韓 國 菌 學 20(4) : 337-346 (1992)
Korean Mycol 20 : 337-346 (1992)

Suppressive Mechanism of Soil-borne Disease Development and its Practical Application

Isolation and Identification of Species of *Trichoderma* Antagonistic to Soil diseases and its activities in the Rhizosphere

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토양병의 발병억제 기작과 그 실용성

길항성 *Trichoderma* spp.의 분리, 동정 및 근권내 활동

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ABSTRACT: *Trichoderma* spp. are an effective control agent for damping-off or other plant diseases. The interaction between. *T. hamatum* and *Rhizoctonia solani* on the rhizosphere or surface soil were examined to assess the possible roles of antibiosis or competition in the mechanisms of biological control agents as a basic reasearch. In a proportional comparison, total bacteria, fungi, actinomycetes and *Trichoderma* spp were 65%, 8.8%, 25.9% and 0.28% respectively in their distribution in the soil. Among *Trichoderma* spp isolated, the 5 species of *Trichoderma* spp were indentified as *T. koninggii, T. pseudokoninggii, T. aureoviridi, T. hamatum* and *T. viride* respectively. In a mycoparasitic test, one isolate of *T. hamatum* strain Tr-5 showed an enzymatic ability to break fungal hyphae into piecies and infected on the *R. solani* hyphae showing a parasitism. Spore germination of the all isolates of *Trichoderma* spp showed a 1.7-7.3% of germination in natural soil conditions, but the percentage was high in sterile soil indicating all the natural soil were fungistatic on conidia of *Trichoderma* spp. In rhizosphere competent assay in pea plant, the antagonistic *T. hamatum, T. viride, T. koninggii, T. pseudokoninggii* showed a colonizing upper soil depth in rhizosphere around 1-3 cm in root zone, but the colonizing ability was much reduced along the deeper the soil depth. Propagule density was decreased in deeper the soil layer. Disease development rate treated alone with plant pathogens, *Fusarium solani, Rhizoctonia solani, Cylindrocarpon destructans* increased, but disease incidence rate reduced in treatment with combinations with antagonistic *T. hamatum* strain Tr-5.

KEYWORDS: *Trichoderma* spp., antibiosis, biological control agents, mycoparasitic test, fungistatic rhizosphere competent assay, rhizosphere colonizing, *Fusarium solani, Rhizoctonia solani, Cylindrocarpon destructans,* disease incidence.

In the light of present-day constraints on plant disease control practices, especially, those imposed on potentially hazardous use of pesticides, biological control measures are increasingly capturing the imagination of pathologist and are gaining as a possible practical agricultural method for soilborne-pathogen control (Papavizas and Lewis, 1981). The possibility of controlling pathogenic fungi with

antagonistic microorganisms introduced to soils, or with combination of them, low doses of fungicide has long been considered and studied for a long time. The potential for use of *Trichoderma* species as biological control agents was suggested by Weindling (1932). Fluorescent Pseudomonads were implicated in take-all disease (Cook and Weller, 1987) and also reported to be an antagonistic organisms for *Fusarium wilt suppressive soils (Baker et al* 1986; Scher and Baker, 1982; Lee, 1977).

The many studies on *Trichoderma* species were implicated in suppressive soils to *Rhizoctonia solani* (Liu and Baker, 1980). Undetected antagonistic components of known microbiological communities were analyzed (Yuen and Schroth, 1986). Ginseng (*Panax ginseng* Meyer) is particularly vulnerable to disease for recultivation previously used. Many problems still remain to be solved in increasing Korean ginseng yields. One of the most important limiting factors in ginseng production would to considered to be soilborne diseases with poor quality. and also low yields (Lee, 1984).

We ultimately concerned with microbial interaction in soil and how these microbial interaction inactivate the infection of pathogen propagules, reduce their potentials, or adversly interfere with germling functioning. In this regardings practical biological management of diseases can be practised in the integrated disease control systems. The objective of this research is to isolate and identify active fungal antagonists against ginseng pathogens, *F. solani, C. destractans* and *R. solani,* and study the survival of these antagonists in soil and rhizosphere in the absence of added nutrients. In this study, an antagonistic strain of *Trichoderma* spp. was selected, and its characteristics were observed.

Materials and Methods

Estimation of microbial population: Microbial population sizes were estimated by a serial dilution plate method (Lee, 1977). Each of the dilution was plate out in triplicate using 0.2 m*l* soil suspension samples. The medium used for the isolation of total bacteria was nutrient agar (Difco), rosebengal agar (Martin, 1950) for general fungi, chitin agar (Hsu and lockwood, 1975) for actinomycetes and Elad *et al* (1981) selective medium for *Trichoderma* spp.

Collection, isolation and identification: From the 15 soil-samples of Kyungi-do, *Trichoderma* spp. were isolated with Elad's selective medium (Elad *et al.,* 1981). *Trichoderma* isolates were transferred on PDA slant and the slant cultivar kept in 4℃ refrigerator for the following experiments. For identification of *Trichoderma* isolates, the characteristics of *Trichoderma* isolates were observed both microscopically and macroscopically. Hyphae, chlamydospore, conidiophore, phialides and phialospore were all stained with cotton-blue in lactic acid for microscopic observation. As a macroscopic characteristics of isolates, we observed the mycelial growing extention, colony color, conidiophore area, light requirement for spore coloration and color production on 2% malt extract agar plate (Rifai, 1969).

