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Isolation of Antibiotic-Producing Actinomycetes Antagonistic to *Phytophthora capsici* from Pepper-Growing Soils

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고추 栽培土壤에서 *Phytophthora capsici*에 拮抗效果가 있는 抗生物質生成 放線菌의 分離

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ABSTRACT: Fifty-three actinomycetes antagonistic to *Phytophthora capsici* and *Magnaporthe grisea* were isolated from rhizosphere soils in six pepper-growing areas and ashore soils. Thirty-two antagonistic actinomycetes, showing inhibition zone larger than 5 mm, were classified into 20 groups according to their colony morphology and color. The antagonistic activity against *P. capsici* greatly varied, which showed inhibition zone sizes in the ranges from 5.7 to 17.5 mm on V-8 juice agar and from 2.5 to 17 mm on tryptic soy agar. The antagonistic activity of some actinomycetes tested was remarkably different between the two test media. The antagonists showed a relatively broad antifungal spectrum, but their antibacterial activity was negligible, except for *Pseudomonas solanacearum*. Butanol extracts of culture filtrates from antagonistic actinomycetes inhibited mycelial growth of *P. capsici* and *M. grisea*, thereby confirming strongly antibiotic production in culture. Culture filtrates of some antagonistic actinomycetes completely inhibited *Phytophthora* blight in pepper plants.

KEYWORDS: *Phytophthora capsici*, Antagonistic actinomycetes, Antibiotics

Phytophthora capsici Leonian, that causes root rot, crown rot, and blight of pepper (*Capsicum annuum* L.), is widely distributed and destructive in many pepper-growing areas over the world(Papavizas *et al.*, 1980). In Korea, *Phytophthora* blight is an important disease in pepper (Kim, 1989), causing considerable yield loss in pepper production (Cho, 1987). Currently, systemic fungicides such as metalaxyl have been available for the effective control of the Phytophthora disease. However, its control is not always effective because of nature of a soil-borne pathogen. The intensive use of fungicides produced fungicide-resistant strains of *P. capsici*, and increased environmental pollution, and health hazards (Lifshitz *et al.*, 1984; Cohen and Coffey, 1986; Gees and Coffey, 1989; Lee *et al.*, 1990). Thus, biological control by antagonists may be an alternative of chemicals for control of *Phytophthora* blight. Recently, a number of researches have been attempted to find possibility of biological control of this disease in Korea (Cho, 1987; Jee *et al.*, 1988; Nam *et al.*, 1988; Kim, 1989; Park and Kim, 1989). In addition, there are several reports on the suppression of *Phytophthora* species in soil by the antagonistic action of soil-inhabiting microbes (Utkhede, 1984a; Gees and Coffey, 1989; Chung and Hong, 1991; Roiger and Jeffers, 1991).

Several attempts have been made to demonstrate the production in soil of antibiotic substances by microorganisms (Utkhede and Gaunce, 1983; Utkhede, 1984b), Antagonism mediated by antibiotic metabolites of microbial origin has created considerable interest in its potential value for the significant disease control (Fravel, 1988; Kim and Kim, 1989; Lee *et al.*, 1990; Kraus and Loper,

1992). Antibiotics, originated from microorganisms as secondary metabolites, have extensively been studied (Woodruff, 1980; Lancini and Parenti, 1982; Shephard, 1987), because they have highly selective activity against some plant pathogens and no public hazards owing to their easily decomposing nature (Kim *et al.*, 1991). The antibiotics as protectants against plant pathogens was a rather novel idea in 1954 (Odake, 1982). However, the success of blasticidin S as an effective fungicide for the control of rice blast disease has stimulated extensive investigations for new antibiotics effective against various plant diseases (Otake, 1982). Capsimycin (Aizawa *et al.*, 1982) and phthoramycin (Omura *et al.*, 1988) produced by *Streptomyces* sp. have been demonstrated to be active against soilborne diseases caused by *Phytophthora* spp.. The former was effective against leaf blight disease of cucumber caused by *P. capsici*, and the latter was effective against *P. parasitica*. Actinomycetes, especially most *Streptomyces*, produce large numbers of antibiotics with a wide variety of chemical structures, though no all actinomycete strains produce antibiotics (Goodfellow *et al.*, 1988).

