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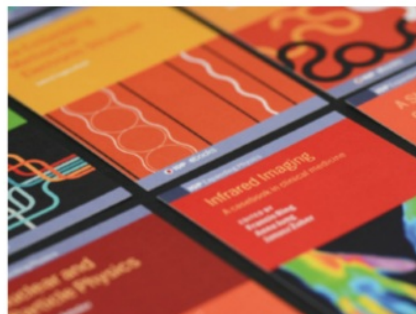
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Isolation and Characterization of Potassium-Solubilizing Bacteria from Paddy Rhizosphere (*Oryza sativa* L.)

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Abstract. Potassium is one of macronutrients required by plants. The concentrations of soluble Potassium in the soil are usually very low, more than 90% of Potassium in the soil are (K)-bearing minerals form, one of them is Feldspar which quite abundant found in Indonesia. Rhizobacteria have been found to solubilize (K)-bearing mineral into a form that plants can access. This study aims to isolate and characterize Rhizobacteria from paddy rhizosphere that potentially dissolve mineral feldspar as potassium source. This study was conducted on February-June 2018 in Microbiology Laboratory, Department of Biology, Universitas Negeri Surabaya. Seven bacteria isolates capable of solubilizing potassium were LJK 1, LJK 2, LJK 4, LBK 4, LBK 5, PSUK 4, and PSIK 6. Based on the potassium-solubilizing index, seventh isolates were capable of solubilizing feldspar in Alexandrov solid medium, the best isolate with the highest score was LJK 2 (5,25 cm in diameter), contrary with the lowest score was LBK 4 (2,37 cm). In amended Alexandrov broth, isolate suspension of LJK 2 caused maximum solubilization (6,846 mg/L), while the minimum solubilization was PSIK 6 (6,724 mg/L). All these isolates were characterized on the basis of morphological and physiological-biochemical test up to 39 characteristics.

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1. Introduction

Potassium (K) is an essential nutrient that plays important roles for plant growth and development, such as activating enzymes, maintaining turgor, transporting of nutrient, and protecting plant from disease and insects. As one of the agricultural commodities, paddy needs Potassium in the generative phase for the developing and filling grain. In such, the availability of potassium become one of the factors that affect the productivity of paddy. Unfortunately, more than 90 to 98 per cent of Potassium found in the soil are K-bearing minerals which are unavailable for plant uptake. Indonesia has some K-bearing minerals of low solubility such as feldspar and mica [1]. Soil microorganisms have been reported play a key role in ion cycling and soil fertility [2]. Many studies have known that microbes decompose silicate minerals such as feldspar and mica, transformed insoluble K into available K so that can be directly absorbed by plants [3]. In light of above facts, the objective of this research was to isolate and characterize of potassium-solubilizing bacteria (KSB) from paddy rhizosphere and its ability to solubilize feldspar as the sole source of insoluble K in Alexandrov medium.

2. Method

2.1. Isolation of Paddy Rhizosphere Bacteria

Sample of rhizosphere was taken from four farming areas, Bangkingan, Jeruk, Sumber Makmur and Siwalan Makmur. Ten grams of rhizosphere were inoculated onto 90 ml *Alexandrov broth* for 4 days. The serial dilutions up to 10^{-7} consisted of 1 ml of sample and 9 ml of 0,85% NaCl solution. Each



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dilution was continuously shaken for two minutes by vortex, and was inoculated on *Alexandrov* agar plate. The isolates were then incubated at 30°C for 4 days.

2.2. Characterization of Potassium Solubilizing Bacterial Isolates

The isolates were cultured on Alexandrov's medium. Cell and colony morphology and physiological-biochemical characteristics were examined. Motility, catalase test, *Ziehl-Nelsen* test, *Methyl Red* test, *Voges-proskauer* test, urease test, nitrate reduction test, indole production test, ornithine utilization test, H₂S production test, citratase utilization, growth in different pH (3, 7, and 10), growth in different temperature (25°C, 30°C, and 45°C), and starch hydrolysis were studied as physiological-biochemical characters.

