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ISOLATION AND SCREENING OF PHOSPHATE SOLUBILIZING BACTERIA FROM RHIZOSPHERE OF TEA (CAMELLIA SINENSIS L.) ON ANDISOLS

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ABSTRACT

Andisols is a soil with high retention of phosphate and cannot be absorbed by plants. Some of soil bacteria have the ability to solubilize P and make it available to growing plants are known phosphate solubilizing bacteria (PSB). The research aims to get the kinds of bacteria potential of PSB in the tea plantation. The experiment was conducted at the Laboratory of Biology, Faculty of Agriculture, Padjadjaran University from February 2016 to April 2016. Selection taken from five point blocks with a depth of 0-30 cm and 30-60 cm decision to the topography peaks and slopes. Obtained 23 isolates taken PSB and five isolates with the highest qualitative P solubility test in the soil. The experimental was conducted using Completely Randomized Design, which consists of ten treatments, sterile and non-sterile soil, and five isolates. Incubation was performed for 21 days and the total test bacteria, pH, and P-available every 7, 14, and 21 days. The results showed the best bacterial isolates that capable of solubilize phosphate was taken from A6 block located in the north area of the plantation with a depth of 0-30 cm and is at the peak topography with the ability to dissolve P in the soil is not sterile at 0.997 ppm on the 14th day.

1. INTRODUCTION

One of well-known commodities in Indonesia is tea. Tea is a plantations that processed for beverages that widely consumed in the world. Tea is widely planted in Andisols soil which is widely spread in West Java. Tea productivity has been undergoing fluctuations development and tends to decrease in the last few years. One of the causes that decreased tea production is land degradation in response to soil conditions.

Andisols is a soil that has problems of a low P availability, although the soil was conducted with intensive P fertilization. Intensive and continous P fertilization can cause environmental pollution and the soil will no longer response to fertilization. To improve fertilizer efficiency and reduce the costs, it is necessary to develop soil biotechnology through application of biofertilizer. Biofertilizer is an amendment material containing beneficial microbes to improve soil fertility and optimal plant growth in order to obtain maximum plant production.

The main obstacle for growing plants on Andisols among others is, it has a low pH, high in organic material and a very high soil P, but low P available for plant as well as poisoned. The P nutrient deficiencies caused by elements that strongly dependent on soil colloids such as clay mineral and oxides of iron and aluminum form Al-P and Fe-P so it becomes unavailable for plants. These events caused by retention of P linking P where these elements still can be extracted with dilute acid, therefore form P can provide quite a lot for plant growth.

Some free-living microbes in the soil have the ability to dissolve P soil that is attached to be available, therefore the plants are able to absorb P to satisfy it needs. Phosphate solubilizing microbes (PSM), this is a group of soil microbes that have the ability to extract P from the bonds with Al, Fe, Ca, dan Mg, so that it can be dissolved P whose origin is not available for plant becomes available for plants. This happens because these microbes secrete organic acids which can form stable complexes with cations binder P in the soil. Microbes play a role in this process of dissolving phosphorus, among others of group bacteria: *Pseudomonas*, *Bacillus*, *Mycobacterium*, *Micrococcus* "phosphobacteria " , while from group of fungi : *Penicillium*, *Aspergillus*, *Fusarium*, *Sclerotium* (Whitelaw, 2000).

Phosphate solubilizing microbes application on the soil showed a good effect on the improvement of nutritional status as well as increased plants yields. PSM application producer of plant growth regulator (*Pseudomonas cepaceae* , *P. mallei* , *Aspergillus niger* and *Penicillium* sp.) can improve the efficiency of P' fertilizer, by reducing the needs for P'fertilizer up to 50% on Ultisols soil, and increase yields of corn plants to 20% (Fitriatin et al., 2013; Fitriatin et al., 2014).

The decline in soil fertility in tea garden can reduce the growth and the results of the tea plant (Rahardjo and Salim, 1995). The soil quality is not only determined by its chemical factors, Biological factors are also important in knowing the quality of the soil. Total population of beneficial microbes in the soil indicates the quality of the soil. Therefore, Selection of indigenus phosphate solubilizing bacteria on the tea plant's Andisol is necessary to help release the element P from linkages between Al and Fe so that it becomes a form that can be absorbed by plants.

2. MATERIAL AND METHOD

Andisol soil samples from the tea plantations in Gambung area, West Java Indonesia was taken using the drill ground. The depth was taken in 0-30 cm and 30-60 cm. Soil samples were collected in two categories topography, which is slopes category and peaks category. The total soil samples taken were 20 samples. Media for PSB's isolation was Pikovskaya.

The experiment was conducted in two phases, which is isolation phase and selection of bacterial phosphate solvent, and dissolution test P on Andisols. Isolation and selection phase, includes sampling, isolation of phosphate solubilizing bacteria, macroscopic observation of bacterial colonies, clear zone and P dissolve test in Pikovskaya 's liquid media.