The purpose of this study was to isolate antibiotic-producing actinomycetes antagonistic to *P. capsici* as the first step of a new antibiotic screening program. Using the subsequent screening procedures, the antagonistic actinomycetes isolated were evaluated for antibiotic-producing ability and for efficacy in controlling Phytophthora blight in the pepper plants.

Materials and Methods

Screening of antagonistic actinomycetes: To isolate the actinomycetes antagonistic to *Phytophthora capsici* and *Magnaporthe grisea*, soils were obtained from the top 10 cm of pepper-growing fields in the six locations, Kuangrung, Kimpo, Suweon, Ansan, Jungup and Kochang, Korea. The soil samples were stored in plastic bags at 4℃. A bulk sample of 5g soil was mixed in 50 m*l* sterile tap water and shaken to suspend the tightly adhering soil with rhizosphere microorganisms in a rotatory shaker (150 rpm) for 30 min. Suspensions were filtered through Whatman No. 1 filter paper and then diluted 1:100 (v/v) with sterile tap water. One m*l* of each dilution was incorporated into 9 m*l* of molten 0.3% tryptic soy agar (TSA, tryptic soy broth 3 g, agar 15 g, H_2O 1 *l*), nutrient agar (NA, beef extracts 3 g, peptone 5 g, agar 15 g, H_2O 1 *l*) or arginine glycerol agar (AGA, arginine·HCl 1 g, glycerol 12.5 g, K_2HPO_4 1 g, NaCl 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $Fe_2(SO_4)_3 \cdot 6H_2O$ 10 mg, $CuSO_4 \cdot 5H_2O$ 0.1 mg, $MnSO_4 \cdot H_2O$ 0.1 mg, H_2O 1 *l*, pH 7.1) (Dhingra and Sinclair, 1986) at 40℃. The seeded agar plates were incubated at 28℃ for 5-6 days until colonies appeared.

Among a number of colonies appearing, only actinomycete-like microorganisms were tested for antagonism to *Phytophthora capsici* and *Magnaporthe grisea*. They were streaked on V-8 juice agar 10 mm from the edges of plates and incubated for 3 days at 28℃. A mycelial disc (7 mm in diameter) of *P. capsici* and *M. grisea* cut from the margin of growing cultures was placed in the center of the media. The size of inhibition zones was measured after incubation for 5 days.

The 150 isolates of actinomycetes from the ashore soils were provided from the Department of Food Technology, Korea University. Antagonistic activity of the isolates was tested by the screening method described above.

Thirty-two actinomycetes which showed good antagonisic activity against *P. capsici* and *M. grisea* were selected and evaluated for antagonistic effect to *P. capsici* on the two media, V-8 juice agar and tryptic soy agar, to compare the levels of antagonistic activity according to the test media.

Each of the antagonstic actinomycetes obtained was maintained on 0.3% TSA in a vial (10×28 mm) for daily use. For a long-term maintenance, pure cultures also were grown in 0.3% TSB (tryptic soy broth) for 1-2 days, mixed with 15% glycerol and stored at −70℃. Thirty-two actinomycetes selected in terms of their antagonistic activity to *P. capsici* were classified into 20 groups on the basis of colony morphology and color (Table 1).

Antifungal activity of antagonistic actinomycetes: The antifungal activity was measured by placing mycelial discs of each of 14 plant pathogenic fungi

Table 1. A list of antagonistic actinomycetes isolated from soils of various locations in Korea