2.3. Potassium Solubilizing Activity in Alexandrov Agar

The selected bacterial isolates from paddy rhizosphere were subjected to a Potassium solubilizing test in *Alexandrov agar*, incubated at 30°C for 4 days. Detection of potassium solubilization by different rhizobacterial isolates based upon the ability of solubilization zone formation. The medium contains of glucose 5.0 g; agar 20 g; MgSO₄.7H₂O 0,5 g; CaCO₃ 0,1 g; FeCl₃ 0,006 g; Ca₃PO₄ 2,0 g; and Feldspar 3 g diluted by 1 L deionized water [4].

2.4. Potassium Solubilizing Activity in Alexandrov Broth

The solubility of Potassium was investigated in 50 ml *Alexandrov* broth, all those isolates incubated at 30°C for 7 days. The growth suspension was centrifuged at 7,000 rpm for 10 minutes in the microcentrifuge to separate the supernatant from the cell growth and insoluble Potassium. One ml of the supernatant was taken and placed into 50 ml volumetric flask, added with distilled water and mixed thoroughly. The solution was fed to *Atomic Absorption Spectrometer* to determine (AAS) K content [5].

2.5. pH Measurement in Alexandrov Broth

pH was measured before and after incubation in *Alexandrov* broth. Before its sterilization, the pH of *Alexandrov* 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.

3. Results and discussion

Twenty two KSB isolates were isolated from rhizospheric soils of paddy (*Oryza sativa* L.). Seven isolates were capable of solubilizing Feldspar in *Alexandrov* agar presented in Table 1. These seventh isolates colonies showed exhibiting zone of clearance indicating Potassium solubilization. The value of clear zone from each isolates examined by using Premono's selection ratio, noted as Solubilizing Index (SI) [6]. Solubilization of K from mineral Feldspar by bacterial strains also examined in *Alexandrov* broth using AAS (Table 1).

Table 1. Potassium solubilization values of selected bacterial isolates in *Alexandrov* agar

Isolates	Solubilizing Index (SI)	Solubilization AAS
LJK 1	3,03	6,803
LJK 2	5,25	6,846
LJK 4	3,00	6,784
LBK 4	2,37	6,759
LBK 5	2,63	6,770
PSUK 4	2,67	6,744
PSIK 6	3,11	6,724

The morphological colony (shape, margin, elevation, surface, pigmentation, and optic) and cell morphology of seventh bacteria are presented in Table 2.

Table 2. Morphological colony, cell shape, gram reaction and spore formation characteristics of Potassium Solubilizing Bacteria

Isolates	Colony characters	Cell shape	Gram reaction	Spore formation
LJK 1	Irregular, undulate, raised, smooth, white, opaque	Coccus	-ve	+ ^a
LJK 2	Irregular, undulate, flat, smooth, white, translucent	Bacil	+ve	- ^b
LJK 4	Irregular, undulate, raised, smooth, white, translucent	Bacil	-ve	+
LBK 4	Circular, entire, raised, smooth, creamy, opaque	Coccus	+ve	+
LBK 5	Irregular, entire, flat, smooth, creamy, opaque	Coccus	+ve	-
PSUK 4	Irregular, undulate, raised, smooth, white, opaque	Coccus	+ve	+
PSIK 6	Irregular, entire, flat, smooth, white, opaque	Coccus	+ve	+

^a positive (+)^b negative (-)

Physiological-biochemical characteristics test include motility, catalase test, Ziehl-Nelsen test, Methyl Red test, Voges-proskauer test, urease test, nitrate reduction test, indole production test, ornithine utilization test, H₂S production test, citratase utilization, growth in different pH (3, 7, and 10), growth in different temperature (25^oC, 30^oC, and 45^oC), and starch hydrolysis (Table 3).

