

Ability of Potassium-Solubilising Microbes to Solubilise Feldspar and Their Effects on Sorghum Growth

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ABSTRACT

Potassium-solubilising microbes (KSM) are microbes that are able to solubilise different kinds of potassium compounds and make their potassium ions available to plant growth. This study undertook to: (1) isolate KSM from soil samples, (2) evaluate their ability to solubilise not-easily soluble potassium sources, and (3) evaluate the effect of selected KSM on the growth of sorghum. KSM were isolated from soil samples taken from agricultural land, ex-tin mining land, and ex-gold mining land. KSM isolates were further selected according to (1) pathogenic characteristics, (2) a potassium solubility index on solid medium, (3) ability of KSM to solubilise not-easily soluble potassium sources in liquid medium, and (4) ability of KSM to stimulate sorghum growth. The best selected KSM were characterised by using molecular analysis. Results showed that KSM isolated from ex-mining land have better ability to solubilise feldspar than KSM from agricultural land. KSB₂ and KSB₆ isolates have the best ability to stimulate sorghum growth. KSB₂ has 97.8% similarity with *Achromobacter xylosoxidans*, and KSB₆ has 99% similarity with *Burkholderia cepacia*.

Keywords: *Achromobacter xylosoxidans*, *Burkholderia cepacia*, feldspar, potassium-solubilizing microbes, sorghum

INTRODUCTION

Total potassium content in soil commonly ranges between 0.5 to 2.5%, depending on soil type and climatic conditions, but usually 90 to 98% of that total potassium is not in available form (Havlin *et al.*, 2005). In many locations, inorganic potassium fertiliser such as KCl is needed to provide enough potassium for plant nutrition. In Indonesia, this fertiliser is expensive since it has to be imported, and often what is available in the market is of low quality. Fortunately, Indonesia has a number of potassium sources of low solubility such as feldspar and mica.

According to a report from the Ministry of Energy and Mineral Resources (2015), potassium rock potential in Indonesia is around 455 million tons. However, such a source has low potassium content and is not very soluble because of a slow weathering process. Roger *et al.*, (1998) notes that a slow weathering processes of potassium rock can be accelerated by using potassium-solubilising microbes (KSM). KSM will accelerate the weathering process of minerals containing

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potassium, thereby making potassium more readily available to plants. KSM include potassium-solubilising bacteria (KSB) such as *Bacillus mucilaginosus* (Han and Lee, 2005), *Microbacterium hominis*, *Flectobacillus* sp., *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis* and *Bacillus megaterium* (Diep and Hieu, 2013), and also potassium-solubilising fungi (KSF) such as *Aspergillus terreus* (Prajapati *et al.*, 2013).

Usable KSM must be non-pathogenic to plants, animals, and humans and also have a strong ability to solubilise not-easily soluble potassium sources. This study was done to (i) isolate KSM from soil samples, (ii) evaluate their ability to solubilise not-easily soluble potassium sources, and (iii) evaluate the effect of selected KSM on the growth of sorghum.

MATERIALS AND METHODS

Potassium-solubilising microbes were isolated from soil samples from three locations and from three different vegetations at each location (Table 1). The medium used for isolation was modified Alexandrov medium (Prajapati and Modi, 2012). Composition of this medium was 0.5% glucose, 0.05% $MgSO_4 \cdot 7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% Ca_3PO_4 , and 0.3% not-easily soluble potassium source. Two kinds of potassium feldspar, representing not-easily soluble potassium sources, were used in this study: feldspar from Cirebon, West Java with a total K_2O content of 1.93% and feldspar from Malang, East Java with a total K_2O content of 1.74%. Both feldspars were crushed and sieved with a 270 mesh sieve before use.

Isolated KSM were further selected by using (i) test for pathogenicity, (ii) a potassium-solubilising index test, (iii) ability to solubilise feldspar, and (iv) ability to stimulate growth of sorghum. Beside potassium-solubilising ability, KSM isolates were tested for their ability to solubilise not-easily soluble phosphate sources. The ability to solubilise phosphate sources was tested by measuring the phosphate-solubilising index and soluble phosphate content in liquid medium. Not-easily soluble phosphate sources used in this experiment were $Ca_3(PO_4)_2$ with total P_2O_5 content of 45.76 % and phosphate rock from Blitar, East Java with a total P_2O_5 content of 26.61% total.

