BIODIVERSITAS
Volume 21, Number 2, February 2020
Pages: 578-586

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210220

Pseudomonas plecoglossicida as a novel bacterium for phosphate solubilizing and indole-3-acetic acid-producing from soybean rhizospheric soils of East Java, Indonesia

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Manuscript received: 11 November 2019. Revision accepted: 17 January 2020.

Abstract. *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: 578-586.* The use of synthetic fertilizers to grow soybean (*Glycine max* (L.) Merrill) in a long time, can increase the risk of environmental damage. Therefore, the current study aimed to find phosphate solubilizing bacteria that can produce indole-3-acetic acid (IAA) hormone to minimize the use of chemical fertilizers. Superior isolates selected through characterization of phosphate solubilization activity on Pikovskaya medium, screening of IAA producing bacteria, quantitative estimation of phosphate solubilizing activity using a spectrophotometer, hypersensitivity assay, antagonist within isolate assay, and molecular identification of selected bacterial isolates using 16S rRNA sequencing with primer *forward* 63f and primer *reverse* 1387r. Among the isolated bacteria, isolate Arj8 showed the highest phosphate solubilizing activity and IAA production. Molecular identification indicated that isolate Arj8 shared 100% similarity with *Pseudomonas plecoglossicida*. The highest phosphate solubilizing activity (75.39 mg/L) and IAA production (38.89 ppm) recorded on day-3. Multiple potentialities of *P. plecoglossicida* as phosphate solubilizing and IAA producing bacterium are a novel finding in the development of bioinoculants as bio-fertilizers that can reduce dependency on synthetic chemical fertilizers.

Keywords: *Pseudomonas plecoglossicida,* phosphate solubilization, rhizobacteria, rhizosphere soybean

INTRODUCTION

East Java is one of Indonesia's soybean [*Glycine max* (L.) Merrill] largest producer with high levels of self-sufficiency (Zainuri et al. 2015; Hasan et al. 2015; Putra et al. 2017). Statistics Indonesia (2016) reported that 35% or 344.99 thousands of tons of the total soybean production in 2015 (963.18 thousands of tons) originated from East Java. Fertilization is one of the determining factors of soybean productivity. Synthetic chemical fertilizers such as NPK, urea, and TSP have used as nitrogen, phosphate, and potassium supply for soybean (Kristanti et al. 2017). However, the use of these chemical fertilizers can bring a negative impact on soil fertility by leaving hazardous residues to the surrounding environment (Kumar 2014). The use of synthetic phosphate fertilizers can be minimized by administering phosphate solubilizing bacteria (PSB) into the soil. PSB plays a key role in the mobilization and transformation of phosphate underground. The benefits of PSB as biofertilizers are alternatives to solving the phosphate availability issue for plants (Wei et al. 2017).

Even though soil contains a large amount of phosphorus, its availability for plants is very low because phosphorus can easily react with other compounds such as iron, aluminum, and calcium to form sediments (Weil and Brady 2017). The availability of phosphate in the soil can increase with the help of soil microbes (Wei et al. 2017). Rhizospheric soils contain more abundant microbes compared to ordinary soils due to the release of root exudates that can attract microorganisms (Li et al. 2016). Root exudates are plant metabolites secreted by the roots of living plants. Root exudates such as isoflavones play a crucial role in biological processes, including nutrient absorption, plant defenses, and signaling for rhizospheric bacteria (Tsuno et al. 2018). During phosphate deficiency, plant root exudates determine changes in the concentration of metabolites produced by rhizobacteria. In mycorrhiza, metabolites produced include phosphatase enzyme which plays a vital role in the release of organic acids in the soil (Tawaraya et al. 2014).

Bacteria that inhabit and colonize plant roots are called plant growth promotion rhizobacteria (PGPR) (Singh 2018). PGPR serve an important function to improve plant growth through various mechanisms including producing phosphatase enzymes to support mineralization of phosphate in soil (Hungria et al. 2015; Fallo et al. 2015) and synthesizing natural compounds as plant growth promoters such as indole acetic acid (IAA) (Li et al. 2018; Qin and Huang 2018). IAA hormone can regulate many aspects of plant growth and development, cell division, and cell elongation (Majda and Robert 2018) and initiates lateral root formation (Jing and Strader 2019). However,

applications of synthetic IAA harmful for plants. Compared to other types of synthetic hormones such as naphthalene acetic acid (NAA) 2,4-D and gibberellic acid (GA), IAA provoked the highest cytotoxic reactions (Solomon and Bradly 2014). An alternative to reducing the use of synthetic compounds is to replace it with natural compounds.

