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The Application of Bioagents to Control *Rhizoctonia solani* and Other Soil-Borne Plant Pathogens

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ABSTRACT

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Besides *Bacillus cereus, Trichoderma pseudokoningii* and *T. viride* which are known antagonists to *Rhizoctonia solani, T. koningii* (isolate #98), *T. harzianum* (isolate #136), and *Penicillium* sp. (isolate #100) were isolated from composted swine manure (CSM) and identified to be antagonistic to *R. solani, Phytophthora capsici, Pythium aphanidermatum*, and *Pythium spinosum* on five different cultural media. *B. cereus* survived well in CSM when it was dried, grounded and stored at room temperature for nine months, but antagonistic *Trichoderma* spp. declined to about one percent of the initial amount, like powder-formulated *B. cereus* and *Trichoderma* spp. A suitable inert material in the powder formulation was kaolin which was selected from eight different inert materials. Although *B. cereus* and *Trichoderma* spp. survived well in alginate beads, the beads shrivelled and dried out after storage for 3 to 5 months at room temperature. The CSM-formulated antagonist was used for treatment of soil. The powder-formulated antagonist was used for treatment of seed and tissue. Soil treated with 0.1% *T. koningii* increased the survival rate of mungbean when the population of *R. solani* was 26 propagules/g soil in pot, but not when the population of *R. solani* was 32 propagules/g soil in the greenhouse and 54 propagules/g soil in the field. Although soil treated with *B. cereus* was unable to increase the survival rate of mungbean when *R. solani* was 28 propagules/g soil in the greenhouse, the yield of cultivar 1628 increased significantly when the population of *R. solani* was 54 propagules/g soil. Mungbean seed treated with *B. cereus* had a significantly greater rate of survival than the control in both the greenhouse and the field where *R. solani* were present. *B. cereus* controlled root knot and the disease complex caused by *Meloidogyne incognita* and *R. solani* on tomatoes in pots and chrysanthemum stem rot significantly in field. It also helped chrysanthemum cuttings to produce normal root systems even better than the effects of the combination of benomyl and 0.1% NAA. Soil treated with *T. koningii* or *T. harzianum* increased the survival rate of cucumber and muskmelon when the soil was infested with *P. capsici, P. spinosum* or *P. aphanidermatum*. whereas soil treated with *T. viride, T. pseudokoningii* or *B. cereus* increased the survival rate of tomatoes, hot and sweet pepper when soil was infested with *P. aphanidermatum*. Except for *B. cereus*, the effectiveness of seed treatment with *T. viride* or *T. pseudokoningii* to protect tomatoes, hot and sweet pepper from infection by *P. capsici* and *P. aphanidermatum* was in consistent. The potential of applying CSM-formulated bioagents to control soil-borne plant pathogens was worthy of exploration.

Key words: soil-borne plant pathogen, biological control, *Rhizoctonia solani, Phytophthora capsici, Pythium aphanidermatum, Pythium spinosum*, mungbean, chrysanthemum, cucumber, muskmelon, tomatoes, peppers, composted swine manure, soil and seed treatment.

INTRODUCTION

Rhizoctonia solani kühn is an important plant pathogen distributed world-wide (19,33). It has a wild host range and causes losses of many economic crops. This pathogen survives in soil effectively and is difficult to be eliminated. Chemical control is commonest method used to control this pathogen (28,33). Because of limitations of use of fungicides, other control methods have to be developed. Physical methods, *e.g.* heat treatment, and cultural method, *e.g.* using resistant variety, are not used commonly and soundly to control *R. solani*. However, the existence of high potential to apply natural microorganisms successfully to control this pathogen have

been demonstrated (6,21,31,34). Biocontrol has many advantages compared to use of fungicides (7). Based upon the standpoints of environmental protection, endurance of controlling measures, food safety, time and cost of developing new chemicals, etc., biocontrol is regarded as a major replacement of chemical control, especially in developed countries in coming decades. Although the population of *R. solani* in the field fluctuates (26), it exists widely in Taiwan and causes many diseases of diverse crops, *e.g.* rice, sorghum, corn, vegetables, flowers and turf (3,20,24,27,29,30,32). *R. solani* is able to infect a plant with other pathogens simultaneously or consecutively. For examples, lily root rot is caused by *Fusarium oxysporum* Schlechtend, *Pythium spinosum* Sawada apud Sawada and Chen and *R. solani* (3). Tomato roots are infected by *Meloidogyne incognita* (Kofoid and White) Chitw. and *R. solani* (22). Other important soilborne plant pathogens, *e.g. Pythium aphanidermatum* (Edson) Fitzp., *Pythium spinosum* (10) and *Phytophthora capsici* Leonian (2,12,15), well known in Taiwan, may infect crops with *R. solani* in the same field.

Each of *Bacillus cereus* Frankland and Frankland, *Trichoderma harzianum* Riai, *T. pseudokoningii* Rifai, and *T. viride* Pers. ex S. F. Gray is antagonistic to *R. solani* and able to control chrysanthemum stem rot (34), pea (6) and soybean (31) seedling damping-off, and potato black scurf (4). Although these antagonists have been tried on several of these crops, the availability of these antagonists to control *R. solani* from other, different crops and other important soilborne plant pathogens need to be evaluated. When one applies these antagonists, suitable formulation of these antagonists also needs to be developed. Beyond these antagonists, other effective antagonists needed to be isolated and screened because each different microorganism had an ecological niche in which it could achieve its best ability to control of the studied pathogens if they were present in the same field. As *R. solani* exists commonly with other plant pathogens in soil, the effectiveness of a bioagent to control the disease complex is worthy of investigation.

