Exploring Phosphate-Solubilizing Bacteria In Paddy Rhizospheres Across Varied Management Systems

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Abstract. Phosphate-solubilizing bacteria (PSB) from diverse paddy field management systems are promising candidates for use as biofilm-based biofertilizers. This study aimed to identify PSB isolates with superior phosphate-solubilizing capacity and to examine soil factors influencing this ability. Bacterial inoculants were isolated from paddy rhizospheres managed under conventional, semi-organic, and organic systems with different durations: 7, 10, and 15 years of organic practice. All fields were located in Inceptisols of Mojogedang, Karanganyar, Indonesia. Results showed that the management systems significantly affected the total bacterial population density. From the total bacterial colonies, 15 isolates were identified as capable of solubilizing phosphate. The 15-year organic farming system (Organic 2009) showed the highest bacterial population (1.24×10^{18} CFU/g), while the lowest was observed in the semi-organic system (1.19×10^{15} CFU/g). The isolate R5 had the highest phosphate solubilization index (PSI) of 5.10, followed by R7 (3.35) and R13 (3.13), while R8 had the lowest (2.12). The result of correlation analysis revealed that organic carbon (r = 0.56*) and available phosphorus (r = 0.83*) were positively correlated with the solubilization index. These PSB isolates have promising potential for the development of innovative biofilm-based biofertilizer formulations.

1 Introduction

Organic farming is a sustainable and environmentally friendly agricultural management system that avoids synthetic chemical inputs to maintain soil and ecosystem health. In contrast, conventional farming relies heavily on external inputs, including synthetic fertilizers, fungicides, insecticides, and herbicides Organic fertilizers not only improve soil fertility but also create a favorable microclimate for beneficial soil microorganisms. Organic farming contributes to improved soil quality, land productivity, and crop quality. Several studies have shown that soils initially degraded by conventional practices undergo significant

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recovery when transitioned to organic management and that long-term organic farming supports sustained microbial activity.

The rhizosphere, which is the narrow zone surrounding plant roots, is a hotspot for microbial activity. This zone is strongly influenced by root exudates and serves as a colonization site for rhizobacteria . Roots exude various nutrient-rich metabolites that attract microbes into the rhizosphere. In the rhizosphere of paddy plants, bacteria are capable of phosphate solubilization. Phosphate-solubilizing bacteria (PSB) play a crucial role in the phosphorus cycle by converting insoluble phosphorus into forms that are more readily available to plants. These bacteria can make up between 1–50% of the total soil microbial population . Through their solubilization activity, PSB enhance phosphorus uptake by plants and contribute to overall plant health, making them valuable candidates for development as biofertilizers.. Phosphorus is essential for plant development, especially in promoting tiller formation, accelerating grain filling and maturation, and enhancing root growth.. Certain genera such as *Ralstonia*, *Rhodanobacter*, and *Rhodococcus* are more commonly found in organically managed fields, while *Acidovorax*, *Methylobacterium*, and *Pleomorphomonas* are typically associated with conventionally managed fields. Meanwhile, *Burkholderia*, *Cupriavidus*, and *Enterobacter* are found in both systems..Different paddy cultivation systems, particularly those that incorporate organic fertilizers and reduce chemical inputs, have a significant influence on the structure and function of microbial communities in the rhizosphere. Previous studies have shown that bacterial abundance is generally higher in organic farming systems than in conventional ones. Organic fertilizers significantly alter the soil microbial community structure and increase microbial abundance, which can indirectly enhance crop yields through improved enzymatic activity and nutrient availability. This study aims to investigate the morphological diversity of bacteria with high potential for phosphate solubilization across various paddy field management systems, including conventional, semi-organic, and organic systems with 7, 10, and 15 years of organic application. Additionally, it seeks to identify the soil properties influencing the phosphate solubilization index. The findings will contribute to the development of biofertilizers containing highly functional phosphate-solubilizing bacterial (PSB) inoculants to support sustainable paddy cultivation.

2 Materials and Method

2.1 Research location

Soil and rhizosphere isolate sampling took place in March 2024, with samples obtained from paddy fields managed under five distinct systems: 15-year organic (organic 2009), 10-year organic (organic 2014), 7-year organic (organic 2017), semi-organic, and conventional practices. The study site was located in Ngampel, Gentungan Village, Mojogedang Subdistrict, Karanganyar Regency, Central Java, Indonesia (7°32'49"S 111°00'38"E to 7°32'51"S 111°00'50"E). Bacterial isolation and subsequent laboratory analyses were carried out at the Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret.