Indole acetic acid (IAA) is the most common and most active hormone found in nature. Around 80% of IAA produced by microbes that live around plant roots can be used to stimulate plant growth (Spaepen and Vanderleyden 2011; Glick 2012). This study aimed to isolate phosphate solubilizing bacteria that can also produce IAA hormone from soybean rhizospheres in East Java. The results of this study expected to provide insights into the development of bio inoculants that can serve as eco-friendly, effective, and efficient fertilizer for plants in the future.

MATERIALS AND METHODS

Area study

The current study was conducted from November 2018 to April 2019 at the Microbiology Laboratory of Faculty of Mathematics and Natural Sciences of Universitas Negeri Malang, Indonesia. Samples of the rhizospheric soybean soil were collecting from soybean plantations in Binangun Village, Blitar Regency, East Java. The GPS data from the sampling location (S 8 º11'.38.9 E 112º 20'03.6) (https://www.google.co.jp/maps).

Materials

A laminar airflow, an autoclave, an Erlenmeyer flask, an oven, a hot plate, a bunsen burner, a spatula, an incubator, a shaker, a spectrophotometer, a petri dish, a micropipette, a microtube, a microscope, a centrifuge, a 1 mL syringe, and a digital camera were the tools used in this study. Materials used were soybean rhizospheric soil samples, *Nicotiana tobaccum* L., alcohol 70%, primer 63F and 1387R, nutrient broth (NB) medium, nutrient agar (NA) medium, Pikovskaya medium containing 10 g glucose; $Ca_3(PO4)_2$, 5 g; $(NH_4)_2SO_4$, 0,5 g; NaCl 0,2 g; $MgSO_4.7H_2O$, 0,1 g; KCl, 0,2 g; yeast extract 0,5 g; $MnSO_4.H_2O$, 0,002 g; $FeSO_4.7H_2O$, 0,002 g (Nautiyal, 1999); Salkowski reagent (150 ml H_2SO_4, 7,5 ml $FeCl_3.6H_2O$ 0,5 M, and 250 ml sterile aqua dest), and L-Tryptophan (Gang et al. 2019).

Procedures

Sample collection

Soil sampling carried out using a composite method where soil samples were collecting from 3 points around the roots of soybean plants with a depth of 5-10 cm. A total of 100 grams of soil sample was taken from the location and then mixed and put in a plastic bag. One gram of soil weighed for bacterial isolation (Chaiharn and Lumyong 2009).

Isolation of phosphate solubilizing bacteria

Ten grams of rhizosphere soil samples were weighed and put into 90 mL sterile physiological saline solution 0.85% NaCl. Dilution performed from 10^{-1} to 10^{-6}. The sample inoculated in Duplo at 10^{-4}, 10^{-5}, 10^{-6} dilution on solid Pikovskaya medium, then incubated at room temperature for three days (Susila et al. 2016). Colonies that formed clear zones on the Pikovskaya medium were marked. Then, the solubilization index (SI) of each colony measured with a ratio (Mubarik et al. 2014):

$$\text{Phosphate Solubilization Index} = \frac{\text{diameter of the clear zone-diameter of the colony}}{\text{the diameter of the colony}}$$

Quantitative estimation of phosphate solubilizing activity

Bacterial isolates were grown in 50 mL liquid Pikovskaya medium and incubated for 48 hours. One mL of inoculant was transferred into 100 mL liquid Pikovskaya medium and incubated. Every 24 hours (in 6 days), 1.5 mL of culture centrifuged. Centrifugated to separate the supernatant. One mL of supernatant was reacted with 2.5 mL of sodium molybdate 2.5% and 1 mL of hydrazine sulfate 0.3% and heated for 10 minutes. A positive reaction was determined by the color change of the mixture from white into the blue. The mixed solution was measured using a spectrophotometer at the 830 nm wavelength (Lynn et al. 2013).

