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Stability studies of free and microencapsulated Lactobacillus caseiunder gastrointestinal conditions for probiotic yogurt preparation

Priyanka G[1], R. Venkata Bhargavi[2], Krishna Satya Alapati
Department of Biotechnology, Acharya Nagarjuna University, Guntur- 522510

Abstract-Probiotics have emerged as powerful agents for promoting gastrointestinal health, with *Lactobacillus casei* being a prominent candidate. In this innovative research, *L. casei*was isolated from milk and thoroughly examined for its probiotic properties. Recognizing the critical role of storage conditions in probiotic effectiveness, we investigated the impact of co-encapsulation with complementary oat starch, a potent prebiotic.The encapsulation process demonstrated a remarkable enhancement in the viability of *L.casei*under simulated gastrointestinal conditions, laying a strong foundation for its therapeutic potential. Microencapsulation technique is quiet a challenging task which involves selection of suitable and nontoxic biopolymers. Through meticulous monitoring and stability evaluation over an 8-week storage period, we unveiled an astonishing revelation - encapsulated *L. casei* exhibited significantly increased viability compared to its non-encapsulated counterpart. Observation of storage at different temperatures plays a crucial role in understanding the shelf life of the probiotic product. The microencapsulated probiotic beads were successfully incorporated in to yogurt. The storage evaluation and stability studies are much helpful for future predictions in the applications, such as probiotic yogurt preparation in the current study. The tolerance to the adverse gastric conditions due to variations in pH and bile concentrations of L.*casei*has been examined and it was proven to be an excellent probiotic. These findings offer a ground breaking approach to preserving the viability of *L. casei* during storage, an essential consideration for its successful application as a probiotic supplement. The synergistic relationship between *L. casei* and oat starch holds the promise of revolutionizing the probiotic industry, paving the way for new opportunities in enhancing gut health and overall well-being.

Keywords- *Lactobacillus casei*, microencapsulation, probiotics, storage, stability studies, probiotic yogurt.

I. INTRODUCTION

Fortified foods have gained popularity as an effective strategy to combat nutrient deficiencies and promote overall health and wellness. By incorporating important nutrients into various food products, manufacturers offer consumers an easily ecosystem. The gastrointestinal tract plays a critical role in nutrient absorption, metabolism, and overall health. Fortified foods can positively impact the gut microbiota, which refers to the complex community of microorganisms residing in the intestines. The consumption of fortified foods that contain prebiotic

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fibers or probiotics can help maintain a balanced microbial ecosystem. Microencapsulation technology offers a promising solution to protect probiotics and ensure their efficacy through the gastrointestinal tract, providing consumers with maximum health benefits. Microencapsulation plays a crucial role in preserving the viability of probiotic bacteria during the journey through the harsh conditions of the gastrointestinal tract. By encapsulating probiotics in protective coatings or evenly dispersing them within microspheres, their survival is enhanced, allowing them to reach their target organs and exert their health-promoting effects effectively.

The study focuses on assessing the survival rate and distribution of the probiotic culture, making it relevant to both the fermentation industry and various product formulations. Synbiotic yogurt can be prepared by adding specific strains of probiotics and prebiotics to regular yogurt. Probiotic strains commonly found in synbiotic yogurt include Lactobacillus acidophilus, Bifidobacterium bifidum, and Streptococcus thermophilus, among others. Prebiotics such as inulin, fructo oligosaccharides (FOS), and galacto oligosaccharides (GOS) are added to provide nourishment for the probiotic bacteria. Synbiotics are categorized as either complementary synbiotics or synergistic synbiotics. Complementary synbiotics contain both prebiotics and probiotics that are independently selected for their beneficial effects on health. Conversely, synergistic synbiotics contain prebiotics that are chosen specifically to support the effects of the selected probiotics.