MATERIALS AND METHODS

Isolation and screening of antagonists

Composted swine manure (CSM) was collected from Department of Animal Science of National Taiwan University and from Tao-Yuan County (桃園縣). Various microorganism were isolated by means of the dilution-plate method on nutrient agar, potato dextrose agar and glucose-asparagine agar. An agar disc of *R. solani* used as a bait was buried in CSM and incubated. The lysed hyphae of *R. solani* were picked up under a microscope and transferred to a potato dextrose agar plate or to a series of sterilized distilled water for purification.

Isolated microorganisms were dual cultured with *R. solani, P. capsici, P. aphanidermatum* or *P. spinosum* on glucose-asparagine agar, King's B medium, nutrient agar, potato dextrose agar and soil extract agar. The microorganisms were selected when they showed the ability to inhibit or overgrow the growing pathogens under test. The selected microorganisms were again concomitantly cultured with these pathogens at the center of agar plates to test their ability to compete with or to inhibit the tested pathogens on those five different media. Each test had five replicated.

Tested antagonists and plant pathogens

Besides the isolated and selected antagonists, *B. cereus, T. pseudokoningii* and *T. viride*, previously as identified antagonists to *R. solani*, were used in this work. *R. solani* was isolated from diseased chrysanthemum cuttings and mungbean seedlings which we collected from Yuan-Lin (員林) and Shan-Hwa (善化), respectively. *R. solani* was identified to be AG-4.

All selected antagonists and *R. solani* were cultured in potato dextrose agar slants and stored at 5 C before testing. *R. solani* was cultured in potato-sand medium (50 g chopped potato and 500 ml sand) and used as inoculum when bioassay was carried out. *P. capsici* was isolated from diseased pepper. *P. aphanidermatum* and *P. spinosum* were isolated from diseased bean seedlings and lily bulbs, respectively. These fungi were cultured on V-8 agar for 7 days at 28 C. Zoospores of these two fungi were released after the mycelial mat was placed at 5 C for 30 min and removed to room temperature afterwards. However, *P. aphanidermatum* grew much faster than *P. capsici*. Zoospores were collected and used to infest the field soil when bioassay was needed, but sporangia were used for *P. spinosum*.

Production and formulation of bioagents

T. harzianum, T. koningii and *Penicillium* sp. isolated from CSM and *B. cereus, T. pseudokoningii*, and *T. viride* were pure cultured in sterilized CSM at 24 C for one month during which the CSM was completely colonized by the cultured antagonists. Then these CSM was air-dried, ground, and sieved through 10-mesh sieve. It was stored in glass jars at room temperature and 5 C and used for soil treatment. The survival of *B. cereus, T. pseudokoningii, T. viride* was tested each month for nine months.

B. cereus was liguid fermented in potato dextrose broth and *T. pseudokoningii, T. viride* were liguid fermented in the medium formulated by Papavizas *et al.* (21), respectively. The biomass of these antagonists was collected by centrifugation and dried by lyophilization. Bagasse, kaolin, bentonite, volcanic ash, chitin, talc, attapulgite or potato starch used as inert meterial was mixed with these harvested antagonists. They were stored at under room temperature. The suitable inert material was determined by measurement of the viability of the

antagonist which was mixed with one of these inert material each month and this test endured nine months. Alginate bead was prepared by following the procedure developed by Fravel *et al.* (15), but 1% skim milk or 1% V-8 juice was added in this study. To delay the drying of these beads, 1 or 5% glycerin was supplemented in the bead. The viability of antagonist was determined by a count of the colony-forming units (cfu) on a specific cultural medium when beads were ground, diluted and plated out on agar plate. Beads were stored at both room temperature and 5 C. The cultural medium used to determine *B. cereus* was nutrient agar supplemented with 100 ppm streptomycin as a streptomycin resistant strain of *B. cereus* was used in this test. A *Trichoderma* selective medium (19) was used for determining the survival of *Trichoderma* spp.

Bioassay in the greenhouse

0.1 or 1% (v/v) of CSM cultured with either *T. koningii, T. harzianum* or *Penicillium* spp. (isolates #100 & 144) was mixed with field soil which was filled in a pot (6.4 cm × 5.8 cm, diam × height). One part of inoculum of *R. solani* was mixed with 9 parts of soil. When *P. capsici, P. aphanidermatum* and *P. spinosum* were tested, zoospores or sporangia were added to the soil. Mungbean, cucumber or muskmelon were sown in the pot when the CSM was tested. The survival of seedlings and disease severity were counted. Seeds of these tested plants were also treated with powder-formulated antagonists and sown in the pathogen-infested soil.

Whereas other than cucumber and mungbean, radish, zinnia, cosmos, and strawflower were the tested plants to evaluate the effectiveness of 0.1 or 1% CSM cultured with *B. cereus, T. pseudokoningii*, or *T. viride* to control *R. solani*, tomatoes, hot and sweet peppers were used for analysis of the efficiency of the powder-formulated bioagent to control *P. capsici* or *P. aphanidermatum*. Ten seeds were sown in each pot. Each treatment consisted of five replicates and was repeated once. The survival of seedlings was counted and analyzed statistically.

Bioassay of biocontrol of disease complex in greenhouse

M. incognita was isolated from Yang-Ming Mt. (陽明山) and cultured on tomato root. *Paecilomyces lilacinus* (Thom.) Samson was supplied by *C. Netscher* and cultured in chopped potato-sand (potato : sand = 1:1 (v/v)).

