Protection of Tomato Seedlings by Pre-treatment with *Pseudomonas glumae* from Infection with *Pseudomonas solanacearum* and Its Mechanisms*

Naruto FURUYA**, Yoshiyuki KUSHIMA†, Kenichi TSUCHIYA††,
Nobuaki MATSUYAMA** and Satoshi WAKIMOTO**

Abstract

Pseudomonas glumae strains showed different antibiotic activities against *P. solanacearum* depending upon kind of media used. When triphenyl tetrazolium chloride medium (TTC medium) was used, all strains of *P. glumae* formed growth inhibition zones around their colonies on the lawn of *P. solanacearum* used as indicator. Pre-treatment of the roots of tomato seedlings with the suspension of antibiotic productive strains of *P. glumae* suppressed the severity of wilt incited by challenge inoculation with *P. solanacearum*. The disease suppression was positively correlated with length of dipping time and concentration of the bacterial suspension used for pre-treatment. Dipping of the roots of tomato seedlings into the bacterial suspension of ca. 10^{10} cfu/ml for 24 hr showed highest suppressiveness. The pre-treatment of tomato roots with non-antibiotic-producing strain or heat-killed cells of *P. glumae* also showed protection, suggesting that some mechanisms other than antibiotic productivity were involved in the suppression of the disease.

(Received October 2, 1990)

Key words: *Pseudomonas solanacearum*, *Pseudomonas glumae*, antibiotic productivity, biological control.

INTRODUCTION

Bacterial wilt of tomato caused by *Pseudomonas solanacearum* (Smith 1896) Smith 1914 is one of the most serious diseases of tomato in the world. The causal bacterium can attack not only various solanaceous plants but also many plants belonging to other families¹¹. *P. solanacearum* multiplies even under the nutritionally limited conditions such as in soil^{20,24} and water^{13,28}, and survives for many years. Thus, the bacterial wilt of tomato is one of the typical soil-borne diseases¹. For control the disease, some measures such as cultivation of resistant varieties¹⁹, crop rotation every several years, and soil disinfection by chemical application have been recommended. All of these procedures, however, are not sufficiently effective. Some other effective methods have been expected to be developed.

Since successful application of the bacteriocin productive strain K84 of *Agrobacterium radiobacter* against crown gall of stone fruits^{15,16}, the studies on biological methods to control bacterial diseases of plant have been emphasized. Chen and Echandi^{2,3} reported the efficacy

* This work was supported by a Grant-in-Aid for Scientific Research No. 01440008 (1989).

** Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan 九州大学農学部

† Present address: Miyazaki Agriculture Research Center, Sadowara, Miyazaki 880-02, Japan
現在: 宮崎総合農業試験場

†† Present address: National Institute of Agrobiological Resources, Tsukuba 305, Japan 現在: 農業生物資源研究所

of an avirulent bacteriocin-producing strains of *P. solanacearum* on the control of bacterial wilt of tobacco. Tanaka *et al.*²³⁾ also showed that tobacco plants were protected from bacterial wilt with an avirulent strain M4S of *P. solanacearum* and its bacteriophages.

The authors previously demonstrated²⁹⁾ that strains of *P. glumae* Kurita *et Tabei* 1967, the causal bacterium of grain rot of rice, produced antibacterial substances showing wide activity spectrum against plant pathogenic bacteria including *P. solanacearum*. The efficacy of *P. glumae* strains on the protection of tomato seedlings from infection with *P. solanacearum* and its mechanisms were investigated.

