한국식물병리학회지 11(4) : 287-291(1995)
Korean J. Plant Pathol. 11(4) : 287-291(1995)

Synergistic Effects of *Gliocladium virens* and *Pseudomonas putida* in the Cucumber Rhizosphere on the Suppression of Cucumber Fusarium Wilt

Yeoung Seuk Bae, Chang Ki Shim[1], Chang Seuk Park[1] and Hee Kyu Kim[1]*
Department of Crop Protection, National Agricultural Science and Technology Institute, Suwon 441-707, Korea
[1]Department of Agricultural Biology, College of Agriculture, Gyeongsang National University, Chinju 600-300, Korea

오이 덩굴쪼김병 抑制에 관한 根圈定着能力이 있는 *Gliocladium virens*와 *Pseudomonas putida*의 協力效果

裵永錫 · 沈昶琪[1] · 朴昌錫[1] · 金喜圭[1]*
農業科學技術院 作物保護部, [1]慶尙大學校 農科大學 農生物學科

ABSTRACT : Biocontrol agents, *Gliocladium virens* G872B and *Pseudomonas putida* Pf3, were compatible each other in colonizing cucumber rhizosphere, which contributed to a long-term inhibition of cucumber Fusarium wilt. G872B colonized successfully on the cucumber root system, irrespective of the introduction of Pf3. Pf3 also colonized well in the cucumber rhizosphere regardless of the presence of G872B. The individual strains effectively suppressed cucumber wilt up to 56 days after transplanting. The combined treatment of G-872B and Pf3 provided a long-term protection of about 80 days with the efficacy greater than that obtained by any individual strains under greenhouse conditions. These results suggest that the colonization of the biological control agents in the rhizosphere could be correlated directly to Fusarium wilt-suppressive potentials.

***Key words* : *Gliocladium virens*, *Pseudomonas putida*, synergistic colonization, cucumber rhizosphere, long-term protection, *Fusarium oxysporum* f. sp. *cucumerinum*.**

Various microorganisms antagonistic to plant root pathogens were isolated from soil and applied to control plant diseases. They are mostly applied as single inoculants; however, sometimes more than one species or isolates are applied as a mixed inoculant, hoping to achieve better control efficacies.

Sneh *et al.* (14) reported that combinations of lytic bacteria and fluorescent *Pseudomonas* isolates added to conducive soil did not increase suppressiveness against Fusarium wilt of wheat. Kloepper (9) also reported that the control effect of the mixtures of two or more rhizobacteria was not greater than that obtained by any single strains. Hubbard *et al.* (5) observed that fluorescent *Pseudomonas* and *Trichoderma* are mutually exclusive to each other in iron deficient soil. However, Park *et al.* (10) reported that combinations of fluorescent *Pseudomonas* and nonpathogenic isolates of *Fusarium oxysporum* showed effective control against the cucumber Fusarium wilt. Their result suggests the possibility of compatible combinations of antagonists having various beneficial attributes for the control of soil-borne diseases. This possibility was supported by Jeong *et al.* (6) who isolated compatible isolates that are synergistically colonizing at rhizosphere.

The objectives of this study were to investigate the possibility of compatible colonization of *Gliocladium virens* and *Pseudomonas putida* on cucumber rhizosphere and to examine the relevancy of the long-term control of cucumber Fusarium wilt by the populations singly or in combination.

*Corresponding author.

MATERIALS AND METHODS

Biocontrol agents. *G. virens* G872B was used in this experiment as in our previous work (2). The wild type of the fungus was previously proved to be effective in the suppression of cucumber wilt caused by *F. oxysporum* f. sp. *cucumerinum* (12). A strain of *Pseudomonas putida* was isolated from the sesame rhizosphere in Fusarium wilt-suppressive soil of the experimental field of Gyeongnam Provincial Rural Development Administration, Chinju, Korea. *P. putida* Pf3 obtained by UV mutagenesis was a mutant tolerant to 100 μg/ml rifampicin (8).

Preparation of inocula. The isolate G872B was grown on potato dextrose agar (PDA) (Difco) medium at 26°C for one week. The conidia were harvested and washed three times in sterile distilled water by centrifugation at 2,500 g for 10 min. Two milliliters of sterile distilled water was added to the pellet. The final concentration of conidia was 10^9 conidia per ml. The Pf3 suspension was transferred to 250 ml Erlenmeyer flasks containing 100 ml of King's B broth, and incubated at 26°C on a rotary shaker for 48 hr. Bacterial cells were harvested by centrifugation at 2,600 g for 10 min, washed and resuspended twice in 0.01 M $MgSO_4$ solution. Bacterial suspension was adjusted to give 10^8 cells per ml.

