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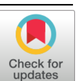
Viability of Plant Growth-Promoting Rhizobacteria from Shallot Rhizosphere Encapsulated in Alginate–Chitosan Matrices



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ABSTRACT

Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial microorganisms that enhance plant growth and health through various biological mechanisms. However, their viability in the open environment is often compromised by abiotic stress factors. Bioencapsulation using natural coating materials such as alginate and chitin offers a promising strategy for maintaining the survival and function of these microorganisms. This study aimed to isolate and evaluate the viability of PGPR from the rhizosphere of red onions (*Allium ascalonicum*) encapsulated in an alginate-chitin matrix. A total of 31 bacterial isolates were obtained from the rhizosphere soil of shallots. Based on plant growth-promoting (PGP) properties—including indole-3-acetic acid (IAA) production, phosphate solubilisation, potassium solubilisation, and antagonistic activity—10 isolates were identified as potential PGPR candidates. These selected isolates were encapsulated using various combinations of alginate and chitosan concentrations. The experiment was arranged in a completely randomised design (CRD) with a 3 × 3 non-factorial layout and three replicates. The parameters observed included emulsion stability, emulsion viscosity, water content, protein solubility, encapsulation efficiency, and bacterial viability. The results showed that the coating formulation had a very significant effect on emulsion stability, water content, protein solubility, and encapsulation efficiency, but did not significantly affect emulsion viscosity. Among the tested formulations, the 1%: 1.5% combination (alginate: chitosan) yielded the highest water content (96.74%) and emulsion stability (145.81 $m^2 \cdot g^{-1}$). The highest protein solubility (56.71%) was observed in the 1% alginate: 0.5% chitosan formulation, while the highest encapsulation efficiency (79.64%) was achieved with the 1% alginate: 1% chitosan combination. Importantly, the viability of encapsulated PGPR is maintained for up to 6 weeks of shelf life, indicating that the alginate–chitosan matrix is effective in maintaining PGPR viability. These findings indicate that the alginate–chitosan matrix is effective in maintaining the viability of encapsulated PGPR and has the potential to be developed as a bioformulation to support sustainable agricultural practices.

1. INTRODUCTION

Plant Growth-Promoting Rhizobacteria (PGPR) are microorganisms that live in the soil and play an important role in enhancing plant growth and resistance through various biological mechanisms, including phytohormone production, phosphate solubilisation, nitrogen fixation, and antagonistic activity against plant pathogens [1, 2]. Their presence in the rhizosphere significantly affects nutrient uptake efficiency and plant adaptation to environmental stress. Among horticultural crops, shallots (*Allium ascalonicum*) are known to have high potential for microbial interactions and are of agronomic importance in tropical regions such as Indonesia [3].

The use of PGPR in free-living form faces various challenges. Their viability and functional activity often decline due to exposure to environmental factors such as temperature fluctuations, humidity, UV radiation, and competition with native soil microorganisms. These conditions lead to a reduction in the effectiveness of PGPR as a plant growth-promoting (PGP) agent when applied directly to the soil. Therefore, strategies are needed to protect and maintain the viability of PGPR so that it remains stable and functional during storage and after application. Bioencapsulation offers a solution capable of overcoming these limitations.

Bioencapsulation is a technique that involves encapsulating bioactive agents or microorganisms in a protective matrix made from natural polymers. This approach improves microbial viability, stability, and controlled release, while reducing the risk of contamination during storage and transport. Encapsulation-based delivery systems have

emerged as a promising tool for the preservation and application of PGPR in agricultural systems [4].

Natural coating materials such as alginate and chitosan are widely used in microbial encapsulation due to their biocompatibility and functionality. Alginate, a biodegradable and non-toxic polymer, is often used for microbial immobilisation and has been shown to enhance microbial performance as a biocontrol agent and growth promoter [5-7]. Chitosan, a cationic polymer, exhibits antimicrobial activity and enhances plant tolerance to biotic and abiotic stress. When combined with alginate, it forms a stable gel suitable for microbial cell immobilisation [8-10].

