BIODIVERSITAS
Volume 21, Number 4, April 2020
Pages: 1614-1624

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210442

The isolation, characterization endophytic bacteria from roots of local rice plant Kamba in, Central Sulawesi, Indonesia

SRI SUDEWI[1,2,♥], AMBO ALA[3,♥♥], BAHARUDDIN[4,♥♥♥], MUH. FARID[3], ♥♥♥♥

[1]Program of Agriculture, Graduate School, Universitas Hasanudin. Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Alkhairaat. Jl. Diponegoro No. 39, Palu 94221, Central Sulawesi, Indonesia. Tel.: +62-451-461123, ♥email: srisudewirahim@gmail.com
[3]Department of Agrotechnology, Faculty of Agriculture, Universitas Hasanudin. Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-586014, ♥♥email: amboala_aa@yahoo.com, ♥♥♥♥farid_deni@yahoo.co.id
[4]Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Hasanudin. Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-586014, ♥♥♥email: baharunhas@yahoo.com

Manuscript received: 6 February 2020. Revision accepted: 23 March 2020.

Abstract. *Sudewi S, Ala A, Baharuddin, Farid M. 2020. The isolation, characterization endophytic bacteria on from roots of local rice plant Kamba in Central Sulawesi, Indonesia. Biodiversitas 21: 1614-1624.* The local "Kamba" rice plants are cultivated by the Bada Valley people for generations by obtaining seeds from previous crops. This rice plant is able to survive even though it is cultivated with traditional cultivation techniques. The ability to survive is suspected because this local rice plant is associated with endophytic bacteria through a variety of mechanisms including being able to produce the IAA hormone and its ability to dissolve phosphate. This study aims to find endophytic bacteria that have the potential to produce IAA and phosphate solvents through morphological and physiological characterization (Gram reaction test with 3% KOH, catalase test, hypersensitivity reaction and hemolysis activity), screening of IAA-producing bacteria qualitative and quantitative, phosphate dissolution activity on Pikovskaya medium, and quantitative using a spectrophotometer. Endophytic bacteria isolates from Gintu give the highest yields in IAA production and phosphate solubility activity, namely RKGU11 and RKGU6, respectively at 4,905 mg L^{-1} and 10.984 mg L^{-1} so that it has the potential to be used as a candidate for biofertilizer agents in developing effective sustainable agriculture, respectively, efficient and environmentally friendly.

Keywords: Endophytic bacteria, IAA hormone, Kamba, phosphate solubilization, rice

INTRODUCTION

Isolation and characterization of endophytic bacteria from various types of plants with various interests has been widely carried out in terms to obtain a superior endophytic bacteria, especially in agriculture. Bacteria that have the potential on improving the plant growth could be developed as an alternative to synthetic fertilizers for sustainable crop production and as a qualified agent for biological fertilizers so that to increase the food crop production such as rice (*Oryza sativa* L.).

The lack of information about the presence of endophytic bacteria in the "Kamba" Local Rice plant and its potential for rice plants itself has encouraged the efforts to explore endophytic bacteria in these local rice plants.

Endophytic bacteria are neutral bacteria, isolated from the surface of sterilized plant tissue, live in plant tissues without causing symptom of disease in their host plant, and directly benefit their host plant by increasing plant nutrient uptake and modulating phytohormones which can induce the growing plant. (Miliute et al. 2015; Santoyo et al. 2016; Afzal et al. 2019). These bacteria are able to produce the growth-promoting substances such as Indole-3-Acetic acid (IAA), Abscisic acid (ABA), Gibberellic acid (GA) and Cytokinin (CTK) which support plants to absorb nutrients so that they can help plants grow better even in unfavorable environmental conditions (Ma et al. 2016). This is also supported by the study by (Etesami and Alikhani 2016; Etesami et al. 2015) which stated that the production of phytohormones by bacteria can stimulate plant growth under both normal and stress environmental condition.

In addition, the endophytic bacteria can stimulate the plant growth through tethering of N2 (diazotrophic bacteria) (Gopalakrishnan et al. 2018; Silveira et al. 2016), phosphate dissolution (Khamwan et al. 2018; Valetti et al. 2018), and their ability as biocontrol agent in increasing plant resistance to plant pest and diseases (Djaya et al. 2019; Abdallah et al. 2016; Mohammed et al. 2019).

The presence of endophytic bacteria in plant tissues is influenced by both biotic and abiotic factors. The endophytic bacteria that are associated with plants depend on the plant genotypes, age, tissue taken and also the season when isolation is carried out. Environmental factors also take an effect such as soil properties, organic matter in the soil, cultivation techniques, fertilization, and the application of pesticides (Munif et al. 2012).

Bada Valley or Napu Valley is located in Poso, Central Sulawesi with an altitude of 1000-1200 meters above sea level (masl) surrounded by mountain forest that is still natural. This valley is part of the Lore Lindu National Park and customary forest. This area is separated by the Lariang River, which divides this area into two sub-districts namely South Lore District (Gintu, Bewa, Badang'kaia, Runde,

Bakekau, Bulili, Pada and Bomba Villages) and West Lore District (Lelio Village, Kolori, Lengkeka, Kageroa, Tomehipi, and Tuare). The identification of microorganisms types in this region is not yet available until now. This study aims to identify the types of endophytic bacteria isolated from the Kamba local rice rhizosphere which has the potential to be used as a source of biological fertilizers including plant growth booster bacteria. The results of this study are expected to be useful in order to support a sustainable agricultural system in the local area.

MATERIALS AND METHODS

Study area

This research was conducted is from October 2018 to January 2019 at the Laboratory of Plant Biotechnology Research Activity Center (PKP) Hasanuddin University Makassar. The endophytic bacteria were isolated from the roots of the Kamba rice plants in the paddy fields of the residents in the Bada Valley, Poso Central Sulawesi, which consisted of six different locations, namely Bakekau Village (1°54'34.01"S120°15'27.18"E), Badang'kaia (1°54'58.56"S; 120°15'20.26"E), Kolori (1°51'35.73"S; 120°16'46.71"E), Bulili (1°53'40.10"S; 120°14'33.13"E), Gintu (1°53'26.21"S; 120°14'45.07"E), and Lelio (1°51'28.62"S; 120°14'31.38"E). The root samples were carried out by removing a healthy rice plant gently from the three sampling points in each location. Then plant samples were transferred into labeled A5 brown envelope and then put into a cooler box and taken to the Laboratory. Samples were analyzed as soon as possible before reaching 48 hours after being taken from the field (Forster 1995).

The isolation and morphology and physiological characterization of endophytic bacteria

5 grams of root sample was washed using sterile water and dried over the petri dish coated with filter paper. The surface was sterilized by soaking in the sterile water for 1 minute, 70% alcohol for 1 minute, and rinsing with sterile water 3 times. Then the root sample was dried using sterile tissue (Strobel and Daisy 2003). Isolation of endophytic bacteria by a multilevel dilution method from 10^{-2} to 10^{-6} using sterile aquadest by grinding the root tissue using mortars. Dilution is carried out by taking 1 ml of the root solution that had been crushed using a micropipette and then put in a test tube containing 9 ml of sterile aquadest, so that a suspension with a dilution rate of 10^{-1} is obtained. Further dilution is carried out in the same manner until a 10^{-6} suspension is obtained. Each bacteria suspension from 10^{-2} to 10^{-6} dilution was taken as much as 0.1 mL and then inoculated in a petri dish containing the nutrient agar (NA) medium with a *spread plate* method and then incubated at room temperature 28ºC for 24 hours. Every single colony that growth, is gradually purified one by one using the *streak plate* method (Jawetz et al. 2013). Individual colonies from plates were analyzed macroscopically for characterizing the morphology. This step was done by observing at size (pinpoint, small, moderate, large); pigmentation (colony color); colony form (circular, irregular, rhizoid, filamentous); colony edge (entire, lobate, undulate, serrate, curled, filamentous/erose); and elevation (flat, raised, convex, umbonate, pulvinate). Individual colonies were also picked to obtain pure isolates.