Screening of Antagonistic Reaction of *Trichoderma* spp. against *R. solani*: Hyphal tips on agar were obtained with cork-borer (0.3 cm i.d.) from the margin colony of 3 days old *Trichoderma* spp. and *R. solani* colony, and then inoculated on PDA plate as dual culture. After 4 days incubation at 27℃ of incubator, we measured the distance from two colonies contacting border to the more transparent *R. solani* mycelium growing end than *R. solani* alone growing part (Tronsmo and Dennis, 1978).

Hyphal interaction and ability of mycoparasitism between antagonistic *Trichoderma* spp. and *R. solani*: For observing mycroparasitic growing inhibition of *Trichoderma* isolates on *R. solani* mycelium, the following system was used to observe interaction points of both organisms. After boiled cellophane membranes with distilled water, the membrane was placed on water agar. An agar disk (0.3 cm i.d.) of the mycelium of *T. hamatum* was placed on one end of the cellophane membrane and a disk one of the *R. solani* mycelium was placed on the other. *T. hamatum* and *R. solani* grow towards each other at 28℃ for 4 days and the hyphae intermingled on the cellophane plates (Grif-

fin, 1972 Jee and Kim 1987).

Sensitivity of propagules to soil fungistasis: Germination of propagules was observed from sterilized soil and nonsterile soil (natural soil) to determine their sensitivities to mycostasis (Hsu and Lockwood, 1973). Fifty gram samples of the soil were adjusted to −0.05 bar matric potential, and were equilibrated from 16~24 hr before use. Soils in 9 cm diameter glass dishes sterilized by autoclaving for 1 hr. Natural soil or amended soil were contained in 9 cm diameter glass dishes. The soil was well mixed with spatula, soil surface smoothed, and allowed to equilibrate for one hr. In each experiment, duplicate nuclepore membranes bearing fungal propagules were placed on duplicate samples of untreated and treat soil. Conidia were incubated on the soils for 12~16 hr prior to germination assay. Membrane bearing propagules were stained with phenolic rose bengal (Hsu and Lockwood, 1973), destained in water, and mounted on glass slide with double sticky tape. Germination was counted microscopically with incident illumination. Three to four experiments were done.

Rhizosphere competence assay: *Trichoderma* spp were grown on PDA slant for 8 days at 25℃. The culture was flooded with sterile distilled water and conidia were gently freed from the culture surface with a loof inoculation needle. The conidia suspension was sieved through 4 layers of gauze and washed for 3 times (at 4℃, 2,500g). Density of conidia was adjusted to 10^8 per mililiter with the aid of haemocytometer.

Seed of pea was surface disinfested for 10 min in 1.0% hypochlorite solution and 70% ethanol, washed in distilled water and air dried in clean bench. Disinfested seeds of pea were placed on water agar plate, and incubated at 27℃ incubator till the seeds germinate. Modified Ahmad and Baker's method (1987) was used in this experiment. Seedlings prepared above were sown in the plastic pots 18×10 cm that were filled with soil. To determine rhizosphere competence 6 pea seedling were placed in each pot with antagonistic *Trichoderm* spp. of 2 m*l* of conidial suspension. The seedling sowed pots were added more soil to cover seedlings to 2 cm depth and placed in the room temperature for the seedling culture. Starting from soil surface, the soil containing pots were excised latitudinary per 1 cm with sterile scapel after 8 days culture. After shake off the loosely adhereing soil, root segments with their rhizosphere soil were air-dried under a 100 W lamp for 30 min. Each segment was weighed and transfered to a 20 m*l* glass vial containing 1 m*l* of sterile distilled water. The colony forming units (cfu) of *Trichoderma* contained in the rhizosphere soil at each centimeter of root were determined by plating a series of 10-fold dilutions on *Trichoderma* selective medium (Elad *et al.*, 1981). Plates were incubated at 25℃ for 5 days. Counts of *Trichoderma* colony forming units per miligram of rhizosphere soil for each root segment were repeated twice.

Pot test for control of pathogens: *Trichoderma hamatum* strain Tr-5 was grown in PDA for days at 25℃ on a incubator. Conidia of this fungus were harvested and centrifuged at 2500 rpm for 20 min and adjust the number of spores to a concentration of 10^9 cells per milliliter. One milliliter of these spores suspension was inoculated into root zone of ginseng. Sterile soil extract was used for producing chlamydospores (Hong and Lee, 1986). Infection of soil with chlamydospores of *F. solani* and *C. destructans* were done with method of Son *et al* (1985). The soil infested with 10^5 chlamydospores per grams of soil by *F. solani* and *C. destructans*. *R. solani* was inoculated in soil with the number of 90 sclerotia per a pot containing 500g soil and mixed well.

