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Isolation and Characterization of Plant Growth Promoting Rhizobacteria From *Ipomoea* sp. Rhizospheres Growing in Iron Sand Soil

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Abstract
The iron sand field is mostly found along the Indonesia coast. It has low organic matter, contains 38-59% iron (Fe) and sand particles. These characteristics can be called extreme environments. However, there are bacteria capable of growing and surviving in such habitats. Several genera are known as PGPR agents, such as *Rhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas*. This research aimed to measure the total bacterial population of *Ipomoea* sp. rhizosphere growing in iron sand soils, to investigate the plant growth-promoting properties, and to identify the plant growth-promoting rhizobacteria candidates. For this purpose, random sampling from *Ipomoea* sp. rhizosphere was performed. Bacteria were isolated by culturing serial on Nutrient Agar, Pikovskaya, Ashby, and Caceres medium. The ability of isolates to fix nitrogen, dissolute phosphate, and produce indole acetic acid (IAA) was investigated. The best growth-promoting isolates were identified based on morphological, biochemical, nutritional, and physiological characters. The population of bacteria was ranged from 1.59 x 10^5 to 5.2 x 10^5 CFU.g^{-1}. There were 22 bacteria isolated from *Ipomoea* sp. rhizosphere growing in iron sand soils. Six isolates (A4, A10, C10, P2, P3, and P4) were selected as plant growth-promoting candidates. One isolate (P4) was able to fix nitrogen, dissolute phosphate, and produce IAA. Based on the bacterial identification, four isolates (C10, P2, P3, P4) were identified as the species members of *Bacillus* and two isolates (A4, A10) were identified as the species members of *Actinomycetes*.
Key Words: *Indole Acetic Acid, iron sand soil, nitrogen fixation, phosphate solubilization, plant growth-promoting rhizobacteria*

INTRODUCTION

Soil as habitat for plants contains various microorganisms, some of which colonize plant roots (rhizosphere) and give beneficial effects. This group of microorganisms is called *Plant Growth Promoting Rhizobacteria* (PGPR), which is a significant component for plant management (Kafrawi *et al*., 2015). Intensive agricultural practices mostly use mineral fertilizers. The use of mineral fertilizers continuously can damage nutrient balance in the soil (Plante, 2007). Excessive use of mineral fertilizers causes air pollution and soil water pollution, and increases nutrient content in waters (*eutrophication*) (Youssef & Eissa, 2014). Other negative impacts on soil ecosystems are hardening of the soil, decrease in organic matter, heavy metal contamination, resistance to certain pests and diseases, and can eliminate predators and parasitoids (Stoate *et al*., 2001). The method that mostly used to solve it might include the introduction of beneficial microorganisms such as biofertilizer.

Biofertilizer is a fertilizer containing microorganisms that promote growth by increasing the nutritional needs of the plant. The use of biofertilizers is to increase yield production, because biological fertilizers contain microorganisms that are capable of producing nutrients for plant growth. Bacteria colonizing rhizosphere and rhizoplane are known as rhizobacteria. According to Sutariati *et al*. (2014), rhizobacteria are a trigger for plant growth through its ability to solubilize phosphate, fix nitrogen and produce growth hormone. Rhizobacteria are a group of bacteria living as a saprophyte in the rhizosphere and some types are capable of promoting plant growth.

Three major roles of Plant Growth Promoting Rhizobacteria (PGPR) are biofertilizer, biostimulant and bioprotectant. PGPR can affects plants directly and indirectly (Zainudin *et al*., 2014). The former is based on the bacterium's ability to provide and mobilize or facilitate the absorption of various nutrients in the soil. Bacteria can synthesize and alter the concentration of various growth phytohormones such as by increasing nitrogen uptake, producing phytohormone (IAA, gibberellin or cytokinin), and solubilizing minerals such as phosphate. The later is related to the ability of PGPR to suppress pathogen activity by producing compounds or metabolites such as antibiotics, cyanides (HCN), or siderophores and reducing the concentration of ethylene by producing ACC-Deaminase (Hussain *et al*., 2013).

Bacteria known as PGPR commonly belong to the genera Rhizobium, Azotobacter, Azospirillum, Bacillus, Pseudomonas, and Arthrobacter (Widawati, 2015). Several of them are capable of growing in an extreme environment such as in iron sand soils. This soil is characterized by high sand, low organic content, and low pH. The iron sand region is a marginal habitat that grown by a few numbers of plant species. The habitat might also limit the growth and development of soil microorganisms, while the microorganisms are needed and very important for plant growth (Oedjijono *et al*., 2014).

