ANTIBACTERIAL AND ANTI-ADHESION STUDIES OF PROBIOTIC STRAINS AGAINST *ESCHERICHIA COLI* IN THE PRESENCE OF SUPPOSITORY BASES WITH GC-MS PROFILES OF THE COCULTURE

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A THESIS WRITTEN IN THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

APRIL, 2026

CERTIFICATION

This is to certify that this work was carried out by Dr. Enosakhare OLOTON in the Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmacy, University of Benin, Nigeria.

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CERTIFICATION OF THESIS ON PLAGIARISM

We the undersigned attest and declare that the thesis of Enosakhare Oloton

Titled: **ANTIBACTERIAL AND ANTI-ADHESION STUDIES OF PROBIOTIC STRAINS AGAINST *ESCHERICHIA COLI* IN THE PRESENCE OF SUPPOSITORY BASES WITH GC-MS PROFILES OF THE COCULTURE**

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DEDICATION

This thesis is dedicated to the supreme God, the creator of wisdom and applied strength. Through his invisible guidance, I was sustained in every challenge and moment of uncertainty in the experimental work that gave birth to this thesis. His mercy and financial provision made the completion of this work possible. I hereby appreciate him for using me as a messenger in improving the health of humanity through the content of this research in probiotics. To God be the honor and continuous praise now and forever.

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LIST OF ABBREVIATIONS

ATCC:	American Type Culture Collection
AV:	Aerobic vaginitis
AMPs:	Antimicrobial proteins
Caco- 2:	Cancer coli-2
cas:	Chemical abstracts service.
DAL:	Double agar layer
DLA:	Double-layer agar
DNA:	Deoxyribonucleic acid
ECM:	Extracellular matrix
EPEC:	Enteropathogenic E. coli
EPS:	Extracellular Polymeric Substances
EI:	Electron ionization
EMB:	Eosin methylene blue
FAPS:	Functional abdominal pain syndrome
FAO/WHO:	Food and Agriculture Organization of the United Nations (FAO) and the World
FDA	Food and Drug Administration
GER:	Gastroesophageal reflux
GC-MS:	Chromatography- mass spectrometry
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
GPB:	Gram positive Bacillus
GNB:	Gram-negative bacillus
h:	Hour
IgA:	Immunoglobulin A
IL 6:	Interleukin-6
Log_{10} CFU/mL:	Logarithm base 10 of Colony Forming Units per milliliter
LTA:	Lipoteichoic Acid.
LPxTG:	Leu-Pro-x-Thr-Gly
MTX:	Methotrexate
MRS:	de Man, Rogosa and Sharpe
Mm:	Milimeter
MR:	Methyl red
Mub:	Mucus-binding proteins

NGP:	Next-generation probiotics
NIST:	National Institute of Standards and Technology
PROM:	Preterm rupture of membranes.
PEG:	Polyethylene glycol
PBS:	Phosphate buffer saline
p-value:	Probability value
RNA:	Ribonucleic acid
S-layer:	Surface-layer
SCFAs:	Short-chain fatty acids
SpaCBA:	SpaC (tip), SpaB (basal), and SpaA (backbone).
SPSS:	Statistical Package for the Social Sciences
Th17:	T helper 17
TNF α:	Tumor necrosis factor-alpha
UC:	Ulcerative colitis
UTI:	Urinary Tract Infections
VP:	Voges Proskauer

ABSTRACT

Probiotics are products containing live microorganisms which when administered in adequate amount confer health benefits to the host. *Lactobacillus* can confer therapeutic benefits in aerobic vaginitis and ulcerative colitis. The aim of this study was to evaluate the antibacterial, anti-adhesion, and comparative GC-MS profiling of some probiotic strains against *Escherichia coli* in the presence of some suppository bases. Gram staining reaction and biochemical test were conducted to authenticate all microorganisms. Double-layer agar method was used for the evaluation of the antibacterial effect of *Lactobacillus* strains. Solidified albumen was prepared and afterward the weight, surface area, and linearity studies were examined across all microorganisms. The de-adhesion of *Lactobacillus* strains from solidified albumen was evaluated using Tween 20. Co-cultivation assay of individual *Lactobacillus* strains against *Escherichia coli* was determined in the absence and presence of Glycerogelatin, Polyethylene glycol and Theobroma. Survival of *Lactobacillus* strains in the presence of *Escherichia coli* and bases was quantified. Adhesion assay of individual *Lactobacillus* strains against *Escherichia coli* was determined in the absence and presence of the bases. The anti-adhesion of *Lactobacillus* strains against *Escherichia coli* in the absence and presence of the bases was determined. A comparative GC-MS analysis profiled compounds produced in *Lactobacillus*-*Escherichia coli*-bases interactions. Regression plots and P-values were determined using one way analysis of variance (ANOVA) via SPSS version 27. Gram staining reaction and biochemical test confirmed *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-VO3, *Lactobacillus rhamnosus* LMS2-1 and *Escherichia coli* ATCC 25922. Double-layer agar method showed a bacteriostatic range of 30.8 to 31.5 mm. Solidified albumen showed: 1.86 g, 817.15 mm with a strong linearity (R^2 = 0.83-0.96) for all microorganisms. Tween 20 exhibited a 50% de-adhesion concentration. There was a significant reduction of *Escherichia coli* in the presence of *Lactobacillus* strains ($p < 0.001$). Similarly, a significant reduction of *Escherichia coli* in the presence of *Lactobacillus* strains combined with Glycerogelatin base ($p < 0.001$) and in combination with Polyethylene glycol base ($p = 0.001$) were observed. No detectable reduction of *Escherichia coli* in the presence of *Lactobacillus* strains combined with Theobroma ($p = 0.879$). *Lactobacillus* strains survived (0-40h) in the presence of *Escherichia coli* combined with Glycerogelatin ($p = 0.001$); Polyethylene glycol ($p = 0.566$) and Theobroma ($p = 0.614$) respectively. The differences were not significant for the anti-adhesion effect of *Escherichia coli* in the presence of *Lactobacillus* strains ($p = 0.96$). A significant anti-adhesion effect of *Escherichia coli* in the presence of *Lactobacillus* strains-Glycerogelatin ($p = 0.001$) and *Lactobacillus*-Polyethylene glycol ($p = 0.001$) were demonstrated.

Anti-adhesion effect of *Escherichia coli* in the presence of different strains of *Lactobacillus* and Theobroma were consistent with control ($p = 0.523$). *Lactobacillus* strains showed poor adhesion in glycerogelatin ($p = 0.137$) while, polyethylene glycol ($p = 0.492$) and Theobroma ($p = 0.302$) showed detectable presence of *Lactobacillus* strains between 25-48 h. *Lactobacillus–Escherichia coli*-polyethylene glycol interactions produced compounds such as n-Tetracosanol-1, Hexadecanoic acid, methyl ester, 2-Piperidinone, and Hexacosane. This study showed glycerogelatin and polyethylene glycol as pharmaceutical formulation bases can positively affect the antibacterial and anti-adhesion properties of *Lactobacillus* probiotic strains. The study also revealed some bioactive lead compounds for further studies.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The word probiotics have had series of chronological modifications by different authors before the recommended definition. The focal points of some definitions comprise: Metchnikoff, 1907 (bacteria in fermented milk that replace gut microbes and gives longevity); Werner Kollath, 1953 (substances essential for life); Lilly and Stillwell, 1965 (metabolites that is produced by an organisms and interact with another microbe); Packer, 1974 (dual effect of both microbes and metabolites that contribute to enhance intestinal stability); Fuller,1989 (feed supplements with positive effect on the host by enhancing gut homeostasis) and while FAO/WHO, 2001 and Hiller *et al.,* 2014 (living microbes which when prescribed and administered in sufficient quantity confer a specific health attributes on the host).

Therefore, microorganisms used as probiotics must be *alive* at the duration of administration. Their viability is important because only living cells can proliferate and exert the expected effects. Probiotics are delivered as ingredients in foods, nutraceuticals, pharmaceuticals, but when it is intentionally prepared to prevent, manage or treat a disease condition, it becomes a 'drug.' FDA resolved by classifying probiotics as live biotherapeutics (Sreeja and Prajapati, 2013).

Different route of administrations such as oral, nasal, transdermal, vaginal, and rectal have been utilized for optimal therapeutic effectiveness of this product (Baral *et al.,* 2021). The organisms must be sufficient to achieve the target health benefit. It should be noted that the administered dose should fall within the range of 10^6 to 10^{10} colony-forming units per day (Champagne *et al.,* 2011; Martinez *et al.,* 2015). The health benefits conferred by the probiotic confer health benefits in different ways depending on the specific strain administered: probiotics are health specific; strain specific and not specie specific

(Gbadamosi *et al.,* 2020). The host can be human, animal, or plant, depending on the intended health application (Song *et al.,* 2012). This conferred benefits can be of great application to the host: which could be human, animals and also in plant science (Song *et al.,* 2012).

1.2 Types of probiotics organisms for humans

1.2.1 *Lactobacillus* Species

Gram-positive, non-spore-forming rods called *Lactobacilli* make up a large portion of the normal human bacterial microbiota. *Lactobacillus* species are classified into obligatory homofermentative, facultatively heterofermentative and obligately heterofermentative. The obligatory homofermentative such as the *Lactobacillus acidophilus* are known to break down carbohydrates to produce lactic acid. The second group known as the facultatively heterofermentative such as the *Lactobacillus plantarum* breaks down carbohydrates to produce acetic acid, ethanol and lactic acid, ethanol or acetic acid, and carbon (IV) oxide depending on the sugar fermented. The last group, the obligate heterofermentative group (*Lactobacillus fermentum* and *Lactobacillus reuteri*) continuously converts carbohydrate to produce carbon dioxide, lactic acid, and ethanol/acetic acid as final products (Dempsey *et al.,* 2022; Hammes *et al.,* 2015). An example of a common probiotic strains, is *Lactobacillus crispatus* FSCDJY67L3 used for the treatment of *H. pylori* infection (Hong *et al.,* 2023), while *Lactobacillus reuteri* CF48-3A has immunostimulatory property (Jones and Versalovic, 2009).

1.2.2 *Bifidobacterium* Species

They are Gram-positive, non-motile and non-spore-forming bacteria with a curved or clubbed shape, often branched with Y and V forms (Cunningham *et al.,* 2021). *Bifidobacteria* constitute one of the predominant groups of human intestinal microbiota and fulfil a lot of

well documented probiotic functions (Mitsuoka, 1996; Gibson, 1998). Most *Bifidobacterial* species are strict anaerobes, with a few exceptions, such as *B. boum*, *B. thermophilum*, *B. dentium* and *B. psychraerophilum* and they tolerate microaerophilic condition. (Chen *et al.,* 2021). *Bifidobacterium breve* M-16 V (Kosuge *et al.,* 2021) is a specific strain with ability to prevent social interaction impairment.

1.2.3 *Saccharomyces* Species

Typical features of this species are the dual utilization of both nitrate and carbohydrates. Saccharomyces has a unique capacity for ethanol production, and they are highly suitable yeasts for large-scale fermentation (Zhao and Bai, 2009; Seyidoglu and Aydin, 2021). The probiotic medicinal: *Saccharomyces boulardii* CNCM I-745), is used for the prevention and treatment of diarrhea. This therapeutic effects are associated with multiple mechanisms such immunological effects, pathogen-binding and antitoxin effects (Moré and Swidsinski, 2015).

1.2.4 *Streptococcus* species

Streptococci are Gram-positive, nonmotile, non-spore forming, catalase-negative cocci that occur in pairs or chains. They are divided into three groups by the type of hemolysis on blood agar namely: β-hemolytic (clear, complete lysis of red cells), α hemolytic (incomplete, green hemolysis), and γ hemolytic (no hemolysis) (Patterson,1996). In safety evaluation, strains displaying β-hemolytic and α hemolytic are excluded from probiotic use, while γ hemolytic strains are considered safe (Binda *et al.,* 2020). *Streptococcus salivarius* K12 has shown instant beneficial effect in pharyngotonsillitis (Little *et al.,* 2017: Di Pierro *et al.,* 2016).

1.2.5 Next-Generation Probiotics species

Resent scientific search has been concentrated on other microorganism, referred to the next-generation probiotics (NGPs). These can demonstrate targeted beneficial outcomes and

constitute personalized treatment strategies for managing specific diseases, with the all-inclusive optimization of therapeutic outcomes. (Loo *et al.,* 2024). In comparison to the traditional probiotics such as *Lactobacilli, Bifidobacteria, Saccharomyces, Streptococcus* (discussed above), NGPs are bacteria species that were not previously investigated and used as probiotics. *Christensenella minuta*, *Bacteroides fragilis, Faecalibacterium prausnitzii, Prevotella copri, Parabacteroides goldsteini, Eubacterium limosum and Burkholderia cepacian* are in the route to make a pathway for future for beneficial microorganisms. (Fakruddin *et al.,*2022).

1.3 Basic mechanisms of probiotic microorganisms.

1.3.1 Suppression of quorum-sensing mechanism.

Microorganisms tend to interact with other microbes and the environment via chemical signaling molecules known as the auto-inducers. This communicative mechanism is called quorum sensing. The use of this cell-to-cell signaling mechanism facilitates the regulation of important traits of enteric microbes that allow them to successfully colonize and/or start infection in their host (Gogineni *et al.,* 2013) and leads to the formation of biofilms or the expression of virulence factors. In accordance with Medellin-Pena *et al.* (2007) *Lactobacillus acidophilus* exhibit quorum quenching by secreting bioactive substances that interfere with quorum sensing network or sometimes interconnect with transcription of *E. coli* O157 gene, which is known to be associated with colonization and the eventual pathogenicity of bacteria cells.

1.3.2 Production of Antimicrobial Substances

Probiotics secrete metabolites, comprising: bacteriocins (Lactacin B, plantaricin, and nisin); organic acids (acetic, formic, succinic, and lactic acids); hydrogen peroxide; biosurfactants; enzymes; and siderophores. Antimicrobial substances, disrupt specific aspect of the

pathogenic organisms such as the cell membranes, DNA, RNA, and protein synthesis pathways. Similarly, *Lactococcus* and *Lactobacillus* strains inhibit *E. coli* O157:H7 through lactic acid production and pH reduction (Vinayamohan *et al.,*2024). In addition, Probiotic bacteria can also produce rutarin antibiotics (Aleman and Yadav, 2024).

1.3.3 Intestinal Barrier: stabilization of integrity

Intestinal barrier in healthy persons contain: the mucous layer, antimicrobial peptides, secretory IgA and epithelial cells that form tight junctions. Any slight disruption of the intestinal barrier function may result in enteric infections leading to diseased conditions (Caballero-Franco *et al.,* 2007). Administration of probiotic bacteria can contribute to strengthen intestinal barrier function by: enhancing the expression of genes involved in tight junction signaling; modulates protein phosphorylation; counteract the disruptive effects of enteropathogenic *E. coli* (EPEC) and prevents cytokine-induced epithelial damage (Goudarzi *et al.,* 2014).

1.3.4 Intestinal Barrier: mucous secretion

This beneficial microorganisms had been attributed to mucus production, as a mode of enhancing both the integrity of the intestinal walls and the removal of pathogen. This probiotic mechanism, demonstrated an upgrade of basal luminal musin content by 60% after treatment. The Lactobacillus probiotic species were the most documented potentiator of mucin secretion in an experiment as demonstrated by Caballero-Franco *et al.,* 2007.

1.3.5 Intestinal Barrier: antimicrobial proteins (AMPs)

The α defensins, β defensins, cathelicidins, C-type lectins and the ribonucleases from epithelial cells are some antimicrobial proteins which play a major role in the homeostasis in creating a functional intestinal barrier. The defensins protein has shown anti-infective effects against a large variety of microorganisms. An investigative study by Goudarzi *et al.,*2014

showed that cathelicidin regulation in some beneficial microorganisms have shown correlation with protection against some pathogenic organisms.

1.3.6 **Competitive Exclusion of Pathogenic Microorganisms**

In this situation, a specific microorganism vigorously competes for binding sites in the gastrointestinal region: thereby, inhibiting the attachment of other microbes. The formation of unsupportive environment, saturation of receptor sites, production of inhibitory metabolites and competitive usage of important nutrients are all cumulative approaches by which bacteria exclude the growth of pathogens as shown by Bermudez-Brito *et al.,* 2012.

1.3.7 Appendages aided adhesion

The first attachment of some beneficial microorganisms is driven by general physicochemical interactions to specific substratum. The second mode of bonding aids a stronger attachment which is usually as a result of adhesins or adhesion factors. Some scientific literatures identified these structures as "cell-derived components" (Gorreja and Walker ,2022). These are simply called appendages in this Ph.D. work. Some of these derived components have reshaped the precise understanding of: probiotic -host adhesion; Probiotic-pathogen adhesion (coaggregation); probiotic-probiotic species adhesion (coaggregation) probiotic-probiotic strain adhesion (autoaggregation).

1.4 **Probiotic adhesion**

1.4.1 Probiotic adhesion with host.

Probiotic adhesion to the host tissues (intestinal, vaginal and oral epithelium) is crucial in ensuring colonization, persistence and functional activity of the probiotics on the host. The critical study of the appendages involved will lead to an understanding of probiotic

effectiveness and also support strain selection and product development. Table 1.1 gives a concise overview of some of the probiotic-host aided interactions.

Table 1.1: Characteristics of Probiotic-host interactions

S/N	Type of Probiotic mediated Adhesion	Probiotic Appendages	Host binding site	Nature of interaction	Probiotic Species
1	Pili / Fimbriae	Hair-like protein (e.g., SpaCBA pili, fimbriae)	Mucins (MUC2, MUC3), epithelial glycoproteins	protein–carbohydrate binding; hydrophobic & hydrogen bonds	*Lactobacillus rhamnosus* (well-illustrated in Kankainen *et al.,* 2009)
2	Surface-layer.	Crystalline arrays on cell wall	Epithelial cells; mucin glycoproteins	Electrostatic & hydrophobic; receptor–ligand binding	*Lactobacillus. acidophilus* (Sengupta *et al.,* 2013)
3	Mucus-binding proteins (Mub) and LPxTG-anchored proteins	Large surface-associated proteins with mucus-binding domains	mucin oligosaccharides.	Lectin-like protein–carbohydrate binding	*L. plantarum, L. reuteri* (Pretzer *et al.,* 2005)
4	Lipoteichoic acid (LTA	Amphiphilic molecules anchored in cell wall	Epithelial cell membrane phospholipids	Electrostatic and hydrophobic interactions	*L. casei, L. fermentum* (Granato *et al.,*1999)
5	Extracellular polysaccharides (EPS) and exopolysaccharide capsules	Polysaccharide layer surrounding cell wall	Mucin and epithelial glycocalyx	Non-specific (steric, hydrophobic) & weak carbohydrate–carbohydrate interactions	*L. johnsonii, B. breve* (Lebeer *et al.,* 2009)
6	Adhesion via moonlighting proteins	Cytoplasmic proteins expressed on surface (e.g., enolase, GAPDH, GroEL)	Mucin and epithelial receptors	Protein–protein and electrostatic interactions	*L. plantarum, L. crispatus* (Kainulainen and Korhonen, 2014)
7	Hydrophobic and electrostatic (non-specific) adhesion	Overall cell surface hydrophobicity and charge	Intestinal epithelial surface (non-specific regions)	Weak physical forces (van der Waals, hydrophobic)	*Lactobacillus and Bifidobacterium spp.* Kos *et al.,* (2003).
8	Biofilm formation-associated adhesion	EPS matrix and cell surface proteins	Intestinal mucosa or abiotic surfaces	Multivalent binding and aggregation	*L. reuteri, L. rhamnosus GG* (Jones and

Versalovic, 2009).

1.4.2 Probiotic adhesion with pathogens (coaggregation)

Probiotic adhesion with pathogens (coaggregation) is the ability of probiotic microorganisms to bind directly to harmful microbes. This is one of the most important inhibitory mechanisms by which probiotics help protect the host in the gastrointestinal, oral cavity, and the urogenital tract. Table 1.2: presents some of the possibilities for interaction between common probiotics and a variety of pathogens. This coaggregating interaction will eventually lead to binding of pathogens, blocking colonization, enhancing removal, and finally suppressing infection.

Table 1.2: Characteristics of Probiotic-Pathogen interactions

S/N	Probiotic Organism	Pathogen	Appendages	Mode of binding	Outcomes
1	*L. rhamnosus GG*	*Escherichia coli*, *Salmonella Typhimurium*	SpaCBA pili, adhesins	Pili bind to pathogen receptors	Blocks pathogen adhesion (Kankainen *et al.,* 2009)
2	*L.reuteri*	*Streptococcus mutans*, *Candida albicans*	Lectin-like adhesins, EPS	Lectin–carbohydrate recognition	Reduces oral pathogen colonization. (Båth *et al.,* 2005).
3	*Lactobacillus. Acidophilus*	*Helicobacter pylori*	S-layer proteins (SlpA)	S-layer binds to pathogen surface	Prevents *H. pylori* attachment to gastric cells. (Buck *et al.,* 2005)
4	*B. bifidum*	*Clostridium difficile*	Exopolysaccharides, adhesins	Coaggregation and steric blocking	Inhibits *C. difficile* colonization (Collado *et al.,* 2008)

1.4.3 Probiotic adhesion with another probiotic strain. (Autoaggregation)

Autoaggregation (Probiotic adhesion with another probiotic strain) refers to the property of cells of the same probiotic strain to adhere to each other and form stable, visible clumps. It is an important surface property of probiotic bacteria because it can further strengthen colonization, persistence, pathogen exclusion, and resistance to environmental stress in the human substratum. Table 1.3 gives a summary of the appendages, unique mechanisms, and overall actions of these interactions in the study of probiotics in humans

Table 1.3: Characteristics of Probiotic-monoculture interactions

S/n	Probiotic Microorganism	Appendages	Mechanism	Eventual Outcome
1	*L. plantarum*	Surface proteins AggL, Msa	Protein-mediated same-strain adhesion	Biofilm formation, mucosal persistence (Maldonado *et al.,* 2011)
2	*L. crispatus*	S-layer proteins	Protein–protein surface recognition	Dense aggregates on mucosa (Sillanpää *et al.,* 2000)
3	*L. johnsonii*	EPS, LTA	EPS-dependent cell clumping	Gut colonization stability (Ventura *et al.,* 2012)
4	*B. bifidum*	Extracellular polysaccharides	Sugar-mediated aggregation	Acid and bile tolerance (Castro-Bravo *et al.,* 2018)

1.4.4 Probiotic adhesion with probiotic species. (coaggregation)

Probiotic adhesion with probiotic species (coaggregation) is the ability of a probiotic microorganism to adhere to cells of another probiotic species or strain, forming a firmly visible cluster of cells. Table 1.4: shows the combinations of genetically distinct probiotic microorganisms to holistically enhance multi-strain activity against other offending microbes. It should be noted that autoaggregation discussed earlier in this thesis is different from this type of binding.

Table 1.4: Characteristics of Probiotic-mixed culture interactions

S/N	First probiotic Organism	Second Probiotic organism	Appendages	Mechanism of Adhesion	Outcomes
1	*Lactobacillus rhamnosus* (e.g., GG)	*Lactobacillus reuteri* (Collado *et al.,* 2007; Lebeer *et al.,* 2008)	Pili (e.g., SpaCBA) & surface proteins	Protein-protein binding, pilus-mediated adhesion	Mixed biofilm, enhanced adhesion to epithelium
2	*Lactobacillus plantarum*	*Bifidobacterium longum* (Collado *et al.,* 2008; Gueimonde *et al.,* 2006)	Exopolysaccharides (EPS), S-layer proteins	EPS-mediated bridging & hydrophobic interactions	Improved persistence, metabolic cooperation
3	*Lactobacillus acidophilus*	*Lactobacillus casei* (Kos *et al.,* 2003)	Surface layer proteins, lipoteichoic acids	Cell surface protein interactions	Formation of multi-species clusters, pathogen exclusion
4	*Bifidobacterium bifidum*	*Bifidobacterium breve* (Gueimonde *et al.,* 2006)	Pili/surface proteins, EPS	Lectin-carbohydrate binding & co-aggregation via polysaccharides	Cooperative colonization & carbohydrate utilization
5	*Lactobacillus fermentum*	*Lactobacillus salivarius* (Del Re *et al.,* 2000)	Adhesins, EPS	Hydrophobic interactions & surface matching	Competitive exclusion of pathogens
6	*Lactobacillus rhamnosus.*	*Bifidobacterium animalis.* (Collado *et al.,* 2008)	Pili, EPS	EPS-protein interactions and co-adhesion	Enhanced gut persistence & synergy
7	*Saccharomyces boulardii*	*Lactobacillus rhamnosus* Collado *et al.,* 2008; Czerucka *et al.,* 2007	Yeast mannan, bacterial adhesins	Lectin–mannan binding	Stabilized probiotic consortium, pathogen binding
8	*Lactobacillus plantarum*	*Lactobacillus rhamnosus* Del Re *et al.,* 2000	Mucus-binding proteins, pili	Co-adhesion to mucus shared sites	Improved mucosal protection & immune effects

1.5 General health beneficial effects of probiotic organisms

1.5.1 Cancer prevention and support

Cancer arises from faulty DNA repair mechanisms or from mutations acquired during DNA replication. Its principal risk factors include: genetic predisposition, environmental factors, and personal lifestyle choices. Some of these predisposing factors are infectious agents, toxic substances and UV radiation. (Legesse *et al.,* 2020). *Lactobacillus acidophilus*, has been demonstrated to stop the functionality of bacterial nitroreductases, which are responsible for converting nitrosamines into more potent carcinogens (Ahmad *et al.,* 2025)

1.5.2 Urinary tract Infection

Urinary Tract Infections (UTI) result from an imbalance in vaginal microbiota. The infection can affect both the upper and lower urinary tract. It frequently recurs, and while antibiotics therapy are beneficial, their repeated use increases the risk of antibiotic resistance strains. The presence of lactic acid bacteria in the vaginal microbiota from healthy women plays a crucial role in reducing vaginal pH. Therefore, in treatment of UTI there are over 50 probiotics that can effectively treat this condition, all these are based on *Lactobacillus* species. (Van *et al.,* 2023).

1.5.3 Respiratory disease

Inflammation in Bronchial, Paranasal sinus, pharynx, and the ear are associated with respiratory infections. Probiotics exhibit documented anti-inflammatory and anti-infective tendencies which paved the way in their therapeutic prevention of a number of respiratory disorders. Administration of *Lactobacillus rhamnosus* has shown documented effect patients

with cystic fibrosis presented with recurrent pneumonia. Soccol *et al.*, 2014 further documented the promising effect of *L. casei* and *Lactobacillus fermentum* in treatment of some respiratory related conditions.

1.5.3 Detoxification

Chemically contaminated foods (heavy metals, acrylamide, polycyclic aromatic hydrocarbon—benzopyrene) or biologically contaminated (microorganisms, pathogens, fungi—molds, and yeasts) have been shown to be hazardous to one's health, causing disease and death annually (Kirk *et al.,*2017; Fung *et al.,* 2018). These toxic contaminants originate from raw materials or by-products resulting from food processing (Srednicka *et al.,*2021). The microbiota-probiotic have been effective in deactivating toxins via enzymatic degradation (deamination, decarboxylation hydrolysis) or by biotransformation which convert toxins into non-toxic or reduced-toxicity compounds (Sionek *et al.,* 2025).

1.5.4 Dermatological Diseases

In vitro studies have demonstrated that *L. salivarius LS03, Lactococcus, and Streptococcus salivari* secrete bacteriocins that inhibit the growth of *Cutibacterium acnes*. Similarly, *B. adolescentis* SPM0308, possesses antimicrobial activity against both *C. acnes and S. aureus*. Additionally, *S. thermophiles* has been shown to be beneficial in the production of lipids (ceramides in the stratum corneum), which help the skin to maintain moisture, and also phytosphingosine, which helps inhibit *C. acnes.* (Sánchez-Pellicer *et al.,* 2022)

1.5.5 Immune Regulation

Immunomodulation refers to the ability of certain agents (drugs, natural compounds, cells, biologicals) to modify the immune response. That modification may be in the direction of enhancing (immunostimulation) and suppressing (immunosuppression) as identified by Yeap,

*et al.,*2011. Primary mechanisms of Immunostimulation of probiotics are usually via: *Lactobacillus casei* Shirota enhanced NK activity & IL 12 induction (Shida *et al.,* 2013), *L. plantarum* NCIMB8826 induced NK cell activation, pro inflammatory cytokine production (Aziz and Bonavida, 2016) while immunosuppression are usually through the down regulation of pro inflammatory cytokines (TNF α, IL 6), modulate NF κB signalling, reduced Th17 polarization and increased anti-inflammatory markers (Cristofori *et al.*,2021).

1.5.6 Gut-brain Axis and Mental Health

The gut-brain axis signifies the communication network between the gastrointestinal tract (including the microbiota) and the central nervous system. Key defined pathways include: neural (the vagus nerve), endocrine (hormones), immune (cytokines) and short chain fatty acids (microbial substances). Holistically, probiotics results in significant reductions in depression and moderate reductions in anxiety (Asad *et al.,* 2025) Additionally, meta-analysis found combination of probiotics and prebiotics significantly reduced anxiety, depression and improved cognitive function respectively (Zandifar *et al.,* 2025).

1.5.7 Male reproductive system

Probiotics have shown promising effects in prostate cancer, prostatitis and improve overall semen integrity. In prostate cancer, *Bifidobacterium animalis* Bb12, *Lactobacillus acidophilus* La-05 and *L. rhamnosus* GG strongly induced apoptosis (Rosa *et al.,* 2020 and Celebioglu *et al.*,2021) . Administration of probiotics in prostatitis condition has led to a decreased bioburden of *Escherichia coli* and *Enterococcus faecalis* in urine cultures: indicating the potential of these beneficial organism to suppress prostatitis (Pacifici *et al*., 2021). Animal model using aging mice treated with probiotics can restore testosterone levels and seminiferous tubule profiles as well as spermatogenesis, thus indicating that probiotics may affect semen quality by through testicular function (Poutahidis *et al.,* 2014).

1.5.8 Female reproductive system

In this system, probiotics have shown some beneficial effect on the follicle, placenta, vagina, endometrial microbiome configuration and polycystic ovary syndrome (PCOS). Probiotics can regulate follicle development by increasing reproductive hormones while decreasing adrenal cortical hormone (Zhou *et al.,* 2020; Zhao *et al.,* 2019). These beneficial organisms reduce inflammatory responses in the placenta thereby decreasing the risk of severe preeclampsia (Brantsaeter *et al.,* 2011). A systematic review of vaginal probiotics concluded that *Lactobacillus* probiotics show promising potential for both the treatment and prevention of bacterial vaginitis (Van de Wijgert *et al.,* 2020). In addition, modulation of gut microbiota composition through probiotics has been recommended for the management of polycystic ovary syndrome (PCOS), a common endocrine disorder affecting women of reproductive age (Martinez Guevara *et al*., 2024). Furthermore, probiotics may help alleviate endometritis and endometriosis by influencing the endometrial microbiome, suppressing inflammatory responses, and strengthening uterine barrier integrity (Feng *et al.,* 2022).

1.5.9 Early life development

Numerous studies have investigated the role of probiotics in infant development (Catania *et al.,* 2021). For example, *Lactobacillus rhamnosus* Lcr35 has been shown to relieve constipation by improving bowel movement frequency and stool consistency (Wojtyniak *et al.,* 2017). In addition, evidence suggests that the *Lactobacillus reuteri* DSM 17938 strain can

reduce mean crying time by 56 minutes per day in exclusively breastfed infants at three weeks of age, supporting the use of probiotics in the management of infantile colic (Sung *et al.,* 2018). There are other interesting information to support the use of probiotics *L. reuteri* DSM 17938 for gastroesophageal reflux (GER) (Sandipan and Sudipta, 2025); *L. rhamnosus* GG ATCC53103 for functional abdominal pain syndrome (FAPS) (Horvath *et al.,*2011) and the use of mixed probiotic combination for necrotizing enterocolitis (Morgan *et al.,* 2020).

