Gut reaction: Survival of Lactobacillus rhamnosus GG through the digestive gauntlet

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Gut Reaction: Survival of *Lactobacillus rhamnosus GG* Through the Digestive Gauntlet

KEY WORDS

Lactobacillus rhamnosus GG (LGG)

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ABSTRACT

The United States probiotics industry is a growing multi-billion-dollar industry. With promises of improving overall digestive health, more research is needed to assess survivability of probiotic strains in reaching target colonization sites. Understanding the survivability of probiotics throughout the gastrointestinal (GI) tract is crucial for optimizing their therapeutic efficacy. This study focuses on the survival of *Lactobacillus rhamnosus GG* (LGG), a commonly used probiotic, while it progresses through each phase of a simulated GI tract. Using a modified in vitro model, this study simulated physical, chemical, and biological conditions of the mouth, stomach, and small intestine to measure LGG survival. This modified in vitro model is a mechanical process that allows for the study of the GI tract outside of the human body. Environmental factors such as pH, enzymatic activity, and mechanical forces were implemented to mimic digestion. Results revealed a decline in LGG viability, as visualized by microscopy and growth measured as CFU/mL, particularly in the stomach phase (mean 5.2 X 10^3 CFU/mL) as compared to the control (mean 1.17 X 10^9 CFU/mL). This reduction is most likely due to the harsh conditions of

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this region (low pH and enzymatic activity). It is hypothesized that LGG can survive passage through the GI tract, with attrition in CFU/mL due to harsh simulated environmental conditions. Our findings show that LGG is capable of surviving in a simulated digestive system to reach the small intestines albeit in smaller numbers which could influence intestinal colonization and thus probiotic effectiveness.

INTRODUCTION

Background

Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer a health benefit to the host (National Institutes of Health, 2023). These beneficial microbes can be found naturally in fermented foods, added to other foods, and in dietary supplements (National Institutes of Health, 2023). There are many benefits these microbes provide, including but not limited to: maintaining gut health, modulating the immune system, and preventing colonization of harmful pathogens (Matera, 2024). There are seven main microbial strains used most often in probiotics: *Bacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia*, and *Lactobacillus* (National Institutes of Health, 2023). Some probiotic strains of *Lactobacillus* spp. and *Bifidobacterium* spp. suppress the colonization of several pathogens including *E. coli*, *Salmonella* spp., *Helicobacter pylori*, *Listeria monocytogenes* and Rotavirus by outcompeting for adhesion sites and nutrients; many Lactic Acid Bacteria (LAB) produce antimicrobial substances that target these pathogenic microbes (Das et al., 2022).

The genus *Lactobacillus* comprises of a diverse group of gram-positive, lactic acid-producing bacteria recognized for their role in food fermentation and human health (Bernardeau et al., 2007). Lactic acid production helps maintain a pathogen-inhibiting environment, contributing to host health and food preservation (Alakomi et al., 2005). Consequently, *Lactobacillus* strains are

frequently used in a probiotic setting. One *Lactobacillus* strain that has extensive research backing is *Lactobacillus rhamnosus* GG (LGG), one of the most used probiotics in clinical studies and probiotic supplements (Yan et al., 2012).

LGG, first isolated from fecal samples of a healthy human in 1900 by Ernst Moro, has been shown to have stronger colonization of the small intestine as compared to other *Lactobacillus* species (Segers & Lebeer, 2014) which allows LGG to outcompete pathogenic microbes. The presence of LGG within the intestines and its stronger intestinal colonization capabilities indicate a potential role of this microbe in a healthy human gut microbiome. LGG was chosen as a specific strain of *Lactobacillus* utilized in this study due to its effectiveness in surviving under a variety of challenging physiological conditions, combined with a strong adherence characteristic towards intestinal mucosa (Segers & Lebeer, 2014).

Implications

Currently, there is a rapidly increasing market for probiotics, which were consumed by 3.9 million US adults in 2015, a staggering quadruple increase of probiotic consumption from 2007 (Parker et al., 2018). This widespread use drove $1.4 billion in probiotic supplement sales in 2014 (Parker et al., 2018). The global market for probiotics was valued at $73.65 billion USD in 2023 and is expected to rise to $80.48 billion USD in 2024 according to a Probiotics Global Market Report (The Business Research Company, 2025). Some of the factors driving consumer probiotic consumption include a desire for relief of gastrointestinal symptoms and overall improvement to health and longevity (Lynch et al., 2021).

