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Alginate - Biochar PGPR Beads: Preparation, Characterization, and Effect on *Zea mays* Growth

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Abstract An experiment was conducted to examine the efficiency of plant growth promoting rhizobacteria (PGPR) encapsulated in sodium alginate (SA) with either oak biochar (OK-BC) or sawdust biochar (SD-BC) at concentrations of 0. 2.5 and 5% on the growth of *Zea mays*. The PGPR strain SK1, isolated from the rhizosphere of *Rhizophora mucronata*, exhibited the potential to produce indole-3-acetic acid, siderophores, and solubilize phosphate. Based on 16S rDNA sequencing, strain SK1 was determined to be a member of the genus *Bacillus*. Characterization of the encapsulated beads revealed that the SA+SD-BC5%+SK1 formulation showed superior encapsulation efficiency, moisture retention, water absorption potential, and gradual release of SK1 cells compared to other treatments. Furthermore, the survivability of strain SK1 was remarkably increased in the SA+SD-BC5%+SK1 beads, maintaining viability after 80 days of storage even at room temperature. In pot experiments, *Z. mays* treated with SA+SD-BC5%+SK1 beads showed the greatest improvement in shoot length, root length, fresh weight, and dry weight relative to other treatments. Additionally, SA+SD-BC5%+SK1 beads improved the colonization of SK1 in the rhizosphere of *Z. mays*, which likely contributed to enhanced plant growth. In conclusion, the SA+SD-BC5%+SK1 formulation provided better survival and activity of PGPR compared to SA+SK1 beads alone, making it a promising and more effective carrier system for delivering beneficial microbes to the plant rhizosphere and thereby promoting plant growth.

Keywords: *PGPR, Sawdust biochar, Encapsulation, Zea mays, Indole-3-acetic acid, Encapsulation efficiency*

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1. Introduction

Improving soil health and crop productivity has become a global priority, particularly in the context of climate change and environmental degradation. These challenges intensify the risk of food insecurity, making it increasingly difficult to provide adequate food in both quantity and quality for a rapidly growing population [1]. To address this issue, both chemical and biological soil management strategies have been explored. Among biological approaches, the bioaugmentation of plant growth-promoting rhizobacteria (PGPR) has proven especially effective. PGPR can stimulate plant growth directly by solubilizing nutrients (P, K, Zn) and producing growth-regulating compounds including indole-3-acetic acid (IAA), or indirectly by improving plant stress tolerance [2]. However, a major limitation of PGPR application is the reduction or loss of its activity under fluctuating soil conditions [3]. To overcome this challenge, immobilization technology involving encapsulating microorganisms within polymeric matrices, such as sodium alginate (SA) combined with suitable additives has gained attention in recent years [4]. Immobilization protects beneficial bacteria from environmental stress, enhances mechanical stability, and allows for higher cell densities, thereby improving their persistence and effectiveness in agricultural soils [5].

Biochar (BC), is a carbonaceous material generated through the pyrolysis of biomass such as crop residues, wood, or animal manure under limited oxygen conditions [6]. It is increasingly recognized as a sustainable soil amendment, with its effectiveness influenced by factors such as feedstock type, pyrolysis parameters, and application rate. Due to its porous structure and mineral composition, BC improves soil physicochemical characteristics and nutrient cycling. It can raise soil pH, increase organic carbon content, enhance nutrient availability, and boost cation exchange capacity, thereby supporting nutrient retention and reducing leaching losses [7]. These changes contribute to improved soil structure, greater nutrient use efficiency and enhanced plant growth. Beyond its direct effects on soil properties, BC has gained recognition for its synergetic interactions with soil microbiota [8]. By providing a conducive microenvironment, BC facilitates microbial colonization and activity, which play a vital role in nutrient cycling and plant growth. Recent studies have reported that BC amendments can increase the survival and activity of

PGPR by promoting microbial establishment and improving nutrient availability [9]. In turn, PGPR enhance plant growth through multiple mechanisms, including nutrient solubilisation, phytohormone production, pathogen suppression, regulation of ion availability, and improvement of soil structure [2]. The multifaceted benefits of BC on soil physico-chemical properties and microbial proliferation underscore its potential as a valuable additive in microbial encapsulation technologies, thereby reinforcing its importance in sustainable agriculture [10]. These advantages prompted us to investigate the feasibility of incorporating BC into SA-based encapsulation systems with the objective of enhancing bead performance.

Thus, the aim of this study was to determine the effect of incorporating BC into SA and PGPR-encapsulated beads on their properties, including encapsulation efficiency, PGPR survival during storage, moisture content, water absorption and cell release profile. Additionally, the study assessed whether these BC-enriched beads improve the PGPR colonization in the rhizosphere and thus the growth of *Zea mays* (maize).