Characterization and identification of bacteria are required stages to investigate their identities. Bacteria capable of growing in plant roots in iron sand soil have a high competitive value so that the opportunity to use them as a biofertilizer agent is promising. Conditions in iron sand soil is quite extreme, so that the bacteria are most possibly have high stimulant for plant growth.

The objectives of this research were to : 1) measure total populations of the bacteria from rhizosphere of Ipomoea sp. in iron sand soils, 2) investigate the ability of the bacterial isolates in fixing nitrogen, solubilizing phosphate, and producing plant growth hormone (IAA), 3) taxonomically identify plant growth-promoting rhizobacteria isolated from the plant rhizospheres.

MATERIAL AND METHOD

Materials used in this research were iron sand soils from the rhizosphere of *Ipomoea* sp. in Sodong Beach, Cilacap, Nutrient Agar (NA) medium, Pikovskaya medium, Ashby medium, Caceres medium, Nitrogen free Bromothymol blue (NfB) medium, *L-Tryptophan*, *Salkowski* Reagent, *Sulphide Indole Motility Agar* (SIMA), mineral medium, phenol red, glucose 1%, sucrose 1%, lactose 1%, arabinose 1%, mannitol 1%, and O/F medium.

Sample Collection

Soil samples of plant rhizosphere were taken randomly at four spots in Sodong beach, Cilacap. The samples were collected with a spoon, wrapped with plastic and put into the box. They were brought to Laboratory Microbiology, Faculty of Biology, Universitas Jenderal Soedirman, and samples were stored in a refrigerator with temperature 4^{o}C. Parameters observed were number and variety of isolates, capability of the isolate in fixing nitrogen, solubilizing phosphate, and producing IAA.

1. Bacterial Isolation

Ten grams of iron sand soil were diluted with 90 mL sterile distilled water to make a dilution of 10-1, the serial dilution was continued up to 10-4. About 1 mL of 10-2 and 10-3 dilutions were inoculated onto Nutrient Agar medium using the pour plate method to know the total population of rhizobacteria. About 1 mL of 10-2 and 10-3 dilutions were inoculated onto Pikovskaya medium (Elias *et al*., 2016), Ashby medium, and Caceres medium. Assay on Pikovskaya medium was done to isolate bacteria capable of solubilizing phosphate, Caceres medium was done to isolate bacteria member of Azospirillum that capable of fixing N2, and Ashby medium was done to isolate bacteria member of Azotobacter that also capable of fixing N2 (Elias *et al.*, 2016). All plates were incubated at room temperature and each distinct colony appears in the medium. Colonies grown on Pikovskaya, Ashby, and Caceres medium were purified to make a pure culture. The selected isolates from Pikovskaya medium are capable forming a clear zone around the colony. The selected isolates from Ashby medium have yellow or creamy color, while from Caceres medium have pinkish or red color.

2. Assay of phosphate solubilizing ability (Mukamto *et al*., 2015)

A loopfull of the selected isolates colonies were inoculated onto Pikovskaya medium with spot inoculation and incubated for 4-7 days at room temperature. Isolates capable of solubilizing phosphate produced a clear zone around the colony on the medium. The clear zone appeared around the colonies was observed and calculated based on the formula (Wuryanto *et al*., 2015):

$$Index\ Phosphate = \frac{Solubilization\ Diameter}{Colony\ Diameter}\ x\ 100$$

3. Assay of nitrogen-fixing ability (Erfin *et al*., 2016)

The selected dominant isolates from Pikovskaya medium, Cacares medium or Ashby medium were tested for N_2 fixing in NfB semisolid medium. One loop of the isolates was stab inoculated onto NfB semisolid medium and incubated for 4-7 days at room temperature. All tubes were observed in the presence of a turbid ring on the subsurface of the NfB medium.

4. Assay of IAA production by bacteria (Gravel *et al*., 2007)

The ability of isolates which were able to produce IAA qualitatively was done by the colorimetric method using *Salkowski* reagent (Gordon & Weber, 1951). A loop of selected isolates was inoculated on Nutrient Agar medium that have been added with tryptophan, incubated for 5 x 24 hours at room temperature. After the incubation period, the medium was added with *Salkowski* reagent and incubated back in the dark for 30 minutes. The positive interpretation was observed when the medium color changes into pink.

5. Colony morphology observation

Macromorphology of potential isolates colony observed included size, shape, color, optical characteristic, surface layer, elevation, and margin. The chemical observation was conducted under the light microscope.

