

Unveiling the BPF9 Isolate as a Potential of Phosphate-Solubilizing Bacteria Through In Vitro Characterization

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Abstract

The growing demand for food in Indonesia has prompted the intensification of agricultural practices, notably through the application of phosphate fertilizers. Despite this, the efficiency of insoluble phosphorus uptake by plants remains low due to its prevalence in forms within the soil. The utilization of phosphate-solubilizing bacteria (PSB) presents a promising strategy to address this limitation. This study aimed to evaluate the solubilization potential of the BPF9 isolate through qualitative and quantitative assessments, as well as growth curve profiling. BPF9 was selected from the laboratory's microbial collection due to its survival capabilities under diverse environmental conditions. The research was conducted at the Soil and Environmental Biotechnology Laboratory, IPB University. Qualitative phosphate solubilization was assessed on Pikovskaya agar by measuring halo zone formation to calculate the Solubilization Index (SI). Quantitative analysis was carried out using spectrophotometry at 660 nm. The bacterial growth curve was analyzed using the haemocytometer method. Results showed that BPF9 had a solubilization index of 2.0 (moderate category) and was able to solubilize phosphate up to 128.5 ppm, which indicates a considerable solubilization capacity. The growth curve indicated that BPF9 entered the exponential phase at 26 hours and reached its maximum population at 42 hours. These findings demonstrate that BPF9 possesses strong phosphate-solubilizing capability and stable growth characteristics, making it a promising candidate for biofertilizer development.

1. Introduction

Indonesia's ongoing population expansion has intensified the need to ensure an adequate food supply, recognizing food as a basic necessity for human life. To address this challenge, agricultural intensification has been promoted as a key strategy to boost food production. One of the most common practices in this effort is the use of synthetic fertilizers to support optimal plant growth. However, long-term and intensive use of synthetic fertilizers has been shown to negatively impact soil quality and health. These adverse effects include soil acidification, loss of soil organic matter, and nitrate leaching (Hong et al., 2019). This condition adversely affects the soil's function in supporting vegetation, ultimately resulting in suboptimal crop production.

Phosphorus (P), together with nitrogen (N) and potassium (K), is one of the most extensively used macronutrients in agriculture, given its vital role in floral development, mitotic activity, and the overall physiological advancement of plants. However, it is also one of the nutrients with the lowest uptake efficiency by plants, with only about 10-30% of applied phosphorus being absorbed (Naomi et al., 2021; Rech et al., 2018). Phosphorus in soil commonly interacts with various soil constituents and is predominantly taken up by plants through diffusion. Its movement within the soil profile is restricted due to its strong tendency to associate with mineral elements, forming compounds with low solubility. Under acidic conditions, phosphorus undergoes

fixation as aluminum-phosphate (Al-P), iron-phosphate (Fe-P), or occluded forms, whereas in alkaline environments, it reacts with calcium to generate sparingly soluble calcium-phosphate (Ca-P) complexes (Subaedah et al., 2018). Moreover, the naturally available phosphorus in soils typically does not exceed 0.01% of the total phosphorus content. The majority of phosphorus in the soil, ranging from 95% to 99%, is retained in insoluble fractions, limiting its immediate availability to plants. This high degree of phosphorus immobilization significantly reduces its diffusion rate toward plant roots. The amount of phosphorus that ultimately reaches the rhizosphere depends on several factors, including soil mineralogy, clay content, phosphorus concentration, bulk density, and soil moisture (Rech et al., 2018).

