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Solubilization of silicate from quartz mineral by potential silicate solubilizing bacteria

Pelarutan silika asal mineral kuarsa oleh bakteri pelarut silika potensial

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Abstrak

Silika (Si) merupakan unsur kedua paling banyak dijumpai di dalam tanah dengan konsentrasi yang sangat beragam antara 50-400 g Si per kg tanah. Silika memberikan pengaruh positif pada ketahanan tanaman terhadap berbagai cekaman abiotik dan biotik seperti salinitas, kekeringan, toksisitas logam berat, dan penyakit. Meskipun berlimpah, sebagian besar sumber Si tidak tersedia bagi tanaman, karena kelarutan senyawa Si di dalam tanah cukup rendah. Untuk meningkatkan kelarutan Si di dalam tanah, bakteri pelarut silika berperan penting melarutkan silika dalam bentuk tidak larut. Penelitian ini bertujuan untuk menetapkan: (i) kemampuan melarutkan silika dari magnesium trisilika dan kuarsa oleh Burkholderia cenocepacia *KTG,* Aeromonas punctata *RJM3020, and* B. vietnamiensis *ZEO3, (ii) karakterisasi secara biokimia bakteri pelarut silika, dan (iii) kelarutan silika, Ca, dan Mg dari kuarsa oleh aktivitas bakteri pelarut silika. Kegiatan penelitian dilakukan di Laboratorium Mikrobiologi dan Lingkungan, Pusat Penelitian Bioteknologi dan Bioindustri Indonesia, Bogor, tahun 2016. Bakteri pelarut silika potensial ditumbuhkan di dalam medium Bunt dan Rovira yang mengandung masing-masing 0, 25% magnesium trisilikat atau kuarsa sebagai substrat. Aktivitas pelarutan silika oleh bakteri pelarut silika ditetapkan dengan spektrofotometer emisi atom dan produksi asam organik diukur dengan kromatografi cair kinerja tinggi (HPLC). Hasil penelitian ini menunjukkan bahwa tidak ada korelasi antara aktivitas pelarutan silika dengan zona jernih yang terbentuk dalam medium padat Bunt dan Rovira*. Isolat B. cenocepacia *KTG,* A. punctata *RJM3020, dan* B. vietnamiensis *ZEO3 dapat menghasilkan asam sitrat, oksalat, dan asetat dengan waktu inkubasi optimum yang bervariasi. Ketiga isolat tersebut juga dapat melarutkan* SiO_2 *yang berasal dari mineral kuarsa.*

[*Kata kunci:* *bakteri pelarut silika,* Burkholderia cenocepacia *KTG,* A. unctate *RJM 3020,* B. vietnamiensis *ZEO3, asam silikat.*]

Abstract

Silicon (Si) is the second most abundant in the soil varies from 50 to 400 g Si per kg of soil. Silicon has positive influence on the plant resistance to various abiotic and biotic stressors such as salinity, drought, heavy metal toxicities and diseases. Although Si is abundant in soil, most of its sources are not available for plant uptake due to low solubility of Si compounds in soil. To improve plant-available Si in the soil, silicate solubilizing bacteria (SSB) are potentially important in solubilizing insoluble forms of silicate. The objectives of this study were to determine: (i) silicate solubilizing activity of *Burkholderia cenocepacia* KTG, *Aeromonas punctata* RJM3020, and *B. vietnamiensis* ZEO3 on magnesium trisilicate and quartz, (ii) biochemical characteristics of SSB, and (iii) solubilization of silicate, Ca, and Mg from quarts by SSB activity. A laboratory study was conducted at Microbiology and Environment Laboratory, the Indonesian Research Institute for Biotechnology and Bioindustry, Bogor, in 2016. All SSB were grown on Bunt and Rovira media containing 0.25% magnesium trisilicate or quartz as substrate, respectively. Silica solubilizing activities by SSB were determined by using inductively coupled plasma-atomic emission spectrometry while organic acid concentration in the culture were measured by using high-performance liquid chromatography (HPLC). The results indicate that no correlation between solubilizing silicate activity and clear zone on solid Bunt and Rovira media. *B. cenocepacia*

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KTG, *A. punctata* RJM 3020, and *B. vietnamiensis* ZEO3 isolates were capable of producing citric, acetic, and oxalic acid in various optimum incubation time and accelerating the solubilization of SiO_2 originated from quartz.