Probiotics have varying beneficial effects dependent on strain and dosage, involving differences in competitive exclusion of pathogens, enzymatic activity, gut barrier reinforcement, and immunomodulation (Hill et al., 2014). Beneficial effects of

probiotics include the use of probiotics as a treatment for reducing severity of acute gastroenteritis (Szajewska et al., 2019) and for the management of irritable bowel syndrome (IBS) symptoms (Dale et al., 2019). Probiotics have been shown to regulate levels of appetite-stimulating hormones (Noormohammadi et al., 2023) and have been associated with improvements in mood and cognition in older adults (Kim et al., 2020). Despite studies showing the wide-ranging benefits of probiotic application, there is evidence that the benefits of probiotic supplementation and changes to intestinal flora occur without direct colonization of the gastrointestinal (GI) tract by the probiotic species (Grazul et al., 2016). The microbes found in probiotics must travel from the mouth and survive through the harsh conditions of the stomach to colonize the intestines of the digestive tract (Han et al., 2021). Figure 1 shows this pathway for digestion from the mouth (oral cavity) to stomach to small intestine. This raises the question of how many

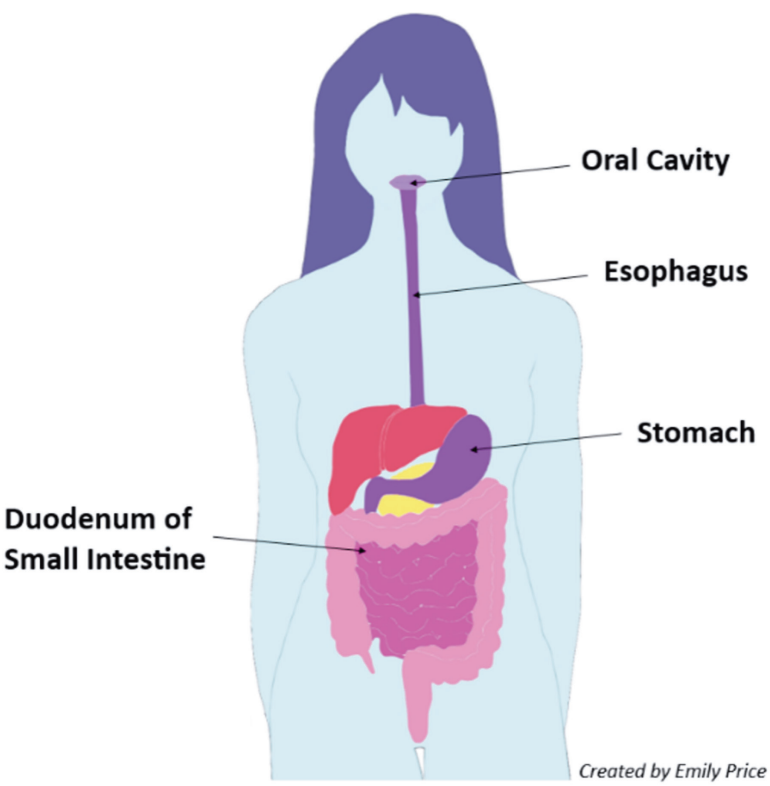

FIGURE 1

Pathway for Digestion. (Price, 2025). Once foods or microbes like probiotic microorganisms enter the oral cavity, they will go down the esophagus to reach the acidic conditions of the stomach. After a few hours/minutes of enzymatic exposure, the partially digested items will travel to the small intestines and be exposed to bicarbonate, enzymes and bile. Lactobacilli are common normal flora of the small intestine.

microbes in probiotic supplements remain viable when reaching the intestines following digestion.

This study seeks to assess the survivability of LGG in tablet form under simulated gastrointestinal conditions. Specifically, the impact of physical, chemical, and biological factors on the viability of LGG as it transitions through the digestive system was examined.

Materials and Methods

Organism Used in the Study

The probiotic used in the study was Culturelle Probiotic Digestive Daily Probiotic Chewable with an expiration date of January 2026, which has one bacterium, *Lactobacillus rhamnosus* GG (LGG). According to the manufacturer, each tablet contains 53 mg, or 10 billion Colony Forming Units (CFUs), of LGG (Culturelle, n.d.). This probiotic was chosen as its singular bacterium allows for ease of experiment set up and analysis, as compared to probiotics with multiple strains. Other contributing factors include the effectiveness of LGG survivability under various challenging physiological conditions and its strong adherence characteristic towards intestinal mucosa (Segers et al., 2014).