Natural soil was infested with the *F. solani, C. destructans* and *R. solani,* and then the soil was also infested with 1 m*l* of a conidia suspension of candidate antagonist T. hamatum.

To determine root rot incidence, 6 roots of 2 years old ginseng were placed in 8×12 cm pots containing the soil infested with *R. solani, F. solani* and *C. destructans.* Four pots were used as a replicate and two replicate were used for each treatment. Pot were incubated in a growth room at 25℃. Counts of emergence and rate of rotting were taken after 21 days and were expressed as

a percentage of these plant emerging and roting rate from non-infected soil (Son *et al.*, 1985).

Results

Numbers of microorganisms in soil sample collected from different site in Kyunggi-do are shown in Table 1. Numbers of bacteria and actinomycetes were higher than total fungi and *Trichoderma* spp.. Proportions of total bacteria, general fungi, actinomycetes and *Trichoderma* spp. in soil sample were 65%, 8.8%, 25.9% and 0.28% respectively. The ratio in general fungi and *Trichoderma* spp. was 31 times less propagules of *Trichoderma* spp. presented in sampled soil.

125 strains of isolates were isolated from the *Trichoderma* selective medium for a identification of *Trichoderma* spp., 15 isolates among the isolated strains were selected for identification by cultural and macroscopic observation. In culture, colonies usally grow rapidly at first smooth-surfaced and almost transmitted or watery white, later becoming floccose or compactly tufted of green or pure white coloration. Pigments may be released into the medium or the reverse of the colony remains unchanged and the mycelium is compared to hyphae, septate, much-branched and smooth walled hyphae.

The conidiopores are highly ramified, loosely or very compactly tufted and generally they are formed in distinct concentric ring-like conidia producing zone, or borne highly and irregulary on the aerial hyphae. The strains of *Trichoderma* Tr-1, 7, 8, 9 and 12 were similar in their morphology; conidial shape was loose tuft, color was green, growing form smooth or moderate, pigment production was none and phialospore color was green. whereas strains *Trichoderma* Tr-4, 5, 10 and 11 were different in their morphological characteristics comparing with *Trichoderma* Tr-1, 8, 9 and 12. Strains 2, 3, 13, 14 and 15 were different slightly in color and shape in their morphology with other strains. Morphological characteristics of *Trichoderma* isolates was in Table 2. Conidiophore's width and shape ranged in 3.0-4 μm in the strains of all isolates. The form of side branch was conical in the strains *Trichoderma* Tr-1, 8, 9 and 12 but others was variable in branch-spores were not formed in culture (Table 2). The antagonistic activity of isolates of *Trichoderma* spp. agai-

Table 1. Numbers of microorganisms in the soils collected from different sites in Kyunggi-do.

Location		Microorganisms ($X10^4$ cfu/g soil)			
		Bacteria	Fungi	Actinomycetes	*Trichoderma* spp.
Goysan	ri	126(28.6%)	21.9(5.0%)	290(65.8%)	3.0(0.7%)
Mangual	ri	646(61.1%)	185.4(17.5%)	225(21.3%)	2.3(0.2%)
Ligang	ri	717(68.0%)	33.0(3.1%)	298(28.3%)	7.0(0.7%)
Gochun	ri	1046(70.7%)	247.8(16.8%)	185(12.5%)	0.1(0.0%)
Daepo	ri	1895(58.8%)	221.0(6.9%)	1105(34.3%)	3.0(0.1%)
Onsoo	ri	367(37.8%)	368.8(38.0%)	230(23.7%)	5.6(0.6%)
Bulwoop	ri	423(44.1%)	100.3(10.5%)	423(44.1%)	12.6(1.3%)
Daemyung	ri	9317(89.1%)	206.1(2.0%)	930(8.9%)	4.0(0.0%)
Sukjung	ri	2356(75.5%)	101.9(3.3%)	659(21.1%)	2.0(0.1%)
Galsan	ri	4988(84.2%)	257.7(4.4%)	675(11.4%)	2.0(0.0%)
Suktan	ri	3482(47.6%)	348.7(4.8%)	3482(47.6%)	3.0(0.0%)
Magok	ri	5527(84.7%)	216.6(3.3%)	771(11.8%)	10.0(0.2%)
Yanggok	ri	1296(88.5%)	147.6(10.1%)	18(1.2%)	3.3(0.2%)
Gumgok	ri	3157(73.6%)	229.3(5.4%)	899(21.0%)	0.3(0.1%)
Daebyug	ri	4696(63.2%)	72.2(1.0%)	2663(35.9%)	4.0(0.1%)

Table 2. Morphological characteristics of the *Trichoderma* spp. isolates.

