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Isolation and identification of phosphate solubilizing bacteria from the rhizosphere of rice in organic and non-organic rice fields in Sukoharjo District, Indonesia

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Abstract. *Purwanti D, Setyaningsih R, Susilowati A. 2019. Isolation and identification of phosphate solubilizing bacteria from the rhizosphere of rice in organic and non-organic rice fields in Sukoharjo District, Indonesia. Bonorowo Wetlands 9: 108-115.* One of the essential nutrients in the soil is phosphate. Phosphate fertilization is often inefficient because phosphate is bound and difficult for plants to absorb. The use of phosphate solubilizing bacteria (PSB) can be used as an alternative to the availability of P in the soil. This study aimed to obtain PSB isolates with high phosphate solubilization ability and identify these PSB isolates molecularly. Soil samples were taken from organic and non-organic rice (*Oryza sativa* L.) fields in Sukoharjo, Central Java, Indonesia, namely Grogol Village, Menuran Village, and Pondok Village. The research activity was carried out in several stages: isolation of PSB on Pikovskaya medium, characterization and testing of PSB in dissolving P, and Gram staining. PSB colonies with a phosphate solubilization index and showed different colony colors were taken, and then DNA was isolated. DNA isolation was carried out by extraction method using a DNA extraction kit. The gene encoding 16S rRNA amplified the obtained DNA samples. Sequencing was carried out using a genetic analyzer. Data analysis by comparing sequence data on the BLASTN program. Based on the isolation of rhizosphere samples from organic paddy fields, 14 isolates of PSB were obtained, and ten isolates of non-organic paddy were PSB. P13, P1, Q7, and Q8 isolates had the highest P solubilization ability with dissolution index (IP), namely 3.35, 2.13, 1.82, and 1.47. The 16S rRNA gene sequence analysis showed that isolates P1 and P13 had 99% similarity to *Acinetobacter* sp. ADP1 strain. The isolate Q7 had a 96% similarity with the genus *Clavibacter* strain NCPPB 382, while Q8 had a 99% similarity with *Pseudomonas aeruginosa* strain PAO1.

Keywords: Gene encoding 16S rRNA, organic and non-organic rice fields, phosphate, phosphate solubilizing bacteria

INTRODUCTION

Indonesia is an agricultural country with a broad agricultural sector and has a vital role in supporting the national economy. According to Simamora (2006), challenges in the agricultural sector will continue to increase. It is estimated that by 2025 the population growth will reach 8.5 billion, and most of them will come from developing countries. Technology and agricultural innovation are needed to support increased food production, especially rice.

Fertilizer is a material used to change soil's physical, chemical, and biological properties to improve plant growth. Chemical fertilizers played an essential role during the green revolution, but the imbalance reduced soil fertility and environmental damage (Gyaneshwar et al. 2002). Thus, humans are aware of the negative impact of chemical fertilizers on the environment, thereby encouraging the development of organic farming systems (Andoko 2008). Organic agriculture avoids chemicals, including chemical fertilizers, that are toxic to the environment to obtain a healthy environment (Sutanto 2002; Andoko 2008).

In organic farming, the fertilizers used are organic fertilizers and biological fertilizers. Organic fertilizers are fertilizers in which all organic materials come from animals or plants, while biological fertilizers are derived from all functional groups of soil microbes. The advantages of biological/organic fertilizers in implementing organic farming are that they are more environmentally friendly than chemical fertilizers, can increase land productivity, and preserve the environment (Suriadikarta and Simanungkalit 2006). Biofertilizers also leave no residue on crop yields, so they are safe for human health (Musnamar 2003).

One element that is often given during fertilization is phosphorus. Phosphorus is an element that is needed in large amounts (macronutrients). Plants absorb phosphorus in primary orthophosphate ions (H_2PO_4-) and secondary orthophosphate ions (HPO_4^{2-}). Generally, P is not entirely soluble in water because P can react with other ions and form compounds whose solubility is reduced to become compounds that are not easily washed. Most of them become compounds unavailable to plants (Rosmarkam and Nasih 2002).

