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Control of Fusarium Wilt of Chili With Chitinolytic Bacteria

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Biological control of plant disease using antagonistic microorganism has been obtaining much attention and implemented for decades. One of the potential microorganisms used in this strategy is chitinolytic bacteria. Utilization of this bacteria ranges from cell life, enzymes, genes, or other metabolites. In this study, we examined the ability of chitinolytic bacteria as a biocontrol agent of Fusarium wilt of red chili (*Capsicum annuum* L.) seedlings. The ability of chitinolytic bacteria to suppress the disease was evaluated by soaking red chili seeds in the bacterial isolates solution for 30 minutes prior seedling. Percentage of seedling of treated chili seed at end of study (4-weeks) ranging from 46 to 82.14%. Relative reduction of the seedling damping-off was observed in all bacterial treatment ranged from 28.57 to 60.71%. Furthermore, manifestation of bacterial suppression to Fusarium wilt was also exhibited by increasing of seedling height (ranged from 7.33 to 7.87 cm compared to 6.88 cm) and dry-weight (ranged from 2.7 to 4.3 mg compared to 2.3 mg). However, no significant effect was observed in leaf number. Then, from all chitinolytic isolates tested, BK08 was the most potential candidate for biological control agent of Fusarium wilt in chili seedling.

Key words: biological control, chitinolytic bacteria, chitinase activity, Fusarium wilt

INTRODUCTION

For the last two decades, many research results have provided convincing evidence that root health and vigor are directly related to plant productivity. As a consequence, root disease control has become one of the most challenging research areas in the context of plant productivity improvement (Benhamou *et al.* 1990). Soilborne pathogen *Fusarium oxysporum* is one of common disease causing Fusarium wilt in crop of Solanaceae: tomato, potato, eggplant, and chili. This disease causes serious seedling dampingg-off. *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 (Miller *et al.* 1986).

Biological control using microorganism has been studied intensifely since not many alternatives to control are available (Duffy *et al.* 1995). Health, environmental concern, development of resistance in target populations also contribute to developing biological control using natural enemies (Martin & Loper 1999). Nonetheless, the vast array of antimicrobial molecules produced by diverse soil microbes remains as a reservoir of new and potentially safer biopesticides (Kang *et al.* 1998).

Certain strain of microorganism has been reported to succesfully suppress the growth of plant pathogen. Fusarium wilt particularly can be suppressed through the activity of fluorescent *Pseudomonas* spp. strains and nonpathogenic strains of *F. oxysporum* (Larkin *et al.* 1996; de Boer *et al.* 2003). Nonpathogenic *Trichoderma piluliferum* and *T. viridae* reduced Fusarium wilt manifestation in banana (Getha & Vineswary 2002). Strains of *Streptomyces*, *Nocardia*, and *Pseudomonas* were capable of lysing hyphae of *F. solani* or *Neurospora crassa* (Potgieter & Alexander 1966). *Gliocladium solani* and *Aspergillus oryzae* are the potential biological control agents of the disease (Purnomo 2006).

Chitinolytic bacteria such as *Aeromonas hydrophila*, *A. caviae*, *Pseudomonas maltophila*, *Bacillus licheniformis*, *B. circulans*, *Vibrio furnissii*, *Xanthomonas* spp., and *Serratia marcescens* (Gohel *et al.* 2006) have been reported and played important role as biological control agents. Our previous study have shown that several local chitinolytic bacterial isolates inhibited the growth of pathogenic fungi *Ganoderma boninense*, *Penicillium citrinum* and *F. oxysporum*, *in vitro* (manuscript has been sent for a publication). One possible approach in biological control of soilborne plant diseases is to apply potential isolates to seeds or plant material. This paper reports the supression of Fusarium wilt disesease of chili (*Capsicum annuum* L.) seedling by chitinolytic bacteria.

MATERIALS AND METHODS

Isolates and Chili Seeds. Five chitinolytic bacterial isolates (BK07, BK08, BK09, LK08, KR05) were obtained from the previous study (Irawati 2008) and *F. oxysporum* was the stock collection of Laboratory of Microbiology, Department of Biology, University of Sumatera Utara. All bacteria were grown and maintained on modified salt

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medium supplemented with 2% (w/v) chitin colloidal (MSMC) agar (Suryanto & Suwanto 2003). *Fusarium oxysporum* was maintained in potato dextrose broth (PDB) or agar (PDA). All cultures were incubated at 30 °C.