Screening of Indole Acetic Acid (IAA) producing bacterial isolate

Bacterial isolates indicating phosphate solubilizing activity underwent screening for the potential for indole acetic acid (IAA) production. One loop of the selected bacterial isolate was inoculated in 50 ml NB medium added with 1.0 mM L-tryptophan and incubated in a shaking incubator until its cell density reached 10^8 cells/ml. One milliliter of bacterial culture was grown on 100 ml NB medium and incubated at an agitation speed of 100 rpm. Estimation of IAA was conducted by collecting 1mL of supernatant and pouring 4mL of Salkowski reagent into it. The culture incubated in a dark room for 15 minutes. The level of IAA production was measured using a spectrophotometer at 520 nm (Mahmoud et al. 2015).

Hypersensitivity assay

Bacterial hypersensitive reaction to tobacco (*Nicotiana tobaccum L.)* was examined through suspension injection using a 1 mL syringe. The response was observed from 24h to 72 h after inoculation time. A positive reaction was indicated by the appearance of brown spots or necrotic symptoms around the injected area, whereas the absence of symptoms of necrosis considered a negative reaction (Abdallah et al. 2016).

Antagonist within isolate assay

Bacterial culture was grown in NB for 24 hours. 400 µL of culture put in a Petri dish containing a semi-solid nutrient agar medium. Wells made using a sterile straw on the homogeneous culture so that other isolates can be tested. Isolates and sterile aqua dest were used as a negative control, while kanamycin (1 mg/mL) used as a positive

control. 15-20 µL of kanamycin was dropped onto the culture and incubated for 24 hours at room temperature (Fitriyanti et al. 2017).

Bacterial identification

Morphological and physiological identification of bacteria

Bacterial identification performed by observing the morphological shape of the colony, gram staining, and physiological testing. The observation of the colony morphology included the shape, margin, elevation, and color of the colony. Gram staining conducted to investigate the type and shape of the bacterial cell (Hadioetomo 1993). Physiological testing used *Microbact* 12A/12E or 24E Kit. Wells on the kit contained testing media. A hundred µl of bacterial suspension was dripped into the wells. Then, the Microbact incubated at 37 ºC for 24 hours. Number seven well on the Microbact added with two drops of Nitrate A and B reagent, while number eight well on the Microbact added with two drops of Indole Kovacs. VP I and VP II were dripped onto number ten well, and TDA was dripped onto number twelve well. Carbohydrate fermentation testing on Microbact twelve B conducted without the addition of a reagent. Reading for positive and negative results was performed by comparing the color table (Oxoid, 2004), while bacterial identification conducted using the Bergeys Manual of Determinative Bacteriology ninth ed (Holt et al. 2000).

Isolation of bacterial genomic DNA

Bacterial culture (1.5 mL) was collected and move into an *Eppendorf* 1.5 ml tube, then centrifuged at 8000 rpm for 10 minutes. The supernatant was separated, and the pellet cleaned with STE buffer (composition: 0.3 M sucrose; 25 mM Tris-HCL; 25 mM EDTA.2Na pH 8), centrifuged at 8000 rpm for 10 minutes. The pellet rinsed three times. The supernatant was discarded and added with 200 µL STE buffer and 45 µL lysozyme (20 mg/mL), shaken slowly, and incubated at 55 ºC for an hour until protoplasts there formed. 20 µL of proteinase-K (20 mg/mL) was added into the solution and incubated at 55 ºC for 60 minutes. After that, 400 µL of CTAB 10% was added into 0.7 M NaCl and incubated at 65 ºC for 30 minutes. Then, a 1x volume of 1 phenol: chloroform (25:24) was poured into the solution and centrifuged for 10 minutes. The clear phase was transferred into a new tube and added with a 0.6x volume of isopropyl alcohol and 20 µL sodium acetate, incubated at-20ºC overnight. The solution centrifuged at 12000 rpm for 10 minutes. The supernatant removed and 1 mL pellets washed using alcohol 70%. The DNA was let dry for an hour so that the alcohol removed. After that, it was dissolved into 50 µL sterile ddH_2O. The result of the DNA isolation stored at a temperature of 4ºC or-20 ºC (Modified from Sambrook and Russel 2001).

