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## Solubilization of Potassium Containing Mineral by Microorganisms From Sugarcane Rhizosphere

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### Abstract

Potassium solubilizing microorganisms (KSM) isolated from sugarcane rhizosphere and their capability on solubilization from some insoluble potassium were examined. Isolation of potassium solubilizer was carried out from three sugarcane plantations area, on Alexandrov's agar medium. From the 41 isolated microorganisms were selected 15 isolates potassium solubilizing microorganisms which exhibiting highest potassium solubilization (solubility index) on solid medium. All the KSM were found to be capable of solubilizing K from insoluble K-bearing minerals source, and the solubilization zone ranging from 0.15 to 4.5 cm. 13.3% isolate has Solubility Index (SI) more than four on solid medium. Quantitative test result of KSM conducted on liquid medium containing potassium mineral trachyte, feldspar, leucite (Pati and Situbondo), released water soluble-K (exchangeable-K) ranging from 0.13 to 12.25 mg.L<sup>-1</sup> (Feldspar); 1.24 to 15.57 mg L<sup>-1</sup> (Leucite pati); 1.01 to 4.59 mg L<sup>-1</sup> (Leucite Situbondo); and 0.16 to 6.34 mg L<sup>-1</sup> (Trachyte). KSM strain Sbr3 caused maximum solubilization on feldspar (12.25 mg L<sup>-1</sup>), KSM strain Asb3 on leucite Pati (18.17 mg L<sup>-1</sup>), KSM strain Prj3 on leucite Situbondo (16.14 mg L<sup>-1</sup>), whereas KSM strain Prj5 caused maximum solubilization on trachyte (6.92 mg L<sup>-1</sup>). All isolates produced organic acid citric, ferulic and coumaric, some isolates also produced malic and syringic acid. Total organic acid produced by isolated ranging from 130.42 to 434.44 mg.L<sup>-1</sup>. Ferulic acid was produced by all isolates on all of K sources higher than other organic acid.

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Keywords: Leucite, Trachyte, Feldspar, Aleksandrov's agar medium, Potassium solubilizing microorganisms

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## 1. Introduction

Among the three essential nutrients required by plants, one of them is Potassium. It is available in four forms in the soil which are K ions ( $K^+$ ) in the soil solution, as an exchangeable cation, tightly held on the surfaces of clay minerals and organic matter, tightly held or fixed by weathered micaceous minerals, and present in the lattice of certain K-containing primary minerals. Potassium though present in as abundant element in soil or is applied to fields as natural or synthetic fertilizers, only one to two percent of this is available to plants, the rest being bound with other minerals and therefore unavailable to plants. The most common soil components of potassium, 90 to 98%, are feldspar and mica (McAfee, 2008).

Certain soil microorganisms (bacteria, fungi) are capable to dissolve insoluble potassium. *Enterobacter hormaechei* has capability as Potassium-solubilizing bacteria (KSB) since they produce Oxalic acid and citric acids and specific enzyme (Prajapati and Modi, 2012). KSB could serve as inoculants. They convert insoluble potassium in the soil into a form that plants can access. Inoculation with A potassium-releasing bacterial strain *Bacillus edaphicus* NBT was found to increase root and shoot growth of cotton and rape. Strain NBT was able to mobilize potassium efficiently in both plants when illite was added to the soil (Sheng, 2005)

Research and Development Centre for Mineral and Coal Technology or known as tekMIRA conducted a number of researches regarding the beneficiating K-bearing silicate rocks during 2012 (Wahyudi et al., 2012). The researches focused on upgrading the K content available for plant within the rocks. Some minerals, for example K-feldspar ( $KAlSi_3O_8$ ); Leucite ( $KAlSi_2O_6$ ) and trachyte, were used as raw materials for K-fertilizers. Based on data from Center for Geological Resources, there is a K-mineral reserve in Situbondo Regency, East Java and Payak (Jepara) Central Java. The mineral is leucite or  $KAlSi_2O_6$ . In addition to K- mineral resources, trachyte that spreads out in South Sulawesi is available in District of Barru. In this research, the insoluble potassium sources refer to tekMIRA analysis.

## 2. Materials and Methods

### 2.1. Sample collection

Potassium containing minerals (Feldspar, leucite, trachyte) was taken from each deposit. Physically, the preparation of the source K was through the process of comminution (by downsizing), by grinding natural rock up to the size of the fine powder of 200 mesh (53  $\mu m$ ) and then analyzing the total content of macro and micro elements using X-ray fluorescence (XRF), which was conducted at the Centre for Mineral and Coal Technology (tekMIRA), Bandung.

### 2.2. Potassium solubilizing microorganisms (KSM) isolation.

Forty-one rhizobacterial isolates were obtained from the sugarcane rhizosphere. Soil samples were collected randomly from the rhizosphere of sugarcane in 90 days of plant growth after *Ratoon* from 3 different locations. From each location, samples were collected from five different sites.

