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Isolation of Potential Plant Growth Promoting Rhizobacteria (PGPR) from Cassava (*Manihot esculenta*) Rhizosphere Soil

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Abstract. Cassava is the third most important calorie source in tropical area after rice and corn. The microorganism associated with cassava roots may be potent and useful for application to promote plant growth. Plant growth promoting rhizobacteria is a group of benefical bacteria that live in rhizosphere. The aim of this study was to isolate and to identify the potential Plant Growth Promoting Rhizobacteria (PGPR) from cassava rhizosphere soil. The study consisted of isolation and identification of bacteria based on morphological and biochemical characters, hypersensitive reaction test, the ability to solubilize potassium and phosphate, and the ability to inhibit the growth of pathogen *Sclerotium rolfsii*. A total of nine bacteria isolates were succesfully isolated from Cassava rhizosphere soil. Those isolates suspected as *Micrococcus* sp.1, *Micrococcus* sp.2, *Micrococcus* sp.3, *Micrococcus* sp.4, *Micrococcus* sp.5, *Micrococcus* sp.6, *Neisseria* sp.1, *Neisseria* sp.2 and *Bacillus* sp. All nine isolates did not show hypersensitivity reactions. Only *Neisseria* sp.1 and *Neisseria* sp. 2 were able to solubilize potassium and phosphate. All isolates were able to inhibit the growth of *S. rolfsii*. The highest inhibition was done by *Micrococcus* sp.4 (51.46 %). The physical and chemical properties of cassava rhizosphere soil affected the type of bacteria found in this study. The result confirmed that the potential isolates obtained from cassava rhizosphere soil can be plant growth promoters. The present study suggested that PGPR isolates might have potential in future field applications as plant growth promoters or biocontrol agents.

Key words: isolation; plant growth promoting rhizobacteria (PGPR); phosphate; potassium; *Sclerotium rolfsii*

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a plant originating from South America. Cassava is a food source of carbohydrate which is preferred by the public (Bradbury et al., 2013). Cassava is also a raw material for products such as tapioca flour, cassava flour, and bioethanol (Devy et al., 2018). Cassava plants grow widely in the community's environment and are used as a cheap food source and contain an important proportion of carbohydrate. Cassava is the third most important source calory in the tropic after rice and corn in Indonesia. As cassava is a long duration crop and faces many biotic and abiotic stresses during the growth phase, the microorganism associated with cassava roots may be potential and useful for application to promote plant growth.

One environmentally friendly alternative to promote plant growth and reduce chemical input in agriculture is by using microorganisms. Microorganisms play an important role in regulating the dynamics of organic matter, decomposition, and availability of plant nutrients. In the soil, it is possible to find various types of microorganism such as bacteria, fungi, actinomycetes, protozoa, and algae, which bacteria are by far the most common (Glick, 2012). Some of bacteria are associated with plant rhizosphere, and those that able to promote plant growth, known as Plant Growth Promoting Rhizobacteria (PGPR). Some known PGPR include *Azospirillum*, *Pseudomonas*, *Klebsiella*, *Azotobacter*, *Enterobacter*, *Alcaligenes*, *Bacillus*, *Burkholderia*, and *Serratia* (Babu et al., 2015). PGPR affect plant growth in two different ways, directly and indirectly. The direct promotion of plant growth such as providing nutrient by solubilizing potassium, phosphate and synthesizing phytohormone, and the indirect promotion plant growth is by reducing the impact of pathogen (Glick, 2012).

Plants play an important role in attracting a bacterial community by exuding root exudate. The bacterial community in the rhizosphere has an efficient

system for absorption and catabolism of organic compounds present in the exudate. Plants can effectively communicate with the rhizosphere bacteria by exuding chemicals or signals (Bais et al., 2006). While these bacteria can relate to plant by triggering host functional signals (Ma et al., 2016). Here we tried to isolate rhizobacteria that promote plant growth which have the potential as a potassium solubilization, phosphate solubilization, and as a biocontrol agent for pathogens.

This study provides information about bacterial species from the cassava rhizosphere that have the potential to promote plant growth. These isolates may have potential in future field application as plant growth promoting agents as a solution to reducing the use of chemical fertilizers and pesticides that affect the ecological balance and biodiversity.

METHODS

This research was conducted from July 2019 to January 2020 at two laboratories. 1.) Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. The study conducted consisted of bacterial isolation, colony morphological characterization, Gram staining, biochemical tests, identification of bacteria based on morphological and biochemical characters, hypersensitivity reaction test, potassium solubilizing test, phosphate solubilizing test, and a test to determine the ability to inhibit the growth of pathogen *Sclerotium rolfsii*. 2.) Chemistry laboratory of Agriculture faculty, Syiah Kuala University. The study consisted of soil texture analysis and determination of pH, organic carbon, N total, available P, and exchangeable K. Research data were analyzed descriptively and presented in tables and figures.

Soil Sampling

The soil samples were collected from rhizosphere of 5 healthy cassava planted in Lamleupung, Kuta Cot Glie, Aceh Besar. The five cassava were uprooted and the soil around the roots of each cassava was taken and put into sterilized plastics. Collected rhizosphere soil samples were used for bacterial isolation and soil analysis.

