Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

Shelf-Life Storage Conditions Dependent Anti-S. Aureus Efficacy of Live and Attenuated Lactobacillus Rhamnosus: An in Vitro Study

Varun Sureja[1]*, Dharmeshkumar Kheni[1], Shrikalp Deshpande[1]

[1] Faculty of Pharmaceutical Science, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India.

***Corresponding author:** Varun Sureja, Faculty of Pharmaceutical Science, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India.

(Received: 16 March 2025 Revised: 20 April 2025 Accepted: 01 May2025)

KEYWORDS

Probiotic, Lactobacillus rhamnosus, Storage condition, Efficacy, S. aureus, Viability.

ABSTRACT:

Introduction: The effect of different storage condition and duration on probiotic viability, cell membrane compositional characteristics, and its antibacterial efficacy is not well studied.

Objectives: The present study evaluated the impact of different storage conditions on the viability, membrane characteristics, and antibacterial effect of live and heat attenuated *Lactobacillus rhamnosus* at various shelf life stages.

Methods: Live and heat attenuated *L. rhamnosus* probiotic was prepared and stored at -20°C, 2-8°C, 25°C ± 60% RH, and 40°C ± 75% RH for 12 months. Representative samples from each storage condition at baseline, months 3, 6, and 12 were utilized for evaluating viability, intactness, and antibacterial efficacy using *in vitro Staphylococcus aureus* growth inhibition method.

Results: Progressive reduction in the viability and number of intact cells of live and attenuated *L. rhamnosus* was observed. After 12 months, probiotic at -20°C showed 3.23% viability reduction, while probiotics at 25°C and 40°C showed 64.52% and 100% viability reduction. Heat attenuated probiotic cell count showed similar trend in its intactness. Significant reduction in antibacterial efficacy was observed for live probiotic stored at 25°C (16.27 to 9.77 mm; $p < 0.05$) and 40°C (16.40 to 4.77 mm; $p < 0.05$), compared to baseline antibacterial strength. Heat attenuated *L. rhamnosus* showed similar trend.

Conclusions: In conclusion, the present study underscores the potential impact of storage condition on the overall stability and therapeutic efficacy of probiotics throughout shelf-life.

1. Introduction

The "human gut microbiome" comprises several microbial habitats that reside in the human gut. Maintaining the general health of the gastrointestinal tract (GIT) depends heavily on the human gut microbiota [(1,2)]. An increase in the population of foreign (pathogenic) bacteria and a decrease in the number of local (good) microbes are characteristics of gut dysbiosis. Gut dysbiosis has been implicated in a number of acute (such as acute watery diarrhea and gastroenteritis) and chronic (such as irritable bowel syndrome, inflammatory bowel disease, obesity, and diabetes) disorders, according to numerous studies [(2–5)].

According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are live bacteria that, when taken in sufficient amounts, can offer a host of health advantages [(6,7)]. Through a variety of mechanisms, probiotics have been shown to improve health. These include: preventing pathogens from binding to mucosal surfaces through competition; producing different antimicrobial compounds to prevent pathogenic overgrowth; improving the structure and activity of the intestinal barrier; modifying immune system activity; producing secondary metabolites that can positively influence the activity of organ systems; and influencing the activity of various signaling pathways at the cellular

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

and molecular level [8,9]. Products derived from probiotics are known as postbiotics, and they have the potential to improve a person's health [10]. The three main categories of postbiotics that are currently available are: probiotic-derived metabolites, which include short-chain fatty acids (SCFA), vitamins, nutrients, and enzymes; probiotic cellular fragments, which include exopolysaccharides, cell wall fragments, and probiotic lysates; or dead probiotic cells in their entirety, which are also referred to as paraprobiotics, ghost probiotics, or ghostbiotics [10,11]. Postbiotics are superior to probiotics in a number of ways, such as improved and consistent production yield, improved safety for patients with compromised intestinal barriers, decreased risk of antibiotic resistance or other genetic mutations, and improved and consistent formulation development in addition to other useful ingredients [10]. In a similar vein, despite postbiotics' recent surge in popularity, there are comparatively fewer clinical trials demonstrating their safety and effectiveness for people than probiotics.

