

The ability of potassium solubilizing rhizo-bacteria isolated from maize rhizosphere for microbial fertilizer

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Abstract. Potassium is an essential macro nutrient for plants but most of them are not available because it is fixed in the soil in primary or secondary silicate minerals such as K-feldspar or 2:1 type clay form, so trigger the excessive use of potassium fertilizer that can damage the environment. Potassium solubilizing rhizo-bacteria (KSRB) which is a microbial fertilizer can overcome this problem by changing the non available into available soil potassium. This is a promising strategy for improving of plant absorption of potassium and reducing the use of chemical fertilizers. The objective of this study was to evaluate the ability of fifteen KSRB isolates that isolated from maize rhizosphere to dissolve potassium. Potassium dissolution activity by KSRB isolate was observed using Khandeparkar's selection ratio (modified) by measuring the diameter of the clear zone around the colony compared with the colony diameter on Aleksandrov solid medium (K-feldspar powder as sole potassium source) and pH measurement in Aleksandrov liquid medium. All KSRB isolates were capable of solubilizing K-feldspar powder in solid and liquid medium. There was a high negative correlation between Khandeparkar's selection ratio and pH ($r = -0.90^{**}$). The highest potassium dissolution activity was shown by RBPK-DHJ3-3150, RBPK-DHJ1-4125, and RBPK-DHJ2-5250 KSRB isolates, which showed Khandeparkar's selection ratio of 5.97, 5.40, and 4.00 while pH 3.34, 3.28, and 3.64 respectively, so these three KSRB isolates were selected as the superior isolates based on the simple scoring and ranking method.

1. Introduction

One of the essential macro nutrients of plants is potassium which functions as an activator of several enzymes, related to the regulation of water, synthesis of starches and proteins, and the energy system of plants (photosynthesis and respiration) [1]. The total potassium level in the soil is quite high (2.6%) but the available is very low (1-2%) because it is in the form of primary silicate minerals (feldspar, biotite, muscovite, and mica) or secondary silicate minerals (2:1 type clay such as montmorillonite, vermiculite, and illite) [2]. The natural supply of potassium for plants that comes from weathering of rocks or minerals that contain potassium is very low so that fertilization of potassium tends to be carried out in large quantities, which triggers environmental damage [3].

Potassium solubilizing bacteria have the ability to decompose silicate minerals containing potassium through the organic acids they produce [4-6] so that the need for potassium for their various metabolic activities can be met [7-8]. Therefore, potassium solubilizing bacteria can be used as



microbial fertilizer which can facilitate the availability of potassium in the soil for plants by changing the form of unavailable to be available potassium so that it can help increase plant growth and yield [9, 6, 10-12, 3, 13].

The twenty-five isolates of potassium solubilizing bacteria called potassium solubilizing rhizobacteria (KSRB) were isolated from maize rhizosphere around the Agriculture Faculty Experimental Station, Padjadjaran University, Jatinangor, Indonesia [14]. The purpose of this study was to evaluate the ability of these KSRB isolates to dissolve potassium so that the superior selected isolates were obtained to be potentially used as microbial fertilizer which could facilitate the availability of potassium in the soil. The results of this KSRB research could enrich the study of soil biotechnology in Indonesia, especially microbial fertilizer which is currently focused on nitrogen-fixing bacteria, phosphate solubilizing microbes and plant growth promoting rhizobacteria (PGPR).

2. Materials and methods

2.1. Observation of potassium dissolution activity by potassium solubilizing rhizo-bacteria

The fifteen KSRB isolates that have been obtained in the previous study were used in present study [14]. Potassium dissolution activity by the KSRB isolates was further tested on Aleksandrov solid medium [15] and Aleksandrov liquid medium (K-feldspar powder as sole potassium source).