Bacteria Isolations

A total of 1 g rhizosphere soil was placed into 9 mL distilled water (10^{-1}), shaken for few minutes, then 1 ml sample solution of dilution 10^{-1} was transferred into 9 mL distilled water (10^{-2}). This serial dilution was continued up to 10^{-7}. A total of 100 µL soil sample solution from each dilution was spread on Tryptic Soy Agar (TSA) medium. The plates were incubated at room temperature for 48 hours. The individual bacterial colonies were then selected and subcultured on TSA medium.

Morphological Characterization of Colonies and Gram Staining

Pure bacterial colonies that had grown on TSA were characterized for the following traits: form, margin, elevation, color, and texture. The Gram staining was performed as standart procedures described by (Mudili, 2007). A drop of the bacterial broth was placed on a glass object and heat-fixed. The bacteria were stained with crystal violet as a primary stain for 1 minute and rinsed with water. Then the iodine solution was dropped as a mordant for 1 minute and rinsed with water. The bacterial cells were then decolorized rapidly with alcohol and rinsed. Safranin was dropped for 1 minute and rinsed, then the bacteria were observed under a microscope.

Biochemical Characterization

Biochemical characters of the isolates were examinated according to methods described in Cowan and Steel's Manual for the Identification Medical Bacteria (Cowan, 2004). The isolates were characterized for the following traits: catalase, oxidase, simmons citrate, methyl red, voges proskauer, oxidative-fermentative, and carbohydrate fermentation (glucose, sucrose, fructose, maltose, xylose, arabinose, trehalose, rhamnose, mannitol, sorbitol, inositol).

Hypersensitivity Reaction (HR)

Hypersensitivity reactions were tested on healthy tobacco plant (*Nicotiana tabacum*). The isolates were cultured on Tryptic Soy Broth (TSB) for 24 hours. The cultures were shaken for few minutes, then 100 µL of broth culture were injected into the leaves tissue of tobacco. The HR was observed after 48 hours. Positive result showed by necrotic at infiltrated leaves tissue. Only the isolates that showed negative result were used for further research (Wulandari et al., 2012).

Potassium Solubilizing Test

The isolates were checked for potassium solubilizing ability on Aleksandrov medium ($C_6H_{12}O_6$ 5 g/L; $MgSO_4$. $7H_2O$ 0.1 g/L; $FeCl_3$ 2.0 g/L; $CaCO_3$ 0.006g/L; Ca (PO_4) 3 g/L; $KAlSi_3$ 20 g/L; agar 20 g/L). A 24 hour old colony was taken using a sterile plastic pipette 7 mm in diameter, then inoculated on solid Aleksandrov and incubated at room temperature for 5 days. Formation of a clear zone around the growing colony indicates potassium solubilizing ability. The clear zone was measured using calipers, then the solubilization index was calculated using the following formula (Sadiq et al., 2013).

$$IP = \frac{\text{total diameter (colony + clear zone) (mm)}}{\text{diameter of the colony (mm)}}$$

Posphate Solubilizing Test

The isolates were checked for posphate solubilizing ability on solid Pikovskaya medium (glucose 10 g/L; $Ca_3(PO_4)_2$ 5 g/L; NaCl 0,2 g/L; $MgSO_47H_2$ 0,1 g/L; yeast extract 0,5 g/L; $MnSO_47H_2O$ 0,002 g/L; $FeSO_47H_2O$ 0,002 g/L; agar 15 g/L) (Thakuria et al., 2004). A 24 hour old colony was taken using a sterile plastic pipette 7 mm in diameter, then inoculated on solid Pikovskaya and incubated at room temperature for 5 days. Formation of a clear zone around the growing colony indicates phosphate solubilizing ability. The clear zone was measured using calipers, then the solubilization index was calculated using the following formula (Pande et al., 2017).

$$IP = \frac{\text{total diameter (colony + clear zone) (mm)}}{\text{diameter of the colony (mm)}}$$

The ability to inhibit the growth of pathogen *Sclerotium rolfsii*

The ability of bacteria to inhibit the growth of pathogen *Sclerotium rolfsii* was conducted using dual culture assay method. This test was carried out by growing pathogenic *S. rolfsii* and bacterial colonies in pairs on Potato Dextrose Agar (PDA) medium that had been added by YE 1%. Each isolate was placed at a distance of 3 cm from the edge of Petri dish. The culture was incubated at room temperature for 72 hours. The inhibition percentage was calculated using the following formula:

IP (%): $\frac{R1-R2}{R1} \times 100\%$

Information:

IP (inhibition percentage)

R1 (pathogenic diameter without antagonistic isolate)

R2 (pathogenic diameter with antagonistic isolate)

RESULTS AND DISCUSSION

Morphological Characteristics of Colonies and Gram Reactions

A total of nine bacterial isolates were successfully isolated from rhizosphere soil of cassava and designated as RSC1 to RSC9 (RSC = rhizosphere soil cassava). All nine isolates have different colony morphological characteristics (Table 1). Microscopic observations were performed to investigate the characteristics of isolates such as Gram reactions and cells shape. Seven isolates were Gram positive and two isolates Gram negative. Eight isolates were cocci and one isolate was bacilli.