Group	Antagonistic actinomycete	Location of isolation	Colony morphology and color
1	A3	Kochang	Abundant aerial mycelia, dark gray
	A4	Kochang	
	A11	Suweon	
	A13	Ansan	
	A17	Ansan	
	A39	Jungup	
	A41	Jungup	
	A47	Ansan	
2	A6	Kuangnung	Rough, dark olive
3	A9	Suweon	Abundant aerial mycelia, white
4	A10	Suweon	Smooth, dark French gray
	A12	Suweon	
5	A15	Ansan	Abundant aerial mycelia, light raw umber
	A16	Ansan	
6	A18	Ansan	Rough, white or dark gray
7	A20	Ansan	Abundant aerial mycelia, white
8	A22	Ashore[a]	Smooth, dark yellow
	A24	Ashore	
	A30	Ashore	
	A37	Ashore	
9	A25	Ashore	Aboundant aerial mycelia, white
10	A28	Ashore	Rough, light umber
11	A38	Jungup	Abundant aerial mycelia, burnt unber
12	A40	Jungup	Rough, dark gray
13	A42	Jungup	Rough, black and white
14	A43	Jungup	Rough, dark gray
15	A44	Jungup	Rough, dark gray and white
16	A48	Kochang	Rough, dark gray
17	A49	Ansan	Smooth, dark gray
18	A50	Jungup	Abundant aerial mycelia, white and yellow
19	A51	Jungup	Abundant aerial mycelia, gray
20	A53	Jungup	Rough, white

[a]The strains of actiomycete groups 8, 9 and 10 from the ashore soils were obtained from the Department of Food Technology, Korea university.

on both sides of V8-juice agar plates 30 mm from streak-inoculation of antagonistic actinomycetes in the middle of the plates. Inhibition of mycelial growth of each fungus was rated 7 days after inoculation of the test fungi, but 14 days and 21 days after inoculation for *Colletotrichum kikuchi* and *Cladosporium cucumerinum*, respectively.

Antibacterial activity of antagonistic actinomyce-

tes: The antagonistic actinomycetes streak-inoculated in the middle of TSA was incubated at 28℃ for 72 hr. Test bacteria were streaked on both sides of the medium at right angles to the actinomycetes streak-inoculated. Inhibitory activities of the actinomycetes to various bacteria were evaluated as the presence of a zone of inhibition.

Antibiotic production in liquid cultures: Fermentation of actinomycetes was conducted using shaking flask-cultures. Three-day-old cultures grown in 2% tryptic soy broth was used as inocula for antibiotic production. A 500-m*l* volume of tryptic soy broth were inoculated with the precultured broth (1% of the total volume) and incubated at 28℃ for 8 days with agitation at 150 rpm on a rotatory shaker. After removing the mycelia from the culture media by vacuum filtration, each of culture filtrates was extracted twice with n-butanol. The upper layer partitioned was concentrated at 40℃ *in vacuo* using a rotatory evaporator. The resulting residue was dissolved in a minimum volume of dichloromethane and stored in a vial at −20℃ until used for bioassay.

Bioassay of antifungal activity in culture filtrates: All bioassays were conducted on the agar media seeded with each of the two plant pathogenic fungi, *P. capsici* and *M. grisea*, by using a paper disk method. The assay plates were prepared by mixing a 10 m*l* molten V8-juice agar with a zoospore suspension of *P. capsici*. In the case of *M. grisea*, 10 m*l* of a spore and fragmented mycelial suspension was mixed with a 10 m*l* molten potato dextrose agar. The sterile filter-paper disc (7 mm) soaked with the concentrated culture filtrates were dried for 2 hr at room temperature and then placed on the surface of the seeded media. The plates were incubated at 28℃ for 42 to 72 hr. Sizes of inhibition zones in the treated plates were measured from the extreme edges of clearing against the background lawn.

Inhibitory effect of culture filtrates on *P. capsici* infection: Pepper seeds (cultivar Hanbyul) tested were sown in a plastic tray (55×35×15 cm) containing steam-sterilized sand and loam soil (1:1, v/v). Six seedlings at the four-leaf stage were transplanted in each of plastic pots (5×15×10 cm) containing steam-sterilized loam soil, sand, and peat (1:1:1, v/v/v).

After evaporation of dichlorometane, the butanol extracts equivalent to m*l* culture filtrates were mixed with a zoospore suspension (10^6 spores ml^{-1}) of *P. capsici*. The mixture of the extracts and 30 m*l* zoospore suspension was drenched in the soils of pots with pepper plants at the first-branch stage.