P leaching test phase in Andisols carried out by using experimental methods of Complete Random Design. The experiment consisted of ten treatments and five repetitions. This experiment was conducted on Andisols which consists of ten treatments which were five sterile soils and five non-sterile soils also five isolates. Each treatment was repeated five times, and in one repetition there were 10 units of experiments, so the total were 50 units experiments. The observed variable response were P available and the population of PSB 7, 14 and 21 days after inoculation.

Qualitative analysis includes visual data that were analyzed using descriptive methods, while quantitative data were analyzed using analysis of variance by the F test level of 5% and if there is a significant difference continued with Duncan Multiple Range Test at 5% (Gaspersz, 1994). Test statistical analysis performed using IBM SPSS Statistics 23 software.

3. RESULTS AND DSICUSSION

3.1. Soil pH

The results showed no significant difference between the treatment of the soil pH, however the change of pH occurred with incubation time (Table 1). Every week the average pH decreases by 0,05 until 0,1 with a final pH 5,51 for sterile soil and 5,28 for non-sterile soil.

Table-1. Data on average pH of dy 7, 14, 21

Treatment	Day 7	Day 14	Day 21
isolate 1 S*	5.38	5.57	5.61
isolate 2 S	5.44	5.46	5.52
isolate 3 S	5.40	5.48	5.50
isolate 4 S	5.31	5.45	5.50
Isolate 5 S	5.25	5.36	5.42
isolate 1 NS*	5.08	5.13	5.17
isolate 2 NS	5.28	5.33	5.39
isolate 3 NS	5.08	5.10	5.16
isolate 4 NS	5.17	5.26	5.29
Isolate 5 NS	5.23	5.30	5.41

S (sterile soil) dan NS (non steril soil)

Soil acidity can affect microbial growth and species diversity. In general, the cells always maintain the pH conditions within the cell so that it constants near neutral to be able to do some of the biochemical process by enzymes in the cell organelles. Some microbes can remove a number of protons into the cytoplasm by using energy. The ability of bacteria to remove these ions which can distinguish certain groups of bacteria can be resistant to acid (low pH) or not acid resistant.

According to Widawati *et al.* (2005) that the maximum pH for phosphate availability in the soil is 6.5 whereas phosphate solubilizing bacteria still has the ability to dissolve phosphate at pH below 5.5. According to Hardjowigeno (1993) that the availability of phosphate in the soil maximum at near neutral pH of 5.5 to 7.

A variety of beneficial bacteria activities will be hampered if not introduced into it natives habitat. Acidity factors (pH) greatly limits the activity of these bacteria, therefore selection of the beneficial bacteria in growth media which according to the media that grows tea plants will be very useful to capture the potential of bacteria from which applied to Andisol soils (Pranoto and Setiawati, 2014).

3.2. Population of Phosphate Solubilizing Bacteria

Total population of PSB tend to increase from day 7 to day 14 (Table 2). The highest changes occur in bacterial isolates 1 and lowest in 4 isolates for treatment using sterile soil. In non-sterile soil the highest changes occured in isolates 1 and the lowest occurred in isolates 2.

On day 21, bacterial population has decreased considerably compared to day 14. This is presumably because the organic matter in the soil used for living bacteria has decreased and leaving only the best phosphate solvent bacteria to survive. The highest bacterial population in sterile soil occurred in isolates 1, and the lowest occurred in isolates 2. Meanwhile, the highest population for non-sterile soil occurred in isolates 1 and the lowest occurred in isolates 3.

The bacterial population of non-sterile soil was higher than the population of bacteria in sterile soil. It is suspected that there are still other bacteria besides phosphate solvent bacteria that grows in the petridish. A decrease in bacterial populations phosphate solu from day 14 to day 21 to be expected because the bacteria lost their natural state. In these circumstances, population of indigenous bacteria lack of optimum condition, such as temperature, humidity, content of organic material, and the competition factor.

According to statistical data analysis, the entire treatment gives effect, it's just a different treatment clearly shows only on treatment on day 7, whereas on day 14 and 21 were not clearly shows the differences.