[*Key words: Silicate solubilizing bacteria (SSB); Burkholderia cenocepacia* KTG, *A. punctata* RJM 3020, *B. vietnamiensis* ZEO3, and silisic acid]

Introduction

Drought stress is one of the major causes for crop loss in Indonesia agriculture, reducing average yields by 50% and even more. Some studies indicate that silicon (Si) application is able to avoid the damage of plant when grown under drought stress condition (Sacala, 2009; Boecharnikova & Benes, 2011; Son *et al*., 2011; Mauad *et al*., 2016; Hajiboland *et al*., 2017). Unfortunately, many soils in Indonesia are considered lack of available silicon that has positive impact for plant by stimulate nutrient uptake and plant photosynthesis, decrease susceptibility to disease and insect damage, alleviate water and various mineral stresses and decrease the toxic effects of aluminum and heavy metals (Currie & Perry, 2007; Heckman, 2013; Adrees, 2015; Ng *et al*., 2016; Sakr, 2016; Khalifa *et al*., 2017). In nature, silicon occurs generally in the form of silicates, including ferromagnesian silicates (e.g. olivine, pyroxenes, and amphiboles), aluminosilicates (e.g. feldspar, mica, and clays), and silicon dioxide (e.g. amorphous silica, quartz). In general, the silicon in silicate minerals is surrounded by four oxygen atoms in tetrahedral fashion (Gauger *et al*., 2016). Silicon content in soil varies from 50 to 400 g Si per kg of soil (Balakhnina & Borkowska, 2013). In soil, Si compounds mainly present as SiO_2, about 50–70 % of the soil mass, and in various aluminosilicate forms (Sommer *et al.*, 2006). Although Si is abundant in soil, but most of its sources are not available for plant uptake due to low solubility of Si compounds in soil (Guntzer *et al*., 2012; Rizwan *et al*., 2012; Peera *et al*., 2016).

Silicon is never be found in a plant as available form and is always combined with other elements, usually forming oxides or silicates (Gunes *et al*., 2007). Silicon is absorbed by plants in the form of uncharged silicic acid, $Si(OH)_4$, and is ultimately irreversibly precipitated throughout the plant as amorphous silica (Ranganathan *et al.*, 2006). Therefore, although silicon is plentiful, most sources of silicon are insoluble and in a plant-unavailable form. Typical concentrations of silicic acid in soil solution range from 0.1 to 0.6 mM. In soil, Si compounds exist in two forms viz. liquid and solid. The solid form mainly consists of crystalline, poorly crystalline, and amorphous silica (ASi) (Sauer *et al*., 2006). The liquid form generally consists of mono- and polysilicic acids, complexed with organic and inorganic compounds (Cornelis *et al.*, 2011). Wollastonite is a naturally occurring mined $CaSiO_3$ and can be a useful Si source when finely ground. However, this mineral is not found in Indonesia.

Bangka Island is the largest and best quality deposit of quartz in Indonesia, therefore can be used as locally sources for silicate fertilizer. Quartz is the second most abundant mineral in Earth's continental crust, after feldspar. Quartz is a compound of one part silicon and two parts of oxygen, silicon dioxide, SiO_2. Its crystal structure is a continuous framework of SiO_4 silicon–oxygen tetrahedra, with each oxygen being shared between two tetrahedra, giving an overall chemical formula of SiO_2 and will naturally dissolve very slowly. Therefore inoculation of SSB into quartz as silica fertilizer or soil may benefit the crop by releasing several nutrients and available silica form from soil and quartz (Brindavathy *et al*., 2012; Meena *et al*., 2014).