1.5.10 **Oral health**

A double-blind study involving 31 children demonstrated that *Lactobacillus casei* significantly reduced the progression of dental caries by inhibiting pathogenic bacteria, thereby providing strong support for the use of probiotics in promoting oral health (Chugha *et al.,* 2020; Yadav *et al.,* 2014). It was noted that a regular administration of *L. reuteri* prepared lozenges proved to be a beneficial in the modulation of pregnancy-linked gingivitis and plaque in healthy gravid women (Schlagenhauf *et al.,* 2016). Probiotics adhere to dental tissues as a part of the biofilm (or plaque) and compete with the growth of cariogenic bacteria or periodontal pathogens (Penala *et al.,* 2016). Periodontitis is another oral health issue that can benefit from this beneficial organisms because probiotics can adhere to dental tissue and compete with the progression of periodontal pathogens (Penala *et al.,* 2016). Additionally, the replacement of bacteria implicated in halitosis with probiotic strains may have clinical application as adjuncts for the prevention, management, or treatment of halitosis condition (Chugha *et al.,* 2020).

1.6 Vaginal infection induced by *Escherichia coli.*

1.6.1 Aerobic vaginitis (AV)

Aerobic vaginitis (AV) is an infectious condition of the vagina marked by pronounced inflammation and epithelial atrophic changes. The reduction and elimination of the beneficial

Lactobacillus species and a proliferative increase in enteric bacteria such as predominant *Escherichia coli* is a likely reason for this condition. (Serretiello *et al.,*2021). This condition was found to be predominant within the age range between of 55–64. Among the pathogenic strains, *Escherichia coli* (32.5%) was the most predominant strain, followed by *Enterococcus faecalis* (29.4%), *Klebsiella pneumoniae* (7.8%) and *Enterococcus faecium* (7.7%) (Serretiello *et al.,*2021). The consequences of this condition in the vagina will aid in understanding the severity to woman health.

1.6.2. Evidenced based consequences of *Escherichia coli* in the vagina

1.6.2.1 Infertility

Infertility is the lack of conception after at least one year of constant unprotected sexual intercourse (Pellati *et al.,* 2008). It is an increasing medical problem seen in 13-15% of reproductive age couples (Kim *et al.,* 2016). *E. coli* can affect both female and male fertility (Cools, 2017). Agrawal *et al.,* 2013 and Vander and Prabha, 2019 documented that Lipopolysaccharide appears to alter the concentration levels of the cytokines TNF-α as well as follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). This hormonal and immunological disturbance most significantly result to loss in early pregnancy.

1.6.2.2 Miscarriage

This condition is usually considered the loss of pregnancy within the first 22 weeks of gestation. Cools reported that in 85 (66%) cases, bacteria were recovered from the placenta and fetus. *Escherichia coli* was isolated in one-fourth of the cases and was identified as the main organism in eight cases (Cools, 2017). The *E. coli* infection group exhibited a higher proportion of miscarriages before 28 weeks, a lower proportion of full-term deliveries, and fewer cesarean sections than the non -*E. coli* infection group. (Shi *et al.,* 2025)

1.6.2.3 Still birth

Stillbirth can be defined as loss of pregnancy after 22 weeks of gestation (Lawan *et al.,*2011) Infection is more clearly correlated with early stillbirth (22 to 28 weeks) than with late stillbirth after 28 weeks (Goldenberg *et al.,* 2000). Studies have confirmed *E. coli* intrauterine (subclinical) infection as a major contributor to intrauterine fetal mortality (Cools, 2017). Despite the gains in perinatal medicine, this issue is one of the most common causes of pregnancy, with an estimated 3.2 million cases occurring worldwide annually (McClure, 2009).

1.6.2.4. Preterm rupture of membranes (PROM)

In approximately 8-10% of pregnancies, fetal membranes will rupture before the onset of labor. (Caughey *et al.,* 2008). When this happens at above 37 weeks, this is known as premature rupture of the membranes (PROM). While, PROM before 37 weeks is considered a preterm premature rupture of membranes (PPROM) (Goldenberg *et al.,* 2008), PPROM is difficult to manage and is responsible for about 20% of perinatal deaths. (Caughey *et al.,* 2008). In PPROM cases, the causative factor is unknown, but intrauterine infection is a frequent precursor (Mercer *et al.,* 2000). Pettker and co-workers isolated *E. coli* in 8% of amniotic fluid and placental samples of cases of PPROM and preterm labor (Cools, 2017).

1.6.3 Problems associated with conventional treatment aerobic of vaginitis.

Women with AV can develop a negative clinical outcome following failure of antibiotic therapy. (Kaambo, 2018). Ingestion of antibiotics provides selective pressure ultimately leading to a higher prevalence of resistant bacteria (Okeke *et al.,* 2000). This is further buttressed in a work by Blancarte-Lagunas *et al.,* 2020 in which they demonstrated how vaginal-origin *E. coli* strains harbor class 1 integrons that serve as reservoir of antibiotic resistance genes, with the potential to acquire additional resistance cassettes. When the

condition and the eventual therapy is a problem, there is need for evidence-based alternative therapy to handle the condition.

1.6.4. Effect of Vaginal *Lactobacillus* probiotics on *Escherichia coli.*

The inhibition of infections caused by microbial pathogens such as *E. coli* by *L. fermentum* SK5 that produces antimicrobial substances leads benefits to health. (Kaewnopparat *et al.,* 2013). *Lactobacillus rhamnosus* HN001 and *Lactobacillus acidophilus* GLA-14 strains demonstrated inhibitory effect against *Escherichia coli* in a co-culturing assay experimental model (Bertuccini *et al.,* 2017). Comparatively, Hütt *et al.* revealed that *L. crispatus* displays better antagonistic activity against *E. coli* than *L. gasseri* and *L. jensenii* (Hütt *et al.,* 2016). Precise information on *Lactobacillus* species strengthens the use of probiotics as alternative therapy.

1.7 Rectal infection induced by *Escherichia coli.*

1.7.1 Ulcerative colitis

This is usually triggered by changes in the intestinal microbiota and consequentially followed by an abnormal immune response. (Head *et al.,* 2003). The increasing prevalence of *Escherichia coli* has been deduced to play a role in the pathogenesis of Ulcerative colitis (UC). (Petersen *et al.,*2009; Mirsepasi-Lauridsen*, et al.,* 2016). UC can be classified either according to disease severity or by the extent of colonic involvement. Based on the extent colonic movement, it is categorized into proctitis (confined to the rectum), proctosigmoiditis (involving the rectum and sigmoid colon), left-sided colitis (extending up to, but not beyond, the splenic flexure), and extensive colitis (spreading beyond the splenic flexure) (Kornbluth and Sachar, 2010). The extent of rectal involvement, together with the total length of bowel segments affected, plays a key role in determining the clinical severity of the disease and its potential health consequences in affected individuals.

1.7.2. Evidenced based consequences of *Escherichia coli* in the vagina

1.7.2.1 Increased risk of colorectal cancer

A Meta-analysis of strictly population-based cohort studies shows 2.4-fold increased risk of induced colorectal cancer in Ulcerative colitis (Jess *et al.,* 2012). Ulcerative colitis is a known risk factor for the development of colorectal cancer (Ekbom *et al.,* 1990). It should be noted in this thesis that cancers superimposed on ulcerative colitis are often multifocal (20%), sometimes poorly differentiated (50%), and present in advanced Stage III or more (van Heerden and Beart,1980; Connell *et al.,* 1994).

1.7.2.2 Extraintestinal manifestations

These manifestations are seen in 15–25% of the patients and usually involve the eyes, liver, skeleton, and the skin. (Greenstein *et al.,* 1985; Talbot *et al.,*1986). Generally, they include: ankylosing spondylitis and sacroiliitis; cardiac complications (pericarditis); hepatobiliary disease (pericholangitis, primary sclerosing cholangitis); ocular (anterior uveitis, iritis, episcleritis); peripheral arthritis and skin lesions (pyoderma gangrenosum, erythema nodosum) (Kaiser and Beart, 2001).

1.7.2.3 Toxic megacolon

Toxic megacolon is an uncommon but severe complication of ulcerative colitis, occurring primarily in patients with acute severe or extensive colitis. Contemporary studies estimate an incidence of approximately 1–2.5% in UC overall, although higher proportions are reported in hospitalized severe cases (Dao *et al.,* 2024). Clinically, these patients often present with sudden high fever, an elevated white blood cell count, rapid heart rate, abdominal pain or tenderness, and progressive abdominal swelling. Mortality of toxic megacolon has historically been reported to range from 15–30% but has fallen drastically in recent years in parallel with a more aggressive clinical approach. (Kaiser and Beart, 2001).

1.7.3 Problems associated with conventional treatment of ulcerative colitis

Despite the clinical gains associated with these agents in management, each has its limitations. Aminosalicylates are effective for managing mild to moderate ulcerative colitis, but they are often insufficient in severe cases. Glucocorticoids provide rapid anti-inflammatory relief, yet continuous use can result in adverse effects such as elevated blood sugar, reduced bone density, and increased risk of infections. Immunosuppressants, which may require several weeks to months to take full effect, carry potential risks including bone marrow suppression and liver or kidney toxicity. This scenario underscores the need for complementary strategies such as use of probiotics to address the dysbiosis of the gut microbiota (Xu *et al.,* 2025)

1.7.4 Effect of Rectal *Lactobacillus* probiotics on *Escherichia coli*

Beneficial activities of probiotic such as *L. casei* DG can aid manipulation of mucosal microbiota and its enhance effects on the mucosal immune system will be of benefit in UC patients. (D'Incà *et al.,* 2011). Additionally, *E. coli* Nissle 1917 displayed comparative efficacy with mesalazine in patients with ulcerative colitis as documented by Kruis *et al.,*2004.

1.8 Adsorbents in determination of adhesion of probiotics.

In vitro assessment of probiotic adhesion is a key functional property influencing colonization, persistence, and strain-specific health benefits in the host. These experimental systems should provide a simulation of host surfaces, ensure standardization and reproducibility, and allow for the elucidation of strain-specific adhesion characteristics. They also facilitate understanding of mechanisms of action, prediction of colonization potential, evaluation of antipathogenic activity, and assessment of environmental effects within controlled laboratory models.

1.8.1 Abiotic substrates *in vitro* adhesion study of probiotics

Abiotic substrates are artificial or non-biological surfaces designed to simulate environmental or host-contact surfaces. Common examples and their distinct characteristics are presented in Table 1.5. An *in vitro* adhesion study of probiotics using abiotic substrates examines the ability of probiotic microorganisms to adhere to non-living surfaces under controlled laboratory conditions. This approach is widely used to evaluate properties associated with colonization potential, biofilm formation, survivability in delivery systems, and suitability for industrial applications. Many researchers use different abiotic substrates as proxies to elucidate probiotic adhesion.

Table 1.5: Characteristics of some Abiotic adsorbents

S/N	Type	Application	Modification or treatment	Merit	Limitations	References
1	Polystyrene: petri dishes, microtiter	Bacterial Adhesion and Bioform assay	Untreated*	Quantitative, reproducibility and cost-effective assay	Poor correlation with *in-vivo* condition Due to hydrophobicity of substrate	Krausova *et al.,* 2019.
2	Polystyrene or glass	Simulate effect of intestinal mucus layer	Mucin from porcine stomach or bovine submaxillary gland	Prediction of effect of mucosal surfaces;	Variation in mucin purity and complex coating protocol.	Nishiyama *et al.,* 2019
3	Polystyrene	Simulate extracellular matrix (ECM) for intestinal epithelial adhesion	Human fibronectin or collagen type I or IV	Enhance study of specific adhesin–ligand interactions	Comparatively expensive	Muscariello *et al.,* 2020.
4	Glass slides	Adhesion study in Hydrophilic surface	Untreated	Designed for microscopic evaluation	Poor correlation with *in vivo* condition and different surface charge when compared to mucosal layer.	Leska *et al.,* 2022

5	Stainless steel, glass, plastic, and coupons	study of biofilm formation on industrial surfaces and food-contact.	Untreated	Stimulating probiotic survival in processing condition	Poor correlation to physiological-host-adhesion	Kumar and Anand, 1998

1.8.2 Biotic substrates in vitro adhesion study of probiotics

In vitro adhesion evaluation of probiotic microbes utilizes a variety of biotic substrates to evaluate quantitatively strains adhesion as shown in Table 1.6. This is a key feature in colonization and probiotic functionality. Categorized below are well-documented biotic substrates used, which may enhance further designs and modifications in experimental determinations.

Table 1.6: Characteristics of some Biotic adsorbents

S/N	Type	Application	Modification or treatment	Merit	Limitations	References
1	Epithelial cell lines (Caco- 2, HT29- MTX, or co-cultures)	Probiotic adhesion study	Living cell coated with mucin.	More physiologically relevant: has living cells with cell-surface receptors, cell junctions, metabolic activity. Allows measurement of probiotic adhesion to host cells.	lack of immune cells, Caco-2 lines lack mucus secretion.HT29-MTX produce mucus but still imperfect. Results may vary widely between labs and strains-strain specificity is	Laparra *et al.,* 2009;Krausova *et al.,* 2019
2	Raisins / natural supports	Assess adhesion and immobilization potential	Untreated	Natural, food-compatible, improves viability	Surface heterogeneity; not standardized	Mileriene *et al.,* 2022,

1.9 Physicochemical properties of some suppository bases.

1.9.1 Coco butter base

Cocoa butter also known as Theobroma is extracted from cocoa beans. It is non-toxic, biocompatible and used as an excipient in formulations of suppository base. It is a pale-yellow to creamy-white solid with a chocolate odor. It is brittle at low temperature and melts readily on contact with the skin (Rowe *et al.,* 2009; Aulton and Taylor, 2018). It consists mainly of triglycerides of oleic stearic and palmitic acids and linoleic acid **(**Gunstone *et al.,* 2007**;**Aulton and Taylor, 2018**;** Othman *et al.,* 2007**).**

It is insoluble in water but soluble in organic solvents such as chloroform, and miscible with oils and fats. (Rowe *et al.,* 2009; Aulton & Taylor, 2018) The lipophilicity due to the triglyceride composition makes it suitable for encapsulating lipophilic substances and for protecting probiotics from acidic environments. (Gunstone *et al.,* 2007; Anal and Singh, 2007; Arslan and Erbas, 2015). Cocoa butter has mild antimicrobial and protective activity mainly due to its minor components such as polyphenols and free fatty acids. However, cocoa butter is not a primary antimicrobial agent (Othman *et al.,* 2007).

1.9.2 Polyethylene glycol base

Polyethylene glycol (PEG), also known as macrogol, is a synthetic, non-ionic, biocompatible, hydrophilic polymer of ethylene oxide nucleus. PEG is widely applied as a solvent, plasticizer, base, and carrier system. Its biologically inert nature, makes it suitable for oral, topical, and parenteral preparations (Rowe *et al.,* 2009; Aulton & Taylor, 2018). Common pharmaceutical grades include PEG 200, 400, 600 (liquids) and PEG 1500, 4000, 6000, 8000 (solids) (Rowe *et al.,* 2009; Allen *et al.,* 2011). It is highly hydrophilic, and poorly lipophilic and forms hydrogen bonds with water to enhance aqueous dispersion. PEG-based matrices allow rapid hydration and faster release of encapsulated probiotics. However, it provides less protection against gastric acidity compared with lipid carriers. (Rowe *et al.,* 2009; Anal and Singh, 2007)

PEG is chemically stable under normal conditions. However, it may undergo oxidation at high temperatures, degrade in the presence of strong acids or bases, and absorb moisture (hygroscopicity). It does not protect probiotics from gastric acidity but readily combines with enteric polymers or lipid carriers to ensure protection (Anal and Singh, 2007; Arslan and Erbas, 2015). PEG is considered microbiologically inert and compatible with probiotics (Knop *et al.,* 2010).

1.9.3 Glycerogelatin base

Glycerogelatin base is a hydrophilic, semi-solid base composed from the addition of glycerol, gelatin, and water. It is commonly used in pharmaceutical preparations like suppositories and topical gels. It dissolves slowly in body fluids rather than melting like fatty bases. After preparation, it is usually pale yellow or colorless, jelly-like elastic with sticky consistency and faintly sweet odor (Aulton and Taylor, 2018; Rowe *et al.,* 2009). Glycerogelatin base typically contains gelatin (10–20%) (which provides a structural framework and gel formation); glycerol (50–70%) (which acts as a plasticizer and humectant), and purified water

(20–30%) (to aid dissolution and consistency) (Rowe *et al.,* 2009; Allen *et al.,* 2011). It softens and dissolves in body fluids at body temperature (37°C). The gelatin network swells in moisture, gradually releasing the active ingredient. (Aulton and Taylor, 2018; Allen *et al.,* 2011). It is moderately suitable for vaginal and rectal probiotic preparation rather than oral probiotic capsules. It requires preservatives and is less protective against gastric acidity compared to lipid bases (Anal and Singh, 2007; Arslan and Erbas, 2015). Glycerogelatin base has no strong intrinsic antimicrobial activity. The formed base is considered microbiologically inert (Rowe *et al.,* 2009).

1.10 Methods used in the determination of anti-*Escherichia coli* effect of probiotics

1.10.1 The agar overlay method

This is a microbiological technique used to detect inhibitory metabolites. A primary set of sterile agars is first inoculated with a producer organism and pre-incubated to allow growth, production, and diffusion of metabolites. Subsequently, a secondary layer of soft sterile agar seeded with a specified pathogenic organism is overlaid on the primary plate. Following incubation, zones of inhibition on the overlay indicate the presence and activity of diffusible-inhibitory metabolites released by the producer microorganisms inoculated in the primary agar (Hockett and Baltrus, 2017). This method is sometimes documented as Agar double layer (ADL), Double agar layer (DAL), or Double-layer agar (DLA) technique or method. The diameter of the zone of inhibition is an indication of the strength of antagonistic activities of metabolites: strong antagonistic activity (> 20 mm); moderate activity (10-20 mm), and weak activity (< 10 mm) (Halder *et al.,* 2017) (Fijan *et al.,* 2018).

1.10.2 Cocultivation Assay

In the co- culture assay, a microorganism secreting metabolite and a pathogenic organism are in a predetermined growth medium that supports the growth of both organisms. At specific intervals, an aliquot from the broth is taken and spread on selective agar media that exclusively supports the growth of the organisms. A low concentration of the pathogen relative to the negative control indicates the production and secretion of antimicrobial compounds by the metabolite secreting microbe (Hossain *et al.,* 2024). Cocultivation assay is also known as mixed culture assay, dual culture assay or interaction assay.

1.11 **Justification of study**

Probiotics are widely known to confer health benefits, particularly in gastrointestinal and vaginal health. Certain strains have been shown to kill, inhibit, or modulate the presence of pathogens such as *Escherichia coli* associated with induced aerobic vaginitis and ulcerative colitis (Zhang *et al.,* 2024; Zuniga *et al.,* 2024; Ismael *et al.,* 2025). However, the impact of suppository bases on the antibacterial activity and adhesion-inhibiting properties of these probiotic organisms remains poorly understood. The outcomes of this research will help identify optimal formulation bases that maximize antibacterial effectiveness; support the design of adsorbent for study of competitive, exclusive and displacement assay of probiotics-pathogen interactions; provide evidence for experimental models amenable to the presence of suppository bases. These strengthen the need to study the antibacterial and anti-adhesion studies of probiotic strains against *Escherichia coli* in the presence of suppository bases with GC-MS profiles of the coculture.

1.12 **Aim and objectives**

The aim of the study was to investigate the antibacterial and anti-adhesion studies of probiotic strains against *Escherichia coli* in the presence of suppository bases with GC-MS profiles of the coculture.

The objectives of the study were to:

1. authenticate the probiotic strains and *Escherichia coli,*
2. design and prepare an adsorbent,
3. evaluate the de-adhesion effect Tween 20 solubilizing agents,
4. determine the antibacterial effect of *Lactobacillus* strains on *Escherichia coli* in the presence of suppository bases,
5. determine the effect of suppository bases on growth of *Lactobacillus* strains,
6. investigate the anti-adhesion effect of *Lactobacillus* strains on *Escherichia coli* in presence of Suppository bases,
7. investigate the effect of suppository bases on the adhesion of *Lactobacillus* strains,
8. comparative GC-MS studies of the interactive effects of suppository bases on the *Lactobacillus* stains.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents and Chemicals

Methylated spirit, crystal violet, Lugol's iodine, acetone,

Ethanol (JHD (China) Lot 20100408

hydrogen peroxide CDH (India)

Ethyl acetate JHD (China) Lot 20100408

Liquid Paraffin JHD (China) Lot 20210408

Polyethylene Glycol 300 (Qualikems) #L121941603

Polyethylene Glycol 600 (Qualikems) Batch No: ACA140711

Zeolite 19904 Batch No: 19112901

Dipropylene Gycol Lot: 28061000

Disodium hydrogen phosphate anhydrous .GH TECH (China) Lot No:20200618

Safranine (Qualikems) Batch No: PSG030611

Raffinose pentahydrate. Trust chemical Laboratory. (China)

D-fructose. Trust chemical Laboratory. (China)

Sodium dihydrogen Phosphate dihydrate GH TECH (China) Lot No: 2018015

Potassium Dihydrogen Phosphate GH TECH (China) Lot No 20120922

Sodium Chloride.GH TECH (China) Lot No: 20190523

Sucrose. Laboratory Burgoyne reagent (India)

Lugol's Iodine. Bema scientific and chemical Co.LTD.

Lactose. Laboratory Burgoyne reagent (India)

Cellulose powder. Laboratory Burgoyne reagent (India).Batch no: 32127

Galactose. Trust chemical Laboratory. (China)

L-arabinose.Trust chemical Laboratory. (China) Cas No: 5328-37-0

Dimethylsulfoxide. GH TECH (China) Lot No: 20200417

Kaolin. Loba Chemie LTD. (India) Batch No: Batch No: L239701712

Activated Charcoal. Molychem. (India). Batch no: MCR-17878

Tween 20 GH TECH (China) Lot no: 20180710

Crystal violet. Scharlau. (Spain). Batch no: 13338101

2.1.2 Culture Media

Mueller Hinton agar.Titan Biotech LTD. (India)

Muller Hinton broth. Titan Biotech LTD. (India).

Eosine Methylene Blue agar. Ready MED. (India).

De Man-Rogosa-Sharpe (MRS)Agar. Titan Biotech (India)

De Man-Rogosa-Sharpe Broth. Titan Biotech (India)

Eosin Methylene Blue agar. ReadyMED. (India)

2.1.3 Equipment

Refrigerator. Haier Thermocool (Nigeria)Model:HR-1700T

Incubator.Dai Han Scientific LTD (South Korea). Model: LIB-211-E

Hot air oven, Incubator,

Micropipette. 1000 and 200 µL capacity. Microlit. (India)

Autoclave. Prestige series 2100.(UK)

Microscope.Biobase (China) Model: BMB-300M

Weighing balance. Ohaus (USA) Model: 02A.2.00. OD0B

Shimadzu GCMS-QP2010. Shimadzu Corporation (Japan)

Rotary evaporator. Stuart. (UK) Serial No: R000100875

PH meter. Hanna instruments.(Italy)

Water distiller.SZ-96 Biochemistry instrument factory (China)

2.1.4 Glassware

Universal bottles, Beakers, 500mL Borosilicate glass, measuring cylinder, microscopic glass slides, glass stirrer and conical flask, separating funnel. All glass wares were products of Pyrex England

2.1.5 Biological materials

Lactobacillus reuteri CF48-3A, *Lactobacillus gasseri* JV-VO3, *Lactobacillus rhamnosus* LMS2-3A, *Escherichia coli* ATCC 25922 and eggs of Isa brown breeds. All *Lactobacillus* strains were obtained through BEI Resources, NIAID, NIH.

2.1.6 Suppository base constituents

Theobroma base, (Nigeria)

Gelatin. Molychem (India) Batch No: MCR-24111

Glycerol,

Polyethylene glycol 6000, Qualikems (India) Batch No: L217821707

Polyethylene glycol 4000 Qualikems (India) Batch No: ACA140711

Sterile distilled water

2.1.7 Other materials

Petri dishes (Italy), cotton wool (Nigeria), Bunsen burner, gas, sterile swab sticks (Nigeria), surgical gloves (Thailand) foil papers and anaerobic jar, 5ml syringes (Nigeria), sterile distilled water, Mathematical sets, Inoculating loop, Surgical blade, Aluminum foil (China), detergent.

2.2 Methods

2.2.1 Gram Staining Reaction

Dried smears of the probiotic organisms (*Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-3A) and the pathogenic organism (*Escherichia coli* ATCC 25922) were prepared on properly labelled, grease-free slides. The three *Lactobacillus strains* are publicly available: *Lactobacillus reuteri* CF48-3A (NCBI, n.d.-a), *Lactobacillus gasseri* JV-V03 (NCBI, n.d.-b), and *Lactobacillus rhamnosus* LMS2 strain (NCBI, n.d.-c). The slides were treated with crystal violet, Lugol's iodine, acetone–alcohol (1:1) and Neutral red stain. Relevant precautions such as timing and rinsing were adhered to in accordance with Cheesbrough, 2006. The dried slides were examined microscopically and results documented.

2.2.2 Biochemical Test

A forty-eight-hour (48 h) culture from a de Man, Rogosa and Sharpe (MRS) Agar plate containing the probiotic organism were submerged into three percent (3%) of hydrogen peroxide in a test tube with the aid of a sterile glass rod. Results were documented as positive for presence of active bubbling and negative for absence of bubbling (Cheesbrough, 2006).

Sugar fermentation test was carried out for the following sugars; cellobiose, sucrose, raffinose, lactose, maltose, D-fructose, D-glucose, D-mannitol, L-rhamnose, D-xylose and L-arabinose (Cheesbrough, 2006).

2.2.3 Antibacterial Studies

The Agar overlay method was employed to evaluate the attribute of the *Lactobacillus* strains to interfere with the growth pattern of the *Escherichia coli*. Fifty microlitres of probiotic organisms were grown in the center MRS agar for 48 h and subsequently overlaid with a 24 h culture of the *Escherichia coli* in Mueller Hinton Agar (Rahalison *et al.,*1991). The Zone of inhibitions was determined after incubation at 37℃ as a reflection of the antibacterial-bacteriostatic nature of the *Lactobacillus* strains. Subcultures were obtained from the zone of inhibitions and subcultured in Mueller Hinton broth to determine the antibacterial-bactericidal potential of the *Lactobacillus* strains.

2.2.4 Preparation of Adsorbent

An adsorbent was prepared from twenty-four hours (24 h) egg. The albumen of an egg from an Isa brown breeds were extracted and used in the formation of a cylindrical albumen. The weight, height, diameter, and stability to autoclaving were determined. Linearity study was conducted to determine the suitability of the solidified albumen to be used for *L. reuteri* CF48-3A, *L. gasseri* JV-VO3, *L. rhamnosus* LMS2-3A and *E. coli* ATCC 25922 (Kluepfel and Pueppke, 1985). The values of the coefficient of determination (R^2) were used to predict the strength of using the adsorbent for further experiments.

2.2.5 Determination of De-adhesion Concentration

A qualitative inhibitory effect of various concentration of solubilizing agents on *L. reuteri* CF48-3A, *L. gasseri* JV-VO3, *L. rhamnosus* LMS2-3A for 2.5 minutes to 30 minutes were determined for various concentrations of control (sterile distilled water), 12.5%, 25%, 50%,

75%, and 100%. Subsequently, a specific time of 4 hours was considered in the quantitative determination of the optimal de-adhesion concentration of *Lactobacillus* strains detached from the sterile solidified albumen.

2.2.6 Co-cultivation Assay

Co-cultivation assay in accordance with Lin *et al* (2025) was conducted in the absence and presence of some suppository bases. An equal concentration of *Lactobacillus* strains and *Escherichia coli* were incubated in sterile MRS broth, Mueller-Hinton (MH) broth and base in a ratio of 1:1:1. One hundred microliters from the individual tubes were subjected to 1:10 serial dilution at intervals of 4 h up to 48 h and plated in MRS Agar (supplemented with sorbic acid) and Eosin methylene blue (EMB) Agar. Control experiments were carried out with only *Escherichia coli* ATCC 25922. A regression of the survival against time (h) of the various combinations were plotted to compare various inactivation rate constant with the control.

2.2.7 Adhesion Assay

Adhesion Assay were conducted in the absence and presence of suppository bases. An equal concentration of *Lactobacillus* strains and *Escherichia coli* were incubated in MRS broth, MH broth and base in a ratio of 1:1:1 containing sterile solidified albumen. The solidified Albumen were aseptically withdrawn, rinsed twice in 10 mL phosphate buffer saline (PBS) and put in 5 mL Tween 20 (50%) for 10 minutes. One hundred microliters from the Tween 20 were subjected to 1:10 serial dilution and subsequently plated in MRS Agar (supplemented with sorbic acid) and Eosin methylene blue (EMB) Agar. Control experiments were determined with the *Escherichia coli*. A regression of the adhesion ($\log_{10}$ CFU/mL) against time (h) of the various combinations were plotted to compare various desorption rate constant with the control.

2.2.8 GC-MS Studies

2.2.8.1 Sample preparation

Comparative Gas chromatography- mass spectrometry (GC-MS) studies of various probiotic (*Lactobacillus* strains) and *Escherichia coli* were done in the presence and absence of suppository bases (Ibrahim *et al.,* 2021). The various individual and combined microorganisms were grown in the broth-base which consist of De man, Rogosa and sharpe (MRS), Mueller-Hinton (MH) and base in a ratio of 1:1:1. After 48 h, metabolites were separated using sequential extraction in Ethyl-acetate in a separating funnel. All samples were concentrated with the aid of a rotary evaporator and subjected to GC-MS analysis

2.2.8.2 **GC-MS analysis protocol**

GC–MS analysis was performed using a Shimadzu system comprising a GC-2010 gas chromatograph coupled to a GCMS-QP2010 single-quadrupole mass spectrometer (Shimadzu Corporation, Japan). Chromatographic separation was achieved on a fused-silica capillary column (e.g., DB-5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The ethyl acetate metabolic extract was reconstituted in ethyl acetate prior to analysis. An aliquot of 1.0 µL of the extract was injected in split mode (split ratio 10:1) at an injector temperature of 250°C. The oven temperature program was set from an initial temperature of 40 °C (held for 2 min), ramped at 10°C min^{-1} to 200°C, and held for 5 min at the final temperature. Mass spectrometric detection was performed in electron ionization (EI) mode at 70 eV. The ion source and interface temperatures were maintained at 230°C and 250°C, respectively. Data were acquired in full-scan mode over a mass range of m/z 40–300, with a solvent delay of 2.0 min. Metabolite identification was based on comparison of acquired mass spectra with those

in the NIST mass spectral library, as well as evaluation of retention times and characteristic fragment ions (Ibrahim *et al.,* 2021).

2.2.8.3 **Comparative GC-MS analysis evaluation**

The analysed broth without any organism was separately compared with: broth containing probiotic organism; broth containing pathogenic organism and broth containing both pathogenic and probiotic organism. The outcome of the GC-MS analysis was compared to identify constitutive, induced or toxic metabolites.

2.2.9 Data Analysis

All Data were discretely labeled and entered into SPSS version 27. Regression plot of various survival or adhesion in Log $_{10}$ CFU/mL against time (h) were determined. Inferential analysis was comparatively conducted using one away analysis of variance ANOVA and P values of < 0.05 were considered significant for the individual experiment.

CHAPTER THREE

3.0 RESULTS

3.1 Gram staining and biochemical test for *Lactobacillus* strains

Table 3.1 shows the Gram staining and some biochemical characteristics of *Lactobacillus* strains used for these studies: *Lactobacillus gasseri* JV-V03, *Lactobacillus reuteri* CF48-3A, and *Lactobacillus rhamnosus* LMS2-1.

Lactobacillus gasseri JV-V03, the organism is Gram-positive bacilli (GPB) but catalase-negative. It demonstrated a positive reaction for cellobiose, salicin, sucrose, lactose, maltose, D-fructose and D-glucose in carbohydrate fermentation. However, it was negative for raffinose, D-mannitol, L-rhamnose, D-xylose, and L-arabinose.

Lactobacillus reuteri CF48-3A exhibited GPB and catalase-negative nature. The carbohydrate utilization showed positive reactions for sucrose, raffinose, lactose, maltose, D-fructose, and D-glucose but negative for cellobiose, salicin, D-mannitol, L-rhamnose, D-xylose and L-arabinose in carbohydrate fermentation.