2. Methodology

2.1. Isolation and Characterization of PGPR

The bacterial strains were isolated from the rhizosphere soil of *Rhizophora mucronata* in the Pitchavaram mangrove forest, Tamil Nadu, India following previously established method [11]. To select efficient PGPR, the isolates were screened using the roll towel assay. Briefly, seeds of *Z. mays* were subjected to surface-sterilization by immersing in 70% ethanol for 1 min, followed by the treatment with 5% sodium hypochlorite for 5 min, and washed thoroughly with sterile distilled water. The bacterial strains were cultured in Luria–Bertani broth at 28 ± 2°C for 18 h. Cells were harvested by centrifugation at 5000 rpm for 10 min and adjusted to a concentration of ~10^8 CFU/mL. For bacterial inoculation, seeds previously sterilized were immersed in the bacterial suspension for 2 h, whereas the control group was soaked in sterile distilled water. Both bacterial treated and untreated seeds were placed in moist blotters, and after 21 days of incubation, seedling growth parameters including root length (RL) and shoot length (SL) were recorded. The vigor index was calculated using the following formula:

Vigor index = (Mean root length + Mean shoot length) × Germination%

Additionally, the plant growth promoting (PGP) properties of the selected bacterial strains were evaluated, including production of IAA [12], siderophore [13], ammonia [14], solubilization of P [15], and hydrogen cyanide (HCN) activity [16], using standard protocols. The molecular characterization of the isolates was performed through 16S rRNA gene sequence analysis [17].

2.2. Preparation of Biochar

Biochar was generated from agricultural residues, specifically oak and sawdust, through slow pyrolysis [18]. The biomass was subjected to pyrolysis under oxygen-restricted conditions for 2 h at temperatures of 550 °C. The resulting material was finely ground and passed through a 0.5 mm sieve to obtain uniform BC powder.

2.3. Preparation of PGPR and BC Encapsulated Beads

To evaluate colonization efficiency in pot experiments, mutants of the SK1 isolate were generated by culturing in LB broth supplemented with ampicillin (300 mg/L) [17]. For encapsulation, the ampicillin-resistant SK1 mutants were grown in LB broth for 18 h at 28 ± 2°C. In parallel, SA (2%) was combined with oak BC (OK-BC) or sawdust BC (SD-BC) separately at concentrations of 0, 2.5 and 5%. Each mixture was stirred for 1 h and sterilized by autoclaving at 121 °C. The bacterial suspension was standardized to an optical density of 1.0 at 600 nm (OD_{600}) and aseptically incorporated into the SA–BC mixture. Using a syringe, the prepared suspension was carefully dispensed into a 2% $CaCl_2$ solution to form beads. These beads were then rinsed with sterile water and dried for 2 h.

2.4. Characterization of BC and PGPR Encapsulated Beads

The encapsulation efficiency (EE) of the beads was determined by comparing the CFU initially added to the suspension (No) with the CFU retained within the beads (Nu). EE was expressed as Nu/No × 100 [19]. The moisture level of freshly prepared beads was determined by recording their weight before and after drying for 3 h in a hot-air oven at 105 °C as detailed by [20]. The swelling behaviour was assessed by immersing 5 g of dried beads in sterile deionized water for 24 h. After blotting, excess surface moisture removed with tissue paper, the beads were weighed, and the swelling rate was calculated as (Wf - Wi)/Wi x 100, where Wi is the initial dry weight and Wf is the final weight. Similarly, the shelf life of PGPR encapsulated in beads stored at 4 °C and at room temperature (27 ± 2 °C) was determined at set intervals (0, 4, 8, 20, 40, 60, 80 days) over 80 days using plate count analysis as detailed by [21]. Cell release profile of the beads was assessed following the protocol described by [22]. Briefly, 0.75 g of beads were suspended in 37.5 mL of saline solution and incubated in a shaking incubator set to 150 rpm at 30 °C. Colony-forming units (CFUs) were enumerated at specific time intervals (0 min, 30 min, 4 h, 12 h, 24 h, 48 h, and 96 h). Aliquots collected at each interval were analyzed using the standard plate count method to determine CFU values.

