ISOLATION OF POTENTIAL PROBIOTIC YEASTS FROM FRUITS AND VEGETABLES.

A Project Report

By

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DECLARATION PAGE

This is to declare that this research project titled "Isolation of Potential Probiotic Yeasts from Fruits and Vegetables", carried out by me, Daniel Faith Toluwanimi, in the Department of Medical Laboratory Science, Faculty of Basic Medical and Health Sciences, Thomas Adewumi University Oko-Irese, Kwara State, is solely the result of my work except where acknowledged as being derived from other person(s) work or resources.

Signature:_____ _____ **Date:** **09-08-2025**

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CERTIFICATION

I declare that this project report is my original work and has not been previously submitted to any other institution of higher learning.

I further certify that all sources cited or quoted are duly acknowledged by means of a comprehensive list of references.

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ABSTRACT

Antimicrobial resistance (AMR) is a global health crisis that was directly responsible for 1.27 million deaths in 2019 and contributed to an additional 4.95 million deaths globally in 2019. Probiotics have emerged as a promising alternative to antibiotics, providing a natural approach to maintaining gut health and preventing infections. This study aimed to isolate and characterize potential probiotic yeasts from commonly consumed fruits and vegetables in Ilorin, Nigeria. Samples of lettuce, tomato, garden egg, watermelon, cucumber, cabbage, and grapes were collected, processed under aseptic conditions, and cultured on yeast extract peptone dextrose (YEPD) media. Morphological and biochemical analyses were conducted, followed by assessments of probiotic properties, including bile salt tolerance, acid and alkaline resistance, osmotic stress tolerance under glucose concentrations (30%, 40%, and 50%), and antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. During this submission, the Yeast isolates are still undergoing molecular identification, so they are referred to as Yeast A, B, and C. The results revealed that several yeast isolates, particularly those labeled YEAST A and YEAST C, demonstrated high tolerance to acidic pH (3.0), alkaline pH (8.0), and bile concentrations up to 0.5%. They also showed strong growth under high osmotic stress. Antimicrobial activity was observed against *S. aureus*, though not significantly against *E. coli*. These findings suggest that selected yeast strains from local produce possess promising probiotic traits and may serve as potential candidates for use in functional foods or probiotic formulations. Further in vivo evaluation is recommended to confirm safety and efficacy.

Keywords:
Non-pathogenic yeasts, probiotics, fruits and vegetables, bile tolerance, osmotic stress, and antimicrobial activity.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Antimicrobial resistance (AMR) is a global health crisis that threatens to undermine decades of medical progress. In 2019, AMR was directly responsible for 1.27 million deaths and contributed to an additional 4.95 million deaths globally (Christaki *et al.,* 2020). This growing crisis has been fueled by the overuse and misuse of antibiotics in healthcare, agriculture, and animal husbandry. As bacteria develop resistance to antibiotics, infections become increasingly difficult to treat, leading to higher mortality rates, prolonged hospital stays, and rising healthcare costs (Abdus-Salam *et al.,* 2023).

Probiotics have emerged as a promising alternative to antibiotics, providing a natural approach to maintaining gut health and preventing infections. The concept of probiotics has a long and fascinating history, rooted in ancient practices and evolving alongside scientific advancements. The term "probiotic," derived from the Latin phrase "pro-life," highlights its beneficial role in promoting health. Historically, fermented foods such as beer, bread, cheese, yogurt, and kefir were commonly consumed for their therapeutic and nutritional properties. These foods were often discovered through spontaneous fermentation processes, as seen in the Middle East and Central Asia, where the fermentation of milk in animal skin bags likely gave rise to early forms of yogurt (Mcfarland, 2015).

Probiotics play a critical role in maintaining human health by contributing to the balance of the gut microbiota. The gastrointestinal tract is home to trillions of microorganisms, and a disruption in this balance, often caused by antibiotics, infections, or poor dietary habits can lead to conditions like diarrhea, irritable bowel syndrome (IBS), and inflammatory bowel diseases (Ouwehand *et al.,* 2002). Probiotics restore this equilibrium by introducing beneficial microbes that help prevent the overgrowth of harmful

bacteria, improve digestion, and enhance nutrient absorption. They have also been shown to modulate the immune system, reducing the risk of infections and autoimmune conditions (Marco *et al.,* 2006).

Beyond gastrointestinal health, probiotics may have systemic effects, including lowering cholesterol levels, managing weight, and improving mental health through the gut-brain axis. (Cryan and Dinan, 2012).

The systematic study of probiotics began with Élie Metchnikoff in the early 20th century. Metchnikoff, a Nobel Prize-winning scientist, proposed that certain fermented dairy products could promote longevity by fostering beneficial gut microbiota. His work inspired further research into identifying specific microbial strains that could confer health benefits (Mcfarland, 2015).

Probiotics gained global recognition in the mid-20th century as advancements in microbiology allowed for the isolation and identification of specific strains. These discoveries paved the way for modern probiotic products, which are now widely available and used for a variety of health conditions. Despite their popularity, challenges remain in areas such as strain-specific functionality, regulation, and clinical validation (Ozen and Dinleyici, 2015).

1.1.1 Mechanism of Action of Probiotics

Probiotics exert their benefits through multiple mechanisms. Firstly, they compete with pathogenic bacteria for nutrients and adhesion sites on the intestinal lining, effectively reducing the colonization of harmful microbes. They also produce antimicrobial substances such as bacteriocins and organic acids, which inhibit the growth of pathogens. Additionally, probiotics strengthen the gut barrier by enhancing the production of mucins and tight-junction proteins, preventing the translocation of harmful bacteria into the bloodstream (Ouwehand *et al.,* 2002).

Probiotics are known to modulate immune responses by interacting with gut-associated lymphoid tissue (GALT). This interaction helps regulate inflammatory responses and promotes the production of protective antibodies like immunoglobulin A (IgA). Such immune-modulating properties are particularly beneficial in reducing the severity of allergies and autoimmune disorders (Marco *et al.,* 2006).

1.1.2 Sources of Probiotics

Probiotics can be obtained from a variety of dietary and supplemental sources. Fermented foods are among the most traditional and accessible sources, including:

Dairy-based products*:* Yogurt, kefir, and certain cheeses contain strains like Lactobacillus and Bifidobacterium, which are well-documented for their health benefits.

Non-dairy options: Fermented vegetables such as kimchi, sauerkraut, and pickles provide probiotics for those with lactose intolerance or dietary restrictions.

Beverages: Kombucha and other fermented teas are rich in probiotics and antioxidants.

Supplements: Probiotic capsules or powders are formulated to contain specific strains for targeted health benefits (Sanders, 2008).

These diverse sources make probiotics widely accessible, allowing individuals to incorporate them into their diets for improved health outcomes. While bacterial probiotics are well-researched and widely used, they are not without limitations. One significant disadvantage is the potential for horizontal gene transfer, where antibiotic resistance genes present in bacterial probiotics may be transferred to harmful bacteria in the gut. This poses a risk of exacerbating antimicrobial resistance (AMR), a global health crisis. Additionally, bacterial probiotics are often sensitive to environmental conditions, such as gastric acidity and bile salts, which can reduce their survival and effectiveness in the gastrointestinal tract (Gueimonde *et al.,* 2013; Sharma *et al.,* 2014; Zmora *et al.,* 2018).

In contrast, non-pathogenic yeasts remain an underexplored yet promising area of study. Yeasts like *Saccharomyces boulardii* offer unique advantages, including resilience to harsh gastrointestinal conditions and an inability to transfer resistance genes. Moreover, they have demonstrated significant probiotic potential through pathogen inhibition, immune modulation, and anti-inflammatory properties (*Pais et al.,* 2020). This highlights the need to expand research beyond bacterial probiotics to explore the untapped potential of yeast-based alternatives for gut health and infection prevention.

Non-pathogenic yeasts naturally present in fruits and vegetables offer several advantages over bacterial probiotics. They are more resilient to harsh gastrointestinal conditions and do not carry antibiotic-resistant genes, reducing the risk of contributing to AMR. This research focuses on isolating and evaluating the probiotic potential of these yeasts to provide a sustainable and safe alternative to conventional probiotics (Pais *et al.,* 2020).

1.2 Statement of the Problem

The global rise in AMR has rendered many antibiotics ineffective, creating a dire need for alternative strategies to manage infections and maintain gut health. Probiotic bacteria, while beneficial, are not without risks. Studies have highlighted concerns regarding the horizontal transfer of antibiotic-resistance genes from bacterial probiotics to pathogenic bacteria in the gut (Alvarez *et al.,* 2023). This risk underscores the need for non-bacterial alternatives.

Yeasts offer a promising solution due to their unique attributes. Unlike bacteria, yeasts are less likely to transfer resistance genes, and their robust cell walls make them better suited to su1rvive the acidic and bile-rich conditions of the gastrointestinal tract (Zahoor *et al.,* 2021). Despite their potential, the use of non-pathogenic yeasts as probiotics remains underexplored, particularly in the context of fruits and vegetables commonly consumed in Nigeria. Addressing this gap in research could pave the way for innovative solutions to combat AMR while promoting gut health.

1.3 Justification of the Study

This research is significant for several reasons:

Antimicrobial Resistance Mitigation: By exploring yeast-based probiotics, this study offers a potential solution to reduce dependency on antibiotics, contributing to the global effort to combat AMR (Pais *et al.,* 2020).

Novel Probiotic Development: The findings will expand the understanding of non-pathogenic yeasts as probiotics, creating opportunities for their integration into dietary supplements or functional foods.

Public Health Impact: The research addresses the growing demand for safe, natural, and sustainable alternatives to conventional probiotics and antibiotics.

Economic Benefits: Developing yeast-based probiotics from locally sourced fruits and vegetables could lead to cost-effective solutions, benefiting both producers and consumers in Nigeria.

1.4 Research Questions

This study seeks to answer the following questions:

I. Can non-pathogenic yeasts be successfully isolated from commonly consumed vegetables and fruits?
II. Do the isolated yeast strains exhibit the ability to survive under harsh gastrointestinal conditions?
III. How effective are these yeast strains at inhibiting pathogens such as *Escherichia coli* and *Staphylococcus aureus*?

1.5 Research Hypothesis

Null Hypothesis (H_0):

Non-pathogenic yeasts isolated from selected vegetables and fruits sold in Ilorin, Nigeria, do not exhibit significant probiotic properties such as bile and acid tolerance, antimicrobial activity, and osmotic stress resistance**.**

Alternative Hypothesis (H_1):

Non-pathogenic yeasts isolated from selected vegetables and fruits sold in Ilorin, Nigeria, exhibit significant probiotic properties, including bile and acid tolerance, antimicrobial activity, and osmotic stress resistance.

1.6 Aim of the Study

The aim of this research is to assess the presence of potential probiotic yeasts in fruits and vegetables vended in Ilorin, Nigeria,

1.7 Objectives of the Study

This study aims to achieve the following objectives:

- Isolate and identify non-pathogenic yeasts from commonly consumed vegetables and fruits, such as lettuce (*Lactuca sativa*), garden egg (*Solanum aethiopicum*), tomatoes (*Solanum lycopersicum*), and watermelon (*Citrullus lanatus*).
- Assess probiotic potential through testing yeast survival in simulated gastric juice and osmotic stress conditions.
- Investigate pathogen inhibition by testing the ability of yeast strains to inhibit *Escherichia coli* and *Staphylococcus spp*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspective on Probiotics

The history of probiotics dates back to ancient times, with the consumption of fermented foods being a common practice across various civilizations. Different types of fermented milk products were invented in various generations, such as Kefir in approximately 5000 BC and the use of yeast in 5000 BC. Their history can be traced to the first use of cheese and fermented products, which were well-known to the Greeks and Romans who recommended their consumption (Gismondo *et al.,* 2022). The fermentation of dairy foods represents one of the oldest techniques for food preservation (Tortora *et al.,* 2019).

The term "probiotic" originally referred to microorganisms that have effects on other microorganisms (Lilly and Stillwell, 1965). The concept of probiotics involved the notion that substances secreted by one microorganism stimulated the growth of another microorganism. The term was used again by Sperti (1971) to describe tissue extracts that stimulated microbial growth. The term probiotics was taken up by Parker (1974), who defined the concept as, "Organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance." Later, the definition was improved by Fuller (1989), whose explanation described probiotics as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." He stressed two important claims for probiotics: the viable nature of probiotics and the capacity to help with intestinal balance.

Fermented foods such as yogurt, kefir, sauerkraut, and kimchi are natural reservoirs of probiotics, particularly lactic acid bacteria (LAB) and yeasts. These microorganisms play a crucial role in fermentation, enhancing the flavor, texture, and shelf life of foods while contributing to their health-promoting properties (Saxena and Gupta, 2020).

In ancient times, fermentation was primarily used as a preservation method. However, over time, the health benefits of consuming fermented products became widely recognized. For instance, yogurt, which originated in the Middle East and Central Asia, was traditionally made using bacterial strains like Lactobacillus bulgaricus and Streptococcus thermophilus. These microorganisms are still widely used today for their probiotic benefits and ability to support gut health (Aryana and Olson, 2017).

Kefir, another popular fermented product, has a history dating back to the Caucasus Mountains. It is made using kefir grains, which are a complex symbiotic matrix of bacteria and yeasts. Kefir has been studied extensively for its ability to improve gut microbiota, boost immunity, and provide antimicrobial effects (Lillie *et al.,* 2022). Similarly, fermented vegetables like sauerkraut and kimchi have been staples in European and East Asian diets, known for their rich probiotic content and role in improving digestive health (Yuan *et al.,* 2023).

Over the past century, scientific advancements have deepened our understanding of probiotics in fermented foods. Researchers have identified the mechanisms through which probiotics in these foods promote gut health, including pathogen inhibition, modulation of the immune system, and production of bioactive compounds. Additionally, the commercial production of fermented foods has evolved, with an increasing focus on the selection of specific probiotic strains for targeted health benefits (Grujović *et al.,* 2022).

2.1.1 Early Practices in Fermentation

Evidence of fermentation practices has been found in ancient civilizations across the globe.

Mesopotamia and Egypt (6600 BCE): Early records indicate that beer and bread were produced through fermentation, likely involving wild yeasts. These foods were staples, providing nourishment and long shelf life.

India and China (3000 BCE*):* Fermented milk products like yogurt and fermented soy products like soy sauce were part of traditional diets. These foods were valued for their perceived health benefits and their ability to improve digestion (Grujović *et al.,* 2022).

Greek and Roman Empires*:* Greek physicians, including Hippocrates, extolled the virtues of sour milk, while Romans developed fermented fish sauces and wines. These practices underscored fermentation's role in enhancing food and health (Yuan *et al.,* 2023).

2.1.2 Scientific Recognition of Fermented Foods as Probiotic Sources

The transition from traditional knowledge to scientific exploration of probiotics began in the early 20th century with Élie Metchnikoff, a Nobel Prize-winning scientist. He observed the longevity of Bulgarian peasants who consumed fermented milk and hypothesized that certain bacteria in yogurt (e.g., *Lactobacillus bulgaricus*) could prevent harmful gut microbes and extend lifespan. His landmark publication, The Prolongation of Life: Optimistic Studies (1908), laid the foundation for the scientific study of probiotics (Aryana and Olson, 2017).

Kefir, originating from the Caucasus region, is another fermented food with ancient roots. Known as the "drink of the prophet," kefir grains are a symbiotic community of bacteria and yeast that ferment milk into a tangy, effervescent beverage. The probiotic benefits of kefir include modulation of gut microbiota, enhanced immunity, and reduction of gut inflammation, making it one of the earliest examples of a functional food with therapeutic potential (Lillie *et al.,* 2022).

2.1.3 Cultural Staples of Fermented Foods Globally

Fermented foods like sauerkraut (fermented cabbage) and kimchi (a Korean staple) are rich in lactic acid bacteria (LAB), which enhance gut health. These foods evolved not only as preservation methods but also

as sources of essential nutrients. Sauerkraut, for example, became critical during long sea voyages to prevent scurvy due to its high vitamin C content and probiotic activity (Yuan *et al.,* 2023).

In African and Asian cultures, non-dairy fermented foods, such as fermented cassava (*gari*) and soy products (*tempeh*), provide probiotics. These foods often feature LAB and yeasts that contribute to digestive health and nutrient bioavailability (Swain *et al.,* 2024).

