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Biocontrol of *Phytophthora* Root Rot of Angelica Trees by *Enterobacter cloacae* and *Serratia ficaria* Strains

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ABSTRACT

Bacterial strains isolated from the rhizosphere of angelica trees were evaluated for their antagonistic activity against *Phytophthora cactorum*, a causal agent of *Phytophthora* root rot. Of these, three bacterial strains, designated as T-1-8, T-1-14 and T-1-23, strongly inhibited mycelial growth of *P. cactorum* ARE-862 in a dual-culture plate assay. Biocontrol activity of these strains was then examined by dipping root of young seedlings of angelica trees into a bacterial suspension. The incidence of *Phytophthora* root rot was markedly suppressed for at least 79 days in pot tests when treated seedlings were planted in naturally infested soil. The suppression was maintained through June of the next year. In addition, these strains significantly reduced the development of *Phytophthora* root rot up to 47 days in naturally infested field and up to 63 days (the last day of testing) in an artificially (moderately) infested field. Based on their main bacteriological properties, strain T-1-14 was identified as *Enterobacter cloacae* and T-1-8 and T-1-23 were identified as *Serratia ficaria*.

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Key words : *Enterobacter cloacae*, *Serratia ficaria*, *Phytophthora cactorum*, biocontrol, *Phytophthora* root rot of angelica tree.

INTRODUCTION

The angelica tree (*Aralia elata* Miq. Seem.), or "tara-no-ki" in Japanese, has edible buds and is one of the most popular edible wild plants ("san-sai") in Japan. Cultivation of angelica trees in agricultural fields started in Yamanashi prefecture in 1978, and has since developed in various districts, including Kyoto and Fukui prefectures. Uchida *et al.*[27,28] first identified a disease of angelica characterized by shoot (stalk) blight and resulting in dieback (Plate I-1,2). They determined that a *Phytophthora* sp. (later identified as *Phytophthora cactorum*) was responsible and named the disease *Phytophthora* root rot of angelica trees.

Although this disease can be controlled for a given period of time using metalaxyl and other chemicals, such chemical treatment is unsuitable for angelica trees because their buds are used for food. Moreover, plants resistant to this pathogen have not yet been bred. Therefore, biological control is a desirable strategy for *Phytophthora* root rot of angelica trees.

Although more than 10 genera of rhizobacteria have recently been reported to have potential for the biological control of plant diseases[25], no antagonistic ones were identified for use in the control of *Phytophthora* root rot of angelica trees prior to our preliminary study in 1988[17].

Here, we present the isolation and characterization of bacteria antagonistic to *P. cactorum*, the causal agent of *Phytophthora* root rot of angelica trees. In addition, we describe the biological control of this disease by these strains in both pot and field tests. Several previously reported strains antagonistic to *P. capsici* P-9-2 (damping-off disease of cucumber), as well as *Fusarium oxysporum* f.sp. *raphani* F-1-6-3 (yellows of Japanese radish), were also evaluated for their usefulness against

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Table 1. Bacterial strains tested as biocontrol agents against *Phytophthora cactorum* ARE-862

Bacterial strain	Isolation origin	Biocontrol use	Reference
T-1-8	rhizosphere of *Aralia elata*	*Phytophthora* root rot of angelica tree	this study
T-1-14	*Ibid*	*Ibid*	*Ibid*
T-1-23	*Ibid*	*Ibid*	*Ibid*
M-1-1	"rhizosphere" of *Pleurotus ostreatus*	damping-off of cucumber	Okamoto and Isaka[18]
M-6-1	"rhizosphere" of *Steccherinum rhois*	*Ibid*	*Ibid*
M-6-2	*Ibid*	*Ibid*	*Ibid*
Me-1-4	rhizosphere of *Coptis japonica*	*Ibid*	*Ibid*
V-2-2	rhizosphere of *Allium fistulosum*	*Ibid*	*Ibid*
V-2-3	*Ibid*	*Ibid*	*Ibid*
V-3-1	rhizosphere of *Brassica campestris* (pekinensis group)	yellows of Japanese radish	Isaka and Okamoto[7]

the disease. Preliminary results have been reported elsewhere[17,19].

