Research article

Use of Bacillus as a Plant Growth-Promoting Rhizobacteria to Improve Phosphate and Potassium Availability in Acidic and Saline Soils

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Abstract.
Bacillus is a rhizobacterium that can help with the nutrient cycle in the soil. Plant growth-promoting rhizobacteria (PGPR) can be found in the rhizosphere of many plants. This research was divided into two parts: (1) a test of the ability of *Bacillus* genus isolates to withstand various NaCl concentrations in saline and acidic soils; and (2) quantification of the secondary metabolites produced by the rhizobacteria in the form of hormones and organic acids. *Bacillus valezensis* (BPF2), which can dissolve phosphate, *Bacillus sp* (BPK1), and *Bacillus subtilis* (BPK2), which can dissolve potassium, were the isolates tested. *Bacillus* increased the availability of phosphate and potassium in the saline and acidic soils and the secondary metabolites produced, such as organic acids and the hormone indole acetic acid. The results showed that the three isolates could still dissolve phosphorous and potassium with a 3% NaCl addition, but the concentration decreased as the incubation time increased to H+15. On the 30th day, *Bacillus valezensis* inoculation improved soil phosphate availability by up to 88% in the acidic soil and 73% in the saline soil compared to the control. On the other hand, *Bacillus sp. and Bacillus subtilis* raised the potassium concentration in the acidic soil until day 10, reaching a maximum of 0.37 me.100g^{-1}. The three PGPRs (*Bacillus valezensis, Bacillus sp., and Bacillus subtilis*) produced 13.25, 11.97, and 14.97 g.mL^{-1} of indole acetic acid metabolites, respectively. Acetic, lactic, citric, malic, and oxalic acids were among the organic acids produced. *Bacillus valezensis* produced the most lactic acid at 4.94 mg.L^{-1}, while *Bacillus sp.* and *Bacillus subtilis* produced the most acetic acid, at 2.91 and 2.55 mg,L^{-1}, respectively.

Keywords: Bacillus, Organic acid, Indole acetic acid

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1. Introduction

Microorganisms in the soil, especially in the rhizosphere, play an essential role in soil formation, biogeochemical cycles, macro and micronutrient cycles, organic matter decomposition, and the transformation of toxic forms into non-toxic forms. One that dominates the rhizosphere area is a group of bacteria (Rhizobakteria). The composition

of the bacterial community depends on the root zone, plant species, plant phenological phase, stress, and the presence of disease[1]. The beneficial rhizobacteria were declared as Plant growth promotion rhizobacteria (PGPR). Generally, around 2–5% of rhizosphere bacteria are PGPR. Among the beneficial rhizobacteria in soil is the phylum Firmicutes, genus Bacillus. The general characteristics of the genus Bacillus are rod-shaped[2], Gram-positive, aerobic or facultatively anaerobic, catalase-positive, and endospore-forming. *Bacteroidetes, Betaproteobacteria*, and *Alphaproteobacteria* are bacterial groups abundantly found in four different soil types[3].

Several members of the genus Bacillus have beneficial roles, directly or indirectly, assisting plants in acquiring nutrients, producing phytohormones, protecting against pathogens and other abiotic stresses[4]. Various direct and indirect mechanisms by *Bacillus spp* as PGPR include nitrogen fixation, dissolution and mineralization of phosphorus and other nutrients, phytohormones production, siderophores, antimicrobial compounds, and hydrolytic enzymes, induced systemic resistance (ISR), and tolerance to abiotic stress[5]. Direct mechanisms include mechanisms that affect the balance of growth regulators, either because microbes release natural growth regulators in plants or because microbes act as absorbents for plant-released hormones and induce plant metabolism, which leads to increased adaptability. In comparison, the indirect mechanism requires plant defense metabolic processes, which respond to signals sent from bacteria. Two important indirect mechanisms are the induction of systemic resistance to plant pathogens (biotic stress) and protection against unhealthy environmental conditions (abiotic stress).

Several in vitro studies have proven that PGPR can directly or indirectly produce several phytohormones/ZPT, including auxins, gibberellins (GAS), cytokinins (CK), and nitric oxide (NO), as well as other compounds. Therefore, PGPR regulation in assisting plant growth is expected to support the tolerance level of plants in overcoming abiotic (environmental) stresses, especially stress with salinity (salinity stress) and acid (acidity stress) for plants. In particular, several Bacillus species such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus, B. subtilis* have been reported as phosphate solubilizing bacteria[6].

In addition, Bacillus species bacteria can also produce IAA hormones; the research results [7] *Bacillus sp. strain JH 2□2* produced IAA with a 6.44 $mg.ml^{-1}$ in the Cr bioremediation experiment. Another study tested *Bacillus aryabhattai SK1-7,* which can release potassium from the surface of feldspar minerals, and the potassium concentration reached 10.8 g/mL; the percentage of potassium released is 32.6%[8]. Four hundred forty isolates of *Bacillus sp* from various sources were tested in dissolving

phosphate and producing organic acids[9]. The results showed that the dissolution of phosphate ranged from 6.9 ±1.00 to 95.5 ± 1.83 g.mL^{-1} for Bacillus isolates, and most of the isolates were able to produce more than one organic acid, namely acetic acid, propionic acid, isobutyric acid, isocaproic acid, caproic acid, and heptanoic acid with the total acid concentration produced in the range between 70.70 ± 1.90 and 619.20 ± 1.40 (ng.µL^{--1}). In addition, these bacteria can produce secondary metabolites Bacillomycin and fengycin, which are proven to Figureht phytopathogenic fungi[10].