MATERIALS AND METHODS

***Pseudomonas glumae*.** Forty-seven strains of *P. glumae* shown in Table 1 were used in this experiment. These strains were collected from various localities of Japan and preserved in the Laboratory of Plant Pathology, Kyushu University. Most of these strains were virulent to rice seedlings while some others were avirulent. The avirulent strains were considered to be the mutants which appeared during preservation by means of successive transfers on the medium in the past. These strains were cultured on the slants of PSA medium (decoction of 300 g potato 1 liter, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 2 g, $\text{Ca}(\text{NO}_3)_2$ 0.5 g, peptone 5 g, sucrose 15 g, agar 15 g, pH 7.0) at 30°C for 2 to 3 days, suspended in sterile distilled water at the concentration of *ca.* 10^7 cfu/ml in test tubes, and preserved at 22–25°C during this experiment. For long term preservation, the bacteria were lyophilized with PS broth and kept at 4°C.

The bacterial cells were preliminary grown on the PSA slants at 30°C for 48 hr, suspended in 10 ml of sterile distilled water, added the suspension to 200 ml of PS broth in Sakaguchi flask, and shaken at 30°C for 48 hr. The bacterium was harvested by centrifugation at $6,000 \times g$ for 20 min, and resuspended in sterile distilled water at the concentration of *ca.* 10^{10} cfu/ml. The suspension was diluted 10 and 100 times just before use. Viable cells were counted by plating on PSA medium. Bacterial suspensions thus prepared were used for protecting tomato seedlings from the infection with *P. solanacearum*.

***Pseudomonas solanacearum*.** Of six *P. solanacearum* strains²⁸⁾ (N6211, N6275, KT-2, Ku7501-1, Ku7502-1, Ku7601-1), the strain KT-2^{28,29)} was used most frequently because of its strongest virulence to tomato plants. These strains suspended in sterile distilled water were maintained at 25–27°C. Each stock suspension was spread on TTC medium¹²⁾ (peptone 10 g, casein hydrolysate (Difco) 1 g, glucose 5 g, agar 17 g, triphenyl tetrazolium chloride (1% solution) 5 ml, distilled water 1 liter), and the plates were incubated at 30°C for 48 hr for selecting virulent colonies. Typical virulent colonies were isolated and grown in PS broth on a rotary shaker at 30°C for 48 hr. Cells were harvested by centrifugation at $6,500 \times g$ for 20 min and resuspended in sterile distilled water to give concentrations of *ca.* 10^8 , 10^9 and 10^{10} cfu/ml to be used as inoculum. Viable cells were counted by plating method on TTC medium. If the inoculum concentration was lower than 10^8 cfu/ml, the disease did not occur sufficiently (data not shown).

***In vitro* antibiotic activity of *P. glumae* to *P. solanacearum*.** To assess the antibiotic activity of *P. glumae* to *P. solanacearum* *in vitro*, the fresh culture of *P. glumae* grown on the PSA slant for 1 to 2 days was spotted at the center or at 3 points keeping distance from one another on the plates of various media such as YPDA²⁹⁾, PSA and TTC medium, in petri dishes 9 cm in diameter. After incubation at 30°C for 72 hr, bacterial cells were killed by chloroform vapor as reported previously²⁹⁾. The suspension (0.2 ml) of *P. solanacearum* (conc. *ca.* 10^8 cfu/ml) mixed with 5 ml of plain agar (0.5%) was poured over the bottom layer, and the plate was incubated at 30°C for 48 hr. The antibiotic activity was evaluated by measuring the widths of inhibition zones.

Induction of non-antibiotic-producing mutants of *P. glumae* by NTG treatment. A wild strain of *P. glumae*, N750, was cultured on the YPDA medium containing streptomycin

sulfate (500 $\mu\text{g/ml}$) to obtain streptomycin resistant (SR) mutants. The streptomycin resistant parental strain, N750-SR, thus obtained was grown in YPD broth under shaking conditions at 30°C for 24 hr. The bacterial cells were washed twice in sterile phosphate buffer (0.067 M, pH 7.0) by centrifugation. The final pellet was resuspended to make the concentration of *ca.* 10^7 cfu/ml, which was treated with *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine (TNG) at the concentration of 50 $\mu\text{g/ml}$ in the same buffer at 30°C for 20 min. After the treatment, cells were washed twice in the same buffer to remove NTG. A part of bacterial suspension was transferred to YPD broth and incubated overnight for multiplication of surviving cells. The culture was then diluted, spread on YPDA plates containing streptomycin sulfate (500 $\mu\text{g/ml}$) and incubated for 3 to 4 days at 30°C. Single colonies were isolated and each isolate was measured for antibiotic activity by plating chloroform method using various media containing *P. solanacearum* as indicator. Thus, streptomycin resistant and non-antibiotic-productive strain, N750-SR-NP, was obtained.