Amplification of bacterial isolate 16S rRNA gene

Amplification of bacterial isolate 16S rRNA gene was conduct using polymerase chain reaction (PCR) and prokaryotic specific primers (Marchesi et al. 1998), which are forward 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse 1387r (5′-GGG CGG WGT GTA CAA GGC-3′). PCR reaction was composed of 0.5 µL of La Taq DNA polymerase enzyme, 2x 25 µL of GC buffer, 8 µL of dNTP mixture, 1.5 µl of primer (10 pmol), 9.5 µL of ddH_2O, and 4 µL of DNA template. The PCR conditions used were pre-denaturation (94 ºC, 4 minutes), denaturation (94 ºC, 45 seconds), annealing (55 ºC, 1 minute), elongation (72 ºC, 1 minute 10 seconds), and post PCR (72 ºC, 7 minutes), 30 cycles in total. The separation of DNA was conducted on mini-gel Electrophoresis Equipment using 1% agarose gel, 75 Volt for 45 minutes. DNA visualization was performed on UV transilluminator using Ethidium Bromide (EtBr) stain.

DNA sequencing and phylogenetic tree construction

Raw data on sequencing were trimmed and assembled using ChromasPro program version 1.5. The assembled data were analyzed using BLAST containing gene registered at the NCBI/ National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). The result of the BLAST sequencing analysis considered as species with the closest kinship. The species strain type from GenBank data at the NCBI. The DNA sequence was aligned using MEGA 6.1 program (Tamura et al. 2011). Phylogenetic tree showing kinship of the Xyl_22 bacterial isolates constructed using Actinomycete, while other non-actinomycete microbes were analyzed using the Neighbor-Joining Tree method with 1000 bootstrap replications (Felsenstein 1985).

RESULTS AND DISCUSSION

Isolation of phosphate solubilizing and IAA producing bacteria

Based on the result of the bacterial isolation, eight bacterial isolates found to be able to grow on a solid Pikovskaya medium. These bacterial isolates purified for identification of colony shape, margin, elevation, and color. Table 1 shows that the colony shape of the eight isolates was dominated by circular shape, while the margin was mostly entire and the elevation was mostly flat. Six isolates were white color and two isolates were cream. The eight isolates were assumed to have the ability to dissolve phosphate, indicated by the clear zones formed around the bacterial colony.

The ability of the isolates to solubilize phosphate was re-examined qualitatively by dripping bacterial cultures on solid Pikovskaya medium. The bacterial phosphate solubilizing activity expressed in the phosphate solubilization index (SI). Six isolates were proven to be able to form clear zones around the bacterial colonies on the Pikovskaya medium (Figure 1).

Table 1. Characteristics of bacterial isolate

Isolate code	Morphological characteristics			
	Shape	Margin	Elevation	Color
Arj1	Circular	Entire	Convex	Milky white
Arj2	Circular	Entire	Flat	Cream
Arj3	Circular	Entire	Flat	White
Arj4	Circular	Entire	Flat	White
Arj5	Circular	Entire	Flat	Transparent
Arj6	Irregular	Undulate	Umbonate	Transparent
Arj7	Circular	Entire	Flat	Transparent
Arj8	Concentric	Entire	Convex	Cream

Figure 1. Testing the ability of eight isolates to solubilize phosphate on Pikovskaya medium (1) Arj1, (2) Arj2, (3) Arj3, (4) Arj4, (5) Arj5, (6) Arj6, (7) Arj7, and (8) Arj8

Pikovskaya medium normally contains tricalcium phosphate and is a turbid white color. Therefore, isolates that can grow and form clear zones on the medium are isolates that can dissolve phosphate elements. Similarly, Atekan et al. (2014) reported that the ability of isolates to dissolve phosphate characterized by the formation of clear zones around bacterial colonies. Clear zones are an indication of bacterial activity in dissolving phosphate contained on the medium.

The solubilization index (SI) of each bacterial isolate ranged from 0.125 to 0.61 (Table 2). Solubilization index (SI) may vary depending on the ability of bacterial isolates to secrete organic acids. Phosphate solubilizing mechanisms are often associated with the release of organic acids by phosphate solubilizing bacteria (Ricardshon et al. 2009). Microorganisms produce different organic acids, such as acetate, lactate, malate, oxalate, succinate, citrate, gluconate, and ketogluconate. Organic acids can form stable compounds through reactions with calcium (Ca), iron (Fe), and aluminum (Al) (Gyaneshwar et al. 2002). Besides organic acids, phosphate solubilization released by phosphatase and phytase enzymes. The availability of organic phosphate depends on microbial activity to produce enzymes. Phosphatase enzymes play an important role in organic phosphate mineralization (Joner et al. 2000; Mubarik et al. 2014).

Table 2. Characteristics of isolate growth on the Pikovskaya medium.

