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European Journal of Biology

European Journal of Biology,
EJB 2025

https://doi.org/10.26650/EurJBiol.2025.1634721
Submitted: 06.02.2025
Revision Requested: 18.03.2025
Last Revision Received: 17.06.2025
Accepted: 27.06.2025
Published Online 15.09.2025

Research Article

Open Access

Evaluation of the Phosphatase Activities of the *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1

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Abstract

Objective: Phosphatase is primarily derived from soil microorganisms, and the composition of soil microbial communities greatly influences the availability of this enzyme source. Therefore, it is crucial to research soil bacteria capable of producing phosphatase. This study evaluated the phosphatase production ability of two bacterial strains, *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1, isolated from agricultural soil under different conditions. This study was conducted *in vitro* to assess the effects of shaking, pH level, salt conditions, and carbon and nitrogen nutrient sources on phosphatase production ability.

Materials and Methods: Experiments were conducted under varying environmental conditions, including different shaking speeds (0, 60, 90, 120, and 150 rpm), medium pH levels (3, 5, 7, and 9), and NaCl concentrations (0, 1, 2, 3, 4, and 5%). The carbon sources used in the experiment were lactose, sucrose, maltose, and glucose, each at a concentration of 1%. The inorganic nitrogen sources were KNO_3, $(NH_4)_2SO_4$, $(NH_4)_6Mo_7O_{24}$, and $CO(NH_2)_2$ at all concentrations of 10%. Each experimental condition was tested in four replications.

Results: *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 exhibited the highest phosphatase production when cultured at a shaking speed of 150 rpm, pH 7, and a NaCl concentration of 1%, with glucose and urea as the carbon and nitrogen sources, respectively.

Conclusion: These findings indicate that optimising the culture conditions of these two bacterial strains significantly enhances phosphatase production.

Keywords *Burkholderia vietnamiensis* 1.7 · Phosphatase · *Priestia aryabhattai* 6.1 · Soil bacteria

Citation: Khanh, ND & Tat, TQ. Evaluation of the Phosphatase Activities of the *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1. Eur J Biol. 2025; Advance Online Publication. DOI: 10.26650/EurJBiol.2025.1634721

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INTRODUCTION

Phosphorus is essential for all living organisms, and cells have developed mechanisms to acquire inorganic phosphate for various biological processes. Phosphorus is the second limiting nutrient in soils that affects plant growth and yield after nitrogen.[1-4] In the phosphorus cycle, phosphatases play crucial roles in the hydrolysis of organophosphorus compounds to inorganic phosphate. Phosphatases catalyse the hydrolysis of both esters and anhydrides of phosphoric acid.[5] Phosphatases can be classified into four main categories: phosphoric monoester hydrolases or phosphomonoesterases (EC 3.1.3), phosphoric diester hydrolases or phosphodiesterases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5), and enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1) and on P–N bonds (EC 3.9). There are two major groups of phosphatases: acid phosphatases, which have an optimal activity at pH 5.0-6.0, and alkaline phosphatases, which exhibit an optimal activity at pH 7.5-8.5.[6-8] Various factors influence phosphatase activity, including soil properties, interactions with soil organisms, plant coverage, leachate input, and the presence of inhibitors and activators.[9]

Among soil microorganisms, Phosphate Solubilising Bacteria (PSB) are considered multidimensional beneficial bacteria.[10,11] Their important features include enhancing plant-available phosphorous content, reducing plant pathogens, promoting plant growth, and improving the response of rhizobacteria.[12] As a result, they are associated with the benefits of sustainable farming practices, increased productivity, cost savings, and improved soil health.[13,14] These multidimensional PSBs are called plant growth-promoting rhizobacteria (PGPR). PGPR are soil bacteria that live around or on the surface of roots and are directly or indirectly involved in stimulating plant growth and development through the production and secretion of various chemicals around the root zone.[15] Among the PGPRs, *Burkholderia* sp. and *Priestia* sp. are considered potential multidimensional PSBs. They increase crop yields by increasing phosphorus solubility, fixing nitrogen in soil, and producing plant growth hormones such as gibberellins, auxins, and cytokinins.[16-18]

