Exploration and effectiveness test of the potassium solvent bacteria originating from limestone mountain of Bahorok Langkat

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Abstract. Plants need potassium in large quantities. The dissolved potassium concentrations are only 1-2%, meanwhile more than 90% of potassium in the soil is in the form of soluble rock. Potassium solvent bacteria facility in dissolving K. From several studies, potassium solvent bacteria can be found in limestone quarries. One of the limestone mountains in North Sumatera is the mountains in Laudamak village, Bahorok sub-district, Langkat District. This study aimed to obtain the potassium solvent bacteria and examine their potential of superior isolates from Bahorok Langkat mountain limestone. Sampling was conducted by the purposive random sampling method which was in two different locations and two different soil depths, namely on the mountain slop and foot of the limestone mountain. The ability to dissolve K was tested qualitatively by calculating the dissolution index (IP). The hypersensitivity test was carried out on tobacco leaves. The result showed that 18 isolates found from 10 soil sample from Bahorok Langkat limestone mountain were able to dissolve K in the Aleksandrov medium, there were 11 isolate that were able to dissolve K and 11 of selected isolates were not pathogenic to plants.

1. Introduction

Potassium is needed by plants in large quantities and is one of the third macro nutrients after nitrogen and phosphorus. Generally, K is absorbed by plants in the form of soluble K which is in a balance reaction with K interchangeable and K cannot be exchanged [1]. Potassium that cannot be exchanged are including the fixed K and the structural K [2]. The concentration of dissolved potassium is very low at only 1-2% and more than 90% of potassium in the soil is in the form of soluble rock [3].

The low availability of K in soil causes farmers to use inorganic fertilizers, such as potassium chloride (KCl) and muriate of potassium (MOP) to add the K elements to the soil. Excessive application of fertilizer can increase the costs, reduce the efficiency of K fertilizer and damage the environment. If this condition is left unchecked, it can cause widespread land damage and result in a decrease in land and plant productivity. An alternative to replace the inorganic K fertilizer is needed for sustainable agricultural development.

One of alternative is by using biotechnology such as potassium solvent bacteria to replace or reduce the usage of inorganic fertilizers. Potassium solvent bacteria, have been found in Navsari India, got the two best bacterial strains, namely Bacillus licheniformis and Bacillus subtilis [4]. The mechanism of
dissolving potassium rocks occurred through the process of acidolysis, chelation, exchange reactions, complexolysis and organic acid production [5]. *Burkholderia cepacia* isolates of the Palimanan Cirebon quarry was able to dissolve the potassium from feldspar rock on a laboratory test scale [6].

One of the limestone hills in North Sumatra is limestone mountain of Lau Damak Village in Bahorok Subdistrict which is located on 3.47770167 North Latitude and 98.1804500 East Longitude and has an area of 11.19 km² [7]. Therefore, it is suspected that in the limestone ecosystems such as the Bahorok Langkat limestone, the potassium solvent bacteria can be found. This study aimed to obtain the types and test the potential of superior isolates of potassium solvent bacteria from Bahorok Langkat limestone mountain land.

2. Material and methods

2.1. Research design
The soil samples were obtained from two different locations at Lau Damak Village in Bahorok Subdistrict i.e. the mountain slope and foot of the limestone mountain and two different soil depths (0-5 cm and >5-10 cm). The sampling was conducted by purposive random sampling method. Then, the observations and qualitative tests were carried out

2.2. Material and tool
Materials used this research were Aleksandrov Media (feldspar, CaPO4, glucose, yeast extract, MgSO4.7H2O, FeCl3, CaCO3, aquades, NaCl) [1]. Nutrient broth media (peptone, extra beef, NaCl, yeast extract), physiological solution of NaCl 0.85%, KCl and soil material from limestone mountains.

2.3. Observed parameters

2.3.1. Index of dissolution. Dissolution index was measured using calliper, potassium dissolving index (IP) to determine the ability of bacteria to dissolve the potassium using the following equation.

\[ IP = \frac{a - b}{b} \]

Information:
IP = Dissolution index
a = Diameter of colonies and clear zones
b = Diameter of colonies

2.3.2. Test of hypersensitivity on tobacco leaves. Test of hypersensitivity was carried out on the tobacco leaves (*Nicotiana tabacum* L.) which were 3 months old. Then, the selected isolates were injected (10⁸ cells-ml⁻¹) on the lower surface of the tobacco leaf in the mesophyll section between the bones of the tobacco leaf and on the leaves that were not too young and old, on the leaves found in the middle of the tobacco tree. If the colour of the place where the bacterial culture has been injected turned into yellow or the symptoms of necrosis after 24-48 hours, it means that the test bacteria were categorized as plant pathogens. Aquades were used as negative controls and *Xanthomonas oryzae* pv. *oryzae* was used as a positive control indicate.