Table 1. Morphological characteristics and Gram reactions

Isolate code	Colony Form	Edge	Elevation	Color	Texture	Gram Reactions	Cells shape
RSC1	irregular	undulate	slightly umbonate	beige	opaque	+	cocci
RSC2	circular	curled	raised	milky white	opaque	+	cocci
RSC3	irregular	irregular	flat	beige	translucent	+	cocci
RSC4	circular	undulate	umbonate	beige	moist	+	cocci
RSC5	circular	curled	umbonate	beige	moist	+	cocci
RSC6	circular	undulate	raised	beige	moist	-	cocci
RSC7	circular	entire	convex	beige	translucent	-	cocci
RSC8	circular	curled	raised	beige	opaque	+	cocci
RSC9	filamentous	filamentous	flat	beige	opaque	+	bacilli

RSC: rhizosphere soil cassava

The dominant form of bacterial colonies found in this study was circular with raised elevation. Colony colors are generally beige with opaque texture. The appearance of bacterial colonies could be seen in figure 1. Bacterial colonies from rhizosphere soil of cassava in this study had more varied morphology compared to the colonies from rhizosphere of *Hevea brasiliensis.* (Gofar et al., 2016) reported that all isolates from rhizosphere of *H. brasiliensis* have circular colony form with entire edge and white color. (Kaufmann & Schaible, 2005) stated that colony morphology was a basic means of identifying a bacterium. The ways to identify different bacteria species based on colony morphology generally described in terms of qualitative measure of form, elevation, edge, surface, and pigmentation.

Biochemical Characteristics

Biochemical characteristics are very important criteria for identifying a bacterium, because each bacterium has different abilities in breaking down or synthesizing a particular compound. Biochemical

characteristics of all nine bacterial isolates could be seen in Table 2.

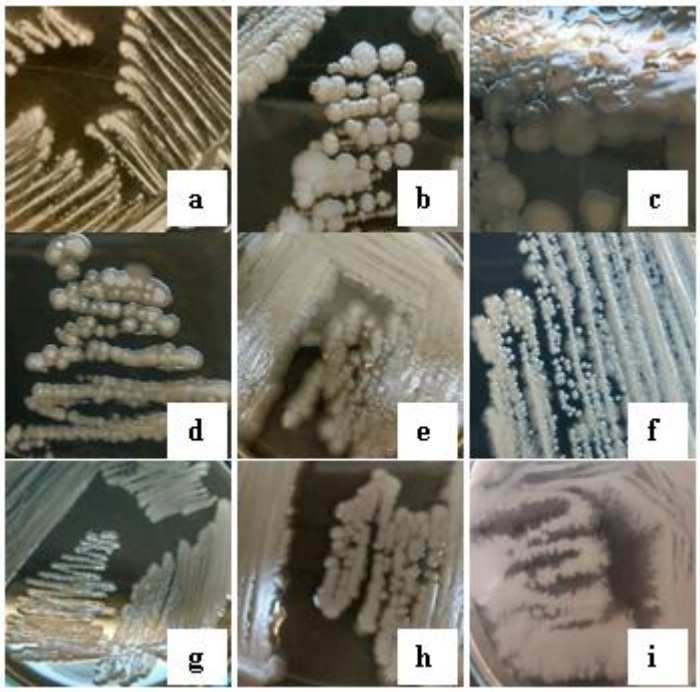

Figure 1. Bacterial colonies (a) RSC1, (b)RSC2, (c)RSC3, (d)RSC4, (e)RSC5, (f)RSC6, (g)RSC7, (h)RSC8, (i)RSC9.

Table 2. Biochemical characteristics of bacteria

Type of Test	Isolates								
	RSC1	RSC2	RSC3	RSC4	RSC5	RSC6	RSC7	RSC8	RSC9
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-	+
Oxidative/ fermentative	-/+	+	-	-/+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	-
Methyl Red	-	-	-	-	+	-	-	-	+
Voges Proskaeur	-	-	-	+	-	+	-	+	-
Glucose	-	+	-	-	+	+	+	+	+
Fructose	+	+	-	-	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-
Sucrose	-	+	-	-	+	+	+	-	-
Maltose	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	+	-	-
Arabinose	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	+	-	-
Trehalose	-	+	+	-	+	+	+	+	+
Mannitol	-	-	-	-	-	-	+	-	-
Sorbitol	-	-	-	-	-	-	+	-	-
Inositol	-	-	-	-	-	-	-	-	-

RSC : rhizosphere soil cassava; +: positive; - : negative; -/+ : negative/positive

Based on the morphological and biochemical characteristics of nine bacterial isolates, according to the Book Cowan and Steel's Manual for the Identification of Medical Bacteria (Cowan, 2004), six isolates were suspected as *Micrococcus* sp., two isolates as *Neisseria* sp. and one isolate as *Bacillus* sp. (Table 3).

