The Ability of Potassium-Solubilizing Bacteria to Dissolve K-Feldspar

Laily Mutmainnah, Iswandi Anas and Budi Nugroho

***Abstract*—Potassium is the third macro nutrient elements that serve as activators of enzymes and plays a role in the biophysical processes of plants. Potassium fertilizer that use frequently in Indonesia is KCl. Fulfillment of potassium fertilizer in Indonesia is through imports from various countries. It is caused Indonesia does not produce potassium fertilizer and Indonesia agriculture system is depend on KCl. The other hand, Indonesia has deposits of potassium i.e. mica and K-Feldspar. Potassium containing in mica is up to 10% and containing in K-Feldspar is up to 14%. Those potassium is unavailable for plants. It is can be available through weathering process for a long time. Dissolving of K-Feldspar can be accelerated through the role of the Potassium Solubilizing Bacteria (KSB). The mineral grain size be an important to facilitate application on agricultural lands. The aim of this study were to test the capability of KSB to dissolving K-Feldspar and to know the best size of K-Feldspar to dissolve by KSB. Four isolates of KSB, namely KSB2, KSB3, KSB4 and KSB5, was inoculated on liquid medium containing 0,5% K-Feldspar at ±34oC for 15 days. After the incubation period, the media is centrifuged to separate between dissolved and unsolved potassium. The soluble potassium was measured using a flame-photometry. The finer the size of the K-Feldspar grains the easier it will be dissolved by the KSB. KSB was able to dissolve K-Feldspar by 19%. In this study, the best K-Feldspar grain size is the fine size of 20-74 µm. KSB dissolves K-Feldspar through the mechanism of organic acid production. The higher the organic acid produced, the higher the chance K-Feldspar dissolves.**

***Keywords*— Organic Acid, KSB, K-Feldspar.**

I. Introduction

Potassium is one of the elements of a very important macro nutrient for plant life. Potassium is absorbed by plants in amounts approaching or sometimes exceed the amount of nitrogen absorption. Potassium serves as an activator of the enzyme and was instrumental in the biophysical processes of plants. Plants absorb potassium in the form of ion k. Plants absorbing potassium in quantities approaching or sometimes exceed the amount of nitrogen absorption. One of the widely used potassium fertilizer was KCl. Until this year, the fulfillment of KCl fertilizer in Indonesia through imports from various countries. Import KCl performed because Indonesia does not produce KCl (Hadi et al. 2007). On the other hand, Indonesia has deposits of the mineral form of potassium in potassium that are scattered in various parts of Indonesia, namely K-Feldspar and Mica (Tekmira 2017). Potassium content in mica could reach 10% and in K-Feldspar can reach 14% (Tisdale et al. 1985). Feldspar mineral reserves in Indonesia reached 8857 million tons, but the potassium contained in mineral form is not available to plants. Potassium in minerals can become available to plants one of them through the process of weathering in a very long time. The process of dissolving potassium can be accelerated through Potassium-Solubilizing Bacteria (KSB).

The purpose of this study was to assess the capability of the KSB in the dissolving residual K-Feldspar minerals and to know grain size of K-Feldspar best able to dissolve the KSB. This research is useful for providing information sources of fertilizer potassium in Indonesia so as to reduce the dependence of agricultural system in Indonesia against importing fertilizer KCl.

II. Method

The research was carried out in August – September 2017 on land and environmental Biotechnology Laboratory, IPB. Analysis of organic acids in the Large Hall of research and development for agriculture, Post-harvest Cimanggu, Bogor. The KSB used isolated from sugar cane crop in rhizosfer area of sugar cane belonging to PTPN XIV XI in East Java. 5 isolates are selected based on the nature of the KSB pathogens to plants and humans. The nature of highly pathogenic against crops is done by means of KSB on section isolates inject intracellular tobacco leaves. The nature of highly pathogenic for humans to be tested by means of KSB isolates grow on blood agar media.