Procedure Creating a Simulated Gastrointestinal Tract

The procedure was based on Robic's *in vitro* model for determining probiotic survivability throughout the human digestive tract. In this model, conditions of the GI tract are simulated to mimic stages of digestion, measuring the effects of each condition on the survival rates of the probiotic species (Robic, 2010). Environmental factors that were reviewed and adjusted included pH, digestive enzymes, temperature, and mechanical forces. A total of eight trials were run; however, trials one through four were omitted due to the complete consumption of glucose by autoclave procedures. Glucose was added to the subsequent trials to allow for a correct

simulation of the GI tract, which allows for nutrient availability.

Recreating the Mouth

To simulate saliva, 3.0 mL of 10 mM Na_3PO_4 buffer, pH 6.5 (eCon Lab Supply Store) was added into a sterile 50 mL conical test tube. Since these tablets are chewed prior to digestion, the mass of each Culturelle tablet was recorded and then crushed 11 times with a mortar and pestle, lined with sterile weigh paper, to simulate chewing as determined by the average chew time between three student researchers (average 10.3 chews/tablet). The crushed tablet was then transferred to a 50 mL buffer-containing conical test tube with 0.2 mL Avian Lysozyme (Thermo Scientific, 1 mg/mL in sterile water, filter sterilized) added. The conical tube was vortexed briefly at 2000 rpm to ensure uniform suspension. The solution pH was recorded and found to be 4.0–4.5, varying between trials. After 20 minutes, the solution was neutralized using 1.0 M of $NaOH_{(aq)}$ (Innovating Science) to increase the solution's pH to 7, inactivating the lysozyme before serial dilution and plating on agar.

Recreating the Stomach

To create the conditions found in the stomach, the solution from the mouth simulation was treated with 0.1 M HCl to lower the pH from pH 7 to pH 2. The solution was then transferred to a sterile 15 mL conical tube and 0.2 mL Porcine Pepsin (Sigma-Aldrich, 2 mg/mL in 10 mM HCl, filter sterilized) was added. The solution was briefly vortexed at 2000 rpm before incubating at 37°C shaking for 1 hour at 135 rpm to simulate the movement and churning of the stomach. After incubation, 1.0 M NaOH was added to raise the pH to 7, inactivating pepsin before being serial diluted and plated on agar.

Recreating the Small Intestine

To create the conditions found in the small intestines, the

solution from the stomach simulation was treated with 0.2 mL Bovine Alpha-chymotrypsin (Sigma-Aldrich, 5 mg/mL in 10 mM Na_3PO_4, pH 6.5, filter sterilized) and 0.2 mL Trypsin (Sigma-Aldrich, 5 mg/mL in 10 mM Na_3PO_4, pH 6.5, filter sterilized), and vortexed briefly at 2000 rpm. The treated solution was incubated at 37°C shaking for 1 hour at 65 rpm to simulate the movement and peristalsis of the intestines. After incubation, serial dilutions were performed and plated on agar.

Creating a Control

A positive control was created by adding 3.0 mL of 10 mM Na_3PO_4 buffer with a pH 6.5 into a sterile 50 mL conical test tube. The weight of each Culturelle tablet was recorded. Tablets with similar mass to each experiment sample were used. The control tablet was added to the Na_3PO_4 buffer. The control solution was vortexed for 3 minutes at 2000 rpm until the tablet disintegrated forming a suspension. The control solution pH was recorded at 4.0–4.5, varying between trials. The control solutions were serially diluted and plated on agar to determine the numbers of bacteria at the start of the experiment.

Bacterial Enumeration

To determine both the number of viable bacteria in the control and each treatment stage, the CFUs were calculated as follows:

At the end of each stage, treated probiotic solutions were inoculated and serial diluted in a 1:10 ratio in sterile De Man–Rogosa–Sharpe (MRS) Broth (HIMEDIA) in series from 10^0 to 10^9, with 100 µl of each dilution series spread plated onto sterile MRS agar plates (HIMEDIA). Plates were statically incubated at 37°C for 48–72 hours, with normal atmospheric conditions for the control and mouth samples. The stomach and intestine samples were placed in a candle jar to simulate decreased oxygen and in-creased carbon dioxide levels.

After the final incubation, colony counts were recorded. To calculate colony forming units (CFU) per mL of bacteria on an agar plate, the formula CFU/mL = (colonies formed X dilution factor)/mL plated was used. Plates with more than 300 colonies were labeled "Too Many to Count" (TMTC). Colony counts less than 30 and more than 300 were not included for statistical significance. Comparison of CFU/mL was calculated by using Total Decline = (Average Control – Average Mouth) / (Average Control) X 100.

Microscopy

After each stage and the control, 100 µl samples were stained and viewed under the microscope at 10X, 40X and 100X. Simple stains were performed with methylene blue and observed under various magnifications to assess viability through Brownian motion. The Gram stain was performed to look for lactobacilli as Gram-positive bacilli. Additionally, clumping and relative clump size were recorded to understand the degradation progress of the protective elements in the tablet.