II.METHODOLOGY

Inoculums preparation:
The isolated probiotic bacterium, Lactobacillus casei, was prepared for microencapsulation by first culturing it in MRS broth for 24 hours at 37°C. Following the incubation, the bacterial cells were harvested by centrifugation at 2500 × g for 10 minutes at 4°C. Thorough washing of the cells was performed twice to eliminate any residual broth and contaminants. Subsequently, the washed cells were resuspended in 5 mL of buffer saline to create an inoculum of free cells. These free cells were then utilized to generate microencapsulated beads

Extraction of oligosaccharides
The extraction was carried out with 80% ethanol. The prebiotic source (oats) were dried and made into powder. Hundred mg of the powdered oats was added to 5 mL of 80% ethanol. It was allowed for continuous stirring of 1 h at room temperature. The extract was then evaporated by keeping in hot water bath at 80 °C. Then 10 mL of water was added to dissolve the sugars that were settled. The extract was used for further studies.

Co-Encapsulation
Various ratios of bacteria to slurry of sodium alginate and oat starch (pre-biotic) were employed, including 1:2, 1:4, 1:6, 2:2, 2:4, 2:6, 3:2, 3:4, and 3:6, while utilizing alginate concentrations ranging from 1% to 4% w/v. The objective was to achieve a final concentration of 108 CFU/mL (colony-forming units per millilitre) in each microcapsule. The mixture of alginate and bacterial cells was added drop by drop using a 5ml or 10ml syringe in to chilled calcium chloride solution. The drops were added into separate calcium chloride solutions with varying concentrations of 0.5%, 1%, 2%, and 3% (w/v), each containing a total volume of 40mL. The microencapsulated beads were left in the calcium chloride solution for approximately 30 minutes. Later, the beads were carefully transferred to a saline solution.

Bead solubilization
The survival of the bacteria in the encapsulated beads shows the efficiency of the entrapment procedure. 1gram of calcium alginate beads were dissolved in 0.1M phosphate buffer solution at pH-7. Serial dilution in saline solution was performed and survival was observed on MRS agar plates.

Stability at different storage temperatures
The encapsulated bacterial beads were divided into two batches and placed under separate conditions, with one batch maintained at 4°C and the other at 25°C. Survival of free and encapsulated bacterium was studied at the time intervals of 3, 6, 9, 20 and 30 days. 10 beads were analysed each time whereas, three replications were performed for each temperature. The results were tabulated.

Stability at different pH and bile conditions
Both the free and encapsulated forms of isolated strain were examined for survival rates at different pH and bile concentrations. In test tubes containing 10 mL of MRS broth 1gm of fresh encapsulated beads and 1mL of free cell suspension were separately taken, pH was adjusted 2.0 and 3.0 and

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incubated at 37 ° C for 24 hrs. The bacterial cells were collected, washed, and subsequently used for enumerating viable cells at two time points: 0 hours and 24 hours by plating the cells on MRS agar medium and incubating them at 37°C for 24 hours. Similarly, Bile tolerance was examined by transferring 1 gm of encapsulated beads and 1 mL of free cell suspension in to test tubes with10 mL of MRS broth with 0.3, 0.6 and 0.8 g/100 mL bile salt concentrations and the enumeration of viable cells at 0 h and 24 h was carried out after incubating at 37 ° C. the results were tabulated.

Probiotic yogurt preparation

Fresh cow milk was collected and pasteurized for 30 min at 60°C from a local dairy. Heating was continued to 80-85℃ and then cooled to 45°C. The Yogurt starter culture was added. The microencapsulated probiotic beads were added. Then this mixture was allowed to ferment for 10 hours for probiotic yogurt formation.

III.RESULTS AND DISCUSSION

1.Encapsulation

L. caseiwas cultured in MRS broth medium. The sample was centrifuged and the pellet was collected; washed with the saline solution. This was added to the slurry of sodium alginate and oat starch. The slurry was suspended in chilled calcium chloride solution which resulted in the formation of calcium alginate beads in which the bacteria was encapsulated. After 30min, the beads were collected and transferred to buffered saline for preservation

Figure1: Encapsulated beads

Bead morphology:

The size of the beads was determined by passing them through sieves with openings of 1mm, 500µm, and 150µm. The results indicated that the majority of the beads fell within the range of 0.5 - 1mm, with only a small fraction falling below 500µm in size. As for the shape, most of the beads exhibited a spherical morphology. However, variations in the dropping height during encapsulation led to some beads showing drop-shaped or elliptical characteristics. The incorporation of prebiotic oat starch in the encapsulation process played a crucial role in enhancing the bead quality. The addition of oat starch contributed to the development of beads with improved shape, texture, and viability, particularly under challenging conditions such as low pH and high bile concentrations.

