

# Plant growth promoting characterization of indigenous phosphate solubilizing rhizobacteria and effects on germination of some crops in Vietnam

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## Abstract

Inorganic Phosphate Solubilizing Bacteria (PSB) are widely present in nature and have been successfully applied in fields in many countries. However, researches on indigenous PSB are still very limited in Vietnam. The objective of this study was to isolate and evaluate the growth promoting characteristics and effects on germination of seed of indigenous PSB for fertilizer production from PSB. Thirteen isolates of indigenous PSB were collected using selective isolation medium containing  $\text{Ca}_3(\text{PO}_4)_2$ , of which eight added the ability to dissolve  $\text{AlPO}_4$  and five isolates added the ability to dissolve  $\text{FePO}_4$ . Initial qualitative tests indicated that all thirteen PSB isolates were incapable of HCN and lipase production while other growth promoting activities including amylase, caseinase, cellulase, chitinase, pectinase, indole acetic acid, K solubility, Zn solubility, and N fixation varied according to PSB isolate. The collected PSB isolates had no effect on seed germination rate, root length, and hypocotyl length of plantules of soybean, rice, maize, cucumber, tomato, and chili by plate assay. This study had shown that PSB was also common in the rhizosphere of various crops in Vietnam. Therefore, isolation to enrich the indigenous PSB collection was essential for the screening of suitable PSB strains for subsequent fertilizer production.

**Keywords:** plant growth promoting activity; phosphate solubilizing bacteria; rhizosphere; seed; solubility index

**Abbreviations:** IAA: indole acetic acid; PSB: phosphate solubilizing bacteria; SI: solubility index

## Introduction

Phosphorus is one of the three most important macronutrients for plants. Traditional agricultural cultivation often uses chemical fertilizers to supplement available sources of phosphorus that are deficient for plants. Meanwhile, there are different insoluble inorganic and organic phosphorus in the soil (De Zutter *et al.*, 2022). These sources of phosphorus, if used, will help reduce the need for chemical fertilizers as well as the negative effects caused by chemical fertilizers. Available phosphorus for plant uptake can be released from these insoluble phosphorus-containing compounds by natural physicochemical processes or by microorganisms (Glick, 2012; de Silva *et al.*, 2021).

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Various fungi and bacteria can solubilize insoluble inorganic phosphorus-bearing minerals or mineralize insoluble organic forms of phosphorus. In which, inorganic phosphate solubilizing bacteria (PSB) can directly and indirectly affect the health and nutritional status of crops is not new information. However, because many issues have not been fully elucidated, studies on PSB have been continuously published in recent years. Examples have included the researches on improving methods for isolation (Patel *et al.*, 2021), understanding biochemical mechanism of dissolution (Yu *et al.*, 2022), the relationship between PSB and soil characteristics (Djuuna *et al.*, 2022).

In addition to phosphate solubilization, PSB also possesses many other plant growth-promoting activities. Other plant growth-promoting activities reported in PSB have included nitrogen fixation (Mei *et al.*, 2021), K solubilization (Aliyat *et al.*, 2022), production of indole acetic acid (Kadmiri *et al.*, 2018; Mei *et al.*, 2021), HCN (Aliyat *et al.*, 2022), generation of siderophores (Mei *et al.*, 2021; Aliyat *et al.*, 2022), secreting extracellular hydrolytic enzymes such as amylase, cellulase, lipase, pectinase, and protease (Lelapalli *et al.*, 2021), and aminocyclopropane-1-carboxylate deaminase activity (Mei *et al.*, 2021). These activities have made PSB-using strategies and results diverse and complex.

According to the results of the meta-analysis of De Zutter *et al.* (2022) from PSB publications in Web of Science from 1976 to 2021, the field efficiency of PSB has varied depending on bacterial strain, soil pH as well as host plant, application method (soil drench, seed coating, root dip, and spray inoculation) and cultivation conditions. Also, according to De Zutter *et al.* (2022), trials have sometimes shown that PSB has increased biomass but not the absorbed amount of P in crops. The unknown complexity of PSB has probably made the inconsistent effect of PSB on crops. In addition, like many other microorganisms, indigenous PSB was more effective than PSB from other regions (Li *et al.*, 2021). Therefore, isolation, screening, and field evaluation are being of interest to researchers for collection of suitable new PSB strains to meet sustainable agricultural development for each location.

PSB has also been interested by Vietnamese researchers for many years. However, these studies have focused on assessing the diversity and role of PSBs in different phosphate-rich ecoregions. PSB screening and application on crops have been very limited in literatures. Publications have also not paid much attention to other plant growth-promoting activities of indigenous PSB strains. Besides, many articles have also indicated that PSB inoculation by seed coating and root dip has been more effective than by soil drench and spray inoculation (De Zutter *et al.*, 2022). Therefore, the *in vitro* seed treatment test has been also considered one of the important initial tests for the subsequent PSB application strategies. There have been no publications have used this test to screen indigenous PSBs in Vietnam. So, this study was performed to isolate and survey plant growth-promoting activities of indigenous PSB as well as evaluate effects of PSB on germination of common crops. The indigenous PSB collection would be used for the development of PSB-containing fertilizers in other further studies.

## Materials and Methods

### *Soil samples*

Samples were randomly collected from the rhizosphere of some mono-cultured common crops in Vietnam. Soil samples were collected, preserved, and processed as described by Amaresan *et al.* (2022). Only one soil sample was taken from each farm and recorded information including sample name, the sampling coordinate, location, and date of sampling.

### *Isolation of PSB*

PSB in rhizospheric soils were isolated using the serial dilution technique and spreading on selective agar medium as Li *et al.* (2021). Pikovskaya medium containing  $\text{Ca}_3(\text{PO}_4)_2$  was used as the selective medium.

$\text{Ca}_3(\text{PO}_4)_2$  was an insoluble phosphorus compound and made the medium an opaque white color. After spreading, the plates were incubated at 30-33 °C for 48-72 h. After incubation, colonies that were surrounded by halo zone and had different morphological characteristics from the same soil sample were selected for purification. The selected colonies were streaked on Luria Bertani agar to obtain the pure isolates. The purified isolates were inoculated onto Luria Bertani slant agar tubes for culture and preservation.