RESULTS

The amount of LGG that survived the simulated digestive gauntlet was on average 1.16 x 10^3 CFUs as compared to the control at 1.17 x 10^9 CFUs (Tables 1 and 2, Figure 2). The decrease in CFU

	Plate ID	Volume of Culture Plated (mL)	Dilution of original Culture (mL)	Colony Count	CFU/mL
Trial #6	Mouth #6	0.1	1000000	57	5.70E+08
	Stomach #6	0.1	1	237	2.37E+03
	Intestine #6	0.1	1	178	1.78E+03
Trial #7	Mouth #7	0.1	1000000	70	7.00E+08
	Stomach #7	0.1	1	210	2.10E+03
	Intestine #7	0.1	1	48	4.80E+02
Trial #8	Mouth #8	0.1	1000000	50	5.00E+08
	Stomach #8	0.1	10	109	1.09E+04
	Intestine #8	0.1	1	123	1.23E+03
Control	Control #6	0.1	1000000	57	5.70E+08
	Control #7	0.1	1000000	153	1.53E+09
	Control #8	0.1	1000000	142	1.42E+09

TABLE 1

The CFU/mL of LGG After Each Stage of the Simulated Digestive Tract.

TABLE 2

The Mean CFU/mL of LGG After Each Stage of the Simulated Digestive Tract.

Stage	Trial 6 (CFU/mL)	Trial 7 (CFU/mL)	Trial 8 (CFU/mL)	Mean
Mouth	5.70E+08	7.00E+08	5.00E+08	5.90E+08
Stomach	2.37E+03	2.10E+03	1.09E+04	5.12E+03
Intestine	1.78E+03	4.80E+02	1.23E+03	1.16E+03
Control	5.70E+08	1.53E+09	1.42E+09	1.17E+09

counts supports the progression in the simulated in vitro GI tract as predicted. The control CFUs seen were less than the quantity of bacteria noted by the manufacturer (1.00 x 10^{10} CFUs). The CFU/mL progressively decreased as LGG passed through the GI stages, with the largest reduction noticed during the stomach stage at an average of 5.12 x 10^{3} CFUs from 5.90 x 10^{8} CFUs average count following the mouth stage. All trials showed a consistent trend in CFU/mL reduction across the three stages (Tables 1 and 2, Figure 2). Data from trials 1 to 5 were not described here as slight modifications to the protocol were made during these trials.

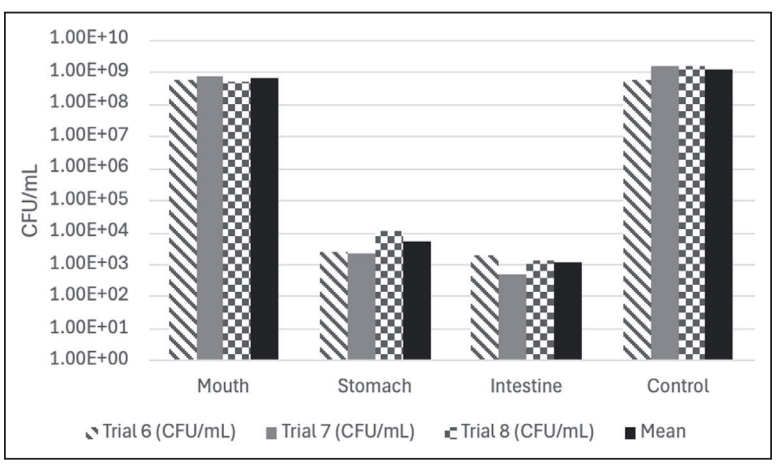

FIGURE 2

Comparison of the Means of Each Trial and the Total Mean for Each Test Stage. The viability of LGG through a simulated digestive tract was determined by serial dilution and spread plating of samples from each stage on sterile MRS agar plates. The control was the probiotic tablet dissolved in a 10 mM Na_3PO_4 buffer with a pH 6.5. The mean for each trial is represented as bars with diagonal lines (Trial 6), solid gray bars (Trial 7), and checkered bars (Trial 8). The mean for the trials for each stage is noted as the total mean and represented as solid black bars.