MATERIALS AND METHODS

Bacterial strains and fungal isolates The bacterial strains used in this study are shown in Table 1. In addition to three strains isolated from the rhizosphere of angelica trees in this study, several previously reported[7,18] strains antagonistic to *P. capsici* P-9-2 (the pathogen of the damping-off disease of cucumber) or *F. oxysporum* f. sp. *raphani* F-1-6-3 (the pathogen of the yellows of Japanese radish) were used.

For isolation and maintenance of the bacteria, PSA medium ($Na_2HPO_4 \cdot 12H_2O$, 2 g ; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 g ; peptone, 5 g ; sucrose, 15 g ; agar, 15 g ; in 1 liter decoction of potato 300 g, pH 7.0) or modified LB medium (polypeptone, 10 g ; yeast extract, 5 g ; NaCl, 10 g ; agar, 15g ; distilled water 1 liter) was used.

Strains of *P. cactorum* ARE-862 and *P. capsici* P-9-2 were used for the bioassay, and were grown and maintained on PDA medium (glucose, 20 g ; agar, 15 g ; in 1 liter decoction of potato 200 g, pH 7.0) at 20°C.

Bacterial properties Identification of the suspected bacterial antagonists were identified using the API 20E kit (Bio MERIEUXS. A. Company, France) according to the manufacturer's protocol. To further confirm the identification, the additional bacterial properties were investigated by the protocol of Cowan[1], Tominaga[26] and Komagata[11] and then compared with the description in Bergey's Manual of Determinative Bacteriology (9th ed.)[5].

Fungal growth inhibition assay The inhibition of mycelial growth of *P. cactorum* ARE-862 was assayed by measuring the inhibition zone produced in dual culture with *P. cactorum* ARE-862 and each test bacterial strain on PDA plates (Plate I-3).

The inhibition of cystospore germination of *P. capsici* P-9-2 was examined as follows. A cystospore suspension of *P. capsici* P-9-2 (10^4/ml) was mixed with an equal volume of bacterial suspension (2.7×10^7 cfu/ml), and the mixture was then dropped on a glass slide. After a 6-hr incubation at 28°C, the number of germinated cystospores was counted using a microscope (×200). A total of about 5000 cystospores was scored in each sample.

Pot tests Young angelica tree seedlings were grown from seeds in sterile soil in pots by the method of Miyajima and Ohki[13] with a slight modification. Briefly, seeds were collected in early November, and one-half of the coat of each seed was removed. The seeds were then surface-sterilized, treated with gibberellin (100 ppm), treated with low temperature (5°C in the dark for 1 week), then germinated by incubation at 25°C under light (4000 lux) for 1 week. Thus, seedlings with a stalk length of 5 cm were used for pot tests 1 and 2.

To establish a suitable micro-ecosystem for pathogen infection, soil was collected from naturally infested fields in Kyoto prefecture (Kuta, Sakyo-ku, Kyoto city) in June and August, 1987, and used to fill 15-cm pots. For uniformity among the pots, soil was mixed with a mixing machine (Kumagai Agricultural Machinery Co., Ltd. Type : K-910). The pathogen in the soil had been confirmed as the same species as the one under investigation here. Roots of young seedlings of angelica tree (stalk length, 5 cm) were dipped into a suspension of the antagonistic bacteria (10^7-10^8 cfu/ml) for 30 min. Each treated seedling was planted in the infested soil in pots, one plant per pot. Treated plants were then grown in a greenhouse for a fixed period of time. In pot test 2, treated plants were transferred from the greenhouse to the open air from the time disease was assessed on November 18, 1987 until June of the next year. One treatment group consisted of 10 pots in a plastic container, dedicated to one antagonistic bacterial strain and the pathogen. Eleven

treatments, including a control without antagonistic bacteria, made up one set of the experiment. Three replications of the set were carried out.

Tested plants were scored for disease incidence (mortality) at several times over 30 days (June 24 to July 24, 1987 : pot test 1) or 290 days (August 31, 1987 to June 16, 1988 : pot test 2).