Isolate	Colony on malt extract plate	Conidiophore			Phialide		Phialospore		
		sterile hyphae	base width(μm)	branching	shape	size (μm)	shape	size (μm)	color
Tr-1	loose tuft	−[a]	4.0~3.7	Complicated dendroid	conical attenuated	7.5~12 x2.5~3.5	smooth surface ellipsoidal	3.0~4.8 x1.9~2.8	dark green
Tr-2	orbicular tuft	−	2.3~2.5	rare branching at top	bent-slended	7.5~8.3 x3.0~3.5	elliptic obovoidal	3.5~4.0 x3.0~3.3	pale green
Tr-3	wide tuft	−	3.8~3.4	complicated dendroid	asymmetric bottle	6.0~7.0 x3.0~3.2	obovid smooth surface	3.1~3.0 x2.9~3.3	yellowish green
Tr-4	local tuft	+[b]	2.8~3.0	short and thick	crowded pear-shaped	7.3~7.0 x3.6~4.0	obovoid	4.0~5.1 x3.0~3.2	green
Tr-5	local tuft	+	2.8~3.3	short and thick	crowded pear-shaped	7.3~7.0 x3.6~4.0	obovoid	4.0~5.1 x3.0~3.2	green
Tr-7	loose tuft	−	2.1~1.5	complicated dedroid	conical attenuated	7.5~12 x2.5~3.5	smooth surface elliipsoidal	3.0~4.8 x1.9~2.8	dark green
Tr-8	loose tuft	−	4.1~3.2	complicated dendroid	conical attenuated	7.5~12 x2.5~3.5	smooth surface elliipsoidal	3.0~4.8 x1.9~2.8	dark green
Tr-9	loose tuft	−	3.9~3.3	complicated dendroid	conical attenuated	7.5~12 x2.5~3.5	smooth surface elliipsoidal	3.0~4.8 x1.9~2.8	dark green
Tr-10	local tuft	+	3.2~3.4	short and thick	crowded pear-shaped	7.3~7.0 x3.6~4.0	obovoid	4.0~5.1 x3.0~3.2	green
Tr-11	local tuft	+	3.2~3.4	short and thick	crowded pear-shaped	7.3~7.0 x3.6~4.0	obovoid	4.0~5.1 x3.0~3.2	green
Tr-12	loose tuft	−	4.1~3.6	complicated dendroid	conical attenuated	7.5~12 x2.5~3.5	smooth surface elliipsoidal	3.0~4.8 x1.9~2.8	dark green
Tr-13	wide tuft	−	4.3~3.7	complicated dendroid	asymmetric bottle	6.0~7.0 x3.0~3.2	obovid smooth surface	3.1~3.0 x2.9~3.3	yellowish green
Tr-14	ring like	−	4.5~3.9	complicated dendroid	curled	11~12 x3.0~3.2	irregular whorlled	3.0~2.8 x3.3~3.5	green
Tr-15	ring like	−	4.1~4.9	complicated dendroid	curled	11~12 x3.0~3.2	irregular whorlled	3.0~2.8 x3.3~3.5	green

[a]sterile hyphae absent, [b]sterile hyphae elongated out of the conidiophore.

nst *R. solani* was in Table 3. Isolates Tr-5 and 8 were shown a strong inhibition ability toward *R. solani,* followed by strains *Trichoderma* Tr-4, 2 and 3. Whereas strains 13, 10 and 12 were showed a weak activity against *R. solani.*

One isoloates Tr-5 showing strong inhibition against *R. solani* selected to test an parasitism on the pathogens. The isolate Tr-5 showed a parasitic action. The isolate begin to coil the hypha of *R. solani* become to swelling in its hyphal tip and after that some of hypha become to break into segment and lysed (Fig. 1-A, C, D, E, F).

Sensitivity of propagules to soil fungistasis was measured in conditions of sterilized and natural soil. All the isolates of *Trichoderma* spp. germinated in 1.7-7.3% in natural soil, whereas the generation rate was high in steril soil conditions. When pea plants were grown in soil treated with *Trichoderma* spp. conidia. *Trichoderma* spp. were recovered from 1-3 cm of root depth, but the population

Table 3. Inhibition zones produced by *Trichoderma* spp. against *Rhizoctonia solani* grown on PD agar plate.

Isolates	Inhibition (mm)
TR- 1	17
TR- 2	35
TR- 3	30
TR- 4	35
TR- 5	40
TR- 7	35
TR- 8	40
TR- 9	29
TR-10	14
TR-11	17
TR-12	14
TR-13	12
TR-14	26
TR-15	27

Table 4. Conidial germination of the isolates of *Trichoderma* spp. incubated in natural and sterilized soils for 14 hours.

	Germination (%)	
Isolate	Natural soil	Sterilized soil
TR- 1	2.3	86.3
TR- 2	3.4	87.6
TR- 3	6.7	92.3
TR- 4	1.5	67.9
TR- 5	3.2	77.3
TR- 7	2.6	89.3
TR- 8	5.1	92.1
TR- 9	3.9	88.3
TR-10	4.7	79.3
TR-11	7.3	92.1
TR-12	7.3	86.9
TR-13	1.7	92.5
TR-14	4.5	87.3
TR-15	6.9	91.4

density significantly reduced as compared with upper layer of soil depth. Numbers of general fungi propagules were also distributed in 1-3 cm of root depth, but it's propagule density was decreased along the lower soil depth (Fig. 3).

Pathogenic organisms cause a root rot of ginseng disease development treated alone in pot, but the incidence rate are reduced in case of treatment with combination of antagonistic Tr-5 isolates (Fig. 4).