Potassium solubilizing microorganisms were obtained from 15 soil samples rhizosphere soils of sugarcane at three plantations located in East Java (Asembagus, Prajekan and Semboro) by serial dilution plate method using modified Alexandrov medium containing (5.0 g Glucose, 0.5 g  $MgSO_4 \cdot 7H_2O$ , 0.1g  $CaCO_3$ , 0.006 g  $FeCl_3$ , 2.0 g  $Ca_3PO_4$ , 3.0 g insoluble potassium source (Feldspar, leucite, and trachyte), and 20.0 g agar) in 1 litre of deionized water. The pH of the medium was adjusted to 7.5 using dilute acid and/or alkali. Spread plate technique was used and incubated at 37°C for 1 week (Sugumaran and Janartham, 2007). Various colonies on the  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  dilution plates, which showed clear zone indicating the ability to solubilize potassium from insoluble potassium source, were selected and grown on Alexandrov agar. Colonies exhibiting clear zone of potassium solubilization were selected as potassium solubilizers. Secondary screening was carried out from the isolates by studying their ability to dissolve potassium (Solubilization Index, SI),  $SI = D/d = \text{Diameter of zone of clearance} / \text{Diameter of growth}$ .

### 2.3. Characteristic of insoluble potassium

Analysis of the total content of macro and microelements in the source of soluble potassium used X-ray fluorescence (XRF).

Table 1. Chemical data on feldspar, trachyte and leucite utilized as potassium source

Component	Feldspar (Jepara) %	leucite (Pati)	leucite (Situbondo)	Trachyte (Baru)
SiO <sub>2</sub>	63.10	50.42	49.40	60.4
Al <sub>2</sub> O <sub>3</sub>	20.57	21.76	22.8	16.19
Fe <sub>2</sub> O <sub>3</sub>	1.30	5.75	5.67	5.25
MnO	0.003	0.16	-	0.1
MgO	0.032	1.34	0.38	1.97
CaO	0.54	4.56	1.29	4.57
Na <sub>2</sub> O	5.62	4.02	0.62	4.26
K <sub>2</sub> O	10.49	11.37	7.86	5.00
TiO <sub>2</sub>	0.071	0.35	-	0.51
P <sub>2</sub> O <sub>5</sub>	0.022	0.26	-	0.14

Sources: Hartatik et al. (2014)

### 2.4. Water soluble Potassium and Organic acid analysis

Potassium dissolved in a liquid Alexandrov medium after 15 days was analyzed using Atomic Adsorption Spectrophotometer (AAS) in each of insoluble potassium source (Feldspar, leucite, and trachyte) for 15 isolates tested. Analysis was carried out in soil laboratory of Soil Research Center, Bogor. Analysis of organic acid produced by some isolates was tested in liquid Alexandrov medium on each insoluble potassium source (feldspar, leucite, and trachyte) using high-performance liquid chromatography (HPLC) at Post-Harvest Laboratory of Bogor.

## 3. Results and discussion

### 3.1. Isolation and screening

Colonies exhibiting zone of clearance indicating Potassium solubilization were selected. The colonies were selected which has potassium solubility index. Total 15 isolates were isolated as potassium solubilizers (Table-2).

Table-2. Potassium solubilization values of isolates at 14-days.

Isolates	Feldspar			Trachyte			Leucite Pati			Leucite Situbondo		
	D	d	D/d ratio	D	d	D/d ratio	D	d	D/d ratio	D	d	D/d ratio
Asb1	2,84	1,13	<b>2,51</b>	2,78	1,28	<b>2,18</b>	2,07	1,32	<b>1,56</b>	3,97	1,13	<b>3,50</b>
Asb2	0,20	1,14	0,18	0,10	1,47	0,07	0,10	0,93	0,11	0,20	1,27	0,16
Asb3	2,27	1,04	2,18	2,17	1,25	1,74	1,35	1,12	1,21	1,40	1,28	1,09
Asb4	6,59	0,69	<b>9,53</b>	3,77	1,36	<b>2,78</b>	2,63	1,21	<b>2,18</b>	5,21	1,14	<b>4,56</b>
Asb5	2,30	1,26	1,82	2,11	1,41	1,50	1,18	1,36	0,87	0,83	1,42	0,58
Prj1	5,15	0,94	<b>5,50</b>	5,27	0,81	<b>6,52</b>	5,19	1,19	<b>4,37</b>	4,17	0,91	<b>4,59</b>
Prj2	2,84	0,68	<b>4,16</b>	3,62	1,04	<b>3,47</b>	4,25	1,15	<b>3,70</b>	3,67	0,97	<b>3,79</b>
Prj3	2,57	1,24	2,07	2,58	1,23	2,09	1,26	1,07	1,18	3,51	1,49	2,35
Prj4	5,09	2,04	2,49	2,10	0,79	2,65	2,05	1,14	1,79	2,67	0,90	2,96
Prj5	2,28	1,35	1,69	1,85	1,23	1,50	1,12	1,18	0,94	1,77	1,23	1,43
Sbr1	1,70	1,04	1,64	2,05	1,58	1,30	1,14	1,44	0,79	1,63	1,23	1,32
Sbr2	3,94	0,58	<b>6,78</b>	4,88	1,23	<b>3,95</b>	3,30	1,25	<b>2,64</b>	3,60	1,33	<b>2,70</b>
Sbr3	2,22	1,11	2,00	1,83	1,24	1,48	1,13	1,01	1,12	1,93	1,23	1,57
Sbr4	2,71	1,12	<b>2,43</b>	2,53	1,49	<b>1,70</b>	2,28	1,10	<b>2,08</b>	1,95	1,03	<b>1,89</b>
Sbr5	1,40	1,31	1,07	1,31	1,89	0,69	0,93	1,21	0,77	1,08	1,53	0,70