Despite the potential of alginate–chitosan matrices, existing studies still show several shortcomings. Most research has focused on non-local or commercial PGPR strains, limiting relevance to specific agroecosystems such as the shallot rhizosphere in Indonesia. Furthermore, encapsulation studies often emphasize alginate alone, without systematically evaluating the synergistic effects of alginate–chitosan combinations. Data on PGPR viability during storage and the physicochemical characterization of encapsulation matrices (e.g., particle size, gel stability, release profile) remain scarce, and few studies have linked laboratory findings to practical applications in tropical horticulture.

To address these gaps, this study integrates the isolation of local PGPR from shallot rhizosphere soil with a systematic evaluation of their viability following encapsulation in various alginate–chitosan formulations. In addition, physicochemical properties of the encapsulation matrices are characterized to provide comprehensive data. The encapsulation formulations tested cover a range of alginate (1%) combined with different chitosan concentrations (0.5–1.5%), allowing identification of optimal conditions for maintaining PGPR viability during storage. The unique contribution of this study lies in its focus on PGPR strains indigenous to shallot rhizospheres in Indonesia, combined with a dual assessment of microbial viability and material properties. The findings are expected to support the development of stable and effective microbial bioformulations that are both scientifically robust and practically relevant for sustainable agriculture in tropical regions.

2. MATERIALS AND METHOD

2.1 Experimental materials and design

This study utilized rhizobacterial isolates obtained from the roots of shallot (*Allium ascalonicum*) plants, along with food-grade alginate, chitosan, Luria broth, nutrient agar, calcium chloride ($CaCl_2$), 0.85% physiological NaCl, distilled water, and ethanol (95% and 70%). A completely randomized design (CRD) without factorial treatment was applied, consisting of three encapsulation formulations: P1: 1% alginate: 0.5% chitosan; P2: 1% alginate: 1% chitosan; P3: 1% alginate: 1.5% chitosan. Each treatment was replicated three times.

2.2 Molecular identification of rhizobacteria

Genomic DNA was extracted from selected rhizobacterial isolates, followed by amplification of the 16S rRNA gene using the Polymerase Chain Reaction (PCR). PCR products were purified and sequenced to obtain nucleotide data. Sequence alignment and species identification were performed using the BLAST tool in the NCBI database.

2.3 Encapsulation procedure

The encapsulation procedure involved the preparation of three primary solutions: chitosan at concentrations of 0.5%, 1%, and 1.5%, each dissolved in 100 mL of 1% acetic acid until homogeneous; sodium alginate prepared by dissolving 1 g of alginate in 100 mL of distilled water heated to 60 ℃; and a 0.1 M calcium chloride ($CaCl_2$) anhydrous (110.98 g/mol) solution prepared by dissolving 11.1 g of $CaCl_2$ in 1 L of distilled water, followed by sterilization at 121 ℃ for 15 minutes. Rhizobacterial suspensions were prepared at a concentration of approximately 1×10^9 CFU/mL ($OD_{600} \approx 1$), and mixed with alginate–chitosan solutions at a ratio of 1:2 (v/v).

2.4 Coating process

The coating process used the extrusion method, employing a syringe pump with a nozzle (diameter 29.70 mm), a flow rate of Q = 1 mL/min, and a voltage of V = 0 kV. The capsules were collected in 125 mL of 0.1 M $CaCl_2$ solution and allowed to harden for 30 minutes before further processing.

