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Original Research

Enhanced Survival of *Lactobacillus* Lg71 from Mangrove Sediment Under Simulated Gastric and Intestinal Conditions

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ABSTRACT

Background: For a probiotic to offer health benefits, it must stay viable through the tough environment of the human gastrointestinal tract. This study assesses the effectiveness of sodium alginate encapsulation in improving the survival of *Lactobacillus* LG71, a new strain from mangrove sediment, under simulated gastric and intestinal conditions. **Objective:** This study aims to evaluate the effectiveness of sodium alginate encapsulation in enhancing the viability and survival of Lactobacillus LG71, a probiotic strain isolated from mangrove sediment, during cold storage and under simulated gastric and intestinal conditions. **Methods:** *Lactobacillus* LG71 was encapsulated in sodium alginate beads. The viability of both encapsulated and free (non-encapsulated) cells was assessed over a 4-week storage period at 4 °C and during sequential exposure to simulated gastric and intestinal environments. **Results:** Encapsulation significantly improved survival rates compared to free cells ($p < 0.05$). Although both groups experienced a decline during the first week of storage, encapsulated cells maintained a high viability of approximately 10^7 CFU/mL, losing only 2.51 log CFU/g over four weeks. Most notably, encapsulated *Lactobacillus* LG71 demonstrated greater resilience during digestion, retaining populations of 2.21 log CFU/mL in gastric simulations and 1.00 log CFU/mL in intestinal simulations. In contrast, free cells were much more vulnerable to these acidic and enzymatic conditions. **Conclusion:** Sodium alginate encapsulation effectively protects *Lactobacillus* LG71 from environmental and biological stressors. These findings indicate that encapsulation is a crucial step for the commercial application of mangrove-derived probiotics, ensuring that a viable dose of living cells reaches the host's lower gastrointestinal tract to promote health benefits.

Keywords: alginate encapsulation; functional foods; Lactobacillus; mangrove sediment; probiotic

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1. INTRODUCTION

Probiotics are live microorganisms that, when taken in sufficient amounts, provide health benefits by influencing the gut microbiota and producing antimicrobial compounds (Binda et al., 2020; Kusharyati et al., 2023; Sarita et al., 2025). However, the commercial and therapeutic effectiveness of any probiotic depends entirely on its viability; it must survive the harsh journey through the gastrointestinal tract to reach the colon in an active state (Binda et al., 2020; Jumazhanova et al., 2023; Sarita et al., 2025).

The low pH of the stomach and high bile salt concentrations in the small intestine are significant physiological barriers that often cause a sharp decline in live cell counts, making many potential probiotics ineffective. To reduce these losses, microencapsulation with sodium alginate has become an essential delivery method. Alginate forms a protective hydrogel matrix that acts as a physical barrier against acidic degradation, helping to maintain the therapeutic minimum of 10^6–10^7 CFU/g necessary for probiotic effectiveness (Łętocha et al., 2024; Wang et al., 2022, 2025).

A probiotic candidate can be isolated from sources such as dairy, fermented foods, vegetables, stool, and water (Kusharyati et al., 2020; Rovik & Kusharyati, 2025; Sornplang & Piyadeatsoontorn, 2016). The most commonly used probiotics include *Lactobacillus, Bifidobacterium, Enterococcus, Lactococcus,* and *Streptococcus* (Kusharyati et al., 2021; Le Morvan De Sequeira et al., 2022; Rovik & Kusharyati, 2025; Sornplang & Piyadeatsoontorn, 2016). To find more effective probiotic candidates, the search for new strains has broadened beyond traditional sources to include microbes adapted to extreme environments. In this context, mangrove ecosystems are a promising, unexplored source because their resident microbes have evolved to endure harsh, variable conditions such as high salinity and anoxia, which may provide them with natural resilience that is beneficial for survival in the gastrointestinal tract (Catozzi et al., 2019; Hwanhlem et al., 2014; Sornplang & Piyadeatsoontorn, 2016).

Kusharyati et al. have isolated and screened *Lactobacillus* sp. from mangrove sediment in Jawa Tengah (Kusharyati et al., 2021; 2023a; 2023b). Despite the promising origin of *Lactobacillus* LG71, its survival ability and the need for encapsulation for practical use remain unconfirmed. This study assesses the viability differences between free and encapsulated *Lactobacillus* LG71 in simulated gastric and intestinal fluids. As the author's knowledge, this is the first to examine the gastrointestinal resilience of a mangrove-derived *Lactobacillus* strain, specifically through an encapsulation delivery system, offering a new approach for using environmental extremophiles as strong candidates for functional probiotic applications.