Sand and field soil (1:1 (v/v)) were mixed and filled in each pot which contained 1.1 L of soil. Three tomato seedlings were planted in each pot. Two hundred larvae of *M. incognita* (Mj) harvested from hatched eggs were added to each seedling. Four hundred eggs of the same nematode (Me) were also used as inoculum to place at the site of the tomato seedling. The treatments were soil only, *R. solani* only, Mj only, Me only, Mj plus *R. solani*, Mj plus *P. lilacinus*, Me plus *P. lilacinus*, Mj plus *B. cereus* (5 ml of *B. cereus*, 3.24 × 10 cfu/ml), *R. solani* plus *B. cereus*, Mj puls *B. solani* and *B. cereus*, etc. Each treatment consisted of four replicates and repeated once. After being planted for three weeks, fresh weight of root and stem, root knot index and survival rate were measured and analyzed statistically.

Bioassay in the field

A field plot at Asian Vegetable Research and Development Center (AVRDC) was selected to plant mungbean. The population of *R. solani* in soil was determined when taking records. Two cultivars, *i.e.* 1628 and 3890, of mungbean were supplied by AVRDC. There were nine treatments when *B. cereus, T. pseudokoningii* and *T. viride* were used. They were divided into seed and soil treatments. Seed treated with monceren (M), *B. cereus* (BC), BC plus M, *T. pseudokoningii* (Tp) or *T. viride* (Tv) at the rate of 190 g bioagent per 100 kg seeds. 330 g of CSM-formulated BC, Tp or Tv was spread along each seed bed as soil treatment. No treatment was regarded as control.

T. koningii cultured in CSM was treated in the soil as previously described. This treatment was compared with soil treated with monceren and control.

The seed bed of each treatment was 2 m long and 1 m wide and consisted of 120 seeds of each cultivar. Each treatment had four replicates. Randomized complete block dasign was applied in this experiment. The survival rate was counted 15 and 40 days after planting. The yield was harvested 85 days after planting.

Chrysanthemum cuttings (Yellow Queen) was not treated, with powder-formulated BC or with monceren for the first field trial and planted in the bed which was naturally infested with *R. solani* (AG 4) for years. Two cultivars (Yellow Queen and White General) were not treated or treated with powder-formulated BC, BC plus 0.1% naphthylacetic acid (*NAA*), BC plus 5% monceren, or benomyl plus 0.1% NAA and planted in the same bed for the second field trial. Each treatment consisted of four replicates and each replicated consisted of 250 cuttings for the first trial, and 1000 cuttings for the second trial in 1991. Disease index and length of roots were recorded after 14 days of planting. Disease index was classified as 0, healthy seedling; 1, few lesion on leaves or stem; 2, lower leaves rotted and brown lesion on stem; 3, only top 2–3 leaves remained alived; 4, seedling decayed and dead.

RESULTS

Isolation and screening of antagonists

One hundred fifty seven different isolates of microorganisms were isolated from CSM on three different media. Among them, 92 were bacteria, 58 were fungi and 7 were actinomycetes. After evaluation by

means of dual and concomitant cultures with tested pathogens. *T. koningii* (isolate #98), *T. harzianum* (isolate #136), and *Penicillium* sp. (isolate #100) were able to inhibit the growth of all tested pathogens on five different media. Another species of *Penicillium* sp. (isolate #144) unable to inhibit the growth of tested pathogens was used as control for further study. Isolates of 98, 136 and 100 were all able to twist around the hyphae of tested pathogens or overgrow the colony of tested pathogens when they were dual cultured. Isolates 98, 136 and 100 were able to inhibit completely the growth of tested pathogens when they were concomitantly cultured.

Production and formulation of bioagents

Among eight different inert materials, *B. cereus* survived steadily in kaolin, talc or chitin. The population of *B. cereus* declined slightly during the first month of storage and remained constant (2.9×10^9 cfu/g) during the remaining eight months of storage. Bagasse was the worst inert material as the population of *B. cereus* decreased gradually form 3.9×10^9 cfu/g to 1.0×10^6 cfu/g. If 0.1% potato dextrose broth was added in kaolin as a supplement, this combination kept the population of *B. cereus* as high as the initial amount for nine months. The population of *Trichoderma* spp. declined to about one percent when it was mixed with kaolin after storage for seven months. The initial amount of *T. viride* and *T. pseudokoningii* were 7.6×10^7 and 4.3×10^7 propagules/g, respectively, and declined to 8.1×10^5 and 5.5×10^5 propagules/g. A similar situation occurred when *B. cereus*, *T. viride* and *T. pseudokoningii* were formulated with CSM.

B. cereus in alginate beads survived well when these beads were stored at 5 C compared to room temperature. The addition of 5% glycerin and nutrients (*i.e.* 1% skim milk or V-8 juice) in the bead was slightly helpful for the survival of *B. cereus*. Contrarily, *Trichoderma* spp. in alginate beads survived better when they were stored at room temperature than at 5 C. The addition of 1% glycerin and nutrients (1% skim milk or V-8 juice) increased survival of *Trichoderma* spp. compared to supplement with 1% glycerin alone. Addition of glycerin was better than plain bead for the survival of tested *Trichoderma* spp. The population of *Trichoderma* spp. increased to 10^6–10^7 propagules/10 beads from 5.0×10^4 and 3.0×10^5 propagules/10 beads of *T. viride* and *T. pseudokoningii*, respectively, in the period of five months of storage at room temperature. However, beads started to shrivel after three months of storage and lost their original appearance after five months of storage at room temperature. Although the appearance of alginate bead did not change dramatically, the beads were contaminated commonly with other microorganisms under storage at 5 C.