TABLE 1
KSM isolates sources

Type of land	Type of vegetation	Coordinate
Agricultural land, Cikabayan, Indonesia	<i>Theobroma cacao</i> L.	S 06°33.144' ; E 106°43.069'
	<i>Elaeis guineensis</i> Jacq.	S 06°33.106' ; E 106°43.008'
	<i>Coffea arabica</i> L.	S 06°33.105' ; E 106°42.977'
Ex-tin mining land, Bangka, Indonesia	<i>Melastoma malabathricum</i> L.	S 01°59.376' ; E 106°08.579'
	<i>Acacia mangium</i> Willd.	S 01°59.401' ; E 106°08.589'
	<i>Dillenia suffruticosa</i> (Griff) Martelli	S 01°59.393' ; E 106°08.389'
Ex-gold mining, Pongkor, Indonesia	<i>Dalbergia latifolia</i> Roxb.	S 06°38.692' ; E 106°34.233'
	<i>Elaeocarpus serratus</i> L.	S 06°38.627' ; E 106°34.219'
	<i>Schima wallichii</i> (DC.) Korth.	S 06°38.745' ; E 106°34.256'

$$\text{P/K-solubilizing index} = \frac{\text{Total diameters (colony + halo zone) (mm)}}{\text{Colony diameter (mm)}}$$

$$\text{Solubility percentage (\%)} = \frac{\text{Soluble level of K/P (mg/L)}}{\text{Total level of K/P in sources (mg/L)}} \times 100 \%$$

Isolation of KSM isolates was done by using a modified Alexandrov medium as used by Prajapati and Modi (2012). Ten grams of soil sample were put into 100 ml Alexandrov broth medium for four days for bacteria and seven days for fungi. Ten-fold dilution series of these cultures were prepared by using 0.85% NaCl solution and plated on Alexandrov agar medium by using the spread plate method. Isolated colonies with hollow zone on Alexandrov agar medium were classified as potassium-solubilising microbes.

Pathogenic selections involved hypersensitivity testing and hemolysis testing. The purpose of hypersensitivity testing is to identify isolates that have pathogenic characteristics for plants. A modified method of Schaad *et al.*, (2001) was used to evaluate the pathogenic characteristics of bacteria isolates. These isolates were grown in nutrient broth (NB) medium for 12 h and 0.1 ml of isolated bacteria (10^9 CFU) was injected into tobacco leaf (Havana cultivar). Symptoms of necrosis were observed 72 h after injection of the bacteria suspension. A modified method of Mahmoud *et al.*, (2013) was used to evaluate the pathogenic characteristics of fungus isolates. These isolates were cultured in potato dextrose broth (PDB, 20% potato and 1% glucose) for 48 h. Rice seeds (Ciherang cultivar) were soaked in a fungus isolate culture and subsequently the seeds were placed on sterile wet cotton in a petri dish for seven days. As a control treatment, rice seeds were soaked in sterile water before placement in a petri dish. The number of germinating seeds was used as an indicator for the hypersensitivity test. When the percentage of germinating seeds was lower than the control treatment, it means that the fungi isolate is testing positive for hypersensitivity.

Hemolysis test was done to identify the pathogenic characteristics of isolates to humans and animals. Isolates were grown on a blood agar medium consisting of 1.4% casein enzymic hydrolysate, 0.45% peptic digest of animal tissue, 0.45% yeast extract, 0.5% sodium chloride, and 2% agar mixed with 5% sheep blood and then incubated for 24 h (Difco, 2009). Formation of a halo zone around the microbial colony shows that the isolate has pathogen potential for humans and animals.