2.1.4 Probiotics in Modern Times

By the mid-20th century, advancements in microbiology enabled the isolation and characterization of probiotic strains from traditional fermented foods. Probiotic research expanded beyond dairy to include non-dairy alternatives, addressing the needs of lactose-intolerant populations. Today, traditional foods remain vital to probiotic research, serving as reservoirs of novel strains with potential health benefits (Murad *et al.,* 2024)

.2.1.5 Early Focus on Bacterial Probiotics and Their Widespread Adoption

The scientific interest in probiotics initially centered on bacterial strains, particularly lactic acid bacteria (LAB), due to their prevalence in fermented foods and their beneficial effects on gut health. Early research by Élie Metchnikoff, a Nobel Prize-winning scientist, identified LAB as a key component of fermented milk that could inhibit harmful bacteria in the gut. This discovery established the foundation for modern probiotic research, positioning bacterial probiotics as a promising therapeutic tool for enhancing human health (Aryana and Olson, 2017).

The dominance of bacterial probiotics in the 20th century can be attributed to their compatibility with dairy-based delivery systems such as yogurt, kefir, and cultured milk. Strains like *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* became the focus of extensive studies due

to their ability to colonize the gut, improve digestion, and restore microbial balance after antibiotic use (Saxena and Gupta, 2020).

As research progressed, bacterial probiotics were shown to exhibit mechanisms such as:

Gut Microbiota Modulation: *Restoring the balance of beneficial and harmful bacteria in the gut (Zmora et al., 2018).*

Pathogen Inhibition: *Competitive exclusion of pathogens through the production of organic acids, hydrogen peroxide, and bacteriocins (Goyal et al., 2022).*

Immune Modulation: *Enhancing the gut's immune response by stimulating the production of immunoglobulins and cytokines (Mazziotta et al., 2023).*

The adoption of bacterial probiotics expanded rapidly with advancements in food processing and biotechnology. By the late 20th century, commercially available probiotics were widely incorporated into dairy products, supplements, and functional foods. This shift was driven by consumer demand for natural and preventive health solutions, as well as increasing scientific evidence supporting the efficacy of bacterial probiotics in managing gastrointestinal disorders, lactose intolerance, and even mental health through the gut-brain axis (Grujović *et al.,* 2022).

Despite their success, bacterial probiotics face limitations, including their sensitivity to harsh gastrointestinal conditions, such as stomach acid and bile. This has spurred interest in alternative probiotics, such as non-pathogenic yeasts, which demonstrate greater resilience and potential for broader applications (Lillie *et al.,* 2022).

2.1.6 The Emergence of Yeast Probiotics in Recent Years

The field of probiotics has long been dominated by bacterial strains such as *Lactobacillus* and *Bifidobacterium*. These bacterial probiotics have been celebrated for their role in enhancing gut health, modulating the immune system, and preventing gastrointestinal disorders. However, advancements in probiotic research have highlighted the potential of yeast-based probiotics, particularly *Saccharomyces boulardii* and other non-pathogenic yeast strains. Yeasts have gained traction as alternative or complementary probiotics, demonstrating unique advantages that extend beyond the capabilities of traditional bacterial strains. Importantly, the emergence of yeast probiotics does not signify a replacement for lactic acid bacteria (LAB) but rather emphasizes the potential for synergistic roles where LAB and yeasts complement one another (Tullio, 2024).

2.2 Properties of Yeast Probiotics

Yeast probiotics offer several distinctive features that make them valuable in probiotic research and applications. Unlike bacterial strains, yeast probiotics are eukaryotic, which endows them with structural and functional differences that provide unique benefits:

Resilience in Harsh Conditions*:* Yeast probiotics can survive extreme gastrointestinal environments, including low pH and bile-rich conditions, where many bacterial probiotics are less effective. This makes yeasts particularly reliable for delivering probiotic benefits in the digestive tract.

Pathogen Inhibition*:* Yeast probiotics produce antimicrobial compounds, such as short-chain fatty acids and bioactive peptides, that inhibit the growth of pathogenic bacteria, including E. coli and Staphylococcus. These mechanisms reduce infection risks without contributing to antimicrobial resistance (Balabekyan *et al.,* 2018).

Immune Modulation: Yeasts are known to stimulate the host's immune response by enhancing the production of immunoglobulins and cytokines. This immune support is particularly valuable in recovering from infections or during antibiotic treatments.

Gut Health Restoration*:* Yeast probiotics are highly effective in mitigating dysbiosis, especially after the use of antibiotics. Unlike bacteria-based probiotics, which may struggle in post-antibiotic conditions, yeasts remain stable and active, aiding in the restoration of gut microbiota (Grujović *et al.,* 2022).

These unique characteristics of yeast probiotics have made them suitable for diverse applications, ranging from improving gut health to treating conditions such as antibiotic-associated diarrhea, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD).

Additionally, their ability to resist environmental stressors during processing and storage has further solidified their position as viable probiotics in both food and pharmaceutical industries (Balabekyan *et al.,* 2018).

2.2.1 Factors Driving Interest in Yeast Probiotics

The growing focus on yeast probiotics is driven by their ability to address some limitations associated with bacterial strains. While LABs are effective in many cases, they are often sensitive to harsh gastrointestinal conditions and require encapsulation or other protective measures to maintain viability. Yeast probiotics, such as *Saccharomyces boulardii*, naturally withstand these conditions, making them a more robust option for oral delivery (Pais, Almeida, Yılmaz, and Teixeira, 2020). Moreover, yeast probiotics have shown promise in addressing global health challenges such as antimicrobial resistance (AMR). By inhibiting pathogenic bacteria without contributing to AMR, yeast probiotics present a sustainable solution for reducing antibiotic dependency and improving infection control (Tullio, 2024).

2.2.2 Complementary Roles of LAB and Yeasts

It is crucial to note that yeast probiotics are not intended to replace LAB but to complement them. LAB remain essential due to their well-documented health benefits, including lactic acid production, gut microbiota modulation, and support for lactose digestion. Yeasts, on the other hand, bring additional advantages, such as enhanced survivability and broader antimicrobial effects. Together, LAB and yeast probiotics can form a holistic approach to gut health and disease prevention, with each contributing unique strengths to probiotic therapy (Balabekyan *et al.,* 2018).

For example, combined cultivation of LAB and yeast strains has been shown to increase antimicrobial activity through synergistic effects. These combinations can inhibit multidrug-resistant bacteria more effectively than either type alone, demonstrating the potential of integrated probiotic formulations (Balabekyan *et al.,* 2018).

2.2.3 Implications for Probiotic Development

The rise of yeast probiotics represents a paradigm shift in the field, expanding the scope of probiotic research beyond bacterial strains. This shift is particularly significant in addressing the limitations of bacterial probiotics and exploring new applications in gut health, immune modulation, and infection prevention (Mazziotta *et al.,* 2023). Yeasts also provide a promising avenue for developing probiotics that are more accessible, sustainable, and versatile, particularly in regions where traditional probiotic delivery methods may be less feasible (Joossens, 2020).

As research into yeast probiotics continues to advance, it is becoming increasingly clear that these eukaryotic microorganisms have the potential to transform the probiotic landscape. By complementing LAB and offering unique benefits, yeast probiotics can contribute to a more comprehensive and effective approach to health and wellness (Pais *et al.,* 2020).

2.2.4Mechanisms of Action of Probiotics

Probiotics influence the human body through various mechanisms, primarily by modulating the gut microbiota and inhibiting the growth of harmful pathogens. These mechanisms underpin their beneficial effects on digestion, immunity, and overall health (Liu *et al.,* 2022).

2.2.5 **Gut Microbiota Modulation**

The human gut microbiota is a complex ecosystem comprising trillions of microorganisms, including bacteria, fungi, and viruses. Maintaining a balance between beneficial and harmful microbes is essential for digestive health and immune function. Probiotics play a pivotal role in modulating this delicate balance by promoting the growth of beneficial bacteria and inhibiting harmful ones (Zmora, Suez, and Elinav, 2018; Ouwehand, Salminen, and Isolauri, 2002).

Enhancing Microbial Diversity*:* Probiotics can restore microbial diversity, which is often reduced due to poor diet, illness, or antibiotic use (Mazziotta *et al.,* 2023). By increasing the abundance of beneficial bacteria such as Lactobacillus and Bifidobacterium, probiotics create a healthier gut environment.

Regulating Metabolite Production: Probiotics produce short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate, which have anti-inflammatory properties and serve as energy sources for gut epithelial cells (Liu *et al.,* 2022).

Strengthening Gut Barrier Function: Probiotics enhance the gut's physical barrier by increasing the production of tight junction proteins, which prevent the translocation of harmful bacteria and toxins into the bloodstream (Walter, 2018).

2.2.6 Competitive Exclusion of Pathogens

Another critical way probiotics benefit the human body is by inhibiting the growth and colonization of harmful pathogens, such as Escherichia coli and Staphylococcus. This is achieved through a process known as competitive exclusion.

Competition for Nutrients*:* Probiotics outcompete pathogens for essential nutrients, limiting their ability to grow and colonize the gut (Bang *et al.,* 2022).

Adhesion to Intestinal Surfaces: Probiotics adhere to the intestinal epithelium, occupying attachment sites that might otherwise be used by pathogens. For example, studies have shown that probiotic coatings can enhance adhesion properties, making it harder for harmful bacteria to establish themselves (Yu *et al.,* 2022).

Production of Antimicrobial Compounds*:* Probiotics secrete antimicrobial peptides and organic acids that inhibit the growth of pathogens. These compounds lower the pH of the gut environment, creating conditions unfavorable for pathogenic bacteria (Ebrahem *et al.,* 2023). Probiotics exert their beneficial effects by producing antimicrobial compounds that inhibit the growth and colonization of harmful microorganisms. These compounds include bacteriocins, organic acids, hydrogen peroxide, and other metabolites, each playing a distinct role in maintaining gut health and preventing infections (Alvarez *et al.,* 2023).

2.2.7 Immune System Modulation by Probiotics

Probiotics interact with the host immune system to enhance its ability to fight infections and maintain homeostasis. This immune modulation is achieved through various mechanisms, including stimulation of immune cells and production of immunomodulatory compounds (Mazziotta *et al.,* 2023; Liu, Wang, and Wu, 2022).

Enhancing IgA Production: Probiotics stimulate the secretion of immunoglobulin A (IgA) in the gut. IgA acts as the first line of defense by neutralizing pathogens and toxins before they penetrate gut tissues (Bang *et al.,* 2022).

Cytokine Regulation*:* Probiotics influence the production of cytokines, signaling molecules that regulate immune responses. For instance, *Lactobacillus reuteri* enhances anti-inflammatory cytokines while suppressing pro-inflammatory cytokines, helping to manage inflammatory diseases (Alvarez *et al.,* 2023).

Dendritic Cell Activation: Probiotics interact with dendritic cells in the gut, promoting the maturation of regulatory T-cells, which maintain immune tolerance and prevent autoimmune responses (Liu *et al.,* 2022).

2.3 Types of Antimicrobial Compounds Produced by Probiotics

Bacteriocins: Bacteriocins are proteinaceous toxins produced by bacterial probiotics, such as *Lactobacillus* and *Bifidobacterium*. These compounds selectively target and kill harmful bacteria by disrupting their cell membranes. For example, *Lactobacillus plantarum* produces plantaricin, a potent bacteriocin that inhibits pathogens like *Listeria monocytogenes* (Goyal *et al.,* 2022).

Organic Acids*:* Probiotics produce organic acids, such as lactic acid and acetic acid, which lower the gut's pH. This acidic environment inhibits the growth of pH-sensitive pathogens like *Staphylococcus* and *Escherichia. coli*. Organic acids also play a role in enhancing the gut barrier and promoting beneficial microbial populations (Liu *et al.,* 2022).

Hydrogen Peroxide: Certain probiotic strains produce hydrogen peroxide, which has broad-spectrum antimicrobial activity. Hydrogen peroxide inhibits the colonization of pathogens in mucosal surfaces, particularly in the vaginal and oral microbiomes (Walter, 2018).

Other Metabolites: Probiotics can produce bioactive compounds like diacetyl and reuterin, which inhibit both Gram-positive and Gram-negative bacteria.

2.4 Health Benefits of Probiotics

The modulation of gut microbiota is fundamental to the preventive and therapeutic benefits offered by probiotics, addressing both acute and chronic health conditions such as:

Improved digestion and nutrient absorption. (Liu *et al.,* 2022).

Reduced risk of gastrointestinal disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).

Enhanced immune response by promoting the development of regulatory T-cells and anti-inflammatory cytokines (Alvarez *et al.,* 2023).

2.5 Comparison of Mechanisms: Bacterial vs. Yeast Probiotics

2.5.1 Gut Colonization and Survival

Bacterial Probiotics:

Bacterial strains like *Lactobacillus* and *Bifidobacterium* are effective colonizers of the gut but are sensitive to acidic and bile-rich environments. Protective delivery systems (e.g., encapsulation) are often required to ensure their viability (Christaki *et al.,* 2020

Yeast Probiotics:

Yeasts such as *Saccharomyces boulardii* demonstrate greater resilience, naturally surviving harsh gastrointestinal conditions without additional protection (Pais, Almeida, Yılmaz, and Teixeira, 2020).

2.5.2 Antimicrobial Activity

Bacterial Probiotics:

Produce bacteriocins and organic acids that inhibit pathogenic bacteria.

Some strains can compete with pathogens for nutrients and adhesion sites in the gut (Alvarez *et al.,* 2023).

Yeast Probiotics:

Yeasts do not produce bacteriocins but generate other antimicrobial compounds, such as ethanol and bioactive peptides, which inhibit both bacteria and fungi.

Yeasts also neutralize toxins, such as those produced by *Clostridium difficile* (Zahoor *et al.,* 2021).

2.5.3 Immune Modulation

Bacterial Probiotics:

Strongly influence IgA production and cytokine responses, directly interacting with the host immune system (Bang *et al.,* 2022).

Specific bacterial strains have been shown to reduce inflammation and enhance immunity.

Yeast Probiotics:

Yeasts stimulate innate immunity, including macrophage activation and dendritic cell regulation.

Unlike bacteria, yeasts do not carry the risk of horizontal gene transfer, making them safer in the context of antimicrobial resistance (AMR) (Zahoor *et al.,* 2021).

2.5.4 Nutritional Contributions

Bacterial Probiotics:

Aid in lactose digestion and vitamin synthesis (e.g., folate).

Yeast Probiotics:

Contribute to the synthesis of B-complex vitamins and detoxification of mycotoxins in the gut (Bang *et al.,* 2022).

2.5.5 Resilience to Gastric Acidity and Bile Salts

Probiotics must demonstrate the ability to survive the harsh conditions of the gastrointestinal tract, including exposure to acidic stomach environments and bile salts in the small intestine. This resilience is a critical determinant of their effectiveness in delivering health benefits (Nandha and Shukla, 2023).

Comparison of Resilience

Bacterial Probiotics:

Many bacterial strains, such as *Lactobacillus* and *Bifidobacterium*, are sensitive to low pH and bile salts, which can significantly reduce their viability during gastrointestinal transit.

Protective measures, such as encapsulation or microencapsulation, are often required to enhance their survival. For example, *Lactobacillus plantarum* has demonstrated tolerance under optimized encapsulated conditions but remains less resilient than yeast probiotics in unprotected states (Nandha and Shukla, 2023).

Yeast Probiotics:

Yeasts such as *Saccharomyces boulardii* exhibit natural resilience, thriving in acidic environments (pH as low as 2.5) and bile salt concentrations of 0.3–0.5%. This inherent robustness is due to their eukaryotic cell structure and thick polysaccharide cell walls (Ebrahimi *et al.,* 2022).

Studies show that yeast strains, including *Saccharomyces cerevisiae*, maintain viability even after prolonged exposure to bile salts, outperforming bacterial probiotics (Marlida *et al.,* 2021)

2.5.6 Significance of Resilience

Effective Colonization: Yeast probiotics can survive gastrointestinal transit intact, ensuring colonization in the gut (Gudiña, Teixeira, and Rodrigues, 2020).

Health Benefits: This resilience makes yeast probiotics particularly effective in managing conditions like antibiotic-associated diarrhea and irritable bowel syndrome (IBS) (Lillie, 2022).