Bioassay in greenhouse

After treatment with 0.1 or 1.0% (v/v) of either antagonist colonized in CSM in soil which was infested by *R. solani*, *P. capsici*, *P. aphanidermatum*, or *P. spinosum*, the tested crops survived significantly ($P = 0.05$) better than the pathogen-infested control (Table 1). The only exception was 0.1% *T. koningii* or *Penicillium* sp. (#100) in soil and seed treated with *Penicillium* sp. (#100) which were unable to increase significantly the survival rate of cucumber when it was sown in *R. solani*-infested soil. Although *Penicillium* sp. (#144) was not lethal to these tested pathogens, it increased significantly ($P = 0.05$) the survival rate of tested plants. The effectivenes of soil and seed treatment did not differ significantly or consistently. With one exception, 0.1%-antagonist colonized CSM in soil was enough to control these soil-borne pathogens.

When *T. viride*, *T. pseudokoningii* and *B. cereus* were applied to soil, they increased the survival of tested crops, except mungbean and zinnia when they were sown soil in which *R. solani* was present at 28 and 34 propagules/g soil, respectively (Table 2). Among the three antagonists, *B. cereus* was effective to increase the survival rate of radish and cosmos. *B. cereus* was also effective to control *P. capsici* and *P. aphanidermatum* and to increase the survival rate of tomatoes, hot and sweet peppers (Table 3).

B. cereus had the ability to control the three tested pathogens when it was treated on the seed of tested crops (Table 4). The effectiveness of *B. cereus* to control *R. solani* and *P. aphanidermatum* was as good as monceren and Aliette, except when it was treated on cucumber and zinnia seeds. Generally, *Trichoderma* spp. were ineffective as seedtreated bioagents.

Bioassay of biocontrol of disease complex in greenhouse

The presence of *M. incognita* enhanced the disease severity caused by *R. solani* (Table 5). *P. lilacinus* and *B. cereus* were able to reduce the disease severity caused by larvae of *M. incognita* as the fresh weight of tomatoes was increased and the root knot index decreased significantly ($P = 0.05$). *B. cereus* decreased the root knot index and increased the survival rate significantly when larvae of *M. incognita* and *R. solani* existed in the soil concurrently.

Bioassay in the field

When the population of *R. solani* in soil was as great as 54 propagules/g soil, only mungbean (*cv.* 1628) seed treated with *B. cereus* emerged significantly ($P = 0.05$) more than the control (Table 6). 85 days of after sowing, mungbean (*cv.* 3890) seed treated with *B. cereus* plus monceren yielded significantly ($P = 0.05$) more than the control (Table 7). Although mungbean (*cv.* 1628) grown in soil treated with *B. cereus* or *T. pseudokoningii* yielded

TABLE 1. Effectiveness of soil treated with composted swine manure containing different bioagents to protect different crops from different soil-borne pathogens

Crop	Pathogen	Propagules/g soil	% Survival[1]													
			CK[2]	P[3]	*Trichoderma koningii*			*Trichoderma harzianum*			*Penicillium* sp. (#100)			*Pencillium* sp. (#144)		
					0.1[4]	1.0[4]	S[5]	0.1	1.0	S	0.1	1.0	S	0.1	1.0	S
Mungbean	*Rhizoctonia solani*	26	72 abc[6]	0 d	72 abc	96 a	88 ab	80 abc	96 a	92 a	64 bc	60 c	80 abc	80 abc	96 a	92 a
Cucumber	*Rhizoctonia solani*	32	84 ab	4 e	8 e	92 a	56 bc	—	—	—	24 de	56 cd	32 cde	—	—	—
Muskmelon	*Rhizoctonia solani*	22	68 b	0 e	64 b	72 b	76 ab	68 b	68 b	100 a	36 cd	72 b	72 b	32 d	60 bc	56 bcd
Cucumber	*Phytophthora capsici*	2×10^3	100 a	32 d	100 a	100 a	100 a	68 c	100 a	100 a	100 a	100 a	92 ab	68 c	100 a	92 ab
Muskmelon	*Phytophthora capsici*	2×10^3	92 a	16 d	60 c	96 a	100 a	84 ab	100 a	96 a	84 ab	96 a	96 a	68 bc	88 a	96 a
Cucumber	*Pythium spinosum*	2×10^3	100 a	36 c	88 a	100 a	100 a	88 a	96 a	96 a	68 b	92 a	88 a	88 a	96 a	96 a
Muskmelon	*Pythium spinosum*	2×10^3	96 a	12 c	68 b	96 a	100 a	92 a	96 a	100 a	24 c	92 a	96 a	88 a	92 a	96 a
Cucumber	*Pythium aphanidermatum*	2×10^3	80 ab	16 c	80 ab	92 ab	96 ab	96 ab	100 a	84 ab	68 b	76 ab	92 ab	76 ab	80 ab	84 ab
Muskmelon	*Pythium aphanidermatum*	2×10^3	96 a	32 b	88 a	88 a	92 a	92 a	88 a	84 a	88 a	88 a	88 a	84 a	80 a	88 a

1. Survival of 100 tested plants was counted after 14 days of sowing.
2. Natural soil only, without pathogen or antagonist.
3. Pathogen was infested in the soil but without antagonist.
4. Soil was amended with 0.1 or 1.0% (v/v) antagonist-colonizing composted swine manure.
5. Seed treated with powder-formulated antagonists at the rate of 1.88 g/1 kg seeds.
6. Data, followed by the same letter in the same lane, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

TABLE 2. Effectiveness of soil treated with composted swine manure containing different bioagents to control *Rhizoctonia solani*