Isolate	Phosphate solubilizing activity	Solubilization Index (SI)	Concentration IAA (ppm)
Arj1	+	3.5	0
Arj2	+	0.33	9.73
Arj3	+	0.125	24.35
Arj4	-	-	0
Arj5	-	-	36.21
Arj6	+	0.125	0
Arj7	+	0.16	0
Arj8	+	0.61	38.39

Note: (+) colony showed phosphate solubilizing activity, (-) colony did not show phosphate solubilizing activity

Morphological characterization of potential bacteria performed to investigate phosphate solubilizing activity and IAA production of the bacteria. The result of the analysis showed that L-tryptophan as the precursor in the NB medium was turned into IAA after overnight incubation. The highest IAA production was showed by isolate Arj3, Arj5, and Arj8 (24.35 ppm, 36.21 ppm, and 38.39 ppm, respectively). *Serratia marcescens* KAHN 15.12 could optimally synthesize IAA (22.37 ppm) at 18 hours (Astriani et al. 2016). The ability of rhizospheric bacteria to produce 15.20 mg/L IAA can affect the growth of primary soybean roots (Wahyudi et al. 2011).

Variation in the IAA level produced by bacteria is caused either by the environmental condition or the main precursor used for IAA biosynthesis (Patten and Glick 2002). Tryptophan, as an inducer, can synthesize IAA in bacterial cultures (Tsavkelova et al. 2006; Mohite 2013). Indole-3-acetamide (IAM) identified as the IAA biosynthesis pathway involves tryptophan-2-monooxygenase (IaaM) enzyme and IAM hydrolase enzyme (Spaepen and Vanderleyden 2011).

Quantitative estimation of bacterial phosphate solubilizing activity

Two isolates (Arj3 and Arj8) that showed multiple potentials to dissolve phosphate and synthesize IAA selected for quantitative analysis of phosphate solubilizing activity (Figure 2). Arj3's phosphate solubilizing activity was at the highest on day-2 (51.45 mg/L), and Arj8 showed the highest P solubilizing activity on day-3 and day-5

(75.39 and 74.33 mg/L, respectively). The bacterial phosphate solubilizing mechanism was based on Ca phosphate (tricalcium phosphate) solubilizing activity in liquid culture. The concentration of phosphate dissolved depends on sources of phosphate, microorganism growth, and condition of culture (Ricardson et al. 2009). Phosphate solubilizing activity in rhizobacteria liquid culture containing 1.5% tricalcium phosphate reached its highest level on day-5 (27.10 µg/mL) at an incubation temperature of 37 °C (Patel et al. 2017). After 7-day incubation, bacterial cultures could dissolve organic phosphate $Ca_3(PO_4)_2.Al_3(PO_4)_2$ and phosphate rock in liquid medium (18.59 L^{-1}, 18.3 L^{-1}, 5.68 L^{-1}) (Widawati 2011). Tricalcium phosphate $Ca_3(PO_4)_2$ contained in the Pikovskaya medium was broken down by Ca-phosphate solubilizing rhizobacteria into the available forms (Richardson et al. 2009).

Hypersensitivity Assay and Antagonist Within Isolate

The two isolates injected on tobacco leaves did not indicate any necrotic symptoms until the fourth day (similar to the negative control reaction) (Figure 3c-d). The leaf injected with isolate Arj3 showed brown and yellow spots on four replicate injection sites as it entered day 14 (Figure 3e). The changes that occurred in isolate Arj3 are the same as those experienced by pathogenic bacteria on positive control. The found was consistent with Klement and Godman (1967), who explained that every time a pathogen isolate attacks the host, the tissue would show a necrosis reaction to prevent further spread of the pathogen. Isolate Arj8 did not show any form of plant defense against pathogens, so this isolate can be considered eco-friendly and thus can as a biofertilizer candidate.

The result of the hypersensitivity assay showed that isolate Arj3 was less effective for fertilizer application due to its pathogenic properties. Therefore, this isolate was excluded from further testing. On the other hand, isolate Arj8 was selected for further testing because it indicated multiple potentials to dissolve phosphate and produce IAA and was not pathogenic to plants. Antagonist within isolate assay of isolate Arj8 can be seen in Figure 4. There was no inhibitor zone shown in the picture as also occurred in positive control using kanamycin (Figure 4). This finding suggests that isolate Arj8 does not exude metabolites nor produce compounds that can inhibit the growth of other isolates, so it called that isolate Arj8 is safe to form a microbial consortium with other superior isolates.

