

Isolation, identification, and characterization of phosphate solubilizing bacteria, *Paenibacillus* sp., from the soil of Danum Valley Tropical Rainforest, Sabah, Malaysia

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Abstract. Lindang HU, Subbiah VK, Rodrigues KF, Budiman C. 2021. Isolation, identification, and characterization of phosphate solubilizing bacteria, *Paenibacillus* sp., from the soil of Danum Valley Tropical Rainforest, Sabah, Malaysia. *Biodiversitas* 22: 4370-4378. Phosphorus (P) is a vital element for plant growth. However, only 0.1% of available soil phosphate is directly utilized by plants. Consequently, P fertilizer, which is mostly sourced from unrenewable resources of phosphate rock, is practically added into croplands. Furthermore, as the demand for this fertilizer increases, its availability and impact on environmental issues raise wide concerns. The use of soil phosphate solubilizing bacteria (PSB) is a promising alternative to be further developed as a biofertilizer to increase the availability of P elements for plant growth. As such, we report here on our efforts to screen and characterize novel PSB from tropical rainforest soil. The soil samples were collected from the Danum Valley tropical rainforest, which is located in Sabah, at the northeastern region of Borneo. Phosphatase solubilizing bacteria were then screened using the NBRIP agar selective media. The result yielded five colonies, designated as PSB1, PSB2, PSB3, PSB4, and PSB5, displaying halos with an average diameter of 10mm. The 16S rRNA gene sequence analysis using BLASTn indicated that PSB1, PSB2, PSB3, PSB4, and PSB5 were mainly *Bacillus* sp. PSB01 (MZ675820), *Pseudomonas oryzyhabitans* PSB02 (MZ675821), *Staphylococcus pasteurii* PSB03 (MZ675822), *Paenibacillus* sp. PSB04 (MZ675823), and *Staphylococcus pasteurii* PSB05 (MZ675824), respectively. Consequently, since *Paenibacillus* has been reportedly used in the global agriculture industry as a promising biofertilizer, we then selected *Paenibacillus* sp. PSB04 for further downstream characterization using Gram staining and scanning electron microscope (SEM). The Gram staining revealed that *Paenibacillus* sp. PSB04 was a Gram-negative bacterium with a rod shape, which was in good agreement with the SEM data. Further analysis revealed that the specific phosphatase activity of the extracellular fraction of this bacterium was 7,378.12 U mg⁻¹. This was the highest activity observed when compared to previous studies. The results here provide an early insight into an excellent phosphate-solubilizing bacterium obtained from a tropical rainforest which could be beneficial to the agriculture industry.

Keywords: Danum Valley, *Paenibacillus*, phosphatase, phosphate solubilizing bacteria, soil bacteria

Abbreviations: DNA: Deoxyribonucleic acid; DV: Danum Valley; PDB: Protein Data Bank; PSB: Phosphate solubilizing bacteria; P: Phosphorus; SDGs: Sustainable Development Goals; SEM: Scanning Electron Microscope

INTRODUCTION

By the year 2050, the global food demand and the phosphorus (P) input into croplands are expected to increase by 70% and 86% respectively. Moreover, developing countries will likely increase the use of fertilizer, including P, to meet the growing agriculture demand and supply (Hunter et al. 2017; Mogollón et al. 2018). Chemical P fertilizers are known as the product of mining non-renewable sources of rock phosphate minerals. Due to the expected global food demand, the cost of production is expected to rise due to the scarcity of rock phosphate minerals, which is the primary source of P fertilizer (Hunter et al. 2017). As a result of the challenges to the global community, stakeholders have developed strategies and policies to address the problems, such as the United Nation Sustainable Development Goals (SDGs), which were established in 2012 (Campagnolla et al. 2019). As stated in the SDGs of the 2030 Agenda for Sustainable

Development, harnessing microbes as a biofertilizer agent to control the critical P macronutrient requirement for plant growth is one of the proposed long-term solutions.

An abundance of 60 – 70% of the P element that is available in the soil exists in both organic and inorganic forms. However, only 0.1% of the available soil phosphate is ready for direct uptake by plants. The remaining phosphate requires assistance from phosphate solubilizing microorganisms to produce inorganic forms of free solubilizing phosphate (Sharma et al. 2013). In nature, plants need the aids of the microbial community to mineralize and solubilize both forms of phosphate (Ushio et al. 2010). Lowering the pH of the soil, enhancing chelation of cations bound to the available phosphate, and forming soluble complexes with the metal ions associated with the insoluble available P are some of the processes used to solubilize the available inorganic forms. Chemical substrates were used in the techniques to overcome the negative effects. Even though chemical fertilizers have

been proven to increase the availability of soluble P in the agricultural soil, but it has long-term adverse effects on the soil, surface water, and estuaries due to its chemical impurities (Komatsuzaki and Ohta 2013). Meanwhile, non-specific acid phosphatases, alkaline phosphatases, and phytases formed naturally by microbes are responsible for the solubilization of organic phosphate by mineralization.