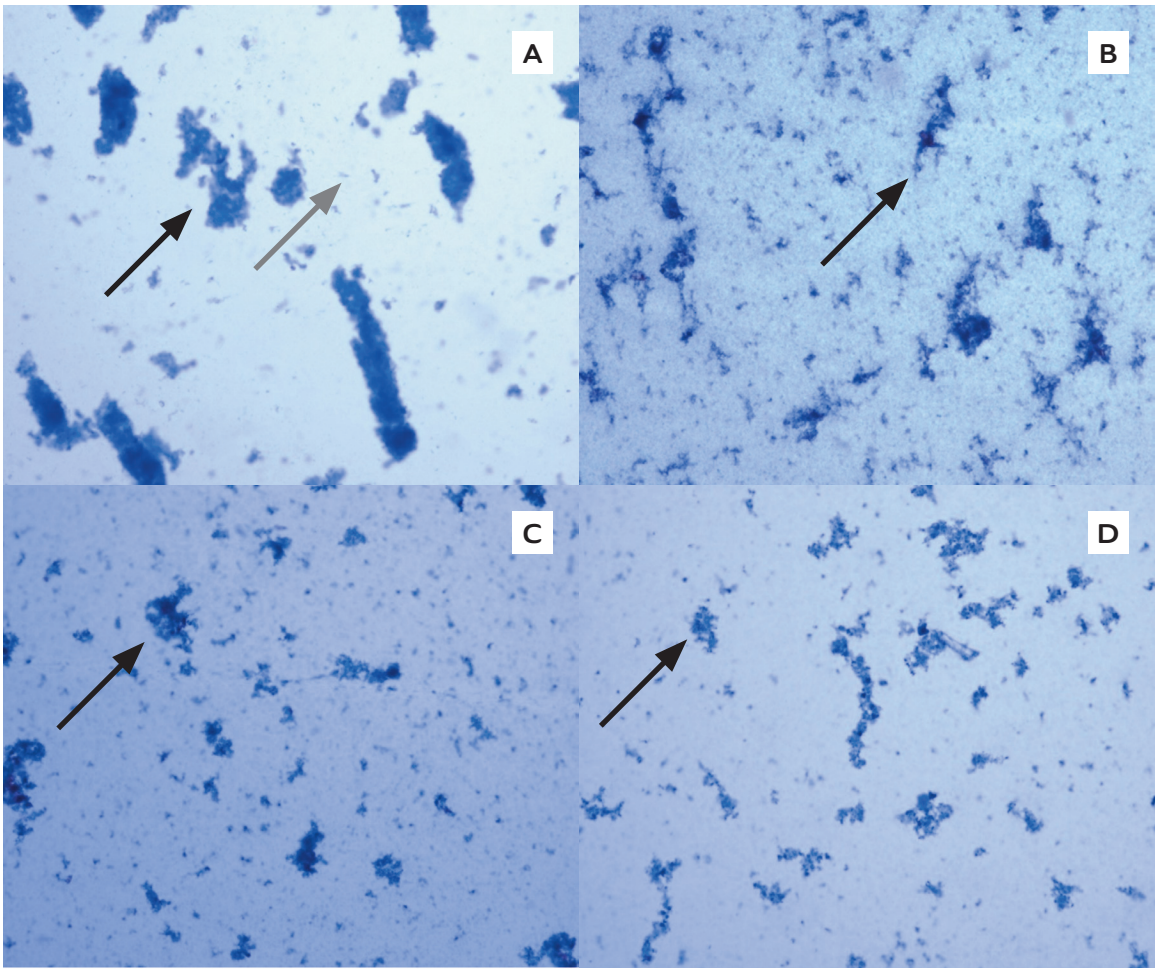

FIGURE 3

Simple Stains of the Experimental and Control Samples. At the end of each stage, a 100 µl sample was collected and stained with methylene blue. Samples were viewed at a magnification of 10x. Clumping, as designated by the black arrows, was noted at the control (A) and at each stage (Mouth B, Stomach C, Small Intestines D). The bacterial numbers and the clump sizes appeared less at each stage as compared to the control. The bacilli in each frame are thought to be LGG that had escaped the tablet. A bacillus is noted by the gray arrow in panel A.

