Research article

The Influence of Different Acidic Conditions on the Plant Growth-Promoting Rhizobacteria Activity of Phosphate Solubilizing Bacteria

Betty Natalie Fitriatin[1]*, Detty Asna Fauziah[2], Reginawanti Hindersah[1], and Tualar Simarmata[1]

[1]Department of Soil Sciences and Land Resouces Management, Agriculture Faculty, Universitas Padjadjaran, Indonesia
[2]Agrotechnology, Agriculture Faculty, Universitas Padjadjaran, Indonesia

ORCID
Betty Natalie Fitriatin: https://orcid.org/0000-0002-3749-5081

Abstract.
The acidity of the soil influences the amount of phosphorus available in the soil; acidic soils have low phosphorus availability. The use of phosphorus solubilizing bacteria improves phosphorus availability in acidic soils. The production of dissolved phosphorus and organic acids is one of the functions of the plant growth-promoting rhizobacteria (PGPR) activity of phosphorus solubilizing bacteria. The purpose of this study was to examine how acidity affects the organic acid production and dissolved phosphorus levels of phosphate solubilizing bacteria. Experiments were carried out in the Laboratory of Soil Biology, Department of Soil and Land Resources, Faculty of Agriculture, Universitas Padjadjaran. Two isolates of PGPR (*Burkholderia* sp. strain WK 11 and *Burkholderia* sp. strain MQ-14W) were used in this study, both of which were isolated from an acidic soil-ecosystem. pH 4.5, normal pH (7) and 10.5 were the levels of acidity. The results revealed that the acidity of the water had an effect on the amount of dissolved phosphorus and the amount of organic acid produced by the phosphate solubilizing bacteria. PSB produced more organic acid (lactic, citric, oxalic, and tartaric acid) and dissolved phosphorus at pH 4.5 than at pH 7 or pH 10.5.

Keywords: Acidity, Burkholderia, organic acid, PGPR, solubilizing P

Corresponding Author: Betty Natalie Fitriatin; email: betty.natalie@unpad.ac.id

Published 07 June 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the PGPR 2021 Conference Committee.

1. Introduction

One of the main problem of Indonesian marginal soil is limited availability of macronutrients, such as nitrogen and phosphate. Phosphate is an important element in soil that can affect growth of plant and soil fertility. The phosphorus in plants plays an important role in the following activities: (1) cell division, fat and albumin formation; (2) the formation of flowers, fruits and seeds; (3) stimulate roots; (4) increase crop yields; (5) stress resistance to pests and diseases [1]

Dissolve of phosphate in the soil is usually no more than 0.01% of the total P. Most of the phosphate forms are bound by soil colloids so they are not available for plants

[2]. The pH is the most factor affecting the availability of posphate in the soil [3]. Soil P availability is generally found in soils with a pH range of 6.0 to 6.5 [4].

Soil P availability is affected by the level of soil acidity. Available P is mostly found in soils with pH around neutral. According to [5], when the soil pH is lower than 5 or higher than 7, phosphorus availability will decrease. Furthermore, the pH value increases, phosphorus will be adsorbed by Ca and Mg which are often found in alkaline soils. Phosphate solubilizing microorganisms are an alternative that is often used to dissolve P which is compounded with other elements so that the P element can be used by plants [6].

The ability of phosphate solubilizing microbes to solubilize of varies phosphate, depends on the organic matter that produced and phosphate source [7]. The ability of phosphate solubilizing microorganisms to dissolve bound phosphate can be identified by growing pure cultures on Pikovskaya media containing insoluble phosphorus, such as $Ca_3(PO_4)_2$. Phosphate solubilizing microbes are characterized by the presence of a clear zone around the colony [8].

The presence of organic matter such as citric, oxalic, lactic, and tartaric which are the result of secretion from PSB is considered very important to reduce the binding of phosphate by the adsorbent and also to reduce the toxicity of aluminum in acid soils. Organic acids are able to increase the available P in the soil through a chelating mechanism, thereby reducing the amount of P fixed by Fe and Al [9 ; 10]. Organic acids produced by phosphate solubilizing microorganisms vary, both in terms of quality and quantity. The ability of bacteria to produce organic acids is influenced by various factors including pH. The organic acid production of two fungi (*Aspergillus niger* and *Penicillium oxalicum*) increased under acidity conditions [11]. Environmental factors greatly affect the ability of phosphate solubilizing bacteria to dissolve phosphate. Therefore, further analysis is needed to determine pH effect on phosphate solubilizing bacteria activity.