Soil is classified as saline if the saturated extract from saline soil has a DHL (electrical conductivity) or EC (electrical conductivity) value greater than four dSiemens/m (equivalent to 40 mM NaCl) and the percentage of exchangeable sodium (ESP = exchangeable sodium percentage) usually less than 15. Saline soils mainly contain soluble salt components, including calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), and bicarbonate (HCO_3), or sulfate (SO_4). In addition, saline soils contain high levels of Na+ ions in the soil's absorption complex, causing disturbance and imbalance in the uptake of water and nutrients for crops and disadvantages in soil physical properties. Soil salinity can also affect crops by causing nutrient deficiencies or nutrient imbalances in plants, such as the imbalance in the ratio of Na^{+}/Ca2^{+}. In particular, the availability of P is reduced due to the ionic strength effect, a high sorption capacity to soil particles, and low solubility of minerals in saline soil. In addition, acid soils have some problems, especially low pH and low phosphorus availability. This study aimed to determine the potential of three Bacillus species in dissolving P and K nutrients under saline and acid soil and quantify the metabolites produced in the form of the hormone indole acetic acid (IAA) and organic acids.

2. Methodology

The research was carried out at the Soil Biology Laboratory, Faculty of Agriculture, University of Jember. The tested isolates of the PGPR genus Bacillus were three species, namely (1) *Bacillus valezensis* (BPF2), which could dissolve phosphate, while (2) *Bacillus sp* (BPK1) and (3) *Bacillus subtilis* (BPK2) could dissolve potassium. The three isolates are the collection of the Soil Biology Laboratory, Faculty of Agriculture, University of Jember. In addition, refreshing Bacillus isolates were carried out on Nutrient broth media and specific media Pikovskaya and Alexandrov's.

2.1. Test of tolerance and dissolving ability at some NaCl concentration

The salinity tolerance test was carried out by growing isolates on specific media containing NaCl with concentrations of 0, 0.3%, 0.5%, and 1.0% for solid media, and continued with quantitative testing with concentrations of 0%, 1%, 2 %, and 3% in liquid media. Qualitatively, the dissolution of P and K was carried out on specific solid media, namely Pikovskaya and Alexandrov modifications. Every day observed and measured the diameter of the colony and diameter of the halo zone to calculate the dissolution index for seven days with the formula Solubilization Index (SI) = halo diameter (mm)/colony diameter (mm). Observation of activity and development to calculate the Solubilization Index of *Bacillus* on Alexandrov solid media (potassium dissolution) used bromothymol blue (BTB) indicator, with a concentration of 100 ppm bromothymol blue; the method is a modified method that was previously with the use of Alexandrov media without BTB[11].

Quantitative testing used 50 ml of Pikovskaya media and modified Alexandrov media (Potassium source from leucite and feldspar minerals) with a predetermined concentration of NaCl. Each was inoculated with 1 ml of Bacillus isolate that had been grown on Nutrient Broth media. The medium was incubated at room temperature, shaken with an orbitary shaker at low speed. Every five days, an analysis on the pH of the solution, available P concentration (water-soluble and Bray 2) using a Spectrophotometer (Shimadzu UV-1800), and available K concentration (2% citric acid extract) by an Atomic Absorption Spectrophotometer (Shimadzu AA 6300).

2.2. Test the dissolving capability of P and K elements from acid and saline soils

The test was carried out on two types of soil, namely, saline Inceptisol soil originating from Situbondo district (Agel sub-district and Banongan sub-district) and acid soil (Ultisol and Inceptisol) originating from Bogor Regency (Table 1). The saline soil has Sodium Absorption Ratio (SAR) characteristics of 4,92 and 5,51; electrical conductivity (EC) 6,23 $dS.m^{-1}$. A total of 250 g of sterile soil at 80% field capacity were inoculated by PGPR isolate with a density ($\pm 10^8$ cfu/g soil). Incubation for 30 days, at the beginning and regularly every ten days, the pH of the solution, the concentration of P, K, Na, and Ca were analyzed. The viability of the isolates was calculated on day 30 using the Plate count method on solid Pikovskaya and Alexandrov selective media. Organic acid measurements were also carried out on inoculation treatments in saline and acid soils

using Shimadzu 20A High-Performance Liquid Chromatography (HPLC) with a flow rate of 0.5 ml.min^{-1} and an injection volume of 5µl.

Table 1: Characteristics of saline and acid soils for dissolution test.

Soil	pH		N-total (%)	P available (ppm)	K available (me.100g^{-1})	CEC cmol.100 g^{-1}	Percentage			Texture
	H_2O	KCl					% clay	% sand	% silt	
Acid: Inceptisol (M1)	5,67	4,77	0,081	4,60	0,44	5,80	78,02	1,49	20,49	clay
Acid: Ultisol (M2)	4,65	3,69	0,106	13,30	0,15	11,59	74,30	3,69	22,01	clay
Saline: Inceptisol Agel (S1)	7,07	6,58	0,097	12,44	15,56	18,58	20,79	31,25	47,97	Loam
Saline: Inceptisol Banongan (S2)	6,73	6,59	0,066	11,74	17,93	44,85	20,16	27,36	52,48	Silt loam

2.3. Quantification of metabolites

2.3.1. Determination of IAA Hormones

To analyze the ability of isolates to produce IAA hormone, sterile 25 ml Tryptic Soy Broth (TSB) media was used in an Erlenmeyer flask enriched with 2% L-Tryptophan. The suspension was inoculated with isolate as much as one ose of bacterial cells from the agar slant. The bacteria were incubated in a dark environment and moved with an orbital shaker with a rotating speed of 120 rpm. TSB media that isolates have inoculated is taken as much as 3 ml and then put into a centrifuge tube. The culture was centrifuged at 10,000 rpm for 10 minutes. A total of 2 ml of the supernatant was added with 1 ml of Salkowski's reagent (2:1), homogenized with a vortex, and incubated for 1 hour. The absorbance of the suspension was measured by a spectrophotometer (Shimadzu UV-1800) at a wavelength (λ) of 535 nm, and then the concentration of IAA was calculated using the IAA standard curve equation. Sampling was carried out at incubation times of 24 and 72 hours[12] and [13].