The potassium-solubilising index measurement was done by growing KSM that had passed through a pathogenicity test on Alexandrov agar medium. KSM isolates on Alexandrov agar medium were then incubated, three days for bacteria and five days for fungi. The best 10 isolates with the highest potassium-solubilising index were taken for phosphate-solubilising index measurement by growing these isolates on Pikovskaya agar medium (0.05% yeast extract, 1% dextrose, 0.5% phosphate source, 0.05% ammonium sulfate, 0.02%, potassium chloride, 0.01% magnesium sulfate, 0.00001% manganese sulfate, 0.00001% ferrous sulfate, and

2% agar) and then incubated for three days for bacteria and five days for fungi (Edi *et al.*, 1996). The potassium and phosphate-solubilising index formula is as follows:

$$\text{P/K-solubilising index} = \frac{\text{Total diameters (colony + halo zone) (mm)}}{\text{Colony diameter (mm)}}$$

The best 10 isolates from solubilising index measurements were tested for their ability to solubilise not-easily soluble potassium and phosphate sources in a liquid medium. This test was a single factor experiment with 11 treatments and 3 replications. The tests were placed in completely randomised design. Treatments that were used were KSM application consisting of control (without isolates) and 10 best KSM isolates according to the potassium-solubilising index test.

KSM ability was tested by growing 10 KSM isolates in 25 ml growth medium (1% glucose, 0.05% yeast extract, and 0.5% not-easily soluble potassium or phosphate sources) for 7 days. The suspension of the isolates was centrifuged for 25 min (4000 rpm) to separate supernatant from residue. The supernatant was taken to measure the soluble potassium or phosphate content.

Soluble potassium measurement was done by using the modified method of Parmar and Sindhu (2013). The supernatant was measured with a flame photometer using K-titrisol as standard solution. A modified method of Susilowati and Syekhfani (2014) was used to measure the content of soluble phosphate. The supernatant (1 ml) was mixed with reagent solution (0.5% H₃BO₃, 0.38% ammonium molybdate, and 7.5% HCl), to which was added 5 drops of reduction solution. The mixed solution was measured by using a spectrophotometer UV-visible with 660 nm wave length and using KH₂PO₄ as a standard solution. Based on potassium and phosphate measurements in liquid medium, potassium and phosphate solubility percentage was calculated. The formula for potassium and phosphate solubility is

$$\text{Solubility percentage (\%)} = \frac{\text{Soluble level of K/P (mg/L)}}{\text{Total level of K/P in sources (mg/L)}} \times 100 \%$$

Observed parameters were soluble potassium and phosphate content (mg/L) and also percentage of potassium and phosphate solubility (%) in liquid medium. The best five isolates were then taken for the next test, KSM ability to stimulate sorghum growth.

The five best isolates from the liquid medium test were tested for their ability to stimulate sorghum growth. The test was a single factor experiment with six treatments and three replications. Tests were conducted in a completely randomised design. Treatments were KSM application to growing media consisting of P0 (without isolates) and 5 best KSM isolates according to the liquid medium tests (P1, P2, P3, P4, and P5).

A modified method of Basak and Biswas (2010) was used to test the ability of KSM to stimulate sorghum growth. Five kg of oven dried soil was taken from an agricultural land in Cikabayan, West Java, Indonesia, and placed into plastic polybags with water content of soil being kept at field capacity. Inorganic fertiliser consisting of nitrogen fertiliser (100 mg/L N) and phosphate fertiliser at a rate of 50 mg/L P_2O_5 was applied into soil medium (1/3 dosage at planting, and 2/3 dosage at 3 weeks after planting), then the suspension of the isolates (20 ml KSM isolate [10^9 CFU] + 250 ml aquadest) was applied into the soil medium. Sorghum seeds (Numbu cultivar) were planted into the soil medium. To maintain field capacity and keep sufficient water content in soil, watering was done. Parameters such as plant height (cm), number of leaves, root dry weight (g), and shoot dry weight (g), and total potassium in plant (%) were observed for 6 weeks after planting. The best two isolates were taken for characterisation of isolates.

KSM characterisation consisted of colony morphology (colony form, colour, elevation, and ledge form), cell shape, gram test, biochemical test (motility, catalase, indole, urease, glucose fermentation, starch test, and citrate testing) (Faddin, 1979) and identity analysis based upon 16s rRNA gene sequences (Santosa, 2001). Sequencing results were compared to sequences from the European Molecular Biology Laboratory (EMBL) using FASTA in the website www.ebi.ac.uk.

Observed data in the liquid medium test and sorghum growth test were analysed using analysis of variance with $\alpha = 0.05$ and continued with Duncan's Multiple Range Test (DMRT), if there were significant responses in the observed treatments.