Crop	Propagules/g soil	Days after sowing	% Survival[1]							
			CK[2]	P[3]	Tv[4]		Tp[4]		Bc[4]	
					0.1	1.0	0.1	1.0	0.1	1.0
Cucmber	45	10	100 a[5]	24 c	72 b	76 ab	76 ab	68 b	64 b	68 b
	45	15	100 a	0 c	60 b	64 b	68 b	56 b	60 b	64 b
	32	10	100 a	0 e	40 d	44 d	60 cd	64 bcd	76 abc	88 ab
Radish	42	10	100 a	12 d	64 bc	48 c	68 bc	80 ab	84 ab	84 ab
	28	10	100 a	36 d	68 bc	68 bc	60 c	64 c	92 a	88 ab
Mungbean	28	10	78 a	0 c	2 c	0 c	2 c	0 c	8 c	8 c
	10	10	78 a	12 d	54 abc	50 bc	34 cd	68 ab	68 ab	66 ab
Zinnia	30	10	96 a	0 d	52 bc	48 c	64 bc	52 bc	52 bc	80 ab
	34	10	34 a	0 d	2 d	12 c	0 d	14 c	26 b	26 b
Cosmos	25	14	100 a	8 d	44 c	44 c	48 c	48 c	76 b	72 b
	25	20	100 a	0 d	36 c	40 c	40 c	44 c	72 b	72 b
Strawflower	12	10	100 a	56 c	96 ab	100 a	80 b	84 ab	96 ab	92 ab

1. Survival of 100 tested plants was counted.
2. Natural soil only without pathogen or antagonist.
3. Pathogen was infested in the soil but without antagonist.
4. Soil was amended with 0.1 or 1.0% (v/v) antagonist-colonizing composted swine manure. Tv, *Trichoderma viride*; Tp, *T. pseudokoningii*; Bc, *Bacillus cereus.*
5. Data, followed by the same letter in the same lane, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

TABLE 3. Effectiveness of soil treated with antagonist-colonized composted swine manure to control *Phytophthora capsici* and *Pythium aphanidermatum*

Crop	Pathogen	Propagules/g soil	Days after sowing	% Survival[1]							
				CK[2]	P[3]	Tv[4]		Tp[4]		Bc[4]	
						0.1	1.0	0.1	1.0	0.1	1.0
Tomatoes	*Phytophthora capsici*	3.0×10^6	13	80 a[5]	20 b	—	68 a	—	56 ab	—	48 ab
Sweet pepper	*Phytophthora capsici*	1.2×10^6	5	100 a	4 c	—	40 b	—	8 c	—	44 b
		3.0×10^5	5	100 a	0 d	8 cd	20 c	4 cd	12 cd	64 b	60 b
Hot pepper	*Phytophthora capsici*	3.6×10^5	5	100 a	0 c	4 c	12 c	16 c	16 c	68 b	76 b
Tomatoes	*Pythium aphanidermatum*	4.1×10^4	10	100 a	8 d	34 c	28 c	28 c	30 c	52 b	52 b
Sweet pepper	*Pythium aphanidermatum*	4.1×10^4	10	66 a	0 c	38 b	46 b	46 b	50 ab	40 b	54 ab
Hot pepper	*Pythium aphanidermatum*	4.1×10^4	10	78 a	2 c	34 b	42 b	36 b	44 b	52 b	54 b

1. Survival of 100 tested plants was counted.
2. Natural soil only, without pathogen or antagonist.
3. Pathogen was infested in the soil but without antagonist.
4. Tv, *Trichoderma viride*; Tp, *Trichoderma pseudokoningii*; Bc, *Bacillus cereus.* Soil treated with 0.1 or 1.0% (v/v) antagonist-colonizing composted swine manure.
5. Data, followed by the same letter in the same lane, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

TABLE 4. Effectiveness of seed treated with bioagents to control three soil-borne pathogens

Crop	Pathogen	Propagules/g soil	Days after sowing	% Survival[1]						
				CK[2]	P[3]	Tv[4]	Tp[4]	Bc[4]	Bc[5]+C	C[5]
Cucumber	*Rhizoctonia solani*	10	12	82 a[7]	0 d	48 b	28 c	52 b	84 a	82 a
Radish	*Rhizoctonia solani*	10	11	86 a	24 c	78 a	64 b	80 a	80 a	84 a
Mungbean	*Rhizoctonia solani*	28	10	78 a	0 c	—	—	46 b	8 c	54 b
	Rhizoctonia solani	10	10	78 a	12 b	—	—	74 a	90 a	82 a
Zinnia	*Rhizoctonia solani*	10	10	82 a	0 e	12 d	18 cd	26 c	58 ab	52 b
Tomatoes	*Phytophthora capsici*	2.5×10^4	10	92 a	0 c	10 c	42 b	48 b	—	—
Sweet pepper	*Phytophthora capsici*	2.5×10^4	10	34 a	0 b	22 ab	8 b	36 a	—	—
Hot pepper	*Phytophthora capsici*	2.5×10^4	10	66 a	0 c	20 b	28 b	34 b	—	—
Tomatoes	*Pythium aphanidermatum*	4.1×10^4	10	88 a	2 d	26 cd	28 cd	48 bc	66 ab	40 bc
Sweet pepper	*Pythium aphanidermatum*	4.1×10^4	10	64 a	4 c	16 bc	44 ab	54 a	52 a	60 a
Hot pepper	*Pythium aphanidermatum*	4.1×10^4	10	28 bc	0 c	38 ab	28 bc	48 ab	58 a	56 a