When the untreated-control pepper plants completely died, disease severity was recorded based on a 0-5 scale: 0=no visible disease symptom, 1=leaves slightly wilted brownish lesions beginning to appear on stems, 2=30-50% of entire plant diseased, 3=50-70% of entire plant diseased, 4=70-90% of entire plant diseased, and 5=plant dead. All the experiments were made with 6 replicate plants.

Results

Screening of antibiotic-producing actinomycetes: To screen antagonistic actinomycetes with the most antibiotic-producing potential, approximately 10,000 actinomycetes isolated from rhizosphere soils in six pepper-growing locations of Korea and 150 actinomycetes from ashore soils were evaluated for their inhibitory effects against *Phytophthora capsici* and *Magnaporthe grisea*. Most of the actinomycetes were ineffective in suppressing mycelial growth of the two pathogenic fungi. Only 53 actinomycetes were antagonistic to *P. capsici* and *M. grisea*. Among them, 32 antagonistic actinomycetes which showed inhibition zone larger than 5 mm were classified into 20 groups according to their colony morphology and color (Table 1).

The 32 selected antagonistic actinomycetes was compared for antagonistic efect to *P. capsici* on V-8 juice agar and tryptic soy agar (Table 2). The antagonistic activity against *P. capsici* greatly varied among the actinomycetes tested, which showed inhibition zone sizes in the ranges from 5.7 to 17.5 mm on V-8 juice agar and from 2.5 to 17 mm on tryptic soy agar. The antagonistic activity of some actinomycetes tested was remarkably different between the two test media. For instance,

Table 2. Inhibition of mycelial growth of *Phytophthora capsici* on the two media by antagonistic actinomycetes isolated from soils of various locations in Korea[a]

Antagonistic actinomycete	Inhibition zone length (mm) on the media	
	V8 juice agar	Tryptic soy agar
A3	14.2± 1.3[b]	10.5± 3.0
A4	14.2± 1.0	10.2± 2.2
A6	15.0± 2.8	13.0± 0.0
A9	6.5± 1.7	10.0± 0.8
A10	6.0± 0.8	13.5± 2.1
A11	17.0± 1.0	7.0± 4.2
A12	6.2± 0.5	12.2± 2.8
A13	12.0± 1.0	3.5± 0.7
A15	6.5± 0.6	7.8± 2.1
A16	8.5± 2.4	6.7± 1.5
A17	14.8± 1.3	6.0± 2.2
A18	17.5± 0.6	2.8± 1.9
A20	7.0± 0.8	0.0± 0.0
A22	12.5± 0.6	8.8± 5.0
A24	9.7± 1.5	10.2± 1.7
A25	9.5± 2.1	17.0± 1.2
A28	9.8± 1.0	6.0± 2.4
A30	10.5± 1.3	15.5± 3.7
A37	9.5± 0.7	3.0± 2.4
A38	6.2± 1.0	8.0± 0.0
A39	12.8± 2.2	6.5± 3.7
A40	9.0± 1.2	12.8± 0.5
A41	13.5± 1.4	9.0± 3.4
A42	11.8± 1.0	11.0± 1.4
A43	10.5± 0.6	12.5± 1.7
A44	10.8± 1.0	15.7± 1.2
A47	14.8± 2.6	2.5± 1.0
A48	8.8± 0.5	12.0± 1.0
A49	8.7± 0.6	9.2± 0.5
A50	9.3± 2.5	13.2± 1.3
A51	9.3± 0.6	8.2± 0.5
A53	5.7± 1.2	11.2± 7.0

[a]A mycelial disk of *P. capsici* was placed in the center of the test media 3 days after streak-inoculation of antagonistic actinomycetes 10 mm from the edges of plates.

