THE SURVIVAL STUDIES OF *Lactobacillus gasseri* IN THE PRESENCE OF SOME SUPPOSITORY BASES AND *Escherichia coli* .

BY

EKHOEVBIYE SIMON OSAYOMWONBOR

PHA1908491

SUPERVISED BY

DR ENOSAKHARE OLOTON

DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY,

FACULTY OF PHARMACY,

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PRESENTED TO THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN.

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, **EKHOEVBIYE SIMON OSAYOMWONBOR,** with matriculation number **PHA1908491,** hereby affirm that this project titled **"The Survival Studies of *Lactobacillus gasseri* in the Presence of Some Suppository Bases and *Escherichia coli* "** is an original research work conducted 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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EKHOEVBIYE SIMON OSAYOMWONBOR **DATE**

CERTIFICATION

This is to certify that this research work is an original study conducted by **EKHOEVBIYE SIMON OSAYOMWONBOR** 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 (Pharm. D) degree.

_____ _____

EKHOEVBIYE SIMON OSAYOMWONBOR **DATE**

(PROJECT STUDENT)

_____ _____

DR E. OLOTON **DATE**

(PROJECT SUPERVISOR)

_____ _____

DR (MRS) UPE BABAIWA **DATE**

(HEAD OF DEPARTMENT)

DEDICATION

This project work is dedicated to God Almighty for His unfailing grace and strength throughout the course of this research. It is also lovingly dedicated to my grandmother of blessed memory, who is no longer here to witness what her favorite grandchild has become. Finally, this work is dedicated to all healthcare professionals and those in training, who selflessly devote parts of their lives to saving the lives of others.

ACKNOWLEDGEMENT

I give all glory and thanks to God Almighty for His grace, wisdom, and strength which have sustained me throughout the completion of this research work. His guidance and blessings have been my constant source of help from the beginning to the end of this journey.

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I deeply appreciate my beloved family, beginning with my parents, Mr. and Mrs. J. Igbinovia, for their prayers, love, and sacrifices throughout my stay in this great institution. My heartfelt thanks also go to my siblings Engr. Eghosa Igbinovia, Engr. Kelly Igbinovia, Dr. Ephraim Ekhoeovbiye, Ade Omorodion, and Joy Igbinovia for their unwavering love, support, and encouragement which continually inspired me to stay focused and finish strong.

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My heartfelt appreciation also goes to my church, Christian Fellowship International (CFI), for being a family and a source of strength to me. I am grateful to the International Officers, Sector Directors, and my Current CEC — Pst. Ella, Pst. Nancy, Pst. Caleb, Pst. CG, Elo, Shalom Mbet, Shalom Godswill, Promise, Destiny, Precious, Pst. Miracle, Excellent, and Treasure — for their encouragement and support. Thank you for making life as both a pastor and a student fulfilling. To all my CFI children, I love and appreciate you all deeply.

Finally, I would like to appreciate myself — Simon, you did it! Thank you for staying consistent, resilient, and committed to the end. Cheers to this achievement!

ABSTRACT

Introduction: The survival and interaction of probiotic microorganisms in pharmaceutical carriers are critical in determining their therapeutic efficacy. This study investigated the survival pattern of *Lactobacillus gasseri* in the presence of selected suppository bases and *Escherichia coli* , with the aim of identifying a suitable base that enhances probiotic viability while inhibiting pathogenic growth.

Methods: Pure cultures of *Lactobacillus gasseri* and *Escherichia coli* were obtained and characterized through Gram staining and biochemical tests. Survival studies were conducted by inoculating the organisms in media containing different suppository bases—glycerogelatin, Polyethylene Glycol (POLYETHYLENE GLYCOL), and Theobroma base—and monitoring their growth patterns over time. The regression equations for the growth curves were determined as follows: control *Lactobacillus gasseri*, $y = 6.81 + 0.18x$; glycerogelatin, *Escherichia coli* , $y = 4.31 - 0.06x$; POLYETHYLENE GLYCOL, *Escherichia coli* , $y = 3.27 + 0.24x$; Theobroma base, *Escherichia coli* , $y = 6.93 + 0.23x$. Statistical analyses were performed to evaluate significance ($p < 0.05$).

Results and Discussion: The control culture of *Lactobacillus gasseri* exhibited a steady positive growth trend, confirming its natural viability. In the presence of glycerogelatin, *Escherichia coli* showed a decline in growth (negative slope), while *Lactobacillus gasseri* maintained its viability, indicating a statistically significant difference ($p < 0.001$). Polyethylene Glycol supported the growth of both organisms but favored *Escherichia coli* proliferation ($p < 0.001$), whereas Theobroma base showed no significant difference between both microorganisms ($p = 0.126$). These results suggest that the hydrophilic nature of glycerogelatin promotes probiotic survival and inhibits pathogenic bacteria, while POLYETHYLENE GLYCOL supports general microbial growth and Theobroma base remains relatively inert.

Conclusion: The type of suppository base significantly influences the survival of *Lactobacillus gasseri* in the presence of *Escherichia coli* . Among the bases tested, glycerogelatin demonstrated the most favorable effect by enhancing probiotic viability and suppressing pathogenic growth. Therefore, glycerogelatin is recommended as a suitable base for probiotic suppository formulations intended to support microbial balance and therapeutic efficacy

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INTRODUCTION

Background of the Study

The use of probiotics in pharmaceutical and clinical applications has attracted increasing attention due to their role in promoting health and restoring microbial balance. Among these beneficial microorganisms, Lactobacillus species are the most widely studied, particularly for their capacity to colonize mucosal surfaces and inhibit the growth of pathogenic bacteria (Ouwehand & Vesterlund, 2004). *Lactobacillus gasseri*, a member of the lactic acid bacteria group, is a prominent probiotic found in the human gastrointestinal and urogenital tracts. It produces lactic acid, hydrogen peroxide, and bacteriocins, all of which contribute to the inhibition of pathogens such as *Escherichia coli,* Gardnerella vaginalis, and Staphylococcus aureus (Dobson *et al*, 2012).

For probiotics to exert their therapeutic effect, viability and stability within the delivery system are crucial. Pharmaceutical formulations such as suppositories offer an alternative route for delivering probiotics directly to mucosal surfaces, such as the vaginal or rectal epithelium, where they can restore microbial balance and combat infections (Baral *et al*, 2021). However, the survival of probiotic organisms during formulation, storage, and administration depends largely on the type of base used. Suppository bases like glycerogelatin, Polyethylene Glycol (POLYETHYLENE GLYCOL), and Theobroma base differ in their physicochemical properties—melting point, hydrophilicity, moisture content, and pH stability—all of which can influence microbial viability and release characteristics (Polova *et al*, 2020).

The concurrent presence of *Escherichia coli* , a common urogenital and intestinal pathogen, poses an additional challenge to probiotic survival. *Escherichia coli* competes with beneficial bacteria for nutrients and adhesion sites and may produce inhibitory metabolites that reduce probiotic viability (Nader-Macías *et al*, 2021). Understanding how *Lactobacillus gasseri* survives when exposed to different suppository bases in the presence of *Escherichia coli* is therefore important for optimizing probiotic formulations intended for infection prevention and mucosal health.

This study investigates the survival pattern of *Lactobacillus gasseri* in various suppository bases (glycerogelatin, Polyethylene Glycol, and Theobroma base) and its interaction with *Escherichia coli* . The findings will provide scientific insight into how formulation components influence probiotic stability, thereby guiding the design of effective probiotic suppositories with improved therapeutic potential for managing microbial imbalances in the genitourinary and intestinal tracts.

1.2 Aim of study

The aim of the study is to determine the Survival of *Lactobacillus gasseri* in the Presence of Some Suppository Bases and *Escherichia coli* .

1.3 Objectives of the Study

1. To confirm the identification and biochemical characteristics of *Lactobacillus gasseri* and *Escherichia coli using* Gram staining and standard biochemical tests.
2. To determine the survival rate and growth pattern of *Lactobacillus gasseri* in

different suppository bases; glycerogelatin, Polyethylene Glycol (POLYETHYLENE GLYCOL), and Theobroma base.

3. To assess the effect of *Escherichia coli on* the survival and growth of *Lactobacillus gasseri* in each suppository base.
4. To statistically compare the influence of the different suppository bases on *Lactobacillus gasseri* viability in the presence of *Escherichia coli.*
5. To identify the most suitable suppository base that maintains the stability and survival of *Lactobacillus gasseri* for potential use in probiotic suppository formulation.

1.4 Justification of the Study

Probiotics, particularly species of Lactobacillus, play an important role in maintaining the microbial balance of the human mucosal surfaces, especially in the vaginal and gastrointestinal tracts. Among them, *Lactobacillus gasseri* is a prominent species known for its ability to produce lactic acid and bacteriocins, thereby inhibiting the growth of pathogenic organisms such as *Escherichia coli* and maintaining a healthy microbial ecosystem (Pan *et al*, 2020). However, for *Lactobacillus gasseri* to be effectively delivered to mucosal sites through pharmaceutical preparations such as suppositories, its viability and stability within the formulation matrix must be ensured.

The choice of suppository base is a critical determinant of probiotic survival and activity. Bases such as glycerogelatin, Polyethylene Glycol, and Theobroma base differ in physicochemical properties including hydrophilicity, melting point, moisture content, and compatibility with biological materials (Baral *et al*, 2021). These

properties may influence not only the release of the probiotic but also its ability to survive during formulation, storage, and co-existence with other microorganisms. Despite increasing interest in probiotic drug delivery systems, limited studies have examined the survival of Lactobacillus species particularly *Lactobacillus gasseri* within different suppository bases and in the presence of competing microorganisms.

