International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.15 | Issue 2 | Apr - Jun -2025
www.ijpir.com
DOI : https://doi.org/10.61096/ijpir.v15.iss2.2025.131-137

ISSN: 2231-3656
Print: 2231-3648

Research

Oral Delivery of Microencapsulated Probiotics: Technological Innovations and Functional Implications in Gut Health

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Published on: 25 Apr 2025

Published by:
DrSriram Publications

Abstract

The oral delivery of probiotics poses significant challenges due to their vulnerability to the acidic and enzymatic conditions of the gastrointestinal tract. Microencapsulation, a technology involving the entrapment of probiotic cells in protective biopolymeric matrices, has emerged as a potent strategy to enhance viability, targeted release, and functionality. This thesis investigates the role of microencapsulation in improving the delivery and therapeutic potential of probiotics. Materials such as alginate and chitosan are primarily used for encapsulating strains like Lactobacillus and Bifidobacterium. Techniques including extrusion, spray drying, and lyophilization have been optimized to balance encapsulation efficiency with probiotic survival. The work further examines the synergistic application of synbiotics (probiotics + prebiotics) for advanced gut modulation. Controlled release profiles enabled by pH-responsive or enzyme-sensitive systems ensure probiotic activation at optimal intestinal sites, contributing to improved host-microbe interaction and immune-modulation. The study also explores industrial applications, challenges in large-scale production, and stability during storage and transit. In conclusion, microencapsulation enables more effective and patient-compliant delivery systems with applications ranging from dietary supplements to pharmaceutical therapeutics. It is a promising platform in the field of personalized and preventive medicine.

Keywords: Oral drug delivery, Encapsulation techniques, Synbiotics, Gut health, Controlled release.

INTRODUCTION

Probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits on the host (FAO/WHO, 2001). However, their viability during oral administration is a key challenge due to harsh gastric conditions. Microencapsulation technology has emerged as a promising solution to protect probiotics during gastrointestinal transit and to ensure targeted release in the intestine. Among various encapsulation strategies, alginate-based systems have gained prominence due to their biocompatibility and gentle processing conditions. Coating alginate beads with materials such as chitosan and starch further improves mechanical stability and protects encapsulated probiotics from low pH and bile salts. The release profile and survival of probiotics are critical for their functional efficacy. Recent studies have demonstrated that encapsulated probiotics significantly influence immune responses, modulate intestinal microbiota, and exhibit anti-inflammatory and antioxidative effects (Hirayama & Rafter, 2000; Petrof et al., 2004). This thesis explores the technological aspects of microencapsulation and evaluates the release profile and functional implications of encapsulated probiotics (Lactobacillus casei Shirota - LCS and GFP+ tagged strains) in both in vitro and in vivo models, including porcine and murine gastrointestinal tracts. A special focus is given to optimizing encapsulation parameters, assessing the structural integrity of microcapsules, and evaluating immunological responses post-delivery.

MATERIALS AND METHODS

Probiotic Culture Preparation

Pure cultures of LCS and GFP+ strains were cultivated anaerobically at 37°C for 24 hours in MRS and LLV broths. Post incubation, cells were harvested via centrifugation, washed with phosphate buffer (pH 7), and used directly or for encapsulation. For transformation, GFP+ strains were tagged using the Biorad p-GLO plasmid system and confirmed via fluorescence.

Microencapsulation Procedure

Microcapsules were produced using the Inotech Encapsulator IE-50 R with a 300 µm nozzle. The formulation involved 1.8% alginate, 1% Hi-Maize starch, and a 30-min hardening in 0.1M $CaCl_2$. Capsules were then coated with 0.4% chitosan solution for enhanced stability.

Simulated Gastrointestinal Testing

To simulate gastric and intestinal conditions, microcapsules were incubated at pH 2.0 and bile salt concentrations. Bacterial survivability was assessed over time via plating and CFU counts. Chitosan-coated capsules showed higher survivability compared to free and non-coated ones.