3. Result and discussion

3.1. Isolation of potassium solvent bacteria
Isolation from 10 soil samples generated 18 isolates dissolve K in the Aleksandrov medium. Potassium solvent isolates were characterized by the presence of the clear zones around the colonies (Figure 1). The clear zone indicated the ability of bacteria to dissolve the potassium.
Figure 1. Growth of bacteria colonies in alexandrov medium. (a). sample of TB3.10^{-3}, (b). sample of TA1.10^{-3}, (c). sample of PB2.10^{-4}, (d). sample of TB3.10^{-2}

Potassium solvent bacteria can be found in the limestone mountain of Bahorok Langkat, but the population of these bacteria was small due to the environmental factors such as nutrients and little organic material which caused inhibited bacterial growth. Environmental factors, such as the soil acidity, soil aeration and temperature affected the microbial growth. Changes in microbial morphology and physiology were caused by differences in environmental factors and also caused the bacterial growth rates to decrease or increase.

Figure 2. Bacteria isolates of TB3.1, PB2.2 and PA2.2 in alexandrov medium

3.2. Dissolution Index
Calculation of dissolution index (IP) of 18 K solvent isolates obtained 11 isolates that were able to dissolve the best K in alexandrov medium.
Table 1. Result of isolates test in potassium dissolution

| No. | Location of Isolates                  | Position      | Vegetation                | Isolate Code | Dissolution Index |
|-----|--------------------------------------|---------------|---------------------------|--------------|-------------------|
| 1.  | Top of the mountain (depth 1-5 cm)   | E: 3.4552°   | *Castanopsis Javanica*    | KBS.PA.1     | 4.29              |
| 2.  | Top of the mountain (depth 1-5 cm)   | E: 3.4552°   | *Castanopsis Javanica*    | KBS.PA.2     | 2.57              |
| 3.  | Top of the mountain (depth 1-5 cm)   | E: 3.4543°   | *Prunus Avium*            | KBS.PA.3     | 2.06              |
| 4.  | Top of the mountain (depth 1-5 cm)   | E: 3.4543°   | *Prunus Avium*            | KBS.PB.1     | 2.89              |
| 5.  | Mountain Slope (depth 1-5 cm)        | E: 3.4521°   | *Piper Aduncum*           | KBS.TA.1     | 0.88              |
| 6.  | Mountain Slope (depth 5-10 cm)       | E: 3.4521°   | *Piper Aduncum*           | KBS.TB.1     | 1.20              |
| 7.  | Mountain Slope (depth 5-10 cm)       | E: 3.4521°   | *Piper Aduncum*           | KBS.TB.2     | 0.64              |
| 8.  | Mountain Slope (depth 5-10 cm)       | E: 3.4521°   | *Piper Aduncum*           | KBS.TB.3     | 2.18              |
| 9.  | Mountain Slope (depth 5-10 cm)       | E: 3.4547°   | *Hevea Brasiliensis*      | KBS.TB.4     | 0.94              |
| 10. | Mountain Slope (depth 5-10 cm)       | E: 3.4547°   | *Hevea Brasiliensis*      | KBS.TB.5     | 2.63              |
| 11. | Mountain Slope (depth 5-10 cm)       | E: 3.4547°   | *Hevea Brasiliensis*      | KBS.TB.6     | 2.61              |

Based on Table 1, it can be seen that the best dissolving index was in isolates KSB.PA.1 and KBS.PB.1. The higher value of the microbial dissolving index, the better ability of these microbes to dissolve potassium [8]. This variation of dissolution index value was due to differences in the ability of bacteria to secrete the extracellular organic acids or the types of organic acids produced. The main mechanism of dissolving P and K which is insoluble was through the production and secretion of organic acids [9].

Figure 3. Dissolution index of KSB.PA.1. Bacteria in Aleksandrov medium
3.3. Test of hypersensitivity on tobacco leaves

Hypersensitivity test results on tobacco leaves of the 11 selected isolates were negative hypersensitivity (not pathogenic to plants), tobacco leaves that were injected by each isolate did not show symptoms of necrosis after incubation for 24-48 hours.