Most of the paddy fields in Indonesia are saturated with phosphate due to the high use of chemical fertilizers. Still, plants cannot absorb the phosphate because it is bound to other compounds, so farmers apply phosphate (P) fertilization to increase the dose, even though the amount of phosphate in the soil is quite large. The phosphate-binding with soil colloids causes phosphate fertilization to

be less efficient and can cause soil pollution (Kurnia et al. 2008). P deficiency can cause the volume of plant tissue to be smaller and the leaf color to be darker. One of the efforts to overcome the availability of P in the soil is to utilize microorganisms (Rosmarkam and Nasih 2002).

Phosphate fertilization efficiency can be done by utilizing phosphate solubilizing bacteria. Phosphate solubilizing bacteria can provide plants with phosphate not previously available in the soil through conversion. The mechanism of phosphate solubilizing bacteria in dissolving inorganic P is by excreting organic acids due to bacterial activity in the rhizosphere (Hardjowigeno and Rayes 2005).

Phosphate solubilizing bacteria can be isolated from around plant roots. The ability of bacteria to dissolve phosphate can be tested by growing it on Pikovskaya media containing Ca_3PO_4. The dissolution of Ca, which binds P in Pikovskaya media, can be seen from the width of the clear zone formed around the colony. The larger the clear zone, the greater its ability to dissolve phosphate. The increase in the width of the clear zone, which was higher by one isolate against another, showed an indication that this bacterial isolate had superior characteristics. An increase also follows the increase in the clear zone in the diameter of the bacterial colony (Maryanti 2006).

In general, studying bacteria's morphology, cell structure, and biochemical properties is done by isolation. Bacterial characterization can be done by identifying 16S rRNA (Reeve 1994). The 16S rRNA gene is relatively constant and does not change for a very long time; in other words, the mutation rate is minimal, so it is relevant when used as an object of research (Janda and Abbott 2007).

The aims of this study were (i) to obtain isolates of phosphate solubilizing bacteria from organic and non-organic paddy soils that have high phosphate solubilization ability in Sukoharjo and (ii) to obtain the names of bacterial species that have a high ability to solubilize phosphate from organic and inorganic paddy soils based on the 16S rRNA gene sequence.

MATERIALS AND METHODS

Ingredient

The main ingredients were soil samples from organic and non-organic rice rhizosphere from three villages in Sukoharjo District, Central Java, Indonesia, namely Grogol, Menuran, and Pondok villages. Pondok village farmers are the pioneers of organic farming in Sukoharjo. Most of the productive/working-age population, especially those aged between 15-50 years, work as farmers in the food agriculture sector. They have carried out the development of organic rice farming, and all farmers have carried out organic rice farming, both those who have followed all the stages or only some of the stages (Rustiono 2008).

Procedures

Rhizosphere sampling

Rice rhizosphere was taken from organic and non-organic farmland in the Sukoharjo area. The roots of the rice plants are cut using scissors to separate them from the stems, then shaken. The collected soil is still attached to rice roots (*Oryza sativa* L.). The rhizosphere sample was then put into a sterile bottle (Prasanna et al., 2011).

Pikovskaya medium production

A total of 0.5 grams of yeast extract, 10 grams of dextrose, 5 grams of $CA_3(PO_4)_2$, 0.5 grams $(NH_4)_2SO_4$, 0.20 grams of KCL, 0.1 grams of $MgSO_4.7H_2O$, 0.0001 grams of $MnSO_4.H_2O$, 0.0001 grams $FeSO_4.7H_2O$, 18 grams agar homogenized in 1000 mL distilled water. Medium ph 6.8 and autoclave sterilization for 20 minutes at 121°C (Prasanna et al. 2011).

Phosphate solubilizing bacteria

Isolation of phosphate solubilizing bacteria (PSB) and purification of isolates were based on the method of Prasanna et al. (2011). The soil from each sample was put into a sterile 250 mL Erlenmeyer with a size of 10 grams and homogenized with 90 mL of sterile distilled water in an orbital shaker (200 rpm, 30 minutes). The sample was left for 10 minutes. A total of one mL of the sample supernatant was put into a flask that already contained nine mL of 0.85% NaCl, then put into 6 test tubes with each dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. Furthermore, 0.1 mL of each dilution was spread in a petri dish filled with Pikovskaya medium. Anaerobic incubation at 28°C ± 2 to 7 days in an incubator. In a petri dish, bacterial colonies will grow. Colonies surrounded by a clear zone indicate the presence of phosphate dissolution. Colonies with a wide clear zone and different colony colors were taken to dissolve phosphate qualitatively. The colonies were counted and expressed in colony-forming units (CFU) per gram of soil, then phosphate solubilizing bacteria were identified.