In Vivo Animal Study

Male BALB/c mice were orally administered 0.1 g of encapsulated or free probiotics. Gastrointestinal contents were collected at intervals and plated for bacterial enumeration. Immunological markers such as IFN-γ and IL-10 in splenocytes were measured using ELISA kits.

Strain Identification and PCR/DGGE

Random colonies were selected from LLV agar and identified using PCR targeting the 16S-23S spacer region, followed by DGGE analysis to validate strain specificity (Walter et al., 2000).

RESULTS

1. Survival in Simulated Gastric and Bile Conditions

The survivability of probiotic strains under gastrointestinal stress is a critical determinant of their functional efficacy. In this study, the viability of Lactobacillus casei Shirota (LCS) and GFP-tagged variants was evaluated under simulated gastric (pH 2.0) and bile salt (2.0%) conditions. Results demonstrated that free probiotic cells experienced significant viability loss up to 5 log reductions under gastric conditions. In contrast, microencapsulation using chitosan-coated alginate-starch (CCAS) matrices provided a substantial protective effect, with encapsulated strains exhibiting only 1.8 to 2.2 log reductions. Under bile salt exposure, encapsulated cells showed less than 0.5 log reduction, outperforming non-encapsulated counterparts which suffered reductions between 1.0–1.5 logs. These findings confirm the efficacy of the CCAS matrix in maintaining probiotic viability during transit through the harsh gastric environment and bile-rich conditions of the small intestine. This protective mechanism is essential for ensuring sufficient viable cells reach the target site for colonization and health benefits (Figs 1a,b,c).