2.6 Antimicrobial Resistance and Probiotics

Antimicrobial resistance (AMR) poses one of the most significant global health challenges of the 21st century. According to the World Health Organization (WHO), AMR could cause up to 10 million deaths annually by 2050 if immediate action is not taken (Baekkeskov *et al.,* 2020). As at 2020, AMR was responsible for over 700,000 deaths each year, with direct mortality from bacterial resistance reported to be 1.27 million deaths in 2019 alone (Christaki *et al.,* 2020). The issue extends beyond healthcare into agriculture and the environment, as resistant pathogens spread through soil, water, and food chains. Common multidrug-resistant pathogens include Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae, all of which pose serious treatment challenges in clinical and community settings (Solanki *et al.,* 2022). The economic burden of AMR is equally alarming. The increased need for expensive second- or third-line antibiotics, prolonged hospital stays, and lost productivity amount to billions of dollars annually in global economic losses (Rabetafika *et al.,* 2023).

2.6.1 The Role of Antibiotics in Driving AMR and the Need for Alternatives

The misuse and overuse of antibiotics are key drivers of AMR. Inappropriate prescribing practices, self-medication, and excessive agricultural use have exacerbated the issue (Leistikow *et al.,* 2022). For instance, antibiotics are frequently used as growth promoters in livestock, with two-thirds of global antibiotic consumption occurring in animal farming. This accelerates the evolution of resistant strains that can easily transfer to humans through food or the environment.

Addressing AMR requires a multifaceted approach, including reducing unnecessary antibiotic use, promoting stewardship programs, and developing alternatives to antibiotics. Alternatives such as bacteriophage therapy, antimicrobial peptides, and probiotics are gaining traction as potential solutions (Sibirtsev *et al.,* 2021).

2.6.2 Probiotics as a Tool to Reduce Antibiotic Dependency

Probiotics represent a promising approach to mitigate AMR by restoring microbial balance and reducing the need for antibiotics. They exert their effects through multiple mechanisms, including:

Gut Microbiota Modulation: Probiotics maintain a healthy gut microbial balance, preventing opportunistic pathogens from dominating.

Pathogen Inhibition*:* Probiotic strains secrete antimicrobial compounds, such as organic acids and bacteriocins, that directly suppress harmful bacteria (Habteweld and Asfaw, 2023).

Immune Enhancement: Probiotics stimulate immune responses, reducing the severity and incidence of infections.

Yeast-based probiotics, such as *Saccharomyces boulardii,* have shown particular promise in reducing the prevalence of resistant bacteria in the gut microbiota. Unlike bacterial probiotics, yeast-based probiotics do not carry transferable resistance genes, further minimizing the risk of amplifying AMR (Chance *et al.,* 2022). For example, clinical studies have demonstrated the efficacy of *S. boulardii* in treating antibiotic-associated diarrhea and Clostridium difficile infections without contributing to resistance (Tullio, 2024).

Probiotics are also proving effective in agricultural applications, reducing the need for antibiotics in livestock by enhancing animal health and immunity. This dual impact on human and animal health makes probiotics an integral part of AMR mitigation strategies (Rabetafika *et al.,* 2023).

2.6.3 Preventing Infections with Probiotics

Probiotics have demonstrated significant efficacy in preventing infections by creating an environment that is inhospitable to pathogenic microbes. Their ability to protect against infections stems from several synergistic mechanisms, including competitive exclusion, antimicrobial compound production, and immune system modulation (Tullio, 2024).

2.6.3.1 Mechanisms of Infection Prevention

Pathogen Exclusion*:* Probiotics compete with pathogens for adhesion sites on the intestinal lining, reducing the ability of harmful microbes to colonize and invade. For instance, *Lactobacillus rhamnosus* has been shown to prevent the adhesion of *Clostridium difficile* and *Staphylococcus Typhimurium* (Bang *et al.,* 2022).

Production of Antimicrobial Compounds*:* By producing bacteriocins, organic acids, and other metabolites, probiotics lower the gut's pH, making it an unfavorable environment for pathogens like *E. coli* and *Helicobacter pylori* (Habteweld and Asfaw, 2023). Yeast probiotics like *Saccharomyces boulardii* neutralize bacterial toxins, directly preventing damage caused by pathogenic bacteria such as *Vibrio cholerae* (Pais *et al.,* 2020).

Immune Modulation: Probiotics enhance the host's immune response by stimulating immunoglobulin A (IgA) production and activating macrophages, improving the body's ability to fight infections (Ebrahimi *et al.,* 2022).

2.6.4 Probiotic Applications in Infection Prevention

Traveler's Diarrhea: Probiotic supplements, including *S. boulardii* and *L. rhamnosus*, are widely recommended for preventing traveler's diarrhea by inhibiting enteric pathogens.

Nosocomial Infections: Probiotics reduce the risk of hospital-acquired infections, especially in patients undergoing intensive care or antibiotic treatment (Ebrahimi *et al.,* 2022).

Urinary Tract Infections (UTIs): Probiotics such as *Lactobacillus crispatus* have been effective in reducing UTI recurrence in women by preventing the colonization of uropathogens (Bang *et al.,* 2022).

2.6.5 Impact on Global Health

The preventive role of probiotics aligns with global efforts to mitigate antibiotic resistance by reducing the need for antibiotics in treating and preventing infections. Their use in both clinical and community settings represents a sustainable strategy for managing infection risks without contributing to AMR (Rabetafika *et al.,* 2023).

2.6.6 Restoring Gut Microbiota After Antibiotic Use

Antibiotic treatments, while essential for eradicating bacterial infections, often disrupt the delicate balance of the gut microbiota. This disruption, known as dysbiosis, can lead to overgrowth of opportunistic pathogens such as Clostridium difficile, resulting in complications like diarrhea and inflammation. Probiotics play a crucial role in restoring gut microbiota to its natural state after antibiotic use (Ebrahimi *et al.,* 2022).

2.6.6.1 Mechanisms of Microbiota Restoration

Repopulating Beneficial Bacteria*:* Probiotic strains such as Lactobacillus acidophilus and Bifidobacterium longum help replenish beneficial bacteria lost during antibiotic therapy, promoting the re-establishment of a healthy microbial ecosystem (Liu *et al.,* 2022).

Inhibiting Pathogen Overgrowth*:* Probiotics prevent the proliferation of antibiotic-resistant pathogens by outcompeting them for nutrients and adhesion sites.

Immune System Recovery*:* Probiotics modulate immune responses that may be weakened due to dysbiosis, aiding the gut in recovering its barrier integrity and immune defense capabilities (Bang *et al.,* 2022).

2.6.7 Clinical Evidence

Antibiotic-Associated Diarrhea (AAD): Clinical trials have shown that probiotics like S. boulardii and L. rhamnosus significantly reduce the incidence of AAD by restoring gut microbial balance and neutralizing bacterial toxins (Tullio, 2024).

Clostridium difficile Infections (CDI): Probiotics reduce the risk of recurrent CDI by reintroducing beneficial microbes and limiting pathogen colonization (Liu *et al.,* 2022).

2.7 Yeasts as a Promising Probiotic Alternative

Yeasts are emerging as a compelling alternative to traditional bacterial probiotics due to their unique properties and advantages in addressing limitations of bacterial strains. Non-pathogenic yeast strains, such as *Saccharomyces boulardii*, have demonstrated significant probiotic potential, making them a viable option for promoting gut health and preventing infections (Balabekyan *et al.,* 2018).

2.7.1 Non-Pathogenic Yeasts with Probiotic Potential

Non-pathogenic yeast strains like *Saccharomyces boulardii* have been extensively studied for their probiotic benefits (Pais *et al.,* 2020). They are well-tolerated in humans and exhibit multiple mechanisms that promote gut health, including:

Pathogen Inhibition: Yeasts produce antimicrobial compounds, such as ethanol and bioactive peptides, which suppress the growth of harmful bacteria and fungi (Tullio, 2024).

Toxin Neutralization: *S. boulardii* is particularly effective in neutralizing bacterial toxins, such as those produced by *Clostridium difficile* and *Vibrio cholerae.*

Anti-inflammatory Effects: Yeasts reduce gut inflammation by modulating immune responses, which is beneficial in conditions like irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) (Liu *et al.,* 2022).

Unlike bacterial probiotics, yeasts do not colonize the gut but instead exert their effects transiently during passage, making them less likely to disrupt the existing microbial balance (Pais *et al.,* 2020).

2.7.2 Better Survival in the Gastrointestinal Tract

One of the most significant advantages of yeasts over bacterial probiotics is their superior ability to withstand the harsh conditions of the gastrointestinal tract:

Resistance to Gastric Acidity: Yeasts can survive at low pH levels, unlike bacterial probiotics, which often require encapsulation for protection.

Tolerance to Bile Salts*:* Yeasts such as *S. boulardii* exhibit high resilience to bile salts, ensuring they remain viable as they transit through the intestines (Liu *et al.,* 2022).

Heat Stability: Yeasts are thermotolerant, making them easier to process and store without losing viability.

This resilience makes yeast probiotics particularly suitable for oral delivery, ensuring consistent efficacy in gut health applications.

2.7.3 Risk of Transferring Antibiotic Resistance Genes

A critical concern with bacterial probiotics is the potential for horizontal gene transfer, where bacteria share genetic material, including antibiotic resistance genes, with other microbes in the gut. This can inadvertently contribute to the spread of antimicrobial resistance (AMR) (Balabekyan *et al.,* 2018).

Yeasts, being eukaryotic, do not engage in horizontal gene transfer with bacteria. This eliminates the risk of transferring antibiotic resistance genes, making them a safer alternative, particularly in settings where antibiotic use is prevalent or where resistant bacterial strains are a concern (Christaki, Marcou, and Tofarides, 2020).

2.7.4 Other Advantages of Yeasts

Broad Antimicrobial Spectrum*:* Yeasts are effective against a wide range of pathogens, including antibiotic-resistant bacteria.

Improved Nutrient Utilization: Yeasts contribute to nutrient synthesis, such as B vitamins, which support overall health.

Diverse Applications: Beyond gut health, yeast probiotics are being explored for use in skincare, animal husbandry, and aquaculture (Pais *et al.,* 2020).

2.7.5 Examples of Yeasts Studied for Probiotic Use

Saccharomyces boulardii:

The most extensively studied yeast probiotic, *S. boulardii*, has demonstrated efficacy in preventing and treating gastrointestinal disorders such as antibiotic-associated diarrhea and *Clostridium difficile* infections. This strain is known for its ability to neutralize bacterial toxins, modulate immune responses, and enhance gut barrier function (Tullio, 2024).

Pichia kudriavzevii:

Pichia kudriavzevii has shown potential in improving gut health and nutrient absorption. It also exhibits antimicrobial properties, particularly against pathogens like *Escherichia. coli*. This yeast is being explored for applications in both human and animal health due to its robustness under gastrointestinal conditions (Pais *et al.,* 2020).

Kluyveromyces marxianus:

Known for its dual role in food fermentation and gut health, *K. marxianus* produces bioactive peptides that inhibit harmful microbes while contributing to improved digestion and immune function. It is increasingly used in research for its probiotic properties in animal feed and dairy products (Liu *et al.,* 2022).

2.7.6 Potential Applications in Human and Animal Health

2.7.6.1 Human Health:

Gut Health: Yeast probiotics like *S. boulardii* are used to treat gastrointestinal conditions, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) (Aryana and Olson, 2017).

Immune Support*:* Yeasts enhance immune responses by stimulating cytokine production and increasing immunoglobulin levels, aiding in infection prevention and recovery (Saxena and Gupta, 2020).

Post-Antibiotic Therapy*:* Yeasts restore gut microbiota disrupted by antibiotics, reducing the risk of opportunistic infections such as *Clostridium difficile*.

2.7.6.2 Animal Health:

Improved Feed Efficiency: Yeast probiotics are included in animal feed to enhance nutrient utilization and growth performance, particularly in poultry and livestock (Ebrahimi *et al.,* 2022).

Disease Prevention*:* By modulating gut microbiota, yeasts reduce the prevalence of enteric diseases caused by pathogens like *Staphylococcus aureus* and *Escherichia coli*.

Antibiotic Replacement: Yeasts provide a sustainable alternative to antibiotics in animal husbandry, addressing the global issue of antimicrobial resistance (AMR) (Tortora *et al.,* 2019).

2.7.7 Other Emerging Sources of Probiotics

While bacterial and yeast probiotics dominate research and applications, several underexplored sources are gaining attention for their potential probiotic benefits. These include fungi, algae, and other unconventional microorganisms that present unique advantages for gut health, immunity, and broader applications (Singhi and Kumar, 2021).

2.7.7.1 Filamentous Fungi

Potential*:*

Filamentous fungi, such as *Aspergillus oryzae* and *Rhizopus oligosporus*, are used in traditional food fermentations and have shown promise as probiotics due to their ability to produce enzymes that aid digestion (Boyle *et al.,* 2023).

Applications:

Enhance nutrient absorption by breaking down complex carbohydrates, proteins, and lipids in the gut.

Produce bioactive compounds with antimicrobial and antioxidant properties (Friedman *et al.,* 2021).

Challenges:

The risk of allergenic responses and potential pathogenicity in immunocompromised individuals limit their broader use as probiotics (Tullio, 2024).

2.7.2.2 Algae and Microalgae

Potential:

Algae, such as Chlorella vulgaris and Spirulina platensis, contain prebiotic fibers that support probiotic growth, while microalgae like *Dunaliella salina* and *Haematococcus pluvialis* exhibit direct probiotic effects (Shiby and Mishra, 2023).

Applications:

Provide antioxidant protection through compounds like beta-carotene and astaxanthin.

Modulate gut microbiota and improve immune responses.

Act as a source of essential nutrients, including vitamins and omega-3 fatty acids (Swain *et al.,* 2024).

Advantages

Algae are sustainable, easy to cultivate, and environmentally friendly, making them attractive for large-scale production (Sahu *et al.,* 2022).

2.7.7.3 Other Non-Conventional Probiotics

Archaea: Some archaea, like *Methanosphaera stadtmanae*, have been explored for their role in gut microbiota modulation, although research is in its infancy (Rijkers *et al.,* 2021).

Spore-Forming Bacteria*:* Strains like *Bacillus subtilis* and *Bacillus coagulans* are being increasingly utilized for their stability under harsh conditions and long shelf life. These bacteria are particularly effective in agricultural and aquaculture applications (Shiby and Mishra, 2023).

Non-Traditional Yeasts: Beyond Saccharomyces, yeasts such as *Candida utilis* and *Debaryomyces hansenii* are being studied for their antimicrobial properties and potential use in food systems (Sahu *et al.,* 2022).

2.7.8 Relevance to Yeast Probiotics

Highlighting these alternative sources provides context for the growing focus on yeast probiotics. Yeasts like *Saccharomyces boulardii* stand out for their resilience, safety, and multifaceted benefits, making them one of the most viable emerging probiotics. The exploration of other sources complements yeast research and broadens the scope of potential applications (Meiling, 2018).

CHAPTER THREE

3.0 OVERVIEW OF THE EXPERIMENTAL DESIGN

This research adopts an experimental design to isolate, identify, and evaluate non-pathogenic yeasts from fruits and vegetables for their probiotic potential. The study involved a series of well-structured laboratory experiments:

3.1 MATERIALS AND METHODS

3.1.2 Sourcing of Test Materials:

Fruits and vegetables {Lettuce (*Lactuca sativa)*, Garden Egg (*Solanum aethiopicum)*, Cabbage (*Brassica oleracea)* Cucumber (*Cucumis sativus)* Tomato (*Solanum lycopersicumi)*, Watermelon (*Citrullus lanatus),* Grape (*Vitis vinifera)*} were purchased from different local markets in Ilorin and prepared under sterile conditions for yeast isolation.

3.1.2.1 Criteria for Selecting Samples

To maintain quality and reliability, the following criteria were applied in selecting the samples:

Freshness*:* All produce items were freshly harvested and displayed for sale within 24 hours of harvest.

Organic and Unprocessed: Priority was given to organic produce that had not been chemically treated or processed to ensure the natural microbial load remained intact.

Physical Condition: Samples were free of visible damage, decay, or bruising, which could influence microbial diversity and favor pathogenic organisms.

Variety Representation: A broad spectrum of fruits and vegetables was included to maximize the chances of isolating diverse yeast strains from different natural niches.

This strategic selection of samples ensures that the study explores a wide range of potential non-pathogenic yeast strains, enhancing the validity of the findings.

3.1.2.2 Processing of Test Materials:

All fruits and vegetables were surface-sterilized by immersing them in a 1% sodium hypochlorite solution for 3 minutes, a method widely used to remove external contaminants from fresh produce (Oliveira *et al.,* 2012). After sterilization, the produce was thoroughly rinsed with sterile distilled water to eliminate any residual disinfectant and then air-dried under aseptic conditions. Samples were homogenized using a sterile blender and suspended in sterile saline solution to prepare for microbial analysis, a standard practice in microbial studies (Cappuccino and Sherman, 2014).