Several probiotic species and postbiotics are being used in a variety of culinary applications as a result of the encouraging findings of several clinical studies assessing their effectiveness in treating various medical diseases. Despite their efficiency, probiotics and postbiotic components are highly susceptible to their surroundings, hence, one of the key factors in preserving their efficacy over time is how they are stored [12–14]. Probiotics and postbiotics' physical characteristics and viability are crucial for the intended effectiveness, so it's critical to assess how storage conditions affect these aspects to guarantee the best possible efficacy throughout product's shelf life. Very few studies, nevertheless, have been done to assess how storage conditions affect probiotics and postbiotics throughout their shelf life.

2. Objectives

The current investigation was carried out to assess how shelf-life storage conditions affect the physical characteristics and survival of *Lactobacillus rhamnosus*, as well as to correlate with its antibacterial efficiency in preventing the growth of *Staphylococcus aureus* in vitro.

3. Methods

3.1. Micro-organisms procurement and storage

Sundyota Numandis Probioceuticals Pvt. Ltd. (Ahmedabad, Gujarat) provided the live and heat-attenuated *L. rhamnosus* powder with the initial strength of 310 billion colony-forming units (CFU) and intact cells per gm powder, respectively. An in-house process was used to produce the live and heat-attenuated *L. rhamnosus*. The probiotic biomass was produced by batch fermentation after the *L. rhamnosus* seed culture was grown in the proper culture to supply the necessary nutrients for growth. Following centrifugation, the biomass was combined with the proper cryoprotective chemicals for the lyophilisation procedure. The live *L. rhamnosus* probiotic powder that was obtained was kept at -20°C in aluminium bags. The probiotic biomass was heated for the ideal amount of time and temperature to produce the heat attenuated probiotic.

After being heated, the resulting slurry was spray-dried to powder and kept at room temperature in aluminium bags. The manufacturer's storage guidelines were followed for both the live and heat-attenuated *L. rhamnosus.* Plate count and flow cytometry were used to assess the potency of the live and heat-attenuated probiotics, respectively. After being separated into four groups, the probiotics were kept for a year at -20°C, 2-8°C, 25°C ± 60% relative humidity (RH), and 40°C ± 75% RH, respectively. According to the evaluation protocol, sufficient quantity of live and attenuated probiotics were taken out from all storage conditions at months 0, 3, 6, and 12 for further analysis. The probiotic strength and antimicrobial efficacy were assessed in order to determine how various storage conditions affected the probiotic's overall survivability, structural makeup, and antimicrobial efficacy.

3.2. Probiotic strength evaluation

The colony counting method was used to assess the live probiotic powder's potency. In short, 100 ml of sterile MRS (deMan, Rogosa, and Sharpe) broth and 1 gm of probiotic sample were put into a sterile flask. To guarantee adequate probiotic dissolution, the solution was kept at 37°C and incubated for around 40 minutes, shaking it appropriately at regular intervals. With a strength of 10^{-2}, the prepared solution is the "primary solution." One milliliter of the primary solution and nine milliliters of the diluent were serially diluted using a sterile 0.1% peptone/MRS broth solution to create a solution with a strength of 10^{-3}. To create a sequence of dilutions up to the final 10^{-10} strength, similar serial dilutions were carried out. To guarantee adequate

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

mixing, the solution was vortexed after each dilution. The cysteine/agar solution was created by mixing 100 ml of molten MRS agar with 1 ml of sterile 1% cysteine HCl solution in a different sterile flask. One milliliter of each serial dilution (10^{-2} to 10^{-10}) and fifteen milliliters of cysteine/agar solution were added to individual sterile petri plates, stirred, and allowed to solidify at room temperature. Lastly, the petri plates were incubated for 48–72 hours at 37°C in an anaerobic environment. Following incubation, each serial dilution's colonies were enumerated, and the probiotic's overall strength was determined using the dilution factor. The entire procedure was carried out twice, and the mean of the two counts was taken into account. Utilizing the flow cytometry approach as described in ISO 19344:2015 [15], the potency of heat-attenuated probiotic was assessed.

