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Augmenting Phosphate Solubilizing Bacterial Survival Using Biochar

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Authors' contributions

This work was carried out in collaboration among all authors. Author AY designed the layout and structure of the study. Author GH performed the tasks as per the guidelines. Author KY was actively involved in critically reviewing and revising the manuscript. All authors read and approved the final manuscript.

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Abstract

This study investigated the survival rate of phosphate solubilizing bacterial (PSB) isolates stored in talcum powder, cocopeat, and biochar, commonly used bioinoculant carrier materials. Four bacterial strains, viz. *Klebsiella aerogenes* LC515412, *Klebsiella pneumoniae* LC515413, *Kocuria flava* LC515414, and *Enterobacter hormaechei* LC515415 were stored in sterilized flasks at 28 °C for 60 days. The results showed a progressive decline in viable counts over time for each carrier material. In talcum powder, *K. pneumoniae* had the highest population retention, while *K. flava* had the lowest. Cocopeat exhibited good bacterial survival initially, but significant population drops were observed after 60 days. Biochar produced encouraging results, and *K. pneumoniae* exhibited the highest initial population, but its viability declined significantly over time. Overall, the findings suggest that biochar-based formulations can enhance the survival of specific bacterial strains; however, performance is strain-dependent. Further research is needed to optimize carrier

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formulations and to validate PSB viability and stability across extended storage durations and varying storage conditions.

Keywords: Phosphate solubilizing bacteria; biochar; cocopeat; talcum powder; survival; carrier.

1. Introduction

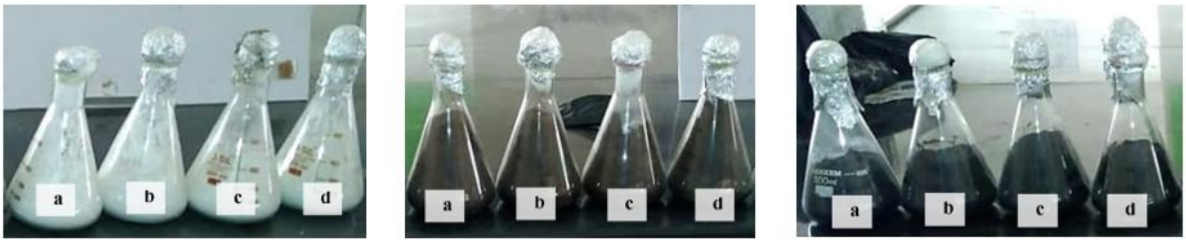
The survival and efficient delivery of phosphate solubilizing bacteria (PSB) are critical factors in developing effective bioinoculant formulations for agricultural applications. Various carrier materials have been explored to protect and sustain PSB during storage and application in the soil. Talcum powder, cocopeat, and biochar are commonly used carriers, each offering unique properties that may impact the survival rates of specific bacterial strains. Understanding the performance of these carriers is essential to optimize their use in promoting soil health and enhancing crop productivity. This study focused on investigating the survival rate of four PSB strains, namely, *Klebsiella aerogenes* LC515412, *Klebsiella pneumoniae* LC515413, *Kocuria flava* LC515414, and *Enterobacter hormaechei* LC515415, when stored in flasks containing talcum powder, cocopeat, and biochar. The temporal reduction in bacterial colony forming units (CFU) was assessed over 60 days at a controlled temperature of 28 °C in an incubator. We evaluated carrier performance to identify those that best support PSB survival and improve the stability of bioinoculant formulations.