Moreover, *Escherichia coli* is a common opportunistic pathogen in the urogenital tract and can antagonize beneficial lactobacilli, leading to infections such as bacterial vaginosis and urinary tract infections (Nader-Macías et al., 2021). Understanding how *Lactobacillus gasseri* survives and interacts with *Escherichia coli in* various suppository environments is therefore essential for developing effective probiotic-based therapeutics that can restore and maintain microbial balance.

This study is justified by the need to provide scientific evidence on the optimal suppository base that supports the survival of *Lactobacillus gasseri* and sustains its antagonistic potential against *Escherichia coli.* The findings will contribute to the formulation of stable, efficacious probiotic suppositories and expand the pharmaceutical application of beneficial microbes in infection prevention and mucosal health maintenance. Furthermore, it will generate baseline data for future studies on the compatibility of probiotics with excipients used in vaginal and rectal dosage forms.

1.5 INTRODUCTION TO PROBIOTICS

The term PROBIOTICS relatively means “for life” which is used as a term for bacteria which have been found to have health benefit by improving the intestinal flora.

Everyday humans ingest a significant number of microorganisms; over the years probiotics microbes have been an essential part of the human diet due to its positive benefit. [Bonfait *et al* 2009, Parvez, 2006] According to The Agricultural Organization of the World Health Organization defines Probiotics in 2001 as bacteria that, when given in the right proportion, improve the health of man. The most commonly used probiotics are; Lactobacillus and Bifidobacterium. [Corsoba, 2001]

Probiotics are live organisms which when introduced into the gastrointestinal tract improve health by enhancing the internal microbial balance. They do so by producing certain chemicals; bacteriocins, siderophores, lysozymes, proteaseses and hydrogen peroxide which inhibit the growth of harmful pathogens. [Fuller, 1992]

Some specific strains of Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus, and Saccharomyces are commonly added to foods because of the health benefits they're believed to offer. In particular, Lactobacillus and Bifidobacterium have been linked to a number of positive effects on health.

For example; they may help people who are lactose intolerant digest dairy more easily (Ouwehand, 1999). They've also been used to prevent and treat different types of diarrhoea, including those caused by infections, antibiotics, or cancer treatments like radiotherapy (Parvez *et al*, 2006). In addition, some studies suggest they can support the immune system (Forsythe and Bienenstock, 2010), reduce the risk of genetic mutations (Chalova *et al*, 2008), help protect against cancer (Liong, 2008), and even lower cholesterol levels in the blood (Ooi and Liong, 2010).

Despite the growing optimism surrounding probiotics, there remains significant skepticism. Many commercial "probiotic" products are criticized for being unreliable in the content and lacking in clinical validation (Hughes and Hillier 1990)

1.5.1 Organisms Known to Be Probiotics

Based on previous studies, there remains significant skepticism as regarding the use of "probiotics" as many commercial products have been criticized for being unreliable in content and lacking in clinical validation (Hughes and Hillier, 1990; Mackay *et al*, 1999; Zhong *et al,* 1998).

Hence, to achieve Probiotics status, microrganism must attain certain standards related to safety, functional effects, and technological properties. (FAO/ WHO, 2001). From the safety point of view, the probiotic microorganisms should not be pathogenic, have no connection causing diarrhoea and no ability to transfer antibiotic resistance genes as these can increase antimicrobial resistance, as well as be able to maintain genetic stability. For them to be recognized as functional food components, they should have certain properties: acid and bile stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, anticarcinogenic and anti-mutagenic activity, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes (Ouwehand *et al,* 1999).

Ellie Metchnikoff's work could be regarded as the pioneer research on probiotics of this century(Fuller, 1989), his work was on a specie of lactobacillus, a very popular specie called the "Bulgarian bacillus" which he isolated from yoghurt starter, this was because he tried to draw a relationship between the life expectancy of the peasants to the consumption of fermented milk, because he found out that majority of the peasants in that area drink this yoghurt and they live long(Fuller, 1989). Ever since then there have been numerous studies on different species. The

main groups of recognized Probiotics include;

I. Lactobacilus spp

The specie *Lactobacillus delbreuckii* isolated by Metchnikoff was later discovered incapable of living and colonizing the human and animal intestinal tract and this is an important factor for an effective probiotic. (Daly, 1991) others found to be highly recognized for their remarkable probiotics qualities include;

a. *Lactobacillus plantarum*:

Also known as *Lantiplantibacillus plantarum* which belongs to the lactic acid bacteria which are a broad group of gram-positive bacteria living in several ecological spectrum (Stefanovic *et al,* 2017; Soleymanzadeh *et al,* 2016). *Lactobacillus plantarum* can adapt and colonize different environments by aiding metabolic flexibility through diverse functional genomes (Saavedra, 2001, Embaby AM *et al,* 2014).

There are 18 strains of the lactobacillus which are each intended to improve ensiling at proposed doses (DSM 23375, CNCM I-3235, DSM 19457, DSM 16568, LMG 21295, DSM 16565, VTT E-78076, CNCM MA 18/5U, NCIMB 30238, ATTC PTA-6139, DSM 18112, ATCC 55058, DSM 18113, DSM 18114, ATCC 55942, ATCC 55943, ATCC 55944 and NCIMB 30094).(Gabriel et. al 2012)

Based on studies on *Lactobacillus plantarum* some of the proven health benefits include; reduction of gastrointestinal infection, reduction in risk of inflammatory bowel disease, and stimulating effects on the immune system. furthermore, the bacterium enhances food shelf life in bio-processed food and prevention of growth of food pathogens. *Lactobacillus plantarum* is a lactic acid producing bacteria which

can also produce antibacterial bio-active compounds and exopolysacccharides EPS, hence, exhibiting antagonistic potential against enteric food borne pathogens activities (Abdelazez *et. al* 2018; Ge *et al,* 2021; Kim *et al,* 2018.)

b. *Lactobacilus acidophilus*:

First named in the 1900s when it was isolated from the human gut, due to its good resistance against acid and bile salts it has broad biological application. *L.acidophilus* is also a lactic acid producing bacteria (LAB). *L.acidophilus* is gram-positive bacilli which does not produce spores and are rod-like in nature. Most *L.acidophilus* strains are microaerobic meaning they grow better in anaerobic conditions. due to the broad application

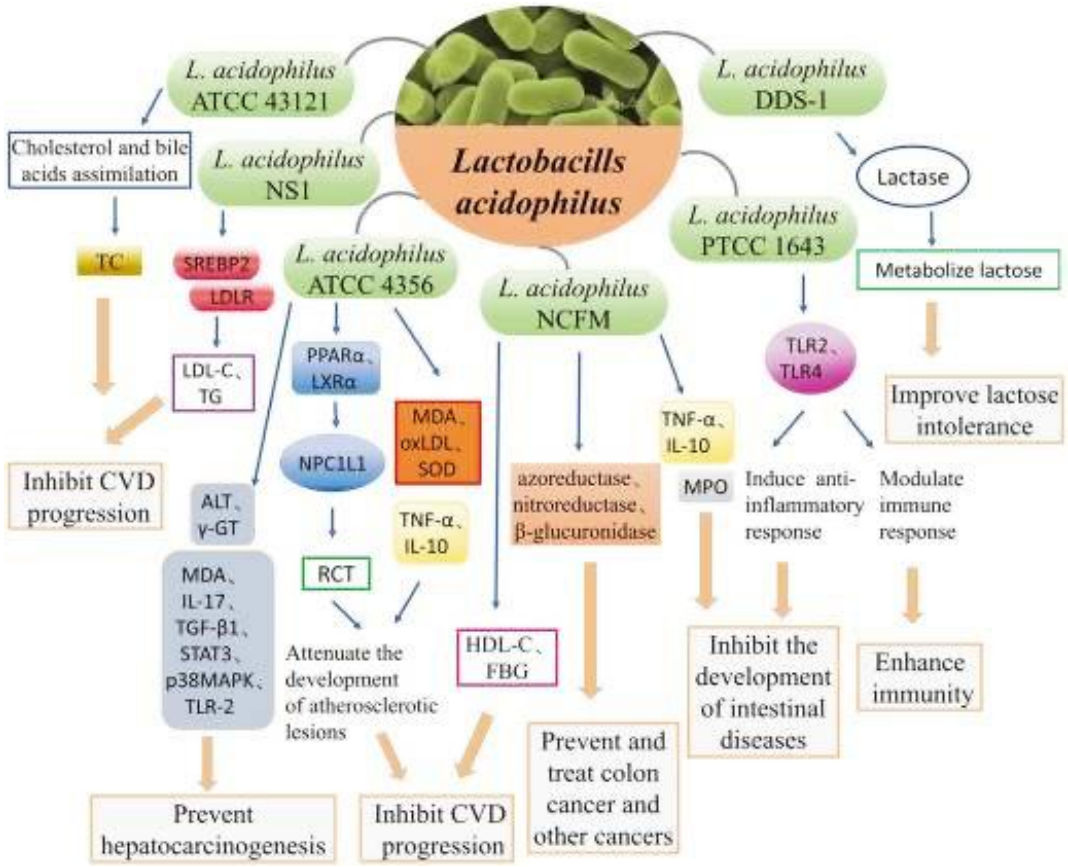

Fig.1. Probiotic properties and biological functions of Lactobacillus acidophilus.

c. *Lactobacillus paracasei:*

This probiotic is present in the human intestine, reproductive tract, initially classified by (collins *et al,* in 1989) has been widely studied for its advantageous effects on lipid metabolism. In 2020, the genus was later renamed to *Lacticaseibacillus paracasei* subsp. paracasei by (Zheng *et al,* 2020). due to advancements in bacterial taxonomy. This reclassification reflects the evolving understanding of the phylogenetic relationships within the Lactobacillaceae family.