6. Cell morphology observation

a. Gram staining

A loop of the isolates was smeared onto an object-glass and fixated using a bunsen burner. Firstly, Gram A solution (*crystal violet)* was dropped to the smear for 60 sec. The object-glass was washed using sterile distilled water and let it dry. Gram B solution (*Lugol's iodine*) was dropped to the smear for 60 sec. The object-glass was washed using sterile distilled water and let it dry. Gram C solution (*ethanol* 96%) was dropped to the smear until no reagent that previously dropped. The object-glass was washed using sterile distilled water and let it dry. Gram D solution (*safranin*) was dropped to the smear for 45 sec. The object-glass was washed using sterile distilled water and let it dry. Finally, the smear was observed under a microscope. Gram-positive bacteria were stained violet, while Gram-negative bacteria were stained red. The cell shape was also observed.

b. Endospore staining

A loop of the isolate was smeared onto an object-glass and covered with straw paper. Drops of the malachite green solution were added to the covered smear. The object-glass was then heated over boiling water for 5 min then washed with sterile distilled water and let dry. Drops of the safranin solution were added for 45 min and washed again. The endospore was observed using a microscope, endospore was stained green and the cell was stained red.

c. Motility assay

The assay was done using the SIMA semi-solid medium. One loop of the isolate was stab inoculated and incubated for 48 hours at room temperature. Positive motility was indicated by the spreading pattern of the bacterial growth in the SIMA medium.

7. Biochemical characteristics

a. Catalase assay

A loop of the isolate was smeared onto an object-glass and added with H_2O_2 reagent. The ability of bacteria to produce catalase enzyme indicated by the production of bubbles.

b. Oxidase assay

A loop of the isolate was smeared onto an object-glass and was covered with tissue. Reagent *tetramethyl d-phenylenediamine dihydrochloride* was added onto the covered smear and the change of color on the tissue was observed. Dark blue color indicates the presence of oxidase enzyme.

c. Oxidative/fermentative assay

A loop of the isolate was inoculated onto the O/F medium. Liquid paraffin was added above the medium to create anaerobic condition then incubated for 5-7 days at room temperature. The fermentative reaction was indicated by the color change of the medium, from green to yellow, due to acid production. And if the medium stays green, the reaction is oxidative.

d. Amylolytic assay

A loop of the isolate was inoculated onto the Starch Agar (SA) medium by streak method and incubated for 2 x 24 hours at room temperature. After incubation, each isolate in the SA medium was flooded with *Lugol's iodine* reagent and was observed. The positive reaction was indicated by blue color in the medium.

e. Proteolytic assay

A loop of the isolate was inoculated onto the Protease Agar medium by streak method, then incubated for 2 x 24 hours at room temperature and was observed. The positive reaction was indicated by a clear zone around the colonies.

8. Nutritional and Physiological characteristics

a. Assay of sugar utilization and the acid production

One ose of the isolate was transferred to sugar medium containing phenol red. The sugar media used were glucose, lactose, sucrose, arabinose and mannitol, then incubation for 5-7 days at room temperature. The color change of the medium from red to yellow indicates sugar utilization and acid production.

b. Data Analysis

The characterization data were analyzed descriptively and the identification refers to *Bergey's Manual of Systematics Bacteriology 3rd edition* (2009).

RESULTS AND DISCUSSION

1. Isolation of Plant Growth Promoting Rhizobacteria

Iron sand soils at Sodong beach contain high iron (Fe), blackish in natural conditions, and low organic matter content. Soil physico-chemical analysis showed the level of acidity with the pH range from 6.5 to 7, temperature 30-35^{o}C, water content was from 4.5 to 12.5 % and humidity 45-50%. Widawati *et al*. (2005) reported that bacteria could be found in any type of soil but their population is varied because of the influence of soil texture and organic substrate in the soil. The ability of bacteria to survive in an extreme environment is due to their character to form spores which have thick strong sheathes. Bacteria can survive in extreme climate condition although temperature, humidity, pH, agriculture practice, fertilizers, pesticide, and the addition of organic matter can influence their population.

The bacterial population was from $1.59x\ 10^5$ to $5.2x10^5$ CFU.g^{-1} . The highest bacterial population was in rhizosphere 4 with an average of $3.61x10^5$ CFU.g^{-1} and for the lowest number was in rhizosphere 2 with an average of $2.1x10^5$ CFU.g^{-1}

(Table 1). These results are lower than reported by Amri (2018), that the average of total viable counts of bacteria in iron sand soil was $1.19x10^6$ CFU.g^{-1}. According to Puwaningsih (2005), the high number of the bacterial population is a sign that the fertility of the soil is high. Soil fertility comes from the activity of microorganisms that capable of degrading organic matter.