![Image of hypersensitivity test results on tobacco leaves]

**Figure 4.** Observation result of hypersensitivity test on tobacco leaves. (A). Leaves that was injected by the *Xanthomonas oryzae pv oryzae* bacteria as positive control, (B). KBS.TB.5 Isolate, (C). KBS.PB.1. Isolate (D). KBS.TB.6, Isolate (E) negative control (aquades)

Positive hypersensitivity reactions (positive control) were demonstrated by the areas of tobacco leaves that were injected with the *Xanthomonas oryzae pv. oryzae* bacteria which causes rice leaf blight. This bacterium was able to induce hypersensitive reactions on tobacco leaves which were indicated by the presence of yellow spots or experiencing symptoms of necrosis in the injection area. Negative hypersensitive reactions were also shown by leaves injected with distilled water. Hypersensitivity reactions were included as rapid and localized cell death programs. This reaction occurred in infected plants when introducing pathogens and together with it, was an attempt to inhibit the growth of pathogens [10].

4. Conclusions

About 11 isolates that were able to dissolve K in Aleksandrov medium were found in Bahorok Langkat limestone area. The best two isolates were KSB.PA.1 and KBS.PB.1. which can dissolve potassium based on the dissolving index. There were 11 isolates that were able to dissolve K and 11 isolates were not pathogenic to plants.

References

[1] Parmar K B, Mehta B P and Kunt M D 2016 Isolation, Characterization and Identification of Potassium Solubilizing Bacteria from Rhizosphere Soil of Maize (*Zea Mays*) *Journal of Science* **5** (5) pp 3030 – 37

[2] Mursyida E 2015 Isolasi dan Identifikasi Bakteri Pelarut Fosfat dan Kalium dari Kawasan Sekitar Tambang Batu Kapur Cirebon [Isolation and Identification of Phosphate and Potassium Solvent Bacteria from the Area Around the Cirebon Limestone Mine] *Thesis* Institut Pertanian Bogor, Bogor

[3] Basyuni Z 2009 Mineral dan Batuan Sumber Unsur Hara P dan K [Minerals and Rocks Sources of P and K Nutrient] *World Journal Agricultural Science* **3** pp 350–5

[4] Wahyudi A T, Meliah S and Nawangsih A A 2011 Xanthomonas oryzae pv. oryzae bakteri penyebab hawar daun pada padi: isolasi, karakterisasi, dan telaah mutagenesis dengan transposon [Xanthomonas oryzae pv. bacterial oryzae causing leaf blight: isolation, characterization, and study of mutagenesis with transposon] *Makara Sains* **15** (1) pp 89-96

[5] Meenaa V S, Bihari R M and Verma J P 2014 Does a rhizospheric microorganism enhance Kavailability in agricultural soils? *Journal of Microbiological Research* **169** pp 337–47
[6] Anggraini E 2015 Kajian Potensi Bakteri Pelarut Kalium Dari Lahan Penambangan Batu Kapur Palimanan Cirebon [Potential Study of Potassium Solvent Bacteria from the Palimanan Limestone Mining Land in Cirebon] Thesis Institut Pertanian Bogor, Bogor

[7] Sebayang B W 2015 Kajian Karakteristik Gua Pintu Air di Desa Lau Damak Kecamatan Bahorok Kabupaten Langkat [Study of the Characteristics of Pintu Air Cave in Lau Damak Village, Bahorok District, Langkat Regency] Thesis Universitas Negeri Medan, Medan

[8] Wahyudi A T, Meliah S and Nawangsih A A 2011 Xanthomonas oryzae pv. oryzae bakteri penyebab hawar daun pada padi: isolasi, karakterisasi, dan telaah mutagenesis dengan transposon [Xanthomonas oryzae pv. bacterial oryzae causing leaf blight: isolation, characterization, and study of mutagenesis with transposon] Makara Sains 15 (1) pp 89-96

[9] Song O R, Lee S J, Lee Y S, Lee S C, Kim K K and Choi Y L 2008 Solubilization of insoluble inorganic phosphate by Burkholderia cepacia DA23 isolated from cultivated soil Brazilian Journal of Microbiology 39 (1) pp 151-6

[10] Premono M E, Moawad A M and Vlek P L G 1996 Effect of phosphate-solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere Indonesian Journal of Crop Science 11 (1) pp 13-23