Bead solubilization

The solubilized beads were tested for viability. 1 gram of beads were taken, to this 9 ml of phosphate buffer was added and subjected to homogenization. Then, 1 ml of the solution was plated on MRS agar medium.

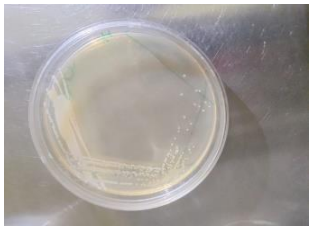

Figure 2 probiotic yogurt

Solubilization of beads

Stability of free and encapsulated bacteria at different storage temperatures, pH and bile concentrations Probiotic bacteria's survival and growth are influenced by factors like low pH and storage conditions. Storage analysis under room and frozen conditions showed no significant decrease in viable count for encapsulated bacteria at different temperatures (Table 1), pH levels, and bile concentrations (Table 2). The survival rate was 75-80% under low pH conditions with prebiotic oat starch, and 75-85% under bile concentration compared to free probiotic bacteria. Encapsulated bacteria demonstrated higher viability in storage compared to free cells. Unlike another study with calcium alginate immobilization, the prebiotic used in this research improved survival rates under low pH and high bile concentrations. These fermentation by-products contribute positively to the host's health.

Table:1- Storage analysis of Encapsulation and survival rate

Storage temperature	Moisture content	7 days	15 days	Survival %
Beads at 4°C	4.31+0.28	8.37+0.32	8.27+0.43	77%
Beads at 25°C	4.21+0.53	9.15+0.18	8.44+0.16	74%

Table: 2- Survival of free and Encapsulated L.casei under gastric condition

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Treatment	Initial mean	Acid concentration					Bile concentration (%)	
		pH2		pH3	pH6.5	0.3	0.5	0.8
Free cells	8.82±0.13	7.23±0.35	8.68±0.30	8.81±0.16	6.54±0.45	8.23±0.10	6.44±0.21	
Encapsulated	9.71±0.18	8.73±0.30	8.67±0.34	9.18±0.39	9.16±0.35	8.16±0.17	9.27±0.52	

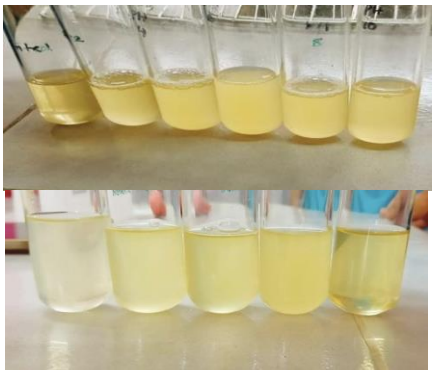

Figure : Survival of free and Encapsulated L.caseiunder gastric condition.

Probiotic yogurt formation

Fresh cow milk was collected from a local dairy of Nambur pasteurized for 30 min at 60°C. Heating was continued to 80-85°C and then milk was cooled to 45°C. The Yogurt starter culture was added along with the encapsulated probiotic beads. Then this mixture was allowed to ferment for 10 hrs. The curd formed was mixed and stirred thoroughly to form yogurt. The probiotic yogurt was stored in glass bottles.

IV.CONCLUSION

Microencapsulation of Lactobacillus casei using calcium alginate beads with a prebiotic proves to be a successful strategy for enhancing stability and survival under adverse conditions. Successful microencapsulation and probiotic yogurt preparation were possible using L. caseiand the stability of the encapsulated probiotic strain was understood. The study demonstrates the value of encapsulated L. caseiin both industrial processes and consumer products, providing opportunities for innovation in the field of dairy fermentation and probiotic supplementation.

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