One promising approach to enhance phosphorus availability and uptake efficiency, both from applied fertilizers and from native soil phosphorus, is the use of phosphate-solubilizing microorganisms (PSMs). Such microorganisms facilitate the solubilization of phosphorus, enabling its conversion into plant-accessible forms. Phosphate-solubilizing microbes can utilize tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$ or apatite) and other insoluble phosphate sources by producing organic acids, which transform them into soluble monovalent (H_2PO_4^-) and divalent (HPO_4^{2-}) phosphate ions. Such microorganisms facilitate the solubilization of phosphorus, enabling its conversion into plant-accessible forms (Beheshti et al., 2022). The application of phosphate-solubilizing bacteria (PSB) in crop cultivation has been shown to promote plant growth and enhance resistance to environmental stresses such as salinity and nutrient deficiency (Wang et al., 2023). Research from Pande et al. (2017) demonstrated that PSB markedly improved maize growth in pot experiments when compared to uninoculated plants. This growth promotion was attributed to the secretion of organic acids such as gluconic, formic, and citric acids, which constitute the primary mechanism of phosphate solubilization. The phosphate solubilizing ability of PSB varies based on their genetic characteristics. The objective of this study was to evaluate the phosphate solubilizing potential of the bacterial isolate BPF9 in converting insoluble phosphorus into forms that plants can absorb. This isolate is expected to be a promising candidate for development as a biofertilizer to support sustainable plant growth.

2. Research Method

2.1. Research Location

This research was conducted at the Soil and Environmental Biotechnology Laboratory, Faculty of Agriculture, IPB University Bogor, Indonesia.

2.2. Research Methods

2.2.1. Rejuvenation of Isolates

The phosphate-solubilizing bacteria used in this study was the BPF9 isolate, originally isolated from agricultural soil in Rejo Agung Madiun. Rejuvenation and purification of the BPF9 isolate were carried out to obtain a pure culture from previously grown stocks. This isolate was selected from the laboratory's microbial collection based on preliminary screening results that indicated its ability to survive under diverse environmental conditions. The process continued until all colonies displayed uniform morphology, indicating cultural homogeneity (Amri et al., 2023). The purification was conducted using Pikovskaya agar medium, composed of 5 g tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), 0.5 g ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 0.2 g sodium chloride (NaCl), 0.1 g magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.2 g potassium chloride (KCl), 10 g glucose, 0.5 g yeast extract, 20 g agar, and 0.0002 ml of a solution containing manganese sulfate and ferrous sulfate ($\text{MnSO}_4 + \text{FeSO}_4$), dissolved in 1 L of aquadest. All procedures were performed under sterile conditions using a Laminar Air Flow Cabinet (LAFC).

2.2.2. Qualitative Assesment of Phosphate-Solubilizing Bacteria

The ability of phosphate-solubilizing bacteria (PSB) to dissolve insoluble phosphate was qualitatively evaluated by examining the formation of clear zones (halo zone) around colonies grown on Pikovskaya agar. The BPF9 isolate was inoculated aseptically onto the medium using a sterile loop and incubated at 28°C for 72 hours. The appearance of a transparent halo surrounding the bacterial growth was considered evidence of phosphate solubilization. The Solubilization Index (SI) was determined using the formula:

$$\text{SI} = \frac{\text{halo zone diameter} - \text{colony diameter}}{\text{colony diameter}}$$

Based on the SI value, phosphate-solubilizing activity was classified into three levels: low ($\text{SI} < 2.00$), moderate ($2.00 < \text{SI} < 4.00$), and high ($\text{SI} > 4.00$) (Oedjijono et al., 2024). Isolates that produced a visible halo zone were further evaluated for quantitative phosphate solubilization using a spectrophotometer at 660 nm.

2.2.3. Quantitative Measurement Based on Optical Density (OD)

Quantification of phosphate solubilized by the BPF9 isolate was carried out based on the procedure outlined by Lynn et al. (2013). A sterile loop was used to transfer a bacterial colony into 100 mL of autoclaved liquid Pikovskaya medium, followed by incubation at ambient temperature under constant agitation for five days. A control was prepared using the same medium without bacterial inoculation. To determine the amount of soluble phosphate, PB reagent (comprising ammonium molybdate, boric acid, and hydrochloric acid) and PC reagent were employed. After incubation, 25 mL of the culture broth was centrifuged at 1000 rpm for 25 minutes. From the resulting supernatant, 5 mL was combined with 5 mL of PB reagent and five drops of PC reagent. This mixture was left at room temperature for 15-30 minutes to allow for color development. The phosphate concentration in the solution was then determined using a Shimadzu UV-Vis 1800 spectrophotometer at a wavelength of 660 nm. A standard calibration curve was generated using KH_2PO_4 with phosphate concentrations of 1, 2, 3, 8, and 10 ppm (Figure 1).