Discussion

The extensive literature that exists on the distribution of soil fungi shows clearly that species of *Trichoderma* are major element in the microflora especially in temperate forest soil. Their abundancy coupled with their know ability to produce a range of antibiotic (Dennis and Webster, 1971).

Widden and Abitbal (1980) found that species of *Trichoderma* isolated from spruce-forest soil and their overall abundance is mediated to a large extent by competition with other species rather than by the direct effect of abiotic factors, and also *T. hamatum* was populated in 0.22% frequency. In our test the *T. hamatum* was populated in 0.28% frequency comparing with other microorganisms (Table 1).

Despite some claims that species of the hyphomycetes genus Tr. Pers, ex F. cannot be easily distinguished from one another but reliable characters which can be used in classifying these fungi, for which, however, the "spiecis aggrigate" concept has to be adopted. Based on the types of branching system of the conidiophores, manner of phialides disposition and the characters of the phialospores it has been possible to recognize species aggregates (Rifai, 1969).

Isolated strains of *Trichoderma* spp. were identified by Rifai method (1969) the based on a cultural or morphological characteristics. Strains of *Trichoderma* Tr-1, 7, 8, 9 and 12 were identified as *T. koninggii,* strains of *Trichoderma* Tr-2 was *T. pseudokuninggii,* strain of *Trichoderma* Tr-3 and 13 were to *T. aureoviride,* strains of *Trichoderma* Tr-4, 5, 10 and 11 were was *T. hamatum* and strains of *Trichoderma* Tr-14 and 15 were *T. viride.*

The biocontrol ability of isolate *T. hamatum* strain Tr-5 was very effective when it is cocultured

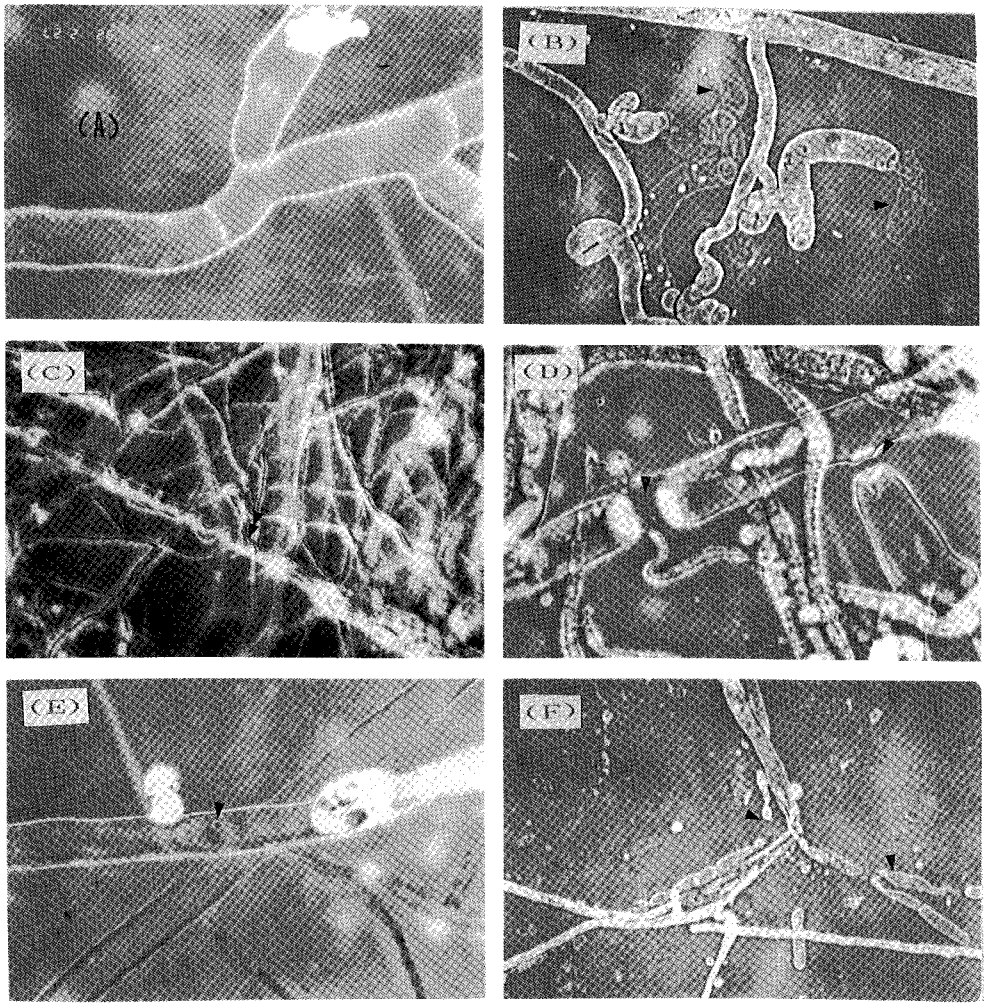


Fig. 1. Antagonistic reaction on *R. solani* by *Trichoderma hamatum* Tr-5 strain. A. Normal hyphal growth of *R. solani.* B. Abnormal growth of *R. solani* in its hyphal tip. C. Network of *T. hamatum* Tr-5 strain hyphae on out-side hyphal surface of *R. solani.* D. Cutting-off of *R. solani* hyphae by *T. hamatum* strain. E. Hyphal growth of *T. hamatum inside* hyphae of *R. solani.* F. Lytic mycelia of *R. solani by the antagonist.*

with *R. solani* and produce cellulase while *T. haziamem* has little cellulolytic ability. This may indicate parasitsm of *R. solani* by *T. hamatum* and also this antagonist may be effective against some soil borne disease of old plants such as roots rot (Harman *et al.*, 1980).