There are two factors in the experiment this time, i.e. a factor of isolates the KSB and grain size of K-Feldspar. Isolates coded KSB KSB2, KSB.3,4 KSB and KSB5 grown on liquid media containing 1% glucose, 0.2% NaCl, 0.05% yeast extract and 0.5% K-Feldspar. Grain size factors K-Feldspar consisted of coarse-size (124-295 µm), rough (74-124 µm) and fine size (20-74 µm). To control treatment is the treatment without inoculation and KSB without the K-Feldspar. Each treatment was repeated as many as 5 times, so there are 100 units of the experiment. Isolates of the KSB in the media's treatment of incubated for 15 days at a temperature of approximately 34oC. After incubation period, the solution containing the KSB centrifuged to separate between the dissolved potassium and not dissolved. Potassium dissolved is measured using

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flame-photometry. Pellet results centrifugation observed under electron microscopy (Scanning Electronic Microscopy/SEM) to see the change of morphology of K-Feldspar.

Analysis of organic acids are intended to find out the type and quantity of organic acids produced by the KSB. There are 8 types of main organic acids (Shanware et al. 2014) that will be analyzed in this study, i.e. citric acid, virulat acid, khumarat acid, siringat acid, Malic acid, acetic acid, pyruvic acid, and oxalic acid. Organic acids will be analyzed using High Performance Liquid Chromatography (HPLC) method by modifying Sitorus et al. (2015). Analysis of organic acids begins with preparing the creation of a standard curve of each organic acid that begins with the creation of the stock solution. Organic acid standards were analyzed by reverse phase chromatography column using Grace smart RP 18 5 µ and read at λ = 210 nm. Before being injected into the HPLC organic acid standards, and sample treatment is filtered beforehand with a 0.2 µm filter paper. The analysis carried out on conditions of isokratik at a temperature of 40oC by using 50 mM phosphate solution phase motion (dissolve 6.8 g potassium hydrogen phosphate in 900 mL water. The value of pH is regulated by adding phosphoric acid to 2.8 using a pH meter, and then added up to 1000 mL), after it is filtered with a 0.45 µm filter paper and the rate of the water phase of motion set 0.7 mL/minute. Then injected in HPLC.

III. Results And Discussion

Based on the results of test 1, there is a hypersensitivity of the known positive KSB are pathogens against plant, namely the KSB1. It can be seen from the brown spots that surround the area of infiltration of the KSB. Brown spots that form a symptom of necrosis in plants. Necrosis is defined as damaged or dead cells on the plant body. When bacteria are injected in the form of tobacco leaf pathogen then it will happen necrosis (Kerr and Gibb 1997). While bacteria that infiltrated on the leaves of tobacco does not induce the hypersensitive response then it can be used as bacteria for the plant growth boosters (Sallytha et al. 2014).