Clear zone measurement

The growth of phosphate solubilizing microbial colonies in Petri dishes from the culture results was observed using a magnifying glass. Then the colony diameter and clear zone diameter were measured with a ruler. The colony diameter and clear zone were measured 2-3 times at different positions, and the measurement results were averaged. The way to measure it is:

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

Colonies with high ratio values were isolates of phosphate solubilizing bacteria taken for identification (Saraswati et al. 2007).

Bacterial colony and cell morphology characterization

The bacterial isolates were identified for their cell morphology by using the Gram stain test and microscopic observation of the shape of the microbe. Gram staining is done by passing a glass object over a Bunsen flame. Then one drop of sterile distilled water was dropped on a glass object, followed by pure isolate. The isolates and aquadest were flattened and then dried and fixed. The surface was

dripped with crystal violet solution and left for 30-60 seconds, and then was washed with running water and dried. Iodine solution is dripped on the surface of the preparation, left for 30-60 seconds, washed with running water, and dried. Next, the preparations were washed with 96% alcohol for 5 seconds, then dripped with safranin and left for 30-60 seconds. Finally, the preparations were washed with running water and observed under a microscope to see (color, shape, surface, edge, size, and optical characteristics) and the microscopic character of the colony in the form of Gram reaction and bacterial cell shape (Prasanna et al. 2011). If the staining results of bacterial cells were red, the cells were Gram-negative, while if they were purple, they were Gram-positive.

DNA extraction

DNA extraction using the GeneJET Genomic DNA Purification kit followed the procedure recommended by the manufacturer with the following steps: 1 mL of the bacterial isolate from 10 mL of previously cultured bacterial culture was put into a tube, then centrifuged at 3,500 rpm; 10 minutes. The supernatant was discarded. 180 µL Digestion Solution was added to the tube. Furthermore, a micropipette mixed 20 µL of Proteinase K Solution by vortexing/resuspension. Samples were incubated in a shaking incubator for ±30 minutes; 100rpm; 56ºC. Then 20 µL of RNase A Solution was added to the tube and suspended using a micropipette. Incubated at room temperature for 10 minutes. 200 µL of Lysis Solution was also added and resuspended using a vortex for 15 seconds until the mixture was homogeneous. Then 400 µL of 50% ethanol was added and resuspended. The resulting lysates were transferred to the GeneJET Genomic DNA Purification Column (ban tube). Centrifuge 4200 rpm; 1 minute. Discard the centrifuged liquid's collection tube and replace it with a 2 mL collection tube. Added 500 l Wash Buffer I (if the Kit used is new, it is necessary to add ethanol according to the manufacturer's procedure). Centrifuge 5600 rpm; 1 minute. Discarded the liquid that fell to the bottom of the tube. Also added 500 µL Wash Buffer II (if the Kit used is new, it is necessary to add ethanol according to the manufacturer's procedure) on the membrane tube. Centrifuge 8,400 rpm; 3 minutes. Optional: if the residue is still visible on the membrane tube, empty the collection tube and centrifuge to full speed. The collection tube containing the fluid was discarded, and the GeneJET Genomic DNA was transferred to a sterile 1.5 mL collection tube. Then 200 µL of Elution Buffer was added to the center of the GeneJET Genomic DNA Purification Column to elute Genomic DNA. Incubation for 2 minutes at room temperature and centrifuge 5,600 rpm; 1 minute. The purification column (membrane tube) is discarded, and the extract can be used immediately, or the extracted DNA can be stored at -20ºC

16S rRNA gene amplification

The amplification of the gene encoding 16S rRNA was carried out with primers 63F (5'CAG GCC TAA CAC ATG CAA GTC) and 1387R (5'GGG CGG WTG GTA CAA GGC) (Marchesi et al. 1998) with the following reaction mixture: DNA 0.1 g, 1x buffer, two µL 10 mM dNTP Mix, two U Taq DNA Polymerase, five pmol each primer, ddH2O to 25 µL. The PCR program consisted of initial denaturation at 95ºC for five minutes, followed by 30 cycles at 95ºC for 30 seconds, 55ºC for one minute, 72ºC for one minute, and then final extension at 72ºC for five minutes. The PCR results were observed by electrophoresis using 1% of agarose gel and 1x TAE buffer.

