Potential of *Capsicum annuum* rhizosphere bacteria in inhibiting the growth of *Fusarium oxysporum* in-vitro

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**Abstract.** The rhizosphere has been known to contain rich microbial community which is directly influenced by secretions of roots. Rhizosphere microbes are known to have various benefits such as protection of plant from pathogens and toxic compounds, producing plant growth hormone, nitrogen fixation, and solubilizing phosphate. The objective of this study was to obtain rhizosphere bacteria capable of inhibiting the growth *Fusarium oxysporum* which is common pathogen attaching *Capsicum annum*. Bacteria were isolated from rhizosphere *C. annum* of local farm in Kabanjahe North Sumatra. Nine isolates based on morphological performance and biochemical analyses were obtained. Antagonistic test showed that two isolates BR6 and BR9 inhibited the growth of *F. oxysporum* with inhibition rate of 60% and 52%, respectively after 7 days incubation. These isolates also exhibited the activity to solubilize phosphate in Pikovskaya media with solubility index of 3.04 and 2.77, respectively. The other isolate BR5 even showed higher phosphate solubility index (3.42). These results suggest these isolates may have important role to support the growth of *C. annum* by providing protection from pathogens and phosphate ion. Then, study on the control of *F. oxysporum* attack on *C. annum* seedlings using these potential isolates is in process in our laboratory, as well as their molecular identification based on 16S rRNA.

1. **Introduction**

Red chili (*Capsicum annuum* L.) is a horticultural commodity of high economic value for domestic needs and the food industry [1]. However, according to the Central Bureau of Statistics of North Sumatera [2] from 2015 to 2016 the production of red chili has decreased compared to that of 2014. Singh et al. [3] reported that one of the causes of the decrease is the presence of plant pathogens that cause diseases; the plants become rotten and wilted. Saraf et al. [4] plant pathogens are very abundant in the rhizosphere plant including virus, nematode, bacteria, and fungi. Fungal pathogen such as *Fusarium oxysporum* f.sp. capsici is commonly found and attacks *C. annum* and causes great production lost.

An alternative to increase production of red chili is using microbial agents which have an ability to inhibit the growth of certain pathogen as well as to produce plant growth hormone, plant growth promoting rhizobacteria known as PGPR has attracted many researcher for the last three decades. The bacteria colonize plant roots and secrete plant growth hormone, give fertility to the land, and inhibit the growth of pathogens [5]. In addition to have antagonistic activity of pathogens and to produce plant growth hormone, rhizosphere bacteria have been reported to exhibit the ability to solubilize
phosphate. These bacteria provide phosphate ion available for the plant from fix phosphate [6]. Widawati [7] reported that the presence of rhizosphere bacteria in their ability as phosphate solvents is an important indication in the biogeochemical cycle of phosphorus and plant growth.

Meanwhile, some results show that each bacterial isolate has a different ability and different mechanism to induce plants [8]. Therefore, it is necessary to know the potential of rhizosphere bacteria as phosphate solvents and have antagonistic activity against pathogen. This study reported the ability of rhizosphere bacteria isolated from chili (Capsicum annuum L.) have an ability to inhibit the growth of Fusarium oxysporum f.sp. capsici and to solubilize phosphate in vitro.

2. Material and Method

2.1. Isolation of Rhizosphere Bacteria

Five hundred grams of soil samples were taken from red chili rhizosphere of local farm in Kabanjahe, North Sumatera using clean shovel and kept in sterile plastic bags for isolation. A total of 10 g of soil was put into 90 ml of sterile distilled water in a 250 mL erlenmeyer and shaken at 100 rpm for 15 minutes. One ml of the suspension was taken into new tube containing 9 ml of sterile distilled water. The process was repeated until dilution of 10⁻¹ was achieved. One ml of sample suspension was spread on nutrient agar (NA) plate media and incubated for 24 hours at 28 ± 2°C. Each growing colony was transferred to new NA plate for purification of isolate. Characteristic of each different isolate including morphology and simple biochemical analyses was recorded as routine analyses.

2.2. Test of phosphate solubilization activity

Test of phosphate solubilization was done using Piskovkaya agar media (PKV) with a composition (g/l) of 10 g glucose; 5 g Ca³⁺ (PO₄)₂; 0.2 g KCl; 0.5 g (NH₄)₂SO₄; 0.2 g NaCl; 0.1 g MgSO₄·7H₂O; 0.002 g MnSO₄·H₂O; 0.002 g FeSO₄·7H₂O; yeast extract 0.5 g and 15 g bacto agar. A paper disc containing 10 µl bacterial suspension was placed on the center of Piskovkaya agar and plates were incubated incubated at 28 ± 2°C for 7 days. The measurement of the phosphate solubilization index was carried out at the end of incubation by measuring the diameter of clear zone and the diameter of the colony. The index was calculated by following:

\[
\text{Phosphate Solubilization Index (SI)} = \frac{\text{diameter of clear zone} - \text{colony diameter}}{\text{colony diameter}}
\]