Biological control of soil borne plant pathogen by the addition of antagonistic microoganism to the soil is a potential nonchemical means for plant disease control. The species of *Trichoderma* capable of hyperparasitize pathogenic fungi are highly efficient antagonists (Barnet *et al.*, 1973; durrell, 1968). In this test, isolate of *T. hamatum* Tr-5 strain showed a ability to attack the fungal hyphae by a supercoiling and lysis and separation at later state of interaction.

Harman *et al.* (1980) proved that *T. hamatum* acts as a mycopaprasite on *Pythium* spp. and *R. solani* and this may be its principal mode of action. *T. hamatum* limitied density of *R. solani* but merely prevented large increase in the number of *Pythium* spp. *T. harzianum* was found to be

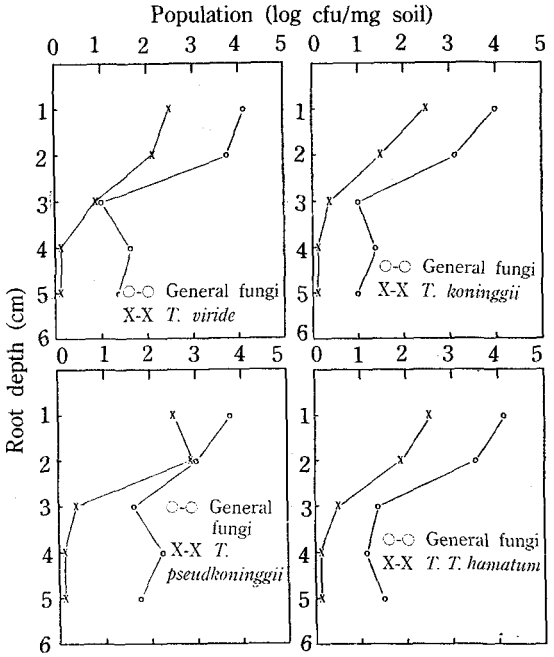
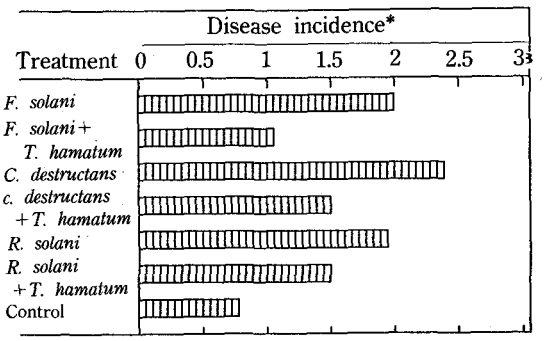


Fig. 2. Population densities of toal fungi and *Trichoderma* spp. in rhizosphere soil of pea root.



Regends: Root rot was rated on a scale 0~3.
0, healthy, no lesion; 1, partly yellowish brown lesion developed
around root; 2, reddish brown lesion girdling the root, and the lesion
under half of the root; 3, root severly rotted and decayed.

Fig. 3. Effect of the antagonist TR-5 isolate of *T. hamatum* on ginseng root-rot caused by several fungal pathogens 21 days incubated for pot test.

an effective bilogical control agent for protecting a crop plants from damage under green house and field condition. The fungus was capable of directly attacting and lysing the pathogens in culture. The ability of other *Trichoderma* spp. to attack different fungi was proved by Durrell (1968).

Spores of most fungal species don't germinate in natural soil except in the vicinity of undercomposed organic matter or in rhizosphere (Lockwood, 1964).

In relation to fungal spore nutrient, Ko and Lockwood (1967) observed that *T. viride* was not germinated well on natural soil and distilled water. Fungistasis is mainly caused by diffusible inhibitory substance in the soil, and also caused by the lack of substances required by the spores for germination (Lockwood, 1964; Lee and choi, 1982).

In our test spores of *T. hamatum* also showed a lower germination rate in natural soil, but the spores germinated high in sterile soil. It indicate that decreased spore germination was correlated with rapid diffusion of nutrient in soil by microbial nutrient sinks.

Various methods to test rhizosphere competance were primarily based on a comparison of the numbers of microoganisms in the soil associated with roots to population density in nonrhizosphere soil. The rhizosphere competence assays was developed to improve measurement in time and space of the activity of potential rhizosphere in habitants.