d. *Lactobacillus rhamnosus*

Lactobacillus rhamnosus has evolved unique characteristics that allow it to thrive in both acidic and alkaline environments within the human body. Its strong ability to adhere to and colonize the intestinal lining contributes to its prolonged probiotic effects. As a result, this strain is commonly incorporated into dairy products such as yogurt, cheese, and milk to boost probiotic content. Additionally, *L. rhamnosus* plays a key role in cheese maturation, enhancing overall flavor. Certain strains have shown health benefits in both adults and children, particularly in the management of irritable bowel syndrome (IBS), eczema, allergies, and immune system support (Gao *et al,* 2022).

e. Lactobacillus fermentum

Lactobacillus fermentum is a probiotic bacterium known for its adhesive properties and anti-infective effects. Frequently found in the ripening process of specific

cheeses, it is classified as a non-starter lactic acid bacterium (NSLAB). These traits suggest its potential in promoting health, particularly in the urinary and reproductive systems. A notable strain, L. fermentum JDFM216, has been found to support cognitive and physical functions while exhibiting immunomodulatory effects. This includes enhanced macrophage phagocytic activity, increased IgA production, and stimulation of immune cells. Furthermore, L. fermentum demonstrates antibacterial, antioxidative, and cholesterol-lowering abilities, suggesting a role in cardiovascular health. Its strong auto-aggregation capacity is essential for epithelial cell adherence and biofilm formation in the gut (Anjum *et al,* 2014).

f. Lactobacillus johnsonii

One of the earliest strains proposed for probiotic dairy supplementation is Lactobacillus johnsonii LA-1, which is used in Nestlé's LC-1 yogurt products. This strain is notable for enhancing immune responses, resisting stressors such as bile salts and antibiotics, and fighting against multidrug-resistant pathogens. It also maintains high viability in food products. Moreover, L. johnsonii can reduce the adhesion and activity of harmful microbes, inhibit the growth of gut pathogens, and help shorten the duration of diarrhea and enterocolitis (Tavasoli *et al,* 2022).

g. Lactobacillus lactis

Lactobacillus lactis, a well-researched lactic acid bacterium, is widely recognized for its probiotic potential in supporting immune function and alleviating inflammatory bowel conditions. Its probiotic efficacy is assessed based on key criteria such as resistance to acid and bile, cholesterol assimilation, and adhesion to intestinal cells. Strains like L. lactis ML-2018 exhibit anti-inflammatory effects by inhibiting the release of pro-inflammatory molecules triggered by lipopolysaccharides (LPS). Additionally,

some L. lactis strains have been associated with immune enhancement and protection against gastrointestinal and respiratory infections (Zanjani *et al,* 2017; Nazia *et al*, 2014).

h. *Lactobacillus reuteri*

Lactobacillus reuteri is associated with multiple health benefits, including the prevention and management of urogenital infections, bacterial vaginosis in women, allergic conditions, food sensitivities, and dental caries. It has also been studied for its role in preventing colitis and reducing leukocyte-platelet interactions with endothelial cells, which are relevant in intestinal diseases. Its broad antimicrobial activity against bacteria, yeast, and other pathogens further supports its application in managing gastrointestinal and urogenital disorders, including infantile colic (Assimos, 2020).

II. BIFIDOBACTERIUM

a. *Bifidobacterium breve*

Bifidobacterium breve, a species within the genus Bifidobacterium, is well-known for its probiotic properties. Naturally residing in the human intestine, it has been used in managing conditions such as constipation, diarrhea, irritable bowel syndrome (IBS), and even the common cold and flu. Research has supported its wide-ranging physiological benefits. This gram-positive, anaerobic, non-motile, rod-shaped bacterium forms branches with neighboring microbes. B. breve strains are particularly common in pediatric applications and dominate the gut microbiota of breastfed infants. Their presence in human breast milk further highlights their natural occurrence in infants' gastrointestinal tracts. Clinical studies have demonstrated their effectiveness in improving digestive health and overall wellness, reinforcing their importance as a probiotic (Zanjani *et al,* 2017).

b. *Bifidobacterium infantis*

Also known scientifically as Bifidobacterium longum subsp. infantis, *Bifidobacterium infantis* is a non-pathogenic strain that naturally colonizes the human mouth and digestive tract. Belonging to the lactic acid bacteria group alongside Lactobacillus, it plays a vital role in maintaining digestive health. The well-studied strain *B. infantis* 35624 has been shown to alleviate symptoms of IBS—such as bloating, abdominal discomfort, and dysbiosis, especially in infants. Its use has been associated with improved gastrointestinal function and better quality of life in pediatric IBS cases (Azad *et al,* 2018; Sanders *et al,* 2018).

c. *Bifidobacterium longum*

A major constituent of the gut microbiota, *Bifidobacterium longum* is especially abundant in the gastrointestinal tracts of infants. It offers numerous health benefits, including the production of bioactive compounds and interactions between surface molecules and the host immune system. This species has been studied extensively for its role in relieving IBS symptoms such as bloating, abdominal pain, and diarrhea. It has also been investigated for its ability to prevent antibiotic-associated diarrhea, induce remission in ulcerative colitis, and maintain mucosal integrity in the gut. Additionally, research has explored its enzymatic functions, cell adhesion ability, anti-cancer potential, immune modulation, and role in reducing allergic responses and managing inflammatory bowel disease (Azad *et al,* 2018).

d. *Bifidobacterium lactis*

The strain Bifidobacterium lactis has been well-characterized in vitro, showing excellent stability in both food products and freeze-dried formulations. Clinical

studies have demonstrated that *B. lactis* HN019 can enhance gastrointestinal health, improve digestion, and support immune function (Sanders *et al,* 2019).

e. Bifidobacterium thermophilum

Bifidobacterium thermophilum is an aerotolerant probiotic that thrives in low-oxygen environments, making it suitable for various probiotic applications. It produces bacteriocin-like compounds with antimicrobial activity against pathogens such as Listeria spp., Salmonella spp., and Campylobacter jejuni, and has shown protective effects against rotavirus infections. These characteristics make it a valuable candidate for inclusion in probiotic products and functional foods (Sanders *et al,* 2019).

III. OTHERS

i. Bacillus coagulans;

Bacillus coagulans is a spore-forming probiotic known for its resilience under harsh environmental conditions, including high gastric acidity. This resilience allows it to survive digestion and deliver health benefits such as relief from gastrointestinal discomfort. It can also regulate gut microbiota composition and suppress the growth of harmful microbes, promoting both digestive and immune health. Naturally present in fermented foods like yogurt, sauerkraut, and kimchi, *B. coagulans* is also commonly used in probiotic supplements due to its industrial viability (Ma *et al,* 2021).

ii. Streptococcus thermophilus

Widely used in the dairy industry, *Streptococcus thermophilus* plays a central role in producing yogurt and various cheeses. It assists in lactose breakdown, contributing to yogurt's texture and flavor, and may reduce fat content in certain cheeses like Swiss

cheese. Beyond food processing, this strain offers probiotic benefits, including immune system support and anti-inflammatory effects in both the gastrointestinal and urogenital tracts. It has shown antimicrobial activity against viruses, fungi, and parasites. Notably, the combined presence of *Bifidobacterium bifidum* and S. thermophilus in infants has been linked to lower incidences of rotavirus diarrhea and may help prevent sepsis-related inflammation (Qu *et al,* 2023).

iii. Enterococcus faecium

Although concerns about antibiotic resistance exist for some strains, probiotic applications of *Enterococcus faecium* involve only those strains classified as safe for food and supplement use. This bacterium can survive digestion and colonize the gut, where it competes with pathogenic microbes for nutrients and adhesion sites, fostering a healthier microbial balance. E. faecium strains also show therapeutic promise in preventing and treating diarrhea in animals and in inhibiting the growth of Listeria spp. (Tilwani *et al,* 2022).

iv. Saccharomyces cerevisiae

The yeast *Saccharomyces cerevisiae*, particularly the *S. boulardii* variant, is well regarded for its probiotic properties. Extensively studied for its role in gastrointestinal health, it is frequently used to manage inflammatory bowel disease and various forms of diarrhea. Its mechanisms include neutralizing pathogens and toxins, reducing gut inflammation, and enhancing IgA secretion. Its probiotic potential is further supported by its ability to aggregate, co-aggregate with pathogens, adhere to intestinal cells, and withstand simulated gut environments. These attributes make it a promising agent for use in functional foods and therapeutic interventions (Fernandez-Pacheco *et al,* 2018).

1.6 Beneficial Properties of Probiotics

Probiotics have been linked to a wide range of health benefits, including the prevention and management of various conditions such as allergic disorders, cancer, hypercholesterolemia, lactose intolerance, inflammatory bowel disease, diarrhea, and irritable bowel syndrome (Grom *et al,* 2020).

i. Anti-allergic Effect of Probiotics

Allergy is a type I hypersensitivity reaction, defined as a disorder resulting from an exaggerated immune response to an otherwise harmless antigen. With its incidence steadily increasing, allergic conditions now affect nearly 50% of the population in Europe and North America (Prakash *et al,* 2014). These immune responses are commonly triggered by environmental allergens, such as pollen, dust mites, certain foods, drugs, or insect stings. Frequently observed allergic manifestations include asthma, allergic rhinitis, atopic eczema, contact dermatitis, urticaria, angioedema, hay fever, and hypersensitivities to food, medications, or insect bites (Lopez-Santamarina *et al,* 2021). Recent studies highlight the gut microbiota as a promising therapeutic target for the prevention and management of allergic diseases (Harata *et al,* 2016). Probiotics, through their ability to regulate immune and inflammatory responses, may influence the development of allergic sensitization and reduce the severity of allergic conditions (Fiocchi *et al,* 2015).