In recent years, the Mekong Delta in Vietnam has been facing a growing problem of salinity intrusion. This abiotic stressor negatively affects soil health and agricultural production in the region.[19] In response, various solutions have been proposed, including the use of biofertilizers containing salt-tolerant PGPR bacteria to support crops in withstanding salt stress and enhance the productivity of agricultural soil ecosystems in stressed salt areas.[20,21] Among these solutions, two isolates, *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1, were identified as promising candidates. These two potential PSB strains were isolated from saline rice soil and have shown good ability to solubilise insoluble phosphate under salt stress of up to 5%.[22] However, their phosphatase production activities have not been evaluated. To further evaluate the results of previous studies, this study aimed to evaluate the influence of environmental factors on phosphatase production. The research results will provide parameters for suitable culture environment conditions for the synthesis of phosphatase enzymes by *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1, laying the groundwork for producing microbial products for sustainable agricultural production.

MATERIALS AND METHODS

Preparation of Bacterial Cultures

Bacterial Cultures: *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 from saline soil samples collected from paddy rice fields in Vietnam were cultured in Tryptone Soya Broth (TSB) medium for 3 days under laboratory conditions.[22] The TSB medium contained 30 g. L^{-1} tryptone soya broth. The bacterial biomass of each strain was harvested separately by transferring the entire culture medium. The bacterial population density was adjusted to OD_{660}=0.7 using sterile distilled water. This standardised bacterial inoculum was used in all experiments.

Evaluation of Phosphatase Production in *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1

This experiment was conducted using 100 mL Erlenmeyer flasks with a completely randomised design, with four replicates per treatment. Each flask contained 49 mL of sterilised National Botanical Research Institute's Phosphate (NBRIP) growth medium, 1% sodium chloride (w/v), and 1 mL of adjusted bacterial culture to OD_{660} = 0.7. The NBRIP medium containing 10 g.L^{-1} glucose, 0.25 g.L^{-1} $MgSO_4{\cdot}7H_2O$, 5 g.L^{-1} $Ca_3(PO_4)_2$/sodium phytate/$FePO_4$/$AlPO_4$, 5 g. L^{-1} $MgCl_2{\cdot}7H_2O$, 0.2 g.L^{-1} KCl, 0.1 g. L^{-1} $(NH_4)_2SO_4$, and 2 mL.L^{-1} trace element solution was applied to acclimate and isolate PSB. The trace element solution contained (g. L^{-1}) EDTA, 10; $MnSO_4{\cdot}H_2O$, 2.2; $FeSO_4{\cdot}7H_2O$, 1.0; $CuSO_4{\cdot}5H_2O$, 0.5; $CoCl_2{\cdot}6H_2O$, 0.3; $Na_2MoO_4{\cdot}2H_2O$, 0.2; and $CaCl_2$, 0.1. The initial pH of all media was adjusted to 7.0. A control experiment was conducted under the same conditions but without bacterial inoculation. The flasks were placed on an orbital shaker at 120 rpm and incubated for 10 days at 30°C in the dark. Phosphatase enzyme activity was measured on days 4, 7, and 10 after inoculation. The following method was used: 1 drop of toluene and 1 mL of bacterial cell-free supernatant were added to a glass tube. Subsequently, 4 mL of buffer solution

(2-Amino-2-(hydroxymethyl)propane-1,3-diol and Maleic acid, pH 6.5) and 1 mL of disodium p-nitrophenyl phosphate (0.5 mM) were added. The mixture was then stirred and incubated at 37℃ for 1 h. After incubation, 4 mL of NaOH (0.5 M) and 1 mL of $CaCl_2$ (0.5 M) were added to the solution to terminate the reaction. Subsequently, the sample was filtered using filter paper. The yellow colour of the filtrate indicates positive phosphatase activity. A p-nitrophenol standard series was prepared using the same procedure, except without toluene and 0.5 mM disodium p-nitrophenyl phosphate. The p-nitrophenol concentration in the positive samples was measured at 420 nm using a spectrophotometer. Phosphatase enzyme activity ($U.mL^{-1}$) in all experiments was determined following the method described by Tabatabai and Bremner.[23]