3.3. *S. aureus* zone of inhibition study

The antibacterial effectiveness of both live and attenuated *L. rhamnosus* was assessed using the agar well diffusion method of in vitro growth inhibition with *S. aureus*. In short, *S. aureus* pathogen cells were cultured at 120 rpm at 37°C for the entire night. Agar plates were prepared concurrently using Tryptic soy agar and Muller Hinton agar, and they were incubated for the entire night at 37°C. Agar plates were prepared, and 100 µl of incubated *S. aureus* were spread out on the plate using sterile cotton swabs. Seven wells with a 1 ml capacity each were made on the agar plate for sample inclusion using sterile tips. To achieve the ultimate concentration of 3*106 CFU/intact cells per 100 µl solution of the baseline probiotic material, the probiotic sample was made using the serial dilution procedure. On the following evaluation period, the same serial dilution was carried out. The plate well was filled with 100 µl of the produced probiotic sample. S1 S2, S3, and S4 were the names given to the samples from -20°C, 2-8°C, 25°C ± 60% RH, and 40°C ± 75% RH, respectively. Sterile water served as the negative control (NC), ciprofloxacin at 500 µg/ml was the standard control (SC), and maltodextrin as a placebo served as the positive control (PC). To guarantee adequate sample diffusion in the agar medium, all of the samples (NC, PC, SC, and S1–S4) were loaded into their corresponding petri plate wells. The plates were then incubated at 2–8°C for 15–30 minutes, allowed to cool to room temperature, and then incubated at 37°C for 18–24 hours. A calibrated scale was used to measure the *S. aureus* zone of inhibition for each sample, and the entire procedure was carried out in duplicate.

3.4. Statistical analysis

At various storage settings, the live and heat-attenuated probiotic strengths were calculated as CFU and the number of intact cells per gram of product, respectively. Live and attenuated probiotics' antibacterial effectiveness in preventing S. aureus growth in vitro under various storage conditions was assessed in millimeters (mm). Values from the data were displayed as mean ± SD. The descriptive analysis was carried out using Microsoft Excel. GraphPad Prism software (Desktop version 9.0.0; GraphPad Software Inc., San Diego, CA, USA) was utilized to implement the post-hoc Tukey test and two-way ANOVA for statistical analysis of the zone of inhibition data. The cutoff point for statistical significance was $p < 0.05$.

4. Results

4.1. Effect of storage condition on probiotic viability and physical attributes

According to the hypothesis, the physical characteristics of heat-attenuated probiotics and their live probiotic viability decreased as the storage temperature and relative humidity increased. As the storage temperature rises, the live probiotic survival gradually decreases, as shown in **Table 1**. Probiotics kept at -20°C did not exhibit any change in viability throughout the course of the storage period as compared to baseline, but as the temperature rose, viability gradually decreased. In comparison to baseline values, the viability decline was only 3.2% and 19.4% at -20°C and 2-8°C storage settings, respectively, however the highest decline was seen at 25°C ± 60% RH and 40°C ± 75% RH storage conditions, where at 12 months the viability decline was 64.5% and 100%, respectively.

Additionally, the physical characteristics of the attenuated probiotics were directly impacted by the storage temperature. The number of heat-attenuated probiotic intact cells gradually decreased as the storage temperature rose, as shown in **Table 2**. When stored at -20°C and 2-8°C, the number of intact cells did not decrease at 12 months; however, when stored at 25°C ± 60% RH and 40°C ± 75% RH, the number of intact cells decreased by 16.1% and 35.5%, respectively.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

Table 1. Effect of storage temperature on live probiotics survivability

	Baseline	Month 3	Month 6	Month 12
-20°C	310	320 (+3.2%)	310 (0.0%)	300 (-3.2%)
2-8°C	310	280 (-9.7%)	260 (-16.1%)	250 (-19.4%)
25°C ± 60% RH	310	250 (-19.4%)	190 (-38.7%)	110 (-64.5%)
40°C ± 75% RH	310	80 (-74.2%)	0 (-100.0%)	0 (-100.0%)

Data presented as mean billion CFU/gm probiotic powder (%change in survival rate compared to baseline value).