Soil contains a large number of bacteria but only few bacteria found release silicon from natural silicates. Studies have shown that these bacteria solubilize silica besides releasing phosphate, potassium, iron and calcium from the soil silicate minerals. Bunt and Rovira medium supplemented with these insoluble silicates have been used to screening SSB. Isolation of SSB from soil and water in Bunt and Rovira medium containing 0.25% magnesium trisilicate was made earlier by Muralikannan & Anthomiray (1998). However, the morphological characteristics of the surface on Bunt and Rovira medium and physiological properties of each isolates needs to be cross-analyzed as data base in advanced or research purposes. The objectives of this study were to determine: (i) silicate solubilizing activity of *B. cenocepacia* KTG, *A. punctata* RJM3020, and *B. vietnamiensis* ZEO3 on quartz, (ii) biochemical characteristics of SSB, and (iii) solubilization of silicate, Ca, and Mg from quarts by SSB activity.

Materials and Methods

Microorganism

Burkholderia cenocepacia KTG, *Aeromonas punctata* RJM3020, and *Burkholderia vietnamiensis* ZEO3 were isolated from a sandy soil (Kotawaringin Barat, Central Kalimantan), Ultisol (Rajamandala Estate, West Java), and zeolite mineral originating from Bayah, West Java, respectively (Santi *et al*, 2010; Santi & Goenadi, 2012b). These bacteria were lodged at Microbiology and Environmental Laboratory of Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), Bogor. As a source of inoculum, three inoculant SSB were grown on medium Bunt and Rovira as described by Vasanthi *et al*, (2013) consisting of (g/L): 20 g glucose; 1.0 g peptone; 1.0 g yeast extract; 0.5 g $(NH_4)_2SO_4$; 0.4 g K_2HPO_4; 0.1 g $MgCl_2$; 0.01 g $FeCl_3$; 250 mL soil extract; 20 g oxoid agar; 750 mL tap water; pH 6.6-7.0. These media supplemented with 0.25% magnesium trisilicate ($2MgO{\cdot}3SiO_2$) and quartz mineral, respectively, as sources of silicate.

Source of quartz mineral

The mineral used was quartz type collected from Bangka area, Sumatera. Quartz samples were air dried and passed through 325 mesh sieve and analyzed for the following: pH, total carbon (spectrophotometer), nitrogen (Kjeldahl), phosphorus (spectrophotometer), potassium (Atomic Absorption Spectrophotometer, AAS), magnesium (AAS), calcium (AAS), total SiO_2 (gravimetry), Sulphur, Zinc, aluminium trioxide, iron trioxide, manganese dioxide, and cation exchange capacity (CEC) by using SNI 15-0346-1989 standard method (Table 1).

Biochemical characteristics

Silicate bacteria isolates were biochemically characterized by using microbacterial identification kits (Oxoid™ Microbact™ GNB Kit). These test were carried out according to 25 types of biochemical reaction, i.e. catalase, indole, hydrogen sulphide production, tryptophan deaminase, beta–galactosidase, arginine hydrolysis, lysine decarboxylase, urease carbohydrate utilization tests, etc. The results were clearly visible as it was shown by distinct color reactions that can be interpreted after 18-24 hours.

Silicate solubilizing activity

Silicate solubilizing activity was assayed in 100 mL liquid and solid Bunt and Rovira medium with 0.25% insoluble magnesium trisilicate. A loopful of the SSB strain containing 10^9 colony forming units (cfu) were inoculated into liquid Bunt and Rovira medium, and then incubated on a mechanical shaker at 100 rpm, 28^{o}C for 24, 96, and 192 hours. Silicate solubilizing activity was determined by using inductively coupled plasma-atomic emission spectrometry (AOAC, 2005).