2.4. Determination of Organic Acids

Bacterial cells of ±1.0 x 10^{10} CFU were inoculated on 100 ml of Pikovskaya and Alexandrov medium and incubated for three days at room temperature on a 100 rpm shaker. At the end of incubation, the culture was centrifuged at 7,500 rpm at 25°C for 20 minutes.

The filtrate obtained was used to determine the levels of organic acids, namely: citric, oxalic, formic, succinic, butyric, acetic, and propionic acids. Determination was carried out according to standards using High-Performance Liquid Chromatography (HPLC) Shimadzu 20A with a flow rate of 0.5 ml.minute^{-1} and an injection volume of 5µl.

3. Result and Discussion

3.1. Test of P and K dissolution by Bacillus under various saline conditions

The three rhizobacteria *Bacillus* that were tested for their capabilities were derived from grass plant rhizosphere, namely *Bacillus valezensis* (BPF2) and *Bacillus subtilis* (BPK2), while *Bacillus sp* (BPK1) came from the rhizosphere of sugarcane plants. *Bacillus sp* and *B. subtilis* isolates could dissolve potassium in saline conditions (Figure 1). The dissolution was characterized by a change in color (yellow) on the potassium-solubilizing bacterial colonies from Alexandrov's media given Bromothymol blue (BTB). The use of BTB dye is a modification of the use of Alexandrov's media to facilitate analyzing the dissolution index through changes in the pH of the media. According to[14] Aleksandrov et al. (1967), screening potassium solubilizing bacteria on Alexandrov media was based on the exopolysaccharide produced. However, Potassium solubilizing-bacteria activity of in dissolving potassium other than through exopolysaccharides can be through the production of protons, siderophores, organic acids, or organic ligands[15], which is very likely to modify media acidity.

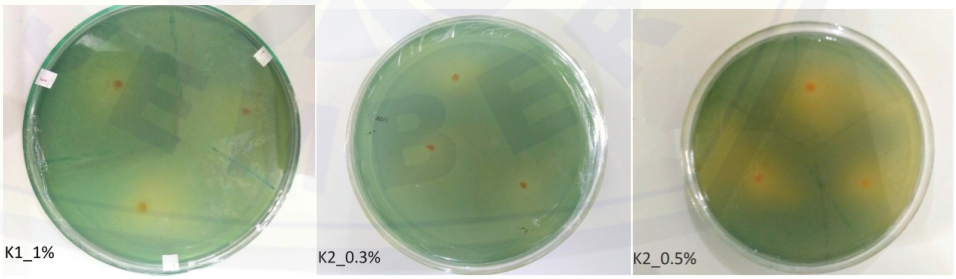


Figure 1: Dissolution of Potassium by *Bacillus sp* and *Bacillus subtilis* on Alexandrov modified agar using Bromothymol blue (BTB) after seven days of incubation at several salinity concentrations.

The dissolution of potassium in solid media with 1% NaCl concentration by both bacteria still showed a halo zone with a dissolution index of 1.70 to 1.93 with an average colony diameter of 2.49 mm to 3.56 mm on the seventh day. In control with 0% NaCl, the dissolution index on Alexandrov media was 1.38 and 1.90. This condition shows the ability of the two bacteria to metabolize well. The use of Bromothymol blue (BTB)

(Figure1) changes the color of the media to yellow around the isolate because the organic acids produced by BPK change the pH of the media[11]. Although the *Bacillus* produce acetic, lactic, citric, malic, and oxalic acids (Table 4), *Bacillus sp* isolates tested on Alexandrov media with agromineral sources also produced polyphenol organic acids (ferulic, syringic, and coumaric acid) with total concentrations ranging from 130.42 to 434.44 mg.L^{-1}[16].

The PGPR test of phosphate solubilizing bacteria *Bacillus valezensis* was able to form a clear zone (hallo zone) on Pikovskaya media of 1.06 and a colony diameter of 8.37mm with a NaCl concentration of up to 1%, through the acidification process due to the production of organic acids (Figure 2 and Table 2).

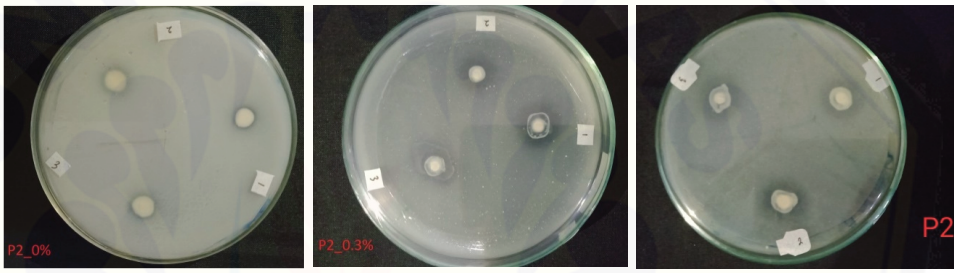


Figure 2: Dissolution of Phosphate by *Bacillus valezensis* on Pikovskaya agar after the 7th day incubation.

