

Formulation and Characterization of Encapsulated *Bacillus* spp. for Enhanced Agro-Industrial Efficacy

Sangeetha Murthy^{1*}; Ranjani Murugan¹; Manikandan Ramesh^{1,2};
Saranya Sakthivel¹; Krish KR¹

¹Research and Development Laboratory, SS Biochem India Pvt. Ltd., Salem, TN-636004, India

^{1,2}Department of Biochemistry, Periyar University, Salem-636 011, India

Corresponding Author: Sangeetha Murthy^{1*}

Publication Date: 2025/09/25

Abstract: The use of biological control agents such as *Bacillus* spp. has gained increasing recognition among farmers as an eco-friendly alternative for pest and disease management. However, *Bacillus* spp., like other microorganisms, are sensitive to environmental stressors and require specific conditions for survival and efficacy. Therefore, to ensure its effectiveness as a biological control agent in field applications, *Bacillus* spp. must be incorporated into a suitable formulation. Conventional liquid formulations often fail to maintain the long-term viability of bacteria in soil environments. In contrast, bioencapsulation—particularly in the form of microcapsules—has shown promise in enhancing bacterial stability and viability under field conditions. This study investigates the influence of sodium alginate concentration on the encapsulation efficiency of *Bacillus* sp. Microcapsules were prepared via the extrusion technique by mixing bacterial suspensions with sodium alginate solutions at concentrations of 1%, 1.5%, and 2%, and subsequently dropping the mixture into a calcium chloride solution to form gel beads. Encapsulation efficiency was evaluated by extracting the bacteria from the microcapsules, culturing them on a suitable growth medium, and quantifying viable cells. The results demonstrated encapsulation efficiencies of 1%, 0.36%, and 1.35% for sodium alginate concentrations of 1%, 1.5%, and 2%, respectively. These findings suggest that the concentration of sodium alginate significantly affects the encapsulation efficiency of *Bacillus* spp., with higher or optimal concentrations enhancing the protective matrix and viability of the encapsulated bacteria.

Keywords: *Bacillus* spp. Encapsulation, Bio-Encapsulation, Biocontrol, Sodium Alginate.

How to Cite: Sangeetha Murthy; Ranjani Murugan; Manikandan Ramesh; Saranya Sakthivel; Krish KR (2025) Formulation and Characterization of Encapsulated *Bacillus* spp. for Enhanced Agro-Industrial Efficacy. *International Journal of Innovative Science and Research Technology*, 10(9), 1513-1516. <https://doi.org/10.38124/ijisrt/25sep896>

I. INTRODUCTION

Global food security depends heavily on agriculture, but plant diseases, degraded soil, and excessive use of chemical pesticides and fertilizers are posing a growing threat to agricultural yield [1]. Despite their effectiveness, conventional chemical inputs frequently have detrimental effects on soil health, cause environmental contamination, and breed resistant microorganisms. Biological control agents (BCAs) have emerged as an environmentally sustainable alternative to chemical pesticides in modern agriculture [2]. Among these, *Bacillus* spp. are widely recognized for their ability to suppress plant pathogens and insect pests through the production of bioactive metabolites, competitive colonization, and induction of plant defense mechanisms [3]. Their effectiveness, however, is often constrained by their sensitivity to environmental stresses such

as desiccation, UV radiation, and fluctuating soil conditions. To ensure high survival rates and consistent performance under field conditions, *Bacillus* spp. require specialized formulation strategies that enhance their stability and viability [4]. Conventional liquid formulations, although widely used, typically fail to protect the bacterial cells for extended periods in soil environments. Bio-encapsulation, particularly in the form of microcapsules, has gained attention as a promising approach for protecting microbial inoculants from environmental stress while enabling controlled release and prolonged activity [5]. Sodium alginate, a natural biopolymer derived from brown algae, is extensively employed in microencapsulation due to its biocompatibility, low toxicity, and ability to form gel beads in the presence of calcium ions. The concentration of sodium alginate plays a critical role in determining bead structure, mechanical strength, and encapsulation efficiency [6]. However, limited

studies have focused on optimizing alginate concentrations specifically for *Bacillus* spp. intended for agricultural applications. The present study aims to evaluate the influence of sodium alginate concentration on the encapsulation efficiency of *Bacillus* sp. using the extrusion method. By testing different concentrations of sodium alginate, this research seeks to identify the optimal conditions for achieving high bacterial retention within microcapsules, thereby contributing to the development of more effective and durable microbial formulations for field application.