ii. Cancer Suppressor Activity of Probiotics

Probiotics have shown promise as adjuvant agents in cancer therapy due to their ability to modulate the intestinal microbiota and strengthen both local and systemic immune responses. Through these mechanisms, probiotics can inhibit the initiation,

progression, and metastasis of both transplantable and chemically induced tumors (Samanta, 2022). The anticancer effects of probiotics are mediated through several mechanisms. They suppress carcinogenesis by competitively excluding pathogenic microbes, enhancing the production of short-chain fatty acids (SCFAs) such as butyrate (Chong, 2014), and reducing the synthesis of carcinogenic bile salts. Additionally, probiotics can bind and neutralize mutagens and carcinogens, downregulate nuclear factor-kappa B (NF-κB)-dependent genes involved in cell proliferation (e.g., Cox-2, cyclin D1) and survival (e.g., Bcl-3, Bcl-xL), and promote apoptosis (Konishi *et al,* 2016). Furthermore, probiotics have been shown to upregulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a key mediator of programmed cell death in cancer cells (Kłłonowska-Olejnik, 2004). They also modulate the cell cycle through pathways such as mTOR/4EBP1 (Islam *et al,*2014) and inhibit the formation of aberrant crypt foci—precursors to colon cancer (Yu and Li, 2016).

iii. Impact of Probiotics on Intestinal Diseases

The gastrointestinal (GI) tract plays a crucial role not only in nutrient digestion and absorption but also in preserving the integrity of the mucosal barrier. It is home to a diverse community of commensal microorganisms that significantly influence host physiology and immune function (Shehata *et al,* 2022). Alterations in gut microbiota composition—whether due to infection, diet, or disease—can disrupt this balance and contribute to the development of various intestinal disorders. Strategies to restore and maintain a healthy gut microbiome include the use of antibiotics, probiotics, prebiotics, and fecal microbiota transplantation (Shahverdi, 2016). Probiotics, in particular, help in re-establishing microbial balance, enhancing barrier function,

reducing inflammation, and modulating immune responses, making them a promising therapeutic option in the management of intestinal diseases.

iv. **Immunomodulatory Properties;**

Probiotic microorganisms have been widely investigated for their ability to modulate both the innate and adaptive components of the immune system. These beneficial bacteria support host immune defenses by promoting the production of cytokines and immunoglobulins, and by activating key immune effector cells. This immunological stimulation enhances resistance to pathogenic infections and helps mitigate systemic inflammatory reactions (Mazziotta *et al* 2023). Moreover, specific probiotic strains have shown promise in boosting vaccine performance, reinforcing their potential role as adjuvants in immunization strategies (Peroni & Morelli, 2021; Abavisani *et al* 2024).

v. Other Industrial Application of Probiotics

a. Probiotics in the Food Industry

Growing public awareness of diet-related health concerns, along with increasing scientific evidence supporting the health benefits of probiotics, has significantly boosted consumer demand for probiotic-enriched foods. As a result, a wide range of food products now serve as carriers for probiotics, including yogurt, powdered milk, fermented frozen desserts, cheeses and cheese-based products, ice cream, infant formulas, breakfast cereals, and fruit juices (Papademas and Kotsaki, 2019). These products not only offer nutritional value but also serve as functional foods that contribute to gut health, immune support, and overall well-being, making probiotics a key focus in the development of health-oriented food innovations.

b. Probiotics in the Dairy Industry

The dairy industry has shown significant interest in probiotic innovation due to the wide consumer acceptance of dairy-based functional foods. Dairy products are often formulated as natural vehicles to promote health and prevent disease (Nami *et al,* 2019). Lactic acid bacteria (LAB), commonly present in these products, not only improve nutritional value but also enhance shelf life through fermentation. Moreover, LAB possess antimicrobial properties, helping to inhibit pathogenic microorganisms in the human body and contributing to overall health (de Souza da Motta *et al,* 2022).

c. Probiotics in the Beverage Industry

There has been a growing demand for non-dairy probiotic alternatives, driven by increased consumer awareness of the side effects associated with conventional medications. Probiotic-enriched beverages are perceived as a more natural and acceptable means of supporting daily probiotic intake (Reque and Brandelli, 2021). Among these, fruit juices fortified with probiotics are gaining popularity as an innovative and effective medium for probiotic delivery in the functional beverage market.

1.7 Safety Concern of Probiotics

i. Bacterial Translocation or Transmigration

The translocation of intestinal bacteria—i.e., their passage from the gut lumen to normally sterile tissues—is influenced by several factors, including damage to the intestinal mucosa, immune suppression, gut immaturity, and imbalances in the microbiota (A. Henriksson, T. Borody *et al,* 2005; Syndman DR. 2008). Bacterial adherence to the mucosal lining further facilitates this process (Borriello SP *et al,* 2003).

(Zhou *et al,* 2000) investigated whether oral administration of three probiotic strains—Lactobacillus rhamnosus HN001 (DR20), Lactobacillus acidophilus HN017, and Bifidobacterium lactis HN019 (DR10)—could lead to bacterial translocation or invasive infection in a mouse model (Karpa KD 2007, RD. Berg *et al,* 1988,). While concerns have been raised, other studies suggest that probiotics do not increase translocation of other bacteria (E.A Deitch *et al,* 1985). In fact, some evidence shows that probiotics may reduce the translocation of pathogenic organisms (B.S Reddy *et al,* 2007). Furthermore, population-based studies have not reported any elevated risk of bacteremia or endocarditis linked to probiotic use (Zhou *et al,* 2000).

ii. **Gastrointestinal Toxicity**

The impact of probiotics on gastrointestinal function warrants careful evaluation, particularly in vulnerable populations. In patients with short bowel syndrome, probiotics may produce undesirable metabolites (Mogensen, 2003). There is also a theoretical risk that probiotics could cause malabsorption by deconjugating bile salts (Salyers, 2008, Ammor, *et al.* 2007), which may, in turn, increase the risk of colon cancer (Ammor, *et al,* 2007, Tompkins *et al,* 2008).

iii. Risk of Bacteremia and Endocarditis

Although rare, some probiotic strains have been associated with cases of bacteremia and endocarditis. Reported organisms include *Lactobacillus rhamnosus, L. plantarum, L. casei, L. paracasei, L. salivarius, and L. acidophilus*, among others (Ammor, *et al,* 2007, Marteau *et al,* 1990). Additionally, Lactococcus lactis, Leuconostoc species, Pediococcus species, and even Bifidobacterium strains have been isolated from blood samples in patients diagnosed with endocarditis (Ammor, et

al, 2007, Midvedt *et al* 1967).

iv. Inadequate Quality Standards in Probiotic Products

The reliability and accuracy of probiotic product labeling is a major concern. Temmerman et al. (Syndman 2008) analyzed 55 European probiotic products—including 30 dried food supplements and 25 dairy-based products—and found inconsistencies in product quality. No viable probiotic strains were found in 11 (37%) of the dried supplements. Moreover, 15 products either contained unlisted species or included more species than indicated on their labels. Only 4 products (13%) accurately reflected the label claims.

v. Transfer of Antimicrobial Resistance

Antibiotic resistance genes can spread via conjugation, transduction, or transformation (Yamazaki *et al,* 1985). Studies have shown that lactic acid bacteria and Bifidobacterium species isolated from human and animal gastrointestinal tracts frequently harbor resistance genes, confirming the potential for gene transfer among commensal microbes (McNaught CE *et al,* 2002). This raise concerns that such organisms may serve as reservoirs of resistance genes, potentially contributing to opportunistic infections (Vesterlund S. *et al,* 2005).

1.8 Mode of Action of Probiotics

Probiotics exert their beneficial effects on the host through a variety of interconnected mechanisms, influencing both gastrointestinal health and systemic

physiology. Their actions involve microbial competition, immunomodulation, barrier function enhancement, and communication via the gut-brain axis. The major mechanisms include:

i. Competitive Exclusion of Pathogens

Probiotics compete with pathogenic microorganisms for nutrients and receptor sites on the gut mucosa. This competitive inhibition reduces the ability of harmful microbes to colonize, survive, and adhere to the intestinal lining. (Vinayamohan *et al* 2024)

ii. Production of Antimicrobial Substances

Certain probiotic strains produce antimicrobial compounds, such as bacteriocins, hydrogen peroxide, and organic acids, which inhibit the growth of pathogenic bacteria, thus maintaining microbial balance in the gut. (Ismael *et al* 2024).

iii. Enhancement of Intestinal Barrier Function

Probiotics strengthen the gut epithelial barrier by stimulating mucus production and increasing the expression of tight junction proteins. These actions help prevent the translocation of pathogens and toxins from the intestinal lumen into the bloodstream. (Latif *et al* 2023).

iv. Immunomodulation

Probiotics play a crucial role in regulating host immunity. They influence the maturation and function of dendritic cells, leading to enhanced activity of T

lymphocytes, which are vital for maintaining immune homeostasis. They also modulate both innate and adaptive immune responses by acting on dendritic cells, macrophages, and B and T lymphocytes. Additionally, probiotics can promote the production of anti-inflammatory cytokines during interactions with intestinal epithelial cells and immune cells such as macrophages and mononuclear cells (Petruzziello *et al* 2023).

v. Neurotransmitter Regulation via the Gut-Brain Axis

Emerging research highlights the ability of probiotics to influence the gut-brain axis. Specific probiotic strains are capable of synthesizing and modulating neurotransmitters like serotonin, dopamine, and gamma-aminobutyric acid (GABA), thereby affecting mood, behavior, stress response, and gut motility (Srivastav *et al,* 2019; Sajedi *et al,* 2021; Gangaraju *et al,* 2022).

1.9 ANTIMICROBIAL PROPERTIES OF PROBIOTICS

Beyond their role in maintaining gut and mucosal homeostasis, probiotics possess well-documented antimicrobial properties that enable them to inhibit, compete with, or eliminate pathogenic microorganisms in the human body. These antimicrobial activities are multifactorial and depend on strain type, environmental conditions, and the physiological state of the microorganisms.