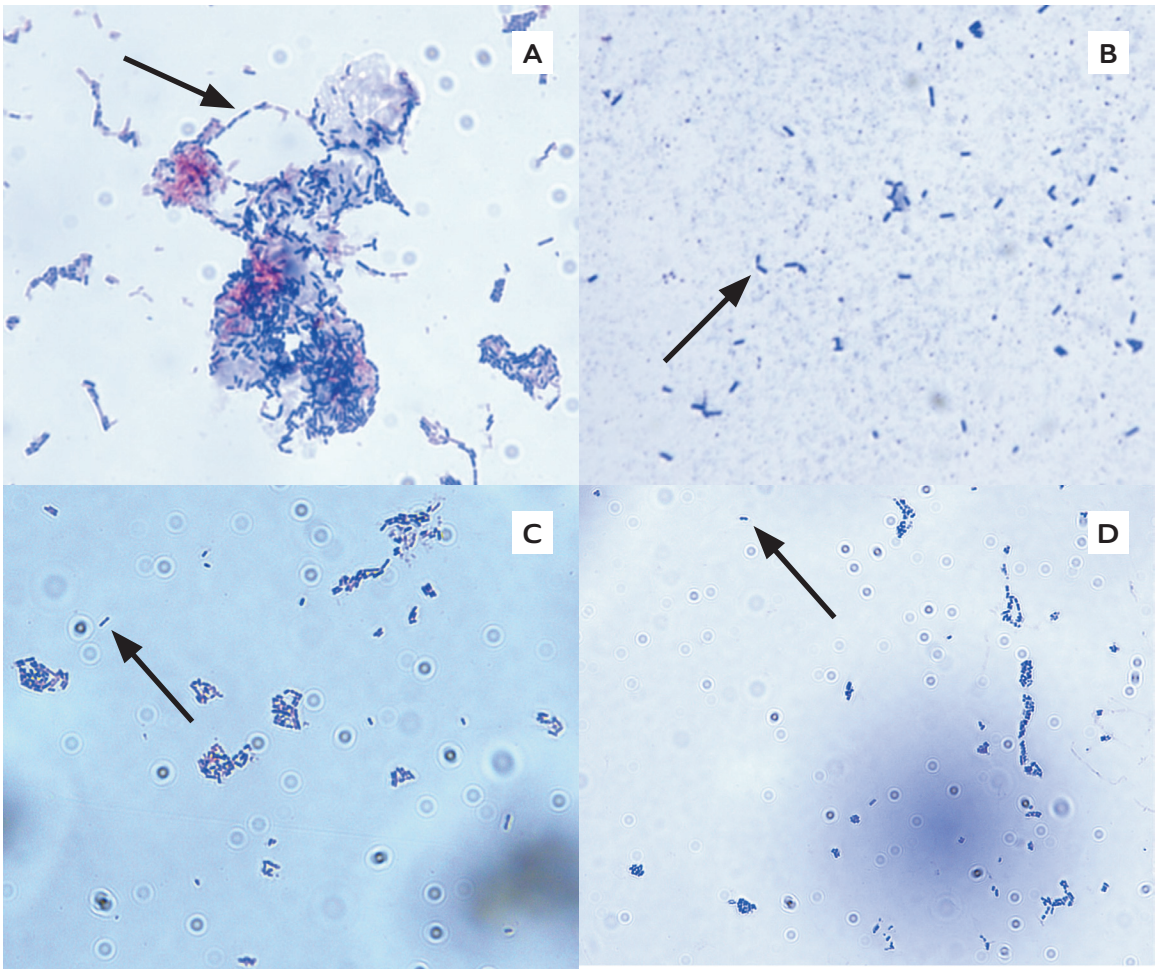

FIGURE 4

Gram Stains of the Experimental and Control Samples. At the end of each stage, a 100 µl sample was collected and stained with the Gram stain. Samples were viewed at a magnification of 100x. Clumping was noted at the control (A) and at each stage (Mouth B, Stomach C, Small Intestines D). The bacterial numbers and the clump sizes appeared less at each stage as compared to the control. Purple bacilli can be observed inside and outside of the clumps. The purple bacilli noted in each panel with an arrow are thought to be LGG that had escaped the tablet.

In Trial 8, there was a (4x) increase of CFU compared to other trials at the end of the stomach stage; however, the count at the end of the intestinal stage was consistent with previous trials. Bacterial numbers and clump sizes visualized by staining (simple, Gram) resembled the CFU counts as decreases were noted for both as the tablets progressed through the simulated in vitro GI tract (Figures 3 and 4).

Discussion

Results demonstrate that LGG survived the simulated GI tract of all trials performed based on viable cell count and microscopy. Microscopy confirmed viability through Brownian motion, while CFU counts on glucose-enhanced MRS agar averaged 1.16×10^3 CFU/mL at the end of the stages (Table 2, Figures 3 and 4). This finding supports the hypothesis that LGG can survive passage through the GI tract, with attrition in CFU/mL due to harsh simulated environmental conditions. The progressive degradation of the nano-crystalline cellulose tablet as observed through microscopy may have assisted LGG in its survival of the stomach and its release in the intestines for colonization.