The synergistic relationships between plants and microbial communities in soil have been the key factor to keep the ecosystem in balance. The microbial community is able to use the P content in soil by producing phosphate solubilizing enzymes and solubilize the inorganic compound of P. The profile of bacterium producing phosphate solubilizing enzymes, or namely phosphate-solubilizing bacteria (PSB), are related to physical, chemical, and biological characteristics of the soil. The most commonly identified PSB belong to the genera of *Pseudomonas*, *Rhizobium*, *Aspergillus*, *Bacillus*, *Staphylococcus*, *Paenibacillus*, and *Penicillium* (Suliasih and Widawati 2005; Hii et al. 2020). Their distribution and diversity in the soil are influenced by factors such as soil type, total biomass, plant type, and nutrient accessibility (Tripathi et al. 2012; Pathania et al. 2020). Interestingly, *Paenibacillus* has the potential to not only able to solubilize phosphate but has nitrogen-fixation ability, antibacterial capacities, release siderophores, and producing indole acetic acid phytohormone (Grady et al. 2016; Liu et al. 2019). These are the essential traits of plant growth-promoting bacteria to facilitate the sustainable development of agriculture goals as biofertilizers. Accordingly, there have been wide attempts to screen and isolate PSB from various sources. Besides, the study on the diversity of PSB is considered as one of the key elements in conservation through understanding sulfur cycling in the ecosystems (Alori et al. 2017).

Danum Valley (DV) is an old-growth primary tropical rainforest in Sabah, Malaysia, with ultisols soils and a year-round low concentration of extractable phosphorus (Green et al. 2005). Accordingly, it is postulated that the phosphatase-producing bacteria are highly abundant in the tropical rainforests of Borneo such as the primary rainforest of DV. As such, it is thought that PSB is widespread in Borneo's tropical rainforests, such as the DV primary rainforest. The dipterocarp rainforest of DV, which is mostly lowland, is a possibly strong source of PSB. The predominantly lowland dipterocarp rainforest of DV may serve as a good source of PSB. Thus, it is hypothesized that DV could harbour diverse microorganisms producing phosphate solubilizing enzymes that can withstand the complex soil condition. It is believed that the native bacteria exhibit unique biological traits and enzymatic activities. Using a metagenomic and direct DNA amplification technique, previous attempts to profile microorganisms from rainforest soil in Sabah discovered some novel bacteria that may be involved in P solubilization (Tin et al. 2017). Despite this, no attempts have been made to isolate and characterize these bacteria further. Accordingly, this study aims to screen and characterize novel PSB from the tropical rainforest soil.

This report described the first PSB isolated from DV tropical forest soil which was then morphologically and molecularly characterized. The detail on the extracellular alkaline phosphatase enzymatic activity of the selected bacteria was also further characterized. As a result, the findings of this study include an early insight into culturable PSB for future utilization on biofertilizers.

MATERIALS AND METHODS

Study site and sampling methods

The study site was located at DV, Sabah, Malaysia whereby, two existed carbon plots designed by the previous study were selected as the sampling plots to collect the soil samples (Riutta et al. 2018). The plot had a planimetric area of 1 ha and was divided into several subplots of 20m x 20m namely Danum Carbon 1 and Danum Carbon 2. Danum Carbon 1 (4.951°, 117.796°) represent steep slope soil topography, and Danum Carbon 2 (4.953°, 117.793°), represent flat soil topography. In this study, 5 subplots were selected from the 2 establish carbon plot. Three sets of 50g subsurface soil samples were collected at a 1m distance from the center of the subplot. The soil was taken using a hand auger. The soil samples were kept at 4°C storage in the field center and were transferred to the laboratory for storage at -80°C within 24 hours until further analysis (Pepper and Gerba 2015; Taylor and Williams 2010).