Lactobacillus rhamnosus LMS2-1 was Gram-positive but catalase-negative. This strain of *Lactobacillus* showed a positive reaction for cellobiose, salicin, sucrose, lactose, maltose, D-fructose, L-arabinose, D-glucose, and D-mannitol but negative for raffinose, L-rhamnose and D-xylose.

The three *Lactobacillus* strains can be differentiated based on their carbohydrate utilization profiles: *L. reuteri* CF48-3A is uniquely characterized by its ability to ferment raffinose while being unable to utilize cellobiose and salicin; *L. rhamnosus* LMS2-1 is distinguished by its capacity to ferment D-mannitol and L-arabinose; whereas *L. gasseri* JV-VO3 shares similarities with *L. rhamnosus* LMS2-1 in fermenting cellobiose and salicin but cannot utilize D-mannitol and L-arabinose, thereby serving as an intermediate profile between the two.

Table 3.1: Gram staining reaction and biochemical test of *Lactobacillus* strains.

Test	**Specific interactions**	***Lactobacillus gasseri* StrainJV-V03**	***Lactobacillus reuteri* Strain CF48-3A**	***Lactobacillus rhamnosus* Strain LMS2-1**
Gram staining	Primary and Secondary Dyes	GPB	GPB	GPB
Catalase test	Hydrogen peroxide	**-**	**-**	**-**

Biochemical Characteristics	Cellobiose	+	-	+
	Salicin	+	-	+
	Sucrose	+	+	+
	Raffinose	-	+	-
	Lactose	+	+	+
	Maltose	+	+	+
	D-Fructose	+	+	+
	D-Glucose	+	+	+
	D-Mannitol	-	-	+
	L-Rhamnose	-	-	-
	D-Xylose	-	-	-
	L-Arabinose	-	-	+

Key
GPB: Gram -positive bacilli
+:Positive
-:Negative

3.2 Gram staining and biochemical test for *Escherichia coli*

Table 3.2 shows the Gram staining reaction and biochemical characteristics of *Escherichia coli* ATCC 25922 used in this experiment. The microorganism was identified as a Gram-negative bacillus (GNB) based and catalase-positive. It demonstrated a positive reaction for cellobiose, salicin, maltose, lactose, D-fructose, D-glucose, D-mannitol, L-rhamnose, D-

xylose, and L-arabinose, but negative for sucrose and raffinose. The other tests showed that the organism was methyl red (MR) positive and Voges-Proskauer (VP) negative. It was also negative for citrate utilization and the urease test. The biochemical profile of *Escherichia coli* ATCC 25922 is characterized by Gram-negative rod morphology, catalase positivity, extensive carbohydrate fermentation (with select exclusions), and a distinct MR-positive/VP-negative pattern. These features are consistent with standard identification criteria for *E. coli* and support its use as a reference strain in microbiological studies.

Table 3.2 : Gram staining reaction and biochemical test of *Escherichia coli* ATCC 25922

Test	**Specific interactions**	***Escherichia coli ATCC 25922***
Gram staining	Primary and Secondary Dyes	GNB
Catalase test	Hydrogen peroxide	+

Biochemical Characteristics	Cellobiose	+
	Salicin	+
	Sucrose	-
	Raffinose	-
	Lactose	+
	Maltose	+
	D-Fructose	+
	D-Glucose	+
	D-Mannitol	+
	L-Rhamnose	+
	D-Xylose	+
	L-Arabinose	+
Other Tests	Methyl Red (MR)	+
	Voges-Proskauer (VP)	-
	Citrate	-
	Urease	-

Key
GNB: Gram-negative bacilli.
+: Positive
-: Negative

3.3 Antibacterial Studies results of *Lactobacillus* strains on *Escherichia coli*

Table 3.3 is the outcomes of the antibacterial activities of *Lactobacillus gasseri* JV-V03, *Lactobacillus reuteri* CF48-3A, and *Lactobacillus rhamnosus* LMS2-1 against *Escherichia coli* ATCC 25922. The *Lactobacillus* strains showed an inhibitory zone diameter of: *L.*

gasseri JV-V03 (30.8 mm); *L. reuteri* CF48-3A (30.8 mm) and *L. rhamnosus* LMS2-1 (31.5 mm). They all exhibited bacteriostatic effect on *E. coli* ATCC 25922. The bactericide determination against *E. coli* ATCC 25922 showed growth for *L. gasseri* JV-V03; *L. reuteri* CF48-3A and *L. rhamnosus* LMS2-1.

Overall, the results indicate that while all three *Lactobacillus* strains possess strong bacteriostatic properties, *L. rhamnosus* LMS2-1 exhibits the highest level of inhibition. However, none of the strains demonstrated bactericidal activity, highlighting their role in suppressing rather than eliminating bacterial growth.

Table 3.3: Antibacterial effects of *Lactobacillus* strains.

Organisms	**Bacteriostatic (mm)**	**Bactericidal**
***L.gasseri* JV-V03**	30.8 mm	Growth

***L.reuteri* CF48-3A**	30.8 mm	Growth
***L.rhamnosus* LMS2-1**	31.5 mm	Growth

3.4 Adsorbent studies

3.4.1 Preparation of Adsorbent

As shown in Table 3.4, the adsorbent designed and prepared was an opaque white solidified albumen. The average weight was 1.86g with a calculated cylindrical surface area of 817.15 mm^2. The solidified albumen was stable to the temperature and pressure of an autoclave. Overall, the solidified albumen showed typical physical properties of denatured protein material, with stable structural and visual characteristics suitable for experimental use.

Table 3.4: Measurements of solidified Albumen.

Characteristics	Measurement/observation
Weight	1.86 g
Surface area	817.15 mm^2
Colour = Cream	Opaque white
Stability	Thermostable

3.4.2 Linearity study of *L. gasseri* JV-VO3 on solidified albumen

Figure 3.1 shows the adhesion of *Lactobacillus gasseri* JV-VO3 on solidified albumen. The scatter plot is indicative of the relationship between *L. gasseri* JV-VO3 concentrations and adhesion on solidified albumen. The x-axis represents the concentration (Log_{10} CFU/mL) while the y-axis represents adhesion (Log_{10} CFU/mL). The blue lines indicate the adhesion at a given concentration of the *Lactobacillus*. Each blue dot corresponds to an experimental observation of adhesion at a given *L. gasseri* JV-VO3 concentration. Linear regression analysis showed adhesion was a significant predicator of concentration. The regression equation was y=1.79+0.71x, indicating adhesion increased by 0.71 units for every unit increase in concentration. A very strong positive relationship was observed between concentration and adhesion (r = 0.910). The coefficient of determination was very high (R^2 = 0.828), indicating that 83% of the variation in survival was explained by plot. The relationship was statistically significant ($p < 0.001$)

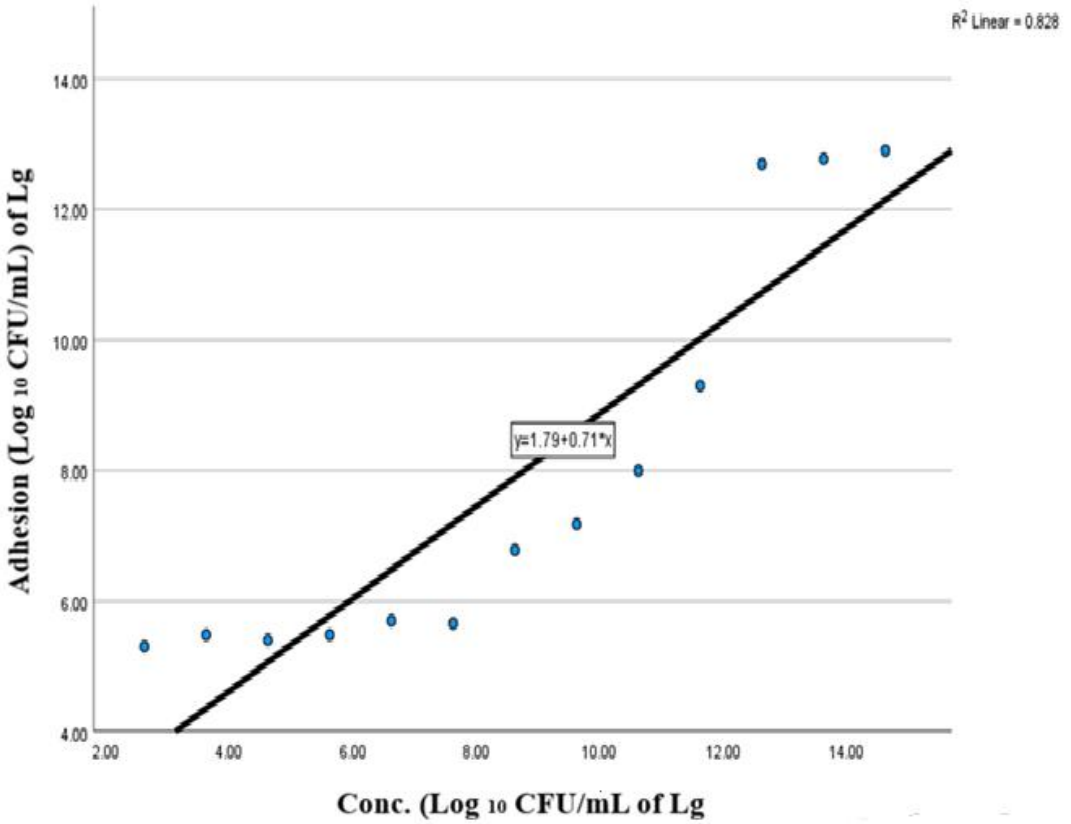

Figure 3.1: Adhesion study of *Lactobacillus gasseri* JV-VO3 on solidified Albumen. (r = 0.910; p < 0.001)

3.4.3 Linearity study of *Lactobacillus reuteri* CF48-3A on solidified albumen

Figure 3.2 shows the adhesion of *Lactobacillus reuteri* CF48-3A on solidified albumen. The scatter plot is indicative of the relationship between *L. reuteri* CF48-3A concentrations and adhesion on solidified albumen. The x-axis represents the concentration in Log_{10} CFU/mL while, the y-axis represents adhesion in Log_{10} CFU/mL. The blue lines indicate the adhesion at a given concentration of the *Lactobacillus*. The blue dot aligns to an experimental observation of adhesion at a given *L. reuteri* CF48-3A concentration. Linear regression analysis showed concentration was a significant predicator of adhesion. The regression equation was $y=3.59+0.71x$, indicating adhesion increased by 0.71 units for every unit increase in concentration. A very strong positive relationship was observed between concentration and adhesion ($r = 0.959$). The coefficient of determination was very high ($R^2 = 0.919$) indicating that 92% of the variation in survival was explained by time. The relationship was statistically significant at $p < 0.001$.

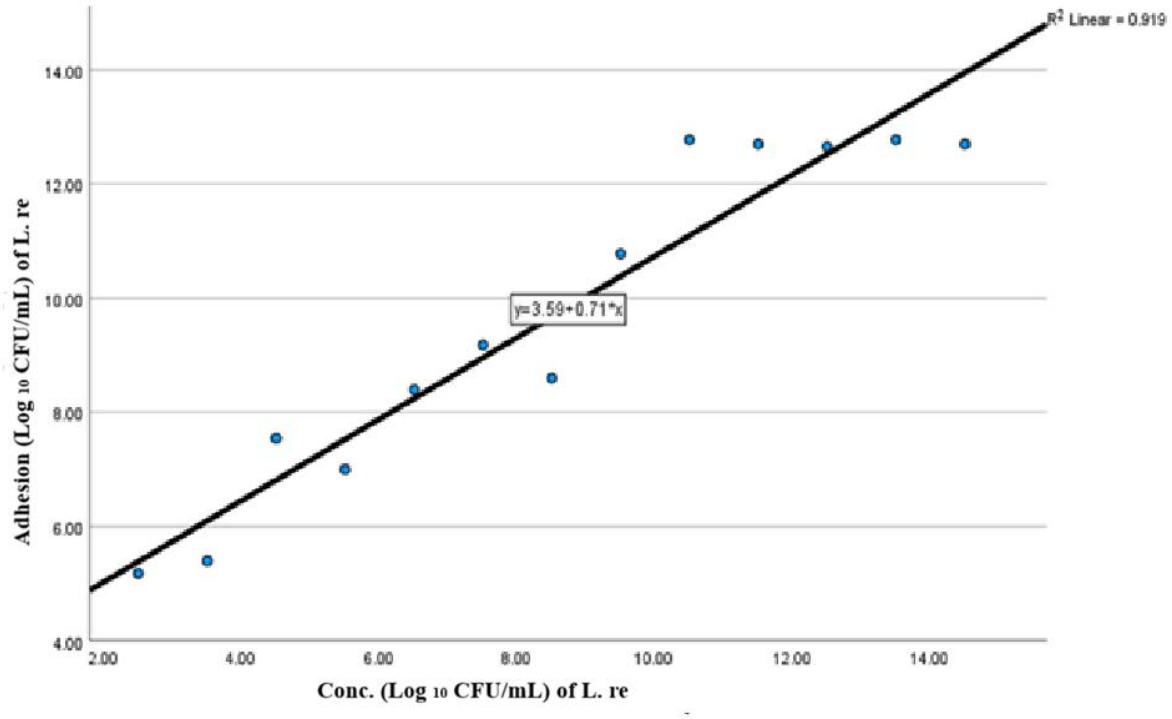

Figure 3.2: Adhesion study of *Lactobacillus reuteri* CF48-3A on solidified Albumen (r = 0.959; p < 0.001)

3.4.4 Linearity study of *Lactobacillus rhamnosus* LMS2-1 on solidified albumen

The figure 3.3 is showing the adhesion of *L. rhamnosus* LMS2-1 on solidified albumen. The scatter plot is indicative of the interaction between *L. rhamnosus* LMS2-1 concentrations and the adhesion on solidified albumen. The x-axis represents the concentration converted to Log_{10} CFU/mL. While, the y-axis represents adhesion converted to Log_{10} CFU/mL. The blue lines indicate the adhesion at a specific concentration of the *Lactobacillus*. The blue dot aligns to an experimental observation of adhesion at a given *L. rhamnosus* LMS2-3A concentration. Linear regression analysis showed concentration was a significant predicator of adhesion. The regression equation was y=1.38+0.69x, indicating adhesion increased by a 0.69 units for every unit increase in concentration. A very strong positive relationship was observed between concentration and adhesion (r = 0.970). The coefficient of determination was very high (R^2 = 0.940) indicating that 94% of the variation in survival was explained by the plot. The relationship was statistically significant as shown by $p < 0.001$.

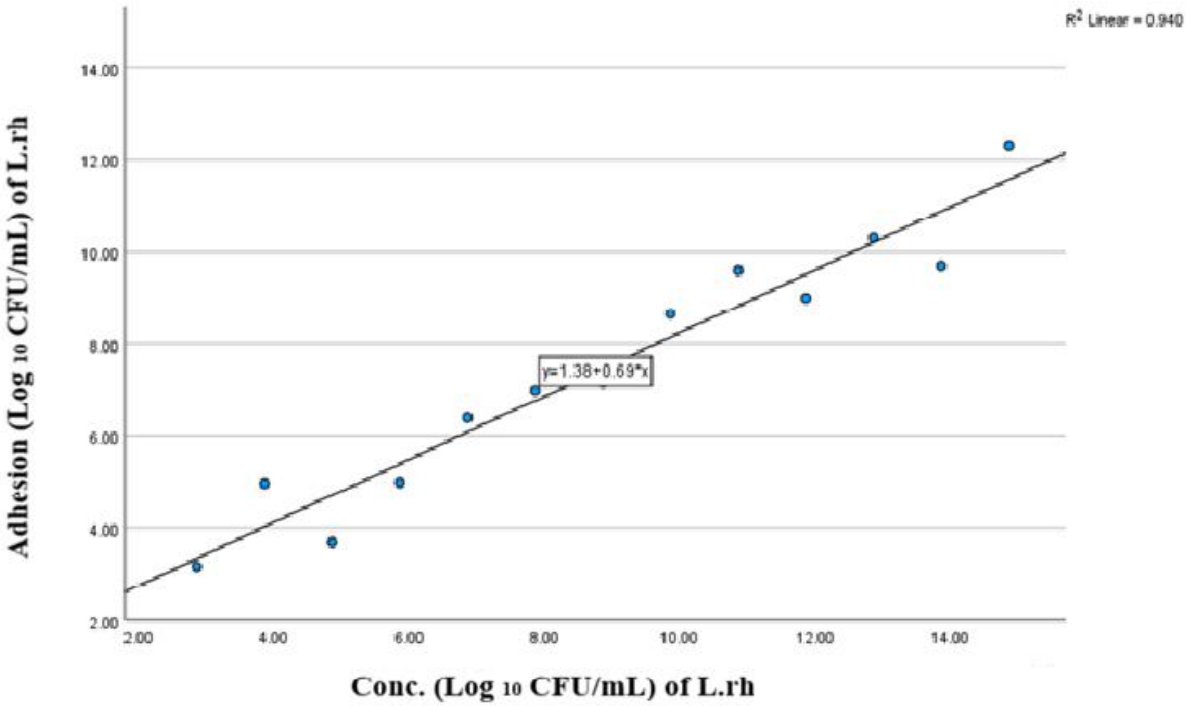

Figure 3.3: Adhesion study of *Lactobacillus rhamnoses* LMS2-1 on solidified Albumen ($r = 0.970$; $p < 0.001$)

3.4.5 Linearity study of *Escherichia coli* ATCC 25922 on solidified albumen

Figure 3.4 shows the adhesion of *E.coli* ATCC 25922 on solidified albumen. The scatter plot is indicative of the relationship between *E.coli* ATCC 25922 concentrations and adhesion on solidified albumen. The horizontal axis (x-axis) represents the concentration in Log_{10} CFU/mL, and the y-axis represents adhesion in Log_{10} CFU/mL. The blue lines indicate the adhesion at a given concentration. The blue dot aligns with an experimental observation of adhesion at a given *Escherichia coli* ATCC 25922 concentration. Linear regression analysis showed concentration was a significant predicator of adhesion. The regression equation was $y = 0.9+0.72x$, indicating adhesion increased by 0.72 units for every unit increase in concentration. A very strong positive relationship was observed between concentration and adhesion ($r = 0.978$). The coefficient of determination was very high ($R^2 = 0.957$), indicating that 95% of the variation in survival was explained by the plot. The relationship was statistically significant at $p < 0.001$.

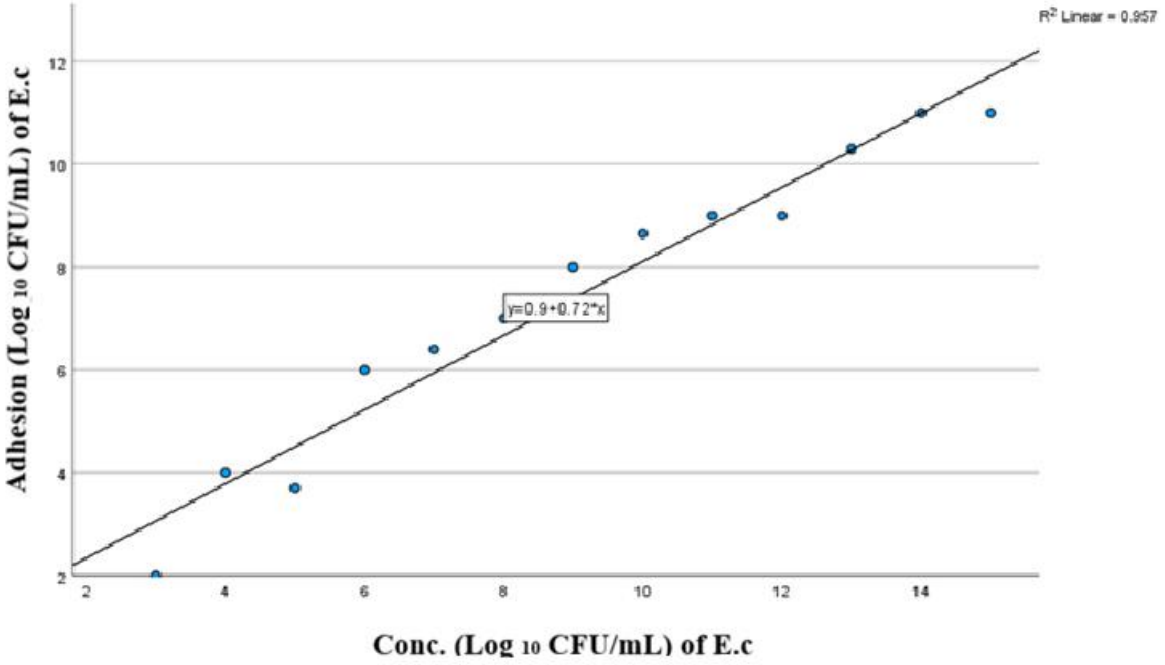

Figure 3.4: Adhesion study of *Escherichia coli* ATCC 25922 on solidified Albumen (r = 0.978; p < 0.001)

3.5 Determination of de-adhesion concentration of Tween 20

Figure 3.5 is a horizontal bar chart that presents the impact of increasing concentrations of Tween 20 as a solubilizing agent on the de-adhesion of *Lactobacillus gasseri* JV-V03, *Lactobacillus reuteri* CF48-3A, and *Lactobacillus rhamnosus* LMS2-1. At the highest concentration (100), *L. rhamnosus* exhibited the greatest de-adhesion (5.77 $\log_{10}$ CFU/mL), followed by *L. gasseri* (5.26 $\log_{10}$ CFU/mL), while *L. reuteri* showed the lowest count (4.50 $\log_{10}$ CFU/mL). At 75% concentration, *L. reuteri* CF48-3A demonstrated a marked increase in de-adhesion (8.74 $\log_{10}$ CFU/mL), surpassing both *L. rhamnosus* LMS2-1 (6.83 $\log_{10}$ CFU/mL) and *L. gasseri JV-VO3* (5.82 $\log_{10}$ CFU/mL), indicating a strain-specific response at this level. At a 50% concentration, all three strains showed peak growth values, with *L. rhamnosus* LMS2-1 (9.68 $\log_{10}$ CFU/mL), *L. reuteri* CF48-3A (9.45 $\log_{10}$ CFU/mL), and *L. gasseri* JV-V03 (9.43 $\log_{10}$ CFU/mL), suggesting that this concentration provides optimal conditions for proliferation. At 25% concentration, *L. rhamnosus* LMS2-1maintained relatively high de-adhesion (8.62 $\log_{10}$ CFU/mL), whereas *L. gasseri* JV-V03 (4.82 $\log_{10}$ CFU/mL) and *L. reuteri* CF48-3A (5.26 $\log_{10}$ CFU/mL) showed a decline, indicating reduced removal at lower concentrations. At the lowest concentration 12.5%, *L. rhamnosus* LMS2-1 still demonstrated comparatively de-adhesion (8.26 $\log_{10}$ CFU/mL), while *L. gasseri* JV-VO3 (5.62 $\log_{10}$ CFU/mL) and *L. reuteri* CF48-3A (5.54 $\log_{10}$ CFU/mL) remained at moderate levels. Notably, all strains achieved maximum de-adhesion at 50% concentration, suggesting this as the optimal condition for detachment of these *Lactobacillus* strains. Tween 20 concentration was identified as a significant factor influencing bacterial recovery ($p < 0.05$). In contrast, *Lactobacillus* strains showed no statistically significant differences in their response ($p > 0.05$), indicating that the effect of Tween 20 was consistent across strains. These findings suggest that optimization of Tween 20 concentration is more critical than strain-specific variation in enhancing *Lactobacillus* recovery.

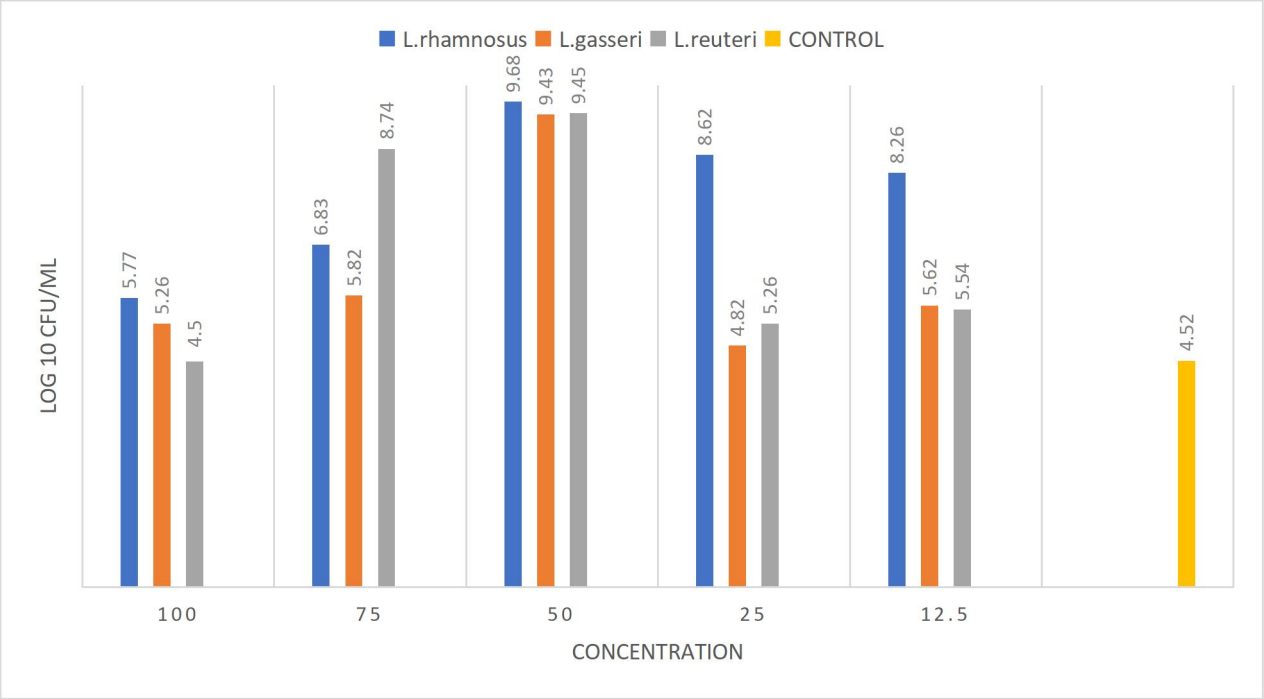

Figure 3.5: De-adhesion effect of Tween 20 on some *Lactobacillus* strains (Concentration: $p < 0.05$; *Lactobacillus* strains: $p > 0.05$)

3.6 Co-cultivation assays

3.6.1 Cocultivation assay of *Lactobacillus* Strains on *Escherichia coli* ATCC *25922*

Figure 3.6 is the outcome of the linear regression analysis for the cocultivation assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922.

Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of survival. The regression equation was y = 6.6+0.21x, indicating survival increased by 0.21 units for every unit increase in time. A strong positive correlation was displayed for the survival-time plot (r = 1.000).

Linear regression analysis of the effect of *L. reuteri* CF48-3A showed time was a significant predicator of survival. The regression equation was y=6.93-0.16x, indicating survival decreased by 0.16 units for every unit increase in time. A moderate negative relationship was displayed for the survival-time plot (r = - 0.590).

Linear regression analysis of the effect of *L. gasseri* JV-V03 showed time was a significant predicator of survival. The regression equation was y = 4.54+0.17x, indicating survival increased by 0.17 units for every unit increase in time. A strong positive relationship was observed between time and survival (r = 0.752)

Linear regression analysis of the effect of *L. rhamnosus* LMS2-1 showed that time was a significant predicator of survival. The regression equation was y = 5.97+0.02x, indicating survival increased by 0.22 units for every unit increase in time. A moderate positive relationship was observed between time and survival (r = 0.580).

The relationship was statistically significant with a p-value of $p < 0.001$ within groups.

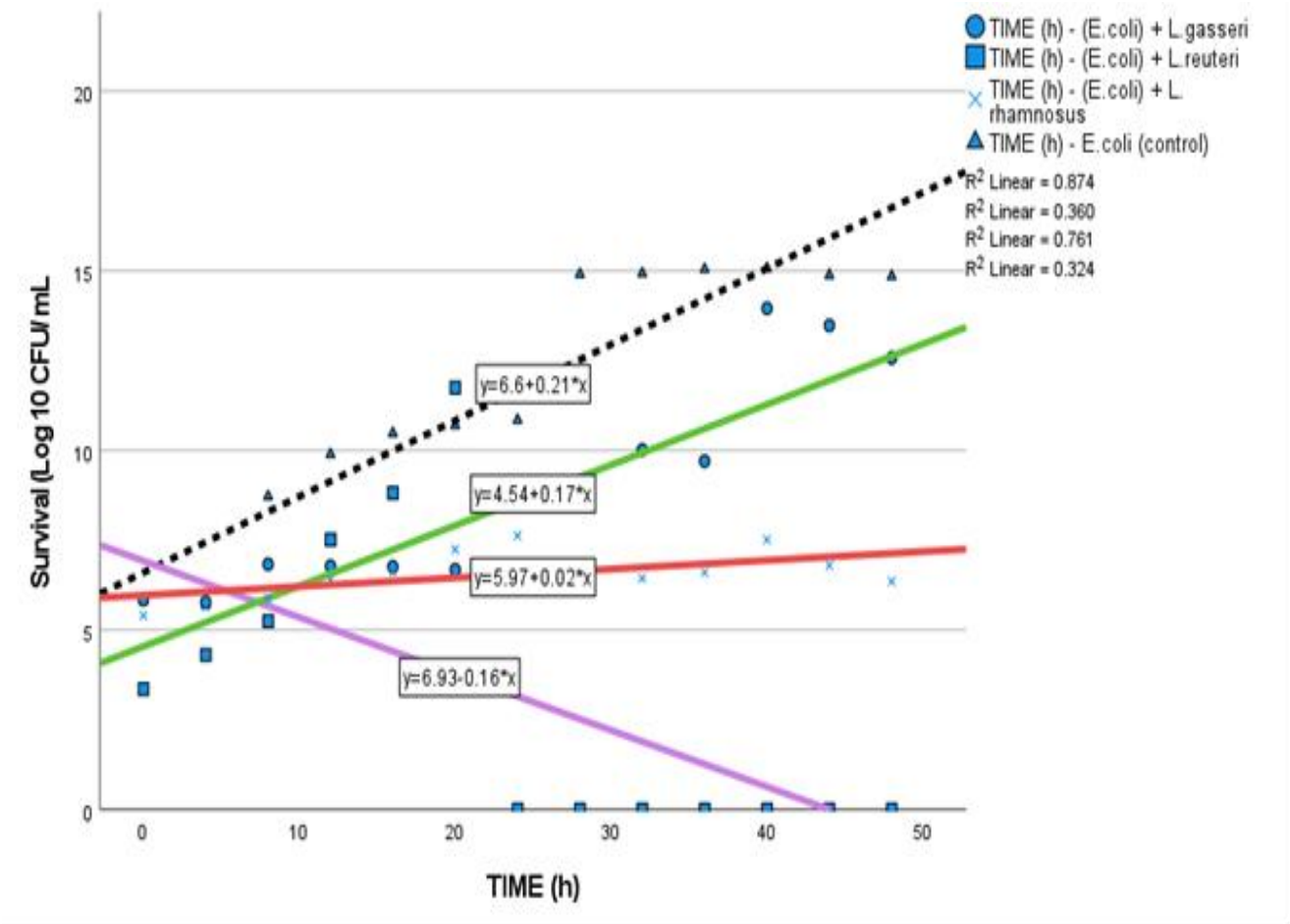

Figure 3.6: Antibacterial effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922 ($p < 0.001$)

Key
Dotted line: *E.coli* ATCC 25922 (r = 1.000)
Purple line: *E.coli* ATCC 25922 + *L.reuteri* CF48-3A (r = - 0.590)
Green line: *E.coli* ATCC 25922 + *L.gasseri* JV-V03 (r = 0.752)
Red line: *E.coli* ATCC 25922 + *L. rhamnosus* LMS2-1 (r = 0.580)

3.6.2 Cocultivation assay of *Lactobacillus* Strains on *Escherichia coli* ATCC 25922 in the presence of Glycerogelatin base

Figure 3.7 is the outcome of the linear regression analysis for the cocultivation assay of *Lactobacillus reuteri* CF48-3A *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922 in the presence of glycerogelatin base. Linear regression analysis of the control (*E.coli* ATCC 25922) showed time was a significant predicator of survival. The regression equation was y = 6.6 + 0.21x, indicating y increased by 0.21 units for every unit increase in time. A perfect positive correlation was observed between time and survival (r = 1.000). Linear regression analysis of the effect of *L. reuteri* CF48-3A showed time was a significant predicator of survival. The regression equation was y = 3.39 + 7.6E-3x, indicating survival decreased by 0.0076 units for every unit increase in time. A weak positive linear relationship was observed between time and survival (r = 0.479). Linear regression analysis of the effect of *L. gasseri* JV-V03 showed time was a significant predicator of survival. The regression equation was y = 3.21+2.53E-3x, indicating survival increased by 0.00253 units for every unit increase in time. A weak positive linear relationship was observed between time and survival (r = 0.315). Linear regression analysis of the effect of *L. rhamnosus* LMS2-1 showed that time was a significant predicator of survival. The regression equation was y = 3.45+1.72E-3x, indicating survival increased by 0.00172units for every unit increase in time. A very weak positive linear relationship was observed between time and survival (r = 0.220).