RESULTS AND DISCUSSION

Based on isolation results, 185 isolates consisting of 162 bacteria isolates and 23 fungi isolates were found from the soil samples (Table 2). Hypersensitivity test results showed that 64 bacteria and 15 fungi isolates did not have pathogenic potential to plants. Hemolysis test results showed 36 isolates from the first-round 64 bacteria isolates and 12 isolates from the initial 15 fungi isolates did not exhibit pathogenic potential to humans and animals. The total number of isolates that did not have pathogenic characteristics for plants, humans and animals were 48 isolates, i.e., 36 bacterial isolates and 12 fungal isolates.

TABLE 2
Total colony of KSM isolated from soil samples

Type of land	Type of vegetation	Feldspar from Cirebon		Feldspar from Malang	
		B	F	B	F
----- Total colony -----					
Agricultural land, Cikabayan, Indonesia	<i>Theobroma cacao</i> L. <i>Elaeis guineensis</i> Jacq. <i>Coffea arabica</i> L.	16 21 13	0 4 0	12 14 10	0 2 3
Ex-tin mining land, Bangka, Indonesia	<i>Melastoma malabathricum</i> L. <i>Acacia mangium</i> Willd. <i>Dillenia suffruticosa</i> (Griff) Martelli	8 17 7	3 2 0	5 7 4	2 1 0
Ex-gold mining, Pongkor, Indonesia	<i>Dalbergia latifolia</i> Roxb. <i>Elaeocarpus serratus</i> L. <i>Schima wallichii</i> (DC.) Korth.	1 5 9	4 0 0	3 2 8	2 0 0
Total Unit		97	13	65	10

Notes: B (Bacteria), F (Fungi)

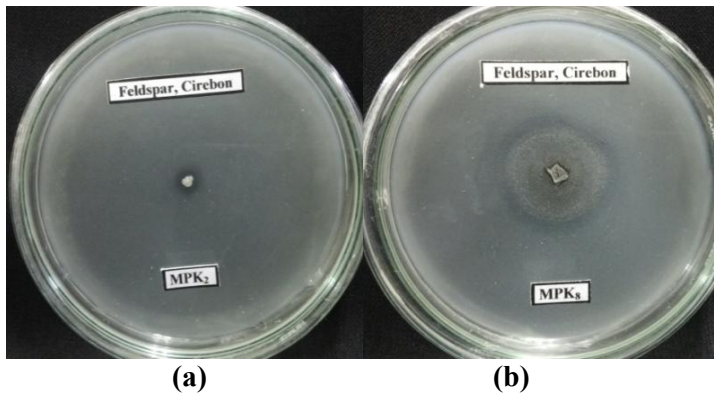


Figure 1: KSM isolates on Alexandrov agar medium, a) potassium-solubilising bacteria and b) potassium-solubilising fungi

Based on the potassium-solubilising index, the best 10 isolates with the highest scores were selected from 48 isolates that had passed through the pathogenicity selection (Table 3). From these KSM isolates, some were found to show ability to solubilise not-easily soluble phosphate sources. Among these 10 isolates, 9 isolates were also able to solubilise $\text{Ca}_3(\text{PO}_4)_2$ and 5 isolates could solubilise phosphate from the phosphate rock from Blitar, East Java (Table 4).

TABLE 3
Isolate code, type of isolate, land and vegetation from 10 best KSM isolates based on potassium-solubilising index

Isolate code	Type of isolate	Type of land	Type of vegetation
KSB ₁	Bacteria	Agricultural land	<i>Theobroma cacao</i> L.
KSB ₂	Bacteria	Ex-gold mining land	<i>Dalbergia latifolia</i> Roxb.
KSB ₃	Bacteria	Agricultural land	<i>Elaeis guineensis</i> Jacq.
KSB ₄	Bacteria	Ex-tin mining land	<i>Acacia mangium</i> Willd.
KSB ₅	Bacteria	Ex-gold mining land	<i>Schima wallichii</i> (DC.) Korth.
KSB ₆	Bacteria	Ex-tin mining land	<i>Dillenia suffruticosa</i> (Griff) Martelli
KSF ₁	Fungi	Agricultural land	<i>Elaeis guineensis</i> Jacq.
KSF ₂	Fungi	Agricultural land	<i>Coffea arabica</i> L.
KSF ₃	Fungi	Ex-tin mining land	<i>Acacia mangium</i> Willd.
KSF ₄	Fungi	Ex-tin mining land	<i>Melastoma malabathricum</i> L.