Table 1. The Bacterial Population Isolated from Rhizospheres of *Ipomoea* sp.

Location	Population (CFU.g^{-1})	Average (CFU. g^{-1})
Plant 1	3.11×10^5	$3.53x10^5$
	3.95×10^5	
Plant 2	1.59×10^5	$2.17x10^5$
	2.75×10^5	
Plant 3	3.25×10^5	$3.6x10^5$
	3.95×10^5	
Plant 4	2.03×10^5	$3.61x10^5$
	5.2×10^5	

There were 22 bacteria isolated from the rhizosphere of *Ipomoea* sp. A total of 7 isolates were isolated from Ashby medium, seven isolates were isolated from Pikovskaya medium, and eight isolates were isolated from Caceres medium (8 isolates). Colony characteristics of the isolates from Ashby medium were small, yellow, opaque, circular, raised, smooth, and entire. Characteristics of the isolates from Pikovskaya medium were moderate, creamy white, opaque, circular, raised, smooth, and lobate. Characteristics of the isolates from Caceres medium were small, pink and red colors, opaque, circular, convex, smooth, and entire.

2. Assay of Nitrogen-Fixing Ability

The 18 isolates were able to fix nitrogen (A1, A2, A4, A9, A10, C2, C3, C4, C6, C7, C10, C12, P2, P3, P4, P6, P7, and P10). It was characterized by the formation of a white ring on NfB medium and changes in the color of medium from the original green color to blue color (Table 3). The color change in the NfB medium is due to the presence of alkaline NH_3, causing the indicator bromothymol blue changes to blue. In the process of nitrogen fixation, bacteria will produce ammonia (NH_3). Kanimozhi & Panneerselvam (2010) reported that *Azospirillum* spp. could fix nitrogen, free-living in the soil or in association with roots of economically important grasses. The ability of the bacteria to fix N means these bacteria can provide N to the rhizosphere that will be used by plants and thus able to fertilize the soil. The positive effects of inoculation with *Azospirillum* were mainly derived from phytohormone production which induced morphological changes in plant roots, resulting in enhanced mineral and water uptake.

3. Assay of Phosphate Solubilizing Ability

Several isolates were capable of forming a clear zone on Pikovskaya medium because they produce organic acids. The organic acids leads to solubilization of insoluble phosphate into an available form. The results showed that 5 isolates (A4, A10, P2, P3, and P4) were able to solubilize phosphate with different clear zone diameters (Table 2).

Table 2. P Solubility Index of Rhizobacteria Isolated from Rhizosphere of *Ipomoea* sp. Growing in Iron Sand Soil

Isolate Codes	Diameter		P Solubilization Index
	Solubilization (mm)	Bacterial Colony (mm)	
A4	1.5	11.5	13.03
A10	2.5	19.5	12.82
P2	1	11.5	8.69
P3	1	15.5	6.45
P4	4	17.5	22.85

Phosphate solubility was measured by the clear zone formed on Pikovskaya medium. Isolate P4 showed the highest value of phosphate solubilization index of 22.85. The wider of the clear zone diameter formed, the higher the ability to solubilize phosphate. Pikovskaya medium is white turbid containing $Ca_3(PO4)_2$ bound (Zulaika, 2015). When phosphate apart in the medium, it will form a clear zone. The clear zone formed on Pikovskaya medium is determined as Phosphate Solubility Index (PSI). Widiawati & Suliasih (2006) state that the greatest ability of *Pseudomonas* and *Bacillus* as biofertilizers and due to they solubilize phosphate elements bound to other elements (Fe, Al, Ca, and Mg), so that the P element becomes available to plants.

