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# THE POTENTIAL OF PHOSPHATE SOLUBILIZING AND PLANT GROWTH PROMOTERS OF *Burkholderia territorii* EF. NAP 1 ISOLATED FROM ACID SOILS FOR THE CONSERVATION OF FORMERLY RUBBER PLANTATION LAND

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***Abstract***

*Phosphorus (P) is an essential element needed by plants. The presence of phosphorus in the environment, especially soil in the form of bound phosphates. This condition can be affected by the acidity of the soil. Available phosphates can be released by phosphate solubilizing bacteria. EF.NAP 1 isolate is a phosphate-solubilizing bacteria that has been successfully isolated from acid soil in the area of the Institut Teknologi Sumatera (ITERA), Lampung, Indonesia, and potency as plant growth-promoting bacteria (PGPB). Based on the results of identification using the 16S rRNA gene, EF.NAP 1 isolates has similarities with Burkholderia territorii. These isolates were able to dissolve phosphate of 104.7 mg/L. P dissolution correlates with the growth phase of bacterial cells. During the phosphate dissolution process, the pH of the medium continues to increase, inversely proportional to the concept in general. The EF.NAP 1 isolate is able to produce six organic acids, i.e, acetic (339.14 mg/L), lactic (260.97 (mg/L), malic (133.24 mg/L), formic (31.52 mg/L), fumaric (19.31 mg/L) and tartaric acids (19.13 mg/L). B. territorii can produce IAA, nitrogenase, siderophore, HCN, chitinase, protease, and cellulose. The potency of B. territorii EF.NAP 1 as phosphate solubilizing bacteria and PGPB properties that have not been reported by others.*

***Keywords****: Acid soils; Organic acids; pH change; IAA; Nitrogenase*

## Introduction

Indonesia has very high biodiversity, one of which is known to be microbes. However, changes in environmental conditions due to human behaviour caused many disturbances to the ecosystem, which greatly affected the microbial community, such as in agriculture. Furthermore, Lampung is a province in Indonesia, which has quite extensive agricultural land. In this area, farmers do not maintain land sustainability, such as excessive use of fertilizers,

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which in turn causes a decrease in productivity. The Sumatra Institute of Technology is also one of the areas affected by the chemical fertilizers previously used, with a former rubber plantation experiencing excessive fertilization for years. This resulted in these areas having a very acidic soil pH of 4.09, which is classified as being water-poor [1]. Therefore, this condition is quite arid, making it difficult to reforest, due to the very low rate of essential nutrients, especially phosphate content.

Phosphate is an essential nutrient needed by plants. It is a limiting factor in plant productivity, due to the lack of dissolved soil concentrations, chemical fixation, and chelating complexes [2, 3]. The soil phosphate presence is also strongly influenced by soil acidity. Based on the soil chemical analysis conducted by *M. Asril and Lisafitri* [4], it showed that the P content available in the soil is very low and bound to various metal types, such as Al and Ca. The low availability of P is due to the high P-fixation by minerals in the form of Ca-P, Fe-P, and Al-P, therefore making it difficult to be absorbed by plants [5–7]. Furthermore, this condition caused the conservation and reforestation process in the area to always be hampered. An alternative solution to this problem is the presence of phosphate solubilizing bacteria, which are capable of dissolving phosphate bonds into available P, through organic acid excretion [8–10]. An acidic environment significantly increases phosphate solubility [11], therefore making this method the most likely way to increase the dissolved availability of P in the soil [12]. Organic acids produced by phosphate solubilizing bacteria are low molecular weight organic molecules, such as citrate, malate, lactate, 2-ketogluconate, gluconate, glyoxylic acid, and more [8], [9], [13–17]. However, the ability for organic acid to be secreted is determined by genes and environmental conditions [18].

Several phosphate solubilizing bacteria that increase plant production include *Pseudomonas*, *Bacillus*, *Rhizobium*, *Flavobacterium*, *Achromobacter*, *Erwinia*, *Micrococcus*, and *Agrobacterium* [19–23]. Several Pseudomonas species have even been reported as highly efficient phosphate solubilizing bacteria and biological fertilizers. These bacteria are very active in increasing plant growth by producing enhancement regulators and suppressing the development of soil infectious diseases [20]. Bacteria also increases plant growth by secreting indole acetic acid (IAA), nitrogenase, siderophore, HCN, and hydrolysis enzymes, such as chitinase, protease [24].