1. Survival of 100 tested plants was counted.
2. Natural soil only, without pathogen or antagonist.
3. Pathogen was infested in the soil but without antagonist.
4. Tv, *Trichoderma viride*; Tp, *Trichoderma pseudokoningii*; Bc, *Bacillus cereus*. Soil treated with 0.1 or 1.0% (v/v) antagonist-colonizing composted swine manure.
5. When *R. solani* was tested, C was monceren (25% WP, Bayer). When *P. aphanidermatum* present, C was Aliette (80% WP, Rhone-Poulenc).
6. The rate of formmulated bioagent to treat seed was 1.88 g/1 kg seeds.
7. Data, followed by the same letter in the same lane, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

TABLE 5. Effectiveness of biocontrol of *Meloidogyne incognita* and *Rhizoctonia solani* on tomato plants

Treatment[1]	Fresh weight (g/pot)		Index of[2] root knot (0–10)	% Survival[3]
	Stem & leaves	roots		
CK	2.36 a[4]	0.45 a	0 d	100 a
R	1.79 bc	0.46 a	0 d	92 ab
Mi	0.85 e	0.19 d	7.3 a	92 ab
Me	0.82 e	0.34 abc	6.8 b	100 a
Mi + R	1.07 de	0.21 cd	7.2 a	67 c
Mi + Pa	2.17 ab	0.38 ab	4.4 c	100 a
Me + Pa	0.79 e	0.34 abc	7.2 ab	100 a
Pa	1.64 c	0.20 cd	0 d	100 a
Mi + B	1.82 bc	0.29 bcd	4.4 c	100 a
R + B	2.13 ab	0.41 ab	0 d	100 a
Mi + R + B	1.43 cd	0.22 cd	4.1 c	83 b
B	2.14 ab	0.39 ab	0 d	100 a

1. CK, no pathogens or antagonists in soil; R, *R. solani* (110 g/pot); Mi, larvae of *M. incognita* (200 larvae/plant); Me, eggs of *M. incognita* (400 eggs/plant); P, *Paecilomyces lilacinus* (50 g/pot); B, *Bacillus cereus* (3.24×10^9 cfu/ml, 5 ml/pot).
2. Index of root knot was based on the description of Bridge & Page's chart. 0, no root knot; 1, few and small root knot; .....; 10, severe root knot, root system absent, and plant usually dead.
3. Twenty four seedlings of each treatment were tested.
4. Data, followed by the same letter in the same column, were not significantly ($P = 0.05$) different based on Duncan's multiple range test.

TABLE 6. Effectiveness of biocontrol on the survival of mungbean in *Rhizoctonia solani*-infested field

Cultivar of Mungbean	Propagules/g soil	Days after sowing	% Survival[1]								
			CK[2]	Seed treatment[3]					Soil treatment[3]		
				M	Bc+M	Bc	Tv	Tp	Bc	Tv	Tp
1628	18	15	62.3 ab[4]	72.3 ab	65.5 ab	81.0 a	58.3 b	66.5 ab	56.0 b	59.3 b	59.0 b
	54	40	38.0 b	50.8 ab	50.5 ab	64.0 a	40.5 b	40.3 b	45.8 ab	45.0 ab	37.0 b
3890	18	15	68.3 a	80.2 a	83.8 a	78.5 a	76.3 a	74.0 a	74.5 a	69.0 a	65.3 a
	54	40	47.3 a	67.3 a	66.0 a	55.5 a	55.8 a	56.3 a	61.0 a	50.3 a	56.3 a

1. Survival from 480 seeds.
2. No control measure applying to the field.
3. Seed treated with specific material at the rate of 1.88 g chemical or powder-formulated bioagent/1 kg seed. Soil treatment was applying CSM-formulated bioagent to seed furrow at the rate of 300 g/plot. M, monceren (25% WP, Bayer); Bc + M, combination of 1/2 amount of Bc and monceren; Bc, *Bacillus cereus*; Tv, *Trichoderma viride*; Tp, *Trichoderma pseudokoningii*.
4. Data, followed by the same letter in the same lane, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

TABLE 7. Effectiveness of biocontrol on the yield of mungbean in *Rhizoctonia solani*-infested field

Cultivar of Mungbean	Yield (g)[1]								
	CK[2]	Seed treatment[3]					Soil treatment[3]		
		M	Bc+M	Bc	Tv	Tp	Bc	Tv	Tp
1628	25.1 b[4]	43.2 ab	47.8 ab	45.1 ab	39.3 ab	38.1 ab	52.7 a	48.7 ab	61.0 a
3890	39.7 b	74.7 ab	82.9 a	66.3 ab	68.5 ab	55.4 ab	64.0 ab	69.4 ab	68.4 ab

1. Average yield of one replicate. Each replicate consisted of 120 seeds for sowing.
2. No control measure applying to the field.
3. Seed treated with specific material at the rate of 1.88 g chemical or power-formulated bioagent/1 kg seed. Soil treatment was applying CSM-formulated bioagent to seed furrow at the rate of 330 g/plot. M, monceren (25% WP, Bayer); Bc + M, combination of 1/2 amount of Bc and monceren; Bc, *Bacillus cereus*; Tv, *Trichoderma viride*; Tp, *Trichoderma pseudokoningii*.
4. Data, followed by the same letter in the same lane, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

significantly ($P = 0.05$) more than control (Table 7), soil treated with *T. koningii* or monceren was unable to increase the yield of mungbean.

B. cereus promoted root formation like the mixture of benomyl and 0.1% NAA and decreased the disease index significantly ($P = 0.05$) for the first trial (Table 8). *B. cereus* alone promoted root formation and development of two tested cultivars of chrysanthemum (Table 9). Chrysanthemum (*cv.* White General) had significantly ($P = 0.05$) longer roots when the cuttings were treated with *B. cereus* compared to treatment with benomyl plus 0.1% NAA. When seedlings were transplanted in the field, chrysanthemum (*cv.* White General) treated with *B. cereus* plus 0.1% NAA survived significantly ($P = 0.05$) better than that treated with benomyl plus 0.1% NAA.

