

Selection of Rhizobacteria to Control Twisted Disease of Shallot

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ABSTRACT

The purpose of this study is to find out the type of antagonistic rhizobacteria and their capability in *F. acutatum* growth suppression on *in vitro* and *in vivo* condition. The study was conducted by selecting of rhizobacteria isolated from Shallot's rhizosphere which was taken from 4 regions; they were Bantul district (Yogyakarta), Nganjuk (East Java), Cirebon (West Java) and Brebes (Central Java) district. Characterization was taken based on physiology and biochemist, PCR based on 16S rRNA and Rep-PCR (BOX-PCR and ERIC-PCR). The *in vivo* test was carried out by factorial experiment with 2 trials using completelly randomized design *i.e.*, 1) soaking shallot seeds into rhizobacteria suspension for 1 hour before planting and 2) watering plants with rhizobacteria suspension in once 2 weeks. Observation variables consists of incubation period, disease incidence, plant high, fresh and dry weight of plant and total *Fusarium* population.

The results showed that there were some beneficial rhizobacteria from shallot's rhizosphere which could be used to control twisted disease on shallot, which consisted of *Bulkholderia* and *Bacillus*. The isolate BrSG.3 closely related to *Burkholderia seminalis*, isolate BrSG.5 and Bp25.6 closely related to *Bacillus amyloliquofaciens*, isolate BrSM.4 closely related to *Burkholderia cepacia* strain NTUIOB TPC6, isolate Bp25.2 closely related to *Bacillus methylothrophicus* and isolate Bp25.7 closely related to *Bacillus subtilis*. The bacterial isolates which are potential for biological control and increased of plant growth were *Bacillus* spp. isolates.

Keywords: Shallot; Twisted disease; Fusarium acutatum; Antagonistic rhizobacteria

INTRODUCTION

Shallot is one of the vegetable commodity which intensively planted by farmers. It has important role can give high contribution to Indonesian economic development. The needs of Indonesian society which cannot separated with shallot cause the demand of this commodity continuously increase. However, there are problems on the efforts to increase onion production. One of the problem is caused by twisted disease [1].

Twisted disease caused by fungus *Fusarium* spp. is one of the most important disease on shallot. The highest severity is caused by *Fusarium acutatum*.

The symptoms of this disease are twisted leaf and rot of stem base, therefore causing dry wilting and continuously die. Twisted disease mostly occurs in rainy season. The pathogen which has infect the roots will growing along roots to stems, then continuously growing across a network of vessels [2].

Various efforts to control twisted disease have been performed mechanically and chemically, by collecting and destroying infected plants and the use of chemical pesticides. In recent study, there is much research of controlling twisted disease of shallot such as the use of biological agent.

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Biological control of soil borne pathogen is alternative approach needs to be developed, because it is relatively inexpensive, easy to do and environmentally friendly. One of the biological control is using the role of rhizobacteria. The rhizobacteria is very important for the growth of plants. They increase itself and active help with the provision of element to plants through a process symbiotic to the release element bound into the available to absorb by root of plant [3]. These microbes also have an active role protect plant against diseases in the soil, thus their existence is expected to suppress the development of soil pathogens such us *F. acutatum*.

Based on the ideas, the objectives of this research are: 1) to find out the antagonistic rhizobacterial capability in *F. acutatum* growth suppression on *in vitro* and *in vivo* condition, 2) to do molecular characterization of rhizobacteria isolates that are potential to be antagonistic against *F. acutatum*.

DESCRIPTION

The research started from 10th April 2013-March 2015 in the laboratory of plant bacteriology, laboratory of plant disease clinic and screen house of plant protection departement, faculty of agriculture, university of Gadjah Mada, Yogyakarta. The materials used in this study consist of shallot rhizosphere soil, antagonistic rhizobacteria isolates, *F. acutatum* (SKM BP isolate) and Biru cultivar which is susceptible to twisted disease [4]. The experiment was conducted in two stages, *i.e., in vitro* and *in vivo* tests. In vitro test was done to obtain the best antagonistic rhizobacteria through antagonistic test with *Fusarium*. While in the *in vivo* test performed with two trials, *i.e.,* soaking and watering using six antagonistic rhizobacteria isolates that have been tested of their antagonistic ability, characterized and identified [5].

The result showed that based on the *in vitro* test and genetic diversity identification using Rep-PCR, there are six different isolates which have high antagonistic ability, consists of BrSG.3, BrSG.5, BrSM.4, Bp35.2, Bp25.6 and Bp25.7 isolates. Based on sequencing analysis 16S rRNA gene, BrSG.3 was identified as *Burkholderia seminalis* strain DSM 2351, BrSG.5 and Bp25.6 was identified as *Bacillus amyloliquofaciens* strain BCRC 11601, BrSM. 4 was identified as *Burkholderia cepacia* strain NTUIOB TPC6, Bp25.2 was identified as *Bacillus subtilis* strain KCTC 3135 [6].

On *in vivo* test, the occurrences of disease in the greenhouse showed that plants which are treated with antagonistic rhizobacteria in both soaking and watering trials better to control. Generally, the control showed the percentage of disease occurrences which was higher than the plant treated with antagonistic rhizobacteria. This can be shown with the AUDPC value (Area under Disease Progress Curve) which was lower than control. Low value indicated that the effort of controlling by using antagonistic rhizobacteria provide effective results. The treatment of antagonistic rhizobacteria also gave good impact toward the growth rate of the plants, fresh weight of foliage and tuber and dry weight of foliage and tuber [7]. It was suspected because *Burkholderia* and *Bacillus* are belongs to PGPR. Therefore, based on their influence on emphasis the development of a disease and growth of plants, all of the isolates potentially can be used as biological control agent. However, *Burkholderia* is susceptible to use because it can be pathogenic in plants.

In addition, the ability antagonistic rhizobacteria had a positive impact to reduce the existence of pathogenic fungus. This was shown by the low population of *Fusarium* in the soil at rhizobacteria treatment. Thus, the application of rhizobacteria be able to suppress growth of *Fusarium* spp. rhizobacteria can suppress the pathogenic fungus by the mechanisms of antibiosis, competition, ISR and the production of enzymes [8].

CONCLUSION

Based on this study, there are some benefit rhizobacteria from Shallot's rhizosphere that can be used to control twisted disease. The isolates consist of *Burkholderiaceae* and *Bacillus*. Moreover, the isolates which are potential for biological control and increasing plant growth were *Bacillus* spp. isolates.

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