2.5 Protein solubility analysis

Protein solubility was assessed using a modified method from research [3]. A 1% (w/v) protein suspension was adjusted to pH 1–13, stirred for 1 hour, and centrifuged at 6,000 rpm for 20 minutes. Soluble protein was quantified using the Bradford assay at 595 nm. Protein solubility was calculated using the following formula:

$$S\,(\%) = \frac{Protein\ concentration\ in\ supernatant}{initial\ protein\ concentration} \times 100$$

2.6 Moisture content determination

Moisture content was measured gravimetrically:

$$Moisture\,(\%) = \left(\frac{W_{microparticle} - W_{dry\ microparticle}}{W_{microparticle}}\right) \times 100$$

where, $W_{\text{microparticle}}$ represents the initial weight of the sample before oven drying, and $W_{\text{dry microparticle}}$ represents the sample weight after drying in the oven until a constant weight was reached. This measurement aimed to determine the moisture level of the microparticles, which can influence the stability and viability of rhizobacteria within the bioencapsulation formulation.

2.7 Emulsion stability and viscosity

Emulsion stability was evaluated by storing 25 mL of emulsion in a graduated cylinder at 20 ± 5 ℃ for 24 hours [11]. Viscosity was measured using a Brookfield viscometer at 30 rpm on a 300 mL sample [12], expressed in centipoise (cP).

2.8 Microcapsule morphology

Surface morphology was examined using Scanning Electron Microscopy (SEM) at 1500× magnification. Samples were dried and coated with a conductive layer to assess

particle shape, integrity, and uniformity.

2.9 Encapsulation efficiency

The efficiency of microencapsulation represents the survival rate of microorganisms during the microencapsulation process and is calculated according to the following equation:

$$\text{Encapsulation Efficiency (\%)} = \frac{N1}{N0} \times 100$$

where *N1* represents the number of viable cells (log 10 CFU mL^{-1} released from the microcapsules and *N0* represents the number of viable cells (log 10 CFU mL^{-1}) from the cell concentrate (emulsion) used for microencapsulation [13].

2.10 Viability of encapsulated rhizobacteria

Capsules were disrupted to release bacterial cells, followed by serial dilution in 0.85% NaCl. Suspensions were plated on nutrient agar and incubated at 37 ℃ for 24 hours. Viability was expressed as colony-forming units (CFU).

3. RESULT AND DISCUSSION

3.1 Molecular identification of rhizobacterial isolates from shallot roots

The molecular identification of ten rhizobacterial isolates from the rhizosphere of shallots (Table 1) showed that most isolates belonged to the genera Bacillus and Pseudomonas, with only one isolate identified as Brevibacillus. The genera Bacillus and Pseudomonas are commonly found as rhizobacteria that have the ability to act as PGPR, playing an important role in enhancing the growth and health of onion plants. Meanwhile, the single Brevibacillus isolate, which is still classified in the Bacillaceae family, also shows similar potential benefits.

Molecular identification of ten rhizobacterial isolates obtained from the rhizosphere of shallots (*Allium ascalonicum*) was performed through analysis of the 16S rRNA gene sequence. The sequence results showed a high degree of similarity with several bacterial species listed in the NCBI database, as summarised in Table 1. Isolates BB 7/1, GB 4/5, and GB 5/1 showed high sequence similarity with *Bacillus subtilis*, while GB 7/1 was identified as *Brevibacillus brevis*. Four isolates—GB 6/1, BB 7/3, BB 4/1, and GB 4/4—were closely related to *Pseudomonas aeruginosa*, while GB 6/3 and BB 6/1 matched *Bacillus amyloliquefaciens*.

The presence of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in the rhizosphere zone of shallots highlights their potential as biological agents, as both species are known to produce antimicrobial compounds and induce systemic resistance in host plants [13]. Similarly, *Pseudomonas* spp. have been extensively documented for their ability to synthesise various enzymes and secondary metabolites that promote plant growth, facilitate bioremediation, and suppress plant pathogens [14]. Although *Pseudomonas aeruginosa* is recognised as a multifunctional bacterium capable of producing siderophores, proteolytic enzymes, and pathogen-inhibiting compounds, some strains are opportunistic pathogens, requiring careful selection and safety evaluation before application in agriculture [15]. On the other hand, *Brevibacillus brevis*, although rarely reported, is known to produce gramicidin and other bioactive compounds with strong biocontrol potential [16].

