The role of rhizobacteria to control rhizoctonia disease and to improvement plant growth of soybean on sub-optimal dry land

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Abstract. Rhizoctonia disease is caused by the Rhizoctonia solani fungus. that is a soil pathogen and is very dangerous because of its ability to persist in soil (soil borne pathogen) for a long time. Controlling by chemical not effective because they are have negative impact for example pollute the environment, harm non-target organism and harmful to humans. For that efforts are required alternative control an effective and environmentally friendly, one of them is by use bacteria PGPR. Some the genus of rhizobacteria often use are Pseudomonas fluorescens and Bacillus subtilis able to control a plant pathogen. The aims of research to understand the role of rhizobacteria potential in controlling rizoctonia disease and improvement plant growth of soybean. The result in vitro research showed that combination bacteria antagonistic P. fluorescens and B. subtillis provide better than single treatment, with inhibition is 88,1 % on 7 days after inoculations. While in vivo research showed that a combination of both the bacteria is also the best results with the disease incidence 12,4 % with frequency application 3 times, the control (not treatment) showed disease incidence 25,6 % with frequency application 4 times. The variables of compliance were N-total of tissues (%) shows that combination treatment bacteria better than the single treatment, with the N percentage is 0,499 % and heavy dry up to 4,12 %.

1. Introduction
Rhizoctonia disease on soybean is caused by the fungus Rhizoctonia solani [1]. This disease has spread almost in all production centers of soybeans in Indonesia with almost of 90%. This fungus can attack soybeans at various growth phases of seed rot, sprout or damping-off, leaf blight and pod blight. This fungus is a soil pathogen and has the ability to survive in soil (soil borne) for a long time in the form of sclerotium, and has a wide range of hosts. This makes it difficult for the disease to be controlled by technical culture and with fungicide [2].

Until now, disease control efforts still using fungicide. But the reality has not given satisfactory results. On the contrary, unwise use of fungicides have a negative impact on environmental pollution, human health, pathogen resistance and can lead to new strains for more vicious pathogens and the erosion of biological agents up to 72% [3]. Therefore, some efforts are needed to overcome the problems of diseases that are effective and environmental friendly, as well as overcome the problem of soil fertility reduction, and one of them is the utilization of Plant Growth Promotion Rhizobacteria (PGPR).
In general, the dry land the tropics is dominated by the type of land that was a alfisol order type, ultisol and oksisol type. The soils included in the order are dominated by clayey mineral caolinit and oxycide iron and aluminum, characterized by a strong level of strongest PH, the low level of Ca, K and Mg and the proportion of complex exchange by Alluminum. Deficiency of elements N, P, K, Ca and Mg in general was found in a field (poor elements). The fixation of P and another Anion levels and save capacity of moist in low ground and vulnerable to erosion. The characteristics as owned by alfisol, ultisol and oxysol. The of soils, causing low soil productivity or fertility. So that is an obstacle in developing this. From the characteristic of dry land, the indispensable technology and some innovations solve that problem. The one of solution is a PGPR (Plant Growth Promoting Rhizobacteria) utilization.

The use of PGPR in the field of agricultural many aimed to correct the quality of growth and the production of plants. Some the genus group bacteria PGPR of them are Pseudomonas, Bacillus, Azopirillum, Azotobacter, Rhizobium, and Agrobacterium. Some of associated research in the use of bacteria PGPR has done of them are the use of the bacterium Pseudomonas fluorescens and Bacillus in plants eucalyptus. Using Pseudomonas fluorescens to control disease blight bacteria and results from can be lowered severity disease up to 70 %, and usage of combination bacteria Psedomonas and Bacillus to increase the growth of plants soy and results from an increase in root formation of 65 %.

General studies have proven that root bacteria such as Pseudomonas fluorescens and B. subtilis are able to control plant pathogens [4]. It is known that strains of P. fluorescens to suppress Rhizoctonia solani on soybean, fusarium wilt on banana [5], fusarium wilt on cucumber [6], and fusarium wilt on chilli. Reported that B. subtilis was effective for controlling root pathogens of cucumber roots and fractured sprouts in tomatoes, as well as bacterial wilt pathogens [7]. The utilization of PGPR (Plant Growth Promoting Rhizobacteria) bacteria that act as biopesticide, biofertilizer and biostimulant can be used as a breakthrough alternative to help solve the problem of low national soybean production. The aim of research is product of biopesticide and biofertiliser formulation active from PGPR bacteria that can increase plant growth and for control of rizoctonia disease in soybean.