Table 3. Physiological-biochemical characteristics of Potassium Solubilizing Bacteria

Characters	LJK 1	LJK 2	LJK 4	LBK 4	LBK 5	PSUK 4	PSIK 6
Motility	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+
<i>Ziehl-Nelsen</i>	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+
VP	+	-	+	-	-	-	-
Urease	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	-
Indole production	-	-	-	+	+	-	-
Ornithine utilization	+	+	+	+	+	+	+
H ₂ S production	+	+	+	-	-	-	-

Characters	LJK 1	LJK 2	LJK 4	LBK 4	LBK 5	PSUK 4	PSIK 6
Citratase utilization	+	-	+	+	+	+	+
Growth in pH 3	+	-	+	+	+	+	+
Growth in pH 7	+	+	+	+	+	+	+
Growth in pH 10	+	+	+	+	+	-	-
Growth in 25°C	+	+	+	+	+	+	+
Growth in 30°C	+	+	+	+	+	+	+
Growth in 45°C	+	+	+	+	+	+	+
Glucose hydrolysis	+	+	+	+	+	+	+
fructose hydrolysis	+	+	+	+	+	+	+
Lactose hydrolysis	+	-	+	+	+	-	-
Sucrose hydrolysis	+	+	+	+	+	+	+
Mannitol hydrolysis	+	+	+	+	+	+	+
Amilum hydrolysis	+	-	+	+	-	+	+

Potassium solubilization by rhizobacterial isolates was studied on Alexandrov agar and Alexandrov broth medium. Solubilizing index is used to measure the K-solubilization zone ability of the KSB on Alexandrov agar. Seventh of KSB were found to be capable of K-solubilization, and the Solubilization Index ranged from 2,37 to 5,25 cm in diameter. Strain LJK 2 showed the highest ability to solubilize K (5,25 cm in diameter) followed by PSIK 6, LJK 1, LJK 4, PSUK 4, LBK 5, and LBK 4. Solubilization of Potassium also conducted on Alexandrov broth then fed to AAS, this aims to determine the concentration of ion K from solubilization activities by KSB after 7 days incubation period. The results exhibited that isolate LJK 2 was consistent with the previous studies on Alexandrov agar as the best isolate compared to other isolates, its dissolved K of 6,846 mg/L at 7 DA.

There were least differences in the solubilization activity of each isolates, it is hypothesized that the release of K may be due to the various organic acids that produced by KSB, besides, the production of organic acids by each isolates have different concentration. Organic matter while solubilize minerals produced by KSB has known as oxalic acid and citric acid [4], ferulic acid and coumaric acid [7], formic acid, malic acid and acetate acid [8], tarttric acid [9]. These organic acids produced by the KSB might enhanced the dissolution of K-bearing minerals by supplying protons, destabilizing surface of K-bearing and complexing Ca^{2+} , Fe^{2+} , and Al^{3+} ions [10,11,12]. In this study, feldspar (KAlSi_3O_8) was the sole source of K-bearing, this reacts with organic acids promotes cation exchange reactions between H^+ and K^+ ions. Furthermore, K released from feldspar into available K for plant uptakes, besides, these solubilizing activity also forms a secondary mineral as kaolinite ($\text{H}_4\text{Al}_2\text{Si}_2\text{O}_9$) [1,13].

Characterization of the KSB were examined by 39 characters: morphological colony (shape, margin, elevation, surface, pigmentation, and optic) and cell morphology. Physiological-biochemical characteristics test include motility, catalase test, Ziehl-Nelsen test, Methyl Red test, Voges-proskauer test, urease test, nitrate reduction test, indole production test, ornithine utilization test, H_2S production test, citratase utilization, growth in different pH (3, 7, and 10), growth in different temperature (25°C, 30°C, and 45°C), and starch hydrolysis. Characterization performed in this study was also conducted in previous studies to characterize Potassium solubilizing bacteria [4,14,15].

4. Conclusion

Isolation and screening processes have found seven isolates from rhizospheric soils of paddy (*Oryza sativa* L.). Seventh isolates were capable of solubilizing Feldspar in Alexandrov agar and Alexandrov broth. The best isolate with the highest score in Alexandrov agar was LJK 2 (5,25 cm diameter), this isolate also caused maximum solubilization in Alexandrov broth (6,846 mg/L). All these isolates were characterized on the basis of morphological and physiological-biochemical test up to 39 characteristics.

Seventh KSB in this study are able to dissolve Potassium from K-bearing mineral have both economic and environmental advantages, so that these isolates potentially to be applied as an alternative bio-fertilizer agent of Potassium, generally the bio-fertilizer can be applied to the seed, soil, or during composting. Microencapsulation engineering and fermentation engineering can be done to ensure an increase in the microbial population and reduce the potential for microbial contamination of other undesirable during storage and transportation [16].

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