Control samples demonstrated an average CFU/mL of 1.17×10^9 (Table 2), validating initial viability of LGG prior to exposure to GI conditions. This established the starting population of LGG at entry to the digestive tract and provided a basis for comparison of survivability of CFU observed in the trials. A decrease in bacterial survivability was observed at each subsequent stage as compared to the control which was expected due to the various environmental conditions of the digestive tract. The starting CFU per tablet was less than what was stated on the manufacturer's website (Culturelle, n.d., 1.00×10^{10} CFUs). This may be due to the number of LGG still trapped in the nano-crystalline cellulose as the tablet was not crushed or chewed during the control preparation compared to the experimental samples. Further, this

may be attributed to human error in pipetting, maximum solubility limits, or due to a variety in sample weights.

Mouth samples saw an average CFU/mL of 5.90×10^9, a decrease of 49.6% from the baseline starting population demonstrated in the control trials. This decrease is expected as lysozyme degrades the peptidoglycan layer of the cell wall that LGG needs to survive. Microscopic samples of the mouth showed less clumping compared to the control sample, indicating the protective nano-crystalline cellulose coating was compromised either through the enzymatic activity of lysozyme or mechanical pulverization with mortar and pestle (Figures 3 and 4).

Stomach samples saw an average CFU/mL of 5.12×10^3, a decrease of over 99% from the starting control population. This sharp decline may be attributed to the simulated conditions of the stomach: Gastric acid, the enzyme pepsin, and the strong acid HCl. This strong acid dropped the pH to around 2, creating an inhospitable environment as LGG cannot survive at a pH less than 3 (Li et al., 2016). This lower pH disrupts Culturelle's protective nano-crystalline cellulose coating (Qi et al., 2019), resulting in the release of bacteria. Bacterial cytoplasmic pH can be disrupted by these extreme conditions affecting cellular integrity (Han et al., 2021). The enzyme pepsin can potentially degrade the exposed proteins of the tablet and the probiotic strain further compromising bacterial integrity. Reduced survivability observed in the stomach trials are consistent with known literature on the sensitivity of probiotic strains to gastric conditions (Segers & Lebeer, 2014).

Intestinal samples saw an average CFU/mL of 1.16×10^3, a decrease of over 99% from the starting control population, but a smaller reduction in CFUs as compared to the stomach stage with 20% surviving. This is likely due to the increase in pH (pH 6) which provided a more hospitable environment for LGG. Additionally, proteolytic enzymes such as trypsin and chymotrypsin require a pH of 7.8 or higher for optimal activity and should not be a factor

in impeding growth within the intestinal phase, which simulated the conditions in the duodenum of the small intestines (pH 7).

Microscopy samples were taken at each stage, and every simple stained sample was deemed viable based on both Brownian motion under microscopy as well as growth on media (Figures 3 and 4). Chemical tests, including catalase and Gram staining, were performed for initial LGG identification, and results were consistent with known literature. Further testing needs to be completed to confirm the presence of LGG over potential contaminants. Simple staining of the samples showed decreased clump size as samples progressed through the simulated GI tract, suggesting some degradation of the nano-crystalline cellulose from chemical and environmental exposures during digestion (Figure 3). Fewer and smaller clumps were observed in the simple stains of the intestinal trial samples, indicating that some of the probiotic microbes may be still encased in protective nano-crystalline cellulose (Figure 3). Reduced colony counts on intestinal plates may result from growth inhibition due to the remaining nano-crystalline cellulose, or to a reduction in available nutrients from the tablet consumed in other stages of digestion. These findings align with previous studies indicating that there is a loss of viable LGG during gastric transit, but the surviving bacteria can grow in the small intestine given glucose availability (Corcoran et al., 2005). Increased survivability in the presence of additional glucose is consistent with studies showing addition of glucose as the key component of LGG survival in acidic environments (Corcoran et al., 2005). The duodenum of the small intestine has a volume of approximately 500 mL, with a bacterial load of 10^3–10^4 bacteria/mL (Judkins et al., 2020), meaning the microbial population of a typical human duodenum would be 500 mL x 10^3 CFU or 5.00 x 10^5 CFU. The calculated probiotic dose that survives the simulated GI gauntlet averages 1.16 x 10^3 CFUs and would constitute about 0.23% of the total microbial

population. Lactobacilli are estimated to constitute 6% of the total bacterial cell numbers in human duodenum (Heeney et al., 2017). Surviving LGG have the potential to colonize and thus increase the existing microbe population upon reaching the intestine due to their improved growth in the favorable environmental conditions and their formation of protective biofilms.