In 2020, *M. Asril et al.* [25] succeeded in isolating phosphate solubilizing bacteria from acid soils in the Sumatra Institute of Technology (ITERA), Lampung, Indonesia, and obtained one isolate with the EF.NAP 1 code, which had the highest phosphate dissolution index in pikovskaya medium. The isolate was identified as *Burkholderia territorii*, which is known as a plant growth-promoting bacterium, with quite a lot of potential, such as *Burkholderia australis* sp. *nov* [26] and *Burkholderia cepacia* [27]. These bacteria fix nitrogen and bio-control agents, to produce potential antifungal compounds [28, 29]. However, studies of the *B. territorii* species as a phosphate solubilizing microbe and its potential as a plant growth-promoting bacterium have not been reported. Until this very period, the only report related to the potential of *B. territorii* is about it being an antimicrobial and antifungal agent against pathogenic fungi, such as *Alternaria alternata*, *Colletotrichum acutatum*, *Phyllosticta citricarpa*, *Phytophthora nicotianae* and *Phytophthora palmivora* [30]. Therefore, this study should be used as an information base related to the potential for bacteria to be used in increasing soil fertility, while also supporting conservation and reforestation programs being carried out by the Institut Teknologi Sumatera. This study aims to determine the ability of the isolate EF.NAP 1 (which is isolated from acidic soil, in the Sumatra Institute of Technology, Lampung, Indonesia) as a phosphate solubilizing and growth-promoting bacteria, with its mechanism of organic acid secretion, therefore making it a potential microorganism to help fertilize plants for conservation and reforestation programs.

## Experimental part

### *Materials*

Phosphate solubilizing isolate EF.NAP 1 has been isolated from acid soils in the Institut Teknologi Sumatera (ITERA) region, Lampung, Indonesia. Ultisols soils are a former rubber plantation with a soil pH of 4.09. Bacteria identified as *Burkholderia territorii* were grown on Nutrient agar medium enriched with $Ca_3(PO_4)_2$ and stored at 30°C.

### *Methods*

*Identification based on 16S rRNA gene, DNA Isolation of bacterial Isolates* (*modified from Sambrook and Russell* [31]).

A total of 1.5mL of bacterial culture was put into eppendorf 1.5mL and then centrifuged at 8000rpm for 10 minutes, the supernatant was removed, and the formed pellet was washed with STE buffer (composition: 0.3M sucrose; 25mM Tris-HCL; 25mM EDTA.2Na pH = 8), then centrifuged at 8000rpm for 10 minutes. Pellets are washed three times repeatedly. Subsequently, the supernatant was removed, and the pellets obtained were added 200µL STE buffer and 45µL lysozyme (20mg/mL) slowly turned and then incubated at 55 ºC for 1 hour to form a protoplast. A total of 20µL proteinase-K (20mg/mL) was added to the mixture and incubated at 55 ºC for 60 minutes. After that, 400µL 10% CTAB was added to the 0.7M NaCl solution and then incubated at 65 ºC for 30 minutes. Then, one time, the phenol volume: chloroform (25:24) was added to the solution and centrifuged at 12000rpm for 10 minutes. The clear phase is transferred to a new tube and added 0.6 times the volume of isopropanol and 20µL sodium acetate, and incubation were carried out at -20ºC for one night. Subsequently, centrifuged 12000rpm for 10 minutes. The section is removed while the pellet is washed using 70% alcohol as much as 1.0mL. The DNA is dried for 1 hour to remove the alcohol and then dissolved in sterile 50µL dd$H_2O$, then the results of DNA isolation are stored at 4ºC or -20ºC.