The isolates suspected as *Micrococcus* sp. had characteristics of Gram positive cocci, catalase positive, oxidase positive, non motile, could use citrate as carbon source, and used glucose oxidatively

or fermentatively. In accordance with the statement of (Wieser et al., 2002), *Micrococcus* sp. is a Gram positive cocci, usually non motile, catalase positive and oxidase positive, lives aerobically or facultatively anaerobically, and can use glucose oxidatively or cannot use glucose at all. *Micrococcus* can be isolated from soil and water.

Table 3. Identified isolates

Isolates	Species
RSC1	*Micrococcus* sp.
RSC2	*Micrococcus* sp.
RSC3	*Micrococcus* sp.
RSC4	*Micrococcus* sp.
RSC5	*Micrococcus* sp.
RSC6	*Neisseria* sp.
RSC7	*Neisseria* sp.
RSC8	*Micrococcus* sp.
RSC9	*Bacillus* sp.

RSC: rhizosphere soil cassava

The isolates suspected as *Neisseria* sp. had characteristics of Gram negative cocci, catalase positive, oxidase positive, non motile, citrate positive, and can ferment several types of carbohydrates. In accordance with the statement of (Bennett et al., 2012), *Neisseria* is a Gram negative cocci, catalase positive, oxidase positive, and all species can produce acids from several carbohydrates oxidatively. *Neisseria* is a bacterium that generally isolated from animal mucosa. However, some previous studies also found *Neisseria* sp. which was isolated from rhizosphere soil of plant. (Pandey & Singh, 2013) found *Neisseria sicca* isolated from the rhizosphere of the *Azadirachta indica*.

The isolate suspected as *Bacillus* sp. had characteristics of Gram positive bacilli, catalase positive, oxidase positive, motile, and could ferment glucose. In accordance with the statement of (Parvathi et al., 2009), B*acillus* is a Gram positive bacilli, can live in aerobic or facultative anaerobes, catalase positive, varied oxidase, motile, can form endospores, so it is easy to adapt to various habitats, and is generally found in soils.

Hypersensitivity Reactions

The pathogenicity of nine bacterial isolates was tested through hypersensitive reactions in tobacco plants (*Nicotiana tabacum*). The results of the nine isolates were negative, no necrosis reaction occurred in the area where the bacteria were injected into tobacco leaves (Figure 2). It could be assumed that all nine isolates have no potential as plant pathogens.

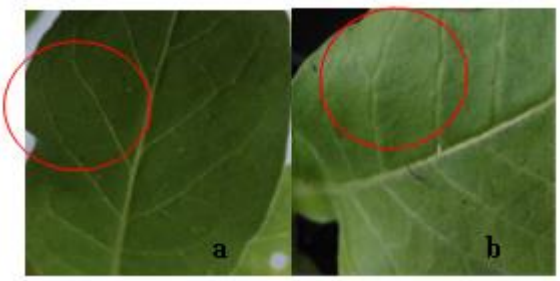

Figure 2. Tobacco (*Nicotiana tabacum*) leaves. The red circle indicates the injection area, (a) injected with distilled water (control), (b) injected with bacteria.

Hypersensitivity reactions are rapid forms of programmed cell death associated with plant defenses, which can be morphologically characterized by the formation of necrotic lesions around the pathogen entry site. Hypersensitive reaction can be induced by fungi, oomycetes, bacteria, and virus (Balint-Kurti, 2019).

Potassium Solubilization Activity

Out of nine isolates, only two isolates were able to solubilize pottasium on Aleksandrov solid medium. The two isolates were *Neisseria* sp.1 and *Neisseria* sp.2. The ability to solubilize potassium was characterized by the present of clear zones around the colonies in Aleksandrov solid medium (Figure 3). According to (Ghevariya & Desai, 2014), bacteria that were able to form clear zones on Aleksandrov medium were considered as potassium solubilizing bacteria.

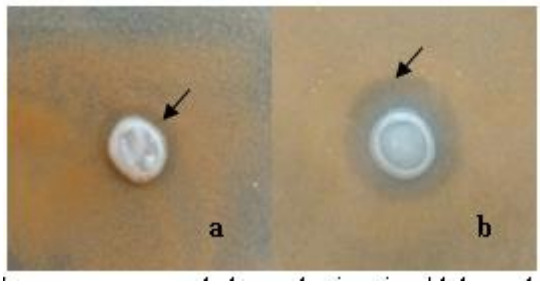

Figure 3. Clear zone around the colonies in Aleksandrov solid medium (a) *Neisseria* sp.1 (b) *Neisseria* sp.2

According to (Maurya et al., 2014) and (Meena et al., 2014), microorganisms generally contribute to the release of K from K-bearing minerals through several mechanisms, including the production of organic and inorganic acids. According to (Huang et al., 2013), organic acids secreted by bacteria could accelerate the weathering process of K-bearing minerals. Organics acids secreted by bacteria also can release of K ions from K-bearing minerals by complex formation

$Al^{3+,}$ $Ca^{2+,}$ Fe^{2+} and Si^{4+} ions which combined with minerals K (Etesami et al., 2017).