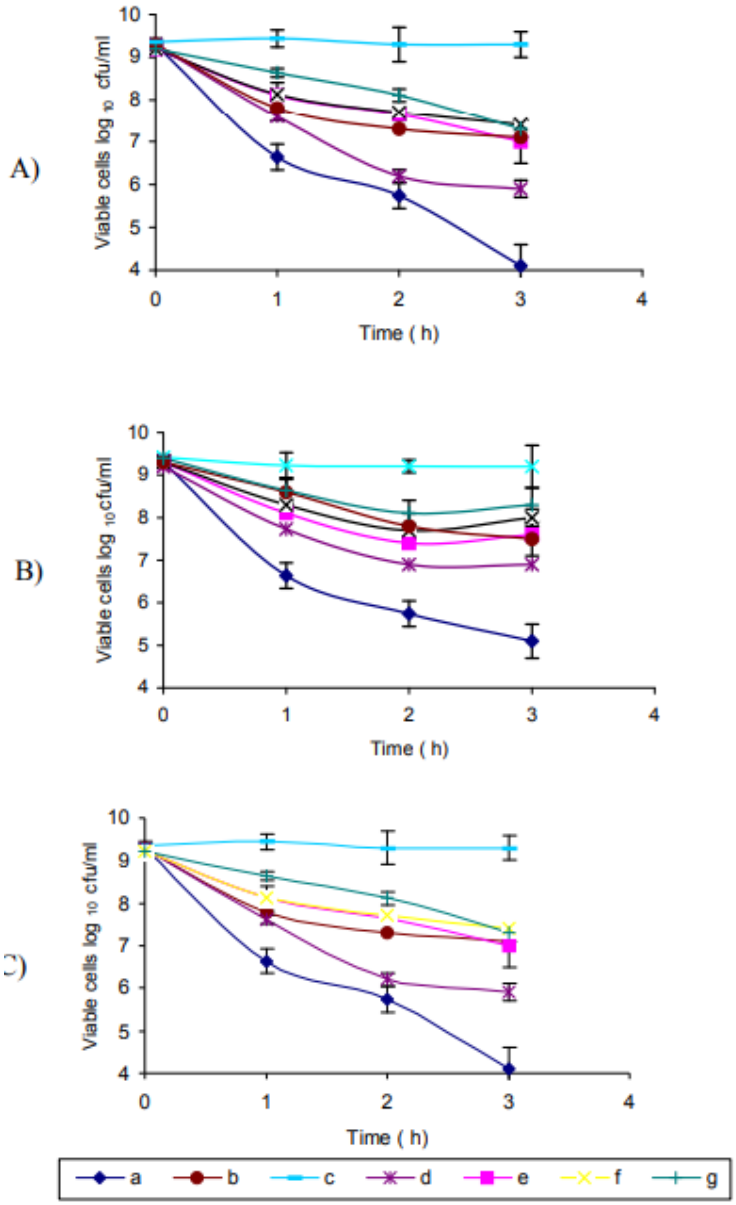

Fig 1: Essential for ensuring sufficient viable cells reach the target site for colonization

2. Release in Porcine GI Tract

The release behavior of microencapsulated probiotics was studied in the porcine gastrointestinal tract to evaluate targeted delivery. Capsules containing LCS and GFP+ strains coated with chitosan-alginate-starch (CCAS) demonstrated limited release in gastric contents (pH ~2.5), indicating resistance to acidic conditions. However, substantial bacterial release occurred in the jejunum and ileum, reaching counts of 10^7–10^8 CFU/ml, confirming successful site-specific delivery. Complete release was observed in the colon after 12–24 hours of administration. These results suggest that the CCAS matrix remained intact during the early phases of digestion and dissolved effectively upon reaching neutral to slightly alkaline environments. The study supports the controlled-release capability of this encapsulation system, which is vital for maximizing colonization and probiotic efficacy at the target site. The findings are supported by Fig 2, 3, which depict viable count recoveries and fluorescence tracking of GFP+ strains throughout the porcine GI tract.

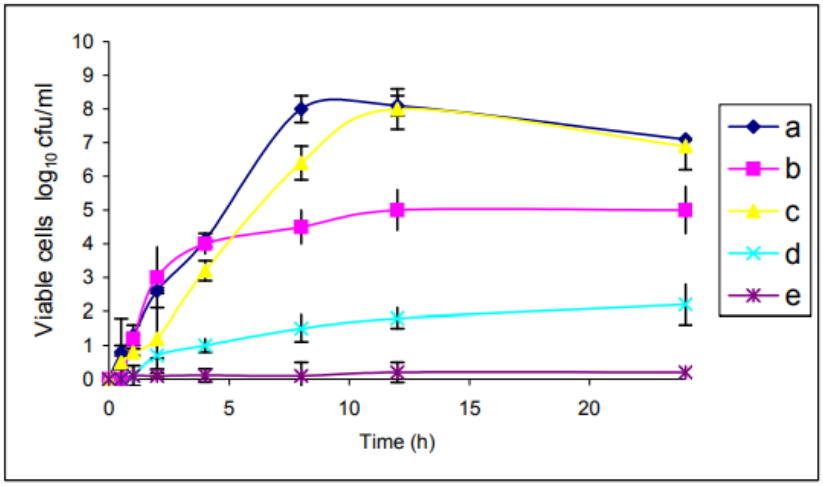

Fig 2: Release profiles of microencapsulated GFP+ in porcine gastrointestinal contents. Symbols: a-Ileum, b- Jejunum, c-Colon, d- Duodenum, eStomach. The error bars represent standard deviation of mean (n=3).