To test whether the biocontrol agents introduced from culture into rhizosphere could compete under the typical ecological conitions, raw soil was used. Therefore, the system allowed rhizosphere competence to be measured on the basis of population density of *Trichoderma* spp. as a function of root depth. Rhizosphere competence assay provides the best bioassay yet developed for the rhizosphere nutrient status at root tips (Rovira, 19 73).

Ahmad and Baker (1987) observed that no species of *Trichoderma* spp. grew to greater depth than 2 cm is as a test of rhizosphere competence and also Papavizas and Lewis (1981) proved that *Trichoderma* spp are not rhizosphere competence. Unlike the result of Ahmad and Baker (1987), the *Trichoderma* spp in our pot test colonized all sections of the rhizosphere to the root tips, When it was cultivated in green house condition around

23-27℃. but recovered numbers were significantly reduced greater depth than 3 cm (Fig. 3).

A possible expalanation for this colonization might be concern to more exudates produced at the higher temperature such as 26℃ than at 19℃, and also all the *Trichoderma* spp. was isolated from all segments the rhizosphere of pea root grown in soil (Rovira, 1973).

Liu and Baker (1980) observed that numbers of *Trichoderma* spp. propagules in the soil increased as surppressiveness increased, whereas inoculum density of *R. solani* was inversely proportional to the density of these *Trichoderma* spp. They found a fact that increase in population and association of Trichoderma with suppressiveness was concerned. Especially *Trichoderma* was isolated with high frequency from mycelial mats of *R. solani* incubated in suppressive soil. Conidia of *Trichoderma* added to conducive soil induced suppressiveness (Henis *et al.*, 1979).

The major problem of applying antagonists to soil, their inability to become established in the ecosystem, and to overcome the resistance of soil microflora to the introduction of new microorganism (Alexandr, 1971; Boosalis and Mankau, 1970). In recent years, several attempts have been made to overcome this obstacle by applying fungal biocontrol agents, grown on suitable food bases to soil (Bakmam and Rodriguez-Kabana, 1975).

It has been assumed that preventive measure to some pathogens are much concerned to enhencing increment of antagoist by suppling a food base and induce a fungistatic condition for these pathogens.

Acknowledgement

This research was supported by the Korea Science and Engineering Foundation in 1989-90 (Grant No. 901-1503-059-2).

적 요

길항균 *Trichoderma* spp.가 입고병 및 식물토양병 방제에 효과적이었으므로 *T. hamatum* strain Tr-5와 *Rhizoctonia solani* 사이의 토양근권내의 상호작용을 구명하고, 생물학적 방제로서의 활용성을 알고저 기초적인 실험을 하였다. 길항성이 우수한 *Trichoderma* spp.의 토양내 분포비를 구명하고저 채집한 토양시료에서 길항균과 토양 미생물의 분포를 조사하였다. 전세균, 전진균, 방선균 및 *Trichoderma* spp.의 분포비는 65%, 8.8%, 25.9% 및 0.28%로 세균이 많고 *Trichoderma* spp.의 분리비는 가장 적었다. *Trichoderma* spp.를 분리하여 동정한 결과 *T. koninggii, T. pseudokoninggii, T. aureoviride, T. hamatum* 및 *T. viride*의 5종이 동정되었고, 이중 균주 Tr-5인 *T. hamatum*을 공시하여 균기생성여부를 관찰한 결과 *R. solani*에 대한 기생성을 관찰하였으며 효소적으로 균사가 용균되어 파괴됨을 관찰하였다. *Trichoderma* spp.의 토양내 발아력을 조사한 결과 자연토양에서 *Trichoderma* spp. 모든 종은 1.7~7.3% 이내의 발아력을 나타내었고, 살균토양에서는 90% 이상의 포자발아력을 나타내어 자연토양에서 *Trichoderma* 정균현상이 관찰되었다. 길항력이 있는 *T. koninggii, T. pseudokoninggii, T. hamatum* 및 *T. viride* 등 4균주의 토양근권내 완두뿌리 근권점유율실험에서 4종 균주 모두는 토양깊이 1 cm 범위까지는 점유율이 크게 나타났으나 2~3 cm에서는 분포수가 감소하였고 4 cm 이하에서는 뿌리 점유분포수가 크게 감소하였다. 인삼근을 심은 폿트실험에서 *Fusarium solani, Cylindrocarpon destructans, Rhizoctonia solani*균의 단독처리구에 비해 길항력이 있는 *T. hamatum* Tr-5 처리구에서는 근부현상이 감소하였다.

References

Ahmad, J. S. and Baker, R. 1987. Rhizosphere competence of *Trichoderma hazianum*. Phytopathology 77: 182-189.

Alexander, M. 1971. Microbial ecology. pages 207-223. John wily and sons, New Tork and London.

Baker, K. F. and Cook, R. J. 1947. Biological control of plant pathogens. W. H. Freema, San Francisco, Ct 433pp.

Baker, R., Elad, Y. and Sneh, B. 1986. Physical, biological and host factors in iron competition in soil. In: Swin burne, T. R. (ed). Iron. Siderophores and plant disease. Plenum, New york. pp. 72-84.