Field tests Young angelica tree seedlings (stalk length, 30 cm) treated with 30-min root dips in an antagonistic bacterial suspension (10^7-10^8 cfu/ml) of strain T-1-8, T-1-23 or V-2-3 and untreated control seedlings were transplanted in infested fields plowed with a tractor to maintain higher uniformity within the experimental field in Kyoto prefecture (Inden-cho, Ayabe city) ; the plants in these preceding fields had been severely infected with *Phytophthora* root rot over several years. Each treated seedling was planted into the infested field, five plants in each area (2.5 m^2).

The granular fungicide Ridomil (metalaxyl 2.0%) was mixed in soil (2 g/2.5 m^2 area) as a chemical control treatment. As an additional control, untreated plants were also assayed. Five treatments made one set of the experiment. Three replications of the set were carried out (field test 1). Plants were scored for disease incidence (mortality) at several times over 2 months (August 12 to October 12, 1988).

Field test 2 was in an artificially infested field at Fukui Prefectural College (Obatake-cho, Fukui city, Fukui prefecture). The field was artificially infested by amending it with infested soil from field test 1. Three strains (T-1-8, T-1-14 and T-1-23) isolated from the rhizosphere of angelica trees were used. Control treatments were prepared as in test 1. Five treatments made one set of the experiment. Three replications of the set were carried out. Tested plants were scored for their disease incidence (mortality) at several times over 2 months (August 15 to October 17, 1988).

RESULTS

Isolation of antagonistic bacteria

Twenty-nine bacterial strains were isolated from the rhizosphere of several living angelica trees rescued from severe infection in fields of Kuta (Sakyo-ku, Kyoto city) in June 1987. These strains were assayed for their antagonistic activity against disease by growth inhibition tests using *P. cactorum* ARE-862. Among them, 16 strains (55.2%) were antagonistic to the pathogen. Three strains designated as T-1-8, T-1-14 and T-1-23 (hereafter, the three angelica strains) were used for further pot and field experiments. In contrast, 5 strains (23.8%) isolated from the rhizosphere of plants with dieback symptoms of the disease were antagonistic to isolate of ARE-862 of *P. cactorum*.

Table 2. Effect of bacterial strains on hyphal growth of *Phytophthora cactorum* ARE-862 and germination of cystospores of *P. capsici* P-9-2

Bacterial strain	Growth inhibition of hyphae of *P. cactorum* ARE-862[a)]	Germination (%) of cystospores of *P. capsici* P-9-2[b)]
T-1-8	+++	6.8
T-1-14	+++	0.0
T-1-23	+++	1.2
M-1-1	++	0.0
M-6-1	+++	0.0
M-6-2	+++	2.8
Me-1-4	+++	0.0
V-2-2	+++	0.0
V-2-3	+++	0.0
V-3-1	+++	0.0
Control	−	88.1

a) The grade of inhibition zone produced in the dual culture of *P. cactorum* ARE-862 and bacterial strains on PDA plates : +++ (10-7 mm)> ++ (7-3 mm)> + (3-1 mm)> − (none).

b) A cystospore suspension of *P. capsici* P-9-2 (10^4/ml) was mixed with an equal volume of bacterial suspension (2.7×10^7 cfu/ml). After incubation for 6 hr at 28°C, the number of germinated cystospores was counted in a total of about 5000 cystospores in each sample.

Inhibition assay of fungal growth

In addition to the three angelica strains, seven bacterial strains, M-1-1, M-6-1, M-6-2, Me-1-4, V-2-1, V-2-2 and V-3-1, which are antagonistic to either *P. capsici* P-9-2 (the pathogen of damping-off disease of the cucumber) and *F. oxysporum* f. sp. *raphani* F-1-6-3 (the pathogen causing yellows of Japanese radish), were used for the fungal growth inhibition assay. As shown in Table 2, all the tested strains were strong antagonists on *P. cactorum* ARE-862 (inhibition of mycelial growth) and *P. capsici* P-9-2 (inhibition of germination of cystospores).

Pot tests

In pot test 1, young seedlings (stalk length, 5 cm) treated or untreated with bacteria were monitored for their disease incidence over a 30-day period from June 24 to July 24. All three angelica strains strongly suppressed the incidence of disease, which was 10 % on day 30 (Table 3, Plate I-4). In contrast, untreated plants were severely infected, and all died within the 30-day period. On the other hand, the other strains antagonistic to damping off, except for V-2-3 and V-3-1, weakly suppressed *Phytophthora* root rot.