Red chili seeds (*Capsicum annum*) were from commercial red chili seeds (PT. Tanindo Subur Prima, Surabaya) sold in Medan. The viable seeds were selected by putting the seeds in sterilize distillated water. Immersed seeds were collected.

Test of Pathogenicity. *Fusarium oxysporum* was taken from 10-days culture of 100 ml PDB. Fungal suspension was blended with 500 g sterilized soil mixed with sterilized compost (3:1) in a 30 x 22 x 10 cm tray. Chili seeds were surface sterilized with 2% aqueous sodium hypochlorite for 60 minutes and rinsed thoroughly with sterile distilled water. The seeds were then planted for 30 days in the trays covered with plastic wrap. Control seeds were treated similarly but in soil without fungal inoculation. Thirty seeds were used for each treatment. Direct observation was conducted to know percentage of infected seedlings. The test was done twice. The percentage of red chili seedling damping-off was observed as [number of seedling dumping-off / number of treated seeds] x 100%.

Fungal reisolation was conducted from sample of infected seedlings. Samples were taken by cutting the collar stem, sterilizing with 2% aqueous sodium hypochlorite, and rinsing thoroughly with sterile distilled water prior inoculating to PDA. Direct and microscope observation was conducted to see the reisolated fungal.

Control of Fusarium Wilt of Chili Seeds. Similar preparation as mentioned previously was done for this examination, except that the chili seeds were soaked with 2-days old culture (≈ 10^6 cell/ml) of each chitinolytic bacterial isolate for 30 min. The experiment was repeated 4 times. Controls were isolate-free seeds planted in fungus-free soil (IFFF) and isolate-free seeds planted in fungus-inoculated soil (IFF).

Observations of plant height and number of leaves were conducted every week by randomly chosen three seedlings for each treatment. Dry-weight was measured at end of study. Number of seedling damping-off were observed from total seedlings and damping off reduction (%) was measured as:

[(number of IFF damping off - number of seed treated damping off) / (number of IFFF seedlings)] x 100%

RESULTS

Test of Pathogenicity. Tests of pathogenicity of *F. oxysporum* to red chili seedling were done twice. The tests showed similar results with 91.6 and 90.47% of red chili seedling damping-off for the first and the second test respectively. Manifestation of the disease was observed as leaf and seedling wilted with yellowing leaf followed by seedling slunted. This clearly confirmed that *F. oxysporum* used was pathogen to red chili.

Control of Fusarium Wilt of Chili Seeds. Red chili seeds were treated by soaking them into bacterial solution of BK07, BK08, BK09, LK08, or KR05 separately for 30 minutes. Treated seeds were planted in soil inoculated with *F. oxysporum*. Seeds planted in Fusarium-inoculated soil were succeptible to Fusarium wilt showed by IFF. On the other hand, Fusarium wilt of seedling was suppressed by soaking the seeds into the isolate solution prior planted. However, the isolate ability to control the disease was varied (Figure 1). BK08 decreased more damping-off rather than others. Seedling dumping off with BK08 treatment was only 17.86%, while percentage of damping off in untreated seeds was relatively high. At the end of study, 82.14% of IFF seedling was damp off. In accordance with its suppression to damping off, BK08 relatively reduced damping off by 60.71% (Figure 2).

One out of thirty seeds did not grow, and one out of growing seedling of IFFF died at the end of study. It is believed that it was not because of Fusarium wilt, but it was their viability. Fusarium wilt was observed after 13 days of seedling in IFF, and between 14-17 days of seedling in the treated seeds. The numbers of seedling damping-off were increased rapidly to 4 weeks of observation. Our observation also showed that late seedlings were observed in the treatments in which the pathogen may take its role.