Ten KSM isolates that were tested in liquid medium showed that isolate KSB6 had the best ability to solubilise not-easily soluble potassium and phosphate sources. Isolate KSB6 showed ability to solubilise potassium sources from feldspar from Cirebon and from Malang with soluble potassium content of 248.10 mg/L and 273.21 mg/L respectively, and with a solubility percentage of 1.29 % and 1.57 %. Isolate KSB6 also had the best ability to solubilise phosphate sources from Ca₃(PO₄)₂ and phosphate rock from Blitar with soluble phosphate content of 893.8 mg/L and 172 mg/L, corresponding to a solubility percentage of 1.95 % and 0.07 % (Table 5).

TABLE 4
Potassium and phosphate-solubilising index on growth medium with application of the best 10 KSM isolates

Isolate code	Feldspar from Cirebon	Feldspar from Malang	Ca ₃ (PO ₄) ₂	Phosphate rock from Blitar
	----- Potassium-solubilizing index -----		---- Phosphate-solubilizing index ----	
KSB ₁	6.60	4.00	1.94	-
KSB ₂	6.77	4.73	1.91	-
KSB ₃	4.29	4.00	1.50	-
KSB ₄	2.59	5.91	3.70	2.32
KSB ₅	2.05	5.46	1.94	1.05
KSB ₆	3.86	3.88	3.56	2.04
KSF ₁	2.23	1.77	1.16	1.67
KSF ₂	2.22	1.94	1.12	1.68
KSF ₃	2.06	1.07	-	-
KSF ₄	1.16	1.46	1.50	-

Notes: Growth medium for test of potassium-solubilizing index is Alexandrov agar and growth medium for test of phosphate-solubilizing index is Pikovskaya agar. The “-” sign means that KSM colonies did not have halo zone on medium.

TABLE 5
Soluble content and solubility percentage of potassium and phosphate in growth medium with application of the best 10 KSM isolates

Isolate code	Soluble K		K solubility		Soluble P		P solubility	
	Feldspar from Cirebon	Feldspar from Malang	Feldspar from Cirebon	Feldspar from Malang	Ca ₃ (PO ₄) ₂	Phosphate rock from Blitar	Ca ₃ (PO ₄) ₂	Phosphate rock from Blitar
	mg/L		%		mg/L		%	
KSM ₀	31.65 d	29.54 e	0.16	0.17	62.50 b	25.00 b	0.14	0.01
KSB ₁	238.80 ab	268.99 a	1.24	1.55	718.80 a	71.88 b	1.57	0.03
KSB ₂	242.19 ab	259.40 ab	1.26	1.49	375.00 ab	31.25 b	0.82	0.01
KSB ₃	240.51 ab	253.16 bc	1.25	1.46	753.10 a	62.50 b	1.65	0.02
KSB ₄	222.78 abc	242.62 c	1.15	1.39	793.80 a	90.63 ab	1.73	0.03
KSB ₅	235.02 ab	259.49 ab	1.22	1.49	456.30 ab	75.00 b	1.00	0.03
KSB ₆	248.10 a	273.21 a	1.29	1.57	893.80 a	172.00 a	1.95	0.07
KSF ₁	235.02 ab	248.31 bc	1.22	1.43	553.10 ab	100.00 ab	1.21	0.04
KSF ₂	228.27 abc	248.95 bc	1.18	1.43	700.00 a	106.25 ab	1.53	0.04
KSF ₃	207.59 c	209.92 d	1.08	1.21	96.90 b	28.13 b	0.21	0.01
KSF ₄	218.99 bc	220.40 d	1.14	1.27	515.30 ab	31.25 b	1.13	0.01

Notes: KSM₀ (without KSM application), numbers in bold showed the biggest result from observed parameter. Means followed by the same letter in the same column were not significantly different result by DMRT test at 5% level of significance. Percentage of solubility calculated from comparison between soluble K/P content with total K/P content in K/P sources.

