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The Effectiveness of Phosphate Solubilizing Rhizobacteria on The Growth and Yield of Several Soybean Varieties on Ultisol Soils

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ABSTRACT

This study aims to obtain the interaction of rhizobacteria and soybean varieties, types of rhizobacteria effective in dissolving phosphate and varieties able to grow well on ultisol soil. The research were rhizobacteria isolation and rhizobacteria selection. Observations made were bacterial isolation, identification of morphological and physiological rhizobacteria (gram reactions, hypersensitive reactions, as phosphate solvents and germination test and rhizobacteria test of phosphate solvents by inplanta. The results showed that rhizobacteria isolates from ultisol soil of chilli plants, eggplant plants and rice roots obtained 32 isolates. The highest number of isolates were obtained from the ultisol soil of chili plants namely 18 isolates. The color of the rhizobacteria isolate colony was obtained by 27 cream isolates and 5 yellow isolates. The surface of the colony in the chili ultisol soil consisted of 3 groups namely arising, flat and convex, the surface of the colony arising obtained 8 isolates, the flat surface obtained 9 isolates and the convex surface obtained 1 isolate. All isolates were gram-negative From 32 rhizobacteria isolates, there were 2 isolates which were able to dissolve phosphate, namely RT1 and RC3. In mung bean plants with RT1 administration, the weight of the seed was 2.76 grams high compared to control and RC3.

INTRODUCTION

Soybean is the third most important food crop after rice and corn. The need for soybean consumption nationally increases every year, in line with increasing population growth Soybean is one source of vegetable protein, generally consumed in the form of processed, namely: tempeh, tofu, soy sauce, '*tauco'*, soy milk and various forms of snacks (Sudaryanto and Swatika, 2007). At present tofu and tempeh are he daily menu of Indonesian people. So soybean is one of the most important commodities in Indonesia, soybean demand is increasing along with population growth, this causes a crisis in soybean production. The trigger for the crisis is the local ability to meet only 48% of the total domestic soybean needs and the rest is met by soybeans originating from imports.

The high demand for soybeans in Indonesia is not matched by slow-growing soybean production. To meet these needs can be done with land expansion, the expansion of the planting area can be done by using marginal land such as Ultisol land. Ultisol is a type of acidic soil, with high saturation of Al, Fe, Mn,

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and poor macronutrient content, especially P, K, Ca and Mg. Although P contained in the soil is abundant, but if the soil does not contain phosphate solubilizing bacteria, only a small amount of phosphate is absorbed by the soil or plants, causing the soil to become infertile and yields from agriculture decline. The P element is an indispensable element of the plant because the P element is an essential macro nutrient. To fulfil the P element, it can come from microbes that are able to dissolve P minerals, microbes that can be used to fulfill the P element are bacteria, fungi and actinomycetes, these microbes can dissolve phosphate. Phosphate solubilizing bacteria can improve soil fertility because phosphate solubilizing bacteria are able to carry out the mechanism of phosphate solvents by excreting several or some or many low molecular weight orgnic acids (Simanungkalit and Suriadikarta, 2006).

Some important characteristics of rhizobacteria in increasing plant growth are producing growth hormones such as IAA (Karnwal, 2009), gibberellins (Joo et al., 2005), fixing N (Hafeez et al. 2006), and dissolving P (Faccini et al., 2004) ; Mehrvraz & Chaichi, 2008). Specifically on the ability to dissolve P, rhizobacteria such as Pseudomonas spp. And Bacillus spp. Can remove organic acids such as formic acid, acetate, and lactate which are able to dissolve the phosphate forms that are difficult to dissolve so that it becomes a form available to plants (Rao, 2007). Suliasih et al (2010) research as life fertilizer can increase BPF population and phosphatase enzyme activity. Microorganism activity is influenced by the availability of P in the soil, so P for the source of nutrition for MPF activities needs to be given to the soil. Based on Hasanuddin's research results (2002) showed that the treatment of inoculation of 15 ml phosphate solvent bacteria per plant inoculum can increase the availability of P (62.21%) and increase the dry weight of soybean plants. Anjasmoro varieties, baluran and grobogan are superior varieties. Anjasmoro and Grobogan varieties are soybeans with large, round and yellow seed characters. The highest economic contribution of superior varieties of soybeans was given by Anjasmoro (Rp. 1.3 trillion), Mahameru (Rp. 0.61 trillion), Grobogan (Rp. 0.61 trillion), Wilis (Rp. 0.56 trillion), and Baluran (Rp. 0.25 trillion). Nationally, the economic contribution of superior varieties of soybean reaches Rp 3.9 trillion (Krisdiana, 2013).