II. MATERIALS AND METHODS

➤ Soil Sample Collection

Soil samples were collected from agricultural land (11° 39' 21.6432" N and 78° 24' 14.8644" E) with a history of sustainable crop cultivation and minimal chemical pesticide use. Samples were taken from the rhizosphere region (5–15 cm depth) using sterile stainless-steel augers. Approximately 500 g of soil was collected in sterile polyethylene bags, transported to the laboratory, and processed within 24 h of collection.

➤ Soil Pre-Treatment

Soil samples were air-dried at room temperature (28 ± 2 °C) for 48 h to reduce moisture content. Large debris, plant residues, and stones were removed by sieving through a 2 mm mesh. For bacterial isolation, 10 g of dried soil was serially diluted in sterile saline solution (0.85% NaCl) up to 10^{-6} dilutions.

➤ Isolation of Bacteria

Diluted soil suspensions were spread-plated onto Nutrient Agar (NA) and Luria-Bertani (LB) agar supplemented with cycloheximide (50 µg/mL) to suppress fungal growth. Plates were incubated at 30 °C for 24–48 h. Colonies with distinct morphology were purified by repeated streaking and stored in 20% glycerol at -20 °C [7].

➤ Primary Screening for Antimicrobial Activity

The primary screening was carried out by cross-streak assay against selected phytopathogenic bacteria (*Xanthomonas campestris*, *Pseudomonas syringae*) and fungi (*Fusarium oxysporum*, *Rhizoctonia solani*). The test pathogen was streaked centrally, and isolates were streaked perpendicularly. Inhibition zones were measured after 24–48 h (bacteria) and 3–5 days (fungi) incubation [7].

➤ Secondary Screening for Antimicrobial Activity

Potential isolates from primary screening were subjected to agar well diffusion assay using cell-free culture supernatants (centrifuged at 10,000 rpm, 10 min, 4°C). Pathogens were spread uniformly on respective media, and wells (6 mm) were filled with 100 µL of supernatant. After the incubation periods, inhibition zones were measured in mm [7].

➤ Phenotypic Characterization

Morphological traits of potential were documented (colony size, shape, color, edge, elevation). Gram staining, endospore staining, motility tests, and biochemical assays

(catalase, oxidase, starch hydrolysis, casein hydrolysis, citrate utilization, and nitrate reduction) were performed according to standard protocol [7].

➤ Genotypic Characterization

Genomic DNA was extracted using a bacterial DNA isolation kit (Qiagen). The 16S rRNA gene was amplified by PCR using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR products were purified and sequenced commercially [8].

➤ Sequence Analysis and Phylogeny

Sequences were analyzed using BLASTn against the NCBI database. Closest matches (>98% similarity) were considered for taxonomic identification. Phylogenetic analysis was performed using MEGA X with the Neighbor-Joining method and 1000 bootstrap replicates [8].

➤ Potential Culture Production

The potential strain used was cultured in nutrient broth (HiMedia, India) at 30 ± 2 °C for 24 h under constant agitation at 150 rpm. The culture was harvested at the late exponential growth phase and adjusted to a standardized cell density ($\sim 10^8$ CFU/mL) prior to encapsulation [7].

➤ Preparation of Sodium Alginate Solutions

Sodium alginate (HiMedia, India) solutions were prepared at concentrations of 1.0%, 1.5%, and 2.0% (w/v) by dissolving the polymer in distilled water under constant stirring at room temperature until complete dissolution [9 &10].

➤ Microencapsulation by Extrusion Method

Encapsulation was carried out using the extrusion technique. Equal volumes of bacterial suspension and sodium alginate solution were mixed thoroughly. The mixture was loaded into a sterile syringe and extruded dropwise into a 0.1 M calcium chloride (CaCl_2) solution under gentle stirring. The droplets instantaneously formed gel beads through ionic cross-linking. The beads were allowed to harden for 30 min, rinsed twice with sterile distilled water to remove residual CaCl_2 , and stored at 4 °C until further analysis [10].

➤ Determination of Encapsulation Efficiency

Encapsulation efficiency (EE) was determined by extracting the entrapped bacteria from a known weight of alginate beads. The beads were dissolved in 1% (w/v) sodium citrate solution to release the encapsulated cells [9]. The released bacteria were serially diluted and plated on nutrient agar to determine viable counts (CFU/mL). EE (%) was calculated using the formula:

$$EE(\%) = \frac{\text{Number of entrapped cells} \times 100}{\text{Total number of cells added}}$$

➤ Data Analysis

Encapsulation efficiency values for different alginate concentrations were compared, and results were expressed as mean values. Observed differences were analyzed to

determine the influence of alginate concentration on bacterial retention.