The Analysis revealed a statistically significant difference within groups ($p < 0.001$).

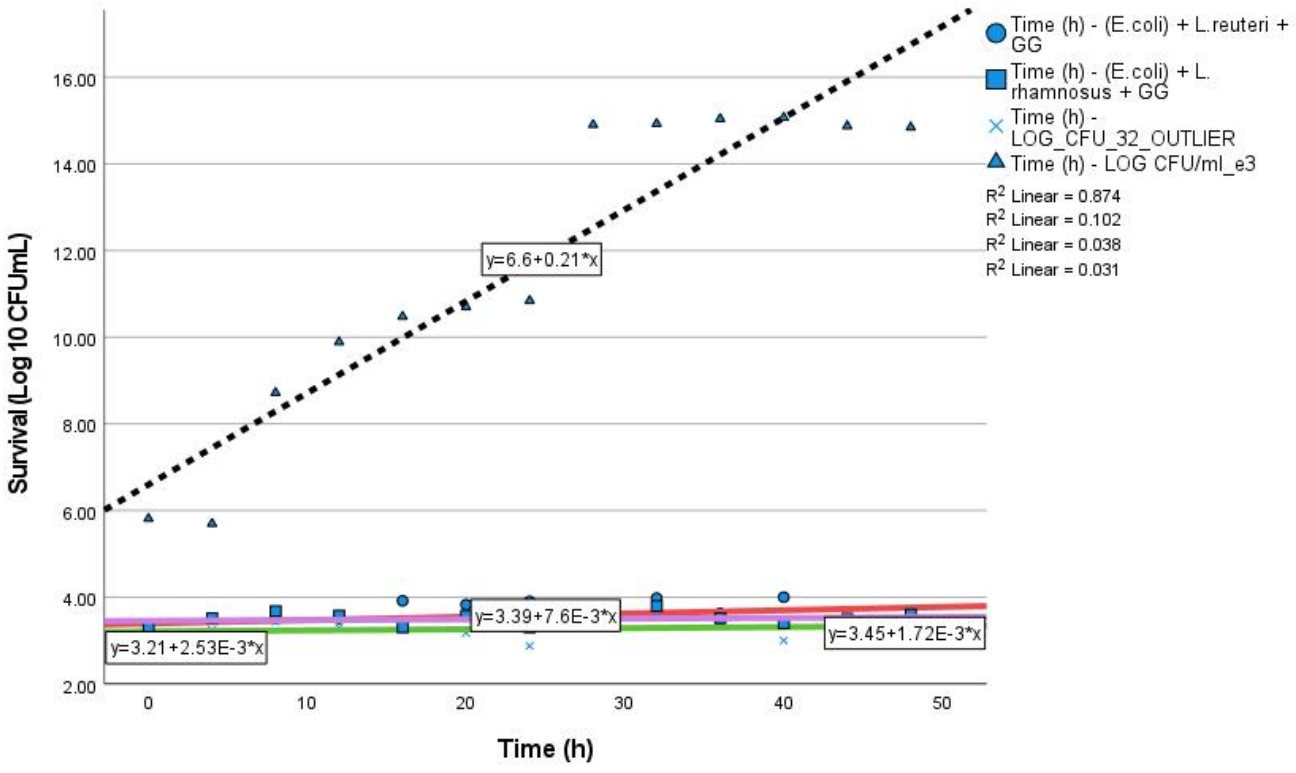

Figure 3.7: Effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of Glycerogelatin Base. ($p < 0.001$)

Key
Dotted line: *E.coli* ATCC 25922 (r =1.000)
Purple line: *E.coli* ATCC 25922 + *L.reuteri* CF48-3A (r = 0.479)
Green line: *E.coli* ATCC 25922 + *L.gasseri* JV-V03 (r = 0.315)
Red line: *E.coli* ATCC 25922 + L. *rhamnosus* LMS2-1 (r = 0.220)

3.6.3 Cocultivation assay of *Lactobacillus* Strains on *Escherichia coli* ATCC 25922 in the presence of Polyethylene glycol base

Figure 3.8 is the outcome of the linear regression analysis for the cocultivation assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922 in the presence of polyethylene glycol base. Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of survival. The regression equation was y = 6.6+0.21x, indicating survival increased by 0.21 units for every unit increase in time. A perfect positive correlation was observed between time and survival (r = 1.000). Linear regression analysis of the effect of *L. reuteri* CF48-3A showed time was a significant predicator of survival. The regression equation was y = 4.23-0.1x, indicating survival decreased by 0.1 units for every unit increase in time. A moderate negative relationship was displayed between the survival-time plot (r = -0.523). Linear regression analysis of the effect of *L. gasseri* JV-V03 showed time was a significant predicator of survival. The regression equation was y = 2.57+9.18E-3x, indicating survival increased by 0.00918 units for every unit increase in time. A very weak positive relationship was observed between time and survival (r = 0.192). Linear regression analysis of the effect of *L. rhamnosus* LMS2-1 showed time was a significant predicator of survival. The regression equation was y = 4.46-3E-3x, indicating survival decreased by -0.003units for every unit increase in time. A very weak negative relationship was observed between time and survival (r = -0.114). The analysis revealed a statistically significant difference within groups (p = 0.001)

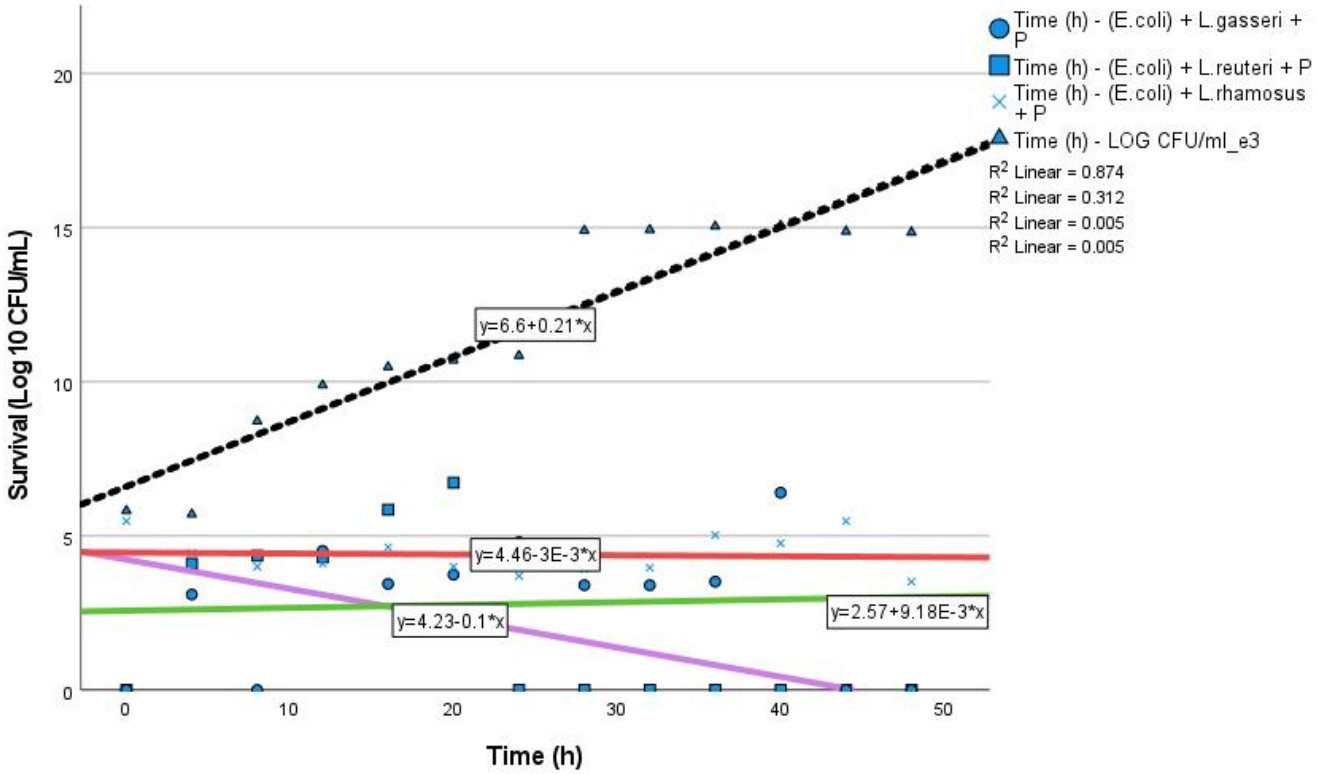

Figure 3.8: Effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of polyethylene glycol base. (p = 0.001)

Key
Dotted line: *E.coli* ATCC 25922 (r = 1.000)
Purple line: *E.coli* ATCC 25922 + *L.reuteri* CF48-3A (r = - 0.523)
Green line: *E.coli* ATCC 25922 + *L.gasseri* JV-V03 (r = 0.192)
Red line: *E.coli* ATCC 25922 + *L. rhamnosus* LMS2-1 (r = - 0.114)

3.6.4 Cocultivation assay of *Lactobacillus* Strains on *Escherichia coli* ATCC 25922 in the presence of Theobroma base

Figure 3.9 is the outcome of the linear regression analysis for the cocultivation assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922 in the presence of theobroma base

Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of survival. The regression equation was y = 6.6+0.21x, indicating survival increased by 0.21 units for every unit increase in time. A perfect positive correlation was observed between time and survival (r = 1.000). Linear regression analysis of the effect of *L.reuteri* CF48-3A showed time was a significant predicator of survival. The regression equation was y = 5.13+0.26x, indicating survival decreased by 0.26 units for every unit increase in time. A very strong positive relationship was observed between time and survival (r = 0.899). Linear regression analysis of the effect of *L. gasseri* JV-V03 showed time was a significant predicator of survival. The regression equation was y = 3.61+0.28x, indicating survival increased by 0.28 units for every unit increase in time. A very strong positive relationship was observed between x and y (r = 0.932). Linear regression analysis of the effect of *L. rhamnosus* LMS2-1 showed time was a significant predicator of time. The regression equation was y=5.18+0.25x, indicating survival decreased by 0.25 units for every unit increase in time. A strong positive relationship was observed between time and survival (r = 0.892). There was no statistically significant association within the groups (p = 0.879).

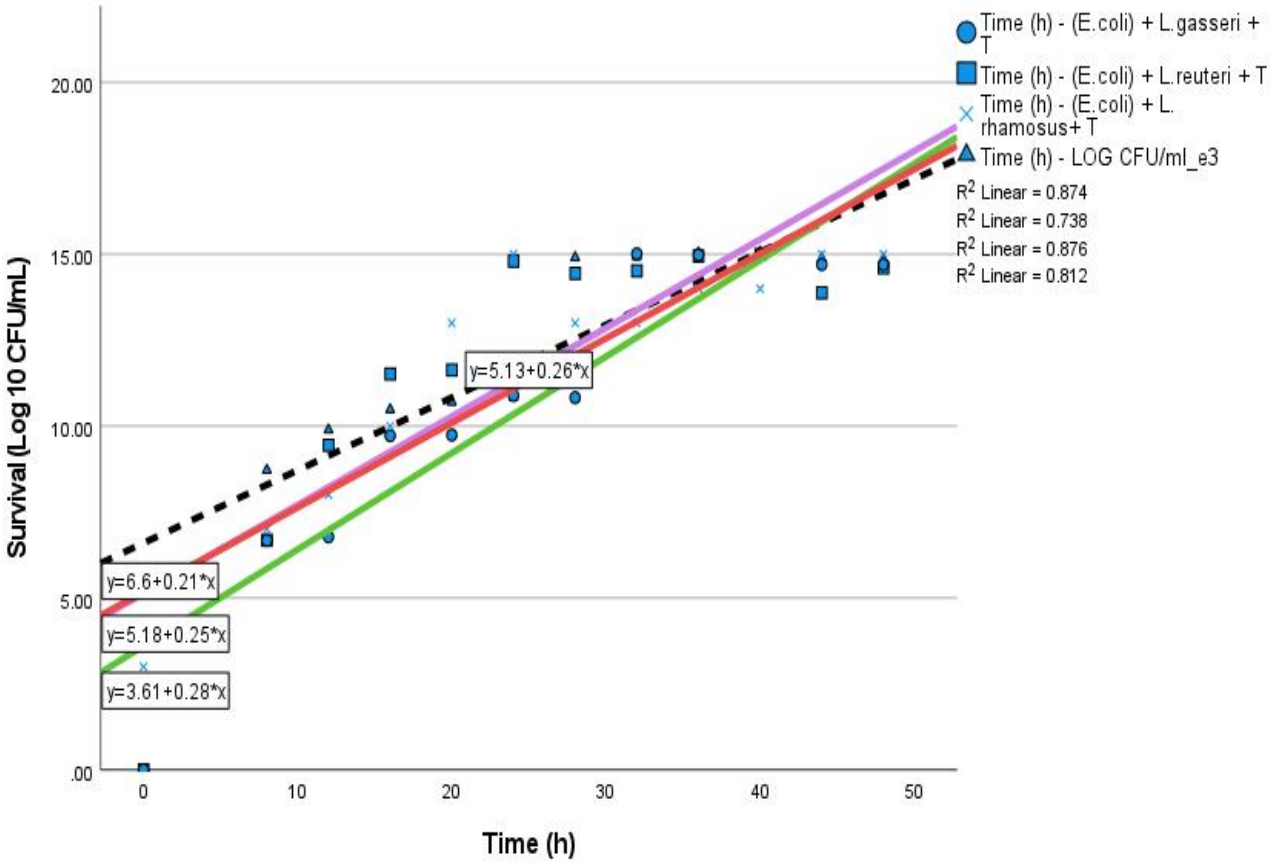

Figure 3.9: Effect of *Lactobacillus strains* on *Escherichia coli* ATCC 25922 in the presence of Theobroma base (p = 0.879)

Key
Dotted line: *E.coli* ATCC 25922 (r = 1.000)
Purple line: *E.coli* ATCC 25922 + *L.reuteri* CF48-3A (r = 0.899)
Green line: *E.coli* ATCC 25922 + *L.gasseri* JV-V03 (r = 0.932)
Red line: *E.coli* ATCC 25922 + *L. rhamnosus* LMS2-1 (r = 0.892)

3.7 Survival of *Lactobacillus* strains in suppository bases

3.7.1 Survival of *Lactobacillus* strains in glycerogelatin bases

Figure 3.10 was the survival pattern of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 in the presence of glycerogelatin base.

L. rhamnosus LMS2-1 and *L. gasseri* JV-V03 survived throughout the entire period, from 0 to 48 hours, maintaining viability across all time points. *L. reuteri* CF48-3A showed survival from 0 to approximately 40 hours, after which there was a sharp decline to 0 Log $_{10}$ CFU/mL, indicating loss of viability at later time points. In summary, both *L. rhamnosus* LMS2-1 and *L. gasseri* JV-V03 exhibited full-time survival during the duration of the experiment. Whereas L. *reuteri* CF48-3A had a shorter survival window due to a complete loss of viability toward the end of the experiment in glycerogelatin base. The survival within groups was significant ($p = 0.001$)

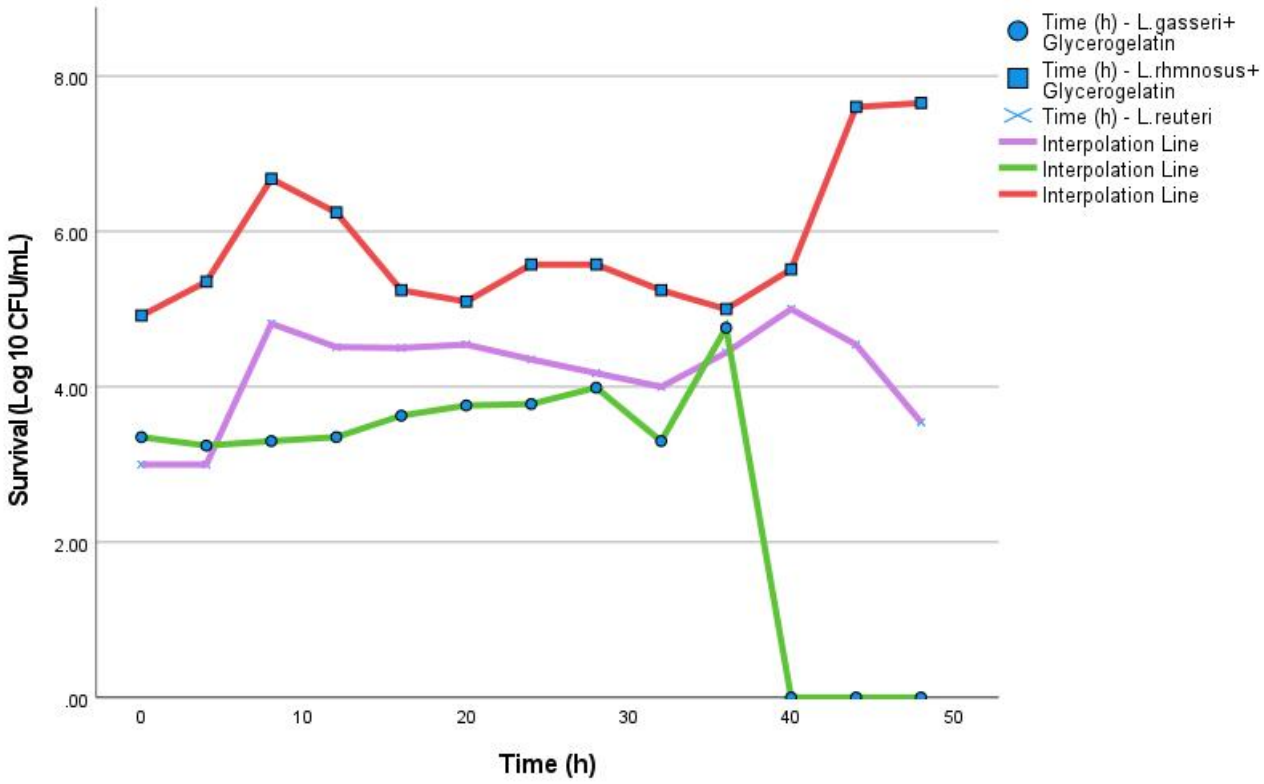

Figure 3.10: Survival of *Lactobacillus* strains in the presence of Glycerogelatin Base. (p = 0.001).

Key
Dotted line*: E.coli* ATCC 25922
Purple line*: L.reuteri* CF48-3A
Green line: *L.gasseri* JV-V03
Red line: *L. rhamnosus* LMS2-1

3.7.2 Survival of *Lactobacillus* strains in polyethylene glycol bases

Figure 3.11 was the survival pattern of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 in the presence of polyethylene glycol base. *L. reuteri* CF48-3A *L. gasseri* JV-V03, and *L. rhamnosus* LMS2-1 survived between 0-48h in the presence of polyethylene glycol base. The survival within groups was not significant ($p = 0.566$). Ultimately, the three strains exhibited complete survival within the time range of the experiment in the presence of polyethylene glycol.

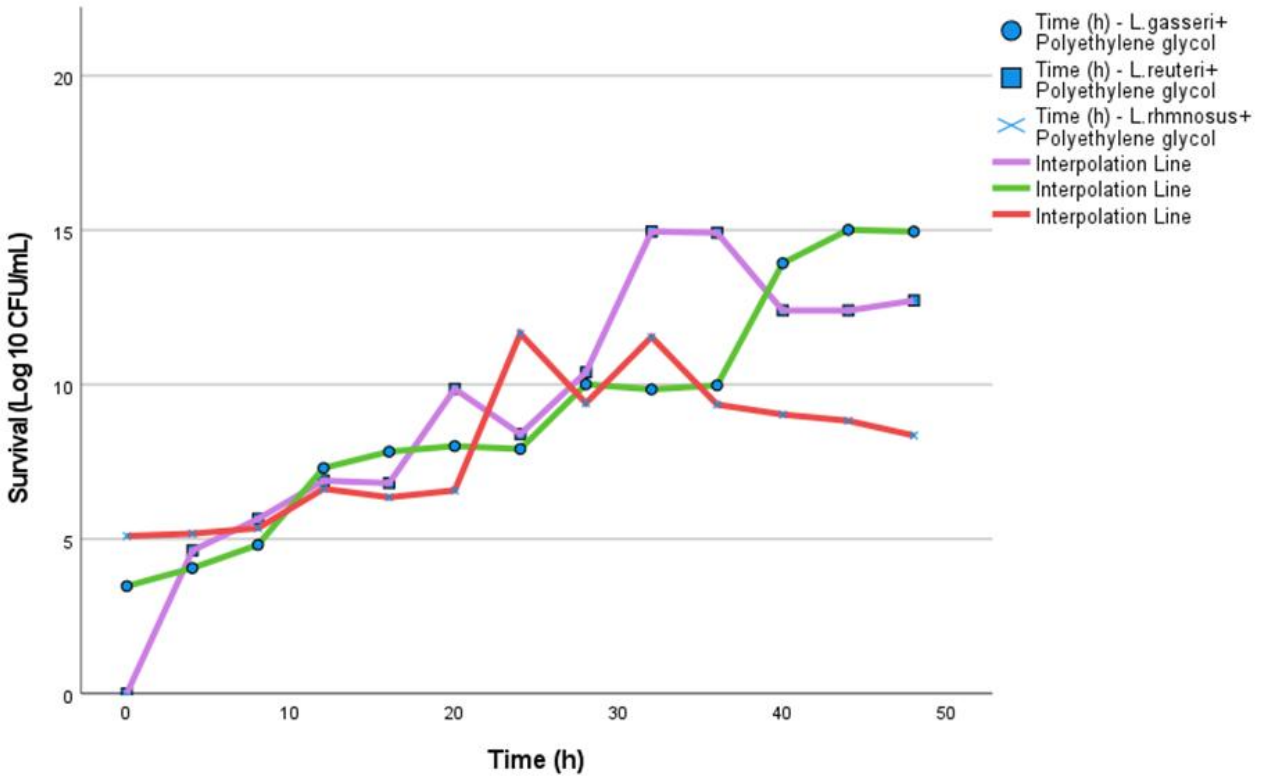

Figure 3.11: Survival of *Lactobacillus* strains in the presence of Polyethylene glycol base. (p = 0.566)

Key
Dotted line*: E.coli* ATCC 25922
Purple line: *L.reuteri* CF48-3A
Green line: *L.gasseri* JV-V03
Red line: *L. rhamnosus* LMS2-1

3.7.3 Survival of *Lactobacillus* strains in Theobroma bases

Figure 3.12 was the survival pattern of *Lactobacillus reuteri* CF48-3A *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 in the presence of Theobroma base. The survival in Log $_{10}$ CFU/mL over time showed that *L. reuteri* CF48-3A *L. gasseri* JV-V03, and *L. rhamnosus* LMS2-1 survived between 0-48h in the presence of Theobroma base. The survival within groups was not significant ($p = 0.614$). In summary, the three strains exhibited complete survival during the experiment in the presence of Theobroma base.

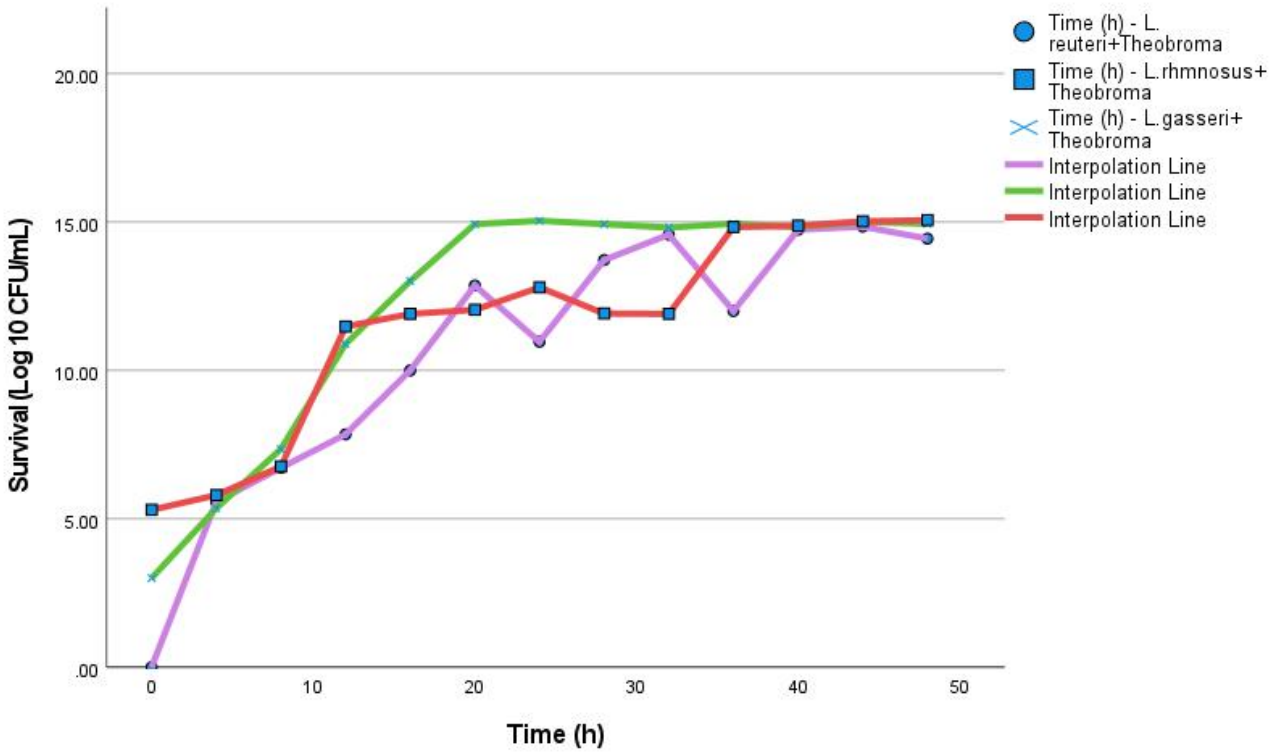

Figure 3.12: Survival of *Lactobacillus* strains in the presence of Theobroma base. (p = 0.614).

Key
Dotted line: *E.coli* ATCC 25922
Purple line: *L.reuteri* CF48-3A
Green line: *L.gasseri* JV-V03
Red line: *L. rhamnosus* LMS2-1

3.8 Adhesion Assays

3.8.1 Adhesion assay of *Lactobacillus Strains* on *Escherichia coli* ATCC 25922

Figure 3.13 is the outcome of the linear regression analysis for the Adhesion assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922.

Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of adhesion. The regression equation was y = 3.59+0.08x, indicating adhesion increased by 0.08 units for every unit increase in time. A perfect positive correlation was observed between time and adhesion (r = 1.000).

Linear regression analysis of the effect of *L. reuteri* CF48-3A showed that time was a significant predicator of adhesion. The regression equation was y = 4.74+0.03x, indicating adhesion decreased by 0.03 units for every unit increase in time. A strong positive relationship was observed between time and adhesion (r = 0.700)

Linear regression analysis of the effect of *L. gasseri* JV-V03 showed time was a significant predicator of adhesion. The regression equation was y = 3.56+0.09x, indicating adhesion increased by 0.09 units for every unit increase in time. A strong positive relationship was observed between time and adhesion (r = 0.788).

Linear regression analysis of the effect of *L. rhamnosus* LMS2-1 showed time was a significant predicator of adhesion. The regression equation was y = 3.99+6.24E-3x, indicating adhesion decreased by 0.00624 units for every unit increase in time. A weak positive relationship was observed between time and adhesion (r = 0.474).