2.2 Research method

This research employed a descriptive-exploratory approach using a field survey followed by laboratory analysis. Rhizosphere and soil samples were collected during the maximum vegetative stage of paddy growth from five management systems: conventional, semi-organic, 15-year organic, 10-year organic, and 7-year organic. Sampling was carried out using purposive random sampling at five distinct locations within each field. Each paddy management system was replicated three times. Rhizosphere samples were obtained by carefully collecting soil adhering to paddy roots. The collected soil samples were then composited, air-dried, and sieved through a 2 mm mesh prior to analysis.

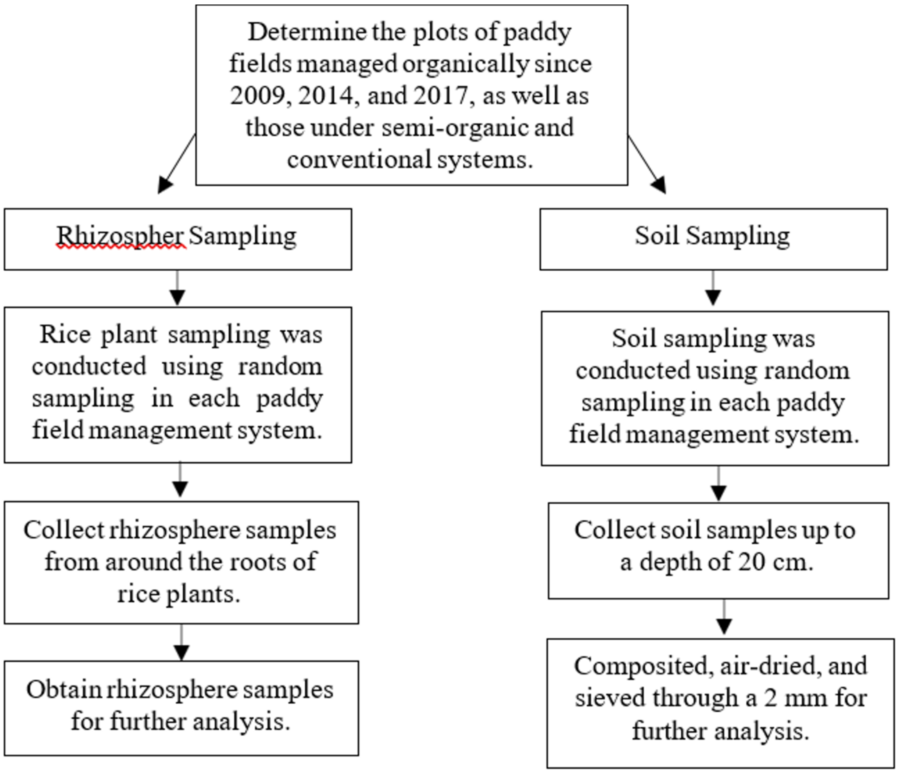

Fig. 1. Sampling workflow for rhizosphere and soil collection.

2.3 Soil chemical analysis

Soil properties analyzed included pH H_2O (1:5), organic Carbon (Walkley and Black method), available Phosphorus (Olsen method), and soil texture (the pipette method). All analyses were conducted at the Soil Chemistry and Fertility Laboratory, Faculty of Agriculture, Universitas Sebelas Maret.

2.4 Isolation of bacteria

Rhizosphere bacteria were isolated using the serial dilution method. Five grams of rhizosphere soil were suspended in 45 mL of sterile physiological salt and mixed thoroughly using a vortex mixer. From serial dilutions ranging from 10^{-11} to 10^{-16}, 0.1 mL aliquots were pipetted onto Nutrient Agar (NA) plates using sterile techniques. The suspension was evenly spread using the spread plate method and incubated for 7 days. Isolates were incubated at room temperature (approximately 25 ± 2 °C). Morphological characteristics of bacterial colonies, including color, shape, margin, elevation, and surface texture, were recorded. The colony-forming units (CFU), or the number of viable cells per gram of soil, were calculated using the following formula.:

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$$CFU = \frac{Mean\ plate\ count\ \times\ Dilution\ factor}{Amount\ of\ dilution} \quad (1)$$

2.5 Phosphate-solubilizing bacteria isolation and analysis

The ability of bacterial isolates to solubilize phosphate was assessed by transferring purified colonies from Nutrient Agar into Pikovskaya's agar medium, which contains the following composition: 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 2H_2O$, trace amounts of $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract, and 15 g agar in 1 liter of distilled water. The inoculated plates were incubated at room temperature (approximately 25 ± 2 °C) for 7 days. The identification of phosphate-solubilizing bacteria was conducted by observing the formation of a halo zone around the colonies that developed on the medium, followed by measurement using the following formula:

$$Phosphate\ Solubilization\ Index = \frac{(Colony\ diameter\ +\ Halo\ zone\ diameter)}{Colony\ diameter} \quad (2)$$

2.6 Data analysis

Data were analyzed using analysis of variance (ANOVA) at a 95% confidence level with R Studio. If the F-test indicated a significant difference among treatments, Duncan's Multiple Range Test (DMRT) was conducted to compare treatment means. Pearson correlation analysis was used to examine the relationship between soil properties and phosphate-solubilizing activity.

3 Results and Discussion

3.1 Characteristics of soils collected from rhizospheres

Soil type for all paddy field management systems in this study are classified as Inceptisols. Different paddy field management practices significantly ($p < 0.05$) influence several soil characteristics (Table 1). The 15-year organic system, established in 2009, exhibited the lowest soil pH, indicating slightly acidic conditions. This decrease in soil pH is likely due to the long-term decomposition and mineralization of organic matter, which generates organic acids that gradually acidify the soil. Soil organic carbon (C-Organic) was consistently higher in all organic systems, regardless of their duration, compared to semi- organic and conventional systems. Organic farming practices maintain higher soil organic matter content than conventional systems, primarily due to the application of organic fertilizers, which enhance soil organic carbon levels [1]. The available phosphorus (P) content was also notably higher in organic systems, with the highest concentration (36.53 ppm) observed in the 7-year organic system (the 2017 organic field). This trend aligns with previous studies demonstrating that organic fertilizer application significantly improves soil phosphorus availability over time [2]. The soil texture across all five paddy management systems is classified as clay to clay loam (Table 2). Organic paddy systems, particularly the 2009 and 2017 organic fields, exhibited a higher clay percentage, leading to a clay-textured classification. In contrast, conventional and semi-organic fields were characterized by a clay loam texture. Long-term organic fertilizer application can also affect soil texture. Organic fertilizer improves soil quality by enhancing structure, texture, and aeration, while also increasing the soil's moisture retention capacity [3].

Table 1. Chemical characteristics of soil in paddy field management systems.

Paddy Field System Management	pH H_2O		C-organic (%)		Available P (ppm)	
Conventional	6.75b	N	2.19c	M	7.77d	L
Semi Organic	6.92a	N	2.2b	M	9.74cd	L
Organic 2009	6.38c	SA	2.61a	M	14.37b	M
Organic 2014	6.82ab	N	2.43ab	M	11.17cd	M
Organic 2017	6.73b	N	2.64a	M	36.53a	VH

Note: Different small letters within the same column indicate significant differences of DMRT ($p < 0.05$). N = Neutral, SA = Slightly Acidic; M = Medium; L = Low, VH = Very High.

Table 2. Soil texture in paddy field management systems.

Paddy Field System Management	Silt (%)	Clay (%)	Sand (%)	Classification
Conventional	27.54	32.58	39.88	Clay loam
Semi Organic	29.72	33.64	36.64	Clay loam
Organic 2009	17.18	47.63	35.19	Clay
Organic 2014	23.7	39.87	36.43	Clay loam
Organic 2017	19.12	46.74	34.14	Clay