MATERIALS AND METHODS

Experiments carried out in the laboratory are rhizobacterial exploration of the rhizosphere and both isolation and identification of rhizobacteria, from August 2018 to February 2019.

Rhizobacteria isolates from samples of rice roots, rhizosphere of red chilli and eggplant plants. 10 g of each sample was taken and put into 100 ml of sterile distilled water in an Erlenmeyer flask (vol 250), the suspension was homogenized using a screw for 10 minutes at a speed of 100 rpm, then let stand for 10 minutes. The suspension was diluted in series with sterile distilled water, the suspension from the 10-5, 10-6 and 10-7 dilutions was taken 1 ml each then grown on Nutrient Agar (NA) medium and incubated at room temperature for 48 hours. Each individual colony that appeared that was morphologically different was transferred with a sterile loop needle on a petri dish containing the same medium, incubated for 24 hours. For storage the pure isolates were transferred to via eppendorf (vol2 ml) containing nutrient media without agar + 10% glyserol and labeled (date and isolate code) then stored in an AC room at 10-12 ^{0}C.

Identification based on morphology is observing colony shape, colony surface and colony color. Physiological identification, namely the gram reaction, the gram test using 3% KOH. Hypersensitivity reaction with bacterial Xaa suspension at a population density of 108 cells / ml was infused on the lower surface of tobacco leaves using a syringe. The leaves were infused incubated for 2x24 hours. If necrotic occurs within 2x24 hours, it means that the bacteria are HR+. Tobacco leaves that were integrated with Xaa isolate showed a hypersensitive reaction in the

form of necrotic symptoms. As a Phosphate Solvent, namely rhizobacterial Phosphate grown on Pikovskaya selective medium (5 g Ca3 (PO4); 0.2 g KCl; 0.2 g NaCl; 0.1 g MgSO4.7H2O; 2.5 g MnSO4.7H2O; 2.5 g feSO4.7H2O; 0.5 g (NH4) 2SO4; 10 g glucose; 0.5 g yeast extract; 15 g agar; 1000 ml distilled water) and incubated 2 x 24 hours. The germination test was carried out by planting mung bean seeds in 250 ml element, where the cotton was moistened with distilled water and put in 250 ml element then sterilized. After that, the green bean seeds were soaked with phosphate solubilizing bacterial isolates for 15 minutes and then planted.

RESULTS AND DISCUSSION

Isolation

Based on the results of rhizobaceria isolation from ultisol soil, chilli plants and eggplant plants and rice roots obtained 25 isolates. In ultisol soil, chili plants obtained 14 isolates, 14 isolates were RC1, RC2, RC3, RC4, RC5, RC6. RC7, RC8, RC9, RC10, RC11, RC12, RC13 and RC14. In ultisol soil, eggplant plants obtained 5 isolates, 5 isolates are RT1, RT2, RT3, RT4 and RT5. At the root of the rice plant 6 isolates were obtained namely AP1, AP2, AP3, AP4, AP5 and AP6. Ultisol soil of chilli plants found many rhizobacteria isolates compared to ultisol soil of eggplant and rice roots. The existence of these microbes is based on the number of organics found in the soil.

Morphological Character Identification

Based on the results of rhizobacterial isolation from the ultisol rhizosphere of chilies, eggplant plants and rice roots, 32 isolates were obtained The distribution and character of rhizobacterial isolates in each varied (Table 1).

The highest number of isolates were obtained from the ultisol soil of chilli plants, namely 18 isolates. Ultisol soil 6 eggplant plant isolates and 8 rice root plant isolates. The morphology of 32 colonies of rhizobacteria isolates varied and could be grouped into 2 forms of colonies, namely 16 isolates (50%) and irregular 16 isolates (50%). The amount of isolates obtained from each soil sample was determined by soil type, soil moisture, pH, soil temperature, plant age and relative humidity. In addition, the number of isolates was also influenced by the availability of energy sources in the form of organic carbon and the ability of the bacteria itself to compete for the same energy source in their habitat (Rohyani et al, 2014).