The three Bacillus isolates on first-day dissolving have started to occur, so it is classified as ”fast,” but the dissolution index <2 indicates low dissolving ability except for *Bacillus valezensis* with a 0.3%NaCl concentration is 2.51. Qualitative test results have not shown the concentration of dissolved phosphate or potassium. Furthermore, the capabilities of the two functional bacteria were tested quantitatively using Pikovskaya selective media for phosphate solubilizing bacteria (BPF) and Alexandrov media for potassium solubilizing bacteria (BPK).

Table 2: Solubility Index on selective media at Day+7.

Isolate	Consentration NaCl			
	0	0,30%	0,50%	1,00%
Bacillus sp	1,38	1,42	2,03	1,70
Bacillus subtilis	1,90	1,98	1,99	1,92
Bacillus valezensis	1,27	2,51	1,57	1,06

Description: *Bacillus sp* and *B. subtilis* on Alexandrovs modified media, *B. valezensis* on Pikovskaya media

The concentration of water-soluble P at 0% NaCl on the 15th day after inoculation was more significant than the concentration of water-soluble P with 1% and 3% NaCl, which was 12.80 ppm (Figure 3a) with a decreasing dissolution pattern at D+10 then increases

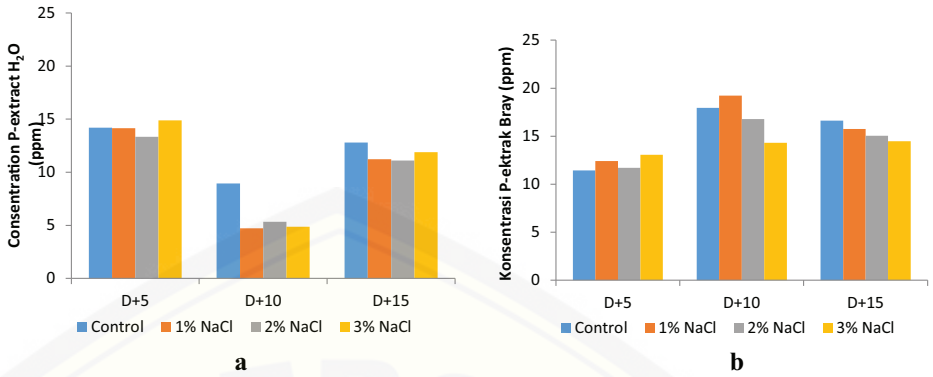


Figure 3: The concentration of water-soluble P (a) and P-available Bray extract (b) at three salinity conditions up to Day 15 inoculated with *Bacillus valezensis*.

to D+15. Meanwhile, the concentration of available P with Bray extract increased at D+10 and decreased at D+15, with the highest concentration at 0% NaCl at 16.63 ppm (Figure 3b).

Changes in the condition of the media to become more saline in the presence of NaCl gave lower concentrations in both water-soluble P and Bray-extracted. Water-soluble P concentration decreased by 9.98% in control, compared with the presence of NaCl, which was decreased >16%. While concentration available phosphate increased by 45.45%, 26.72%, 28 ,51% and 10.82% by added 0%, 1%, 2% and 3% NaCl concentrations, respectively. The addition of NaCl on Pikovskaya media required *Bacillus valezensis* to adapt first. In general, there are at least three mechanisms for microorganisms to adapt to different salt concentrations in the culture medium. The first is passive ions, where the concentration of cytoplasmic ions is always the same as the concentration in the culture medium. The second mechanism used by many organisms involves the concentration of compatible solutes to create an osmotic balance between the cytoplasm and the external environment. The third mechanism involves changes in cell physiology to control the movement of water that allows ionic dilution of the cell cytoplasm[17].

A quantitative test of potassium dissolution using Alexandrov media with K sources from leucite and feldspar minerals was carried out for 15 days showing a decrease in the concentration of water-soluble K and 2% citric acid-soluble K (Figure 4a and 4b).

The salinity conditions (1%, 2%, and 3% NaCl) in media with K sources of leucite and feldspar did not affect the activity of Bacillus in dissolving K, so the concentration of water-soluble K and citric acid-soluble K was not lower than 0% NaCl. *Bacillus sp* and *Bacillus subtilis* can adapt to saline conditions and provide potassium dissolving ability, but with increasing time, the concentration of K decreases. The decrease in

