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Probiotic Potential of *Lactobacillus rhamnosus* BM55 Isolated from *Bongo,* a Traditionally Fermented Milk Beverage from Uganda

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DECLARATION

I, Rhema Bethany Gawaya, hereby do affirm and declare to the best of my knowledge and understanding that the material presented in this report is my own work and contains no material, which has been presented elsewhere for any academic award.

Student

Signed.....

RHEMA BETHANY GAWAYA

Date...3/3/2025.....

This report has been submitted for examination with approval of my supervisor.

Supervisor

Signed.....

DR. STELLAH BYAKIKA

Date...03/03/2025.....

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DEDICATION

I dedicate this to my family for the continued support through the journey.

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LIST OF ACRONYMNS

BSH	Bile Salt Hydrolase
CFCS	Cell-free culture supernatant
CFU	Colony forming units
GIT	Gastrointestinal Tract
GRAS	Generally regarded as safe
LAB	Lactic acid bacteria

ABSTRACT

Lactobacillus rhamnosus BM55, isolated from the traditional Ugandan fermented milk beverage, *Bongo*, was assessed for potential probiotic properties. The organism was evaluated for its survival in simulated gastro intestinal conditions assessed through acid and bile tolerance tests. Its safety was also assessed through antibiotic resistance and biogenic amine production tests. Furthermore, potential probiotic benefits that is, pathogenic inhibition and bile salt hydrolase tests were conducted. Results were compared against a known reference, *Lactobacillus rhamnosus* Yoba 2012. The findings indicated the tolerance of *Lactobacillus rhamnosus* BM55 as well as the reference microorganism in simulated gastrointestinal conditions. The candidate isolate was resistant to 18 of 20 antibiotics. It was susceptible to streptomycin and sulfisoxazole. The reference was resistant to sulphamethoxazole, colistin sulphate, vancomycin, kanamycin and metronidazole. The candidate isolate decarboxylated L-histidine and L-ornithine whereas, the reference did not decarboxylate any amino acids. The candidate and reference isolates exhibited medium and low BSH activity, respectively. *Lactobacillus rhamnosus* BM55 demonstrated minimum inhibitory activity against *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* but not against *E. coli* ATCC 25922. Additional evaluations are required to further ascertain if the candidate possesses probiotic properties.

Keywords: Probiotics, *Lactobacillus rhamnosus*, *Bongo*, fermented dairy beverage, lactic acid bacteria

1 INTRODUCTION

1.1 Background

Probiotics are live microorganisms that confer health benefits to a consumer when ingested in sufficient amounts (Araya et al., 2002). They have sparked global interest in the realms of food, animal feeds, and supplements (Reuter, 2001) These microorganisms have been associated with a number of health advantages, including preventing antibiotic-related diarrhea, alleviating irritable bowel syndrome, enhancing the immune response, and even contributing to the production of essential B vitamins and antioxidants (Kechagia et al., 2013). Their potential impact extends to prolonging life, reducing serum cholesterol levels and addressing conditions like lactose intolerance and *Helicobacter pylori* infection. They are also proven to possess anti-cholesterol, anti-obesity, anti-diabetic and anti-anxiety properties (Mathur et al., 2020; Zoumpopoulou et al., 2017).

Probiotic bacteria are primarily found in fermented foods, with dairy products serving as the primary carriers of these beneficial microorganisms (Heller, 2001). *Bongo* is a traditional fermented milk beverage native to Uganda, and is made by fermentation of either pasteurized or unpasteurized milk (Ogwaro et al., 2023). The milk undergoes natural fermentation as a result of the acidic properties of indigenous microorganisms present in the raw milk (Leone et al., 2022). *Lactobacillus rhamnosus* BM55 was isolated from *Bongo* by Byakika and Mukisa (2024).

Prior to this study, the probiotic potential of the isolate, *Lactobacillus rhamnosus* BM55, remained unexplored. Given its origin in a traditional fermented food, there are questions about its viability and functionality within the human GIT. To address the questions and adhere to established guidelines, this research sought to evaluate its probiotic potential while comparing against a known probiotic, *Lb. rhamnosus* yoba 2012.

1.2 Problem statement

Fermented foods have been an important part of the human diet in nearly every culture on every continent for a very long time (Tamang et al., 2020). These foods are often well-preserved and serve as stable and significant sources of proteins, vitamins, minerals, and other nutrients (Tamang et al., 2020). These foods have exhibited positive effects on health in many ways, for example; lowering blood cholesterol levels, boosting immunity, protecting against pathogens, fighting

carcinogenesis, osteoporosis, diabetes, obesity, allergies, and atherosclerosis, and reducing the symptoms of lactose intolerance (Tamang and Kailasapathy, 2010). Examples of fermented foods include; *Miso*, *tempeh*, soy-sauce, beer, wine, *Bongo* and yoghurt (Chilton et al., 2015)

Fermented dairy products are obtained from fermentation of milk, through the action of suitable and harmless microorganisms (García-Burgos et al., 2020). The biggest percentage of fermented dairy products contain LAB, but others also contain bacteria, yeasts and molds (Wouters et al., 2002). *Bongo* is a traditionally fermented milk beverage from Uganda. It is produced traditionally by fermenting unpasteurized cow's milk (Mugampoza et al., 2011). It is consumed as a side dish (Gershom and Edward, 2017). *Lactobacillus rhamnosus* BM55 is a starter culture that was isolated from *Bongo* by Byakika and Mukisa (2024)

Aside from *L. rhamnosus* BM55 having starter culture properties for *Bongo*, it is probiotic characteristics of remain unknown. Despite the rich diversity of microorganisms in various fermented foods, there is a scarcity of information about the probiotic potential of isolates from *Bongo*. It was, therefore, necessary to investigate whether *L. rhamnosus* BM55 possesses the potential probiotic properties.