[b]Inhibition was scored by measuring the distance between the fungal and the actiomycetic mycelia. Values are means± standard deivations from 4 replicates.

the actinomycete strain A20 was rather antagonistic to *P. capsici* on V-8 juice agar, but not antagonistic on tryptic soy agar. In contrast, the strains A11 and A18 were highly antagonistic on tryptic soy agar, but moderately antagonistic on V-8 juice agar. However, most of the actinomycete strains tested were similar to the two test media in the levels of antagonistic activity to *P. capsici*.

Antimicrobial activity of antagonistic actinomycetes: Eleven actinomycetes selected on the basis of their antagonistic activity to *P. capsici in vitro* were tested for inhibition of mycelial growth of the 14 plant pathogenic fungi (Table 3). Any of these antagonistic actinomycetes did not inhibit the mycelial growth of all fungi tested. In particular, *Cladosporium cucumerinum* was sensitive to all the actinomycetes tested, except for the strain A12, but *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were not inhibited by the antagonistic actinomycetes.

The 11 antagonistic actinomycetes selected through a series of screening procedure were tested for inhibitory activity to 11 plant pathogenic bacteria, *Escherichia coli*, and *Bacillus subtilis* (Table 4). Most of the antagonistic actinomycetes, except for the strains A12 and A24, did not inhibit *E. coli* and *B. subtilis*. None of these antagonistic actinomycetes also inhibited the plant pathogenic bacteria tested. Some strains A15, A25, A43, A44, and A48, however, showed relatively striking inhibition against *Pseudomonas solanacearum*.

Bioassay of antifungal activity of culture filtrates: The inhibition of mycelial growth of *P. capsici* and *M. grisea* by culture filtrates of the selected 11 antagonistic actinomycetes was evaluated by a paper-disk assay (Table 5). Except for the strains A4 and A24, culture filtrates of all actinomycete strains inhibited *P. capsici*. Among them, the strain A50 was most effective in antifungal activity. The inhibitory effect of the strain A15 against *M. grisea* was most conspicuous among all the actinomycetes tested. Other strains also exhibited considerable levels of antifungal effect against *M. grisea*.

Control of Phytophthora blight in pepper plants by culture filtrates: Inhibitory effects of culture

Table 3. Antifungal activity of the selected actinomycetes to various plant pathogenic fungi on the agar media[a]

Plant pathogenic fungus	Inhibition zone length(mm)[b] produced by the actinomycete										
	A4	A12	A15	A24	A25	A40	A43	A44	A48	A50	A53
Alternaria mali	1.0	0.0	0.0	4.0	5.5	5.0	4.5	7.5	6.0	0.0	6.0
Alternaria solani	14.0	0.0	2.5	9.0	5.0	12.0	14.0	12.0	11.0	7.0	10.0
Botryosphaeria dothidea	4.5	3.0	3.0	15.0	4.0	9.5	13.0	10.0	11.5	3.0	9.0
Cercospora capsïci	0.0	0.0	0.0	7.0	5.5	13.5	15.0	13.5	11.5	7.0	9.0
Cercospora kikuchi	9.0	4.0	0.0	4.0	16.5	10.0	6.5	10.0	7.0	12.0	14.0
Cladosporium cucumerinum	14.5	0.0	9.0	15.0	16.0	19.0	19.5	22.0	15.0	7.0	18.0
Colletotrichum gloeosporioides	6.5	0.0	0.0	7.0	7.5	10.5	10.5	10.0	12.0	1.0	6.5
Cylindrocarpon destructans	0.0	0.0	0.5	7.0	7.0	11.0	11.5	12.5	10.5	2.0	5.0
Fusarium oxysporum f. sp. *cucumerinum*	0.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mycosphaerella fragariae	2.0	0.0	6.0	10.0	8.0	15.0	11.5	15.0	15.5	0.0	10.0
Mycosphaerella mellonis	0.0	0.0	0.0	8.0	4.5	13.0	13.0	13.5	0.0	1.0	4.0
Magnaporthe grisea	13.0	0.0	0.5	15.0	10.0	14.0	13.5	16.5	16.0	9.5	10.0
Rhizoctonia solani	9.5	0.0	13.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sclerotinia sclerotiorum	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0

[a]The antifungal activity was measured by placing a mycelial disk of each fungus on a side of V8-juice agar (yeast-malt extract agar in the case of A50) 30 mm from streak-inoculation of antagonistic actinomycetes in the middle of plates.