3.1.3 Transport Conditions for Maintaining Sample Integrity:

Freshly collected produce was placed in sterile polyethylene bags. Transport to the laboratory was completed within 4 hours to minimize environmental exposure and microbial alterations. Upon arrival, the prepared suspensions were processed immediately.

3.2 Isolation and Culturing:

Following the processing of the test materials, 10g of each sample was aseptically transferred into a sterile blender and homogenized with 90ml of sterile 0.85% saline solution, maintaining a 1:10 dilution (10^{-1} dilution) for microbial recovery (Almeida, 2020; Fernández, 2021). The homogenates were left to stand for 5 minutes to allow debris to settle before further processing.

3.2.1 Culturing Techniques

3.2.1.1 Media preparation

A 1ml aliquot of the homogenized sample was inoculated into 9ml of Yeast Extract Peptone Dextrose (YEPD) broth (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose), supplemented with 50 mg/L gentamicin to inhibit bacterial growth (Wang *et al.,* 2021; García *et al.,* 2023). The inoculated broth was incubated at 30°C for 24–48 hours under aerobic conditions.

3.2.1.2 YEPD agar slant preparation

Agar slants were prepared by pouring sterilized YEPD medium into sterile test tubes at a slanted angle and allowing them to solidify.

3.2.1.3 Inoculation in Broth

One thousand microliter (1000µL) of each homogenized sample (prepared in sterile PBS) was inoculated into 9ml of sterilized YEPD broth in sterile plain tube and were incubated at 30 °C in an incubator for 48hours (Raimondi *et al.,* 2023).

3.2.1.4 Preparation of Yeast Extract Peptone Dextrose Agar (YEPDA)

The workbench was swabbed using 80% ethanol. 5 g of yeast extract was weighed and 10 g of dextrose, 10 g of peptone, 10 g of agar, and 625 µl of gentamicin was added. 500 ml of distilled water was added to the conical flask. The flask was plugged using cotton wool wrapped in foil paper and sealed with cello tape, then placed on a hot plate to homogenize. The conical flask containing the YEPD agar was sterilized for 15 minutes at 121 °C in an autoclave and allowed to cool before 20 ml each of agar was dispensed into twenty-five sterile plates aseptically and allowed to solidify for forty minutes. The plates were placed in the incubator overnight to ensure purity. A total of forty plates were prepared (Merck, 2012).

3.2.1.5 Inoculation Procedure:

After incubation in YEPD broth, a loopful of the culture was plated on YEPD agar using the streak plate method under aseptic conditions. Plates were incubated at 30°C for 48–72hours under aerobic conditions (Cappuccino and Sherman, 2014).

3.2.1.6 Sterility Testing of the Media

The sterility of media was confirmed by incubating uninoculated broth and agar controls at 37ºC and were checked after 24hours to detect contamination (Cappuccino and Sherman, 2014).

3.3 Morphological Identification

3.3.1 Macroscopic Examination

After incubation, yeast-like colonies were initially identified based on morphological characteristics, including color, size, texture, elevation, and margin appearance on YEPD agar. Colonies that appeared smooth, creamy, or mucoid were selected for further examination (Bassyouni *et al.,* 2023; Raimondi *et al.,* 2023).

3.3.2 Microscopic Examination

A small portion of each colony was subjected to Gram staining for preliminary microscopic identification: A thin smear of yeast cells was prepared on a clean glass slide. Heat-fixed slides were stained with crystal violet for 60 seconds, followed by iodine treatment for 30 seconds. Decolorization with ethanol was performed for 10–20 seconds, and slides were counterstained with safranin for 30 seconds. The slides were then observed under a light microscope using ×40 and ×100 (oil immersion) objectives (Cappuccino and Sherman, 2014). Morphological screening criteria were based on the descriptions by Bassyouni et al. (2023) and Raimondi et al. (2023). These isolates were carried forward for physiological screening and probiotic characterization.

3.4 Storage and Packaging of Isolates

Short-Term Storage: Freshly isolated yeast colonies were streaked onto YEPD agar slants and incubated at 30 °C for 24–48 hours. Post-incubation, the slants were sealed with parafilm to maintain moisture and prevent contamination and were stored at 4 °C for immediate use (Kurtzman, Fell, and Boekhout, 2011).

Long-Term Storage: Colonies were transferred into 10 mL of sterile YEPD broth and incubated at 30°C for 24 hours with constant agitation (150rpm). After incubation, cultures were mixed with sterile glycerol to achieve a final concentration of 15% (v/v) glycerol. One milliliter (1ml) was aliquoted and then

transferred into sterile cryovials, which were tightly sealed and stored at −80 °C (Kurtzman, Fell, and Boekhout, 2011).

3.5 Biochemical Characterization

Biochemical characterization was performed to identify yeast isolates based on their metabolic activities, including fermentation profiles. This test followed standard protocols (Kurtzman *et al.,* 2011; Deak and Beuchat, 2020).

3.5.1 Sugar Fermentation Tests

The sugars selected for this study were D-glucose, sucrose, lactose, fructose, and galactose, representing a range of monosaccharides and disaccharides to provide a comprehensive fermentation profile (Reiner, 2012).

3.5.1.1 Media Preparation

The fermentation medium used was prepared using peptone (1.0 g), yeast extract (0.3 g), sodium chloride (0.5 g), and bromocresol green (1.6 mg) dissolved in 100 mL of distilled water, with the pH adjusted to 6.5-6.8; individual sugar solutions (1% w/v of D-glucose, sucrose, lactose, fructose, and galactose) were then incorporated into separate portions of the medium for fermentation analysis.

Small, inverted Durham tubes were placed within each test tube to detect gas production during fermentation. The tubes were carefully inserted to avoid trapping air bubbles and were fully submerged to ensure accurate gas detection. The assembled test tubes, along with the Durham tubes, were sterilized using an autoclave at 121°C for 15 minutes, after which they were allowed to cool to room temperature before inoculation. Sucrose and lactose sugars were sterilized at 121°C as they are subject to breakdown by autoclaving (Reiner, 2012).

3.5.1.2 Inoculation Procedure

Pure yeast isolates were obtained under aseptic conditions and used for inoculation. A sterile inoculation loop was employed to transfer a loopful of yeast culture into each test tube containing sugar-supplemented broth. To prevent cross-contamination, the inoculation loop was flamed and cooled between transfers. After inoculation, the tubes were gently agitated to ensure a uniform distribution of yeast cells throughout the medium.

Negative controls were set up by incubating sterile, uninoculated broth to check for contamination. Additionally, positive controls using a known fermenting yeast strain, such as *Saccharomyces cerevisiae*, were included to validate the reliability of the fermentation system (Kurtzman *et al.,* 2011).

3.5.2.3 Incubation Conditions

All test tubes were incubated at 30°C for 5–7 days. This temperature was chosen because it is optimal for yeast growth and metabolic activity. The tubes were observed daily for changes in color and gas production.

3.6: Fruits and Vegetables in relation to the isolate ID.

The isolates were given an identification code that tallied with their source; Lettuce was coded as (LET), Cabbage (CAB), Cucumber (CUC), Garden eggs (GAR), Grape (GRA). Tomatoes (TOMA), Watermelon (WA). See Table 3.1.

Table 3.1: Fruits and Vegetables in relation to the isolate ID.

Samples	Isolate I.D
Lettuce	LET
Cabbage	CAB
Cucumber	CUC
Garden Eggs	GAR
Grape	GRA
Tomatoes	TOMA
Watermelon	WA

3.6.1 Bile Salt Tolerance Test:

Yeast strains were grown in YEPD broth supplemented with 0.3% and 0.5% (w/v) cow bile (Sigma-Aldrich) and incubated at 37°C for 4 hours. Growth was monitored by measuring optical density (OD_{660}) to evaluate bile stress tolerance (Zhou *et al.,* 2018). Yeasts that exhibited high survival rates (>80%) under acidic and bile conditions were considered strong probiotic candidates.

3.6.2: Osmotic Test

Yeast isolates were exposed to 30%, 40%, and 50% (w/v) glucose, which simulate extreme osmotic conditions. The test was carried out by inoculating standardized overnight yeast cultures into YEPD media containing the respective glucose concentrations. The cultures were incubated at 30°C, and growth was monitored at 24 and 48 hours by measuring the optical density at 660 nm. The ability of the yeast to grow under such high osmolarity conditions was taken as an indicator of osmotic stress tolerance.

This method is adapted from Solieri and Giudici (2008), who used similar conditions to assess the osmotolerance of yeasts isolated from traditional balsamic vinegar environments. The capacity of yeast strains to survive and proliferate under hyperosmotic stress is not only vital for their probiotic potential but also enhances their suitability for biotechnological applications where stress resistance is essential.

3.6.3: Acid Tolerance Test

Overnight cultures of yeast were harvested by centrifugation at 10,000 × g for 5 minutes and washed twice in sterile phosphate-buffered saline (PBS, pH 7.0). The cells were resuspended in YEPD broth adjusted to pH 3.0 using 1 M HCl, simulating gastric conditions. A 1mL aliquot of yeast suspension (standardized to approximately 10^9 CFU/mL using McFarland 3.3) was inoculated into 9mL of the acidified medium and incubated at 37°C for 2 hours. Cell viability was assessed before and after incubation using optical density at 660 nm and/or serial dilution followed by plating on YEPD agar (Cho *et al.,* 2022).

3.6.4 Alkaline Tolerance Test

Similarly, yeast suspensions were inoculated into YEPD broth adjusted to pH 8.0 using 1M NaOH, mimicking conditions in the small intestine. After incubation at 37°C for 4 hours, growth and viability were measured as described above (Sui *et al.,* 2023). Yeast isolates demonstrating stable or increased OD_{660} values and high CFU recovery were considered tolerant to alkaline stress.

3.6.5Antimicrobial Activity

The antimicrobial activity of the yeast isolates was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method, following modified protocols from (Younis *et al.,* 2017).

3.6.6 Sourcing of Bacterial Isolates

Bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*) were obtained from the laboratory bank in Thomas Adewumi University, Oko, Kwara State.

3.6.7 Preparation of the Bacterial isolates:

Escherichia coli and *Staphylococcus aureus* were cultured in MacConkey broth and Nutrient broth, respectively, and incubated at 37°C for 18–24 hours. The suspensions were standardized to 0.5 McFarland (approximately 1.5×10^8 CFU/mL) (CLSI, 2021).

3.6.8 Preparation of Yeast Supernatants:

Yeast isolates were grown in YEPD broth at 30°C for 24-48 hours under aerobic conditions. Cultures were centrifuged at 5000 rpm for 10 minutes to separate the supernatant. Instead of membrane filtration, the supernatant was carefully decanted to avoid cellular residues (Kurtzman *et al.,* 2011).

3.6.9 Agar Well Diffusion Assay

Mueller-Hinton Agar (MHA) plates were prepared and inoculated with suspensions of *Escherichia coli* and *Staphylococcus aureus* using the lawn culture method with a sterile pipette. Each organism was applied to separate plates. Three wells (7 mm in diameter) were made in the agar using a sterile cork borer, and 100µL of yeast culture supernatant was added to each well. A control antibiotic disc was also placed on each plate. The plates were then incubated at 37°C for 24 hours (CLSI, 2021).

3.7 Evaluation of Antimicrobial Activity:

A clear inhibition zone around the wells was measured (mm). Yeast strains with >10 mm inhibition zones were considered to have strong antimicrobial activity.

3.8 Data Analysis

Data were analyzed using statistical Package for Social Sciences (SPSS) version 24 and Microsoft Excel. During this submission, molecular results for the isolates are pending; therefore, they are referred to as Yeast A, B, and C (see Table 3.2).

Table 3.2: Designated Codes and Source Origins of Yeast Isolates

Isolate	Code
Cucumber isolates	Yeast A
Grape isolates	Yeast B
Cabbage isolates	Yeast C

Key

Yeast A= CUC (Cucumber)

Yeast B= GRA (Grape)

Yeast C= CAB (Cabbage)

CHAPTER FOUR

4.0 RESULT

4.1 Morphological Characterization of Yeasts

After incubation on agar, numerous colonies with typical **yeast-like morphology** were observed. Colonies appeared predominantly **cream-colored**, **raised**, and **opaque**, with **butyrous or mucoid textures**. Shapes ranged from **irregular** to **round or oval**, with sizes between **4 mm and 12 mm** in diameter. Edges were mostly **serrated**, although some secondary subcultures exhibited **entire margins**, suggesting strain variation. A total of **24 presumptive yeast isolates** were obtained across all samples (See Table 4.1).

Table 4.1 Morphological Characterization of Yeasts

Isolate	Size (mm)	Shape	Color	Elevation	Edge	Texture	Opacity	Gram Reaction
CAB	**9**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**
CUC	**8**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**
GAR	**10**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**
GRA	**7**	**Round**	**Cream**	**Raised**	**Serrated**	**Mucoid**	**Opaque**	**Gram Positive**
GRA-R	**4**	**Oval**	**Cream**	**Undulated**	**Entire**	**Butyrous**	**Opaque**	**Gram Positive**
LET	**10**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**
TOMA	**8**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**
WA (Large colonies)	**12**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**
WA (small colonies	**8**	**Irregular**	**Cream**	**Raised**	**Serrated**	**Butyrous**	**Opaque**	**Gram Positive**

KEY

CUC= Cucumber isolate
CAB= Cabbage isolate
GAR= Garden egg isolate
GRA= Grape isolate A
GRA-R= Grape isolate B
LET= Lettuce isolate
WA= Watermelon isolate A
WA= Watermelon isolate A
TOMA= Tomatoes isolate

4.2 Gram Staining Results of Yeast Isolates

Microscopic examination of the yeast isolates following Gram staining revealed that all isolates appeared **Gram-positive**, displaying **oval to round cells** with visible **budding patterns**. Actively dividing cells showed clear formation of **blastoconidia**, confirming yeast morphology.

Additionally, some isolates exhibited the presence of **pseudohyphae**, which are elongated budding cells characteristic of certain yeast species and useful for preliminary species-level differentiation. These features are consistent with typical characteristics of non-pathogenic, budding yeasts.

4.3 Sugar Fermentation Test for Yeast Isolates

All isolates tested positive for glucose fermentation, indicated by a color change to green and gas accumulation in the Durham tube. This confirms the utilization of glucose as a carbon source by all strains. In contrast, fructose, maltose, sucrose, and lactose showed no color change across all isolates, indicating the absence of fermentation for these sugars. See Table 4.2.

Table 4.2: Sugar Fermentation Test for Yeast Isolates

Isolates	Glucose	Fructose	Maltose	Lactose	Sucrose	Probable Organism
CAB	+	–	–	–	–	*Candida glabrata*
CUC	+	–	–	–	–	*Candida glabrata*
GAR	+	–	–	–	–	*Meyerozyma caribbica*
GRA	+	–	–	–	–	*Pichia spp.,*
LET	+	–	–	–	–	*Pichia kudriavzevii*
TOMA	+	–	–	–	–	*Debaryomyces hansenii*
WA	+	–	–	–	–	*Candida glabrata*

KEY :

Where (+) signifies positive, while (–) represents negative.

4.4 Effect of 0.3% Bile on Yeast Isolates

Across all time points, YEAST B exhibited the highest optical density (OD) at OD660nm values, followed by YEAST C and then YEAST A. At 0 hour, the yeast isolates A had the lowest initial OD (M = 0.017, SD = 0.0047), whereas isolates B and C showed higher values (M = 0.075, SD = 0.0095; M = 0.080, SD = 0.0091, respectively). The yeast isolates C and A exhibited poor bile tolerance as shown by the mean values of their absorbance readings. (M= Mean optical density value). See Figure 1.