4. Assay of IAA Producing Ability

The result showed that 2 bacterial isolates (C10 and P4) were obtained (Table 3), the ability of the isolates to produce IAA qualitatively can be seen by comparing the pink color formed after addition of Salkowski reagent. According to Kholida & Zulaika (2015), changes to the reddish color of the isolates after being dropped by the Salkowski reagent occur because of a reaction between Salkowski reagents and IAA or with several IAA forming compounds. Indole-3-acetic acid (IAA) binds to $FeCl_3$ and $HClO_4$, which are compounds that make up the Salkoswki reagent to form a tris-(indole-3-aceto) iron (III) complex which gives a reddish to red color. The reaction of the occurrence of red discoloration in the isolates after being dropped by the Salkowski reagent indicates the ability of these bacteria to metabolize L-tryptophan into IAA. Several studies showed that Indole 3 acetic acid (IAA) is produced by *Enterobacter* sp., *Pseudomonas* sp. and *Azospirillum* sp. IAA produced by bacteria is phytohormones which can accelerate plant growth. IAA hormone is

an endogenous auxin which plays a role in cell enlargement, inhibits the growth of side shoots, stimulates the stem growth, plays a role in the formation of xylem and phloem tissue, and also affects the development and elongation of roots (Herlina *et al*., 2016).

Table 3. Assay of Nitrogen Fixation, Phosphate Solubilization, and IAA Production

Isolate Codes	Assay		
	Nitrogen Fixation	Phosphate Solubilization	IAA Production
A1	+		
A2	+		
A4	+	13.03	
A5			
A8			
A9	+		
A10	+	12.82	
C2	+		
C3	+		
C4	+		
C5			
C6	+		
C7	+		
C10	+		+
C12	+		
P2	+	8.69	
P3	+	6.45	
P4	+	22.85	+
P6	+		
P7	+		
P10	+		
P11			

Note : + : positive interpretation
\- : negative interpretation

The selected isolates were potential for biofertilizer, due to their ability to fix N. A total of 18 isolates were able to fix nitrogen (A1, A2, A4, A9, A10, C2, C3, C4, C6, C7, C10, C12, P2, P3, P4, P6, P7, and P10), five isolates were able to solubilize phosphate (A4, A10, P2, P3, and P4) with the highest value of phosphate solubilization index of 22.85, and 2 isolates (C10 and P4) were able to produce IAA.

Beside biocontrol, PGPR functions as a biofertilizer because it can trigger plant growth by fixing nitrogen, solubilizing phosphate, and produce phytohormones (Vacheron *et al.,* 2013). Widawati & Suliasih (2006) reported that the addition of bacterial inoculants as biofertilizers increases the population of bacteria that can solubilize phosphates bound in the soil and fix nitrogen from the air. The microbial population in the soil is influenced by several factors, such as the type of nutrients, nutrients, pH, and temperature (Budiyanto, 2004).

Yusron & Azwar (2012) reported that the effect of giving *Pseudomonas fluorescens* and *Bacillus* sp. as biofertilizer could increase the growth and number of sengon leaves. *Bacillus* sp. and *Pseudomonas* sp. are reported could produces growth hormones (Backman *et al.,* 1994).

5. Characterization and Identification of PGPR Isolates

The selected isolates (A4, A10, C10, P2, P3, and P4) were characterized and identified based on their macromorphological, micromorphological, biochemical, nutritional and physiological characteristics (Table 4). All isolates macromorphologically had colony size small to moderate, optical characteristics mostly opaque (A4, A10, C10, P2, P3) and one isolate has translucent optical characteristics (P4), colonial form of five isolates was circular (A4, A10, C10, P2, P3) only one isolate has irregular form (P4), colonial elevation most isolates (A10, C10, P2, P3, P4) were raised, and one isolate was flat elevation (A4), colony surface of three isolates were smooth (C10, P2, P4) one isolate rough (P3) one isolate dry like powder (A10) and one isolate dope (A4), colony colors of two isolates yellow (A4, P2) two isolates creamy white (P3, P4) isolate A10, C10 was white and pink. Observation of micromorphological characteristics, all isolates were Gram-positive, rod-shaped, motile and mostly produce endospores (C10, P2, P3, and P4) while two isolates (A4, A10) did not produce endospores.

The results of biochemical characteristics showed that five isolates (A4, A10, C10, P2, P3) were catalase positive while one isolate (P2) catalase-negative, oxidase of five isolates (A4, A10, C10, P2, P3) negative while one isolate (P2) were oxidase-positive, five isolates (A4, C10, P2, P3, P4) were fermentative while one isolate (A10) oxidative, amylolytic assay of five isolates (A4, A10, C10, P2, P3) were positive and one isolate (P4) negative, proteolytic assay of five isolates (A4, C10, P2, P3 and P4) were positive and one isolate (A10) negative. Nutritional characteristics by sugar utilization assay using five sugars showed all isolates positive on glucose and arabinose, while all isolates negative on mannitol, two isolates positive on sucrose (P3, P4) and four isolates negative (A4, A10, C10, P2), on lactose only one isolate positive (A10) and five isolates negative (A4, C10, P2, P3, P4). Physiological characteristics by acid production assay showed, all isolates were negative on lactose and mannitol sugar, and only one isolate positive on glucose and sucrose (P3), on arabinose four isolates positive (A4, A10, P2, P4) 2 isolates negative (C10, P3).