2.3. Antagonistic test of phosphate solubilizing bacteria to Fusarium oxysporum

Fusarium oxysporum used in this study was obtained from the Laboratory Microbiology, Faculty of Science, University of Sumatera Utara. Fungi was subcultured on potato dextrose agar (PDA) media and incubated at 28 ± 2°C. Rhizosphere bacteria used for this test were bacterial isolates which have higher phosphate solvent index from the previous screening. The antagonist test was carried out using the standard double culture method using the PDA media plus yeast extract. An agar plug of fully growth of F. oxysporum was inoculated in the center PDA plate. Then paper discs containing 10 µl of the suspension of selected phosphate solubilizing bacteria were placed on each side of F. oxysporum agar plug with a distance of 3 cm. The culture was incubated at 28 ± 2°C for 3-5 days. The antagonistic activity was observed during incubation. The antagonistic interaction is characterized by the formation of an inhibition zone between antagonistic bacteria and fungi [9]. Diameters of bacterial colonies and F. oxysporum were measure using calipers. Percentage of inhibition was calculated by formulas Kaiser et al. [10]:

\[
\text{Percentage of inhibition} = \left[\frac{A - B}{A}\right] \times 100\%
\]

A: Normal fungal growth distance
B: Distance of fungal growth inhibition
3. Results and Discussion

3.1. Characteristic of rhizosphere bacteria from C. annuum

Nine different isolates were based on morphological characters obtained from rhizosphere of red chili, three isolates with irregular shape and six isolates with circular shape of colony. Edge of the colony was mostly entire and undulate, but all showed flat colony, and the color of colony were mostly white (seven isolates). Gram staining showed all isolates were gram negative with seven isolates were cocci and two were bacilli. The different characters of observable colony indicated the difference species of each isolate. Kirisits et al. [11] reported that the differences in the morphology of colonies and bacterial properties were caused by different sources of isolates and intracellular pigments, namely carotenoids, anthocyanins, melanin, triphyl methenes and phenazim. Cappucino and Sherman [12] reported the form of bacterial colonies generally in the form of circular, irregular. Elevations are raised, convex, flat, umbonate, crateriform. Edge entire, filamentous, rhizoid, undulate, filiform, curled and lobate forms. This is in line with the research conducted by Ulfa et al. [13] which reported the isolation results of average bacterial isolates in the form of circular, irregular, entire, undulate, flat, pigmented, gram negative.

Characterization of bacterial isolates based on biochemical tests included starch hydrolysis test, gelatin hydrolysis test, citrate test, hydrogen sulfide test, motility test, and catalase test. Like morphological characters, most isolates exhibited different characteristics from the biochemical test. Lay [14] reported that biochemical tests are generally carried out to identify the metabolic activities of bacterial isolates, including the ability to interact between metabolites and chemical reagents and utilize certain compounds as energy and carbon sources. Characters morphology and the Gram nature of the rhizosphere bacteria can be seen in Table 1 and for biochemical tests in Table 2.

### Table 1. Characters of morphology and Gram nature of rhizospheric bacteria

| Isolate Code | Colony Morphology | Gram Morphology cell |
|--------------|-------------------|----------------------|
| BR 1         | Circular          | - Coccus             |
| BR 2         | Circular          | - Coccus             |
| BR 3         | Circular          | - Coccus             |
| BR 4         | Circular          | - Coccus             |
| BR 5         | Irregular         | - Coccus             |
| BR 6         | Irregular         | - Bacill             |
| BR 7         | Circular          | - Coccus             |
| BR 8         | Circular          | - Bacill             |
| BR 9         | Irregular         | - Bacill             |
Table 2. Characteristics of biochemical test of rhizosphere bacteria

| Isolate Code | Starch Hydrolysis | Gelatin Hydrolysis | Citric | Hydrogen Sulfide | Motility | Catalase |
|--------------|-------------------|--------------------|--------|------------------|----------|----------|
|              | Slant              | Butt               |        |                  |          |          |
| BR 1         | +                  | -                  | -      | Yellow           | Yellow   | +        |
| BR 2         | +                  | -                  | -      | Yellow           | Yellow   | +        |
| BR 3         | +                  | -                  | +      | Red              | Yellow   | -        |
| BR 4         | +                  | -                  | +      | Red              | Red      | -        |
| BR 5         | +                  | -                  | +      | Yellow           | Yellow   | -        |
| BR 6         | +                  | -                  | +      | Red              | Red      | +        |
| BR 7         | +                  | -                  | +      | Yellow           | Yellow   | +        |
| BR 8         | +                  | -                  | +      | Yellow           | Yellow   | +        |
| BR 9         | +                  | -                  | +      | Red              | Red      | +        |

3.2. Screening of rhizosphere bacterial isolates

Qualitative testing of phosphate dissolution of rhizosphere bacterial isolates obtained as many as nine bacterial isolates capable of phosphate solubilization. The results of screening for bacterial isolates were then tested for the ability of bacteria to dissolve phosphate in agar Pikovskaya medium, to obtain a phosphate solubilization index (SI). From Table 3, bacterial isolates having the highest SI value was BR5 of 3.42, followed by BR6 and BR9 while the lowest value showed by isolate BR1 (Table 3). The high value of the phosphate SI exhibited by isolates BR5, BR6 and BR9. The isolates were grown in separate Piskovkaya agar media as shown Figure 2.