1.3 Objectives

1.3.1 General objective

To investigate the probiotic potential of *Lactobacillus rhamnosus* BM55 from *Bongo.*

1.3.2 Specific objectives

1. To assess the survival of *Lactobacillus rhamnosus* BM55 in simulated gastrointestinal conditions.
2. To assess the safety of *Lactobacillus rhamnosus* BM55 for human consumption.
3. To determine selected potential probiotic benefits of *Lactobacillus rhamnosus* BM55.

1.4 Hypotheses

1. *Lactobacillus rhamnosus* BM55 can survive simulated gastrointestinal conditions.
2. *Lactobacillus rhamnosus* BM55 is safe for human consumption. 3. *Lactobacillus rhamnosus* BM55 has potential probiotic benefits.

2 LITERATURE REVIEW

2.1 Probiotics

The word “probiotic” was adapted from the Greek word meaning “for life” (Harpreet and Shailly, 2012). The notion of probiotics runs back to 1908 when Ilya Metchnikoff, Ukranian Nobel Prize winner, suggested that the long life of peasants in Bulgaria was a result of their consumption of fermented milk products (Metchnikoff, 1908). These microorganisms contribute to intestinal microbial balance and play a role in maintaining health (Sehrawat et al., 2021). Probiotic microorganisms consist mainly of the LAB strains of *Lactobacillus* and *Bifidobacterium* (Gueimonde et al., 2004). Besides LAB, other non-pathogenic microorganisms with healthpromoting characteristics such as certain strains of *Saccharomyces boulardii* and *Escherichia coli* (*E. coli* Nissle 1917), are also applied (Sonnenborn and Schulze, 2009). Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of beneficial microbes in the intestinal flora (Ricardo *et al.*, 2010).

2.2 Criteria for classification of a probiotic

There are a number of probiotics on the market whose authenticity cannot be proven (Mahasneh and Abbas, 2010). Moreover, there is criteria for classification of probiotics which assesses three major characteristics: survival in GIT conditions, safety to the consumer, and any health benefits conferred to the consumer (Ramos et al., 2013). These health benefits of probiotics vary based on the specific strain, and they cannot be generalized to all probiotic organisms or even those of the same species (Ramos et al., 2013). In figure 1, we see some of the major guidelines for evaluation of probiotics.