The potassium solubilizing bacteria was characterized by the ability to form a clear zone in Aleksandrov medium. Solubilization index was used to measure K-solubilization zone in solid Aleksandrov medium (Table 4). The differences of solubilization index values in each isolates might be caused by the ability of each bacterium in producing organic acids (Sheng & He, 2006). According to (Ismangil & Hanudin, 2005), mineral solubilization was influenced by the type of mineral, pH, and type and amount of organic acids. Some organic acids produced by potassium solubilizing bacteria are oxalic, citric, tartaric, formic, and malic acid (Shanware et al., 2014).

Table 4. Potassium solubilization index

Isolates	Solubilization index
Neisseria sp.1	1.17
Neisseria sp.2	1.52

Phosphate Solubilization Activity

All nine isolates were tested for their ability to solubilize phosphate, only *Neisseria* sp.1 and *Neisseria* sp.2 were able to solubilize phosphate. The ability to solubilize phosphate was characterized by the formation of clear zones around bacterial colonies on Pikovskaya solid medium (Figure 4). (George et al., 2002) stated that the formation of clear zones around the colony can be caused by the presence of organic acids secreted by bacteria, these organic acids bind with Ca ions from $Ca_3(PO_4)_2$ on Pikovskaya medium and release H_2PO4^-, thus forming more colored areas clear compared to the P bound area.

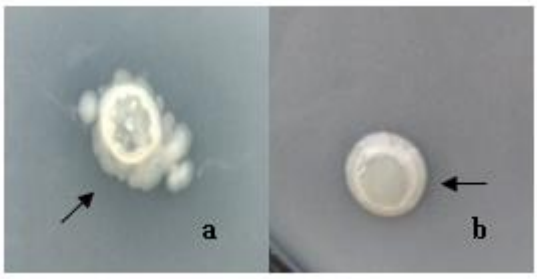

Figure 4. Clear zone around the colonies in Pikovskaya solid medium (a) *Neisseria* sp.1 (b) *Neisseria* sp.2

The ability to solubilize phosphate was expressed in phosphate solubilization index (Table 5). *Neisseria* sp.1 and *Neisseria* sp.2 were Gram negative bacteria that were able to solubilize phosphate compared to seven other Gram positive bacteria. According to (Kumar et al., 2018), Gram negative bacteria were more effective in solubilizing phosphate minerals compared to Gram positive bacteria, because Gram negative bacteria were known to produce more diverse organic acids.

Table 5. Phosphate solubilization index

Isolates	Solubilization index
Neisseria sp.1	1.23
Neisseria sp.2	1.02

The main mechanism of solubilizing phosphate by bacteria was a decrease in pH with the production of organic acids. Organic acid is the result of bacterial metabolism from oxidation or fermentation of glucose which is used as a carbon source. The type and amount of organic acids can be different depend on the species. The efficiency of solubilization is very dependent on the strength and acidic nature. Some organic acids that have been known to solubilize phosphate are citric acid, lactate, gluconate, 2-ketogluconate, acetate, malate, fumarate, succinate, malonic, glutaric, propionate, and butyrate. Gluconate and 2-ketogluconate are the acids most often found in solubilizing phosphate minerals (Kalayu, 2019).

According to (Khan et al., 2009), these organic acids will form a chelate (stable complex) with metal ions in soil minerals such as $Ca^{2+,}$ Mg^{2+}, Fe^{3+}, and Al^{3+}, thus releasing bound phosphates. 2-ketogluconate is a strong chelator in calcium. (Walpola & Yoon, 2012) stated that another mechanism in solubilizing soil phosphate is mineralization. Bacteria can convert organic phosphate from plant and animal residues into inorganic phosphate with the help of the phosphatase enzyme.

Inhibition of the growth of *Sclerotium rolfsii* by rhizosphere bacteria

All nine isolates of cassava rhizosphere bacteria have the ability to inhibit the growth of pathogen *Sclerotium rolfsii*. *S. roflsii* grown with bacterial isolates has fewer hyphae compared to *S. roflsii* grown without bacterial isolates. Growth inhibition was clearly seen in area that interact directly with bacteria (Figure 5).

Microscopic observation with 400 times of magnification showed the damage of *S. roflsii* hyphae that was grown along with bacteria, the hyphae appeared craked and was larger in size than normal hyphae (Figure 6). (Eliza et al., 2007) stated the antifungal compounds produced by bacteria generally cause abnormal growths in pathogenic hyphae, which were characterized by lysis and swelling. Abnormal hyphae structures of *S. rolfsii* resulting from diffusible secondary metabolite compounds from bacteria.

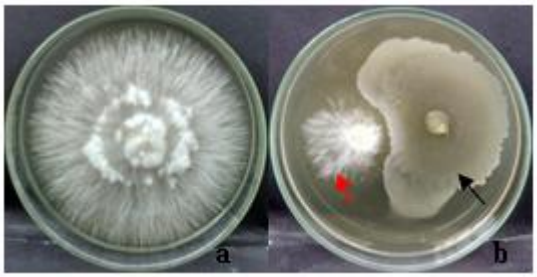

Figure 5. Appearance of *Sclerotium roflsii* growth on PDA medium (a) *S. roflsii* (control), (b) *S. rolfsii* grown with bacteria (dual culture). Red arrow indicates *S. roflsii* and black arrow indicates bacterial isolate.