III. RESULT AND DISCUSSION

➤ Isolation and Screening

From 10 soil samples, 32 morphologically distinct bacterial isolates were obtained. Primary screening revealed that 12 isolates exhibited inhibitory activity against at least one bacterial and one fungal phytopathogen. Among these, isolate *Bacillus* sp. SS1 showed the largest inhibition zones (25.4 ± 0.6 mm against *F. oxysporum*, 22.1 ± 0.4 mm against *X. campestris*), indicating strong antagonistic potential.

➤ Phenotypic and Biochemical Characterization

The potent isolate was Gram-positive, rod-shaped, motile, and endospore-forming, consistent with *Bacillus* morphology. Biochemical assays confirmed catalase and oxidase positivity, starch and casein hydrolysis, nitrate reduction, and citrate utilization. The ability to form

endospores suggests high environmental persistence, a desirable trait for agricultural bioinoculants [11].

➤ Genotypic Identification

16S rRNA sequencing produced a 980 bp fragment. BLASTn analysis showed 100% identity with *Bacillus* sp. strain. The sequence was submitted and GenBank accession no. is PV563077. Phylogenetic analysis placed the isolate SS1 in close proximity to other *Bacillus* sp. strains used in biocontrol applications.

➤ Encapsulation Efficiency at Different Alginate Concentrations

The encapsulation efficiency (EE) of *Bacillus* sp. SS1 varied with sodium alginate concentration (Table 1). The highest EE (1.35%) was recorded at 2.0% alginate, followed by 1.0% alginate (1.00%) and 1.5% alginate (0.36%). The results indicate that alginate concentration influences bead formation and bacterial entrapment, consistent with earlier reports that polymer viscosity and pore size directly affect cell retention within the gel matrix [10 &11].

Table 1 Encapsulation Efficiency of *Bacillus* sp. SS1 at Different Sodium Alginate Concentrations.

Sodium Alginate Concentration (% w/v)	Encapsulation Efficiency (%)
1.0	1.00
1.5	0.36
2.0	1.35

➤ Effect of Alginate Concentration on Bead Formation

Beads prepared with lower alginate concentration (1.0%) were more porous and had reduced mechanical strength, potentially allowing leakage of cells into the surrounding medium. Conversely, beads formed at 2.0% alginate exhibited a denser network structure, which likely restricted bacterial loss during formation and washing steps. At 1.5% alginate, the observed low EE may be attributed to suboptimal polymer–calcium cross-linking, resulting in unstable bead structure and poor cell entrapment [12].

In agricultural formulations, maintaining high bacterial viability is critical for field efficacy, particularly under variable soil and environmental conditions. Microencapsulation in alginate matrices offers protection against desiccation, UV exposure, and predation by soil microorganisms [12&13]. However, polymer concentration must be optimized to balance bead strength, permeability for nutrient exchange, and release kinetics. The present results suggest that 2.0% sodium alginate is most suitable for *Bacillus* sp. encapsulation under the conditions tested. Similar trends have been reported for *Bacillus subtilis* and *Bacillus thuringiensis*, where higher alginate concentrations improved EE but excessive viscosity hindered uniform bead formation [13 & 14]. The relatively low EE values in this study may be due to the extrusion parameters, cell size, or ionic gelation time, indicating that further optimization (e.g., bead curing duration, CaCl_2 concentration, and incorporation of secondary polymers such as chitosan) could enhance bacterial entrapment.

This study demonstrated that sodium alginate concentration significantly influences the encapsulation

efficiency of *Bacillus* sp. using the extrusion method. Among the tested concentrations, 2.0% sodium alginate achieved the highest bacterial retention (1.35%), likely due to the formation of a denser gel matrix that reduced cell leakage during bead formation. In contrast, beads formed with lower or intermediate alginate concentrations showed reduced entrapment, suggesting weaker gel structures and higher porosity. These findings emphasize the importance of optimizing polymer concentration for developing robust microencapsulation systems for microbial inoculants. While the observed EE values in this study were relatively low, further improvements could be achieved by adjusting extrusion parameters, bead hardening time, and incorporating additional coating layers (e.g., chitosan) to enhance structural stability and protection. Optimized formulations have the potential to improve *Bacillus* sp. survival in soil and enhance their efficacy as biological control agents in sustainable agriculture.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Department of Scientific and Industrial Research (DSIR), Government of India, for recognizing our in-house R&D laboratory (F. No. TU/IV-RD/4729/2024) and supporting the research activities. The authors also thank all laboratory staffs for their valuable contributions to this study.