2.5. Effect of BC and PGPR Encapsulated Beads on the Growth of *Z. mays*

Soil was sourced from the experimental field site at Bharathiar University, Coimbatore, air-dried, and subsequently sterilized in an autoclave at 121 °C for 2 h on three consecutive days. *Z. mays* seeds were surface-disinfected as detailed in the previous section, and five sterile seeds were sown per pot. Each seed was treated either with 1 g of encapsulated beads containing approximately 6.6×10^5 CFU or with 1 mL of PGPR suspension standardized to $OD_{600} = 1.0$. The treated and untreated (control) seeds were allowed to grow in a glasshouse at 25 °C with a photoperiod of 16 h light and 8 h dark. After 40 days, plants were harvested, roots were rinsed with distilled water, and growth parameters including SL, RL, fresh weight (FW), and dry weight (DW) were recorded. DW was measured following oven-drying the samples at 80 °C for 48 h. Colonization of SK1 in the rhizosphere of *Z. mays* was assessed using serial dilution plating. 1 g of rhizosphere soil was suspended in 50 mL sterile distilled water, diluted, and cultured on LB agar supplemented with ampicillin (300 mg L^{-1}). Plates were incubated at 37 ± 2 °C for 5–6 days, and CFU were counted [17].

2.6. Statistical Analysis

All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation. Statistical analysis was carried out using analysis of variance (ANOVA) in SPSS software (version 25.0). Differences among treatment means were evaluated using Tukey's honestly significant difference (HSD) post hoc test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Isolation and Characterization of PGPR

Nineteen distinct bacterial isolates were initially obtained from the rhizosphere soil of *R. mucronata*. To identify efficient PGPR, all isolates were evaluated for their potential to improve the growth of *Z. mays* using the roll towel method. Of the 19 isolates tested, 14 strains remarkably increased SL, RL, and vigor index relative to un-inoculated control, highlighting their potential to promote plant growth (Figure 1). Among these, strain SK1 exhibited the strongest growth-promoting activity, producing the greatest increases in SL, RL, and vigor index relative to the control. Based on its superior performance, SK1 was selected for further investigation.

