifteen distinct disease conditions. The strongest evidence supports its use in treating acute pediatric diarrhea, preventing antibiotic-associated diarrhea, managing *Helicobacter pylori* infections, and mitigating the adverse effects associated with *H. pylori* eradication therapies. Additionally, *S. boulardii* shows promising results in treating acute diarrhea in adults and inflammatory bowel disease, although these findings are supported by a smaller number of clinical trials. Other potential therapeutic applications, such as for *Clostridium difficile* infections, giardiasis, traveler's diarrhea, and diarrhea related to enteral nutrition, demonstrate encouraging outcomes but require further rigorous study to confirm efficacy (Shruthi *et al.*, 2022). Across all studied populations and disease conditions, *S. boulardii* was generally well tolerated, indicating a favorable safety profile.

The probiotic functions of *S. boulardii* is as a result of multiple mechanisms. These include cellular adhesion to the gut mucosa, modulation of the gut-brain axis, stimulation of mucin production, antagonism of pathogenic microorganisms, neutralization of toxins, regulation of the resident microbiota, immune modulation, metabolic regulation, alteration of intracellular signaling pathways, physiological protection of the intestinal environment, and competitive inhibition of pathogenic species. Through these diverse mechanisms, *S. boulardii* contributes to maintaining gut homeostasis and defending against infections.

*S. boulardii* demonstrates potent antimicrobial effects against a range of pathogens, which supports its clinical use in conditions such as *Clostridium difficile* infections, inflammatory

bowel diseases, candidiasis, diarrhea, and small intestine bacterial overgrowth, including in patients with multiple sclerosis.

Immunologically, *S. boulardii* acts both as an immune stimulant and as an inhibitor of excessive inflammation. It modulates inflammatory responses by reducing the production of proinflammatory molecules, including cytokine interleukin-8, mitogen-activated protein (MAP) kinases, and signaling through the nuclear factor kappa B (NF- $\kappa$ B) pathway, particularly during infections caused by *Salmonella typhimurium*, *Escherichia coli*, *Clostridium difficile*, and *Candida albicans* (Gao *et al.*, 2021). Moreover, it influences enzyme activity crucial for maintaining gastrointestinal tract health, thereby supporting mucosal integrity and function.

## **1.6 Beneficial Properties of Probiotics**

The physiological effects of probiotics have been extensively documented, particularly in maintaining gastrointestinal homeostasis and modulating immune function. Beyond their established roles in digestion, probiotics exert influence over metabolic processes and contribute to the management of various pathological conditions. The following subsections are the main health-promoting properties attributed to probiotics.

### **1. Gastrointestinal Health**

The gastrointestinal tract constitutes the principal site for probiotic action, where these microorganisms exert various physiological effects. A balanced gut microbiota plays a critical role in maintaining gastrointestinal homeostasis and preventing a spectrum of disorders, notably infectious diarrhoea, irritable bowel syndrome (IBS), and inflammatory conditions such as ulcerative colitis (So *et al.*, 2023). Probiotics contribute to this equilibrium by promoting the growth and multiplication of beneficial commensals while simultaneously suppressing the

growth and colonization of pathogenic microorganisms. Their established functions include the enhancement of digestive processes and the modulation of lipid metabolism, notably through the reduction of serum cholesterol levels (Kumar *et al.*, 2022; Momin *et al.*, 2023). Probiotics are also widely employed in the management of various forms of diarrhoea, including travellers' diarrhoea, diarrhoea associated with antibiotic use, and that induced by rotavirus infection in paediatric populations (Steyer *et al.*, 2022).

Among the complications associated with antibiotic therapy, antibiotic-associated diarrhoea (AAD) is prominent, particularly when the gut microbiome is disrupted. *Clostridioides difficile*, a pathogen that exhibits resistance to carbapenem antibiotics, is frequently implicated in AAD and presents a considerable therapeutic challenge. Probiotic co-administration has been shown to reduce the incidence of AAD, with certain formulations specifically those containing *Lactobacillus rhamnosus* and *Saccharomyces boulardii*, exhibiting high efficacy (Kopacz *et al.*, 2022). Notably, these interventions have been associated with a substantial reduction in AAD occurrence, with no corresponding increase in adverse effects, suggesting a favourable safety and efficacy profile in clinical contexts.

## **2. Immunomodulatory Effects**

Probiotic microorganisms have been extensively studied for their capacity to influence both innate and adaptive branches of the immune system. These beneficial microbes enhance host immunity by stimulating the synthesis of immunoglobulins and cytokines, as well as activating various immune effector cells. Such activity leads to improved resistance to infections and contributes to the attenuation of systemic inflammatory responses (Mazziotta *et al.*, 2023). Certain probiotic strains have further demonstrated the ability to enhance vaccine efficacy which supports their use as vaccine adjuvants (Peroni & Morelli 2021; Abavisani *et al.*, 2024).

### 3. Metabolic and Nutritional Benefits

Probiotic supplementation has gained increasing attention for its role in metabolic regulation, particularly in the context of obesity and type 2 diabetes mellitus (Tao *et al.*, 2020; Mallappa *et al.* 2022). These metabolic disorders are frequently associated with gut microbial dysbiosis, which contributes to their pathophysiology through mechanisms involving inflammation, insulin resistance, and impaired energy homeostasis. Probiotics, through various metabolic and immunomodulatory pathways, appear capable of ameliorating such dysfunctions and may serve as adjuncts in metabolic disease management.

One of the main mechanisms by which probiotics exert their beneficial effects involves the fermentation of dietary fibers, leading to the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Chang *et al.*, 2021). These microbial metabolites play several roles in host physiology. They act as energy substrates for colonocytes, contribute to the maintenance of intestinal barrier integrity, and stimulate the release of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) which are two gut-derived hormones that enhance satiety, delay gastric emptying, and improve glucose metabolism. SCFAs also activate G-protein-coupled receptors such as GPR41 and GPR43, which are involved in regulating systemic energy expenditure, lipid metabolism, and insulin sensitivity. In this manner, SCFAs serve as links between gut microbiota activity and host metabolic regulation, potentially mitigating the risk of cardiovascular and metabolic diseases.

Probiotics may reduce adipogenesis and lipid accumulation by downregulating key transcriptional regulators such as peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and sterol regulatory element-binding protein-1 (SREBP-1) (Zhou *et al.*, 2021). These transcription factors are integral to adipocyte differentiation and triglyceride synthesis.

Suppression of their activity may reduce lipid deposition in adipose tissues, thereby mitigating obesity-related complications. Furthermore, probiotics have been observed to exert anti-inflammatory effects through the reduction of circulating lipopolysaccharides (LPSs) and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ). The resultant decrease in systemic inflammation contributes to improved insulin sensitivity and a reduction in metabolic endotoxemia.

#### **4. Vaginal health**

The maintenance of a stable and acidic vaginal microenvironment is necessary for preventing the proliferation of pathogenic microorganisms, a function primarily attributed to beneficial bacteria, particularly species of *Lactobacillus*. These microorganisms are essential in safeguarding female reproductive tract health. They adhere to the vaginal epithelium, preventing the initial colonization of pathogens through. This defense is further enhanced by the production of key antimicrobial agents, such as lactic acid, which sustains the necessary low pH environment, and bacteriocins and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which directly inhibit the growth of harmful bacteria and yeast. This enables probiotics to be used in both the prevention and treatment of common vaginal infections, including aerobic vaginitis (AV), bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) (Zahedifard *et al.*, 2023).

The supportive role of *Lactobacillus* has also been investigated in complex conditions. During menopause, when declining estrogen levels compromise the integrity of urogenital tissues and increase the susceptibility to infection, probiotics can help mitigate the effects of hormonal changes. Specific probiotic strains such as *L. acidophilus*, particularly in conjunction with ultra-low-dose vaginal estriol, have demonstrated efficacy in alleviating symptoms associated with

Genitourinary Syndrome of Menopause (GSM) by restoring tissue elasticity and the vagina's natural acidity, thereby preventing the overgrowth of opportunistic pathogens (Wu *et al.*, 2024).

Current research also shows a potential role for probiotics in cervical health. The presence of an abundant *Lactobacillus* population is inversely correlated with the detection of high-risk human papilloma virus (HPV) infection, cervical intraepithelial neoplasia, and subsequent cancer development (Mei *et al.*, 2022). As a promising non-chemotherapeutic adjunctive therapy, *Lactobacillus* species have been shown to exert cytotoxic effects on cervical cancer cells through the secretion of metabolites, including peptidoglycan and exopolysaccharides, which inhibit cell proliferation (Mei *et al.*, 2022). This suggests that the administration of vaginal probiotics not only stabilizes the flora and enhances epithelial function but may also influence immune surveillance and directly impede the progression of certain gynecological malignancies.

## **1.7 Safety Concerns of Probiotics**

Although probiotics are generally regarded as safe for the general population, on-going research has highlighted several safety concerns that require careful scrutiny, particularly in clinical and therapeutic contexts. These concerns are due to the intrinsic biological properties of probiotic microorganisms, their interactions with host systems, and the rules that regulate their production and use. The safety profile of a probiotic preparation is determined by several variables, including the specific microbial strain employed, the host's underlying health status, and the environmental or clinical conditions under which administration occurs. The risks and adverse effects associated with probiotic use include:

### **1. Systemic Infections**

One of the most significant safety concerns associated with probiotic use is the potential for systemic infections, particularly in immunocompromised or critically ill individuals (Žuntar *et al.*, 2020). There is accumulating clinical evidence documenting cases of probiotic-associated sepsis, endocarditis, and fungemia, all of which point to the ability of probiotic microorganisms to translocate from their original sites of action into normally sterile anatomical compartments (Katkowska *et al.*, 2021; Liu *et al.*, 2024). These risks are particularly pronounced in hospitalized patients, those undergoing immunosuppressive therapy, and individuals with compromised mucosal integrity.

Although probiotics have demonstrated prophylactic benefits in preventing infections of the respiratory and genitourinary tracts, recent research indicate that they may also act as opportunistic pathogens in certain clinical contexts. Specific probiotic strains have been implicated in the onset of sepsis, bacteremia, and localized infections. For example, while *Bifidobacterium* species are commonly utilized for their health-promoting properties, there are reports linking them to bacteremia under vulnerable physiological conditions. A notable case involved a paediatric patient with severe heart failure who developed *Bifidobacterium* spp. bacteremia following probiotic therapy for antibiotic-associated diarrhoea (Liu *et al.*, 2024).

In addition to the risk of systemic infections, another major safety consideration is the possession of transmissible antibiotic resistance genes by certain probiotic strains. These genes pose a risk of horizontal gene transfer within the host microbiota, potentially contributing to the emergence and dissemination of multidrug-resistant organisms (Zavišić *et al.*, 2023). Such outcomes could seriously undermine the efficacy of conventional antimicrobial therapies, particularly in clinical environments where resistance surveillance and infection control are already challenged.

## **2. Gastrointestinal Effects**

The administration of probiotics has been associated with a range of minor gastrointestinal disturbances. Reported symptoms include abdominal cramping, nausea, soft stools, flatulence, bloating, and alterations in taste perception (Xie et al., 2025). These adverse effects are typically temporary and self-limiting, particularly among healthy individuals. Sensitive populations, including individuals with underlying gastrointestinal disorders, immunocompromised states, or dysregulated gut microbiota, may exhibit heightened susceptibility to these effects. Furthermore, probiotic strains are capable of engaging in metabolic activities that may not always confer benefit to the host. These activities include the generation of toxic metabolites and potential interference with nutrient absorption dynamics, which may result in adverse metabolic outcomes.

### **3. Allergic Reactions**

Although probiotics are widely regarded as safe for the general population, there is documented evidence of adverse immunological responses in certain individuals. Reported allergic manifestations vary in severity, including mild cutaneous hypersensitivity reactions such as urticaria and erythematous rashes, as well as more serious outcomes including asthma, allergic rhinitis, and, in rare instances, anaphylaxis (Xie et al., 2025). These immunological events appear to be modulated by host-specific factors, particularly individual predispositions to atopic conditions, as well as the immunogenic properties of the specific probiotic strain administered.

### **4. Regulatory and Quality Control Issues**

A significant challenge in ensuring probiotic safety lies in the absence of uniform and strict regulatory standards governing probiotic production and labelling. As a result, probiotic products may contain undeclared or misidentified strains, non-viable organisms, or microbial contaminants, thereby compromising product efficacy and safety. Moreover, inconsistencies in

manufacturing quality such as poor strain identification, inadequate storage conditions, and insufficient viability testing have been documented in certain commercial preparations.

The prospect of introducing new genetic elements into the host microbiota via probiotics, including genes conferring antibiotic resistance or metabolic traits, represents an additional biosafety concern, particularly given the increasing use of engineered and next-generation probiotic strains.