For assessment in solid Bunt and Rovira medium, petri dishes containing Bunt and Rovira medium at each initial pH condition were inoculated with each culture strain of SSB. The culture dishes were incubated at 28°C for 7 days. The diameter of the solubilization halo (translucent area surrounding colonies) was measured after the 7th day incubation and the Solubilization Index (SI) expressed as halo diameter (mm)/colony diameter (mm) was calculated (Akintokun *et al.*, 2007). The investigated strains were classified based on
SI as demonstrating low (SI < 2.00), interme 45
(2.00 < SI < 4.00) and high (SI > 4.0) solubilization capacities.

Acid production by SSB isolates

Petri dishes containing Luria Bertani agar plates were inoculated with each culture strain of *B. cenocepacia* KTG, *A. punctata* RJM3020, and *B. vietnamiensis* ZEO3, respectively, and then incubated for 72 hours at temperature of 28^o C. Luria Bertani (LB) medium is consisting of (g/L): 10 g Tryptone, 5 g yeast extract, 5 g NaCl, 12 g agar bacteriological (Oxoid), and amended with 0.2% (v/v) bromophenol blue. Acid production by the isolate was detected as blue media turned yellow around bacterial colonies.

The SSB producing organic acid activity was determined in liquid Bunt and Rovira with 0.25% magnesium trisilicate. Three strains of SSB were grown in Bunt and Rovira medium for 24, 96 and 192 hours, incubated at a mechanical shaker at 100 rpm and in 28^{o}C. Organic acid concentration in the culture were determined by using High-performance liquid chromatography (HPLC). Agilent HP Series 1100 was used to identify and quantify organic acids. Samples were collected, filtered through a 0.45 micellulose membrane, and injected into a Supelcogel C-610H 9 chromatographic column sizing 30 cm x 7.8 mm.

Solubilization of silicate, Ca, and Mg from quarts by SSB activity

The solubilisation of Si, Ca, and Mg was investigated in 100 mL Bunt and Rovira liquid medium to which 0.25 g of quartz was added separately. The flasks were sterilized at 15lb psi for 20 minutes, cooled and inoculated with 0.1 mL single strain of SSB, i.e *B. cenocepacia* KTG, *A. punctata* RJM3020, and *B. vietnamiensis* ZEO3. The flasks were incubated at a mechanical shaker at 100 rpm speed and 28^{0}C temperature for 24, 96, and 192 hours, respectively. After incubation periods, each flasks were withdrawn, the culture was centrifuged at 10,000 rpm for 15

Table 1. Characteristics of quartz mineral from Bangka.

Tabel 1. Karakteristik mineral kuarsa dari Bangka.

Types of analysis *Jenis analisis*	Results *Hasil*	Types of analysis *Jenis analisis*	Results *Hasil*
pH in H_2O/ *pH pada H_2O*	8.2	SiO_2 (%)	97.1-99.1
Carbon / *Karbon* (%)	0.12	Sulphur (ppm)	86
Nitrogen (%)	0.03	Zn (ppm)	53.2
P_2O_5 HCl 25% (ppm)	30.1	Al_2O_3 (%)	0.17
K_2O HCl 25% (ppm)	6.14	Fe_2O_3 (%)	0.06
MgO HCl 25% ppm)	<0.01	MnO_2 (%)	<0.01
CaO HCl 25% (ppm)	0.05	CEC ($cmol^+$/kg)	1.98

minutes to remove the cells and debris, and the supernatant was analyzed for the Silica, Ca, and Mg by using spectrophotometer, Shimadzu UV mini-1240 (AOAC, 2005).