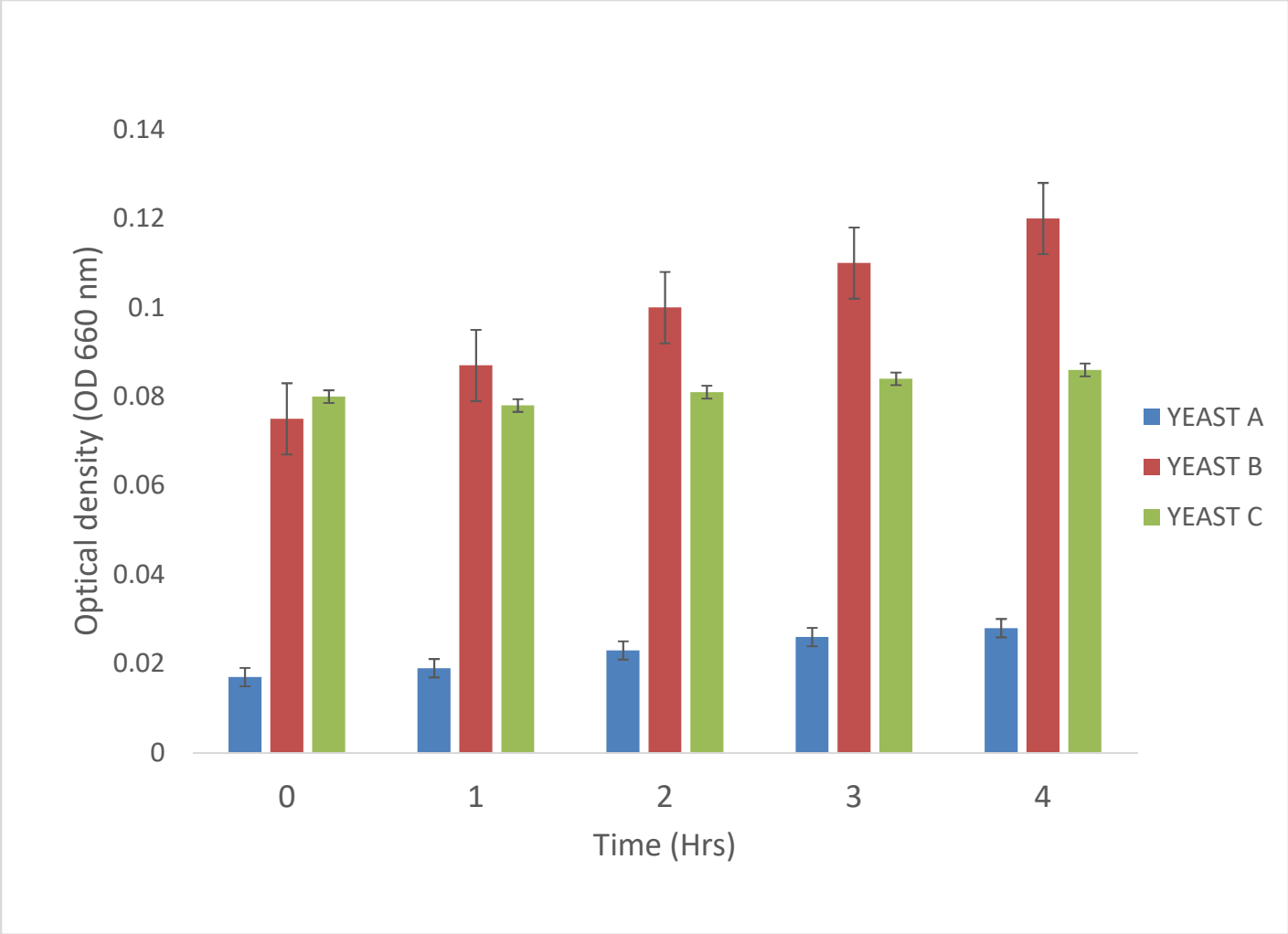


FIGURE 1: Effect of 0.3% Bile on Yeast Isolates

4.5 : Effect of 0.5% Bile on Yeast Isolates

Across all time points, YEAST B exhibited the highest optical density (OD) at OD660nm values, followed by YEAST C and then YEAST A. At 0 hour, the yeast isolates A had the lowest initial OD (M = 0.025, SD = 0.009), whereas isolates B and C showed higher values (M = 0.069, SD = 0.007; M = 0.072, SD = 0.007, respectively). At 4hours the yeast isolate A had the lowest OD (M= 0.050, SD= 0.006),whereas isolates B and C showed higher values (M = 0.168, SD = 0.070; M = 0.099, SD = 0.021, respectively). (M= Mean optical density value). See Figure 2.

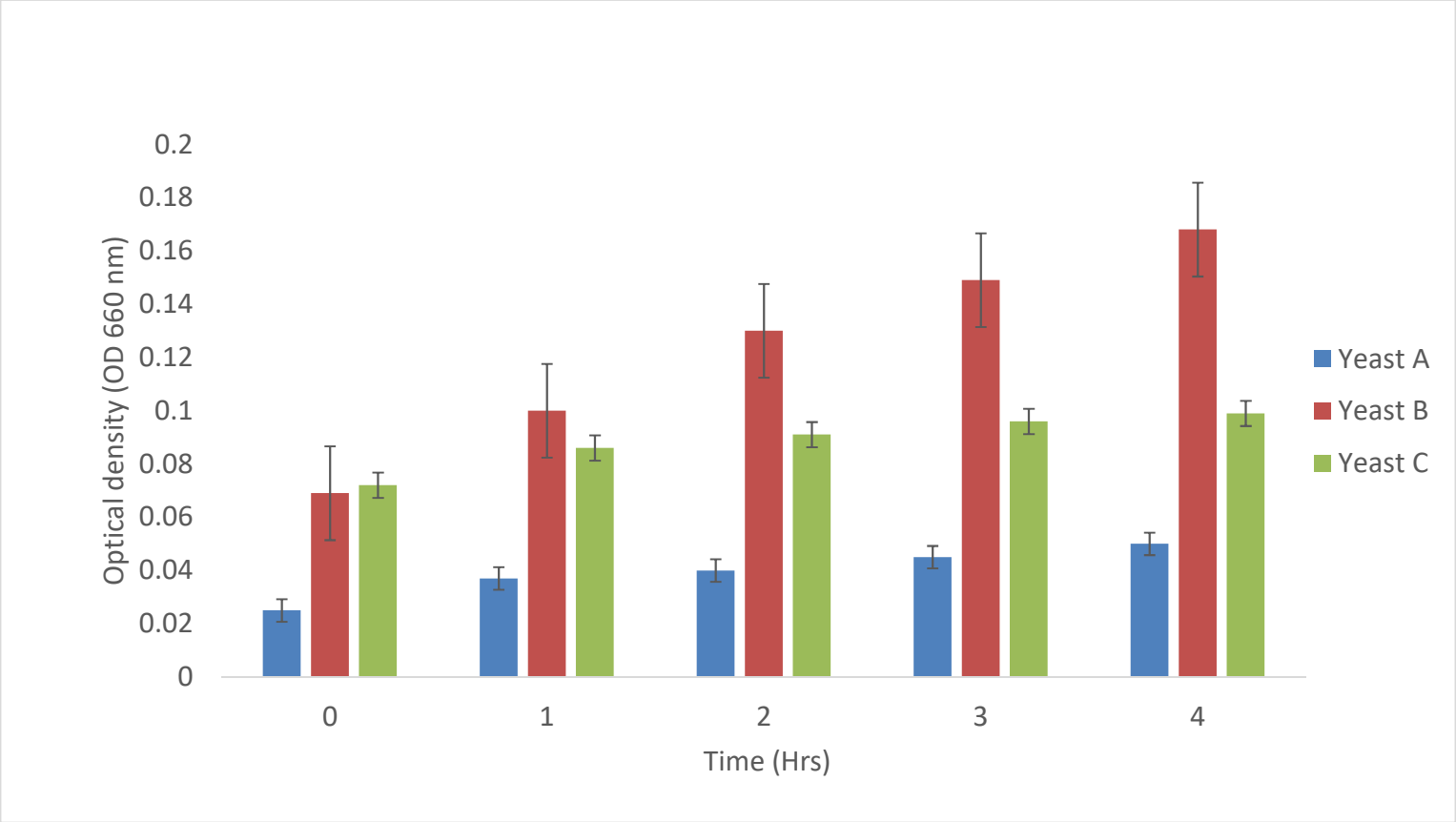


Figure 2: -Effect of 0.5% Bile on Yeast Isolates

4.6 Effect of 30% Glucose on Yeast Isolates' Growth Over Time

Descriptive statistics indicated that OD values increased progressively over the 48-hour incubation period across all isolate groups. At time point 0 hours (T0), the YEAST C isolates exhibited the highest initial OD values (M = 0.191), followed by YEAST A (M = 0.168) and YEAST B (M = 0.082). (M= Mean optical density value). By 48 hours (T48), YEAST A isolates showed the highest OD (M = 0.878), indicating the greatest level of osmotic stress tolerance under 30% glucose. YEAST C isolates followed with a mean OD of 0.731, while YEAST B isolates remained the lowest (M = 0.502).

Estimated marginal means further demonstrated a consistent upward trend in OD over time. The mean OD increased from T0 (M = 0.147, SD = 0.002) to T24 (M = .429, SD = 0.005), and continued rising at T48 (M = 0.703, SD = 0.007. See Figure 3.

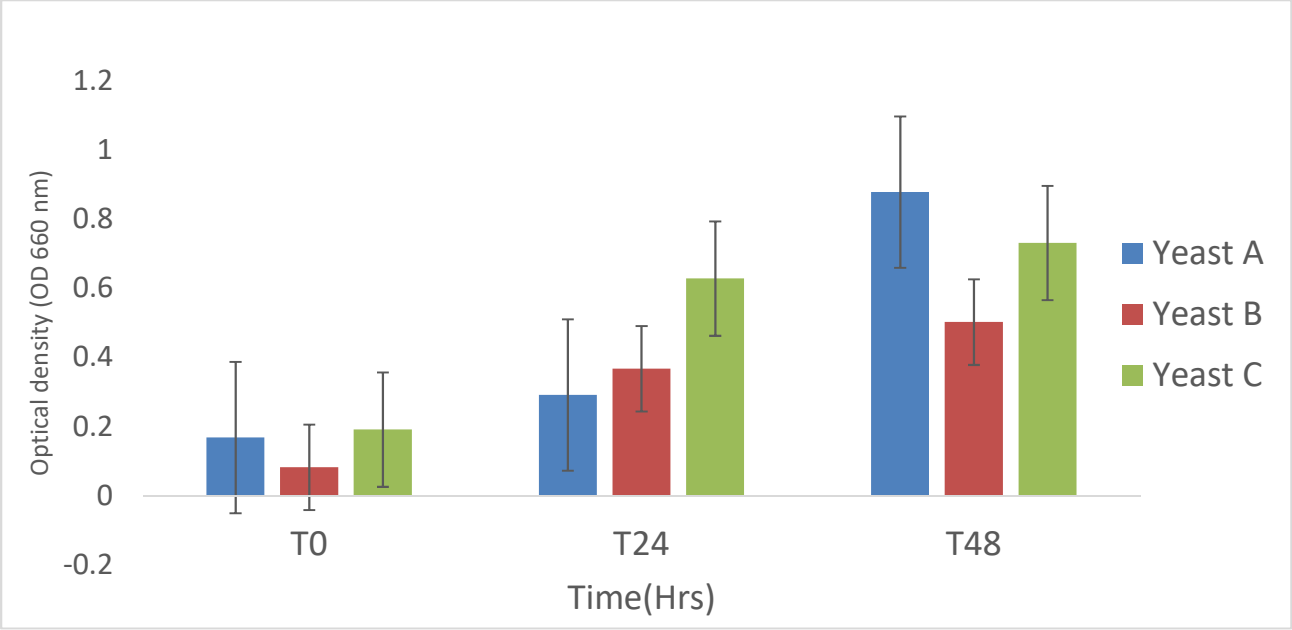


Figure 3: Effect of 30% Glucose on Yeast Isolates' Growth Over Time

4.7 The Effect of 40% Glucose on Yeast Isolates' Growth Over Time

Descriptive statistics indicated a steady increase in OD across time for all isolate groups, suggesting growth despite osmotic stress. At baseline (T0), the mean OD for the YEAST A isolate was highest (M = 0.168, SD = 0.0065), closely followed by the YEAST C (M = 0.165, SD = 0.0065), while YEAST B recorded the lowest OD (M = 0.096, SD = 0.0030). By 24 hours, YEAST C showed the most growth (M = 0.359, SD = 0.017), while YEAST B remained the lowest (M = 0.199, SD = 0.0090). After 48 hours, YEAST A exhibited the highest OD (M = 0.878, SD = 0.0228), followed by YEAST C (M = 0.668, SD = 0.0172), and YEAST B remained the least (M = 0.502, SD = 0.0170), suggesting YEAST A had the best tolerance to 40% glucose-induced osmotic stress. See Figure 4.

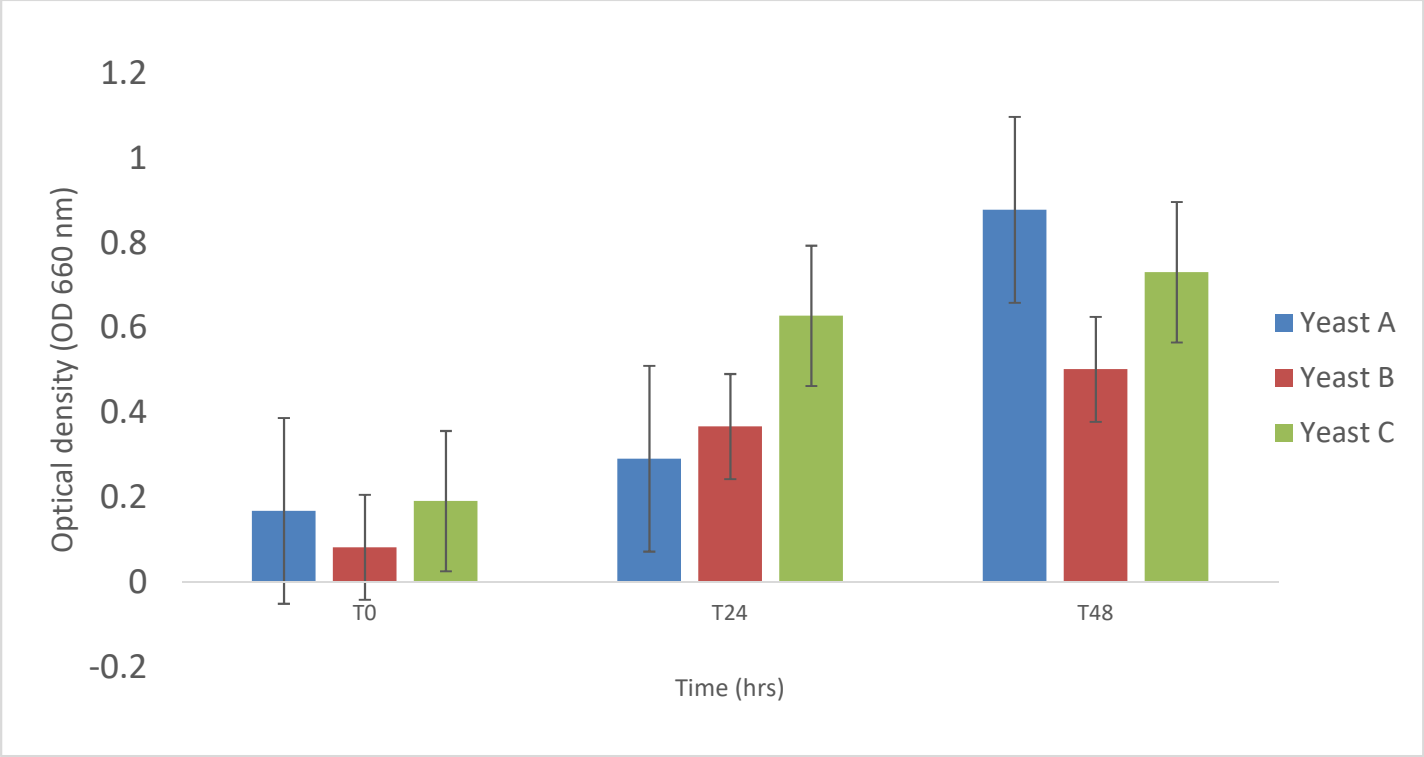


Figure 4: The Effect of 40% Glucose on Yeast Isolates' Growth Over Time

4.8 Effect of 50% Glucose on Yeast Isolates' Growth Over Time

The results of the repeated measures ANOVA at 50% glucose concentration indicate significant changes in yeast growth across time and between isolates. At 0 hour, the average OD660nm values for the isolates were relatively low and closely clustered, with YEAST B showing the lowest mean (M = .117, SD = .0046), while YEAST A and C had slightly higher values. However, by 24 hours, YEAST C demonstrated the greatest increase in growth (M = .261), while YEAST A exhibited a slight decline. By 48 hours, YEAST A showed a substantial increase in OD660 (M = .516), overtaking both YEAST B and YEAST C. See Figure 5.