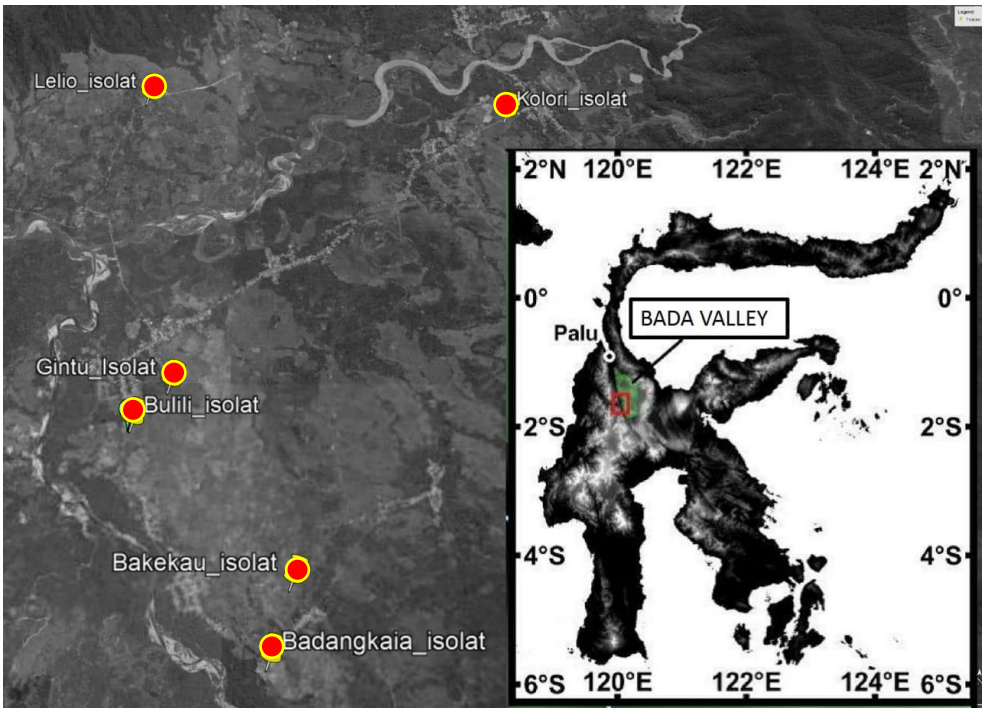

Figure 1. Locations of sampling sites in Bada Valley, Poso District, Central Sulawesi, Indonesia

Gram test

The testing of Gram reaction using 3% KOH was carried out by making a pure culture through picking 1 loop of individual colonies which had been cultivated on NA media using an ose needle and plated on the slide microscope which had been given two drops of 3% KOH solution (*Potassium Hydroxide*) then stirred rotating clockwise repeatedly and lift it slowly. A positive reaction implied that the bacterial colonies were Gram-negative (-) which will appear slimy, sticky like thread when lifted from the surface of the suspension. While a negative reaction implied to Gram-positive (+) which the colony will appear not slimy, and when lifted from the surface of the suspension directly regardless (Suslow et al. 1982).

Catalase test

The catalase test was performed to analyze the ability of the microbial on producing catalase enzyme to degrade Hydrogen Peroxide (H_2O_2). The production of catalase was analyzed by plating 1 (full) loop of pure culture of individual colony on the object-glass which had been applied with two drops of 3% Hydrogen Peroxide. The positive reaction from the catalase test was indicated by the existence of gas bubbles from free Oxygen, while negative reactions did not.

Hypersensitivity reaction test

Endophytic bacteria isolates were tested for hypersensitivity reactions in tobacco plants (*Nicotiana tabacum* L.) aged ± 3 months to determine bacterial isolates with potential pathogens. As much as 1 mL of endophytic bacteria isolate suspension is injected into leaf tissue with a density of 10^8 CFU/mL through secondary leaf bone using a 1 ml syringe (without needle). The positive control is used *Burkholderia glumae*, while the negative control used sterile aquadest. Observations were made after 24 hours to 120 hours to see the hypersensitive reaction in tobacco plants that have been injected. Brownish necrosis spots and dryness in tobacco leaf tissue indicate a positive hypersensitivity reaction, otherwise, if necrosis does not occur then the resulting reaction is a negative hypersensitivity reaction (Abdallah et al. 2016).

Hemolysis activity test

Endophytic bacteria isolates were cultured on Blood Agar media and incubated for 24 to 48 hours at room temperature of 37ºC. Hemolysis testing is carried out to determine the bacteria that have hemolytic activity (which is potentially pathogenic to humans and animals). This method refers to Sorokulova et al. (2008). The formation of a clear zone around the colony in the Blood Agar media is a positive indicator that the bacteria can do lysis on the Blood Agar media so that its use is not safe for humans or animals, and vice versa if the clear zone around the colony does not form a negative indicator.

The analysis of endophytic bacteria ability to produce IAA

The ability of endophytic bacteria on producing IAA hormone was analyzed by growing the bacteria on Nutrient Agar (NA) media containing 200 ppm L-tryptophan. 10 ml suspension of bacteria isolates with a cell number of 10^8 CFU/ mL equivalent to Mc Farland (Bressan and Borges 2004) were transferred into a fresh tube then centrifuged at 8000 rpm for 10 minutes. 2 mL of supernatant was taken and then put into a fresh tube then added with 2 drops of Orthophosphoric acid and 4 mL Salcowski reagent (50 mL, 35% Sulfuric acid (H_2SO_4) 1 mL, 0.5 M Iron Trichloride solution ($FeCl_3$) (Glickmann and Dessaux 1995). The tube was incubated in the darkroom for 24 hours. The color change was observed. The reaction between the Salcowski reagent and the supernatant of endophytic bacteria isolates resulted in a pink color which is an indicator that shows the ability to produce the IAA hormone. The optical solution was then measured at a wavelength of 535 nm using a UV-VIS spectrophotometer (Genesys 10S UV 840208100). IAA level produced by endophytic bacteria were determined from the results of a linear plot of standard IAA absorbance value. The IAA standard curve used a regression equation (Y = 0.0632x + 0.0189; R2 = 0.9897) which was made from serial dilution of the IAA stock solution, with IAA concentrations ranging from 0 to 5.0 mg L^{-1}. The concentration in the culture filtrate was determined and expressed as mg L^{-1}.