Table 2. Effect of storage temperature on heat attenuated probiotics physical attributes

	Baseline	Month 3	Month 6	Month 12
-20°C	310	320 (+3.2%)	320 (+3.2%)	310 (0%)
2-8°C	310	310 (0%)	300 (-3.2%)	310 (0%)
25°C ± 60% RH	310	300 (-3.2%)	290 (-6.5%)	260 (-16.1%)
40°C ± 75% RH	310	280 (-9.7%)	270 (-12.9%)	200 (-35.5%)

Data presented as mean number of intact billion cells/gm heat-attenuated probiotic powder (%change in number of intact cells compared to baseline value).

4.2. Effect of storage condition on antimicrobial efficacy

Using in vitro inhibition of *S. aureus* growth, the impact of storage conditions on the antibacterial activity of live and heat-attenuated probiotics was assessed. As the storage conditions increased, the antibacterial activity of live probiotics gradually decreased, as shown in **Figure 1** and **Table 3**. In comparison to baseline values, the antibacterial efficacy of live *L. rhamnosus* during 12 months of storage decreased by 2.0%, 11.0%, 40.0%, and 70.9% under the storage conditions of -20°C, 2-8°C, 25°C ± 60% RH, and 40°C ± 75% RH, respectively. Similarly, compared to baseline values, the antibacterial efficacy of heat attenuated *L. rhamnosus* decreased by -0.4%, 3.3%, 15.3%, and 60.9% for the storage conditions of -20°C, 2-8°C, 25°C ± 60% RH, and 40°C ± 75% RH, respectively, as shown in **Figure 2** and **Table 4**. **Figure 3** shows how various storage conditions affect the anti-*S. aureus* effectiveness of both live and heat-attenuated probiotics.

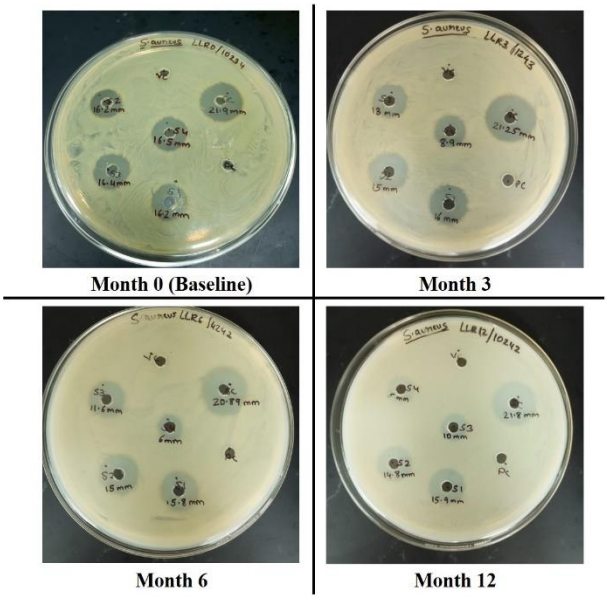

Figure 1. Anti-*S. aureus* efficacy of live probiotics at different storage conditions during shelf life period.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

Table 3. Anti- *S. aureus* efficacy of live probiotics at different shelf life period.

	Baseline	3 month	6 month	12 month
Negative control	0.00	0.00	0.00	0.00
Positive control	0.00	0.00	0.00	0.00
Standard control (Ciprofloxacin)	22.07	21.42	21.23	21.53
-20°C	16.33	16.07	15.87	16.00
2-8°C	16.33	14.77	14.73	14.53
25°C ± 60% RH	16.27	13.30	11.07	9.77
40°C ± 75% RH	16.40	8.40	5.77	4.77

Data presented as zone of inhibition (in mm).

Table 4. Anti- *S. aureus* efficacy of heat attenuated probiotics at different shelf life period.