Table 1. Molecular identification results of rhizobacterial isolates from the shallot rhizosphere based on 16S rRNA sequencing

No.	Isolate Code	Species	% Identify	Accession
1	BB 7/1	*Bacillus subtilis* strain F1	95.78	MT052342.1
2	GB 7/1	*Brevibacillus brevis* strain GT49	98.75	KY312739.1
3	GB 6/1	*Pseudomonas aeruginosa*	97.43	LN558598.1
4	BB 7/3	*Pseudomonas aeruginosa* Strain BBBJ	98.87	MN493755.1
5	BB 4/1	*Pseudomonas aeruginosa* strain FQ6	98.10	MF144459.1
6	GB 4/4	*Pseudomonas aeruginosa* strain AJM12	97.44	OP117243.1
7	GB 4/5	*Bacillus subtilis* strain F1	96.34	MT052342.1
8	GB 6/3	*Bacillus amyloliquefaciens*	97.59	PV017902.1
9	BB 6/1	*Bacillus amyloliquefaciens* strain WUMSB4	97.07	PV017902.1
10	GB 5/1	*Bacillus subtilis* strain ABL 1483	96.01	MW131529.1

These findings are consistent with the study [17], which successfully isolated and characterised indigenous rhizobacteria from the rhizosphere of shallots in Aceh, Indonesia, using quantitative and qualitative approaches, including phosphate solubility tests and colony morphology analysis. Their study confirmed the presence of diverse Gram-positive and Gram-negative PGPR candidates, reinforcing the potential of local microbial resources for sustainable agriculture. The molecular identification results presented here provide a critical foundation for selecting superior rhizobacterial strains for bioencapsulation and the development of environmentally friendly microbial formulations. Validation through 16S rRNA sequencing ensures taxonomic accuracy and biological safety, which are essential prerequisites for large-scale application in agricultural systems.

3.2 Effect of coating material formulation on the physical properties of rhizobacterial microcapsules

The physical properties of biologically encapsulated rhizobium bacteria are greatly influenced by the composition of the coating material used. Changes in the alginate–chitosan formulation significantly affect the emulsion stability, viscosity, and water content of the resulting microcapsules (Table 2). Among the formulations tested, treatment P3 (1% alginate: 1.5% chitosan) exhibited the highest emulsion stability (145.81 $m^2 \cdot g^{-1}$) and the highest water content (96.74%), demonstrating superior performance in maintaining structural integrity and water retention. Although increasing chitosan concentration caused an increase in emulsion viscosity, the effect was not statistically significant. However,

higher chitosan content is known to increase matrix density and mechanical strength, thereby enhancing protection for encapsulated microorganisms under environmental stress.

The increase in viscosity observed at higher chitosan concentrations is associated with the polyelectrolyte properties of chitosan and its ionic interactions with alginate. These interactions form a denser and more stable gel network, as supported by previous studies [10, 18]. Chitosan contributes to resistance to pH and temperature fluctuations, while alginate stabilises the gel structure. The denser matrix formed at higher chitosan concentrations results in stronger cross-links and smaller pore sizes, as reported in the study [19], enhancing the encapsulation barrier and reducing permeability.

Table 2. Mean values of emulsion viscosity, emulsion stability, and moisture content of bioencapsulated rhizobacteria under different alginate–chitosan coating formulations

Coating Formulation (Alginate-Chitosan, % w/v)	Emulsion Viscosity (cP)	Emulsion Stability ($m^2 \cdot g^{-1}$)	Bioencapsulation Moisture Content (%)
P1 (1: 0.5)	181.0	99.13 ab	94.46 a
P2 (1: 1)	171.8	68.02 a	94.84 a
P3 (1: 1.5)	242.6	145.81 b	96.74 b

Note: Values followed by the same letter within the same column are not significantly different according to the HSD test ($p \leq 0.05$).