2. Materials and methods

2.1. Bacterial rejuvanation

Bacterial isolate subculture Sub-cultures of bacterial isolates were carried out on Pikovskaya selective media with a slanted plate using the scratch method, then incubated in an incubator for 24-48 hours. The bacteria that grew were then added with 10 mL of physiological NaCl (0.85% NaCl) and re-grown in Pikovskaya liquid media as an inoculant. After that, the population density in the inoculants was calculated using the serial dilution plate method.

2.2. Medium pH setting

The media used in this experiment is Pikovskaya (glucose, $Ca_3(PO_4)_3$, yeast extract, NaCl, KCl, $MnSO_4.7H_2O$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $(NH_4)SO_4$) with a predetermined pH. Media pH adjustment is done by adding HCl or NaOH. The addition of these materials is done before the media is sterilized in the autoclave. For normal medium pH (pH 7), no added HCl or NaOH.

2.3. Incubation

PSB was cultured in 100 mL of liquid Pikovskaya medium which had modified its pH value. In the medium was given 1 mL of PSB suspension with a density of 10^8 CFU/mL. Incubated for 7 days on a shaker at 80 rpm. The treatments was replicated three times.

2.4. Analysis

The bacterial population was observed on day 3, 5, 7. Observation of the total population of PSB was carried out using the serial dilution plate method. Dissolved P and organic acids were analyzed on day 7 after incubation. Dissolved P was analyzed by Bray and Olsen methods, while organic acids were analyzed by HPLC.

3. Results and Discussion

3.1. Bacterial population

The result showed that the pH of the media influenced the bacterial isolates population. The results of observing the number of bacterial populations on days 3, 5, and 7 can be seen in the Table 1.

Note: The same letter behind the mean value indicated not significantly different based on Duncan Test at the 5% level

Based on data at Table 1, the largest bacterial population was found at pH 10.5. On the 7th day, *Burkholderia* sp. strain MQ-14W at pH 10.5 was significantly different from

Table 1: The effect of pH on PSB population.

Treatments	Day- (CFU/mL)		
	3 (x 10^7)	5 (x 10^8)	7 (x 10^9)
(A) *Burkholderia* sp. strain WK 11 + pH 4.5	5.26 a	4.27 a	0.87 a
(B) *Burkholderia* sp. strain WK 11 + pH 7.0	5.79 a	4.96 a	2.73 a
(C) *Burkholderia* sp. strain WK 11 + pH 10.5	156.33 b	51.53 b	91.50 b
(D) *Burkholderia* sp. strain MQ-14W + pH 4.5	4.11 a	2.39 a	0.52 a
(E) *Burkholderia* sp. strain MQ-14W + pH 7.0	2.81 a	3.27 a	1.44 a
(F) *Burkholderia* sp. strain MQ-14W + pH 10.5	183.83 b	60.87 b	58.00 ab

the treatment at pH 4.5 and 7.0 but not significantly different from *Burkholderia* sp. strain WK 11 at pH 10.5.

Bacterial population reflects the ability of bacteria to adapt to the surrounding environment. The growth and development of microorganisms depends on the sensitivity of microbes to the environment condition i.e., hydrogen ion concentration, humidity, temperature, water activity, and hydrostatic pressure [12]. Based on the results of research [13] that the optimum pH for *Buurkholderia diazotrophica* growth is between 6.0 and 7.0.

3.2. Organic matter production

Organic acid is one indicator of PSB activity. There were 4 organic acids analyzed in this study: citric acid, oxalic acid, tartaric acid, and lactic acid. Based on the ability of organic acids to dissolve phosphate, the four organic acids can be ordered from strongest to weakest: Citric > Oxalic > Tartaric > Lactic

Table 2: Organic acids production by PSB.

Isolates	pH	Organic acids (ppm)			
		Lactic	Citric	Oxalic	Tartaric
Burkholderia sp. strain WK 11	4.5	673.53	859.51	270.09	368.89
	7.0	59.00	210.79	28.94	0.00
	10.5	74.41	281.93	25.78	154.99
Burkholderia sp. strain MQ-14W	4.5	626.93	290.82	443.15	0.00
	7.0	1864.05	98.13	29.84	273.78
	10.5	603.71	96.62	37.01	166.87

The data on Table 2, it can be seen that *Burkholderia* sp. WK 11 strain at pH 4.5 produced the most citric acid. At the same pH, *Burkholderia* sp. strain MQ-14W also produces more citric acid when compared to the other pH.

Organic acid is one indicator of PSB activity. In dissolving phosphate, PSB produces organic acids that will bind Al and Fe so that P in the soil becomes available for plants. Malic, citric and oxalic acids are types of organic acids that have high affinity for metals with 3 valences such as Al^{3+} and Fe^{3+} [14].