Limitations

Several limitations may have influenced this study's findings. Simulated GI conditions may not accurately replicate the complex dynamic of the human digestive system, including mucus barriers, competitive microbial interactions, and bile salt activity. Host-specific factors such as immune response variations and the presence of underlying health conditions, IBS, immunodeficiencies, and diabetes may influence the efficacy and survivability of probiotics like LGG. For example, individuals with immunodeficiencies may present altered immuno-regulatory mechanisms which affect interactions between probiotic and host immune functions, while those with diabetes may experience differences in probiotic survival or activity due to changes in gut microbiota and increased sugar levels (Parker et al., 2017). Furthermore, variations in pipetting accuracy and subsequent inoculum preparation may contribute to differences in colony counts and growth rates. Another limitation concerns access of glucose for the energy needs of LGG. Degradation of glucose via autoclave procedures may have occurred (in Trials 1–5) as the addition of post-autoclave supplementation of MRS agar (in Trials 6, 7, and 8) was needed to support LGG growth. Glucose availability in the tablet would most likely have contributed to enhanced growth had glucose not been degraded, supporting the colony count. LGG may in general need more glucose to survive in oxygen-reduced environments as it may need to shift its metabolism from cellular respiration to fermentation, a metabolic process that produces less ATP energy

per glucose molecule. This methodological adjustment may have influenced CFU counts and colony morphology, as colonies may exhibit size variability potentially linked to nutrient availability and initial cell density. Bacterial entrapment in the nano-crystalline cellulose matrix may also prevent efficient access by LGG for growth on agar plates. Other studies show when probiotics are microencapsulated, the survivability rate significantly increases (Han et al., 2021). Another aspect to consider is the liquid used to consume the probiotic. Our trials were performed with water as the solvent; however, findings may vary when using different liquid types like coffee, tea, milk, and juices that have changes in pH levels, sugar content, temperature, and/or additives as compared to water, all which may influence viability of endpoint probiotics. Without statistical analyses, conclusions on colonization and effectiveness are limited. Performing further trials that include glucose supplementation would provide the additional data needed for statistical analysis and could determine whether or not our findings are significant based on the in vitro model used in our study.

Implications

The ability of LGG to survive simulated GI conditions, even at reduced concentrations, has implications for probiotic therapies and dietary supplementation, such as efficacy over varying durations of time, individual dietary preferences and influences, underlying gut-health conditions, and bile salt tolerance. The findings from this study further support the resilience of LGG under adverse conditions and its use as a potential probiotic for human health. From an academic perspective, this study contributes to the growing body of research on probiotic survival in simulated gastrointestinal environments, and these results emphasize the importance of optimizing delivery systems for probiotics to maximize efficacy.

Future Directions

Further research may resolve how additional factors such as dietary intake (drinking coffee, tea, juices, or milk) affect the survival of LGG, specifically regarding increases or decreases in the pH of the gastric environment. Investigations involving bile salt tolerance and interactions with competing microbiota during this transit would provide a more comprehensive understanding of probiotic efficacy. Additional studies comparing the survival of LGG in different formulations, such as capsules or functional food could inform producers and consumers on better practices for efficacy. Results of probiotic use may differ depending on pre-existing conditions such as Crohn's Disease and Celiac Disease (Sanders et al., 2014). Results of probiotic use may also differ depending on the consumption of medications such as daily proton pump inhibitor drugs which may provide therapeutic intervention of negative bacterial overgrowth (Kiecka & Szczepanik, 2023). Differences in host microbiota and genetic differences may also contribute to the wide variation in probiotic efficacy (including differences seen in longitudinal studies) (Sanders et al., 2014). Methodological improvements, such as testing broader ranges of digestive enzymes, pH conditions, and digestive timing, would better replicate the complexity of the GI tract. Lastly, it is recommended that additional trials be performed to allow for the statistical analysis needed to strengthen the reliability of conclusions on efficacy.

CONCLUSION

This study provides insight into the survivability of LGG under simulated gastrointestinal conditions. Findings show that while LGG survived the simulated environment, viability decreased as stages of the simulated digestive tract progressed. The highest survivability rate was observed in the mouth phase. Factors such as bacterial release from the protective nano-crystalline cellulose encapsulation in the probiotic tablet, enzyme activity, or

pH changes, may affect the probiotics' ability to survive. Notable challenges such as omission of bile salts and maintaining temperature at the mouth phase did not impact alignment with existing literature. These findings contribute to a better understanding of probiotic efficacy from probiotic ingestion to bacterial colonization, and optimal amounts to be added to tablet production for the most beneficial results. By addressing knowledge gaps on the efficacy of LGG during transitions through different gastrointestinal stages, this study provides insight towards optimizing therapeutic potential through enhanced understanding of how probiotics may behave in vivo.

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