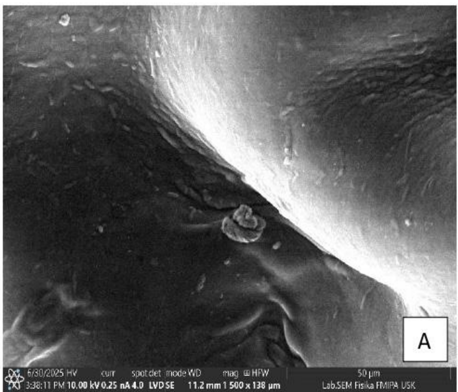
Viscosity measurements showed that formulation P3 produced the highest value (242.6 cP), followed by P1 (181.0 cP) and P2 (171.8 cP). Increased viscosity is beneficial for emulsion stability, as it inhibits droplet coalescence and phase separation [20, 21]. Conversely, the lowest emulsion stability was observed in P2 (68.02 $m^2 \cdot g^{-1}$), possibly due to inadequate polymer interaction and lower viscosity. Emulsion stability is a critical parameter in bioencapsulation, as it affects microcapsule formation efficiency and particle uniformity [22].

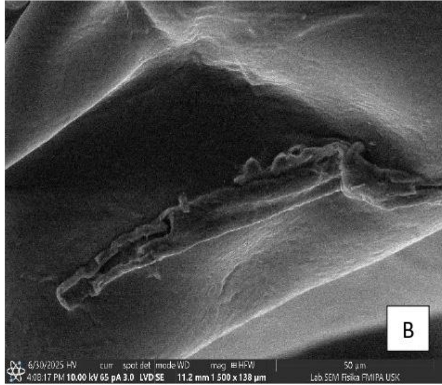
Formulation P3 (1% alginate: 1.5% chitosan) exhibited the highest viscosity and emulsion stability, as well as denser and more uniform microcapsule morphology. However, the observation that P3 exhibited the highest emulsion stability but not the highest encapsulation efficiency can be explained by the effect of chitosan concentration on matrix properties. At 1.5% chitosan, the matrix becomes denser and more rigid, enhancing stability but limiting nutrient diffusion and uniform cell entrapment, which reduces encapsulation efficiency. In contrast, formulation P2 (1% alginate: 1% chitosan) achieved the highest encapsulation efficiency and lowest protein solubility, representing an optimal balance where the matrix is compact enough to retain bacterial cells yet sufficiently permeable to support nutrient exchange. Viability assessment during a six-week storage period showed that P3 provided superior long-term protection, maintaining higher cell viability levels compared to P1 and P2. These findings suggest that although P2 offers optimal chemical performance, P3 excels in physical integrity and microbial viability. Therefore, both formulations possess strategic advantages and can be selected based on specific application objectives, P2 to maximise encapsulation efficiency and P3 to enhance microbial stability, supporting their potential use as biofertilizer carriers or bioprotectants in sustainable agricultural systems.

The enhanced stability in P3 is also associated with chitosan's ability to form a positively charged protective layer around emulsion droplets, thereby reducing degradation. Electrostatic bonds between the carboxylate ($-COO^-$) groups of alginate and the protonated amino ($-NH_3^+$) groups of chitosan at $pH < 6.5$ produce a robust polyelectrolyte complex [23]. This interaction reduces surface tension and strengthens the matrix, improving encapsulation performance [24]. Furthermore, high molecular weight chitosan with a high degree of deacetylation, when combined with glucuronic acid-rich alginate, improves the stability and controlled release of active compounds [25], although excessive molecular weight can inhibit solubility and mixing [26].