Bacterial isolation

The screening and isolation of PSB were done based on the procedures done by Nautiyal (1999) and Mohamed et al. (2019). Firstly, the 5g of soil was suspended with 20ml of autoclaved MiliQ water and was agitated for 1hour at 30°C. The samples were then spread on National Botanical Research Institute's phosphate (NBRIP) growth agar media containing (per litre): 15g agar, 10g glucose, 5g MgCl₂ (Hydrated), 0.25g MgSO₄ (Hydrated), 0.2g KCl, 0.1g (NH₄)₂.SO₄, 5g Ca₃PO₄ (Nautiyal 1999). The plates were incubated at 30°C for 7 days. Identification of PSB was done by observing the formation of a halo zone around the colony as it reflects the hydrolysis reaction of tricalcium phosphate in the media. Meanwhile, well plate screening was also done to determine the size of the halo zone (mm). The colonies of dissimilar morphotypes that appeared on the NBRIP agar were purified and long-term storage was performed *via* cryopreservation with 25% (v/v) glycerol at -80°C until further analysis.

Morphology identification and characterization

Morphology and Gram characterization of five isolates were also done to further characterize the isolates. For morphology identification, the bacteria were grown in Nutrient Broth (Oxoid Ltd., Hampshire, United Kingdom) at 37°C for 16 hours. The potential candidates were then sub-cultured on a new agar plate and observed under the microscope. All isolates were subjected to Gram staining identification using Gram staining reagent kit (Sigma-Aldrich, St. Louis, USA). Meanwhile, the selected bacterium

was then subjected to morphological characterization using scanning electron microscopy (SEM). For this purpose, the gold-coated specimen was prepared according to Arief et al. (2015) and further observed under electron microscope Hitachi S-3400 (Hitachi Ltd, Chiyoda, Tokyo, Japan) at an accelerating voltage of 10kV.

Molecular characterization and phylogenetic analysis of bacteria

Freshly grown pure bacterial culture in Nutrient Broth media was subjected to genome extraction using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instruction. The quantity and integrity of the extracted DNA were determined by agarose electrophoresis and Nanodrop spectrophotometry (Thermo Fisher Scientific, Massachusetts, USA).

The polymerase chain reactions (PCR) were performed using NEB *Taq* PCR (NEB, England) according to the manufacturer's protocol to amplify the conserved bacterial 16S rRNA gene. The components of PCR cocktail mixtures were 1x PCR Buffer, 0.2mM dNTPs, 5mM MgCl₂, 0.5uM of Forward BSF8' and Reverse BSR1541 Primers and 2.5 units/ μ l of *Taq* DNA polymerase in 50 μ l PCR reaction solution. Primer pairs, namely BSF8 (5'-AGA GTT TGA TCC TGG CTC AG-3') and primer BSR1541 (5'- AAG GAG GTG ATC CAG CCG CA-3') were used as the forward and reverse primers, respectively. PCR was carried out using a Bio-Rad Thermal Cycler (Bio-Rad, California, USA) according to the following program: Initial denaturation at 94°C for 2 min; then 30 cycles of denaturation at 98°C for 10 sec, annealing at 49°C for 30 sec and extension at 68°C for 30 sec for the amplification of the full length of 16S rRNA gene. PCR products were resolved by electrophoresis on 1% agarose gels and visualized on a GelDoc Go Imaging System (Bio-Rad, California, USA). The band of interest was then excised, purified using QIAquick Gel (Qiagen, USA) extraction kit and sequenced using BigDye Terminator v3.1. Sequencing services were provided by Firstbase Laboratories Sdn Bhd., Selangor, Malaysia.

The identification of the bacterial isolates and determination of their evolutionary relationships were done using the gene sequences. The essential reference sequences were sourced from the NCBI GenBank database. Prior to phylogenetic tree construction, the sequences were aligned by ClustalW and the BLASTn search program was performed to compare the sequences. The phylogenetic analysis was done using molecular evolutionary genetics analysis (MEGA) software version 10 (Stecher et al. 2020). Meanwhile, the reported phosphate enzyme was obtained from the Protein Data Bank database and the accession number was listed (Berman et al. 2000). The nucleotide sequences of the isolated bacteria were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank>).

Determination of alkaline phosphatase activity

Each bacterial isolate was determined for its phosphate solubilization ability through the phosphatase assay method according to Chu et al. (2019) with slight modification. The selected bacterium was inoculated in a 250ml flask

comprising 100ml of Nutrient broth medium and incubated for 16 hr at 37°C. The overnight cultured was subjected to centrifugation at 10,000 $\times g$ for 10 min. The cell-free supernatant sample was collected for alkaline phosphatase (ALP) assay. The activity of ALP was assayed using a colourless substrate *p*-nitrophenyl phosphate (*p*-NPP) as per the method, which yielded yellow *p*-nitrophenol (*p*-NP). The reaction mixture contained: 1ml of 5mM pNPP, 1ml of 10mM Tris Buffer, 1ml of the crude enzyme, and fixed to a final volume of 5ml with sterile MiliQ water. After incubation at 37°C for 60 min, and the reaction was stopped by adding 3.0ml of termination buffer (0.1M NaOH and 5mM EDTA). Finally, the absorbance at 405nm was measured by GENESYS20 Visible Spectrophotometer (Thermo Scientific, United States of America). One unit (U) of phosphatase activity was defined as the amount of enzyme required to produce 1 μ mole of *p*-NP per minute at 37°C.