DISCUSSION

T. koningii (isolate #98), *T. harzianum* (isolate #136) and *Penicillium* sp. (isolate #100) were isolated from CSM and identified to be antagonistic to *R. solani, P. capsici, P. aphanidermatum,* and *P. spinosum* on five different cultural media. Several microorganisms isolated from CSM were antagonistic to *R. solani.* However, most were unable to inhibit the growth of all four soilborne pathogens. The antagonists specific to inhibit *Pythium* spp. were especially few as the growth rate of *Pythium* spp. was rapid on these five tested media. The five different media represented different levels of nutrient. When isolated microorganisms were able to inhibit the growth of tested pathogens on these media, they had more potential to become successful antagonists to "work" in the soil.

TABLE 8. Effectiveness of *Bacillus cereus* to control chrysanthemum stem rot in field[1]

Treatment[2]	Length of roots (cm)	Disease[3] index
CK	2.02 b[4]	55.4 a
B. cereus	3.54 a	27.0 b
Benomyl + 0.1% NAA	3.05 ab	34.1 b

1. The population of *Rhizoctonia solani* in sand bed was 5 propagules/g soil. 1000 cuttings were used for each treatment.
2. CK, chrysanthemum cuttings were treated nothing; Bc and benomyl + 0.1% NAA, cuttings were treated with powder-formulated *B. cereus* or benomyl + 0.1% NAA.
3. Disease was rated as 0, healthy; 1, one lesion on stem or leaves; .....; 4, damping-off or dead. Disease index was calculated as (disease rate × no. of plant in specific related disease catagory/4 × total tested cuttings) × 100%.
4. Data, followed by the same letter in the same column, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.

Similarly, efficient strain of *Pseudomonas putida* (Trevisan) Migula and *Alcaligenes* sp. were isolated based on their behaviour on several different media (16,36).

CSM is a cheap soil organic additive in Taiwan. To increase the efficiency, CSM was used to culture these three selected antagonists. *T. pseudokoningii, T. viride,* and *B. cereus* were also cultured in CSM. They were previously identified to be antagonistic to *R. solani* (4,14,34) and kept in this laboratory. *T. pseudokoningii, T. viride* and *B. cereus* were selected to test the suitability of CSM as a substrate to maintain their population. *Trichoderma* spp. declined to one precent of the original amount. However, *B. cereus* remained about the same for nine months. This behavior observed was true when they were formulated as powder and mixed with kaolin. The probable reason is that *Trichoderma* spp. were more senstive to desiccation than *B. cereus.* Although *Trichoderma* spp. produced chlamydospores, their chlamydospores may not be as durable as the endospores of *B. cereus* for storage. The population of *Trichoderma* spp. in alginate beads increased within five months. However alginate beads were subject to dryness and unable to be stored for more than six months at room temperature. For this reason alginate bead-formulated antagonists were not bioassayed in this study.

With few exceptions, soil or seed treated with *T. koningii, T. harzianum,* and *Penicillium* sp. (isolate #100) increased significantly the survival rate of cucumber and muskmelon when *R. solani, P. capsici, P. aphanidermatum* or *P. spinosum* existed in soil, and mungbean when *R. solani* was present. These encouraging results indicated that these bioagents were efficient antagonists having a wide spectrum which was also expressed on cultural media. Again with few exceptions, soil treated with *T. pseudokoningii, T. viride* and *B. cereus* protected cucumber, radish, mungbean, zinnia, cosmos and strawflower from infection by *R. solani. B. cereus* also increased the survival rate of sweet and hot peppers when soil was infested with *P. capsici* or *P. aphanidermatum. B. cereus* was used effectively with treatment of soil and seed. Hence *B. cereus* was an unspecific antagonist. The reason may be that it prouced water-soluble antibiotics (perhaps glycopeptide) which had wide spectrum (11). These effects were even applied to control *M. incognita* or the combination of *M. incognita* and *R. solani* on tomatoes. Similarly, introducing *T. harzianum* into soil or pelleting it on seeds, the emergence and vigor of wheat and oats was improved from soil infested with *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Fusarium culmorum* (W. G. Sm) Sacc. and *R. solani*-infested soil (24). *T. pseudokoningii* and *T. viride* were unable to control *P. capsici* and *P. aphanidermatum* consistently in this study. The reason is probable that these two antagonists were screened out to controll one specific pathogen only in my previous studies.

TABLE 9. Effectiveness of *Bacillus cereus* alone and combined with chemical to control chrysanthemum stem rot in field[1]

Treatment[2]	Root length (mm) of cuttings planted in sand bed for 14 days		% Survival in field	
	Yellow Queen[4]	White General	Yellow Queen	White General
CK	9.21 c[3]	13.74 b	90.50 a	57.25 ab
B. cereus (Bc)	18.42 ab	19.74 a	92.38 a	62.75 ab
Bc + 0.1% NAA	12.11 c	18.83 ab	90.63 a	74.13 a
Bc + 5% monceren	19.28 a	19.09 ab	86.63 a	56.00 b
Benomyl + 0.1% NAA	13.67 bc	14.08 b	92.00 a	53.63 b

1. and 2. The same as Table 8, except 4000 cuttings were used for each treatment in this experiment.
3. Data, followed by the same letter in the same column, were not significantly ($P = 0.05$) different analyzed by Duncan's multiple range test.
4. Cultivar of chrysanthemum.