This research aims to know the potential of Rhizobacteria P. fluorescens and B. Subtillis in increasing soybean growth and its potential in controlling rizoctonia disease in soybean. The results of the research will be the leverage of industrial acceleration in the field of biological pesticide products, accelerating the realization of the implementation of constitution No. 12 of 1992 on the implementation of integrated pest control, and environmental safety. Regardless of the world needs, the production of biological agents in Indonesia is very feasible to develop because Indonesia is very rich in biodiversity.

2. Material and Methods
The research was conducted in the Laboratory of Plant Disease and Greenhouse Faculty of Agriculture, University of Jember. Stages of research include exploration of antagonistic bacteria in doing with the method [8]. While the identification of bacteria was done [8, 9]. Effectiveness study of antagonistic bacteria P. fluorescens and B. subtilis on Rhizoctonia solani in soybeans was done through two stages: in vitro research and in vivo research.

2.1 Inhibit Pathogen Inhibition Test
To obtain superior isolate from isolation field, selectivity of in vitro inhibition in laboratory was selected. Testing is done by testing isolates of antagonists singly or in combination. The antagonistic capability test was performed using King's B and YDA medium and dual cultures method [10]. One R. solani pure culture disc (10 mm diameter) is placed in the center of the test medium in a petridis. One pure suspension of the test antagonist bacteria (5 × 10^9 CFU / ml density) was scratched into a circle 6 cm in diameter around the R. solani (Fig. 1). The petridis was incubated for 72 hours at 22\(^\circ\)C and then measured R.solani colony diameter and compared with its control (bacterial suspension replaced with sterile water). Each treatment was repeated four times.
Figure 1. An antagonistic bacterial antagonistic test (b), against pathogenic fungi (a) [9-10]

Percentage inhibition of growth Pathogenic fungus by antagonistic bacteria is calculated based on Formula:

\[ I = \frac{d_1 - d_2}{d_1} \times 100\% \]

I = Percentage inhibition
\( d_1 = \) Colony diameter of fungus \( F. \text{oxysporum} \) on control
\( d_2 = \) Colony diameter of fungus \( F. \text{oxysporum} \) on treatment

2.2 Test for suppression of Rhizoctonia at Greenhouse

The study was conducted at Green House. This test is to determine how much superior isolate ability of laboratory selection results in suppressing rizoctonia in greenhouses. Experiment using Completely Randomized Design (RAL) Factorial, two factors. The first factor is the type of antagonistic bacteria (A) consisting of bacteria \( P. \text{fluorecens} \) (A1), \( B. \text{subtilis} \) (A2), combination of \( P. \text{fluorecens} \) and \( B. \text{subtilis} \) (A3), Dhitane M 45 (A4) as a comparison. The second factor is the application frequency of bacteria (B) consisting of 0 times (B1), 1 time (B2), 2 times (B3), 3 times (B4), and 4 times (B5). The treatment combination was repeated three times. Each superior antagonist isolate was cultured in 250 mL of 0.6% peptone water medium for 48 hours while being shaken. After that the culture was harvested by adding sterile \( \text{H}_2\text{O} \) to a concentration of antagonistic bacteria of 2 x 108 CFU/ml. Furthermore each of the 50 ml suspension of superior antagonist isolate was poured on the root of soybean plant and incubated for 60 days. Observations were made based on the severity of the disease measured using the severity index of the disease as follows:

\[ IP = \sum_{i=1}^{k} \frac{k.nk}{Z.N} \]

0 = no symptoms; 1 = <25\% wilted leaves; 2 = 25 <x <75\% wilted leaves; 3 = all leaves wither, where: nk = number of affected plants; Disease with scale n (n = 0,1,2,3); N = number of inoculated plants; Z = highest disease scale (= 3)

2.3 Data Analysis

Data obtained from observations both in laboratory and in greenhouse in analysis using Completely Randomized Design (RAL). The results of further research were analyzed by variance (ANOVA) done at 95\% confidence level. The real difference test was performed with the Smallest Differential Difference Test (BNT) for the laboratory treatment and Duncan distance test for greenhouse treatment. The combined reliability of antagonistic bacterial treatment against fusarium wilt disease in banana is indicated by the decreasing intensity of wilt disease.
2.4 Observation of N-network Content (%)
N-tissue analysis was performed to determine the level of nitrogen nutrient present in experimental tissue. N-tissue analysis was performed at the end of the observation using the Kjeldahl method.