RESULTS AND DISCUSSION

PSB were found in DV soil samples. The isolated PSB were confirmed by the development of a halo zone around the colony, as seen in Figure 1. The halo zone formed as a by-product of the tri-calcium phosphate hydrolysis reaction in the medium. Initially, numerous colonies were grown on the agar media, but only PSB were subjected to further purification characterization of isolates. The screening revealed five distinct colonies with halos averaging 10mm in diameter among these isolates. The halo zone was observed from 4 days and kept increasing in its size until 7 days. Halos in the 5–10mm range were commonly used as a baseline for determining potential PSB by other researchers (Bononi et al. 2020). The previous study shows the screening of P solubilizing activity varied across bacteria species, and typically 16 hr to 72 hr incubation time for bacteria from forest soil (Song et al. 2021; Sánchez-Cruz et al. 2020; Cabugao et al. 2017). Nonetheless, this discovery revealed that tropical rainforest soil contains culturable PSB that are similar to PSBs found in other types of soil (Tang et al. 2020).

Further, Gram staining revealed that PSB1, PSB2, and PSB4 were found to be Gram-negative rod-shaped bacteria, while PSB3 and PSB5 were Gram-positive coccus-shaped bacteria (Figure 2). There is no consensus so far on the types of Gram bacteria dominance of PSB. Billah et al. (2019) reported that PSB may originate either from a group of Gram-positive or negative bacteria. Subsequently, molecular characterization was done for species identification by looking at the sequence of its 16S rRNA gene. PCR successfully amplified the 16S rRNA band from the isolated PSB at the expected size of around 1,500 bp (Figure 3). The primers used in this study were chosen in order to obtain the full-length of 16S rRNA. Sabat et al. (2017) reported that the use of a full-length sequence of the 16S rRNA gene has proven to be a useful molecular target for bacterial identification since it may cover the variable region among the species.

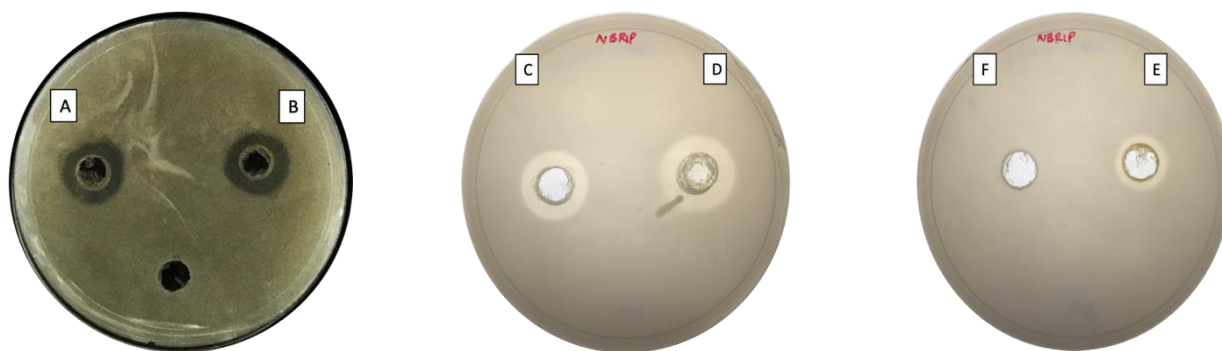


Figure 1. Formation of a clear zone around the isolated bacterium (A)PSB1, (B)PSB2, (C)PSB3, (D)PSB4, (E)PSB5, and (F)Negative Control on NBRIP media 7 days after incubated at 30°C.