*Amplification of 16 sRNA bacteria for bacterial isolates*

The 16S rRNA gene from genomic DNA was amplified by Polymerase Chain Reaction (PCR) machines and prokaryotic specific primers [32], namely 63f forward primer (5′-CAG GCC TAA CAC ATG CAA GTC-3 ′) and reverse primer 1387r (5′-GGG CGG WGT GTA CAA GGC-3 ′). The composition of the PCR reaction consisted of the enzyme La Taq DNA polymerase 0.5µL, 2x GC buffer 25µL, dNTP mixture eight µL, each primer (10pmol) 1.5µl, ddH2O 9.5µL, and DNA template four µL. PCR conditions used were pre-denaturation (94ºC, 4 minutes), denaturation (94ºC, 45 seconds), annealing (55ºC, 1 minute), elongation (72ºC, 1 minute 10 seconds), and post PCR (72ºC, 7 minutes) with a total of 30 cycles. DNA separation of PCR products was carried out on a mini-gel electrophoresis machine using 1% agarose at a 75V mains voltage for 45 minutes. DNA visualization was carried out on a UV transluminator using Ethidium Bromide (EtBr) colouring. The raw data from the sequencing results are then trimmed and assembled using the ChromasPro version 1.5 program. Data that has been assembled subsequently by BLAST with genome data that has been registered by the NCBI/National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Some of the blast sequence data results are the closest species and are the type of strain of each species taken from the GenBank data at NCBI. Furthermore, the data were analyzed again by aligning the sequence using the MEGA 6.1 program [33]. The construction of the phylogenetic tree was carried out to show the kinship level of EF.NAP 1 isolate with other bacterial isolates using the Neighbor-Joining Tree method with 1000 replications (bootstrap used).

*Quantitative Test of Phosphate Solubilizing Bacteria Ability*

A quantitative test of the ability of bacteria to solubilizing phosphate is carried out based on the method of *Lynn* [34]. A total of 1 loop isolate was inoculated in 50mL liquid Pikovskaya media, then incubated in a rocking incubator for 48 hours. After 48 hours, 1.0mL of culture was re-inoculated into 100mL of liquid Pikovskaya media, then incubated in a rocking incubator for

seven days at 37°C. Every 24 hours, 1.5mL of culture is centrifuged at a speed of 10,600g for 10 minutes to separate bacterial cells from the supernatant. A 1.0mL supernatant from centrifugation was taken and reacted with color-forming reagents (2.5mL of sodium molybdate 2.5% and 1.0mL of hydrazine sulfate 0.3%). The mixture was heated for 10 minutes then cooled. After the blue colour is formed, the phosphate concentration is measured by a spectrophotometer at a wavelength of 830nm. Standard curves were made using $KH_2PO_4$ with concentrations of 0, 20, 40, 60, 80, and 100mg/L.

*Organic Acid Production Test*

2mL of bacterial culture was added with 5mL of 20mM $H_3PO_4$ buffer and homogenized. The mixture was sonicated for 10 minutes at room temperature. Then the 20mM $H_3PO_4$ buffer was added again. The sample mixture was centrifuged at 4000rpm for 10 minutes and then proceeded to the Clean-up SPE-C18 process. A total of 10µL of the filtrate was then injected into the UPLC-PDA with a C18 100x3.0mm I.D, 1.9µm column. Conditions were adjusted to 20mM $H_3PO_4$ as a mobile phase and a stationary phase of 0.425mL/min with an isocratic pump system at a wavelength of 210nm.

*Determination of Nitrogenase Activity*

Measurement of nitrogenase activity was carried out by the Acetylene Reduction Assay (ARA) method using a gas chromatography tool [35]. Selected isolates were grown overnight in semisolid NfB media at 30°C. 0.5mL of culture was inoculated into 5mL of NfB media in a 25mL tube [36], then incubated for 5 days at 30°C. After incubating for 5-7 days (until the pellicle is formed), the tube is covered with a rubber stopper and parafilm paper. ARA measurement was done by removing the air in the tube using a sterile syringe as much as 1.0mL, then the acetylene gas ($C_2H_2$) was injected into the tube with the same volume as the volume of air released earlier. After incubating for 2 hours, the ethylene gas formed in the tube was measured by gas chromatography [37]. Ethylene standard curves are made with concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1.0ppm.

*IAA Production*

Indole acetic acid (IAA) production by bacterial isolates was determined following the Calorimetrically assay using ferric chloride acid reagent in the presence of tryptophan [38]. A standard curve was prepared from the serial dilution concentration of IAA stock solutions, a pink colour confirmed the production IAA.

*Cell Wall Degrading Enzyme Production*

Protease activity (Casein degradation) were carried out to detect the protease enzyme activity in degrading protein/casein in skim milk agar media. Chitinase and cellulase activities were also carried out using the Minimum Mineral Salt Chitin medium and CMC Agar. The EF.NAP 1 bacterial colony was grown on a toothpick in each medium and incubated for 96 hours for chitinase and cellulase activity, while proteases for 24 hours incubation. The clear zone formed shows the ability of bacteria to degrade the substrate.