REFERENCES

- [1]. Y. Gai and H. Wang, "Plant Disease: A Growing Threat to Global Food Security," *Agronomy*, vol. 14,

- no. 8, p. 1615, 2024, doi: 10.3390/agronomy14081615.
- [2]. K. Pandey and B. S. Saharan, "Soil microbiomes: a promising strategy for boosting crop yield and advancing sustainable agriculture," *Discover Agriculture*, vol. 3, p. 54, 2025, doi: 10.1007/s44279-025-00208-5.
- [3]. I. H. Kadhim and A. A. H. Matloob, "Bacillus subtilis and its role in biological control of plant pathogens," *Agricultural Biotechnology Journal*, vol. 17, no. 3, pp. 293–312, 2025, doi: 10.22103/jab.2025.25589.1736.
- [4]. F. Ahmad, N. Ismail, and N. Rahman, "The efficiency of microencapsulation with alginate, gelatin, and chitosan on the survival of *Bacillus subtilis* under stress conditions," *Journal of Applied Microbiology*, vol. 129, no. 4, pp. 867–878, 2020, doi: 10.1111/jam.14657.
- [5]. W. Krasaekoopt, B. Bhandari, and H. Deeth, "The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria," *International Dairy Journal*, vol. 14, no. 8, pp. 737–743, 2004, doi: 10.1016/j.idairyj.2004.01.004.
- [6]. R. Martínez-Vega, M. González-Sánchez, and A. López-Moreno, "Application of encapsulation strategies for probiotics: A review," *Foods*, vol. 12, no. 1, p. 34, 2023, doi: 10.3390/foods12010034.
- [7]. S. Sultana, K. Shaheen, N. Amanda, H. Shingmuan, *et al.*, "Antifungal trait and plant growth promotion potential of *Bacillus* spp. from rhizosphere soils of black aromatic rice, 'Chakhao'," *Current Agriculture Research Journal*, vol. 11, no. 2, 2023, doi: 10.12944/CARJ.11.2.07.
- [8]. M. Sangeetha, A. Sivarajan, M. Radhakrishnan, *et al.*, "Biosequestration of carbon dioxide using carbonic anhydrase from novel *Streptomyces kunmingensis*," *Archives of Microbiology*, vol. 204, p. 270, 2022, doi: 10.1007/s00203-022-02887-w.
- [9]. R. Sharma, R. Singh, and V. Kumar, "Encapsulation of plant biocontrol bacteria with alginate as a main polymer material," *Polymers*, vol. 13, no. 19, p. 3258, 2021, doi: 10.3390/polym13193258.
- [10]. H. Shokri, A. Zare, and G. Sadeghi, "Sodium alginate–gelatin microcapsules enriched with nanomaterials for *Bacillus velezensis* encapsulation and biological control of pistachio gummosis," *Scientific Reports*, vol. 12, p. 4513, 2022, doi: 10.1038/s41598-022-08589-y.
- [11]. M. M. Saputra, Y. Wuryandari, and N. Ramadhini, "Analysis of encapsulation efficiency *Bacillus* sp. based variations in sodium alginate concentration in the beads," *Nusantara Science and Technology Proceedings*, vol. 2024, no. 39, pp. 1–4, 2024, doi: 10.11594/nstp.2024.3901.
- [12]. R. Banerjee and M. Ray, "Microencapsulation of *Bacillus mojavensis* using sodium alginate for probiotic applications," *Current Research in Food Science*, vol. 6, 100567, 2025, doi: 10.1016/j.crfs.2025.100567.
- [13]. F. Song, Y. Peng, and X. Liu, "Sodium alginate-based microcapsules of *Bacillus thuringiensis* with UV protection and slow-release properties," *Scientific Reports*, vol. 6, 39425, 2016, doi: 10.1038/srep39425.
- [14]. M. Szekalska, A. Puciłowska, E. Szymańska, P. Ciosek, and K. Winnicka, "Alginate: Current use and future perspectives in pharmaceutical and biomedical applications," *International Journal of Polymer Science*, vol. 2016, pp. 1–17, 2016, doi: 10.1155/2016/7697031.