[b]Inhibition of mycelial growth of each plant pathogenic fungus were rated 7 days after inoculation of the test fungi, 14 days and 21 days after inoculation for *C. kikuchi* and *C. cucumerinum*, respectively.

Table 4. Antibacterial activity of the selected actinomycetes to various bacteria on the agar medium[a]

Bacteria	Inhibition zone length (mm)[b] produced by the actinomycete										
	A4	A12	A15	A24	A25	A40	A43	A44	A48	A50	A53
Bacillus subtilis	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erwinia carotovora subsp. *carotovora*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Escherichia coli	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0
Pseudomonas aurefacines	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas chlororaphis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluoresiens	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas malginalis pv. *malginalis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas putida	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas solanacearum	0.0	0.0	15.0	0.0	10.0	9.5	13.0	7.5	7.5	0.0	0.0
Pseudomonas syringae pv. *syringae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Xanthomonas campestris pv. *oryzae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a]The antibacterial activity was measured 3 days after streaking the test bacteria on both sides of tryptic soy agar (yeast-malt extract agar in the case of A50) at right angles to the antagonistic actinomycetes streak-inoculated in the middle of plates.

[b]Inhibition zones were rated 2 days after inoculation of the test bacteria.

Table 5. Activity of culture filtrates of the selected antagonistic actinomycetes against *Phytophthora capsici* and *Magnaporthe grisea* as indicated by sizes of inhibition zones by paper-disc assay[a]

Antagonistic actinomycete	Diameter of the inhibition zone (mm) of	
	P. capsici	*M. grisea*
A4	0.0± 0.0[b]	0.5± 0.7
A12	1.3± 0.4	2.0± 0.0
A15	0.7± 0.2	17.5± 4.9
A24	0.0± 0.0	2.0± 0.0
A25	5.0± 0.7	9.0± 0.0
A40	0.7± 0.4	5.0± 1.4
A43	1.3± 1.2	5.0± 0.0
A44	1.0± 1.0	9.5± 2.1
A48	0.7± 0.2	6.0± 0.0
A50	7.0± 1.0	3.5± 0.7
A50(Y)[c]	35.0± 2.8	9.0± 0.0
A53	2.3± 0.3	9.5± 0.7

[a]The assay plates were prepared by mixing a 10-m*l* V8-juice agar with a zoospore suspension of *Phytophthora capsici* and a mycelial suspension of *M. grisea*, respectively. The paper-disc (7 mm-diameter) soaked with the butanol extracts equivalent to 5 m*l* culture filtrates was placed on the surface of the assay agar and were incubated at 28℃ for 48 to 72 hr.

[b]Inhibition of fungal growth were estimated by subtracting the diameter of the paper disc (7 mm) from the diameter of inhibition zones. Each value is a mean± standard deviation from 3 replicate plates.

[c]A_{50}(Y) was cultured in yeast-malt extract broth, but others in tryptic soy broth.

filtrates of 11 selected antagonistic actinomycetes on *P. capsici* infection in pepper plants were evaluated when the untreated control plants completely died by the Phytophthora blight (Table 6). The Phytophthora disease was not effectively controlled by applying the butanol extracts equivalent to 10 m*l* culture filtrates of these antagonistic actinomycetes. However, the effective control of the disease was achieved with increasing the application amount of culture filtrates. In particular, antagonistic actinomycete strains A15, A25, A43, and A50 completely inhibited Phytophthora blight at a 50 m*l* culture filtrates. The culture filtrates of the actinomycete strains A24 and A48 were ineffective in inhibiting Phytophthora blight.