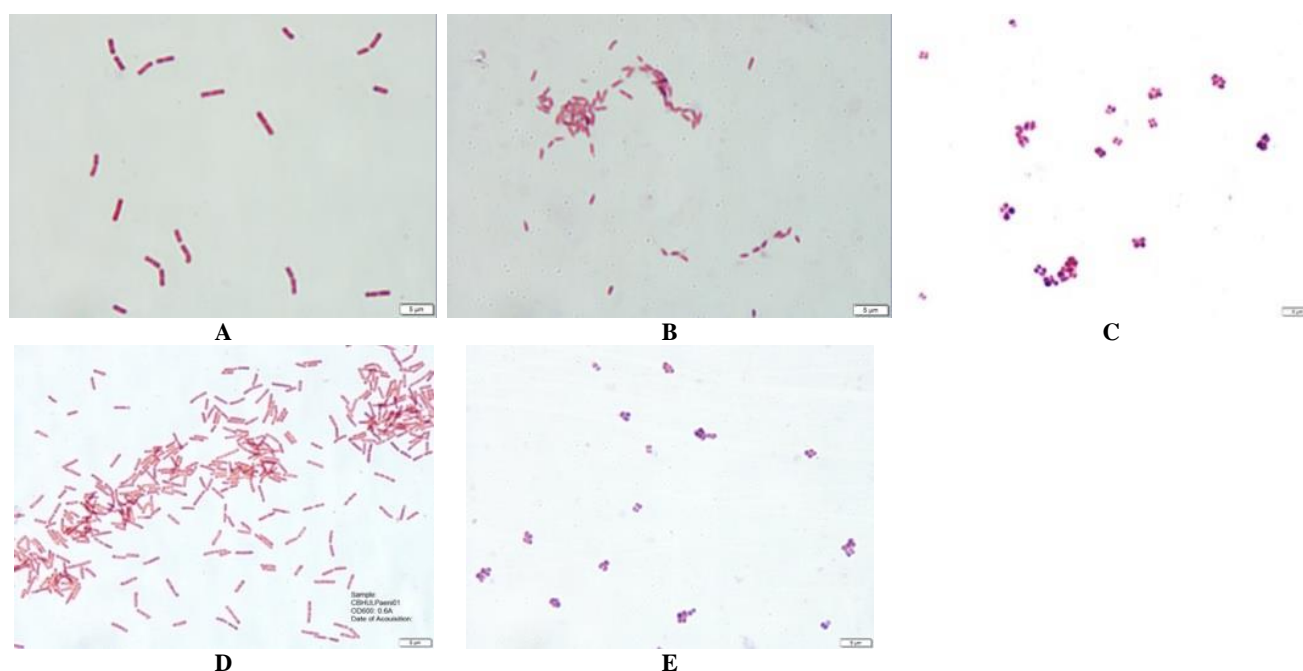


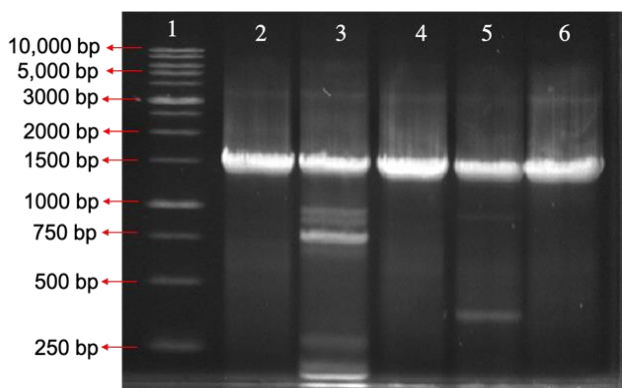
Figure 2. Gram stain of isolated phosphate solubilizing bacteria (A)PSB1, (B)PSB2,(C)PSB3, (D)PSB4 and (E)PSB5

Further sequence analysis on the 16S rRNA amplicon using the BLASTn database is shown in Table 1. The analysis of the 16S rRNA region revealed these strains exhibited high similarity to *Bacillus* sp., *Pseudomonas oryzyhabitans*, *Paenibacillus* sp. and two strains of *Staphylococcus pasteurii*. The sequences of strains were deposited into GenBank and its accession number were shown in Table 1. These identities were 100% matched with BLASTn databases based on their base sequence similarities. All identified isolates have been reported as one of the main microbial communities in the forest soil. Previous research has shown that this community is responsible for keeping the nutrient cycle in the soil stays in balance. Moreover, the isolated Gram-negative *Bacillus* and *Pseudomonas* from the rainforest soil have been shown to support crop plant growth and the well-known model plant, *Arabidopsis thaliana* (Huang et al. 2015). *Pseudomonas*, on the other hand, decomposes fungal mycelia, which returns nutrients to the soil and is absorbed by plants (Lladó et al. 2017). Meanwhile, Gram-negative

Paenibacillus, a member of the Firmicutes phylum, has been found in a variety of environmental studies, including animals and fish, palm oil waste, and plants. Interestingly, only a few species of the *Paenibacillus* genus have been isolated from forest soil, especially in the tropical regions (Ina-Salwany et al. 2015; Grady et al. 2016; Chin et al. 2017; Haruna et al. 2017; Huang et al. 2017; Kim et al. 2021). Gram-positive *Staphylococcus* on the other hand is likely to be found in an animal's intestinal tract instead of the environmental samples. According to previous research, the majority of *Staphylococcus* bacteria present in the soil are phosphate solubilizing bacteria (Panda et al. 2016; Hii et al. 2020). The isolated bacteria were shown to have antifungal and solubilizing phosphatase in a wide range, proving that they were promising plant growth-promoting bacteria. Since the genera were typically present in the soil and most were reportedly able to solubilize phosphate compounds, all isolated strains obtained from our sample support the existing literature.