16S rRNA coding gene sequencing

Detection of nucleotide sequences (sequencing) using the ABIprism™ 310 Automated DNA Sequencer (PE Applied Biosystem), then repeated PCR for the sequencing stage (PT Genetics Science Indonesia – First Base, Singapore).

Data analysis

Sequence data obtained from the selection of isolates with the widest clear zone were then compared with sequences in the National Center for Biotechnology Information (NCBI) data bank in the Basic Local Alignment Search Tool for Nucleotides (BLASTN) program (http://www.ncbi.nlm.gov/BLAST/).

RESULTS AND DISCUSSION

Soil samples from the rhizosphere of rice plants (*Oryza sativa*)

Soil samples that were taken have the potential to develop organic agriculture. Organic farming in the three villages has been certified by an Organic Certification Institute that KAN has accredited. The definition of organic agriculture in the village is agriculture whose processing is done organically, namely by using organic fertilizers. Organic fertilizers used with the composition are as follows: manure (cow), compost, dolomite, husk charcoal, molasses water, and decomposing bacteria. In addition, irrigation in organic rice fields is different from non-organic rice fields, but the water source used is the same, namely springs from Mount Lawu. The irrigation system is treated differently in organic rice fields by making water reservoirs. The reservoir is given water hyacinth to neutralize water that still contains residues of chemical substances. Some organic rice fields do not use dams but make ponds in irrigation channels along with these fields. The pond is filled with water hyacinth and fish, holding water and neutralizing chemical residues. Fish in these ponds are usually sensitive to chemicals, intending to measure the level of chemical residues; if the fish in the pond die, the water may contain high chemical substances. Irrigation of non-organic rice fields is not given special treatment, so it is possible that the water still carries many chemical residues.

Phosphate solubilizing bacteria are often associated with organic and inorganic soil. In the soil, there are plant roots that microbes can utilize as nutrients in the form of exudate released by plants so that bacteria will associate in the plant rhizosphere. Fiantis (2012) said that organic soil

comes from plant remains that accumulate in an area. Conventional methods of non-organic soil are usually carried out for narrow land and have a particular slope. Thus, applying an organic farming system from the point of view of the availability of P nutrients is suitable to implement because it can increase the productivity of rice plants.

Phosphate solubilizing bacteria

Phosphate solubilizing bacteria (PSB) is one type of biological fertilizer that can make inorganic P fertilizers more efficient to overcome the low P-available soil and increase the P concentration of plants. The ability of PSB varies greatly depending on the type of microbe, adaptability, to the ability to produce organic acids and enzymes (Whitelaw 2000).

Colonies with morphological differences, including color, shape, and edges, were taken from all bacterial colonies that grew in each dilution. The entire colony was then purified again by streaking on the surface of the NA medium. Isolates that showed the presence of a clear zone were taken and then grown on Pikovskaya media as a solid test, and the phosphate solubilization index (SI) was measured. The results of observations of colonies with morphological differences (color, shape, and edges) can be seen in Table 2.

Twenty-four (24) bacterial colonies were obtained based on the selection, namely 14 bacterial colonies from organic fields and ten bacterial colonies from non-organic fields. The isolation of phosphate solubilizing bacteria in organic soils was more than in non-organic soils. In addition, bacteria in organic rice fields can dissolve phosphate higher than bacteria in non-organic rice fields. According to Suriadikarta and Simanungkalit (2006), phosphate solubilizing microbes are related to the amount of organic matter contained in the soil.