Seed treatment. Cucumber seeds (cv. Sa-yeup-Oi, Jungang Seed Co.) were surface-disinfected for 5 min in 1% sodium hypochlorite solution, washed and air-dried for 1 hr at room temperature. Two grams of seeds were treated either with 1 ml of the conidial suspension or the bacterial suspension in 0.1% methyl-cellulose solution and air-dried for 1 hr. For the combined treatment of both G872B and Pf3, both conidial and bacterial suspensions were mixed to 1 : 1 (v/v) prior to seed treatment as above. Non-treated seeds were dipped in 0.1% methyl-cellulose solution.

Rhizosphere colonizing test. *In vitro* assessment of rhizosphere competence of G872B and Pf3 was performed according to the method of Ahmad and Baker (1). In greenhouse experiments, population densities of both agents in the rhizosphere soil adhering to the root tip of cucumber were determined three times: one day before, and 40 and 80 days after transplanting. In this experiments, King's B medium amended with 100 μg/ml rifampicin for Pf3 and Trichoderma-selective medium (3) for G872B were used to determine population density in the cucumber rhizosphere.

Evaluation of disease control in greenhouse plots. Soil-chlamydospore inoculum of the cucumber wilt pathogen (4, 12) was mixed thoroughly with sandy loam soil to final concentration of 10^3 cfu/g soil 15 days before transplanting. The cucumber seeds treated with either or both of the antagonists of G872B and Pf3 were sown in 8-cm-d plastic pots containing the contaminated soil, allowed to germinate and maintained in the greenhouse for 2 weeks. Six seedlings were transplanted in each plot with 4 replicates by the completely randomized design. Experimental plots were maintained by common cultural practices in Chinju area. Disease incidence was examined every 2 days up to 80 days after transplanting.

RESULTS

Coexistence of fungal and bacterial antagonists in the rhizosphere. In laboratory tests, *G. virens* G-872B colonized successfully throughout the cucumber rhizosphere in the presence of fluorescent *Pseudomonas putida* Pf3 (Table 1). The population density was not significantly different between the two treatments that were inoculated with or without the fluorescent *Pseudomonas putida* Pf3. Pf3 were also colonized well in the rhizosphere of cucumber plant, from the top to the root tip of cucumber (Fig. 1). The bacterial population was not affected by the simultaneous introduction of G872B.

In greenhouse experiments, the population densities of G872B and Pf3 in root tip of cucumber gradually declined during the plant growth, except the mixed treatment with Pf3 and G872B together (Table 1). The treatment with the mixture of the two antagonists re-

Table 1. Population densities of *Gliocladium virens* G-872B and *Pseudomonas putida* Pf3 in the rhizosphere soil adhering to cucumber root tip after inoculation by seed-coating with either of the antagonists or both

Antagonist treatment	Population density[a] (cfu/g rhizosphere soil)		
	1 day	40 days	80 days
G872B alone	7,110	1,410	3,400
Pf3 alone	18,900	27,000	24,000
G872B+Pf3 mixture			
G872B	9,740	2,500	1,300
Pf3	26,300	33,000	71,000

[a] Population density was determined at different times after transplanting cucumber.

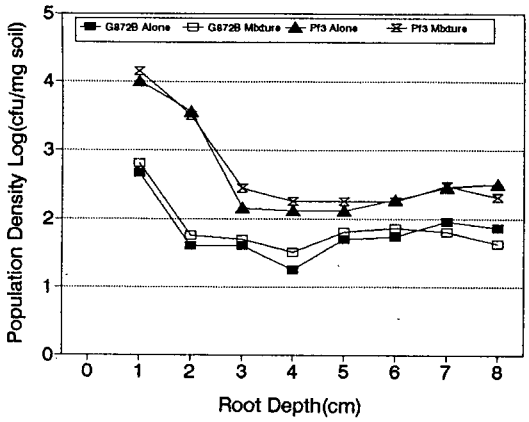

Fig. 1. Population densities of *Pseudomonas putida* Pf3 and *Gliocladium virens* G872B in the rhizosphere soil of cucumber in the treatment of seed-coating with either G-872B or Pf3 alone or with the G872B-Pf3 mixture. Prior to seed treatment (2 g seeds vs. 1 ml suspension), suspensions of G872B (10^9 conidia/ml) or Pf3 (10^8 cells/ml) were used alone or in combination with equal volumes of the suspension of each isolate.