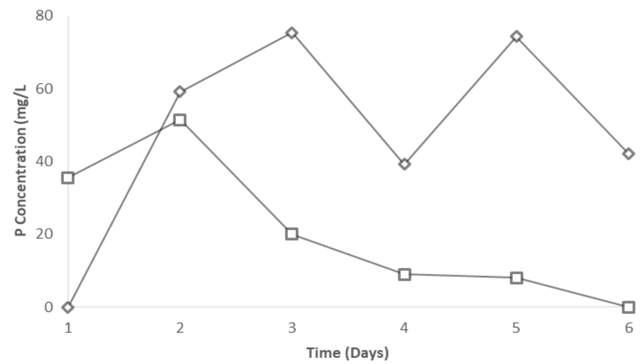

Figure 2. P solubilizing concentration. —□— isolate Arj3, —◇— isolate Arj8

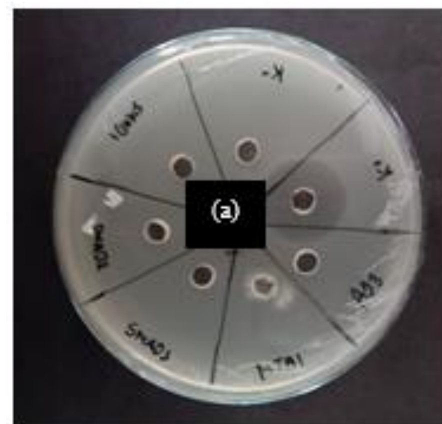

Figure 4. Antagonist Transisolate Assay on semisolid nutrient agar media. Isolate Arj8 (a), positive control (K+) using kanamycin, negative control (K-) using sterile aquadest

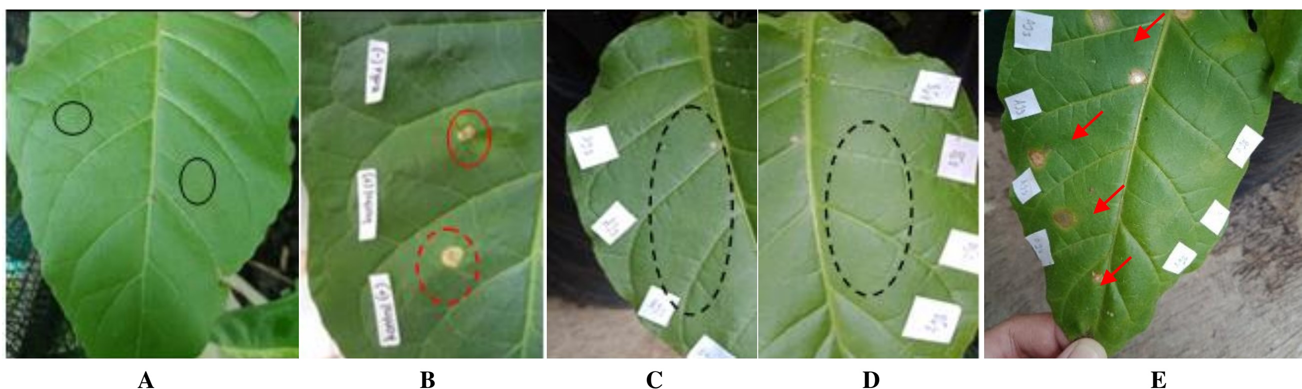

Figure 3. Hypersensitivity Assay on day-4. Negative (A), Necrotic Symptoms (B),), isolate Arj3 after four days (C), isolate Arj8 after four days (D), treatment after 14 days (isolate Arj3 on the left) and (isolate Arj8 on the right) (E), red arrows (+ Necrosis)

Identification of the selected bacterial isolates

After bacterial screening conducted in several stages, bacterial identification performed to the selected isolate, namely isolate Arj8. Isolate Arj8 identified as one of the isolates with the highest IAA production and the highest phosphate solubilizing activity. Arj8 colony has a diameter of 1.11 mm and coccobacilli shape based on negative gram staining (Figure 5). The result of the physiological testing using Microbact on Arj8 showed positive results in motility, lysin, glucose reducing, xylose, citrate, and catalase. Based on the bacterial morphological and physiological identifications, isolate Arj8 shared many similarities with *Pseudomonas* (Holt et al. 1994). Molecular Identification of isolate Arj8 was performed afterward to find out the accuracy of the species name and the kinship of the isolate towards other bacteria by constructing a phylogenetic tree.