The ability of phosphate solubilizing endophytic bacteria

The ability of bacteria to dissolve phosphate is characterized by the formation of clear zones around the colony that can be tested using Pikovskaya media referring to Verma and Srivastav (2017) modified with the addition of Bromophenol blue 0.01 g l^{-1}. Endophytic bacteria isolates were cultured in solid Pikovskaya media which had been added to Bromophenol blue. The spot inoculation method was used in culturing bacterial isolates and then incubating for 3 days at room temperature of 28ºC. Colonies that grow and are able to form clear zones around the colony are indicated as isolates that are able to dissolve phosphate. The measurement of the phosphate solubilization efficiency (PSE) and phosphate solubilization index (PSI) used the following formula (Premono et al. 1996).

$$PSE = \frac{\text{The diameter of clear zone}}{\text{The colony diameter}} \; x \; 100$$

$$PSI = \frac{\text{The colony diameter} + \text{The diameter of clear zone}}{\text{The colony diameter}}$$

Quantitative determination of phosphate concentration

Quantitative determination of phosphate concentration in endophytic bacteria isolates using a modified spectrophotometric method. Endophytic bacteria isolates were cultured in 30 mL liquid Pikovskaya medium consisting of 10 g Glucose ($C_6H_{12}O_6$); Tricalcium Phosphate (Ca_3 $(PO_4)_2$) 5 g; Ammonium Sulfate ($(NH_4)_2SO_4$) 0.5 g; Potassium Chloride (KCl) 0.2 g; Yeast extract 0.5 g; Magnesium Sulfate ($MgSO_4$) 0.1 g; Sodium Chloride (NaCl) 0.2 g; Manganese Sulfate Monohydrate

($MnSO_4.H_2O$) 0.002 g; Ferrous Sulphate Heptahydrate ($FeSO_4.7H_2O$) 0.002 g. All materials were dissolved with sterile aquadest to a volume of 1 L, homogenized and then shaker for 7 days at 28ºC. After 7 days, a culture of 1.5 mL was centrifuged for 15 minutes at 10,000 rpm in order to separate the supernatant. 1 mL of the supernatant was reacted with 3 mL of sterile water and 1 mL of color reagent containing Ammonium Molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) 1.5% (w/v) solution, 5.5% H_2SO_4 Sulfuric acid (v/v) and 2.7% Iron Sulfate ($FeSO_4$) solution (w/v) in the test tube. The change in color of the supernatant to blue indicates a positive reaction (Lynn et al. 2013). Furthermore, the absorbance of the supernatant was measured using a UV-VIS spectrophotometer (Genesys 10S UV 840208100) at 693 nm. Phosphate concentration was measured using (standard Titrisol Curve (PO_4) made from dilution with Titrisol Concentrations ranging from 0 to 2.5 mg L^{-1}. Concentrations in culture filtrate were determined and expressed as mg L^{-1}.

RESULT AND DISCUSSION

The isolation and characterization of endophytic bacteria from the roots sample of kamba rice

There are 35 isolates that have obtained from the isolation of the rhizosphere samples of Kamba Local Rice in Bada Valley, Poso Regency and there were 19 isolates of Kamba root samples taken from six different locations shows various characteristics and potentials on producing IAA. These isolates are 2 isolates from Bakekau Village (RKBA), 3 isolates from Badang'kaia (RKBK), 3 isolates from Kolori (RKBL), 2 isolates from Bulili (RKBU), 3 isolates from Gintu village (RKGU) and 6 isolates from Lelio (RKLE). The results of morphological characterization are performed in Table 1.

The endophytic bacterial isolates obtained generally show different morphological characteristics. The grouping by size results dot size, small size, and medium-size which are 1, 11, and 7 colonies respectively. The grouping by shape is dominated by 16 colonies in circular/rounded shape, while the rest is 3 colonies in irregular shape. The edge of the colony varies flat, wavy, curved and jagged. The colony elevation resulted from macroscopic characterization shows 13 colonies with flat elevation, 1 convex colony, and 5 colonies having elevated elevation. The color of the bacterial colonies varied from yellow, beige, clear, and white, which is dominated by 9 colonies with the cream color (Table 1). The difference in colony color is due to the presence of intracellular pigment produced by bacteria (Mitra et al. 2014; Samyuktha and Mahajan 2016) and the microbial pigments contribute to the pathogenesis of the disease by showing cytotoxic properties (Ramesh et al. 2019).

The morphological characterization shows no similarity among 19 isolates. It means that the isolates obtained are a different type. Further analysis is needed to find out every type of each endophytic bacteria isolates. The morphological characterization of endophytic bacterial isolates can not describe both the genus and species of bacteria, as it requires several advanced biochemical tests and molecular identification.

Table 1. The morphological characterization analysis of endophytic bacteria isolated from Kamba local rice

Isolate code	Colony morphology					Gram	Catalase	Reaction	Hemolysis
	Size	Colony form	Colony edge	Elevation	Colony color	(+/-)	(+/-)	hypersensitive	(+/-)
RKBA 1	Small	Circular	Entire	Umbonate	Yellow	-	+	-	-
RKBA 3	Small	Circular	Entire	Raised	Cream	+	+	-	-
RKBK 1	Small	Circular	Entire	Flat	Cream	+	+	-	-
RKBK 3	Small	Irregular	Undulate	Raised	Yellow	+	+	-	-
RKBK 4	Small	Circular	Entire	Flat	Cream	+	+	-	-
RKBL 5	Small	Circular	Entire	Flat	Yellow	-	-	-	-
RKBL 6	Moderate	Circular	Entire	Flat	Cream	-	-	-	-
RKBL 7	Pinpoint	Irregular	Lobate	Flat	Cream	-	+	-	-
RKBU 1	Small	Circular	Entire	Flat	Transparent	-	+	-	-
RKBU 5	Small	Circular	Serrate	Raised	White	-	+	-	-
RKGU 6	Small	Circular	Entire	Flat	Cream	-	+	-	-
RKGU 11	Moderate	Circular	Entire	Flat	Cream	-	+	-	-
RKGU 12	Small	Circular	Entire	Flat	White	-	+	-	-
RKLE 2	Moderate	Circular	Entire	Flat	Cream	-	+	-	-
RKLE3	Moderate	Circular	Undulate	Flat	Cream	-	+	-	-
RKLE5	Moderate	Circular	Entire	Raised	White	+	+	-	-
RKLE 6	Moderate	Circular	Entire	Flat	White	-	+	-	-
RKLE 7	Moderate	Irregular	Undulate	Raised	White	+	+	-	-
RKLE 15	Small	Circular	Entire	Flat	Yellow	-	+	-	-

Note: RKBA: Isolate of Bakekau, RKBK: Isolate of Badang'kaia, RKBL: Isolate of Kolori, RKBU: Isolate of Bulili, RKGU: Isolate of Gintu, RKLE: Isolate of Lelio

