Biocontrol of soil-borne Fusarium wilts of tomato and cabbage with a root-colonizing fungus, *Penicillium* sp. EU0013

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Abstract
The soil-inhabiting fungal pathogen *Fusarium oxysporum* can result of severe losses in many plants. We investigated the biocontrol effect of a novel root-colonizing fungus, *Penicillium* sp. EU0013, to Fusarium wilt diseases of tomato and cabbage. Application of EU0013 at inoculum concentration of 10^6 conidia /g to the potting mix resulted in 78 and 74% reduction of the diseases in tomato and cabbage, respectively. With the increase of applied EU0013 concentration, disease severity was reduced. Recovery of EU0013 from tomato and cabbage roots was 39 to 81% and 36 to 79%, respectively.

Key Words
Biocontrol, soil-borne wilt diseases, root colonization, plant growth promoting fungi.

Introduction

Soil-borne wilt diseases caused by *Fusarium* spp. are difficult to control. Among the different farm management practices, crop rotation is known to be less effective in controlling this pathogen because of its wide host range. Broad-spectrum biocides, such as methyl bromide have been widely used to manage soil-borne diseases caused by *Fusarium* spp., however such soil fumigants have hazardous effects on the environment (Blancard 1993). Similarly breeding cultivars that are resistant against soil-borne diseases is difficult due to mutation in *Fusarium* spp; therefore research has been focused on the biological control (Fravel *et al*. 2003).

A novel plant growth promoting fungus (EU0013) was recently isolated from eucalyptus roots (Teshima and Sakamoto 2006). From morphological features of the conidiophores and sequence data on the ITS region of rDNA, the EU0013 was identified as *Penicillium* sp. EU0013 colonized cabbage roots and significantly increased seed germination and seedling growth. We investigated EU0013 as a candidate for biocontrol agent to Fusarium wilt of tomato and cabbage in controlled environmental conditions.

Materials and Methods

In vitro antagonism of EU0013
Using dual culture technique, the EU0013 and causal agents of Fusarium wilt of tomato, *Fusarium oxysporum* f.sp. *lycopersici* CU1 and cabbage, *F. oxysporum*. f.sp. *conglutinans* K124F were grown in the same Petri dish by inoculating fungal culture distantly apart from each other on PDA media, and then incubated at 25 ^{o}C in dark for 7 days. The inhibition of the pathogens were recorded either in formation of inhibition zone or overgrowth to the pathogen by the EU0013. The colony diameter of pathogens in dual culture was measured and growth inhibition was expressed as percentage of the control (Baker and Cook, 1974).

Effect of EU0013 on the development of Fusarium wilt in tomato and cabbage
Biocontrol effect of *Penicillium* sp. EU0013 against tomato and cabbage wilt was studied in growth chamber conditions. Seeds of tomato cv. Oogatahukuju were grown in 5x5x5 cm plastic pots containing autoclaved potting mix (peat and vermiculite mixed 1:1 v/v) placed in a lighted growth chamber at 25^{o}C during day (16h) and 18 ^{o}C at night (8h). Different concentrations (10^2, 10^3, 10^4, 10^5, and 10^6 conidia /g of potting mix) of EU0013 were applied 1 week after sowing and 10^5 conidia /g of CU1 was applied at 2 weeks after sowing the seeds. After 10 days of inoculation, seedlings were monitored regularly for external symptoms of Fusarium wilt for a period of 40 days. Disease severity was evaluated by the percentage of leaves with symptoms (yellowing and wilting) to the total number of leaves.

Root colonization by EU0013 was examined by sampling small root sections. Each root segment was surface sterilized by immersion in 70% ethanol, followed by rinsing in sterile water and incubation at 25 ^{o}C in dark for 1 week. Root colonization was scored positive, when a typical EU0013 colony developed. Root staining method reported by Giovanetti and Mosse (1980) was used for hyphal observation of EU0013 in tomato root

tissues under light microscope. Effect of EU0013 on the development of cabbage wilt was studied using same protocol as described above. Cabbage cultivar Shikidori was used in this study.