Twenty-four colonies were streaked onto the surface of the Pikovskaya medium containing 1% Ca_3PO_4. The colonies that formed the clear zone were purified by streak for a single colony on Pikovskaya media. These bacterial colonies that formed a clear zone around them were 20 PSB isolates. The PSB isolates were then re-grown on Pikovskaya media to determine the clear zone produced from each isolate. The diameter of this clear zone is used to calculate the phosphate solubilization index (SI). PSB with SI 1 was taken for further identification.

PSB produces acid during growth. According to Maryanti (2006), the signs that a bacterium can dissolve phosphate are the presence of a clear zone around the bacterial colony and an increase in the size of the bacterial colony on Pikovskaya media; this is because the bacteria can dissolve phosphate ($Ca_3(PO_4)_2$) contained in the Pikovskaya media formulation. The formation of a clear zone indicates that the bacteria can produce organic acids. The solid test results on Pikovskaya media were the 20 isolates with the highest SI with codes P1 and P13 from samples of organic rice fields and Q7 and Q8 from samples of non-organic rice fields (Figure 1).

Based on the resulting SI, it can be seen that PSB has various P-dissolving abilities (Table 1). According to Rachmiati (1995), the clear zone area qualitatively indicates the size of the bacteria's ability to dissolve phosphate. In the research conducted by Nopparat et al. (2007), P solubilizing bacteria and fungi have different abilities depending on the strain type. Superior P solubilizing microorganisms will produce the largest clear strain zone diameter compared to other colonies.

Characteristics of phosphate solubilizing bacteria isolates

Bacterial isolates obtained in the solid test in Pikovskaya media were then carried out in the Gram stain test. The results of the Gram staining test can be seen in Table 3.

Table 1. Characteristics of phosphate solubilizing bacterial colonies in dissolving p at solid Pikovskaya medium

Isolate code	Shape	Color	edge	Solubilization Index (SI)
P1	Light yellow	Round	Flat	2.13
P2	White	Round	Flat	-
P3	Yellow	Irregular	Curvy	-
P4	Yellowish white	Round	Flat	0.58
P5	Yellow	Long	Flat	0.41
P6	Milky white	Round	Flat	0.22
P7	Milky white	Round	Flat	-
P8	Yellow	Round	Flat	-
P9	Milky white	Round	Jagged	0.33
P10	Light yellow	Irregular	Flat	0.17
P11	Yellowish-white	Irregular	Curvy	0.17
P12	Yellowish white	Round	Flat	0.24
P13	Yellowish-white	Round	Flat	3.35
P14	Yellowish white	Irregular	Curvy	0.43
Q1	Milky white	Irregular	Curvy	0.73
Q2	Milky white	Round	Curvy	0.22
Q3	Milky white	Round	Flat	0.93
Q4	Orange	Irregular	Flat	0.39
Q5	Milky white	Irregular	Wavy	0.35
Q6	Milky white	Round	Flat	0.46
Q7	Yellowish white	Round	Flat	1.82
Q8	Light yellow	Round	Flat	1.47
Q9	Yellowish white	Round	Flat	0.41
Q10	Light yellow	Round	Flat	0.14

Note: PSB isolates from organic rice fields, PSB isolates from non-organic rice fields

Table 2. Gram staining of bacterial isolates isolated from the rhizosphere of rice (*Oryza sativa*) in organic and non-organic rice fields in Sukoharjo, Central Java, Indonesia

Isolate code	Gram	Cell shape
P1	-	single rod
P13	-	single rod
Q7	-	single rod
Q8	-	single cocci

The four bacterial isolates showed a red color after Gram staining. It indicates that the four bacterial isolates were Gram-negative bacteria. According to Purwani et al. (2009), Gram-negative bacteria have a relatively more complex cell wall structure, i.e., three layers of the cell wall. They were the outer layer in the form of lipoprotein, the middle layer in the form of lipopolysaccharide, and the inner layer in the form of peptidoglycan. Gram-negative bacteria whose lipid substances will dissolve during washing with alcohol. It causes the pores in the cell wall to enlarge, the permeability of the cell wall becomes large so that the dye that has been absorbed is quickly released, and the bacterial cell becomes colorless (pink). In Gram-negative bacteria, the red color of safranin is the outer membrane. Gram-positive, stained purple or blue by crystal-violet is the peptidoglycan layer. Gram staining was also carried out to determine the cell shape of PSB. Of the four bacterial isolates, three had a single rod form, and one had a single cocci form (Figure 2).