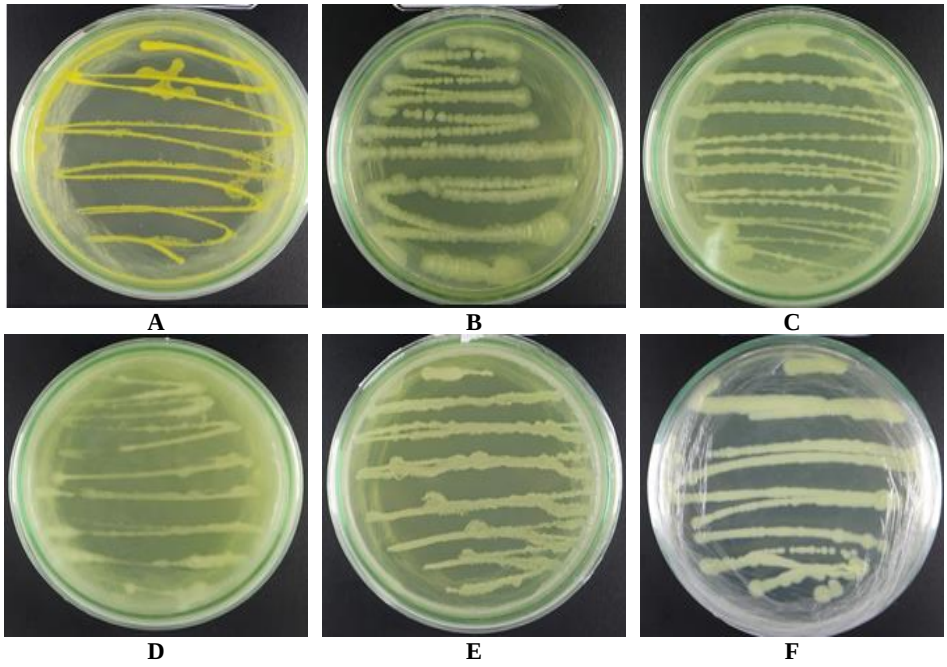

Figure 2. Pure culture of isolation endophytic bacteria from several locations in the Bada Valley, Poso District, Central Sulawesi, Indonesia. A. RKBA1, B. RKBK3, C. RKBL7, D. RKBU5, E. RKGU11, F. RKLE7

The result of Gram test using 3% Potassium Hydroxide (KOH) shows 68.42% Gram-negative group and 31.57% Gram-positive group (See Table 1). The Gram method using Potassium Hydroxide is easier and more practical and provides a better diagnosis than the Gram staining procedure (Dash and Payyappilli 2016).

The group of Gram-negative bacteria is thin cell wall in thick periplasmic space and fat whereas Gram-positive bacteria are thick cell walls and thin fat. The Potassium Hydroxide used will attack fat (lipid bilayer) and make thin cell wall of Gram-negative bacteria break while Gram-positive cell bacteria are not affected (Chandra and Mani 2011). The isolates of non-pathogenic bacteria both Gram-positive and Gram-negative have an important role as biocontrol agents in controlling plant diseases (Amaria et al. 2019).

The catalase test for endophytic bacteria isolates is dominated by positive catalase, and only 2 isolates are negative catalase. 17 endophytic bacterial isolates that have been added with Hydrogen Peroxide solution produce bubbles which indicate that these samples positively produce the catalase enzyme, while the rest 2 isolates do not (See Table 1). The negative catalase bacteria do not have the catalase enzyme which can break Hydrogen Peroxide into water and Oxygen (Pulungan and Tumangger 2018).

The catalase enzyme is commonly found in almost all living organisms that carry out metabolism with the help of Oxygen. This enzyme is very popular in the global market as it is used in various industrial and medical processes (Babiker et al. 2016). In respiration, bacteria produce a variety of components which one of them is Hydrogen Peroxide. This component is a poison that can damage the bacterial metabolic system. Bacteria will experience death if they are not able to break down Hydrogen Peroxide into other harmless compounds. This process can be done if there is a catalase enzyme. Some bacteria that have the enzyme catalase or peroxidase are able to break down Hydrogen Peroxide into water and Oxygen so that it will soon form a defense system from the toxic Hydrogen Peroxide which produces itself (Murali and Patel 2017). The parameter that shows the presence of catalase activity in the presence of oxygen bubbles.

The hypersensitivity reaction test of 19 endophytic bacteria isolates injected into tobacco leaves showed no symptoms of necrosis until 120 hours after inoculation (see Table 1), which had similarities with negative controls (Figure 4). This indicates that the 19 bacteria isolates did not have the potential to be pathogenic to plants, so the isolates could be potential candidates for biofertilizer agents. A very clear change occurred in the positive control that had been inoculated by the isolation of the *Burkholderia glumae* pathogenic bacterium which showed symptoms of necrosis and even the leaf tissue seemed to dry out (Figure 4).

All endophytic bacteria isolates from the hypersensitivity reaction test were used in this hemolysis activity test. Test results obtained for 19 endophytic bacterial isolates that had been incubated in the Blood Agar media for 24 to 48 hours showed negative hemolysis activity results (no clear zone formation around bacterial isolates) (Figure 5). Bacterial isolates with negative hemolysis activity test results (See Table 1) indicate that these bacterial isolates are safe for human and animal use because they have no potential to cause disease.

Figure 3. The result of Gram test using 3% KOH (A) Gram-negative marked with a slimy-looking colony, (B) Gram-positive characterized by dilute and not slimy colonies, (C) Positive reaction Catalase test (+) presence of gas bubbles, Negative reactions (-) no gas bubbles

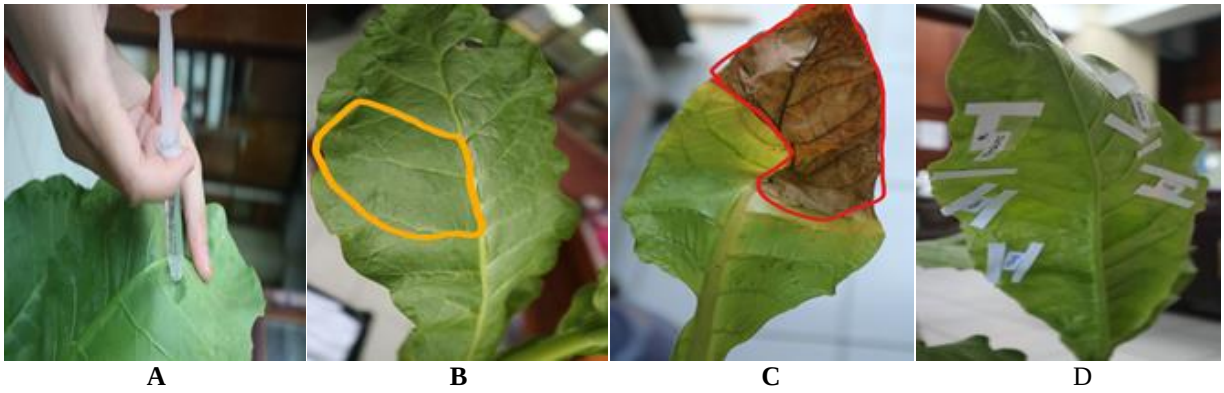

Figure 4. Hypersensitive reactions in *Nicotiana tabacum* L. plant at 72 days after inoculation. (A) Suspension injection using a 1 mL syringe, (B) Negative control reaction (-), (C) Positive control with *Burkholderia glumae* showed necrotic symptoms (+), (D) Endophytic bacteria isolate showed is hypersensitive adverse negative reactions

The production of IAA by endophytic bacteria

The ability of endophytic bacteria to produce IAA varies based on the type of isolate and location of the sampling. The qualitative testing results (See Figure 6) show that the variation in the color change of the suspension becomes pink in the process of testing IAA production in all Kamba endophytic bacterial isolates after the application of Salcowski reagent compared to the control.