Observation of potassium dissolution activity by KSRB isolates on Aleksandrov solid medium was carried out by spot test method (modified) [16] through KSRB colony clear zone diameter measurement formed on Aleksandrov solid medium surface (figure 1). The collection of KSRB isolate on Aleksandrov slant agar was given 10 ml physiological saline solution (0.85% NaCl), the mass of KSRB isolate was dredged using an ose needle and mixed to obtain a homogeneous suspension, then disc paper (0.5 cm in diameter) was immersed in that suspension. The disc paper containing KSRB isolate was then placed in the center of the Aleksandrov solid medium surface with three replications and then placed in incubator (30°C) for three days. Control treatment was also carried out, only disc paper immersed in physiological saline solution was then placed in the center of the Aleksandrov solid medium surface. The diameter of the KSRB colony clear zone formed around the disc paper was measured using a ruler and expressed in centimeters. Potassium dissolution activity on Aleksandrov solid medium was calculated using the Khandeparkar's selection ratio (KSR) formula (modified) [17], which compared the diameter of the colony clear zone with the colony diameter or disc paper. The KSR formula used was as in equation (1):

$$\text{KSR} = \frac{D}{d} \quad (1)$$

where KSR was Khandeparkar's selection ratio, D was diameter of the KSRB colony clear zone (cm), and d was diameter of the KSRB colony or disc paper (cm) (Figure 1).

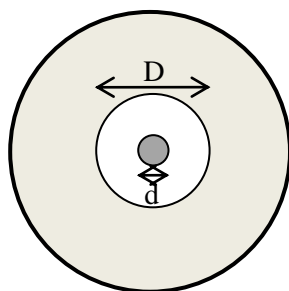


Figure 1. Spot test method (modified) to measure potassium dissolution activity on Aleksandrov solid medium

Observation of potassium dissolution activity by KSRB isolates in Aleksandrov liquid medium was carried out by using pH measurement. One percent suspension of the same physiological saline-KSRB

isolate to be used in testing on Aleksandrov solid medium was inoculated into 50 ml of Aleksandrov liquid medium with three replications. Control treatment was also carried out, by adding only one percent physiological saline solution into 50 ml of Aleksandrov liquid medium. The liquid medium was incubated on a rotary shaker (120 rpm) for one week at room temperature (28°C) and afterwards pH measurement was carried out using a pH meter.

Correlation and regression analysis was conducted to determine the correlation between KSR and pH. The analysis aimed to prove that the potassium dissolution activity by KSRB isolates was mediated by the produced organic acids which was characterized by a decrease in the pH of the medium.

2.2. Selection to determine the superior selected potassium solubilizing rhizo-bacteria isolates

Three superior selected KSRB isolates would be determined through the selection of fifteen KSRB isolates based on their ability to dissolve potassium on Aleksandrov solid medium and Aleksandrov liquid medium by measuring the diameter of the colony clear zone, KSR and pH. The measurement results were calculated by simple scoring and ranking method.

The rules in the simple scoring and ranking method were as followed: (i) The highest diameter of the colony clear zone of KSRB was given a score of 1, the lower was given a score of 2 and so forth; (ii) The highest KSR was given a score of 1, the lower was given a score of 2 and so forth; (iii) The lowest pH was given a score of 1, the higher was given a score of 2 and so forth; and (iv) The scores were summed up so that the smallest number of score was the highest ranking that characterized the ability of selected KSRB isolate to dissolved potassium.

3. Results and discussion

3.1. Potassium dissolution activity by potassium solubilizing rhizo-bacteria

The fifteen KSRB isolates tested in this study were able to dissolve potassium on the Aleksandrov solid medium. The RBPK-DHJ3-3150 KSRB isolate showed the highest KSR value followed by RBPK-DHJ1-4125 and RBPK-DHJ2-5250 respectively (Figure 2).