Table 1. Summary of hits on NCBI Database using BLASTn and reported Phosphatase in PDB

Sample	16S rRNA NCBI BLASTn	Identical (%)	Phosphatases availability in Protein Data Bank	Strain designation for the isolated colony (GenBank Acc. No.)
PSB1	<i>Bacillus</i> sp. strain JUBYHD3-1	100	Yes (3OT9,2YEQ,1EJJ, 3CWF)	<i>Bacillus</i> sp. PSB01 (MZ675820)
PSB2	<i>Pseudomonas oryzyhabitans</i> strain IASSTS7	100	Yes (3ZWU,4ALF,4A9V,4F18)	<i>Pseudomonas oryzyhabitans</i> PSB02 (MZ675821)
PSB3	<i>Staphylococcus pasteuri</i> strain 3I10	100	Yes (6IHT)	<i>Staphylococcus pasteuri</i> PSB03 (MZ675822)
PSB4	<i>Paenibacillus</i> sp. strain UFLA01-923	100	No	<i>Paenibacillus</i> sp. PSB04 (MZ675823)
PSB5	<i>Staphylococcus pasteuri</i> strain 3I10	100	Yes (6IHT)	<i>Staphylococcus pasteuri</i> PSB05 (MZ675824)

**Figure 3.** 16S rRNA gene PCR of the isolated colony on a 1% Agarose Gel; Lane 1: 1 Kb DNA Ladder, Lane 2: PSB1; Lane 3: PSB2; Lane 4: PSB3; Lane 5: PSB4; Lane 6: PSB5

The isolates and associated reference strains were used to create a phylogenetic tree (Figure 4). The tree is grouped into four clusters, each representing different genera. Cluster A, B, C, and D, corresponds to the *Staphylococcus* group, *Bacillus* group, *Paenibacillus* group, and *Pseudomonas* group, respectively. Figure 4 shows the genetic tree generated by the neighbor-joining algorithm method, and a similar result was obtained by using the maximum-likelihood algorithm method (data not shown).

Earlier studies have shown that the phosphatase family from *Staphylococcus* is a promising candidate for anti-virulence targeting protein and a reporter in gene expression. *Staphylococcus* species were also found to be able to effectively dephosphorylate peptides using the phosphate protein crystal structure of Stp1 (du Plessis et al. 2007; Yang et al. 2019). In addition, the class C family of nonspecific acid phosphatase from *Staphylococcus* also proved to be a promising reporter in protein and gene expression studies due to its simple quantitative and qualitative detection. Meanwhile, bacterial phosphatase-encoding genes were also found in the *Pseudomonas* group. Plant growth was found to be supported by a consortium of *Pseudomonas* and *Bacillus*, as well as other genera, particularly in the organic farming system. The co-inoculation of *Bacillus* and *Pseudomonas* had a synergistic effect on enzymatic activity in the soil and increased the biomass of the microbial population in the plantation soil

(Kumar et al. 2016; Angelina et al. 2020). Furthermore, it was revealed that one of the isolated species belongs to the same genus as *Paenibacillus rhizosphaerae*, *P. amyloiticus*, *P. silvae*, and *P. tyrfis*. These findings are also consistent with the previous finding in which our isolated strain was categorized under the same group (Rivas et al. 2005). Noteworthy, PSB04 and *P. silvae* strains were both isolated from a similar type of environmental condition, primarily rainforest soil (Huang et al. 2017). Besides, a strain of *P. tyrfis* was also found previously from the tropical mangrove soil in Malaysia although there were differences in the type of soil sampled. Hence, it is reasonable to conclude that the tropical climate is conducive to the growth of this genus (Aw et al. 2016). Although the BLASTn presented genus or even species-level identification, the established phylogenetic tree based on the 16S rRNA gene sequence of the isolates supported the earlier discovery.