The IAA concentration produced by the isolates of RKGU11 bacteria from Gintu shows the highest production of 4.905 mg L^{-1} followed by the sample from the same location (RKGU6) which is 3.651 mg L^{-1}. But on the contrary, the isolates of RKBK4 endophytic bacteria from Badang'kaia results in the lowest IAA concentration of 0.460 mg L^{-1} (See Figure 7). While the sampling picked from the Lelio village results IAA concentrations varied between 0.762 mg L^{-1} (RKLE15) to 1.254 mg L^{-1} (RKLE7). This difference is allegedly due to the type varies based on the location of the sampling of plants and the type of bacteria. The variation of types and strains in the same genus can produce varies IAA due to environmental factors, growth rate, and availability of substrates such as amino acids and other N sources (Souza et al. 2015; Susilowati et al. 2018).

The IAA production resulted from bacteria plays a role in promoting plant growth so that the synthesis by certain bacteria is the reason that causes an increasing rate in plant growth (Herlina et al. 2016). The endophytic bacteria from plant roots have been shown to be better candidates in inducing plant growth as the complex interactions between bacteria and plant root tissues (Castanheira et al. 2017).

Qualitative and quantitative analysis of phosphate solubilization

The ability of endophytic bacteria isolates to dissolve phosphate was qualitatively determined by culturing bacterial isolates in Pikovskaya solid media modified with the addition of Bromophenol blue 0.01 g L^{-1}. The test results showed that 19 isolates of endophytic bacteria were shown to be able to form clear zones around bacterial colonies on solid Pikovskaya media (Figure 8) with different clear zone diameters and colony diameters (Table 2).

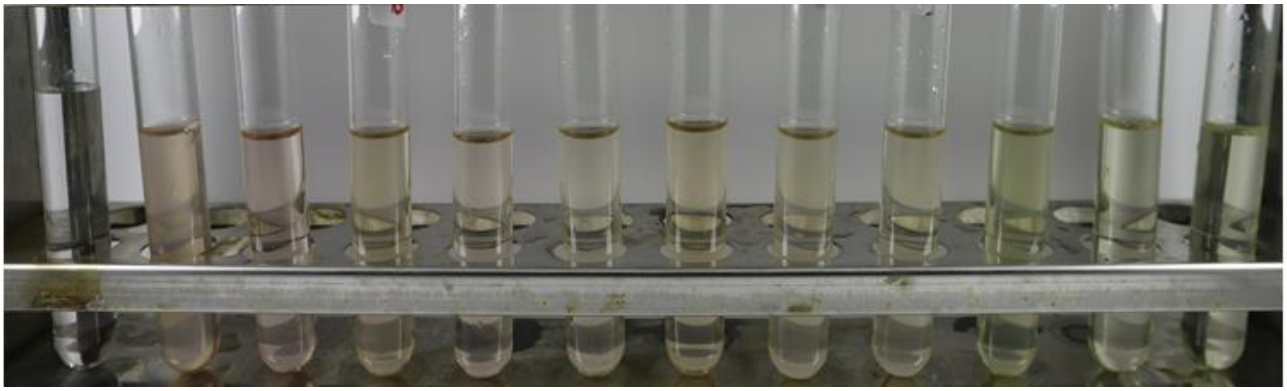

Figure 6. The qualitative analysis of IAA production using Salkowski reagent (discoloration of the supernatant to pink)

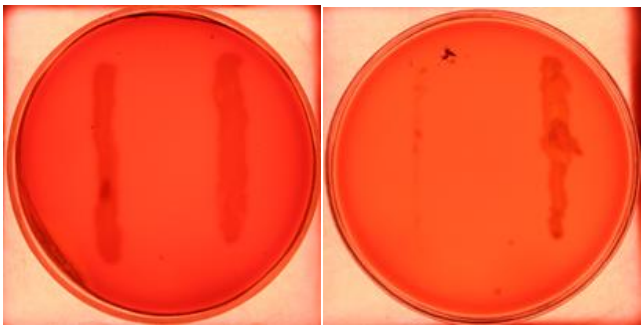

Figure 5. Endophytic bacteria isolates reaction on Blood Agar media showed no symptoms of hemolysis

Indicator of the ability of bacteria to dissolve phosphate is characterized by its ability to grow on solid Pikovskaya media and form clear zones around the colony. Cherchali et al. (2019) reported that the activity of phosphate dissolution depends on the activity of bacteria that can grow and form clear zones on Pikovskaya media.

The activity of phosphate solubilization by bacteria is expressed in the phosphate solubilization index. The results showed that the phosphate solubilization index varied between 2.00 to 2.53 (Table 2). This depends on the ability of each bacteria isolate to release organic acid compounds.

The ability of endophytic bacteria isolates to dissolve phosphate quantitatively was determined by the presence of dissolving Ca-phosphate (Tricalcium Phosphate) activity on liquid Pikovskaya media. The test results showed the highest concentration of phosphate dissolution was found in RKGU6 isolate of 10,984 mg L^{-1} while the lowest was RKBL6 of 6,560 mg L^{-1}. The concentration value of phosphate solubilization of bacteria isolates was measured using a UV-VIS 693 nm spectrophotometer (Figure 9). Types of strains of bacteria isolates, growth of microorganisms in culture, phosphate sources, and environmental conditions are things that greatly affect differences in the level of concentration of phosphate dissolution produced by bacteria isolates.

Table 2. The qualitative analysis of phosphate solubilization ability in endophytic bacteria isolates

Isolate code	Phosphate solubilization ability			
	Diameter of clear zone (cm)	Colony diameter (cm)	Phosphate solubilization efficiency (SE)	Solubilization index (SI)
RKBA 1	1.20	0.91	131.87	2.32
RKBA 3	1.13	1.10	102.73	2.03
RKBK 1	1.00	0.91	109.89	2.10
RKBK 3	1.16	0.86	134.88	2.35
RKBK 4	1.32	1.10	120.00	2.20
RKBL 5	1.01	0.73	138.36	2.38
RKBL 6	1.00	0.70	142.86	2.43
RKBL 7	1.26	0.98	128.57	2.29
RKBU 1	0.75	0.71	105.63	2.06
RKBU 5	1.06	0.91	116.48	2.16
RKGU 6	1.43	1.16	123.28	2.23
RKGU 11	1.36	1.18	115.25	2.15
RKGU 12	1.15	0.75	153.33	2.53
RKLE 2	0.86	0.86	100.00	2.00
RKLE 3	0.88	0.63	139.68	2.40
RKLE 5	0.68	0.65	104.62	2.05
RKLE 6	1.03	0.76	135.53	2.36
RKLE 7	1.35	1.06	127.36	2.27
RKLE 15	0.93	0.88	105.68	2.06

Discussion

One of the characterization activities can be done by testing the morphological characteristics of the colony which includes size, shape, color, and edges. A total of 19 endophytic bacterial isolates originating from the roots of the local Kamba rice plant at six different locations showed different colony morphological characteristics (Table 1). This is in line with (David et al. 2016) reported that differences in sampling locations, rainfall and cultivation aspects are factors that affect the diversity of endophytic fungal characters. The results showed that the size of the colony was dominated by a small size with varying colony colors, yellow, beige, clear and white. Almost all isolates have a circular colony shape, some are irregular with a flat, ascending and convex elevation, while the edges of the

colony are flat, bumpy, curved, and jagged. Endophytic bacterial isolates that were characterized consisted of 6 bacterial isolates classified as Gram-positive, while 13 others were classified as Gram-negative. This difference is due to the cell wall structure of bacterial isolates consisting of peptidoglycan for Gram (+) and cell wall structure consisting of lipids for Gram (-).