3.3. Dissolved P level

The result showed that the pH of the media had a significant effect on dissolved P in the media after being incubated for 7 days. The results of observations of dissolved P can be seen from Table 3.

Table 3: pH Effect on Dissolved P level.

Treatments	Dissolved P (ppm)
(A) *Burkholderia* sp. strain WK 11 + pH 4.5	23.84 c
(B) *Burkholderia* sp. strain WK 11 + pH 7.0	13.70 b
(C) *Burkholderia* sp. strain WK 11 + pH 10.5	8.00 a
(D) *Burkholderia* sp. strain MQ-14W + pH 4.5	23.68 c
(E) *Burkholderia* sp. strain MQ-14W + pH 7.0	14.10 b
(F) *Burkholderia* sp. strain MQ-14W + pH 10.5	10.70 ab

Note: The same letter behind the mean value indicated not significantly different based on Duncan Test at the 5% level

The tendency of dissolved P levels to increase following a decrease in the pH value of the media (Table 3). This is presumably because the pH of the media is able to affect the solubility of P in the soil. At an acid pH below 5.5, the presence of aluminum increases the possibility of phosphate fixation, while at an alkaline pH the phosphate is mostly absorbed by Ca and Mg [3].

The mechanism of phosphate solubilization by bacteria is related to its ability to produce organic acids. Each bacterium has a genetically different ability to produce organic acids both in the amount and organic matter type. The amount and organic matter type are important to determining the high dissolution of P [15].

4. Conclusions

The experiment showed that the acidity of medium affected dissolved P and organic acid production of phosphate solubilizing bacteria. The organic acid (lactic, citric, oxalic and tartaratic acid) produced by PSB and dissolved P were higher at pH 4.5 than at pH 7 and pH 10.5.

5. Acknowledgments

This research was funded by Academic Leadership Grant (ALG) Universitas Padjadjaran. We thank to staff Laboratory of Soil Biology Faculty of Agriculture, Universitas Padjadjaran for their supporting of this work.

References

[1] López-Arredondo DL, Leyva-González MA, González-Morales SI, López-Bucio J, Herrera-Estrella L. Phosphate nutrition: Improving low-phosphate tolerance in crops. Ann. Rev. Plant Biol. 2014; 65: 95–123.

[2] Hanyabui E, Apori SO, Frimpong KE, Atiah K, Abindaw T, Ali M, Asiamah JY, Byalebeka J. Phosphorus sorption in tropical soils. AIMS Agriculture and Food. 2020;5(4):599-616.

[3] Penn CJ, Camberato JJ. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture. 2019;9:120.

[4] Havlin JL, Beaton JD, Tisdale SL, Nelson WL. Soil fertility and fertilizers. 6th ed. New Jersey: Pearson Prentice Hall; 2005.

[5] Cerozi BDS, Fitzsimmons K. The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. Bioresource Technology. 2016;219:778-781.

[6] Tian J, Ge F, Zhang D, Deng S, Liu X. Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. Biology. 2021;10:158.

[7] Serna-Posso EJ, Prager MS, Cisneros-Rojas CA. Organic acids production by rhizosphere microorganisms isolated from a typic melanudands and its effects on the inorganic phosphates solubilization. Acta Agronómica. 2017;66(2):241-247

[8] Pande A, Pandey P, Mehra S, Singh M, Kaushik S. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. Journal of Genetic Engineering and Biotechnology. 2017;15:379-391.

[9] Barker AV, Pilbeam DJ. Hand book of plant nutrition. New York: CRC Press; 2007.

[10] Beebout SEJ, Loeppert RH. Role of organic acids in phosphate mobilization from iron oxide. Soil Science Society of America Journal. 2006;70(1):222-234.

[11] Li Z, Bai T, Dai L et al. A study of organic acid production in contrasts between two phosphate solubilizing fungi: Penicillium oxalicum and Aspergillus niger. Scientific Reports. 2016;6:25313.

[12] Stanaszek-Tomal E. Environmental factors causing the development of microorganisms on the surfaces of national cultural monuments made of mineral building materials—Review. Coatings. 2020;10:1203.

[13] Sheu SY, Chou JH, Bontemps C et al. Burkholderia diazotrophica sp. nov., isolated from root nodules of Mimosa spp. International Journal of Systematic and Evolutionary Microbiology. 2013;63(2):435-441.

[14] Jones DL, Brassington DS. Sorption of organic acid in acid soils and its implications in the rhizosphere. Eur. J. Soil Sci. 1998;49:447-455.

[15] Adeleke R, Nwangburuka C, Oboirien B. Origins, roles and fate of organic acids in soils: A review. South African Journal of Botany. 2017;108:393-406.