Results and Discussion

Selected characteristics of potential SSB

In these research, the morphological characteristics of the surface on Bunt and Rovira medium and physiological properties of each isolates needs to be cross-analyzed as data base in advanced or research purposes. Cultural and some of the biochemical characteristics were studied for three selected isolates of SSB from IRIBB Laboratory collection. Colonies form of *B. cenocepacia* and *A. punctata* were irregular, raised, undulate while *B. vietnamiensis* was irregular, flat, and undulate when grown on Bunt and Rovira silicate bacteria medium. All of the isolates were cream, negative stain, rod shape (Table 2), and produced exopolysaccharide as slimming form colony on solid Bunt and Rovira medium (Figure 1). Naureen *et al.,* (2015) reported that bacterial isolates can accelerate and solubilize insoluble silicate minerals by producing extracellular polysaccharides.

Biochemical characteristics

Biochemical analysis techniques refer to a set of methods, assays, and procedures that enable scientists to analyze the substances found in living organisms and the chemical reactions underlying life processes (Table 3). The potential isolates in this research were lysine, glucose, β–galactosidase, citrate, and malonat positive, while the *B. vietnamiensis* isolate was urease and arabinose negative. Only *A. punctata* isolates capable of producing mannitol, xylose, sucrose, and lactose. All of these isolates were unable to produce ornithine, hydrogen sulfide (H_2S), indole (IND), TDA, gelatin, inositol, sorbitol, rhamnose, adonitol, raffinose, salicin, arginine, and nitrate. The results in this study indicates that *B. cenocepacia*, *A. punctate,* and *B. vietnamiensis* were gram negative SSB.

Silicate solubilizing activity

Three selected strains of SSB i.e. *B. cenocepacia* KTG, *A. punctata* RJM3020, and *B. vietnamiensis* ZEO3 solubilized SiO_2 from initial sources of $2MgO{\cdot}3SiO_2$ in Bunt and Rovira liquid medium. Silicate solubilizing activities were optimum at 24 hours after incubation periods (Table 4). The amount of Si solubilized was directly related to the incubation time periods especially for *A. punctata* RJM3020 and *B. vietnamiensis* ZEO3.

In this research, the highest solubilizing silicate diameter zone in solid Bunt and Rovira solid medium was observed for *B. cenocepacia* KTG, but silicate solubilizing activity in liquid medium from this isolate was less than *A. punctata* RJM3020 and *B. vietnamiensis* ZEO3. However, the diameter of the clear zone resulting from the activity of bacteria in this study was less than that reported by Vasanthi *et al.,* (2012) i.e. 16 mm. Results of this research indicating that no correlation between solubilizing silicate activity and solubilisation zone on solid Bunt and Rovira medium as reported by Vasanthi *et al.* (2013). Therefore, some selective substrates and media for assessing the growth and silicate solubilizing activity were needed for to screening potential SSB.

Acid production by SSB isolates

Several mechanisms of dissolution silicate by SSB have been suggested to be in correlation with acidolysis, alkaline hydrolysis, ligand degradation, enzymolysis, capsule adsorption, extracellular polysaccharides and redox have been shown to play a role in bacterial dissolution of silicate. Acidolysis is the main and largely accepted mechanism of weathering silicate minerals (Sheng *et al.*, 2008). In this research, *B. cenocepacia* KTG, *A. punctata* RJM3020, and *B. vietnamiensis* ZEO3 isolates were capable of

Table 2. Morphology of potential SSB species on Bunt and Rovira solid media.
Tabel 2. Morfologi spesies bakteri pelarut silika potensial pada media padat Bunt dan Rovira

SSB species *Spesies bakteri pelarut silika*	Morphology/ *Morfologi*			
	Colony form *Bentuk koloni*	Colony color *Warna koloni*	Gram stain *Pewarnaan gram*	Cell shape *Bentuk sel*
B. cenocepacia	irregular-raised-undulate *tidak beraturan- muncul ke permukaan media-bergelombang*	cream *krem*	negative *negatif*	rod *batang*
A. punctata	irregular-raised-undulate *tidak beraturan- muncul ke permukaan media-bergelombang*	cream *krem*	negative *negatif*	rod *batang*
B. vietnamiensis	Irregular-flat-undulate *Tidak beraturan-datar-bergelombang*	cream *krem*	negative *negatif*	rod *batang*