Similar results were obtained in pot test 2 using young seedlings (stalk length, 5 cm) over a longer observation period. As shown in Table 4, all three angelica strains remarkably suppressed the incidence of the disease for at

Table 3. Effect of root-dip treatment with bacteria on the suppression of *Phytophthora* root rot of angelica trees in a greenhouse (pot test 1)[a]

Bacterial strain	Disease incidence (%)[b]			
	Days after dip treatment			
	6 days (30 Jun. '87)	15 days (9 Jul. '87)	21 days (15 Jul. '87)	30 days (24 Jul. '87)
T-1-8	0.0	3.3	10.0	10.0 b[c]
T-1-14	0.0	3.3	10.0	10.0 b
T-1-23	0.0	0.0	0.0	0.0 a
M-1-1	10.0	30.0	56.7	56.7 d
M-6-1	0.0	20.0	43.3	46.7 c
M-6-2	0.0	30.0	50.0	53.3 cd
Me-1-4	0.0	30.0	73.3	76.7 e
V-2-2	0.0	30.0	33.3	46.7 c
V-2-3	0.0	0.0	0.0	0.0 a
V-3-1	0.0	10.0	10.0	10.0 b
Control	36.7	93.3	100.0	100.0 e

a) Young seedlings of angelica trees (stalk length, 5 cm) were treated by "root-dipping" and planted in naturally infested soil in pots on June 24, 1987. The soil was collected from a naturally infested field in Kyoto prefecture (Kuta, Sakyo-ku, Kyoto city) on June 21, 1987.
b) One treatment consisted of 10 pots, one seedling per pot, in a plastic container, with one antagonistic bacterial strain and the pathogen. Eleven treatments, including a control without antagonistic bacteria, made one set of the experiment. Data are expressed as the means of three replications of one set.
c) Values followed by the same letter are not significantly different ($p=0.01$) according to Duncan's multiple range test.

Table 4. Effect of root-dipping in antagonistic bacteria on the suppression of *Phytophthora* root rot of angelica trees in a greenhouse and in the open air (pot test 2)[a]

Bacterial strain	Disease incidence (%)[b]		
	Days after dipping treatment		
	38 days (8 Oct. '87)	79 days (18 Nov. '87)	290 days (16 Jun. '88)
T-1-8	6.7	10.0	23.3 a[c]
T-1-14	13.3	23.3	23.3 a
T-1-23	0.0	0.0	13.3 a
M-1-1	56.7	70.0	76.7 bc
M-6-1	63.3	73.3	76.7 bc
M-6-2	46.7	56.7	100.0 c
Me-1-4	66.7	83.3	86.7 bc
V-2-2	43.3	63.3	66.7 b
V-2-3	0.0	13.3	13.3 a
V-3-1	13.3	16.7	23.3 a
Control	70.0	83.3	100.0 c

a) Young seedlings of angelica trees (stalk length, 5 cm) were treated by "root-dipping" and planted in naturally infested soil in pots on August 31, 1987. The soil was collected from naturally infested field in Kyoto prefecture (Kuta, Sakyo-ku, Kyoto city) on August 26, 1987. Treated plants were placed in a greenhouse on August 31, 1987, then placed in the open air after disease assessment on November 18, 1987 until the next June.
b) One treatment consisted of 10 pots, one seedling per pot, in a plastic container, with one antagonistic bacterial strain and the pathogen. Eleven treatments, including a control without antagonistic bacteria, made one set of the experiment. Data are expressed as the means of three replications of one set.
c) Values followed by the same letter are not significantly different ($p=0.01$) according to Duncan's multiple range test.

least 79 days (November 18, 1987), and the suppression was maintained until next year (June 16, 1988). In contrast, the other strains, except for V-2-3 and V-3-1, did not markedly suppress disease.

Field tests

In field test 1 (naturally infested field), strains T-1-23 and V-2-3 significantly suppressed the incidence of the disease through day 47 and day 53, respectively (Table 5). After 61 days, however, 80% of these plants were infected with *Phytophthora* root rot, slightly less than that of untreated plants. On the other hand, T-1-8 significantly suppressed disease only until the earliest observation (day 30). In contrast, metalaxyl remarkably inhibited disease incidence through the last time point (day 61).