#### *Screening of PSB*

A small amount of bacterial biomass from a 2-day-old slant agar was spot cultured on a plate containing Pikovskaya agar (Amarean *et al.*, 2022). P solubility was determined due to the presence of a transparent resolution ring around the bacterial growth zone. After 5 days of incubation at 30-33 °C, the bacterial growth and dissolution zone diameters were measured to calculate the solubility index (SI).  $\text{SI} = (\text{the dissolution zone diameters} - \text{the bacterial growth zone diameter}) / \text{the bacterial growth zone diameter}$ . A PSB strain with a high SI corresponded to a high P solubility.

#### *Evaluation of the ability to dissolve different types of phosphate*

The PSB isolates were inoculated as spots on Pikovskaya agar with  $\text{Ca}_3(\text{PO}_4)_2$  replaced with  $\text{AlPO}_4$  and  $\text{FePO}_4$ . SI was also evaluated after 5 days of incubation at 30-33 °C.

#### *Plant growth promoting characterization*

PSB isolates had been tested for other plant growth promoting activities. Qualitative assays had included nitrogen fixation test, HCN test, K and Zn solubility tests, indole acetic acid (IAA) test, and extracellular hydrolytic enzymes including chitinase test, caseinase test, and cellulase test performed according to Amarean *et al.* (2022). Qualitative assays for remaining extracellular hydrolytic enzymes included amylase test according to Hazarika *et al.* (2021), pectinase test according to Rat *et al.* (2021), and lipase according to Lanka and Latha (2015).

#### *Evaluation of the effect of PSB on seeds by plate assay*

A bacterial loop was inoculated into 50 ml of Luria Bertani broth and was shaken at 200 rpm for 48 h at 30-33 °C. The culture was then adjusted with Luria Bertani broth to an Optical Density (OD) at 600 nm of 0.5 for preparation of PSB suspension. The tested seeds included seeds of important grain crops (rice, soybean, and maize) and seeds of plants commonly used as models in evaluating the efficiencies of plants growth promoting bacteria (tomato, cucumber, and chili pepper). Seeds were surface-sterilized and inoculated with the bacterial suspension using the seed inoculation technique on filter paper plate according to Jasnić *et al.* (2005). Seeds were inoculated Luria Bertani broth was used for the control. The germination percentage of seeds and the biological parameters of the plantules (hypocotyl length and root length) were evaluated after 7 days of inoculation.

#### *Data analysis*

All experiments were arranged in a completely random design and the results were an average of repetitions. Comparisons of means were made using SPSS v.20.1 (IBM, New York, USA) with one-way analysis of variance (ANOVA), Duncan test at a significance level of  $P < 0.05$ .

## Results

### *Isolation of PSB*

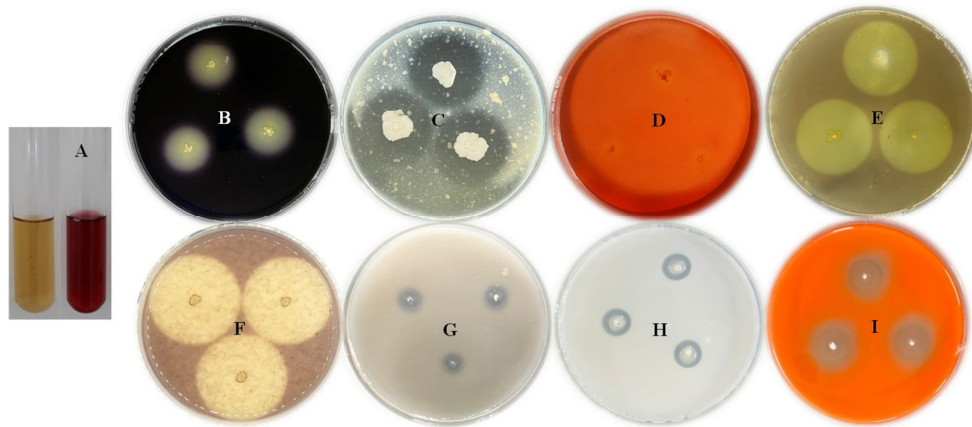
Fifteen soil samples were randomly collected from the rhizosphere of several common crops in different provinces between February 7 and March 3, 2022 (Table 1). After the diluted soil sample was spread on Pikovskaya selective medium, PSB colonies were present with a transparent ring around them due to the solubility of  $\text{Ca}_3(\text{PO}_4)_2$  in the medium (Figure 1). Twenty-three different PSB colony isolates were collected and pureed from 12 out of 15 soil samples (Table 2). Soil samples Đ8, Đ12, and Đ14 did not show the presence of PSB. In general, the number of PSB isolates varied according to the location where the soil sample was taken and the type of crop, ranging from 1 to 3 isolates per sample.

**Table 1.** Information of rhizospheric soil samples

Samples	Locations	Sampling coordinates	Crops	Sampling dates
Đ1	Soc Trang	9°46'12"/105°51'0"	Jackfruit	07/02/2022
Đ2	Long An	10°97'16"/106°32'01"	Sesame	07/02/2022
Đ3	Dong Thap	10°50'32"/105°87'10"	Jackfruit	07/02/2022
Đ4	Dong Thap	10°64'67"/105°78'84"	Jackfruit	07/02/2022
Đ5	Tien Giang	10°40'25"/105°94'46"	Maize	14/02/2022
Đ7	Tien Giang	10°42'67"/105°93'12"	Maize	14/02/2022
Đ8	Binh Duong	10°87'42"/106°78'86"	Green bean	14/02/2022
Đ9	Long An	10°25'48"/106°27'36"	Peanut	14/02/2022
Đ10	Tay Ninh	11°1'48"/106°20'34"	Rice	25/02/2022
Đ11	Tien Giang	10°27'0"/106°10'12"	Rice	25/02/2022
Đ12	Binh Phuoc	11°95'89"/106°79'63"	Cashew	25/02/2022
Đ13	Long An	10°58'12"/106°10'12"	Rice	25/02/2022
Đ14	Long An	11°08'12"/106°05'12"	Rice	25/02/2022
Đ15	Tay Ninh	11°15'36"/106°16'12"	Peanut	03/03/2022
Đ16	Hoc Mon	10°51'0"/106°33'36"	Sesame	03/03/2022