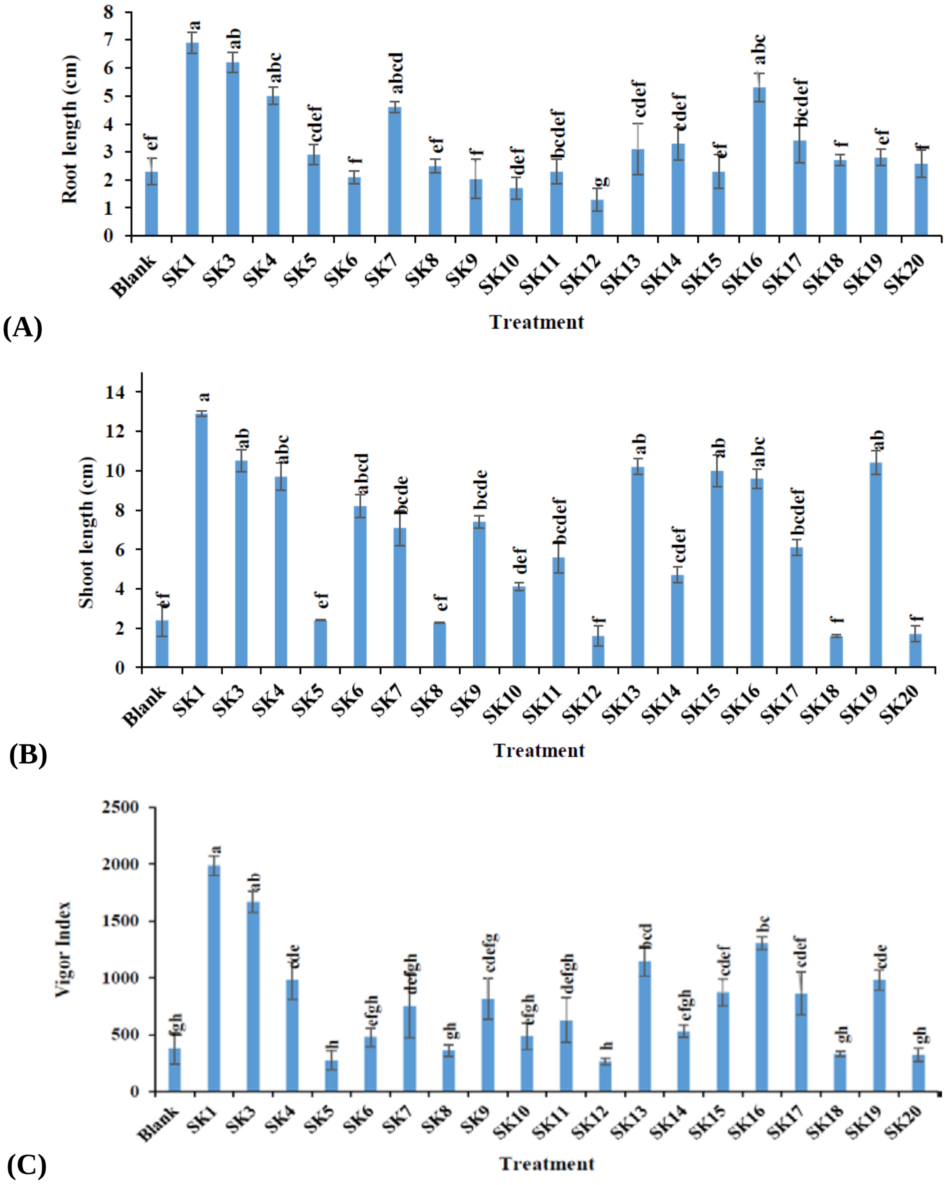


Figure 1. Effect of PGPR on the root length (A), shoot length (B) and vigor index (C) of *Z. mays*. The data represents the mean ± standard deviation of three samples. The different lower case letters represented significance difference ($p<0.05$)

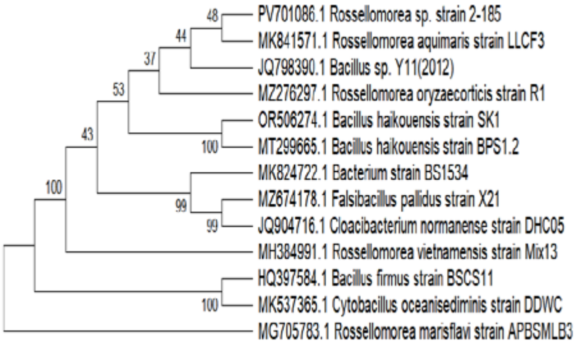


Figure 2. Phylogenetic tree showing the relationship of partial 16S rRNA gene sequences of PGPR strains SK1 with other related sequences obtained from NCBI database

The ability of PGPR to stimulate plant growth is often linked to the synthesis of key PGP metabolites including IAA, siderophores, and P-solubilizing compounds. In general, the IAA plays a crucial part in cell elongation, cell division, and root development, thereby enhancing water and nutrient uptake [23]. Siderophores improve iron availability by forming stable iron–chelate complexes, while P-solubilizing bacteria increase P bioavailability by altering insoluble P into plant-usable forms by the secretion of organic and inorganic acids [24,25]. These mechanisms collectively support root development, plant growth and productivity. In this study, to determine the specific PGP traits of SK1, several biochemical analyses were performed. Interestingly, strain SK1 was found to produce IAA (25.09 ± 1.34 µg mL^{-1}), siderophores (14%), ammonia, and solubilize P (1.88 µg mL^{-1}). These PGP traits confirmed its potential as a promising PGPR candidate for improving plant growth. Our findings are consistent with previous research indicating that the synthesis of PGP metabolites including IAA, siderophores, and P-solubilizing compounds by various PGPR strains contributes to increased plant growth and productivity [2]. Strain SK1 was further identified through a combination of morphological, biochemical (data not shown), and molecular analyses. For molecular identification, a partial sequence of the 16S rRNA gene (1053 bp) was obtained and subjected to BLAST analysis. Phylogenetic tree construction (Figure 2) confirmed that SK1 is affiliated with the genus *Bacillus*. The integration of molecular evidence with morphological and biochemical traits provided a robust basis for its taxonomic classification. The sequence was deposited in GenBank with the assigned accession number OR506274.

3.2. Characterization of BC and PGPR Encapsulated Beads

The SA beads prepared with or without BC and SK1, were further characterized for various parameters including EE, moisture content, swelling behaviour, shelf life of SK1, and cell release profile.

Encapsulation efficiency (EE), an important indicator of bacterial retention within the matrix, varied depending on the composition of the encapsulating material [26]. In this study, beads without BC (SA+SK1) exhibited a lower EE of 79%, whereas BC incorporation remarkably increased EE of the beads (Table 1). Particularly higher concentrations of BC showed greater EE, with SA+OK-BC5%+SK1 and SA+SD-BC5%+SK1 achieving the highest EE values of 86% and 90%, respectively. Moderate increases were noted in SA+SD-BC2.5%+SK1 (82%) and SA+OK-BC2.5%+SK1 (80%). This can be attributed to the reinforcement of the alginate gel network when additives are added, as reported for talc, trehalose [21]. BC, due to its high porosity and large surface area, offers additional binding sites and micro-pores that support microbial entrapment [26]. Overall, increasing BC level to 5%, particularly SD-BC, evidently increased SK1 retention during encapsulation.