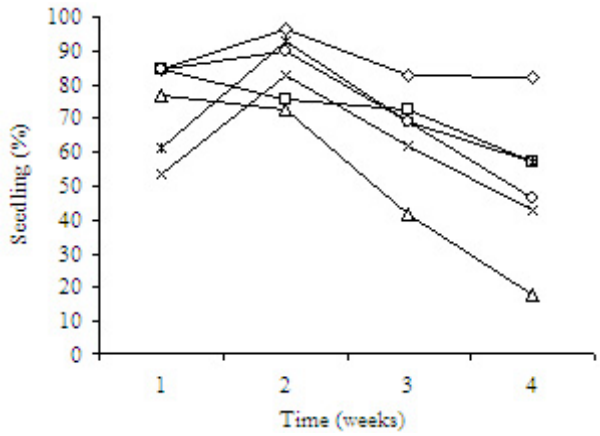

Figure 1. Effect of chili seed soaking treatment with chitinolytic isolates on percentage of seedling. —△—: IFF, —×—: BK07, —◇—: BK08, —□—: BK09, —✳—: KR05, —○—: LK08.

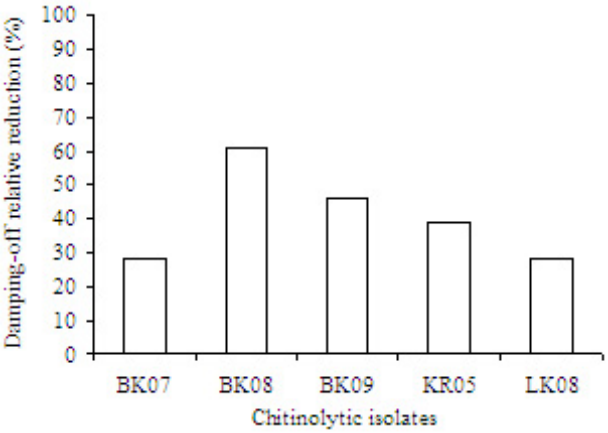

Figure 2. Relative reduction of seedling damping off chili of seed soaking treatment with chitinolytic isolates.

Since the pathogen may alter morphological and physiological traits, seedling height, dry-weight, and leaf number as possible manifestation of *Fusarium* infection in the seedling were also observed. Eventhough they varied, all treated seeds produced seedling higher than that of IFF (Figure 3). Chili seeds treated with BK08 produced 7.86 cm seedling, while seeds of IFF was 6.88 cm in height after 4-weeks.

Dry-weight of treated and untreated seedling were measured at the end of study. Although seedling treated with BK08 produced relatively lower seedling height, the seedling yielded 4.3 mg of dry-weight which was higher than that of both IFFF and IFF (Figure 4). In addition, direct observation of the seedling showed that the seedling was more stout and fresh.

Leaf number was observed by counting total leaves in one seedling. There was no different number of leaves among the treatments. Only two leaves were observed in all seedlings. Planting seeds for 30 days in small trays might influence leaf development, or perhaps, the treatments take in an effect after 30 days.

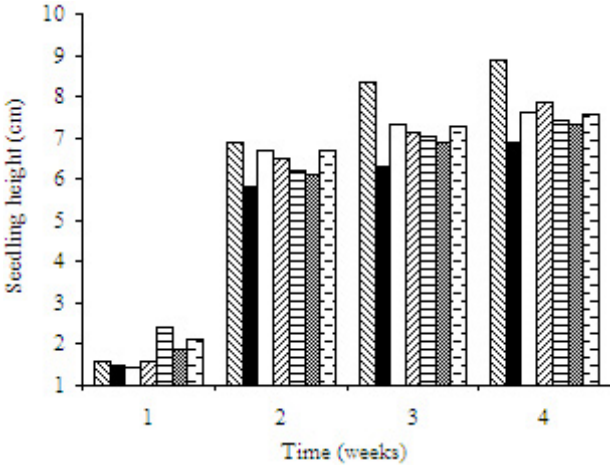

Figure 3. Effect of chili seed soaking treatment with chitinolytic isolates on seedling height. ▨: IFFF, ■: IFF, □: BK07, ▨: BK08, ▤: BK09, ▩: KR05, ⊡: LK08.