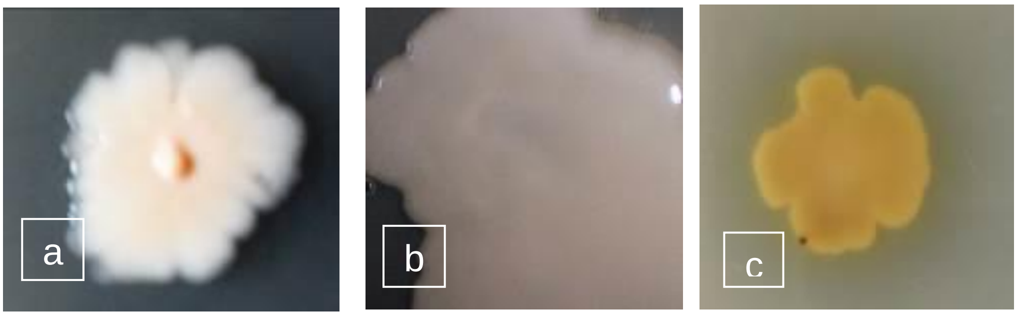

Figure 1 . The growth of *B. cenocepacia* (a); *A. punctata* (b); dan *B. vietnamiensis* (c) on Bunt and Rovira solid media.

Gambar 1 Pertumbuhan B. cenocepacia *(a);* A. punctata *(b); dan* B. vietnamiensis *(c) pada media padat Bunt dan Rovira.*

solubilizing silicates and produced acid as detected by yellow halo formation on solid Luria Bertani media containing 0.2% (v/v) bromophenol blue (Figure 2). As an acid–base indicator, bromophenol blue useful range lies between pH 3.0 and 4.6. It changes from yellow at pH 3.0 to blue at pH 4.6. The color change on solid Luria Bertani medium amended with 0.2% (v/v) bromophenol blue from blue into yellow or greenish-yellow indicating pH drop through the release of organic acid into medium by SSB inoculant.

Many bacteria in soil are able to solubilize unavailable form of silicate mineral such as quartz by excreting organic acid, which either directly dissolves rock potassium or chelate silicon ions to bring the K and Si into solution. It was postulated that the reaction responsible for bacterial promoted K and Si solubilization may involve a combination of proton attack and complexation reaction by organic acid (Sheng *et al*., 2008). Organic acid analyzed in this research was the main organic acid that play important role in dissolving mechanism of silicate. Three selected SSB were high producers of citric, acetic, and oxalic acids. *B. cenocepacia* KTG has high ability in producing acetic acid while *A. punctata* RJM3020 in producing citric and oxalic acid (Table 5). All of these isolates capable of producing citric, acetic, and oxalic acids in various optimum incubation times.

Table 3. Physiological analysis of SSB species.
Tabel 3. Analisis fisiologi spesies bakteri pelarut silika.

Type of analysis *Jenis analisis*	Results *Hasil*		
	Burkholderia cenocepacia KTG	*Aeromonas punctata RJM3020*	*Burkholderia vietnamiensis ZEO3*
Lysine-*Lisin*	+	+	+
Ornithine- *Ornitin*	-	-	-
H_2S-asam sulfida	-	-	-
Glucose- Glukosa	+	+	+
Mannitol- Manitol	-	+	-
Xylose- Silosa	-	+	-
ONPG/β-galactosidase-ONPG/β-galaktosidase	+	+	+
Indole-Indol	-	-	-
Urease-Urease	+	+	-
VP-VP	-	-	-
Citrate-Sitrat	+	+	+
TDA/Indole pyruvate-TDA/Indol piruvat	-	-	-
Gelatin-Gelatin	-	-	-
Malonate-Malonat	+	+	+
Inositol-Inositol	-	-	-
Sorbitol-Sorbitol	-	-	-
Rhamnose-Ramnosa	-	-	-
Sucrose-Sukrosa	-	+	-
Lactose-Laktosa	-	+	-
Arabinose-Arabinosa	+	+	-
Adonitol-Adonitol	-	-	-
Raffinose-Rafinosa	-	-	-
Salicin-Salisin	-	-	-
Arginine-Arginin	-	-	-
Nitrate-Nitrat	-	-	-