3.2 Bacterial population density in rhizosphere

The bacterial population density in paddy rhizospheres varied significantly ($p < 0.05$) across different management systems. Organic paddy management systems exhibited a higher bacterial population density compared to conventional and semi-organic systems (Table 3). The 15-year organic farming system (Organic 2009) recorded the highest bacterial population at 1.24×10^{18} CFU/g soil, while the semi-organic system had the lowest at 1.19×10^{15} CFU/g soil (Table 3). Bacterial isolate diversity was highest in the organic paddy field treatments conducted in 2009 and 2014, and lowest in the semi-organic and organic treatments of 2017 (Table 4). The variation in bacterial population density within organic systems may be attributed to differences in the quality of organic inputs or environmental conditions. These findings align with previous research comparing organic and conventional paddy farming systems, which reported a higher abundance of microorganisms in organic paddy fields than in conventional systems [4]. The increase in microbial populations within organic systems is largely influenced by management practices, such as the application of organic materials and techniques that minimize or eliminate chemical inputs while supporting biological plant protection [5]. Results suggest that the longer an organic system is maintained, the greater the bacterial population density. Furthermore, findings indicate that bacterial population density in semi-organic systems is lower than in conventional systems. Likewise, the total number of bacterial isolates observed in the 2017 organic and semi-organic paddy field treatments was lower compared to the conventional treatment. Various agricultural factors can disrupt microbial community balance, particularly nutrient availability from fertilization, which may alter bacterial activity and composition [6].

Table 3. The bacterial population density in paddy field management systems.

Paddy Field System Management	Population density (CFU/g soil)
Conventional	2.19×10^{15} a
Semi Organic	1.19×10^{15} a
Organic 2009	1.24×10^{18} b
Organic 2014	6.80×10^{17} b
Organic 2017	6.63×10^{17} b

Note: Different letters within the column indicate significant differences based on Duncan's Multiple Range Test.

Table 4. Comparison of the number of rhizosphere isolates across paddy field management systems.

Paddy Field System Management	Isolates	Total Isolates
Conventional	R1, R2, R3, R5, R6, R11, R12, R20	8
Semi Organic	R3, R5, R9, R18, R20	5
Organic 2009	R1, R2, R3, R5, R6, R8, R9, R11, R13, R16	10
Organic 2014	R1, R3, R5, R6, R7, R8, R9, R10, R11, R12	10
Organic 2017	R1, R2, R3, R5, R18	5

3.3 Morphological of phosphate solubilizing bacteria

Bacterial isolates from the rhizosphere were grown on Nutrient Agar (NA) medium for morphological characterization and their phosphate-solubilizing ability was evaluated on Pikovskaya medium. A total of 20 isolates were obtained from paddy fields under different management systems, of which 15 isolates exhibited phosphate-solubilizing ability, as indicated by the formation of clear halo zone around the colony (Figure 2).

Morphological analysis revealed considerable diversity among the isolates. Most isolates displayed white pigmentation, followed by yellow, orange, and cream variants. In terms of colony form, round morphology was dominant, although rhizoid, irregular, and filamentous forms were also observed (Table 5). The colony margin types varied, including entire, lobate, scalloped, undulate, wavy, and filiform, with round and undulate margins appearing most frequently. Elevation of colonies ranged from raised, convex, crateriform, and umbonate to less common forms like draughtsman colony and flat, with raised and convex being dominant. The majority of isolates had a smooth texture, with only three isolates exhibiting rough surfaces. In terms of opacity, isolates were either translucent or opaque, with translucent colonies being more common (Table 5).

Bacteria have diverse morphology because they serve several functions, including mobility, nutrient acquisition, interaction with other organisms, dispersion, and resistance to stress [7]. This morphological diversity suggests that the PSB community in paddy rhizospheres is taxonomically varied and potentially consists of multiple genera, reflecting the influence of different field management systems on microbial diversity. To accurately identify the bacterial genus, several tests are necessary, including relatively simple and inexpensive biochemical assays, or molecular techniques capable of detecting the microorganism's genetic components [8].

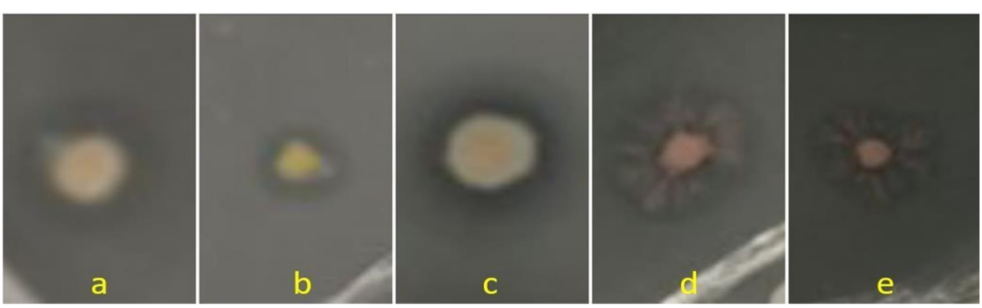

Fig. 2. Appearance of bacterial isolates on Pikovskaya agar medium (a) Isolate R10 from 10-year organic paddy field (b) Isolate R12 from conventional paddy field (c) Isolate R16 from 15-year organic paddy field (d) Isolate R18 from 7-year organic paddy field (e) Isolate R20 from semi-organic paddy field.