**Table 2.** The collected PSB colony isolates

Samples	Crops	No. of isolate	Names of isolates
Đ1	Jackfruit	1	Đ1.1
Đ2	Sesame	1	Đ2.1
Đ3	Jackfruit	1	Đ3.1
Đ4	Jackfruit	2	Đ4.1, Đ4.2
Đ5	Maize	2	Đ5.1, Đ5.2
Đ7	Maize	3	Đ7.1, Đ7.2, Đ7.3
Đ9	Peanut	3	Đ9.1, Đ9.2, Đ9.3
Đ10	Rice	2	Đ10.1, Đ10.2
Đ11	Rice	2	Đ11.1, Đ11.2
Đ13	Rice	2	Đ13.1, Đ13.2
Đ15	Peanut	2	Đ15.1, Đ15.2
Đ16	Sesame	2	Đ16.1, Đ16.2



**Figure 1.** Some positive PSB isolates on the plant growth promoting characteristics  
 A: IAA of D2.1 isolate (left – negative control); B: amylase of D15.1 isolate; C: caseinase of D13.1 isolate; D: cellulase of D10.1 isolate; E: pectinase of D15.2 isolate; F: chitinase of D3.1 isolate; G: nitrogen fixation of D5.1 isolate; H: Zn solubility of D9.1 isolate; I: mica solubility of D15.2 isolate

*The ability to dissolve  $Ca_3(PO_4)_2$  on agar medium*

Only 13 out of 23 collected PSB isolates (56.52%) were able to dissolve  $Ca_3(PO_4)_2$ . SI of the PSB isolates was statistically significantly different (Table 3). The SI varied randomly depending on the PSB isolate but independent of the isolation source of the isolate and the type of crop.

**Table 3.** The ability of PSB isolates to dissolve  $Ca_3(PO_4)_2$  on agar medium

Names of isolates	SI
D1.1	-
D2.1	1.08 <sup>cf</sup>
D3.1	1.73 <sup>bc</sup>
D4.1	-
D4.2	0.98 <sup>ef</sup>
D5.1	0.20 <sup>fg</sup>
D5.2	-
D7.1	-
D7.2	1.61 <sup>bcd</sup>
D7.3	-
D9.1	1.16 <sup>df</sup>
D9.2	-
D9.3	-
D10.1	2.21 <sup>a</sup>
D10.2	0.37 <sup>gh</sup>
D11.1	-
D11.2	-
D13.1	1.96 <sup>ab</sup>
D13.2	0.93 <sup>cf</sup>
D15.1	1.89 <sup>abc</sup>
D15.2	1.61 <sup>bcd</sup>
D16.1	-
D16.2	1.40 <sup>cd</sup>
Avarage	1.35

-: No appearance of halo zone.

In the same column, means followed by the same letter(s) indicated insignificant differences (Duncan test,  $p < 0.05$ ).

*The ability to dissolve other insoluble inorganic forms of phosphate*

Although all thirteen isolates of PSB were able to dissolve  $\text{Ca}_3(\text{PO}_4)_2$ , only eight were able to dissolve  $\text{AlPO}_4$  and five were able to dissolve  $\text{FePO}_4$  (Table 4). In general, the average SI of PSB isolates on  $\text{AlPO}_4$ ,  $\text{FePO}_4$ , and  $\text{Ca}_3(\text{PO}_4)_2$  did not have a big difference and ranged from 1.18 to 1.35. The D10.2 isolates has the highest SI among the  $\text{AlPO}_4$  solubility isolates. Meanwhile, most of  $\text{FePO}_4$ -solubilizing isolates have similar SI.

**Table 4.** The ability to dissolve  $\text{AlPO}_4$  and  $\text{FePO}_4$  of PSB isolates

Isolates	SI	
	$\text{AlPO}_4$	$\text{FePO}_4$
D2.1	-	-
D3.1	1.22 <sup>bc</sup>	-
D4.2	-	-
D5.1	1.41 <sup>b</sup>	1.48 <sup>a</sup>
D7.2	0.85 <sup>cd</sup>	-
D9.1	-	-
D10.1	0.98 <sup>c</sup>	1.35 <sup>a</sup>
D10.2	2.01 <sup>a</sup>	-
D13.1	1.44 <sup>b</sup>	1.27 <sup>ab</sup>
D13.2	0.55 <sup>d</sup>	-
D15.1	-	1.33 <sup>a</sup>
D15.1	0.96 <sup>c</sup>	-
D16.2	-	0.94 <sup>b</sup>
Average	1.18	1.30

In the same column, means followed by the same letter(s) indicated insignificant differences (Duncan test,  $p < 0.05$ ).

*Plant growth promoting characteristics of the PSB isolates*

PSB may possess many other activities that directly or indirectly regulate plant growth. The PSB isolates in this study were evaluated for some common activities that are often of interest to researchers. The results showed that all 13 PSB isolates were incapable of lipase and HCN production (Table 5). For the remaining surveyed activities, it changed according to the PSB isolate. Some isolates possessed potential activity such as the six PSB isolates that had high IAA production with a positive expression as dark red (Figure 1A), the D15.2 isolate and D3.1 isolate had large halo zones on respectively pectin and chitin substrates (Figures 1E and 1F). However, quantitative data were not included in this study.

**Table 5.** Plant growth promoting characteristics of the PSB isolates

Activities	Isolates												
	Đ2.1	Đ3.1	Đ4.2	Đ5.1	Đ7.2	Đ9.1	Đ10.1	Đ10.2	Đ13.1	Đ13.2	Đ15.1	Đ15.2	Đ16.2
Amylase	-	-	-	-	-	-	+	-	+	-	+	-	-
Cascinase	-	-	-	-	-	-	+	-	+	-	+	-	-
Cellulase	-	-	-	-	-	-	+	-	-	-	+	-	-
Chitinase	-	+	+	-	+	-	+	-	+	+	+	+	-
Lipase	-	-	-	-	-	-	-	-	-	-	-	-	-
Pectinase	-	+	-	-	-	-	-	-	-	-	+	+	-
IAA	+	+	-	-	-	-	+	-	+	-	+	-	+
HCN	-	-	-	-	-	-	-	-	-	-	-	-	-
K solubility	+	-	-	+	+	-	-	+	-	+	-	+	-
Zn solubility	+	-	-	-	-	+	-	+	-	-	-	-	-
N fixation	+	-	-	+	+	+	-	+	-	-	-	-	+

+: positive; -: negativic

*Effects of PSB isolates on seeds in plate assay*

The results of seed inoculation on filter paper plate showed that none of the thirteen PSB isolates had any effect compared with the control on the percentage of seeds germinated (Table 6), root length (Table 7), and the length of hypocotyl of plantule (Table 8) of soybean, rice, maize, cucumber, tomato, and chili. There were not insignificant difference at  $P < 0.05$ . It was interesting that there was no any abnormality (compared to the control) of the seeds that did not germinate and the plantules (Figure 2).