Table-2. Population of phosphate solubilizing bacteria days 7, 14, and 21

Treatment	Population of PSB (CFU/g) x 10^6		
	Day 7	Day14	Day21
isolate 1 S*	85,40 a	131 a	4,00 a
isolate 2 S	91,00 a	87 a	2,00 a
isolate 3 S	88,70 a	92 a	3,10 a
isolate 4 S	91,80 a	85 a	2,20 a
Isolate 5 S	80,10 a	112 a	3,10 a
isolate 1 NS*	104,30 ab	130 a	4,50 a
isolate 2 NS	124,90 b	93 a	3,90 a
isolate 3 NS	94,70 a	102 a	1,40 a
isolate 4 NS	102,70 ab	93 a	2,30 a
Isolate 5 NS	102,70 ab	120 a	2,00 a

The numbers followed by the same letter are not significantly different according DMRT at the level of 5%

Effect of quantitative and qualitative composition of soil microbial populations on environment highly dependent on natural conditions of the soil and the relative composition of organic and inorganic materials in soil (Rao, 1994). The population of microbes in the soil is influenced by the level of sensitivity of microbes, soil fertility, humidity, and light intensity. The highest population of soil microbes normally located at the rhizosphere's layer (Lynch, 1989).

Phosphate solubilizing microorganism growth is also strongly influenced by soil acidity. On acid soils, activities of microorganisms is affected by the fungi because the fungi growth optimum at pH 5-5.5. The growth of fungi decreases with increasing pH. Otherwise, groups of bacteria optimum growth at approximately neutral pH and increases with increasing soil pH (Ginting *et al*., 2006). This is in line with a rise in pH experienced by soil that were incubated with the growth of bacterial populations. The increase of the pH soil, followed by the increase of the population of bacteria.

3.3. Soil P-Available

The experimental results showed that the inoculation PSB significantly affect towards P available soil on day 7 and 14 in the incubation (Table 3).

Table-3. The results of the analysis of soil P-available

Treatment	P-available (ppm)		
	Day 7	Day 14	Day 21
isolate 1 S*	0.08 c*	0.39 a	0.12 ab
isolate 2 S	0.07 abc	0.38 a	0.13 abc
isolate 3 S	0.06 ab	0.33 a	0.17 abc
isolate 4 S	0.08 bc	0.37 a	0.12 ab
Isolate 5 S	0.07 a	0.37 a	0.10 a
isolate 1 NS*	0.08 bc	1.00 d	0.19 bc
isolate 2 NS	0.07 bc	0.56 abc	0.20 c
isolate 3 NS	0.07 abc	0.74 c	0.17 abc
isolate 4 NS	0.07 abc	0.47 ab	0.17 abc
Isolate 5 NS	0.07 bc	0.71 bc	0.15 abc

*The numbers followed by the same letter are not significantly different according DMRT at the level of 5%

On day 14, soil P-available on the soil that were incubated with phosphate solubilizing bacteria has increased compared with day 7. It is suspected caused by phosphate solubilizing bacteria which starts working at its optimum and has been able to adapt to the surrounding environment. Here is a table of Duncan's P-available on day 7. The best result was shown by isolates 1.

Soil treatment without sterilization has P available levels higher than the sterilized soil. This situation shows the influence of microbes indigenus in providing nutrients P to the soil without sterilization. Dissolution of P not only can be done by phosphate solubilizing microbes, but also other microbes that are capable of producing an inorganic acid (Sudadi *et al*., 2013). The microbial biomass and cell density can affect the availability of phosphate through immobilization, that is orthophosphate ion fastened to inorganic forms that are bound in the organism.

Activities phosphate solubilizing bacteria can be induced when the amount of P is limited in growth media and microbial growth media, it also characterizes the high demand for P (Savin et al., 2000). Marlina (1997) stated that P-available content in the soil was positively correlated with the number of phosphate solubilizing bacteria contained in the soil. According to Margaretha *et al.* (1999) differences in population of phosphate solubilizing bacteria causes the difference in the availability of P in soil. The population differences lead to differences in the number of organic acids produced by phosphate solubilizing bacteria.

Widyati (2007) stated that the ability of bacterial isolates in dissolving P and produce organic acids depends on the metabolic processes of bacteria isolates itself. The process of bacterial metabolism is influenced by the activity of the enzyme. This indicates that the solvent P bacteria is not optimal to secrete enzymes so that the organic acids that can improve the availability of P becomes less available.

Noorhadi and Sudadi (2003) stated that P dissolution is not only depends on the microbial population but more on the ability of microbes that produce an organic acid for dissolving phosphate. Each species of microbes phosphate solvent have the ability to be genetically different to produce organic acids, both in number and kind during growth. Activity of bacteria in dissolving P on solid and liquid media is not absolutely the same. Clear zone criteria is not enough to determine the ability of bacteria in dissolving P. A lot of numbers of microbes also does not necessarily have a high ability to dissolve P (Fankem *et al*., 2006).

4. CONCLUSION

Phosphate solubilizing superior isolates obtained from tea plants rhizosphere on Andisols that is isolate 1 that have potential in dissolving P Andisols is superior compared to other isolates. The best bacterial isolates that capable of solubilize phosphate was taken from A6 block located in the north area of the plantation with a depth of 0-30 cm and is at the peak topography with the ability to dissolve P in the soil is not sterile at 0.997 ppm on the 14th day.

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