The observed relationship was statistically non-significant within groups (p = 0.096)

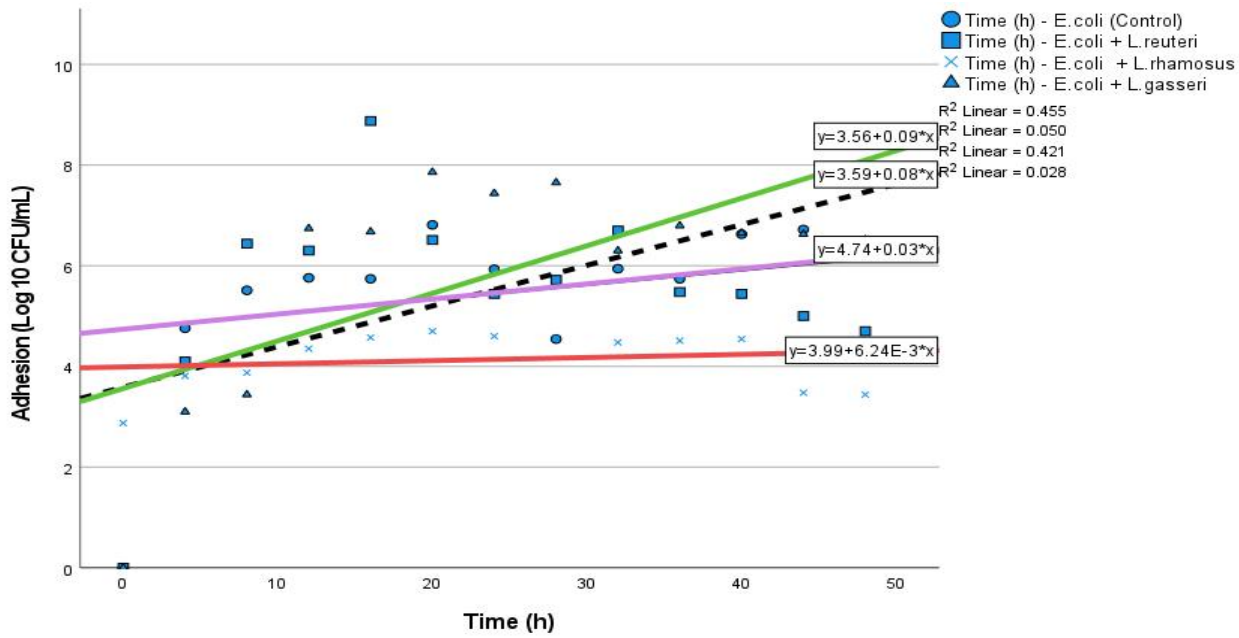

Figure 3.13: Showing the effect of *Lactobacillus* strains on the adhesion of *Escherichia coli* 25922 (p = 0.096)

Dotted line: *E.coli* ATCC 25922 (r = 1.000)
Purple line: *E.coli* ATCC 25922 + *L.reuteri* CF48-3A (r = 0.700)
Green line: *E.coli* ATCC 25922 + *L.gasseri* JV-V03 (r = 0.788)
Red line: *E.coli* ATCC 25922 + *L. rhamnosus* LMS2-1 (r = 0.474)

3.8.2 Adhesion assay of *Lactobacillus* Strains on *Escherichia coli* ATCC 25922 in the presence of Glycerogelatin base.

Figure 3.14 shows the linear regression analysis for the Adhesion assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922 in the presence of glycerogelatin base. Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of adhesion. The regression equation was y = 3.59 + 0.08x, indicating adhesion increased by 0.08 units for every unit increase in time. A perfect positive correlation was observed between time and adhesion (r = 1.000). Linear regression analysis of the effect of *L. reuteri* CF48-3A showed time was a significant predicator of adhesion. The regression equation was y = 0.56-0.01x, indicating adhesion decreased by 0.01 units for every unit increase in time. There was no linear relationship observed between time and adhesion (r = -0.002). Linear regression analysis of the effect of both *L. gasseri* JV-V03 and *L. rhamnosus* LMS2-1 was not plottable. The relationship was statistically significant between groups (p = 0.001)

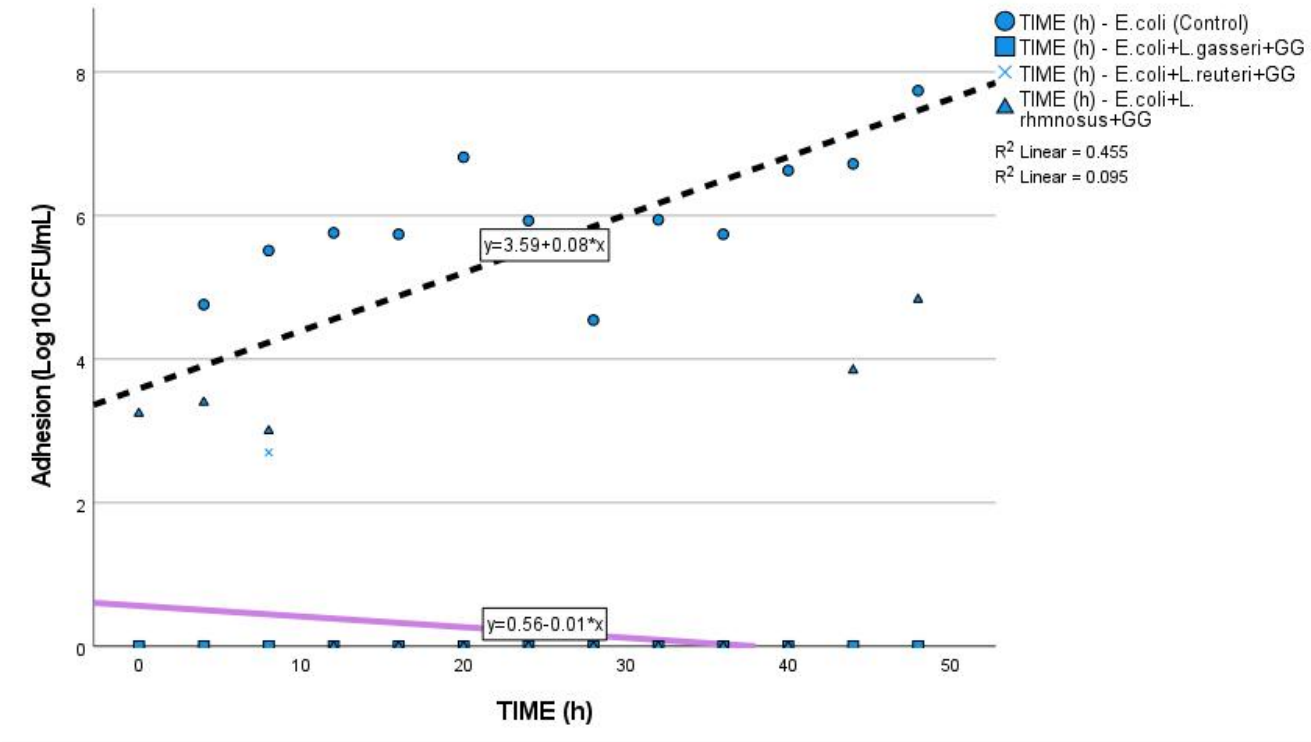

Figure 3.14: Effect of *Lactobacillus* strains on the adhesion of *Escherichia coli* 25922 in the presence of Glycero gelatin base. (p = 0.001)

KEY
Dotted line: *E.coli* ATCC 25922 (r = 1.000)
Purple line: *E.coli* ATCC 25922 + *L.reuteri* CF48-3A (r = - 0.002)
Green line: *E.coli* ATCC 25922 + *L.gasseri* JV-V03
Red line: *E.coli* ATCC 25922 + *L. rhamnosus* LMS2-1

3.8.3 Adhesion assay of *Lactobacillus* Strains on *Escherichia coli* ATCC 25922 in the presence of Polyethylene glycol base

Figure 3.15 shows the linear regression analysis for the Adhesion assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922 in the presence of polyethylene glycol base. Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of adhesion. The regression equation was $y = 3.59 + 0.08x$, indicating adhesion increased by 0.08 units for every unit increase in time. A perfect positive correlation was observed between time and adhesion ($r = 1.000$). Linear regression analysis of the effect of all the *Lactobacillus* strains was not plottable. The overall analysis revealed a statistically significant relationship within the groups ($p = 0.001$).

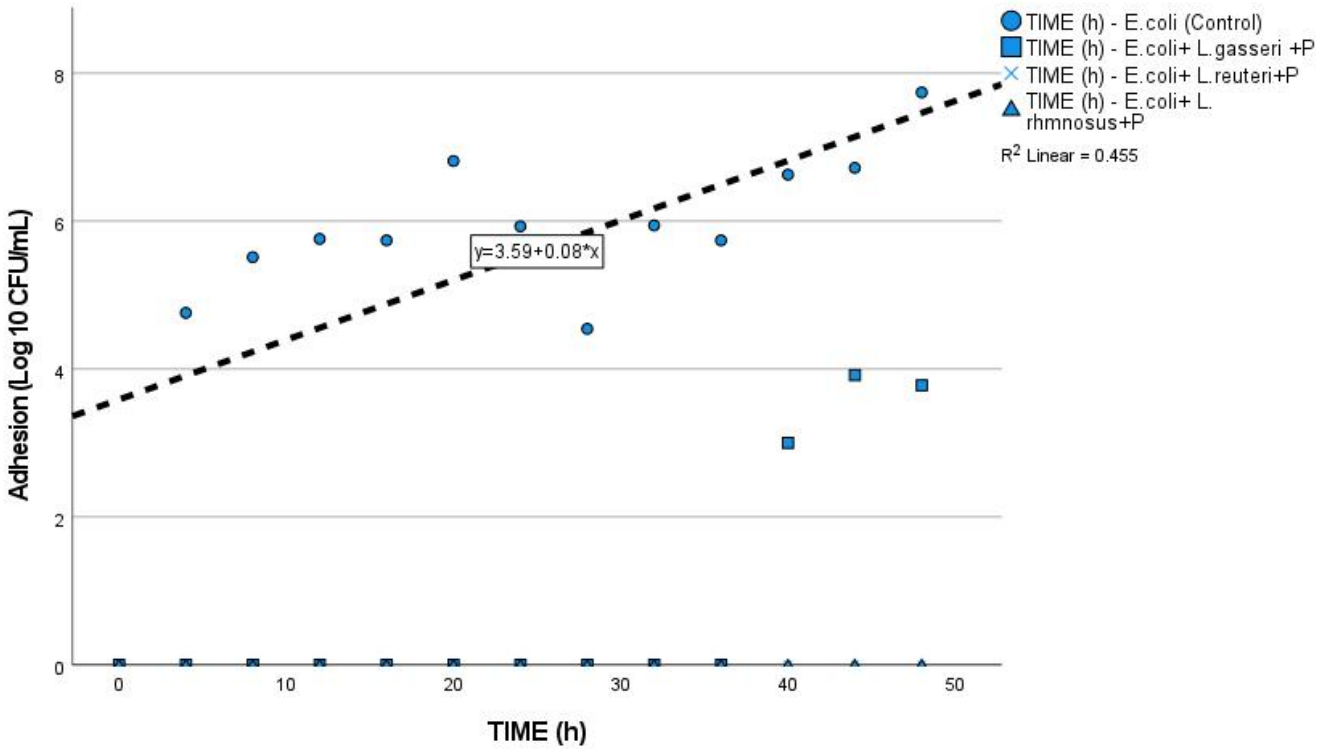

Figure 3.15: Effect of *Lactobacillus* strains on the adhesion of *Escherichia coli* 25922 in the presence of polyethylene glycol base (p = 0.001)

Key
Dotted line: *E. coli* ATCC 25922
Purple line: *E. coli* ATCC 25922 + *L. reuteri* CF48-3A
Green line: *E. coli* ATCC 25922 + *L. gasseri* JV-V03
Red line: E. coli ATCC 25922 + *L. rhamnosus* LMS2-1

3.8.4 Adhesion assay of *Lactobacillus* Strains on *Escherichia coli* ATCC 25922 in the presence of Theobroma base

Figure 3.16 is the outcome of the linear regression analysis for the adhesion assay of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 individually against *Escherichia coli* ATCC 25922 in the presence of theobroma base

Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of adhesion. The regression equation was y = 3.59 + 0.08x, indicating adhesion increased by 0.08 units for every unit increase in time. A perfect positive correlation was observed between time and adhesion (r = 1.000). Linear regression analysis of the effect of *L. reuteri* CF48-3A showed that time was a significant predicator of adhesion. The regression equation was y = 1.15 + 0.19x, indicating that adhesion decreased by 0.19 units for every unit increase in time. A moderate positive relationship was observed between time and adhesion (r = 0.591).

Linear regression analysis of the effect of *L. gasseri* JV-V03 showed time was a significant predicator of adhesion. The regression equation was y = 3.32 + 0.15x, indicating adhesion increased by 0.15 units for every unit increase in time. A strong positive relationship was observed between time and adhesion (r = 0.707). Linear regression analysis of the effect of *L. rhamnosus* LMS2-1 showed that time was a significant predicator of adhesion. The regression equation was y = 4.93 + 0.04x, indicating adhesion decreased by 0.04 units for every unit increase in time. A strong positive relationship was observed between time and adhesion (r = 0.718). There was no statistically significant association within groups (p = 0.523)

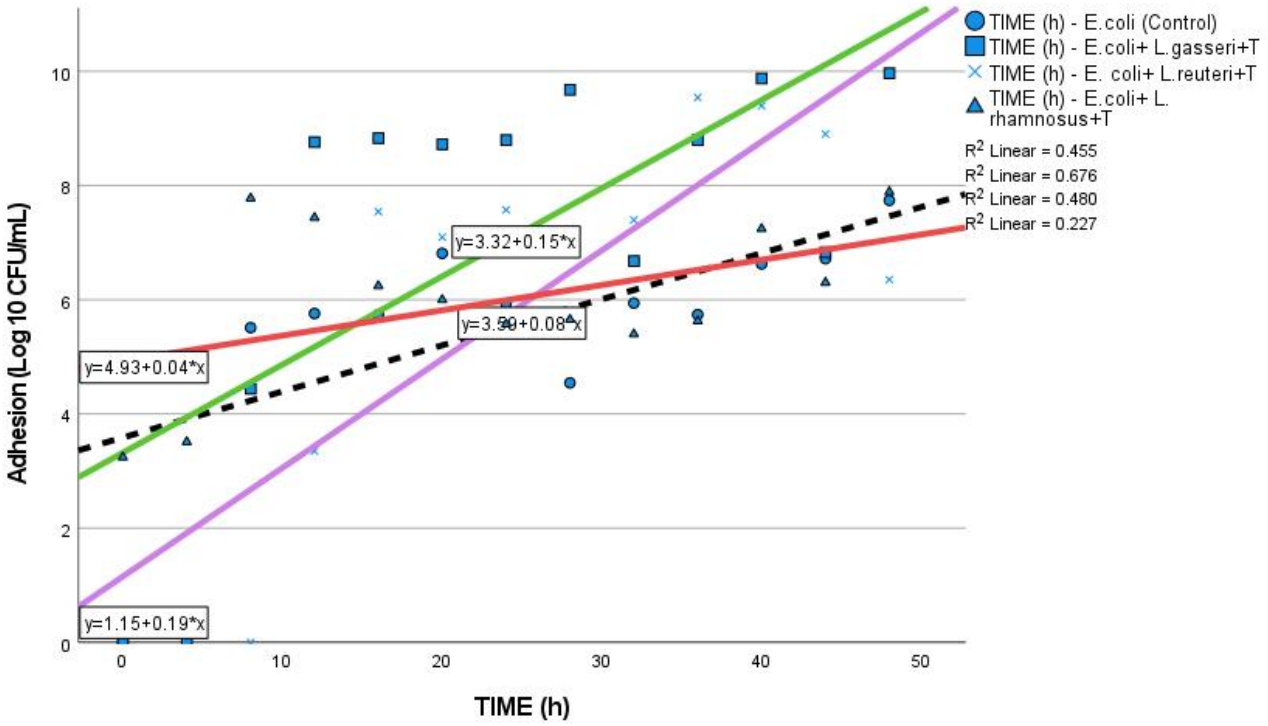

Figure 3.16: Effect of *Lactobacillus* strains on the adhesion of *Escherichia coli* ATCC 25922 in the presence of Theobroma base. (p = 0.523)

KEY
Dotted line: *E. coli* ATCC 25922 (r = 1.000)
Purple line: *E. coli* ATCC 25922 + *L. reuteri* CF48-3A (r = 0.591)
Green line: *E. coli* ATCC 25922 + *L. gasseri* JV-V03 (r = 0.707)
Red line: *E. coli* ATCC 25922 + *L. rhamnosus* LMS2-1 (r = 0.718)

3.9 Adhesion of *Lactobacillus* strains in the presence of suppository bases.

3.9.1 Adhesion of *Lactobacillus* strains in glycerogelatin bases

Figure 3.17 shows the adhesion pattern of *Lactobacillus reuteri* CF48-3A *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 in the presence of glycerogelatin base. *Lactobacillus reuteri* CF48-3A *and Lactobacillus gasseri* JV-V03 did not bind to the solidified albumen during the experiment. In contrast, *L. rhamnosus* LMS2-1 showed binding in the presence of glycerogelatin base between 40- 48h ($p = 0.137$). This outcome indicates the interactive inhibition of adhesion of *L. reuteri* CF48-3A *and L. gasseri* JV-V03. The *L. rhamnosus* LMS2-1 indicates no-detachable adhesion during the first 40h. Later, a sharp increase occured up to 48h. The impact of this experiment showed that Glycerogelatin base, interestingly, prevents adhesion of the two strains (*Lactobacillus reuteri* CF48-3A *and Lactobacillus gasseri* JV-V03). *L. rhamnosus* LMS2-1 exhibited a prolonged onset and a short duration of adhesion.

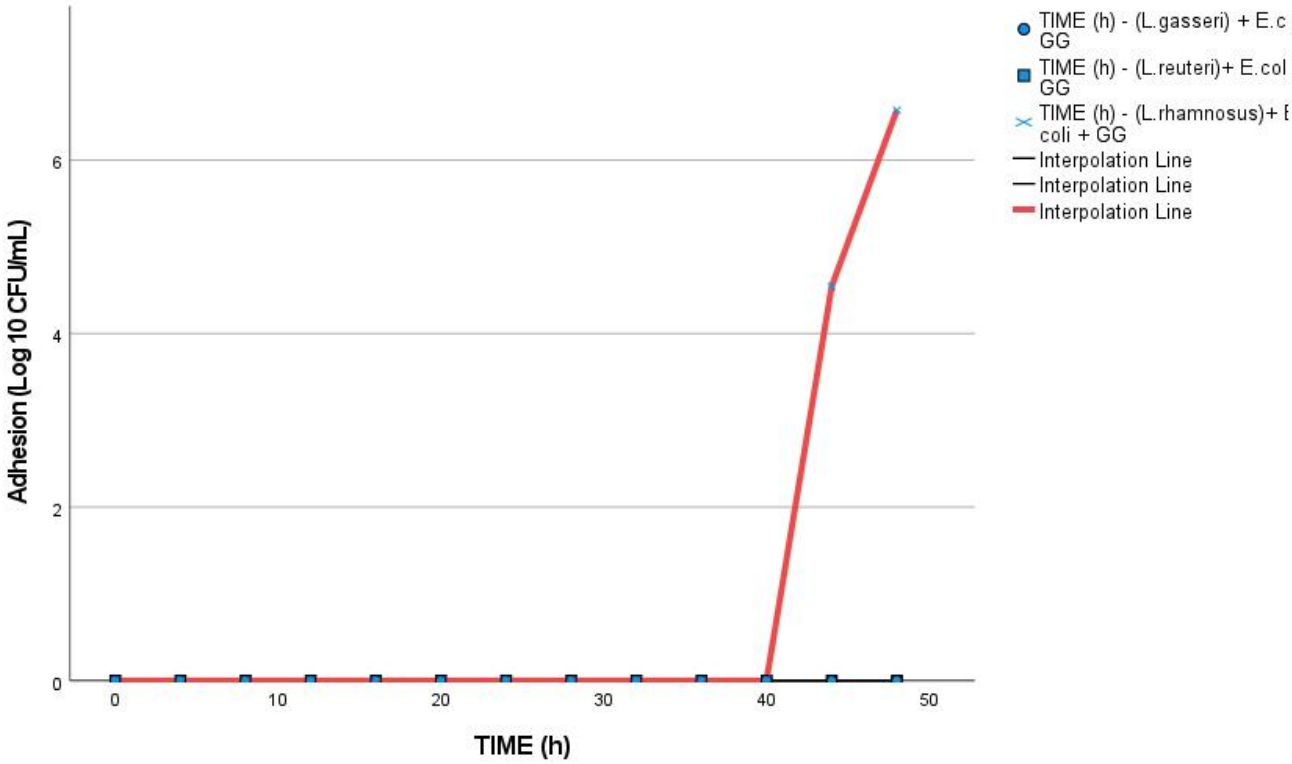

Figure 3.17: The effect of Glycerogelatin base on the adhesion of *Lactobacillus* strains (p = 0.137)

Key
Dotted line: *E. coli* ATCC 25922
Purple line: *L. reuteri* CF48-3A
Green line: *L. gasseri* JV-V03
Red line: *L. rhamnosus* LMS2-1

3.9.2 Adhesion of *Lactobacillus* strains in polyethylene glycol bases

Figure 3.18 shows the adhesion pattern of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 in the presence of polyethylene glycol.

L. reuteri CF48-3A showed adhesion to solidified albumen between 18-48h. *L. gasseri* JV-V03 showed adhesion between 25-48h. The *L. rhamnosus* LMS2-1 was between 18-48h. The survival within groups was not significant at $p = 0.492$. These plots demonstrated a detectable presence of the three *Lactobacillus* in polyethylene glycol. The detectable presence is notable despite the prolonged onset of adhesion.

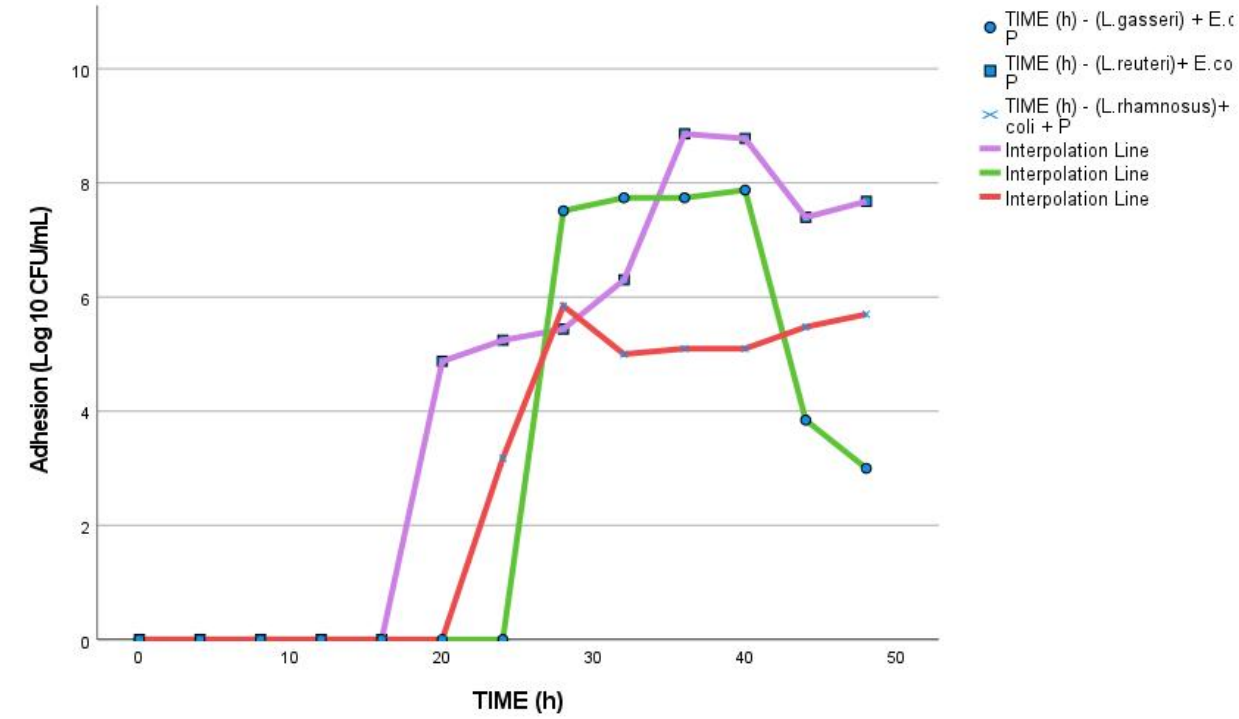

Figure 3.18: The effect of Polyethylene glycol base on the adhesion of *Lactobacillus* strains (p = 0.492)

Key
Dotted line: *E. coli* ATCC 25922
Purple line: *L.reuteri* CF48-3A
Green line: *L.gasseri* JV-V03
Red line*: L. rhamnosus* LMS2-1

3.9.3 Adhesion of *Lactobacillus* strains in Theobroma bases

Figure 3.19 shows the adhesion pattern of *Lactobacillus reuteri* CF48-3A, *Lactobacillus gasseri* JV-V03, and *Lactobacillus rhamnosus* LMS2-1 in the presence of Theobroma base. L. reuteri CF48-3A showed adhesion to solidified albumen between 12 and 48h. *L. gasseri* JV-V03 showed adhesion between 4 and 48h. The survival time of *L. rhamnosus* LMS2-1 ranged from 0 to 48 hours. However, survival differences within groups were not statistically significant ($p = 0.302$). In summary, the *Lactobacillus strains* can adhere to the sterile solidified albumen in the presence of Theobroma base. The process is characterized by a different onset time.

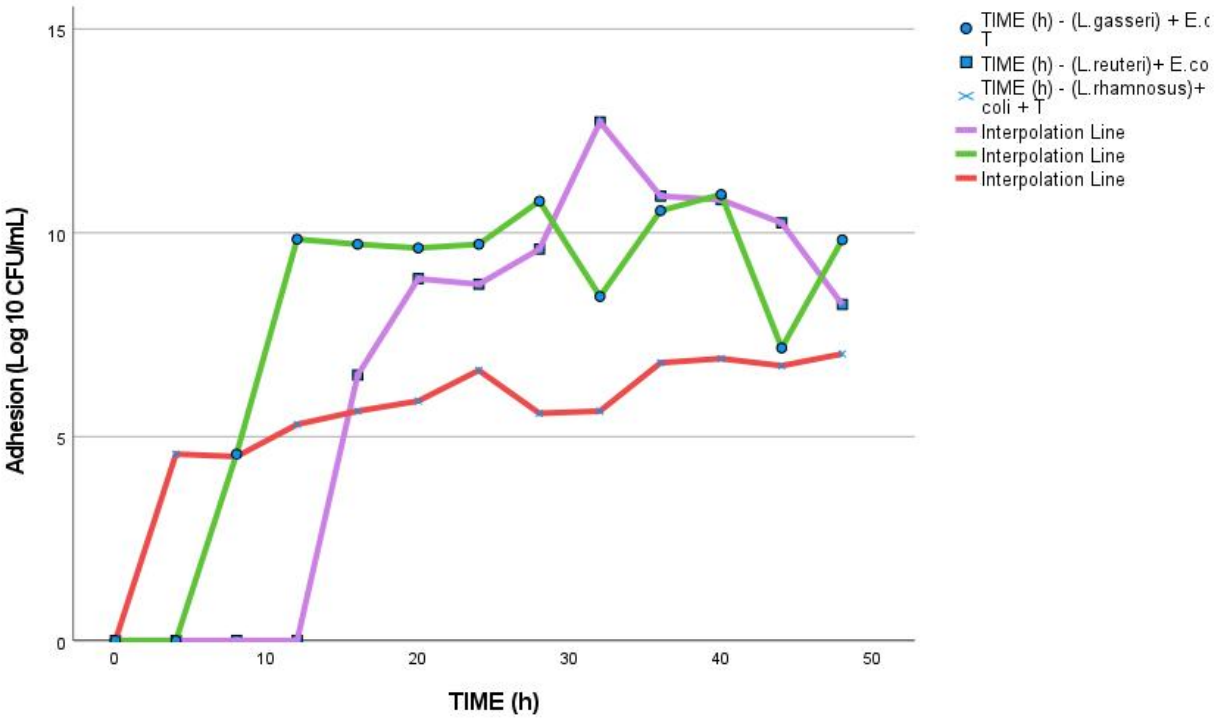

Figure 3.19: Effect of Theobroma base on the adhesion of *Lactobacillus* strains (p = 0.302)

Key
Dotted line: *E. coli* ATCC 25922
Purple line: *L. reuteri* CF48-3A
Green line: *L. gasseri* JV-V03
Red line: *L. rhamnosus* LMS2-1

3.10 GC-MS analysis studies

3.10.1 Comparative GC-MS studies *of Lactobacillus gasseri* JV-VO3.

Table 3.5 was the outcome of the GC-MS analysis of the following combinations: *Lactobacillus gasseri* JV-VO3 and polyethylene glycol; and *Lactobacillus gasseri* JV-VO3, *Escherichia coli* ATCC 25922, and polyethylene glycol. The *L. gasseri* JV-VO3 and polyethylene glycol combination produced octyl-cyclohexane, decyl-cyclohexane and undecyl-cyclohexane with known antimicrobial properties. While the *L.gasseri* JV-VO3, *E. coli* ATCC 25922, and polyethylene glycol combination comparatively produced n-Tetracosanol-1 (antimicrobial, anticancer and antioxidants) and 1,1-(1,3-propanediyl) bis-cyclohexane with known antioxidant. Figure 3.20 presents the GC-MS analysis spectrum of an *Escherichia coli* strain that induced an antimicrobial compound known as n-Tetracosanol-1. The chromatographic peak appears at a retention time of 16.432 min. Library NIST database confirms n-Tetracosanol-1 (long chain fatty alcohols) identity with a molecular formular of $C_{24}H_{50}O$; molecular weight of 354 g/mol and a retention index of 2650.

Table 3.5: *Lactobacillus gasseri, Escherichia coli,* and polyethylene glycol interactions

***L. gasseri* +Polyethylene glycol**	**Beneficial properties**	***L. gasseri*+ *E. coli*+ Polyethylene glycol +**	**Beneficial Properties**
Octyl-cyclohexane	Antimicrobial (Shoaib *et al.,* 2019)	n-Tetracosanol-1	Antimicrobial and Anticancer. Antioxidants. (Kuppswamy *et al.,* 2013)
Decyl-cyclohexane,	Antimicrobial (Shoaib *et al.,* 2019)	1,1'-(1,3-propanediyl) bis-cyclohexane,	Antioxidant (Shoaib *et al.,* 2019)
Undecyl-cyclohexane	Antimicrobial (Shoaib *et al.,* 2019)	-	-

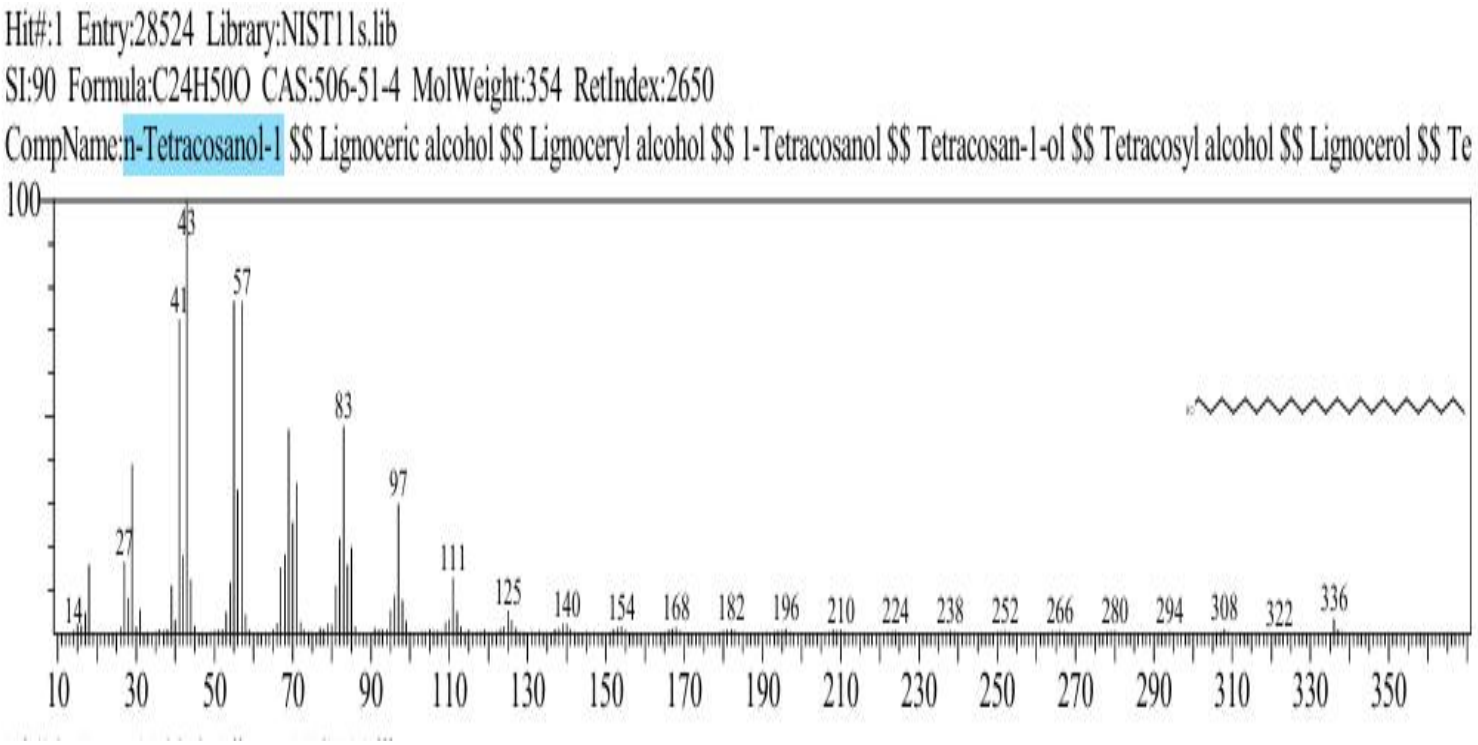

Figure 3.20 GC-MS spectrum and library identification of n-Tetracosanol-1

Peak 5 had a retention time of 16.432 minutes, an area of 2,185,910 representing 0.48% of the total area, a height of 582584, representing 0.45% of total height and an area-to-height (A/H) ratio of 3.75. The compound was identified as n-Tetracosanol.

3.10.2 Comparative GC-MS studies of *Lactobacillus reuteri* CF48-3A.

Table 3.6 was the outcome of the GC-MS analysis of the following combinations: *Lactobacillus reuteri* CF48-3A and polyethylene glycol; and *Lactobacillus reuteri* CF48-3A, *Escherichia coli* ATCC 25922, and polyethylene glycol. The *L. reuteri* CF48-3A and polyethylene glycol did not produce a compound with a known beneficial property. While the *L. reuteri* CF48-3A, *E. coli* ATCC 25922, and polyethylene glycol combination comparatively produced 1-Dodecanol (antimicrobial), Lauryl acetate (anticancer), acid tridecyl ester 2-Propenoic (anticancer), and Hexacone with a known antimicrobial property. Figure 3.21 presents the GC-MS analysis spectrum of an *Escherichia coli* strain *that* induced an antimicrobial compound known as Hexacosane. The chromatographic peak appears at a retention time of 22.664 min. Library NIST database confirms Hexacosane (long chain saturated aliphatic hydrocarbon) identity with a molecular formular of $C_{26}H_{54}$; molecular weight of 366 g/mol and a retention index of 2606.