Moisture content analysis showed values exceeding 96%, which does not indicate free water but rather water entrapped within the hydrogel network formed by alginate–chitosan interactions. The hydrogel structure, stabilized by electrostatic cross-linking between alginate and chitosan, ensures that the microcapsules maintain mechanical strength and integrity despite high moisture levels. This trapped water plays a beneficial role in maintaining microbial viability and controlled release, while SEM images confirm the presence of a dense and compact outer surface, consistent with the structural stability of the encapsulation system. The high-water content in P3 was likely due to the gel-forming and film-forming properties of chitosan, which retained water in the matrix. High viscosity limits water evaporation during drying, contributing to moisture retention [27]. Although moisture is essential for maintaining bacterial viability, excessive water content can accelerate degradation and microbial contamination if not properly managed [21]. Nevertheless, the P3 formulation demonstrated optimal performance in terms of viscosity and emulsion stability, indicating that increasing chitosan concentration enhances microcapsule structure and encapsulation efficiency—findings consistent with previous studies on microbial encapsulation for agricultural and pharmaceutical applications [28]. Importantly, the high moisture content observed does not indicate free water but rather water entrapped within the hydrogel network of alginate–chitosan. This trapped water contributes to bacterial viability while the dense polyelectrolyte matrix, formed by electrostatic interactions between alginate and chitosan, ensures structural integrity. This explains why SEM images show compact and rigid surfaces despite the high moisture values, confirming that the microcapsules remain stable and protective during storage.

Morphological analysis using SEM at 1500× magnification confirmed that increasing chitosan concentration resulted not only in a thicker and stiffer surface layer in the P3 formulation, but also in smaller pore sizes, smoother surface morphology, and more uniform particle size distribution compared to P1 and P2. These structural features indicate that higher chitosan content enhances matrix compactness and mechanical strength, thereby improving bacterial protection during storage. (Figure 1). This structural improvement is due to electrostatic interactions between the positively charged amino groups on chitosan and the negatively charged carboxyl groups on alginate. The resulting polyelectrolyte complex reduces pore size and strengthens the matrix against mechanical stress, thereby improving bacterial protection during storage. The dense matrix also contributes to higher bacterial viability by minimising exposure to adverse environmental conditions. These findings highlight the potential of alginate–chitosan encapsulation systems to improve microbial resistance and functional performance in bioformulation products [29].





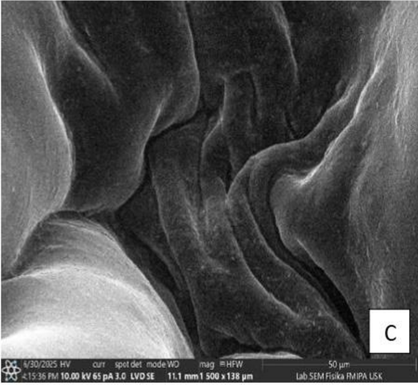


Figure 1. Scanning electron microscope (SEM) images at 1500× magnification showing (A) P1 (alginate 1%: chitosan 0.5%), (B) P2 (alginate 1%: chitosan 1%), and (C) P3 (alginate 1%: chitosan 1.5%)

3.3 Effect of coating material formulation on the chemical properties of rhizobacterial bioencapsulation

The chemical properties of rhizobacterial bioencapsulation were evaluated based on two main parameters: protein solubility and encapsulation efficiency. As shown in Table 3, variations in the alginate–chitosan layer formulation significantly affected both parameters. Formulation P1 (1% alginate: 0.5% chitosan) showed the highest protein solubility (56.71%) but the lowest encapsulation efficiency (17.19%). Conversely, formulation P2 (1% alginate: 1% chitosan) showed the lowest protein solubility (19.81%) and the highest encapsulation efficiency (79.64%).

Table 3. Mean values of protein solubility and bioencapsulation efficiency across different alginate–chitosan coating material formulations

Coating Material Formulation	Protein Solubility (%)	Bioencapsulation Efficiency (%)
Alginate Chitosan 1%:0.5% (P1)	56.71 c	17.19 a
Alginate Chitosan 1%:1% (P2)	19.81 a	79.64 b
Alginate Chitosan 1%:1.5% (P3)	24.76 b	66.83 b

Note: Values followed by the same letter within the same column are not significantly different according to the BNJ test ($p \leq 0.05$).