**Table 6.** Effects of PSB isolates on germination rate (%) of seeds

PSB isolates	Soy	Ric	Mai	Cuc	Tom	Chi
Đ2.1	61.7	68.3	98.3	91.7	88.3	88.3
Đ3.1	48.3	73.3	100.0	96.7	81.7	98.3
Đ4.2	40.0	71.7	95.0	83.3	70.0	95.0
Đ5.1	46.7	48.3	95.0	93.3	90.0	96.7
Đ7.2	55.0	65.5	100.0	96.7	93.3	90.0
Đ9.1	56.7	76.7	91.7	83.3	96.7	100.0
Đ10.1	45.0	80.0	95.0	88.3	90.0	100.0
Đ10.2	51.7	88.3	86.7	91.7	90.0	93.3
Đ13.1	67.7	71.7	86.7	95.0	85.0	98.3
Đ13.2	40.0	73.3	100.0	96.7	93.3	96.7
Đ15.1	60.0	78.3	95.0	88.3	91.7	98.3
Đ15.2	58.4	73.3	93.3	90.0	90.0	98.3
Đ16.2	41.7	80.0	90.0	95.0	93.3	100.0
Control	43.3	56.7	91.7	91.7	88.3	98.3

Soy: soybean, Ric: rice, Mai: maize, Cuc: cucumber, Tom: tomato, and Chi: chili.

In the same column, means were not significantly different (Duncan test,  $p < 0.05$ ).

**Table 7.** Effects of PSB isolates on root length (cm) of plantules

PSB isolates	Soy	Ric	Mai	Cuc	Tom	Chi
Đ2.1	1.60	3.33	9.64	4.51	2.27	1.69
Đ3.1	1.43	3.75	10.03	4.61	3.59	1.85
Đ4.2	1.86	3.15	7.87	4.91	3.81	2.36
Đ5.1	3.76	2.49	8.16	6.31	3.96	2.31
Đ7.2	2.56	3.47	8.52	6.56	4.45	1.86
Đ9.1	3.39	3.85	7.41	6.42	4.67	2.36
Đ10.1	1.84	4.00	8.32	6.08	4.47	1.99
Đ10.2	2.58	3.22	8.68	7.21	3.27	1.80
Đ13.1	1.19	4.02	7.74	8.59	4.29	1.72
Đ13.2	2.97	3.69	8.94	6.78	4.94	1.58
Đ15.1	2.56	3.91	8.57	6.66	4.37	2.14
Đ15.2	3.45	2.81	7.57	5.65	4.38	2.07
Đ16.2	1.31	3.28	8.91	5.89	4.55	2.08
Control	1.24	3.42	6.11	6.67	3.63	1.96

Soy: soybean, Ric: rice, Mai: maize, Cuc: cucumber, Tom: tomato, and Chi: chili.

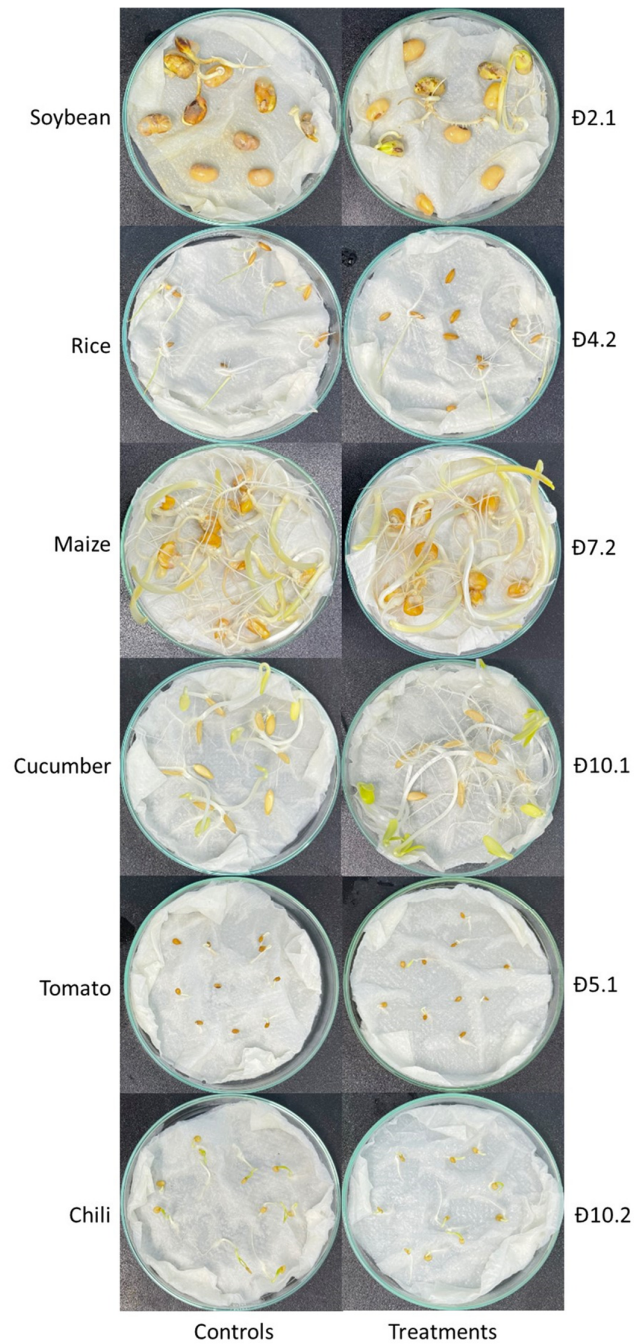
In the same column, means were not significantly different (Duncan test,  $p < 0.05$ ).