Heat-killed cells of *P. glumae*. The bacterial cells of *P. glumae* strain N750 grown on the slant of PSA medium at 30°C for 48 hr were suspended in sterile distilled water (conc. *ca.* 10^{10} cell/ml). The suspension was incubated at 100°C for 10 min in a water bath and used for testing its suppressiveness to tomato wilt.

Tomato plants. To evaluate the suppressiveness of *P. glumae* cells to bacterial wilt, the tomato cultivar Toko was used. The seeds were disinfected by dipping in a solution of 3% hypochlorite (Antiformin) at room temperature for 15 min, rinsed with tap water, sown on the autoclaved soil in the pots of 10 cm in diameter, and incubated in the air-conditioned greenhouse at 25–30°C. Two weeks later, the plants grown up to 10 cm in height were used.

Pot assay for bacterial wilt suppression. After pulling out the tomato seedlings, their roots were rinsed with tap water and dipped in the suspension of *P. glumae* (conc. *ca.* 10^8 , 10^9 and 10^{10} cfu/ml) for a certain period from 10 min to 24 hr (pre-treatment). The roots thus treated were then dipped in *P. solanacearum* suspension (conc. *ca.* 10^8 , 10^9 and 10^{10} cfu/ml) for 10 min (post-inoculation). Each plant thus treated was transplanted in the plastic pot (6×6×4.5 cm) containing fertilized nursery soil which was heat sterilized previously. The plants were grown in the air-conditioned greenhouse at 25–30°C. The soil in the pots was kept uniformly under wet conditions. The disease severity was recorded 5-day intervals on a scale of 0 to 3, where 0=no wilt; 1=1–50% leaves wilted; 2=more than 50% leaves wilted; 3=entire plant wilted or dead.

RESULTS

Antibiotic activity of P. glumae in vitro

All strains of *P. glumae* used in this study showed antibiotic activity against *P. solanacearum* KT-2 on TTC medium. The activities, however, were different depending upon the kind of media used (Table 1). The antibiotic activity of *P. glumae* exhibited on YPDA medium did not show remarkable variation among six strains of *P. solanacearum* used (data not shown).

Protection efficacy shown by P. glumae

The suppressiveness of the strain N750 of *P. glumae* to bacterial wilt varied depending upon the duration of the dipping for pre-treatment of tomato seedlings. The suppression was slight when seedlings were dipped in bacterial suspension for 10 min, while it increased in parallel with the duration of dipping. When the seedlings were dipped for 12 or 24 hr, the control was remarkable (Table 2). The suppressive effect also varied depending upon bacterial concentrations of both pre-treatment and post-inoculation. The effect was the highest at *ca.* 10^{10} cfu/ml of *P. glumae*, while it decreased at 10^8 and 10^9 cfu/ml. When inoculum concentration was *ca.* 10^8 cfu/ml, the higher suppressive effect was obtained than that with 10^{10} cfu/ml (Table 3). Although *P. glumae* strains Ku8117, 805, 752, P1-22-4 and YN7825 showed the different extent of antibiotic activity *in vitro*, they did not show any significant variation in the disease suppress-

siveness (Table 4). The non-antibiotic-producing strain (N750-SR-NP) also suppressed disease to the same level as that shown by antibiotic producing wild strain (N750) (Table 5). Furthermore, the heat-killed cell suspension of *P. glumae* N750 also showed protection similar to those incited by living bacteria (Table 6).

