Biological Control of *Ralstonia solanacearum* Causes of Bacterial Wilt Disease with *Pseudomonas putida* and *Streptomyces* spp. on Some Tomato Varieties

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Abstract. *Ralstonia solanacearum* is one of the main pathogens in tomato plants that cause bacterial wilt disease. *R. solanacearum* in the field is difficult to control, because some phytopathogenic bacterial species produce EPS in large quantities. The practices of cultivation, soil fumigation and development of resistant varieties have been carried out as an effort to control these pathogens. Therefore, biological control is another alternative in controlling this pathogen. The research was conducted at the Plant Bacteriology Laboratory and in the Greenhouse of the Plant Protection Department, Faculty of Agriculture, Gadjah Mada University, Yogyakarta. In this study, the bacteria *Pseudomonas putida* strain Pf-20 and *Streptomyces* spp (isolates S-4 and S-16), 9 varieties of tomatoes and *R. solanacearum* were used. This study uses a Completely Randomized Design consisting of 3 treatments of biological control bacteria and 3 replications. The parameters observed were incubation time and disease intensity. Then also calculated the rate of disease progression and the value of the Area Under Disease Progress Curve (AUDPC). Data obtained were analyzed descriptively for all parameters except incubation time was analyzed statistically. Statistical analysis data were then further tested with LSD at 5% level. Based on the results, the application of antagonistic bacteria can suppress the development of bacterial wilt disease. The best suppression of bacterial wilt disease development by biological control agents was seen in amelia and tombatu varieties. Further research is needed to observe the relationship between varieties and the ability of antagonistic bacteria to control *R. solanacearum* in tomato plants.

Keywords: Biological Control, *Ralstonia solanacearum*, Bacterial Wilt Disease, Tomato

1. Introduction

*Ralstonia solanacearum* is one of the main pathogens in tomato plants that cause bacterial wilt disease. This bacterium is a soil-borne bacterium that is found in many subtropical and tropical regions which naturally infects roots and reproduces itself in xylem tissue. Besides tomatoes, this bacterium also attacks other plants besides the Solanaceae family.

*Ralstonia solanacearum* in the field is difficult to control, because some phytopathogenic bacterial species produce EPS in large quantities, both in culture in the laboratory and when in plant tissue. EPS is always associated with virulence. Withering that occurs quickly in plants infected with *R. solanacearum* due to xylem vessel bundles blocked by EPS and bacterial cells so that transportation of...
water and nutrients becomes disrupted. In addition to blocking the flow of water and nutrients, EPS also serves to protect R. solanacearum from detection by host plants due to the presence of foreign matter that enters the plant tissue [1].

The practices of cultivation and development of resistant varieties have been carried out as an effort to control these pathogens, but all have shown limited success [2]. Soil fumigation with methyl bromide or chloropicrine also shows the same thing. Because of the limited effectiveness of some of these controls, bacterial wilting remains an economically serious problem [3]. Therefore, biological control is another alternative in controlling this pathogen.

The use of biological control bacterium has shown many successes. Pseudomonas putida strain Pf-20 shows a high ability in suppressing the development of bacterial wilt disease in tobacco [4]. Streptomyces sp. S57 and S67 isolates also can increase the growth and production of ginger and reduce bacterial wilt disease index in the field [5].

2. Methods

The research was conducted at the Plant Bacteriology Laboratory and in the Greenhouse of the Plant Pests and Diseases Department, Faculty of Agriculture, Gadjah Mada University, Yogyakarta. In this study, the bacteria Pseudomonas putida strain Pf-20 and Streptomyces spp (isolates S-4 and S-16) were used (isolation from the tomato plant rhizosphere), 9 varieties of tomatoes (Amelia, Melinda, Lentana, Permata, Tombatu, Precious, King Kong, King Kong 2 and H-7996) and Ralstonia solanacearum bacteria isolated from the eggplant roots.

This study uses a Completely Randomized Design consisting of 3 treatments of biological control bacteria and 3 replications, are Pseudomonas putida strain PF-20 and Streptomyces spp. (S-4 and S-16 isolates). The parameters observed were incubation time and disease intensity. Then also calculated the rate of disease progression and the value of the Area Under Disease Progress Curve (AUDPC). The incubation time was observed by observing the first symptom of bacterial wilt disease in tomato plants which were observed every day until the plants were 30 day after inoculation (dai), while the intensity of the disease was calculated based on the symptoms of the disease observed every seven days for 30 days by looking at the symptoms of wilting in each plant and the percentage symptoms are made scoring and then the intensity of the disease is calculated using the scores as according to Arwiyanto and Hartana [4]. AUDPC is calculated using trapezoidal integration with the formulas of Jeger and Viljanen-Rollinson [6]. Data obtained were analyzed descriptively for all parameters except incubation time was analyzed statistically. Statistical analysis data were then further tested with LSD at 5% level.