**Table 8.** Effects of PSB isolates on hypocotyl length (cm) of plantules

PSB isolates	Soy	Ric	Mai	Cuc	Tom	Chi
Đ2.1	2.14	1.37	4.44	4.48	2.62	1.55
Đ3.1	1.90	1.44	5.43	5.00	2.72	1.62
Đ4.2	2.04	1.64	4.68	4.01	2.66	2.52
Đ5.1	3.56	1.27	3.89	3.84	2.94	1.50
Đ7.2	2.93	1.54	4.16	5.29	2.81	1.00
Đ9.1	3.32	2.05	3.87	4.27	2.73	1.73
Đ10.1	1.78	1.44	4.37	4.32	2.89	1.61
Đ10.2	2.63	1.73	4.62	4.05	2.71	1.17
Đ13.1	1.69	2.84	3.09	4.89	2.72	1.29
Đ13.2	2.33	1.59	5.61	5.28	3.22	1.55
Đ15.1	2.24	1.60	3.85	5.17	2.97	1.34
Đ15.2	3.49	1.29	4.07	4.24	2.89	1.46
Đ16.2	2.39	1.99	4.05	4.34	2.90	1.39
Control	1.87	1.67	4.40	4.78	2.92	1.37

Soy: soybean, Ric: rice, Mai: maize, Cuc: cucumber, Tom: tomato, and Chi: chili.

In the same column, means were not significantly different (Duncan test,  $p < 0.05$ ).



**Figure 2.** PSB-inoculated seeds after 7-day incubation

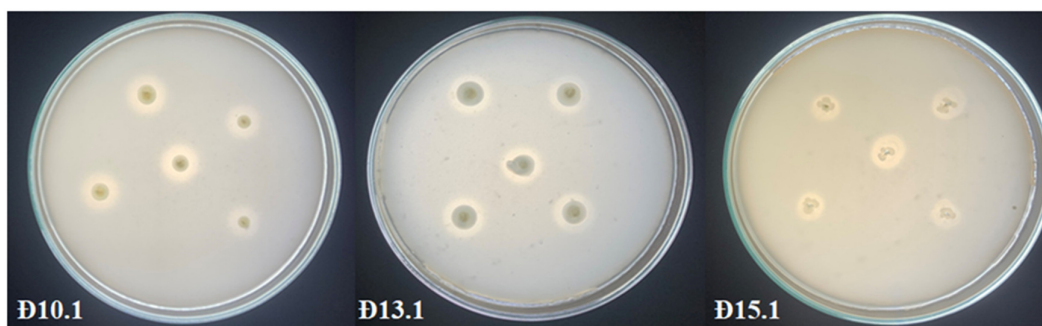
### Discussion

The number of PSB isolates that were collected varied from soil-to-soil sample as has been previously reported by many authors. Notably, in this study, 23 PSB isolates were collected from 12 soil samples (Table 3) and the average was two isolates per sample, much lower than previously publications. For example, Li *et al.* (2021) collected 17 PSB isolates from the soil with saline condition at sea ox-eye daisy site and 4 PSB isolates

collected from the soil from the rhizosphere of bluestem at the rocky land site. Besides, for the same crop, the number of PSB isolates obtained in this study was significantly lower than which published in the world. For example, with maize, Vinod Babu *et al.* (2017) obtained an average of 6 PSB isolates per sample while the results here had only obtained an average of 2 isolates per sample. Thereby, there was the low diversity of PSB in the surveyed soil samples. The cause was possibly due to the natural conditions of the surveyed samples, such as soil type, growing pattern, soil pH, availability nutrients, and climatic conditions (Zhou *et al.*, 2016). In addition, biodiversity loss may have occurred due to the influence of chemical fertilizers and pesticides. Long-term chemical fertilization, such as in traditional agricultural farms in Vietnam, has been reported to lower soil pH and affecting microbial diversity (Sun *et al.* 2015). These results also partly reflected the degradation situation in the agricultural ecosystems in Vietnam even though only a few soil samples were surveyed. This evidence also indicated that sustainable agricultural production as well as preparation of products to support sustainable agricultural production, including microbial fertilizers such as PSB, should be paid more attention.

After isolation and purification, some PSB isolates did not exhibit the activity (solubility of  $\text{Ca}_3(\text{PO}_4)_2$ ) which may have been due to an inappropriate storage medium (Angshumanjana *et al.*, 2016). The repeated subcultures decreased the activity of two bacterial strains antagonizing *Staphylococcus aureus* was mentioned by Neha *et al.* (2019). In addition, the activity of bacterial strains could be also affected by cold storage conditions. In 2015, Kandil and El Soda showed the intracellular enzymatic activity of 26 strains of *Lactobacillus* spp. significantly decreased after 6 months of frozen storage. Abdelsamei *et al.* (2015) reported that the antibacterial activity of *Lactobacillus acidophilus* decreased after 90 days of storage at 4 °C. This was supposed to be because there was an osmotic imbalance that caused physiological and biochemical changes and cold lesions that killed the bacterial cells.

Several components synthesized by PSB involved in the dissolution of inorganic phosphate have been identified such as low-molecular-weight organic acids, inorganic acids, protons, and other chelating compounds. Some PSBs already have had exopolysaccharides that have been also thought to be involved in phosphate solubilization. However, the phosphate solubilization of PSB strains on agar was probably absent from exopolysaccharides because a direct contact between PSB and the phosphate source was required. Meanwhile, the remaining components were able to diffuse in the agar to form a soluble ring around the growth region of PSB (Figure 3). The difference in the phosphate solubility of the PSB isolates was possible because of the variety and content of these components as well as the source of insoluble phosphate as discussed by Vyas and Gulati (2009), Yu *et al.* (2022), etc.