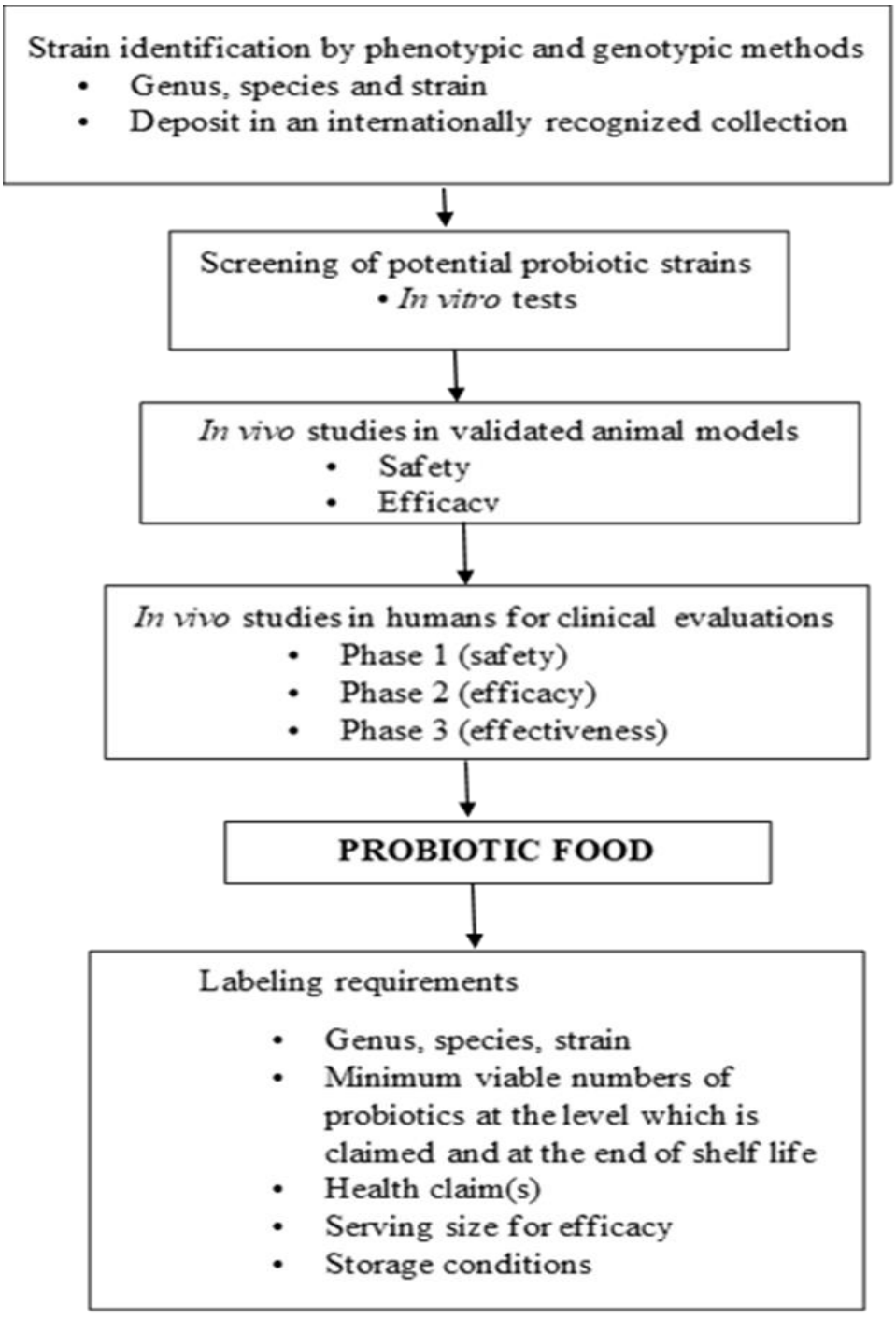

Figure 1: Guidelines for evaluation of candidate probiotic strains (Ganguly et al., 2011)

2.2.1 Survival in the Gastro-intestinal tract

For probiotics to provide their beneficial effects, they must survive the harsh conditions of the gastrointestinal tract and reach the large intestine in sufficient numbers to allow colonization and

growth (Da Silva et al., 2021). However, the low pH of gastric juice prevents most probiotics from surviving in large quantities, limiting their effectiveness in many functional foods (Shori, 2017). Resistance to stomach acid and tolerance to bile salts are essential properties for microorganisms to be classified as probiotics, enabling them to survive the acidic conditions of the stomach and the bile salts present in the small intestine during gastrointestinal transit (De Carvalho Lima et al., 2009; Saad, 2006). FAO/WHO (2002) also recommends bile salt hydrolase (BSH) activity as one of the tests for screening of survival of candidate strains in the GIT.

2.2.1.1 Acid and bile salt tolerance

The selection of a new probiotic strain mainly depends on the *in vitro* tolerance of physiologically related stresses including low pH and bile, to ensure that the potential probiotic microorganism can survive the harsh conditions of the GIT (Ayyash et al., 2021). Many assessment methods are *in vitro* due to the high costs, ethical concerns, and safety issues of *in vivo* studies (Ritter et al., 2009). However, the varied experimental conditions used in these assessments (different types of media, bile, pH, and incubation time) hinder the comparison of the results of these investigations (Ayyash et al., 2021).

While some growth studies have examined the acid tolerance of probiotic strains, survival studies involve counting the cells before and after exposing them to conditions that simulate the stressful environments of the stomach (Martín et al., 2006; Nguyen et al., 2007). Majority of the *In vitro* assays include stressful conditions such as extreme low pH (pH 2-3) (Byakika et al., 2019). Bile tolerance can be evaluated by examining growth in culture media with varying concentrations of bile (Mirelahi et al., 2009). This assessment can be conducted by measuring changes in optical density and pH of the broth culture or by analyzing growth on solid culture media (Liong and Shah, 2005; Morelli, 2007; Succi et al., 2005).

2.2.1.2 Bile Salt Hydrolase (BSH) activity

The ability of probiotic strains to hydrolyze bile salts has often been included among the criteria for probiotic strain selection (Begley et al., 2006). BSH activity is assessed by spotting freshly grown culture on agar plates supplemented with bile salts and incubated at appropriate conditions depending on the candidate strains (Yadav et al., 2016). BSH activity in probiotic strains is usually correlated with the ability to lower serum cholesterol levels in hypercholesterolemic patients

(Hernández-Gómez et al., 2021). Several mechanisms for cholesterol removal by probiotics have been shown, such as deconjugation of bile salts by the enzyme bile salt hydrolase (Tsai et al., 2014). However, the activity of microbial bile salt hydrolase has been suggested to potentially harm the human host, raising uncertainty about whether BSH activity is truly a beneficial trait in probiotic bacteria (Begley et al., 2006).

2.2.2 Evaluation of probiotic safety

The safety of novel probiotic strains must be evaluated through *In vitro* and *In vivo* animal models before being approved for human consumption (Pradhan et al., 2020). Although most safety studies have not demonstrated toxic effects of probiotic bacteria, it is widely accepted that these bacteria are not entirely free of pathogenic traits, as infections have been reported (Pradhan et al., 2020). The Joint FAO/WHO Working Group recommended that antimicrobial resistance patterns and opportunistic virulence properties should be tested to document the safety of probiotic strains. It suggests conducting at least the following tests: antibiotic susceptibility, toxin production, hemolysis, and evaluation of side effects in human studies (FAO/WHO, 2002)

2.2.2.1 Antibiotic susceptibility

Probiotic organisms should not be inhibited by antibiotics, as those with intrinsic resistance can help restore gut microbiota after antibiotic therapy (Gueimonde, Sanchez, et al., 2013). Antibiotic resistance is only a concern if there is a risk of transferring resistance genes to pathogens (Gueimonde, Sanchez, et al., 2013). Many LAB possess intrinsic, non-transmissible resistance to antibiotics (Adams and Marteau, 1995). Some LAB, like certain strains of *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacillus fermentum* may carry plasmid-encoded, transmissible antibiotic resistance genes (Ahn et al., 1992; Fons et al., 1997; Ishiwa and Iwata, 1980). The transmission of antibiotic resistance genes to pathogenic gut bacteria is a major health concern, affecting the selection and safety of probiotic strains (Zhou et al., 2005).