*HCN Determination*

HCN (Cyanogen) production is carried out using the Bakker and Schippers method [39]. Exponential phase bacterial culture is inoculated on a solid medium enriched with 4.4mM Glycine with simultaneous addition of filter paper that has been soaked with 0.5% picric acid in 1% $Na_2CO_3$ in the upper lids of plates and uninoculated as a control. Petri was coated with parafilm and incubated at 28±1°C for 24 hours. A positive test is indicated by a change in colour from yellow to light brown, moderate brown, or firm brown, which indicates HCN production.

*Siderophore production*

Siderophore production is tested using the Chrome Azurol S (CAS) medium so that the medium follows Schwyn and Neilands method [40]. 24-hour isolates were grown in CAS agar medium and incubated 28±1°C for 48-72 hours. Positive testing is marked by a change in colour from orange to yellow around the colony.

## Results and discussion

### *Identification of EF. NAP1 based on the 16S rRNA gene*

Based on the 16S rRNA gene amplified with primers 63f and 1387r produce an amplicon measuring 1300bp. Phylogenetic tree analysis showed that the EF.NAP 1 isolate was closely related to *Burkholderia territorii* with a 99.52% similarity level (Table 1 and Fig. 1). The application of the *Burkholderia* type bacteria in agriculture has been widely used, due to its numerous advantages. Due to no reports about negative effects on human health, these bacteria have been observed to be safe for use [41]. The *Burkholderia* species existence was very easy to locate in the environment, due to the presence of bacteria habitat, which is influenced by soil pH [42, 43]. This type of *Burkholderia* species was very tolerant of acidity, causing it to compete well in a soil having an acidic pH [41]. This condition was like the soil in the Institut Teknologi Sumatera area, which had a pH of 4.09 [1] and is also the sampling location for this study.

Sequence of 16 SRNA gene sequences EF.NAP 1 isolate (1047 bp):

GGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGAC
CAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGG
CCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATCCTTGGCTCTAATACAGTC
GGGGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCA
GGCGGTTTGCTAAGACCGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTGG
TGACTGGCAGGCTAGAGTATGGCAGAGGGGGGTAGAATTCCACGTGTAGCAGTGA
AATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGCCAATA
CTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGT
CCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCATTTCCTTAGTAACGTAGC
TAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGG
AATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGC
GAAAAACCTTACCTACCCTTGACATGGTCGGAATCCTGCTGAGAGGCGGGAGTGCT
CGAAAGAGAACCGGCGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCCACGAGCGCAACCCTTGTCCTTAGTTGCTACGCAAGA
ACACTCTAAGGAGACTGCCGGTGACAAACCGGAAGAAGGGGGGGATGACGTCAAG
TCCTCAGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGAACAGAGGG
TTGCCAACCCGCGAGGGGGAGCTAATCCCAGAAAACCGATCGTAGTCCGGATTG

**Tabel 1.** Alignment results of 16s rRNA gene sequence EF.NAP 1 isolate against data available on NCBI (BLAST N)

| Description | Max score | Total score | Quary cover | E value | Identity | Acces number |
|---|---|---|---|---|---|---|
| *Burkholderia territorii* strain SCAT001 | 1906 | 1906 | 100% | 0.0 | 99.52% | MN540845.1 |
| *Burkholderia territorii* strain yy02 | 1906 | 1906 | 100% | 0.0 | 99.52% | MN177180.1 |
| *Burkholderia cepacia* strain KBB3 | 1906 | 1906 | 100% | 0.0 | 99.52% | MN032405.1 |
| *Burkholderia seminalis* strain BPMR | 1906 | 1906 | 100% | 0.0 | 99.52% | MK139542.1 |
| *Burkholderia cenocepacia* strain DC1 | 1906 | 1906 | 100% | 0.0 | 99.52% | MH762172.1 |

The *Burkholderia* bacteria had potential as plant growth promoters [44] such as nitrogen fixation and bio-control agents in inhibiting pathogenic fungi, through the production of antifungal compounds [29], one of which is *Burkholderia cepacia* [27]. The *Burkholderia territorii* is a new bacterium species, which was discovered in the environment. It is one of two new isolates, which is a complex species with *B. cepacia*, located in groundwater environments [45]. Until this very period, there had been no reports regarding the potential of *B. territorii* as a phosphate solubilizing bacterium and plant growth promoter. This was important in exploring the new potential of *B. territorii*, which played a role in increasing plant growth through the

provision of nutrients in a degraded soil system, due to human activities, such as the occurrence in the Institut Teknologi Sumatera area, where the excessive use of fertilizers during the agroforestry process of rubber plants several decades earlier had caused various changes.