Table 1. Effect of various treatment on encapsulation efficiency of beads

Treatment	%
SA + SK1	79
SA + OK-BC 2.5% + SK1	80
SA + SD-BC 2.5% + SK1	82
SA + OK-BC 5% + SK1	86
SA + SD-BC 5% + SK1	90

SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA+SD-BC 5%- sodium alginate + sawdust BC 5%, SK1-*Bacillus haikouensis.*

The optimum moisture content of beads is vital as it greatly alters gel stability, bacterial shelf life and viability. In our study, BC incorporation increased moisture retention in a dose-dependent manner (5% > 2.5%), with SD-BC outperforming OK-BC when combined with SK1 (Figure 3). In general, BC improves water retention through its micro porosity, that increase the water-holding capacity of composite matrices [27]. SD-BC, in particular, contains large surface area and porosity that promotes water retention [28]. Interestingly, SK1 inoculation further improved moisture levels of the beads, likely attributed to microbial mechanisms including biofilm formation, and metabolites, all of which increase water-holding capacity [29]. It is well known that BC also offers a microhabitat, supporting bacterial survival and metabolic activity, which contributes to moisture retention through matrix–microbe interactions [30]. Overall, SA and BC systems demonstrate strong potential as microbial carriers with improved hydration properties, offering clear benefits for agricultural applications.

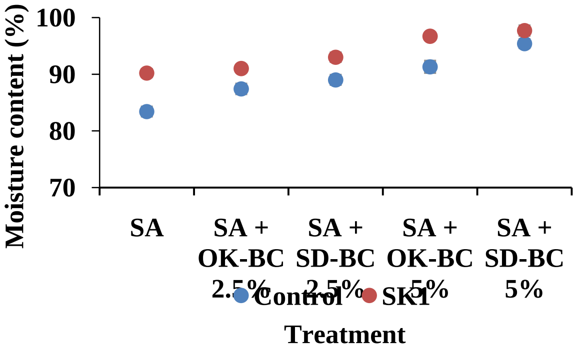


Figure 3. Effect of various treatments on moisture content of beads. Bars represent the standard deviation of three samples. SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% - sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA + SD-BC 5%- sodium alginate + sawdust BC 5%, SK1-*Bacillus haikouensis*

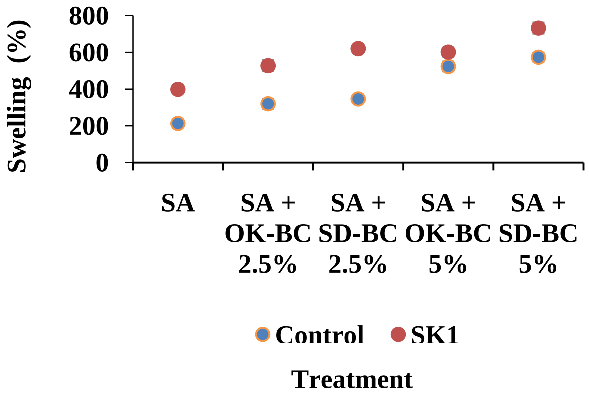


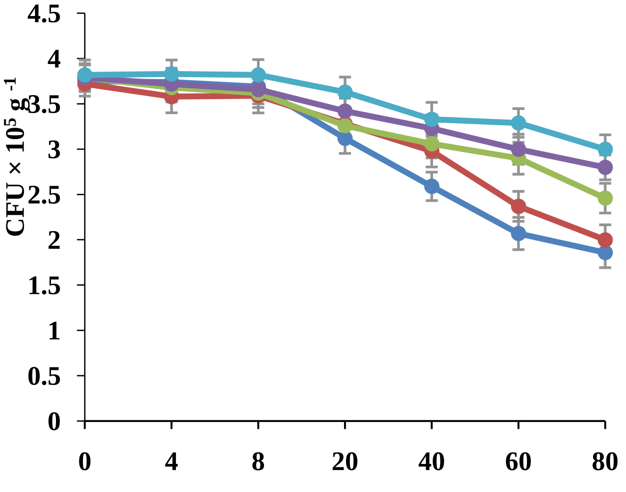
Figure 4. Effect of various treatment on swelling ratio of beads. Bars represents the standard deviation of three samples. SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% - sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA + SD-BC 5%- sodium alginate + sawdust BC 5%, SK1-*Bacillus haikouensis*

The swelling behaviour was further evaluated because it plays a vital role in water retention and the growth of encapsulated microbes. In this study, the swelling ratio of the beads increased with the incorporation of both BC and SK1 (Figure 4). Among the treatments, 5% BC incorporation resulted in a higher swelling ratio compared to 2.5%. Specifically, SA+SD-BC5% exhibited the highest swelling values, followed by SA+OK-BC5%, regardless of SK1 inoculation. This enhanced swelling can be attributed to the high-water absorption capacity of BC within the hydrogel matrix [31]. Additionally, SK1-inoculated treatments showed a further increase in swelling, which may be linked to microbial metabolic activity that modifies the internal structure of the beads and facilitates water diffusion [29,32]. The porous structure, large surface area, and functional groups of BC also contribute to the expansion of the hydrogel matrix and improved water absorption [33]. Overall, these findings indicate that BC-amended beads possess superior swelling capacity, which enhanced water retention and supports microbial growth.

