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Biocontrol of *Phytophthora capsici* by *Serratia marcescens* F-1-1 and Analysis of Biocontrol Mechanisms Using Transposon-insertion Mutants

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Abstract

An antagonistic bacterium against *Phytophthora capsici*, the pathogen of damping-off of cucumber, was isolated from the rhizosphere of scarlet sage (*Salvia splendens* F. Sellow ex Roem. et Schult.) in Fukui Prefecture. The red-pigmented strain F-1-1 was identified as *Serratia marcescens* based on its bacteriological properties. The strain F-1-1 strongly inhibited the germination of cystospores and zoosporangia of *P. capsici* P-9-2 in *in vitro* tests, and also it suppressed the damping-off of cucumber seedlings inoculated with a cystospore suspension of *P. capsici* P-9-2 in pot tests. Moreover, all the other red-pigmented *S. marcescens* strains isolated from the rhizosphere of Japanese holly (*Ilex crenata* Thunb.) were antagonistic, unlike six non-pigmented *Serratia* spp. strains. Four mutants defective red pigment biosynthesis were obtained by transposon (Tn*7*) mutagenesis among 5967 transconjugant clones. These mutants simultaneously lost their antagonistic ability in *in vitro* and in pot tests. Thus, *S. marcescens* F-1-1 was found to be a potential biocontrol agent for damping-off of cucumber seedlings and its antagonistic ability was based on the production of antibiotic red pigments.

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Key words: *Serratia marcescens*, *Phytophthora capsici*, damping-off of cucumber seedlings, biocontrol, transposon mutagenesis.

INTRODUCTION

Damping-off of cucumber seedlings caused by *Phytophthora capsici* is widespread and very destructive in Japan. Control of this disease has been done by using chemicals. However, these treatments are not always effective. In addition, breeding of plants resistant to the pathogen has not been successful. Therefore, biological control is a suitable strategy for the control of damping-off. Biological control with rhizobacteria has recently been reported for more than ten genera of bacteria in relation to various soil-borne plant pathogens of crops. However, there have been no reports on antagonistic rhizobacteria for biocontrol of damping-off of cucumber seedlings.

On the other hand, molecular techniques have been used to analyze the relationship between specific toxic metabolites and disease suppression in some plant diseases[7,15]. Especially transposon mutagenesis is the most useful technique for the analysis of biocontrol mechanisms, since mutants showing an impaired production of biochemically or phenotypically distinguishable metabolites can be easily obtained by transposon insertion.

In the present study, we describe the isolation, identification and *in vitro* and *in vivo* biocontrol activity of strain F-1-1. Furthermore, we obtained useful mutants generated by transposon insertion to analyze the mechanisms of biocontrol, and we examined the role of the metabolites produced by the strain in its antagonism.

MATERIALS AND METHODS

Bacterial strains and culture conditions The bacterial strains used in this study are listed in Table 1. For the isolation and culture of 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, 300 g of potato slices extracted in 1 liter of distilled water, pH 7.0) was used. Rifampicin-

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resistant of F-1-1 strain was spontaneously obtained from cultures on modified LB medium (polypeptone 10 g, yeast extract 5 g, NaCl 10 g, agar 15 g, distilled water 1 liter) supplemented with 20 ppm of rifampicin.

Bacterial properties Main bacterial properties were investigated based on the protocol of Nishiyama[17]. For the identification of *Serratia marcescens*, API 20E kit (BIO MERIEUX S.A. Company) was used according to the protocol indicated by the company.

Fungal inhibition assay Inhibition of *P. capsici* P-9-2 by the bacterial strain *in vitro* was assayed on a glass slide. A cystospore suspension of *P. capsici* P-9-2 (10^4 spores/ml) was mixed with an equal volume of bacterial suspension (2.3×10^7 cfu/ml), and the mixture was dropped on a glass slide. After incubation for 6 hr at 28°C, the number of germinated cystospores was counted. About 5000 cystospores in total were scored in one sample.

For the inhibition assay to zoosporangia, zoosporangia were collected after incubation of cystospores under the light at 25°C. Suspension of zoosporangia (10^4 spores/ml) was mixed with an equal volume of bacterial suspension (2.3×10^7 cfu/ml), and cultured by shaking (90 rpm/min) overnight at 25°C. Thereafter, the number of germinated zoosporangia was counted.