Table 3. Ability of the rhizosphere bacteria in solubilizing phosphates

| Isolate Code | Average colony diameter (mm) | Average diameter of clear zone (mm) | SI    |
|--------------|------------------------------|-------------------------------------|-------|
| BR 1         | 6.5                          | 8.44                                | 2.29  |
| BR 2         | 6.0                          | 7.9                                 | 2.31  |
| BR 3         | 6.0                          | 9.6                                 | 2.6   |
| BR 4         | 6.5                          | 8.4                                 | 2.29  |
| BR 5         | 5.0                          | 12.1                                | 3.42  |
| BR 6         | 6.0                          | 12.26                               | 3.04  |
| BR 7         | 6.0                          | 9.7                                 | 2.67  |
| BR 8         | 6.0                          | 9.7                                 | 2.67  |
| BR 9         | 6.0                          | 10.3                                | 2.77  |

Figure 1. Photograph of screening rhizosphere bacterial isolates to solubilize phosphate in Piskovkaya agar media. Clear zones around colonies indicate that bacterial isolates have the ability to solubilize phosphate.
Figure 2. Ability of Rizosphere Bacteria in Dissolving Phosphate by Measuring the Phosphate Dissolution Index (a) BR5, (b) BR6, and (c) BR9.

Figure 2 shows that three selected and potential isolates enabled to solubilize phosphate indicating with significant and clear zone around the colony. Rachmiati [15] reported that the size of the bacteria's ability to solubilize phosphate from insoluble phosphate is indicated by the presence of a clear zone around the isolates in the petri dish in pikovskaya agar media. Tatiek [16] also reported clear zones in the media so as not to show the ability of each bacterium to donate the amount of dissolved phosphate, even though the area of the clear zone showed a small size of phosphate dissolving bacteria. Based on the results of the phosphate solvent test, then nine bacterial isolates were tested antagonistically against *F. oxysporum*.

3.3. Antagonism activity of rhizosphere bacteria against *F. oxysporum*
Antagonism activity of rhizosphere bacterial isolates against fungal pathogen *F. oxysporum* was done for four selected isolates, results are depicted in Table 4. Two bacterial isolates have high ability to inhibit *F. oxysporum* growth, namely BR6 and BR9 with inhibition values of 60 % and 52 % followed by BR5 (38 %) after the 7th day.

| Code of Isolate | Incubation time (days) |
|-----------------|------------------------|
|                 | 3                      | 5          | 7          |
| BR 3            | 11                     | 16         | 18         |
| BR 5            | 23                     | 28         | 38         |
| BR 6            | 39.6                   | 46         | 60         |
| BR 9            | 47.5                   | 50.9       | 52         |

Table 4 also shows that value of inhibition activity increased as the incubation period which further indicates bacterial isolates continuously secrete antimetabolites to the media. Figure 3 clearly shows the antagonistic activity of rhizosphere isolates. Shehata et al. [17] reported that one of the characteristics of antagonistic microbes is faster growth than pathogens and / or produces antibiotic compounds that can inhibit pathogen growth. Low inhibitory activities of bacterial isolates can be caused the fungi also produce secondary metabolites to reduce the effect of antagonistic isolate.
Based on Figure 3 above, it can be observed three rhizosphere bacterial isolates strongly inhibit the growth of *F. oxysporum*, compared to BR 4 (far right) with almost no inhibition. Inhibition activity could be caused several possibilities, including production of antibiotics, enzymes, and other organic compounds. Saxena et al. [18] utilization of rhizobacteria as plant biocontrol and plant growth boosters due to antibiosis or mycoparasitism, create competition for nutrients in the soil or by inducing defense responses in host plants. These results indicates the potential of rhizosphere bacteria in inhibiting the growth of plant pathogens. The in vivo test is under our investigation as well as the molecular analyses to identify potential isolates.

In conclusion, nine different bacterial isolates were isolated from rhizosphere red chili plants (*Capsicum annuum*). Bacterial isolates exhibited the activity to solubilize phosphate with SI index ranging 2.29 to 3.04 on pikovskaya media. BR 5 had the highest SI index (3.04) followed by BR 6 and BR 9. Isolate BR 6 showed highest antagonistic activity against *F. oxysporum* with percentage of inhibition reach 60%, followed by BR 9 (52) and BR 5 (38) at 7 days incubation.

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