D= Diameter of zone of clearance (cm); d= Diameter of growth (cm); Asb= isolate from Asembagus; Prj= isolate from Prajekkan; Sbr= isolate from Semboro

Solubilization index (SI) of 15 isolates, if analogized with the classification of microbial strains of phosphate solvent Berraquero et al. (1976) in Marra et al. (2011) based on the speed of dissolution, is categorized as "fast" if dissolution occurred before the third day, "slow" if dissolution  $> 3$  days and "non-solubilizing" if after the fifth it could not dissolve, then all isolates of KSM included in rapidly dissolving potassium. In addition, based on the value of SI from all potassium sources (Table 2), K dissolution capability was classified as low ( $SI < 2.00$ ) by 53.33%, intermediate ( $SI \leq 2.00 \leq 4.00$ ) by 33.33% and high by only 13.33% isolate ( $SI \geq 4.00$ ). Diameter of clear zone ranged 0.1 cm to 6.69 cm. This study showed a positive correlation in that the greater the diameter of the colony, the greater the size of clear zone with a correlation coefficient ( $r$ ) of 0.93. Variations in SI value of isolates among others were influenced by the speed of microbial growth and the ability of microbial metabolism. The value of SI could not be used to calculate the concentration of K dissolved by the microorganisms' activity.

At the source of feldspar, 5 isolates (33.33%) had the low ability to dissolve with value of  $SI < 2$ ; the source of trachyte and Leucite Situbondo had 8 isolates (53.33%) and the leucite Pati had 10 isolates (66.67%). Capability of isolates with high dissolving potassium ( $SI > 4$ ) was very low, that is, 4 isolates (26.67%) for the source of feldspar, each with 1 isolate (6.67%) for trachyte and leucite Pati and 2 isolates (13.33%) for leucite Situbondo. The highest SI in Alexandrov's medium with feldspar as Potassium sources was recorded for isolate Asb4, while isolate Prj1 for trachyte and leucite (Pati and Situbondo).

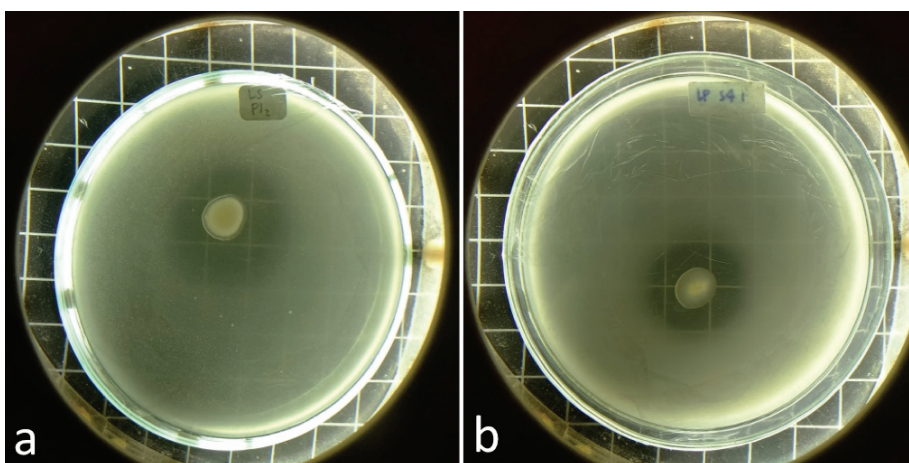


Figure 1. The growth of KSM in the media Aleksandrov with insoluble potassium Leucite Situbondo (a); Leucite Pati (b) characterized by the clear zone around the colony.