Results and Discussion

Efficacy of Penicillium sp. EU0013 on the growth of Fusarium wilts pathogens.

In dual culture experiment, EU0013 formed an inhibition layer near CU1 and K124F without any physical contacts. EU0013 resulted in 30.6% and 29.6% reduction radial of colony growth in CU1 and K124F, respectively, compared to the control (Table 1). This inhibition zone formation indicates that EU0013 produces some antifungal compounds which inhibit the growth of these pathogens. The nature and composition of such compounds is not yet known.

Table 1 Effect of EU0013 on the growth of *Fusarium oxysporum* f.sp. *lycopersici* CU1 and *Fusarium oxysporum*. f.sp. *conglutinans* K124F on Potato Dextrose Agar (PDA) after 10 days of incubation.

EU0013	Growth of CU1		Inhibition zone (mm)	Growth of K124F		Inhibition zone (mm)
	Colony diameter (mm)	inhibition[A] (%)		Colony diameter (mm)	inhibition[A] (%)	
EU0013 (+)	55.5± 0.43	30.6±0.95	3.5 ± 0.05	53.42±0.34	29.56 ±0.59	3.44 ±0.07
EU0013 (-)	80.3± 0.76	-	-	75.84 ±0.21	-	-

Data represent mean±SE (n=5); (+) with EU0013; (-) without EU0013

[A]Inhibition (%) = (1-colony diameter of CU1 or K124F in EU0013 (+)/ colony diameter of CU1 or K124F EU0013 (-)) x100

Efficacy of Penicillium sp. EU0013 on the disease development of tomato and cabbage wilt

Disease severity and percent disease reduction varied significantly (P <0.05) with the concentration of EU0013 (Table 2). In the absence of EU0013, both CU1 and K124F exhibited severe disease (Figure 1). However, prior application of EU0013 significantly reduced the disease severity in tomato and cabbage. The highest disease reduction (78%) in tomato plants was obtained by prior application of EU0013 at 10^6 conidia/g of potting mix, followed by application at 10^5 conidia/g. Disease reduction at these two levels was not significantly different from each other. Application of the same levels of conidial concentration of EU0013 to cabbage resulted in 74 and 70% disease reduction, respectively.

EU0013 recovery from roots of tomato and cabbage varied 39 to 81% and 36 to 79%, respectively (Table 3). The root colonization by EU0013 decreased with decreasing inoculum concentration. Suppression of Fusarium wilt diseases by root colonizing fungi with several mechanisms have been reported (Hossain *et al.* 2007, Narisawa *et al.* 2000, Meera *et al*. 1995).

Table 2. Effect of inoculum concentration of Penicillium sp. EU0013 on the disease severity caused by F. oxysporum f.sp. lycopersici CU1 to tomato plants and F. oxysporum f.sp. conglutinans K124F to cabbage plants.

EU0013: Pathogen (conidia/g of potting mix)	0:0	10^4:0	0:10^5	10^2: 10^5	10^3: 10^5	10^4: 10^5	10^5: 10^5	10^6: 10^5
Disease severity (%)[A] of tomato wilt	0	0	72.4 (e)	39.0 (d) (46)	33.1 (cd) (54)	26.4(bc) (64)	21.1 (ab) (71)	16.9(a) (78)
Disease severity (%)[A] of cabbage wilt	0	0	77.3 (e)	52.8 (d) (32)	44.1 (c) (43)	31.1(b) (60)	22.3 (a) (70)	19.9(a) (74)

Fisher's LSD for tomato wilt at (P <0.05) = 8.30; Fisher's LSD for cabbage wilt at(P <0.05) = 9.97

Inocula of both fungi were added as a water drench. Statistical comparisons between treatments were performed by F test (P <0.05). Letters in common in parenthesis indicate a lack of significant difference (comparisons are valid within each row).