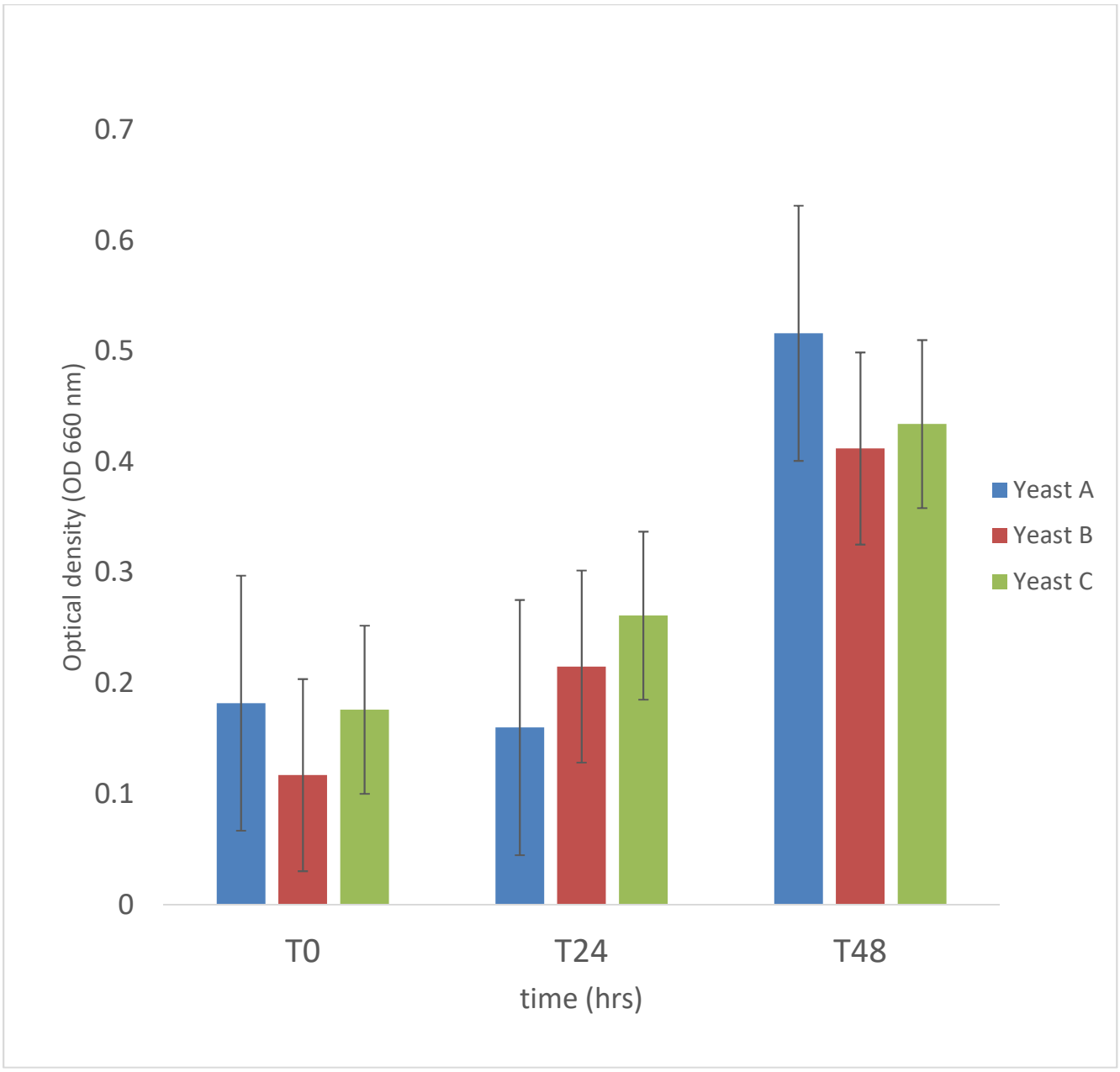


Figure 5: Effect of 50% Glucose on Yeast Isolates' Growth Over Time

4.9 Effect of Acid medium pH3.0, and Alkaline medium pH8.0 on Yeast Isolates' Growth Over Time

The acid and alkaline tolerance results provide insight into the survival capabilities of yeast isolates obtained from YEAST A, YEAST B, and YEAST C when exposed to extreme pH conditions, important indicators of their potential as probiotics.

Under acidic conditions (pH 3.0), which simulate the gastric environment, most yeast isolates showed strong tolerance. The viability of the isolates was measured using optical density at OD660nm. The YEAST A isolates recorded the highest optical density of 0.21, indicating not only survival but active growth over the 2 hours. YEAST B isolates showed more variability, exhibiting an optical density of 0.077. YEAST C isolates performed consistently well, with an optical density of 0.131. See Figure 6.

In the alkaline tolerance test (pH 8.0), measured after 4 hours, YEAST A isolates again demonstrated robust performance, reflecting sustained viability with an Optical density of 0.201, indicating tolerance to alkaline environment. YEAST B isolates consistently increased in OD_{660} from 0 hour to 4hours with an optical density of 0.135 at 4hours, suggesting good adaptation to alkaline conditions. Similarly, YEAST C isolate maintained or improved its OD value of 0.143 at 0 hour to 0.222 at 4hours, highlighting excellent alkaline resilience. See Figure 7.

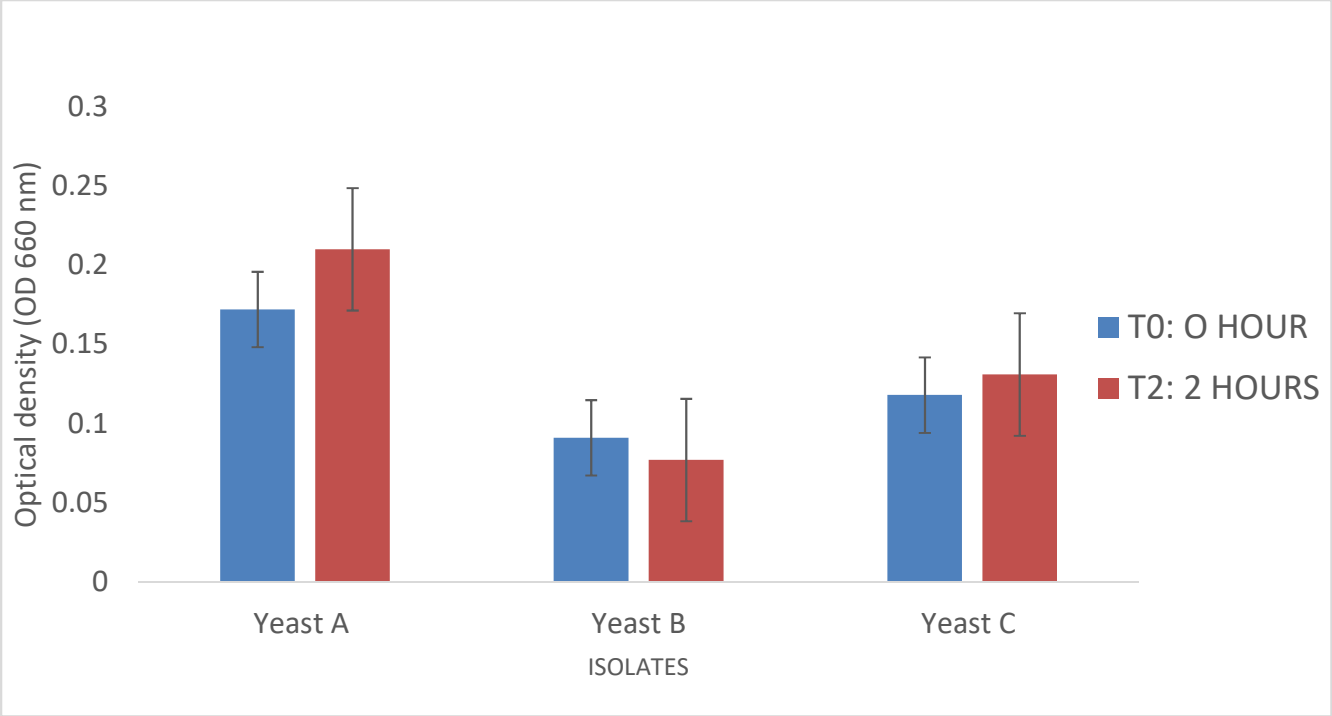


Figure 6: Effect of Acid medium pH3.0 on Yeast Isolates' Growth Over Time

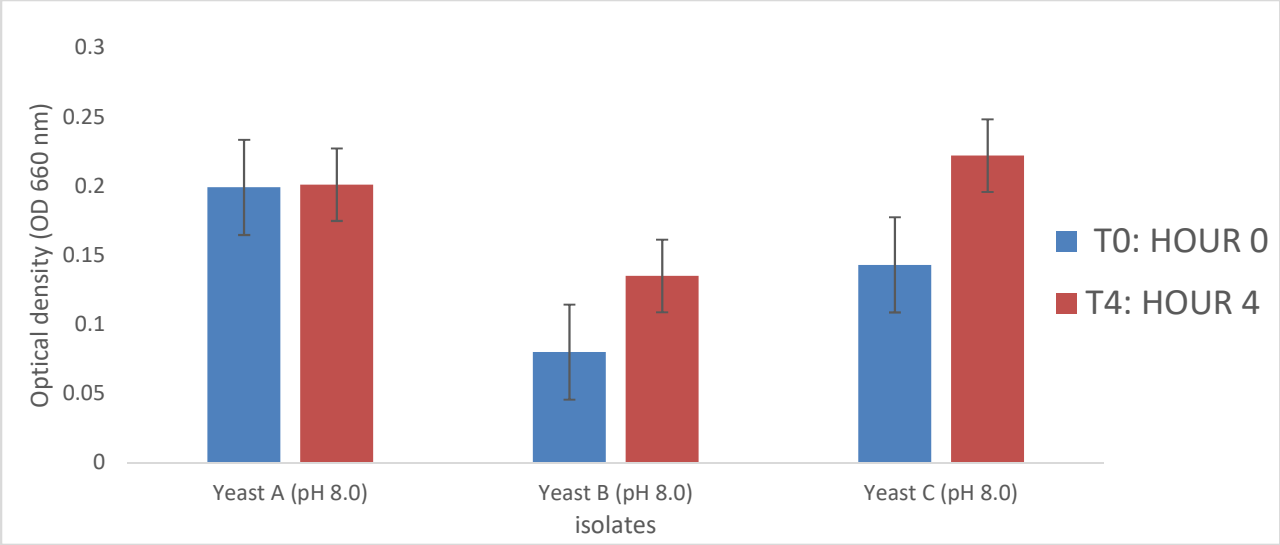


Figure 7: Effect of Alkaline Medium pH8.0 on Yeast Isolates' Growth Over Time

4.9 Antimicrobial Activity

Against *Staphylococcus aureus*:

All isolates exhibited inhibitory effects, successfully resisting bacterial growth. Oxacillin was used as a positive control. See Table 4.3.

Against *Escherichia coli*:

All the isolates did not exhibited inhibitory effects against *Escherichia coli*. See Table 4.3.

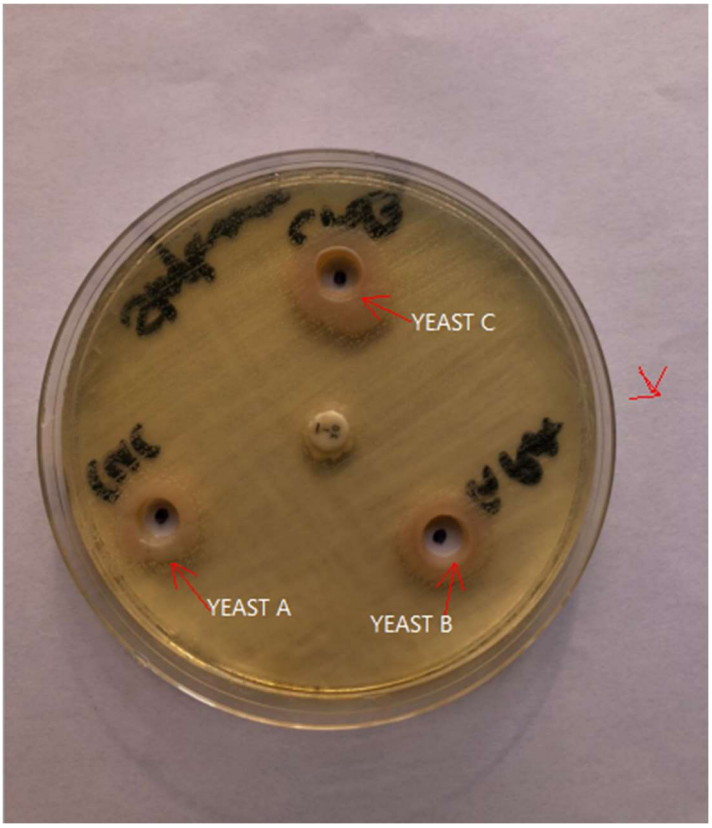


Plate 1: Antimicrobial activity of Yeast A, B, and C against *Staphylococcus aureus.*

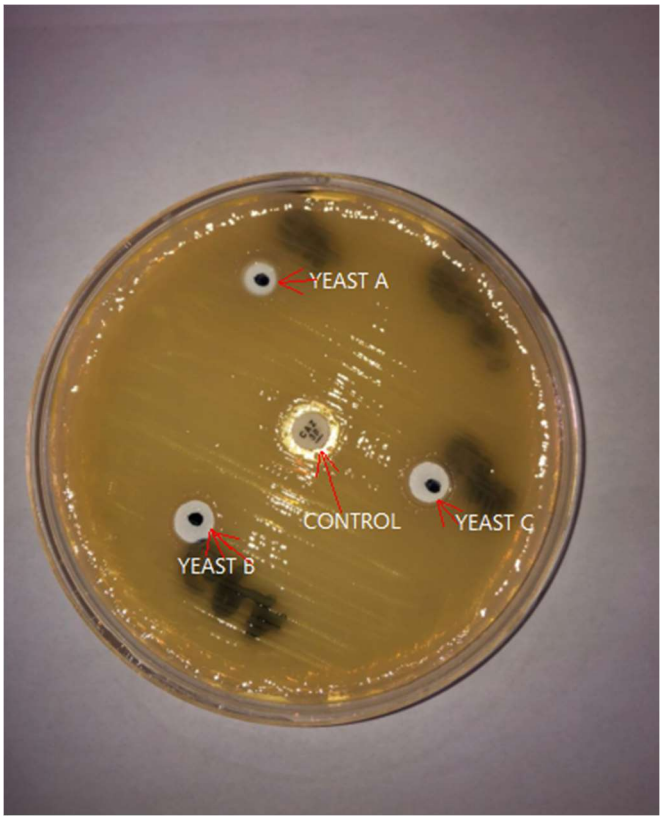


Plate 2: Antimicrobial activity of Yeast A, B, and C against *Escherichia coli.*

Table 4.3: Antimicrobial activity of Yeast A, B, and C against *Staphylococcus aureus* and *Escherichia coli*

Bacteria	Zone of Inhibition in Diameter (mm)			
	Yeast A	Yeast B	Yeast C	Control
Escherichia coli	12	11	11	9
Methicillin Resistant *Staphylococcus aureus*	15	15	10	6

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

Yeasts, particularly non-pathogenic strains, have gained increasing attention as promising candidates for probiotic applications due to their resilience in gastrointestinal conditions and their ability to confer health benefits to the host. The yeasts were sourced from natural sources, including fruits and vegetables {Lettuce (*Lactuca sativa)*, Garden Egg (*Solanum aethiopicum)*, Cabbage (*Brassica oleracea),* Cucumber (*Cucumis sativus),* Tomato (*Solanum lycopersicumi)*, Watermelon (*Citrullus lanatus),* Grape (*Vitis vinifera)*}. This aligns with the study carried out by Chandimala, Rajawardhana, Liyanage, and Hewajulige (2022). Unlike bacterial probiotics, yeasts such as *Saccharomyces cerevisiae* and related species are naturally more resistant to antibiotics and extreme environmental stresses, making them suitable for functional food formulations and therapeutic uses (Hatoum, Labrie, and Fliss, 2012).

In this study, selected yeast isolates from fruits and vegetables demonstrated varying levels of bile salt tolerance, with Yeast B showing the highest survival in both 0.3% and 0.5% bile concentrations. This suggests an ability to withstand the hostile bile-rich environment of the small intestine, a key criterion for probiotic viability (Zhou *et al.,* 2018).

