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Isolation and Characterization of Potassium-Solubilizing Bacteria from Paddy Rhizophere (*Oryza sativa* L) A review

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Abstract **–** Potassium (K) is a very important macronutrient required by plants. Soil contains enormous quantity of potassium, but most of the K are in insoluble form. However lower concentration of K in the soil is in the dependence or bearing minerals form, one such bearing mineral is Feldspar, which is widespread in Indonesia. This bearing feldspar can be solubilized by different strains of Rizobacteria, so that plants can readily take up the solubilized potassium from the soil. This article provides an overview of isolation and characterization of different strains of Rhizobacteria from paddy rhizosphere, which will solubilize the bearing feldspar into available form. Among different strains of Rhizobacteria seven strains LJK 1. LJK 2, LJK 4, LBK 4, LBK 5, PSUK 4, PSIK 6, isolates had ability to solubilize the potassium from the feldspar in Alexandrov solid medium. In this seven strains LJK 2 cost the highest (5, 25cm in diameter) contrary with the lowest score was LBK 4 (2, 37 cm in diameter) In alter Alexandrov broth, isolate suspension of LJK 2 caused maximum solubilization (6, 846mg/l) while the minimum solubilization was PSIK 6 (6, 724mg/l). Hence all strains were characterized on the basis of morphological and physiological biochemical tests. This study was conducted on February – June 2018 in Microbiology Laboratory, Department of Biology, University Negeri Surabaya.

Index Terms – **Biofertilizers, Potassuim solubilizing Bacteria, Feldspar, Rhizobacteria.**

I. INTRODUCTION

As the world population increases demand for the food also increases, a projected to reach 9 billion by 2050, so this creates demand of food which is one of the toughest challenges faced by the increasing global population[1][2].Concerning this growing populations, agriculture must be intensive and sustainable in the future. Soil is the natural body on the earth's crust[1], it is composed of minerals, organic matter, living organisms, water. However it is well known that the food production by agriculture cannot be generally sustained unless the nutrients removed from soil as a result of increased crop production are separated [2].Many agricultural soils lack a sufficient amount of one or more of essential plant nutrient so that plant growth is suboptimal. To obviate this problem and obtain higher plants yields, forms have become increasingly dependent on chemical sources of fertilizers [4]. White the chemical fertilizers helped plant grow, they did not improve the

properties of soil. It is well known that the content use of chemical fertilizer, mainly phosphorous, nitrogenous and potassic fertilizers have harmful effects on the environment [5]. After nitrogen (N), and phosphorous (P), photassium (K) is the most important plant nutrient that has a key role in the growth, metabolism, and development of plants. In addition to increasing plant resistance to diseases, pests and abiotic stresses, K is required to activate over 80 different enzymes responsible for plant and animal processes example such as energy metabolism, starch synthesis, nitrate reduction, photo synthesis and sugar degradation[6 a, b, c, d]. K is the seventh most abundant element in earth's crust. Total K content in soils range between 0.04 and 3% K. Although K is present as an abundant element in soil, only 1 to 2% of this element is available to plants [7]. The rest are bound with other minerals and therefore are unavailable to plants, but more than 90 to 98% of potassium found in the soil are K bearing minerals which are unavailable for plant uptake. As paddy is one of the important agricultural commodities, it requires potassium in the generative phase for the developing and filling grain. So the availability of potassium becomes one of the factors that affect the productivity of paddy [1].

Indonesia has some K- bearing minerals of low solubility such as feldspar and mica [8]. Soil microorganisms have been reported to play a key role in ion cycling and soil fertility[9].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[10]. Taking into consideration of above facts the objectives of this research was to isolate and characterize of Potassium-solubilizing bacteria (KSB) from paddy rhizosphere and its ability to solubilize feldspar ad the sole source of insoluble K in Alexandrow medium.

II. METHODS

Isolation of Paddy Rhizosphere Bacteria

Bacterial samples were collected from the rhizosphere of four farming areas- Bangkingan, Jeruk, Sumber Makmur and Siwalan Makmur. In 90 ml of Alexandrov broth about 10g of rhizosphere were inculated for 4 days. The serial dilutions were made up to 10^{-7} which consist of 1ml of sample and 9ml of 0.85% Nacl solution. Each dilution was continuously shaken for two minutes by Vortex which was then inoculated on Alexandrov agar plate. All these isolates were then incubated at 30ºC for 4 days.

Potassium solubilizing activity in Alexandrov agar

Potassim solubilizing test in Alexandrov agar was performed to the selected bacterial isolates from paddy rhizospher which was incubated at 30ºC for 4 days the rhizobacterial isolates which had ability to solubilize could form zone of layer hence the detection is based on the zone formation. The medium contains of glucose 5.0g, agar 20g, MgSo4, 7H2O 0.5g, CaCo3 0.1g, Fecl3 0.006g, CaPo 2,0g and feldspar 3g diluted by 1L deionized water[11].