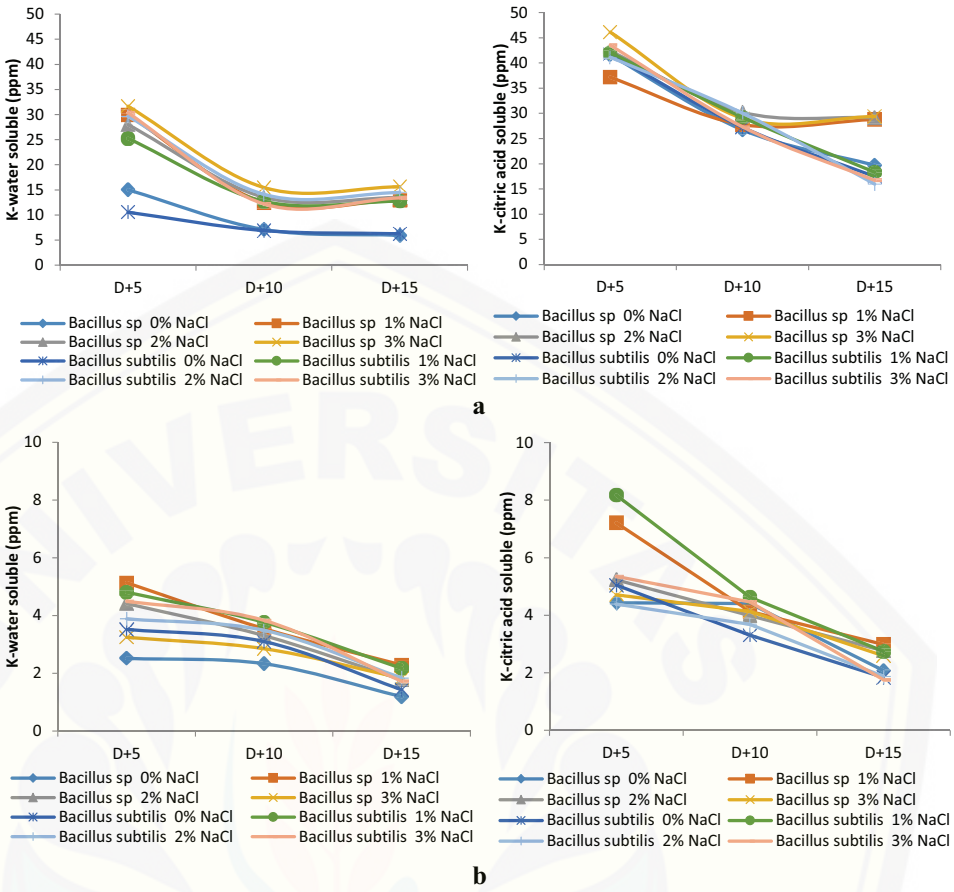


Figure 4: Potassium water-soluble and citric acid-soluble concentration at several salinity conditions up to D+15 inoculated by *Bacillus sp* and *Bacillus subtilis* with potassium sources (a) Leucite and (b) Feldspar.

concentration from D+5 to D+15 was >40% in both K leucite and feldspar sources. The concentration of K dissolved from the mineral leucite is higher than the dissolution of the mineral feldspar because the potassium content of the mineral leucite is greater than feldspar; besides, the structure and position of K in the leucite mineral is more easily separated.

In general, potassium solubilizing bacteria dissolve K from minerals or soil (clay minerals) in the same as the mechanism for dissolving phosphate, including through organic acids (acidolysis mechanism). Carboxylic acids such as citric acid can acidify the medium so that the mineral becomes unstable and allows the release of K_2O[18]. The released H^+ ions can directly dissolve K minerals due to the slow release of exchangeable K so that exchangeable K is readily available[19].

3.2. Quantitative test on acid and saline soil

The dissolution ability test of *Bacillus* isolates was carried out on acid soils and saline soils with different soil characteristics (Table 3), two types of acid soils (Inceptisols and Ultisols), and two types of saline soils (Inceptisols in Banongan village and Inceptisols in Agel villages, Situbondo Regency).

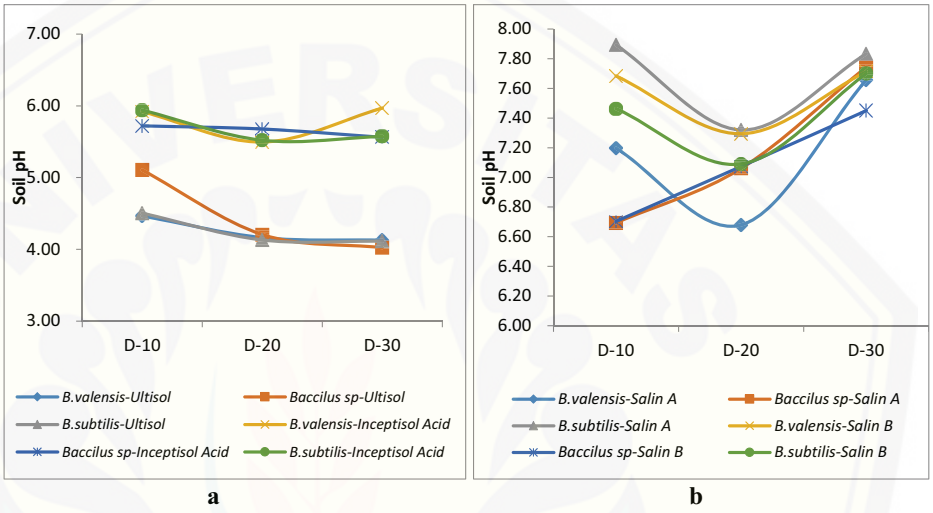


Figure 5: Changes in pH (a) acid soil and (b) saline soil.

In acid soils, *Bacillus* inoculation generally decreased the pH of Ultisol and Inceptisol soils (Figure 5a), whereas, in saline soils, it decreased on the 20th day and increased again on the 30th day. The lower pH changes in acidic soils due to the acidulation process of the organic acids produced, the three Bacillus produce organic acids, including acetic acid, lactic acid, malic acid, and oxalic acid. The research results of [20] prove lactic acid, oxalic acid, and citric acid's ability to reduce the soil pH of Plagic Anthrosols. However, the organic acids produced, such as lactic acid, which is a weak acid but its presence in soil solution can lower soil pH. On the other hand, the pattern of decreasing soil pH in saline soils occurred from the 10th day to the 20th day. However, there was an increase in pH on the 30th day (Figure 5b). The pH changes in saline soils are due to the dissociation of organic acids produced, which causes an increase in H^+ ions in the soil solution. With increasing time in saline soils, there will be an increase in the ionic strength of the solution. As the ionic strength of the soil solution increases, cations such as Na, K, Ca, Mg or anions such as Cl, and SO_4 become dominant in the saline soil solution, and partial exchange of H+ or OH^- from the clay surface will occur. Cation exchange with H^+ or OH^- causes a decrease and increase in pH in saline soils.