## **1.8 Mode of Action of Probiotics**

Probiotics, particularly those belonging to the genera *Lactobacillus* and *Bifidobacterium*, their beneficial effects through multiple, often overlapping, biological mechanisms. These effects are mediated by interactions with the host immune system, intestinal microbiota, and epithelial barrier. The modes of action are:

### **1. Immune Modulation**

Upon reaching the intestinal mucosa, orally administered probiotic microorganisms engage with pattern recognition receptors (PRRs) expressed on epithelial and immune cells. Among these, Toll-like receptors (TLRs) are particularly critical. The interaction between probiotics and TLRs initiates intracellular signaling cascades, notably the nuclear factor-kappa B (NF- $\kappa$ B) pathway (Darma *et al.*, 2020; Paladino *et al.*, 2023). This pathway regulates the transcription of genes involved in immune defense, leading to the production of cytokines, chemokines, and antimicrobial peptides that coordinates both local and systemic immune responses.

Probiotics exert significant antiviral effects through multiple mechanisms. They inhibit viral entry and replication within host cells, enhance immune surveillance, and suppress key pro-inflammatory pathways, including the NLRP3 inflammasome. Their activity results in the downregulation of several pro-inflammatory cytokines and mediators. Notably, reductions have been observed in the levels of tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 $\beta$ ) (Vincenzi *et al.*, 2021). Inflammatory mediators such as nitric oxide (NO) and inducible nitric oxide synthase (iNOS), and are also reduced, indicating a broad anti-inflammatory effect (Kang *et al.*, 2021)

Probiotics also modulate additional signaling pathways that are important to immune regulation. These include the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway and the mitogen-activated protein kinase (MAPK) cascade (Shi *et al.*, 2021). Through the regulation of these pathways, probiotics influence the expression of genes responsible for antimicrobial activity and immune modulation, further supporting both mucosal immunity and systemic immune responses.

## **2. Competitive Exclusion of Pathogens**

The major mechanism through which probiotics offer protection against infections is by competitive exclusion. This enables probiotic organisms to inhibit the colonization and proliferation of pathogenic bacteria by occupying shared ecological niches within the gastrointestinal tract and vaginal epithelium (Mei *et al.*, 2022; Vinayamohan *et al.*, 2024)

There are two forms of competitive exclusion: Exploitation competition and Interference competition. Exploitation competition operates indirectly, with probiotic strains rapidly consuming essential nutrients to limit their availability to competing pathogens. This nutrient

depletion restricts the metabolic activity and replication of harmful microorganisms. Interference competition, in contrast, involves direct antagonistic interactions, including the secretion of antimicrobial compounds or the production of surface molecules that inhibit pathogen adherence.

A critical aspect of competitive exclusion involves the capacity of probiotics to adhere to the mucosal surfaces. By attaching to epithelial cells and colonizing mucosal binding sites, probiotic strains effectively block pathogen access to these critical adhesion niches. This occupation not only impedes the initial binding of enteropathogens but also disrupts the cascade of events required for successful colonization and subsequent infection. Some probiotic species enhance this exclusionary process through the production of adhesins and specific receptor-binding proteins, which confer a selective advantage in the colonization of uninhabited or weakly occupied epithelial surfaces (Gorreja & Walker, 2022).

In addition to preventing pathogen attachment, probiotic adherence to the mucosal surface facilitates the formation of biofilms which are structured microbial communities that are attached to epithelial tissues (Yang *et al.*, 2024; Yao *et al.*, 2025). Expansion of adhesive probiotic cells within biofilms can further outcompete non-adhesive or weakly adherent microbial populations, excluding opportunistic or pathogenic bacteria from the niche.

The implications of these processes are significant. Pathogenic bacteria must pass through the mucus layer and adhere to epithelial cells in order to establish infection within the gastrointestinal tract. By occupying these binding sites, probiotics act as a biological barrier, obstructing pathogen access to the host epithelium.

### **3. Production of Antimicrobial Compounds**

Probiotic microorganisms exhibit antimicrobial properties through the biosynthesis of a several inhibitory compounds, which play a central role in their ability to suppress or eliminate pathogenic competitors within the gastrointestinal and other mucosal environments. Among these bioactive agents are organic acids such as lactic and acetic acid, hydrogen peroxide, and proteinaceous bacteriocins (Ismael *et al.*, 2024). These compounds function through distinct but often complementary mechanisms, including acidification of the local environment, disruption of microbial cell membranes, and inhibition of critical enzymatic functions. The resultant effects may be bacteriostatic or bactericidal, depending on the concentration, environmental context, and susceptibility of the target organism.

Lactic acid bacteria (LAB), in particular, are well-characterized producers of antimicrobial metabolites. Their inhibitory activities extend across a range of pathogenic organisms and have been demonstrated in both *in vitro* and *in vivo* settings. For instance, several LAB strains have shown efficacy in suppressing the growth of *Vibrio* species, *Aeromonas hydrophila*, and *Pseudomonas fluorescens* pathogens commonly associated with aquatic infections, suggesting the broad-spectrum applicability of these probiotic agents. The antagonistic effects are often mediated by extracellular products secreted into the culture medium, known collectively as cell-free culture supernatants. These supernatants have been subjected to targeted assays using catalase treatment and pH neutralization to determine the specific contributions of hydrogen peroxide and organic acids, respectively, to their antimicrobial activity. These studies have revealed that hydrogen peroxide, in particular, plays a dominant role in growth inhibition in certain strains, including specific isolates of *Lactobacillus plantarum* (Miko & Barakonyi, 2023).

Beyond their direct antimicrobial actions, probiotics exert a second line of defense by modulating inter-bacterial communication systems. One such system is quorum sensing, a

regulatory network based on cell-density-dependent signalling that controls the expression of genes related to virulence, motility, and biofilm formation in many pathogenic bacteria (Salman *et al.*, 2023). Probiotic strains can interfere with these signalling pathways, thereby altering the expression of pathogenic traits. This disruption reduces the capacity of pathogens to coordinate infection processes and to form stable biofilms, which are frequently resistant to host immune responses and antimicrobial agents.

#### **4. Enhancement Mucosal Integrity**

Another critical function of probiotics is the reinforcement of the mucosal barriers. This is achieved through upregulation of tight junction proteins, increased mucin production, and modulation of epithelial cell turnover, all of which contribute to improved mucosal integrity. A robust epithelial barrier prevents translocation of pathogens and their toxins into systemic circulation, thereby reducing the risk of systemic infections and chronic inflammation (Latif *et al.*, 2023).

### **1.9 Antimicrobial Properties of Probiotics**

Probiotics possess notable antimicrobial properties, contributing significantly to host defence against a wide spectrum of pathogenic microorganisms. Through multiple mechanisms, probiotics prevent infections, reduce microbial pathogenicity, and serve as adjuncts or alternatives to conventional antimicrobial agents (Ali *et al.*, 2023).

#### **Mechanisms of Antimicrobial Action**

##### **1. Metabolite Production**

Probiotic microorganisms synthesize a range of antimicrobial metabolites, including lactic acid, hydrogen peroxide, and bacteriocins. These compounds exert their effects by reducing environmental pH, disrupting microbial membranes, and interfering with essential metabolic pathways in pathogens, thereby creating conditions that are unfavourable for their survival and proliferation (Monika *et al.*, 2021; Sadaqat, 2024).

## **2. Inhibition of Biofilm Formation**

Certain probiotic strains, such as *Bacillus licheniformis*, demonstrate anti-biofilm activity against clinically significant pathogens. For instance, inhibition of *Acinetobacter baumannii* biofilm formation has been associated with the downregulation of biofilm-related gene expression in the presence of probiotics. This anti-biofilm property is particularly relevant in the context of persistent infections and device-associated complications (Ghanipour *et al.*, 2022).

## **3. Immunomodulatory Activity**

Probiotics contribute indirectly to antimicrobial defence by enhancing host immune responses. This includes upregulation of immunological mediators that facilitate pathogen clearance and inhibit infection. Through such immunomodulatory effects, probiotics support innate and adaptive immune mechanisms against microbial invasion (Sadaqat, 2024).

### **1.10 Methods of Determining the Antimicrobial Properties of Probiotics**

The assessment of antimicrobial activity is important to characterize probiotic strains intended for therapeutic use. Determining the extent to which probiotics inhibit the growth of pathogenic microorganisms not only validates their clinical utility but also guides strain selection for

targeted use in gastrointestinal, urogenital, and systemic infections. Various in vitro methods are employed to evaluate the antimicrobial efficacy of probiotics.

These methods can broadly be categorized into:

A. Solid media methods:

1. Agar well diffusion assay
2. Spot-on-lawn assay

B. Liquid media methods for example, broth inhibition assay

C. Other analytical approaches:

1. Co-culture assays
2. Metabolite profiling and quantification

**A. Solid Media Methods**

**1. Agar Well Diffusion Assay**

This technique involves the inoculation of an agar medium with pathogenic microorganisms, followed by the creation of wells into which probiotic cell-free supernatants or live cultures are introduced. Following incubation, zones of inhibition around the wells are measured to assess the antimicrobial activity. This method is particularly useful for detecting the presence of diffusible antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide. Studies have demonstrated that *Lactobacillus* species exert measurable inhibitory effects against

clinically relevant pathogens such as *Staphylococcus aureus* and *Escherichia coli* using this assay format (Cizeikiene & Jagelavičiute, 2021; Rushdi, 2022).

## **2. Spot-on-Lawn Assay**

The spot-on-lawn assay is a widely utilized, agar-based method for assessing the antimicrobial activity of probiotic bacterial strains. The assay begins with the preparation of a lawn of the target microorganism, also referred to as the indicator strain. A suitable agar medium is selected based on the growth requirements of the pathogen. For example, brain heart infusion (BHI) agar is often used for cultivating *Staphylococcus aureus*, whereas de Man, Rogosa, and Sharpe (MRS) agar is preferred for lactic acid bacteria (LAB) such as *Lactobacillus* species. The target microorganism is first cultured in a liquid medium and subsequently adjusted to an appropriate optical density. It is then either mixed with molten soft agar and poured over the surface of the base agar plate or inoculated directly onto the solidified agar surface to form a uniform bacterial lawn (Christensen *et al.*, 2021).

Following the preparation of the lawn, small volumes (typically 10–20  $\mu\text{L}$ ) of the probiotic cultures or their corresponding cell-free supernatants are spotted onto the surface. If cell-free supernatants are used, they are generally obtained by centrifugation and filtration of overnight probiotic cultures to remove viable cells, ensuring that observed effects are due to secreted factors rather than active colonization.

Upon completion of the incubation period, agar plates are examined for the appearance of inhibition zones, clear, circular regions surrounding the probiotic application site, where the growth of the target microorganism has been visibly suppressed. The presence of such zones

indicates antimicrobial activity and serves as a qualitative confirmation of the inhibitory potential of the tested probiotic strain.

To facilitate standardized evaluation, inhibition zones are assessed semi-quantitatively using a scoring system based on the radius of the clear zones surrounding each spot. This system classifies inhibition into four categories: score 4 corresponds to a radius of 4 mm or more; score 3 encompasses radii between 2 mm and 3.9 mm; score 2 includes radii from just above 0.5 mm to under 2 mm; and score 1 applies to zones with radii between 0 mm and 0.5 mm. This approach allows for comparative analysis of antimicrobial efficacy across multiple probiotic isolates, especially when exact measurements are not feasible or necessary at early screening stages.

The diameter of the inhibition zone is commonly interpreted as the strength of antimicrobial activity; larger zones are generally associated with greater inhibitory capacity. However, several factors can influence zone size, including the rate at which antimicrobial compounds diffuse through the agar, the physicochemical characteristics of those compounds, and the growth dynamics of the indicator organism. These variables should be considered when comparing results across different experimental conditions or bacterial strains.

## **B. Liquid Media Methods**

- **Broth Inhibition Assay**

In the broth co-culture technique, probiotic and pathogenic strains are incubated simultaneously in liquid nutrient media. The growth kinetics of the pathogen are monitored over time, commonly through optical density measurements or viable colony counts, to determine the inhibitory effect exerted by the probiotic. This method can measure both bacteriostatic and

bactericidal activities. For example, *Lactobacillus acidophilus* has been shown to markedly suppress the growth of enteropathogens when assessed in liquid culture systems (Denkova *et al.*, 2022).

## **C. Other Analytical Approaches**

### **1. Co-Culture Assays**

The co-culture assay is an *in vitro* technique employed to evaluate the antimicrobial activity of probiotic strains against target pathogenic microorganisms. This method involves the simultaneous cultivation of a probiotic and a pathogenic organism within a shared medium, thereby allowing for direct and sustained interaction under controlled environmental conditions. (Fredua-Agyeman *et al.*, 2023)

Typically, the co-culture is established in a broth medium that is selectively optimized to support the growth of both microbial populations. The experimental design may involve the inoculation of the probiotic and the pathogen either concurrently or in a defined sequence, depending on the desired investigative focus. Incubation parameters such as temperature, pH, and oxygen availability are tailored to meet the physiological requirements of both species.

Quantitative and qualitative assessments of antimicrobial activity within co-culture systems rely on a variety of complementary analytical techniques. Enumeration of colony-forming units on selective or differential media at predefined time intervals enables the monitoring of each microorganism's population dynamics. Optical density measurements, while not organism-specific, offer a general overview of biomass accumulation and can be used to track overall growth kinetics. Monitoring pH variation during co-incubation may reveal the production of organic acids, a common antimicrobial mechanism of many probiotic strains.