2. METHODS

2.1. Bacterial Strain and Culture Conditions

A small amount of *Lactobacillus* LG71 was inoculated into 10 mL of MRS broth and incubated for 24-48 hours at 37°C. Then, 1 mL of the activated *Lactobacillus* LG71 was transferred to 100 mL of MRS broth and incubated under the same conditions for another

24-48 hours. The final cell concentration in the culture was adjusted to over 10^9 CFUs/mL.

2.2. Alginate Encapsulation of *Lactobacillus* LG71

Lactobacillus LG71 isolates were encapsulated using sodium alginate, following the procedure of Yeung et al. (2016) with modifications. A 2% (w/v) sodium alginate solution totaling 96 mL was sterilized. The sterile solution was mixed with 4 mL of a liquid culture of *Lactobacillus,* which was concentrated to 0.85% (w/v) NaCl, yielding a bacterial population of approximately 10^9 CFUs/mL. The mixture was homogenized to ensure even dispersion of the suspended cells, then allowed to stand for 5 minutes to remove any dissolved air. Encapsulation was performed by applying pressure to the mixture through a syringe with a 0.4-0.8 mm diameter to generate droplets. These droplets were collected in 300 mL of a 0.1 M $CaCl_2$ solution and agitated continuously for 1 hour to allow proper cross-linking and formation of the encapsulation beads. The resulting beads were then vacuum-filtered, rinsed with 200 mL of sterile deionized water, and filtered again. The encapsulated cells were subsequently stored at 4°C.

2.3. In Vitro Viability Assessment

A 0.1 g sample of alginate beads was suspended in 9.9 mL of a 10% (pH 8.2) $Na_3C_6H_5O_7.2H_2O$ solution and vortexed for 10 minutes at room temperature to release the encapsulated cells. The number of viable cells in the free and encapsulated treatments was determined by the TPC method on MRSA media. Serial dilutions up to 10−7 were prepared in duplicate and incubated at room temperature for 24-48 hours. Sampling was conducted weekly for four weeks. Before encapsulation, the initial cell number (prior to bead formation) was determined from the original alginate-bacterial mixture. Serial dilutions to 10−8 were prepared in duplicate on MRS agar, incubated at room temperature for 24-48 hours, and then counted for colonies. For comparison with the encapsulated cells, free cells (the non-encapsulated treatment) were also maintained under aerobic conditions at room temperature. Viable cell counts were determined using the TPC method on MRSA media. Serial dilutions up to 10−8 were prepared in duplicate, and the plates were incubated at room temperature for 24-48 hours before colony enumeration.

2.4. Encapsulation Efficiency and Stability in Simulated Gastric Juice

The viability of free and encapsulated cells was tested following the method of Damodharan et al. (2017) with modifications. A simulated gastric juice solution was prepared in a warm water reaction tube by adding 0.03 M phosphate-buffered saline and 10 mg/mL pepsin. The pH of the simulated solution was adjusted to 2 with 1 M HCl. The simulated solution was mixed with both free and encapsulated cells and incubated at 37°C. The number of viable cells in each treatment was measured at 0, 30, 60, 90, and 120 minutes. Viable cell counts for both free and encapsulated treatments were determined using the TPC method on MRSA media. After incubation at room temperature for 24-48 hours, colonies were counted to quantify viable cells.

2.5. Encapsulation Efficiency and Stability in Simulated Intestinal Juice

The viability of free and encapsulated cells was tested following the method of Damodharan et al. (2017) with modifications. A simulated intestinal fluid was prepared by adding 0.03 M phosphate-buffered saline, 10 mg/mL trypsin, and 0.3% bile salt to sterile deionized water. The pH of the solution was adjusted to 7.5 with 1 M NaOH. The prepared solution was mixed with both free and encapsulated cells and incubated at 37°C. The number of viable cells in each treatment was determined at 0, 30, 60, 90, and 120 minutes. Viable cell counts for both free and encapsulated treatments were measured using the TPC method on MRSA media. After incubation at room temperature for 24-48 hours, colonies were counted to quantify viable cells.