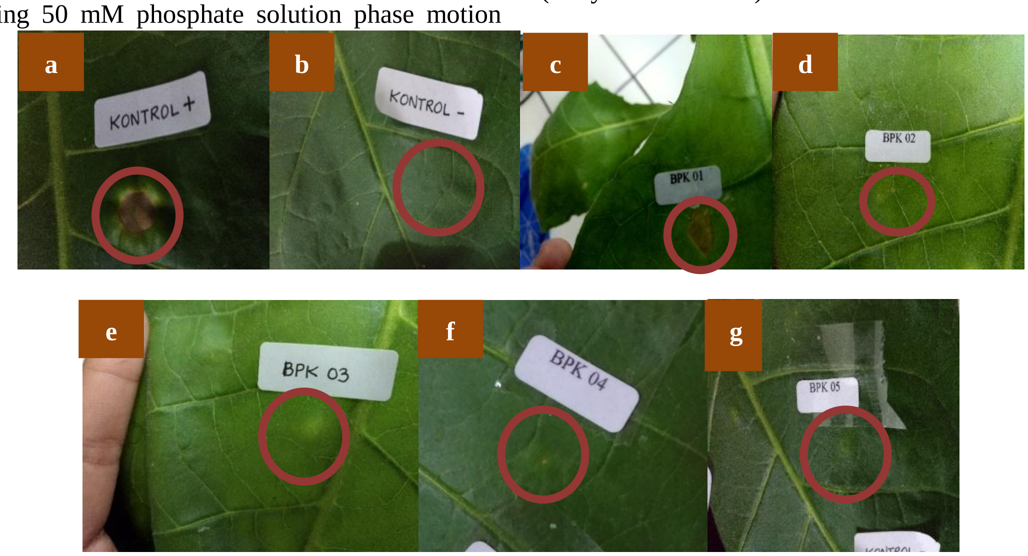


Figure 1 Hypersensitivity test of Potassium-Solubilizing Bacteria (KSB) in tobacco leaves

Based on the results of the test isolates KSB hypersensitivity against tobacco thus isolates pathogenic against the nature of plants, namely the KSB1 not used in test capabilities in dissolving residual the K-Feldspar. Figure 2 shows the KSB isolates are hemolytic or non-hemolytic. There are 3 types of hemolysis on blood agar by bacteria, i.e., β-hemolysis, α-hemolysis and γ-hemolysis. β-hemolysis is complete lyse of red blood cells and hemoglobin. α-hemolysis refers to partial lyse or partial lyse of red blood cells and hemoglobin. On the β-hemolysis will happen clear discoloration on the media or will form clear zones are the result of the activity of the isolates. Whereas in α-hemolysis colonies produce discoloration around the isolates to gray greenish. γ-hemolysis that is not the case where there were no hemolysis color change in the media and or isolates are not capable of developing in the media so that the blood (Wiliam et al. 1980). When isolates of the KSB indicates the occurrence of β-hemolysis and or α-hemolysis then it can be presumed that such harmful or pathogenic isolates against man. This is because both types of hemolysis indicates the ability isolates in Lyse red blood cells and hemoglobin either complete or in part. If the KSB they isolate the hallmark of γ-hemolysis packed the isolates can be applied to the research activities.

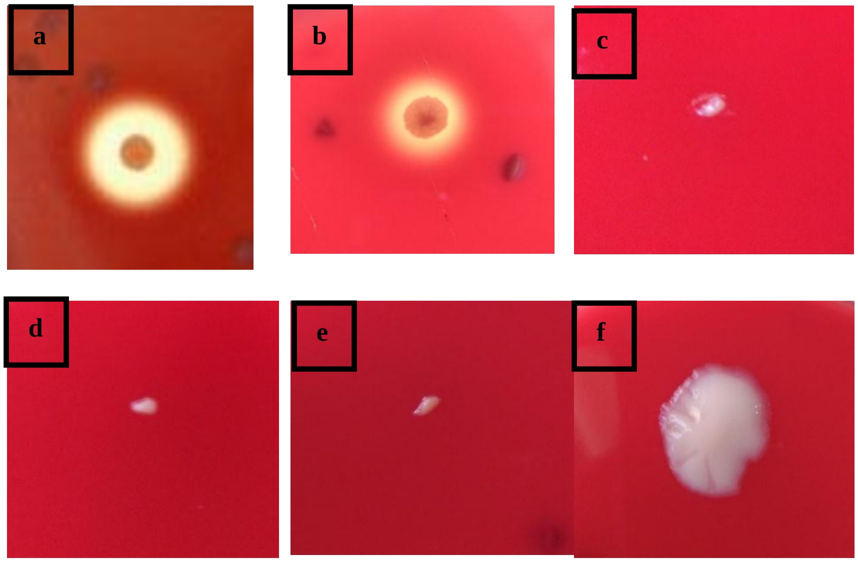


Figure 2 Hemolysis test of Potassium-Solubilizing Bacteria (KSB) on blood agar media, a) positive Control, b) KSB1, c) KSB2, d) KSB3, e) KSB4, f) KSB5