Additional methods include viability assays that utilize fluorescent dyes or metabolic indicators to discern live and dead cells within the culture. More sophisticated molecular tools, such as quantitative Polymerase Chain Reaction (PCR), offer the advantage of species-specific quantification and enhanced sensitivity, thereby overcoming the limitations of culture-based methods in complex or low-abundance microbial systems (Pinto *et al.*, 2024).

Longitudinal co-culture studies have demonstrated the efficacy of various probiotic strains, including *Lactobacillus*, in reducing the viability of pathogenic microorganisms during co-incubation (Kim *et al.*, 2022). Longitudinal co-culture is a time-extended variant of the co-culture method used to evaluate the antimicrobial activity of probiotics against pathogenic microorganisms. Unlike conventional co-culture experiments that are typically short-term (e.g. 24 to 48 hours), longitudinal co-culture involves continuous or repeated co-incubation over an extended period, often spanning several days or weeks.

## **2. Metabolite Profiling and Quantification**

The antimicrobial effects of probiotics are often attributable to bioactive metabolites such as bacteriocins, lactic acid, acetic acid, and hydrogen peroxide. Quantitative metabolomic analysis, employing techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), or enzyme-linked immunosorbent assays (ELISA), enables the identification and quantification of these compounds (Gao *et al.*, 2024; Kyei-Baffour *et al.*, 2025). This approach not only confirms antimicrobial potency but also elucidates the specific metabolic pathways involved in probiotic action.

## 1.11 Future Applications of Probiotics

The therapeutic potential of probiotics is undergoing rapid expansion, driven by advances in microbial genomics, host–microbiota interaction studies, and systems biology. Emerging evidence suggest that probiotics may serve as bioactive agents across a spectrum of medical domains, including oncology, neuropsychiatry, metabolic diseases, and personalized nutrition. Areas of future probiotic application and the scientific rationale supporting their development.

### 1. Probiotics in Cancer Therapy

Recent investigations have highlighted the relationship between the gut microbiome and tumour biology, revealing opportunities for probiotics in both cancer prevention and adjunctive therapy.

**Modulation of Carcinogenic Pathways:** Probiotic-mediated modulation of gut microbiota can influence carcinogenesis through multiple mechanisms, including suppression of pro-inflammatory pathways, regulation of intestinal permeability, and detoxification of carcinogens (Mishra *et al.*, 2021). Certain strains are capable of reducing the genotoxicity of dietary mutagens, thereby mitigating oncogenic risk.

**Oncobiotics:** The emerging class of *oncobiotics* refers to probiotic strains with anti-neoplastic properties. These microorganisms may enhance the efficacy of chemotherapeutic agents, improve tolerability, and modulate immune responses to promote tumour suppression (Howarth, 2024).

### 2. Psychobiotics and Mental Health

The gut–brain axis is promising for probiotic intervention, particularly in the context of neuropsychiatric disorders.

- **Psychobiotic Mechanisms:** The term *psychobiotics* describes live microorganisms that, when ingested in adequate amounts, confer mental health benefits through neurochemical modulation, including the production of  $\gamma$ -aminobutyric acid (GABA), serotonin precursors, and short-chain fatty acids. These metabolites can influence mood regulation, stress responses, and cognitive function (Howarth, 2024).
- **Therapeutic Implications:** Preliminary studies suggest that specific probiotic strains may alleviate symptoms of depression, anxiety, and stress-related disorders. As psychobiotics evolve into clinically viable agents, they may serve as adjuncts or alternatives to traditional psychotropic drugs, especially in patients with comorbid gastrointestinal disturbances.

### 3. Others

- **Atopic dermatitis**

Probiotics have attracted considerable scientific interest for their potential therapeutic applications in the context of atopic dermatitis (AD) (Emokpae *et al.*, 2024). Central to the emerging interest in probiotics is the concept of the gut-skin axis, which describes the relationship between gut microbiota and skin health (Thye *et al.*, 2022). A growing body of evidence suggests that disturbances in the gut microbiome and impairments in intestinal barrier function may contribute to systemic inflammation and exacerbate dermatological conditions, including atopic dermatitis. Microbial metabolites and immune mediators originating in the gut are believed to influence cutaneous immune responses, highlighting the relevance of intestinal health in the pathophysiology of skin disorders.

Among the various probiotic candidates, species within the *Lactobacillus* genus have received particular attention due to their resilience in the gastrointestinal environment and their capacity

to maintain viability during passage through the digestive tract. These properties make them suitable for oral supplementation. In this context, several strains have been identified for their potential utility in managing AD symptoms, including *Lactobacillus salivarius*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus rhamnosus*.

These *Lactobacillus* strains are thought to exert beneficial effects on the course of AD through multiple mechanisms. They may enhance gut barrier integrity, modulate systemic immune responses, and regulate the production of pro-inflammatory cytokines. Clinical studies have indicated that the administration of these strains may lead to improvements in disease severity, as reflected in reduced SCORAD (Scoring Atopic Dermatitis) indices, lower circulating levels of inflammatory cytokines, and increased populations of regulatory T cells, which are central to immune tolerance.

Although these findings underscore the therapeutic potential of probiotics as adjunctive or alternative strategies in atopic dermatitis management, further investigation is warranted. Future research should aim to clarify the specific immunological mechanisms underlying these effects, determine the most effective strains and dosages, and establish the clinical relevance of probiotics in long-term disease management. While their favorable safety profile makes them attractive for therapeutic use, the integration of probiotics into standard care protocols for atopic dermatitis is dependent on more robust clinical evidence.

- **Covid-19 management**

Emerging evidence suggests that specific strains of *Lactobacillus* may offer therapeutic benefits in the management of COVID-19. There are several potential mechanisms for this activity. They are thought to play a role in modulating the host immune response, reducing systemic

inflammation, and possibly exerting direct antiviral effects through the production of antiviral compounds or through interference with viral replication and entry (Taufers *et al.*, 2024).

Despite promising observations, the reviewed literature presents mixed findings, particularly in relation to clinical, biochemical, hematological, and immunological outcomes.

Although preliminary findings support the potential of lactobacilli in reducing symptom burden and improving clinical outcomes in COVID-19, the current body of evidence remains heterogeneous. Future research should aim to address methodological limitations, standardize probiotic interventions, and further explore the mechanistic basis of these observed effects. The ongoing investigation into lactobacilli as a complementary therapeutic strategy offers promising avenues for enhancing host resilience against COVID-19 and other viral illnesses.

- **Management of Alzheimer's disease**

Probiotics have emerged as promising agents in enhancing neurological function, particularly in the improving memory and cognition. Among various probiotic strains, oral administration of *Bifidobacterium breve* MCC1274 has demonstrated significant potential in improving cognitive function. Notably, this strain has shown efficacy in both animal models and human subjects, particularly in those exhibiting mild cognitive impairment (MCI) (Zhu *et al.*, 2022).

Existing preclinical and clinical evidence demonstrates the therapeutic potential of probiotics in slowing the progression of Alzheimer's disease. Not only have *in vivo* studies demonstrated meaningful neurobiological effects, but clinical trials have also confirmed the usefulness of probiotics in improving cognitive outcomes without significant adverse effects. This favorable safety profile enhances their appeal as adjunctive or even primary therapeutic options in the management of Alzheimer's disease.

Future research should prioritize clinical trials designed to identify Alzheimer's-specific alterations in gut microbiota composition. Such studies could reveal critical microbial signatures associated with disease onset and progression, thereby refining the selection and application of probiotic strains. Furthermore, large-scale cohort studies examining the correlation between microbial diversity and cognitive decline could yield important prognostic insights. An interdisciplinary framework that integrates microbiology, neurology, and systems biology will be essential to fully elucidate the host-microbiota interactions underlying Alzheimer's disease pathogenesis. This could potentially pave the way for the development of microbiome-centered strategies in both the treatment and prevention of Alzheimer's disease.

## CHAPTER TWO

### 2.0 MATERIALS AND METHODS

#### 2.1 Materials

All materials used for this experiment were sterilized before and after use.

##### **Microorganisms**

*Lactobacillus rhamnosus*, *Escherichia coli*

##### **Chemicals and Reagents**

Glycero-gelatin, Polyethylene glycol, Theobroma, Phosphate Buffered Saline (PBS), Safranin, Crystal violet, Gram iodine, Acetone, Hydrogen peroxide (3%), Peptone water, Bromothymol blue, Sodium hydroxide, Sucrose, Lactose, Maltose, Glucose, Distilled water

##### **Growth Media**

de Man, Rogosa and Sharpe (MRS) Broth, Eosin Methylene Blue (EMB) Agar, Mueller-Hinton Broth

##### **Equipment**

Incubator, Microscope, Autoclave, pH meter, Refrigerator, Bunsen burner, Digital weighing balance, Pipettes and teats, Red cover tubes

##### **Glassware**

Petri dishes, Beakers, Measuring cylinders, Universal bottles, Bijou bottles, Glass slides, Coverslips, Sterile test tubes, Durham tubes.

## **Analytical and Statistical Tools**

GraphPad Prism, SPSS, Microsoft Excel, Spearman's correlation analysis

## **Safety Equipment**

Laboratory coat, Gloves, Waste disposal containers

## **Miscellaneous Materials**

Marker, Masking tape, Tripod stand, Spatula, Foil paper, Cotton wool, Surgical blades, Candles, Forceps.

## **2.2 Methods**

### **2.2.1 Gram Staining Procedure**

All the microorganisms were gotten from Pharmaceutical Microbiology and Biotechnology Department.

A smear was created on the glass slide using a sterile drop of water and *Lactobacillus rhamnosus*. Drops of crystal violet were then applied for 60 seconds, the stain was poured off, and excess stain was rinsed with water. Iodine was then added and left to stand for 60 seconds, after which the slide was rinsed with water. Acetone was then used for 30 seconds to decolorize the slide, and it was again rinsed with water. Safranin was applied for counterstaining, and after 60 seconds, it was rinsed with water. The slide was then left to air-dry after which it was examined under a microscope. The outcome was then noted.

This same procedure was then repeated for *Escherichia coli* all according to the method used by Paray *et al.*, (2023).

### **2.2.2 Biochemical Tests**

#### **Catalase Test Procedure:**

Following the procedure used by Khatoon *et al.*, (2022), a small portion of a pure colony of *Escherichia coli* was transferred onto a clean, dry microscope slide using a sterile glass rod. Two drops of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution were then added, and the preparation was observed for the rapid evolution of oxygen bubbles. The result was observed and recorded.

This procedure was repeated for *Lactobacillus rhamnosus*.

#### **Carbohydrate Fermentation Tests:**

Based on the procedure utilized by Gunkova *et al.*, (2021), the basal medium was prepared using peptone water, to which bromothymol blue neutralized with sodium hydroxide was added as a pH indicator. The pH was adjusted to 7.3 before the addition of carbohydrates. The test carbohydrates, Raffinose, Lactose, Maltose, D-Fructose, D-Glucose, D-Mannitol, L-Rhamnose, D-Xylose, L-Arabinose, were each incorporated into separate portions of the base medium at a final concentration of 1% (w/v). Control tubes containing only the base medium without any carbohydrate were also prepared. Five millilitres of each preparation were dispensed into sterile test tubes, each containing an inverted Durham tube for detecting gas production, followed by sterilization at 121°C in an autoclave.

After cooling to room temperature, each tube was aseptically inoculated with a 5% (v/v) inoculum from an active bacterial culture. Separate sets were prepared for *Escherichia coli* and *Lactobacillus rhamnosus*. The inoculated media were incubated at 37°C, and observations were made after 24 and 48 hours to confirm fermentation reactions.

### **2.2.3 MRS Broth**

The manufacturer's instruction for the preparation of 1000ml of MRS Broth was to dissolve 55.1g of the dehydrated microbiological media in 1000ml of distilled water. However, since only 200ml of the broth was required, 11.03g of the dehydrated microbiological media was dissolved in 200ml of distilled water. Before inoculating with the organisms, 10 ml of broth was pipetted into universal bottles and then sterilized by autoclaving for 15 minutes at 121°C, and allowed to cool.

### **2.2.4 Phosphate Buffer Saline Preparation.**

8g of sodium chloride, 0.2g of potassium chloride, 1.44g of disodium hydrogen phosphate, 0.24g of potassium dihydrogen phosphate were weighed, dissolved and mixed with 800ml of distilled water and stirred until they were completely dissolved. More distilled water was then added to make the solution up to 1000ml.

### **2.2.5 Mueller Hilton Broth (MH Broth) Preparation**

According to the manufacturer's instructions, 21g of the Mueller Hilton broth was to be dissolved in 1000ml of distilled water. However, since 50ml of the broth was required, 1.051g was instead weighed and dissolved in 50ml of distilled water. Before inoculating with the organisms, 10 ml of broth was pipetted into universal bottles, and sterilized by autoclaving for 15 minutes at 121°C, and allowed to cool.