Except for *Paenibacillus*, the phosphatase enzymes for PSB01, PSB02, PSB03 and PSB05 strains were reported to exist (deposited in the Protein Data Bank (PDB) as listed in Table 1). The availability of phosphatase enzymes in PDB indicated that the enzymes from those strains were extensively studied, particularly in their structures and functions (Cavalli et al. 2007). The absence of a phosphatase structure for *Paenibacillus* (Table 1) indicated that no previous structural studies on the phosphatase from this group and, therefore, contains great potential findings. Nevertheless, with very limited information on its phosphatase enzymes, many strains of *Paenibacillus* sp. have been previously used as biofertilizer with a consortium of bacterial species and its functional traits was only based on its genome analysis (Khalid et al. 2009; Shtark et al. 2010; Passera et al. 2018). Nevertheless, no studies have been reported on the catalytic and structural properties of phosphatase. As a result, *Paenibacillus* sp. PSB04, an isolated *Paenibacillus* sp., was chosen for further investigation.

Firstly, morphological property of *Paenibacillus* sp. PSB04 was confirmed under SEM. The isolated *Paenibacillus* sp. PSB04 showed a rod-shaped structure based on the micrograph obtained by SEM (Figure 5) with the length ranging from 1.85 μ m to 3.45 μ m. Furthermore, the PSB04 strain had the highest specific alkaline phosphatase activity of all the strains previously studied, at 7,378.12 U mg⁻¹ (Table 2).

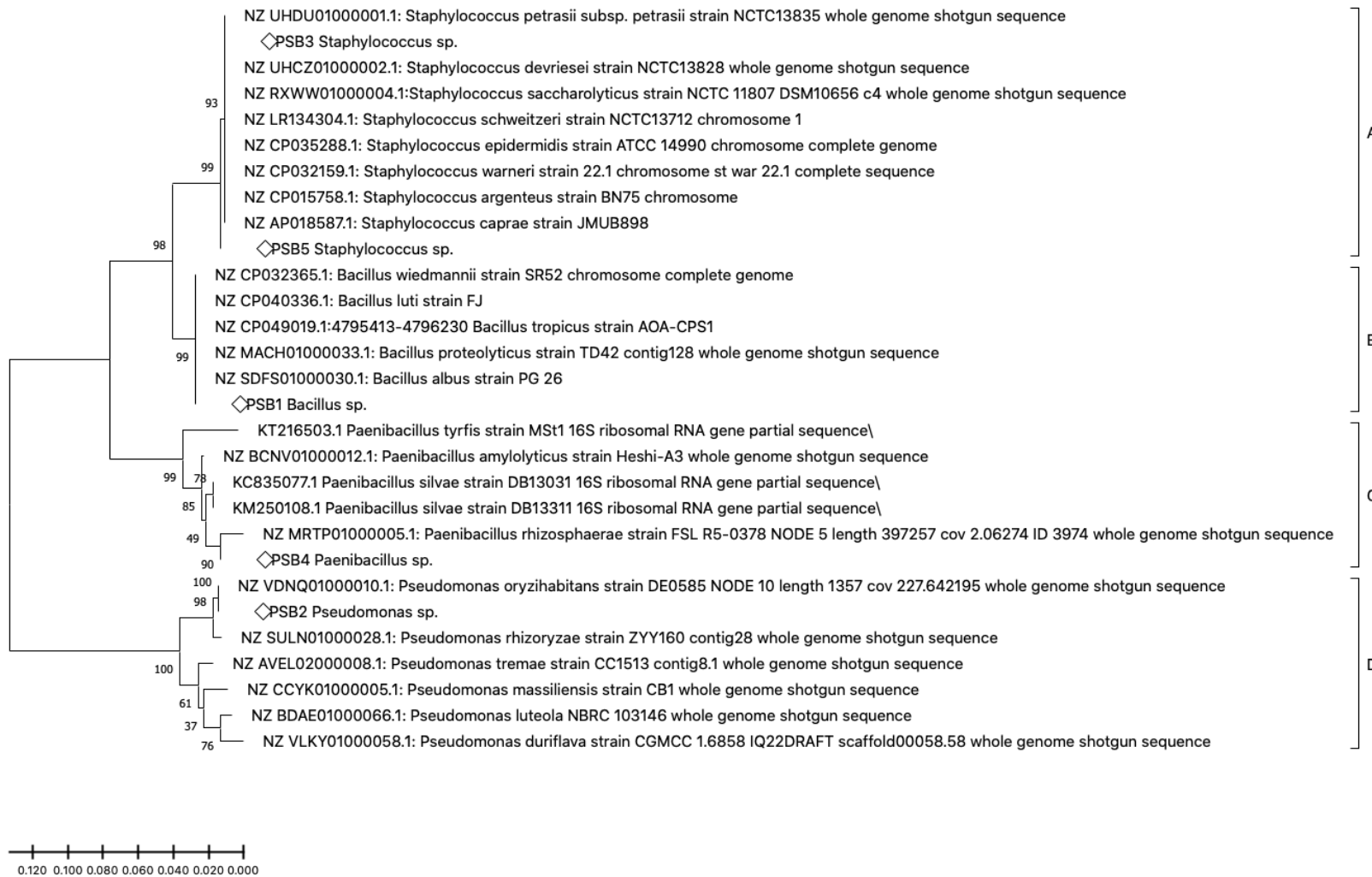
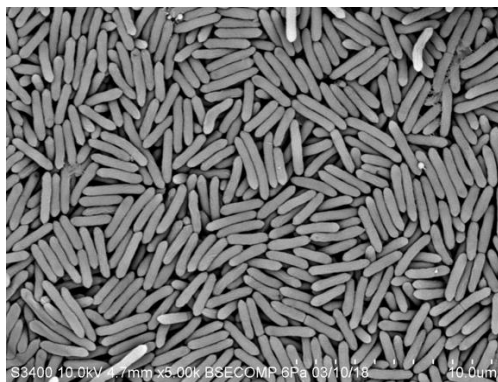