1. Production of Organic Acids

A major antimicrobial mechanism of probiotics, especially Lactobacillus species, is the production of organic acids such as lactic acid and acetic acid during carbohydrate fermentation. These acids lower the environmental pH to levels (around pH 4.0–4.5) that are unfavorable for the growth of pathogens like *Escherichia coli*,

Staphylococcus aureus, and Candida albicans (García-Cayuela et al., 2014). The acidic conditions also destabilize bacterial membranes, disrupt proton gradients, and interfere with enzyme systems required for energy metabolism. This mechanism is particularly relevant in the vaginal environment, where lactic acid–producing lactobacilli play a central role in preventing bacterial vaginosis and urinary tract infections (O'Hanlon et al., 2013).

2. Production of Bacteriocins and Antimicrobial Peptides

Many probiotic strains secrete bacteriocins, which are ribosomally synthesized antimicrobial peptides that inhibit closely related or pathogenic bacteria. For instance, *Lactobacillus gasseri* produces gassericin A, a heat-stable bacteriocin with strong inhibitory activity against *Escherichia coli*, Listeria monocytogenes, and Clostridium perfringens (Dobson et al., 2012). These bacteriocins act by forming pores in target cell membranes, leading to leakage of cytoplasmic contents and cell death. Such targeted inhibition contributes to the maintenance of a balanced microbiota by limiting the overgrowth of harmful organisms.

3. Competitive Exclusion and Adhesion Inhibition

Probiotics also exert antimicrobial effects through competitive exclusion, a process by which they outcompete pathogens for nutrients and adhesion sites on epithelial cells. Lactobacillus species can form biofilms and adhere strongly to mucosal surfaces via surface proteins and exopolysaccharides, effectively blocking pathogen attachment (Lebeer et al., 2008). This mechanism reduces colonization by enteropathogens such as *Escherichia coli*, Salmonella spp., and Helicobacter pylori, thereby preventing infection initiation.

4. Production of Hydrogen Peroxide and Other Metabolites

Several Lactobacillus species, including *Lactobacillus gasseri*, produce hydrogen peroxide (H_2O_2), which has broad-spectrum antimicrobial activity. H_2O_2 acts synergistically with lactoperoxidase and halides to produce reactive oxygen species that oxidize bacterial cell components (Ouwehand & Vesterlund, 2004). Additionally, probiotics produce other inhibitory metabolites such as diacetyl, reuterin, and short-chain fatty acids that suppress the proliferation of both Gram-positive and Gram-negative pathogens.

5. Modulation of Host Immune Response

Apart from direct inhibition of pathogens, probiotics enhance the host's immune defense mechanisms. They stimulate mucosal immunity by increasing the production of secretory immunoglobulin A (sIgA), macrophage activation, and cytokine regulation (de Moreno de LeBlanc & LeBlanc, 2014). This immune modulation helps the host resist infection and accelerates clearance of invading pathogens.

6. Synergistic and Therapeutic Relevance

The antimicrobial properties of probiotics make them promising alternatives or adjuncts to antibiotics, particularly in the era of increasing antibiotic resistance. Their ability to inhibit *Escherichia coli* and other uropathogens underscores their potential use in probiotic suppositories, vaginal formulations, and oral therapies aimed at restoring microbial balance. In pharmaceutical formulations, maintaining probiotic viability is crucial to preserving these antimicrobial benefits (Baral *et alkp*, 2021).

1.10 ADHENSION PROPERTIES OF PROBIOTIC ORGANISM

The adhesion of probiotic bacteria to intestinal surfaces typically occurs in two

stages: an initial non-specific interaction—primarily hydrophobic in nature—followed by specific binding mediated by cell wall-associated components (Haddaji *et al.,* 2015). Although the ability of probiotics to adhere to the host does not directly guarantee a health benefit, it may confer a protective advantage by preventing the colonization of enteropathogens through competitive exclusion for binding sites on host epithelial cells. Furthermore, strong adhesion may facilitate prolonged retention in the gut, enhancing the opportunity for probiotics to interact with host tissues and exert beneficial effects during temporary colonization.

In vitro models have been widely employed to evaluate the adhesion potential of probiotics. These models often utilize mucin-coated abiotic surfaces and human intestinal epithelial-like tumorigenic cell lines such as Caco-2 and HT-29 (Lebeer *et al,* 2012; Monteagudo-Mera *et al,* 2012; Tuo *et al,* 2013; Garriga *et al,* 2015). Such models are instrumental in mimicking the intestinal epithelium and have been critical in elucidating probiotic adhesion mechanisms and identifying key surface molecules involved in host interactions.

For instance, (Wang *et al,* 2017) identified a novel surface layer protein—choline-binding protein A—as essential for the adhesion of Lactobacillus salivarius REN to HT-29 cells. Discoveries like this hold promise for the genetic enhancement of probiotic adhesion. (Hsueh *et al,* 2010) demonstrated that the low adhesion capability of Lactobacillus casei ATCC 393 could be significantly improved by introducing a collagen-binding protein gene from *L. reuteri* Pg4, thereby enhancing its competitiveness against pathogens in Caco-2 cell assays. Similarly, (Zhang *et al,* 2015) supported the potential of such genetic approaches.

Moreover, in vitro systems using epithelial cell lines have proven valuable in

assessing how different gastrointestinal conditions influence the adhesive performance of probiotic strains (Deepika *et al,* 2012; Nivoliez *et al,* 2014). These studies underscore the importance of adhesion in determining probiotic efficacy and offer strategies to improve probiotic formulations for enhanced clinical benefits.

1.11 METHODS FOR ASSESSING THE ANTIMICROBIAL PROPERTIES OF PROBIOTICS

Evaluating the antimicrobial potential of probiotic strains is a critical component in determining their suitability for therapeutic or functional applications. These assessments help verify the efficacy of probiotics in inhibiting pathogenic microorganisms and guide the selection of strains for targeted use in gastrointestinal, urogenital, and systemic infections. Several in vitro techniques have been developed to measure this antimicrobial activity.

i. Solid Media Methods

a. Agar Well Diffusion Assay

In this method, a pathogen is evenly spread on the surface of an agar plate, and wells are made into which either probiotic cultures or cell-free supernatants are added. After incubation, inhibition zones around the wells indicate antimicrobial action. This assay is particularly effective for detecting diffusible antimicrobial agents such as organic acids, hydrogen peroxide, and bacteriocins. Research has shown that Lactobacillus strains demonstrate inhibitory effects against clinically significant pathogens such as Staphylococcus aureus and *Escherichia coli* using this assay (Cizeikiene & Jagelavičiute, 2021; Rushdi, 2022).

b. Spot-on-Lawn Assay

This agar-based technique involves creating a lawn of a target pathogen on a suitable agar medium, such as Brain Heart Infusion (BHI) for S. aureus or MRS agar for lactic acid bacteria. The probiotic strains or their supernatants are then spotted onto the surface. Clear zones around the application spots signify inhibitory activity. These zones are graded based on their radius: ≥4 mm (score 4), 2–3.9 mm (score 3), 0.5–2 mm (score 2), and ≤0.5 mm (score 1), enabling semi-quantitative comparison (Christensen *et al,* 2021).

ii. Liquid Media Methods

Broth Inhibition Assay: In this technique, probiotics and pathogens are co-cultured in a liquid medium, and pathogen growth is monitored over time via optical density measurements or viable colony counts. This approach can help distinguish between bacteriostatic and bactericidal effects. For example, Lactobacillus acidophilus has

shown significant inhibitory effects on enteric pathogens in broth systems (Denkova *et al,* 2022).

iii. Additional Analytical Approaches

a. Co-Culture Assays

Co-culturing probiotic and pathogenic strains in the same medium allows direct interaction under controlled conditions. Both simultaneous and sequential inoculations can be used to monitor the population dynamics via colony counts, optical density, or molecular methods like qPCR. These assays help assess probiotic effects on pathogen viability and metabolic activity over time (Fredua-Agyeman *et al,* 2023; Kim *et al,* 2022; Pinto *et al,* 2024).

b. Metabolite Profiling and Quantification

Probiotic antimicrobial effects are often due to bioactive compounds like organic acids and bacteriocins. Advanced techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and ELISA are employed to detect and quantify these metabolites, revealing the biochemical pathways involved (Gao *et al,* 2024; Kyei-Baffour *et al,* 2025).

1.12 METHODS FOR ASSESSING THE ADHESION PROPERTIES OF PROBIOTICS

Adhesion to intestinal mucosa is a key feature influencing the persistence and efficacy of probiotics. Several in vivo and in vitro approaches are used to evaluate adhesion capacity.

i. In Vivo Methods

a. Animal Models

Rodent models such as Sprague Dawley rats are commonly employed to study probiotic colonization under physiological conditions. These models allow for realistic evaluation of host-microbe interactions. For instance, Lactobacillus plantarum strains have demonstrated effective colonization in rodent colons after oral administration (Darmastuti *et al,* 2021).

b. Caenorhabditis elegans Model

Caenorhabditis elegans serves as a simple yet effective model for high-throughput screening of probiotic adhesion. Its transparent body and conserved innate immune system enable observation of colonization and host–microbe interactions (Poupet *et al,* 2020).

ii. In Vitro Methods

a. Microbial Adhesion to Hydrocarbons (MATH)

This assay evaluates cell surface hydrophobicity by suspending bacterial cells in water and mixing them with a hydrocarbon (e.g., hexadecane). The proportion of cells adhering to the hydrocarbon phase reflects hydrophobicity, which correlates with adhesion potential. Strains of Lactobacillus acidophilus have shown strong hydrophobicity, suggesting effective mucosal adhesion (Farid *et al,* 2021; Salas-Tovar *et al,* 2024).

b. Surface Plasmon Resonance (SPR) Assay

SPR is a label-free technique that quantifies molecular interactions in real-time. By immobilizing human colonic mucins onto a sensor chip, the binding affinity of probiotic strains can be evaluated. Sensorgrams generated during the assay provide kinetic data such as association (k_on), dissociation (k_off), and equilibrium

constants (K_D), offering detailed insights into adhesion mechanisms (Gaudreault *et al,* 2021).

iii. Physicochemical Surface Characterization

Contact angle measurement and surface tension analyses are used to determine surface energy and adhesion work between bacteria and mucus. These techniques provide additional insights into strain-specific adhesion profiles and help predict colonization potential (Phùng *et al,* 2025).