Table 3.6: *Lactobacillus reuteri, Escherichia coli* and polyethylene glycol interactions

***L. reuteri* +Polyethylene glycol**	**Beneficial properties**	**L.gasseri+ *E. coli*+ Polyethylene glycol +**	**Beneficial Properties**
-	-	1-Dodecanol	Antimicrobial (Naoko *et al.,* 2007)
-	-	Lauryl acetate	Anticancer (Mohanas *et al.,* 2021)
-	-	tridecyl ester 2-Propenoic acid,	Anticancer (Sahu *et al.,* 2023)
-	-	Hexacosane	Antimicrobial (Rkaiyat *et al.,* 2015)

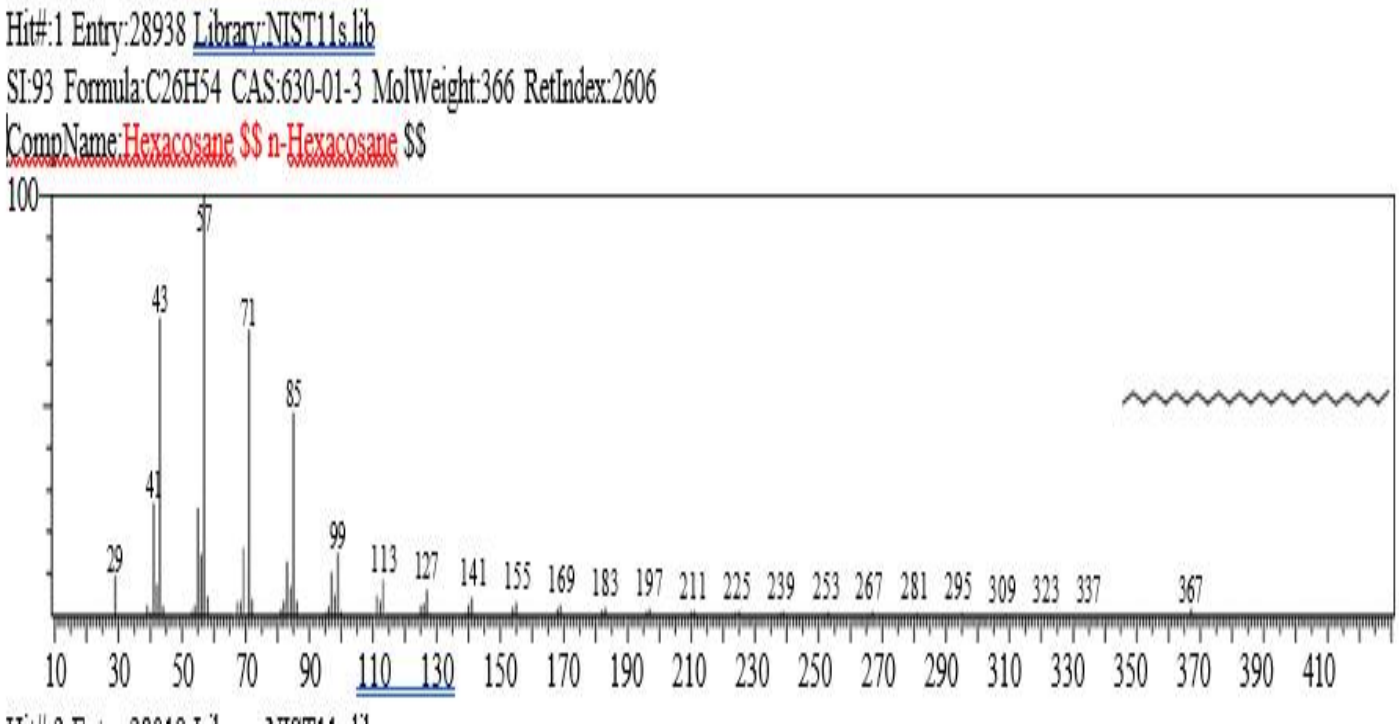

Figure 3.21 GC-MS spectrum and library identification of Hexacosane.

Peak 19 had a retention time of 22.664 minutes, an area of 1,915,140 representing 0.97% of the total area, a height of 540558, representing 0.71% of total height and an area-to-height (A/H) ratio of 3.54. The compound was identified as Hexacosane.

3.10.3 Comparative GC-MS studies of *Lactobacillus rhamnosus* LMS2-1.

Table 3.7 was the outcome of the GC-MS analysis of the following combinations: *Lactobacillus rhmnosus* LMS2-3A and polyethylene glycol; and *Lactobacillus rhmnosus* LMS2-1, *Escherichia coli* ATCC 25922, and polyethylene glycol. The *L. rhamnosus* LMS2-1, and polyethylene glycol produced compound with known beneficial properties such as: 2,3-dihydro-3,5-dihydroxy-6-4H-Pyran-4-one (antioxidant and anti-inflammatory); Stigmasterol (anti-inflammatory, antiviral and antiosteoarthritis); 3,5-dehydro-6-methoxy, 2,5- Cholest-22-ene-21-ol (antimicrobial, anti-inflammatory and antiasthmatic) and 3,6-bis(2-methylpropyl)-Piperazinedione (Antifungal). While *L. rhamnoses* LMS2-3A, *E. coli* ATCC 25922 and polyethylene glycol combination, comparatively produced: 2-Piperidinone and methyl ester Hexadecanoic acid (antimicrobial and anti-inflammatory); 2-hydroxy-1-(hydroxymethyl) ethyl ester Hexadecanoic acid (antioxidant) and methyl ester 9-Octadecenoic acid (Z)-(antioxidant)

Figure 3.22 presents the GC-MS analysis spectrum of an *Escherichia coli* strain that induced an antimicrobial compound known as methyl ester Hexadecanoic acid. The chromatographic peak appears at a retention time of 21.201 min. Library NIST database confirms methyl ester Hexadecanoic acid (fatty acid methyl ester) identity with a molecular formular of $C_{17}H_{34}O_2$; molecular weight of 270 g/mol and a retention index of 1878.

Figure 3.23 presents the GC-MS spectrum of an *Escherichia coli* that induced an antimicrobial compound known as 2-piperidinone. The chromatographic peak appears at a retention time of 13.583 min. Library NIST database confirms methyl ester 2-piperidinone (a lactam ring containing a nitrogen and carbonyl group) identity with a molecular formular of C_5H_9NO; molecular weight of 99 g/mol and a retention index of 883.

Table 3.7: *Lactobacillus rhamnosus, Escherichia coli* and polyethylene glycol interactions

***L. rhamnosus* +Polyethylene glycol**	**Beneficial properties**	***L.gasseri*+ *E. coli*+ Polyethylene glycol +**	**Benefical Properties**
2,3-dihydro-3,5-dihydroxy-6-4H-Pyran-4-one,	Antioxidant and Antiinflamatory (Chen *et al.,*2021;Shkla *et al.,*2021)	2-Piperidinone	Antimicrobial and Antiinflammatory (Santhi *et al.,*2013)
Stigmasterol	Antininflamatory Antiviral Antiosteroarthritis (Verma *et al*.,2015; Abdelgadir and Van staden, 2013)	2-hydroxy-1-(hydroxymethyl)ethyl ester Hexadecanoic acid	Antioxidant (Singh *et al.,* 2008)
3,5-dehydro-6-methoxy Cholest-22-ene-21-ol	Antimicrobial Antinflamatory Antiasthmatic (Arora *et al.,* 2017)	methyl ester Hexadecanoic acid	Antimicrobial and Antiinflammatory (Tulika and Agarwal 2017; Hema *et al.,* 2011)
3,6-bis(2-methylpropyl)- 2,5-Piperazinedione	Antifungal (Altaee, 2017)	methyl ester 9-Octadecenoic acid (Z)-	Antioxidant (Rahamouz-Haghighi *et al.,* 2023)

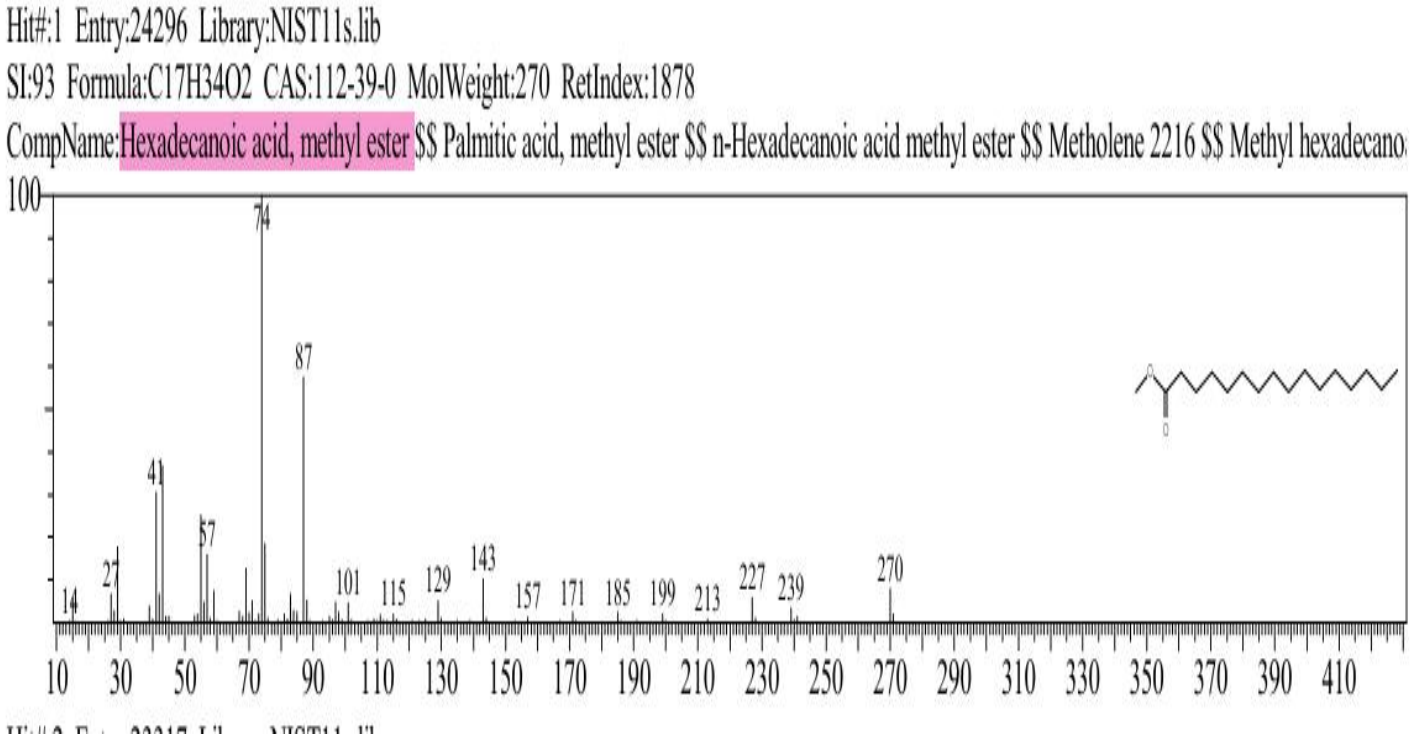

Figure 3.22: GC-MS spectrum and and library identification of methyl ester Hexadecanoic acid

Peak 8 had a retention time of 21.201 minutes, an area of 1890140 representing 0.62% of the total area, a height of 791570, representing 1.24% of total height, and an area-to-height (A/H) ratio of 2.39. The compound was identified as Hexadecanoic,methyl ester.

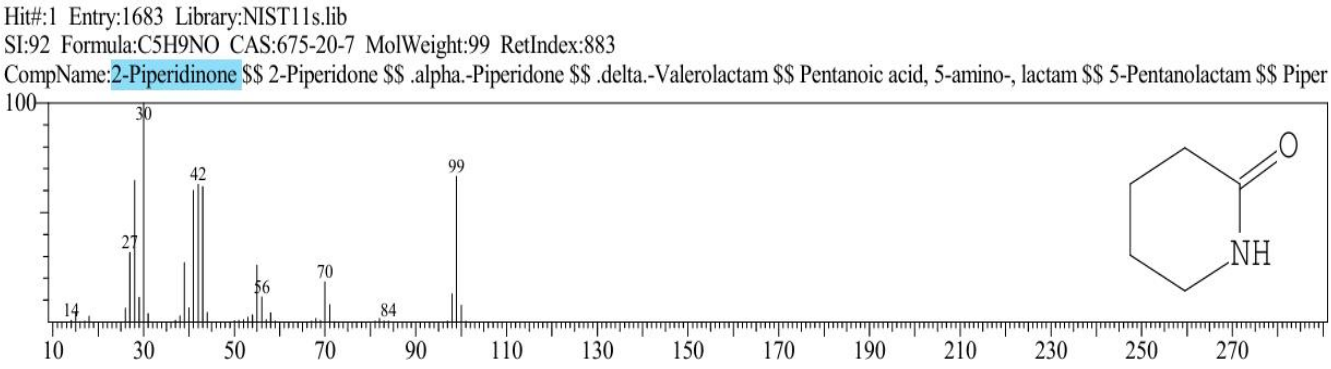

Figure 3.23: GC MS spectrum and library identification of 2-Piperidinone

Peak 2 had a retention time of 13.583 minutes, an area of 2723591 representing 0.89% of the total area, a height of 573955, representing 0.90% of total height and an area-to-height (A/H) ratio of 4.75. The compound was identified as 2-Piperidinone

CHAPTER FOUR

4.0 DISCUSSION

4.1 Authenticity of *Lactobacillus* strains and *Escherichia coli* ATCC 25922

The three *Lactobacillus* strains were differentiated based on their sugar utilization patterns and the unique enzymes involved in metabolizing these substrates. *Lactobacillus reuteri* CF48-3A fermented raffinose, indicating the presence of α-galactosidase for hydrolysis into galactose and sucrose (Axelsson, 2004; Gänzle and Follador, 2012), but did not utilize cellobiose or salicin, suggesting absence of β-glucosidase activity required for β-linked glucoside breakdown (de Vos and Vaughan, 1994). *Lactobacillus rhamnosus* LMS2-1 was distinguished by its ability to ferment D-mannitol and L-arabinose. D-mannitol metabolism involved mannitol dehydrogenase, which converts it to fructose/fructose-6-phosphate for glycolysis (Wisselink et al., 2002), while L-arabinose utilization involved enzymes such as L-arabinose isomerase, ribulokinase, and L-ribulose-5-phosphate epimerase that feed into the pentose phosphate pathway (Saulnier et al., 2007). In contrast, *Lactobacillus gasseri* JV-VO3 shared with *L. rhamnosus* LMS2-1 the ability to ferment cellobiose and salicin, indicating β-glucosidase activity (de Vos and Vaughan, 1994). However, it lacked the capacity to utilize D-mannitol and L-arabinose, suggesting the absence of mannitol dehydrogenase and arabinose-metabolizing enzymes. Overall, it exhibited an intermediate metabolic profile between *L. reuteri* CF48-3A and *L. rhamnosus* LMS2-1. The microorganism was identified as *Escherichia coli*, a Gram-negative bacillus, based on its morphology and biochemical profile. It was catalase-positive, indicating the presence of catalase, which breaks down hydrogen peroxide into water and oxygen (Madigan et al., 2021). It also demonstrated a positive reaction for the fermentation or utilization of cellobiose, salicin, maltose, lactose, D-fructose, D-glucose, D-mannitol, L-rhamnose, D-xylose, and L-arabinose, indicating the presence of β-glucosidase, salicin hydrolase, maltase (α-glucosidase), β-galactosidase,

fructokinase, hexokinase, mannitol dehydrogenase, rhamnose isomerase, xylose isomerase, and L-arabinose isomerase, respectively. These enzymatic activities reflect the metabolic versatility characteristic of *Escherichia coli* (Cappuccino and Welsh, 2017; Madigan *et al.*, 2021). The isolate's positive lactose reaction is characteristic of *E. coli*, which produces β-galactosidase that hydrolyses lactose into glucose and galactose, often with acid and gas formation (Holt *et al.,* 1994). It was negative for sucrose and raffinose fermentation, consistent with many *E. coli* strains that lack sucrase and α-galactosidase needed for their metabolism (Forbes *et al.,* 2007). Further biochemical testing showed the organism was MR-positive and VP-negative, indicating stable acid production from glucose via the mixed-acid fermentation pathway and absence of acetoin production via the butylene glycol pathway, consistent with *E. coli* (MacFaddin, 2000). The isolate was also citrate-negative, showing inability to utilize citrate due to lack of citrate permease, and urease-negative, indicating absence of urease activity for urea hydrolysis (Cheesbrough, 2006). Overall, these carbohydrate fermentation profiles, together with their associated enzymatic systems, provide valuable biochemical pointers for distinguishing the three *Lactobacillus* strains and *Escherichia coli*. This primary authentication validates its suitability for use as a probiotic and pathogenic organism for an interactive study.

A limitation of this study is that the authentication of *Lactobacillus strains and Escherichia coli* using Gram staining and biochemical tests may not provide absolute strain-level accuracy. Closely related bacteria may exhibit similar morphological and biochemical characteristics in sensitive research of this nature. Molecular techniques (16S rRNA gene sequencing, PCR, or MALDI-TOF MS), which offer higher precision, were not employed due to resource constraints.

4.2 Design and preparation of adsorbent

This aspect of this experiment was designed to evaluate the linearity of microorganism binding with a sterile solid albumen of weight of 1.86g and a surface area of 817.15mm^2. Establishing linearity is important in adsorption studies, as it highlights a predictable, concentration-dependent pattern and possibly supports the interpretation of microbial procedure (Foo and Hameed, 2010; Limousin *et al.,* 2007) for further experiments in probiotics. The high coefficients of determination (R^2 = 0.83–0.96) demonstrate that a large proportion of the variability in all microbial adhesion can seamlessly be explained by modification in concentration. In biological adsorption systems, such high R^2 values are indicative of strong process control and limited interference (Beveridge and Murray, 1980). The regression equations exhibited positive slopes (0.69-0.72), indicating that microbial adhesion increased consistently with increasing exposure of microorganisms. This pattern showed that adhesion occurred in a dose-dependent manner, which is consistent with adsorption phenomena where microbial cells interact with available binding sites on the adsorbent surface (Ramasamy *et al.,* 2017). The strong correlation coefficients (r = 0.91–0.98) further support the applicability of the exhibited linear relationships. The consistency between high r values and high R^2 values confirms that the relationships were not only statistically strong but also predictive, satisfying the assumptions of linear regression modeling by Montgomery *et al.,* 2012. Additionally, Standard cycle of temperature (121 °C), Pressure (15 psi) and Time (15–20 minutes) of the autoclave helps to create a sterile adsorbent that is free from any interfering contaminants.

The analysis revealed a statistical significance ($p < 0.001$) which reinforces the validity of the experimental design and analytical approach for further interactive studies in probiotics. The preliminary physical evaluation of the sterile solidified albumen, together with the results

from the linearity studies, indicates that the material has acceptable characteristics for further application. These findings suggest that it is suitable for continued use in subsequent adsorption experiments and coculture-interactive studies. The limitation of this study was that advanced analyses, such as texture analysis to measure firmness, moisture content determination, protein denaturation assessment, and microscopic structural examination of the solidified albumen, were not conducted due to the limited availability of equipment and laboratory facilities. As a result, the study relied mainly on physical observations, which may not provide detailed quantitative information on the internal structural and compositional changes caused by heating time.

4.3 **De-adhesion of Tween 20 solubilizing agent**.

This experiment was designed to identify the Tween 20 concentration that produces the highest recovery (yield) of de-adhered *Lactobacillus* cells from a sterile solid albumen. The outcome showed the highest yield: *L. rhamnosus* LMS2-1(9.68 $\log_{10}$ CFU/mL); *L. gasseri* JV-V03 (9.43 $\log_{10}$ CFU/mL), and *L. reuteri* CF48-3A (9.45 $\log_{10}$ CFU/mL) at an optimal concentration of 50% of Tween 20. The magnitude of recovery differed slightly among strains, showcasing known variation in *Lactobacillus* surface structures such as the surface proteins, exopolysaccharides, and lipoteichoic acids (Kandler and Weiss, 1986; Lebeer *et al.,* 2008). The concentration-dependent deadhesion of Tween 20 in this study therefore aligns with van Loosdrecht *et al.* (1987) and Neu (1996) and is consistent with surfactant-mediated removal of lactic acid bacteria. This demonstrated that bacterial de-adhesion is maximized at intermediate surfactant concentrations, while excessive levels reduce recoverable viability. Tween 20 is useful for detaching microorganisms from sterile solidified albumen, but one important limitation is interference during sample withdrawal. Tween 20 is a surfactant, and it can generate foam and bubbles during mixing or shaking. This foam may cause inconsistent withdrawal of liquid samples with a micropipette, leading to inaccurate sample volumes and

poor reproducibility in subsequent analyses. Air bubbles may also enter the pipette tip and further reduce precision and affect downstream experimental results.

4.4 Antibacterial effect of *Lactobacillus* strains on *Escherichia coli* in the presence of suppository bases

4.4.1 Preliminary antibacterial evaluation

The three *Lactobacillus* strains assessed in this study showed measurable growth inhibitory characteristics on *Escherichia col*i ATCC 25922, as visualised by the clear zones of inhibition. *L. gasseri* JV-V03 produced an inhibition zone diameter of 30.8 mm, *L. reuteri* CF48-3A produced 30.8 mm, while *L. rhamnosus* LMS2-1 exhibited the largest zone at 31.5 mm. This preliminary investigation confirms the ability of these strains to inhibit the growth of *E. coli,* indicating anti- *E. coli* activity as a result of diffusible metabolites released (Axelsson, 2004; Servin, 2004). Bactericidal determination assays revealed that the effect of all the *Lactobacillus* strains on *E. coli* ATCC 25922 exhibited bacteriostatic rather than bactericidal activity. Comparative studies of inhibition assays, time–kill studies, and neutralisation experiments reported by Servin, 2004; Ouwehand *et al.,* 2002, and Todorov *et al.,* 2011 estimated that 70–90% of *Lactobacillus* strains exhibit bacteriostatic activities. While 10–30% exhibit bactericidal activity. Therefore, the three strains selected for this study are among the predominant *Lactobacillus* strains known for their bacteriostatic properties. Based on these characteristics, this study considers them suitable to be synonymously described as probiotic strains. Their ability to inhibit the growth of pathogenic microorganisms supports their potential role in promoting microbial balance and improving host health. Consequently, the inclusion of these strains in this study is justified, as they represent promising candidates for probiotic application.

4.4.2 *Lactobacillus* strains on *Escherichia coli* ATCC 25922

In the control group (*E. coli* ATCC 25922), the positive regression slope (0.21) is indicative that *E. coli* survival increased proportionally with time. This implies the predictable and unhindered growth of *E. coli* across the experimental range, while the strong positive correlation (r=1.000) shows a perfect linear relationship between time and survival. This linearity confirms the suitability of the assay conditions and validates the baseline growth kinetics of *E. coli* in the absence of antagonistic influences seen in the control antibacterial cocultivation assay. The strong linearity and perfect correlation was an essential benchmark to ensure that deviations observed in subsequent co-cultivation groups are comparatively attributable to biological interactions rather than methodological variability. Similar sustained growth trends in monoculture controls over 24–48 h are routinely reported in time–kill and growth-kinetic studies by Madigan *et al.,*2019. Upon co-cultivation of *E. coli* ATCC 25922 with *L. reuteri* CF48-3A, the negative regression slope (- 0.16) observed indicates a decrease in *E. coli* survival with increasing experimental presence of *L. reuteri* CF48-3A with time when compared with the control. This inverse relationship strongly suggests an antibacterial effect, while the moderate negative relationship (r = - 0.590) was observed between time and survival, this is indicative of suppressive effects of the introduced *Lactobacillus* leading to this relationship. Similar multi-log reductions *of E. coli* within 24–48 h, attributed to organic acid accumulation, reuterin production, and pH-dependent killing (Spinler *et al.,* 2017). The co-cultivation with *L. gasseri* JV-V03 demonstrated a positive (0.17) but reduced slope compared to the control above. This confirms partial inhibition or growth modulation rather than complete suppression. Such a result consists of competitive exclusion, mild acidification of the medium, or depletion of nutrients required for *E. coli* proliferation, rather than direct bactericidal activity as shown by Servin, 2004 and Lebeer *et al.,* 2008. The reduced correlation strength (0.752) relative to the control indicates that *E. coli* growth pattern was increasingly constrained by *L. gasseri* JV-V03. Similar reduced-growth patterns have been

described for *L. gasseri*, where inhibition of *E. coli* is linked to competitive exclusion, moderate acidification, and interference with adhesion, rather than strong antimicrobial metabolite production (Servin, 2004; Matijašić *et al.,* 2006). Co-interference of *E. coli* ATCC 25922 with *L. rhamnosus* LMS2-1 resulted in a weak positive slope (0.02), revealing minimal increase in *E. coli* survival across the tested time. This suggests a stronger inhibitory influence than that observed for *L. gasseri* JV-V03, potentially demonstrating enhanced production of antibacterial compounds or inhibition of the quorum-sensing pathway. The Pearson correlation coefficient ($r = 0.58$) presents a moderate linear relationship, which is weaker than both the control and L. gasseri groups. This reduction in correlation strength reflects increased biological interference with *E. coli* growth patterns. Similar findings by Lebeer *et al.,* 2010, and Tejero-Sariñena *et al.,* 2012 have been documented in co-cultivation studies in which pathogen counts remained constant over 24–48 h, reflecting growth inhibition via nutrient competition, acidification, and quorum-sensing interference rather than outright lethality.

Overall, this co-cultivation assay provides strong quantitative evidence of a statistically significant difference between groups ($p < 0.001$), showing *Lactobacillus* strains modulate *E. coli* growth in a strain-dependent manner, with *L. reuteri* showing the most pronounced inhibitory effect, followed by *L. rhamnosus*, while *L. gasseri* exhibits moderate growth-modulating activity.

The findings of this experiment indicate that *E. coli* 25922 exhibited a reduced growth pattern in the presence of *L. gasseri* JV-V03 compared to the control. *L. rhamnosus* LMS2-1 demonstrated a bacteriostatic effect against *E. coli*, suggesting inhibition of bacterial growth without complete eradication. In contrast, *L. reuteri* CF48-3A exerted a bactericidal effect on *E. coli*, indicating a reduction in viable bacterial cells leading to cell death.

4.4.3 *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of Glycerogelatin base.

In the *E. coil* ATCC 25922, the positive regression slope (0.21) and strong positive correlation (r=1.000) show strong linearity and perfect correlation, which is an essential control to ensure that deviations observed in co-cultivation groups are attributable to the combined effect of biological interactions and specific modifications.

Cocultivation of *E. coli* ATCC 25922 combined with *L. reuteri* CF48-3A and Glycerogelatin produced a small regression slope (7.6×10^{-3}) suggesting a limited change in *E. coli* survival with increasing time. The weak positive correlation (r = 0.479) further indicates that the linear association between time and survival was weak. This suggests that while *L. reuteri* influenced the response of *E. coli*, the effect was modest under the experimental combination with Glycerogelatin base.

Cocultivation of *E. coli* in the presence of *Lactobacillus gasseri* JV-V03 and glycerogelatin base also produced a small regression slope (2.53×10^{-3}), indicating a slight increase in the survival variable with increasing time. The weak positive correlation (r = 0.315) indicates that the linear association between time and survival was also weak. The small slope implies that *all the Lactobacillus strains* exerted strong inhibition when compared with the control.

Cocultivation of *E. coli* in the presence of *Lactobacillus rhamnosus* JV-V03 and glycerogelatin base also produced a small regression slope (1.73×10^{-3}), indicating a slight increase in the survival variable with increasing time. The weak positive correlation (r = 0.220) indicates that the linear association between time and survival was also weak. The

small slope implies that *all the Lactobacillus strains* exerted strong inhibition when compared with the control.

Overall, this co-cultivation assay provides a strong quantitative evidence with statistical significant difference between groups ($p < 0.001$). The *Lactobacillus* strains inhibited *E. coli* growth in a *Lactobacillus* strain-supported approach in the presence of Glycerogelatin base.

The impact of this experiment showed that all the strains of *Lactobacillus* exhibited a bacteriostatic effect against *E. coli* 25922 in the presence of glycerogelatin

4.4.4 *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of Polyethylene glycol base.

Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of survival. The regression slope was y = 6.6 + 0.21x, indicating survival increased by 0.21 units for every unit increase in time. A perfect positive correlation was observed between time and survival (r=1.000).

The moderately negative slope (-0.10) indicates that *L. reuteri* exerted a clear inhibitory effect on *E. coli*, reducing viable counts in combination with Polyethylene glycol by approximately 0.1 $\log_{10}$ CFU/mL per hour. This showed a bacteriostatic to weakly bactericidal effect, rather than complete eradication. The Pearson correlation coefficient (r = −0.523) indicates a moderate negative relationship between time and survival.

The regression model slope for *L. gasseri* JV-V03 effect on *E. coli* (9.18×10^{-3}) showed a very small positive slope with a weak Pearson correlation (r = 0.192). This indicates a very weak positive association between time and bacterial counts, suggesting that *E. coli* growth was only reduced.

For *L. rhamnosus* LMS2-1, the regression equation slope (-3×10^{-3}) revealed an almost flat negative slope, with a very weak negative Pearson correlation (r = −0.114). This indicated inhibitory activity when compared to the control. A holistic view suggests that the strain neither significantly suppresses nor promotes *E. coli* growth over time. Such an outcome may reflect strain-specific differences in metabolite production or reduced competitive exclusion capacity.

The relationship revealed a statistically significant relationship within groups (p=0.001). When compared across groups, the control demonstrated unrestricted growth, while *L. reuteri* CF48-3A exhibited the strongest inhibitory effect, as evidenced by the most negative slope and moderate inverse correlation. *L. gasseri* JV-V03 and *L. rhamnosus* LMS2-1 showed weak associations, indicating limited antibacterial impact. The statistically significant difference

between groups (p = 0.001) confirms that the revealed variations in regression behavior were unlikely due to chance.

Overall, the regression slope serves as a quantitative indicator of antibacterial activity, where more negative slopes correspond to stronger inhibitory effects. Pearson correlation complements this by describing the strength and direction of the time-dependent response. The findings highlight the strain-specific nature of *Lactobacillus* antibacterial effects, with *L. reuteri* CF48-3A demonstrating superior antagonistic activity against *E. coli* in cocultivation.

The findings of this study showed that *L. gasseri* JV-V03 in the presence of polyethylene glycol exhibited a bacteriostatic effect against *E. coli*, while *L. reuteri* CF48-3A demonstrated a bactericidal effect that led to eventual cell death. In contrast, *L. rhamnosus* LMS2-1 showed a slow bactericidal effect that did not result in death.

4.4.5 *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of Theobroma base

Linear regression analysis of the control (*E. coli* ATCC 25922) showed time was a significant predicator of survival. The regression equation slope of 0.21 units and r = 1.000 were perfect positive baseline. The regression equation slope of *L. reuteri* CF48-3A against *E.coli* ATCC 25922 in combination with Theobroma demonstrated positive slope (0.26) with a strong Pearson correlation (r = 0.899).

Although bacterial growth still increased with time, the lower correlation coefficient compared to the control suggests increased variability in growth dynamics, likely due to metabolic interactions or competition during cocultivation. The positive slope indicates growth persistence, but the altered linearity reflects growth modulation rather than unrestricted proliferation.

The regression equation slope of *L. gasseri* JV-V03 against *E. coli* ATCC 25922 in combination Theobroma displayed a regression slope positive slope (0.28) and a very strong Pearson correlation (r = 0.932). The relatively steeper slope indicates a more rapid increase in survival over time compared with the control. The strong correlation suggests highly consistent growth. This pattern implies that cocultivation with *L. gasseri* JV-V03 did not suppress *E. coli* growth and may have allowed enhanced proliferation, due to reduced antagonism or favorable environmental conditions for the pathogen.

The regression equation slope of *L. rhamnosus* LMS2-1 against *E. coli* ATCC 25922 combined with Theobroma displayed positive regression slope (0.25) and a strong Pearson correlation (r=0.892). While growth remained positive, the slightly lower slope and correlation compared with *L. gasseri* suggest partial growth moderation. The reduced linear strength indicates that *L. rhamnosus* LMS2-1 may exert subtle competitive or metabolic effects, slowing but not preventing *E. coli* population increase.

The none statistically significant difference within groups (p = 0.879) suggests that while trend differences exist, they are not sufficient to demonstrate statistically distinct growth behaviors at the group comparison level. The results of this work revealed that *Theobroma* interfered with the established bactericidal and bacteriostatic activities of all *Lactobacillus* strains evaluated in this study. This interference promoted the proliferation and survival of *E. coli*, with growth patterns comparable to those observed in the control group.