The color of rhizobacteria isolate colony was obtained by 27 cream isolates and 5 yellow isolates. In ultisol soil, chili plant isolate cream color was obtained 16 isolates and yellow color 2 isolates. Ultisol soil of eggplant was obtained by the color of cream colony, 5 isolates and yellow color 1 isolate. Rice roots obtained 6 colonies cream color and 2 yellow isolates.

Physiology Based Identification

Figure 1 shows that the tobacco leaves were infiltrated with HR bacterial suspension, this is indicated by the absence of necrotic tobacco leaves after 48 hours of infiltration with RT1 and RC3 isolates. So that the RT1 and RC3 isolates were not classified as pathogens.

Gram reactions of 32 isolates showed gram-negative. Based on the results of hypersensitivity reaction (HR) 2 phosphate solubilizing bacteria isolates were HR negative on tobacco leaves after infiltration of bacterial suspension (106 cells ml-1). The two isolates consisted of RC3 and RT1. RC3 comes from the ultisol soil of the chili plant and RT1 comes from the ultisol soil of the eggplant plant.

Figure 2 shows the phosphate solubilizing bacteria which is characterized by the

Table 1. Identification of rhizobacteria based on morphological characters in some samples from isolates

Origin of Isolates	JI	Morphology of the Colonies						
		Shape		Surface			Color	
		B	I	T	D	C	Ke	Ku
Soil Ultisol chilies	18	9	9	8	9	1	16	2
Soil Ultisol eggplant plants	6	5	1	4	2	0	5	1
Rice Roots	8	2	6	6	2	0	6	2

Note: IF: Amount of isolate, B: Round, I: Irregular, T: Embossed, D: Flat, C: Convex, To: Creamy, Ku: Yellow

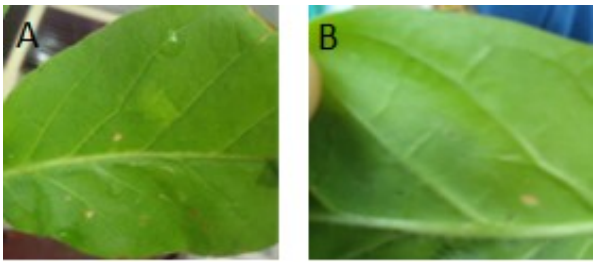

Figure 1. Hypersensitivity reactions in tobacco plants treated with isolates RT1 (A) and RC3 (B)

formation of a clear zone around the colony on Pikovskaya medium. According to Maryanti (2006), a sign that a bacterium can dissolve phosphate is a clear zone around the bacteria. the colony and the increase in the size of the bacterial colony on the pikovskaya media, this is because the bacteria can dissolve the phosphate contained in the pikovskaya media formulation. In addition, the clear zone around the colony shows that the isolate is able to produce organic acids which bind to Ca ions to form Ca3 (PO4) 2 compounds in the media so that they are pikovskaya and form H2PO4 ions so that they are formed more clearly area (Larasati et al, 2018).

Phosphate solvent bacteria was a bacterium that had a very large ability as a biofertilizer by dissolving phosphates that were still trapped in the soil such as Fe, Al, Ca and Mg so that these elements could be dissolved by bacteria and then become available elements for plants (Marbun, *et al,* 2015).

Germination Test

Table 2 shows the emergence days of germination without giving the emergence

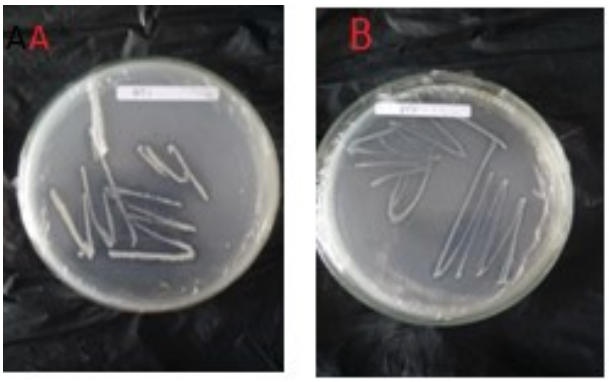

Figure 2. Rhizobacterial isolates of phosphate solvents were characterized by clear zones around the colony. RT1 (A) and RC3 (B)

days of 1.25 days of germination, with the provision of RPF RT1 and RC3 the sprouts appeared 1.00 days. The provision of RPF can spur green bean germination. The ability of phosphate solvent is one of the direct growth enhancement mechanisms by PGPR and IAA.