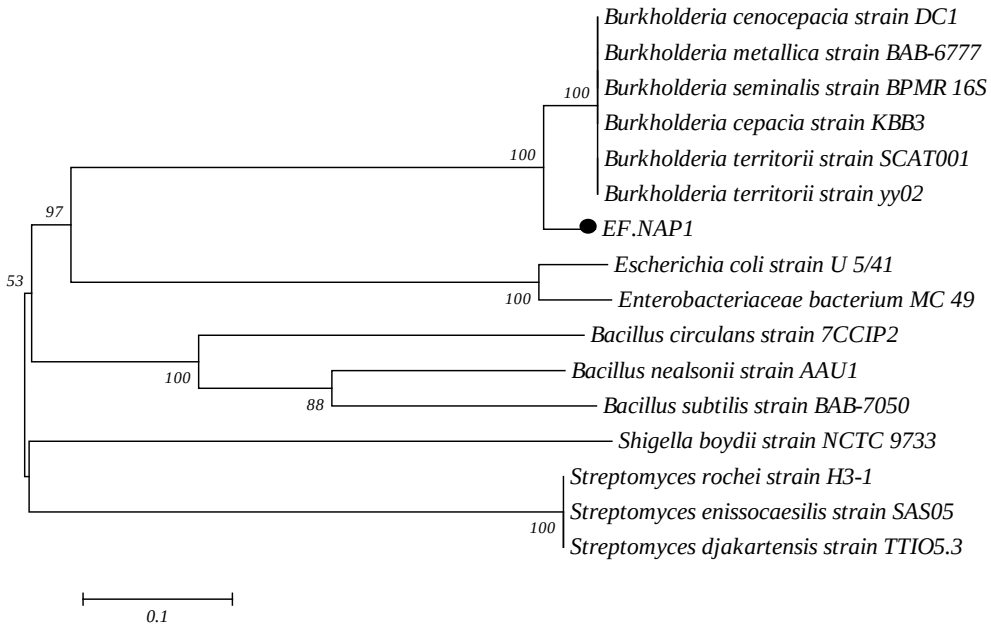


**Fig. 1.** The phylogenetic tree which describes the proximity of EF.NAP 1 isolate to other bacteria in one clade and another clade (outer group). Construction is based on the Neighbor-Joining Tree method with a bootstrap value of 1000x replication

Furthermore, the growth measurement of *B. territorii* EF.NAP 1 isolate was carried out on a liquid nutrient medium, as the growth of the bacteria was observed by increasing the number of cells during incubation. *B. territorii* EF.NAP 1 had a logarithmic time on day 2 of incubation. This logarithmic phase also showed the same correlation with the increased dissolution of phosphate by B. *territorii* EF.NAP 1. The isolate showed increased P dissolution on the second day, in the cell logarithmic phase. The P dissolution was observed until the 7th day of incubation and showed the maximum phosphate breakdown on the 6th day, with a value of 104.7mg/L (Fig. 2).

The potential of the *B. territorii* EF.NAP 1 was shown by its ability to dissolve phosphate in liquid media. The bacteria's presence in the medium greatly affected their ability to dissolve phosphate bound to Ca ions in the medium. This was observed from the phosphate dissolving activity that began to occur in the logarithmic phase of cell growth. The dissolved phosphate concentration in the growth medium occurred, due to the phosphate deposition from organic metabolism, or the formation of organophosphate compounds, based on organic acid secretion. Organic acids are used as an energy source or nutrition for bacterial cells. This condition is likely to recur in the culture medium, with the length of the fermentation process [46]. According to *Rodríguez and Fraga* [19], changes in concentration were related to dissolved P-uptake as a source of energy or nutrition for bacterial cells. When the dissolved phosphate uptake rate is higher than the dissolution level by bacteria, a decrease in the P-concentration within the medium is likely to occur. However, when the dissolved P-uptake rate

decreases (for example, because the bacteria enter a decreased growth phase or enters a stationary phase), the amount of P broken down in the media tends to increase again.