2.5 Dry weight of plant (g)
Observation of plant dry weight was done at the end of observation by weighing all plant organs (roots, stems, and leaves) in an analytical scale with a precision of 0.01 g. Plants that have been cleaned then done drying first using sunlight for 2-3 days. Furthermore the plant can be dried using 70-80°C oven to constant weight (± 48 hours).

3. Results and Discussion
The result of symptoms disease shows the incubation period is different between control and treatment by antagonist application. The incubation period shows antagonist bacteria has been to constrained the pathogens, they using mechanism antibiotics and induction resistance to disease. Bacteria antagonistic can constrained the pathogens with produced an antibiotic and siderofor. On healthy plants (antagonist bacteria application) is better than without antagonist bacteria application. This happen because antagonist bacteria can produce several compounds wich functions as bioprotektan, biostimulan and biofertilizer to stimulate plant growth.

3.1 In Vitro Experimental
The results of in vitro test bacteria antagonist *P. fluorescens* and *Bacillus subtilis* either applied singly or combination of both bacteria can see on picture 2.

![Figure 2](image)

**Figure 2.** Average percentage of inhibition antagonist bacteria to *F. oxysporum* fungi on in vitro testing.

Figure 2 shows that *P. fluorescens* and *B. subtilis* as well as the combination of both can inhibit the growth of *R. solani* fungus colonies on petri dishes. However, in each treatment did not show a significant difference between single application and treatment of antagonistic combination and different significantly when compared with control. *P fluorescens* treatment, *B. subtilis* and combination of both can inhibit pathogens 70.2%, 74.6% and 88.1% at 7 days after inoculsion while control treatment did not inhibited.

Inhibition mecanism of pathogens by antagonist microbial is generally due to antibiotic processes, nutritions competition and parasitism. According to Kurniawan [11], activity of *P. fluorescens* inhibiting the growth of pathogenic fungi in the culture medium is caused *P. fluorescens* can take a iron form media with forming a pigmented iron complex. The pigment shows more fungistatic
properties. Than suggests that the ability to inhibit the growth of the *R. Solani* fungus is due to the presence of antibiotic substances produced by bacteria and diffusion through the medium can inhibit the growth of pathogenic fungi as well as siderophores’s production can binding iron ions required by pathogens.

### 3.2 In Vivo Experimental

The results of *P. fluorescens* antagonism testing, *B. subtilis* and combination of *P. fluorescens* and *B. subtilis* against the incidence of *Rhizoctonia disease* in soybean can be seen in table 1.

**Table 1.** Incidence of Rhizoctonia in soybean on various combinations of treatments on observations 10; 20; and 30 days after inoculation (HSI).

| Observation of Treatment | 10  | 20  | 30  |
|--------------------------|-----|-----|-----|
| Antagonist 1 time        |     |     |     |
| Without an antagonist    | 2.1 | 22.4| 78.8| a   |
| *P. fluorescens*         | 1.4 | 18.5| 50.4| b   |
| *B. subtilis*            | 1.4 | 21.8| 54.7| b   |
| *P. fluorescens dan B. subtilis* | 1.2 | 13.0| 39.8| c   |
| Application antagonist 2 times |     |     |     |
| Without an antagonist    | 1.4 | 26.4| 80.4| a   |
| *P. fluorescens*         | 1.4 | 13.6| 53.3| b   |
| *B. subtilis*            | 1.4 | 21.9| 50.7| b   |
| *P. fluorescens dan B. subtilis* | 0.0 | 10.8| 40.5| c   |
| Application antagonist 3 times |     |     |     |
| Without an antagonist    | 2.5 | 24.8| 85.1| a   |
| *P. fluorescens*         | 0.0 | 12.5| 23.5| d   |
| *B. subtilis*            | 1.4 | 12.5| 20.4| df  |
| *P. fluorescens dan B. subtilis* | 1.4 | 9.8 | 14.6| f   |
| Application antagonist 3 times |     |     |     |
| Tanpa antagonist         | 1.4 | 21.0| 83.5| a   |
| *P. fluorescens*         | 0.0 | 9.9 | 25.2| d   |
| *B. subtilis*            | 0.0 | 10.9| 20.1| df  |
| *P. fluorescens dan B. subtilis* | 1.4 | 7.9 | 12.4| f   |
| Dithane 45 Application (Comparison) | 0.0 | 1.4 | 25.6| d   |

The same letter in the same column shows no significant difference in the Duncan 5% Duncan Multiple Test.