Hemolysis test results in Figure 2 shows that the KSB1 indicate the characteristics of β-haemolytic colonies which well-formed the clear zones clearly around the KSB1isolates. KSB1 may be referred to as a dangerous KSB isolates for man and animal due to able to lyse red blood cells and hemoglobin. The formation of a clear zone surrounding the KSB isolates1 because KSB.1 indicated by producing hemolysis, i.e., an extracellular products which can lyse red blood cells (Mudatsir 2014). KSB2, KSB3, KSB4 and KSB5 indicate the characteristics of γ-hemolysis. KSB2, KSB3, KSB4 does not show the ability of the growth in the media so that the blood. Whereas KSB5 was able to grow on blood agar media but was unable to form a clear zone or change the color of the media. It has the sense that KSB2, KSB3, KSB4 and KSB5 harmless or not against human and animal pathogens, so that based on the test results of highly pathogenic isolates the KSB against the plant the humans and animals, then the KSB1 not used in subsequent research activities. While KSB2, KSB3, KSB4 and KSB5 used to test the ability of dissolving residual potassium containing in K-Feldspar.

The KSB has been noted in some of the mechanisms contributing to the dissolving residual of potassium contained in K-Feldspar minerals. The release of H+ by the KSB is capable of directly dissolving residual minerals K-Feldspar with changes of potassium is unavailable becomes available for plants (Etesami et al. 2017). In table 1 it can be seen that the KSB5 capable of dissolving residual the K-Feldspar with the results of the most high dissolved potassium, namely 44.94 ppm with the size of K-Feldspar 20-74 µm. the different Treatment is not real by inoculation treatment KSB2 with K-Feldspar size 20-74 µm and 74-124 µm. Inoculation isolates KSB3 and4 of the KSB on liquid media shows the result of the solubility of potassium is lower than KSB2 and KSB5 different each other and not real at any granule size treatment K-Feldspar. These results indicate that the more refined grain size of K-Feldspar then the easier it is to dissolve by the KSB.

TABLE I. INFLUENCE OF POTASSIUM-SOLUBILIZING BACTERIA (KSB) INOCULATION TO DISSOLVED POTASSIUM (K_2O) IN LIQUID MEDIA WITH 3 K-FELDSPAR GRAIN SIZE

KSB Isolate	Grain size (µm) ⊖			
	K_0	124-295	74-124	20-74
	----- K_2O(ppm)^a -----			
KSB_0	0.56 a	14.94 b	16.42 b	18.40 b
KSB_2	1.89 a	40.84 cd	42.21 cde	42.59 de
KSB_3	1.70 a	39.38 cd	40.25 cd	39.88 cd
KSB_4	1.78 a	40.12 cd	41.11 cd	38.89 c
KSB_5	1.80 a	40.25 cd	41.36 cd	44.94 e
R^b = -0,566				

The role of the KSB in the dissolving residual K-Feldspar in line with the results of the research of Sheng et al. (2002), i.e. the mineral potassium is dissolving one of them caused by inoculation of the KSB. Solubility potassium negatively correlates with the size of K-Feldspar, that is to say the more coarse grain size of K-Feldspar then the smaller the potassium that is able to dissolve by the KSB. The third K-Feldspar grain size indicates the correlation relationship quite a yawn by value correlation was calculated. The KSB is capable of dissolving residual the K-Feldspar one through the mechanism of production of organic acids, mainly citric acid, oxalic acid and tartaric acid (Basak and Biswas 2009).