Penicillium sp. (isolate #144) showed no antagonistic activity to tested pathogens on different media. This fungus was able to control tested pathogens and increased significantly the survival rate of mungbean, cucumber and muskmelon (Table 1). The reason may be that some other antagonistic activity (*e.g.* competing colonizing site or some essential nutrients) exhibited in soil that could not be shown from dual and concomitant cultures.

Treatment of CSM-*B. cereus* increased the yield of mungbean (*cv.* 1628) significantly in fields which were infested with much (*i.e.* 54 propagules/g soil) *R. solani.* Besides *R. solani, Macrophomina phaseolina* (Tassi) Goid. was common in the same field and infected mungbean with *R. solani* simultaneously. These two pathogens caused severe pre- and post emergence damping-off in the mungbean field plot. Although seed treatment with *B. cereus* did not significantly increase the yield of mungbean, seed treated with *B. cereus* plus monceren produced a better yield than monceren or *B. cereus* alone and significantly better than the control (Table 7). This result indicated that *B. cereus* could be an active ingredient in integrated control and offers a synergistic effect with fungicide to control disease. The same situation was also true for mungbean (*cv.* 1628) because the seed treated with *B. cereus* plus monceren produced a greater yield than monceren and *B. cereus* alone. Chrysanthemum cuttings treated with powder-formulated *B. cereus* produced longer and better root systems than controls, significantly and even better than chemicals (*i.e.* benomyl plus 0.1% NAA). The reason may be that *B. cereus* colonized on these treated cuttings (data not shown) and produced some growth regulators other than antibiotics to promote the formation of root systems. Stimulation of plant growth was an indirect benefit of the use of a bioagent to control plant diseases. Plant growth-promoting rhizobacteria (13) and *Trichoderma* spp. (1) promoted plant growth due to their ability to colonize on plant tissues, to produce some growth regulators, etc. Besides, *Trichoderma* spp. were able to coil around and penetrate the hyphae of *R. solani* (4,5,25,35) and *Pythium ultimum* Trow (9). These characteristics may indicate in part why these bioagents showed their beneficial effects to crops.

From the bioassay in both greenhouse and field, *B. cereus* and *Trichoderma* spp. were able to control several important soil-borne plant pathogens on several crops. The potential of using CSM- and powder-formulated antagonists is encouraging at present. For the purpose of practical and commercial usage, the formulation, effective spectrum and toxicology of these bioagents may require further investigation.

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摘　　要

吳文希．1992. 生物防治立枯絲核菌及其他土媒植物病原的效應．植病會刊 1:1-12. (台北市　國立台灣大學植物病蟲害學系病理組)

由豬糞堆肥中所分離並篩選出之 *Trichoderma Koningii* (98號分離株)、*T. harzianum* (136號分離株)、及 *Penicillium* sp. (100號分離株)，在五種不同培養基上對 *Rhizoctonia solani*、*Phytophthora capsici*、*Pythium aphanidermatum* 和 *Pythium spinosum* 均有拮抗作用；以豬糞堆肥培養已知拮抗菌 *Bacillus cereas* (實驗室中已知之 *R. solani* 拮抗菌)、*Trichoderma pseudokoningii* 及 *T. viride*，以及目前所分離之 *T. koningii* 及 *T. harzianum*，待生長完成後，將其風乾及過篩並貯備之，*B. cereus* 可以持續存活至少九個月，且無存活率降低的情形，但各種 *Trichoderma* 的數量卻會減少 100 倍。這些拮抗菌和高嶺土作成粉劑後，其存活情形如同在豬糞堆肥中一般。如將此等拮抗菌作成藻粒劑，它們在五個月的貯存期間均存活良好，但是藻粒在室溫下，三個月後就開始乾縮。以豬糞堆肥型的拮抗菌作土壤處理，而粉型作種子及繁殖組織處理，

當盆栽綠豆時，土壤如以0.1%量的 *T. koningii* 處理，在每克土壤中含有26個立枯絲核菌繁殖體的環境下，綠豆的存活率仍可增加；但當病原族群增加至每克土壤中含有32個繁殖體，以及田間土壤中每克含有54個繁殖體時，其存活率並無顯著增加。*B. cereus* 雖然在溫室內的生物檢定時，無法藉土壤處理的方法，在每克土壤含有28個繁殖體的環境下增加綠豆的存活率，但卻可在田間每克土壤中含有54個繁殖體的情形下，增加1628號綠豆品種的產量。另外，綠豆種子以 *B. cereus* 處理後，在溫室及田間的生物檢定下，均可提昇其存活率。除此之外，*B. cereus* 尚可防治根瘤線蟲及立枯絲核菌在蕃茄根上所造成的複合感染，以及在田間防治菊花莖腐病，同時並具有促進菊花扦插苗發根的效果，其功效比農民慣用的億力加0.1%發根素的效果還好。土壤處理 *T. koningii* 或 *T. harzinnum* 後，可使胡瓜及香瓜在含有 *P. capsici, P. aphanidermatum* 或 *P. spinosum* 的土壤中，增加其存活率。而土壤處理了 *T. pseudokoningii*、*T. viride* 或 *B. cereus*，也可增加在含 *P. aphanidermatum* 土壤中的蕃茄、甜椒、及辣椒的存活率。但是利用種子處理的方式施用這兩種拮抗菌，卻無法獲得一致地有效結果。以上結果顯示，利用拮抗菌在豬糞堆肥中的形式，防治一些重要土媒植物病原，甚具潛力與開發利用的價值。

關鍵字：土媒植物病原、生物防治、立枯絲核菌、蕃茄及甜椒疫病菌、*Pythium aphanidermatum*、*Pythium spinosum*、綠豆、菊花、胡瓜、香瓜、蕃茄、甜椒、豬糞堆肥、土壤及種子處理。