The increase in protein solubility observed in P1 indicates that the resulting microcapsule matrix is less dense, allowing greater protein diffusion into the surrounding medium. This phenomenon may be due to the lower chitosan concentration, which is insufficient to form strong electrostatic interactions with alginate, resulting in a more porous and degradable polymer network [30]. Conversely, the addition of higher chitosan concentrations in formulations P2 and P3 promotes the formation of polyelectrolyte complexes between the amino groups of chitosan and the carboxyl groups of alginate, thereby increasing matrix integrity, reducing protein solubility, and improving encapsulation efficiency [28, 31].

Encapsulation efficiency varied significantly among the three formulations. P1 (17.19%) showed the lowest efficiency due to insufficient chitosan concentration, which failed to establish strong electrostatic interactions with alginate, resulting in a porous and less compact matrix. In contrast, P2 (79.64%) achieved the highest efficiency, as the 1% chitosan concentration provided an optimal balance between matrix compactness and permeability, allowing effective entrapment of bacterial cells while maintaining nutrient diffusion. Meanwhile, P3 (66.83%) exhibited lower efficiency than P2, likely because the higher chitosan concentration (1.5%) increased viscosity and rigidity, leading to reduced nutrient diffusion and uneven bacterial distribution.

The slightly lower efficiency in P3 (66.83%) may be due to increased viscosity and rigidity, which can inhibit nutrient diffusion and microbial release [21]. In general, higher chitosan concentrations contribute to improved encapsulation performance by increasing matrix density and protective capacity. This is consistent with previous findings showing that encapsulation systems with higher chitosan content support higher microbial stability, bioactivity, and bioavailability [32]. For example, it has been reported that encapsulation of Lactobacillus spp. using an alginate–chitosan system with 2% chitosan increased bacterial viability and reduced release under acidic conditions compared to formulations without chitosan [2]. These results highlight the importance of optimising the composition of coating materials to achieve the desired chemical properties in microcapsules. Balanced formulations, such as P2, offer an effective compromise between matrix strength and permeability, making them suitable for microbial bioencapsulation applications in sustainable agriculture.

3.4 Bioencapsulation viability

The viability of encapsulated rhizobacteria was evaluated to determine the effectiveness of different alginate–chitosan formulations in maintaining microbial survival during storage. The results showed that formulation P3 (1% alginate: 1.5% chitosan) consistently exhibited higher viability values compared to P1 and P2 in several rhizobacteria isolates. This indicates that an increase in chitosan concentration enhances the protective capacity of the bioencapsulation matrix.

Chitosan is known for its ability to form biofilms and its function as a physical barrier against oxygen, moisture, and temperature fluctuations, thereby protecting microbial cells from environmental stress during storage [21, 28]. Electrostatic interactions between the amino groups of chitosan and the carboxylate groups of alginate result in the formation of a solid polyelectrolyte matrix with small pore sizes, which effectively slows down the diffusion of external molecules that could threaten cell viability [30].