Figure 1. Standard KH_2PO_4 solution for quantitative phosphate solubilization measurement

2.2.4. Growth Curve Analysis Using the Haemocytometer Method

Bacterial population density was determined through direct observation using a haemocytometer (Petroff-Hauser Counting Chamber). This device allows for cell quantification within a fixed volume, as it has a precisely known distance between the grid and the cover slip. The grid consists of nine large squares, each with an area of 1 mm^2 . The central square is subdivided into 25 intermediate squares ($0.2 \text{ mm} \times 0.2 \text{ mm}$), and each of these contains 16 smaller divisions, totaling 400 small squares. The chamber has a depth of 0.1 mm, which defines the volume in which cells settle. Bacterial cells suspended in liquid medium are evenly distributed within this space, making it possible to calculate the cell concentration per milliliter. The number of cells counted within the grid was then used to estimate the total cell concentration, following the protocol outlined by Wijaya et al. (2017).

2.3. Data Analysis

All data were processed and presented in descriptive form through tables and figures. No inferential statistical tests were applied, as the study focused on characterizing the isolate's solubilization potential and growth pattern under controlled laboratory conditions.

3. Result and Discussion

3.1. Qualitative Assessment of BPF9 through Halo Zone Observation

The qualitative evaluation of the BPF9 isolate's capacity to solubilize inorganic phosphate was conducted using Pikovskaya agar medium. Evidence of phosphate solubilization was observed as a clear zone (halo zone) surrounding the bacterial colony. This zone is produced through the breakdown of tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), the insoluble inorganic phosphorus compound incorporated in the medium. The appearance of this clear area suggests that BPF9 can convert insoluble phosphate into soluble forms that are potentially available for plant uptake (He and Wan, 2022) (Figure 2). In this process, the primary mechanism is not phosphatase activity but rather the secretion of low molecular weight organic acids such as gluconic, citric, or oxalic acids, which chelate cations (Ca^{2+} , Fe^{3+}) and reduce the pH, thereby releasing soluble phosphate. These organic acids may also compete with phosphate ions for adsorption sites on soil particles, leading to the release of soluble orthophosphate species (HPO_4^{2-} , H_2PO_4^-) (Pan and Cai, 2023; Sharma et al., 2013). Thus, the halo zone observed in Pikovskaya medium indicates that BPF9 functions as a phosphate-solubilizing bacteria primarily through acidification and chelation mechanisms.

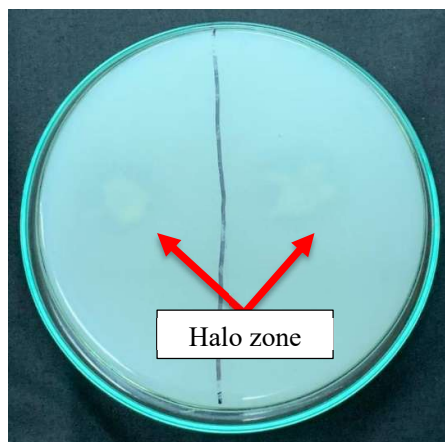


Figure 2. Formation of a halo zone around BPF9, indicating solubilization activity

The comparative size of the clear zone (halo zone) (Figure 2) in relation to the bacterial colony provides a visual measure of the organism's ability to solubilize phosphate. In this study, the solubilization index (SI) of the BPF9 isolate was recorded as 2.0, which classifies it as having an intermediate phosphate-solubilizing ability (Oedjijono et al., 2024). The solubilization of tricalcium phosphate in Pikovskaya medium is primarily attributed to the secretion of low molecular weight organic acids, such as gluconic, citric, or oxalic acids, which reduce pH and chelate metal cations bound to phosphate, thereby releasing soluble orthophosphate (Pande et al., 2017; Paul and Sinha, 2017). Although some studies report that phosphatase enzymes contribute to phosphorus cycling in soils, their activity is generally directed toward the hydrolysis of organic phosphorus compounds rather than inorganic phosphate such as $\text{Ca}_3(\text{PO}_4)_2$ (Situmorang et al., 2015). Thus, in the present study, the clear zone formation is best explained by acidification and chelation mechanisms mediated by organic acids rather than phosphatase enzyme activity. While the halo zone provides a preliminary assessment of phosphate-solubilizing potential, it does not fully represent the actual quantity of phosphate released into the medium. Therefore, a quantitative analysis is necessary to confirm and further evaluate the phosphate-solubilizing efficiency of the isolate.