Several studies shed light on the role of talcum powder and vermiculite as carriers, indicating that the choice of carrier significantly influences bacterial survival (El-Fattah et al., 2013; Sarma et al., 2011). Similarly, investigations into cocopeat as a carrier material have highlighted its potential as a good growing media component with acceptable pH and electrical conductivity, offering a high bacterial survival rate (Dzionek et al., 2016). Furthermore, biochar, a common soil amendment in agriculture, has been extensively studied for its beneficial effects on soil properties, water holding capacity, and nutrient retention (Laghari et al., 2016). Some studies have also reported its potential to enhance the colonization of specific bacterial strains in the rhizosphere of plants (Saxena et al., 2013). The results of this study will contribute valuable insights into the effectiveness of talcum powder, cocopeat, and biochar as carrier materials for promoting bacterial survival. The implications of these findings extend beyond laboratory experiments, as the formulation of bioinoculants plays a crucial role in the successful implementation of sustainable agricultural practices. Moreover, understanding the performance of these carriers can guide agricultural practitioners in selecting the most suitable carrier material for specific bacterial strains, leading to improved soil health and enhanced crop productivity.

2. Materials and Methods

The four PSB isolates, viz. *Klebsiella aerogenes* LC515412, *Klebsiella pneumoniae* LC515413, *Kocuria flava* LC515414, and *Enterobacter hormaechei* LC515415 used in the study were isolated by the authors in a previous study (Yadav et al., 2022). In the same report, the isolates' OD-based semi-logarithmic growth curves and key functional traits were documented. Fig. 1 shows the bacteriological carriers used in the study, including talcum powder, cocopeat, and biochar. The talcum powder, sourced from the market, had an average particle size of 50–80 µm. The cocopeat cake was also purchased from the market. The cocopeat washed with distilled water had a pH of 6.8 and an electrical conductivity (EC) of 0.5 mS cm^{-1}. On the other hand, biochar was produced from mango tree wood through pyrolysis at 350°C for six hours. The resulting coke chunks were blended into a medium-fine powder.

For each 200 g formulation prepared in a 500 mL pre-sterilized conical flask, 173.74 g of the respective carrier material (talcum powder, cocopeat, or biochar) pre-adjusted to 15% moisture (w/w) was taken and amended with 2.26 g carboxymethyl cellulose (CMC), 2.00 g calcium carbonate ($CaCO_3$), and 2.0 mL glycerol (Jorjani et al., 2011). The carrier was then inoculated with 20 mL nutrient broth containing an overnight-grown PSB culture with a density of at least 10^{11} CFU mL^{-1}. The final formulation mass was standardized to exactly 200 g by adding sterile carrier material or sterile broth as needed after accounting for the mass contribution of glycerol and inoculum (Sundaramoorthy and Balabaskar, 2012). Each carrier × strain formulation was prepared in five independent replicate flasks (n = 5), CFU values were recorded from each replicate flask and expressed as mean ± SE. The conical flasks were stored at 28 °C for 1, 15, 30, 45, and 60 days in a BOD incubator.



i) talcum powder ii) cocopeat iii) biochar

Fig. 1. The conical flasks containing talcum powder, cocopeat and biochar inoculated with a) *K. aerogenes* LC515412, b) *K. pneumoniae* LC515413, c) *K. flava* LC515414, and d) *E. hormaechei* LC515415

The same set of replicate flasks (n = 5) was aseptically subsampled repeatedly at each storage interval (1, 15, 30, 45, and 60 days). After the desired storage period, one gram of bioinoculant containing carrier material was taken in culture tubes, serially diluted, and spread plated onto nutrient agar plates. The Petri plates were incubated for 48 h at 30 ºC (Packialakshmi and Riswana, 2014). After incubation, the CFU g^{-1} was calculated based on colony counts, dilution factor, and plated volume.