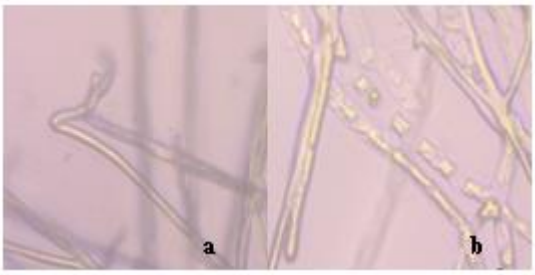

Figure 6. Morphology of *Sclerotium rolfsii* under microscope observation with 400 times of magnification (a) a normal hyphae of *S. rolfsii* on the PDA medium, (b) hyphae was grown in PDA medium with rhizosphere bacterial isolate.

The ability of all nine bacterial isolates to inhibit *S. rolfsii* growth was calculated in percent. The highest percentage of *S. rolfsii* growth inhibition was performed by *Micrococcus* sp.4 and the lowest percentage by *Bacillus* sp. (Table 6). The results of this study were supported by (Abidin et al., 2015), that bacteria isolated from the rhizosphere include *Bacillus* sp. are able to inhibit the growth of pathogenic *S. rolfsii*.

Table 6. Rhizobacterial isolates showed inhibition against mycelial growth of *Sclerotium rolfsii*

Isolates	PI (%)
Micrococcus sp.1	34.14
Micrococcus sp.2	22.97
Micrococcus sp.3	27.06
Micrococcus sp.4	51.46*
Micrococcus sp.5	23.66
Neisseria sp.1	31.10
Neisseria sp.2	28.51
Micrococcus sp.6	25.21
Bacillus sp.	10.92

PI (percentage inhibition)
* (the highest inhibition)

According to (Conrath et al., 2015), the differences in the ability of antagonistic bacteria to inhibit the growth of pathogenic fungi are likely due to differences in type and amount of antifungal compounds produced. According to (Ahemad & Kibret, 2014), bacteria in the rhizosphere as biological control agents can protect plants from damage by pathogens in various ways including by inducing resistance to pathogenic infections in plant tissue, competing for nutrients and niche, and producing antifungal metabolites.

Physical and Chemical Properties of Soil

The physical and chemical properties of cassava rhizosphere soil can be seen in Table 7. The soil has silty loam texture wich silt fraction is more dominant than sand and clay. (Chau et al., 2011) reported that bacterial diversity is more abundant found in coarse textured soils (sandy soils) compared to fine textured soils, because small particles adsorbed on the sand grains provide binding sites for adsorption of organic material and bacterial cells.

Table 7. Physical and Chemical Properties of Soil

Properties	Value
Soil Texture:	
Sand (%)	3
Silt (%)	77
Clay (%)	20
Texture class	H *
Organic carbon (%)	1.26
N total (%)	0.18
pH (H_2O)	6.81
P available (mg kg^{-1})	4.20
Exchangeable K (cmolkg^{-1})	1.46

* H (silty loam soil)

Cassava rhizosphere soil has low organic C and N contents of 1.26% and 0.18% respectively. According to (Eviati & Sulaeman, 2009), organic C content of 1-2% and total N content of 0.1-0.2% are included in low criteria. (Silva-Sánchez et al., 2019) stated that organic C and N are the sources of nutrients that affect bacterial growth. Cassava rhizosphere soil has relatively neutral pH of 6,81. Neutral pH is the optimal growth pH for most groups of bacteria, including *Bacillus* and *Micrococcus*, as found in this study.

According to (Eviati & Sulaeman, 2009) P content available in cassava rhizosphere (4.20 mg kg-1) soils is classified as a low category, while the exchangeable K (1.46 cmol kg^{-1}) is included as a very high category. Low levels of available P may be caused the soil analyzed only contained little organic material and is also formed from material (rocks / minerals) that has low P elements. Biotic and abiotic factors are assumed to affect the structural and functional diversity of bacterial communities. (Berg

& Smalla, 2009) explained that the factors affecting microbial communities in the rhizosphere are plant species and soil types.

Many bacteria from the rhizosphere contributing to plant growth and health, facilitating plant growth by regulating hormonal balance, solubilizing nutrients for easy uptake by plants, and inducing resistance against plant pathogens which are described in the literature (Vejan et al., 2016). Plant growth promoting bacteria can also be found in healthy plant tissues such as roots, stems and leaves are known as endophytic bacteria. These bacteria were able to produce phytohormones such as indole acetic acid (IAA), fix nitrogen, and inhibit the growth of plant pathogenic bacteria, as reported by (Putriani et al., 2019) and (Simarmata et al., 2020).

The results of this study provide information about bacterial species from the rhizosphere of cassava which have the potential to promote plant growth, with the ability of these bacteria to solubilize potassium and phosphate and also to inhibit the growth of *Sclerotium rolfsii* which is one of the pathogens in cassava plants. These isolates may have potential in future field applications as growth promoting agents or as biocontrol agents. The use of PGPR in cassava plantation practices may be a solution to decrease the use of chemical fertilizers and pesticides which affect ecological balance and biodiversity. Applying this cassava rhizosphere bacterial isolate for plants is a further research that needs to be done.