2.2.2.2 Production of biogenic amines

Spano et al. (2010) defined biogenic amines as organic compounds formed in foods by the decarboxylase activity of microorganisms, primarily LAB. They are produced as a defense mechanism against acidic environments (Shalaby, 1996). Ingesting food with high levels of biogenic amines poses a health risk, as these compounds can cause headaches, heart palpitations,

vomiting, diarrhea, and hypertensive crises (Alvarez and Moreno-Arribas, 2014; Hungerford, 2010; Shalaby, 1996). High levels of 2-phenylethylamine, putrescine, cadaverine, agmatine, spermine, and spermidine can cause toxicity and enhance histamine and tyramine toxicity by inhibiting their metabolizing enzymes (Pegg, 2013).

Many LAB are used as starter cultures in the dairy and meat industries, so testing their decarboxylase activity and potential to increase biogenic amine levels is valuable for food manufacturers (Lorencová et al., 2012). Micro-organisms producing significant amounts of biogenic amines in manufacturing conditions should not be used as starters (Suzzi and Gardini, 2003).

2.2.3 Evaluation of probiotic benefit

Probiotic products have been shown to provide a variety of documented health benefits to a wide range of consumers (Salminen et al., 1998). Their health benefits must be scientifically established by clinical studies in humans performed by several independent research groups and published in peer-reviewed journals (Heczko et al., 2006). The probiotic benefits have mainly been related to the health of the gastrointestinal tract (Obioha et al.), immune and the urogenital system (Hill et al., 2014; Vandenplas et al., 2015). In order for a potential probiotic strain to be able to exert its beneficial effects, it must exhibit certain desirable properties. Key factors, as determined by *in vitro* tests, include acid and bile tolerance, which is crucial for oral administration, and adhesion to mucosal surfaces for immune modulation and pathogen prevention (FAO/WHO, 2002). Additionally, probiotics should display antimicrobial activity against pathogens and bile salt hydrolase activity (Mercenier et al., 2008; Saarela et al., 2000).

2.2.3.1 Adhesion to gastrointestinal mucosa

Adhesion to the intestinal mucosa is one of the main selection criteria for probiotics (Kirjavainen et al., 1998). It is considered a prerequisite for successful colonization (Finlay and Falkow, 1997). It is also important for immune modulation by the probiotics (Famularo et al., 1997). It has been shown previously that the composition of the intestinal *Lactobacillus* population changes with time (Kimura et al., 1997). This may influence the ability of a probiotic to adhere and colonize the intestinal mucosa, which can, in turn, affect the efficacy of the probiotic preparation (Ouwehand et al., 1999). Additionally, understanding the adhesive properties of probiotic organisms can

provide insight into their potential to colonize and modulate the immune system (Ouwehand et al., 1999).

2.2.3.2 Antimicrobial activity

One of the main requirements for probiotic is to exhibit antimicrobial activity against pathogenic microorganisms (Collado et al., 2007). The antimicrobial activity of probiotics is strain-specific and operates through direct mechanisms like competitive exclusion for adhesion sites, competition for resources, synthesis of antimicrobial substances, and inhibition of pathogen toxin expression (Denkova et al., 2017). Antimicrobial activity is determined through *In vivo* methods like animal models and clinical trials, or *in vitro* methods such as the spot-on lawn, agar well-diffusion, and disc diffusion assays (Denkova et al., 2017). *In vitro* studies have shown that probiotics can inhibit pathogens like *Salmonella Typhimurium* and *Escerichia coli*, compete for adhesion, and displace enteropathogens from Caco-2 cells. (Tejero-Sariñena et al., 2012).

2.2.3.3 Bile Salt Hydrolase (BSH) activity

The ability of probiotic strains to hydrolyze bile salts has often been included among the criteria for probiotic strain selection (Begley et al., 2006). BSH activity is assessed by spotting freshly grown culture on agar plates supplemented with bile salts and incubated at appropriate conditions depending on the candidate strains (Yadav et al., 2016). BSH activity in probiotic strains is usually correlated with the ability to lower serum cholesterol levels in hypercholesterolemic patients (Hernández-Gómez et al., 2021). Several mechanisms for cholesterol removal by probiotics have been shown, such as deconjugation of bile salts by the enzyme bile salt hydrolase (Tsai et al., 2014). However, the activity of microbial bile salt hydrolase has been suggested to potentially harm the human host, raising uncertainty about whether BSH activity is truly a beneficial trait in probiotic bacteria (Begley et al., 2006).

2.2.3.4 Lactose intolerance

Lactose intolerance is a genetic disorder occurring due to deficiency of beta-galactosidase resulting in the inability of the body to hydrolyze lactose into its constituent monosaccharides (Kechagia et al., 2013). In lactose-intolerant individuals, undigested lactose is broken down by bacterial enzymes in the large intestine, leading to symptoms like osmotic diarrhea, abdominal discomfort, and flatulence after consuming dairy products (Kechagia et al., 2013). When probiotic bacteria are

ingested orally or in a food product, the active microbial β-galactosidase in the microorganisms survives gastric passage and is released by bile salts into the small intestine, where it supports lactose digestion hence relieving lactose intolerance (de Vrese et al., 2001). Therefore, improved lactose metabolism is a recognized health benefit linked to probiotics, with certain strains and specific concentrations showing greater effectiveness (de Vrese et al., 2001). Given that some individuals have responded well to probiotic supplementation, clinicians should consider it as a potential therapeutic option (Levri et al., 2005)

2.2.3.5 Probiotics and allergy

In the last few decades, the incidence and prevalence of allergic disorders have increased progressively throughout the world, especially in western societies (Fishbein and Fuleihan, 2012). Probiotics, administered as supplements or in infant foods, have been assessed in randomized controlled trials for allergy prevention, with numerous studies investigating specific probiotic strains for managing atopic dermatitis and other skin-related allergic conditions (Castellazzi et al., 2013; West, 2016). *L. rhamnosus* GG has effectively prevented atopic eczema in high-risk infants when given to pregnant mothers with a family history of atopic eczema and asthma (Isolauri et al., 2000). However, probiotics have had limited success in alleviating asthma symptoms (Wheeler et al., 1997). Research on various probiotic strains for treating and preventing allergic diseases has however, yielded controversial and inconsistent results (Grüber, 2012). Expert bodies generally do not recommend probiotics for allergy prevention, but the World Allergy Organization suggests considering them for pregnant women, breastfeeding mothers, or high-risk infants (West, 2016).