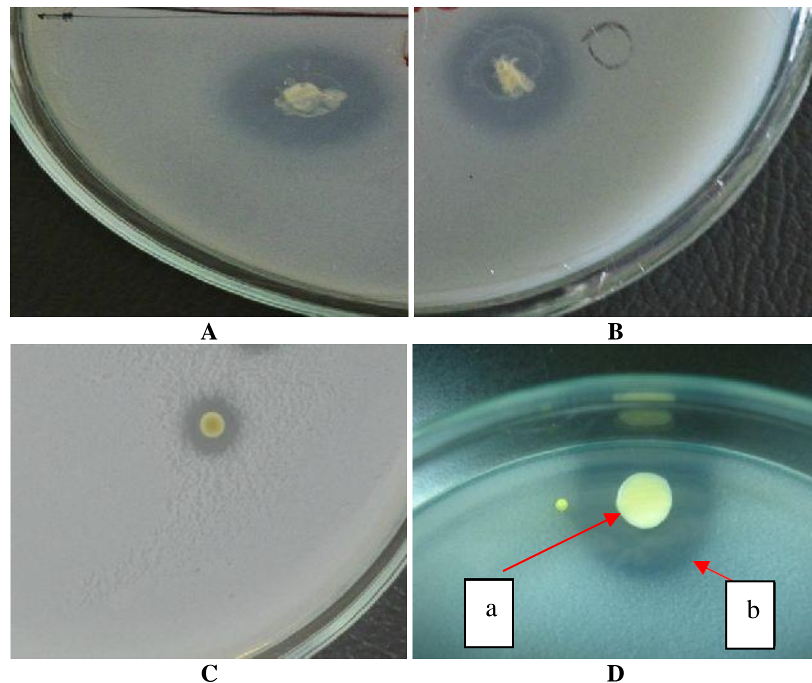

Figure 1. Clear zone by PSB isolates on Pikovskaya media. A. Isolate Q7, B. Isolate Q8, C. Isolate P1, D. Isolate P13. Sections marked with arrows: a. PSB isolates, b. clear zones

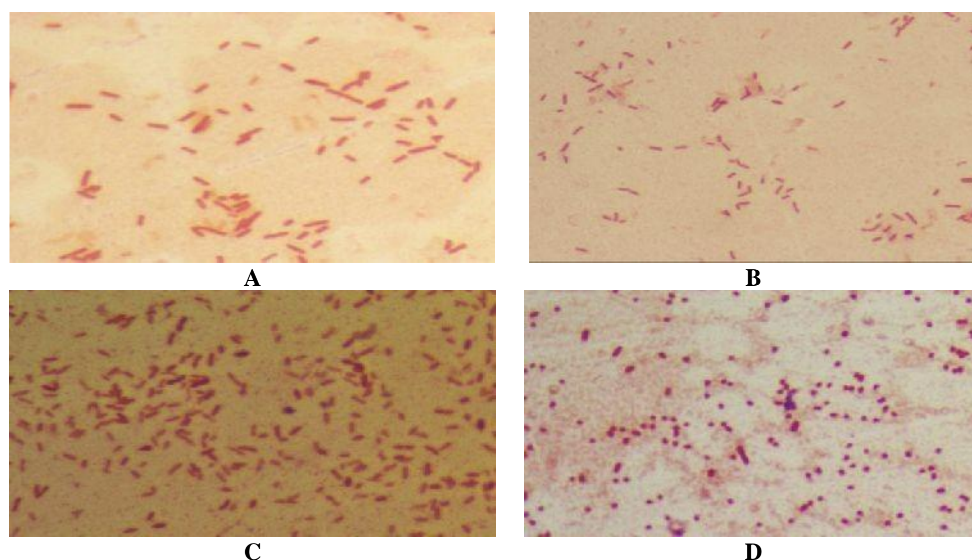

Figure 2. Gram stain test results on four bacterial isolates from the rhizosphere of rice plants (*Oryza sativa*). A. Isolate P1, B. Isolate P13, C. Isolate Q7, and D. Isolate Q8