### **2.2.6 Eosin Methylene Blue Agar Preparation**

The manufacturer's instruction for the preparation of 1000ml of nutrient agar is to dissolve 35.96g of the dehydrated media in 1000ml of water. However, since 80ml of the agar was required for this experiment, 2.88g of the dehydrated media was instead dissolved in 80ml of distilled water. Following this, the agar was heated and constantly stirred for five minutes to make it gel-like. After that, it was autoclaved for 15 minutes at 121°C to disinfect it. Each petri

dish received 10 milliliters of nutrient agar, which was let to solidify before being dried in a hot air oven.

### **2.2.7 Inoculating Media Preparation**

To make 10 ml, 3.3 ml of the Glycero-Gelatin base was mixed with 6.7 ml of Miller Hinton broth and MRS broth in a 1:1 ratio. The same was done for Polyethylene Glycol and Theobroma respectively.

### **2.3 *Escherichia coli* Growth Experiment**

A universal bottle containing 10ml of Mueller Hinton's broth was utilized. The broth was then inoculated with a loopful of *Escherichia coli* using a flamed sterile inoculating loop and stirred vigorously. The universal bottle was then incubated at 37°C for 48 hours. Twelve red cover tubes containing 0.9ml of phosphate buffered saline were used to perform a 1 in 10 dilution beginning with the addition of 0.1ml of the broth inoculated with *Escherichia coli* into the first red cover tube. Using the drop plate method, 20 microliters of the various dilutions were plated out on Eosin Methylene Blue Agar. Within 48 hours, the experiment was repeated every four hours. After being allowed to dry at room temperature, the inoculated plates were incubated for 48 hours at 37°C. The results were noted and recorded.

### **2.4 *Escherichia coli* Growth in the presence of *Lactobacillus rhamnosus* experiment.**

A 10ml 1:1 mixture of Mueller Hinton broth and MRS Broth was prepared by adding a sterile 5ml of each broth into a Universal Bottle. Loopfuls of *Escherichia coli* and *Lactobacillus rhamnosus* respectively were inoculated into the broth mixture. The universal bottle was then incubated at 37°C for 48 hours. Twelve red cover tubes containing 0.9ml of phosphate buffered saline were used to perform a 1 in 10 dilution. Using a drop plate method, 20 microliters of the various dilutions were plated out in Eosin Methylene Blue agar. Within 48 hours, the experiment

was repeated every four hours. After being allowed to dry at room temperature, the inoculated plates were incubated for 48 hours at 37°C. The results were noted and recorded.

### **2.5 *Escherichia coli* Growth in the presence of *Lactobacillus rhamnosus* and suppository bases experiment**

To make 10 ml, 3.3 ml of the Glycero-Gelatin base was mixed with 6.7 ml of a 10ml 1:1 mixture of Miller Hinton broth and MRS broth in a universal bottle. Loopfuls of *Escherichia coli* and *Lactobacillus rhamnosus* respectively were then inoculated into universal bottle. The universal bottle was then incubated at 37°C for 48 hours. Twelve red cover tubes containing 0.9ml of phosphate buffered saline were used to perform a 1 in 10 dilution. Using a drop plate method, 20 microliters of the various dilutions were plated out in Eosin Methylene Blue agar. Within 48 hours, the experiment was repeated every four hours. After being allowed to dry at room temperature, the inoculated plates were incubated for 48 hours at 37°C. The results were noted and recorded.

The above experiment was then repeated then repeated using 3.3ml of Theobroma and Polyethylene Glycol respectively.

## CHAPTER THREE

### 3.0 RESULTS

Table 3.1: Gram staining and biochemical tests for *Escherchia coli*

Test	Specific interactions	<i>Escherchia coli</i>
Gram staining	Primary and Secondary Dyes	GNB
Catalase test	Hydrogen peroxide	Positive
Biochemical Characteristics	Sucrose	Negative
	Raffinose	Negative
	Lactose	Positive
	Maltose	Positive
	D-Fructose	Positive
	D-Glucose	Positive
	D-Mannitol	Positive
	L-Rhamnose	Positive
	D-Xylose	Positive
L-Arabinose	Positive	

Key

GNB: Gram Negative Bacilli

Table 3.2: Gram staining and biochemical tests for *Lactobacillus rhamnosus*

Test	Specific interactions	<i>Lactobacillus rhamnosus</i>
Gram staining	Primary and Secondary Dyes	GPB
Catalase test	Hydrogen peroxide	Negative
Biochemical Characteristics	Sucrose	Positive
	Raffinose	Negative
	Lactose	Positive
	Maltose	Positive
	D-Fructose	Positive
	D-Glucose	Positive
	D-Mannitol	Positive
	L-Rhamnose	Negative
	D-Xylose	Negative
	L-Arabinose	Positive

Key

GPB: Gram Positive Bacilli

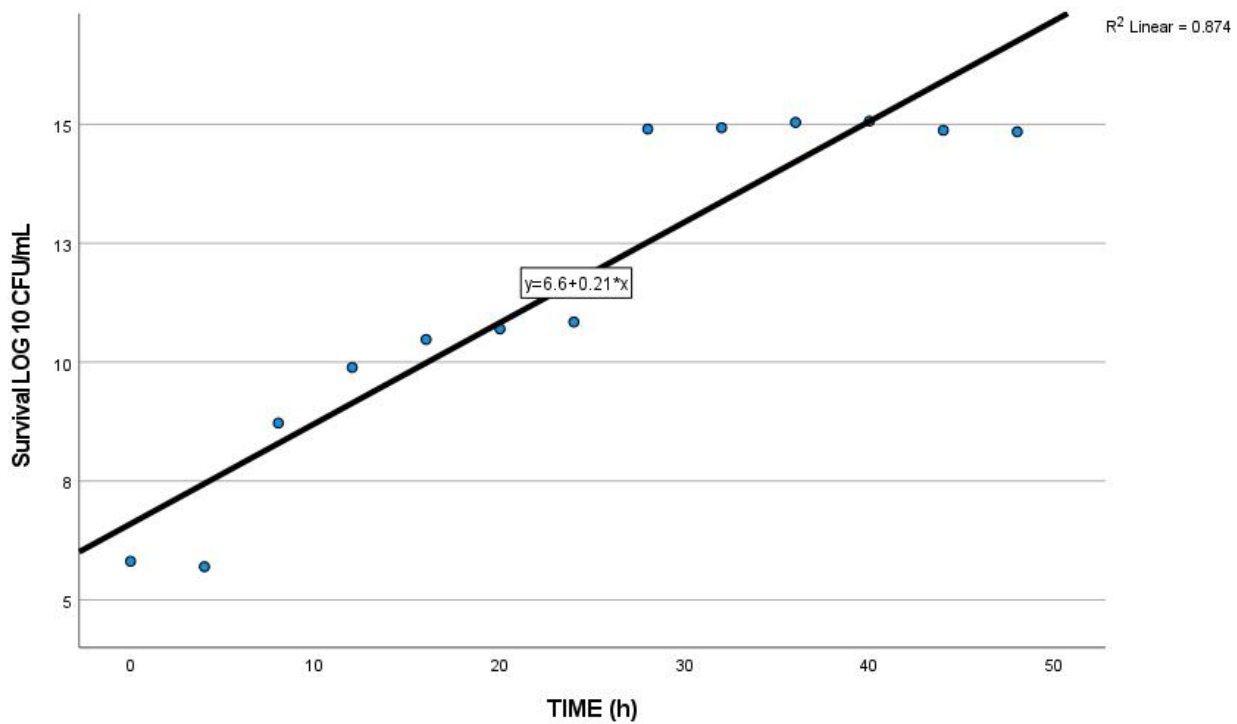


Figure 3.1: Growth pattern of *Escherichia coli*

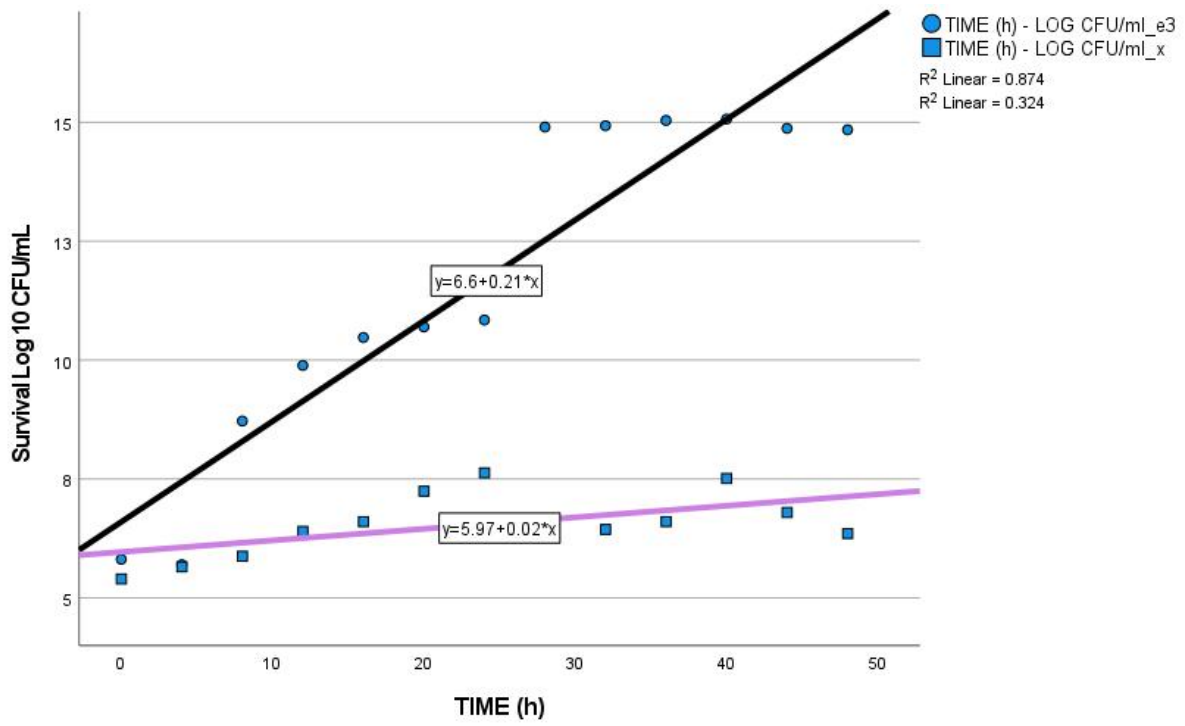


Figure 3.2: Growth pattern of *Escherichia coli* in the presence of *Lactobacillus rhamnosus*

P<0.001

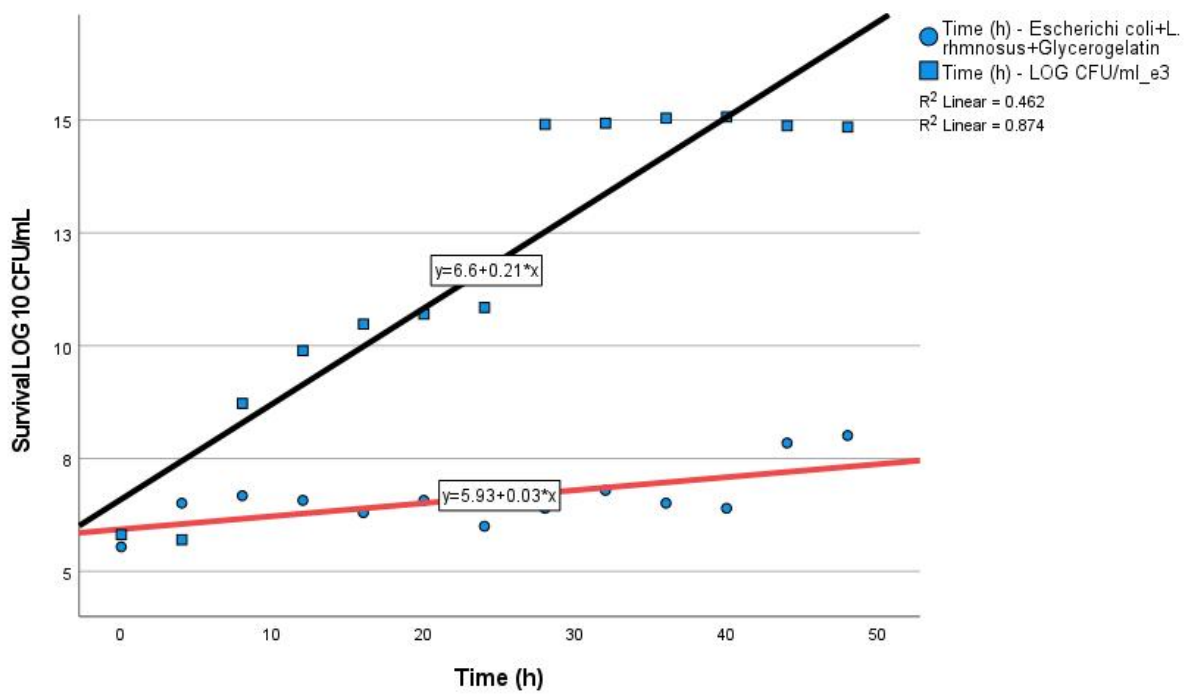


Figure 3.3: Growth pattern of *Escherichia coli* in the presence of *Lactobacillus rhamnosus* and Glycerogelatin