K dissolution rate in modified Alexandrov medium by KSM isolates was generally good; 100% isolate dissolved potassium in the first observation of  $< \text{day } 4$  after inoculation (Fig2). In general, the length of time of observation of SI dissolving ability model varied based on the value of the six tested isolates. SI value for isolates Asb4 and Prj1 on all sources of potassium always increased while the SI value of isolates Prj2 and Sbr2 increased in sources of Feldspar and Leucite Situbondo and decreased in trachyte and Leucite Pati sources. Isolate Sbr4 did not dissolve drastically after the 7th day, except in Situbondo Leucite source (Fig. 2). Quantitative measurement of potassium solubilising activity by AAS method is shown in Figure 3. No correlation between SI value and concentration of potassium solubilising of water on all K sources. Although some strains gave clear zone formation (SI value) when screened on media less than 2, they could optimally solubilize insoluble potassium; on the contrary, the high SI value did not always give high K solubilizing.

### 3.2. Potassium solubilizing activity of the KSM

The ability of microorganism to release K largely depends on the nature of the mineral compounds. The variability among the isolates indicates the importance of exploration of different mineral potassium solubilizing

microorganisms and their solubilizing mechanisms. Dissolution K by 15 isolate strains on four K sources varies greatly. However, in this study, the effectiveness of isolate dissolution was low (Fig.3).

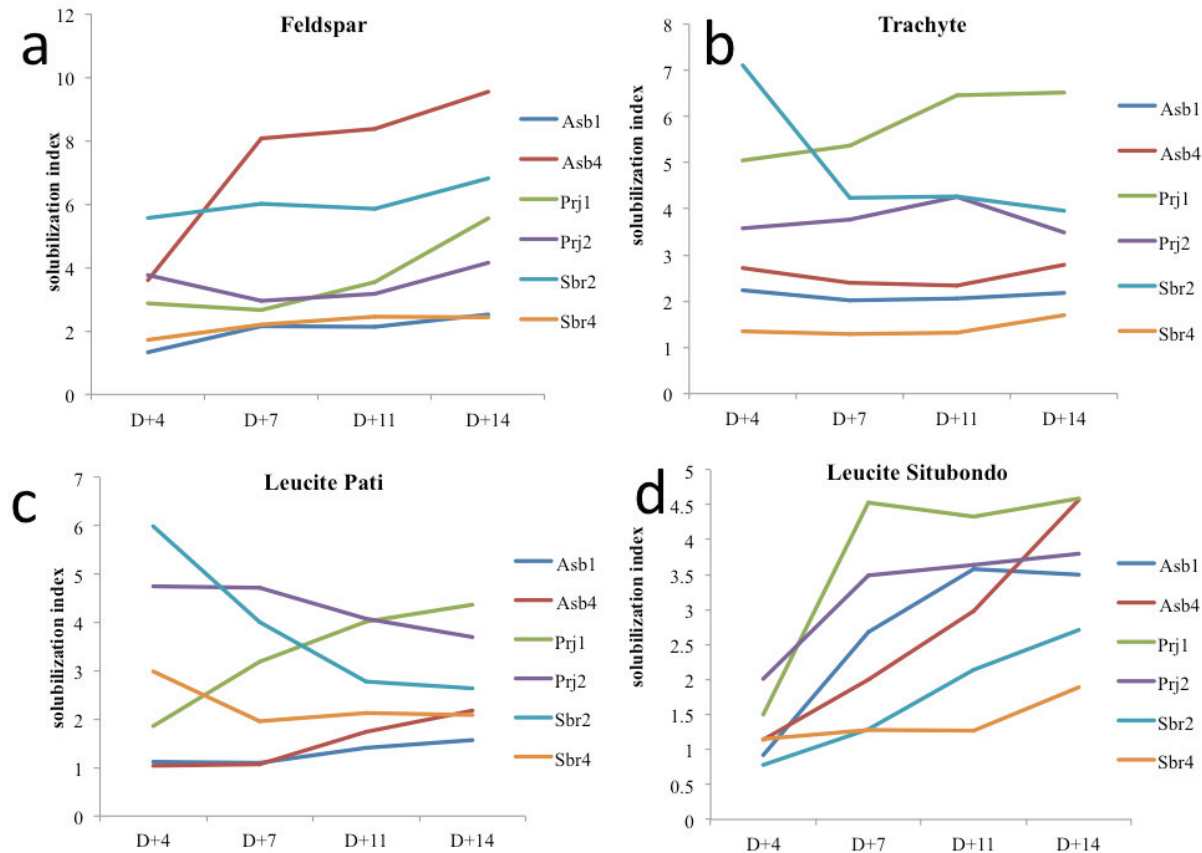


Figure 2: Potassium solubilization index of six isolates at Days+4 to Days+14 on insoluble potassium sources (a) feldspar; (b) Trachyte; (c) Leucite Pati and (d) Leucite Situbondo