Amplified 16S rRNA gene result indicated a 1300 bp DNA band (Figure 6). The length of the fragment was as expected; therefore, 16S rRNA sequencing was continued to obtain the order of nitrogen bases. The result of the sequencing analysis showed that Arj8 consisted of 1136 base pairs (Figure 7). The data were aligned and analyzed using BLAST and then compared to the DNA GenBank database. The result of the analysis showed that Arj8 shared 100% similarity with *Pseudomonas plecoglossicida*, and 99.91% similarity with *Pseudomonas putida* (Table 4).

The phylogenetic tree constructed will describe the kinship between isolate Arj8 and other bacteria that come from the same clade or the outer group. The phylogenetic tree constructed using the Neighbor-Joining Tree method with 1000x bootstrap replications. It showed that isolate Arj8 was closest in kinship with *Pseudomonas plecoglossicida* (Figure 8). *Pseudomonas* is a rhizobacteria genus frequently reported as phosphate solubilizing bacteria that can help repair plant growth mechanisms (Tahir et al. 2015). *Pseudomonas* has also recognized as the best IAA producing bacteria candidate that can be identified using tryptophan (Pattern and Glick, 2002; Apine and Jadav 2011). Furthermore, research shows that *Pseudomonas* spp. can bind nitrogen (Li et al. 2017) and control plant pathogens.

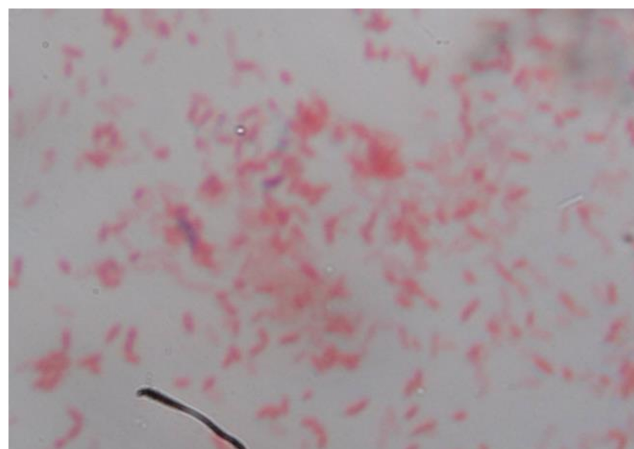

Figure 5. Arj8 Gram Staining at 1000 x magnification

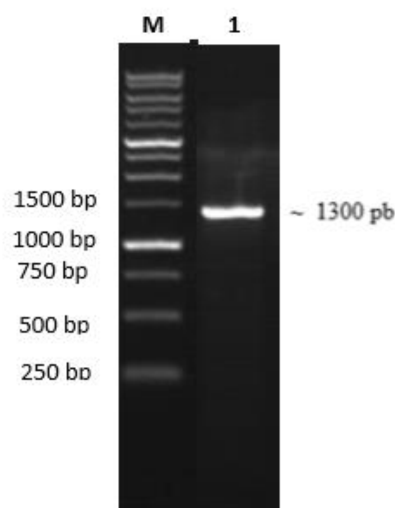

Figure 6. PCR Amplification of the 16S rRNA gene using primer 63f and primer 1378r. Note: M. Marker 1 kb, 1. DNA band of the sample isolate Arj8

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GCGAGTTGCAGACCTGACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCTTACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATTGT
AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCACCAGAGTG
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ACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATCTCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGGTTCTTCGCGTAGCTTCG
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GCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAG
ACTTAAAAAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTA
GCCGTGGCTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAACC
CTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTC
TCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGTTACCCCACCATCTAGCTAATACCCGGGG
GACCATCCAAAAGTGATAGCGAAGGCATCTTT
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Figure 7. 16 SRNA gene sequence of isolate Arj8 consisting of 1136 base pairs

Table 4. The result of 16 SRNA gene sequencing analysis of isolate Arj8 compared to the NCBI (BLASTX) database