1.13 FUTURE OF PROBIOTICS

Over the decades, the role of probiotics has evolved significantly—from being regarded merely as general wellness supplements to becoming central to advanced biomedical research and therapeutic applications. This study explores one of the many breakthroughs achieved in probiotic science, highlighting their transition from simple food-associated microbes to potent antimicrobial agents. It is remarkable to consider that the same bacteria once used solely in yogurt fermentation are now being investigated for their ability to combat pathogenic organisms. These developments strongly suggest that the future of probiotics is only just beginning, with immense potential yet to be unlocked.

The subsequent sections highlight emerging areas of probiotic application, supported by advancing scientific understanding and growing clinical interest;

i. Probiotics in Oncology

Recent research has underscored the complex interplay between the gut microbiome

and tumor biology, revealing the potential of probiotics in both cancer prevention and as supportive agents in cancer therapy.

Modulation of Carcinogenic Mechanisms: Probiotic interventions have shown promise in modulating the gut microbial environment in ways that reduce carcinogenic risk. Mechanistically, this involves downregulating pro-inflammatory signaling, enhancing gut barrier integrity, and facilitating the detoxification of dietary and environmental carcinogens (Mishra *et al,* 2021). Certain probiotic strains have also been found to reduce the genotoxic effects of mutagens, thereby lowering the likelihood of malignant transformation.

Oncobiotics: The concept of "oncobiotics" has emerged to describe probiotic strains exhibiting anti-neoplastic properties. These strains may synergize with chemotherapeutic agents, enhancing their efficacy, reducing adverse effects, and modulating host immune responses to favor tumor suppression (Howarth, 2024).

ii. Psychobiotics and Neuropsychiatric Health

The gut-brain axis presents a compelling avenue for probiotic research, particularly in the context of neuropsychiatric conditions.

Psychobiotic Mechanisms: Psychobiotics are defined as live microorganisms that, when administered in appropriate doses, exert beneficial effects on mental health. These effects are mediated through neurochemical pathways involving the synthesis of key metabolites such as γ-aminobutyric acid (GABA), serotonin precursors, and short-chain fatty acids. These compounds are known to modulate stress reactivity, mood, and cognitive function (Howarth, 2024).

Clinical Potential: Preliminary findings suggest that select probiotic strains may

help alleviate symptoms of anxiety, depression, and stress-related disorders. As psychobiotics advance through clinical development, they hold potential as adjunctive therapies or alternatives to conventional psychotropic medications—particularly in individuals with concurrent gastrointestinal dysfunction.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Materials

All substances utilized in this experiment were sterilized both prior to and following their use.

Microorganisms: *Lactobacillus gasseri*, *Escherichia coli*

Chemicals and Reagents: Glycero-Gelatin, Polyethylene Glycol, Theobroma, Phosphate Buffered Saline (PBS), Safranin, Crystal violet, gram iodine, Acetone.

Growth Media: de Man, Rogosa and Sharpe (MRS) Broth, Nutrient Broth, MRS Agar, Eosin Methylene Blue Agar, Muller Hilton Broth

Equipment: Incubator, Microscope, Glass Slides/Coverslips, Autoclave, Pipettes and Teats, Refrigerator, Bunsen burner, Digital weighing balance, Red cover tubes.

Glassware: Petri dishes, beakers, measuring cylinders, universal bottles, bijou bottles

Analytical Tools: Statistical Software (e.g., GraphPad Prism, SPSS), Excel, Spearman's Correlation

Safety Equipment: Lab Coat, Gloves, Waste Disposal Containers

Additional Items: Marker, masking tape, tripod stand, spatula, foil paper, cotton wool, surgical blades, candles, forceps.

Carbohydrate Fermentation Test Materials: Phenol red broth base, sucrose (analytical grade), raffinose (analytical grade), lactose (analytical grade), maltose (analytical grade), D-fructose (analytical grade), D-glucose (analytical grade), D-mannitol (analytical grade), L-rhamnose (analytical grade), D-xylose (analytical grade), L-arabinose (analytical grade), Durham tubes (small inverted glass tubes), sterile distilled water, sterile inoculating loops, incubator.

2.2 Methods

2.2.1 Gram Staining Procedure

Microorganisms were obtained from the Pharmaceutical Microbiology and Biotechnology Department.

A smear was prepared on a glass slide using a sterile water drop and *Lactobacillus gasseri*. Crystal violet was applied for 60 seconds, then rinsed off with water. Iodine was added and left for 60 seconds before rinsing again. Acetone was used for 30 seconds to decolorize, followed by rinsing. Safranin was applied for counterstaining for 60 seconds, then rinsed. The slide was air-dried and examined under a microscope. The procedure was repeated for *Escherichia coli* (Paray *et al*., 2023).

2.2.2 Carbohydrate Fermentation Tests

Phenol red broth base was prepared and dispensed into sterile test tubes with inverted Durham tubes, each containing 5ml. Carbohydrates such as sucrose, raffinose, lactose, maltose, D-fructose, D-glucose, D-mannitol, L-rhamnose, D-xylose, and L-

arabinose were added to achieve 1% (w/v). The media were sterilized at 121ºC and cooled before inoculation.

Each tube was inoculated with a single colony or a standardized loopful of *Escherichia coli* . Incubation was at 37°C, with observations at 24 and 48 hours to confirm reactions (Sultana *et al*., 2021).

The same process was repeated for *Lactobacillus gasseri*.

2.2.3 Catalase Test

The catalase test was performed on each microorganism separately to obtain clear and distinct results. A clean, grease-free microscope slide was placed on a stable working surface, and a small visible portion of a pure, isolated colony of the first test organism *Escherichia coli* was aseptically transferred onto the slide using a sterile wooden applicator stick. Care was taken to avoid contact with the agar medium, as some components of the medium may cause false-positive reactions.

Following this, two drops of 3% hydrogen peroxide (H_2O_2) solution were added directly onto the colony on the slide. The preparation was immediately observed for the rapid appearance of gas bubbles, which indicated a positive catalase reaction. Observations were made within 30 seconds, after which the result was recorded as either positive or negative.

The same procedure was repeated for the other test organism *Lactobacillus gasseri*. Upon completion of the test, all slides were properly discarded following standard laboratory safety protocols.

2.2.4 MRS Broth and MRS Agar

For 200ml of MRS Broth, 11.03g of media was dissolved in distilled water, sterilized by autoclaving at 121°C for 15 minutes, then cooled. For MRS Agar, 6.715g was dissolved in 100ml of water, heated, stirred, autoclaved, and poured into petri dishes to solidify.

2.2.5 Phosphate Buffer Saline Preparation

8g sodium chloride, 0.2g potassium chloride, 1.44g disodium hydrogen phosphate, 0.24g potassium dihydrogen phosphate were dissolved in 800ml distilled water, stirred, then topped up to 1000ml.

2.2.6 Mueller Hilton Broth (MH Broth) Preparation

21g of MH Broth was dissolved in 1000ml water; for 50ml, 1.051g was used. Sterilization was by autoclaving at 121°C for 15 minutes, with 10ml aliquots prepared for inoculation.

2.2.7 Eosin Methylene Blue Agar Preparation

35.96g of dehydrated media was dissolved in 1000ml water; for 80ml, 2.88g was used. The mixture was heated, stirred, autoclaved, and poured into petri dishes to solidify.

2.2.8 Inoculating Media Preparation

To prepare 10ml, 3.3ml of Glycero-Gelatin was mixed with 6.7ml of Miller Hinton broth or MRS broth in a 1:1 ratio. The same process was applied for Polyethylene Glycol and Theobroma.

2.3 Growth Pattern of *Lactobacillus gasseri*

A 10ml aliquot of Mueller Hinton's broth was inoculated with a loopful of ***Lactobacillus gasseri*** and incubated at 37^{o}C for 48 hours. Dilutions were performed using red cover tubes with phosphate buffered saline, and samples were plated on Eosin Methylene Blue Agar using the drop plate method. Incubation and observation were conducted over 48 hours, with results recorded accordingly.

2.4 Growth Pattern of *Lactobacillus gasseri* in the Presence of *Escherichia coli*

A mixture of Mueller Hinton broth and MRS Broth (5ml each) was prepared in a universal bottle. Loopfuls of *Escherichia coli* and *Lactobacillus gasseri* were inoculated into the mixture, which was then incubated at 37^{o}C for 48 hours. Dilutions and plating procedures followed the same method as above, with incubation and results documented.

2.5 Growth Pattern of *Lactobacillus gasseri* in the Presence of *Escherichia coli* and suppository bases

To prepare 10ml, 3.3ml of Glycerolgelatin was combined with 6.7ml of a 10ml mixture of Miller Hinton broth and MRS broth in a universal bottle. Loopfuls of *Escherichia coli* and *Lactobacillus gasseri* were inoculated, and the mixture was incubated at 37^{o}C for 48 hours. Dilutions and plating were performed at an interval of four hours, with incubation and recording of outcomes. The experiment was repeated using 3.3ml of Theobroma and Polyethylene Glycol, respectively.