CONCLUSION

A total of nine bacterial isolates were isolated from cassava rhizosphere soil. The nine isolates were suspected as *Micrococcus* sp.1, *Micrococcus* sp.2, *Micrococcus* sp.3, *Micrococcus* sp.4, *Micrococcus* sp.5, *Micrococcus* sp.6, *Neisseria* sp.1, *Neisseria* sp.2, and *Bacillus* sp. All isolates did not show hypersensitivity reactions. Two of nine isolates were able to solubilize potassium and phosphate, those isolates were *Neisseria* sp.1 and *Neisseria* sp.2. The highest potassium solubilization index was 1.52 by *Neisseria* sp.2 and the highest phosphate solubilization index was 1.23 by *Neisseria* sp.1. The nine isolates were able to inhibit the growth of pathogen *Sclerotium rolfsii*. The highest inhibition was done by *Micrococcus* sp.4 (51.46%). The isolates obtained from cassava rhizosphere soil have the potential as plant growth promoters. Physical and chemical properties of cassava rhizosphere soil affected the type of bacteria found in this study.

REFERENCES

Abidin, Z., Aini, L. Q., & Abadi, A. L. (2015). Pengaruh bakteri *Bacillus* sp. dan *Pseudomonas* sp. terhadap pertumbuhan jamur patogen *Sclerotium rolfsii* Sacc. penyebab penyakit rebah semai pada tanaman kedelai. *Jurnal Hama dan Penyakit Tumbuhan*, *3*(1), 2338–4336.

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University-Science*, *26*(1), 1–20.

Babu, A. N., Jogaiah, S., Ito, S., Nagaraj, A. K., & Tran, L. S. P. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Science*, *231*, 62–73.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233–266.

Balint-Kurti, P. (2019). The plant hypersensitive response: Concepts, control and consequences. *Molecular Plant Pathology*, *20*(8), 1163–1178.

Bennett, J. S., Jolley, K. A., Earle, S. G., Corton, C., Bentley, S. D., Parkhill, J., & Maiden, M. C. J. (2012). A genomic approach to bacterial taxonomy: An examination and proposed reclassification of species within the genus Neisseria. *Microbiology*, *158*(Pt 6), 1570.

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, *68*(1), 1–13.

Bradbury, E. J., Duputié, A., Delêtre, M., Roullier, C., Narváez-Trujillo, A., Manu-Aduening, J. A., Emshwiller, E., & McKey, D. (2013). Geographic differences in patterns of genetic differentiation among bitter and sweet manioc (*Manihot esculenta* subsp. *esculenta*; Euphorbiaceae). *American Journal of Botany*, *100*(5), 857–866.

Chau, J. F., Bagtzoglou, A. C., & Willig, M. R. (2011). The effect of soil texture on richness and diversity of bacterial communities. *Environmental Forensics*, *12*, 333–341.

Conrath, U., Beckers, G. J. M., Langenbach, C. J. G., & Jaskiewicz, M. R. (2015). Priming for enhanced defense. *Annual Review of Phytopathology*, *53*, 97–119.

Cowan, S. T. (2004). *Cowan and Steel's manual for the identification of medical bacteria* (3rd ed.). Cambridge university press.

Devy, N. F., Syarif, A. A., & Aryawaita, A. (2018). Identifikasi penciri morfologi dan kualitas plasma nutfah lokal ubi kayu (*Manihot esculenta* Crantz) Sumatra Barat. *Buletin Plasma Nutfah*, *24*(1), 53–62.

Eliza, Munif, A., & Djatnika, I. (2007). Karakter fisiologis dan peranan antibiosis bakteri perakaran *Graminae* terhadap *Fusarium* dan pemacu pertumbuhan tanaman pisang. *Jurnal Hortikultura*, *17*(2), 150–160.

Etesami, H., Emami, S., & Alikhani, H. A. (2017). Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects - a review. *Journal of Soil Science and Plant Nutrition*, *17*(4), 897–911.

Eviati & Sulaeman (2009). *Analisis kimia tanah, tanaman, air dan pupuk*. Balai Penelitian Tanah.

George, T. S., Gregory, P. J., Robinson, J. S., & Buresh, R. J. (2002). Changes in phosphorus concentrations and pH in the rhizosphere of some agroforestry and crop species. *Plant and Soil*, *246*(1), 65–73.

Ghevariya, K. K., & Desai, P. B. (2014). Rhizobacteria of sugarcane: in vitro screening for their plant growth promoting potentials. *Research Journal of Recent Sciences*, *3*, 52–58.

Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*, *2012*.

Gofar, N., Munawar, M., Widjajanti, H., & Mulya, A. P. (2016). Eksplorasi bakteri antagonis asal jaringan dan rizosfer tanaman karet untuk menekan pertumbuhan bakteri proteolitik pada bahan olahan karet (BOKAR). *Jurnal Ilmu Tanah dan Lingkungan*, *16*(2), 61–66.

Huang, Z., He, L., Sheng, X., & He, Z. (2013). Weathering of potash feldspar by *Bacillus* sp. L11. *Wei Sheng Wu Xue Bao= Acta Microbiologica Sinica*, *53*(11), 1172–1178.