[A]Disease severity was assessed as proportion of leaves with symptoms (yellowing and wilting) compared with the total number of leaves (n=9) at 40 days post-inoculation with the pathogen. Numbers in parenthesis indicate % disease reduction compared to pathogen inoculated plants alone.

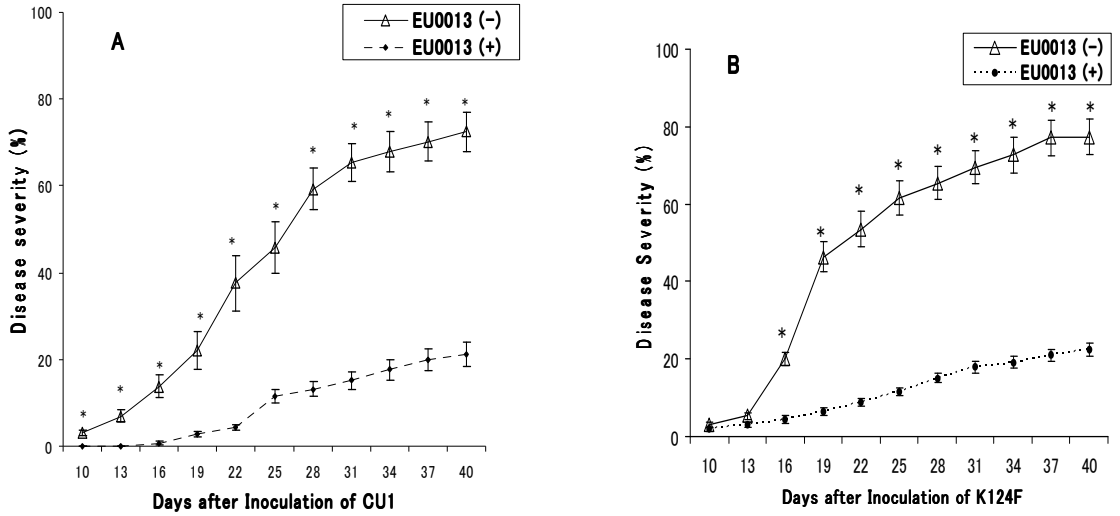

Figure 1. Effect of EU0013 on the development of Fusarium wilt diseases. (A) Tomato cultivar Oogatahukuju inoculated with *Fusarium oxysporum* f.sp. *lycopersici* CU1. Disease progress curves in tomato plants grown on potting mix in the absence (-) or presence (+) of 10^5 conidia /g of potting mix of EU0013. (B) Cabbage cultivar Shikidori inoculated with *Fusarium oxysporum*. f.sp. *conglutinans* K124F. Mean values that were statistically different at each time point ($P \leq 0.01$) are indicated by an asterisk. Bars indicate standard errors of means.

Table 3. Effect of inoculum concentration of *Penicillium* sp. EU0013 on the colonization of tomato and cabbage roots at 40 days post inoculation.

EU0013: Pathogen (conidia/g of potting mix)	10^4:0	10^2: 10^5	10^3: 10^5	10^4: 10^5	10^5: 10^5	10^6: 10^5
EU0013 recovery from tomato roots (%) [a]	81	39	45	53	58	64
EU0013 recovery from cabbage roots (%) [a]	79	36	41	45	55	60

[A]EU0013 recovery from roots tissues of inoculated seedlings= Number of EU0013 recovered roots segments/number of total roots segments incubated (n=30).

Conclusions

This study reports the biocontrol potential of a novel root-colonizing fungus, *Penicillium* sp. EU0013 against soil-borne Fusarium wilt diseases of tomato and cabbage under controlled environmental conditions. Prior application of EU0013 effectively reduced the development of Fusarium wilt in tomato and cabbage.

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