Figure 3 shows with the initial phosphate solvent rizobacterial appearing germinated on green beans faster than without the administration of phosphate solvent rizobacteri. RPF isolates are capable of producing IAA and ZPT, in general the IAA stimulates the germination of seeds and tubers and controls vegetative growth. growth regulatory substances (auksin, giberalin) especially those living around rooting Indications of the high response of isolates RT1 and RC3 to the day sprouts appear may also be influenced by the growth regulator.

A B C

Figure 3. Germination of mung beans without Isolate (A), Germination of mung beans by giving RT1 (B) and germination of mung beans by administration of RC3 (C).

Table 2 shows the weight of green bean seedlings without RPF produces 2.52 g while with the provision of RPF RT1 produces a seed weight of 2.76 g and RC3 produces a seed weight of 2.57 g. RPF can affect the weight of green bean seedlings. PGPR is able to increase plant growth because it produces IAA, Gibberellin and Cytokines. In general the IAA stimulates germination of seeds and tubers and controls vegetative growth.

Table 2 shows that the administration of phosphate solvent rhizobacterials at the root length and shoots of green bean crops does not differ much. Without BPF giving is no different from the provision of RPF of 2.66. RPF RT1 produces 2.66 cm long roots and

Table 2. Days of emergence germination. seed weight. root length and shoots. and percentage of the number of germination of green bean plants due to administration of phosphate solvent rizobacteria.

Rhizobacteria Phosphate Solvents	Day Appears to Germinate (day)	Seed Weight (gram)	Root and Root Length (gram)	Percentage of Amount Sprouted (%)
Control	1.25	2.52	2.66	91.50
RT1	1.00	2.76	2.93	95.00
RC3	1.00	2.57	2.23	96.58
KK (%)	26.62	13.38	13.38	9.03

buds of green beans and BPF RC3 produces 2.23 cm long roots and buds of green beans.

Figure 4 shows the length of the roots and buds of green beans is no different, but the difference between green bean root, green bean root without isolate is softer while the administration of isolates RT1 and RC3 is tighter. Isolate RT1 and RC3 are able to produce vitamins and phytohormones so as to increase root growth (Widawati and Suliasih, 2006).

Table 2 shows the percentage of bean sprouts without RPF provision, namely 91.50%. The percentage of RPF RT1 from the number of sprouts was 95.00% and RPF RC3 for the number of sprouts was 96.58%. However, with RPF RC3 which affects the percentage of sprouts, RBF is able to secrete the enzyme phosphatase which plays a role in the hydrolysis of organic P to inorganic P and can produce growth regulators (Ilham, et al, 2014).

CONCLUSION

We found 32 isolates from ultisol soil of chilli plants, eggplant plants and rice rhizosphere. The chili plant soil obtained more isolates, namely 18 isolates. Eggplant soil obtained 6 isolates and rice roots obtained 8 isolates. The colony forms of 32 rhizobacterial isolates varied and could be grouped into 2 colony forms, namely 16 isolates (50%) round and 16 irregular isolates (50%). Colony surfaces were grouped into 3 surfaces, namely 16 isolates, 13 flat isolates and 1 convex isolate. Colony colors were grouped into 2 colors, 27 isolates beige and 5 isolates yellow. All isolates were gram negative. Of the 32 rhizobacterial isolates, there were 2 isolates that were able to dissolve phosphate, namely RT1 and RC3, by giving RT1 the seed weight was 2.76 grams higher than control and RC3

REFERENCES

Faccini, G., S. Garzo'n, M. Marti'nes, and A. Varela. 2004. Evaluation of the effects of a dual inoculum of phosphate-solubilizing bacteria and *Azotobacter chroococcum*, in creolo potato (Papa "Criolla") yema de huevo variety (*Solanum phureya*). First International Meetingon Microbial Phosphate Solubilization (pp. 301-308). Springer. Berlin, German.