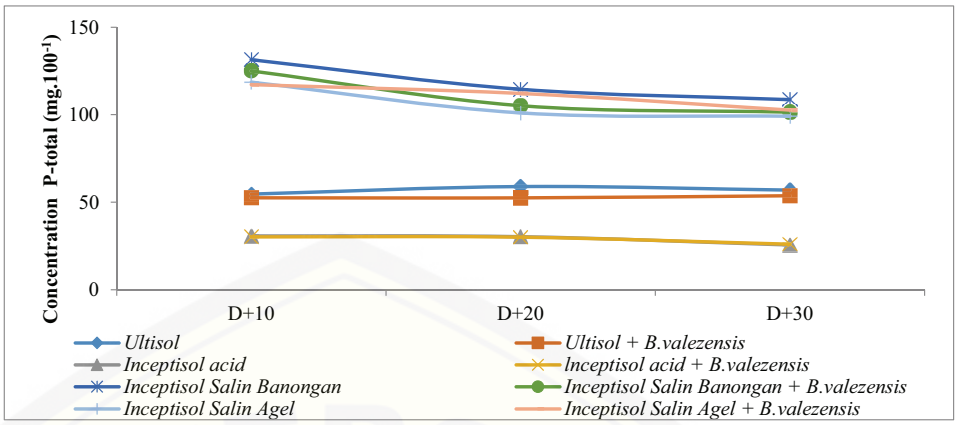


Figure 6: The concentration of P-total in acid and saline soils by *Bacillus valezensis*.

In general, the total P concentration in both acid and saline soils decreased with the length of time in all treatments with and without *Bacillus valensenziz* (Figure 6). Therefore, chemically and biologically, P dissolution will occur to reduce the total P concentration of the soil. In soil, phosphorus is unavailable P-organic, unavailable P-inorganic, and P-inorganic in soil solution. Generally, the total phosphorus content in the soil is about 0.05% (w/w), and plants consume only 0.1% of bioavailable P.

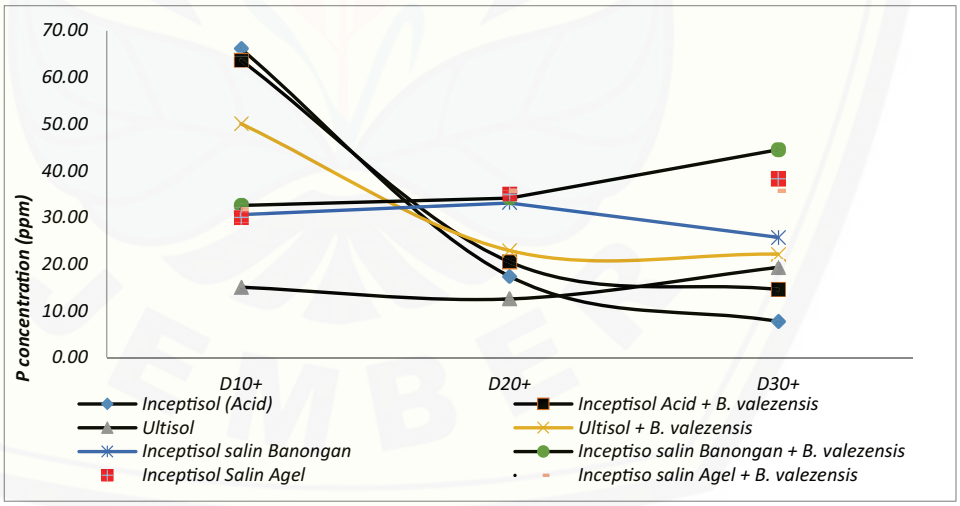


Figure 7: The concentration of available P (Bray) in acid and saline soils by *Bacillus valezensis*.

Bacillus valezensis on acid Ultisol increased phosphate availability up to 231.54% on the 10th day compared to without *B. valezensis* inoculation, but on the 30th day, the increase decreased to 14.56%. The available phosphate concentration with *B. valezensis* was 22.15 ppm (Figure 7). On the other hand, in acid Inceptisol, the activity of *B. valezensis* increased phosphate availability by 88.00% on the 30th day of 14.66 ppm (Figure 8). The mechanism of increasing P solubility that occurred with the application