P<0.001

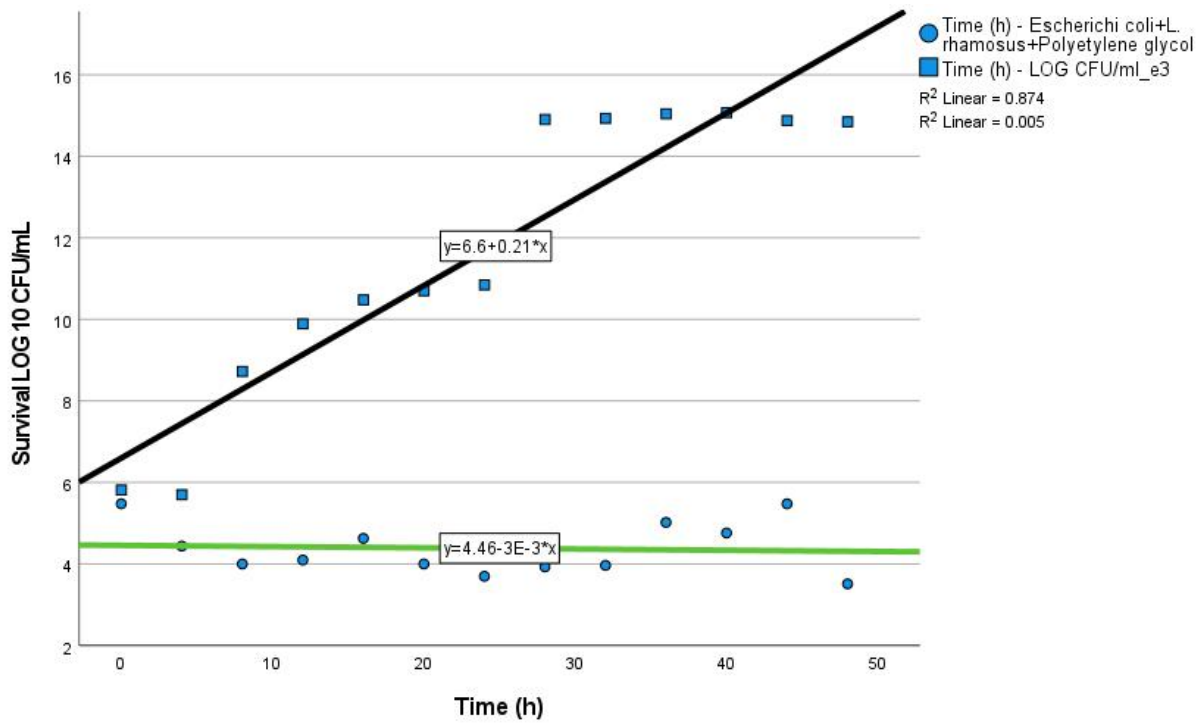


Figure 3.4: Growth pattern of *Escherichia coli* in the presence of *Lactobacillus rhamnosus* and Polyethylene glycol

P<0.001

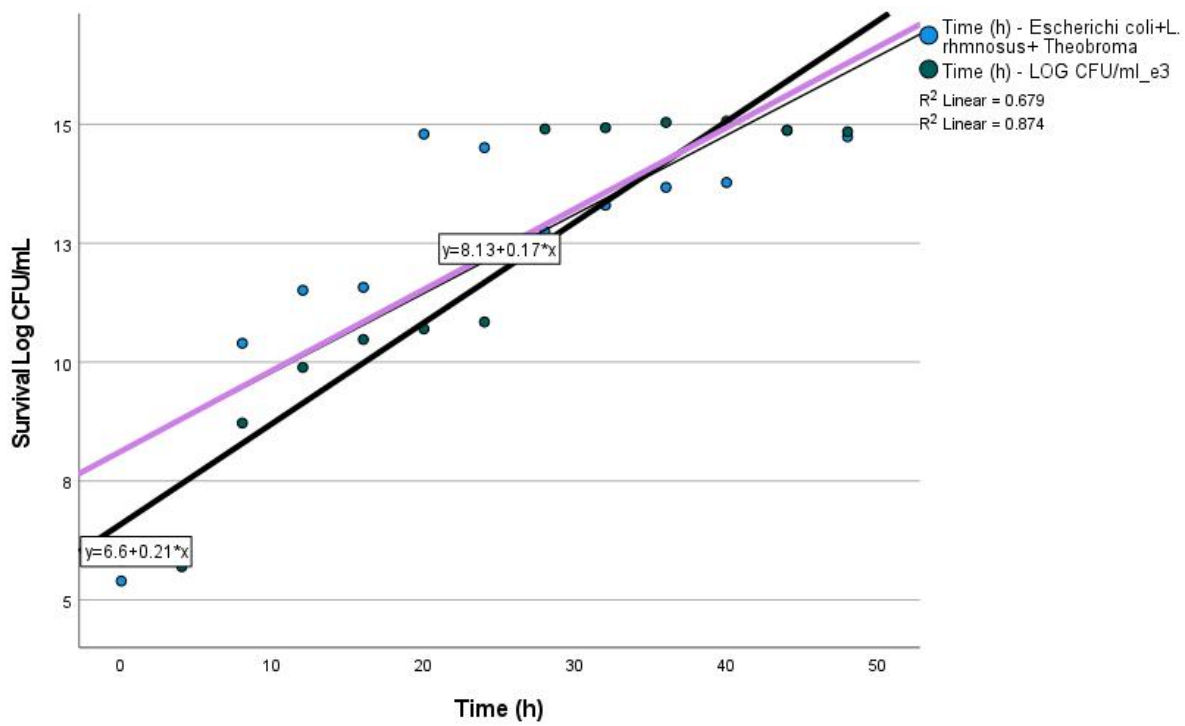


Figure 3.5: Growth pattern of *Escherichia coli* in the presence of *Lactobacillus rhamnosus* and Theobroma

P=0.436

## CHAPTER FOUR

### 4.0 DISCUSSION

#### 4.1 Gram Staining and Biochemical Tests

##### 4.1.1 Gram Staining

The biochemical profiling results successfully differentiated *Escherichia coli* from *Lactobacillus rhamnosus* and confirmed their expected phenotypic characteristics. *E. coli* was identified as a Gram-negative bacillus (GNB) with a positive catalase test, while *L. rhamnosus* was confirmed as a Gram-positive bacillus (GPB) and was catalase-negative, consistent with its classification as a fermentative bacterium.

##### 4.1.2 Biochemical Tests

Fermentation of a carbohydrate by the test organism results in enzymatic hydrolysis of sugars and accumulation of organic acids, which lowers the pH and changes the indicator from blue to yellow. Acid production alone was recorded as a positive result (A), while the presence of both acid and gas within the Durham tube indicated acid and gas production (AG). If the organism failed to utilize the carbohydrate, the medium retained its original blue color, signifying a negative result.

The carbohydrate fermentation profiles further distinguish the two species. Key differentiators include the fermentation of L-Rhamnose and D-Xylose, which is positive for *E. coli* but negative for *L. rhamnosus*. Conversely, *L. rhamnosus* ferments sucrose, which *E. coli* does not. These distinct patterns provide a reliable basis for identification and are consistent with their respective metabolic pathways (Sultana *et al.*, 2021).

## **4.2 *Escherichia coli* Growth Experiments**

The results of this study show how the growth of *Escherichia coli* in the presence of *Lactobacillus rhamnosus* is influenced by polyethylene glycol, glycerogelatin, and Theobroma. The growth curve of *E. coli* in monoculture as seen in Figure 1, revealed a strong positive linear relationship between time and bacterial density, expressed as colony-forming units per millilitre (CFU/mL). The regression model ( $y = 6.6 + 0.21x$ ) indicated that each hour of incubation resulted in an increase of approximately 0.21 log CFU/mL, confirming a steady exponential growth phase. The coefficient of determination ( $R^2 = 0.874$ ) showed that 87.4% of the variation in growth could be attributed to time, reflecting a consistent and predictable growth pattern.

### **Impact of *L. rhamnosus* in a Co-culture System**

When *E. coli* was co-cultured with *L. rhamnosus*, a marked suppression of growth was observed in Figure 2. The regression equation ( $y = 5.97 + 0.02x$ ) demonstrated a tenfold reduction in growth rate relative to the control, indicating near-complete inhibition rather than active proliferation, a finding that was statistically significant ( $p < 0.001$ ). The low  $R^2$  value (0.324) revealed high variability and weak dependence on time, suggesting that the inhibitory effect of *L. rhamnosus* created an unstable microenvironment with fluctuating stress conditions.

### **Impact of Glycerogelatin in a Co-culture System**

In contrast, when *E. coli* was co-cultured with *L. rhamnosus* and glycerogelatin, growth remained strongly suppressed, though the regression model ( $y = 5.93 + 0.03x$ ) indicated a slightly higher rate than with *L. rhamnosus* alone as was observed in Figure 3, and this suppression was also highly significant ( $p < 0.001$ ). The  $R^2$  value improved to 0.462, implying that glycerogelatin contributed to environmental stabilization without diminishing the probiotic's inhibitory activity. These results suggest that glycerogelatin can function as a

stabilizing matrix for probiotic applications, maintaining antimicrobial efficacy while reducing variability in bacterial response.

### **Impact of Polyethylene Glycol (PEG) in a Co-culture System**

In the presence of polyethylene glycol (PEG), *E. coli* exhibited an even more pronounced reduction in viability when co-cultured with *L. rhamnosus* as can be seen in Figure 4. The regression equation ( $y = 4.46 - 0.003x$ ,  $R^2 = 0.005$ ) reflected a static population, indicating that growth was suppressed prior to or at the start of measurement, a result that was also highly significant ( $p < 0.001$ ). This pattern contrasts with the control culture ( $y = 6.6 + 0.21x$ ,  $R^2 = 0.874$ ), where time explained most of the growth variation. The flat slope and negligible  $R^2$  in the PEG treatment confirm that *L. rhamnosus* in combination with PEG produced a stable, low-level bacterial population with no subsequent proliferation. This finding implies a strong synergistic effect, where PEG potentiates the inhibitory influence of the probiotic. The p-values obtained, all being less than 0.001 across the co-culture conditions above, indicated that the suppression of *Escherichia coli* growth was statistically significant. This finding confirmed that the observed reduction in growth was not attributable to random variation but represented a true effect of the treatments applied.

### **Impact of Theobroma in a Co-culture System**

A different outcome was observed with Theobroma, a cocoa-derived extract. When *E. coli* was co-cultured with *L. rhamnosus* and Theobroma as observed in Figure 5, its growth mirrored that of the control. The regression model ( $y = 8.13 + 0.17x$ ,  $R^2 = 0.679$ ) indicated a statistically insignificant difference in growth rate compared with the uninhibited control ( $p = 0.436$ ). The

higher intercept value suggested a substantially larger initial population, implying that Theobroma may stimulate early bacterial proliferation or inhibit *L. rhamnosus* activity. This result contrasts with the strong suppression observed under PEG and glycerogelatin conditions, revealing that Theobroma neutralizes the probiotic's antagonistic effect, possibly through interference with its metabolic pathways or through the action of bioactive polyphenols that alter the physicochemical environment.

Overall, the study demonstrates that *E. coli* growth dynamics are highly dependent on the interactions between probiotic activity and the surrounding chemical environment. While *L. rhamnosus* alone exerts a potent but unstable inhibitory effect, this can be modulated by specific adjuvants. PEG enhances inhibition and maintains a low, static bacterial population; glycerogelatin stabilizes but does not diminish the inhibitory environment; and Theobroma reverses suppression entirely, restoring normal bacterial proliferation.

#### **4.3 Limitations of the Study**

This experiment determined the suitability of the bases in the formulation of *Lactobacillus rhamnosus* based on the activity of the probiotic against *Escherichia coli* alone. Studies on whether polyethylene glycol, glycerogelatin and Theobroma affect the antimicrobial effect of *Lactobacillus rhamnosus* against other microorganisms was not conducted as well as studies on the effect of the formulation bases on the growth of *Lactobacillus rhamnosus*.

Also, the effect of the bases on the adhesion properties of *Lactobacillus rhamnosus* was not also determined in the scope of this study.

## CHAPTER 5

### 5.0 CONCLUSION

This study shows that the growth of *Escherichia coli* is significantly affected by both *Lactobacillus rhamnosus* and the physicochemical properties of the bases. *L. rhamnosus* inhibited *E. coli* growth, with the strongest suppression observed in polyethylene glycol (PEG), which stabilized the environment and prevented population increase. Glycero-gelatin maintained inhibitory activity with moderate stability, while Theobroma reversed the effect, promoting *E. coli* proliferation.

Overall, PEG is identified as the most suitable base for *L. rhamnosus* pessary formulation, with glycero-gelatin as a viable alternative, whereas Theobroma is unsuitable due to its antagonistic influence.