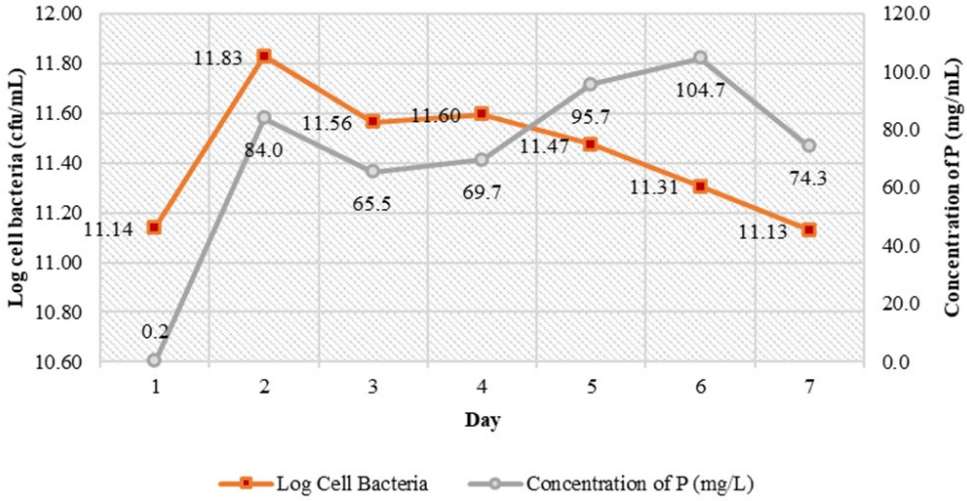


**Fig. 2.** Growth curve of cell and phosphate solubility concentration of *B. territorii* EF.NAP 1 for 7 days observation

During the 7 days of phosphate dissolving process in liquid Pikovskaya medium, *B. territorii* EF.NAP 1 isolate showed an impressive potential to increase the solubility of P within a short time. The organic acids secreted by the bacteria contributed to the dissolution of phosphate. Moreover, three factors play a role in this process, namely, pH, organic acid , and environmental acidity feedback [18]. Based on the observation during the phosphate dissolving process, the pH of the media did not decrease, due to the secretion of organic acids. The phosphate dissolution process in Pikovskaya medium also showed that the pH of the medium increased from 5.35 to 6.55 (Fig. 3). The conditions during this process improved, although not significantly. Generally, during the phosphate dissolving process, the bacteria release organic acids, therefore lowering the pH of the medium. This indicated that the phosphate dissolving process was going well [47]. The EF.NAP 1 isolate was able to secrete organic compounds such as acetic, lactic, malic, formic, fumaric, and tartaric acids with varying concentrations. The main organic acids secreted by *Burkholderia territorii* EF. NAP 1 was acetic, lactic, and malic acids with 339.14, 260.97 and 133.24mg/L, respectively. Organic acids are indicated by higher concentrations than other organic acids such as formic, fumaric, and tartaric acids, which are only 31.52, 19.31 and 19.13mg/L, respectively (Fig. 4). Citric and succinic acids were not detected in the culture medium.

This phenomenon is still required to be studied in further studies. Increasing the pH of the medium during the phosphate dissolving process was considered an advantage when bacteria were applied to acid soils. It increased pH level, in order to make the soil become more fertile and non-acid-resistant to plants. Other conditions also reported that there was no correlation between phosphate dissolution and changes in medium pH [48]. *Pseudomonas sp.* is a bacterium capable of dissolving 31.0mg/L of phosphate, without changing the pH of the medium, with that of the culture enclosure remaining at 6.0 [49]. This phosphate dissolution depends on a decrease in the pH of the medium, and also other factors, namely the production of exopolysaccharides by microorganisms [48].

The ability of phosphate solubilizing bacteria to produce organic acids varies greatly, depending on the type of microbe, adaptability, and ability to produce enzymes [50]. Not only changes in the pH of the medium, basically, phosphate solubilizing microbes also release a

number of organic compounds, including formic, acetic, propionic, lactonic, glycolic, fumarate, lactate, and succinic acids, which form chelates with cations, such as Al and Fe. This kind of binding affects the effectiveness of phosphate dissolution, which then results in readily available P, to be absorbed by plants [15, 51].