CHAPTER 3

3.0 RESULT

Table 3.1 GRAM STAINING AND BIOCHEMICAL TEST FOR *Lactobacillus gasseri*

Test	**Specific interactions**	***Lactobacillus gasseri*.**
Gram staining	Primary and Secondary Dyes	GPB
Catalase test	Hydrogen perxoide	Negative
Biochemical Characteristics	Sucrose	Positive
	Raffinose	Negative
	Lactose	Positive
	Maltose	Positive
	D-Fructose	Positive
	D-Glucose	Positive
	D-Mannitol	Negative
	L-Rhamnose	Negative
	D-Xylose	Negative
	L-Arabinose	Negative

Table 3.2 GRAM STAINING AND BIOCHEMICAL TEST for *Escherichia coli*

Test	**Specific interactions**	***Escherichia coli ATCC 25922***
Gram staining	Primary and Secondary Dyes	GNB
Catalase test	Hydrogen perxoide	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
	Sucrose	Negative
	Raffinose	Negative
	Lactose	Positive

Key

GPB: Gram Positive Bacilli

GNB: Gram Negative Bacilli

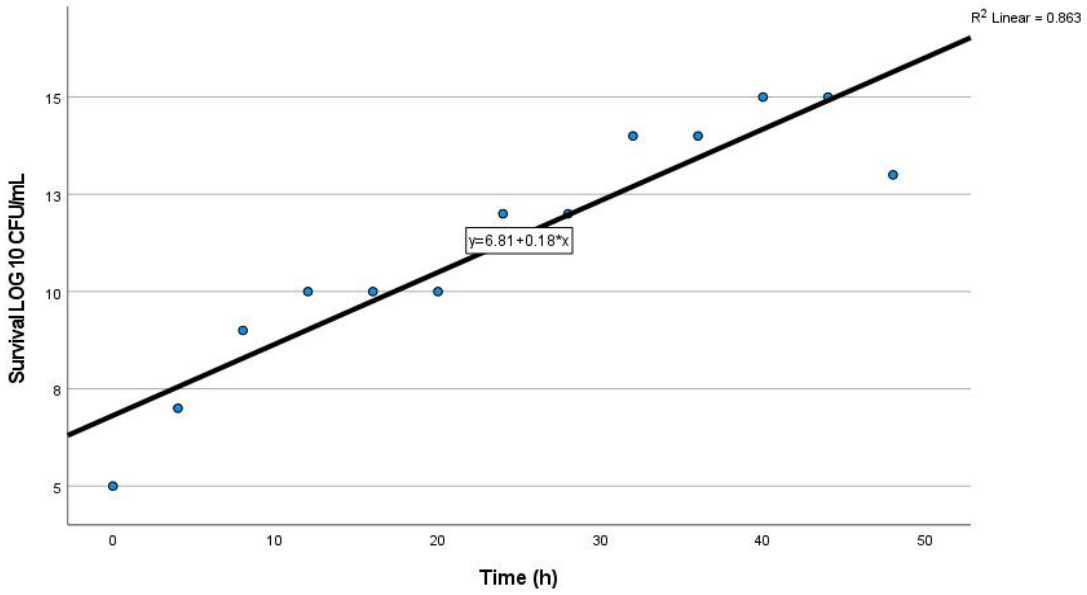

Fig 3.1 Growth pattern for *Lactobacillus gasseri*

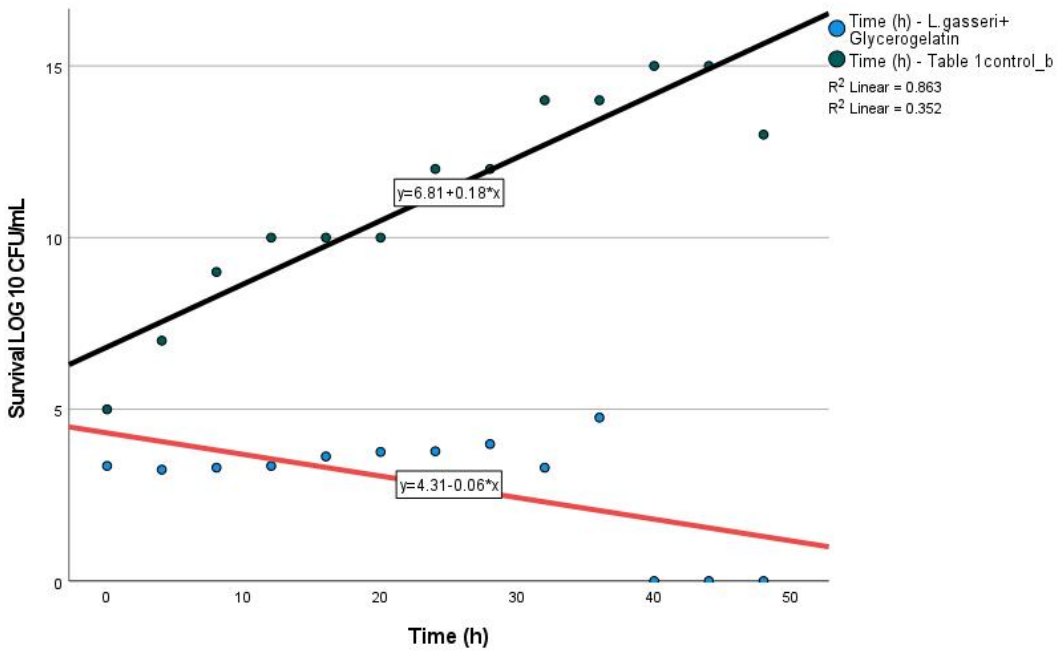

Fig 3.2 Growth pattern for *Lactobacillus gasseri* in the presence of glycerogelatin and *Escherichia coli* *P<0.001*

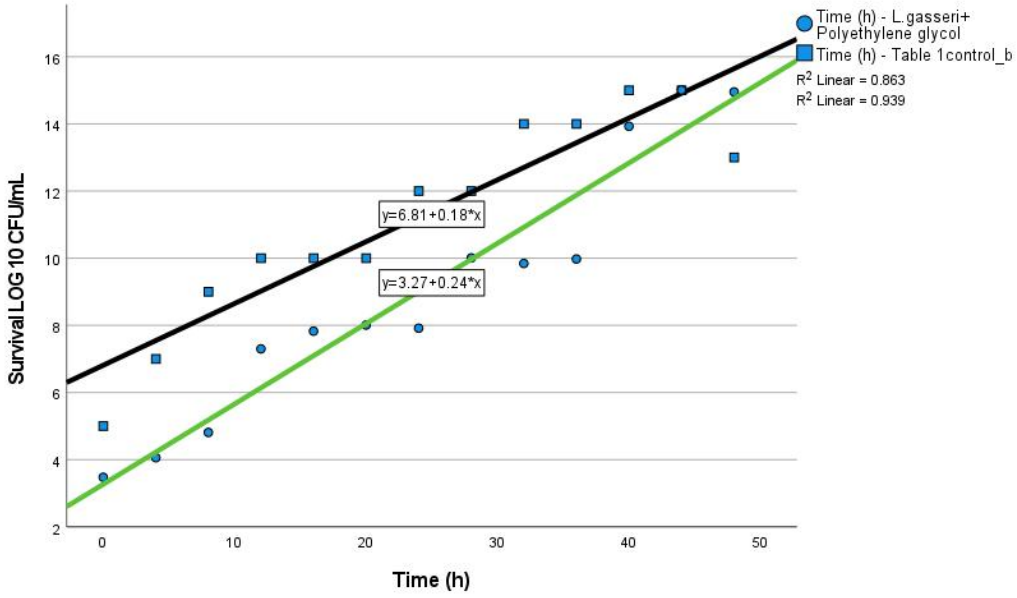

Fig 3.3 Growth pattern for *Lactobacillus gasseri* in the presence of Polyethylene Glycol and *Escherichia coli* *P<0.001*

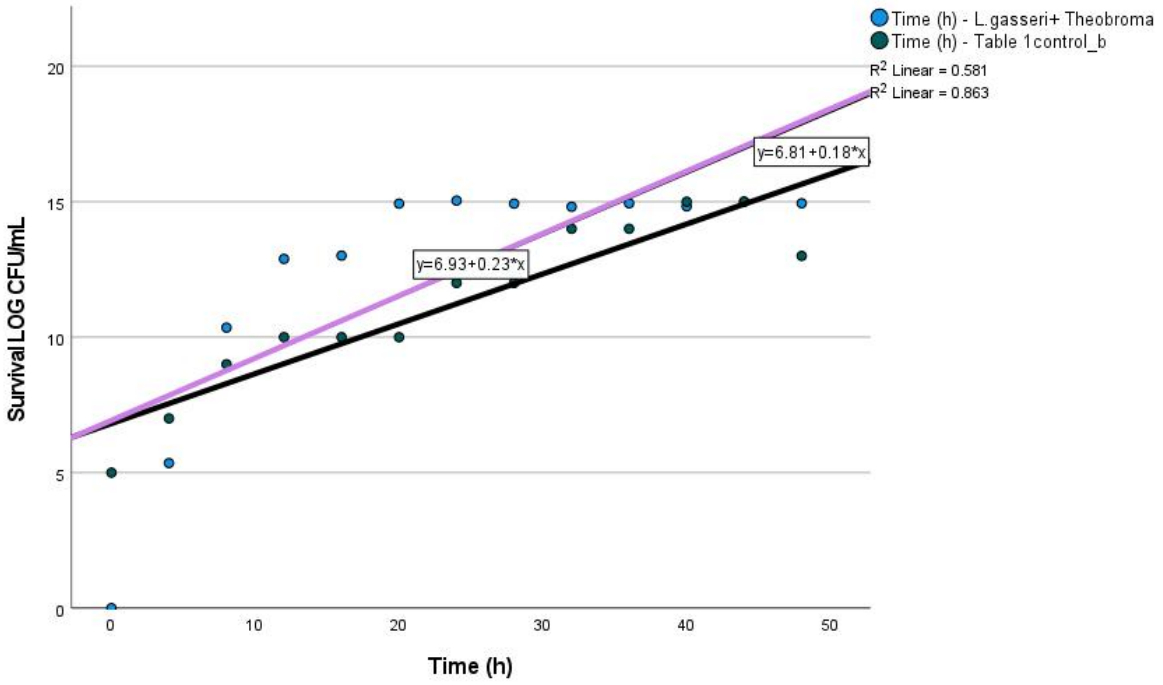

Fig 3.3 Growth pattern for *Lactobacillus gasseri* in the presence of Theobroma and *Escherichia coli* $P = 0.126$

CHAPTER 4

DISCUSSION

4.1 Introduction

This chapter presents and discusses the results obtained from the survival studies of *Lactobacillus gasseri* in the presence of selected suppository bases and *Escherichia coli.* The discussion focuses on the growth patterns derived from regression equations and p-values to interpret microbial interactions and base effects.