3.2. Quantitative Estimation of Phosphate Solubilization by Spectrophotometry

The amount of phosphate solubilized by the BPF9 isolate was determined using spectrophotometry, with measurements interpreted through a standard curve prepared from KH_2PO_4 solutions. The curve was established by correlating absorbance readings with predefined phosphate concentrations, allowing the calculation of soluble phosphate levels in parts per million (ppm) within the culture medium. The absorbance measurements reflect both the bacterial growth and phosphate solubilization potential, as the increasing turbidity of the medium corresponds to higher bacterial density and enzymatic activity (Mengesha and Legesse, 2024). In this study, the absorbance was measured at a wavelength of 660 nm, which had been confirmed through preliminary analysis to be the optimal wavelength for detecting phosphate ions. The formation of a blue-colored complex during the reagent reaction indicates the presence of total phosphate in the sample. Phosphate levels were determined by matching the absorbance readings of the samples to the standard curve, with the absorbance of the uninoculated medium used as a baseline to calculate the net phosphate solubilized by the isolate. The standard curve obtained from KH_2PO_4 solutions is presented in Figure 3.

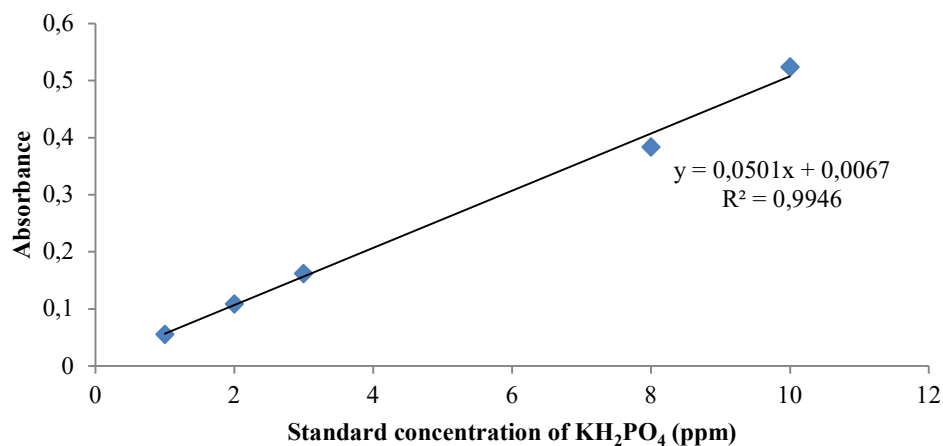


Figure 3. Linear regression curve for quantifying soluble phosphate (KH_2PO_4 standard)

The linear regression equation derived from the curve was $Y = 0.0501X + 0.0067$, with a coefficient of determination ($R^2 = 0.9946$), indicating a very strong linear relationship between phosphate concentration and absorbance. A regression value close to 1.0 confirms the reliability and accuracy of the calibration model. According to Al-zboon and Alharayzeh (2023), an R^2 value between 0.9 and 1.0 indicates a near-perfect positive correlation, making the equation suitable for predicting phosphate concentration based on absorbance data.

Based on this regression model, the BPF9 isolate was found to solubilize phosphate up to 128.5 ppm. This concentration reflects a high solubilization capacity, as isolates capable of releasing more than 100 ppm of soluble phosphate are considered highly efficient and suitable for biofertilizer development (Elfiati et al., 2021). While no direct statistical comparison with other strains was performed in this study, the result suggests that BPF9 possesses a strong metabolic capability for organic acid production, which plays a key role in phosphate solubilization. These findings indicate the potential of BPF9 as a phosphate-solubilizing bacterium that may improve phosphorus availability in soil and support the sustainability of agricultural production systems.