All isolates had the ability to dissolve K toward Feldspar source, with the concentration of dissolved K of 0.17 until 12.25 mg.L<sup>-1</sup>, but only 5 isolates dissolved K with concentration > 1 mg.L<sup>-1</sup>. Strains Sbr3 and Prj2 dissolved K from the highest feldspar source. In this study, the effectiveness of dissolving by KSM was maximally 3.89%. Meanwhile, with the source of trachyte, the effectiveness of maximum K dissolving was 4.23% by isolate Prj5 at 6.34 mg.L<sup>-1</sup>, and only 5 isolates were capable of dissolving K with concentration > 1 mg.L<sup>-1</sup>. The research by Zhang and Kong (2014), also demonstrated the ability of the potassium dissolution by KSB with a concentration of 0.59 mg L<sup>-1</sup> to 4.4 mg L<sup>-1</sup> with a feldspar source. Similarly, the results of research by Sugumaran and Janartham (2007) showed isolates of *Bacillus mucilaginosus* MCRCp1 dissolving K of muscovite mica mineral source, microcline and orthoclas at 0.85 until 4.29 mg.L<sup>-1</sup>. The amount of K<sup>+</sup> released from muscovite mica in a broth by the isolates was studied by Chishi (2010) in 7, 15, 20 days after incubation (DAI). The results indicated that the amount of K<sup>+</sup> released from mineral K by all strains increased with increased maximum incubation time at 20 DAI. The K<sup>+</sup> released from muscovite mica by strain at 20 DAI ranged from 2.41µg/ml to 44.64µg/ml. Other research showed that K feldspar from Egypt also could be dissolved by KSB up to 490 mg.L<sup>-1</sup> (Badr, 2006).

All isolates tested dissolved K from leucite source (Pati and Situbondo) with a concentration >1 mg.L<sup>-1</sup>, but the effectiveness of dissolution was very low. Only four isolates were with the dissolving effectiveness of > 1% (leucite Pati) and only one isolate for leucite Situbondo. Isolate Asb3 and Sbr3 which mostly dissolved K was respectively

18.17 mg.L<sup>-1</sup> or 5.28% (Leucite Pati) and 4.59 mg.L<sup>-1</sup> or 1.92% (Leucite Situbondo). Altered mineralogy resulted in morphological changes of the solid materials and, in particular, in increased specific surface areas (Chiang et al, 2013). The efficiency of potassium solubilization by different bacteria was found to vary with the structure and chemical composition of the potassium bearing minerals.

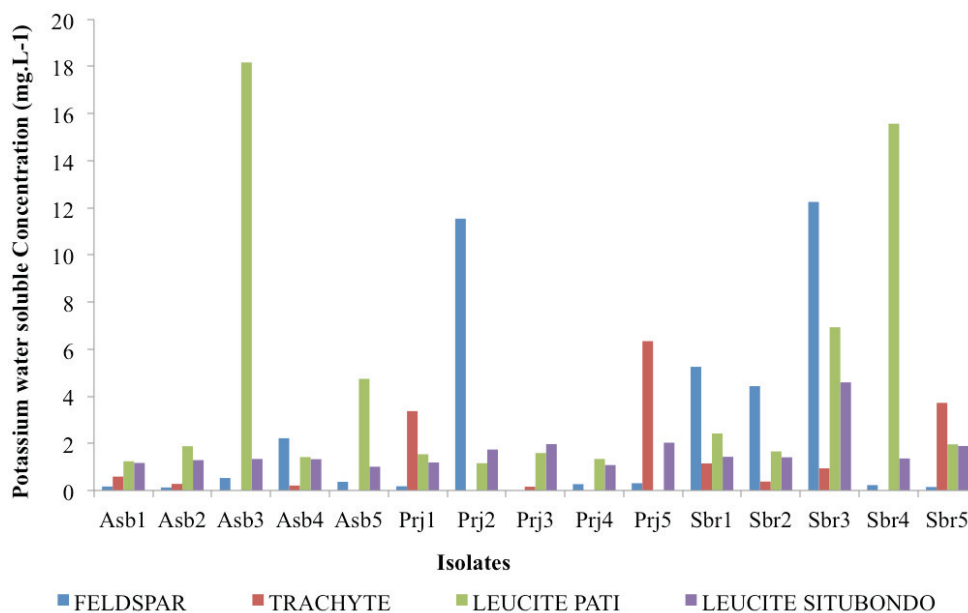


Figure 3. Quantitative analysis of water soluble of potassium by isolates on insoluble potassium

Based on the solubilizing ability of KSM isolates, 6 isolates with strong K solubilizing ability, i.e. Asb1, Asb4, Prj1, Prj2, Sbr3 and Sbr4, were selected to test their ability on organic production.