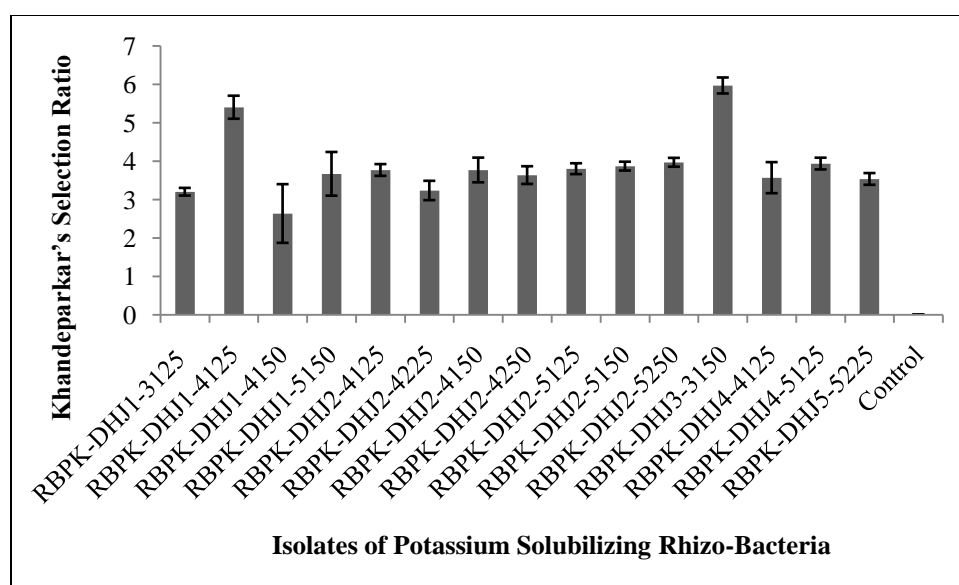


Figure 2. Effect of KSRB isolates on the Khandeparkar's selection ratio

Note: Error bars represent mean \pm sd of three replications.

As in the Aleksandrov solid medium, a similar phenomenon occurred in the observation of the pH in the Aleksandrov liquid medium. All tested KSRB isolates was able to reduce the pH in the liquid medium. The lowest pH indicated by RBPK-DHJ1-4125 KSRB isolate and followed by RBPK-DHJ3-3150 and RBPK-DHJ2-5150 respectively (Figure 3).

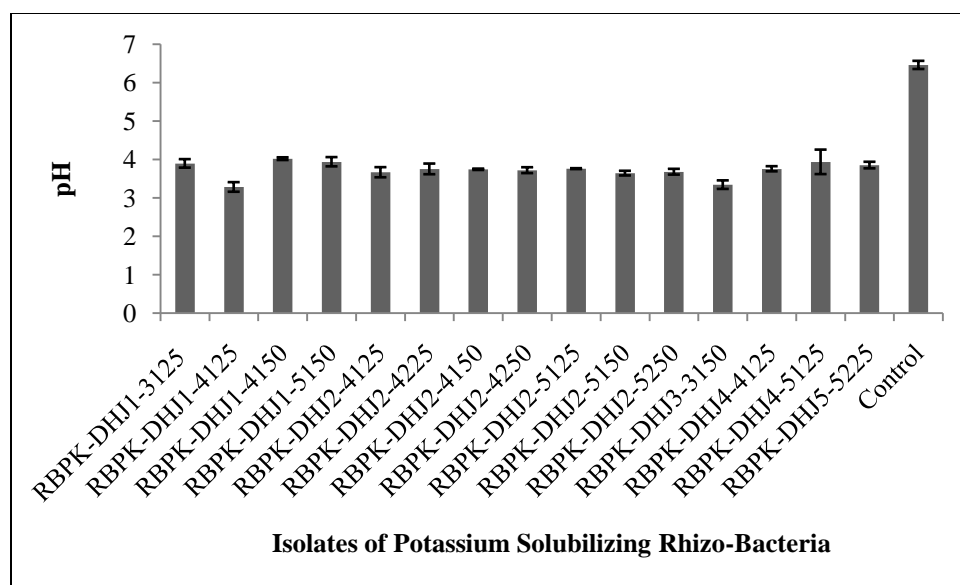


Figure 3. Effect of KSRB isolates on the pH

Note:Error bars represent mean \pm sd of three replications.

There was a high negative correlation between KSR and pH ($r = -0.90^{**}$). It meant that if the KSR value increased, it caused a decrease in pH (Figure 4).

