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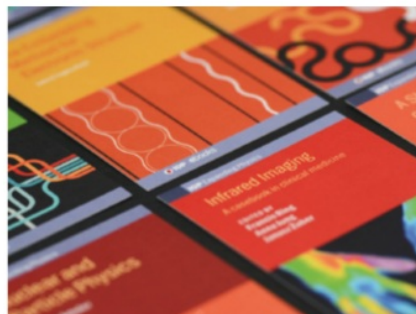
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1 Isolation and characterization of silicate-solubilizing bacteria from paddy rhizosphere (*Oryza sativa* L.)

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Abstract. Silicate is known as a beneficial element that has important role for growth and development of paddy. Paddy is the greatest accumulator of Si up to 10% of its dry weight, but this plant can't absorb that element by itself. The uses of paddy rhizosphere bacteria as a silicate solubilizing agent should be studied furthermore. This study aims to isolate and characterize silicate bacteria from paddy rhizosphere that solubilize silicate optimally in *Bunt and Rovira* medium. The isolation, qualitative, quantitative test and characterization of silicate bacteria up to 39 characters, which is include of colony morphological, cell morphological and physiological-biochemical test. This study was conducted on February-June 2018 in Microbiology Laboratory and Plant Physiology Laboratory, Faculty of Mathematics and Natural Sciences, State University of Surabaya. Five isolates silicate solubilizing bacteria were found OS4, OS5, OS7, OS12 and OS13. The highest Solubilizing Index was gained by OS7 on 1,10, while the highest silicate concentration was solubilized by OS12 on 1,053 ppm in *Bunt and Rovira* broth.

1. Introduction

Optimization for the growth of paddy plant as a stable Indonesian food could be through by nutritional selection [1]. Three groups of element divided by its function were essential, beneficial and toxic element. Essential element has its function to enhance growth from all type of stress in plant so that has main role in growth and development phase. Toxic element capable to inhibit growth and development of plant, while beneficial element influence growth and development stage in some plants, mainly found in monocots [2]. Silica as beneficial element has function for its cell strength in growth and development of paddy, which is accumulated 10% of its dry weight [3]. Epidermis layer strength by silica gel, make paddy is more resistant from any phytopathogenic, such as bacteria and fungi [4]. Desilication of silica-dioxide was mayor problem in soil, influences the availability of silica in paddy plant is lower than the occurrence of silicate in nature 220-240 billion/tons [1]. Silica-dioxide chelation found higher around roots, which included of main inhibition factor the uptake of nutrient by paddy through lateral root [22]. Rhizosphere bacteria play a vital role in the functioning of plants by influencing their physiology and development in rhizosphere [5]. Symbiosis of rhizosphere between rhizosphere bacteria influences availability nutrient uptake by organic acid productions [24]. Although the one of role rhizosphere bacteria as a silica-weathering agent had been recognized, the isolation and characterization of silicate-solubilizing bacteria in paddy rhizosphere should be studied furthermore.

2. Method



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2.1. Isolation of Paddy Rhizosphere Bacteria

Sample of rhizosphere was uprooted from six rice fields, Bangkingan, Jeruk, Kapasan, Sawo, Sumber Makmur and Siwalan Makmur. Ten grams of rhizosphere were diluted by 5% NaCl, shaken two minutes by vortex. The serial dilutions up to 10^{-7} which the composition was 4 ml of sample into 9 ml of 0,85% NaCl, each dilution continuously shaken two minutes by vortex. The diluted inoculum is then added to a molten *Bunt and Rovira Agar* in a Petri dish, mixed and allowed to solidify. The isolates paddy rhizosphere bacteria incubated at 30°C for 4 days in *Bunt and Rovira Agar* [6,7].

2.2. Characterization of Silicate-Solubilizing Bacteria

Colony characteristics such as shape, elevation, margin, texture, pigmentation and optic were examined. Cell morphology characteristics such as cell shape, cell arrangement, Gram staining and spore formation. Physiological and biochemical test of silicate solubilizing bacteria include of catalase test, motility, indole production, H₂S production, ornithine test, acid fast staining, *Methyl Red* (MR), *Voges Proskauer* (VP), urease test, nitrate reduction, citrate utilization, fermentation of glucose, fructose, sucrose, lactose, starch, mannitol, growth at pH 3, 7, 10 and temperature 25°C, 30°C, 45°C [8,9,10,11,12,13].