In field test 2 (artificially infested field), the disease was significantly suppressed through the last observation (day 63) in plants treated with T-1-8, T-1-14 or T-1-23. In this test, however, the disease incidence (mortality) of control plants was lower than that in test 1, because the population of the pathogen was lower in the artificially infested soil relative to that of the naturally infested soil of test 1 (Table 6).

Bacteriological properties

Strains T-1-14, T-1-8 and T-1-23 were gram-negative rods, motile with peritrichous flagella (Plate I-5, 6) and formed white colonies. Since they were thought to belong to the *Enterobacteriae*, their bacterial properties were assessed with an API 20E-Identification Kit (Bio MERIEUX S.A.Company, France) suitable for the identification of *Enterobacteriae*. The "profile index", which consisted of "indicator numbers" corresponding to the results for 27 biochemical properties, were 1,3,0,5,5,7,3,5,7 (T-1-14), 1,2,0,6,5,6,3,5,7 (T-1-8) and 1,2,0,7,5,6,3,5,7 (T-1-23). From these data, T-1-14 was identified as *Enterobacter cloacae* (93.3% significant taxa), and both strains of T-1-8 and T-1-23 were identified as *Serratia ficaria* (87.8% and 78.8%, respectively). To verify the

Table 5. Effect of root-dip treatment with bacteria on the suppression of *Phytophthora* root rot of angelica trees in naturally infested fields (field test 1)[a)]

Treatment	Disease incidence (%)[b)]			
	Days after dipping treatment			
	30 days (11 Sep. '88)	47 days (28 Sep. '88)	53 days (4 Oct. '88)	61 days (12 Oct. '88)
T-1-8	53.3 b[c)]	66.7 ab	93.3 a	100.0 a
T-1-23	13.3 c	33.3 bc	66.7 a	80.0 a
V-2-3	13.3 c	26.7 c	46.7 b	80.0 a
Metalaxyl[d)]	0.0 c	0.0 c	13.3 c	13.3 b
Control	73.3 a	73.3 a	93.3 a	93.3 a

a) Young seedlings of angelica trees (stalk length, 30 cm) were treated with "root-dipping" and were planted into the naturally infested fields in Kyoto prefecture (Inden-cho, Ayabe city) on August 12, 1988.
b) One treatment consisted of five seedlings per area (2.5 m^2). Five treatments made one set of experiment. Data are expressed as the means of three replications of one set.
c) Values followed by the same letter are not significantly different ($p=0.01$) according to Duncan's multiple range test.
d) A fungicide, Ridomil granules (metalaxyl 2.0%), was mixed in the soil (2 g/2.5 m^2 area).

Table 6. Effect of root-dip treatment with bacteria on the suppression of *Phytophthora* root rot of angelica trees in artificially infested fields (field test 2)[a)]

Treatment	Disease incidence (%)[b)]			
	Days after dipping treatment			
	31 days (15 Sep. '88)	48 days (2 Oct. '88)	56 days (10 Oct. '88)	63 days (17 Oct. '88)
T-1-8	13.3 b[c)]	33.3 b	46.7 b	46.7 b
T-1-14	13.3 b	26.7 b	33.3 b	46.7 b
T-1-23	0.0 c	33.3 b	33.3 b	33.3 b
Metalaxyl[d)]	0.0 c	0.0 c	0.0 c	0.0 c
Control	53.3 a	53.5 a	66.7 a	66.7 a

a) Young seedlings of angelica trees (stalk length, 30 cm) were treated with "root-dipping" and were planted into the artificially infested fields at Fukui prefecture (Obatake-cho, Fukui city) on August 15, 1988. The artificially infested field was constructed by carrying infested soil from the field (Inden-cho, Ayabe city, Kyoto prefecture) used in field test 1.
b) One treatment consisted of five seedlings per area (2.5 m^2). Five treatments made one set of experiment. Data are expressed as the means of three replications of one set.
c) Values followed by the same letter are not significantly different ($p=0.01$) according to Duncan's multiple range test.
d) A fungicide, Ridomil granules (metalaxyl 2.0%), was mixed in the soil (2 g/2.5 m^2 area).

identification, other bacterial properties were examined and compared with data from Bergey's Manual of Determinative Bacteriology[4)]. As shown in Table 7, the properties of T-1-14 were almost identical to those of *E. cloacae*, and T-1-8 and T-1-23's were almost identical to those of *S. ficaria* in Bergey's description. Thus, these strains were identified as *E. cloacae* and *S. ficaria*.