Table 1 shows that incidence of Rhizoctonia disease in soybean observations 10 days after inoculation has not shown significant levels of attack on all treatment applications of antagonistic bacteria or controls. The average incidence of disease is still around 1%. Likewise on the treatment frequency of both applications 1,2,3 and 4 times also did not show significantly different results. On the other hand, on treatment with fungicide Dithane M. 45 all plants have not been attacked by the fungus *R. Solani*.

While the treatment with the frequency of different applications also tend to affect the incidence of existing diseases. At the frequency of the application 1 and 2 times incidence of the disease may decrease, but the decrease almost shows no difference between the two treatments. Meanwhile, the treatment with the frequency of application of antagonist bacteria 3 and 4 times the incidence of disease is lower compared to 1 and 2 times the application. However, if the comparison between application frequency antagonist bacteria 3 and 4 times did not show the difference in decreasing incidence of disease. This means that the application of antagonistic bacteria is not enough to be given only once but it needs to be done repeatedly up to as much as 3-4 times the application. The frequency of this application is felt to be effective enough to suppress the incidence of disease, so the next application is no longer necessary because it can lead to in efficiency, it is given that the application
with frequency 4 times did not increase the effectiveness of antagonistic bacteria. Meanwhile, in the application of dithane fungicide M45 incidence of new disease began to occur on observation 20 days after inoculation with a much lower percentage compared with the treatment of antagonistic bacteria that is only at 1.4%. While on the other treatment symptoms of the disease has begun to appear at 10-17 days after inoculation. On observation 30 days after inoculation showed a very clear difference between all treatments with controls. In this observation there is an increased incidence of disease in all treatment combinations, and shows a marked difference. The highest incidence of disease occurred in the controls that reached 78.8 - 85.1%. While the lowest incidence of disease was in combination of treatment of P.fluorescens and B. subtilis application which was applied simultaneously with application frequency 3 and 4 times with disease incidence respectively 12.4% and 14.6%. This was significantly different when compared with single application of each antagonist bacteria with higher incidence of disease than if the bacteria were combined ie reaching 20.3-25.6% at the application frequency 3 and 4 times. This means that the application of antagonistic bacteria can significantly reduce the incidence of Rhizoctonia disease in soybeans.

In the table it is also known that the frequency of application of antagonistic bacteria may have an effect on decreasing the incidence of the disease and showing a noticeable difference especially between 1 and 2 apps when compared with 3 and 4 times. At the application frequency 1 and 2 times the incidence of the disease ranged from 39.8% - 54.7%. While at the application frequency 3 and 4 times the incidence of disease is lower ranging from 12.4% - 25.2%. The observational data on the effect of antagonistic bacteria used showed significant differences.

Based on figure 3 shows that the biggest N nutrition on the soybean plant is combination treatment with 3 (three) applications 0.499 %. While the lowest N nutritions on 1 (one) times is 0.269 % an application as on 7 days before. Interactions of plant with rhizobacteria have best growth because N supported. Availability of nitrogen will improving protein synthesis and growth hormone as auksin [12]
Figure 4. Dry weight of the Plan

Description: The numbers followed by the same letter are not significant in the Duncan test of 5% level.

The frequency of the provision of three times using bacteria combination *P. fluorescence* and *B. subtilis* is the best treatment to improve weights dry plants. Dry weight plants are indicators of the accumulation of fotosintat, so that the higher value is also better to growth of plants [13]. Fixation nitorgen by rhizobacteria as biofertilizer be done by formation root pustule mecanism. Rhizobacteria on observation is PGPR who can affecting growth plants by fixing nitrogen, dissolving phosphate, siderophores production and growth hormone [14].

4. Conclusion

The Rhizobacteria can be used a biofungicides, bioprotectant and biostimulant in plants. The combination of *Pseudomonas fluorescens* and *Bacillus subtilis* having value the percentage of inhibition highest In vitro experiment. That combination in application frequency 3 and 4 times, it has *Rhizoctonia* disease Incidence 12.4 % and 14.6% in the In vivo experiment. And the biggest N nutrition on the soybean plant is a combination of *Pseudomonas fluorescens* and *Bacillus subtilis* in application frequency 3 times than another treatments. The best treatment in dry weight indicator of the soybean plant is a combination of *Pseudomonas fluorescens* and *Bacillus subtilis* in application frequency 4 times than another treatments.

5. References

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