4.5 Suppository bases on the growth of *Lactobacillus* strains

L. reuteri CF48-3A, *L. gasseri* JV-V03, and *L. rhamnosus* LMS2-1 remained viable in a glycerogelatin base for up to 40 hours, with a statistically significant time-dependent effect (p = 0.001). The ability of all three *Lactobacillus* strains to survive within the glycerogelatin base indicates that the formulation environment is non-toxic and compatible with probiotic

bacteria

This observation is consistent with reports showing that gelatin-based and polyol-containing semi-solid excipients do not exert bactericidal effects on lactic acid bacteria (Champagne *et al.,* 2005; Burgain et al., 2011). The statistically significant within-group variation observed in this study ($p = 0.001$) therefore reflects normal probiotic survival kinetics in a semi-solid base rather than formulation-induced toxicity. This aspect of the study indicated that the presence of glycerogelatin does not inhibit the growth of the three *Lactobacillus* strains tested. The observed growth trends remained relatively similar, suggesting that glycerogelatin neither enhanced nor inhibited the development of these strains under the experimental conditions.

L. reuteri CF48-3A, *L. gasseri* JV-V03, and *L. rhamnosus* LMS2-1 in a Polyethylene glycol (PEG) survived within a period of 0–48 hours. All three *Lactobacillus* strains remained viable throughout the experimental duration, with no statistically significant differences observed within groups ($p = 0.560$). This indicates that the PEG base lacks any detrimental effect on the survival of probiotic organisms. The absence of a significant within-group difference suggests that PEG showed that strain-specific differences were minimal, reinforcing the notion that PEG exerts a uniformly compatible effect across multiple *Lactobacillus* strains. The ability of *L. reuteri, L. gasseri*, and *L. rhamnosus* to survive in PEG aligns with literature describing the successful incorporation of these species into PEG-containing vaginal and rectal dosage forms aimed at microbiota restoration (Reid *et al.,* 2001; Petricevic and Witt, 2008).

L. reuteri CF48-3A, *L. gasseri* JV-V03, and *L.rhamnosus* LMS2-1 in a Theobroma (cocoa butter) base over a period of 0–48 hours, expressed as survival. The outcome showed that all *Lactobacillus* strains remained viable throughout the experimental duration, with no statistically significant differences observed within groups ($p = 0.614$). This finding

demonstrates that the Theobroma base did not adversely affect the survival of the probiotic organisms. Minimal *Lactobacillus* strain-dependent differences were displayed within the *L. reuteri, L. gasseri,* and *L. rhamnosus*, indicating that the Theobroma base exerts a uniformly compatible effect across different *Lactobacillus* strains. This is particularly important for formulation development, as excipient compatibility must be demonstrated across multiple probiotic strains intended for therapeutic use. Similar observations have been reported in studies where lipid-based suppository matrix preserved probiotic viability without inducing significant time-dependent loss (Petricevic and Witt, 2008; *Reid et al.,* 2011).

4.6 Anti-adhesion effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of suppository bases.

4.6.1 *Lactobacillus* strains on the adhesion of *Escherichia coli* ATCC 25922

Linear regression analysis of the control (*E.coli* ATCC 25922) demonstrated a positive slope (0.08) and a perfect positive Pearson correlation (r = 1.000), indicating rapid, continuous, and highly predictable adhesion over time. This reflects unrestricted surface colonisation, where bacterial attachment strengthens as exposure time increases. Such behaviour is consistent with classical adhesion models in which prolonged contact enhances physicochemical interactions, leading to stable attachment and early biofilm formation.

Linear regression analysis of the effect of *L. reuteri* CF48-3A on *E. coli* ATCC 25922 showed positive regression slope (0.03) with a smaller magnitude, and the Pearson correlation (r = 0.700) indicates a strong time-dependent relationship. The reduced slope reflects slower accumulation of adherent cells compared with the control, suggesting that *L. reuteri* effectively limits the rate of attachment of the *E. coli* ATCC 25922. The strong value supports a consistent anti-adhesion effect across time. Singh *et al.* (2017) support that *L. reuteri* can

reduce *E. coli* ATCC 25922 adhesion/colonisation through competition/exclusion/displacement assays.

Linear regression analysis of the effect of *L. gasseri* JV-V03 on *E. coli* ATCC 25922 exhibited a positive but higher slope (0.09) compared with the control, alongside a strong Pearson correlation (r = 0.788). This suggests that adhesion increased with time but at a higher rate, indicating no marked interference from the *Lactobacillus*. The strong correlation implies that adhesion progressed in a controlled and reproducible manner, possibly due to seamless binding by the *E. coli.* This pattern is characteristic of non-interference with the binding of this organism. This is contrary to Matijašić et al., 2006 whereas an anti-adhesion study for *L. gasseri* was demonstrated.

L. rhamnosus LMS2-1 showed the smallest positive slope, (6.24×10^{-3}) with a moderate Pearson correlation (r = 0.474). This indicates minimal net increase in adherent bacterial population over time, consistent with strong adhesion inhibition or destabilisation of attachment. The weaker correlation suggests greater biological variability, potentially reflecting dynamic attachment–detachment cycles, where cells adhere transiently but fail to establish stable binding. Such behaviour aligns with reports describing *L. rhamnosus* as an effective competitor for epithelial and abiotic adhesion sites. Petrova et al. (2016) proposed that such a reduced adhesion rate could be because of competitive exclusion, receptor blocking, and anti-adhesive surface interactions. The relationship was not statistically significant relationship between groups showed a strong indication of similarity in the variation in the *Lactobacillus* strains' interference with this pathogen.

The anti-adhesion activity of *L. reuteri* CF48-3A and *L. rhamnosus* LMS2-1 demonstrated that these strains can competitively reduce *E. coli* to the adsorbent surface. In contrast, *L.*

gasseri JV-V03 showed no reduction but rather exhibited higher adhesion of the *E. coli* ATCC 25922.

4.6.2 *Lactobacillus* strains on the adhesion of *Escherichia coli* ATCC 25922 in the presence of Glycero gelatin base

Linear regression analysis of the control (*E. coli* ATCC 25922) exhibited a positive regression slope (0.08), indicating a progressive increase in detectable adhered cells with time. This observation was reinforced by the perfect and positive correlation (r = 1.000), demonstrating a strong linear relationship between time and adhesion. Such behaviour is consistent with well-documented adhesion kinetics of *E. coli*, which readily attach to surfaces through fimbriae, outer membrane proteins, and electrostatic interactions, particularly during prolonged contact (Donlan, 2002; Tuson and Weibel, 2013).

In contrast, *L. reuteri* CF48-3A effects *E. coli* ATCC 25922 in combination with glycerogelatin base showed a negative regression slope (- 0.01), indicating a gradual reduction in adhered cells over time. This suggests progressive detachment or weak surface affinity, while the near-zero negative Pearson correlation (r = −0.002) indicates that time was not a strong determinant of adhesion stability. This may reflect poor surface compatibility, competition with matrix components, or auto-aggregation of cells rather than sustained surface binding as shown by combined literature by Lebeer *et al.,* 2008; Petrova and Sauer, 2012).

In contrast, both effects of *L. gasseri* JV-V03 and *L. rhamnosus* LMS2-1 on *E. coli* ATCC 25922 in presence of glycerogelatin base, regression analysis was not plottable, suggesting minimal or inconsistent adhesion across the evaluated time points. This outcome may indicate negligible attachment, rapid detachment, or complete saturation of the surface at early time points, resulting in insufficient variability for linear modelling. In adhesion studies, such

patterns are often interpreted as poor adhesive affinity or strain-specific surface properties that limit interaction with the adsorbent in accordance with Bos *et al.,* 1999.

Statistically significant difference within groups (p = 0.001) confirms that adhesion behaviour was strain-dependent and influenced by specific cell–surface interactions in combination with glycerogelatin base rather than random variation.

This study indicated that all three *Lactobacillus* strains were able to competitively inhibit the adhesion of *E. coli* ATCC 25922 in the presence of glycerogelatin base. This finding suggests that the glycerogelatin formulation did not impair the anti-adhesion properties of the probiotic strains, allowing them to effectively compete with the pathogen for binding sites.

4.6.3 *Lactobacillus* strains on the adhesion of *Escherichia coli* ATCC 25922 in the presence of polyethylene glycol base

The positive slope (0.08) signifies that for every one-unit increase in time, *E. coli* adhesion increased by 0.08 Log_{10} CFU/mL for the control. The Pearson correlation coefficient (r = 1.000) indicates a perfect positive correlation, displaying a highly consistent adhesion behavior across the experimental duration. This confirms the expected adhesion capacity of *E. coli* ATCC 25922 to the adsorbent surface, considering the absence of competing *Lactobacillus* strains which in summary validate the experimental model.

In contrast, regression analysis for *L. reuteri* CF48-3A, *L. gasseri* JV-V03, and *L. rhamnosus* LMS2-1 against *E. coli* was reported as not plottable. From an adhesion perspective, non-plottable regression lines are indicative of effective competitive exclusion or displacement mechanisms, where *Lactobacillus* strains occupy binding sites or modify surface properties, thereby preventing stable *E. coli* attachment. The statistical significance within groups (p = 0.001) further supports the conclusion that the observed reduction or disruption in *E. coli*

adhesion in the presence of *Lactobacillus* strains is not due to random variation but represents a true *Lactobacillus* effect. These findings provide quantitative and statistical evidence supporting the anti-adhesive potential of these *Lactobacillus* strains, reinforcing their relevance in preventing pathogen attachment and subsequent infection. This study indicated that all three *Lactobacillus* strains were able to competitively inhibit the adhesion of *E. coli* ATCC 25922 in the presence of polyethylene glycol base. This finding suggests that this base did not impair the anti-adhesion properties of the probiotic strains, allowing them to effectively compete with the pathogen for binding sites.

4.6.4 Effect of *Lactobacillus* strains on the adhesion of *Escherichia coli* ATCC 25922 in the presence of Theobroma base

In the control alone (*E. coli* ATCC 25922), the positive slope (0.08) together with the perfect positive pearson correlation (r = 1.000) indicates a steady, uniform, and highly predictable increase in recoverable adhered *E. coli* with time. This behaviour is characteristic of unhindered bacterial adhesion, where initial attachment is followed by stable accumulation and surface colonization as displayed by Bos *et al.,* 1999 and Tuson and Weibel, 2013.

In the presence of *Lactobacillus reuteri* CF48-3A, a higher positive slope (0.19) was observed alongside a moderate positive correlation (r = 0.591). Although adhesion increased with time, the reduced correlation coefficient suggests increased variability in attachment dynamics which must have been induced by the Theobroma base. In adhesion systems, such variability is commonly associated with partial competition for binding sites, surface conditioning, or transient attachment, where adhesion occurs but lacks the stability of a control system as demonstrated by Ouwehand *et al.,* 2002; Servin, 2004.

Similarly, *Lactobacillus gasseri* JV-V03 exhibited a positive slope (0.15) with a strong positive correlation (r = 0.707). This pattern indicates a consistent, but higher increase in

adhered *E. coli* cells over time. Compared with the control, the reduced r value reflects altered adhesion kinetics, which may result from co-aggregation or modified surface network within *E. coli* and *Lactobacillus* cells in combinations with Theobroma base, as signified by Lebeer *et al.,* 2008

In contrast, *L. rhamnosus* LMS2-1 effects on the pathogen demonstrated the smallest positive slope (0.04) while maintaining a strong positive correlation (r = 0.718). The low slope magnitude indicates a lower recovery of *E. coli* adhered to the adsorbent. Suggesting reduced attachment efficiency in the presence of this Theobroma. Despite the consistent trend indicated by r, the diminished slope implies that *L. rhamnosus* effectively limited the extent of *E. coli* surface accumulation over time, a phenomenon widely reported for *Lactobacillus* strains with strong surface-modifying or competitive adhesion characteristics in accordance with Lebeer *et al.,* 2008 and Donlan, 2002. The relationship was not statistically significant between groups (p = 0.523), which is an indication of similar variation in strain-specific nature interaction in the presence of the introduced base.

An overall assessment of the study, when compared with the trends observed in similar studies, demonstrated that the *Theobroma* base interferes with the anti-adhesion effects of *Lactobacillus* strains. This suggests that the *Theobroma* formulation may alter or reduce the ability of these probiotic strains to competitively inhibit the attachment of pathogenic bacteria, thereby diminishing their protective anti-adhesive activity.

4.7 Suppository bases on the adhesion of *Lactobacillus* strains

4.7.1 Glycerogelatin base

The absence of binding by *L. reuteri* CF48-3A and *L. gasseri* JV-V03 implies that the glycerogelatin base inhibited the effective cell–albumen interactions. The glycerogelatin may have exerted a dual effect by forming a viscous matrix that can physically coat the organism

surfaces and albumen surface area, thereby interfering with adhesion-related surface interactions. (Rosenberg, 2006; Sánchez *et al.,* 2008). Matrix-related interference has been seen in scientific literatures where semi-solid or polymeric carriers reduced measurable adhesion despite bacterial viability within the system. (Collado *et al.,* 2007). The glycerol and gelatin may have induced electrostatic repulsion, steric hindrance, and altered surface charge. Ultimately, reducing the possibility of any stable adsorption (Bos *et al.,* 1999). In contrast, *L. rhamnosus* LMS2-1 exacted a binding pattern between 40 and 48 hours, although not statistically significant ($p = 0.137$), signifying a low-affinity interaction. This differs from previous studies by Tuomola and Salminen, 1998 and Vesterlund *et al.,* 2005 showing that *L. rhamnosus* strains possess comparatively enhanced adhesion potential to other *Lactobacilli*, of which such interactions are usually time-dependent and sensitive to environmental factors. Hence, the lack of binding and low-affinity binding does not negate their probiotic functionality, but rather shows incompatibility among strains, albumen, and the glycerogelatin base.

4.7.2 Polyethylene glycol base

L. reuteri CF48-3A exhibited adhesion to the polyethylene glycol between 18 – 48 h, *L. gasseri* JV-V03 showed adhesion (25–48 h), while *Lactobacillus rhamnosus* LMS2-1 survived consistently from 18–48 h, indicating a higher tolerance to the experimental conditions. These strain-specific patterns are in accordance with documented reports indicating that probiotic bacteria differ in their ability to withstand environmental factors, due to differences in cell surface, membrane, and exopolysaccharide framework. (Lebeer *et al.,* 2008; Ruiz *et al.,* 2013).

The survival of the *Lactobacillus* strains in polyethylene glycol indicates that the base is inert and does not interact adversely with the active ingredient, the *Lactobacillus* culture. This

demonstrates the compatibility of polyethylene glycol as a suitable carrier base for probiotic suppositories. Its non-reactive nature helps maintain the viability of the probiotic organisms, thereby supporting the stability, efficacy, and therapeutic potential of the final dosage form.

4.7.3 Theobroma base

Lactobacillus reuteri CF48-3A exhibited adhesion between 12 and 48 hours, *L. gasseri* JV-V03 showed adhesion between 4 and 48 hours. While, *L. rhamnosus* LMS2-1 also demonstrated adhesion between 0-48h. Despite these observed adhesion periods, survival and adhesion within groups were not statistically significant ($p = 0.302$). The survival of the *Lactobacillus* strains in Theobroma indicates that the base is inert and does not interact with the *Lactobacillus* culture. This demonstrates the compatibility of this base as a suitable carrier for probiotic suppositories.

4.8 GC-MS analysis guided interactive effects.

4.8.1 *Lactobacillus gasseri* JV-VO3, *Escherichia coli* ATCC 25922, and Polyethylene glycol interactions

L. gasseri and polyethylene glycol yielded long-chain alkyl-substituted cyclohexanes, including octyl-cyclohexane, decyl-cyclohexane, and undecyl-cyclohexane. Previous experiments have reported that *Lactobacillus* strains secrete some constitutive antimicrobial substances, including organic acids, fatty acids, and hydrophobic secondary metabolites, which collectively contribute to pathogen exclusion (Ouwehand & Vesterlund, 2004; Servin, 2004).

Most of the compounds are known to disrupt microbial cell membranes and permeability, significantly leading cell death due to loss intracellular contents.

Compounds such as n-tetracosanol-1 and 1,1′-(1,3-propanediyl) bis-cyclohexane specifically in the *Lactobacillus–E. coli* co-interference in polyethylene glycol indicates inducible metabolite production. These bioactive substances have been associated with antimicrobial, antioxidant, and anticancer properties. Some *Lactobacilli* are known to upregulate genes leading to secondary metabolite biosynthesis, and ultimately enhancing their antagonistic activity (De Vuyst and Leroy, 2007). The compound, such as n-tetracosanol-1, has been reported to exert antimicrobial activity through integration into bacterial cell membranes, leading to membrane destabilization and disruption of essential enzymatic and energy-generating systems required for cell survival (Desbois and Smith, 2010; Casillas-Vargas et al., 2021). The coexistence of constitutive and inducible substances observed in this experiment demonstrates that *Lactobacillus* employs a dual antimicrobial effect.

4.8.2 *Lactobacillus reuteri CF48-3A, Escherichia coli* ATCC 25922, and Polyethylene glycol interactions

The presence of *L. reuteri* CF48-3A in combination with polyethylene glycol alone did not exhibit any compound of interest. The undetectable bioactive compounds under non-competitive environment suggested that this strain maintains a low-baseline metabolic output in the absence of pathogenic organisms. This correlate with past reports indicating that some *Lactobacillus* strains conserve energy by reducing secondary metabolite production in stable environments (De Vuyst and Leroy, 2007). The obvious absence of any compound based on this experiment suggests that *L. reuteri* may rely less on constitutive chemical antagonism. In contrast, the co-culture with *E. coli* and *L. reuteri* in polyethylene glycol resulted in the production of some biologically active compounds, including 1-dodecanol, lauryl acetate,

tridecyl ester of 2-propenoic acid, and hexacosane. These compounds are linked with antimicrobial and anticancer properties. Long-chain alcohols such as 1-dodecanol are known to showcase antimicrobial activities via disruption of bacterial membrane integrity, which will eventually lead to increased permeability and cell lysis.

Additionally, lauryl acetate and hexacosane have been associated with antimicrobial activity primarily through interactions with microbial cell membranes. These interactions can disrupt membrane integrity, increase permeability, and impair membrane-bound enzymatic systems involved in energy generation and metabolic regulation, leading to growth inhibition or cell death (Desbois and Lawlor, 2013; Kumar *et al.,* 2020; López-Romero *et al.,* 2021).

In accordance to Servin, 2004 and De Keersmaecker and Vanderleyden, 2003 this inducible response aligns with past studies showing that *Lactobacilli* increase production of antibacterial metabolites in the presence of pathogens, thereby buttressing competitive exclusion, pathogen suppression, and ecological stability within the GIT environment.

4.8.3 *Lactobacillus rhamnosus LMS2-1, Escherichia coli ATCC 25922,* and Polyethylene glycol interactions

An auxenic culture of *L. rhamnosus* in polyethylene glycol was found to produce compounds of interest. These biologically active compounds were 2,3-dihydro-3,5-dihydroxy-6-H-pyran-4-one, stigmasterol, 3,5-dehydro-6-methoxy cholest-22-ene-21-ol, and 3,6-bis(2-methylpropyl)-2,5-piperazinedione. These compounds exhibited known beneficial effects: antioxidant, anti-inflammatory, antimicrobial (antifungal, antiviral), and antiasthmatic activities, indicating that this strain maintains constitutive bioactive metabolites, despite the absence of pathogenic stress. The 2,3-dihydro-3,5-dihydroxy-6-H-pyran-4-one is a pyranone derivatives which is widely reported to demonstrate antioxidant and anti-inflammatory attributes via radical quenching and modulation of inflammatory mediators. The constitutive

antioxidant potential possesses dual effects on both the probiotic organism and the host against oxidative stress (Wang *et al.,* 2018). The 3,6-bis(2-methylpropyl)-2,5-piperazinedione are known as the diketopiperazines. Quorum sensing interference and membrane disruption (De Vuyst & Leroy, 2007) are the known mechanism of action by which they exact their antimicrobial and anti-fungal effects. Stigmasterol is a well-documented phytosterol group. Stigmasterol further supports the anti-inflammatory and immunomodulatory potential of *L. rhamnosus*, as these compounds have been shown to inhibit pro-inflammatory cytokines and possess antiviral activity (Bouic, 2001). In a nutshell, some constitutive-compounds capability seen supports the antimicrobial effects, even in the absence of pathogenic presence.

E. coli induced the appearance of additional bioactive compounds, including 2-piperidinone, 2-hydroxy-1-(hydroxymethyl)ethyl hexadecanoate, methyl ester of hexadecanoic acid, and methyl ester of (Z)-9-octadecenoic acid. These compounds are significantly associated with antimicrobial, anti-inflammatory, and antioxidant activities.

Fatty acid methyl esters, hexadecanoic acid (palmitic acid), and octadecenoic acid derivatives, have been reported to possess disruption of the outer membrane and inhibition of key metabolic enzymes as mode of action against Gram-negative bacteria (Desbois and Smith, 2010). In summary, some of the induced compounds' capabilities support the upregulation of the antimicrobial ability in the presence of a pathogen.

CHAPTER FIVE

5.1 CONCLUSION

Antibacterial and anti-adhesion studies of probiotic strains against *Escherichia coli* in the presence of suppository bases with GC-MS profiles of the coculture were conducted, thereby improving existing knowledge of the influence of probiotic strains on *Escherichia coli* in the presence of selected suppository bases. The basic authentication tests confirmed the presence of all *Lactobacillus* strains and *Escherichia coli*, serving as a foundational pre-experimental quality control measure before the interactive phases. The preparation of the sterile solidified albumen fulfilled some quantifiable requirements expected for a designed, concentration-dependent evaluation of adhesion capacity. Tween 20 at 50% made the de-adhesion of all interactive microorganisms on the sterile solidified albumen seamlessly computable for a comparative evaluation in the presence of a control. *Lactobacillus* strains in combination with Glycerogelatin and Polyethylene glycol bases revealed quantitative antibacterial activity

against *Escherichia coli*. Meanwhile, there was no antibacterial effect in the presence of Theobroma. The suppository bases were inert when combined with the *Lactobacillus* strains, as demonstrated by the detectable viability of the strains throughout the time range of this experimental model. This indicates that the bases did not exert any inhibitory effect on the probiotic *Lactobacillus* strains used in this study. A notable anti-adhesion effect against *E. coli* ATCC 25922 was observed when the *Lactobacillus* strains were combined with either glycerogelatin or polyethylene glycol, while a contrary outcome was observed using the Theobroma base. Antiadhesion and the antibacterial assessment revealed a trend of the enhanced probiotic effect of both Glycerogelatin and Polyethylene base in this research. *Lactobacillus* strains adhered to the solidified albumen in combination with either Polyethylene glycol or Theobroma, where as a poor adhesion was shown using Glycerogelatin base. In summary, these experiments selectively revealed polyethylene glycol as the most suitable suppository base in combination with all the *Lactobacillus* strains, while Theobroma supports both proliferation and adhesion in the adhesion and cocultivation models used. The n-Tetracosanol-1, Hexadecanoic acid, methyl ester and Hexacosane were some induced compounds secreted by the *Lactobacillus–Escherichia coli*-Polyethylene glycol combination. This shows the interactive combination generates induced compounds against *Escherichia coli*. The absence of toxic compounds further buttresses the expected inert nature of Polyethylene glycol.

A holistic view of this study established that suppository bases can enhance the antibacterial and anti-adhesion properties of some probiotic strains against *Escherichia coli*. The specific outcomes from the objectives will seamlessly enhance understanding of Probiotics-Pathogen-Base interactions for optimal care of patients with aerobic vaginitis and ulcerative colitis triggered by *Escherichia coli.*

5.2 Contribution to knowledge

The study has contributed to knowledge in the following ways:

1. Shown that Tween 20 solubilizing agent can be used in de-adhesion of *Lactobacillus* strains. This is pivotal in the further competitive, exclusive and displacement assay of probiotic-pathogen interactions.

2. Established that solidified albumen was a heat-stable substratum which will further enhance investigations in probiotic-pathogen-formulation base interactions.

3. Revealed that polyethylene glycol was the most suitable base for formulation of a probiotic suppository for the treatment of *Escherichia coli* induced aerobic vaginitis and ulcerative colitis.

4. Showed that induced compounds such as n-Tetracosanol-1, Hexadecanoic acid, 2-Piperidinone, methyl ester and Hexacosane will pave the way for further understanding the bacteriostatic effects of some probiotic strains.

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APPENDICES

Appendix 6.1: Qualitative inhibitory effect of various concentration of Tween 20 on *Lactobacillus* strains

TIME (min)	*L. reuteri* CF48-3A	*L. gasseri* JV-VO3	*L. rhamnosus* LMS2-1
2.5	G	G	G
5.0	G	G	G
7.5	G	G	G
10.0	G	G	G
12.5	G	G	G
15	G	G	G
17.5	G	G	G

20.0	G	G	G
22.5	G	G	G
25	G	G	G
27.5	G	G	G
30.0	G	G	G

Key
G: Growth
NG: No growth

Appendix 6.2

Appendix 6.2.1: Antibacterial effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922

Correlations

		***E.coli* (Control)**	***(E. coli) + L.reuteri***	***(E. coli) + L.gasseri***	***(E.coli) + L.rhamnosus***
*E. coli (*Control)	Pearson Correlation	1	-.590*	.752**	.580*
	Sig. (2-tailed)		.034	.003	.038
	N	13	13	13	13
(*E. coli*) + *L.reuteri*	Pearson Correlation	-.590*	1	-.576*	-.152
	Sig. (2-tailed)	.034		.039	.620
	N	13	13	13	13
(*E. coli)* + *L.gasseri*	Pearson Correlation	.752**	-.576*	1	.366
	Sig. (2-tailed)	.003	.039		.218
	N	13	13	13	13

(*E. coli*) +	Pearson Correlation	.580*	-.152	.366	1
L.rhamnosus	Sig. (2-tailed)	.038	.620	.218	
	N	13	13	13	13

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

ANOVA

conc

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	491.106	3	163.702	16.916	.000
Within Groups	464.510	48	9.677		
Total	955.616	51			

Appendix 6.2.2: Effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of Glycerogelatin Base

		E. coli (Control)	(*E. coli*) + *L.reuteri* + GG	(*E. coli*) + *L.gasseri* + GG	(*E. coli*) + *L. rhamnosus* + *GG*
E. coli (Control)	Pearson Correlation	1	.479	.315	.220
	Sig. (2-tailed)		.098	.295	.470
	N	13	13	13	13
(*E. coli*) + *L. reuteri* + GG	Pearson Correlation	.479	1	.055	.060
	Sig. (2-tailed)	.098		.857	.846
	N	13	13	13	13
(*E. coli*) + *L. gasseri* + GG	Pearson Correlation	.315	.055	1	.651*
	Sig. (2-tailed)	.295	.857		.016
	N	13	13	13	13
(*E. coli*) + *L. rhamnosus* + GG	Pearson Correlation	.220	.060	.651*	1
	Sig. (2-tailed)	.470	.846	.016	
	N	13	13	13	13

*. Correlation is significant at the 0.05 level (2-tailed).

ANOVA

Conc_GG

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	661.056	3	220.352	69.742	.000
Within Groups	151.657	48	3.160		
Total	812.713	51			

Appendix 6.2.3: Effect of *Lactobacillus* strains on *Escherichia coli* ATCC 25922 in the presence of polyethylene glycol base

Correlations

		E.coli (Control)	(*E. coli*) + L.reuteri + PP	(*E. coli*) + L.gasseri + PP	(*E. coli*) + L.rhamosus + PP
E. coli (Control)	Pearson Correlation	1	-.523	.192	-.114
	Sig. (2-tailed)		.067	.531	.710
	N	13	13	13	13
(*E. coli*) + *L. reuteri* + PP	Pearson Correlation	-.523	1	.099	-.188
	Sig. (2-tailed)	.067		.748	.538
	N	13	13	13	13
(*E. coli*) + *L. gasseri* + PP	Pearson Correlation	.192	.099	1	-.206
	Sig. (2-tailed)	.531	.748		.501
	N	13	13	13	13
(*E. coli*) + *L. rhamosus* + PP	Pearson Correlation	-.114	-.188	-.206	1
	Sig. (2-tailed)	.710	.538	.501	
	N	13	13	13	13

ANOVA

Conc_P

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	767.022	3	255.674	41.986	.000
Within Groups	292.296	48	6.090		
Total	1059.318	51			

Appendix 6.2.4: Effect of *Lactobacillus* strains on *E. coli* in the presence of Theobroma base

Correlations

		E. coli (Control)	*(E. coli) + L.reuteri + T*	*(E. coli) + L.gasseri + T*	*(E. coli) + L.rhamosus+ T*
E.coli (Control)	Pearson Correlation	1	.899**	.932**	.892**
	Sig. (2-tailed)		.000	.000	.000
	N	13	13	13	13
(E.coli) + L.reuteri + T	Pearson Correlation	.899**	1	.941**	.952**
	Sig. (2-tailed)	.000		.000	.000
	N	13	13	13	13
(E.coli) + L.gasseri + T	Pearson Correlation	.932**	.941**	1	.912**
	Sig. (2-tailed)	.000	.000		.000
	N	13	13	13	13
(E.coli) + L.rhamosus+ T	Pearson Correlation	.892**	.952**	.912**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	13	13	13	13

**. Correlation is significant at the 0.01 level (2-tailed).

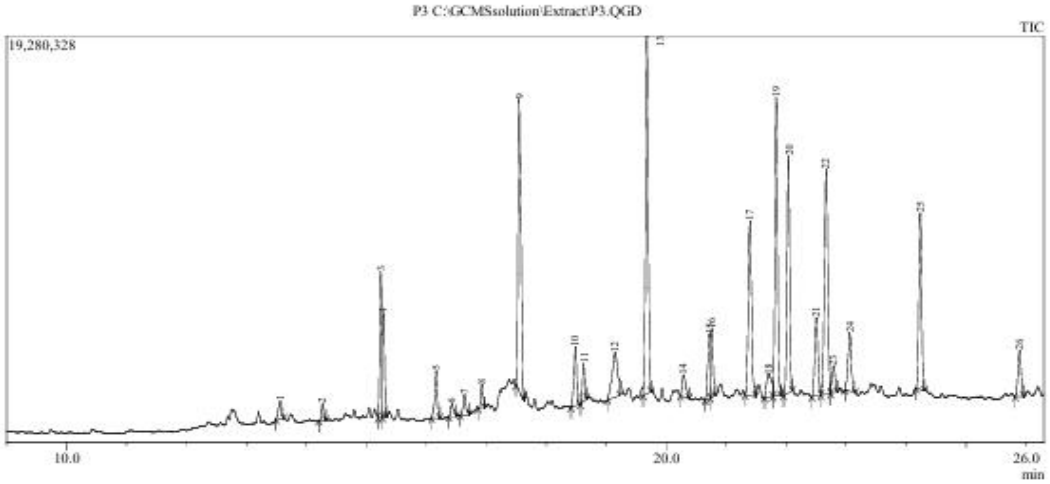
ANOVA

Conc_T

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.464	3	4.155	.224	.879
Within Groups	889.367	48	18.528		
Total	901.832	51			

Appendix 6.3

Appendix 6.3.1: *Lactobacillus gasseri* JV-VO3, and polyethylene glycol interactions