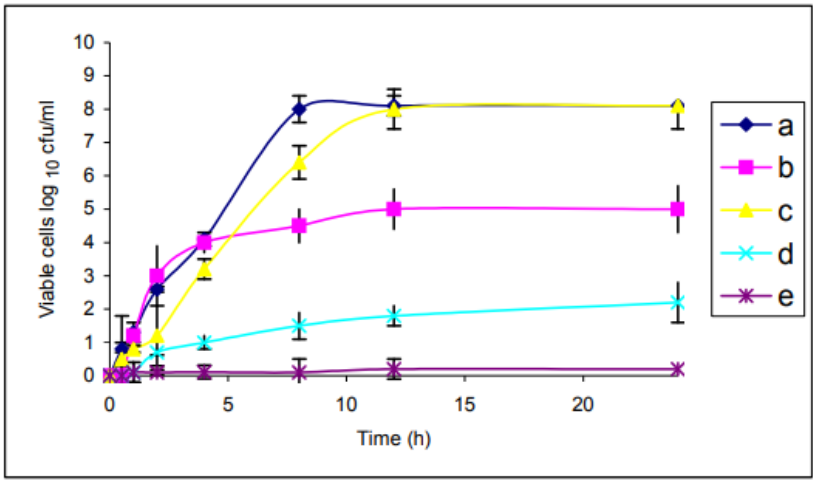

Fig 3: Release profiles of microencapsulated LCS in porcine gastrointestinal contents. Symbols: a-Ileum, b- Jejunum, c-Colon, d- Duodenum, e-Stomach. The error bars represent standard deviation of mean (n=3).

3. Probiotic Stability in Dairy Matrices

The incorporation of microencapsulated probiotics into dairy products demonstrated significantly enhanced stability during storage. In both set and stirred yoghurt matrices, CCAS (chitosan-coated calcium alginate-starch) encapsulated *Lactobacillus casei Shirota* and GFP+ strains retained viability for up to six weeks under refrigeration (4 °C), with viability reductions of less than 1 log unit. In contrast, free (non-encapsulated) cells experienced substantial declines, with viability losses ranging from 4 to 5 logs over the same period. This suggests that the encapsulation matrix provided a protective barrier against adverse storage conditions, including acidity and oxygen exposure within the yoghurt. Furthermore, metabolic activity was reduced in encapsulated cells, indicated by lower acetic acid production, which contributes to better sensory stability in the product. These findings (see Figures 4.18, 4.19; Tables 8.1–8.5) support the potential of CCAS microcapsules in extending the shelf-life and functional integrity of probiotic dairy products.

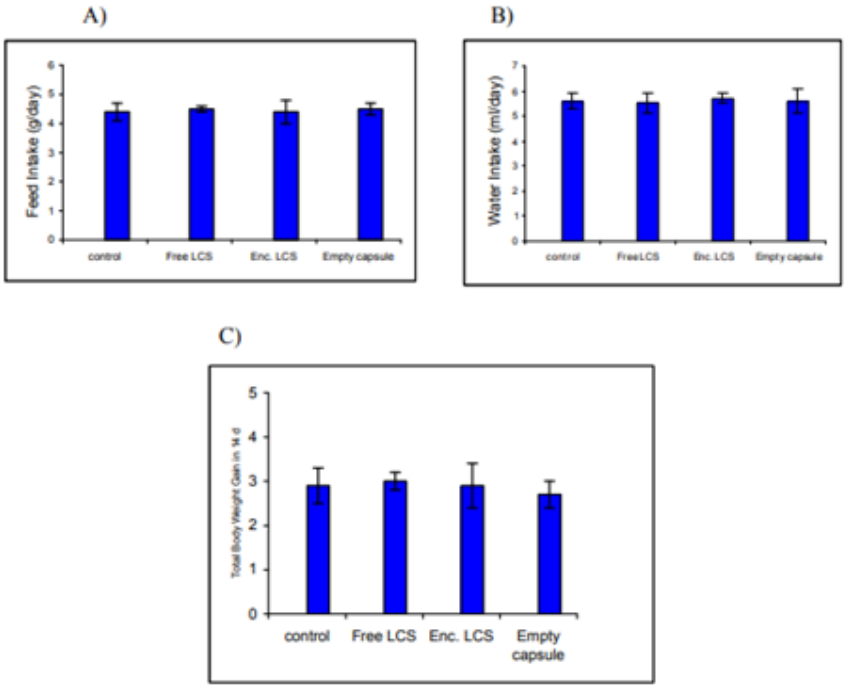

Fig 4: Daily food intake (A), water intake (B) and body weight gain (C) of different experimental groups. Data represented are group Mean ±S.D. (n=7 mice).