**Figure 3.**  $\text{Ca}_3(\text{PO}_4)_2$  solubility of some PSB isolates on Pikovskaya medium after 5 days of incubation

To date, there has been no literature showing the minimum SI has been required for a potential PSB. El Habil-Addas *et al.* (2017) randomly selected the top ten PSB isolates with SI > 1.44 for further research. The reason may have been that the SI-based screening of PSB on agar was not entirely consistent with the PSB screening based on the phosphate content released in the liquid medium as reported by El Habil-Addas *et al.* (2017), Suleman *et al.* (2018), and more recently by Amy *et al.* (2022). The second may also be because of the

efficiencies, when PSB was applied to crops, did not correspond to SI as reported by De Zutter *et al.* (2022). Shahinur *et al.* (2014) suggested that bacteria with SI > 2 were considered PSB. Meanwhile, Amy *et al.* (2022) suggested that bacteria with an SI > 1 when tested on at least one Pikovskaya agar or the National Botanical Research Institute's phosphate growth agar medium were considered potential PSBs. Most of the 13 collected bacterial isolates that were able to solubilize  $\text{Ca}_3(\text{PO}_4)_2$  (Table 4) were considered PSBs according to Amy *et al.* (2022) while only the Đ10.1 isolate was considered PSB according to Shahinur *et al.* (2014). It was clear that PSB screening criteria were inconsistent and highly subjective.

In this study, the number of isolates that were able to dissolve  $\text{FePO}_4$  and  $\text{AlPO}_4$  was lower than that of  $\text{Ca}_3(\text{PO}_4)_2$ . The solubilization efficiency of PSB isolates was also dependent on the insoluble phosphate form. For example, the Đ10.1 isolate had the highest SI on  $\text{Ca}_3(\text{PO}_4)_2$  (Table 3) while the Đ05.1 isolate had the highest SI on  $\text{AlPO}_4$  (Table 4). SIs of each PSB isolate on  $\text{FePO}_4$  and  $\text{AlPO}_4$  were lower on  $\text{Ca}_3(\text{PO}_4)_2$  showed that  $\text{Ca}_3(\text{PO}_4)_2$  was dissolved more readily than  $\text{FePO}_4$  and  $\text{AlPO}_4$ . Previously, Wasoontharawat *et al.* (2017) and (Aliyat *et al.*, 2022) also mentioned that  $\text{Ca}_3(\text{PO}_4)_2$  was a more readily solubilized phosphate form than  $\text{FePO}_4$  and  $\text{AlPO}_4$  by bacteria both in agar and in liquid media. The higher amount of soluble phosphorus from  $\text{Ca}_3(\text{PO}_4)_2$  compared with  $\text{FePO}_4$  and  $\text{AlPO}_4$  was also reported by de Oliveira Mendes *et al.* (2014) on many fungal strains of the genera *Aspergillus*, *Penicillium*, and *Eupenicillium*. Therefore,  $\text{Ca}_3(\text{PO}_4)_2$  was probably suitable for initial detection of PSBs. However, PSB screening based on other phosphate forms should be performed after primary screening on  $\text{Ca}_3(\text{PO}_4)_2$  agar to obtain suitable target PSB strains. PSB screening based on only one phosphate source ( $\text{Ca}_3(\text{PO}_4)_2$ ) was not suitable because there are different sources of insoluble phosphate in nature (Bashan *et al.*, 2013). The  $\text{Ca}_3(\text{PO}_4)_2$ -solubilizing isolates were suitable for application in alkaline soils while the  $\text{FePO}_4$ - and/or  $\text{AlPO}_4$ -solubilizing isolates were suitable for application in acid soils. The isolates including Đ5.1, Đ10.1, and Đ13.1 were the more potential isolates because of their ability to dissolve all three forms of phosphate. However, which PSB isolate was appropriate depended on the specific intended use.

The results in this study again showed that PSB could simultaneously possess different plant growth promoting activities depending on the bacterial strain. These results were consistent with many previous literatures. For example, Lelapalli *et al.* (2021) had shown that PSB strains were able or not to produce amylase and/or lipase. Walpola *et al.* (2012) reported no PSB strains producing protease and chitinase. Meanwhile, Iyer *et al.* (2017) reported that all strains secreted IAA and 11 strains hydrolyzed chitin among 24 evaluated PSB strains. Padder *et al.* (2022) also mentioned that all 10 strains of PSB were able to secrete chitinase. Also, according to Padder *et al.* (2022), out of 10 PSB strains, there were two siderophore-producing strains, four HCN-producing strains, and three nitrogen-fixing strains. In this study, out of thirteen isolates, there were three amylase and caseinase co-synthesizing isolates, two cellulase producing isolates, three pectinase producing isolates, and eight chitinase producing isolates.

Extracellular hydrolytic enzymes have played an important role in the metabolism of organic polymers that improve soil health (da Silva *et al.*, 2021). These enzymes, especially chitinase, cellulase and protease, have also been important in the biological control of fungal and bacterial phytopathogens (Mishra *et al.*, 2020), insects (Lee *et al.*, 2022), and nematodes (Migunova and Sasanelli, 2021). The isolates that had the ability to fix nitrogen or dissolve K and Zn-bearing minerals could produce available N, K, and Zn nutrients for plants (Glick, 2012). Also, according to Glick (2012), IAA were one of the important phytohormones that stimulate plant growth. There were six PSB isolates were able to produce IAA. In addition to the activities investigated in this study, other activities such as biological control of pathogenic fungi and bacteria, nematodes, ect., could also need to be evaluated on the selected PSB isolates. An overall assessment of different plant growth promoting traits helps to effectively exploit PSBs' potential.

Statistics from PSB publications by De Zutter *et al.* (2022) showed that the application of PSB on crops had different effects. In which, root dip gave the highest efficiency, then seed coating. However, different effects

on germination rate of seed and on biological parameters of plantule when seeds have been treated with PSB have also been mentioned in many literatures. For example, positive or neutral effects on the germination rate of wheat seeds treated with different strains of PSB was reported by Batool and Iqbal (2018). PSB also had a negative effect on treated seeds such as *Pseudomonas fluorescens* KACC 10327 reduced the germination rate of tomato seeds compared with untreated control (Pathak et al, 2017). Despite this, *P. fluorescens* KACC 10327 still had positive effects on tomato seedlings when the bacteria were applied to the soil.

Therefore, evaluation of the effect of PSB on seeds by plate assay could provide preliminary information on the application method of PSB strains. In this study, thirteen PSB isolates were examined on the germination of six different crops in plate assay. Soybean, rice, maize, cucumber, tomato, and chili are popular crops in Vietnam. Except for rice, the remaining crops have also been considered as plant models for various studies related to the application of beneficial microorganisms including PSB. All thirteen PSB isolates had a neutral effect compared with the control on parameters including germination rate of seed, root length and hypocotyl length of plantule. Although there were no positive effects, the PSB isolates also did not cause any negative effects on germination of the seeds. This has shown that these isolates could be applied for seed treatment before sowing. Seed treatment may make it easier for PSB to colonize the rhizosphere of plants, then contribute to PSB's efficiency in later growth stages (De Zutter *et al.*, 2022).