Table 1. Effect of the media on the formation of growth-inhibition zone by *Pseudomonas glumae* strains on the lawn of *P. solanacearum*

<i>P. glumae</i>	YPDA	PSA	TTC	<i>P. glumae</i>	YPDA	PSA	TTC
N7401	— ^{a)}	—	+	8028	+++	—	+
N7501	++	—	+	1	+++	++	+
N7502	—	+	+++	111	++	++	+++
N7503	+	—	++	2	+++	+	++
N7504	—	—	++	Ku8101	+	+	++
N7505	++	++	+++	Ku8102	+	+	+
YN7805	+++	—	++	Ku8103	++	—	++
YN7810	—	++	++	Ku8104	+	—	+
YN7825	—	+	++	Ku8105	++	++	++
742	+++	—	+++	Ku8106	+++	+	++
N750	+++	—	+++	Ku8111	+	+	+++
752	+	++	+++	Ku8112	++	+++	+
805	+++	+++	+++	Ku8113	+++	+	+++
806	+	++	++	Ku8114	+++	++	++
P1-22-1	+++	—	+++	Ku8115	+	—	+
P1-22-2	+	—	++	Ku8116	+	++	+
P1-22-3	+	—	+	Ku8117	+	—	+
P1-22-4	+	—	+	Ku8119	++	—	+
So-1	+++	—	++	Ku8120	+++	—	+++
8001	+	—	+	Ku8121	+++	+++	+++
8012	+	+	+	Ku8122	+++	++	++
8015	—	—	++	Ku8123	++	+	++
8017	+++	—	+	Ku8124	+++	+	++
8020	+	—	++				

a) Width of inhibition zone: — not detected, + below 5 mm, ++ 5–10 mm, +++ above 10 mm.

Table 2. Protection of tomato seedlings from bacterial wilt^{a)} by pre-treatment with the suspension of *Pseudomonas glumae* strain N750

Time of pre-treatment (dipping)		Disease index ^{c)}		
		10	20	30 days
10 min	Treated ^{a)}	1.15 NS ^{d)}	1.85 NS	2.10 NS
	Control ^{b)}	1.10	2.00	2.15
60 min	Treated	1.30 NS	2.00 NS	2.30 NS
	Control	1.05	2.00	2.20
12 hr	Treated	0.05**	0.15***	0.15***
	Control	1.20	2.00	2.25
24 hr	Treated	0.00**	0.15***	0.15***
	Control	1.00	1.80	2.40

a) Tomato seedlings were pre-treated with a suspension of *P. glumae* (ca. 10^{10} cfu/ml) and inoculated with *P. solanacearum* strain KT-2 (ca. 10^8 cfu/ml) by dipping their roots just after pre-treatment.

b) Sterile distilled water was used instead of a suspension of *P. glumae* in the case of pre-treatment.

c) Disease index based on a scale from 0, no visible symptom to 3, entire plant wilted or dead.

d) NS: Not significantly different by paired *t*-test, ***, **: Statistically different by paired *t*-test, $P=0.001$ and 0.01 , respectively.

DISCUSSION

Non-pathogenic and bacteriocin productive strains of *P. solanacearum* have been reported to be applicable for biological control of bacterial wilt of solanaceous plants caused by *P. solanacearum*^{2,3,26}. The mechanism of the suppression of the disease was considered to be the inhibition of the multiplication of pathogenic bacteria by bacteriocin producing non-pathogenic

Table 3. Protection of tomato seedlings from infection of *Pseudomonas solanacearum* KT-2 by pre-treatment with suspension of *P. glumae* N750