2.2.3.6 Infectious diarrhea

Infectious diarrhea is a global public health issue and remains a leading cause of illness and death among infants and children in many developing countries (Cheng et al., 2005). Treatment and prevention of infectious diarrhoea are probably the most widely accepted health benefits of probiotic microorganisms, rotavirus being the most common cause of acute infantile diarrhoea in the world (Kechagia et al., 2013). Probiotic supplementation in infant formulas aims to prevent rotaviral infections and treat established disease, with well-controlled clinical studies showing that probiotics like *L. rhamnosus* GG, *L. reuteri*, *L. casei* Shirota, and *Bifidobacterium. animalis* Bb12 can reduce the duration of acute rotavirus diarrhea (Isolauri et al., 2002; Shah, 2007; Szajewska

and Mrukowicz, 2001). Although the benefits of probiotics are modest by reducing the duration of illness by one or two days can be important, they are not a cure-all for diarrheal diseases (Saavedra, 2000).

2.2.3.7 Probiotics and liver diseases

Micro flora resident in intestinal lumen plays a significant role in hepatocytes function (Abatenh et al., 2018). Changes in the types and quantities of microorganisms in the intestinal tract can lead to significant liver dysfunctions, including cirrhosis, nonalcoholic fatty liver disease, alcoholic liver disease, and hepatic encephalopathy (Abatenh et al., 2018). Probiotics are being explored as a novel treatment strategy for liver disease by regulating, restoring, and altering gut microbiota and immune function (Javadi et al., 2017; Lunia et al., 2014). Probiotics are beneficial in treating chronic liver diseases by enhancing the intestinal barrier, which helps block the entry of microorganisms into the bloodstream and consequently, the liver (Cesaro et al., 2011).

2.3 *Lactobacillus* spp.

Lactobacillus is a genus of rod-shaped, gram-positive, non-spore-forming, facultatively anaerobic bacteria that metabolize carbohydrates into lactic acid. They are the largest genus within the lactic acid bacteria (LAB) group (Dempsey and Corr, 2022; Hammes and Hertel, 2015; Ibrahim, 2016). Lactobacilli are extensively utilized in food fermentation processes (Dempsey and Corr, 2022). Moreover, specific strains within this genus have been identified for their probiotic attributes (Pot et al., 2014). Probiotic lactobacilli confer various benefits to the host for example *Lactobacillus rhamnosus* GG helps in prevention and relief of various types of diarrhea such as traveler's diarrhea and acute diarrhea (Pace et al., 2020).

2.3.1 *Lactobacillus rhamnosus*

Lactobacillus rhamnosus is a lactic acid bacterium present in various environments, such as dairy products, the oral cavity, intestines, and vagina (Douillard et al., 2013). Certain strains of *Lactobacillus rhamnosus* are widely utilized as probiotics in food products, as well as in health and functional foods (Holzapfel et al., 1998; Kalliomäki et al., 2001). A well-known probiotic strain, *L. rhamnosus* GG, is commonly used to ferment dairy products (Saxelin, 1997). Other potential probiotic *lactobacillus* strains that are used to ferment dairy products have been studied. They include; *L. rhamnosus* E-97800 and *L. rhamnosus* LC-705 (Kontula et al., 1999; Lehto and

Salminen, 1997; Suomalainen et al., 1995). This study focuses of assessing the probiotic potential of *L. rhamnosus* BM55 isolated by Byakika and Mukisa (2024) from *Bongo*, a traditional fermented dairy product.

2.5 Fermented dairy products as sources of probiotics

Fermented dairy products are widely consumed globally, with recent years showing a significant increase in consumption and trends suggesting continued growth (Garcia-Burgos et al., 2020). Consumer interest in fermented dairy products is rising due to their nutritional and health benefits, as they positively impact intestinal microbiota and promote a healthier, longer life (Bourrie et al., 2016; Chen et al., 2019).

Different LAB produce various fermentation products, yet all remain alive in the product and can interact with microbiota and intestinal wall cells during transit (Granier et al., 2013). The most common LAB used for fermentation of milk are *Streptococcus thermophilus*, usually in association with *Bifidobacteria*, such as *Bifidobacterium breve* C50, *Bifidobacterium lactis*, *Bifidobacterium longum* and *Bifidobacterium animalis*, or with Lactobacilli such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus johnsonii* and *Lactobacillus casei* (Granier et al., 2013). Recent trends in the fermented food industry led to the development of products providing functional components such as probiotic bacteria (Homayouni et al., 2012). Fermented dairy products include yogurt, the most studied among them, as well as *Kumys*, *Skyr*, *Yakult*, and *Kefir* (Bourrie et al., 2016; Chen et al., 2019). *Bongo* is also a fermented dairy product native to Uganda (Mugampoza et al., 2011).