### 3.3. Organic acids productions

All the six bacterial isolates solubilized the potassium from the insoluble source of potassium i.e. feldspar, leucite, and Trachyte through the acid production. Organic acids produced included citric, ferulic, coumaric, syringic, and malic acid. Not all detected isolates produced 5 types of these organic acids; only citric, ferulic and coumaric were produced by all isolates tested, while malic acid was produced only by isolate Sbr4.

Total organic acid produced by all isolates was mostly by liquid Alexandrov medium with K feldspar source, except isolate Asb4. Total organic acids produced by the three isolates Asb4, Prj2 and Sbr3 had a positive correlation with the dissolution of potassium from insoluble potassium, whereas the other three isolates were negatively correlated.

Positive correlation of the total acid with concentration of K water-soluble of all isolates was only by source of K trachyte ( $r = 0.85$ ); the higher the total organic acids produced, the greater K originated from trachyte that could be dissolved; meanwhile, that by the source of feldspar and leucite had a negative correlation. The mineralogy and chemical composition of the minerals may determine their susceptibility to microbial potassium and silicon mobilization.

Organic acids resulted from the metabolic activity of isolates were mostly polyphenol organic acids (ferulic, syringic and coumaric acid) containing hydroxyl group (OH) compared with the organic carboxylic acid (COOH) (Fig 5). The presence of organic acids in general will lower the medium pH (Badr et al., 2006; Sheng and He, 2006); however, the decrease in pH resulted in increase in total acidity is not the only reason for the release of the soluble K. Solubilization of the minerals is achieved through the production of metabolites containing organic acids as the

active ingredients. Several previous studies showed that K organic acids increased the solubility K of mineral. Results of Bevan and Savage (1989) showed that addition of oxalic acid increased the solubility of K-feldspar on pH 4 and 9, which indicated that the mechanism solubility of feldspar are increased was not due to preferential complexation of aluminium, but rather by an increase in the overall solubility of feldspar in the neutral pH region. Organic acid in medium can be as buffering agent to keep on neutral pH.

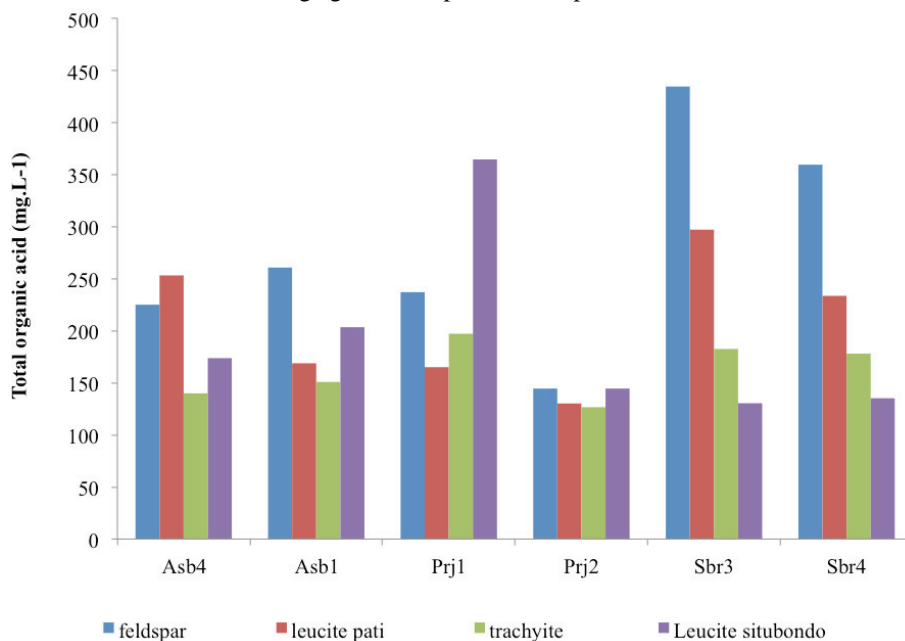


Fig 4. Concentration of total organic acid produced by six isolates on liquid modified Alexandrov medium with insoluble potassium sources