Gene encoding 16S rRNA

The four bacterial isolates with the most potency to dissolve phosphate, namely isolates P1, P13, Q7, and Q8, were identified using the gene encoding 16S rRNA. The PCR product fragment has a size of about 1500 basepair (bp) which is the expected size using a primer combination of 63F (5' CAG GCC TAA CAC ATG CAA GTC3 3') for the *forward direction,* and 1387R (5' GGG CGG WGT GTA CAA GGC 3') for directions *reverse* (Marchesi et al. 1998). Primer is an essential component in the PCR reaction because this primer will determine the target region of the genome to be amplified. The electrophoresis results of PCR amplification of the 16S rRNA region of phosphate solubilizing bacteria are shown in Figure 3.

The electropherogram results showed that the 16S rRNA region of phosphate solubilizing bacteria was about 1300 bp in size. A single band was seen, indicating that the primer pair was specific to the desired region. The single band contains a collection of genes encoding 16S rRNA from bacterial isolates. The thickness and thinness of the bands indicate the quantity of amplified DNA. Electropherogram amplification of the genomic DNA of phosphate solubilizing bacteria in this study is clear and thick, indicating that the concentration of separated molecules in this region is high.

Phosphate solubilizing bacteria identity

Nucleotide base sequences were analyzed and compared with the gene database from GenBank DNA using the BLAST program. Stephen et al. (1990) mentioned that the BLAST program was used to determine the identity and level of homology with previously known gene fragments.

Based on the results of BLASTn analysis, there are three sequences of phosphate solubilizing bacteria which have partial sequences similar to the data from GenBank with a similarity level of 99% (Table 3). It indicates that the bacteria are in the same species as the isolates that have been identified previously by Drancourt et al. (2000). The analysis results also showed that one sequence of phosphate solubilizing bacteria had a similarity value of <97%, meaning that the isolate was a new species for which data were not available in GenBank. The percentage of similarity <97% of the 16S rRNA gene fragment from the sequence indicated that the identified bacteria had a close resemblance to bacteria from the GenBank data but were not bacterial species from the GenBank data. Drancourt et al. (2000) stated that isolates with a 16S rDNA sequence similarity of 99% could represent the same species. Isolates with a sequence similarity of 97% could represent identity at the genus level.

Based on the results of the BLASTn analysis, it was found that the bacterial isolates in the organic rice field samples were *Acinetobacter* sp. while in non-organic rice fields, namely *Clavibacter* and *Pseudomonas aeruginosa. Acinetobacter* sp. is a bacterium of the same genus, namely *Acinetobacter,* a Gram-negative bacterium that grows optimally at a temperature of 33-35°C, stems in the form of stem cells, often found in soil and water (Doughari 2011). Bacteria of this genus can utilize glucose, mannitol, maltose, and sucrose by oxidation (Barrow et al. 1993). Pramono (1994) revealed that phosphate solubilizing bacteria create organic acids by glucose catabolism in the tricarboxylic acid (TCA) cycle, which continues glycolysis. These organic acids are primary metabolites used for cell survival so that the phosphate dissolution mechanism is more straightforward and can be absorbed by plants (Rodrigues et al. 2006).

Table 3. The percentage of DNA similarity encoding the 16S rRNA gene of phosphate solubilizing bacteria from the rhizosphere of rice plants in organic and non-organic rice fields with GenBank sequences

Isolate	Best friend	Access no.	Query cover (%)	% Similarity
P1	*Acinetobacter sp.* ADP1. strains	NC005966.1	97	99
P13	*Acinetobacter sp.* ADP1. strains	NC005966.1	98	99
Q7	*Clavibacter* strain NCPPB 382	NC009480.1	99	96
Q8	*Pseudomonas aeruginosa* strain_PAO1	NC002516.2	99	99