<i>P. glumae</i> ^{a)} (cfu/ml)	<i>P. solanacearum</i> ^{b)} (cfu/ml)	Disease index ^{c)}		
		10	20	30 days
10 ⁸	10 ⁸	0.65 B ^{d)}	0.60 BD	0.95 BC
10 ⁹	10 ⁸	0.50 B	0.50 CD	0.75 C
10 ¹⁰	10 ⁸	0.15 B	0.15 D	0.15 C
0	10 ⁸	1.45 A	1.90 A	2.10 A
10 ⁸	10 ¹⁰	1.35 A	1.60 A	1.85 AB
10 ⁹	10 ¹⁰	1.05 A	1.70 A	1.75 AB
10 ¹⁰	10 ¹⁰	0.90 B	1.10 ABC	1.05 BC
0	10 ¹⁰	1.35 A	1.90 A	2.10 A

a) Pre-treatment for 24 hr.

b) Dip inoculation for 10 min.

c) Disease index based on a scale from 0, no visible symptom to 3, entire plant wilted or dead.

d) Within columns, means with the same letter are not significantly different ($P=0.05$) according to the Duncan's multiple range test.

Table 4. Protection of tomato seedlings from bacterial wilt by pre-treatment with the suspension of various *Pseudomonas glumae* strains

<i>P. glumae</i> ^{a)} (strain)	Disease index ^{b)}		
	5	15	30 days
Ku8117	0.00 B ^{c)}	0.33 B	0.50 B
805	0.20 B	0.80 B	0.93 B
752	0.00 B	0.39 B	0.67 B
P1-22-4	0.00 B	0.44 B	0.50 B
YN7825	0.27 B	0.80 B	0.87 B
Control	2.20 A	3.00 A	3.00 A

a) The roots were dipped in the suspension of ca. 10¹⁰ cfu/ml for 24 hr.

b) Disease index based on a scale from 0, no visible symptom to 3, entire plant wilted or dead.

c) Within columns, means with the same letter are not significantly different ($P=0.05$) according to the Duncan's multiple range test.

Table 5. Effect of the non-antibiotic-producing mutant (N750-SR-NP) of *P. glumae* on the protection of tomato seedlings from bacterial wilt

Strain ^{a)}	Antibiotic productivity	Disease index ^{b)}		
		5	15	30 days
N750	+	0.00 B ^{c)}	0.35 B	1.20 B
N750-SR-NP	-	0.00 B	0.90 B	0.90 B
Control		1.55 A	2.85 A	3.00 A

a) The roots were dipped in the suspension of ca. 10¹⁰ cfu/ml for 24 hr.

b) Disease index based on a scale from 0, no visible symptom to 3, entire plant wilted or dead.

c) Within columns, means with same letter are not significantly different ($P=0.05$) according to the Duncan's multiple range test.

Table 6. Effect of the heat-killed cells of *P. glumae* N750 on the protection of tomato seedlings from bacterial wilt

Pre-treatment ^{a)}	Disease index ^{b)}		
	5	15	30 days
Living bacteria	0.00 B ^{c)}	0.56 B	0.67 B
Heat-killed bacteria	0.00 B	0.77 B	0.87 B
Water control	0.91 A	2.73 A	2.78 A

a) Pre-treatment for 24 hr.

b) Disease index based on a scale from 0, no visible symptom to 3, entire plant wilted or dead.

c) Within columns, means with same letter are not significantly different ($P=0.05$) according to the Duncan's multiple range test.

bacteria³⁾.

A matter of great interest in the characteristics of *P. glumae* is the productivity of antibiotics which inhibit growth of various plant pathogenic bacteria including *P. solanacearum*²⁹⁾. In this experiment, we found that the antibiotic productivity varied depending upon bacterial strains and the kind of media used. However, all strains of *P. glumae* showed antibiotic activity against *P. solanacearum* on TTC medium *in vitro*.

The productivity of antibiotics effective to *P. solanacearum* leads us to make an attempt to use *P. glumae* as an agent for biological control. Dipping the roots of tomato seedlings in a suspension of *P. glumae* just before inoculation with *P. solanacearum* showed a remarkable disease suppression. The efficacy of suppression varied with concentrations of *P. glumae* used for pre-treatment and post-inoculation and duration of dipping for pre-treatment.