DISCUSSION

In this study, three isolated bacterial strains were found to be antagonistic to *P. cactorum*, the pathogen of *Phytophthora* root rot of angelica trees, and identified as *E. cloacae* (T-1-14) and *S. ficaria* (T-1-8 and T-1-23). These two bacterial species constitute the first recorded biocontrol agents for *Phytophthora* root rot of angelica trees, with the exception of a *Pseudomonas* sp., which was reported by Hashimoto and Yoshikawa[4)]. *E. cloacae* has been reported to be an effective biological control agent against seeds and seedling rots in cucumbers, peas, beets and cottons by *Pythium* spp.[3,15)], dollar spot in bentgrass by *Sclerotinia homoecarpa*[16)] and *Fusarium* dry rot of potatoes[24)]. On the other hand, *Serratia ficaria* has not been documented to be antagonistic to any species of plant-pathogenic fungi, although other species of *Serratia*, including *S. marcescence*[8,20–22)], *S. entomophila*[2)], *S. liqufaciens*[9)], *S. plymuthica*[9,12)] and *S. rubidaea*[9)], have been reported.

E. cloacae T-1-14 and *S. ficaria* T-1-8 and T-1-23 clearly suppressed the incidence of *Phytophthora* root rot of angelica trees in pot tests. In pot test 2, they suppressed the disease for at least 79 days (November), and the suppression lasted until the next June. Because we found evidence of strong antagonistic activity of these strains against *P. cactorum* in pot tests, we did two kinds of field tests. The first was a test in naturally infested fields (with a high natural population of the pathogen in soil), and the second was a test in artificially infested fields (with a lower population of the pathogen than the former). In severely infested fields, a significant suppression was detected at 30 (T-1-8) and 47 days (T-1-23). On the other hand, in the moderately infested field, T-1-8, T-1-23 and T-1-14 continued to significantly suppress the disease until the last observation (day 63). These findings suggest that the level of biocontrol activity of these strains was dependent on the population level of the pathogen in soil. The reason disease suppression in the field tests was weaker than in pot tests is still unknown. The ability of these bacterial strains, such as producing an antifungal substance or surviving in the rhizosphere may

Table 7. Bacteriological properties of the strains isolated from the rhizosphere of angelica trees

Characteristic	T-1-14	T-1-8	T-1-23	*Enterobacter cloacae*[a)]	*Serratia ficaria*[a)]
Gram stain	−[b)]	−	−	−	−
Oxidase	−	−	−	−	−
Indole production	−	−	−	−	−
Methyl red	−	+	+	−	d
Voges-Proskauer	+	−	+	+	d
Citrate (Simmons)	+	+	+	+	+
H_2S production	−	−	−	−	−
Urea hydrolysis	−	−	−	d	−
Phenylalanine deaminase	−	−	−	−	−
Lysine decarboxylase	−	−	−	−	−
Motility	+	+	+	+	+
Gelatin hydrolysis	−	+	+	−	+
D-Glucose, acid production	+	+	+	+	+
D-Glucose, gas production	+	−	−	+	−
Acid production :					
D-Adonitol	−	−	−	−	−
L-Arabinose	−	+	+	+	+
Cellobiose	+	+	+	+	+
Dulcitol	−	−	−	−	−
Glycerol	+	+	+	d	−
myo-Inositol	−	+	+	−	d
Lactose	+	+	+	+	−
Maltose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
D-Mannose	+	+	+	+	+
Melibiose	+	+	+	+	d
Raffinose	+	+	+	+	d
L-Rhamnose	+	+	−	+	d
Salicin	+	+	+	+	+
D-Sorbitol	+	+	+	+	+
Sucrose	+	+	+	+	+
Trehalose	+	+	+	+	+
D-Xylose	+	+	+	+	+
Esculin hydrolysis	+	+	+	d	+
Nitrate reduction	+	+	+	+	+
Deoxyribonuclease	−	+	+	−	+
Lipase	−	+	+	−	+
ONPG	+	+	+	+	+
Pigment	−	−	−	−	−
Flagella arrangement	P[c)]	P	P	P	P
Catalase production	+	+	+	+	+
Oxidation-fermentation	F[c)]	F	F	F	F

a) Data from Bergey's Manual of Determinative Bacteriology[5)].