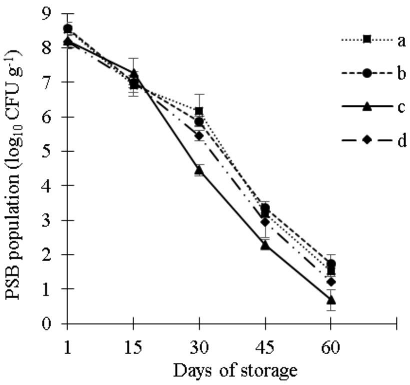
3. Results and Discussion

The viability of PSB in formulations is a significant bottleneck in agricultural microbiology that needs to be addressed (Bansal et al., 2025). Key determinants of bacterial survival in bioformulations include strain robustness, carrier physicochemical properties, processing stresses, storage conditions, and compatibility with formulation additives and contaminant microbiota. Better carrier formulations are the key to success in biofertilizer preparation, which must also ensure carrier compatibility with the inoculum. Chemical and biological compatibility testing of bacteria with a carrier is required for biofertilizer formulation development to improve bacterial survival (Figiel et al., 2025; Yang et al., 2025). Fig. 2 displays the survival rate of PSB isolates stored in sterilized flasks containing talcum powder, cocopeat, and biochar. Observations show a temporal reduction in populations (log_{10} CFU g^{-1}) of *K. aerogenes* LC515412, *K. pneumoniae* LC515413, *K. flava* LC515414, and *E. hormaechei* LC515415 in flasks containing various formulations kept at 28 °C in an incubator for 60 days.

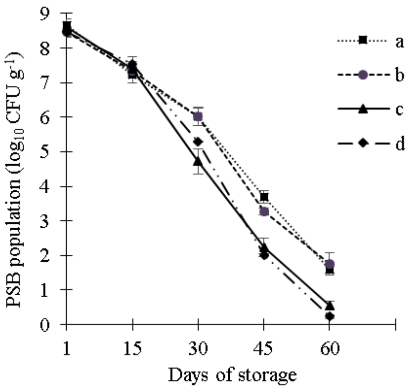
During the investigation of talcum powder-containing flasks, on day 60, *K. pneumoniae* LC515413 exhibited the highest population, reaching 1.73 log_{10} CFU g^{-1}, while *K. flava* LC515414 showed the lowest population of 0.69 log_{10} CFU g^{-1} in talcum powder. The population levels varied substantially during the initial 1–15 days of observation. Another study explored the development of talcum powder and vermiculite-based bioinoculant formulations using culture broths of pseudomonads R62 and R81 and inorganic phosphorus, and vermiculite proved to be a superior carrier with an extended shelf life compared to talcum powder (Sarma et al., 2011). In the same study, the viable CFU g^{-1} of the carrier decreased from 10.36 log to reach 7.80 log CFU g^{-1} over time for vermiculite, whereas, for talcum powder, the population dropped from 10.79 log to 6.65 log CFU g^{-1} for the same duration. The study concluded that talcum-based consortium formulation of pseudomonad R81 and inorganic phosphorus in the field experiment demonstrated higher effectiveness (Sarma et al., 2011).

Cocopeat, a commonly used carrier component, offers favorable attributes for bacterial survival, including acceptable pH, electrical conductivity, and other chemical properties (Chromkaew et al., 2023; Dzionek et al., 2016). Its physical properties, such as air capacity and water retention, depend significantly on the processing techniques and handling, resulting in 11–53% variations and 50–81%, respectively (Awang et al., 2009; Prabhu and Thomas, 2002). Our study involving cocopeat-containing flasks showed distinct population dynamics over time. After one day of storage, *K. aerogenes* LC515412 exhibited the highest population of 8.67 log_{10} CFU g^{-1} on cocopeat, closely followed by *K. flava* LC515414 with 8.60 log_{10} CFU g^{-1}. However, after 60 days, all strains had a 6–7 log_{10} reduction in CFU g^{-1}. Among the strains tested, *K. pneumoniae* LC515413 showed the highest bacterial population at the end of the storage period, with 1.76 $\log_{10}$ CFU g^{-1}, followed by *K. aerogenes* LC515412 with 1.59 $\log_{10}$ CFU g^{-1}. Meanwhile, *K. flava* LC515414 and *E. hormaechei* LC515415 experienced a considerable reduction in population, reaching 0.55 $\log_{10}$ and 0.23 $\log_{10}$ CFU g^{-1}, respectively. The depletion of bioinoculants impregnated on carriers is a well-documented phenomenon. Similar to our findings, El-Fattah et al. (2013) measured the effect of different agro-wastes as carrier materials for bacterial formulations. They assessed the survival and biological efficiency of *Azotobacter chroococcum* after the storage period, finding a considerable decrease in the population of introduced bioinoculants.