	Baseline	3 month	6 month	12 month
Negative control	0.00	0.00	0.00	0.00
Positive control	0.00	0.00	0.00	0.00
Standard control (Ciprofloxacin)	22.27	22.13	21.97	22.17
-20°C	8.13	7.97	8.10	8.17
2-8°C	8.17	8.00	7.87	7.90
25°C ± 60% RH	8.07	7.83	7.00	6.83
40°C ± 75% RH	8.27	6.43	4.13	3.23

Data presented as zone of inhibition (in mm).

5. Discussion

Probiotic micro-organisms are known to have a number of health advantages supported by multiple efficacy and safety studies. Few studies have assessed and emphasized the significance of storage conditions on the survivability and overall efficacy of probiotics, despite the fact that many have primarily concentrated on the therapeutic advantages of probiotics on a variety of illness indications. By assessing the impact of varying storage temperatures on the survivability and antibacterial efficiency of live *L. rhamnosus* probiotics, the current study sought to close the research gap. The current study's findings support the theory that probiotics' overall efficiency and durability are significantly impacted by storage conditions.

Over the past few decades, probiotics—defined as live bacteria that benefit the host when given in sufficient quantities—have drawn a lot of interest from the scientific community and the public health authorities [2,16]. Probiotics have gained recognition for their potential therapeutic effects on a variety of physiological systems after first being made popular for their function in gut health [16]. These helpful bacteria are frequently found in fermented foods, dietary supplements, and some medicinal formulations; the most well researched strains are *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* [16]. A complex ecosystem of bacteria, fungi, viruses, and other microbes, the human microbiota is essential for preserving homeostasis and affecting general health. Numerous health problems can result from disturbances to this delicate equilibrium brought on by things like

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

stress, nutrition, or the use of antibiotics [16]. Probiotics have been investigated in this context as a way to modify immunological responses, metabolism, and gastrointestinal processes, as well as to support or restore a healthy microbiome [16]. Probiotics' ability to remain viable over the course of their shelf life is a major factor in how effective they are as medicinal agents. Probiotics are sensitive to a number of environmental factors, including temperature, humidity, light, and oxygen, which can have a substantial impact on their survival and, ultimately, their therapeutic efficacy and health benefits [13]. As a result, maintaining probiotic viability is a complex challenge. The current study's findings indicate that the live *L. rhamnosus* probiotic's survival gradually decreased as the storage temperature rose. A steady deterioration in the probiotic antibacterial activity to stop *S. aureus* growth was also linked to this loss in viability. Maintaining the viability and potency of probiotics requires ideal storage conditions. Probiotic strains can degrade due to improper storage conditions, such as exposure to high temperatures, dampness, or direct sunlight, which lessens their capacity to provide health benefits. To guarantee that goods containing live probiotics retain their viability and, consequently, their full therapeutic potential, probiotic product manufacturers must also carefully consider storage conditions during production, distribution, and storage at the point of sale.

In the realm of microbiome research and therapeutic applications, heat-attenuated probiotics—also referred to as inactivated or attenuated probiotics—represent a possible substitute for conventional live probiotics [17]. In contrast to live probiotics, which provide health benefits through direct microbial activity, heat-attenuated probiotics maintain many of the positive effects of live microorganisms even though they are not viable. This makes them a desirable alternative for people with weakened immune systems or those who are susceptible to infections, where using live probiotics may present risks [18,19]. The use of heat-attenuated probiotics stems from the theory that the beneficial qualities of probiotics may depend more on the components of their cell surface than just their viability, and that the bioactivity of the probiotics may be retained when the cellular structure is preserved [20–22].

