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Examination of Antagonistic Bacteria in Reducing Tomato Seedling-off Caused by Fusarium

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An alternative to reduce chemical control of plant pathogenic fungi is by utilizing biological control agent like antagonistic bacteria. In this study, ten antagonistic bacterial isolates were examined to know their ability in suppressing *Fusarium* in vitro and in vivo. In vitro examination of fungal growth inhibition was conducted by dual culture test. To examine bacterial ability to reduce seedling-off, tomato seed was dipped in bacterial solution for 30 minutes and planted in soil infested with *Fusarium* culture. Six *Fusarium* isolates were tested for their pathogenicity in tomato seed, in which two of the isolated were newly isolated from infected egg-plant and banana. Bacterial isolate ability to inhibit *Fusarium* growth was varied to some extent, however in general *Alcaligenes* sp. BSO2 inhibited *Fusarium* spp. the most. Unlike in vitro examination, controlling Fusarium wilt in tomato seed showed that *Serratia* sp. AW10 was more active to suppress the disease. This isolate was also to contribute to higher seedling height.

Keywords: antagonistic bacteria, coated seed, plant pathogen, seedling-off.

Chemical control of plant diseases has been contributing to environmental pollution, resistance of disease-causative organisms to fungicides, and health hazards to humans (Ningthoujam *et al*., 2009). To reduce the impacts of chemical application, biological control has been proposed to suppress many diseases in plant, in which antagonistic microorganisms can be utilized (Khan *et al*., 2011; Ningthoujam *et al*., 2009). In these interactions microorganisms produce secondary metabolites such as antibiotics, enzymes, antifungal proteins, or other antimicrobial compounds to inhibit the growth of other microorganisms (Khan *et al*., 2011; Ramos-Solano *et al*., 2010; Chaiharn *et al*., 2009).

One of the most devastating diseases in crop and other plants throughout the world is Fusarium wilt that may cause seedling-off. Extensive commercial losses by reducing both quality and yield has been reported (Farhan *et al*., 2010; Gangadara *et al*., 2010; Anitha & Rabeeth., 2009; Okeniyi, 2007). *Fusarium* is a common phytopathogenic fungus causing Fusarium wilt in chili, tomato, potato, and tobacco, infecting the plant seeds as soilborne disease (Gajbhiye *et al*., 2010). Biological control using antagonistic fungi and bacteria has been reported to suppress Fusarium wilt (Suryanto *et al*., 2010; Farhan *et al*., 2010; Gangadara *et al*., 2010; Gajbhiye *et al*., 2010; Pereira *et al*., 2009).

To protect plant from disease ones may directly apply antagonistic bacteria to soil (Gajbhiye *et al*., 2010), while others utilize as seed coating (Farhan *et al*., 2010; Pareira *et al*., 2009). In this study we examined the use of antagonistic bacterial isolates to reduce Fusarium wilt in tomato seedling by dipping tomato seed to the bacterial solution as seed coating. The isolate effectiveness to control Fusarium wilt was evaluated through the isolates ability in reducing seedling-off.

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MATERIALS AND METHODS

Bacterial and fungal isolates and tomato seed

Antagonistic bacterial isolates *Alcaligenes* sp. S2T16-1, *Pseudomomas* sp. S3T32-3, *Serratia* sp. S3T33-3, *Pseudomonas* sp. AW02, *Alcaligenes* sp. BS02, *Pseudomonas* sp. KM01, *Alcaligenes* sp. KM02, and *Serratia* sp. AW08, *Serratia* sp. AW10, *Serratia* sp. KM04 were from our previous study. *Fusarium oxysporum* and *Fusarium* sp. C2 were collection of our laboratory, *F. lycopersicum* and *Fusarium* sp.1 were collection of Laboratory of Plant Pest and Disease, Faculty of Agriculture, Universitas Sumatera Utara, and *Fusarium* sp.2 and *Fusarium* sp.10 were newly isolated from infected egg-plant and banana, respectively. Tomato seeds were obtained from agricultural market in Medan, Indonesia.

Characterization of *Fusarium* spp.