Examination of the Effect of Culture Conditions on Phosphatase Production in *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1

Effect of the Shaking Speed

To investigate the impact of shaking speed on phosphatase synthesis by *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1, an experiment was conducted using different shaking speeds: 0, 60, 90, 120, and 150 rpm. Each shaking speed was considered an experimental treatment with a corresponding control treatment without bacterial inoculation. Each treatment had 4 replicates (n=4), with 4 separate incubation flasks. Bacterial cultures were prepared at an OD_{660}=0.7. For each treatment, 1 mL of the bacterial suspension was added to 49 mL of NBRIP liquid medium (LM). Phosphatase activity was measured 7 days after inoculation.

Effect of NaCl Concentration

This experiment aimed to evaluate the effects of different NaCl concentrations on the phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1. The tested NaCl concentrations were 0%, 1%, 2%, and 3%, with each concentration being treated as an independent experimental condition. A control treatment without microbial inoculation was also included for each concentration of NaCl. Each treatment was conducted in 4 replicates using 4 separate incubation flasks. The optimal shaking speed, as determined in the previous section, was applied in this study. Phosphatase activity was measured 7 days after inoculation.

Effect of the pH Value

An experiment was conducted using different pH levels (3, 5, 7, and 9) in the NBRIP to evaluate the impact of pH variation on phosphatase activity. Each treatment was performed in four replicates using four separate incubation flasks. The optimal shaking speed and NaCl concentration, as determined in the previous section, were applied in this study. Cultures were incubated in the dark for 7 days to assess phosphatase activity.

Effect of the Carbon Source

An experiment was conducted using lactose, sucrose, maltose, and glucose, each at a concentration of 1%, to evaluate the effect of different carbon sources on phosphatase activity. Each treatment was performed in 4 replicates using 4 separate incubation flasks. A control experiment was also conducted without bacterial inoculation. The optimal NaCl concentration and pH value, as determined in the sections "Effect of NaCl concentration" and "Effect of pH value", were applied in this study.

Effect of the Nitrogen Source

The nitrogen sources tested included KNO_3, $(NH_4)_2SO_4$, $(NH_4)_6Mo_7O_{24}$, and $CO(NH_2)_2$, each at a concentration of 10%. A control treatment without bacterial inoculation was also included. The experiment was conducted using optimal shaking, pH, carbon source, and NaCl concentration, in addition to nitrogen sources, as determined in the previous sections.

Statistical Analysis

All the tests were performed four times, and the results are presented as mean ± standard deviation (SD). Statistical significance at the 95% confidence level (p<0.05) was assessed using the Kruskal-Wallis non-parametric test and Minitab 16 software (version 16.2). The Mann-Whitney test was used to determine whether there were significant differences in phosphatase production between the two bacterial strains. Graphs were plotted using Microsoft Excel 2019.

RESULTS

Production of Phosphatase in *Burkholderia vietnamiensis* 1.7 and *Priestia aryabhattai* 6.1 in NBRIP Liquid Culture Medium

Twelve samples for each tested bacterial strain. Figure 1 shows the phosphatase production ability of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 after 4, 7, and 10 days of incubation. The results indicated that the phosphatase activity produced by each strain at different times was statistically different (p<0.05). During the first 7 days, the phosphatase activity of *B. vietnamiensis* 1.7 increased, peaking on day 7 after incubation. However, there was a subsequent decrease in activity from days 7 to 10 after incubation. Despite this decrease, the phosphatase activity on day 10 remained higher than that on day 4. The phosphatase activity of *P. aryabhattai* 6.1 showed

a similar trend to that of *B. vietnamiensis* 1.7. Furthermore, the phosphatase activity between the two strains was not significantly different (p>0.05).