2.3. Silica Solubilizing Activity in *Bunt and Rovira Agar*

The selected bacterial isolates from paddy rhizosphere were subjected to a silica solubilizing test in *Bunt and Rovira Agar*, incubated at 30°C for 4 days. Silica source (quartz) were air dried and passed through 325 mesh-sieve. The medium contains of (g/750 mL): 20 g glucose; 20 g agar; 1 g peptone; 1 g yeast extract; 0,5 g (NH₄)₂SO₄; 0,4 g K₂HPO₄; 0,1 g MgCl₂; 0,01 g FeCl₃; 250 ml soil extract; 750 ml aquades; pH 6,6-7,0 [11]. Isolates that produces clear zone was recognized has its capability to solubilize 0,25% quartz in *Bunt and Rovira Agar*. Clear zone from each isolates was measured by Solubilizing Index [14].

2.4. Silica Solubilizing Activity in *Bunt and Rovira Broth*

The solubility of silica was investigated in 100 ml *Bunt and Rovira broth*, which each of isolates supplemented by 0,25% quartz. Source of silica (0,25% quartz) were add separately into *Bunt and Rovira broth*. One ml of the bacteria cell 10^8 cfu/ml was inoculated into *Bunt and Rovira broth* for 7 days. After incubation periods, the culture was centrifuged at 10.000 rpm for 15 minutes to remove supernatant from debris. One ml of supernatant was added reagents into urine container and analyzed by silico-molybdate's method [11,15].

2.5. pH Measurement in *Bunt and Rovira Broth*

pH was measured before and after incubation in *Bunt and Rovira broth*. Before its sterilization, the *Bunt and Rovira broth* was measured by pH roll (6,6-7,0). After seven days incubation, each pH culture measured, then compared against pH before incubation periods [11].

3. Results and discussion

Sixteen isolates were isolated from paddy rhizosphere, then five of sixteen isolates have a capability to solubilize 0,25% quartz in *Bunt and Rovira Agar*. Characterizations of colony morphology silica solubilizing bacteria included of shape, margin, elevation, texture, pigmentation and optic were explained on Table 1.

Table 1. Colony morphology of silicate solubilizing bacteria.

Isolat	Shape	Margin	Elevation	Texture	Pigmentation	Optic
OS4	Round	Entire	Raised	Smooth and glossy	Creamy	Opaque
OS5	Irregular and spreading	Irregular	Raised	Smooth	Milky white	Opaque
OS7	Irregular and	Undulate	Flat	Smooth and glossy	Creamy	Opaque

OS12	spreading Irregular and spreading	Irregular	Flat	Smooth	Milky white	Opaque
OS13	Irregular and spreading	Undulate	Flat	Smooth and glossy	Milky white	Opaque

Five isolates were characterized in terms of cell morphology (cell shape and arrangement), physiological-biochemical test (catalase test, motility, indole production, H₂S production, ornithine test, acid fast staining, MR, VP, urease test, nitrate reduction, citrate utilization, fermentation of glucose, fructose, sucrose, lactose, starch, mannitol, growth at pH 3, 7, 10 and temperature 25^oC, 30^oC, 45^oC).

Table 2. Characterization of silicate solubilizing bacteria.

Characteristic	OS4	OS5	OS7	OS12	OS13
Cell shape	Bacil	Bacil	Bacil	Bacil	Bacil
Cell arrangement	Streptobacil	Streptobacil	Streptobacil	Streptobacil	Streptobacil
Gram	-ve ^a	-ve	-ve	-ve	-ve
Spore	-	-	+	+	+
Catalase	+ ^b	+	+	+	+
Motility	+	+	+	+	+
Indole production	+	+	+	-	+
H ₂ S	-	-	-	+	-
Ornithine	+	+	-	+	+
<i>Acid Fast</i>	+	+	+	+	+
MR	-	-	-	+	+
VP	-	-	-	+	-
Urease	- ^c	-	-	-	-
Nitrate	-	+	+	+	+
Citrate utilization	+	+	-	+	+
Glucose	++ ^d	++	++	++	++
Fructose	++	++	++	++	++
Sucrose	++	++	++	++	++
Lactose	-+ ^e	--	--	--	-+
Starch	++	++	++	++	++
Mannitol	-- ^f	++	--	++	--
pH 3	+	-	+	+	+
pH 7	+	+	+	+	+
pH 10	-	+	+	+	+

25 ^o C	+	+	+	+	+
30 ^o C	+	+	+	+	+
45 ^o C	+	+	+	+	+

^a Gram negative.