Table 5. Morphological of phosphate solubilizing bacteria.

Morphology						
Isolate	**Color**	**Form**	**Margin**	**Elevation**	**Texture**	**Opacity**
R1	White	Rhizoid	Entire	Raised	Smooth	Translucent
R2	White	Irregular	Lobate	Raised	Smooth	Translucent
R3	White	Round	Round	Convex	Smooth	Opaque
R5	Yellow	Round	Round	Convex	Smooth	Opaque
R6	Yellow	Filamentous	Wavy	Crateriform	Rough	Translucent
R7	White	Round	Scalloped	Draugtsman Colony	Rough	Translucent
R8	Orange	Round	Undulate	Umbonate	Smooth	Opaque
R9	White	Rhizoid	Filiform	Umbonate	Rough	Opaque
R10	Yellow	Round	Undulate	Crateriform	Smooth	Translucent
R11	Cream	Round	Round	Convex	Smooth	Translucent
R12	Yellow	Round	Round	Convex	Smooth	Translucent
R13	White	Round	Undulate	Raised	Smooth	Opaque
R16	White	Round	Entire	Flat	Smooth	Translucent
R18	White	Round	Undulate	Flat	Smooth	Translucent
R20	White	Round	Round	Raised	Smooth	Opaque

3.4 Phosphate solubilization index of rhizosphere

Fifteen bacterial isolate from twenty isolates successfully formed clear halo zones around their colonies after seven days of incubation on Pikovskaya agar, indicating their ability to solubilize inorganic phosphate. The Phosphate Solubilization Index (PSI) was calculated as the ratio between the total halo zone diameter and the colony diameter, representing each isolate's effectiveness in converting insoluble phosphate (e.g., tricalcium phosphate) into soluble forms.

The largest colony diameter was observed in isolate R20 (7.14 mm) followed by R8 (6.63 mm). The smallest colony belonged to R12 (3.24 mm). Regarding halo zone diameter, R5 showed the widest clear zone (14.93 mm), followed by R13 (9.82 mm). Whereas the smallest halo was observed in R12 (4.93 mm). Among all isolates, R5 exhibited the highest PSI (5.10), indicating strong phosphate-solubilizing potential, followed by R7 (3.35) and R13 (3.13). In contrast, R8 and R20 showed the lowest PSI values, at 2.12 and 2.13, respectively (Table 5).

The variation in PSI values suggests that phosphate-solubilizing efficiency is not solely dependent on colony size but also on the metabolic activity of each isolate. Isolates with higher PSI values, such as R5 and R7, are promising candidates for development as biofertilizer inoculants, particularly in enhancing phosphorus availability in low-input or organic farming systems. The ability of PSB to dissolve phosphate can be utilized by plants for growth and development [9]. A study revealed that PSB used as biofertilizers can enhance rice yield, reduce chemical fertilizer usage, and improve fertilizer use efficiency [10]. Therefore, isolates R5 and R7 require further analysis to determine their genera and assess their potential as biofertilizer inoculants. Identifying the genus allows for application adjustments based on crop type, soil type, or agricultural systems (organic and semi-organic).