The increasing percentage of soluble potassium with feldspar from Cirebon as a potassium source is between 556 to 684 %, while the percentage increase of soluble potassium with feldspar from Malang as a potassium source is between 611 to 825%. The increasing percentage of soluble phosphate with Ca₃(PO₄)₂ as a phosphate source is between 55 to 1330 %, while the percentage increase of soluble phosphate with phosphate rock from Blitar is between 25 to 588 %.

Based on Tables 4 and 5, potassium and phosphate-solubilising indexes do not always have a correlation with ability to solubilise not-easily soluble potassium and phosphate sources in liquid medium. The solubilising index can serve as an indication of the ability of microbes to solubilise not-easily soluble potassium and phosphate sources, but it cannot become a single standard by which to determine the best ability to solubilise potassium and phosphate sources. Tests in liquid medium are needed to determine the ability of microbes to solubilise not-easily potassium and phosphate sources.

Potassium-solubilising mechanisms of KSM include organic acids such as oxalate acid, citrate acid (Liu *et al.*, 2006), tartaric acid (Sheng and He, 2006), citric acid, formic acid, and malic acid (Shanware *et al.*, 2014). Organic acids accelerate the weathering process of minerals containing potassium, causing potassium to become detached and available for plants (Sugumaran and Janarthanam, 2007). The potassium source used in the liquid medium test was potassium feldspar (KAlSi₃O₈). This reacts with organic acids causing ion exchange reactions between ion H⁺ from organic acid and ion K⁺ from potassium minerals. This

reaction, besides releasing ion K^+ that bind with silicate, also forms a secondary mineral, i.e., kaolinite ($Al_2Si_2O_5(OH)_4$) (Fu *et al.*, 2009).

Based on the test of isolates' ability in liquid medium, isolates KSB₁, KSB₂, KSB₃, KSB₅, and KSB₆ were found to be the five isolates that have the best ability to solubilise feldspar in liquid medium. Test results from these five isolates for stimulating sorghum growth showed that KSB₂ significantly had the best effect for plant height and shoot dry weight, while KSB₆ significantly had the best effect for root dry weight and total potassium in plant (Table 7).

KSB isolates from ex-mining land (KSB₂, KSB₅, and KSB₆) were found to have better ability to assist sorghum growth than KSB isolates from agricultural land (KSB₁ and KSB₃). There are no studies related to agricultural effects of KSM isolates from ex-tin mining or ex-gold mining lands that compare KSM from agricultural land as far as we know, although, research has shown that application of microbes from ex-coal mining land increased the growth of *Acacia crassicarpa* (Widyati, 2007).

Adaptation factors are presumably the main factors affecting the different abilities of bacteria. The ability of bacteria isolates to obtain food, grow, and compete with indigenous microbes will affect their ability to survive and solubilise potassium sources in soil. Roszak and Colwell (1987) have written that bacteria living in critical environments that have lower nutrient content have a higher survival rate than bacteria from a higher nutrient environments.

The higher ability of bacteria from ex-mining land could be caused by increased nutrition content from their environment. Soil from agricultural land used for testing of ability of microbes to stimulate sorghum growth had higher nutrient content than soil from ex-mining land. When bacteria from ex-mining land were applied to that soil medium, the nutrient improvement automatically increased food supply for those bacteria.

Egamberdiyeva (2007) has reported that total nutrient levels in soils have an effect on bacterial growing phase and ability. This finding is supported by Bren *et al.*, (2013) who have reported that bacteria from a lower nutrient environment have better ability because when nutrition content increases, the growth of these microbes will increase and cause their ability to increase as well, in this case the ability to solubilise not-easily soluble potassium sources. When growth rate of microbes increases, it will increase their ability to produce organic acids. Increased ability to produce organic acids can accelerate weathering processes of minerals containing potassium and make available their potassium for plants. Increased content of available potassium in the soil can increase the potassium content that is absorbed by plants. Increased content of potassium in sorghum is reflected in an increase in the total potassium in sorghum itself. Sorghum grown in soils applied with KSB isolates from ex-mining land have higher potassium content than the sorghum grown on soils applied with isolates from agricultural land (Table 6).