## REFERENCES

- Abavisani, M., Ebadpour, N., Khoshrou, A. & Sahebkar, A., 2024. Boosting vaccine effectiveness: the groundbreaking role of probiotics. *Journal of Agriculture and Food Research*, 16, p.101189. <https://doi.org/10.1016/j.jafr.2024.101189>
- Abid, R., Waseem, H., Ali, J., Ghazanfar, S., Muhammad Ali, G., Elsbali, A.M. & Alharethi, S.H., 2022. Probiotic yeast *Saccharomyces*: Back to nature to improve human health. *Journal of Fungi*, 8(5), p.444. Available at: <https://doi.org/10.3390/jof8050444>
- Achdiat, P.A., Saraswati, A.L., Handayani, Y., Rowawi, R. and Maharani, R.H., 2025. Aerobic vaginitis caused by *Streptococcus β-hemolyticus* and *Staphylococcus epidermidis* in a 26-year-old woman with a history of frequent antibiotics administration. *International Journal of Women's Health*, 17, pp.1437–1444. <https://doi.org/10.2147/IJWH.S516616>
- Ali, M.S., Lee, E.B., Hsu, W.H., Suk, K., Sayem, S.A.J., Ullah, H.M.A., Lee, S.J. & Park, S.C., 2023. Probiotics and postbiotics as an alternative to antibiotics: an emphasis on pigs. *Pathogens*, 12(7), p.874. <https://doi.org/10.3390/pathogens12070874>
- Al-Mujahidy, S.M.J., Kryukov, K., Ikeo, K., Saito, K., Uddin, M.E. and Ibn Sina, A.A., 2024. Functional genomic analysis of the isolated potential probiotic *Lactobacillus delbrueckii* subsp. *indicus* TY-11 and its comparison with other *Lactobacillus delbrueckii* strains. *Microbiology Spectrum*, 12, e03470-23. <https://doi.org/10.1128/spectrum.03470-23>
- Anupama, S., 2021. *Lactobacillus acidophilus* – An Overview. *Microbe Notes*. <https://microbenotes.com/Lactobacillus-acidophilus/>
- Araújo, M.M., Vogado, C.O., Mendes, M.M., Gonçalves, V.S.S. and Botelho, P.B., 2022. Effects of *Bifidobacterium animalis* subsp. *lactis* supplementation on gastrointestinal symptoms: systematic review with meta-analysis. *Nutrition Reviews*, 80(6), pp.1619–1633. <https://doi.org/10.1093/nutrit/nuab109>
- Armstrong, E., Hemmerling, A., Miller, S., Burke, K.E., Newmann, S.J., Morris, S.R., Reno, H., Huibner, S., Kulikova, M., Nagelkerke, N., Coburn, B., Cohen, C.R. and Kaul, R., 2022. Sustained effect of LACTIN-V (*Lactobacillus crispatus* CTV-05) on genital immunology

following standard bacterial vaginosis treatment: results from a randomised, placebo-controlled trial. *The Lancet Microbe*, 3(6), pp.e435–e442. [https://doi.org/10.1016/S2666-5247\(22\)00043-X](https://doi.org/10.1016/S2666-5247(22)00043-X)

Asgari, S., Pourjavadi, A., Licht, T.R., Boisen, A. and Ajalloueiian, F., 2020. Polymeric carriers for enhanced delivery of probiotics. *Advanced Drug Delivery Reviews*, 161–162, pp.1–21. <https://doi.org/10.1016/j.addr.2020.07.014>

Baek, M.G., Kim, K.W. and Yi, H., 2023. Subspecies-level genome comparison of *Lactobacillus delbrueckii*. *Scientific Reports*, 13(1), p.3171. <https://doi.org/10.1038/s41598-023-29404-3>

Bahaddad, S.A., Almalki, M.H.K., Alghamdi, O.A., Sohrab, S.S., Yasir, M., Azhar, E.I. & Chouayekh, H., 2023. *Bacillus* species as direct-fed microbial antibiotic alternatives for monogastric production. *Probiotics and Antimicrobial Proteins*, 15(1), pp.1–16. Available at: <https://doi.org/10.1007/s12602-022-09909-5>

Bodke, H. and Jogdand, S., 2022. Role of Probiotics in Human Health. *Cureus*, 14(11), p.e31313. <https://doi.org/10.7759/cureus.31313>

Chang, Y.H., Jeong, C.H., Cheng, W.N., Choi, Y., Shin, D.M., Lee, S. & Han, S.G., 2021. Quality characteristics of yogurts fermented with short-chain fatty acid-producing probiotics and their effects on mucin production and probiotic adhesion onto human colon epithelial cells. *Journal of Dairy Science*, 104(7), pp.7415–7425. <https://doi.org/10.3168/jds.2020-19820>

Christensen, I.B., Vedel, C., Clausen, M.L., Kjærulff, S., Agner, T. & Nielsen, D.S., 2021. Targeted screening of lactic acid bacteria with antibacterial activity toward *Staphylococcus aureus* clonal complex type 1 associated with atopic dermatitis. *Frontiers in Microbiology*, 12, 733847. <https://doi.org/10.3389/fmicb.2021.733847>

Cizeikiene, D. and Jagelaviciute, J., 2021. Investigation of Antibacterial Activity and Probiotic Properties of Strains Belonging to *Lactobacillus* and *Bifidobacterium* Genera for Their Potential Application in Functional Food and Feed Products. *Probiotics and Antimicrobial Proteins*, 13(5), pp.1387–1403. <https://doi.org/10.1007/S12602-021-09777-5>

Darbandi, A., Asadi, A., Mahdizade Ari, M., Ohadi, E., Talebi, M., Halaj Zadeh, M., Darb Emamie, A., Ghanavati, R. and Kakanj, M., 2022. Bacteriocins: Properties and potential use as

antimicrobials. *Journal of Clinical Laboratory Analysis*, 36(1), e24093. <https://doi.org/10.1002/jcla.24093>

Darma, A., Athiyah, A.F., Ranuh, R.G., Endaryanto, A., Budiono, B. & Sudarmo, S.M., 2020. Effects of probiotics on the enhancement of the innate mucosal immune response against pathogenic bacteria. *Iranian Journal of Microbiology*, 12(5), pp.445–450. <https://doi.org/10.18502/ijm.v12i5.4606>

de Souza de Azevedo, P.O., Mendonça, C.M.N., Moreno, A.C.R. et al., 2020. Antibacterial and antifungal activity of crude and freeze-dried bacteriocin-like inhibitory substance produced by *Pediococcus pentosaceus*. *Scientific Reports*, 10, p.12291. <https://doi.org/10.1038/s41598-020-68922-2>

Denkova, Z., Yanakieva, V., Goranov, B., Tomova, T., Denkova-Kostova, R., Oulahal, N., Kostov, G. and Degraeve, P., 2022. In vitro examination of the antimicrobial activity of a potentially probiotic *Lactobacillus acidophilus* strain against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. *BIO Web of Conferences*, 45, p.02010. <https://doi.org/10.1051/bioconf/20224502010>

Dong, W., Wang, S., Wang, X., Xu, G., Liu, Q., Li, Z., Lv, N., Pan, Y., Xiong, Q., Liu, D. and Zhu, B., 2024. Characteristics of Vaginal Microbiota of Women of Reproductive Age with Infections. *Microorganisms*, 12(5), p.1030. <https://doi.org/10.3390/microorganisms120>

Emokpae, I., Tobia, D.L., Stamm, S.D., Lundy, P., Weimer, D.S. & Demory Beckler, M., 2024. Examining the efficacy of five *Lactobacillus* species in treating and preventing atopic dermatitis: a systematic literature review. *Cureus*, 16(7), p.e64833. <https://doi.org/10.7759/cureus.64833>

Falzone, L., Lavoro, A., Candido, S., Salmeri, M., Zanghì, A. and Libra, M., 2024. Benefits and concerns of probiotics: an overview of the potential genotoxicity of the colibactin-producing *Escherichia coli* Nissle 1917 strain. *Gut Microbes*, 16. <https://doi.org/10.1080/19490976.2024.2397874>

Fredua-Agyeman, M., Stapleton, P. & Gaisford, S., 2023. Growth assessment of mixed cultures of probiotics and common pathogens. *Anaerobe*, 84, p.102790. <https://doi.org/10.1016/j.anaerobe.2023.102790>

- Gan, L., Xu, W.H., Xiong, Y. and others, 2021. Probiotics: their action against pathogens can be turned around. *Scientific Reports*, 11, p.13247. <https://doi.org/10.1038/s41598-021-91542-3>
- Gao, H., Li, X., Chen, X., Hai, D., Wei, C., Zhang, L. and Li, P., 2022. The Functional Roles of *Lactobacillus acidophilus* in Different Physiological and Pathological Processes. *Journal of Microbiology and Biotechnology*, 32(10), pp.1226–1233. <https://doi.org/10.4014/jmb.2205.05041>
- Gao, H., Li, Y., Sun, J., Xu, H., Wang, M., Zuo, X., Fu, Q., Guo, Y., Chen, Z., Zhang, P., Li, X., Wang, N., Ye, T. and Yao, Y., 2021. *Saccharomyces boulardii* Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice by Regulating NF- $\kappa$ B and Nrf2 Signaling Pathways. *Oxidative Medicine and Cellular Longevity*, 2021, p.1622375. <https://doi.org/10.1155/2021/1622375>
- Gao, Z., Zhou, M.C., Lin, J., Lu, Y. & Liu, S.Q., 2024. Metabolomics analysis of okara probiotic beverages fermented with *Lactobacillus gasseri* and *LimosiLactobacillus fermentum* by LC-QTOF-MS/MS. *Food Chemistry: X*, 21, p.101178. <https://doi.org/10.1016/j.fochx.2024.101178>
- Garcia-Gonzalez, N., Battista, N., Prete, R. and Corsetti, A., 2021. Health-Promoting Role of *Lactiplantibacillus plantarum* Isolated from Fermented Foods. *Microorganisms*, 9(2), p.349. <https://doi.org/10.3390/microorganisms9020349>
- Gavzy, S.J., Kensiski, A., Lee, Z.L., Mongodin, E.F., Ma, B. and Bromberg, J.S., 2023. *Bifidobacterium* mechanisms of immune modulation and tolerance. *Gut Microbes*, 15(2), p.2291164. <https://doi.org/10.1080/19490976.2023.2291164>
- Ge, Y., Yu, X., Zhao, X., Liu, C., Li, T., Mu, S., Zhang, L., Chen, Z., Zhang, Z., Song, Z., Zhao, H., Yao, S. and Zhang, B., 2024. Fermentation characteristics and postacidification of yogurt by *Streptococcus thermophilus* CICC 6038 and *Lactobacillus delbrueckii* ssp. *bulgaricus* CICC 6047 at optimal inoculum ratio. *Journal of Dairy Science*, 107(1), pp.123–140. <https://doi.org/10.3168/jds.2023-23817>
- Ghanipour, F., Nazari, R., Aghaee, S. and Jafari, P., 2022. Evaluation of the Antimicrobial Ability of Probiotics Against Nosocomial Infections by Inhibiting Ompa Gene Expression in

Acinetobacter Baumannii. *Journal of Arak University of Medical Sciences*, 24(6), pp.854–867. <https://doi.org/10.32598/jams.24.6.6662.1>

Gorreja, F. & Walker, W.A., 2022. The potential role of adherence factors in probiotic function in the gastrointestinal tract of adults and pediatrics: a narrative review of experimental and human studies. *Gut Microbes*, 14(1), p.2149214. <https://doi.org/10.1080/19490976.2022.2149214>

Gu, M., Nguyen, H.T., Cho, J.H., Suh, J.W. & Cheng, J., 2023. Characterization of *Leuconostoc mesenteroides* MJM60376 as an oral probiotic and its antibiofilm activity. *Molecular Oral Microbiology*, 38(2), pp.145–157. Available at: <https://doi.org/10.1111/omi.12397>

Gumustop, I. and Ortakci, F., 2022. Comparative genomics of *Leuconostoc lactis* strains isolated from human gastrointestinal system and fermented foods microbiomes. *BMC Genomic Data*, 23, p.61. <https://doi.org/10.1186/s12863-022-01074-6>

Gunkova, P., Buchilina, A., Maksimiuk, N., Bazarnova, Y. & Girel, K., 2021. Carbohydrate fermentation test of lactic acid starter cultures. *IOP Conference Series: Earth and Environmental Science*, 852, 012035. <https://doi.org/10.1088/1755-1315/852/1/012035>.