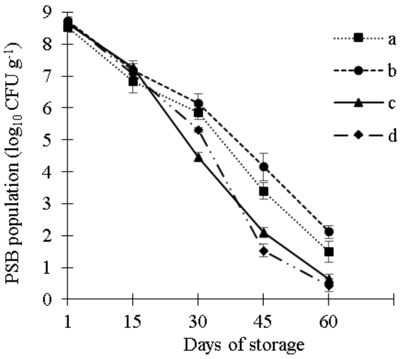
Biochar, a common agricultural amendment, offers various benefits that enhance the soil environment for crop cultivation (Pokharel et al., 2025). It decreases soil bulk density, increases water-holding capacity, and improves cation exchange capacity while adsorbing heavy metals from the soil (Shyam et al., 2025). In our study, in biochar-containing flasks, bacterial populations showed a distinct temporal pattern. The highest initial count was observed for *Klebsiella pneumoniae* LC515413 (8.73 $\log_{10}$ CFU g^{-1} biochar), which declined to 2.11 $\log_{10}$ CFU g^{-1} by day 60. *E. hormaechei* LC515415 had the second-highest initial bacterial population of 8.71 $\log_{10}$ CFU g^{-1} biochar after *K. pneumoniae* LC515413, the population dwindled to 0.44 $\log_{10}$ CFU g^{-1} after 60 days, the lowest among the four isolates. On the other hand, *K. aerogenes* LC515412 and *K. flava* LC515414 showed moderate reductions in their final populations (1.48 $\log_{10}$ and 0.62 $\log_{10}$ CFU g^{-1}, respectively). Recent mechanistic work with *Pseudomonas* sp. NK2 shows that biochar can function as a carrier matrix for PSB, but its effectiveness is strongly biochar-type dependent; among seven biochars evaluated, cotton-straw biochar–immobilized NK2 produced the greatest increase in soil available P (30-day soil assay) and significantly improved tomato biomass, plant height, and root development under P stress (Bai et al., 2024).



(i) talcum powder



(ii) cocopeat



(iii) biochar

Fig. 2. Survival of PSB isolates during storage in (i) talcum powder, (ii) cocopeat, and (iii) biochar. Populations are expressed as $\log_{10}$ CFU g^{-1} at 1, 15, 30, 45, and 60 days for (a) *K. aerogenes* LC515412, (b) *K. pneumoniae* LC515413, (c) *K. flava* LC515414, and (d) *E. hormaechei* LC515415. Values are mean ± SE (n = 5)

In previous studies, different biochar sources, like acacia wood and coconut shells, were evaluated for their potential as commercial biofertilizer carriers. Biochar derived from coconut shells proved the most effective, maintaining a maximal bacterial population of 10.79 log_{10} CFU g^{-1} when stored at 25.22% moisture for 180 days (Saranya et al., 2011). This particular biochar even enabled the storing of a requisite population of *Azospirillum lipoferum* for the same duration (Saranya et al., 2011). Another study compared two rice biochar preparations (biochar alone and biochar-vermiculite 50:50) with a peat moss-based carrier (peat moss: vermiculite 50:50) over 180 days (Ghazi, 2017). At 20% moisture content, biochar-based carriers supported the largest bacterial populations, reaching 9.98 log CFU g^{-1} (Ghazi, 2017). Furthermore, biochar addition has been shown to improve the colonization of *Bacillus* sp. in the rhizosphere of French beans, resulting in improved N and P content in the plant shoots (Song et al., 2022). Similarly, when applied to the soil at 2% and 10% concentrations, heartwood biochar shifted the microbial population toward increased bacteria and fewer fungi. (Ippolito et al., 2014). However, in our study, it was found that the application of talcum powder, cocopeat, and biochar-based formulations of *K. aerogenes* LC515412, *K. pneumoniae* LC515413*, K. flava* LC515414, and *E. hormaechei* LC515415 increased bacterial survivability during storage with strain and carrier-dependent efficiencies.