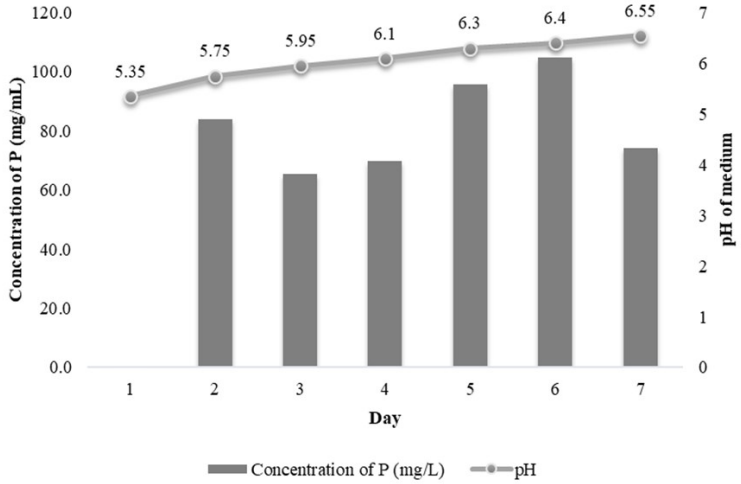


**Fig. 3**. Changes in pH of *B. territorii* EF.NAP 1 culture on pikovskaya medium

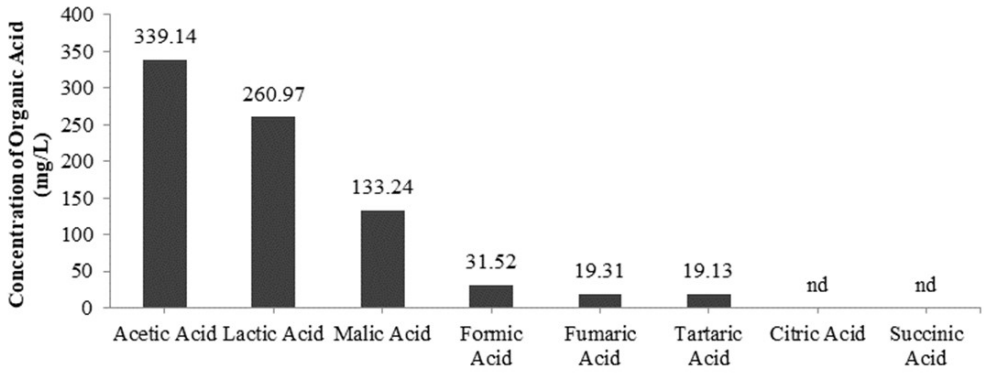


**Fig. 4.** Concentration of organic acids produced by *B. territorii* EF.NAP 1

*B. territorii* EF.NAP 1 had a fairly good ability to secrete organic acids, compared to *Rhizobium tropici* in the form of malic (18.90mmol/L) and lactic acid (0.37mmol/L). Also, *Paenibacillus kribbensis* UFLA 03-10 was only able to produce tartaric acid of 4.44mmol/L at the same pH as the EF.NAP 1 culture [48]. The high production of organic acids by the isolate was an important key in the process of dissolving phosphate in the soil. Considering that the soil conditions in the ITERA area, this region was only overgrown with grasses, and used by local residents as cassava gardens and rice fields, with poor drainage. The existence of growth-promoting bacteria has an important role in increasing land productivity, as one of their conservation efforts. The meaning of conservation is not only in terms of protection (preservation), but also in the exploration and utilization of the surrounding potential.

Furthermore, plant growth-promoting bacteria grow around the roots or rhizosphere of plants, which increases growth. Mechanisms that promote plant development include nitrogen fixation, phosphate dissolution, growth hormone production, lytic enzymes, antibiotics, siderophore, HCN, and induced systemic resistance of plants. The ability of *B. territorii*

EF.NAP 1 isolate to dissolve phosphate was a form of plant growth promoter. Isolate *B. territorii* EF.NAP 1 can produce nitrogenase activity per hour. Nitrogenase activity of EF.NAP 1 isolate was 4.33mmol ethylene/hour, which indicates the ability of bacteria to fix nitrogen in the environment. Besides that, the ability of bacteria to fix nitrogen is also needed, because P and N are essential elements for plants. The ability of a bacterium to fix nitrogen is not separated from the main role of the nitrogenase enzyme complex. Nitrogenase activities of EF.NAP 1 isolate were measured by the Acetylene Reduction Assay (ARA) method. Acetylene in the ARA method was used as an analog substrate for $N_2$ replacement. The nitrogenase complex reduces acetylene ($C_2H_2$) to ethylene ($C_2H_4$). The nitrogenase activity of a bacterium is also measured, based on the amount of ethylene gas produced [52]. EF.NAP 1 isolate had nitrogenase activity of 4,332 ppm/hour, with others such as *Acinetobacter baumannii* GPC3.7 and *Beijerinckia fluminensis* having it at 0.291 and 0.094ppm/hour, respectively [23, 37]. In addition to nitrogenase activity, IAA (Indole Acetic Acid) production is also found in EF.NAP 1 isolate. IAA level produced by EF.NAP 1 isolate was 3.47µMol/ml. Moreover, the production of indole acetic acid was substantial, therefore classifying the isolate as a plant growth promoter. The production of IAA by microbes aimed to root growth, by directly stimulating cell expansion or division [53]. The *Pseudomonas* group is known to have the ability to produce IAA like *P. fluorescent* K-34 and *P. trivalis* [54].