Table 4. The ability of SSB isolates in solubilizing silicate in a liquid and solid Bunt and Rovira medium using 0.25% magnesium trisilicate as a substrate.

Tabel 4. Kemampuan bakteri pelarut silika dalam melarutkan silika di dalam medium cair dan padat Bunt dan Rovira dengan menggunakan 0.25% magnesium trisilika sebagai substrat.

SSB species *Spesies bakteri pelarut silika*	Solubilizing silicate (ppm) *Kelarutan silika (ppm)* Incubation time (hours) *Waktu inkubasi (jam)*			Clear zone (mm) *Zona jernih (mm)* Incubation time (7 days) *Waktu inkubasi (7 hari)*
	24	96	192	
B. cenocepacia	14.5	19.8	11.7	5.0
A. punctata	97.6	17.8	14.4	3.8
B. vietnamiensis	68.0	29.3	0.6	2.9

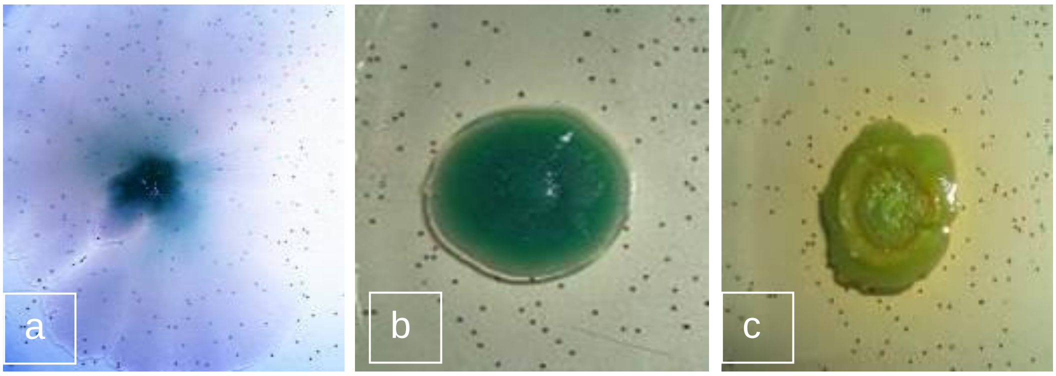

Figure 2. The growth of *Burkholderia cenocepacia* (a); *Aeromonas punctata* (b); and *Burkholderia vietnamiensis* (c) on solid Luria Bertani medium amended with 0.2% (v/v) Bromophenol Blue. The yellow zone indicates the ability of the isolates to generate organic acids.

Gambar 2. Pertumbuhan Burkholderia cenocepacia *(a);* Aeromonas punctata *(b); dan* Burkholderia vietnamiensis *(c) pada media Luria Bertani yang diperkaya dengan 0,2% (v/v) Bromofenol Blue. Zona berwarna kuning menunjukkan kemampuan isolat dalam menghasilkan asam organik.*

Table 5. The organic acid produced by SSB in modified liquid Bunt and Rovira medium.

Tabel 5. Produksi asam organik oleh bakteri pelarut silika di dalam medium cair Bunt dan Rovira yang dimodifikasi.