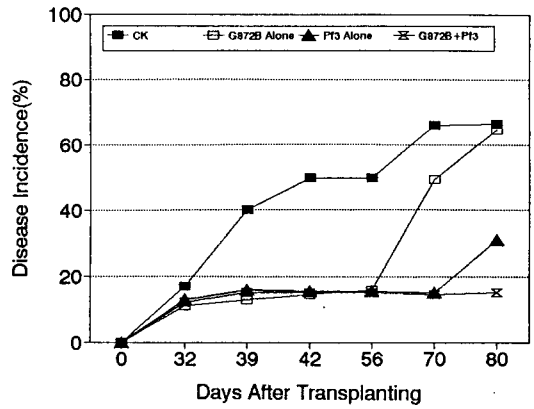

Fig. 2. Disease incidence of cucumber wilt in greenhouse soil infested with *Fusarium oxysporum* f. sp. *cucumerinum* when *Pseudomonas putida* Pf3 and *Gliocladium virens* G872B, or both were seed-coated. The greenhouse soil was artificially infested with soil chlamydospore inoculum (10^6 cfu/g soil) of *F. oxysporum* f. sp. *cucumerinum* to carry final concentration of 10^3 cfu/g soil. Untreated check; CK. *G. virens* G872B alone; G872B. *P. putida* Pf3 alone; Pf3. G872B-Pf3 mixture; G872B+Pf3.

vealed higher populations of antagonists than those of single isolate treatments.

Biocontrol of cucumber wilt. The two antagonist treatments used in this experiment suppressed effectively cucumber wilt until 56 days after transplanting, whereas disease incidence was continuously increased and very high in the untreated plots (Fig. 2). The disease incidence in the plots with treatment of G-872B alone increased abruptly after 56 days, finally reaching as high as in the untreated plots. Pf3 alone significantly suppressed the disease incidence until 80 days after transplanting, and the disease incidence was 33% compared to 66% in the untreated plots after 80 days. The combined treatment with G872B and Pf3 provided more long-term suppression of disease incidence of cucumber wilt than by any single treatments under greenhouse conditions.

DISCUSSION

Microorganisms that can grow in the rhizosphere soil are valuable for use as biocontrol agents, because the rhizosphere provides the front line defense for roots against attack by pathogens (15). Park *et al.* (10) reported that a more efficient strain of fluorescent *Pseudomonas* in the biocontrol of Fusarium wilt of cucumber had higher rhizosphere-competence than an inferior strain of the same species. Kang and Kim (7) reported that rhizosphere-competent *G. virens*, applied to sesame seed, reduced significantly the disease incidence of Fusarium wilt and damping-off of wheat. Moreover, control efficacy of rhizosphere-competent isolates on the the Fusarium wilt was greater than the benomyl seed treatment in the field experiments. Our study revealed that *G. virens* G872B colonized successfully throughout the cucumber rhizosphere, regardless of the introduction of fluorescent *Pseudomonas* Pf3, not only in laboratory tests (Fig. 1) but also in greenhouse experiments (Table 1).

Sivan and Chet (13) reported that rhizosphere-competent *T. harzianum*, when applied to seeds, reduced population density of pathogenic *Fusarium* spp. in the rhizosphere. In our study, rhizosphere-competent *G. virens* G872B suppressed successfully cucumber wilt until 56 days after transplanting. However, the disease incidence increased in the plots with the treatment of G872B alone. The combined treatment of G872B and Pf3 provided a high long-term efficacy for reducing the disease incidence of the cucumber wilt than that obtained by any single treatments under greenhouse conditions (Fig. 2), suggesting that co-treated compatible biocontrol agents may work better for the disease control of soil-borne pathogens. Especially, the Pf3 po-

pulation increased on the rhizosphere by the combined treatment with both antagonistic microorganisms. The increase of the microbial colonization may be related to the increase of control efficacy against cucumber Fusarium wilt in our study.

Elad and Baker (4) reported the spore germination of some isolates of *Fusarium* spp. was inhibited at low levels by a mutant of *Pseudomonas* spp. producing no siderophores, and such inhibition was not reversed by addition of Fe. They suggested that competition for carbon by the bacterial biomass was instrumental in inhibition. Hubbard *et al.* (5) reported that growth of *T. hamatum*, together with *Pseudomonas* spp. on a medium with little available iron resulted in fluorescent zones around *Pseudomonas* spp. colonies that were inhibitory to *T. hamatum*. This inhibition could be overcome by addition of 100 μM Fe^{+2} or Fe^{+3}. Generally, iron is sufficiently available in the soil of plastic film houses in Chinju area (11). Therefore, fluorescent pseudomonads could be used together with *Trichoderma* or *Gliocladium*.