The results of the shelf-life analysis showed that, irrespective of BC incorporation, beads stored at 4 °C exhibited higher survival relative to those stored at room temperature (Figure 5). The enhanced viability at 4 °C may be due to the reduced metabolic activity and slower nutrient depletion, which together diminish stress on SK1. Similar results have been documented by previous works [34], highlighting that low-temperature storage extends bacterial viability in alginate beads by reducing desiccation stress and oxidative injury. Interestingly, across all treatment, maximum viability was maintained up to 20 days of storage, followed by a gradual decline, particularly at room temperature storage. Although viability decreased progressively at room temperature after extended incubation, the incorporation of BC in beads remarkably improved the persistence of SK1 cells. For instance, in the SA+SK1 treatment, the viable count of SK1 was initially 3.75×10^5 CFU g^{-1} but decreased to 1.56×10^5 CFU g^{-1} by the 80th day storage at room temperature. In contrast, BC-incorporated beads showed superior survival. Notably, SD-BC5% incorporation maintained the highest viable count after 80 days of storage at room temperature, outperforming the OK-BC5% treatment. These results indicate that SD-BC provided conducive environment than OK-BC, likely attributed to differences in physico-chemical properties including porosity and functional group composition [35]. The enhanced survival in BC-amended beads can be due to the protective role of encapsulation against desiccation, combined with the intrinsic properties of BC. With its high porosity, large surface area, and abundance of functional groups, BC creates a conducive habitat that supports bacterial persistence and metabolic activity during storage [26]. These findings highlight BC's potential as a stabilizing agent in microbial formulations, ensuring extended shelf life and functional viability.

We further assessed the effect of incorporating SD-BC or OK-BC into SA beads on the release profile of SK1 over a 96 h incubation period (Figure 6). All formulations exhibited a gradual release of cells over time, demonstrating sustained-release behaviour. However, the rate and extent of release varied with BC type and concentration. For instance, SA+SK1 exhibited a steady but comparatively higher SK1 release profile, suggesting that while SA beads provide protection and controlled diffusion, their dense polymer cross-linking and limited porosity restrict rapid cell liberation [36]. Interestingly, the incorporation of BC remarkably reduced cell release compared to SA alone. However, among the treatments, SA+SD-BC5%+SK1 exhibited the lowest release, followed by SA+OK-BC5% + SK1, SA + SD-BC2.5% + SK1, and SA+OK-BC2.5%+SK1. The reduced release of SK1 in BC-incorporated beads may be attributed to the interaction between BC and the alginate matrix, which likely changed the structural uniformity of the polymer network [37]. Between the two BC, SD-BC exhibited slightly lower release rates than OK-BC at comparable concentrations. This difference may be associated with variations of BC characteristics including surface area, pore architecture, and particle morphology [38]. SD-BC generally exhibits higher water-holding capacity, which may support microbial retention and reduce diffusion compared to OK-BC [39,35]. Overall, our findings suggest that BC-incorporated alginate matrices are promising microbial delivery systems.

(A)



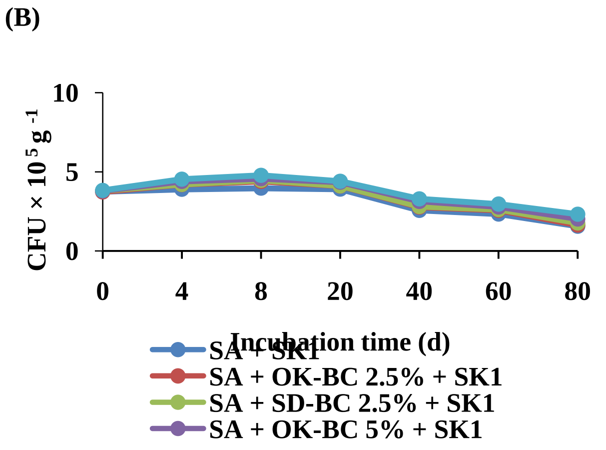


Figure 5. Effect of various treatments on the survival of SK1 encapsulated in beads stored at 4˚C (A) and room temperature (B). SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% - sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA + SD-BC 5%- sodium alginate + sawdust BC 5%, SK1-*Bacillus haikouensis*