Figure 4. Phylogenetic tree of the isolated PSB. All bootstrap values (expressed as a percentage of 1,000 replications) were shown at branch points

Table 2. Comparison of enzymatic activity of *Paenibacillus* sp. PSB04 with some well-studied alkaline phosphatases

Origin of bacteria	Type of enzyme	Total activity (U)	Specific activity (U mg ⁻¹)	Reference
<i>Paenibacillus</i> sp. PSB04	Crude Enzyme	1193.37	7378.1	This Study
<i>Halomonas</i> sp. 593	Crude Enzyme	167.50	6700.0	(Ishibashi et al. 2005, 2011)
<i>Shewanella</i> sp. T3-3	Expressed Enzyme	5380000.00	35.4	(Aiba et al. 2017)
Marine bacterium	Expressed Enzyme	2595.60	12.6	(Balabanova et al. 2014)
<i>Alcaligenes faecalis</i>	Crude Enzyme	3576.55	8.9	(Behera et al. 2017)
<i>Lactobacillus casei</i> 355	Crude Enzyme	1876.00	3.4	(Chu et al. 2019)
<i>Thermotoga maritima</i>	Expressed Enzyme	4.00	2.0	(Wojciechowski et al. 2002)

**Figure 5.** Scanning electron microscopy image of strain *Paenibacillus* sp. PSB04

Even though the assay used unpurified proteins (crude), the specific activity had a similar activity with the phosphatase activity from *Halomonas* sp. 593. To note, the activity of crude enzyme does not reflect the real activity of phosphatases PSB04 since the crude enzyme contains various contaminants protein or enzymes (Ishibashi et al. 2005; Obeng et al., 2017). Other enzymes may interfere with the activity of phosphatase in the crude extract. Nevertheless, empirically, the purified enzyme should exhibit higher specific activity as compared to the unpurified one. Budiman et al. (2011; 2018) and Razali et al. (2021) reported that the increase of enzyme purity is accompanied by the increase in its specific activity. During purification, antagonist enzymes such as proteases are removed, allowing the protein of interest to be in the best possible state to produce its actual catalytic activity. This implies that the real specific activity of the PSB04 strain is expected to be higher after purification. This, nevertheless, should be experimentally confirmed.

In conclusion, we have isolated and characterized five (5) strains of PSB from the DV tropical rainforest soil. The strains are designated as *Bacillus* sp. PSB01 (MZ675820), *Pseudomonas oryzyhabitans* PSB02 (MZ675821), *Paenibacillus* sp PSB04 (MZ675823) and two strains of *Staphylococcus pasteurii* PSB03 (MZ675822) and PSB05 (MZ675824). Morphologically, *Bacillus* sp., *Pseudomonas oryzyhabitans*, *Paenibacillus* sp. were rod-like, while *Streptococcus pasteurii* was spherical shaped. Among these five strains, the phosphatase enzyme from the *Paenibacillus* group was the least studied and, therefore, was selected for further characterization. For the first time,

the current study revealed that the phosphatase enzymatic activity of *Paenibacillus* sp. PSB04 strain was remarkably higher than that of other reported strains. Uncovering the true potential of the phosphatase is in our best interests and may hold key important prospects, as *Paenibacillus* was the only one of the five bacteria whose phosphatase enzyme was not documented in the protein data bank, indicating that it is a promising candidate for further research. This is an essential aspect in a further attempt to develop a sustainable consortium of biofertilizers, especially to reduce non-renewable rock phosphate mining.

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