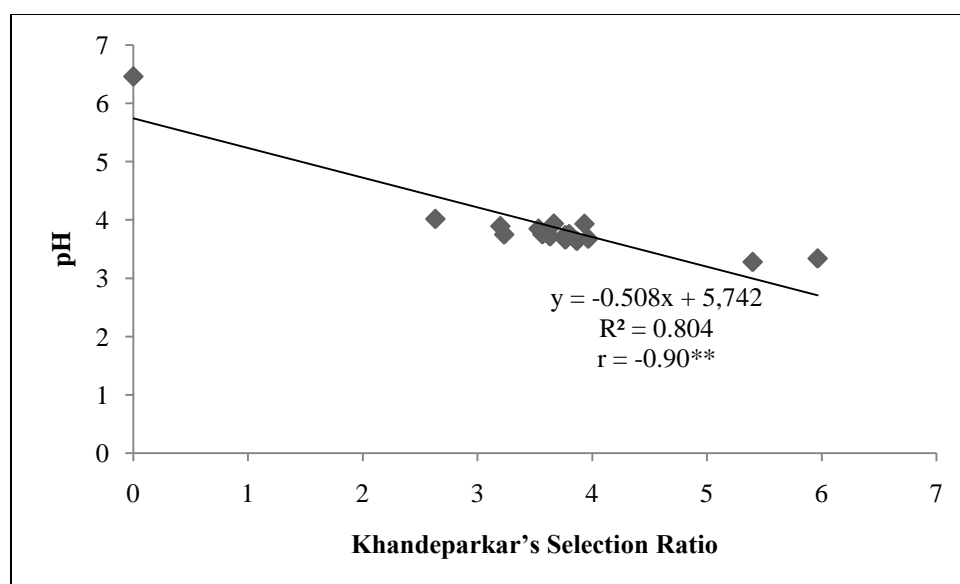


Figure 4. Correlation between Khandeparkar's selection ratio and pH

3.2. The superior selected potassium solubilizing rhizo-bacteria isolates

Three superior selected KSRB isolates were obtained based on their ability in dissolving potassium both in Aleksandrov solid medium and Aleksandrov liquid medium (Figure 5). They were RBPK-DHJ3-3150, RBPK-DHJ1-412, and RBPK-DHJ2-5250 KSRB isolates, respectively, based on the calculations using the simple scoring and ranking method of the fifteen KSRB isolates tested in this study (Table 1).

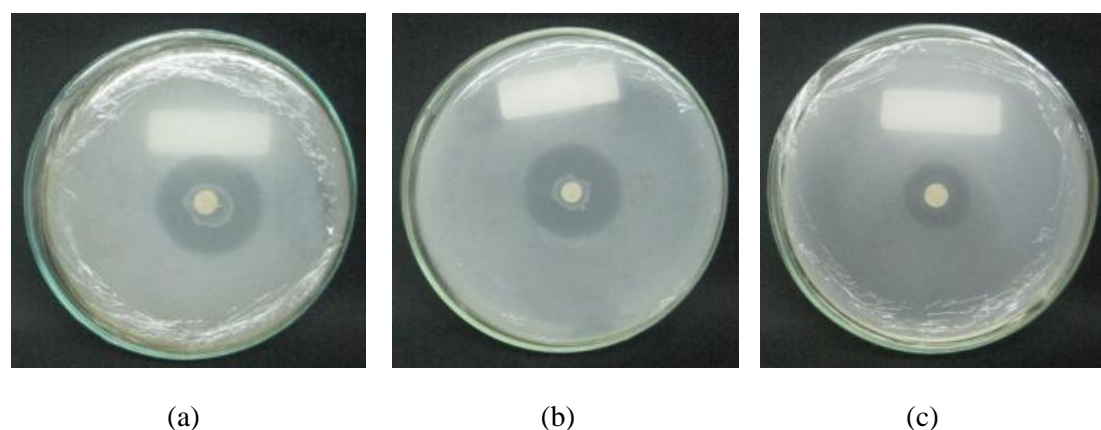


Figure 5. Clear colony zone shown on Aleksandrov solid medium of RBPK-DHJ3-3150 (a), RBPK-DHJ1-4125 (b), and RBPK-DHJ2-5250 (c) superior selected KSRB isolates

Table 1. Simple scoring and ranking method for determining the superior selected KSRB isolates.