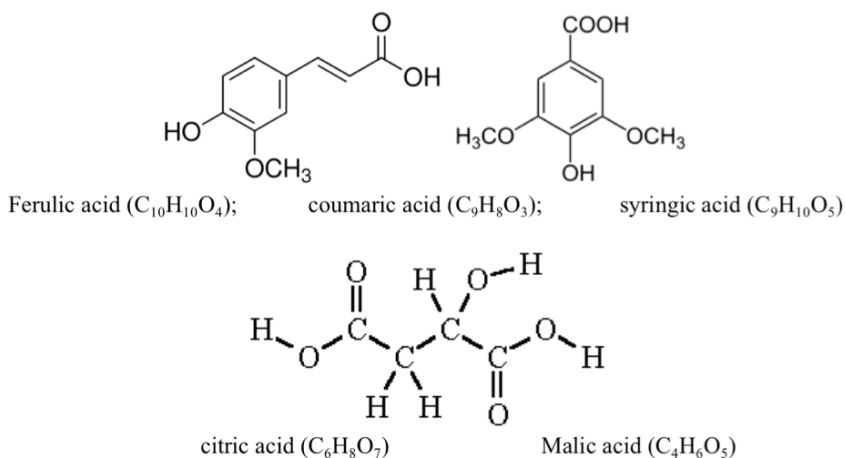


Fig. 5. Molecular structures of organic aromatic acids (ferulic coumaric and syringic acid) and aliphatic acids (citric, pyruvic and malic acid) found in this research.

All isolates of produce citric, ferulic and coumaric acid are on modified Alexandrov liquid medium in all sources of K (Fig 6). The type of organic acids produced by the isolates in this study differed from some previous studies (Sheng and He, 2006) that found Oxalic and tartaric acids produced by KSB *Bacillus edaphicus*, and Prajapati and

Modi (2012) found Citric, Oxalic, Malic, and Tartaric Succinic acid from the activities of some KSB strains. Differences of the production of organic acid depended on the characteristics of microorganisms; in addition, the type of acid production was mainly dependent on the carbon source. Large amount of oxalic, citric, and gluconic acid is produced by *Penicillium frequentans* which are strong solubilizing agents of feldspar, biotite, and phyllosilicates (Torre et al., 1993). Generally, ferulic acid was produced by all isolates on all of K sources higher than other organic acid. This organic acid is one of the potassium dissolution factors. The addition of ferulic acid to the soil material substantially increased the activity of the soil microbe (Blum et al, 1987).