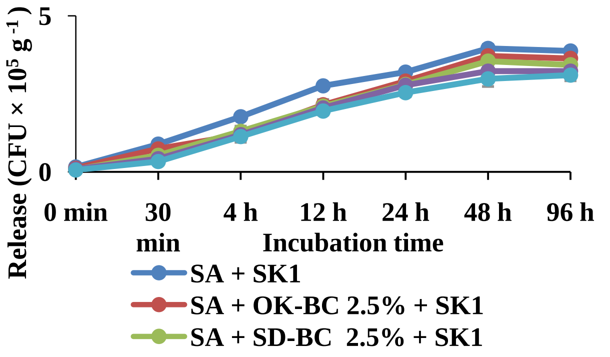


Figure 6. Effect of various treatments on cell release profile of beads. Bars represents the standard deviation of three samples. SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% - sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA + SD-BC 5%- sodium alginate + sawdust BC 5%, SK1-*Bacillus haikouensis*

3.3. Effect of Encapsulated Beads on *Z. mays* Growth and SK1 Colonization

Encapsulation of SK1 in SA-BC beads remarkably improved the *Z. mays* growth relative to both the untreated control and SK1 inoculation alone (Table 2). Although SA+SK1 beads increased plant growth, with SL and RL increasing by 43% and 27%, respectively, compared to the control, the incorporation of BC into the beads exhibited the greatest PGP potential. In particular, the incorporation of 5% BC resulted in stronger growth enhancement than 2.5% BC in both OK-BC and SD-BC treatments. For instance, SA+OK-BC5%+SK1 and SA+SD-BC5%+SK1 increased SL by 102% and 111%, and RL by 46% and 63%, respectively, compared to the untreated control. FW also increased by 86% in SA+OK-BC5%+SK1 and 90% in SA+SD-BC5%+SK1, while DW increase by 134% and 165%, respectively. These findings are consistent with the patterns observed in encapsulation efficiency (Table 1), moisture absorption (Figure 3) and cell release profile (Figure 6) of the beads. Beads containing encapsulated BC retained a larger population of SK1 cells, released them gradually over an extended period, and maintained higher moisture content, collectively resulting in a significant enhancement of plant growth. In addition, BC improves soil nutrient availability (N, P and K) and cation exchange capacity, while PGPR improve nutrient solubilisation and plant uptake, collectively improving plant growth [40,41]. Similarly, they produce PGP metabolites including IAA, siderophores and solubilize P, thereby promoting root and shoot development [2]. Supporting this, [9] have also recorded that pine wood biochar combined with PGPR *Enterobacter cloacae* UW5 improved *Cucumis sativus* growth by improving the physico-chemical and biological properties of soil.

The enhanced plant growth observed with bead treatments suggests that BC-mediated encapsulation improved the survival and activity of SK1, thereby improving plant growth. To verify this, SK1 survival was assessed in the rhizosphere soil of *Z. mays* (Figure 7). SA+SK1 beads significantly increased the viable cell count to 3.09 × 10^{-5} CFU g^{-1}, confirming the protective role of SA in maintaining microbial viability. However, the incorporation of BC into beads further improved SK1 colonization in the rhizosphere. Specifically, the treatments SA+OK-BC5%+SK1 and SA+SD-BC5%+SK1 recorded the highest viable counts of 4.39 × 10^{-5} CFU g^{-1}, and 4.72 × 10^{-5} CFU g^{-1} respectively, which were significantly greater than all other treatments. The improved survival with BC-incorporated beads is explained by the large surface area, and functional groups of BC, which provide conducive habitats and enhance moisture retention, thereby supporting the persistence of SK1 [42,43]. In addition, BC may improve nutrient retention within the bead matrix, further sustaining microbial viability. Overall, these findings demonstrate that SA encapsulation combined with BC, particularly at a 5% concentration, substantially improved SK1 survival. This highlights BC-enriched SA beads as a promising carrier system for delivering PGPR in sustainable agriculture.

Table 2. Effect of encapsulated SK1 on the growth of *Z. mays*

Treatment	Shoot Length (cm)	Root Length (cm)	Fresh weight (g plant $^{-1}$)	Dry weight (g plant $^{-1}$)
Control	8.86±0.899^{c}	7.86±0.449^{c}	1.44±0.074^{c}	0.185±0.045^{e}
SA	11.13±1.555^{bc}	8.66±0.205^{bc}	1.67±0.0952^{c}	0.208±0.03^{de}
SA + OK-BC 2.5%	13.73±1.033^{abc}	10.3±1.373^{abc}	2.134±0.057^{ab}	0.261±0.337^{bcde}
SA + OK-BC 5%	15.16±0.555^{ab}	10.5±1.080^{abc}	2.308±0.050^{a}	0.271±0.038^{bcde}
SA + SD-BC 2.5%	14.7±1.023^{abc}	10.7±0.880^{abc}	2.563±0.188^{a}	0.333±0.048^{abcde}
SA + SD-BC 5%	15.36±0.498^{ab}	11.3±1.564^{ab}	2.595±0.073^{a}	0.385±0.094^{abcde}