No	KSRB Isolates	Clear Zone Diameter		KSR		pH		Σ Scoring	Ranking
		\bar{x} (cm)	Scoring	\bar{x}	Scoring	\bar{x}	Scoring		
1	RBPK-DHJ1-3125	1,60	14	3,20	14	3,89	12	40	14
2	RBPK-DHJ1-4125 ^b	2,70	2	5,40	2	3,28	1	5	2
3	RBPK-DHJ1-4150	1,32	15	2,63	15	4,02	15	45	15
4	RBPK-DHJ1-5150	1,83	9	3,67	9	3,94	14	32	11
5	RBPK-DHJ2-4125	1,88	7	3,77	7	3,66	4	18	5
6	RBPK-DHJ2-4225	1,62	13	3,23	13	3,75	8	34	12
7	RBPK-DHJ2-4150	1,88	7	3,77	7	3,74	7	21	6
8	RBPK-DHJ2-4250	1,82	10	3,63	10	3,72	6	26	9
9	RBPK-DHJ2-5125	1,90	6	3,80	6	3,76	10	22	8
10	RBPK-DHJ2-5150	1,93	5	3,87	5	3,64	3	13	4
11	RBPK-DHJ2-5250 ^c	1,98	3	3,97	3	3,68	5	11	3
12	RBPK-DHJ3-3150 ^a	2,98	1	5,97	1	3,34	2	4	1
13	RBPK-DHJ4-4125	1,78	11	3,57	11	3,75	9	31	10
14	RBPK-DHJ4-5125	1,97	4	3,93	4	3,93	13	21	6
15	RBPK-DHJ5-5225	1,77	12	3,53	12	3,85	11	35	13
16	Control	0,00	16	0,00	16	6,46	16	48	16

^aRBPK-DHJ3-3150 was the superior selected KSRB isolate with the highest potassium dissolution activity.

^bRBPK-DHJ1-4125 had the second highest potassium dissolution activity.

^cRBPK-DHJ2-5250 had the third highest potassium dissolution activity.

The KSRB isolates tested in this study were able to dissolve potassium in the form of K-feldspar powder which was characterized by the presence of clear zones around the KSRB colonies on the Aleksandrov solid medium [3] and a decrease in pH in Aleksandrov liquid medium. The KSR values

obtained from semi-quantitative measurement of the diameter of clear zones produced by KSRB colonies which were then compared with KSRB colonies diameter (in this study disc papers containing KSRB isolate was used) could describe potassium dissolution activity by these isolates. The higher KSR value, the higher potassium dissolution activity [17].

The KSR value affected the decrease in pH by 80.4% ($R^2 = 0.804$). The higher KSR value, the higher pH decrease which linearly showed the high potassium dissolution activity. Potassium containing mineral dissolution activity by potassium solubilizing bacteria were mediated by organic acids produced by these bacteria [6, 18, 16]. Weathering occurred when potassium ions as balancing cations in the mineral structure were replaced by hydrogen ions from organic acids through a hydrolysis process [19]. Organic acids produced by potassium solubilizing bacteria caused decrease in pH of the growing medium [20, 18]. Although the results of this study had proven that there was a strong correlation between KSR value and pH, it should be strengthened by quantitative measurements to determine the quantity of dissolved potassium and the types of organic acids involved in the weathering of potassium-containing mineral.

Selection of fifteen KSRB isolates that had been isolated from the rhizosphere of maize plants in the previous study had three superior selected KSRB isolates in present study. They had high potassium dissolution activity based on KSR values and pH in the order of RBPK-DHJ3-3150 (5.97; 3.34) > RBPK-DHJ1-4125 (5.40; 3.28) > RBPK-DHJ2-5250 (4.00; 3.64). The three superior selected KSRB isolates had the potential to be effective as potassium solubilizing microbial fertilizer. Further research needs to be carried out to make microbial fertilizer formulation from these superior selected KSRB isolates and determine their effect on dissolution of mineral containing potassium to be absorbed by plants and increase crop yields.

4. Conclusion

The RBPK-DHJ3-3150, RBPK-DHJ1-4125, and RBPK-DHJ2-5250 were the superior selected KSRB isolates which have the potential to be used as potassium solubilizing microbial fertilizer.

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