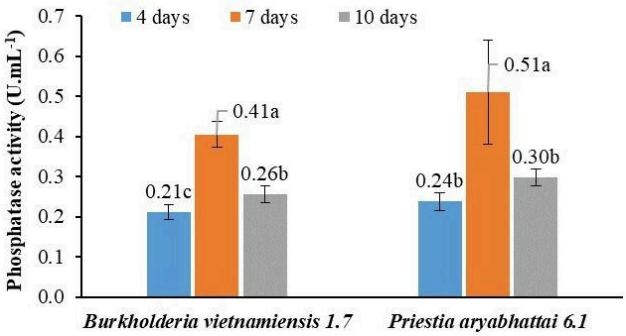


Figure 1. Phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 in NBRIP LM (n = 4). Values are the mean of four independent experiments. Different lowercase letters following the values indicate statistically significant differences at the 95% confidence interval between incubation times for each strain (p<0.05).

Effect of the Shaking Speed

The impact of shaking speed on the phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 in the NBRIP medium (20 samples for each tested strain) is illustrated in Figure 2. The results presented in Figure 2 indicate that the shaking speed significantly influenced the phosphatase activity in both bacterial strains. A positive correlation was observed between shaking speed and enzyme activity, that higher shaking speeds enhance phosphatase production. Specifically, phosphatase activity gradually increased with shaking speed, reaching its highest level at 150 rpm (p<0.05). Although the result in Figure 2 showed that the phosphatase activity of *B. vietnamiensis* 1.7 was consistently lower than that of *P. aryabhattai* 6.1 across all shaking speeds, they were not statistically different (p>0.05).

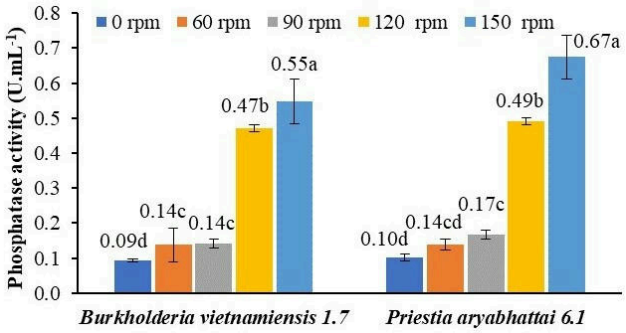


Figure 2. Phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 at different shaking speeds (rpm) in a liquid culture medium (n = 4). Values are the mean of four independent experiments. Different lowercase letters following the values indicate statistically significant differences at the 95% confidence interval between shaking speeds for each strain (p<0.05).

Effect of NaCl Concentration

The impacts of NaCl concentration on the phosphatase activities of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 in the NBRIP medium (16 samples for each tested strain) are illustrated in Figure 3. The results presented in Figure 3 indicate that the NaCl concentration significantly influences the phosphatase activity of the two tested bacterial strains (p<0.05).The highest enzyme activity was observed in a liquid medium supplemented with 1% NaCl, followed by 2%, 0%, and 3% NaCl. The phosphatase activity pattern of *P. aryabhattai* 6.1 was similar to that of *B. vietnamiensis* 1.7. Thus, the two tested bacteria can produce phosphatase enzyme in a liquid medium supplemented with up to 3% NaCl. The optimal NaCl concentration for phosphatase activity was 1%, which was not significantly different (p>0.05).

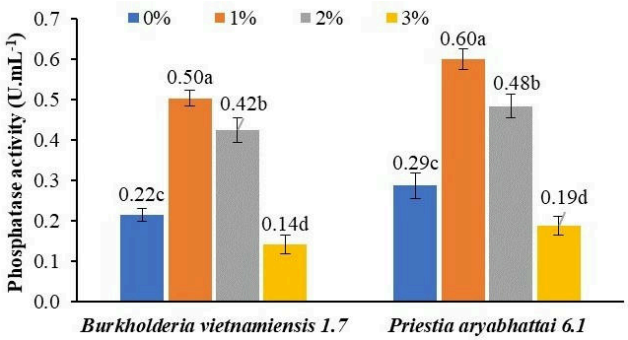


Figure 3. Phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 at different NaCl concentrations (%) in a liquid culture medium at 150 rpm (n = 4). Values are the mean of four independent experiments. Different lowercase letters following the values indicate statistically significant differences at the 95% confidence interval between NaCl concentrations for each strain (p<0.05).