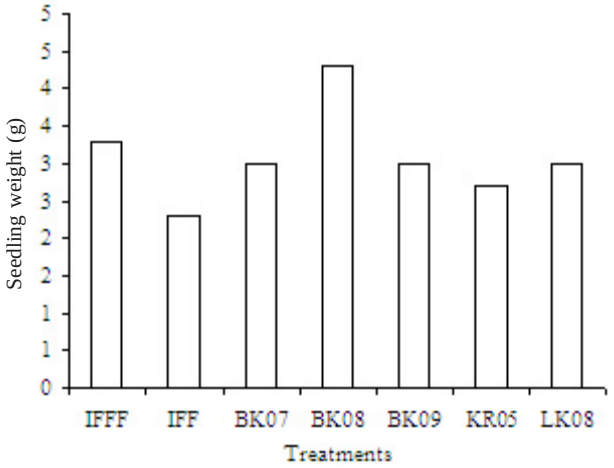

Figure 4. Effect of chili seed soaking treatment with chitinolytic isolates on seedling weight.

DISCUSSION

Reisolation and reinoculation of *Fusarium* were to prove that the fungal was the only causative agent of the Fusarium wilt in the chili seedlings. All samples of seedling with Fusarium wilt symptoms showed that the pathogen was in the seedling. The symptoms were yellowing leaves, which turn brown and brittle, wilted occasionally desiccated stem, collars appeared slunted and discolored with yellowish gray to brownish. Reinoculation of isolated *Fusarium* from infected seedling caused similar symptoms. It was clearly proved that the fungal isolate was responsible to the disease.

Antagonistic microorganisms, by their interactions with various soil-borne plant pathogens, play a major role in microbial equilibrium and serve as powerful agents for biological disease control (Lim *et al.* 1991). Chitinolytic bacteria often show antagonistic association with fungi (Lim *et al.* 1991; Kang *et al.* 1998). In this study, we did not examine antagonistic manner in detail. It is often difficult to gain a complete understanding the mechanisms of which individual microorganisms function to control disease (Kobayashi *et al.* 2002). Antagonism may operate by antibiosis, competition, predation, or parasitism (Ozbay & Newman 2004).

Parasitism involving the production of several hydrolytic enzymes that degrade cell walls of pathogenic fungi might take part of the fungal suppression (Ozbay & Newman 2004). The lytic activity of bacteria is one of a number of mechanisms that has been implicated in biocontrol for several years (Benhamou *et al.* 1990). A number of fungi are particularly susceptible to lyse by microorganisms (Potgieter & Alexander 1966). Five chitinolytic bacteria (BK07, BK08, BK09, LK08, KR05) were utilized to suppress chili seedling damping off. Chitinolytic bacteria were often characterized by their ability to produce clear zone around their colony in chitin containing media. The clear zone formed during cultivation of bacteria in agar containing chitin indicated the bacteria secreted chitinase hydrolizing into its soluble monomer or derivates (results being submitted for a publication).

Chitinase is known as one of antifungal protein (Gohel *et al.* 2006). Chitinase produced by the isolates should be considered as enzyme responsible in lysing chitin polymer of *Fusarium* hyphae. Total chitin in fungal cell wall varied between 4-9% of cell dried weight depend on fungal species/strain (Rajarathnam *et al.* 1998).

Different effect in controlling fungal growth might be caused by different chitinase produced by the isolates. Molecular and biochemical characterizations have revealed that chitinases, similar to other glycosyl hydrolases, are molecular in nature and can differ according to their structural organization. Enzymes can vary both within and between microbes (Kobayashi *et al.* 2002). Fungal cell wall usually is composed not only with chitin but also with other sugar such as â-1,3 glucan which binds to chitin in amorf stucture. Chitinase dan â-1,3 glucanase therefore are key enzymes in cell wall lysis (Benhamou *et al.* 1990). Other antifungal protein and

metabolites such other glycosyl hydrolase, chitin-binding protein, and antibiotics might also be involved in Fusarium suppression in chili seedling.

Fusarium wilt effects plant growth by blocking water and nutrient transport of infected plant. This may consequently alter morphological and physiological traits of the plant. The potential control of Fusarium wilt in this study included the altering of seedling height, dry-weight, and leaf number of chili seedling. By inhibiting Fusarium growth in the seedling, all chitinolytic isolates treatments increased seedling height and dry-weight. This means that the treatment effected plant healthy. However, no effect was for leaf number; two leaves were observed each seedling. Grown in small tray for 30 days might effect to plant growth overall. A 10-11 days-old chili seedling is usually moved and individually grown in polybag. Seedling infected by Fusarium wilt showed smaller stem and petite leaf, in turn wilted and desiccated.

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