The isolate of *B. territorii* EF.NAP 1 showed that protease activity on skim milk agar medium, chitinase on MGMC medium, and cellulase on CMC agar media (Table 2).

**Table 2**. Plant Growth Promoting attributes of *Burkholderia territorii* EF.NAP 1.

| Isolate | Nitrogenase Activity (mmol etilen/hour) | IAA Production (µMol/ml) | Enzyme type (Diameter of Halo Zone in cm) | | | HCN | Siderophore |
|---|---|---|---|---|---|---|---|
| | | | Protease | Chitinase | Cellulase | | |
| EF.NAP 1 | 4.332 | 3.47 | 1.75±0.05 | 1.43± .05 | 1.00±0.08 | + | + |

The isolates' activity in producing hydrolyze enzyme was not too high, but these results indicate that there is potential for these bacteria as candidates for producing degradation enzymes. The isolate of *B. territorii* EF.NAP 1 can produce HCN by forming colour changes in the filter paper from yellow to moderate brown. Changes in colour to brown on the filter paper clearly show that EF.NAP 1 isolate are capable of producing HCN. Isolate of *B. territorii* EF.NAP 1 can produce siderophores on CAS agar medium. This condition is indicated by the existence of an orange halo formation around a bacterial colony (Table 2.). PGPR is another important thing to know, as it is the activity of hydrolysis enzymes, such as protease, chitinases, and cellulase. The existence of this enzyme is important, because of its inhibitory mechanism against the cell walls of pathogenic fungi containing chitin [55, 56]. Inhibition of pathogenic fungi is through the production mechanism of antimicrobial substances, such as chitinase, laminarinase, cellulose, HCN, siderophore, and antibiotics [24, 57–59].

Besides that, the protease enzyme is also involved in the process of inhibiting pathogenic fungi [60]. These results indicated that *Burkholderia territorii* EF.NAP 1 should be used as a plant growth promoter, due to its dissolution of phosphate, production of organic acids, possession of nitrogenase activity, production of IAA, and secretion of metabolites, which suppresses pathogens, such as lytic enzymes, HCN, and siderophore. The ability of *B. territorii* EF.NAP 1 isolate also had great potential in increasing plant growth. The ability of these bacteria to increase soil fertility was a major obstacle in the reforestation and area conservation program at the Institut Teknologi Sumatera. Therefore, fertility due to microbial interactions in the soil increased the number of growing plants populations and another biodiversity in the region.

## Conclusions

Phosphate solubilizing bacteria were isolated from the acid soil of an old rubber plantation at the Institut Teknologi Sumatera in Lampung, Indonesia. The EF.NAP 1 isolate was able to dissolve phosphate on the 6th day in a liquid medium from Pikovskaya at 104.7mg/L. In the process of phosphate dissolution, EF.NAP 1 can produce various organic acids, such as acetic acid (339.14mg/L), lactic acid (260.97mg/L), malic acid (133.24mg/L), formic acid (31.52mg/L), fumaric acid (19.31mg/L) and tartaric acid (19.13mg/L). In the EF.NAP 1 phosphate dissolution process, the pH of the medium increased throughout the fermentation period, with the average pH value 5.75-6.55.

On the basis of the identification of the 16S rRNA gene, it has been shown that the isolate of EF.NAP 1 is closely related to *Burkholderia terrtorii*. These bacteria also have plant growth promoters such as IAA (3.47µMol/ml), nitrogenase (4.33mmol etilen/hour), siderophores, HCN, lytic enzymes (chitinase, protease, cellulase). EF.NAP 1 has all the capacity to be used as an alternative to improving the soil condition of the Institut Teknologi Sumatera and is developing a programme of reforestation and conservation.

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