Under osmotic stress conditions induced by 30–50% glucose, all isolates showed progressive growth over time; particularly Yeast A in osmotic conditions of 40% and 50% concentrations. This indicates strong osmoadaptive capabilities, which are important for yeast survival in sugar-rich food products and in the gut, where osmolarity can fluctuate Solieri and Giudici, 2008. The other Yeast (C) also performed well under moderate stress, while Yeast B showed comparatively lower tolerance. This indicates strong osmoadaptive capabilities, which are important for yeast survival in sugar-rich food products and in the gut, where osmolarity can Solieri and Giudici, 2008.

Acid and alkaline tolerance assays revealed that all isolates remained viable under simulated gastric (pH 3.0) and intestinal (pH 8.0) conditions. Notably, Yeast A isolates displayed exceptional survival under acidic conditions, with survival rates exceeding 150%, suggesting not only resistance but also possible proliferation. This level of acid tolerance is critical for surviving passage through the stomach (FAO/WHO, 2002). Similarly, all isolates showed modest to strong adaptation to alkaline pH, further reinforcing their probiotic potential.(Cho *et al.,* 2022: Sui *et al.,* 2023).

In terms of antimicrobial activity, the yeast isolates inhibited the growth of *Staphylococcus aureus* but did not exhibit any observable inhibition against *Escherichia coli*. This selective effect may be due to the differences in cell wall structures between Gram-positive and Gram-negative bacteria, with yeasts typically producing killer toxins, volatile fatty acids, and other antimicrobial metabolites that are more effective against Gram-positive species (Younis *et al.,* 2017). These results align with earlier findings that some non-*Saccharomyces* yeast species can secrete bioactive compounds, such as organic acids, ethanol, hydrogen peroxide, and killer toxins, which exert selective antimicrobial activity, particularly against Gram-positive bacteria like *S. aureus* (Branco *et al.,* 2023)The absence of inhibition against *Escherichia coli* in previous tests may be attributed to the structural differences in Gram-negative bacteria, which are generally more resistant due to their outer membrane barrier (Nikaido, 2003). Although the lack of activity against *E. coli* limits the spectrum of effectiveness, the inhibition of *S. aureus* remains a valuable trait in the context of probiotic functionality.

The results align with previous studies that have reported the presence of probiotic traits such as bile and acid resistance, osmotic stress adaptation, and antimicrobial activity in yeast strains isolated from plant-based sources and fermented foods (Zhou *et al.,* 2018). The observed differences among isolates (Yeast A, B, and C) further emphasize the strain-specific nature of probiotic properties, which is a recurring theme in probiotic research.

5.2 Conclusion

Yeasts were isolated from a variety of commonly consumed fruits and vegetables, including tomato, lettuce, cucumber, and grape, which are known to harbor diverse microbial communities suitable for probiotic screening. Despite promising findings, this study was limited by its scope to in vitro assays and a narrow pathogen spectrum. Molecular identification, although part of the research scope, was not fully detailed in this report, and in-vivo validation remains necessary for confirming health benefits. Nevertheless, the data provides strong preliminary evidence that selected yeast isolates, particularly from Yeast A and C, exhibit desirable probiotic characteristics and may serve as potential candidates for future development into functional food ingredients or probiotic supplements.

5.3 Recommendation

1. Molecular identification of yeast isolates should be carried out to confirm their species and detect genes associated with probiotic traits.
2. In-vivo studies using animal models or human trials are recommended to verify the probiotic efficacy and safety of the most promising isolates.
3. The ability of yeast strains to adhere to intestinal cells should be investigated to assess their colonization potential.

REFERENCES

Abdus Salam, M. A., Al-Amin, M. Y., and Tabassoom Salam, M. T. (2023). *Antimicrobial Resistance: A Growing Serious Threat for Global Public Health*. Healthcare, 11(13), Article 1946

Almeida, P. (2020). Isolation techniques for yeasts in environmental and food samples. *Applied Microbiology and Biotechnology*, 104(4), 1497–1510

Alvarez, S. C. V. et al. (2023). Non-Saccharomyces yeast probiotics: revealing relevance and potential. *FEMS Yeast Research*, volume 23 (2023)

Aryana, K. J., and Olson, D. W. (2017). A 100-Year Review: Yogurt and Other Cultured Dairy Products. *Journal of Dairy Science*, 100(12), 9987–10013

Balabekyan, T. R. et al. (2018). Antimicrobial activity of preparations after combined cultivation ... *Journal of Animal Physiology and Animal Nutrition*, 102(4), 933–938

Bang, W. et al. (2022). A quadruple coating of probiotics for enhancing intestinal adhesion… *Journal of Medicinal Food*, 25(2), 213–218

Bassyouni, R. (2023). Recent advancements in yeast isolation from food matrices. *Journal of Microbiological Methods, 208,* 106662.

Belletti, N., Gatti, M., Bottari, B., Neviani, E., & Tabanelli, G. (2020). Antimicrobial activity of probiotic yeasts against foodborne pathogens. *International Journal of Food Microbiology, 335*

Branco, P., Maurício, E. M., Costa, A., Ventura, D., Roma-Rodrigues, C., Duarte, M. P., Fernandes, A. R., & Prista, C. (2023). Exploring the multifaceted potential of a peptide fraction derived from *Saccharomyces cerevisiae* metabolism: Antimicrobial, antioxidant, antidiabetic, and anti-inflammatory properties. *Antibiotics, 12*(8), 1332.

Cappuccino, J. G., and Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson Education

Carvalho, R. (2022). Methods for yeast recovery and identification in food samples. *International Journal of Food Microbiology, 377,* 109828.

Chance, J., DeRouchey, J., and Amachawadi, R. (2022). Influence of yeast-based pre- and probiotics on antimicrobial resistance of fecal *Escherichia coli*. *Journal of Animal Science, 100*(6).

Chandimala, U. R., Rajawardhana, D. U., Liyanage, P. L. N., & Hewajulige, I. G. N. (2022). Isolation and characterization of yeasts from locally available foods. *Journal of Agro-Technology and Rural Sciences*, *2*(2), Article 43.

Chandimala, U. R., Rajawardhana, D. U., Liyanage, P. L. N., & Hewajulige, I. G. N. (2022). Isolation and characterization of yeasts from locally available foods. *Journal of Agro-Technology and Rural Sciences, 2*(2), Article 43. https://doi.org/10.4038/jarts.v2i2.43

Cho, H.-Y., Kim, S. R., and Ko, G. (2022). Optimizing conditions in the acid tolerance test for potential probiotics. *Foods, 11*(18), 2806.

Christaki, E., Marcou, M., and Tofarides, A. (2020). Antimicrobial resistance in bacteria: Mechanisms, evolution, and persistence. *Journal of Molecular Evolution*, 88(1), 26–40.

Clinical and Laboratory Standards Institute (CLSI). (2021). *Performance standards for antimicrobial susceptibility testing* (31st ed.). CLSI supplement M100.

Cryan, J. F., and Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience, 13*(10), 701–712.

Ebrahem, A. F., El-Demerdash, A. S., Orady, R. M., and Nabil, N. M. (2023). *Modulatory effect of competitive exclusion on the transmission of ESBL E. coli in chickens. Probiotics and Antimicrobial Proteins*.

Ebrahimi, N. A., Salehi Jouzani, G., and Ebrahimi, M. (2022). Native chicken-derived *Lactobacillus* spp. strains with high probiotic capabilities. *Iranian Journal of Microbiology, 14*, 227–237.

Fernández, A. (2021). Optimized isolation of yeasts from fresh produce. *Food Research International, 145,* 110395.

Friedman, Y., Hugenholtz, J., & De Vos, W. M. (2021). Safe use of genetically modified lactic acid bacteria in food: Bridging the gap between consumers, green groups, and industry. *Electronic Journal of Biotechnology, 52,* 1–8.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology, 66*(5), 365–378.

García, L. (2023). Enrichment and selective culturing of yeasts for food safety applications. *Frontiers in Microbiology*, 14, 1156394.

Gismondo, M. R., Drago, L., and Lombardi, A. (1999). Review of probiotics available to modify gastrointestinal flora. *International Journal of Antimicrobial Agents, 12*(4), 287–292.

Grujović, M. Ž. (2022). *Advantages and disadvantages of non-starter lactic acid bacteria from traditional fermented foods: Potential use as starters or probiotics. Comprehensive Reviews in Food Science and Food Safety, 21*(2), 1537–1567.

Gudiña, E. J., Teixeira, J. A., and Rodrigues, L. R. (2020). Isolation, characterization, and probiotic potential of yeasts from traditional fermented foods. *Journal of Functional Foods, 73*, 104080.

Gueimonde, M., Sánchez, B., G. de los Reyes-Gavilán, C., and Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology, 4*, 202.

Habteweld, H. A., & Asfaw, T. (2023). Novel dietary approach with probiotics, prebiotics, and synbiotics to mitigate antimicrobial resistance. *Frontiers in Nutrition, 10,* 1123456.

Hatoum, R., Labrie, S., & Fliss, I. (2012). Antimicrobial and probiotic properties of yeasts: From fundamental to novel applications. *Frontiers in Microbiology*, 3, 421.

Joossens, M. (2020). Unlocking the full potential of probiotics: Refocusing on microbial demands. *Chinese Medical Journal, 133*(15), 1765–1767.

Kurtzman, C. P., Fell, J. W., and Boekhout, T. (2011). *The Yeasts: A Taxonomic Study* (5th ed.). Elsevier.

Lachance, M. A. (2020). Yeasts and their biotechnological applications: A review. *FEMS Yeast Research, 20*(2), foaa008. https://doi.org/10.1093/femsyr/foaa008

Leistikow, K. R., Beattie, R. E., and Hristova, K. (2022). Probiotics beyond the farm: Benefits, costs, and considerations.

Lillie, A. (2022). *Kefir use in critically injured patients.* Journal of Burn Care and Research.

Lilly, D. M., and Stillwell, R. H. (1965). *Probiotics: Growth-promoting factors produced by microorganisms. Science, 147*(3659), 747–748.

Liu, Y., Wang, J., and Wu, C. (2022). Modulation of gut microbiota and immune system by probiotics, prebiotics, and postbiotics. *Frontiers in Nutrition*.

Malashree, L. & Anushree, Akshaykumar, (2024). Article no.EJNFS.118821 Original Research Article Malashree et al. European Journal of Nutrition & Food Safety. 110-116.

Marco, M. L., Pavan, S., and Kleerebezem, M. (2006). *Towards understanding molecular modes of probiotic action. Current Opinion in Biotechnology, 17*(2), 204–210.

Marlida, Y. (2021). *Potential probiotic yeast isolated from Indonesian fermented fish (Ikan Budu).* Journal of Microbiology and Biotechnology, 15, 460–466.

Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., and Rotondo, J. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, 12(2), 327.

McFarland, L. (2015). *From yaks to yogurt: The history, development, and current use of probiotics.* Clinical Infectious Diseases.

Meiling, C. (2018). *Yogurt ferments the dairy segment. The Daily Telegraph* and *China Daily*.

Merck. (2012). *Merck Microbiology Manual* (12th ed., pp. 515–516). Merck KGaA.

Murad, A. S., Zuhairi, N. A., Shahlan Mohd-Aspar, M. A., & Azhar, M. A. (2024). Isolation and characterisation of potential probiotic yeast strains from local fermented foods: Gastrointestinal tolerance and antimicrobial activity assessment. *Asia-Pacific Journal of Molecular Biology and Biotechnology, 32*(1), 116–125.Goyal, A., Mishra, R., and Rishi, P. (2022). Antibacterial properties of bacteriocins produced by lactic acid bacteria. *Frontiers in Microbiology*.

Nandha, M. C., and Shukla, R. M. (2023). *Exploration of probiotic attributes in lactic acid bacteria isolated from fermented Theobroma cacao L. fruit using in vitro techniques.* Frontiers in Microbiology.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews, 67(4), 593–656. https://doi.org/10.1128/MMBR.67.4.593-656.2003

Oliveira, M., Viñas, I., Usall, J., Anguera, M., Abadias, M. (2012). Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables – A review. *Trends in Food Science and Technology*, 46(1), 13–26.

Ouwehand, A. C., Salminen, S., and Isolauri, E. (2002). Probiotic and other functional microbes: From markets to mechanisms. *Current Opinion in Gastroenterology, 18*(3), 305–309.

Ozen, M., and Dinleyici, E. C. (2015). *The history of probiotics: The untold story.* Beneficial Microbes, 6(2), 159–165.

Pais, P., Almeida, V., Yılmaz, M., and Teixeira, M. C. (2020). *Saccharomyces boulardii*: What makes it tick as a successful probiotic? *Journal of Fungi (Basel)*, 6(2), 78.

Parker, R. B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health*, 29, 4–8.

Pérez, P., Lozano, C., & Molinero, N. (2021). Yeasts as probiotics: Evaluating acid and bile tolerance for gut health applications. *International Journal of Food Microbiology, 337,* 108953.

Piatek, J., Krauss, H., Ciechelska-Rybarczyk, A., Bernatek, M., Wojtyla-Buciora, P., & Sommermeyer, H. (2020). In-Vitro Growth Inhibition of Bacterial Pathogens by Probiotics and a Synbiotic: Product Composition Matters. International journal of environmental research and public health, 17(9), 3332. https://doi.org/10.3390/ijerph17093332

Rabetafika, H. N., Razafindralambo, A., and Ebenso, B. (2023). *Probiotics as antibiotic alternatives for human and animal applications.*

Raimondi, S. (2023). *Selective culturing and identification of yeasts from fruits and vegetables.* Journal of Applied Microbiology.

Reiner, K. (2012). *Carbohydrate fermentation protocol*. American Society for Microbiology.

Rijkers, G. T., de Vos, W. M., and Brummer, R. J. (2021). *Health benefits and health claims of probiotics: Bridging science and marketing*. *British Journal of Nutrition*, 106(9), 1291–1296.

Sahu, N., Pradhan, C., and Thakur, V. (2022). *Role of algae as probiotics: A sustainable approach to improve gut health*. *Algal Research*.

Sanders, M. E. (2008). *Probiotics: Definition, sources, selection, and uses*. *Journal of Food Science*, 73(4), R8–R15.

Saxena, R., and Gupta, N. (2020). *Health benefits of probiotic fermented foods*. Retrieved from https://www.semanticscholar.org/paper/Health-benefits-of-probiotic-fermented-foods-Saxena-Gupta

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics—Approaching a definition. *The American Journal of Clinical Nutrition, 73*(2), 361S–364S. https://doi.org/10.1093/ajcn/73.2.361s

Sharma, R., Kapila, R., Dass, G., Kapila, S. (2014). Improvement in survival of probiotic strains in simulated gastric fluid using calcium alginate and chitosan. *International Journal of Food Sciences and Nutrition, 65*(8), 1007–1013.

Shiby, V. K., and Mishra, H. N. (2023). *Fermented milks and milk products as functional foods – A review*. *Critical Reviews in Food Science and Nutrition*.

Singhi, S., and Kumar, A. (2021). Emerging sources of probiotics: Beyond bacteria and yeast. *Journal of Applied Microbiology*, 131(5), 2145–2158.

Solanki, R., Anand, S., and Kumar, P. (2022). *Antibiotic resistance: A global health crisis*. Retrieved from https://www.semanticscholar.org/paper/Antibiotic-resistance%3A-A-global-health-crisis-Solanki-Anand

Solieri, L., and Giudici, P. (2021). *Yeast associated with traditional balsamic vinegar: Ecological features*. *International Journal of Food Microbiology*, 339, 109027.

Sui, Y., Liu, Y., Song, B., and Wang, Q. (2023). *Assessment of yeasts as potential probiotics: Survival under simulated gastrointestinal conditions*. *Journal of Microbiological Methods*, 212, 106784.

Swain, M. R., Anandharaj, M., Ray, R. C., and Parveen Rani, R. (2014). *Fermented fruits and vegetables of Asia: A potential source of probiotics.* Biotechnology Research International, 2014, Article ID 250424, pp. 1–19.

Tortora, G. J., Funke, B. R., and Case, C. L. (2019). *Microbiology: An Introduction* (5th ed., p. 135). San Francisco, CA: Pearson Benjamin Cummings.

Tullio, V. (2024). Probiotic yeasts: A developing reality? *Journal of Fungi, 10*(7), 489.

Tumbarski, Y. D., Yanakieva, V. B., Denkova-Kostova, R. S., & Denkova, Z. R. (2021). Isolation, identification and comparison of some properties of Lactobacillus delbrueckii subsp. Bulgaricus strains from traditional Bulgarian and Italian yogurts.