4. Summary and Conclusion

This study assessed the use of biochar as a feasible and low-cost alternative carrier for PSB, where talcum powder and cocopeat were used as reference carriers that are commonly used in the formulation of bioinoculants. Under controlled storage conditions (28 °C; up to 60 days), we monitored the temporal viability of four PSB isolates (*Klebsiella aerogenes* LC515412, *Klebsiella pneumoniae* LC515413, *Kocuria flava* LC515414, and *Enterobacter hormaechei* LC515415) within the three carrier matrices. Across carriers, the survival was strain-dependent, reinforcing that carrier choice cannot be generalized across taxa.

In talcum powder, the end of storage retention (day 60) was highest for *K. pneumoniae* with around 1.73 log_{10} CFU g^{-1}, and lowest for *K. flava* with around 0.69 log_{10} CFU g^{-1}. Cocopeat supported high initial populations (day 1; 8.46–8.67 log_{10} CFU g^{-1}); however, significant declines were observed by day 60, with final populations ranging from 0.23–1.76 log_{10} CFU g^{-1}, suggesting that while cocopeat is a useful benchmark carrier, there may be stability limitations under the tested storage regime. Critically, biochar showed clear promise as an alternative carrier, with two added benefits over 'storage-only' carriers: (i) it can be produced locally from low-value biomass, thereby reducing reliance on commercial inputs, and (ii) it is a soil beneficial amendment at application. In our biochar formulation, *K. pneumoniae* had the highest initial population (8.73 log_{10} CFU g^{-1}) and highest retention at day 60 (2.11 log_{10} CFU g^{-1}), indicating it had the best survival compared to all strain-carrier combinations tested. By contrast, day-60 populations for *K. aerogenes* and *K. flava* were 1.48 log_{10} and 0.62 log_{10} CFU g^{-1}, respectively, while the final population of *E. hormaechei* was the lowest in biochar (0.44 log_{10} CFU g^{-1}). These results suggest that biochar performance is strain-specific, and formulation design variables that include biochar type, particle size, and moisture conditioning are likely to be important determinants of shelf-life for targeted strains.

Taken together, the results provide support for the inference that biochar can be used as a viable carrier alternative to talcum powder and cocopeat if the variables of formulation are optimized for the target strain(s). This conclusion is consistent with previously discussed reports, which indicate that the biochar carrier performance is strongly biochar-type dependent and can sustain high viable count under appropriate moisture and storage conditions.

5. Further Research

The study on the survival rates of bacterial isolates in talcum powder, cocopeat, and biochar provides a basis for understanding that has many avenues for further research. One important aspect to explore is the optimization of carrier materials, which could mean looking into combinations of the materials studied or modifications of them (chemical or physical) to improve the survival of bacteria. Strain-specific studies may account for why some bacterial strains, such as *K. pneumoniae,* have better survival rates in certain carriers. Genomic analyses could be used to further identify the genes that cause this survival advantage. Environmental factors, such as temperature and humidity, also need to be further investigated, as this study was conducted at a constant temperature (28 °C). The interaction between carrier materials and soil types could provide additional complexity and relevance to real-world applications. Long-term studies are needed to determine how these carrier materials affect soil health and microbial community structure, as well as how they affect plant growth and yield. Field trials may be a more practical way of assessing the effectiveness of the carrier materials, and research may also concentrate on making commercial formulations based on the most promising combinations of carrier material and bacterial strain. Comparative studies with other commonly used carriers and comparison with international standards could allow a more complete view of the survival of bacteria in carrier materials. We can take these initial findings a step further by exploring these directions of research to create better and more sustainable agricultural practices.

Disclaimer (Artificial Intelligence)

Authors hereby declare that no generative AI technologies, such as large language models and text-to-image generators, have been used during the writing or editing of this manuscript.

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Competing Interests

Authors have declared that no competing interests exist.

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