Discussion

We isolated many actinomycetes from the top 10 cm of soils of pepper-growing fields, because the actinomycetes known to be efficient producers of antibiotics are abundant in soil and also diverse in morphology and color (Singh and Mehrotra, 1980). Approximately 10,000 actinomycetes from the rhizosphere of pepper-growing soils were further examined for antagonism to *P. capsici*. In the subsequent screening procedures, the antagonistic actinomycetes were evaluated for antibiotic-producing ability and for efficacy in controlling Phytophthora blight in pepper plants. A number of actinomycete-like colonies appeared on soil-dilution plates of arginine glycerol agar. This selection media, possibly with a favorable environment for the growth of actinomycetes, provided more chances for isolating actinomycetes from soil than did tryptic soy agar or nutrient agar used (Tsao *et al.*, 1960; Dhingra and Sinclair, 1986). Appearance of bacterial colonies greatly decreased on the arginine glycerol agar plates, although various actinomycete colonies were yielded. To isolate actinomycetes as many as possible, soil-dilution plates should be incubated for more than 5 days, because the growth of actinomycetes was much slower than bacteria. The antagonistic activity of actinomycetes from soil-dilution plates was examined by streak-inoculating randomly chosen actinomycetes on the opposite site to the test fungal pathogens such as *P. capsici* and *M. grisea*. In fact, this screening procedure was time-consuming and laborious, since all kinds of actinomycetes should be tested for antagonism. A triple-agar-layer plate technique (Herr, 1959), which was developed for isolating soil actinomycetes antagonistic to plant pathogenic fungi, has been known to be more rapid and less laborious than the counter-streaking technique used in the present study. However, our use of the triple-agar-layer plate technique failed to screen effectively antagonistic actinomycetes from soils, possibly due to the diffusion of low quantity of antifungal substances into the test agar media and more rapid growth of the test fungi than the antagonists. Therefore, using the

Table 6. Effects of culture filtrates[a] of the selected antagonistic actinomycetes against *Phytophthora capsici* infection in pepper plants

Antagonistic actinomycete	Disease severity[b], as treated with culture filtrate		
	10 ml	50 ml	100 ml
A4	5.0± 0.0	4.6± .0	0.4± 0.8
A12	4.8± 0.4	3.8± 2.0	0.0± 0.0
A15	5.0± 0.0	0.0± 0.0	0.0± 0.0
A24	5.0± 0.0	4.3± 1.6	4.2± 1.4
A25	4.4± 0.6	0.0± 0.0	0.0± 0.0
A40	4.3± 1.0	0.0± 0.0	0.0± 0.0
A43	3.7± 2.1	0.0± 0.0	0.0± 0.0
A44	5.0± 0.0	4.5± 0.6	2.0± 1.6
A48	4.9± 0.2	4.1± 1.1	3.7± 2.0
A50	3.1± 2.4	0.0± 0.0	0.0± 0.0
A53	2.8± 1.7	2.8± 2.2	1.0± 0.0

[a]The butanol-extracts equivalent to ml culture filtrates were mixed with a 30 ml zoospore suspension (10^6 ml^{-1}) of *P. capsici*. The mixture of the extracts and *P. capsici* suspension was drenched in the soils of pots with pepper plants (cv. Hanbyul) at the first-branch stage.

[b]Disease severity was recorded based on a 0-5 scale (see in materials and methods) when the untreated-control pepper plants completely died. Each value represents a mean± standard deviation of 6 replicate plants.

counter-streaking method, we could successfully isolate 32 actinomycetes antagonistic to *P. capsici* and *M. grisea*.

The 32 selected actinomycetes were compared for antagonistic activity to *P. capsici* on V-8 juice agar and tryptic soy agar (Table 2). The antagonistic activity of some actinomycetes tested was remarkably different between the two test media. These data indicated that the evaluation of antagonistic activity could be different based on the test media used. Appropriate test media should be chosen to use in a screening-procedure.

Attempts have been made to evaluate the antagonistic ability of the selected actinomycetes against various plant pathogens. The antagonists showed a relatively broad antifungal spectrum, but their antibacterial activity was negligible, except for *P. solanacearum*. The broad range of antifungal activity of antagonists suggests that a complicated mechanism of action may involve more than one antifungal metabolite and also that the metabolites have the potentials to control not only Phytophthora blight but also other plant diseases (Lumsden and Locke, 1989).