3.3. Growth Curve Profiling of BPF9 Isolate

Understanding the bacterial growth rate is essential for optimizing the use of microbial inoculants in downstream applications such as biofertilizer development. In this study, the growth rate of the phosphate-solubilizing bacterial isolate BPF9 were analyzed using the Haemocytometer method. Growth rate, defined as the relationship between incubation time and bacterial population, helps determine the optimal phase for harvesting cells for further application (Kumakura et al., 2023). Bacteria should be used as working cultures during their optimal growth phase because this affects their performance. Microbial growth can be observed through increases in both cell number and biomass, while the growth rate depends on the physical and chemical conditions of the environment (Jufri, 2020).

Microbial growth occurs through binary fission and typically follows four distinct phases: lag, exponential (log), stationary, and death (Ughy et al., 2023). Microbes in the lag phase initiate metabolic recovery and enzyme synthesis as they adjust to new conditions prior to commencing active proliferation. Although the number of cells does not significantly increase in this phase, cell volume and metabolic readiness improve. In the exponential phase, cells rapidly divide and consume nutrients efficiently. This is the optimal phase for harvesting bacterial cultures due to their high viability and metabolic activity. The stationary phase is characterized by a plateau in growth due to nutrient depletion and accumulation of toxic metabolites. Finally, in the death phase, bacterial populations decline as cellular energy decreases and autolysis occurs (Ughy et al., 2023).