He, T., Lykov, N., Luo, X., Wang, H., Du, Z., Chen, Z., Chen, S., Zhu, L., Zhao, Y. and Tzeng, C., 2023. Protective Effects of *Lactobacillus gasseri* against High-Cholesterol Diet-Induced Fatty Liver and Regulation of Host Gene Expression Profiles. *International Journal of Molecular Sciences*, 24(3), p.2053. <https://doi.org/10.3390/ijms24032053>

Helmy, Y.A., Kassem, I.I. and Rajashekara, G., 2021. Immuno-modulatory effect of probiotic *E. coli* Nissle 1917 in polarized human colonic cells against *Campylobacter jejuni* infection. *Gut Microbes*, 13(1), pp.1–16. <https://doi.org/10.1080/19490976.2020.1857514>

Hemmerling, A., Govender, V., Dong, K., Dong, M., Pillay, S., Ndung'u, T., Bhoola, A., Moodley, J., Casillas, G., Lagenaur, L., Mitchell, C.M., Kwon, D.S. and Cohen, C.R., 2025. Acceptability of the live biotherapeutic LACTIN-V (*Lactobacillus crispatus* CTV-05) among young women at high risk of HIV acquisition in South Africa: data from the phase 2 placebo-controlled trial. *Frontiers in Reproductive Health*, 7, p.1544458. <https://doi.org/10.3389/frph.2025.1544458>

- Howarth, G.S., 2024. Probiotaceuticals: back to the future? *Journal of Nutrition*. <https://doi.org/10.1016/j.tjnut.2024.09.006>
- Huang, Z., Zhou, X., Stanton, C., Ross, R.P., Zhao, J., Zhang, H., Yang, B., Chen, W., 2021. Comparative Genomics and Specific Functional Characteristics Analysis of *Lactobacillus acidophilus*. *Microorganisms*, 9(9), p.1992. <https://doi.org/10.3390/microorganisms9091992>
- Hudson, P.L., Hung, K.J., Bergerat, A. and Mitchell, C., 2020. Effect of Vaginal *Lactobacillus* Species on *Escherichia coli* Growth. *Female Pelvic Medicine & Reconstructive Surgery*, 26(2), pp.146–151. <https://doi.org/10.1097/SPV.0000000000000827>
- Im, E.J., Lee, H.H., Kim, M. & Kim, M.K., 2023. Evaluation of enterococcal probiotic usage and review of potential health benefits, safety, and risk of antibiotic-resistant strain emergence. *Antibiotics*, 12(8), p.1327. Available at: <https://doi.org/10.3390/antibiotics12081327>
- Ioannou, P., Ziogou, A., Giannakodimos, I., Giannakodimos, A., Baliou, S. and Samonis, G., 2024. Infective Endocarditis by *Lactobacillus* Species—A Narrative Review. *Antibiotics*, 13(1), p.53. <https://doi.org/10.3390/antibiotics13010053>
- Ismael, M., Huang, M. & Zhong, Q., 2024. The bacteriocins produced by lactic acid bacteria and the promising applications in promoting gastrointestinal health. *Foods*, 13(23), p.3887. <https://doi.org/10.3390/foods13233887>
- Jayamurali, D., Saminathan, G., Varier, K.M., Acharya, P.S., Manoharan, N., Parasuraman, R., Gajendran, B. and Govindarajulu, S.N., 2023. Revealing the hidden heights of microbial metabolites on reproductive physiology. In: *Recent Advances and Future Perspectives of Microbial Metabolites*. Academic Press, pp.217–248. <https://doi.org/10.1016/B978-0-323-90113-0.00004-3>
- Jin, X., Zhang, L., Cao, Y., Dai, Z., Ge, X., Cai, R., Wang, R. and Hu, Z., 2025. Antibiotic resistance characterization, virulence factors and molecular characteristics of *Bacillus* species isolated from probiotic preparations in China. *Journal of Global Antimicrobial Resistance*, 43, pp.35–39. <https://doi.org/10.1016/j.jgar.2024.06.015>

Kang, C.H., Kim, J.S., Kim, H. et al., 2021. Heat-killed lactic acid bacteria inhibit nitric oxide production via inducible nitric oxide synthase and cyclooxygenase-2 in RAW 264.7 cells. *Probiotics and Antimicrobial Proteins*, 13, pp.1530–1538. <https://doi.org/10.1007/s12602-021-09781-9>

Katiku, M.M., Matofari, J.W. and Nduko, J.M., 2022. Preliminary evaluation of probiotic properties and safety profile of *Lactiplantibacillus plantarum* isolated from spontaneously fermented milk, *Amabere amaruranu*. *Heliyon*, 8(8), p.e10342. <https://doi.org/10.1016/j.heliyon.2022.e10342>

Katkowska, M., Garbacz, K. & Kusiak, A., 2021. Probiotics: Should all patients take them? *Microorganisms*, 9(12), p.2620. <https://doi.org/10.3390/microorganisms9122620>

Khatoon, H., Chavan, D., Anokhe, A. & Kalia, V., 2022. Catalase test: a biochemical protocol for bacterial identification. *V*.

Khorshidian, N., Khanniri, E., Mohammadi, M., Mortazavian, A.M. and Yousefi, M., 2021. Antibacterial Activity of Pediocin and Pediocin-Producing Bacteria Against *Listeria monocytogenes* in Meat Products. *Frontiers in Microbiology*, 12, p.709959. <https://doi.org/10.3389/fmicb.2021.709959>

Kim, R., Wang, Y., Sims, C.E. & Allbritton, N.L., 2022. A platform for co-culture of primary human colonic epithelium with anaerobic probiotic bacteria. *Frontiers in Bioengineering and Biotechnology*, 10, p.890396. <https://doi.org/10.3389/fbioe.2022.890396>

Kopacz, K. & Phadtare, S., 2022. Probiotics for the prevention of antibiotic-associated diarrhea. *Healthcare*, 10(8), p.1450. <https://doi.org/10.3390/healthcare10081450>

Krawczyk, B., Wityk, P., Gałęcka, M. & Michalik, M., 2021. The many faces of *Enterococcus* spp.—Commensal, probiotic and opportunistic pathogen. *Microorganisms*, 9(9), p.1900. Available at: <https://doi.org/10.3390/microorganisms9091900>

Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., Chakraborty, C., Singh, B., Marotta, F., Jain, S. & Yadav, H., 2022. *Corrigendum to "Cholesterol-Lowering Probiotics as Potential Biotherapeutics for Metabolic Diseases"*. *Journal of Diabetes Research*, 2022, p.3952529. <https://doi.org/10.1155/2022/3952529>

- Kyei-Baffour, V.O., Vijaya, A.K., Burokas, A. & Daliri, E.B.M., 2025. Psychobiotics and the gut-brain axis: advances in metabolite quantification and their implications for mental health. *Critical Reviews in Food Science and Nutrition*, pp.1–20. <https://doi.org/10.1080/10408398.2025.2459341>
- Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M.W., Rehman, A., Riaz, T., Aadil, R.M., Khan, I.M., Özogul, F., Rocha, J.M., Esatbeyoglu, T. & Korma, S.A., 2023. Probiotics: mechanism of action, health benefits and their application in food industries. *Frontiers in Microbiology*, 14, 1216674. <https://doi.org/10.3389/fmicb.2023.1216674>
- Lee, H.J., Cho, J.H., Cho, W.J., Gang, S.H., Park, S.H., Jung, B.J., Kim, H.B. and Song, K.H., 2022. Effects of Synbiotic Preparation Containing *Lactobacillus gasseri* BNR17 on Body Fat in Obese Dogs: A Pilot Study. *Animals*, 12(5), p.642. <https://doi.org/10.3390/ani12050642>
- Liu, X., Zhao, H. & Wong, A., 2024. Accounting for the health risk of probiotics. *Heliyon*, 10(6), p.e27908. <https://doi.org/10.1016/j.heliyon.2024.e27908>
- LPSN, 2025. *Lactobacillus gasseri*. Available at: <https://lpsn.dsmz.de/species/Lactobacillus-gasseri> [Accessed 12 Jul. 2025].
- Luise, D., Bosi, P., Raff, L., Amatucci, L., Viridis, S. and Trevisi, P., 2022. *Bacillus* spp. Probiotic Strains as a Potential Tool for Limiting the Use of Antibiotics, and Improving the Growth and Health of Pigs and Chickens. *Frontiers in Microbiology*, 13, p.801827. <https://doi.org/10.3389/fmicb.2022.801827>
- Ma, X., Wu, M., Wang, C., et al., 2022. The pathogenesis of prevalent aerobic bacteria in aerobic vaginitis and adverse pregnancy outcomes: a narrative review. *Reproductive Health*, 19(21). <https://doi.org/10.1186/s12978-021-01292-8>.
- Mallappa, R.H., Balasubramaniam, C., Amarlapudi, M.R., Kelkar, S., Adewumi, G.A., Kadyan, S., Pradhan, D. & Grover, S., 2022. Role of probiotics in the prevention and management of diabetes and obesity. In: M.K. Dwivedi, N. Amaesan, A. Sankaranarayanan & E.H. Kemp, eds. *Probiotics in the prevention and management of human diseases*. 1st ed. Academic Press, pp.321–336. <https://doi.org/10.1016/B978-0-12-823733-5.00006-4>

- Martínez, B., Rodríguez, A., Kulakauskas, S. and Chapot-Chartier, M.P., 2020. Cell wall homeostasis in lactic acid bacteria: threats and defences. *FEMS Microbiology Reviews*, 44(5), pp.538–564. <https://doi.org/10.1093/femsre/fuaa021>
- Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E. & Rotondo, J.C., 2023. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, 12(1), p.184. Available at: <https://doi.org/10.3390/cells12010184>
- Mei, Z. and Li, D., 2022. The role of probiotics in vaginal health. *Frontiers in Cellular and Infection Microbiology*, 12, 963868. <https://doi.org/10.3389/fcimb.2022.963868>
- Miko, E. & Barakonyi, A., 2023. The role of hydrogen-peroxide (H<sub>2</sub>O<sub>2</sub>) produced by vaginal microbiota in female reproductive health. *Antioxidants*, 12(5), p.1055. <https://doi.org/10.3390/antiox12051055>
- Mishra, A., Dash, P.P., Usmani, A., Singh, S.P. and Sirbaiya, A.K., 2021. Probiotics as Next Generation Strategy for Cancer Therapy. In: *Probiotic Research in Therapeutics*. Springer, Singapore, pp.69–94. [https://doi.org/10.1007/978-981-15-8214-1\\_4](https://doi.org/10.1007/978-981-15-8214-1_4)
- Momin, E.S., Khan, A.A., Kashyap, T., Pervaiz, M.A., Akram, A., Mannan, V., Sanusi, M. & Elshaikh, A.O., 2023. The effects of probiotics on cholesterol levels in patients with metabolic syndrome: a systematic review. *Cureus*, 15(4), p.e37567. <https://doi.org/10.7759/cureus.37567>
- Monier, M.N., Kabary, H., Elfeky, A. et al., 2023. The effects of *Bacillus* species probiotics (*Bacillus subtilis* and *B. licheniformis*) on the water quality, immune responses, and resistance of whiteleg shrimp (*Litopenaeus vannamei*) against *Fusarium solani* infection. *Aquaculture International*, 31, pp.3437–3455. Available at: <https://doi.org/10.1007/s10499-023-01136-1>
- Monika, K., Malik, T., Gehlot, R., K, R., Kumari, A., Sindhu, R. and Rohilla, P., 2021. Antimicrobial Property of Probiotics. *Environment Conservation Journal*, 22, pp.33–48. <https://doi.org/10.36953/ECJ.2021.SE.2204>
- Muscariello, L., De Siena, B. and Marasco, R., 2020. *Lactobacillus* Cell Surface Proteins Involved in Interaction with Mucus and Extracellular Matrix Components. *Current Microbiology*, 77(12), pp.3831–3841. <https://doi.org/10.1007/s00284-020-02243-5>

- Muthusamy, K., Han, H.-S., Soundharrajan, I., Jung, J.-S., Valan Arasu, M. & Choi, K.-C., 2023. A novel strain of probiotic *Leuconostoc citreum* inhibits infection-causing bacterial pathogens. *Microorganisms*, 11(2), p.469. Available at: <https://doi.org/10.3390/microorganisms11020469>
- Nilsen, T., Swedek, I., Lagenaur, L.A. and Parks, T.P., 2020. Novel Selective Inhibition of *Lactobacillus iners* by *Lactobacillus*-Derived Bacteriocins. *Applied and Environmental Microbiology*, 86, e01594-20. <https://doi.org/10.1128/AEM.01594-20>
- Paladino, L. et al., 2023. NF- $\kappa$ B regulation and the chaperone system mediate restorative effects of the probiotic *Lactobacillus fermentum* LF31 in the small intestine and cerebellum of mice with ethanol-induced damage. *Biology*, 12(11), p.1394. <https://doi.org/10.3390/biology12111394>
- Paray, A.A., Singh, M., Mir, M.A. and Kaur, M., 2023. Gram staining: a brief review. *International Journal of Research and Review*, 10, pp.336–341. <https://doi.org/10.52403/ijrr.20230934>
- Payne, J., Bellmer, D., Jadeja, R. and Muriana, P., 2024. The Potential of *Bacillus* Species as Probiotics in the Food Industry: A Review. *Foods*, 13(15), p.2444. <https://doi.org/10.3390/foods13152444>
- Peroni, D.G. & Morelli, L., 2021. Probiotics as adjuvants in vaccine strategy: is there more room for improvement? *Vaccines*, 9(8), p.811. <https://doi.org/10.3390/vaccines9080811>
- Petrova, M.I., Reid, G. & ter Haar, J.A., 2021. *Lactocaseibacillus rhamnosus* GR-1, a.k.a. *Lactobacillus rhamnosus* GR-1: past and future perspectives. *Trends in Microbiology*, 29(8), pp.747–761. <https://doi.org/10.1016/j.tim.2021.03.010>.
- Pinto, P.I.S. et al., 2024. Quantitative PCR assays as a monitoring tool for bacterial genera in fresh fish fillets. *Food Bioscience*, 62, p.104928. <https://doi.org/10.1016/j.fbio.2024.104928>
- Pradhan, S. and Weiss, A.A., 2020. Probiotic Properties of *Escherichia coli* Nissle in Human Intestinal Organoids. *mBio*, 11, p.e01470-20. <https://doi.org/10.1128/mbio.01470-20>