2.5.1 *Bongo*

Traditional fermented dairy products are highly valued worldwide for their unique flavors, textures, health-promoting properties, and cultural significance, making them integral to many diets (Zinno et al., 2022). Fermented dairy products support a healthy gut microbiome and serve as a rich source of probiotics (Shiby and Mishra, 2013). *Bongo* is a popular fermented dairy product commonly consumed in central and western Uganda (Mukisa et al., 2020). *Bongo*, though originating from Uganda's rural cattle-keeping regions, is gaining popularity nationwide (Mukisa et al., 2020). It is spontaneously fermented, implying that it may contain a wide range of microbiota. Indeed, several microorganisms such as lactic acid bacteria including *Lactobacillus*,

Pseudomonas, *Listeria*, *Salmonella*, *Shigella*, and *Staphylococcus* have been reported in spontaneously fermented foods (Izah et al., 2016; Mugampoza et al., 2011; Oyelana and Coker, 2012). Spontaneous fermentation can lead to variations in taste, aroma, and texture between batches, influencing the overall consumer experience (Franz et al., 2014).

Byakika and Mukisa (2024) isolated a number of LAB from *Bongo* which the candidate isolate was part of as well as others namely; *Lactococcus lactis* BM01, *Leuconostoc pseudomesenteroides* BM70, which were reported to possess starter culture properties for *Bongo*.

3 MATERIALS AND METHODS

3.1 Microorganisms

Lactobacillus rhamnosus BM55 was isolated from *Bongo* by Byakika and Mukisa (2024). *Lactobacillus rhamnosus* yoba 2012 was obtained from the Uganda Industrial Research Institute (UIRI), Kampala. The pathogens, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *S. enterica*, were obtained from the College of Veterinary Medicine, Animal Resources and Bio-security of Makerere University, Kampala. The stock cultures were stored in Ringer's solution at -40°C in 15% glycerol. The LAB were propagated according to the procedure described by Mukisa et al. (2017). Briefly, 0.1 mL of the culture was transferred into 100 mL of sterile MRS broth (Laboratorios CONDA, Madrid, Spain) and incubated at 30°C for 24 h. The pathogens *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were each inoculated with 0.1 mL of stock cultures into 100 mL of sterile brain heart infusion broth (Laboratorios CONDA, Madrid, Spain) and incubated at 30°C for 24 h. The cells were washed and recovered by centrifugation (7,500 x g for 10 min). The cell pellets were suspended in 100 mL of sterile Ringer's solution (Oxoid Ltd, Basingstoke, Hampshire, England) and used for the different screening assays.

3.2 Survival in simulated gastrointestinal conditions

3.2.1 Acid and bile tolerance

One milliliter of the overnight grown culture was transferred into MRS broth (Laboratorios CONDA, Madrid, Spain) acidified to pH of 3 using concentrated HCl and incubated at 37°C for 3h. Cell counts were determined at 0 and 3 h. To assess bile tolerance, 1 ml of acidified MRS broth containing isolate after 3 h of incubation was transferred into MRS broth containing 1% Ox-bile (Oxoid Ltd, Basingstoke, Hants, England). The pH of this medium was adjusted using 1M sodium hydroxide to 7.8 and incubated at 30°C for 9 h. Cell counts were determined at 0, 3, 6 and 9 h of incubation at 37°C for 48 h.

3.3 Evaluation of safety

3.3.1 Antibiotic susceptibility

This was investigated using the Kirby-Bauer disk diffusion procedure as described by CLSI

(2013). A total of 20 antibiotics were used. Sterile cotton swabs were used to swab the isolate of concentration 10^8 CFU/mL onto plates with sterile pre-poured agar MRS agar. Antibiotic discs were placed onto the inoculated agar and incubated at 30°C for 48 h. The inhibition zone diameter was measured in millimeters. Isolates were categorized as resistant or susceptible to the respective antibiotics (Charteris et al., 1998).

3.3.2 Biogenic amine production

Following the procedure by Bridson (1990), six amino acids; L-histidine, L-tyrosine, L-lysine, Lphenylalanine, L-arginine, and L-ornithine were investigated. A decarboxylation medium was formulated using 3 g/L yeast extract (Merck, Darmstadt, Germany), 1 g/L glucose, 0.016 g/L bromocresol purple, and 5 g/L of the corresponding amino acid. The pH of the media was adjusted to 6.1 ± 0.2 using 1M NaOH. The medium was then autoclaved at 121°C for 15 min. Ten milliliters of the sterile medium was separately inoculated with 0.1 mL of each isolate, and 1 mL of sterile paraffin was added to the tubes to create anaerobic conditions and avoid false positives. Decarboxylation medium without added amino acids was used as a control. The tubes were incubated at 37°C for 5 days. A deep purple coloration indicated decarboxylase activity.

3.4 Potential probiotic benefits

3.4.1 Pathogenic inhibition

The candidate and pathogens were subcultured in respective broths at 30° C for 48h and thereafter centrifuged at 4500 rpm for 10 min and suspended in sterile diluent. One milliliter of *Staphylococcus aureus* ATCC 25923 and 0.1mL of *E. coli* ATCC 25922 and *Salmonella enterica* were each co-cultured with the LAB in 100mL of UHT milk for 24h at 30°C. Plating. was done on selective media at 0h and 24h to determine counts of the pathogens and the LAB at those intervals. Incubation was done for 24h.

3.4.2 Bile Salt Hydrolase activity

BSH activity was determined according to the method described by Borah et al. (2016). Freshly grown cultures were spotted on Bile Esculine Agar plates containing 1% ox bile (Laboratorios CONDA, Madrid, Spain). Plates were then incubated at 30°C for 48 h. A dark brown coloration on the agar was produced due to hydrolysis of the bile esculine. BSH activity was categorized

based on the diameter of hydrolysis zones as: low BSH activity (≤ 10 mm), medium BSH activity (11–15 mm), and high BSH activity (>16 mm).