## Conclusions

PSB also were prevalent in the rhizosphere of various crops in Vietnam. Of the thirteen collected PSB isolates that were able to dissolve  $\text{Ca}_3(\text{PO}_4)_2$ , eight were able to dissolve  $\text{AlPO}_4$  and five were able to dissolve  $\text{FePO}_4$ . The PSB isolates also possessed one or more other directly or indirectly plant growth promoting activities including amylase, caseinase, cellulase, chitinase, pectinase, indole acetic acid, K solubility, Zn solubility, and N fixation. None of these thirteen PSB isolates were capable of producing lipase and HCN. These PSB also had no effect on seed germination percentage, root length, and hypocotyl length of plantules of crops including soybean, rice, maize, cucumber, tomato, and chili. Further evaluations such as identification, bio-safety, efficiency in crop in pot and in field, etc. needed to be performed in order to assess potential applications of the indigenous PSB isolates in the collection.

## Authors' Contributions

HQN sampled, performed the experiments, data analysis, and wrote the results. MN sampled, performed the seed experiments, and participated in the data analysis. NNN designed the experiments, wrote the protocol, managed the literature searches, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Abdelsamei HA, Ibrahim EM, Sohaimy SA, Sad MA (2015). Effect of storage on the activity of the bacteriocin extracted from *Lactobacillus acidophilus*. Benha Veterinary Medical Journal 28(1):216-222.
- Aliyat FZ, Maldani M, El Guilli M, Nassiri L, Ibjibjen J (2022). Phosphate-solubilizing bacteria isolated from phosphate solid sludge and their ability to solubilize three inorganic phosphate forms: calcium, iron, and aluminum phosphates. Microorganisms 2022(10):980. <https://doi.org/10.3390/microorganisms10050980>
- Amarean N, Patel P, Amin D (2022). Practical handbook on agricultural microbiology. Humana Press. New York.
- Amy C, Avice J-C, Laval K, Bressan M (2022). Are native phosphate solubilizing bacteria a relevant alternative to mineral fertilizations for crops? Part I. When rhizobacteria meet plant P requirements. Rhizosphere 21(2022):100476. <https://doi.org/10.1016/j.rhisp.2022.100476>
- Angshumanjana A, Dipjitdey A, Jayantabikashdey TN (2016). Selection of storage methods for maintenance of different stock cultures. International Journal of Current Microbiology and Applied Sciences 5(10):1097-1104. <http://dx.doi.org/10.20546/ijcmas.2016.510.115>
- Bashan Y, Kamnev AA, de-Bashan LE (2013). Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. Biology and Fertility of Soils 49:465-479. <https://doi.org/10.1007/s00374-012-0737-7>
- Batool S, Iqbal A (2018). Phosphate solubilizing rhizobacteria as alternative of chemical fertilizer for growth and yield of *Triticum aestivum* (Var. Galaxy 2013). Saudi Journal of Biological Sciences 26:1400-1410. <https://doi.org/10.1016/j.sjbs.2018.05.024>
- da Silva GJ, Rivadavea WR, de Lima JD, Monteiro PHR, da Silva FB (2021). Microbial enzymes and soil health. In: Mohamed HI, El-Beltagi HEDS, Abd-Elsalam KA (Eds). Plant growth-promoting microbes for sustainable biotic and abiotic stress management. Springer, Cham pp 133-155.
- de Oliveira Mendes G, Moreira de Freitas AL, Liparini Pereira O, Ribeiro da Silva I, Bojkov Vassilev N, Dutra Costa M (2014). Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Annals of Microbiology 64:239-249. <https://doi.org/10.1007/s13213-013-0656-3>
- De Zutter N, Ameye M, Bekaert B, Verwaeren J, De Gelder L, Audenaert K (2022). Uncovering new insights and misconceptions on the effectiveness of phosphate solubilizing rhizobacteria in plants: A meta-analysis. Frontiers in Plant Science 13:858804. <https://doi.org/10.3389/fpls.2022.858804>
- Djuuna IAF, Prabawardani S, Massora M (2022). Population distribution of phosphate-solubilizing microorganisms in agricultural soil. Microbes and Environments 37(1):ME21041. <https://doi.org/10.1264/jsme2.ME21041>
- El Habil-Addas F, Aarab S, Rfaki A, Laglaoui A, Bakkali M, Arakrak A (2017). Screening of phosphate solubilizing bacterial isolates for improving growth of wheat. European Journal of Biotechnology and Bioscience 2(6):7-11.
- Glick BR (2012). Plant growth promoting bacteria: Mechanisms and applications. Scientifica 2012:963401. <https://doi.org/10.6064/2012/963401>
- Hazarika SN, Saikia K, Borah A, Thakur D (2021). Prospecting endophytic bacteria endowed with plant growth promoting potential isolated from *Camellia sinensis*. Frontiers in Microbiology 12:738058. <https://doi.org/10.3389/fmicb.2021.738058>
- Iyer B, Rajput MS, Rajkumar S (2017). Effect of succinate on phosphate solubilization in nitrogen fixing bacteria harbouring chick pea and their effect on plant growth. Microbiological Research 202:43-50. <https://doi.org/10.1016/j.micres.2017.05.005>
- Jasnić SM, Miloš VB, Bagi F, Djordjevic V (2005). Pathogenicity of *Fusarium* species in soybean. Matica Srpska Proceedings for Natural Sciences 109:113-121. <https://doi.org/10.2298/ZMSPN0519113J>
- Kadmiri IM, Chaouqui L, Azaroual SE, Sijilmassi B, Yaakoubi K, Wahby I (2018). Phosphate-solubilizing and auxin-producing rhizobacteria promote plant growth under saline conditions. Arabian Journal for Science and Engineering 43:3403-3415. <https://doi.org/10.1007/s13369-017-3042-9>