SK1	11.1±0.2^{bc}	8.4±0.4^{bc}	1.5±0.06^{bc}	0.217±0.04^{de}
SA + SK1	12.7±2.475^{abc}	9.96±0.939^{abc}	1.575±0.098^{bc}	0.22±0.039^{cde}
SA + OK-BC 2.5%+ SK1	17.06±3.574^{ab}	11.2±0.543^{ab}	2.321±0.463^{a}	0.409±0.0271^{abcd}
SA + OK-BC 5% + SK1	18.033±2.254^{a}	11.5±0.509^{ab}	2.69±0.111^{a}	0.432±0.043^{abc}
SA + SD-BC 2.5 + SK1	17.33±1.126^{ab}	12.2± 1.471^{a}	2.641± 0.092^{a}	0.458± 0.001^{ab}
SA + SD-BC 5% + SK1	18.73±2.375^{a}	12.85±6.058^{a}	2.74±0.212^{a}	0.490±0.093^{a}

Control – without PGPR, SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% - sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA + SD-BC 5% - sodium alginate + sawdust BC 5%, SK1-*Bacillus haikouensis.* The data represents the mean and standard deviation of three Samples. The different lower case letters represented significance difference ($p<0.05$)

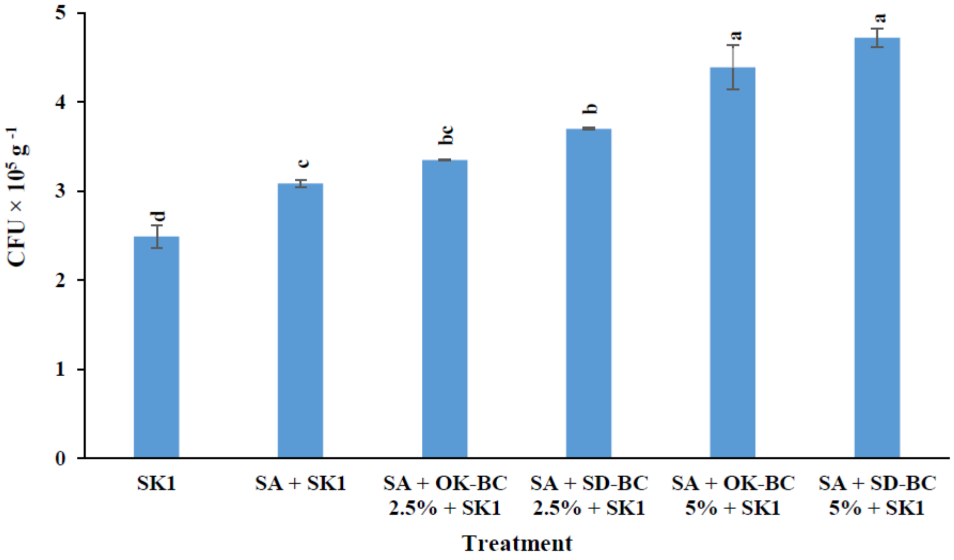


Figure 7. Colonization efficiency of *B. haikouensis* in the rhizosphere of *Z. mays.* SA - sodium alginate, SA + OK-BC 2.5% - sodium alginate + oak BC 2.5%, SA + OK-BC 5% - sodium alginate + oak BC 5%, SA + SD-BC 2.5% - sodium alginate + sawdust BC 2.5%, SA + SD-BC 5% - sodium alginate + sawdust BC 5%, SK1 - *Bacillus haikouensis.* The data represents the mean and standard deviation of three Samples. The different lower case letters represented significance difference ($p<0.05$)

4. Conclusion

In conclusion, this study demonstrates the potential of PGPR, *Bacillus* sp SK1 encapsulated in SA with BC to improve the growth of *Z. mays*. The results revealed that incorporating BC during the encapsulation of *Bacillus* sp SK1 with SA not only improved bead characteristics, including EE, moisture retention, water absorption potential, shelf life of SK1 during storage, and controlled release of SK1 cells, but also improved *Z. mays* growth. The findings suggest that the enhanced bead characteristics created a more supportive environment for PGPR, which in turn contributed to improved plant growth outcomes. Among the tested formulations. SA+SD-BC5% + SK1 showed the most promising results, indicating its potential as an effective bio-formulation for sustainable crop development. Looking ahead, further studies should examine the performance of this formulation across different plant species and under field conditions to confirm its broader applicability and scalability in sustainable agriculture soils.

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