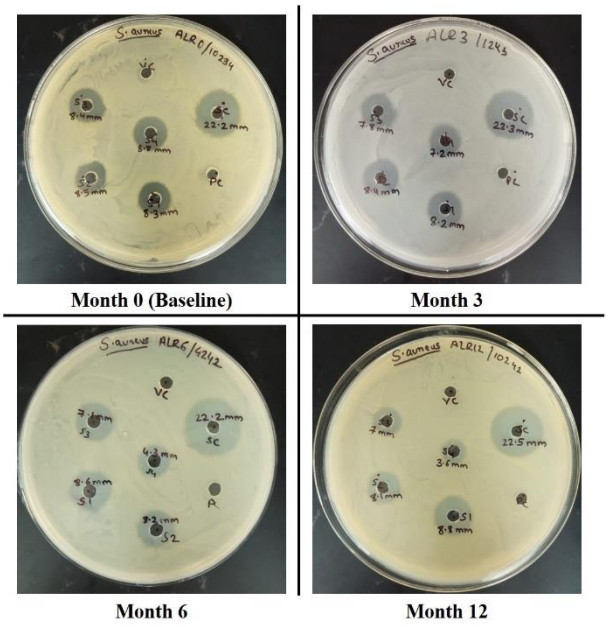

Figure 2. Anti-*S. aureus* efficacy of heat attenuated probiotics at different storage conditions during shelf life period.

Similar to their living counterparts, the preserved cellular components of heat attenuated probiotics have been demonstrated to alter the composition of the gut microbiota, interact with the host's immune system through the intestinal lymphoid tissues, and exhibit anti-inflammatory, antioxidant, and protective properties [20–22]. The potential of heat-attenuated probiotics in a range of clinical illnesses, such as metabolic syndromes, inflammatory diseases, and gastrointestinal disorders, has been highlighted by recent studies [20–22]. Heat-attenuated probiotic formulations are more acceptable for a wider variety of populations than live probiotics because they are frequently more stable, easier to store, and less likely to induce negative side effects [20–22]. Given the significance of cellular components in heat-attenuated probiotic activity, it is imperative to determine if storage conditions affect the postbiotics' physical characteristics and effectiveness. The current study's findings indicate that the amount of intact heat-attenuated cells decreases significantly with increasing storage temperature, indicating that a change in storage conditions may affect the postbiotics' physical characteristics and its antimicrobial efficacy.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