TABLE 6
Growth and total potassium content in sorghum with application of the five best KSM isolates

Treatments	Plant height (cm)	Number of leaves	Shoot dry weight (g)	Root dry weight (g)	K content (%)
P ₀	48.33 d	5.00	0.27 e	0.04 d	0.88 d
P ₁ (KSB ₁)	77.13 c	5.33	0.47 d	0.12 d	0.93 c
P ₂ (KSB ₂)	105.33 a	5.67	2.09 a	0.54 b	1.18 a
P ₃ (KSB ₃)	53.17 d	5.33	0.41 d	0.07 d	0.91 cd
P ₄ (KSB ₅)	81.07 bc	5.67	0.83 c	0.27 c	1.02 b
P ₅ (KSB ₆)	92.07 b	6.33	1.62 b	0.67 a	1.20 a

Notes: P₀ (without KSM application). Numbers in bold showed the biggest result from observed parameter. Means followed by the same letter in the same column were not significantly different result by DMRT test at 5% level of significance.

Characterisation results from the best two KSB isolates showed that each isolate has different characteristics from the other. Molecular analysis showed that KSB₂ has 97.8% similarity to *Achromobacter xylosoxidans* and KSB₆ has 99.0% similarity to *Burkholderia cepacia* (Table 7). Drancourt *et al.*, (2000) reports that molecular analysis results that have a similarity of more than or equal to 99% indicates that the isolates are of the same species, and similarity between 97 to 99% indicates that the isolate are of a different species but belong to the same genus. Based on this statement, KSB₂ isolate is possibly a new species from genus *Achromobacter*, and isolate KSB₆ probably belongs to the same species as *Burkholderia cepacia*.

TABLE 7
Characterisation results from the best two KSM isolates

Parameters	Observation result	
	----- BPK ₂ -----	----- BPK ₆ -----
Colony form	Round	Irregular
Colony color	Whitish	White
Colony elevation	Convex	Convex
Colony ledge form	Slick	Irregular
Cell shape	Coccus	Bacillus/Rod
Gram test	Negative	Negative
Motility test	-	+
Catalase test	+	+
Indole test	-	-
Urease test	-	-
Glucose fermentation test	-	-
Starch test	-	-
Citrate test	-	+
Species similarity	<i>Achromobacter xylosoxidans</i>	<i>Burkholderia cepacia</i>
Strain	2001038723	BAB-2826
Homology	97.8%	99.0%

Notes: + (positive reaction), - (negative reaction).

CONCLUSION

Isolation processes identified 185 KSM isolates from three different sets of soil samples from agricultural land, ex-tin mining land, and ex-gold mining land. Based on the pathogenicity test, 48 isolates of 185 isolates were found to have non-pathogenic characteristics. Of these 48 isolates, 10 KSM isolates were found to have the highest solubilising indexes. Based on the ability of isolates in liquid medium, five best isolates were found to have the highest ability to solubilise feldspar rock. Of these five isolates, two of the best KSM isolates were found to have the best ability to stimulate sorghum growth. KSM isolates from ex-mining land demonstrated better ability to stimulate sorghum growth than isolates from agricultural land. The two best isolates were KSB2 which had 97.8% genetic similarity to *Achromobacter xylooxidans* and KSB6 which had 99% similarity to *Burkholderia cepacia*.

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REFERENCES

- Basak, B.B., and Biswas, D.R. 2010. Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. *Biology and Fertility of Soil*. 46(6): 641 – 648.
- Bren, A., Yuval, H., Erez, D., Daniel, K., and Uri A. 2013. The last generation of bacterial growth in limiting nutrient. *BMC Systems Biology*. 7(1): 27.
- Diep, C.N., and Hieu T.N. 2013. Phosphate and potassium solubilizing bacteria from weathered materials of denatured rock mountain, Ha Tien, Kiên Giang province, Vietnam. *American Journal of Life Sciences*. 1(3): 88 – 92.
- Difco. 2009. Manual Microbiological Culture Media, Zimbardo MJ, Powder DA, Miller SM, Wilson GE, and Johnson JA (eds). 2nd ed. Maryland (US): Becton, Dickinson and Company.
- Drancourt, M., Bollett, C., Carlouz, A., Martelin, R., Gayral, JP., and Raoult D. 2000. 16S Ribosomal DNA Sequence Analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *Journal of Clinical Microbiology*. 38(10): 3623 – 3630.
- Edi, P.M., Moawad, A.M., and Vlek, P.L.G. 1996. Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science*. 11: 13 – 23.