Description	Max score	Total score	Query cover	E value	Identity	Access number
Pseudomonas plecoglossicida strain R19 16S ribosomal RNA genes, partial sequence	2098	2098	100%	0.0	100%	KJ819577.1
Pseudomonas putida strain Xh91 16S ribosomal RNA gene, partial sequence	2093	2093	100%	0.0	99.91%	MK064155.1
Pseudomonas guariconensis strain LBQSKF6 16S ribosomal RNA gene, partial sequence	2093	2093	100%	0.0	99.91%	KX364073.1
Pseudomonas monteilii strain CSR2 16S ribosomal RNA gene, partial sequence	2093	2093	100%	0.0	99.91%	KJ522791.1
Pseudomonas taiwanensis 16S ribosomal RNA gene, partial sequence	2093	2093	100%	0.0	99.91%	GU124496.1

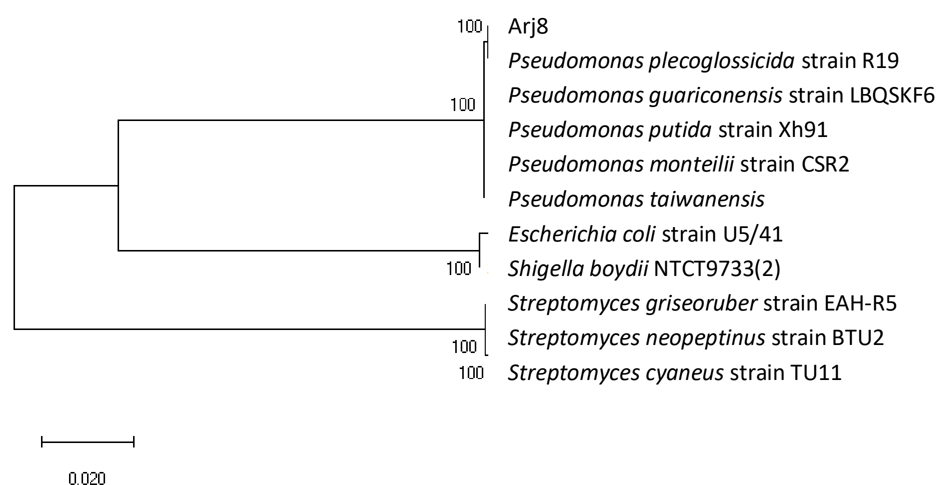

Figure 8. Phylogenetic Tree showing the kinship between isolate Arj8 and other bacteria from the same clade or the outer group. The phylogenetic tree construction was done using the Neighbor-Joining Tree method with 1000x bootstrap 1000x replications

Pseudomonas plecoglossicida is a bacterium that is specifically and genetically related to *Pseudomonas putida* (Mulet et al. 2010). *P. plecoglossicida* possesses the characteristics of motile bacteria, which are non-fluorescent, gram-negative, and bacillus. *P. plecoglossicida* can be used for bioremediation of cypermethrin (Boricha and Fulekar 2009) and degradation of organophosphate (OP) and carbamate pesticide compounds (Chanika et al. 2010). *P. plecoglossicida* activity in phosphatase mechanisms and phytohormone biosynthesis has not reported. Studies merely found *P. putida* as a plant growth-promoting trigger with several mechanisms including the production of indole acetic acid and siderophore (Patten and Glick 2002; Montiel et al. 2017), phosphate solubilization (Kalayu 2019), bioremediation (Liu et al. 2016), and biopesticides (Chanika et al. 2010). Therefore, this study can provide a new insight related to *P. plecoglossicida* activity as a superior bacterial species that possess multiple potentialities that are to produce IAA hormone and solubilize phosphate. This bacterium needs to be studied further so that it can be used as a biofertilizer candidate in the future.

In conclusion, six bacterial isolates found on the soybean rhizosphere were able to dissolve phosphate, indicated by the formation of clear zones. Among them, there were two isolates, namely isolate Arj3 and Arj8, that showed the highest phosphate solubilizing activity and IAA production. Besides performing the highest phosphate solubilizing activity and IAA production, isolate Arj8 also found to be non-pathogenic to plants. Isolate Arj8 has closely related to *Pseudomonas plecoglossicida*. The potential of P. *plecoglossicida* as a phosphate-solubilizing bacterium and an IAA producing bacterium is a novel finding that can contribute to the development of biofertilizers that are environmentally friendly, effective, and efficient in reducing the use of synthetic chemical fertilizers.

ACKNOWLEDGMENTS

The authors would like to thank DRPM DIKTI for the funding support on Doctoral Dissertation Research with contract no. 074/SP2H/L/DRPM/2019 and the Gogodeso

village chief as well as Binangun farmers for their kind assistance in collecting soil samples from soybean plantations in Blitar, East Java, Indonesia.

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