Hafeez, F.Y., S. Yasmin, D. Ariani, M. Rahman, Y. Zafar, and K.A. Malik. 2006. Plant growth promoting bacteria as biofertilizer. J Agron. Sustain. Dev 26:143-150.

Ilham, B.G.D. Ida, M.O.N. Gusti, and K. Retno. 2014. Isolates and identification of potential phosphate solvent bacteria in conventional soils and organic soils.

A B C

Figure 4. Root length and shoots of mung beans without Isolate (A), Root length and shoots of mung beans by giving RT1 (B) and Root length and shoots of mung beans by giving RC3 (C)

Journal of Symbiosis II (1): 173-183.

Hasnudin. 2002. Improved soil fertility and soybean yield due to microbial inoculation of phosphate and azobacterial solvents in Ultisol. Indonesian Journal of Agricultural Sciences 4(2): 97-103.

Joo, G.J., Y.M. Kim, J.T. Kim, I.K. Rhee, J.H. Kim, and I.J. Lee. 2005. Gibberellins-producing rhizoba cteria increase endogenous gibberellins content and promote growth of red peppers. J. Microbiol 43: 510-515.

Karnwal, A. 2009. Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L- tryptophan and rice root exudates. J. Plant Pathol. 91: 61-63.

Khamdanah, R.A. Tiara, and P. Jati. 2014. Effectiveness of phosphate solubilizing bacteria from the Lebak Ultisol Land of Banten against soybean growth. Proceedings of the seminar on Organic Agriculture Journals. Bogor, Indonesia.

Krisdiana, R. 2014. Distribution of soybean superior varieties and their impact on the rural economy. J Food Crop Agriculture Research 33(1): 61-69.

Larasati, E.D., M.G.I. Rukmi, E. Kusdiyantini, and R.C.B. Ginting. 2018. Isolation and identification of phosphate solubilizing bacteria from peat soil. Biomes. 20(1): 1-8.

Marbun, S., M. Sembiring, Bintang. 2015. The Application of microbial phosphate solvents and organic materials to increase P absorption and potato growth in Andisols affected by eruption of Mt. Journal of Agroecotechnology 4(1) :1651 – 1658.

Maryanti, D. 2006. Isolation and test the ability of phosphate solvent bacteria from rhizosphere food plants and shrubs. Skripsi. Andalas University Faculty of Agriculture. Padang, Indonesia.

Mehrvraz, S., and M.R. Chaichi. 2008. Effect of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on forage and garin quality of barley. American-Eurasian J.Agric. & Environ. Sci. 3(6): 855-860.

Prasetyo, B.H., and D.A. Suriadikarta. 2006. Characteristics, Potential and technology of Ultisol land management for the development of dry land agriculture in Indonesia. Agricultural Research and Development, 25(2): 39-47.

Rao, N.S.S. 2007. Microorganisms and Plant Growth. UI Press, Jakarta.

Rohyani, D. Zul, and B.L. Fibrianti. 2014. Potential indigenus bacteria isolates as biofertilizer agents from peat soils in the Zambrud Region and Tesso Nilo National Park, Riau. JOM FMIPA 1(2): 417-429.

Simanungkalit, R.D.M., and D.A. Suriadikarta. 2006. Organic Fertilizers and Biofertilizers. Indonesian Center for Agricultural Land Resources. Bogor, Indonesia

Sudaryanto, T., and D.K.S. Swastika. 2007. Soybean Economy in Indonesia. In: Sumarno, Suyamto, A. Widjono, K. H.Hermanto, (Eds). Soybean: Production and Development Techniques (pp. 1-27). Agency for Research and Development of Agricultural Plants. Bogor, Indonesia.

Suliasih, S. Widawati, and A. Muharam. 2010. Application of organic fertilizers and phosphate solvent bacteria to increase tomato plant growth and soil microbial activity. J. Hort. 20(3): 241-246.

Widiawati, S., and Suliasih. 2005. Augmentation of phosphate solubilizing bacteria (BPF) as a Potential for growth of caisin (*Brasica caventis* Oed.) in marginal soils. J Biodiversitas 7(1): 10-14.