2.6. The Total Number of Lactic Acid Bacteria (LAB) Measurement

The total LAB was measured using a serial dilution and plating method on MRSA medium. After incubation at room temperature for 24-48 hours, the colonies were counted to determine viable cell counts, following the formulation:

$$\text{CFU/mL} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume of inoculum (mL)}}$$

2.7. Statistical Analysis

The mean cell counts from the encapsulation and simulated gastrointestinal tests were analyzed using a two-sample t-test ($\alpha = 0.05$).

3. RESULTS

Lactobacillus LG71 was successfully encapsulated in sodium alginate, yielding beads 45 mm in diameter (Figure 1). To assess performance, the viability of immobilized *Lactobacillus* LG71 cells was monitored during storage at 4 °C and compared with that of the free-cell control.

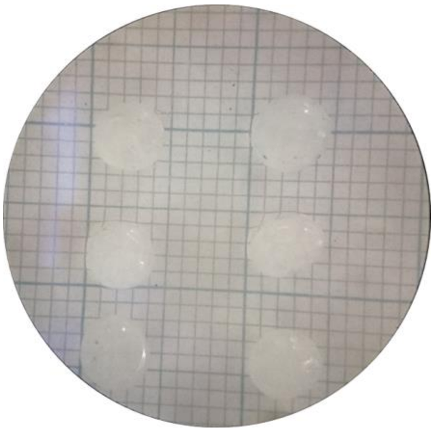

Figure 1. Visual characteristics of sodium alginate-encapsulated *Lactobacillus* LG71 beads. Six hydrogel beads containing Lactobacillus LG71 are placed on a millimeter grid paper to allow size estimation and uniformity analysis.

Both groups experience a sharp decline in the first week, with a reduction of 2.26 log CFU/g (Figure 2). However, free cells decline more rapidly, falling to approximately 10^6 CFU/ml, whereas encapsulated cells maintain a higher viability of approximately 10^7 CFU/ml. Encapsulated cells show remarkable stability after the first week, remaining consistently within the 6.5–7.0 log range. Sodium alginate encapsulation produced a significantly different viability response in *Lactobacillus* LG71 compared with free cells ($p < 0.05$). After four weeks of storage at 4°C, viable encapsulated LG71 cells decreased by 2.51 log CFU/g, whereas free LG71 cells showed a greater reduction of 4.32 log CFU/mL.

Table 1. Morphological description of sodium alginate-encapsulated *Lactobacillus* LG71 beads

Parameter	Description
Shape and symmetry	The beads exhibit a spherical to slightly ovoid geometry. They appear well-formed, suggesting that the viscosity is well optimized.
Colour and opacity	The beads are semi-translucent, with a milky white or cloudy appearance. This opacity indicates a high concentration of trapped *Lactobacillus* cells or a dense calcium alginate matrix.
Surface texture	The surfaces appear smooth and moist. There is no visible "tailing" (the teardrop shape that occurs when the alginate is too viscous).
Size uniformity	Each bead appears to be approximately 34 mm in diameter.

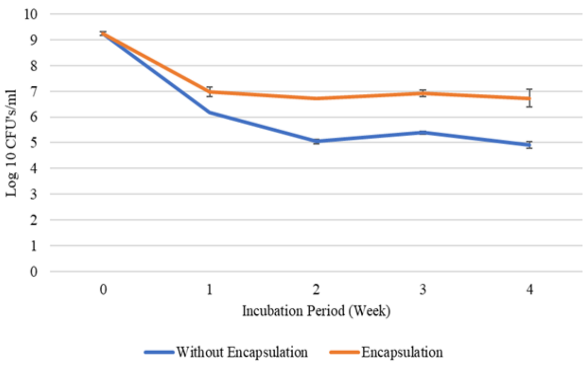

Figure 2. The survival rate of *Lactobacillus* LG71 over a 4-week storage period, comparing free (non-encapsulated) cells with encapsulated cells

Results showed a decrease in viability of both free and encapsulated LG71 isolates under simulated gastrointestinal conditions (Figure 3). Specifically, non-encapsulated LG71 was unable to survive, as the initial population of 6.30 ± 0.20 log CFU/mL decreased to 0 log CFU/mL after exposure to gastric and intestinal fluids. In contrast, encapsulated LG71 retained populations of 2.21 log CFU/mL and 1.00 log CFU/mL after gastric and intestinal simulations, respectively.