Bakman, P. A. and Rodriguez-Kabana, R. 1975. A System for growth and delivery of biocontrol agents to the soil. Phytopathology **65**: 819-821.

Barnett, H. L. and Binder, F. L. 1973. The fungal

host parasite relationship. *Ann. Rev. phytopathol.* **11**: 273-292.

Boosalis, N. G. and Mankau, R. 1970. Parasitism and predation of soil microorganisms paper 374-389. in: Ecology of soil-borne plant pathogens. Baker, K. F. and Williams, C. S. eds. Univ. of California Press, Berkelely.

Cook, R. J. and Weller, D. M. 1987. Management of take-all in consective crops of wheat or barley. In Chet, I (ed.), innovative approaches to plant disease control. Wily and Sons. New York. pp. 41-76.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma* 1: production of non-volatile antibiotics. *Trans. Brit. Mycol. Sco.* **57**: 25-39.

Durrell, I. W. 1968. Hyphal invasion by *Trichoderma viride. Mycophathol et Mycol. Appl.* **35**: 138-144.

Elad, Y., Chet, I. and Henis, Y. 1980. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma hazianum.* Plant Soil **60**: 245-254.

Griffin, D. M. 1972. Ecology of Soil fungi. Terre Haute; Syracuse. 193 pp.

Harman, G. E., Chet, I. and R. Baker, 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp or *Rhizoctonia solani.* Phytopathology **70**: 1167-1172.

Henis, Y., Ghaffa, A. and Baker, R. 1979. Factors affecting suppressiveness to *Rhizoctonia solani* in soil. Phytopathology **69**: 1164-1169.

Hong, E. P. and Lee, M. W. 1986. A study on the selection of a medium for chlamydospores formation in Fusarium spp. Dongguk Univ. *J. Agr.* and *Forestry Science* **11**: 42-52.

Hsu, S. C. and Lockwood, J. L. 1973. Soil fungistasis; Behavier of nutrient independent spores and Sclerotia in a model system. Phytopathology **63**: 334-337.

Hsu, S. C. and Lockwood, J. L. 1975. Powdered chitin agar as a selective madium for enumeration of actinomycetes in water and soil. *Appl. Microbiology* **29** (3): 422-426.

Jee, H. J. and Kim, H. K. 1987. Isolation, identification and antagonism of rhizospheric antagonists to cucumber with pathogen, *Fusarium oxysporum* f. sp. *cucumerinum Owne. Kor. Plant Pathol.* **3**(3): 18-19.

Ko, W. H. and Lockwood, J. L. 1967. Soil fungistasis: relation to fungal spore nutrition. Phytopathology **6**: 894-901.

Lee, M. W. 1977. Studies on the root rot ginseng (VII) on pathogenic microbial population in continous culture and first time culture of ginseng. *Kor. Jor. Microbial.* **15**(1): 20-30.

Lee, M. W. 1984. Diease problems in ginseng (*Panax ginseng* Meyer) caused by soil-borne microorganisms in korea. FFTC Book science No **26**: 120-136.

Lee, M. W. and Choi, H. J. 1982. The nature of fungistasis in sterile and glucose-peptone amended soil on *Helminthosporium victoriae* and *Morthiorella n. sp. Kor. J. Mycol.* **10**(3): 119-124.

Liu, S. and Baker, R. 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani.* Phytopathology **70**: 404-412.

Lockwood, J. L. 1964. Soil fungistasis. *Annu. Rev. Phytopathol.* **2**: 341-362.

Martin, J. P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *soil Sci.* **69**: 215-232.

Papavizas, G. C. and Lewis, J. A. 1981. Introduction and augmentation of microbial antagonists for the control of soil-borne plant pathogens. in BARC. Symposium 5. G. C. Papavizas ed. Allanheld Osman. Totowa.

Rifai, M. A. 1969. A revision of the genus *Trichoderma.* Mycol. Papers 116. CMI. Kew.

Rovira, A. D. 1973. Zones of excudation along plant roots and spatial distribution of microorganisms in the rhizosphere. *Pestic. Sci.*

Scher, F. H. and Baker, R. 1982. A florescent microscopic technique for viewing fungi in soil and its appliation to studies of a *Fusarium* suppresive soil. *Soil Biol. Biochem.* **15**: 715-710.

Son, S. K. Shim, H. S. and Lee, M. W. 1985. Effects of amendment on ginseng root rot caused by *Fusarium solani* population changes of the microorganisms in soil. *Kor. J. Mycol.* **13**(1): 41-47.

Trosmo, A. and Dennis, C. 1978. Effect of temperature on antagonistic properties of *Trichoderma* species. *Trans. Br. mycol. Soc.* **71**(3): 469-474.

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. Phytophathology. **22**: 837-846.

Widden, P. and Abitbol, J. J. 1980. Seasonality of *Trichoderma* species in a sprace-forest soil. *Mycologia* **72**: 775-784.

Yuen, G. Y. and Schroth, M. N. 1986. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* by iron competition with an Alcaligenses sp. Phytopathology. **76**: 171-176.

Accepted for Publication on November 30, 1992