With regard to the mechanisms of the disease suppression by biological agents, many reports attributed it to antibiotic activity of antagonists^{6-10,17,25,27,30)}, contrasting to other results^{4,5,18,21)}. Tanaka²²⁾ revealed that tobacco and tomato plants treated with heat-killed cells of *P. solanacearum* suppressed bacterial wilt. Kempe *et al.*¹⁴⁾ demonstrated that the induced resistance by pre-treatment with pseudomonads may be involved in the mechanism of suppression of bacterial wilt of potato. To investigate the mechanisms of disease suppression, non-antibiotic-producing mutant of *P. glumae* (N750-SR-NP) induced by NTG treatment and heat-killed bacterial cells were used. The fact that both non-antibiotic-producing living bacteria and heat-killed bacteria suppressed disease severity as in the case of antibiotic producing viable wild strain, suggesting that the role of antibiotic produced by *P. glumae* seems to be slight in the mechanisms of disease suppression. Further studies are needed to clarify the mechanisms in detail.

Literature cited

1. Buddenhagen, I.W. and Kelman, A. (1964). Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Ann. Rev. Phytopathol. 2: 203-230.
2. Chen, W., Echandi, E. and Spurr, H.W., Jr. (1981). Protection of tobacco plants from bacterial wilt with avirulent bacteriocin-producing strains of *Pseudomonas solanacearum*. In Proc. Fifth Int. Cont. Plant Path. Bact., Cali. pp. 482-492.
3. Chen, W.Y. and Echandi, E. (1984). Effect of avirulent bacteriocin-producing strains of *Pseudomonas solanacearum* on the control of bacterial wilt of tobacco. Plant Pathol. 33: 245-253.
4. Cooksey, D.A. and Moore, L.W. (1980). Biological control of crown gall with fungal and bacterial antagonists. Phytopathology 70: 506-509.
5. Cooksey, D.A. and Moore, L.W. (1982). Biological control of crown gall with an agrocin mutant of *Agrobacterium radiobacter*. Phytopathology 72: 919-921.
6. Cook, R.J. (1985). Biological control of plant pathogens: theory to application. Phytopathology 75: 25-29.