Rizzello, C.G. and De Angelis, M., 2022. *Lactobacillus delbrueckii* Group. In: *Encyclopedia of Dairy Sciences* (Third Edition). Academic Press, pp.287–292. <https://doi.org/10.1016/B978-0-12-818766-1.00141-0>

Rossi, F., Santonicola, S., Amadoro, C., Marino, L. and Colavita, G., 2024. Recent Records on Bacterial Opportunistic Infections via the Dietary Route. *Microorganisms*, 12(1), p.69. <https://doi.org/10.3390/microorganisms12010069>

Rushdi, R., 2022. Antimicrobial Activity of Probiotic *Lactobacilli* Against Some Pathogenic Bacteria. *Annals of Scientific Research in Healthcare and Rehabilitation*, 1(1). <https://doi.org/10.33140/asrhr.01.01.09>

Sadaqat, M.H., 2024. Antibacterial, antiviral, antifungal, and immunomodulatory properties of probiotics. *Afghanistan Journal of Basic Medical Sciences*, 2(2), pp.111–120. <https://doi.org/10.62134/ajbms/v2.i2.khatamuni.10>

Sahu, M., 2025. Antibacterial activity of *Lactobacillus* species against some pathogenic and food spoilage bacteria. *Food Biology*, 14, pp.1–5. <https://doi.org/10.25081/fb.2025.v14.9224>

Salman, M.K., Abuqwider, J. & Mauriello, G., 2023. Anti-quorum sensing activity of probiotics: the mechanism and role in food and gut health. *Microorganisms*, 11(3), p.793. <https://doi.org/10.3390/microorganisms11030793>

Shi, H., Deng, X., Deng, Q., Liu, Z. & Liu, N., 2021. Probiotic *Lactobacilli* improved growth performance and attenuated *Salmonella Typhimurium* infection via Jak/Stat signaling in broilers. *Brazilian Journal of Poultry Science*, 23(1), p.eRBCA-2020-1328. <https://doi.org/10.1590/1806-9061-2020-1328>

Shruthi, B., Deepa, N., Somashekaraiah, R., Adithi, G., Divyashree, S. & Sreenivasa, M.Y., 2022. Exploring biotechnological and functional characteristics of probiotic yeasts: A review. *Biotechnology Reports*, 34, p.e00716. Available at: <https://doi.org/10.1016/j.btre.2022.e00716>

So, D., Quigley, E.M.M. & Whelan, K., 2023. Probiotics in irritable bowel syndrome and inflammatory bowel disease: review of mechanisms and effectiveness. *Current Opinion in Gastroenterology*, 39(2), pp.103–109. <https://doi.org/10.1097/MOG.0000000000000902>

Steyer, A., Mičetić-Turk, D. & Fijan, S., 2022. The efficacy of probiotics as antiviral agents for the treatment of rotavirus gastrointestinal infections in children: an updated overview of literature. *Microorganisms*, 10(12), p.2392. <https://doi.org/10.3390/microorganisms10122392>

Su, H., Guo, Y., Cheng, H., Hu, S., Zhang, P. & Yang, Z., 2024. Probiotic and fermentation properties of *Leuconostoc mesenteroides* strain I1/53 from sugarcane juice by a multi-omics approach. *LWT*, 211, p.116897. Available at: <https://doi.org/10.1016/j.lwt.2024.116897>

Sultana, T., Tuhin, M., Rabbi, M.F., Sarker, B.R., Islam, M., Begum, M.I.A. and Hossain, K.M.M., 2021. Prevalence and antibiotic resistance patterns of *Escherichia coli* isolated from milk and milk products. *Journal of Bio-Science*, 29(2), pp.81–91. <https://doi.org/10.3329/jbs.v29i2.54957>.

Sun, M., Li, Q., Zhang, F., Yao, D., Huang, W., Lv, Q., Jiang, H., Kong, D., Ren, Y., Chen, S., Jiang, Y. and Liu, P., 2025. The Genomic Characteristics of Potential Probiotics: Two *Streptococcus salivarius* Isolates from a Healthy Individual in China. *Microorganisms*, 13(3), p.694. <https://doi.org/10.3390/microorganisms13030694>

Taghizad, F., Kazerani, H.R., Dehghani, H., Asoodeh, A. and Yaghubi, D., 2021. A novel approach towards obesity: The use of a bacterial product, gassericin A, in 3T3-L1 cells. *Obesity Research & Clinical Practice*, 15(5), pp.499–505. <https://doi.org/10.1016/j.orcp.2021.08.005>

Tang, H., Huang, W. and Yao, Y.F., 2023. The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. *Microbial Cell*, 10(3), pp.49–62. <https://doi.org/10.15698/mic2023.03.792>

Tao, Y.W., Gu, Y.L., Mao, X.Q., Zhang, L. & Pei, Y.F., 2020. Effects of probiotics on type II diabetes mellitus: a meta-analysis. *Journal of Translational Medicine*, 18(1), p.30. <https://doi.org/10.1186/s12967-020-02213-2>

Taufer, C.R., da Silva, J. & Rampelotto, P.H., 2024. The influence of probiotic Lactobacilli on COVID-19 and the microbiota. *Nutrients*, 16(9), p.1350. <https://doi.org/10.3390/nu16091350>

Thye, A.Y., Bah, Y.R., Law, J.W., Tan, L.T., He, Y.W., Wong, S.H., Thurairajasingam, S., Chan, K.G., Lee, L.H. & Letchumanan, V., 2022. Gut-skin axis: Unravelling the connection between

the gut microbiome and psoriasis. *Biomedicines*, 10(5), p.1037.  
<https://doi.org/10.3390/biomedicines10051037>

Todorov, S.D., Dioso, C.M., Liong, M.T., Nero, L.A., Khosravi-Darani, K. & Ivanova, I.V., 2022. Beneficial features of *Pediococcus*: From starter cultures and inhibitory activities to probiotic benefits. *World Journal of Microbiology & Biotechnology*, 39(1), p.4. Available at:  
<https://doi.org/10.1007/s11274-022-03419-w>

Tsai, C.C. and Lai, Z.Y., 2024. Rapid identification and application of *Lactobacillus plantarum*, *Lactobacillus paracasei* and *Lactobacillus pentosus* using multiplex polymerase chain reaction and species-specific primers, targeting 16S ribosomal RNA and *recA* genes. *Applied Food Biotechnology*, 11(1), p.e24. <https://doi.org/10.22037/afb.v11i1.44863>

Vinayamohan, P., Joseph, D., Viju, L.S., Baskaran, S.A. & Venkitanarayanan, K., 2024. Efficacy of probiotics in reducing pathogenic potential of infectious agents. *Fermentation*, 10(12), p.599.  
<https://doi.org/10.3390/fermentation10120599>

Vincenzi, A., Goettert, M.I. & Volken de Souza, C.F., 2021. An evaluation of the effects of probiotics on tumoral necrosis factor (TNF- $\alpha$ ) signaling and gene expression. *Cytokine & Growth Factor Reviews*, 57, pp.27–38. <https://doi.org/10.1016/j.cytogfr.2020.10.004>

Wu, L.Y., Yang, T.H., Ou, Y.C. and Lin, H., 2024. The role of probiotics in women's health: An update narrative review. *Taiwanese Journal of Obstetrics and Gynecology*, 63(1), pp.29–36.  
<https://doi.org/10.1016/j.tjog.2023.09.018>

Xie, Q., Liu, J., Yu, P., Qiu, T., Jiang, S. & Yu, R., 2025. Unlocking the power of probiotics, postbiotics: targeting apoptosis for the treatment and prevention of digestive diseases. *Frontiers in Nutrition*, 12, p.1570268. <https://doi.org/10.3389/fnut.2025.1570268>

Yang, S., Hu, X., Cang, W., Ji, S., Wu, R. & Wu, J., 2024. Biofilm-based probiotic delivery system and its application in the food industry. *Food Bioscience*, 62, p.105172.  
<https://doi.org/10.1016/j.fbio.2024.105172>

Yao, B., Yang, Z., Zhao, X., Han, Z., Li, P. & Shang, N., 2025. Biofilm-state probiotics: advanced alternatives to traditional probiotics. *Trends in Food Science & Technology*, 156, p.104854. <https://doi.org/10.1016/j.tifs.2024.104854>

- Yilmaz, B., Bangar, S.P., Echegaray, N., Suri, S., Tomasevic, I., Lorenzo, J.M., Melekoglu, E., Rocha, J.M. and Ozogul, F., 2022. The Impacts of *Lactiplantibacillus plantarum* on the Functional Properties of Fermented Foods: A Review of Current Knowledge. *Microorganisms*, 10(4), p.826. <https://doi.org/10.3390/microorganisms10040826>
- You, X., Rani, A., Özcan, E., Lyu, Y. and Sela, D.A., 2023. *Bifidobacterium longum* subsp. *infantis* utilizes human milk urea to recycle nitrogen within the infant gut microbiome. *Gut Microbes*, 15(1), p.2192546. <https://doi.org/10.1080/19490976.2023.2192546>
- Zaccaria, E., Klaassen, T., Alleleyn, A.M.E., Boekhorst, J., Chervaux, C., Smokvina, T. and Kleerebezem, M., 2023. *L. rhamnosus* CNCM I-3690 survival, adaptation, and small bowel microbiome impact in human. *Gut Microbes*, 15(1). <https://doi.org/10.1080/19490976.2023.2244720>
- Zahedifard, T., Khadivzadeh, T. and Rakhshkhorshid, M., 2023. The role of probiotics in the treatment of vulvovaginal candidiasis: A systematic review and meta-analysis. *Ethiopian Journal of Health Sciences*, 33(5), pp.881–890. <https://doi.org/10.4314/ejhs.v33i5.18>
- Zavišić, G., Popović, M., Stojkov, S., Medić, D., Gusman, V., Jovanović Lješević, N. & Jovanović Galović, A., 2023. Antibiotic resistance and probiotics: knowledge gaps, market overview and preliminary screening. *Antibiotics*, 12(8), p.1281. <https://doi.org/10.3390/antibiotics12081281>
- Zhang, A., Zhang, Z., Zhang, K., Liu, X., Lin, X., Zhang, Z., Bao, T. and Feng, Z., 2021. Nutrient consumption patterns of *Lactobacillus plantarum* and their application in *suancai*. *International Journal of Food Microbiology*, 354, p.109317. <https://doi.org/10.1016/j.ijfoodmicro.2021.109317>
- Zhang, X., Al-Dossary, A., Hussain, M., Setlow, P. and Li, J., 2020. Applications of *Bacillus subtilis* Spores in Biotechnology and Advanced Materials. *Applied and Environmental Microbiology*, 86, e01096-20. <https://doi.org/10.1128/AEM.01096-20>
- Zhang, Z., Guo, Q., Yang, Z., Sun, Y., Jiang, S., He, Y., Li, J. and Zhang, J., 2025. *Bifidobacterium adolescentis*-derived nicotinic acid improves host skeletal muscle mitochondrial

function to ameliorate sarcopenia. *Cell Reports*, 44(2), 115265. <https://doi.org/10.1016/j.celrep.2025.115265>

Zhang, Z., Guo, Q., Yang, Z., Sun, Y., Jiang, S., He, Y., Li, J. and Zhang, J., 2025. *Bifidobacterium adolescentis*-derived nicotinic acid improves host skeletal muscle mitochondrial function to ameliorate sarcopenia. *Cell Reports*, 44(2), p.115265. <https://doi.org/10.1016/j.celrep.2025.115265>

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G. and Lebeer, S., 2020. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, published 15 April. <https://doi.org/10.1099/ijsem.0.004107>

Zheng, N., Guo, R., Wang, J., Zhou, W. and Ling, Z., 2021. Contribution of *Lactobacillus iners* to Vaginal Health and Diseases: A Systematic Review. *Frontiers in Cellular and Infection Microbiology*, 11, p.792787. <https://doi.org/10.3389/fcimb.2021.792787>

Zhou, X., Shang, G.S., Tan, Q., He, Q., Tan, X., Park, K.Y. & Zhao, X., 2021. Effect of *Lactobacillus fermentum* TKSNO41 on improving streptozotocin-induced type 2 diabetes in rats. *Food & Function*, 12(17), pp.7938–7953. <https://doi.org/10.1039/d1fo01571k>

Zhu, G., Guo, M., Zhao, J., Zhang, H., Wang, G. & Chen, W., 2022. *Bifidobacterium breve* intervention combined with environmental enrichment alleviates cognitive impairment by regulating the gut microbiota and microbial metabolites in Alzheimer's disease mice. *Frontiers in Immunology*, 13, p.1013664. <https://doi.org/10.3389/fimmu.2022.1013664>

Žuntar, I., Petric, Z., Bursać Kovačević, D. & Putnik, P., 2020. Safety of probiotics: functional fruit beverages and nutraceuticals. *Foods*, 9(7), p.947. <https://doi.org/10.3390/foods9070947>