- Egamberdiyeva, D. 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology*. 36(2): 184 – 189.
- Faddin, J.F.M. 1979. *Biochemical Tests for Identification of Medical Bacteria* (4th ed). Baltimore (US): Waverly Press, Inc.
- Fu, Q., Lu, P., Konishi, H., Dilmore, R., Xu, H., Seyfried, W.E., and Zhu, C. 2009. Coupled alkali-feldspar dissolution and secondary mineral precipitation in batch systems: 1. New experiments at 200^o C and 300 bars. *Chemical Geology*. 258(3): 125 – 135.
- Han, H.S., and Lee, K.D. 2005. Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. *Research Journal of Agriculture and Biological Sciences*. 1(2): 176 – 180.
- Havlin, J.L., Tisdale S.L., Beaton J.D., and Nelson W.L. 2005. *Soil Fertility and Fertilizer: An Introduction to Nutrient Management*. New Jersey (US): Pearson Education.
- Liu, W., Xu, X., Wu, S., Yang, Q., Luo, Y., and Christie, P. 2006. Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. *Environmental Geochemistry and Health*. 28(1-2): 133-140.
- Mahmoud, S.Y.M., Hosseney, M.H., EL-Shaikh, K.A.A.A., Obiadalla, A.H.A., and Mohamed, Y.A. 2013. Seed borne fungal pathogens associated with common bean (*Phaseolus vulgaris* L.) seeds and their impact on germination. *Journal of Environmental Studies*. 11: 19 – 26.
- Ministry of Energy and Mineral Resources. 2015. *Neraca Mineral* [internet]. Available in: <http://psdg.bgl.esdm.go.id/Neraca/NeracaMineral214.pdf> (accessed on 20th December 2015)
- Parmar, P., and Sindhu, S.S. 2013. Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. *Journal of Microbiology Research*. 3(1): 25 – 31.
- Prajapati, K., and Modi, H.A. 2012. Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. *CIBTech Journal of Microbiology*. 1: 8 – 14.
- Prajapati, K., Sharma, M.C., and Modi, H.A. 2013. Growth promoting effect of potassium solubilizing microorganisms on okra (*Abelmoscus Esculentus*). *International Journal of Agricultural Science and Research*. 3(1): 181 – 188.

- Rogers, J.R., Bennett, P.C. and Choi, W.J. 1998. Feldspars as a source of nutrients for microorganisms. *American Mineralogy*. 83(11): 1532-1540.
- Roszak, D.B., and Colwell, R.R. 1987. Survival strategies of bacteria in the natural environment. *Microbiological Reviews*. 51(3): 365 – 379.
- Santosa, D.A. 2001. Rapid extraction and purification of environmental DNA for molecular cloning applications and molecular diversity studies. *Molecular Biotechnology*. 17(1): 59 – 64.
- Schaad, N.W., Jones, J.B., and Chun W. 2001. *Plant Patogenic Bacteria* (3th ed). The Minnesota (US): American Phytopathological Society.
- Shanware, A.S., Kalkar, S.A., and Trivedi, M.M. 2014. Potassium solubilisers: occurrence, mechanism and their role as competent biofertilizers. *International Journal of Current Microbiology and Applied Sciences*. 3(9): 622 – 629.
- Sheng, X.F., and He, L.Y. 2006. Solubilization of potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiology*. 52(1): 66 – 72.
- Sugumaran, P., and Janarthanam B. 2007. Solubilizing of potassium containing minerals by bacteria and their effect on plant growth. *World Journal of Agricultural Science*. 3(3): 350 – 355.
- Susilowati, L.E., and Syekhfani. 2014. Characterization of phosphate solubilizing bacteria isolated from Pb contaminated soils and their potential for dissolving tricalcium phosphate. *Journal of Degraded and Mining Lands Management*. 1(2): 57 – 62.
- Widyati, E. 2007. Formulasi inokulum mikroba: MA, BPF dan Rhizobium asal lahan bekas tambang batubara untuk bibit *Acacia crassicarpa* Cunn. Ex-Benth. *Biodiversitas*. 8(3): 238 – 241.