TABLE II ABILITY OF 4 POTASSIUM-SOLUBILIZING BACTERIA (KSB) IN GENERATING 8 KINDS OF ORGANIC ACIDS ON *NUTRIENT BROTH* MEDIA AGE 15 DAYS

Organic Acid	KSB_2[a]	KSB_3	KSB_4	KSB_5[b]
	----------ppm----------			
Citric acid	65.9	61.2	65.8	71.6
Virulat acid	76.0	45.5	57.6	82.7
Khumarat acid	88.9	21.6	71.8	87.2
Siringat acid	76.1	62.8	67.4	72.8
Acetic acid	10.1	9.0	8.9	18.4
Malic acid	35.5	27.9	29.3	41.9
Pyruvic acid	30.2	22.4	26.3	29.5
Oxalic acid	39.7	30.2	28.3	42.5

[a]KSB_2 is *Burkholderia cepacia*
[b]KSB_5 is *Bacillus mucilaginosus*

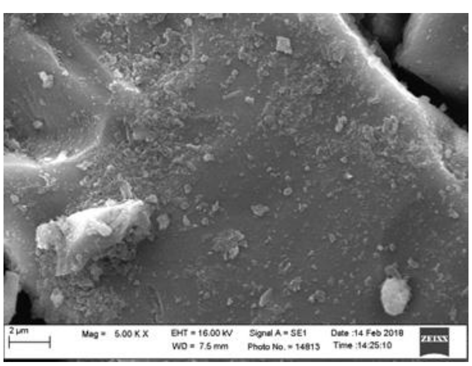
Organic acids are a source of H+ which helps dissolve the K-Feldspar (Reyes et al. 2006). KSB2 isolates producing khumarat acid, siringat acid, and pyruvic acid, whereas the highest organic acid produced by KSB5 isolates. Both of these isolates is superior in producing organic acids than isolates the KSB and KSB3 4. It is directly proportional to the capability of the KSB in the dissolving residual K-Feldspar are presented in table 1. The statement in accordance with the research Ambikadevi and Lalithambika (2000) which concluded the high organic acid production by the KSB influences the increase in solubility of mineral potassium in laboratory experiments.

Organic acids produced isolates KSB dissolving residual K-Feldspar through the formation of ionic bond with The so potassium regardless and mixed together a solution of (Lin et al. 2002). Oxalic acid is known to one of the ingredients that help the process of weathering biochemistry that many occur in the minerals plagioclase especially K-Feldspar (Sheng 2005). Potassium on K-Feldspar naturally will be a form available through the process of hydrolysis. Some chemical reactions that indicate the release of potassium contained in K-Feldspar follows:

(1)$KAlSi_3O_8 + NaCl \rightleftarrows NaAlSi_3O_8 + KCl$

(2)$KAlSi_3O_8 + 8H_2O \rightleftarrows K+ + Al(OH)-_4$

(3)$KAlSi_3O_8 + 2\,H^+ + H_2O \rightleftarrows Al_2Si_2O_5(OH)_4 + 2K^+ + 4SiO_2$.



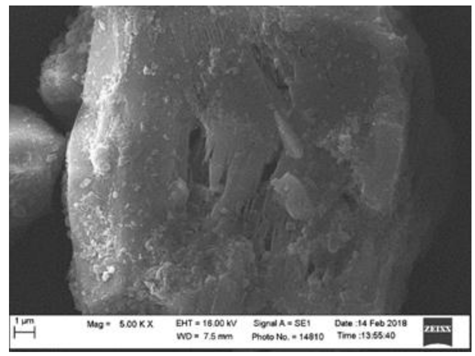


Figure 3 Surface of K-Feldspar (a) without the KSB and treatment (b) treatment with the KSB on the zoom 5000x.

The KSB in addition to producing organic acids, supposedly also capable of producing a biofilm. Biofilms help KSB attach themselves to the K-Feldspar to create a controlled microenvironment. In Figure 3 it can be seen that the surface of K-Feldspar with KSB's treatment shows the surface perforated. It indicates that there has been a typical mechanism of the KSB.

IV. CONCLUSION

Based on the results of the research, it can be concluded that the KSB is capable of dissolving residual the K-Feldspar through organic acid production. K-Feldspar grain size best for dissolved the KSB is smooth, i.e. size 20-74 µm. The KSB is capable of dissolving residual residual the K-Feldspar up to 19%.

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