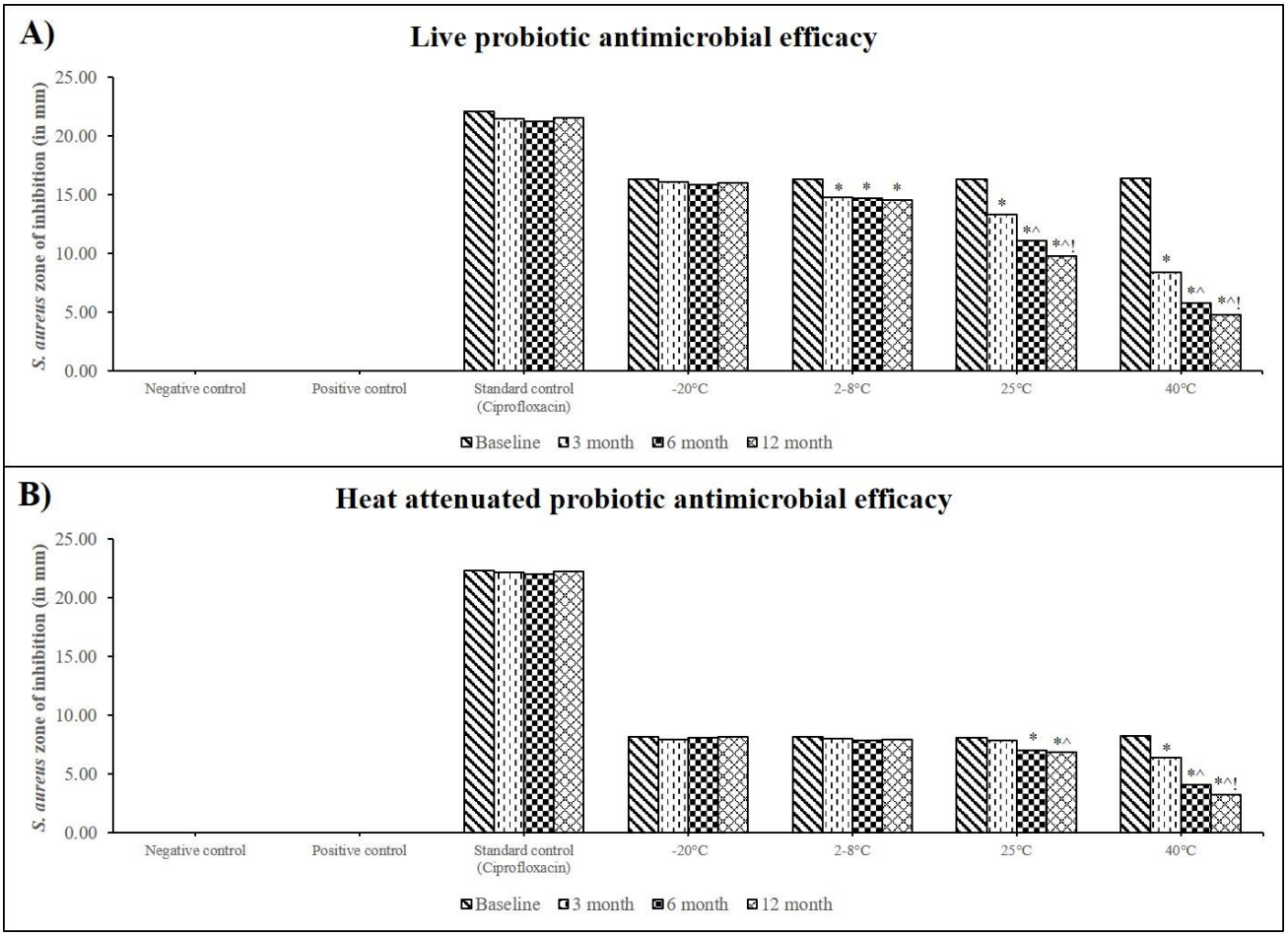

Figure 3. Effect of different storage temperatures on the antimicrobial efficacy of (A) live *L. rhamnosus* and (B) heat-killed *L. rhamnosus* during shelf life period.

Data presented as mean zone of inhibition (in mm). *p < 0.05 v/s baseline, ^p < 0.05 v/s month 3, and ¦p < 0.05 v/s month 6 values, respectively.

There are several advantages to the current study. First off, the current study is unique in that it assessed how various storage temperatures affected the physical characteristics and viability of live and heat-attenuated *L. rhamnosus* probiotics during the course of their shelf life. According to the hypothesis and the observed data, the quantity of intact heat-attenuated probiotics and live probiotics decreased gradually as the storage temperature rose throughout the course of the shelf life. Secondly, the finding of the study supports the hypothesis: less live and heat-attenuated probiotics were found to have the same antibacterial activity in preventing *S. aureus* growth in vitro. The use of an in vitro antimicrobial test rather than an in vivo experimental evaluation approach, the use of only the *L. rhamnosus* probiotic despite the fact that there are numerous well-established probiotics on the market, and, finally, the inability to comprehend the molecular effects of higher storage temperatures on the probiotics that result in decreased efficacy are some of the limitations of the current study.

In conclusion, utilizing both live and heat-attenuated probiotics can enhance gut microbiota health and yield additional health benefits. The current study's findings demonstrate how storage temperature may affect the general health, physical characteristics, and activity of both live and heat-attenuated probiotics during the course of their shelf life. In light of the present study's advantages and disadvantages, the findings are encouraging and may serve as a foundation for further research. Furthermore, the current study's findings can

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 1882-1889 | ISSN:2251-6727

assist producers of health food and supplements containing probiotics in comprehending the significance of ideal storage conditions for such products.