Fusarium was grown on Potato Dextrose Agar for 72 hours at 28-30°C. Observation and identification of suspected pathogenic fungi were carried out macroscopically and microscopically. Determination of fungal isolates used the book of Barnes (1997).

Examination of *Fusarium* pathogenicity

A 100 ml conidial suspension of each *Fusarium* was mixed with 500 g sterilized soil and compost (3:1) in a plastic tray. Thirty tomato seeds were sterilized with 2% aqueous sodium hypochlorite for 60 minutes and rinsed thoroughly with sterile distilled water prior planting in the soil. Seedling-off was observed after 30 days of planting. Negative (-) control was isolate-free seeds soaked in sterile distilled water. Pathogenicity was measured as percentage of seedling-off.

In vitro examination of bacterial isolate inhibition to Fusarium growth

Antifungal activity assay was carried out to *Fusarium* spp. using disc diffusion method. An agar plug (Ø 5-mm) of *Fusarium* spp. from the margin of an actively growing mycelia was inoculated in the center of plate of Muller-Hinton agar and incubated for 3 days. Two pieces of paper discs immersed with bacterial suspension (H" 10^8 cells/ml) were placed in the opposite direction about 3.5 cm from the center toward the edge of plate. Inhibitory activity was determined based on the inhibition zone formed around bacterial colonies, measured at 6-7 days of incubation as the radius of the normal fungal growth subtracted to the radius of the inhibited fungal growth.

Examination of seedling-off reduction with bacterial isolates

To test of effect of bacterial isolates to reduce Fusarium wilt in tomato seedling, similar preparation to that of test of *Fusarium* pathogenicity was carried out, except that the seeds were soaked with bacterial cultures (H" 10^8 cells/ml) for 30 minutes prior planting. Negative (-) control was isolate-free seeds planted in fungus-free soil, and positive (+) control was isolate-free seeds planted in fungus-inoculated soil. *Fusarium* sp.2 was used in this test since it caused relatively high seedling-off.

Seedling off and seedling height

Percentage of seedling-off was measured as: [total seedling-off/total seed] x 100%. All remain seedling were taken at the end of study and measured for seedling height.

RESULTS

Characterization of *Fusarium* spp.

Fusarium is known as pathogens to many important crops attacking both seedlings and mature plants and causing severe economic loss (Farhan *et al*., 2010; Gangadara *et al*., 2010; Anitha & Rabeeth, 2009; Okeniyi, 2007). *Fusarium* also causes plant to grow abnormally, or uses the plant as agent of the pathogen transmission to other host plants. The pathogen infects young root, growing, developing and spreading in root and stem vessel, inhibiting water and nutrient transport. In this study pathogenicity of the fungal isolates were tested in tomato seedling.

Six *Fusarium* isolates namely *F. oxysporum*, *Fusarium* sp.C2, *F. lycopersicum*, *Fusarium* sp.1, *Fusarium* sp.2, and *Fusarium* sp.10 were used in this study. Fungal colony color varied from white to purple. The fungal isolates showed to produce 2-5 macroconidia (Table 1).

Pathogenicity of Fusarium isolates to tomato seedling

Test of pathogenicity of *Fusarium* isolates showed that their pathogenicity varied (Figure 1). It showed that *Fusarium* sp.2 was more pathogenic to tomato seedling by 91.3% seedling-off, while *F. lycopersicum* was a weak pathogen to cause only 14.8% seedling-off compared to others.

Manifestation of the disease caused by *Fusarium* sp.2 was observed as leaf and seedling wilted with yellowing leaf followed by seedling slunted (Suryanto *et al*., 2010). This clearly confirmed that *Fusarium* sp.2 was more pathogen to tomato seedling.

Antagonistic activity of bacterial isolates to *Fusarium* spp.

To know bacterial isolate ability to inhibit growth of *Fusarium* spp., examination was conducted by growing bacterial isolates next to the fungi. All bacterial isolates showed to inhibit *Fusarium* growth. However, the ability to inhibit the fungal growth was varied (Table 2). The ability of such an microorganism to control growth of others is due to their capability to produce antimicrobial compounds such as antibiotics, enzymes, antifungal proteins, or other antimicrobial compounds (Khan *et al*., 2011; Ramos-Solano *et al*., 2010; Chaiharn *et al*., 2009).