4 RESULTS AND DISCUSSION

4.1 Acid and bile tolerance

The microbial counts of *Lactobacillus rhamnosus* BM55 and *Lactobacillus rhamnosus* yoba 2012, subjected to pH 3 and 1% bile salts, are presented in Table 1. *Lactobacillus rhamnosus* BM55 exhibited fairly stable counts at pH = 3 within the 3 h of its exposure to acid and then a gradual increase when incubated in 1% bile for 9 h. In contrast, *L. rhamnosus* yoba 2012 counts a declined when exposed to the same extreme acid and bile conditions.

Table 1: Microbial Counts of *Lactobacillus rhamnosus* BM55 and *Lactobacillus rhamnosus* yoba 2012 after exposure to acid (pH = 3) and 1% bile

Culture	Conditions	0 h	3 h	6 h	9 h
Lactobacillus rhamnosus BM55 (cfu/mL)	MRS broth (pH=3)	$6.1^{a} \pm 0.1$	$5.9^{a} \pm 0.3$		
	MRS broth (1% bile)	$5.2^{a} \pm 0.1$	$5.2^{a} \pm 0.0$	$5.9^{a} \pm 0.1$	$6.6^{b} \pm 0.1$
Lactobacillus rhamnosus yoba 2012	MRS broth (pH=3)	$8.7^{a} \pm 0.0$	$7.7^{b} \pm 0.1$		
	MRS broth (1% bile)	$8.2^{a} \pm 0.1$	$8.1^{ab} \pm 0.0$	$7.6^{b} \pm 0.0$	$7.2^{c} \pm 0.0$

Values are means ± SD of three independent determinations. Mean values in the same column with the same superscripts are not significantly different ($p > 0.05$).

Probiotic microorganisms must adapt to various stressors, such as acidic environments and bile salts, to ensure their viability and effectiveness in the human gastrointestinal tract (Ayyash et al., 2021). The resistance to gastrointestinal conditions varies depending on the genus and species. For example, lactobacilli typically exhibit high resistance (de Melo Pereira et al., 2018). Lactobacilli maintain a consistent pH differential between their internal and external environments, thereby enabling them to withstand acidic conditions (de Melo Pereira et al., 2018). This could be the reason why the study microorganism survived the simulated gastric conditions.

Bacteria have various strategies to survive in acidic conditions. These include mechanisms that pump out protons (H+-ATPases), modify their outer layer, neutralize internal acidity (glutamate

decarboxylation), or produce alkaline substances (urease or arginine deiminase activities) (Cotter and Hill, 2003; Wang et al., 2018).

Bacteria must employ various protective strategies to survive in the gut. These include active transport systems (efflux pumps) that expel bile salts, bile salt hydrolase that breaks down bile salts, homeostasis, and alterations in the structure and composition of their cell membrane (Bustos et al., 2018). Therefore, the isolates in this study may have employed any of the above mechanisms to survive the exposure to bile salts. This implies that the candidate isolate may have the ability to survive the harsh gastrointestinal environment in humans.

4.2 Bile Salt Hydrolase (BSH) activity

The BSH activity of the lactic acid bacteria (LAB) is shown in Table 2. *Lactobacillus rhamnosus* BM55 and *Lactobacillus rhamnosus* yoba 2012 exhibited medium and low BSH activity, respectively.

Table 2: BSH activity of *Lactobacillus rhamnosus* BM55 and *Lb. rhamnosus* yoba 2012

Lactic acid bacteria	Hydrolysis zone (mm)	Bile Salt Hydrolase activity
Lactobacillus rhamnosus BM55	$12.3^{a} \pm 1.2$	Medium
Lactobacillus rhamnosus yoba 2012	$6.3^{b} \pm 1.1$	Low

Values are means ± SD of three independent determinations. Values in the same column with the same superscripts are not significantly different. BSH activity was categorized based on diameter of zones of hydrolysis as low BSH activity (up to 10 mm), medium BSH activity (11–15 mm).

Many probiotic bacteria produce BSH that catalyzes the deconjugation of bile salts (Begley et al., 2006). Unconjugated bile acids are more potent to antibacterial activity compared to conjugated bile acids because the former passively pass across the membranes of pathogens and cause intracellular toxicity (Tian et al., 2020). BSH activity of probiotic microorganisms residing in the GIT has also been associated with cholesterol-lowering effects (Kumar et al., 2012). Therefore, with the observed medium BSH activity of the candidate isolate, it could possess antimicrobial and cholesterol-lowering effects which are desired probiotic benefits.

4.3 Antibiotic susceptibility

Table 3 summarizes the susceptibility of the isolates to different antibiotics. *Lactobacillus rhamnosus* BM55 was susceptible to 2 of 20 antibiotics, which were sulfisoxazole and

streptomycin. The reference probiotic was resistant to 5 of 20 antibiotics: vancomycin, kanamycin, metronidazole, sulphamethoxazole-trimethoprim, and colistin.