^b positive test.

^c negative test.

^d represents acid and gas formulation.

^e represents no acid, gas formulation.

^f represents no acid and gas formulation.

The characterization was included of colony, cell morphological, physiological and biochemical test belong to silicate bacteria characteristic. Colony morphological showed that irregular and spreading, round shape; undulate, entire, regular margin; flat, raised elevation; smooth, smooth and glossy texture; creamy, milky white pigmentation; opaque optic. Cell morphology of five isolates was found bacil, streptobacil, Gram negative, spore or no spore formation. Physiological and biochemical test in silicate solubilizing bacteria include of catalase positive test, motile, did not produce urease enzyme, highly ability to ferment glucose, fructose, sucrose, starch, capable to growth at temperature 25^oC, 30^oC, 45^oC, optimal growth at neutral pH 7, positive or negative test in H₂S production, ornithine, MR, VP, nitrate reduction, citrate utilization, fermentation of lactose, mannitol, growth at pH 3 and 10 [8,9,10,11,12,13].

Silica solubilizing activity were examined on *Bunt and Rovira* Agar and Broth. Each of five isolates were determined its capability in *Bunt and Rovira* Agar by Solubilizing Index (SI), while the other activity of silica solubilized determined by *Bunt and Rovira* broth by concentration of silica.

Table 3. Silica solubilizing activity of five isolates in *Bunt and Rovira* medium.

Isolates	Solubilizing Index	Concentration of Silica (ppm)
OS4	1,08	0,745
OS5	1,07	0,796
OS7	1,10	0,662
OS12	1,05	1,053
OS13	1,05	0,663

Each of five isolates were found its capability in solubilize silica by *Bunt and Rovira* medium. The highest solubilizing index was gained by OS7, vary size of clear zones showed that each of five isolates capable to solubilize silica by their own activities. Vasanthi [7] explained that evaluation media of silica solubilizing bacteria by using *Bunt and Rovira* Agar contains silica source 0,25% showed a high capability silica solubilization. Inoculant in *Bunt and Rovira* Agar optimally growth at range pH 6,7-7,0; incubated at 30^oC for 4 days. Many factors can influence halozone formation in media agar, such as substrate, inoculant size, volume (thickness) of agar layer, medium composition, pH and incubation temperature [16,17,18,19].

Five isolates paddy rhizosphere bacteria solubilize 0,25% quartz determined by silico-molybdate's method, while the concentration of silica was higher than control (0,599 ppm). The highest silica solubilization was 1,053 ppm by OS12 in *Bunt and Rovira* broth. Decrease of pH was compared between before incubation at 6,8 and after incubation period in *Bunt and Rovira* broth at range pH 3,3-4,1 related to mechanism of bacteria solubilizing silica by organic acid production. Organic acid productions by silica solubilizing bacteria break the silicon-dioxide bond (O-Si-O) in quartz, so the soluble silica will release. Role of organic acid by producing of cation (H⁺) help to dissolves silicate minerals. Vary acid organics such as acetic acid and citrate acid that found in quartz solubilization take a vital role as protonation molecule, cation barrier. Such organic acid include of carboxylate acid group, categorized to weak acid which is easily ionized by cation. Cation's excess can influence pH in silica solubilization. Excess of cation pull the anion hydroxide (OH⁻) as a quartz substituent through

organic acid production by bacteria. Chelation of anion in order to solubilize quartz in soluble form, monosilicic acid ($\text{Si}(\text{OH})_4$) absorb by paddy plant through lateral roots [20,21,22].

4. Conclusions

Five paddy rhizosphere isolates were found solubilize silica optimally in *Bunt and Rovira* Agar and broth. The highest capability of silica solubilization in *Bunt and Rovira* Agar was gained by solubilizing index of OS7 by 1,10, while the highest silica solubilizing activity in *Bunt and Rovira* broth were found in OS12 with silica concentrate 1,053 ppm. Characteristic of paddy rhizosphere were identified as silicate bacteria.

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