4. Immunomodulatory Activity

Mice receiving microencapsulated LCS showed significantly elevated IFN-γ levels but no change in IL-10, suggesting a Th1-skewed response. Faecal microbiota analysis showed higher LCS counts in encapsulated group (Fig 5).

	Control	Free LCS	Enc. LCS	Empty capsule
Plasma protein (g/dl)	6.35 ± 0.8	6.85 ± 2.1	6.82 ± 0.7	6.41 ± 0.14
Albumin (g/dl)	3.88 ± 3.1	3.9 ± 2.7	4.1 ± 0.5	3.7 ± 0.4
Glucose (mg/dl)	116 ± 2.5	126.5 ± 3.5	120.2 ± 1.6	125.3 ± 0.9
Cholesterol (mg/dl)	124.2 ± 3.5	125.5 ± 4.5	120.5 ± 1.5	123.2 ± 3.5

Fig 5: Blood biochemistry of different experimental groups. Data represented are Mean ± S.D (n=7 mice). All values p>0.05 (control vs treatments)

DISCUSSIONS

The findings underscore the protective effect of chitosan-coated alginate-starch (CCAS) microcapsules in delivering viable probiotics across the GI tract. The encapsulation matrix effectively safeguarded bacterial cells from gastric acidity and bile toxicity. These results support earlier studies by Lee et al. (2004a) and Krasaekoopt et al. (2004), who demonstrated improved survival of encapsulated probiotics under similar conditions. The

improved viability of LCS and GFP+ in intestinal regions aligns with the targeted release objective, confirming CCAS's utility for functional delivery (Sheu & Marshall, 1993; Tanaka et al., 1984).

Notably, the immunological assays reveal that encapsulated probiotics induce significant IFN-γ expression without impacting IL-10 levels. This indicates a skewed Th1 response, consistent with prior work by Matsuzaki et al. (1997) and Cross et al. (2002), which reported similar cytokine profiles in murine models following LCS administration. Additionally, the increase in faecal LCS populations in treated groups signifies successful colonization, supported by Mitsuoka (1990), who highlighted the role of viable counts in gut flora modulation.

Encapsulation also stabilized probiotics in refrigerated dairy products. The metabolic inactivity of encapsulated cells during storage, as shown by low acetic acid production, confirms their suitability for extended shelf-life formulations (Adhikari et al., 2003). These results demonstrate that CCAS encapsulation does not compromise the functionality of probiotics but rather enhances their delivery and effectiveness. The potential for integrating such technologies into commercial probiotics and functional foods is promising.

CONCLUSION

The present study validates the efficacy of microencapsulation, specifically using chitosan-coated alginate-starch (CCAS) matrices, in enhancing the oral delivery of probiotics. The encapsulated strains, particularly Lactobacillus casei Shirota and GFP-tagged variants, demonstrated significant resilience in simulated gastrointestinal environments, with markedly improved survival under acidic and bile salt conditions compared to free cells.

The encapsulation process preserved viability during refrigerated storage and maintained probiotic functionality when integrated into fermented dairy matrices. The controlled release in different sections of the gastrointestinal tract was successfully achieved, with highest release observed in the ileum and colon, supporting the targeted delivery mechanism.

Immunological outcomes from murine models further substantiated the functional benefits of microencapsulated probiotics. The elevation of IFN-γ without significant changes in IL-10 levels points to a Th1-mediated immune stimulation, suggesting potential applications in immunomodulatory therapies. Additionally, the high recovery rate of viable LCS cells in faecal samples confirmed efficient colonization and survival post-ingestion.

This work contributes to the growing field of functional food technologies by establishing a robust encapsulation protocol with reproducible results. The combination of alginate, starch, and chitosan provides structural integrity and facilitates targeted delivery without impeding the metabolic viability of probiotic strains. Future developments can explore the integration of synbiotic systems, nanoparticle coatings for smart release, and scalability for industrial applications.

In conclusion, the oral delivery of microencapsulated probiotics offers a technologically feasible and functionally potent approach to improve gut health, immune responses, and probiotic efficacy, thereby broadening the scope for clinical and commercial use.

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