Control Fusarium wilt of tomato seedling by bacterial isolates

Efficacy of bacterial isolates to reduce infestation of Fusarium wilt was conducted on tomato seedling by soaking tomato seeds into bacterial solution separately for 30 minutes. Treated seeds were planted in soil inoculated with *Fusarium* sp. 2. Seeds planted in Fusarium-inoculated soil were succeptible to Fusarium wilt showed by positive (+) control. On the other hand, Fusarium wilt of seedling was reduced by soaking the seeds into bacterial solution prior planted (Figure 2).

AW10 decreased more seedling-off rather than others by 34.5%, while seedling-off in positive (+) control reached 81.8%. Although BS02 showed higher fungal suppression growth in vitro (Table 1), this isolate together with KM01 and S3T32-3 might contribute to more severe disease in tomato seedling by killing 100, 96.2, and 87% of seedlings, respectively. Hence, this in vitro test of bacterial ability to control fungal disease was important prior application in the field.

Interaction between the pathogen and bacterial isolates produced varied seedling performance. Direct observation of the seedling showed that negative (-) control seemed to be a healthy seedling (Figure 3a), while positive (+) control showed wilted seedling caused by *Fusarium* sp.2 (Figure 3b). On the other hand, the seedling treated with AW10 looked more stout and fresh with more leaves even compared to negative (-) control (Figure 3c).

DISCUSSION

Soilborne pathogen *Fusarium* is one of common diseases causing Fusarium wilt in crop of Solanaceae: tomato, potato, eggplant, and chili. This disease causes serious seedling-off. *Fusarium* also causes plant to grow abnormally, or uses the plant as agent of the pathogen transmission to other host plants. Lower pathogenicity of Fusarium in the tested plants might be due to its host specificity. It was known that different species/ strains of *Fusarium* infect different host. *F. oxysporum* f.sp. *cubense* is the causal pathogen of wilt disease of banana (Getha & Vikineswary, 2002), *F. moniliforme* commonly occurs in maize seed (Bressan, 2003), and Fusarium wilt, caused

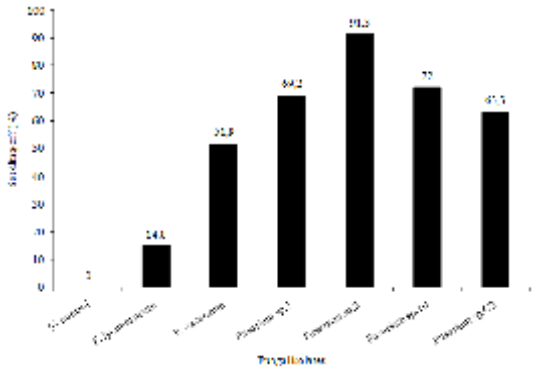

Fig. 1. Pathogenicity of *Fusarium* isolates in tomato seedlings

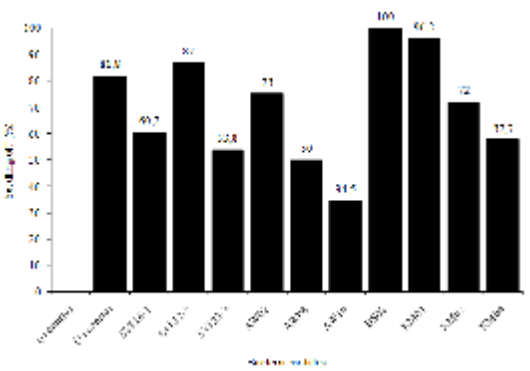

Fig. 2. Effect of antagonistic bacterial isolate treatment on tomato seedling-off reduction

by *F. oxysporum* f. sp. *lycopersici*, is a serious problem for tomato production (Fravel *et al*., 2005).