Another characteristic of endophytic bacteria is the biochemical physiology test of bacterial isolates in producing catalase enzymes. In general, 17 isolates of endophytic bacteria gave a positive reaction in the presence of gas bubbles in bacterial isolates that were given two drops of Hydrogen Peroxide 3%, while 2 isolates showed negative reactions (no gas bubbles formed). This indicates that a positive reaction in the endophytic bacterial isolate is capable of producing the enzyme catalase. Hydrogen Peroxide is the end product produced by bacteria during aerobic metabolism. Sahu et al. (2019) reported that if Hydrogen Peroxide in bacterial cells is allowed to accumulate, it can damage the metabolic system of bacteria and even cause death. Hydrogen Peroxide can be broken down if a bacterium can produce a catalase enzyme. The catalase enzyme will convert Hydrogen Peroxide into water and oxygen, so that it is not harmful to bacteria.

The response of plants to pathogens that arise in plant tissue can be determined by conducting hypersensitivity reaction tests on tobacco leaves. Hypersensitivity reaction test to 19 endophytic bacterial isolates showed negative necrotic symptoms on tobacco leaves (Figure 4). Symptoms of necrosis, leaf drying will be seen if the cellular membrane of tobacco leaves has contact with pathogenic bacteria. The response shows that bacteria have potential as plant pathogens. Isolates with positive hypersensitivity reactions cannot be used in further testing because they have the potential to cause disease to plants.

The same thing was also shown in hemolysis activity testing that all 19 endophytic bacterial isolates did not produce dangerous hemolysis activity because no clear zone was formed around the bacterial colony that had been cultured on Blood Agar media (Figure 5).

IAA hormone production by endophytic bacteria quantitatively shows varying degrees of concentration, from 0.460 mg L^{-1} to 4.905 mg L^{-1} (Figure 7). This difference is thought to be due to the condition of each endophytic bacteria sampling site consisting of six locations of rice fields. In addition, it is suspected that the type of bacteria strain from each location is also different so there is a difference in the ability to convert tryptophan contained in the media into IAA. Auxin is the most common phytohormone secreted by endophytic bacteria in plants that play a role in stimulating the formation of lateral roots, adventitious roots and root hairs, providing nutrition for roots so as to enhance root growth and development (Hilbert et al. 2012; Sahu et al. 2019).

The best phosphate solvent bacteria's ability to produce the diameter of the halo zone with the largest area compared to other bacteria isolates colonies, while isolates that do not form clear zones are unable to dissolve phosphate. The results showed that all endophytic bacteria isolates were able to dissolve phosphates with different colony diameters and clear zone diameters produced (Figure 8; Table 2).

Phosphate is a macronutrient that is very important for plant growth and development. Its presence in the soil is abundant in the form of organic P and inorganic P, but most are in insoluble form (around 95-99%) so that it becomes unavailable for plants to be absorbed so that the use of phosphate solvent endophytic bacteria is needed in helping to provide the phosphate nutrients needed by plant (Sanjotha et al. 2011; Oteino et al. 2015; Wiratno et al. 2019).

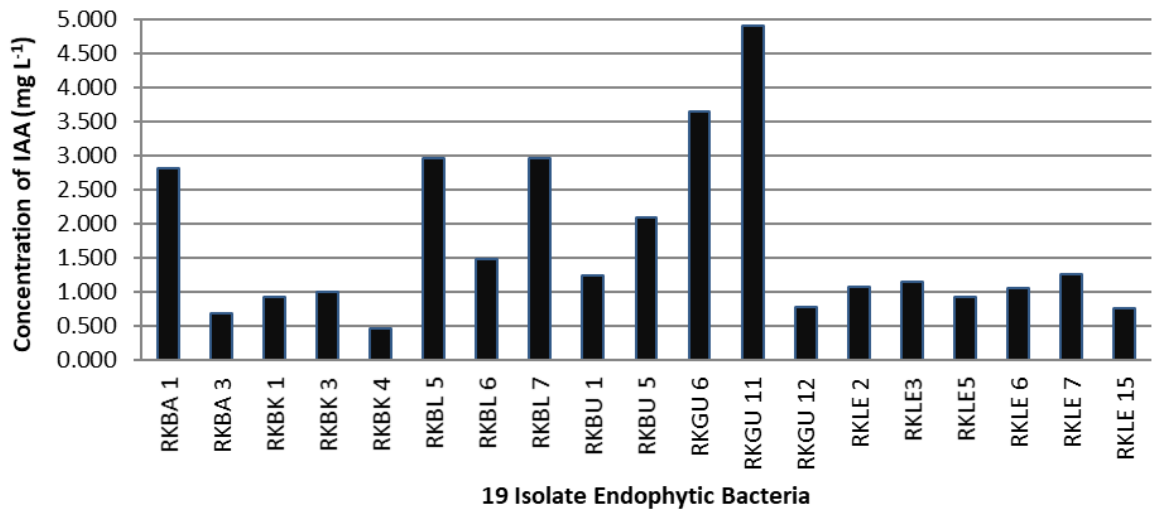

Figure 7. The quantitative analysis of IAA production in several endophytic bacterial isolates using a UV-VIS spectrophotometer 535 nm

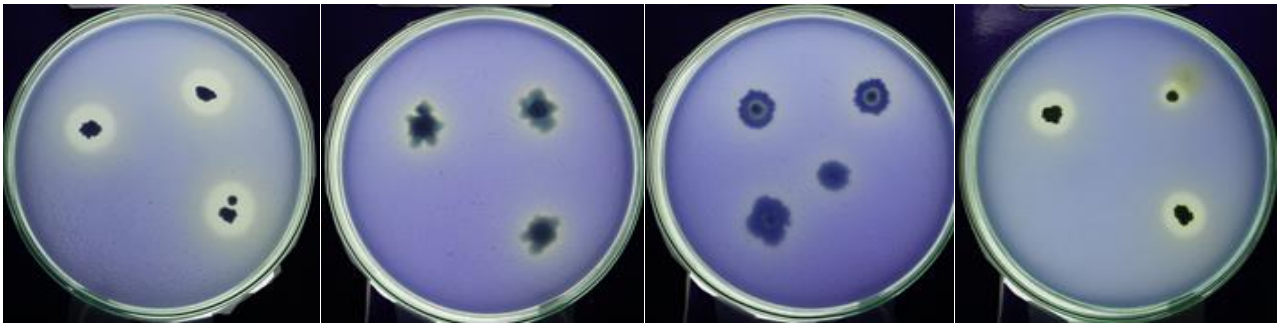

Figure 8. The qualitative analysis of endophytic bacteria isolates to solubilize phosphate on Pikovskaya medium with the addition of Bromophenol blue 0.01 g L^{-1}