Effect of pH Levels

The impacts of pH on the phosphatase activities of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 in the NBRIP medium (16 samples for each tested strain) are illustrated in Figure 4. The results in Figure 4 indicate that pH significantly influences phosphatase activity in both strains (p<0.05). However, this activity between strains was not statistically different (p>0.05). The highest enzyme activities in both strains were observed at pH 7, followed by pH 9. In contrast, phosphatase activity was considerably lower at pH 5 and reached a minimum at pH 3. This result indicates that neutral pH is the optimal pH for phosphatase activity. It exhibits relatively strong activity at an alkaline pH of 9. However, it is considerably less active in an acidic environment (pH 5), especially when it is almost inactive in an extremely acidic environment (pH 3).

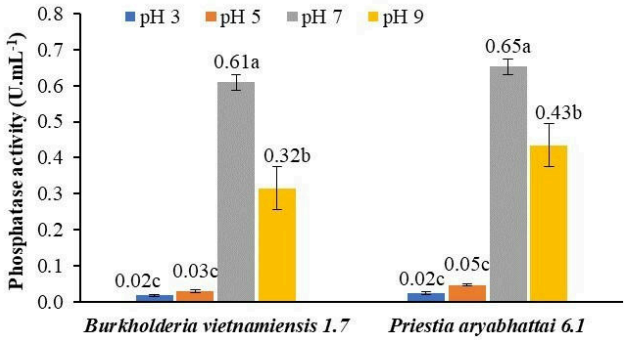


Figure 4. Phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 at different pH levels in a liquid culture medium supplemented with 1% NaCl at 150 rpm (n=4). Values are the mean of four independent experiments. Different lowercase letters following the values indicate statistically significant differences at the 95% confidence interval between pH levels for each strain (p<0.05).

Effect of the Carbon Source

The impacts of carbon sources on the phosphatase activities of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 in the NBRIP medium (16 samples for each tested strain) are illustrated in Figure 5. The results in Figure 5 indicate that the phosphatase activity varied depending on the carbon source used. Both bacterial strains exhibited a similar pattern of carbon source use for phosphatase production. Specifically, glucose was the most suitable carbon source, followed by sucrose, maltose, and lactose. Similarly, to the above results, the enzyme activity between the two tested strains was not significantly different (p>0.05).

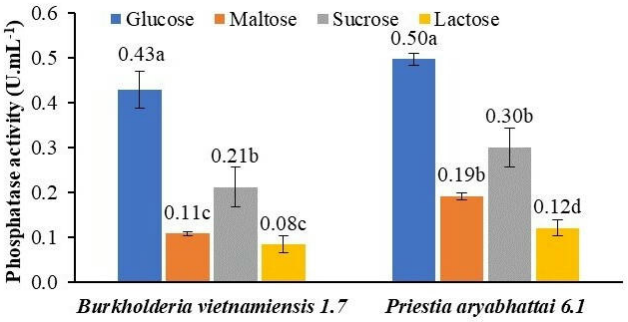


Figure 5. Phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 at 150 rpm and different carbon sources in a liquid culture medium supplemented with 1% NaCl (pH 7) (n=4). Values are the mean of four independent experiments. Different lowercase letters followed the values indicate statistically significant differences at the 95% confidence interval between carbon sources for each strain (p<0.05).

Effect of the Nitrogen Source

The impacts of nitrogen sources on the phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 in the NBRIP medium (16 samples for each tested strain) are illustrated in Figure 6. The results presented in Figure 6 indicate that phosphatase activities varied depending on the nitrogen source used. The phosphatase activity between strains was also not statistically different (p>0.05). They exhibited similar responses to various nitrogen sources. Urea was the most suitable nitrogen source, followed by potassium nitrate, ammonium sulphate, and ammonium molybdate. These findings show that the nitrogen source type plays a crucial role in influencing the phosphatase activity of these bacterial strains.