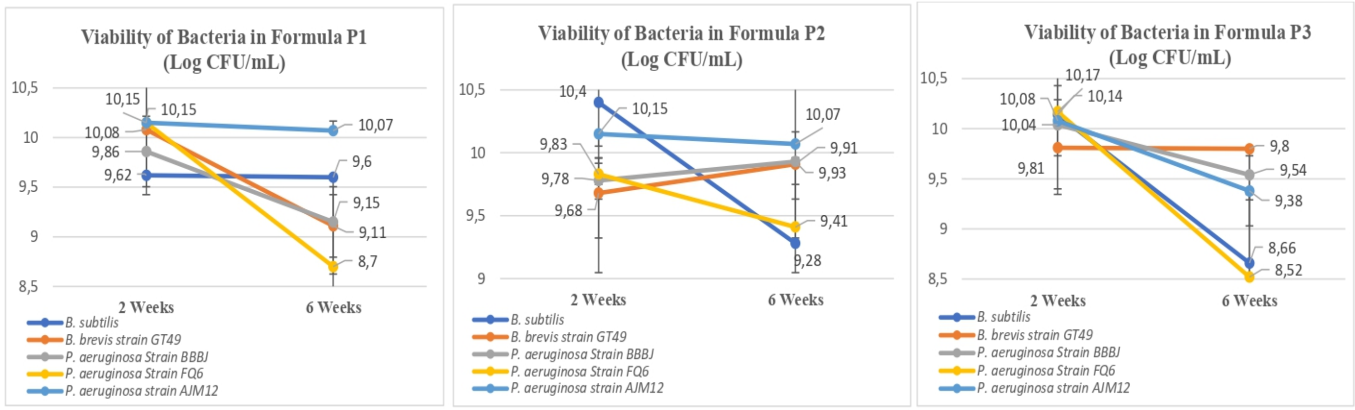


Figure 2. Viability of encapsulated rhizobacteria over two storage periods: 2 weeks and 6 weeks
Note: Data are presented as mean SD (n = 3). Error bars represent standard deviation (SD).

Figure 2 illustrates the viability of encapsulated rhizobacteria during two storage periods—2 weeks and 6 weeks—using three coating formulations. Although all treatments maintained relatively high viability in the second week, a slower decline was observed in P3, indicating better structural integrity and superior microbial protection. In contrast, formulations P1 and P2 showed a more rapid decline in the number of viable cells, possibly due to a less dense matrix structure that allowed greater oxygen and water penetration.

These findings are consistent with a previous study [33], which shows that increasing the concentration of chitosan in the encapsulation system can extend the shelf life of microbes by strengthening capsule integrity. Overall, formulations with higher chitosan content—especially P3—showed the most promising results in maintaining rhizobacterial viability during extended storage. This highlights its potential application as a carrier for biofertilisers and bioprotectants in sustainable agricultural systems, where microbial stability and function are critical for field performance.

4. CONCLUSION

Molecular identification of ten rhizobacterial isolates from the shallot (*Allium ascalonicum*) rhizosphere confirmed the presence of key PGPR species, including *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Pseudomonas aeruginosa*, and *Brevibacillus brevis*, all of which are known for their roles in plant growth promotion and biological control. Evaluation of three alginate–chitosan coating formulations (1% alginate combined with 0.5%, 1.0%, and 1.5% chitosan) demonstrated that increasing chitosan concentration significantly influenced the physical and chemical properties of the bioencapsulation system. Formulation P3 (1% alginate: 1.5% chitosan) exhibited the highest emulsion viscosity and stability, as well as denser and more uniform microcapsule morphology. However, the observation that P3 exhibited the highest emulsion stability but not the highest encapsulation efficiency can be explained by the effect of chitosan concentration on matrix properties. At 1.5% chitosan, the matrix becomes denser and more rigid, enhancing stability but limiting nutrient diffusion and uniform cell entrapment, which reduces encapsulation efficiency. In contrast, formulation P2 (1% alginate: 1% chitosan) achieved the highest encapsulation efficiency and lowest protein solubility, representing an optimal balance where the matrix is compact enough to retain bacterial cells yet sufficiently permeable to support nutrient exchange. Viability assessments over a six-week storage period revealed that P3 provided superior long-term protection, maintaining higher levels of viable cells compared to P1 and P2. These findings suggest that P2 is particularly suitable for applications requiring high initial loading and rapid colonization (e.g., seed coating or short-term biofertilizer use), while P3 is more appropriate for long-term storage, gradual release, and field stability (e.g., soil amendment or bioprotectant formulations). Therefore, both formulations present strategic advantages depending on application goals, P2 for maximizing encapsulation efficiency and P3 for enhancing microbial stability and supporting their potential use as biofertilizer carriers or bioprotectants in sustainable agricultural systems.

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