Many bacteria have been reported to control plant pathogenic fungi (Adekunle & Ogbebor, 2009; Kim *et al*., 2008; Mahadtanapuk *et al*., 2007; Soytong *et al*., 2005). Our study on utilization of chitinolytic bacterial isolates as potential biological control agent to reduce Fusarium wilt in chili seedling showed that the isolates were capable to reduce infestation of the fungi (Suryanto *et al*., 2010). *Fusarium* particularly can be suppressed through the activity of *Bacillus*

Fig. 3. Tomato seedlings: (a). Negative (-) control was looked healthy, (b). Positive (+) control was showed Fusarium wilt, and (c). AW10 treatment was looked more stout and fresh with more young leaves

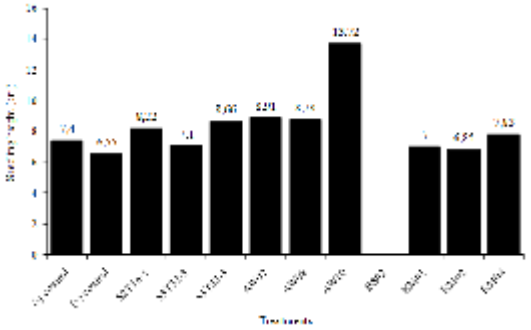

Fig. 4. Effect of chili seed soaking treatment with chitinolytic isolates on seedling height

Table 1. Characterization of *Fusarium* isolates

Species	Properties	Species	Properties
F. lycopersicum	White colony, macroconidia 2-3 septate, hyphae septate, purple at the base of medium, white at the surface of medium	*Fusarium* sp.2	Purple cottony colony, macroconidia 2-3 septate, hyphae septate, white at the base of medium, purple and smooth at the surface of medium
F. oxysporum	White cottony colony, macroconidia 2 septate, hyphae septate, purple at the base of medium, purple and smooth at the surface of medium	*Fusarium* sp.10	White colony, macroconidia 3–5 septate, hyphae septate, white at the base of medium, white at the surface of medium
Fusarium sp.1	Grey colony, macroconidia 3-5 septate, hyphae septate, grey at the base of medium, grey at the surface of medium	*Fusarium* sp.C2	White cottony colony, macroconidia 2-3 septate. hyphae septate, white at the base of medium, white and smooth at the surface of medium

Table 2. Inhibition zone of antagonistic assay of bacterial isolates to *Fusarium* spp.

Fusarium isolates	Antagonistic bacterial isolate inhibition zone (mm)									
	S2T16-1	S3T32-3	S3T33-3	AW02	AW08	AW10	BS02	KM01	KM02	KM04
F. lycopersicum	8.5	13.5	7.0	13.0	13.0	12.5	12.5	10.5	13.0	7.0
F. oxysporum	14.0	11.0	20.0	4.0	8.5	9.0	9.0	14.0	4.5	11.5
Fusarium sp.1	12.5	20.0	18.0	4.0	10.0	10.0	25.0	15.5	21.5	9.0
Fusarium sp.2	5.0	9.5	18.0	1.0	11.5	11.5	16.5	7.5	11.0	8.0
Fusarium sp.10	12.0	6.0	15.5	7.0	13.0	13.0	19.0	2.0	22.0	2.5
Fusarium sp.C2	12.0	16.5	10.0	13.5	17.0	17.0	7.5	7.0	15.0	9.0

subtilis from cotton rhizospheric soil against *F. oxysporum* (Gajbhiye *et al*., 2010). *Streptomyces violaceusniger* strain G10 showed antagonistic effects on *F. oxysporum* f.sp. *cubense* race 4 (Getha & Vikineswary, 2002).

Antagonistic effects responsible for disease suppression in biological control results either from microbial interactions directed against the pathogen, mainly during its saprophytic phase, or from an indirect action through induced resistance of the host plant. The antagonism may operate through antibiosis, competition, predation, or parasitism (Alabouvette *et al*., 2006). Interestingly, many antagonistic bacteria have been reported to enhance plant performance by producing plant growth regulator. Application of these bacteria may result in controlling plant disease and in improving plant growth as well (Hernández-Suárez *et al*., 2011; Farhan *et al*., 2010; Ramos-Salona *et al*., 2010). In vitro assay of antagonism in this study showed that bacterial isolates inhibited fungal growth to some extent, and might also contribute to plant performance as showed by AW10. Different ability of the bacterial isolates to inhibit fungal growth might be due to different antifungal compound producing by the isolates.

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