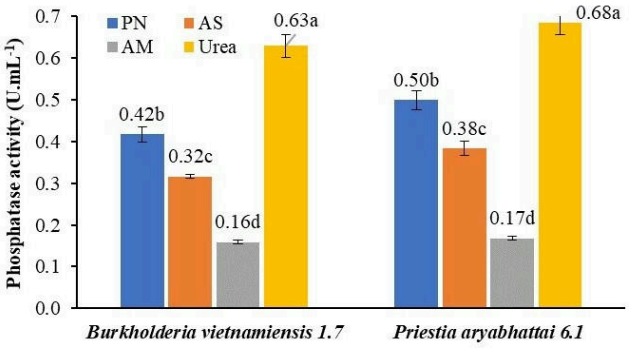


Figure 6. Phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 at different carbon sources in a liquid culture medium supplemented with 1% NaCl, 1% glucose, pH 7, and at 150 rpm (n=4). Values are the mean of four independent experiments. Different lowercase letters following the values indicate statistically significant differences at the 95% confidence interval between nitrogen sources for each strain (p<0.05); PN: potassium nitrate; AS: ammonium sulphate; AM: ammonium molybdate.

DISCUSSION

Various organic substrates and phosphate ions are derived from the hydrolysis of esters, and they catalyse the anhydrides of phosphoric acid through the action of phosphatases.[24,25] Phosphatases are present in the rhizosphere, and these enzymes mineralise organic phosphorus in soil.[26] Organic phosphate is a substrate that is converted into an inorganic form using the catalysis of alkaline and acid phosphatases. The salt concentration, pH, and temperature of the culture medium greatly influence phosphatase enzyme activity. Therefore, determining the optimal conditions for these parameters is essential.[27] In most bacterial species, phosphatase activity is highest at pH 4.0–5.5 and temperatures ranging from 40°C to 70°C.[28-30] However, a study by Behera et al.[31] found that *Serratia* sp. exhibited maximum phosphatase production at 45°C, 100 rpm, and pH 5.0, using glucose as the carbon source and ammonium sulphate as the nitrogen source. Our research indicates that the optimal conditions for phosphatase enzyme activity in *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 occur after 7 days of culture in NBRIP medium at a pH of 7.0, with 1% NaCl, glucose, and urea as the carbon and nitrogen sources, and a shaking speed of 150 rpm.

The speed at which the liquid culture is agitated affects the mixing of oxygen and nutrient availability. Research has shown that varying agitation or shaking speeds can also affect the production of enzymes.[32] Specifically, increasing the shaking speed can increase the oxygen levels in the culture medium; however, this may not necessarily lead to an increase in enzyme production, as the agitation rate can alter the enzyme structure.[33] Conversely, reducing the aeration rate in liquid culture can decrease enzyme production.[34] In the present study, the optimal production of phosphatase by B. *vietnamiensis* 1.7 and *P. aryabhattai* 6.1 was observed at a shaking speed of 150 rpm. In a previous report, the optimal shaking speed for phosphatase production by the *Bacillus megaterium* strain, which was isolated from saline soils in a rice-shrimp farming system in the Mekong Delta, Vietnam, was determined to be 120 rpm.[35] Their shaking speed was lower than the applied speed. The difference in results can depend on the bacterial strain tested.

The results show that the NaCl concentration plays a crucial role in enhancing phosphatase activities in a liquid culture medium. These findings are consistent with those of previous studies, which also demonstrated the significant effect of NaCl concentration on enzyme activity. For example, Bylund et al.[36] the optimal phosphatase activity in *Halomonas elongata* occurred at NaCl concentrations of 1.37 M and 3.4 M. Similarly, Aqel[37] found that a NaCl concentration of 0.5–1% provided the optimal range for phosphatase activity in all tested bacterial strains. Another result supported this view: the optimal NaCl concentration for *B. megaterium* was 1-2%.[35] In the present study, the phosphatase activity of *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 was highest in the 1% NaCl-supplemented liquid culture medium.