References

1. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823–36.
2. Kheni DB, Sureja VP, Deshpande SS, Dubey VP, Kansagra JJ. A systematic mapping review of in-vitro and in-vivo evidences exploring the role of strain-specific probiotic Bifidobacterium longum W11. Int J Pharm Sci Drug Res. 2024;16(1):127–34.
3. Li Y, Xia S, Jiang X, Feng C, Gong S, Ma J, et al. Gut microbiota and diarrhea: An updated review. Front Cell Infect Microbiol. 2021;11:625210.
4. Santana PT, Rosas SLB, Ribeiro BE, Marinho Y, Souza HS de. Dysbiosis in inflammatory bowel disease: Pathogenic role and potential therapeutic targets. Int J Mol Sci. 2022;23(7):3464.
5. Sadagopan A, Mahmoud A, Begg M, Tarhuni M, Fotso M, Gonzalez NA, et al. Understanding the role of the gut microbiome in diabetes and therapeutics targeting leaky gut: A systematic review. Cureus. 2023;15(7):e41559.
6. Appanna V. Dysbiosis, Probiotics, and Prebiotics: In Diseases and Health. In: Appanna V, editor. Human Microbes - The Power Within. Singapore: Springer Nature Singapore Pte Ltd.; 2018. p. 81–122.
7. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health. 2014;11(5):4745–67.
8. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. Adv Nutr. 2019;10(Suppl_1):S49–66.
9. Latif A, Shehzad A, Niazi S, Zahid A, Ashraf W, Iqbal MW, et al. Probiotics: mechanism of action, health benefits and their application in food industries. Front Microbiol. 2023;14:1216674.
10. Żółkiewicz J, Marzec A, Ruszczyński M, Feleszko W. Postbiotics—A step beyond pre- and probiotics. Nutrients. 2020;12(8):2189.
11. Kerksick CM, Moon JM, Jäger R. It's dead! Can postbiotics really help performance and recovery? A systematic review. Nutrients. 2024;16(5):720.
12. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol. 2021;18(9):649–67.
13. Cabello-Olmo M, Oneca M, Torre P, Díaz JV, Encio IJ, Barajas M, et al. Influence of storage temperature and packaging on bacteria and yeast viability in a plant-based fermented food. Foods. 2020;9(3):302.
14. Gurunathan S, Thangaraj P, Kim JH. Postbiotics: Functional food materials and therapeutic agents for cancer, diabetes, and inflammatory diseases. Foods. 2023;13(1):89.
15. ISO 19344:2015 [Internet]. [cited 2025 Mar 20]. Milk and milk products — Starter cultures, probiotics and fermented products — Quantification of lactic acid bacteria by flow cytometry. Available from: https://www.iso.org/standard/64658.html
16. Kheni DB, Sureja VP, Deshpande SS, Dubey VP, Kansagra JJ. Lactobacillus rhamnosus GG in paediatric acute diarrhoea: A clinical perspective and systematic review of clinical studies published in the last decade. Chettinad Heal City Med J. 2024;4(13):135–45.
17. Kheni D, Sureja V, Deshpande S. Potential role of Lactobacilli spp. postbiotics in atopic dermatitis. Int J Res Publ Rev. 2024;5(4):10001–4.
18. Wegh CA, Geerlings SY, Knol J, Roeselers G, Belzer C. Postbiotics and their potential applications in early life nutrition and beyond. Int J Mol Sci. 2019;20(19):4673.
19. Vinderola G, Sanders ME, Salminen S. The concept of postbiotics. Foods. 2022;11(8):1077.
20. Piqué N, Berlanga M, Miñana-Galbis D. Health benefits of heat-killed (Tyndallized) probiotics: An overview. Int J Mol Sci. 2019;20(10):2534.
21. Koh YC, Chang YC, Lin WS, Leung SY, Chen WJ, Wu SH, et al. Efficacy and mechanism of the action of live and heat-killed Bacillus coagulans BC198 as potential probiotic in ameliorating dextran sulfate sodium-induced colitis in mice. ACS Omega. 2024;9(9):10253–66.
22. Ditengou JICP, Ahn SI, Chae B, Choi NJ. Are heat-killed probiotics more effective than live ones on colon length shortness, disease activity index, and the histological score of an inflammatory bowel disease-induced murine model? A meta-analysis. J Appl Microbiol. 2023;134(3):lxad008.