Potaasium solubilizing activity in Alexandrov Broth

The solubility of potassium was investigated in 50ml Alexandrov broth, all those isolates incubated at 30ºC for 7 days. The growth suspension was centrifuged at 7,000rpm for 10 min in the microcentrifuge to separate the supernatant from the cell growth and insoluble potassium 1ml of the supernatant was taken and placed into 50ml volumetric flask, added with distilled water and mixed thoroughly. The solution was fed to Atomic Absorption spectrometer to determine K content [12].

P^H measurement in Alexandrove Broth

P^H was measured before and after incubation in Alexandrov broth. Before its sterilization the $p^H$of Alexandrove broth was measured by p^H rol (6.6-7.0). After seven days incubation, each p^H culture measured, then compared against p^H before incubation period.

III. RESULTS AND DISCUSSION

All together twenty two KSB isolates were isolated from rhizospheric soils of paddy, among them seven isolates were capable of solubilizing feldspar in Alexandrove agar medium.

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

All these seven isolates colonies were 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[13].Solubilization of K from mineral feldspar by bacterial strains also examined in Alexandrov broth using (AAS table 1).

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	Irreguler, undulate, raised, smooth, white, translucent	Bacil	-ve	+
LBK 4	Circular, entire, raised, smooth, creamy, opaque	Coccus	+ve	+
LBK 5	Irreguler, entire, flat, smooth, creamy, opaque	Coccus	+ve	-
PSUK 4	Irreguler, undulate, raised, smooth, white, opaque	Coccus	+ve	+
PSIK 6	Irreguler, entire, flat, smooth, white, opaque	Coccus	+ve	+

[a] positive (+)
b negative (-)

Physiological- biochemical characteristics test include motility, catalase test, Zichl- Nelson test, Methyl red test, Voges-Proskaver test, Urase test, Nitrate reduction test, Indole production test, Ornithine utilization test, H2S production test, Citratase utilization test, Growth in different p^H(3,7 and 10), growth in different temperature (25ºC, 30ºC and 45ºC) and starch hydrolysis were performed.

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	+	+	+	+	+	+	+
Ziehl- Nelseen	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+
VP	+	-	+	_	-	-	-
Urease	-	-	-	_	-	-	-
Nitrate reduction	+	+	+	+	+	+	-
Indole production	-	-	-	+	+	-	-
Ornithine utilization	+	+	+	+	+	+	+
H_2S production	+	+	+	-	-	-	-
Citratase utilization	+	-	+	+	+	+	+
Growth in p^H3	+	-	+	+	+	+	+
Growth in p^H7	+	+	+	+	+	+	+
Growth in p^H10	+	+	+	+	+	-	-
Growth in 25	+	+	+	+	+	+	+
Growth in 30	+	+	+	+	+	+	+
Growth in 45	+	+	+	+	+	+	+
Glucose hydrolysis	+	+	+	+	+	+	+
Fructose hydrolysis	+	+	+	+	+	+	+
Lactose hydrolysis	+	-	+	+	+	-	-
		+	+				

Sucrose hydrolysis	+	+	+	+	+	+	+
Mannitol hydrolysis	+	–	+	+	+	+	+
Amilum hydrolysis	+			+	-	+	+

Potassium solubilization by rhizobactrial isolates was studied on studied on Alexandrov and Alexandrov broth medium. Solubilizing index was used to measure the K- solubilization zone ability of KSB on Alexandrov agar. Seven KSB among them were found to be capable of K- solubolization and the solubilization index ranged from 2.37 to 5.25cm 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, the 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 6.846mg/l at 7 days.

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 solubilization minerals produced by KSB has known as oxalic acid and citric acid [11] ferulic acid and coumaric acid[14] formic acid, malic acid and acetate acid[15] tartic acid[16]. 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 AI $^{3+}$ ions[17,18,19].So in this study feldspar was the sole source of K- bearing this reacts with organic acids promotes cataion exhange reaction between H^+ and K^+ ions. Furthermore K released from feldspar into available K for plant uptakes besides, these solubilizing activity also forms a secondary minerals as Kaolinite[8,20].

Characterization of KSB were examined by 39 characters, morphological colony, (shape, margin, elevation, surface, pigementation and optic) and cell morphology. Physiological- biochemical characteristic test include motility, catalase test, Ziehl – Nelson test, Methyl red test, Voges proskaver test, urease test, nitrate reduction test indole production test, ornithene utilization test, H2S production test, citratase utilization, growth in different p^H(3,7 and 10) growth in different temperatures (25ºC,30ºC and 45ºC) and starch hydrolysis characterization performed in this study was also conducted in previous studies to characterize potassium solubiling bacteria[11,21,22].

1V. CONCLUSION

Isolation and screening processes have fund seven isolates from rhizosphere soils of Paddy (Oryza sativa L). Seventh isolates were capable of solubilizing feldspar in Alexandrov agar and Alexandrov broth. The best isolates with the highest score in Alexandrov agar was LJK 2 (5. 25cm diameter), this isolate also caused maximum solubilization in Alexandrov broth (6.846mg/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 biofertilizer agent of potassium generally the biofertilizer can be applied to the seed, soil or during composting. Micro encapsulation engineering and fermentation engineering can be done to ensure an increase in the microbial population and reduce the potential for microbial contamination of others undesirable during storage and transportation[22].

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