- Kandil S, El Soda M (2015). Influence of freezing and freeze drying on intracellular enzymatic activity and autolytic properties of some lactic acid bacterial strains. *Advances in Microbiology* 5:371-382. <http://dx.doi.org/10.4236/aim.2015.56039>
- Lanka S, Latha NL (2015). A short review on various screening methods to isolate potential lipase producers: Lipases-the present and future enzymes of biotech industry. *International Journal of Biological Chemistry* 9:207-219.
- Lee JH, Anderson AJ, Kim YC (2022). Root-associated bacteria are biocontrol agents for multiple plant pests. *Microorganisms* 10:1053. <https://doi.org/10.3390/microorganisms10051053>
- Lelapalli S, Baskar S, Jacob SM, Paranthaman S (2021). Characterization of phosphate solubilizing plant growth promoting rhizobacterium *Lysinibacillus pakistanensis* strain PCPSMR15 isolated from *Oryza sativa*. *Current Research in Microbial Sciences* 4(2):100080. <https://doi.org/10.1016/j.crmicr.2021.100080>
- Li KS, Zeghbrouck JV, Liu Q, Zhang S (2021). Isolating and characterizing phosphorus solubilizing bacteria from rhizospheres of native plants grown in calcareous soils. *Frontiers in Environmental Science* 9:802563. <https://doi.org/10.3389/fenvs.2021.802563>
- Mei C, Chretien RL, Amaradasa BS, He Y, Turner A, Lowman S (2021). Characterization of phosphate solubilizing bacterial endophytes and plant growth promotion *in vitro* and in greenhouse. *Microorganisms* 9(9):1935. <https://doi.org/10.3390/microorganisms9091935>
- Migunova VD, Sasanelli N (2021). Bacteria as biocontrol tool against phytoparasitic nematodes. *Plants* 10(2):389. <https://doi.org/10.3390/plants10020389>
- Mishra P, Mishra J, Dwivedi SK, Arora NK (2020). Microbial enzymes in biocontrol of phytopathogens. In: Arora N, Mishra J, Mishra V (Eds). *Microbial enzymes: Roles and applications in industries*. microorganisms for sustainability 11. Springer, Singapore pp. 259-285. [https://doi.org/10.1007/978-981-15-1710-5\\_10](https://doi.org/10.1007/978-981-15-1710-5_10)
- Neha VH, Visnuvinayagam S, Murthy LN, Jeyakumari A, Sivaraman GK, Prasad MM (2019). Characterization of vibriocin and prospect of co-culture method to overcome the diminishing antibacterial activity. *International Journal of Current Microbiology and Applied Sciences* 8(10):67-681. <https://doi.org/10.20546/ijcmas.2019.810.076>
- Padder SA, Rather RA, Bhat SA, Shah MD, Baba TR, Mubarak NM (2022). Dynamics, phylogeny and phyto-stimulating potential of chitinase synthesizing bacterial root endosymbiosome of North Western Himalayan *Brassica rapa* L. *Scientific Reports* 12:6742. <https://doi.org/10.1038/s41598-022-11030-0>
- Patel HK, Vyas RV, Shelat HN (2021). Selective enrichment method for isolation of efficient phosphate solubilizing bacteria from soil. *Communications in Soil Science and Plant Analysis* 53(12):1532-1541. <https://doi.org/10.1080/00103624.2022.2055054>
- Pathak R, Paudel V, Shrestha A, Lamichhane J, Gauchan DP (2017). Isolation of phosphate solubilizing bacteria and their use for plant growth promotion in tomato seedling and plant. *Kathmandu University Journal of Science, Engineering and Technology* 13(II):61-70.
- Rat A, Naranjo HD, Krigas N, Grigoriadou K, Maloupa E, Alonso AV, ... Willems A (2021). Endophytic bacteria from the roots of the medicinal plant *Alkanna tinctoria* Tausch (*Boraginaceae*): Exploration of plant growth promoting properties and potential role in the production of plant secondary metabolites. *Frontiers in Microbiology* 12:633488. <https://doi.org/10.3389/fmicb.2021.633488>
- Shahinur MR, Quadir QF, Rahman A, Asha MN, Chowdhury MAK (2014). Screening and characterization of phosphorus solubilizing bacteria and their effect on rice seedlings. *Research in Agriculture Livestock and Fisheries* 1(1):27-35. <https://doi.org/10.3329/ralf.v1i1.22353>
- Suleman M, Yasmin S, Rasul M, Yahya M, Atta BM, Mirza MS (2018). Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. *PLoS One* 13(9):e0204408. <https://doi.org/10.1371/journal.pone.0204408>
- Sun M, Xiao T, Ning Z, Xiao E, Sun W (2015). Microbial community analysis in rice paddy soils irrigated by acid mine drainage contaminated water. *Applied Microbiology and Biotechnology* 9(9):2911-2922. <https://doi.org/10.1007/s00253-014-6194-5>
- Vinod Babu S, Triven S, Reddy RS, Sathyanarayana J (2017). Screening of maize rhizospheric phosphate solubilizing isolates for plant growth promoting characteristics. *International Journal of Current Microbiology and Applied Sciences* 6(10):2090-2101. <https://doi.org/10.20546/ijcmas.2017.610.249>

- Vyas P, Gulati A (2009). Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. BMC Microbiology 9:174. <https://doi.org/10.1186/1471-2180-9-174>
- Walpola BC, Song J-S, Yoon M-H (2012). Assessment of plant growth promoting activities of phosphorus solubilizing bacteria. Korean Journal of Soil Science and Fertilizer 45(1):66-73. <https://doi.org/10.7745/KJSSF.2012.45.1.066>
- Wasoontharawat M, Jatarasa R, Duangpaeng A (2017). Isolation and screening phosphoric solubilizing bacteria from organic anthill fertilizer and phosphorus release capacity. Sakon Nakhon Rajabhat University Journal of Science and Technology 9(2):454-464.
- Yu H, Wu X, Zhang G, Zhou F, Harvey PR, Wang L, ... Zhang X (2022). Identification of the phosphorus-solubilizing bacteria strain JP233 and its effects on soil phosphorus leaching loss and crop growth. Frontiers in Microbiology 13:892533. <https://doi.org/10.3389/fmicb.2022.892533>
- Zhou J, Deng Y, Shen L, Wen C, Yan Q, Ning D, ... Brown JH (2016). Temperature mediates continental-scale diversity of microbes in forest soils. Nature Communications 7(1):1-10. <https://doi.org/10.1038/ncomms12083>



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