b) + : positive reaction, − : negative reaction ; In the case of reference strains (Bergey's), the following standard was used : + : 80% or more of strains positive, − : 20% or less of strains positive, d : 21–79% of strains positive.

c) P : Peritrichous flagella, F : Fermentative.

be different in pot tests and field tests. For practical use of these strains, the relationship between the level of disease suppression and the population level of pathogen in field soil will need to be examined more in detail.

Antagonistic bacteria were frequently isolated from the rhizosphere of angelica trees rescued from severe infection, but not from severely infected trees. This finding suggests that biological control by these bacteria occurs continually under natural conditions. Since *E. cloacae* is one of the most abundant bacteria found in the rhizo-

sphere of a variety of plants[10], a high population of *E. cloacae* could accumulate in the rhizosphere of some angelica trees and protect them from severe infection. This phenomenon may be significant from the viewpoint of practical use of *E. cloacae* (and also, probably, of *S. ficaria*), since these strains may have the ability to survive for long periods of time in rhizospheres under natural conditions.

We previously demonstrated that all the strains that were isolated as antagonists to *P. capsici* P-9-2 (the pathogen of damping-off disease of the cucumber) or *F. oxysporum* f. sp. *raphani* F-1-6-3 (the pathogen of yellows of Japanese radish) inhibited mycelial growth of *P. cactorum* ARE-862 (*in vitro* tests). However, most of these strains, except for V-2-3 and V-3-1, were much less antagonistic in pot tests than were the three angelica strains. The mechanism for inhibition of mycelial growth *in vitro* may be different from that for disease suppression in plants. Or, these strains may be inferior to the three angelica strains in terms of their fitness in the rhizosphere of angelica trees.

The mechanisms of disease suppression by *E. cloacae* have been clarified for various other diseases. Howell *et al.*[6] indicated that ammonia production by *E. cloacae* may be involved in the suppression of *Pythium* preemergence damping off. In addition, Nelson *et al.*[14,16] showed that the growth inhibition of *Pythium ultimum* was associated with the binding of *E. cloacae* to fungal hyphae. Similar mechanisms may have been involved in the disease suppression by *E. cloacae* T-1-14 in this study. On the other hand, disease suppression by *S. ficaria* and its mechanisms have not been reported. However, the mechanisms of disease suppression by other species of *Serratia* have been reported. For example, prodigiosin[20,21] and chitinase[8,23] produced by *S. marcescens* are known to be involved in disease suppression. Whether our strains T-1-8 and T-1-23 produce chitinase or any other antifungal substances was undetermined, although we did determine that they do not produce prodigiosin (*i.e.*, do not form red colonies). To verify the suppressive mechanisms of these strains, further studies will be needed on their antifungal substance productivity and their fitness-ability on roots.

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Explanation of plate

Plate I

1. Symptoms of *Phytophthora* root rot of angelica tree naturally infected with *Phytophthora cactorum* in Kyoto prefecture (Kuta, Sakyo-ku, Kyoto city).
2. Severe *Phytophthora* root rot of angelica trees in summer in Kyoto prefecture (Inden-cho, Ayabe city).
3. A screening method for antagonistic bacteria : Inhibition zones of *P. cactorum* ARE-862 on PDA medium induced by *Enterobacter cloacae* T-1-14 after 7 days of incubation.
4. Suppression of the incidence of *Phytophthora* root rot of angelica trees by treatment with *Enterobacter cloacae* T-1-14 (root dip). Right, treated plant ; Left, untreated plant.
5. Antagonistic bacteria : *Serratia ficaria* T-1-8 (Bar represents 1 μm).
6. Antagonistic bacteria : *Enterobacter cloacae* T-1-14 (Bar represents 1 μm).

Plate I