Ismangil, & Hanudin, E. (2005). Degradasi mineral batuan oleh asam-asam organik. *Ilmu Tanah dan Lingkungan*, *5*(1), 1–17.

Kalayu, G. (2019). Phosphate solubilizing microorganisms: Promising approach as biofertilizers. *International Journal of Agronomy*, *2019*, 1–7.

Kaufmann, S. H. E., & Schaible, U. E. (2005). 100th anniversary of Robert Koch's Nobel Prize for the discovery of the tubercle *Bacillus*. *Trends in Microbiology*, *13*(10), 469–475.

Khan, A. A., Jilani, G., Akhtar, M. S., Naqvi, S. M. S., & Rasheed, M. (2009). Phosphorus solubilizing bacteria: Occurrence, mechanisms and their role in crop production. *J. Agric. Biol. Sci.*, *1*(1), 48–58.

Kumar, A., Kumar, A., & Patel, H. (2018). Role of microbes in phosphorus availability and acquisition by plants. *International Journal of Current Microbiology and Applied Sciences*, *7*(5), 1344–1347.

Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2016). Biochemical and molecular mechanisms of plant-microbe-metal interactions: Relevance for phytoremediation. *Frontiers in Plant Science*, *7*, 918.

Maurya, B. R., Meena, V. S., & Meena, O. P. (2014). Influence of inceptisol and alfisol's potassium solubilizing bacteria (KSB) isolates on release of K from waste mica. *Vegetos*, *27*(1), 181–187.

Meena, V. S., Maurya, B. R., & Bahadur, I. (2014). Potassium solubilization by bacterial strain in waste mica. *Bangladesh Journal of Botany*, *43*(2), 235–237.

Mudili, J. (2007). *Introductory practical microbiology*. Alpha Science International Limited.

Pande, A., Pandey, P., Mehra, S., Singh, M., & Kaushik, S. (2017). Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology*, *15*(2), 379–391.

Pandey, A., & Singh, A. (2013). A comparative study on secondary metabolites producing microbes isolated from rhizospheric & non-rhizospheric region of *Azadirachta Indica* and *Oscimum tenuiflorum*. *International Journal of Pharmaceutical Research & Allied Sciences*, *2*(1), 36–48.

Parvathi, A., Krishna, K., Jose, J., Joseph, N., & Nair, S. (2009). Biochemical and molecular characterization of *Bacillus pumilus* isolated from coastal environment in Cochin, India. *Brazilian Journal of Microbiology*, *40*(2), 269–275.

Putriani, P., Fitri, L., & Ismail, Y. S. (2019). The potential endophytic cacteria isolated from rice (*Oryza sativa*) as biofertilizer. *Biosaintifika: Journal of Biology & Biology Education*, *11*(2), 178–185.

Sadiq, H. M., Jahangir, G. Z., Nasir, I. A., Iqtidar, M., & Iqbal, M. (2013). Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere soil. *Biotechnology & Biotechnological Equipment*, *27*(6), 4248–4255.

Shanware, A. S., Kalkar, S. A., & Trivedi, M. M. (2014). Potassium solublisers: Occurrence, mechanism and their role as competent biofertilizers. *International Journal of Current Microbiology and Applied Sciences*, *3*(9), 622–629.

Sheng, X. F., & He, L. Y. (2006). Solubilization of potassium-bearing minerals by a wild-type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiology*, *52*(1), 66–72.

Silva-Sánchez, A., Soares, M., & Rousk, J. (2019). Testing the dependence of microbial growth and

carbon use efficiency on nitrogen availability, pH, and organic matter quality. *Soil Biology and Biochemistry*, *134*, 25–35.

Simarmata, R., Widowati, T., Dewi, T. K., Lekatompessy, S. J. R., & Antonius, S. (2020). Isolation, screening and identification of plant growth-promoting endophytic bacteria from *Theobroma cacao*. *Biosaintifika: Journal of Biology & Biology Education*, *12*(2), 155–162.

Thakuria, D., Talukdar, N. C., Goswami, C., Hazarika, S., Boro, R. C., & Khan, M. R. (2004). Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of assam. *Current Science*, *86*(7), 978–985.

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Boyce, A. N. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability-A review. *Molecules*, *21*(5), 1–17.

Walpola, B. C., & Yoon, M. H. (2012). Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: A review. *African Journal of Microbiology Research*, *6*(37), 6600–6605.

Wieser, M., Denner, E. B. M., Kämpfer, P., Schumann, P., Tindall, B., Steiner, U., Vybiral, D., Lubitz, W., Maszenan, A. M., & Patel, B. K. C. (2002). Emended descriptions of the genus *Micrococcus*, *Micrococcus luteus* (Cohn 1872) and *Micrococcus lylae* (Kloos et al. 1974). *International Journal of Systematic and Evolutionary Microbiology*, *52*(2), 629–637.

Wulandari, H., Zakiatulyaqin, Z., & Supriyanto, S. (2012). Isolasi dan pengujian bakteri endofit dari tanaman lada (*Piper nigrum* L.) sebagai antagonis terhadap patogen hawar beludru (*Septobasidium* sp.). *Perkebunan dan Lahan Tropika*, *2*(2), 23–31.