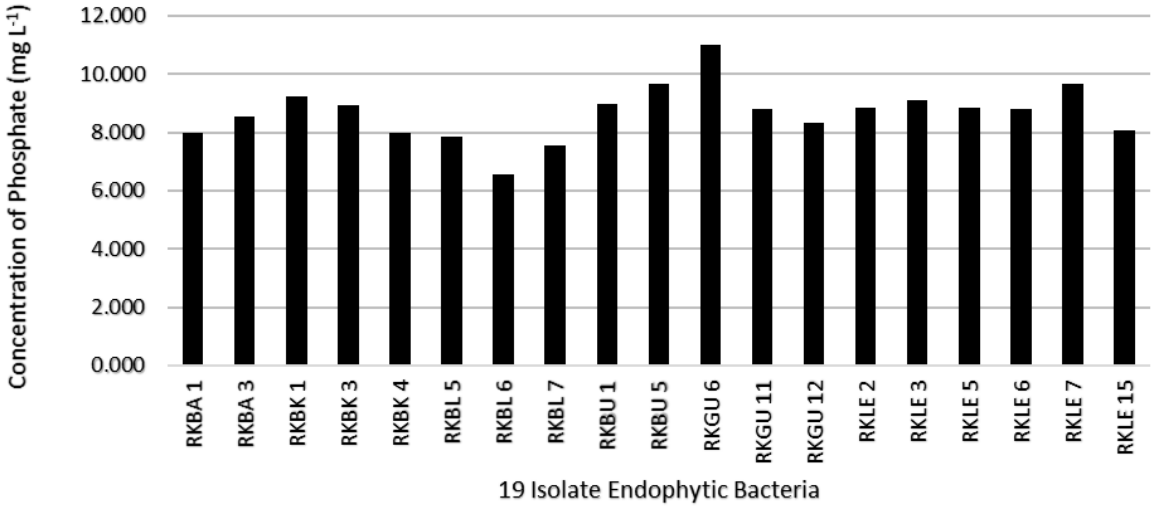

Figure 9. The quantitative analysis of phosphate dissolution ability on endophytic bacteria isolates using spectrophotometer UV-VIS 693 nm

Table 2 shows that the phosphate solubilization index (PSI) by endophytic bacterial isolates ranged from 2.00 to 2.53, while the phosphate solubilization efficiency (PSE) ranged from 100.00 to 153.33. The index of phosphate dissolution and the efficiency of phosphate dissolution give different values. This is influenced by the ability of each different bacterial isolate. This is in line with Astriani et al. (2020) that endophytic bacteria isolates have different abilities in releasing organic compounds. The phosphatase enzyme can be produced by phosphate solvent bacteria associated with plant roots if the availability of phosphate in the soil is low. The role of the phosphatase enzyme is to release phosphates bound by organic compounds such as *citric acid*, *glutamate*, *succinic*, *lactic*, *ocsalate*, *glycoocsalat*, *fumarate*, *tartaric* and *alpha-ketobutiric acids* into forms available to plants so that they can be easily absorbed by plant roots.

A total of 19 endophytic bacteria isolates from a total of 35 bacteria isolates found in the roots of the local Kamba rice plant have the ability to produce IAA hormones and dissolve phosphate. This is indicated by the change in color of the supernatant to pink and the different levels of IAA hormone concentrations found in each isolate of endophytic bacteria. Likewise, the formation of clear zones around the colonies on solid Pikovskaya media indicates the ability of endophytic bacteria isolates to dissolve phosphate. The highest levels of IAA concentration and phosphate dissolution concentration were shown by RKGU11 and RKGU6 endophytic bacteria isolates. Both isolates were from the same location, namely Gintu Bada Valley, Central Sulawesi. RKGU11 and RKGU6 endophytic bacteria isolates also exhibit hypersensitivity reactions and negative hemolysis activity, so their use does not cause pathogens for plants or endanger humans and animals. This indicates that the endophytic bacteria isolates from the rhizosphere of the local Kamba rice plant can be used as candidates for biofertilizer agents to be further developed to support sustainable agriculture that is environmentally friendly, effective and efficient in reducing the use of synthetic chemical fertilizers.

ACKNOWLEDGEMENTS

The author would like to show gratitude to the Kemenristekdikti-LPDP (Lembaga Pengelola Dana Pendidikan) for funding this research; the local farmer in the Bada Valley, Poso District, Central Sulawesi, Indonesia and all parties involved in helping and facilitating the processes from research sampling, analyzing in the Laboratory until the process of publishing this article.

REFERENCES

Abdallah R, Trabelsi B, Nefzi A, Khiareddine H, Remadi D. 2016. Isolation of endophytic bacteria from *Withania somnifera* and assessment of their ability to suppress Fusarium wilt disease in tomato and to Promote Plant Growth. J Plant Pathol Microbiol 7: 5. DOI: 10.4172/2157-7471.1000352.

Afzal I, Shinwari ZK, Sikandar S, Shahzad S. 2019. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res 221: 36-49. DOI: 10.1016/j.micres.2019.02.001.

Ahmad WA, Ahmad WYW, Zakaria ZA, Yusof NZ. 2012. Isolation of pigment-producing bacteria and characterization of the extracted pigments. In: Application of Bacterial Pigments as Colorant. Springer, Berlin.

Amaria W, Kasim NN, Munif A. 2019. Kelimpahan populasi bakteri filosfer, rizosfer dan endofit tanaman kemiri sunan (*Reutealis trisperma* (Blanco) Airy Shaw), serta potensinya sebagai biokontrol. Tabaro 3 (1): 305-317. [Indonesian]

Astriani M, Zubaidah S, Abadi AL, Suarsini E. 2020. *Pseudomonas plecoglossicida* as a novel bacterium for phosphate solubilizing and indole-3-acetic acid-producing from soybean rhizospheric soils of East Java, Indonesia. Biodiversitas 21 (2): 578-586. DOI: 10.13057/biodiv/d210220.

Babiker MB, Ahmed M AE, H Ibrahim. 2016. Isolation &identification of catalase producing *Bacillus spp*: a comparative study. Intl J Adv Res 4 (2): 1206-1211.

Bressan W, Borges MT. 2004. Delivery methods for introducing endophytic bacteria into maize. Biocontrol 49 (3): 315-322. DOI: 10.1023/B: BICO.0000025372.51658.93.

Castanheira NL, Dourado AC, Pais I. 2017. Colonization and beneficial effects on annual ryegrass by mixed inoculation with plant growth-promoting bacteria. Microbiol Res 198: 47-55. DOI: 10.1016/j.micres.2017.01.009.

Chandra TJ, Mani PS. 2011. A study of 2 rapid tests to differentiate Gram-positive and Gram-negative aerobic bacteria. J Med Allied Sci 1 (2): 84-85.

Cherchali A, Boukhelata N, Kaci Y, Abrous-Belbachir O, Djebbar R. 2019. Isolation and identification of a phosphate-solubilizing *Paenibacillus polymyxa* strain GOL 0202 from durum wheat (*Triticum durum* Desf.) rhizosphere and its effect on some seedlings morphophysiological parameters. Biocatal Agric Biotechnol 19: 101087. DOI: 10.1016/j.bcab.2019.101087.

Dash C, Payyappilli JR. 2016. KOH string and *Vancomycin* susceptibility test as an alternative method to Gram staining. J Int Med Dent 3 (2): 88-90. DOI: 10.18320/jimd/201603.0288.

David AS, Seabloom EW, May G. 2016. Plant host species and geographic distance affect the structure of aboveground fungal symbiont communities, and environmental filtering affects belowground communities in a coastal dune ecosystem. Microb Ecol 71 (4): 912-926. DOI: 10.1007/s00248-015-0712-6.