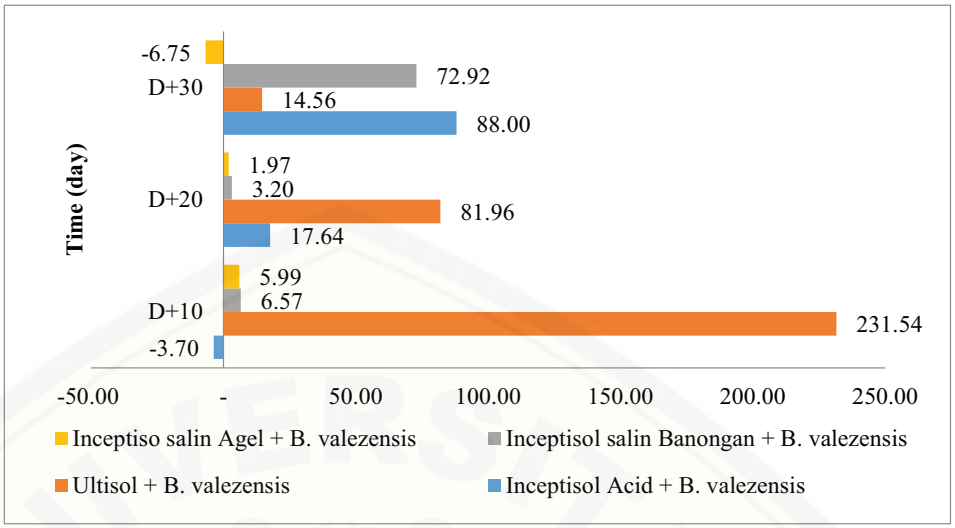
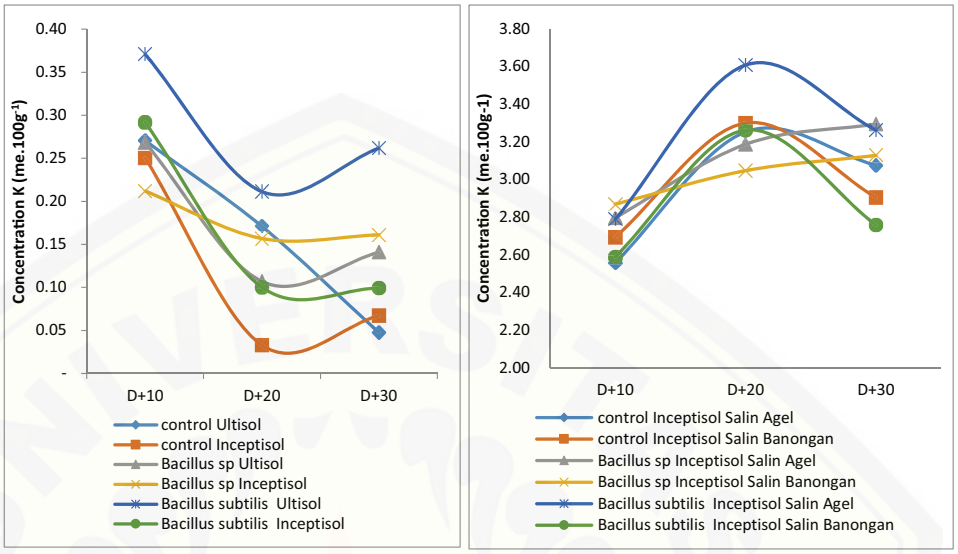


Figure 8: Percentage increase in phosphate concentration of Bray extract against the non-inoculated treatment of *Bacillus valezensis* on acid and saline soil.

of PGPR included (i) competition, Organic anions are produced during mineralization at the same adsorption site as the P adsorption site, and (ii) Al or Fe complexing is exchanged with organic acids[21]. The presence of organic acids with low-molecular weights effectively chelates Al and Fe from Fe-P and Al-P in acid soils to dissolve P [22]. Organic ligands such as tartaric, oxalate, malic, and citric acids containing carboxyl (COOH), aliphatic-OH, phenolic-hydroxyl groups are very effective in dissolving minerals and forming chelates with Al, Fe, Ca, and other elements, and lowering the pH of the media[23], and affect the P fractionation of acid soils[24]. However, the concentration of P with the overtime incubation period decreased (Figure 7); this was partly due to the desorption of phosphate anions on the clay surface or the precipitation process by other cations such as Fe or Al.

The increase in phosphorus concentration in saline soils is less than in acidic soils. The highest increase was 72.92% on the 30th day in Banongan saline Inceptisol of 25.75 ppm. Although the increase in phosphate availability was lower than in acid soils, the P concentration was higher. In general, the longer the incubation time, the P concentration increased with the inoculation of *B. valezensis*. The increase in available P in saline soils due to the presence of organic acids occurs through several mechanisms, namely (a) acidification, the presence of organic acids that have the potential to dissolve inorganic phosphate through (i) a decrease in pH; (ii) compete with P anions at adsorption sites; and (iii) form soluble complex ions (Ca, Al, and Fe) which bind to insoluble P and thereby release soluble P[25]; (b) protonation, among others through proton excretion after

assimilation of NH_4^+ and production of H_2CO_3 due to respiration by PSB; (c) chelating by organic cations.



a b

Figure 9: Potassium concentration in acid and saline soil by *Bacillus sp* and *Bacillus subtilis*.

The pattern of changes in potassium availability in acidic and saline soils differs with the length of incubation time (Figures 9a and 9b). The increase in potassium concentration in saline soils was only <30%, while on acid soils increased >100% up to Day 30. On acid soils, the activity of *Bacillus sp* and *Bacillus subtilis* gave more significant potassium dissolution than control up to D+30, but the concentration at D+20 and D+30 was lower than the concentration at D+10. Percentages of decreased potassium availability are partly due to the fixation or desorption by clay minerals. The fixation and release of K ions are influenced by the concentration of K^+ ion in soil solution, the type of clay minerals, wetting and drying, pore size distribution, clay content, and clay mineral type[26]. Desorption of non-exchangeable potassium (NEK) in the soil depends on the potassium release kinetic, which is mainly attributed to the difference in particle size distribution, clay content, and the type of clay minerals [27]; this is will supply available K in the soil.

Bacillus sp and *B. subtilis* on acid soils increased potassium availability more than on saline soils, under the larger population (Table 3) and higher respiration rates. Respiration indicates the activity of microorganisms, including the dissolution of nutrients.

In general, the population of all *Bacillus* decreased after 30 days of inoculation on acid soil and saline soil (Table 3), with the density of inoculated cells for *B. velezensis* $\pm 65.10^8$cfu to $2.88.10^8$ cfu in Ultisol and $66.23.10^8$ cfu in acid Inceptisol. In Inceptisol saline, the population decreased from 1.83 to $2.07.10^8$ cfu. Saline conditions in the soil

Table 3: Bacillus population at 30^{th} days in acid and saline soil.