Table 3: Susceptibility of the lactic acid bacteria to antibiotics

Antibiotic	*Lactobacillus rhamnosus* BM55	*Lactobacillus rhamnosus* yoba 2012
Penicillin G, 10 μg	R	S
Erythromycin, 15 μg	R	S
Nitrofurantoin, 300 μg	R	S
Rifampicin, 30 μg	R	S
Sulphamethoxazole, 25 μg	R	R
Colistin sulphate, 10 μg	R	R
Chloramphenicol, 30 μg	R	S
Vancomycin, 30 μg	R	R
Gentamicin, 10 μg	R	S
Ceftriaxone, 30 μg	R	S
Levofloxacin, 15 μg	R	S
Tetracycline, 30 μg	R	S
Cephalexin, 30 μg	R	MS
Kanamycin, 30 μg	R	R
Metronidazole, 10 μg**	R	R
Novobiocin, 30 μg	R	S
Amoxicillin, 25 μg	R	S
Streptomycin, 300 μg**	S	S
Ciprofloxacin, 5 μg	R	S
Sulfisoxazole, 300 µg	S	S

R = Resistant, S = Susceptible, MS = Moderately Susceptible. **Antibiotic concentration used was different from the value indicated by Charteris et al. (1998).

One of the most important selection criteria for bacterial isolates intended for use in the food industry is concern for their safety (Gueimonde, Sánchez, et al., 2013). The development of antimicrobial resistance in bacteria presents a threat to human and animal health (EFSA, 2008).

Antibiotic resistance in beneficial microbes, when attributed to mutations or inherent resistance mechanisms, is not a safety concern (Gueimonde, Sánchez, et al., 2013). Certain probiotics with intrinsic antibiotic resistance may be advantageous for re-establishing gut microbiota following antibiotic therapy, therefore, antibiotic resistance is not a safety issue but only becomes one when the risk of resistance transfer is present (Gueimonde, Sánchez, et al., 2013).

Antibiotics can either destroy microbial cells or inhibit their replication, preventing them from multiplying without necessarily leading to cell death (Nemeth et al., 2015). Since probiotics are live microorganisms, concurrent administration of antibiotics could eliminate a large number of the organisms, reducing the efficacy of the microorganism (Williams, 2010). Therefore, probiotic microbes should be resistant to the common antibiotics to reduce chances of their elimination from the GIT. The high level of antibiotic resistance of the candidate isolate can be attributed to the excessive and improper use of these medications in humans and animals (Gould and Bal, 2013).

4.4 Production of biogenic amines

The reference did not decarboxylate any amino acid but *Lactobacillus rhamnosus* BM55 decarboxylated L-Histidine and L-Ornithine.

Detecting bacteria with amino acid decarboxylase activity is crucial for estimating the potential presence of biogenic amines in foods and for preventing their accumulation in food products (Spano et al., 2010). High concentrations of biogenic amines and interrupted detoxification ability pose a risk to health (Doeun et al., 2017). Histamine is considered as one of the most toxic, and food safety-relevant biogenic amines (BIOHAZ, 2011), and as seen, the candidate microorganism produces histamine from L-histidine decarboxylation. Symptoms of biogenic amine toxicity include diarrhea, food poisoning, vomiting, sweating or tachycardia etc. (Wójcik et al., 2021).

4.5 Pathogenic inhibition

Table 4 summarizes the pathogenic inhibition exhibited by the candidate microorganism against *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Salmonella enterica*. *Lactobacillus rhamnosus* BM55 inhibited growth in *Salmonella enterica* by 1.1 log cfu/mL and *Staphylococcus aureus* ATCC 25923 by 2.8 log cfu/mL but did not inhibit *E. coli* ATCC 25922.

Table 4: Pathogenic inhibition by *Lactobacillus rhamnosus* BM55

Treatment	0 h	24 h
Lactobacillus rhamnosus BM55	$6.7^{a}\pm 0.0$	$8.1^{b}\pm 0.3$
E. coli ATCC 25922	$6.1^{a} \pm 0.7$	$8.7^{b} \pm 0.0$
Lactobacillus rhamnosus BM55	$6.1^{a} \pm 0.7$	$8.5^{b} \pm 0.0$
Salmonella enterica	$6.8^{a} \pm 0.0$	$5.7^{b} \pm 0.7$
Lactobacillus rhamnosus BM5*5*	$7.2^{a} \pm 0.2$	$7.8^{a} \pm 0.1$
Staphylococcus aureus ATCC25923	$7.8^{a} \pm 0.8$	$5.4^{b} \pm 0.1$

Values are means ± SD. Values in the same column with the same superscripts are not significantly different ($p > 0.05$)

Many studies have reported that LAB can have significant antimicrobial effects (Gao et al., 2019). Remarkably, LAB secrete a number of antimicrobial compounds such as organic acids, bacteriocins, and hydrogen peroxide (Kanmani et al., 2013). Sufficient levels of LAB are required to release bacteriostatic metabolites that inhibit pathogenic bacteria, while insufficient LAB levels leave the growth of pathogens largely unaffected (Gao et al., 2019).

Lactobacillus species are reported to effectively inhibit the growth of certain strains of *E. coli* (Imani et al., 2014). This trait may be a strain-specific since *Lactobacillus rhamnosus* BM55 did not inhibit growth of *E. coli* ATCC 25922 but inhibited the growth of *Salmonella enterica* and *Staphylococcus aureus* ATCC 25923 (Table 4).

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Lactobacillus rhamnosus BM55 exhibited tolerance to extreme simulated gastro intestinal conditions, was resistant to many antibiotics but produced histamine. It displayed moderate bile salt hydrolase activity and to some extent inhibited by *Salmonella enterica* and *Staphylococcus aureus* ATCC 25923 though not *E. coli* ATCC 25922. These findings are inconclusive with regard to probiotic classification. They strongly suggest further rigorous research to determine if *Lactobacillus rhamnosus* BM55 may be a probiotic or not.

5.2 Recommendations

Conduct other comprehensive *in vitro*, *in vivo* and *ex vivo* studies to evaluate the probiotic potential of *Lactobacillus rhamnosus* BM55.

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