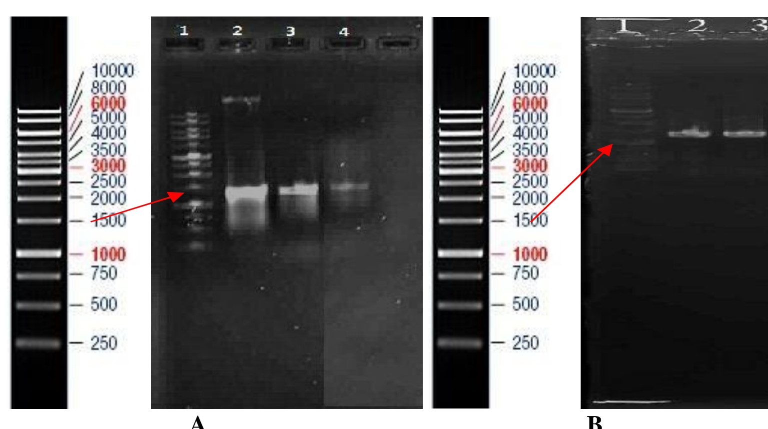

Figure 3. Electropherogram of the 16S rRNA encoding gene amplicons. A. lane one marker, lane two bacterial isolates Q8, lane three isolates bacteria P13, and lane four isolates bacteria Q7; B. lane one marker, two and three isolates of P1 bacteria

According to Bergey's manual, *P. aeruginosa* includes aerobic rods and cocci, Gram-negative bacteria, which are motile because they have flagella, and some are non-motile (Pelczar and Chan 2006). These bacteria grow optimally at 37ºC (Stover et al. 2000). Bacteria of the genus *Pseudomonas* can produce catalase enzymes (Barrow et al. 1993). Besides that, *Pseudomonas* has been widely studied as a biocontrol agent because of its ability to produce antimicrobial metabolites. The phosphate solubilizing bacteria (PSB), *P. aeruginosa,* and *Bacillus* sp. were evaluated for antibiotic resistance to various antibiotics, including ampicillin, chloramphenicol, penicillin, and streptomycin at concentrations of up to 400 µg/mL medium (Setiawati 2000). According to Purwaningsih (2003), bacteria that act as phosphate solubilizers in soil have been found, including the genera *Pseudomonas*, *Micrococcus*, *Bacillus*, *Azotobacter*, *Microbacterium,* and *Flavobacterium*. The research results by Widiawati and Suliasih (2006) also stated that *Pseudomonas* and *Bacillus* bacteria were phosphate solubilizing bacteria that had the most remarkable ability as biofertilizers. It is done by dissolving phosphate elements bound to other elements (Fe, Al, Ca, and Mg) to make the P elements available to plants.

Continuous use of chemical fertilizers can cause damage to the soil and reduce nutrients. According to Jones (1982), inorganic phosphate fertilization on ultisol soils has the main problem: the low effectiveness of P fertilizer, namely 10% to 30%, so that 70% to 90% of P fertilizer remains in the soil and is difficult for plants to absorb. Low fertilizer efficiency causes the amount of inorganic P fertilizer to be applied by farmers to increase so that it has the potential to reduce productivity, so its use needs to be reduced by utilizing biological fertilizers. The use of phosphate solubilizing bacteria can increase the efficiency of P fertilizer.

The use of phosphate solubilizing bacteria (PSB) as an agent to reduce pathogen attacks can increase phosphate availability due to the production of organic acids and phosphatase enzymes; besides, it also functions as a biocontrol agent (Setiawan 2008). Thakuria et al. (2004) stated that phosphate solubilizing bacteria isolated from the rice rhizosphere could increase rice production from 5.4 to 21.6%. Isolation and identification of phosphate solubilizing bacteria from the rhizospheres of rice plants (*O. sativa*) in organic and non-organic rice fields in the Sukoharjo area revealed that Isolates P1 and P13 were 99 % identical to *Acinetobacter* sp. Isolate Q7 had a 96% similarity with the genus *Clavibacter*, and isolate Q8 had a 99% similarity with *P. aeruginosa*.

In conclusion, 24 isolates of phosphate solubilizing bacteria were obtained from organic and non-organic rice fields in the Sukoharjo area. The two lines of *Acinetobacter* sp. with the highest IP were phosphate solubilizing bacteria isolated from organic rice fields. *Pseudomonas aeruginosa* and one isolate with a 96% similarity to *Clavibacter* were the most phosphate solubilizing bacteria isolated from non-organic rice fields with the highest IP.

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