요 약

생물적 방제균 *Gliocladium virens* G872B와 *Pseudomonas putida* Pf3을 오이 종자에 혼합처리시 근권에서 공존 가능성을 검정하고, 실제포장 하에서 이들 균주의 오이 덩굴 쪼김병 방제효과를 검정하였다. G-872B와 Pf3은 오이 종자에 혼합처리시 오이 근권에 잘 정착하여, 두 균주 모두 오이 근권에 공존하여 협력작용이 있었다. 오이 덩굴쪼김 병원균이 감염된 토양에, 이들이 단독 또는 혼합 처리된 오이를 이식했을 때, 이식 후 56일까지 길항균 처리구 모두 우수한 발병 억제효과를 나타내었다. 그러나, 그 이후 G872B 단독 처리구에서는 병 발생이 급속히 증가하여 80일에는 무처리구와 차이가 없었다. 반면, 혼합처리구에서는 80일까지 현저한 억제효과를 나타내는 상승 작용이 나타났다.

REFERENCES

1. Ahmad, J. S. and Baker, R. 1985. Induction of rhizosphere competence in *Trichoderma harzianum*. *Phytopathology* 75 : 1302 (Abstr.).
2. Bae, Y. S., Kim, H. K. and Park, C. S. 1990. An improved method for rapid screening and analysis of root colonizing ability of biocontrol agent. *Korean. J. Plant. Pathol.* 6(3) : 325-332.
3. Cho, C. T., Moon, B. J. and Ha, S. Y. 1989. Biological control of *Fusarium oxysporum* f. sp. *cucumerinum* causing cucumber wilt by *Gliocladium virens* and *Trichoderma harzianum*. *Korean. J. Plant Pathol.* 5(3) : 239-249.
4. Elad, Y. and Baker, R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. Phytopathology 75 : 1053-1059.
5. Hubbard, J. P., Harman, G. E. and Hadar, Y. 1983. Effect of soil borne *Pseudomonas* spp. on the biological agent, *Trichoderma hamatum*, on pea seeds *Phytopathology* 73 : 655-659.
6. Jeong, M. J., Park, C. S. and Kim, H. K. 1993. Compatibility and Synergism of *Gliocladium virens* and *Pseudomonas putida* and their improved competitive potential with *Fusarium oxysporum* f. sp. *cucumerinum*. *Korean J. Plant Pathol.* 9(1) : 12-18.
7. Kang, S. W. and Kim, H. K. 1989. *Gliocladium virens*, a potential biocontrol agent against damping-off and Fusarium wilt of sesame (*Sesamum indicum* L.) in problem field in Korea. *Res. Rep. Rural Development Administration* 31 : 19-26. Suwon, R.O.K.
8. Kim, H. K. and Jee, H. J. 1988. Influence of rhizosphere antagonists on suppression of cucumber wilt; Increased cucumber growth and density fluctuation of *Fusarium oxysporum* f. sp. *cucumerinum* Owen. *Korean J. Plant Prot.* 4 : 10-18.
9. Kloepper, J. W. 1983. Effect of seed piece inoculation with plant growth promoting rhizobacteria on populations of *Erwinia carotovora* on potato roots and daughter tubers. *Phytopathology* 73 : 217-219.
10. Park, C. S., Paulitz, T. C. and Baker, R. 1988. Attributes associated with increased biocontrol activity of fluorescent *Pseudomonas*. *Korean J. Plant Pathol.* 4 : 218-225.
11. Park, J. C. and Cho, J. L. 1986. Studies on the growing condition, disease and pest occurrence on horticultural crops of plastic film houses in Chinju suburbs, in Korea. 3. Soil properties and nutrients of vegetable leaves. *J. Inst. Agric. Res. Util. Gyeongsang Natl. Univ.* 20 : 29-48.
12. Shim, G. Y., Kim, H. K. and Kang, S. W. 1992. Predisposing effect of soil salinity on the cucumber wilt caused by growth of *Fusarium oxysporum* f. sp. *cucumerinum*. *Korean J. Plant Pathol.* 8(1) : 14-21.
13. Sivan, A. and Chet, I. 1989. The possible role of competition between *Trichoderma harzianum* and *F. oxysporum* on rhizosphere colonization. *Phytopathology* 79 : 198-203.
14. Sneh, B., Dupler, M., Elad, Y. and Baker, R. 1984. Chlamydospore germination of *Fusarium oxysporum*

f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from Fusarium-suppressive soil. *Phytopathology* 74 : 259. (Abstr.)

15. Weller, D. M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol.* 26 : 379-407.