Isolate PGPR	Soil	microorganisms (10^8) (cfu/g)	Soil	microorganisms (10^8) (cfu/g)
Bacillus valezensis	Inceptisol Salin Banongan	1,83	Inceptisol Salin Agel	2,07
Bacillus sp		2,02		0,78
Bacillus subtilis		1,47		2,01
Bacillus valezensis	Ultisol	2,88	Inceptisol (Bogor)	66,23
Bacillus sp		2,12		16,98
Bacillus subtilis		2,94		24,03

increase the osmotic potential in the soil solution so that it will cause plasmolysis of *B. valezensis* cells.

Table 4: The concentration of organic acids produced by PGPR in Pikovskaya and Alexandrov's selective liquid media.

No	Isolate PGPR	Source	Concentration ($mg.L^{-1}$)				
			Acetic acid	Lactic acid	citric acid	maleic acid	Oxalic acid
1	*Bacillus sp*	K_2HPO_4	2,907	1,757	0,021	0,342	0,059
2	*Bacillus subtilis*		2,547	1,630	0,015	0,362	0,064
3	*Bacillus sp*	Leucite	0,346	1,183	0,009	0,280	0,076
4	*Bacillus subtilis*		0,499	0,941	0,015	0,358	0,059
5	*Bacillus valezensis*	Ca_3HPO_4	2,705	4,943	0,056	0,740	0,063
		Rock phosphate	0,487	2,391	0,022	0,384	0,075

During the dissolution of phosphate sources ($Ca_3HPO_4$and Rock phosphate) by *B.valezensis,* the production of acetate, lactate, citrate, malic, and oxalate was detected (Table 4) with a total acid release of 8,507 $mg.L^{-1}$ for Ca_3HPO_4 and 3,359 $mg.L^{-1}$ for Rock phosphate sources respectively. Thus, the highest production of lactic acid, while the least amount of citric acid produced by *B. valezensis*.

The quantitative difference concentration in organic acid production was found during potassium's solubilization by *Bacillus* and *Bacillus subtilis* (Table 4). The quantities of organic acids produced ranged from 0.009 – 2.907 $mg.L^{-1}$. During the dissolution of the Leucite agromineral, the two Bacillus isolates produced the highest amounts of lactic acid, namely 1.183 and 0.941 $mg.L^{-1}$.

Table 5: The concentration of the hormone Indole Acetic Acid (IAA) produced by PGPR.

Isolate PGPR	Concentration (µg.mL^{-1})	
	24 hours	72 hours
Bacillus valezensis	13,67	13,25
Bacillus sp	13,02	11,97
Bacillus subtilis	12,71	14,97

The three Bacillus tested could produce IAA hormone, tested at 24 hours and 72 hours at concentrations of 11.97 to 14.97 g.mL^{-1} (Table 5). Bacillus tested were bacteria classified as ”medium” in producing IAA with concentrations in the range of 10-20 ppm[28], and high category if the concentration of IAA produced was >20 ppm. The concentration of IAA produced by the three isolates in the range of 10-20 ppm did not show maximum IAA production. The formation of IAA is influenced by environmental pH, which is generally optimum at near-neutral pH, optimum temperature of 37°C, carbon source, and presence of precursors. The use of Tryptic Soy Broth (TSB) media with a pH of 7.3 provides a reasonably good environment for Bacillus to produce IAA, and the administration of 2% L-Tryptophan on TSB media also stimulates the formation of IAA. A neutral pH stimulates enzyme performance to be optimal in converting L-Tryptophan precursors into IAA. The mechanism or pathway influences the concentration produced in producing IAA, cell activity and density, nutrient availability, and L-Tryptophan substrate concentration. The research [29] isolates *Bacillus cereus,* and *Bacillus subtilis* produced an IAA of 35.8 and 36.6 g.mL-1 with L-tryptophan to yeast extract mannitol broth (YEMB) media at 37°C.

4. Conclusion

The dissolution index in the control H+7, while the addition of NaCl by phosphate solubilizing PGPR ranged from 1.06 to 2.51, while inoculation by potassium solubilizing PGPR from 1.38 to 2.03. The three isolates were still able to dissolve both P and K on a liquid medium with the addition of NaCl up to 3%, but the concentration decreased with incubation time up to H+15.

Inoculation with *Bacillus valezensiz* bacteria in Ultisol acid soil increased phosphate availability up to 232% on the 10th day, while the Inceptisol acid soil increased the maximum 88,00% on the 30th day. In saline soil, the P concentration increase was 72.92% on day 30 in Inceptisol Banongan soil, with a concentration of 25.75 ppm.

The potassium concentration on the 30th day was increased relatively high with Bacillus inoculation on acid soil, with a concentration of 0.26 me.100g^{-1} while on saline

soil, Bacillus inoculation increased 29.09%. Thus, the maximum Potassium concentration on day 30 by Bacillus activity was 3.29 me.100g^{-1}

The three PGPRs (*Bacillus valezensis, Bacillus sp,* and *Bacillus subtilis*) can produce metabolites of Indole Acetic acid, respectively, 13.25 g.mL^{-1}, 11.97, and 14.97 g.mL^{-1}. Organic acids produced include acetic, lactic, citric, malic, and oxalic acids. *Bacillus valezensis* produced lactic acid with the highest concentration of 4.94 mg.L^{-1}, while *Bacillus sp* and *Bacillus subtilis* produced acetic acid with the greatest concentration of 2.91 and 2.55 mg.L^{-1}.

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