Table 4. Characteristics of Selected Plant Growth Promoting Rhizobacteria Isolates

Characters	Isolates code					
	A4	A10	C10	P2	P3	P4
Macromorphology						
Size	Small	Small	Small	Small	Moderate	Moderate
Color	Yellow	White	Pink	Yellow	Cremy white	Creamy white
Optical characteristics	Opaque	Opaque	Opaque	Opaque	Opaque	Trans lucent
Shape	Circular	Circular	Circular	Circular	Circular	Irregular
Elevation	Flat	Raised	Raised	Raised	Raised	Raised
Surface	Dope	Dry like powder	Smooth	Smooth	Rough	Smooth
Margin	Entire	Entire	Undulate	Entire	Lobate	Lobate
Micromorphology						
Gram staining	+	+	+	+	+	+
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod
Endospore staining	-	-	+	+	+	+
Motility	+	+	+	+	+	+
Biochemical						
Catalase	+	+	+	-	+	+
Oxidase	-	-	-	+	-	-
O/F	F	O	F	F	F	F
Amylolytic	+	+	+	+	+	-
Proteolytic	+	-	+	+	+	+
Nutritional and Physiological						
Sugar utilization						
Glucose	+	+	+	+	+	+
Sucrose	-	-	-	-	+	+
Lactose	-	+	-	-	-	-
Arabinose	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-
Acid production						
Glucose	-	-	-	-	+	-
Sucrose	-	-	-	-	+	-
Lactose	-	-	-	-	-	-
Arabinose	+	+	-	+	-	+
Mannitol	-	-	-	-	-	-

Note : + : positive interpretation
- : negative interpretation
F : fermentative
O : oxidative

Based on *Bergey's Manual of Systematic Bacteriology*, the six potential isolates were belong to *Bacillus* (C10, P2, P3, and P4) and member of Actinomycetes (A4 and A10). According to De Vos *et al.* (2009), characteristics of the genus *Bacillus* are rod-shaped cells, straight or slightly curved, occurring singly and in pairs, some in chains, and occasionally as long filaments. Endospores are formed, Gram-positive, or Gram-positive only in early stages of growth, motile, aerobes or facultative anaerobes, but a few species are anaerobic. Catalase is produced by most species and oxidase can be positive or negative. The colonies of species vary from moist and glossy through granular to wrinkled, colony shapes vary from round to irregular, sometimes spreading, with entire through undulate or crenate to fimbriate edges. Color commonly ranges from buff or creamy-gray to off-white, but occasional strains may produce black, brown, orange, pink or yellow pigments; such pigmentation tends to be characteristic of species or subspecies. Elevations range from effuse through raised to convex.

Actinomycetes are bacteria that can produce antibiotics. They are filamentous Gram-positive bacteria, form spores, and have a high of G + C content 57 to 75%. Actinomycetes are often considered to be transitions between bacteria and fungi but are now better known as prokaryotic organisms. Identified actinomycetes are one of the main groups in soil populations (Kuster & Williams 1964). According to Ferfinia (2010), actinomycetes have branched hyphae which often develop into mycelium and have rod-shaped, with various colony colors because of their different pigment content in each constituent cell. According to Rao (1994), soils filled with water are not suitable for the growth of actinomycetes, while soils in dry and semi-arid regions can maintain a large enough population due to spore resistance to drought.

Further research is needed especially the application of the selected isolates to the growth of cultivated plants, because we know that biofertilizer is really needed to improve soil fertility and is expected to create the soil conditions which are safer

and avoid soil damage or pollution, besides that the use of biofertilizers must be accompanied by the given of sufficient organic fertilizer so that bacterial isolates are made as a biofertilizer can work optimally. In addition, complete characterization of the selected isolates is valuable.

CONCLUSIONS

A large number of bacteria from Rhizosphere of *Ipomoea* sp. growing in iron sand soil has been isolated. There are six bacterial isolates selected as biofertilizer candidate due to their ability to fix nitrogen, solubilize phosphate, and produce IAA. They were identified as *Bacillus* (C10, P2, P3, P4) and Actinomycetes (A4, A10).

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