Walker, G. M., & White, N. A. (2017). Yeasts in food and beverages. *Advances in Applied Microbiology, 100,* 215–265.

Walter, J. (2018). The role of hydrogen peroxide in the antimicrobial activity of probiotics. *Current Opinion in Microbiology, 42*, 68–72.

Wang, Y., et al. (2021). Advances in yeast isolation and characterization. *Critical Reviews in Biotechnology, 41*(2), 312–328.

Wulan, R., Astuti, R. I., Rukayadi, Y., and Meryandini, A. (2021). Evaluation of indigenous *Pichia kudriavzevii* from cocoa fermentation for a probiotic candidate. *Biodiversitas, 22*(4), 2242–2252.

Younis, G., Awad, A., Dawod, R. E., & Yousef, N. E. (2017). Antimicrobial activity of yeasts against some pathogenic bacteria. Veterinary world, 10(8), 979–983. https://doi.org/10.14202/vetworld.2017.979-983

Yuan, Y. (2023). Advancing insights into probiotics during vegetable fermentation. *Foods, 12*, 1234. [Retrieved via Semantic Scholar]

Zahoor, F., Sooklim, C., Songdech, P., Duangpakdee, O., and Soontorngun, N. (2021). Selection of potential yeast probiotics and a cell factory for xylitol or acid production from honeybee samples. *Metabolites, 11*(3), 162.

Zhou, Y., Wang, H., Zhang, X., and Yang, R. (2018). Screening and characterization of probiotic yeast strains for acid and bile salt resistance. *Applied Microbiology and Biotechnology, 102*(12), 5245–5254.

Zmora, N., Suez, J., and Elinav, E. (2018). You are what you eat: Diet, health and the gut microbiota. *Nature Reviews Gastroenterology and Hepatology, 15*, 35–56. Abdus Salam, M. A., Al Amin, M. Y., & Tabassoom Salam, M. T. (2023). Antimicrobial resistance: A growing serious threat for global public health. *Healthcare, 11*(13), 1946. https://doi.org/10.3390/healthcare11131946

APPENDIX

Appendix I: Effect of 0.3% Bile on Yeast Isolates

Optical density (OD) is used to assess the viability of cells, after exposure to the modified medium, over time.

Note. SD = standard deviation. *n* = Number of replicates per group.

Time (Hrs)	Sample	Mean	SD
T0	YEAST A	0.017	0.0047
	YEAST B	0.075	0.0095
	YEAST C	0.080	0.0091
T1	YEAST A	0.019	0.0038
	YEAST B	0.087	0.0080
	YEAST C	0.078	0.0036
T2	YEAST A	0.023	0.0042
	YEAST B	0.100	0.0146
	YEAST C	0.081	0.0017
T3	YEAST A	0.026	0.0060
	YEAST B	0.110	0.0225
	YEAST C	0.084	0.0015
T4	YEAST A	0.028	0.0087
	YEAST B	0.120	0.0316
	YEAST C	0.086	0.0025

Appendix II: Effect of 0.5% Bile on Yeast Isolates

Optical density (OD) is used to assess the viability of cells, after exposure to the modified medium, over time. **Note**. *M* = Mean; *SD* = Standard deviation. Optical density measured at 660 nm across five time points (T_0–T_4) under 0.5% bile concentration for each sample.

Time Point	Sample	M (Optical Density)	SD
T_0	YEAST A	0.025	0.009
	YEAST B	0.069	0.007
	YEAST C	0.072	0.007
T_1	YEAST A	0.037	0.005
	YEAST B	0.100	0.029
	YEAST C	0.086	0.012
T_2	YEAST A	0.040	0.005
	YEAST B	0.130	0.052
	YEAST C	0.091	0.015
T_3	YEAST A	0.045	0.005
	YEAST B	0.149	0.061
	YEAST C	0.096	0.018
T_4	YEAST A	0.050	0.006
	YEAST B	0.168	0.070
	YEAST C	0.099	0.021

Appendix III : Effect of 30% Glucose on Yeast Isolates' Growth Over Time

Note. M = Mean, SD = Standard Deviation

Optical density (OD) is used to assess the viability of cells, after exposure to the modified medium, over time

Time (hr)	Group	M	SD
T0	YEAST A	0.168	0.0065
	YEAST B	0.082	0.0030
	YEAST C	0.191	0.0060
T24	YEAST A	0.291	0.0145
	YEAST B	0.367	0.0155
	YEAST C	0.628	0.0155
T48	YEAST A	0.878	0.0228
	YEAST B	0.502	0.0145
	YEAST C	0.731	0.0228

Appendix IV: Effect of 40% Glucose on Yeast Isolates' Growth Over Time.

Optical density (OD) is used to assess the viability of cells, after exposure to the modified medium, over time.

Time Point	Group	Mean OD660	Standard Deviation
Time 0	YEAST A	0.168	0.0065
	YEAST B	0.096	0.0030
	YEAST C	0.165	0.0065
Time 24	YEAST A	0.291	0.0145
	YEAST B	0.199	0.0090
	YEAST C	0.359	0.0165
Time 48	YEAST A	0.878	0.0228
	YEAST B	0.502	0.0170
	YEAST C	0.668	0.0172

Appendix V: Effect of 50% Glucose on Yeast Isolates' Growth Over Time

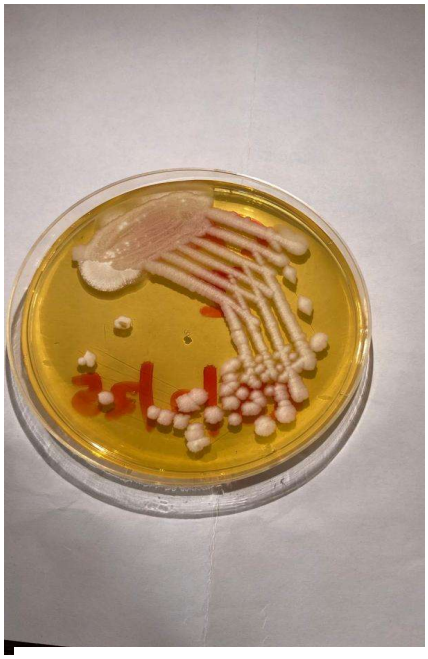
Optical density (OD) is used to assess the viability of cells, after exposure to the modified medium, over time.

Time Point	Group	Mean	Standard Deviation
T0	YEAST A	0.182	0.0066
	YEAST B	0.117	0.0046
	YEAST C	0.176	0.0061
T24	YEAST A	0.160	0.0106
	YEAST B	0.215	0.0093
	YEAST C	0.261	0.0106
T48	YEAST A	0.516	0.0127
	YEAST B	0.412	0.0140
	YEAST C	0.434	0.0145

Appendix VI: Effect of Acid Medium pH3.0, and Alkaline Medium pH8.0 on Yeast Isolates' Growth Over Time

Optical density (OD) is used to assess the viability of cells after exposure to the modified medium over time.

ISOLATES	pH 3.0		pH8.0	
	T0 (hrs)	T2 (hrs)	T0 (hrs)	T4 (hrs)
YEAST A	0.172	0.21	0.199	0.201
YEAST B	0.091	0.077	0.080	0.135
YEAST C	0.118	0.131	0.143	0.222

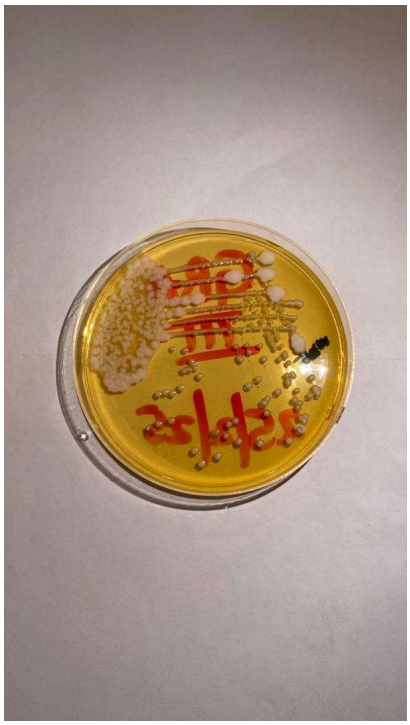


Appendix VII: Morphological characteristics of Isolate LET

Appendix VIII: Morphological characteristics of Isolate CAB

Appendix IX: Morphological characteristics of Isolate GAR

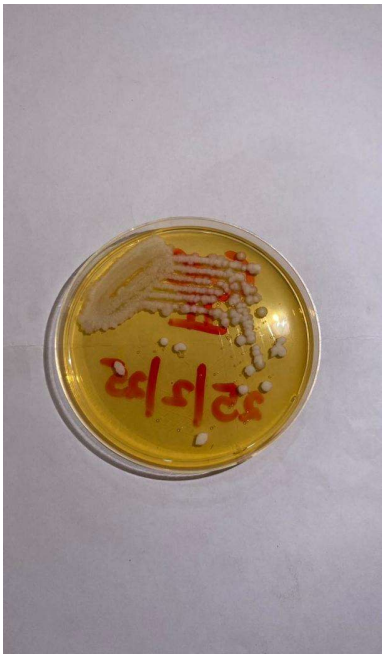
Appendix X: Morphological characteristics of Isolate CUC



Appendix XI: Morphological characteristics of Isolate GRA-B

Appendix XII: Morphological characteristics of Isolate WA

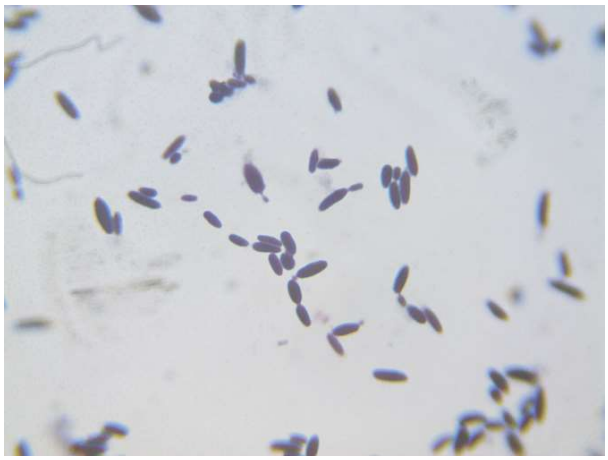
Appendix XII: Morphological characteristics of Isolate TOMA



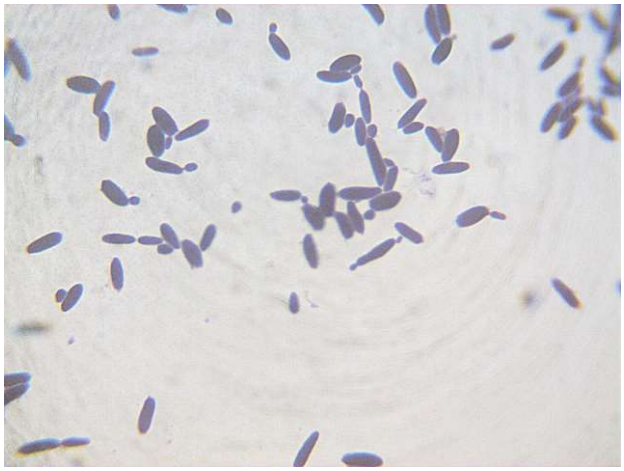
Appendix XIV: Morphological characteristics of Isolate GRA-B

KEY

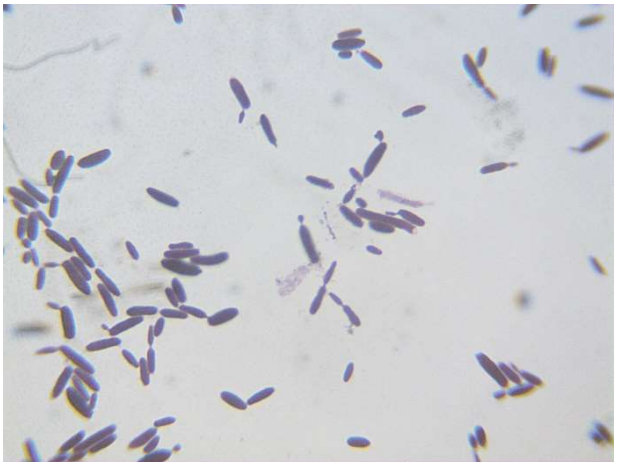
CUC= Cucumber isolate
CAB= Cabbage isolate
GAR= Garden egg isolate
GRA= Grape isolate A
GRA-R= Grape isolate B
LET= Lettuce isolate
WA= Watermelon isolate A
WA= Watermelon isolate A
TOMA= Tomatoes isolate



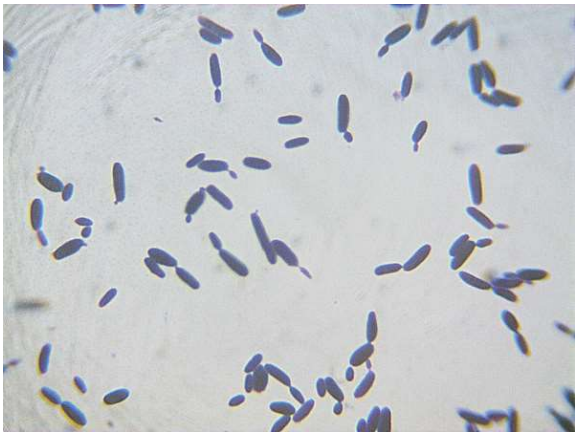
Appendix XV: Gram reaction of CAB



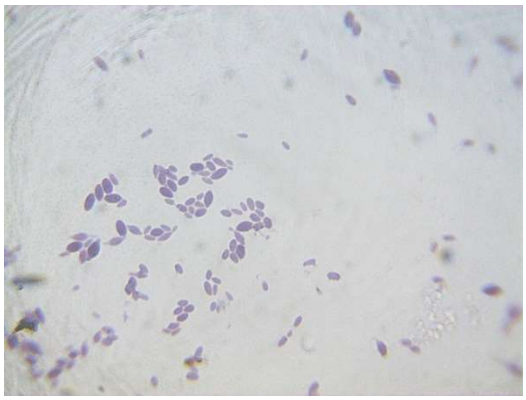
Appendix XVI: Gram reaction of CUC



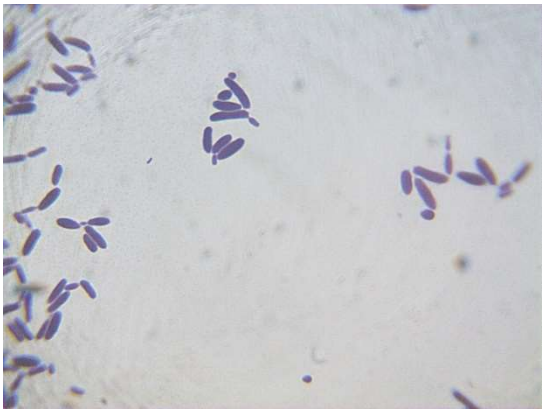
Appendix XVII: Gram reaction of GAR



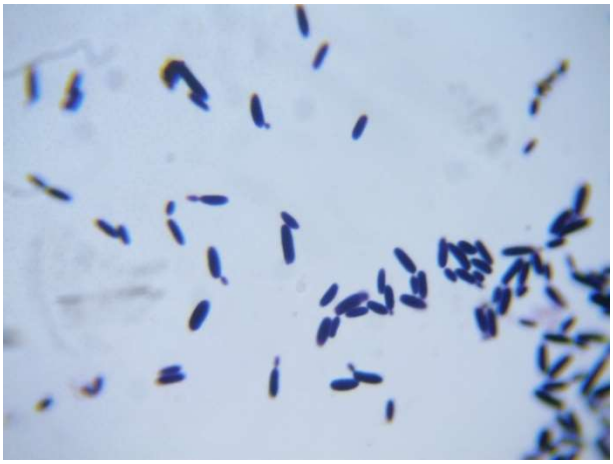
Appendix XIX: Gram reaction of GRA-A



Appendix XX: Gram reaction of GRA-B



Appendix XXI: Gram reaction of TOMA



Appendix XXII: Gram reaction of LET

KEY

CUC= Cucumber isolate
CAB= Cabbage isolate
GAR= Garden egg isolate
GRA= Grape isolate A
GRA-R= Grape isolate B
LET= Lettuce isolate
WA= Watermelon isolate A
WA= Watermelon isolate A
TOMA= Tomatoes isolate