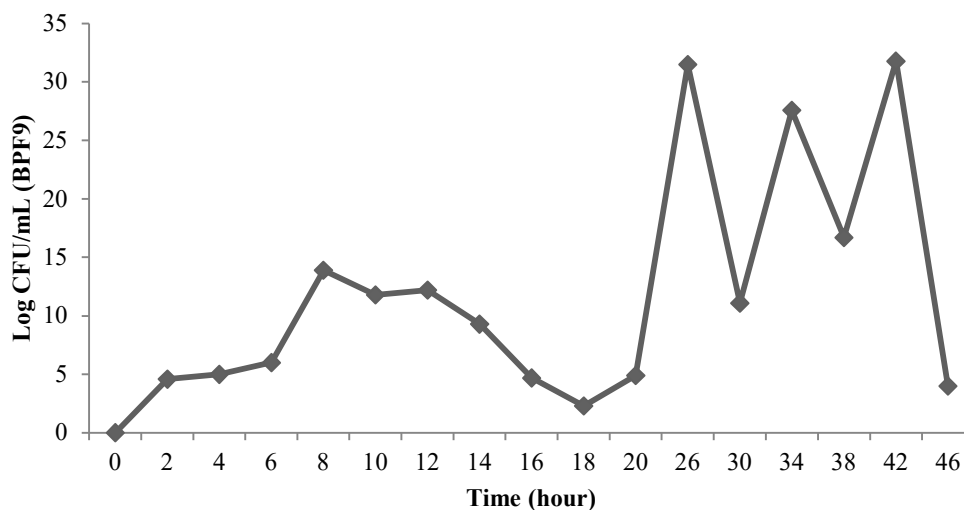


Figure 4. Growth Curve of BPF9 Isolate expressed as log CFU/mL

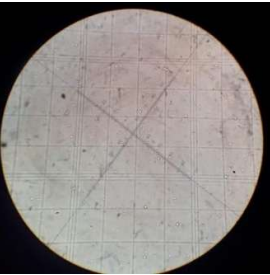
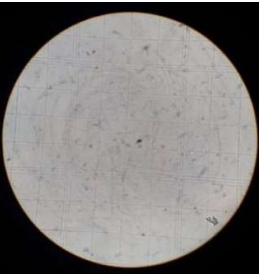
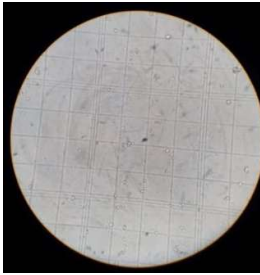
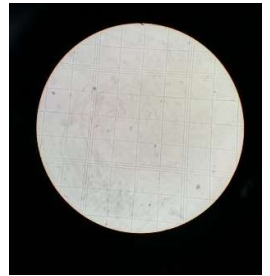
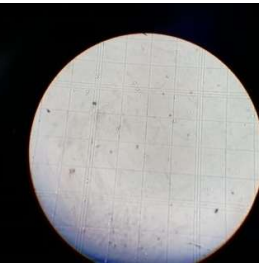
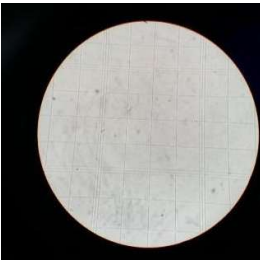
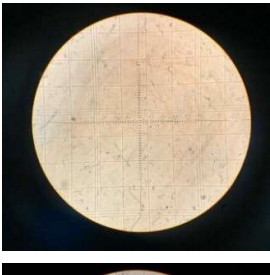
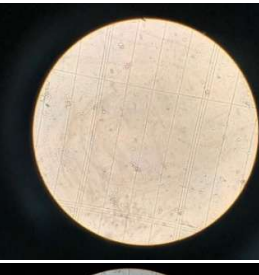
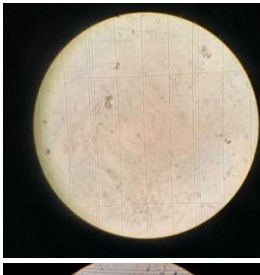
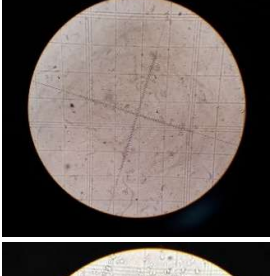
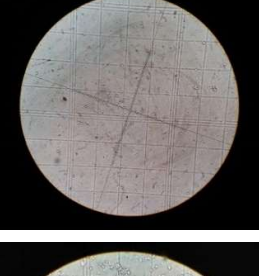
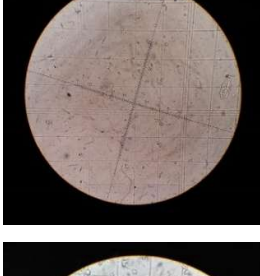
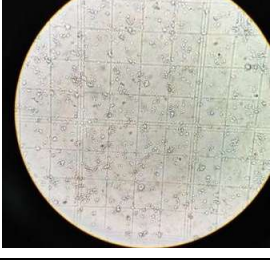
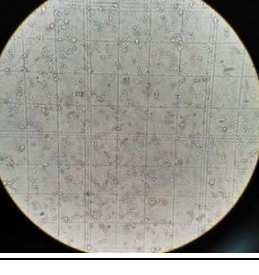
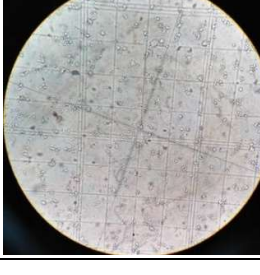
The growth profile of the BPF9 isolate presented in Figure 4 and Table 1, followed the expected bacterial growth curve although with notable fluctuations. In the first 2 hours post-inoculation, a modest increase in cell number was observed (4.6×10^5 CFU/mL), indicating an adaptation period. Similar gradual increases were noted at hours 4 and 6, suggesting that the isolate was still in the lag phase, adjusting to the liquid Pikovskaya medium. Between hours 8 and 18, a temporary decline in cell population was recorded, which may have been influenced by factors such as nutrient imbalance or limited aeration, as continuous shaking was not applied. These fluctuations suggest that while BPF9 does not maintain perfectly consistent growth, it demonstrates resilience and the ability to adapt and recover under changing culture conditions. From hour 18 onward, a marked increase in cell population was observed, reaching 3.15×10^6 CFU/mL at hour 26, indicating the beginning of the exponential phase. The highest cell density was recorded at hour 42, with 3.18×10^6 CFU/mL, representing the peak of active cell division. This period can be considered the optimal harvesting window for further applications, such as biofertilizer formulation, as cells are at their most viable and active state. Thus, despite transient declines, the overall growth dynamics of BPF9 highlight its adaptability and capacity to achieve high population densities, reinforcing its potential as a viable candidate for biofertilizer development. Its adaptability under fluctuating conditions combined with strong phosphate-solubilizing activity, suggests that BPF9 could be effectively incorporated into carrier media and applied in agricultural systems to improve phosphorus availability and promote sustainable crop productivity.