SSB species *Spesies bakteri pelarut silika*	Organic acid production (g/L) *Produksi asam organik* (g/L)								
	Citric acid *Asam sitrat*			Acetic acid *Asam asetat*			Oxalic acid *Asam oksalat*		
	Incubation time (hours) *Waktu inkubasi (jam)*								
	24	96	192	24	96	192	24	96	192
B. cenocepacia	0.26	0.17	0.24	2.67	3.11	3.14	0.53	0.78	0.94
A. punctata	0.42	0.50	0.53	1.04	1.28	1.73	0.79	0.25	1.32
B. vietnamiensis	0.18	0.17	0.60	2.67	3.02	1.38	0.84	1.17	0.45

For the purposes of supplying silica for plant, there is a growing interest in the effectiveness of bacteria and fungi as mineral weathering agent (Santi & Goenadi, 2012 a&b; Goenadi & Santi, 2013). In Indonesia, Bangka and Belitung Islands are the largest deposits and have best quality of quartz that containing 97-99% of SiO_2. Therefore their quartz can be used as locally sources of raw material for silicate fertilizer. In this research, three potential SSB were inoculated in liquid Bunt and Rovira medium amended with 0.25% quartz mineral 325 meshes on particle size. *In vitro* studies showed that all of these SSB can solubilize Si, Ca, and Mg from quartz mineral. *B. cenocepacia* KTG, *A. punctata* RJM3020, and *B. vietnamiensis* ZEO3 grew well (10^7-10^8 cfu) on quartz mineral under *in vitro* conditions at 192 hours incubation periods. The results from this research showed that SSB isolates in Bunt and Rovira medium containing silicate (quartz) could increase the solubility of Ca and Mg as a secondary nutrient. It may due to the ability of the SSB to act the native mineral silicate releasing Ca and Mg.

The lowest solubilizing silica compared to Ca and Mg was observed in the culture filtrate (Table 6), because crystalline quartz will dissolve only very slowly in hot watery alkaline solutions, while *amorphous* SiO_2 will be readily dissolved at room temperatures. The ability of SSB to grow on quartz as substrate in liquid Bunt and Rovira medium can alter silicate solubility directly by perturbing mineral–water equilibrium and reaction dynamics at the point of attachment by producing proton, hydroxyl or metal-chelating metabolic by products (Rogers & Bennett, 2004). The amount of Si, Ca, and Mg solubilized were

Table 6. The ability of SSB isolates in solubilizing silicate in Bunt and Rovira liquid medium by using 0.25% quartz mineral as substrate.
Tabel 6. Kemampuan isolat bakteri pelarut silika dalam melarutkan silika di dalam medium cair Bunt dan Rovira dengan menggunakan 0,25% mineral kuarsa sebagai substrat.

Species of SSB *Spesies bakteri pelarut silika*	Solubilizing of Si, Ca, and Mg (ppm) *Kelarutan dari Si, Ca, dan Mg (ppm)*								
	Incubation time (hours) *Waktu inkubasi (jam)*								
	24			96			192		
	Si	Ca	Mg	Si	Ca	Mg	Si	Ca	Mg
B. cenocepacia	0.72	2.9	1.12	0.76	15.8	1.41	0.84	13.2	2.49
A. punctata	0.82	12.0	1.03	0.86	15.6	1.10	0.32	7.0	0.36
B. vietnamiensis	0.69	12.0	0.78	0.70	13.6	0.92	0.81	10.6	1.00
Without inoculant	0.03	0.01	0.01	0.26	0.01	0.01	0.27	0.05	0.01

Conclusions

Burkholderia cenocepacia KTG, *A. punctata* RJM 3020, and *B. vietnamiensis* ZEO3 have been shown to accelerate the solubilization of SiO_2 originated from quartz. The amount of Si, Ca, and Mg solubilized from quartz mineral were optimum in 96 hours incubation periods. All isolates were gram negative, rod shape, producing exo polysaccharide, organic acid (citric, acetic, and oxalic acid), and had differences of biochemical reaction which differentiate from each other. Acetic acid was a predominant substance produced by SSB isolates in liquid Bunt and Rovira media.

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