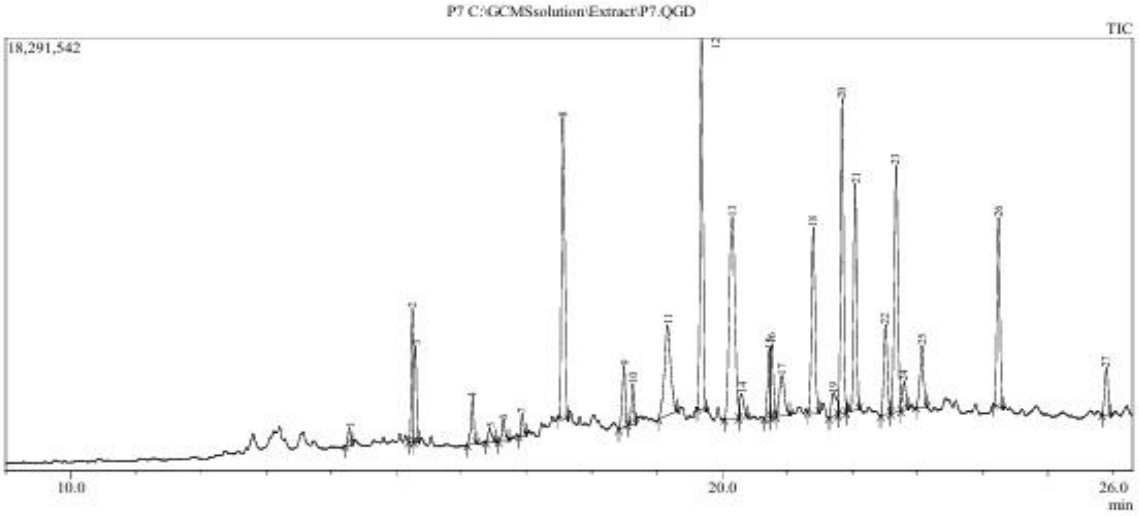


Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	13.565	3047589	0.73	866546	0.68	3.52	Cyclopentane, 1-ethyl-3-methyl-, trans-
2	14.274	2101006	0.50	807894	0.64	2.60	2-Undecanone
3	15.241	17790221	4.27	6921053	5.44	2.57	Cyclododecane
4	15.287	9912409	2.38	4908765	3.86	2.02	Tridecane
5	16.166	6758387	1.62	2260005	1.78	2.99	Cyclohexane, octyl-
6	16.426	2274673	0.55	628630	0.49	3.62	1-Dodecanol
7	16.640	3305356	0.79	983886	0.77	3.36	Dimethyl phthalate
8	16.927	3104576	0.74	1149411	0.90	2.70	Phenol, 3,5-bis(1,1-dimethylethyl)-
9	17.548	46886218	11.24	13839383	10.88	3.39	Cetene
10	18.488	8779354	2.11	2801375	2.20	3.13	Cyclohexane, decyl-
11	18.621	4672712	1.12	1808074	1.42	2.58	8-Pentadecanone
12	19.146	13828732	3.32	2123737	1.67	6.51	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
13	19.675	52823342	12.67	16963665	13.33	3.11	1-Decanol, 2-hexyl-
14	20.287	4424779	1.06	1038253	0.82	4.26	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-di
15	20.720	8544459	2.05	3042747	2.39	2.81	Cyclohexane, 1,1'-(1,4-butanediyl)bis-
16	20.759	8876875	2.13	3238447	2.55	2.74	8-Pentadecanone
17	21.395	31343532	7.52	8303125	6.53	3.77	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
18	21.695	6310570	1.51	1062839	0.84	5.94	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
19	21.835	43364671	10.40	14168127	11.14	3.06	1-Heptacosanol
20	22.037	34008544	8.15	11262156	8.85	3.02	Dibutyl phthalate
21	22.502	14170445	3.40	3705700	2.91	3.82	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
22	22.666	41252885	9.89	10665427	8.38	3.87	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
23	22.793	4622681	1.11	1284698	1.01	3.60	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
24	23.060	10580295	2.54	2781344	2.19	3.80	Cyclohexane, undecyl-
25	24.236	26545932	6.37	8407350	6.61	3.16	1-Dodecanol, 2-octyl-
26	25.891	7725465	1.85	2199527	1.73	3.51	n-Pentadecylcyclohexane
		417055708	100.00	127222164	100.00		

Appendix 6.3.2: *Lactobacillus gasseri* JV-VO3, *Escherichia coli* ATCC 25922 and polyethylene glycol interactions



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	14.278	2316111	0.50	750476	0.58	3.09	2-Undecanone
2	15.246	13715883	2.99	5653427	4.41	2.43	1-Tridecene
3	15.291	9198998	2.00	4105251	3.20	2.24	Tridecane
4	16.171	5816117	1.27	1952002	1.52	2.98	Cyclohexane, octyl-
5	16.432	2185910	0.48	582584	0.45	3.75	n-Tetracosanol-1
6	16.644	3285666	0.72	891056	0.69	3.69	Dimethyl phthalate
7	16.932	2646814	0.58	947973	0.74	2.79	Phenol, 3,5-bis(1,1-dimethylethyl)-
8	17.553	43564505	9.49	12708664	9.91	3.43	Cetene
9	18.493	8144503	1.77	2564380	2.00	3.18	Cyclohexane, decyl-
10	18.626	4860382	1.06	1714939	1.34	2.83	8-Pentadecanone
11	19.162	25423043	5.54	3818274	2.98	6.66	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
12	19.680	48446108	10.55	15704820	12.24	3.08	1-Decanol, 2-hexyl-
13	20.154	51699266	11.26	8527878	6.65	6.06	Di-n-octyl phthalate
14	20.298	4528781	0.99	1043183	0.81	4.34	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-di
15	20.725	9867856	2.15	2880119	2.24	3.43	Cyclohexane, 1,1'-(1,3-propanediyl)bis-
16	20.763	6848616	1.49	3061473	2.39	2.24	8-Pentadecanone
17	20.920	9286630	2.02	1703130	1.33	5.45	Heneicosyl heptafluorobutyrate
18	21.398	29117569	6.34	7823066	6.10	3.72	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-

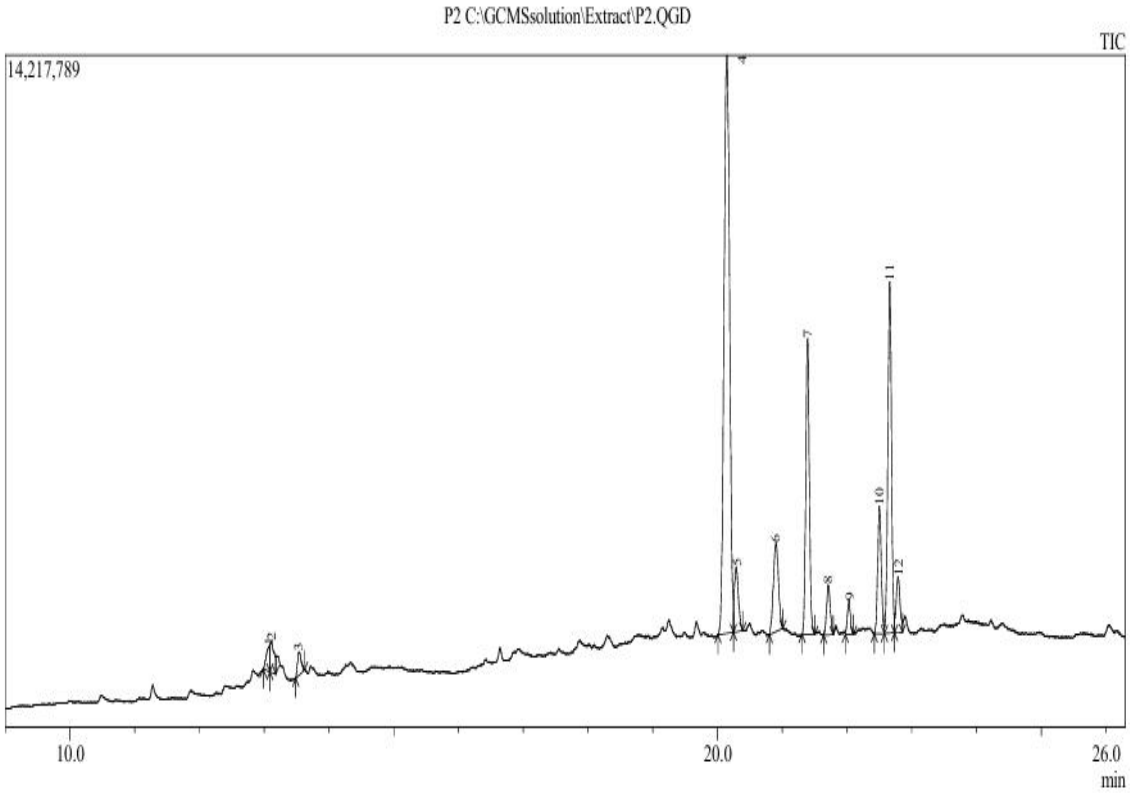
Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
19	21.700	5588862	1.22	961919	0.75	5.81	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
20	21.841	40998506	8.93	13312880	10.38	3.08	1-Heptacosanol
21	22.040	29782191	6.49	9555222	7.45	3.12	Dibutyl phthalate
22	22.509	14439057	3.15	3779835	2.95	3.82	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
23	22.669	40379715	8.80	10431333	8.13	3.87	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
24	22.792	4374854	0.95	1221788	0.95	3.58	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
25	23.066	9871851	2.15	2598137	2.03	3.80	n-Pentadecylcyclohexane
26	24.241	25512696	5.56	7989163	6.23	3.19	1-Dodecanol, 2-octyl-
27	25.898	7196134	1.57	2011709	1.57	3.58	n-Pentadecylcyclohexane
		459096624	100.00	128294681	100.00		

Library

<< Target >>

Line#:1 R.Time:14.280(Scan#:1057) MassPeaks:87

Appendix 6.3.3: *Lactobacillus reuteri* CF48-3A and polyethylene glycol interactions

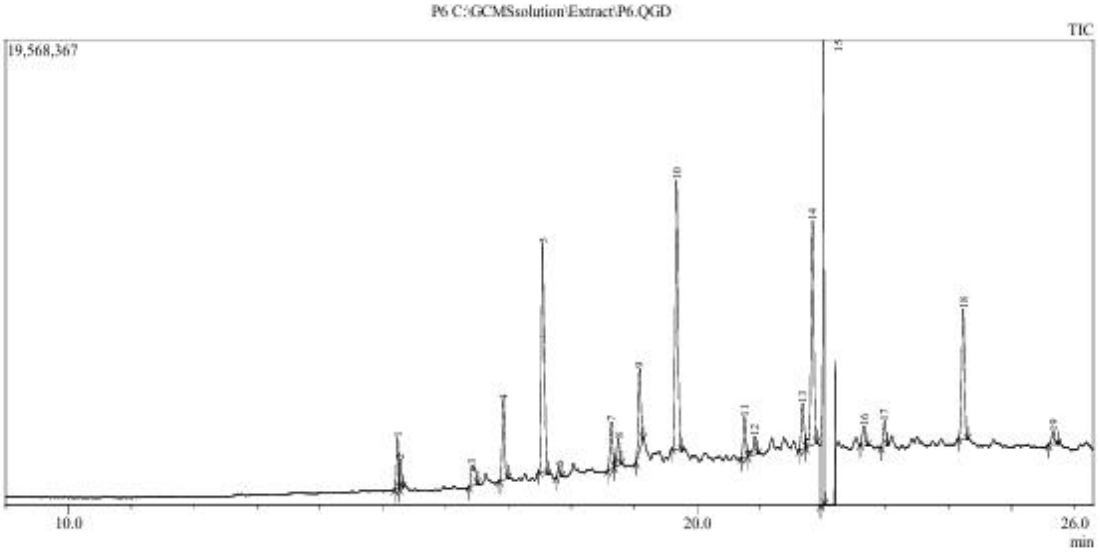


Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	13.065	1945907	1.14	517245	1.42	3.76	Dichloroacetic acid, heptadecyl ester
2	13.104	2837918	1.66	651752	1.79	4.35	Triacontyl pentafluoropropionate
3	13.535	1960124	1.15	493530	1.35	3.97	2-Piperidinone
4	20.144	73654640	43.03	12232320	33.56	6.02	Di-n-octyl phthalate
5	20.289	5932240	3.47	1372857	3.77	4.32	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-di
6	20.901	10342290	6.04	1870565	5.13	5.53	Trichloroacetic acid, hexadecyl ester
7	21.390	23904920	13.97	6252159	17.15	3.82	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
8	21.711	4157818	2.43	1065615	2.92	3.90	5-Pyrrolidino-2-pyrrolidone
9	22.033	2241438	1.31	694582	1.91	3.23	Dibutyl phthalate
10	22.500	10257070	5.99	2693051	7.39	3.81	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
11	22.659	29068294	16.98	7428330	20.38	3.91	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
12	22.786	4857606	2.84	1179683	3.24	4.12	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
		171160265	100.00	36451689	100.00		

Library

Appendix 6.3.4: *Lactobacillus reuteri* CF48-3A, *Escherichia coli* ATCC 25922 and polyethylene glycol interactions



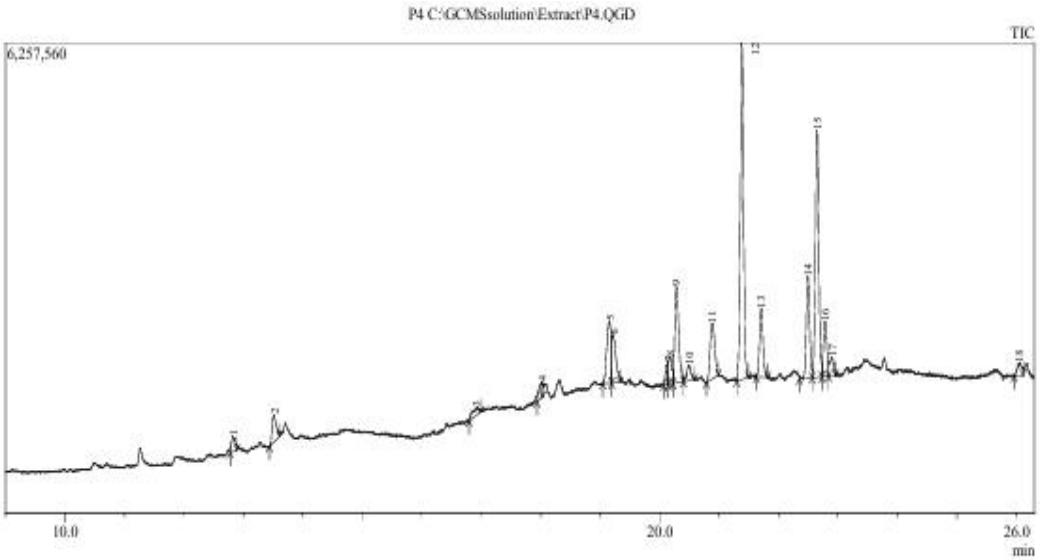
Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	15.235	5414918	2.73	2161748	2.82	2.50	Cyclododecane
2	15.285	2342721	1.18	1184743	1.55	1.98	Tridecane
3	16.422	3951177	2.00	857088	1.12	4.61	1-Dodecanol
4	16.917	9212837	4.65	3322924	4.34	2.77	Phenol, 3,5-bis(1,1-dimethylethyl)-
5	17.541	29950036	15.13	9613237	12.55	3.12	Cetene
6	17.797	1035953	0.52	426633	0.56	2.43	Lauryl acetate
7	18.636	5818243	2.94	1959999	2.56	2.97	Heptadecane
8	18.746	3247579	1.64	1136208	1.48	2.86	2-Propenoic acid, tridecyl ester
9	19.086	9586605	4.84	3444454	4.50	2.78	Phenol, 2,4-di-t-butyl-6-nitro-
10	19.669	34729730	17.54	11318308	14.77	3.07	1-Heptacosanol
11	20.753	4862439	2.46	1691012	2.21	2.88	Octadecane
12	20.915	2872641	1.45	763992	1.00	3.76	1,2-Benzenedicarboxylic acid, bis(2-methylpro
13	21.677	6061704	3.06	1908002	2.49	3.18	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
14	21.834	28839804	14.57	9427048	12.30	3.06	1-Heptacosanol
15	22.013	25203888	12.73	19568367	25.54	1.29	Dibutyl phthalate
16	22.654	2658806	1.34	778030	1.02	3.42	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3
17	22.980	3031960	1.53	1063285	1.39	2.85	Tetracosane
18	24.228	17253692	8.71	5458602	7.12	3.16	1-Dodecanol, 2-octyl-

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
19	25.664	1915140	0.97	540558	0.71	3.54	Hexacosane
		197990173	100.00	76624238	100.00		

Library

Appendix 6.3.5: *Lactobacillus rhamnosus* LMS2-3A and polyethylene glycol interactions



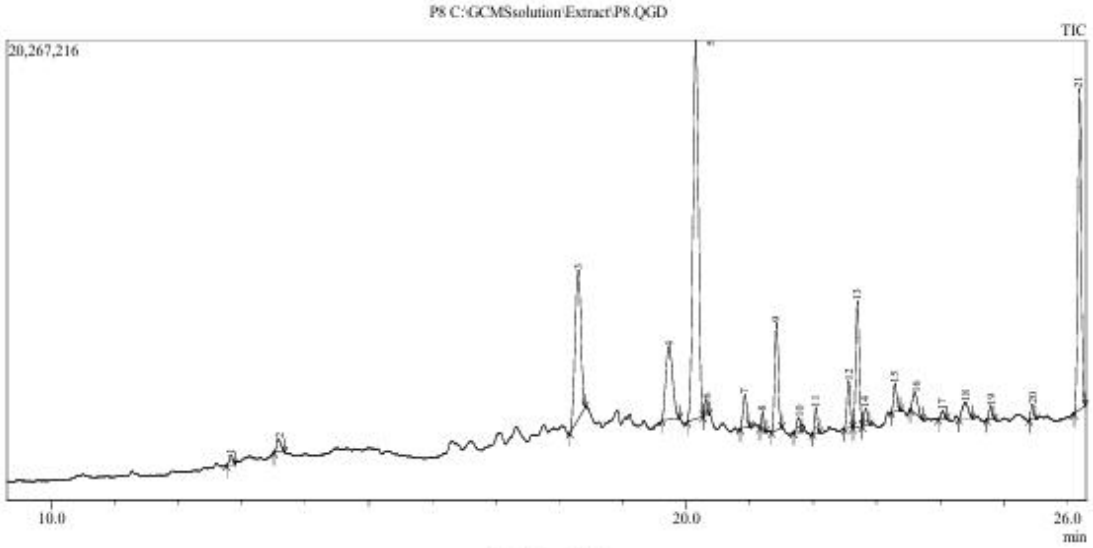
Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	12.829	903609	1.31	206498	1.26	4.38	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
2	13.517	1784660	2.59	363511	2.21	4.91	2-Piperidinone
3	16.926	819732	1.19	108377	0.66	7.56	Stigmasterol
4	18.018	911475	1.32	215432	1.31	4.23	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy
5	19.155	4246138	6.16	829635	5.05	5.12	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
6	19.225	2943958	4.27	637436	3.88	4.62	dl-Alanyl-l-leucine
7	20.140	891179	1.29	298490	1.82	2.99	Di-n-octyl phthalate
8	20.172	1362903	1.98	359921	2.19	3.79	dl-Alanyl-l-leucine
9	20.281	5905814	8.56	1282270	7.80	4.61	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-di
10	20.487	1010600	1.47	210469	1.28	4.80	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-di
11	20.889	4039481	5.86	737241	4.48	5.48	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
12	21.382	17082739	24.77	4476746	27.23	3.82	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
13	21.708	3679058	5.34	914974	5.57	4.02	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
14	22.492	5304179	7.69	1355099	8.24	3.91	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
15	22.648	13094197	18.99	3285455	19.99	3.99	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
16	22.777	2977872	4.32	727275	4.42	4.09	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
17	22.894	1083926	1.57	251023	1.53	4.32	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
18	26.051	912091	1.32	178099	1.08	5.12	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
		68955611	100.00	16437951	100.00		

Library

Appendix 6.3.6: *Lactobacillus rhamnosus* LMS2-3A, *Escherichia coli* ATCC 25922 and polyethylene glycol interactions



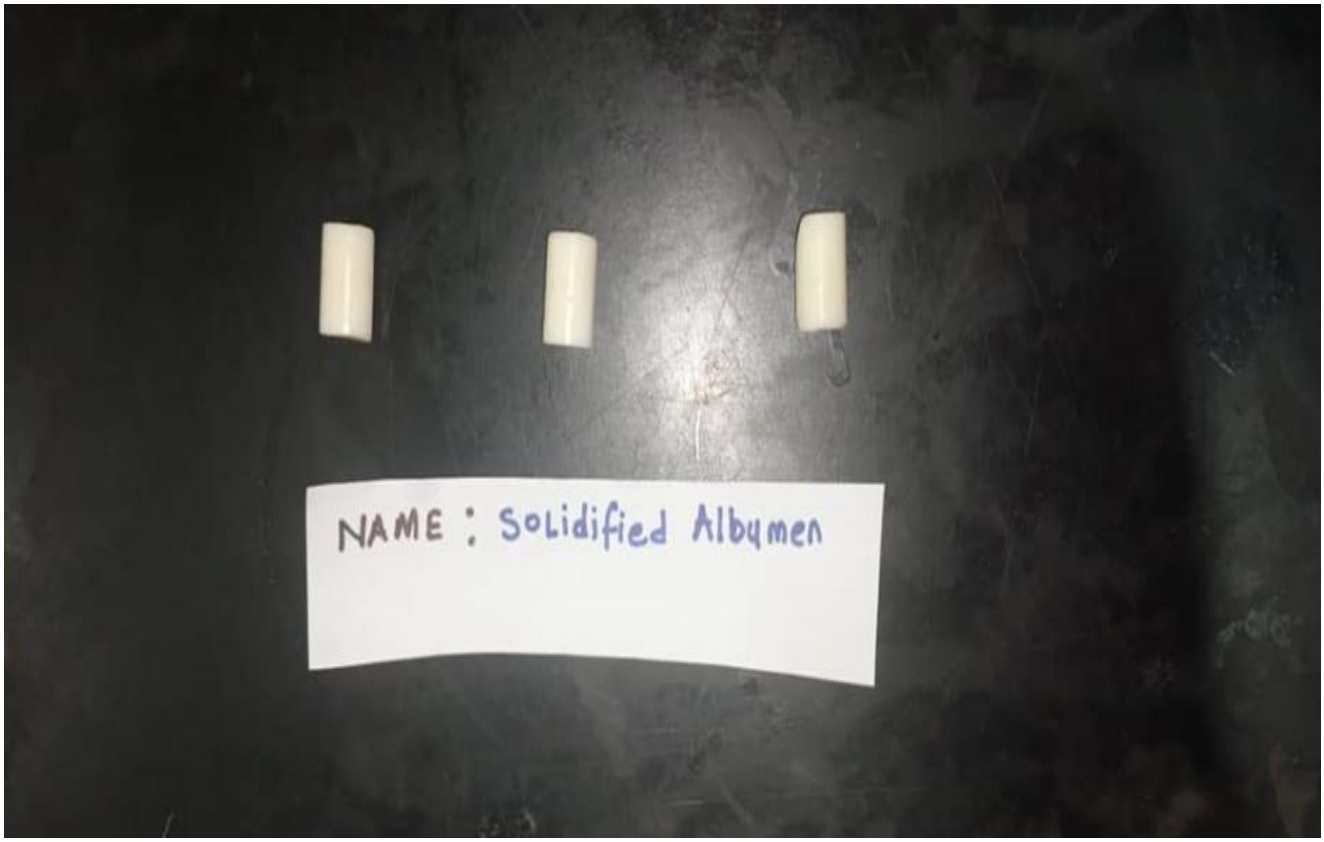
Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	12.825	1817234	0.60	452158	0.71	4.02	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
2	13.583	2723591	0.89	573955	0.90	4.75	2-Piperidinone
3	18.302	42006497	13.79	6547149	10.25	6.42	cis-9-Hexadecenal
4	19.725	23792401	7.81	3195420	5.00	7.45	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
5	20.149	96436693	31.66	16673740	26.12	5.78	Di-n-octyl phthalate
6	20.312	2220309	0.73	718470	1.13	3.09	3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-di
7	20.928	5929193	1.95	1446816	2.27	4.10	1,2-Benzenedicarboxylic acid, diundecyl ester
8	21.201	1890140	0.62	791570	1.24	2.39	Hexadecanoic acid, methyl ester
9	21.422	18154589	5.96	4734243	7.41	3.83	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
10	21.774	2526704	0.83	645207	1.01	3.92	5-Isopropylidene-3,3-dimethyl-dihydrofuran-2
11	22.043	3226751	1.06	1113334	1.74	2.90	Dibutyl phthalate
12	22.556	7545345	2.48	2071241	3.24	3.64	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
13	22.699	20770031	6.82	5528426	8.66	3.76	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
14	22.825	2800480	0.92	750102	1.17	3.73	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-
15	23.286	4765130	1.56	1244329	1.95	3.83	9-Octadecenoic acid (Z)-, methyl ester
16	23.604	4847475	1.59	892224	1.40	5.43	6-Ethyl-3-trimethylsilyloxydecane
17	24.036	1237214	0.41	350713	0.55	3.53	2(3H)-Furanone, dihydro-5-tetradecyl-
18	24.394	4832564	1.59	726143	1.14	6.66	Tetracosane

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
19	24.794	2109881	0.69	599041	0.94	3.52	Hexadecanamide
20	25.447	2211021	0.73	661343	1.04	3.34	Hexadecanoic acid, 1-[[(2-aminoethoxy)hydr
21	26.196	52762976	17.32	14131693	22.13	3.73	Hexadecanoic acid, 1-[[(2-aminoethoxy)hydr
		304606219	100.00	63847317	100.00		

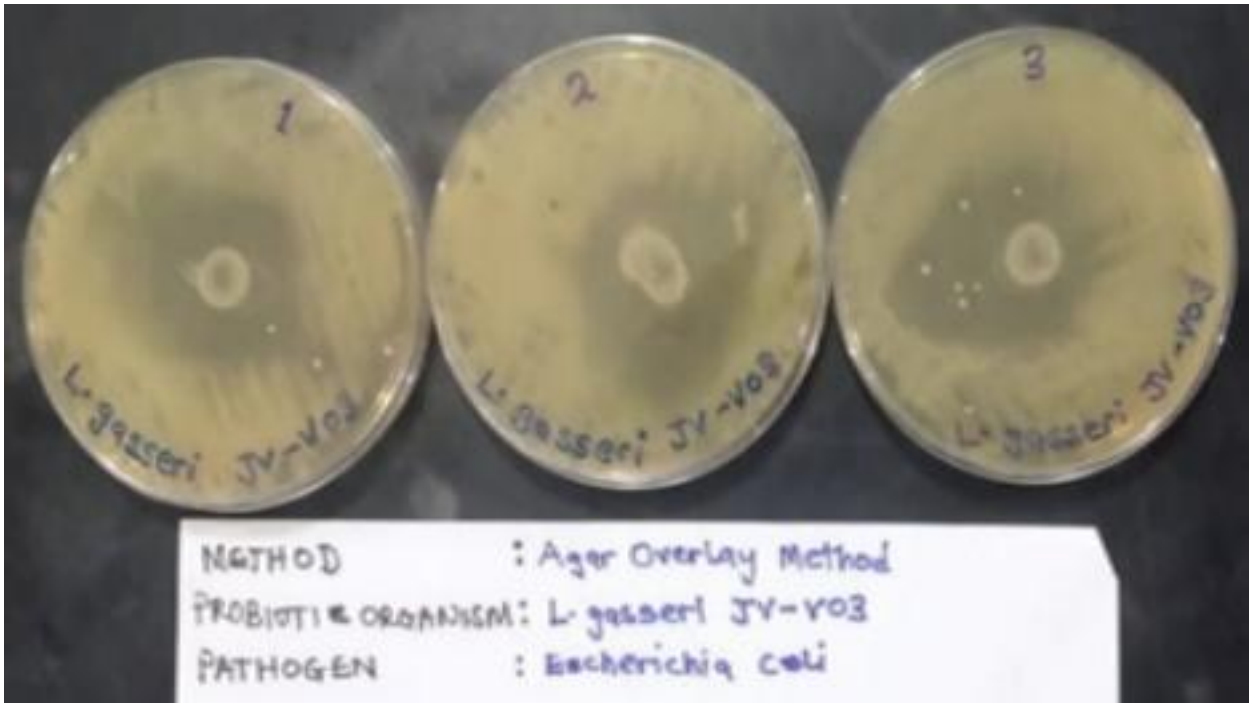
Appendix 6.4

Appendix 6.4: Pictures of solidified albumen



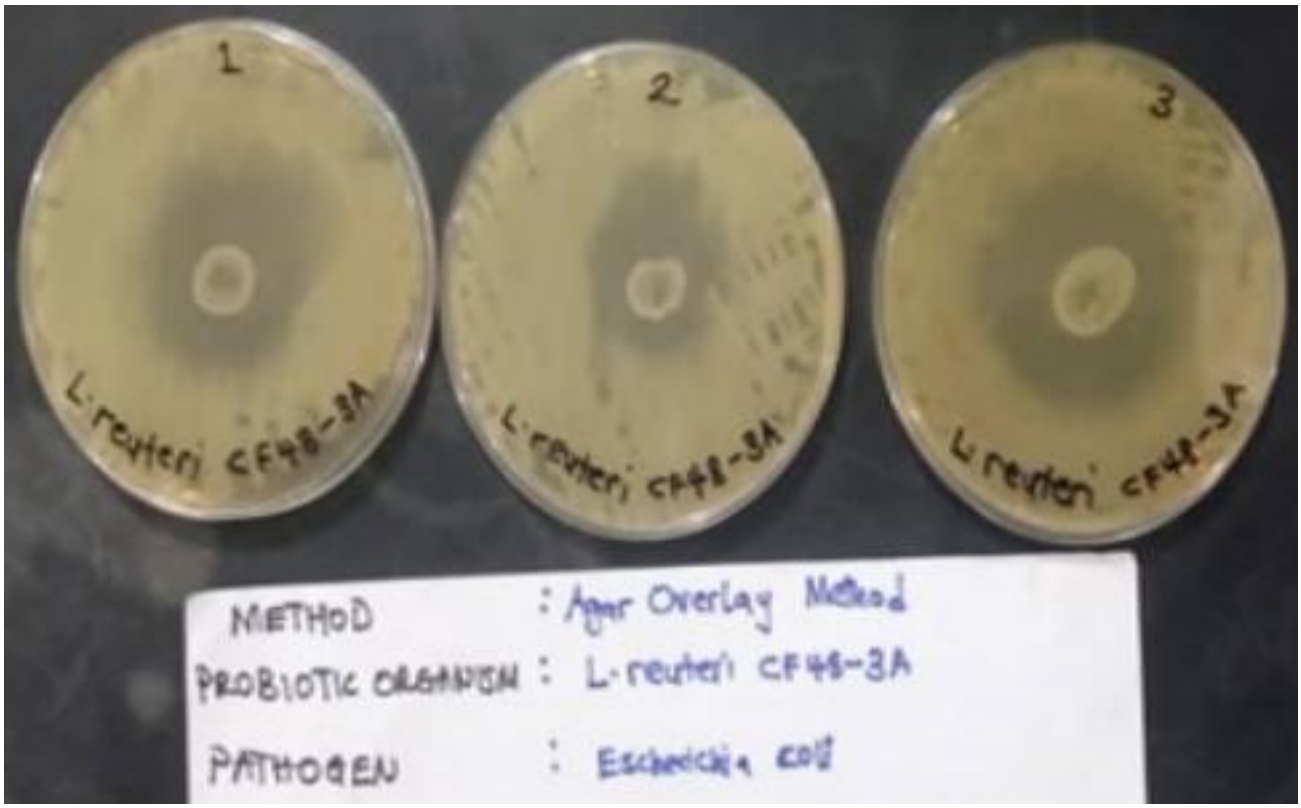
Appendix 6.5

Appendix 6.5.1: Pictures of Agar overlay results of *L. gasseri* JV-VO3 against *E. coli* 25922



Appendix 6.5.2: Pictures of Agar overlay results of *L. rhamnosus* LMS2-1 against *E. coli* 25922

Appendix 6.5.3: Pictures of Agar overlay results of *L. reuteri* CF48-3A against *E. coli* 25922



Appendix 6.6

Appendix 6.6.1: Two-way ANOVA for the de-adhesion effect of *Lactobacillus* strains by Tween20

CONCENTRATION	L.rhamnosus	L.gasseri	L.reuteri
100	5.77	5.26	4.5
75	6.83	5.82	8.74
50	9.68	9.43	9.45
25	8.62	4.82	5.26
12.5	8.26	5.62	5.54

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
100	3	15.53	5.176667	0.408433
75	3	21.39	7.13	2.1991
50	3	28.56	9.52	0.0193
25	3	18.7	6.233333	4.320533
12.5	3	19.42	6.473333	2.395733
L.rhamnosus	5	39.16	7.832	2.36877
L.gasseri	5	30.95	6.19	3.4253
L.reuteri	5	33.49	6.698	4.99582

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	31.54033	4	7.885083	5.42899	0.020608	3.837853
Columns	7.066973	2	3.533487	2.432855	0.149494	4.45897
Error	11.61923	8	1.452403			
Total	50.22653	14				