3.4. Development Prospect of BPF9 as Phosphate Solubilizing Bacteria

The phosphate-solubilizing potential of the BPF9 isolate has been thoroughly validated through both qualitative and quantitative assessments, along with growth curve profiling. The growth curve showed an initial sequence of lag, log, stationary, and death phases from 0 to 18 hours, followed by a re-initiation of lag, log, and stationary dynamics between 18 and 42 hours before entering the death phase. This fluctuation may be attributed to the metabolic flexibility of BPF9, including the ability to utilize secondary nutrient sources or adapt to changing culture conditions, which temporarily supports regrowth. Although the pattern appears fluctuating, BPF9 demonstrated the capacity to sustain population density over time without abrupt collapse, indicating a degree of resilience and adaptability in liquid culture. These traits, combined with its strong phosphate-solubilizing ability, highlight the potential of BPF9 as a promising candidate for biofertilizer development in sustainable agricultural systems.

In addition to their ability to solubilize phosphate, phosphate-solubilizing bacteria (PSB) are known to possess a range of plant growth-promoting characteristics. These include the synthesis of phytohormones like indole-3-acetic acid (IAA), production of siderophores to facilitate iron uptake, and secretion of antifungal agents that improve plant health and tolerance to environmental stress (Aliyat et al., 2020). This broad functionality positions BPF9 not only as a biofertilizer but also as a biostimulant capable of enhancing crop performance under diverse growing conditions. While the present study focused on phosphate solubilization and growth profiling, further research could be directed toward assessing additional plant growth-promoting traits of BPF9 to better understand its full agricultural potential.

Table 1. Observation of BPF9 Isolate Growth During 46-Hour Incubation

Time (hour)	Observation 1	Observation 2	Observation 3
0			
2			
18			
26			
42			
46			

Looking ahead, BPF9 may also be formulated as part of a microbial consortium with nitrogen-fixing or potassium-solubilizing bacteria to produce multi-nutrient biofertilizers. Such combinations can offer a broader range of benefits for plant nutrition and soil health, potentially enhancing overall agricultural productivity (Kaur et al., 2022). Overall, BPF9 demonstrates excellent potential as a phosphate-solubilizing inoculant with dual advantages, which are its ability to efficiently solubilize phosphate and its capacity to maintain growth under fluctuating conditions in liquid culture. Its application in agricultural systems could help reduce the reliance on chemical phosphorus fertilizers, lower production costs, and promote environmentally sustainable farming practices. To fully harness its potential, future research should prioritize formulation development, compatibility with various carrier materials, and field-level performance validation.

4. Conclusion

The BPF9 isolate demonstrated considerable potential as a phosphate-solubilizing bacteria, supported by its moderate solubilization index (2.0), high quantitative phosphate release (128.5 ppm), and the ability to maintain growth under fluctuating conditions. The exponential phase was reached at hour 26 and peaked at hour 42, suggesting an optimal window for culture harvesting and inoculant production. The ability of BPF9 to convert insoluble phosphate into bioavailable forms makes it suitable for use as a biofertilizer to enhance phosphorus use efficiency in agricultural soils. Its capacity to maintain growth also supports its compatibility with various carrier formulations. While the present study was limited to laboratory-scale evaluations, further field trials and formulation studies are essential to validate its long-term viability and functional performance under diverse soil conditions. By focusing on these agricultural applications, BPF9 can be further developed as a sustainable input to reduce reliance on chemical fertilizers and promote environmentally friendly farming practices.

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