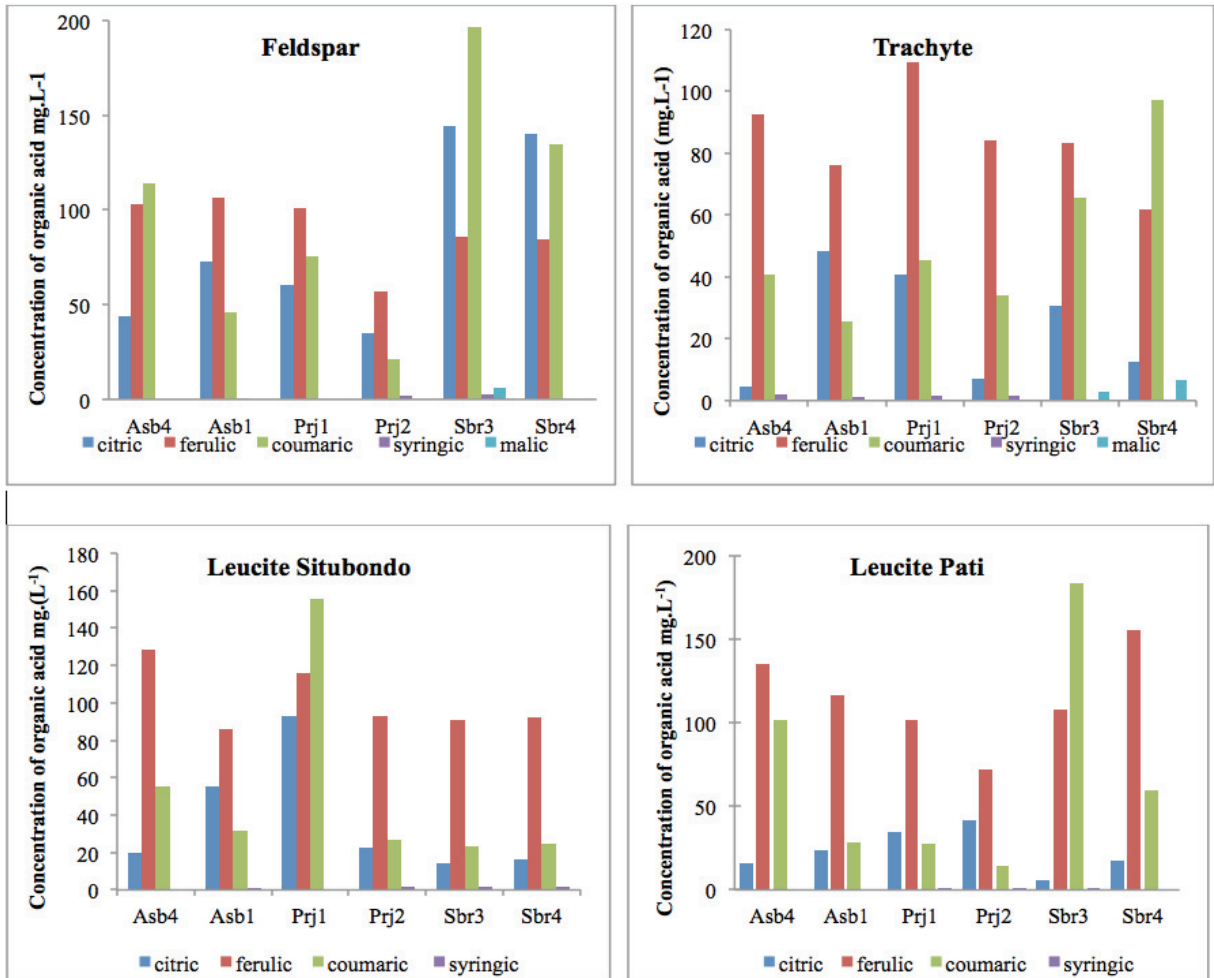


Fig 6. Different organic acids produced by isolates in various K sources.

Some organic acids produced in the periplasm could easily be diffusible into adjacent environment and subsequently dissolve insoluble forms of minerals such as muscovite mica, microcline and orthoclase (Sugumaran and Janartham, 2007), feldspar and illite (Sheng and He, 2006), feldspar, biotite, and phyllosilicates (Torre et al., 1993), ability of the isolates to reduce the pH of the growth medium was taken as an indication of medium acidification. While those that dissolve water insoluble K were assumed to have the capability of producing organic acids in high quantity. The result of Chen et al. (2000) explain that generally the great effect of oxalic acid on dissolution of rocks and minerals is attributed to the presence of hydrogen ions and the formation of cation-complexes. The structural cations, released from minerals as a result of the attack of hydrogen ions, tend to form cation-organic complexes



with oxalic acid, which has OH<sup>-</sup> and COOH<sup>-1</sup> groups in the ortho position. The chemisorption of the cation–organic complexes on the mineral surfaces causes a shift of electron density toward the framework of the mineral. This charge transfer increases the electron density of the cation–oxygen bonds and makes them more susceptible to hydrolysis.

Review by Sand (1997) suggested on weathering process induced by microbial communities, although many microorganisms are known to participate in weathering processes, their action may be summarized by nine main categories 1) physical presence of microbial cells (connection of electric contacts); (2) attack by mineral acids like sulfuric, nitric, carbonic acid (hydrolysis of materials); (3) attack by organic acids like acetic, citric, oxalic, gluconic, and other acids (hydrolysis of materials); (4) attack by organic solvents like acetic or butyric acid or alcohols like ethanol or propanol or ketons (swelling and hydrolysis of materials); (5) salt stress because of reaction products of mineral acids and organic acids (retaining water in porous materials causes increased susceptibility against freeze-thaw attack and furthers crystallization); (6) production of noxious compounds like hydrogen sulfide, nitrogen oxides (production of mineral acids or precipitation of metal sulfides and oxidants/reductants); (7) effect of biofouling and biofilm (exopolymers cause localized corrosion cells; retainment of water in porous materials; hydrophobic effects on surfaces; reduced heat transfer efficiency; reduced (flow) velocity or increased pressure); (8) attack by exoenzymes (cleavage of insoluble organic compounds to small, water soluble molecules); and (9) production of chelating agents of emulsifying compounds (increased solubility of ‘insoluble’ and/or hydrophobic substances).

The results also indicated great variation between the isolates to solubilize the same or different sources of insoluble potassium. The ability of KSM to release K largely depends on the nature of the mineral compounds, the type and concentration of organic acid production and the characteristic of microbe. The variability among the microbe indicates the importance of exploration of different mineral potassium solubilizing bacteria and their solubilizing mechanisms.

#### 4. Conclusion

All the KSM were found to be capable of solubilizing K from insoluble K-bearing minerals source, and the solubilization zone ranged from 0.15 to 4.5 cm. 13.3% isolate has Solubility Index (SI) more than four on solid medium. Quantitative test result of KSM conducted on liquid medium containing potassium mineral trachyte, feldspar, leucite (Pati and Situbondo), released exchangeable-K ranging from 0.13 to 12.25 mg.L<sup>-1</sup> (Feldspar); 1.24 to 15.57 mg L<sup>-1</sup> (Leucite Pati); 1.01 to 4.59 mg L<sup>-1</sup> (Leucite Situbondo); and 0.16 to 6.34 mg L<sup>-1</sup> (Trachyte). All isolates produce organic acid citric, ferulic and coumaric, some isolates also produce malic and syringic acid. Total organic acid produced by isolated ranging from 130.42 to 434.44 mg.L<sup>-1</sup>.

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