4.2. Identification and Characterization of the Test Organisms

The biochemical testing and Gram-staining of *Lactobacillus gasseri* and *Escherichia coli* provided a crucial foundation for the survival and interaction studies. The Gram-positive, catalase-negative profile of *Lactobacillus gasseri* aligns well with the known attributes of lactic acid bacteria, and its carbohydrate fermentation profile is consistent with other studies highlighting its utilization of simple sugars (Pan *et al*, 2020). For *Escherichia coli,* the Gram-negative, catalase-positive profile and broad sugar fermentation spectrum are typical of Enterobacteriaceae and support its use as a competitor organism in this study. Correct identification of both organisms therefore strengthens the validity of subsequent survival and interaction data.

4.3 Control Experiment (Baseline)

The control culture of Lactobacillus gasseri produced the regression equation $y = 6.81 + 0.18x$, showing a steady increase in cell population over time. The positive slope (+0.18) indicates normal, uninhibited growth under ideal nutrient conditions without competition or external stressors. This growth pattern serves as the baseline for comparing the influence of glycerogelatin, Polyethylene Glycol, and Theobroma

base on *Lactobacillus gasseri* survival in the presence of *Escherichia coli.*

4.4 Comparison of Glycerogelatin with the Control Experiment

In the glycerogelatin environment, *Lactobacillus gasseri* maintained the same growth equation as the control ($y = 6.81 + 0.18x$), showing no reduction in its normal growth ability. However, *Escherichia coli* produced a declining regression equation ($y = 4.31 - 0.06x$) with $p < 0.001$, indicating a statistically significant reduction in viability.

When directly compared with the control experiment:

- *Lactobacillus gasseri* showed identical growth performance to the control, confirming that glycerogelatin preserves probiotic viability.
- *Escherichia coli* showed a negative growth slope, opposite to what would occur under normal conditions.

This means glycerogelatin is more favourable than the control environment because it supports *Lactobacillus gasseri* and suppresses *Escherichia coli.* Its hydrophilic nature likely enhances nutrient diffusion for the probiotic, while allowing acid and bacteriocin accumulation that inhibits pathogens.

4.5 Comparison of Polyethylene Glycol (polyethylene glycol) with the Control Experiment.

In the polyethylene glycol environment, *Lactobacillus gasseri* again showed the same growth equation as the control ($y = 6.81 + 0.18x$), meaning polyethylene glycol did not improve or reduce its survival. However, *Escherichia coli* produced a rapid growth trend ($y = 3.27 + 0.24x$) with $p < 0.001$, indicating a statistically significant increase in growth compared to what would be expected in a natural environment.

Compared with the control:

- *Lactobacillus gasseri* showed no improvement from control conditions.
- *Escherichia coli* grew faster than in the control, shown by its steeper positive slope.

This demonstrates that Polyethylene Glycol creates a more favourable environment for *Escherichia coli* than the control, due to its hydrophilic properties and moisture retention that enhance nutrient access. Therefore, Polyethylene Glycol performs worse than the control in supporting probiotic dominance.

4.6 Comparison of Theobroma base with the Control Experiment

In Theobroma base, the regression equations were *Escherichia coli:* $y = 6.93 + 0.23x$ and *Lactobacillus gasseri*: $y = 6.81 + 0.18x$, with a non-significant p-value ($p = 0.126$).

Compared with the control:

- *Lactobacillus gasseri* shows the same growth rate as in the control, indicating no enhancement or inhibition.
- *Escherichia coli* also shows a slight increase, but it is not statistically significant, meaning the difference from control is minimal.

Theobroma base therefore acts as a neutral base, offering no selective advantage to either organism. Its lipophilic nature restricts moisture and nutrient movement, resulting in minimal microbial interaction.

4.7 General Interpretation

The variations observed among the different suppository bases highlight the

influence of base composition on microbial survival and competition. Hydrophilic bases such as glycerogelatin and polyethylene glycol significantly affected microbial interactions ($p < 0.001$), while the lipophilic base (Theobroma base) showed minimal influence ($p = 0.126$).

Glycerogelatin enhanced *Lactobacillus gasseri* survival while inhibiting *Escherichia coli,* making it the most promising base for probiotic suppository formulation. Polyethylene glycol, although beneficial for *Lactobacillus gasseri*, simultaneously promoted *Escherichia coli* proliferation, which may reduce its selectivity. Theobroma base maintained both microorganisms without significant impact.

These results corroborate findings by (Dunne *et al*. 2001), who emphasized that probiotic survival and activity are highly dependent on carrier material and environmental factors. Therefore, understanding base–microbe interactions is crucial in designing effective probiotic delivery systems.

CHAPTER 5

CONCLUSION

This study demonstrated that the survival of *Lactobacillus gasseri* in the presence of *Escherichia coli* is significantly influenced by the type of suppository base used. Glycerogelatin provided the most favorable environment, promoting *Lactobacillus gasseri* growth while suppressing *Escherichia coli,* as indicated by the regression slopes and significant p-value ($p < 0.001$). Polyethylene Glycol also supported growth but favored *Escherichia coli* more strongly, while Theobroma base exhibited no significant effect ($p = 0.126$).

Overall, glycerogelatin appears to be the most suitable base for probiotic suppository formulations aimed at enhancing Lactobacillus survival and therapeutic efficacy.

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APPENDIX I

GROWTH PATTERN FOR *LACTOBACILLUS GASSERI* IN THE PRESENCE OF GLYCEROGELATIN AND ESCHERICHIA COLI

Author: Ekhoeovbiye O. Simon
Supervisor: Dr. Oloton

University of Benin, Faculty of Pharmacy, Department of Pharmaceutical Microbiology

Mat. No.: PHA1908491
Class No.: 72

Introduction

The balance between probiotic (*Lactobacillus gasseri*) and pathogenic (*Escherichia coli*) species within the human microbiome is essential for gut and mucosal health. Suppository bases such as **glycerogelatin** can influence microbial viability and interaction. This study investigates the **growth dynamics of *L. gasseri* in the presence of glycerogelatin and *E. coli***, with the goal of understanding potential probiotic–pathogen interactions and base effects on microbial survival.

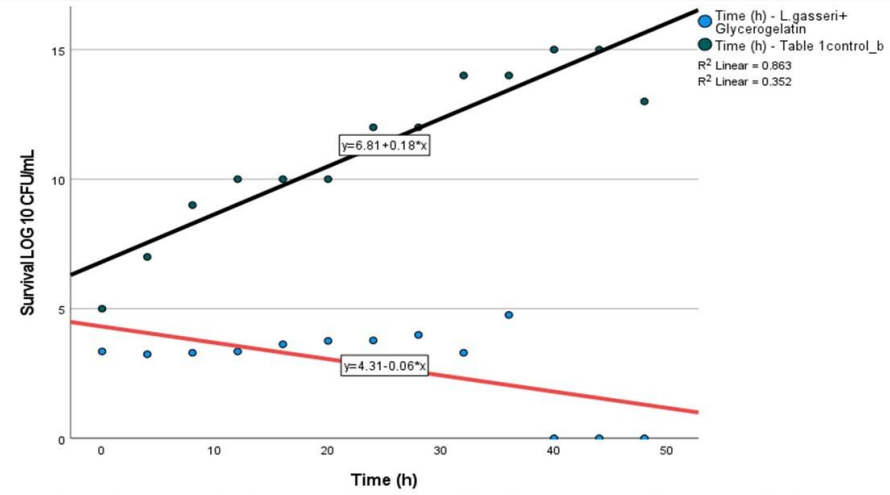
Objectives

To determine the growth pattern of *Lactobacillus gasseri* in the presence of glycerogelatin and *Escherichia coli*.

Materials & Method

Microorganisms: *Lactobacillus gasseri*, *Escherichia coli*
Chemicals & Reagents: Glycerogelatin, Polyethylene glycol, Theobroma oil, PBS, Safranin, Crystal violet, Gram iodine, Acetone.
Growth Media: MRS broth/agar, Nutrient broth, Eosin Methylene Blue agar, Muller-Hinton broth.
Equipment: Incubator, Autoclave, Microscope, Bunsen burner, Digital balance, Pipettes, Refrigerator.
Glassware: Petri dishes, Beakers, Cylinders, Universal and Bijou bottles.
Analytical Tools: GraphPad Prism, SPSS, Excel (Spearman's correlation).
Safety: Lab coat, gloves, proper waste disposal containers.
Method: To prepare 10 mL of the test mixture, **3.3 mL of glycerogelatin** was combined with **6.7 mL of a mixed broth** (Muller-Hinton + MRS) in a universal bottle. Loopfuls of *E. coli* and *L. gasseri* were inoculated and incubated at **37°C for 48 hours**. Dilutions and plating were done post-incubation, and colony counts were recorded to monitor bacterial growth patterns.

Result



Growth pattern for Lactobacillus gasseri in the presence of glycerogelatin and Escherchia coli

Conclusion

L. gasseri exhibited **inhibitory influence** on *E. coli* growth in co-culture. **Glycerogelatin** base maintained *L. gasseri* viability, suggesting potential use in probiotic formulations

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