The results indicate that the optimal pH for phosphatase activity is neutral, with relatively strong activity at an alkaline pH of 9. Enzyme activity decreased significantly in acidic conditions (pH 5) and was nearly absent at pH 3. A decrease in pH below the optimum value may prevent the formation of the enzyme-substrate complex, as lower pH levels can alter the active sites of amino acids in the enzyme.[38] Padan et al.[39] Suggested that determining optimal growth conditions for bacteria is crucial for maximising crude enzyme production. Generally, most aquatic bacteria exhibit optimal growth within a pH range of 7.0–8.5. In this study, the highest phosphatase production by *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 was observed at pH 7. Similar to this result, a pH 7-adjusted liquid medium was suitable for phosphatase production by the *B. megaterium* strain.[35]

In this study, the parameters for carbon and nitrogen sources were optimised for phosphatase production by *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1. Previous studies have shown that microorganisms' type and concentration of carbon sources greatly influence enzyme production. Glucose and sucrose are suitable carbon sources for phosphatase production in phosphate-solubilising bacteria. These sugars are commonly found in rhizosphere exudates, making them highly compatible with bacteria.[40] In addition, Stefanoni et al.[41] have shown that glucose is a primary substrate for gluconic acid and oxalic acid synthesis, which can increase phosphate-solubilising activity by acidifying the medium and acting as a calcium chelator. In terms of nitrogen, urea is the preferred source, promoting both microbial growth and phosphatase production. This is supported by a study by Nghia et al.[35], who found that the highest phosphatase activity was achieved when urea was used as the main nitrogen source for the *B. megaterium* bacteria strain. However, as noted by Behera et al.[31], not all nitrogen sources have the same effect on enzyme production. Therefore, determining the optimal nitrogen source for each organism is important.

Amongst the various conditions, pH significantly affects acid phosphatase activity. Research has shown that enzyme activity increases with increasing acidity. For instance, Mclachlan[42] reported that phosphatase activity was highest for all species within the pH range of 5.0-6.0. This indicates that changes in pH can alter the charges on amino acids at the active site, hindering the formation of the enzyme-substrate complex. Consequently, enzyme activity decreases.[43] The optimum pH for AG41 phosphatase was determined to be 5.0, and it remained relatively stable within a pH range of 3.0-7.0. Rombola et al.[44] discovered that acid phosphatases isolated from *B. gladioli* maintained stability after 6 h of incubation at 45℃ in 100 mM acetate buffer at pH 6.0. The enzyme hydrolysis rate peaked at pH 6.0. Boyce and Walsh[45] reported that an acid phosphatase produced by Mucor hiemalis Wehmer exhibited maximum activity at 55℃ and a pH range of 5.0-5.5. Another study by Mahesh et al.[43] revealed that the alkaline phosphatase produced by Bacillus spp. isolated from soil samples reached optimum activity at pH 8.8 and 65℃. This study examined the biological production of phosphatase by *Bacillus* sp. AG41, which withstands high temperatures and acidic pH levels. The strain also demonstrated a strong capacity for phosphatase production, activity, and stability under various conditions, as well as the ability to solubilise phosphate from carbon and phosphorus sources. These findings indicate that this strain has significant potential as a biofertilizer. Furthermore, efforts have been made to encapsulate PS bacteria for agricultural use, as these bacteria play a role in both mineral and organic PS, leading to an increase in soluble phosphorus in the soil, stimulation of

root growth, and encouragement of sprouting in various plant species through enhanced P uptake.

CONCLUSION

The highest phosphatase enzyme activity was observed in *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 after 7 days of culture in NBRIP medium at pH 7.0 with 1% NaCl and a shaking speed of 150 rpm. Among the carbon sources tested, glucose had the highest phosphatase activity, whereas urea was the most effective nitrogen source. This indicate that *B. vietnamiensis* 1.7 and *P. aryabhattai* 6.1 have the potential to be used in microbial fertiliser production, thereby reducing the need for chemical phosphate fertilisers and promote safe and sustainable agricultural practices.

Ethics Committee Approval	Ethics committee approval is not required for the study.
Peer Review	Externally peer-reviewed.
Author Contributions	Conception/Design of Study- T.Q.T., N.D.K.; Data Acquisition- T.Q.T., N.D.K.; Data Analysis/Interpretation- T.Q.T., N.D.K.; Drafting Manuscript- T.Q.T., N.D.K.; Critical Revision of Manuscript- T.Q.T., N.D.K.; Final Approval and Accountability- T.Q.T., N.D.K.
Conflict of Interest	Authors declared no conflict of interest.
Financial Disclosure	Authors declared no financial support.

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