Our results provide a substantial evidence for antibiosis, as a mechanism for antagonism of the selected actinomycetes to various plant pathogens including *P. capsici* and *M. grisea*. Butanol extracts of culture filtrates from 11 antagonistic actinomycetes inhibited mycelial growth of *P. capsici* and *M. grisea*, thereby confirming strongly antibiotic production in culture. The culture extracts of actinomycete strain A50 cultured in tryptic soy broth were considerably inhibitory against *P. capsici* and *M. grisea*, but highly effective when cultured in yeast-malt extract broth. In the cases of other actinomycete strains, the difference in inhibition between the production media was not significant (no data presented). The actinomycete strains A4 and A24 did not show any antagonistic activity against *P. capsici*, probably due to the absence of antifungal substances against *P. capsici* in the butanol extracts.

The dependability of pot tests in assessing the potential of the selected antagonists in the field was essential to the screening process (Burr *et al.*, 1978). By using steamed soils in pot tests, we attempted to eliminate the possibility of significant

direct effects of soil microbes other than the added crude extracts of antagonistic actinomycetes. In the preliminary experiments, the efficacy of individual culture filtrates as biological control agents often was not consistent among trials. The inconsistent greenhouse results may be due in part to plant growth stage, the amount of culture filtrate applied, soil types, and fluctuations in greenhouse temperature (Roiger and Jefes, 1991). One of the most important factors causing inconsistant biological control by individual agents among trials could be temperature in the greenhouse, which may affect plant growth and resistance. Soil type may strongly affect the result of greenhouse test, because the crude culture extract applied in the soil of pot with pepper plants may be adsorbed to the soil particles. As yet, it has not been definitely established whether an antibiotic substance maintains its antimicrobial activity in an adsorbed state, or whether it should be released first to exhibit activity. The behavior of antibiotics in soil, a matter of much scientific and practical interest, is still only partially understood. Culture filtrates of some antagonistic actinomycete strains A15, A25, A40, A43, and A50 efficiently managed Phytophthora blight of pepper plants at a 50 m*l* culture filtrate (Table 6). The culture filtrates of the actinomycete strains A24 and A48 were inefficient in inhibiting Phytophthora blight. Since no correlation was observed between inhibition zone on agar plate and disease severity in a greenhouse test, no evidence was found in these antagonistic actinomycetes to suggest that antibiotic production is involved in protection against *P. capsici* infection. However, the actinomycete strain A50 was most antagonistic to *P. capsici* in the culture media and in the greenhouse, as demonstrated in the present study. Therefore, we will use the antagonistic actinomycete A50 for further studies of mass production, purification, and structure determination of antifungal components.

摘 要

6개 고추재배지의 근권 및 해안가 토양으로부터 고추 역병균(*Phytophthora capsici*)과 벼 도열병균(*Magnaporthe grisea*)에 길항효과가 있는 53개의 방선균을 분리하였다. 분리된 방선균 중에서 고추 역병균의 균사생장을 억제시켜 5 mm 이상 저지원을 형성하는 32균주를 선발하여 이들 균주의 균총형태, 색깔 등에 따라 20균으로 유별하였다. 이들 길항방선균의 고추 역병균에 대한 길항효과는 균주간에 매우 다양하여 V-8 juice agar에서 5.7-17.5, tryptic soy agar에서는 2.5-17 mm의 저지효과를 보였다. 몇가지 방선균의 길항효과는 두 배지에서 상당히 다르게 발현되었다. 길항균들은 비교적 넓은 항진균성 스펙트럼을 나타내었으나, 세균에 대해서는 *Pseudomonas solanacearum*을 제외하고는 항균작용이 거의 없었다. 길항방선균 배양여액의 butanol 추출액이 고추 역병균과 벼도열병균의 균사생장을 억제하는 것으로 보아서 이들 길항균이 항균물질을 생성함을 강하게 시사하고 있다. 몇가지 길항방선균의 배양여액은 고추 역병방제 효과가 뚜렷하였다.

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