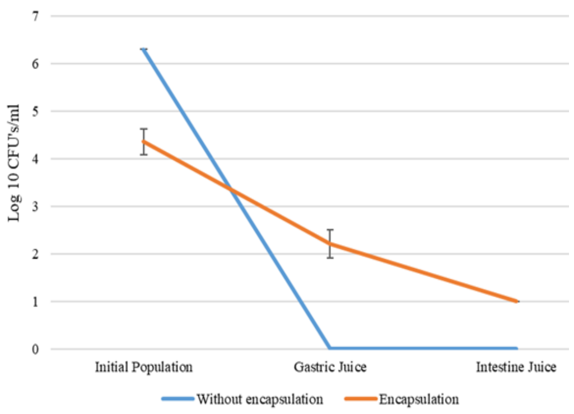

Figure 3. Survival of encapsulated and free LAB LG71 cells under simulated gastrointestinal conditions

4. DISCUSSION

The encapsulation of *Lactobacillus* LG71 in sodium alginate resulted in beads measuring 4–5 mm, a size that balances protective physical barriers with industrial processing needs (Figure 1). From a food science perspective, bead diameter is important: while larger beads provide greater insulation against acid, they must not be so large as to impart a texture in functional foods or hinder enzymatic degradation in the host's small intestine (Afzaal et al., 2019; Shi et al., 2013). The stability of these beads was optimized at 1.5–3% alginate, as lower concentrations risk mechanical rupture during food processing. In comparison, higher concentrations may hinder the diffusion of essential nutrients to the bacteria during storage.

An important observation was the initial decrease in viable counts from 6.3 log CFU/mL (free) to 4.4 log CFU/mL (encapsulated). This initial reduction highlights a common issue in probiotic manufacturing: encapsulation efficiency (EE). While the alginate matrix may protect some cells from immediate detection on culture media, the decline indicates that the extrusion process—involving calcium chloride exposure and osmotic shock—is a stressor that must be carefully managed to ensure a high starting dose for commercial products. Although another study suggests that alginate concentration (1–3%) does not significantly impact EE, choosing the hydrogel material remains essential to ensuring a high initial cell population (Ayama et al., 2014).

Throughout the four-week storage period, the encapsulated *Lactobacillus* LG71 showed significantly higher stability than free cells, losing only 2.51 log CFU/g. This data indicates that alginate provides a strong protective barrier against adverse environmental factors (Luca & Oroian, 2021). We noticed a characteristic decline in viability during the first week. However, the population stabilizes afterward. This stabilization indicates that the alginate matrix effectively hardens the probiotic population against environmental stressors, a crucial trait for extending the shelf life of probiotic-fortified products (Pradeep Prasanna & Charalampopoulos, 2019; Qi et al., 2019).

The most important finding for industrial use is the survival of *Lactobacillus* LG71 during simulated gastrointestinal transit. The gastric environment (pH 2.0) usually destroys free cells by inducing HCl-mediated protein damage and denaturation. However, the alginate matrix acts as a protective barrier, slowing H^+ ion penetration and helping to maintain the internal pH (Andriamanantoanina & Rinaudo, 2010). Upon entering the small intestine (pH 6–7), the matrix responds with pH-triggered swelling and disintegration (Pradeep Prasanna & Charalampopoulos, 2019; Qi et al., 2019). It releases the viable *Lactobacillus* LG71 at the precise site required for colonization.

To be considered a functional probiotic, a product must deliver 10^6 to 10^7 CFU/mL to the consumer (Champagne et al., 2011). Although *Lactobacillus* LG71 demonstrates high resilience, the relatively low EE observed here suggests that, for large-scale production, the formulation should be optimized—possibly through double-coating with chitosan or adding prebiotic fillers (symbiotic). These findings confirm that mangrove-derived *Lactobacillus* LG71 is a strong candidate for the supplement industry, provided that the encapsulation process is refined to maximize the initial cell load.

5. CONCLUSION

Sodium alginate encapsulation effectively protects *Lactobacillus* LG71 from environmental and biological stressors. These findings suggest that encapsulation is a critical step for the commercial use of mangrove-derived probiotics, ensuring that a functional dose of viable cells reaches the host's lower gastrointestinal tract to provide beneficial effects.

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CONFLICT OF INTEREST

The author reports no conflict of interest.

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