Djaya L, Hersanti, Istifadah N, Hartati S, Joni IM. 2019. In vitro study of plant growth-promoting rhizobacteria (PGPR)and endophytic bacteria antagonistic to *Ralstonia solanacearum* formulated with graphite and silica nanoparticles as a biocontrol delivery system (BDS). Biocatal Agric Biotechnol 19. DOI: 10.1016/j.bcab.2019.101153.

Etesami H, Alikhani HA. 2016. Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant (*Oryza sativa* L.). Rhizosphere 2: 5-12. DOI: 10.1016/j.rhisph.2016.09.003.

Etesami H, Alikhani HA, Hosseini HM. 2015. *Indole-3-Acetic acid* (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth-promoting agents. MethodsX 2: 72-78. DOI: 10.1016/j.mex.2015.02.008.

Forster JC. 1995. Soil sampling, handling, storage and analysis. In: Methods in applied soil microbiology and biochemistry (pp. 49-121). Academic Press. DOI: 10.1016/b978-012513840-6/50018-5.

Glickmann E, Dessaux Y. 1995. A critical examination of the specificity of the Salcowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61 (2): 793-796.

Gopalakrishnan S, Srinivas V, Vemula A, Samineni S, Rathore A. 2018. Influence of diazotrophic bacteria on nodulation, nitrogen fixation, growth promotion and yield traits in five cultivars of chickpea. Biocatal Agric Biotechnol 15: 35-42. DOI: 10.1016/j.bcab.2018.05.006.

Herlina L, Pukan KK, Mustikaningtyas D. 2016. Kajian bakteri endofit penghasil IAA (*Indole Acetic acid*) untuk pertumbuhan tanaman. Sainteknol 14 (1): 51-58. DOI: 10.15294/sainteknol.v14i1.7616. [Indonesian]

Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A. 2012. Indole derivative production by the root endophyte Piriformospora indica is not required for growth promotion but for biotrophic colonization of barley roots. New Phytol 196 (2): 520-534.

Jawetz, Adelberg's M. 2013. Medical microbiology. Mc Graw Hill Med. 26th ed. The McGraw-Hill Companies: United States DOI: 10.1016/B978-0-323-40181-4.00114-6.

Khamwan S, Boonlue S, Riddech N, Jogloy S, Mongkolthanaruk W. 2018. Characterization of endophytic bacteria and their response to plant growth promotion in *Helianthus tuberosus* L. *B*iocatal Agric Biotechnol 13: 153-159. DOI: 10.1016/j.bcab.2017.12.007.

Lynn TM, Win HS, Kyaw EP, Latt ZK, and San San Yu. 2013. Characterization of phosphate solubilizing and potassium decomposing strains and study on their effects on tomato cultivation. Int J Innov Appl Stud 3 (4): 959-966.

Ma Y, Rajkumar M, Zhang C, Freitas H. 2016. Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manag 174: 14-25. DOI: 10.1016/j.jenvman.2016.02.047.

Miliute I, Buzaite O, Baniulis D, Stanys V. 2015. Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste-Agric DOI: 10.13080/z-a.2015.102.060.

Mitra D, Arup Kumar Mondal, Saikat Acharya, Arijit Mukhopadhyay. 2014. Isolation and characterization of some intracellular pigmented bacteria from soil & coal powder. Res Biotechnol 5 (6): 24-32.

Mohammed BL, Toama N. 2019. Biological control of Fusarium wilt in tomato by endophytic rhizobacteria. Energy Procedia 157: 171-179. DOI: 10.1016/j.egypro.2018.11.178.

Munif A, Wiyono S, Suwarno S. 2012. Isolasi bakteri endofit asal padi gogo dan potensinya sebagai agens biokontrol dan pemacu pertumbuhan. J Fitopatol Indones 8 (3): 57-64. DOI: 10.14692/jfi.8.3.57.

Murali A, Patel S. 2017. The effect of different heavy metal acetate solutions on the inhibition of catalase enzyme. J South Carolina Acad Sci 15 (2): 68-74.

Otieno N, Culhan J, Germaine KJ, Brazil D, Ryan DA, Dowling DN. 2013. Screening of large collections of plant associated bacteria for effective plant growth promotion and colonisation. Aspects Appl Biol 120: 23-28.

Premono ME, Moawad AM, Vlek PLG. 1996. Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere (No. REP-12113. CIMMYT.). Indones J Crop Sci 11: 13-23.

Pulungan AS, Tumangger DE. 2018. Isolation and characterization of endophytic bacteria producing catalase enzyme from buasbuas (*Premna pubescens* Blume) leaves. J Biologi Lingkungan dan Kesehatan 5 (1): 72-80. [Indonesian]

Ramesh C, Vinithkumar NV, Kirubagaran R, Venil CK, Dufossé L. (2019). Multifaceted applications of microbial pigments: current knowledge, challenges and future directions for public health implications. Microorganisms 7 (7): 186. DOI: 10.3390/microorganisms7070186.

Sahu AK, Kar BRK, Sahu SS, Achary, Pragnya P, Harichandan PS. 2019. Isolation, characterization of endophytic bacteria from *Psidium guajava* plant and screening of their antibiotic activities. Int J Res Appl Sci Eng Technol 7 (1): 345-355. DOI: 10.22214/ijraset.2019.1061.

Sanjotha P, Mahantesh P, Patil CS. 2011. Isolation and screening of efficiency of phosphate solubilizing microbes. Int J Microbiol Res 3 (1): 56-58. DOI: 10.9735/0975-5276.3.1.56-58.

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR. 2016. Plant growth-promoting bacterial endophytes. Microbiol Res 183: 92-99. DOI: 10.1016/j.micres.2015.11.008.

Silveira APD da, Sala VMR, Cardoso EJBN, Labanca EG, Cipriano MAP. 2016. Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation. Appl Soil Ecol 107: 313-319. DOI: 10.1016/j.apsoil.2016.07.005.

Sorokulova IB, Pinchuk IV, Denayrolles M. 2008. The safety of two *Bacillus* probiotic strains for human use. Dig Dis Sci 53 (4): 954-963. DOI: 10.1007/s10620-007-9959-1.

Souza de R, Ambrosini A, Passaglia LMP. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38 (4): 401-419. DOI: 10.1590/S1415-475738420150053.

Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67 (4): 491-502. DOI: 10.1128/mmbr.67.4.491-502.2003.

Susilowati DN, Riyanti EI, Setyowati M, Mulya K. 2018. *Indole-3-Acetic acid*-producing bacteria and its application on the growth of rice. AIP Conf Proc 2002 (1): 020016. 10.1063/1.5050112. [Indonesian]

Suslow T, Schroth M, Isaka M. 1982. Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. Am Phytopathol Soc 72 (7): 917-918.

Valetti L, Iriarte L, Fabra A. 2018. Growth promotion of rapeseed (*Brassica napus*) associated with the inoculation of phosphate solubilizing bacteria. Appl Soil Ecol 132: 1-10. DOI: 10.1016/j.apsoil.2018.08.017.

Verma DK, Srivastav PP. 2017. Proximate composition, mineral content and fatty acids analyses of aromatic and non-aromatic Indian rice. Rice Sci 24 (1): 21-31. DOI: 10.1016/j.rsci.2016.05.005.

Wiratno, Syakir M, Sucipto I, Pradana AP. 2019. Isolation and characterization of endophytic bacteria from roots of Piper nigrum and their activities against Fusarium oxysporum and Meloidogyne incognita. Biodiversitas 20 (3): 682-687. DOI: 10.13057/biodiv/d200310.