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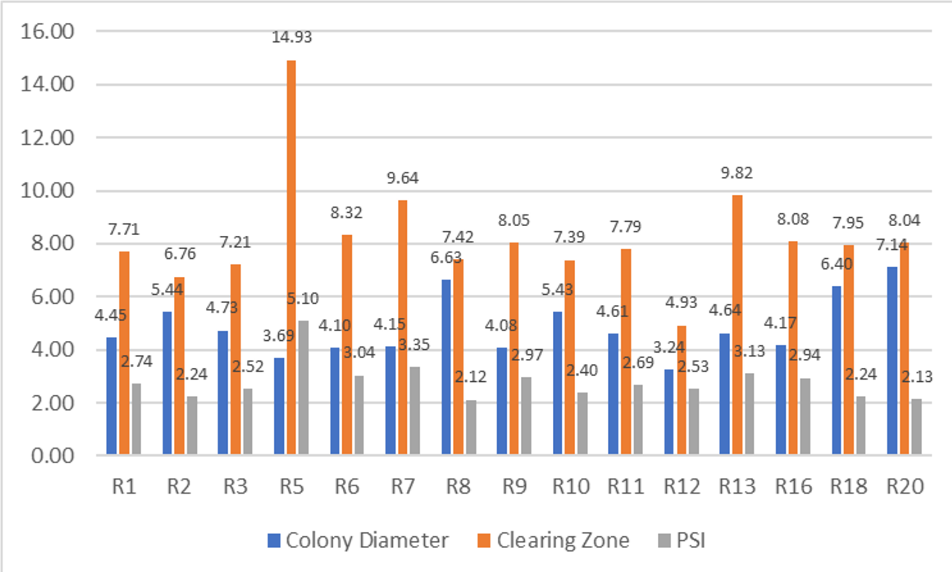

Fig. 3. The Phosphate Solubilization Index (PSI) in a plate assay using Pikovskaya's agar is determined by measuring the Colony Diameter (mm) and the Clearing Zone diameter (mm), with PSI serving as an indicator of phosphate solubilizing activity.

3.5 Determinant factors of the phosphate solubilization index

Correlation analysis was conducted to examine the relationship between PSI, organic carbon (C-organic), and available phosphorus (P). The results revealed a positive correlation between both soil chemical parameters and PSI. Specifically, the correlation coefficient between C-organic and PSI was 0.56, indicating a moderate positive relationship, while the correlation between available phosphorus and PSI was stronger, at 0.83, demonstrating a very strong positive correlation (Table 6).

Microbial activity in phosphate solubilization depends on the breakdown of organic carbon, which supports the growth of phosphate-solubilizing bacteria (PSB) [11]. The primary mechanism underlying phosphate solubilization by PSB involves the production of organic acids, which convert insoluble phosphate compounds (e.g., tricalcium phosphate) into bioavailable forms for plants [12, 13]. Consequently, higher levels of available phosphorus reflect increased PSB activity, leading to higher PSI values [14].

Additionally, a positive correlation was observed between C-organic and available phosphorus ($r = 0.61$). Supporting previous findings that organic matter plays a crucial role in nutrient dynamics, particularly in enhancing phosphorus availability through microbial mediation and soil buffering capacity [15].

Table 6. Results of Correlations Analysis.

Variable	Available P	PSI
C organic	0.61*	0.56*
Available P		0.83*

Note: * indicates statistically significant correlation.

4 Conclusion

Different paddy field management systems influence rhizosphere bacterial populations and phosphate-solubilizing capacity. The highest bacterial density was observed in the 15- year organic paddy management system, while the lowest density was recorded in the semi-organic system. Organic paddy field treatments in 2009 and 2014 showed the highest total bacterial counts, while the lowest levels were recorded in the semi-organic and organic treatments in 2017. Among the bacterial population, 20 isolates exhibited distinct colony morphologies. Of these, 15 isolates demonstrated phosphate-solubilizing ability, with R5 showing the highest phosphate solubilization index (PSI) of 5.10, followed by R7 with a PSI of 3.35.

The key factor affecting PSI is soil organic carbon. which showed a moderate positive correlation (r = 0.56). A higher PSI indicates greater phosphate-solubilizing bacterial (PSB) activity. leading to increased phosphorus availability (r = 0.83). These findings highlight the importance of maintaining soil organic carbon levels to support the growth of highly functional bacteria, which have the potential to be used as inoculants in biofertilizer development. These biofertilizer inoculants have the potential to enhance soil fertility, particularly by increasing the availability of phosphorus nutrients and reducing the use of chemical fertilizers, making them environmentally friendly. The limitations of using biofertilizers for commercial development include challenges such as selecting a suitable carrier material to support microbial viability and achieving large-scale production with high-quality control. Therefore, further research is needed to identify appropriate carrier materials and to develop standardized mass production methods to ensure high-quality outcomes.

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