3. Results and Discussion

Based on the results of observations on the ability of antagonistic bacteria in inhibiting the development of bacterial wilt disease in several varieties of tomatoes showed mixed results. This is shown in the incubation period, the intensity of the disease and the development of various diseases in different varieties after antagonistic bacteria was applied.

The application of antagonistic bacteria to the 9 tomato varieties used showed mixed results. The giving of antagonistic bacteria can prolong the incubation period compared without the antagonistic bacteria seen in tomatoes of Melinda, Tombatu, Precious, King Kong and H7996 varieties. However, the Amelia variety shows that without antagonistic bacteria, the incubation period is quite long. This is presumably due to the variety of Amelia tomatoes, including resistance.

Figure 1 shows the application of antagonistic bacteria can reduce the intensity of the disease up to 0% in the Amelia variety by giving Pseudomonas putida strain pf-20 and Streptomyces sp. S-4 isolates, in Melinda variety by giving of Streptomyces sp. S-16 isolate, in Tombatu variety by giving of Pseudomonas putida strain pf-20 and Streptomyces sp. S-16 isolates and in H-7996 variety with Streptomyces sp. S-4 isolate. In the varieties of Lentana, Permata, Precious, King Kong and King Kong 2 show that application of antagonistic bacteria is not able to suppress the development of bacterial wilt disease. This is seen more clearly on the graph of disease progression in Figure 2.
**Table 1.** The incubation period of bacterial wilt in several tomato varieties after the application of antagonistic bacteria

| Antagonistic Bacteria Application | Amelia | Melinda | Lentana | Permata | Tombatu | Precious | King Kong | King Kong 2 | H7996 |
|----------------------------------|--------|---------|---------|---------|---------|----------|-----------|------------|-------|
| Pf-20                            | 30 a   | 9 b     | 14 a    | 18 a    | 30 a    | 19 a     | 15 a      | 5 a        | 6 b   |
| S-4                              | 30 a   | 25 a    | 7 a     | 6 a     | 22 a    | 20 a     | 20 a      | 13 a       | 30 a  |
| S-16                             | 28 a   | 30 a    | 13 a    | 14 a    | 30 a    | 28 a     | 20 a      | 7 a        | 22 ab |
| K (+)                            | 19 a   | 5 b     | 10 a    | 13 a    | 13 a    | 5 a      | 7 a       | 4 a        | 10 ab |

Note: The numbers followed by unequal lowercase letters are significantly different according to the LSD test at the 5% level. Pf-20 = *Pseudomonas putida* strain Pf-20; S-4 and S-16 = *Streptomyces* spp. isolate; K (+) = giving of *Ralstonia solanacearum* without antagonistic bacteria.

**Figure 1.** Intensity of bacterial wilt in several tomato varieties 30 days after inoculation of *Ralstonia solanacearum* after application of antagonistic bacteria.
Figure 2. Development of bacterial disease after application of antagonistic bacteria to some varieties of tomatoes: Amelia (a), Melinda (b), Lentana (c), Permata (d)

Figure 3. Development of bacterial disease after application of antagonistic bacteria to some varieties of tomatoes: Tombatu (a), Precious (b), King Kong (c), King Kong 2 (d) and H-7996 variety (e)

In general, the application of antagonistic bacteria can suppress the development of bacterial wilt disease. This can be seen from the small value of AUDPC in the giving of antagonistic bacteria compared to without antagonistic bacteria (Figure 4). The AUDPC value in this experiment reflects the effectiveness of a treatment in suppressing pathogens [7]. However, in some varieties such as Lentana, Permata, King Kong 2 and H-7996 variety, the application of antagonistic bacteria shows high AUDPC values as without antagonistic bacteria.

Pseudomonas and Streptomyces have been widely reported as biological control agents. A study report that P. putida A1 could colonization the root surface, delayed the appearance of wilt symptoms and reduced wilt disease severity and in vitro assay, A1 showed antimicrobial activity against R. solanacearum [8]. Streptomyces plurilocolorescens which are endophytic actinomycetes isolated from tomato plants in Brazil showed 86.6% of antimicrobial activity against the pathogens in tomato plants [9].
Figure 4. AUDPC value of bacterial disease after applying antagonistic bacteria to tomatoes of Amelia (a), Melinda (b), Lentana (c), Permata (d), Tombatu (e), Precious (f), King Kong (g), King Kong 2 (h) and H-7996 variety (i). (Note: The vertical line shows the standard deviation of three replications)

4. Conclusion
The use of antagonistic bacteria such as Pseudomonas putida strin Pf-20 and Streptomyces sp. S-4 and S-16 isolates were able to suppress the development of bacterial wilt disease with diverse abilities in several varieties of tomatoes. The best suppression of bacterial wilt disease development by biological control agents was seen in Amelia and tombatu varieties. Further research is needed to observe the
relationship between varieties and the ability of antagonistic bacteria to control *Ralstonia solanacearum* in tomato plants.

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