7. Homma, Y. and Suzui, T. (1989). Role of antibiotic production in suppression of radish damping-off by seed bacterization with *Pseudomonas cepacia*. Ann. Phytopath. Soc. Japan 55: 643-652.
8. Howell, C.R. and Stipanovic, R.D. (1979). Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69: 480-482.
9. Howell, C.R. and Stipanovic, R.D. (1980). Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70: 712-715.
10. Howell, C.R. and Stipanovic, R.D. (1983). Gliovirin, a new antibiotic from *Gliocladium virens*, and its role in the biological control of *Pythium ultimum*. Can. J. Microbiol. 29: 321-324.
11. Kelman, A. (1953). The bacterial wilt caused by *Pseudomonas solanacearum*. North Carolina Agr. Expt. Sta. Tech. Bull. 99: 1-194.
12. Kelman, A. (1954). The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44: 693-695.
13. Kelman, A. (1956). Factors influencing viability and variation in cultures of *Pseudomonas solanacearum*. Phytopathology 46: 16-17.
14. Kempe, J. and Sequeira, L. (1983). Biological control of bacterial wilt of potatoes: attempts to induce resistance by treating tubers with bacteria. Plant Dis. 67: 499-503.
15. Kerr, A. (1972). Biological control of crown gall: seed inoculation. J. Appl. Bacteriol. 35: 493-497.
16. Kerr, A. and Htay, K. (1974). Biological control of crown gall through bacteriocin production. Physiol. Plant Pathol. 4: 37-44.
17. Kloepper, J.W. and Schroth, M.N. (1981). Relationship of *in vitro* antibiotics of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology 71: 1020-1024.
18. Lindow, S.E. (1985). Integrated control and role of antibiosis in biological control of fire blight and frost injury. In Biological Control of the Phylloplane (Windels, C.E. and Lindow, S.E. eds.). American Phytopathological Society, St. Paul. pp. 83-115.
19. Matsuda, T. (1977). Fundamental studies on the breeding of bacterial wilt resistant varieties in tobacco. Bull. Utsunomiya Tobacco Expt. Sta. 15: 1-90.
20. Okabe, N. (1971). Population changes of *Pseudomonas solanacearum* and soil microorganisms in artificially infested natural soil. Rev. Plant Protec. Res. 4: 105-108.
21. Rovira, A.D. and Wildermuth, G.B. (1981). The nature and mechanisms of suppression. In Biology and Control of Take-All (Asher, M.J.C. and Shipton, P.J. eds.). Academic Press, London. pp. 385-415.
22. Tanaka, H. (1983). Protection of tobacco and tomato against root infection of *Pseudomonas solanacearum* by heat-killed bacterial cells. Ann. Phytopath. Soc. Japan 49: 66-68.
23. Tanaka, H. (1990). Control of tobacco bacterial wilt by an avirulent strain of *Pseudomonas solanacearum* M4S and its bacteriophage. Ann. Phytopath. Soc. Japan 56: 243-246.
24. Tanaka, Y. and Noda, N. (1973). Studies on the factors affecting survival of *Pseudomonas solanacearum* E.F. Smith, the causal agent of tobacco wilt disease. Bull. Okayama Tobacco Expt. Sta. 32: 81-91.
25. Thomashow, L.S. and Weller, D. (1988). Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bact. 170: 3499-3508.
26. Trigalet, A. and Trigalet-Demery, D. (1990). Use of avirulent mutants of *Pseudomonas solanacearum* for the biological control of bacterial wilt of tomato plants. Physiol. Mol. Plant Pathol. 36: 27-38.
27. Utkhed, R.S. and Rahe, J.E. (1983). Interactions of antagonist and pathogen in biological control of onion white rot. Phytopathology 73: 890-893.
28. Wakimoto, S., Utazu, I., Matsuo, N. and Hayashi, N. (1982). Multiplication of *Pseudomonas solanacearum* in pure water. Ann. Phytopath. Soc. Japan 48: 620-627.
29. Wakimoto, S., Hirayae, K., Tsuchiya, K., Kushima, Y., Furuya, N. and Matsuyama, N. (1986). Production of antibiotics by plant pathogenic pseudomonads. Ann. Phytopath. Soc. Japan 52: 835-842.
30. Xu, G.-W. and Gross, D.C. (1986). Selection of fluorescent pseudomonads antagonistic to *Erwinia carotovora* and suppressive of potato seed piece decay. Phytopathology 76: 414-422.

和 文 摘 要

古屋成人・櫛間義幸・土屋健一・松山宣明・脇本 哲：イネもみ枯細菌病菌 (*Pseudomonas glumae*) によるトマト青枯病の発病抑制とその機作

イネもみ枯細菌病菌のトマト青枯病菌に対して示す抗菌活性は供試する培地の種類によって異なり、TTC 培地上ではすべてのイネもみ枯細菌病菌株がトマト青枯病菌に対して阻止帯を形成した。抗菌物質産生性のイネもみ枯細菌病菌株の懸濁液でトマト幼苗の根部を浸漬処理することによって青枯病の発病が抑制された。この発病抑制効果と浸漬処理時間および浸漬処理に用いた細菌懸濁液の濃度との間には高い相関が認められた。トマト幼苗の根部を濃度約 10^{10} cfu/ml のイネもみ枯細菌病菌懸濁液中へ 24 時間浸漬した場合に最も高い抑制効果が得られた。この発病抑制効果は抗菌物質非産生菌株および熱処理死菌によっても認められ、イネもみ枯細菌病菌の産生する抗菌物質以外の要因もこの発病抑制効果の機作に強く関与していることが明らかとなった。