Damping-off suppression assay The ability of the bacteria to protect damping-off of cucummber seedlings was determined by pot test. Pot soils of seedlings were treated with a suspension of strain F-1-1 (2.3×10^4–2.3×10^7 cfu/ml) and they were inoculated with a suspension of cystospores of *P. capsici* P-9-2 (10^4 spores/ml, 20 ml/pot) after two days. Tested plants were scored for their survival 15 days after inoculation. Nine cucumber seedlings were used for one experiment (protective-treatment style).

Transposon mutagenesis Transposon Tn*7* was used to generate mutations in chromosomal DNA of strain F-1-1. The plasmid pBPW1::Tn*7*[11,12] was used as a suicidal vector for introducing Tn*7* into recipient cells. Strain F-1-1^{rif} was mated with *Pseudomonas syringae* pv. *tabaci* BR2 (pBPW1::Tn*7*) overnight by using the filter method described previously[12]. The mixture was diluted and plated on modified LB agar containing rifampicin (20 ppm) and streptomycin (50 ppm) to select transconjugants (F-1-1^{rif}::Tn*7*). Transconjugants were purified by single colony isolation and their properties such as trimethoprim (500 ppm)-resistance which was another marker of Tn*7* and absence of plasmid (pBPW1::Tn*7*) were investigated.

Molecular genetic technique Plasmid DNA was extracted by the method described by Birnboim and Doly[2], and detected by electrophoresis at 80 mA and 80 volts in 0.8% agarose gel. Chromosomal DNA was extracted according to a slight modification of the method of Staskawicz *et al.*[13]. For the isolation, all the DNA were subjected to purification on CsCl-ethidium bromide gradients.

Dot blot hybridization using Tn*7* DNA probe was performed as follows; *Hin*dIII cleavage site (1.55 and 1.75 Mdal) of Tn*7*[10] was selected as a probe. The DNA fragment was isolated from DNA bands appearing by low melting agarose gel electrophoresis by using a standard method[16]. The DNA fragments were labeled with photobiotin by using the photobiotin-labeling and detection kit of BRESA company.

The labeling and colorimetric detection of the biotinylate probe were performed according to the protocol of the company. Dot blot hybridization was carried out as follows; Five μl of DNA solution (250 ng and 25 ng of DNA) of each sample was spotted on nitrocellulase followed by baking (80°C, 2 hr). The DNA probe was denatured by incubation at 95°C for 5 min. The biotinylated probe concentration in the hybridization solution was *ca.* 50 ng/ml.

Table 1. Bacterial strains used in the present study

Bacterial strain	Relevant properties[a]	Source/reference
Serratia marcescens		
F-1-1	Red$^+$	Rhizosphere[b]
F-1-1^{rif}	Red$^+$, Rif^r	This study
F-1-1^{rif}::Tn*7* W15	Red$^-$, Rif^r, SM^r, TP^r	This study
F-1-1^{rif}::Tn*7* W18	Red$^-$, Rif^r, SM^r, TP^r	This study
F-1-1^{rif}::Tn*7* W21	Red$^-$, Rif^r, SM^r, TP^r	This study
S-1-1-1	Red$^+$	Matsuda, I.
JCM 1239	Red$^+$	JCM[c]
T1-T17	Red$^+$	Rhizosphere[d]
Serratia spp. (6 species)	Red$^-$	See Table 2
Pseudomonas syringae pv. *tabaci* BR2 (pBPW1::Tn*7*)	SM^r, TP^r	See reference[11]

a) Red$^+$, red-pigmented colony; Red$^-$, non-red pigmented (white) colony; Rif^r, Resistant to rifampicin (20 ppm); SM^r, Resistant to streptomycin (50 ppm); TP^r, Resistant to trimethoprim (500 ppm).
b) Isolated from the rhizosphere of scarlet sage (*Salvia splendens*).
c) Japan Collection of Microorganisms (RIKEN).
d) Isolated from the rhizosphere of Japanese holly (*Ilex crenata*).

RESULTS

Isolation of bacteria

Over 1000 bacterial strains were collected from the rhizosphere and rhizoplane of various plants and from a mushroom, and were screened for the ability to inhibit the germination of cystospores of *P. capsici* P-9-2 and to protect cucumber seedlings from damping-off. Among them, several potential strains for biocontrol were detected. Especially one red-pigmented strain designated as F-1-1 which was isolated from the rhizosphere of scarlet sage (*Salvia splendens*) in Fukui Prefecture was selected for this study. Thereafter, seventeen red-pigmented bacterial strains designated as T1–T17 were

isolated from only the rhizosphere and rhizoplane of Japanese holly (*Ilex crenata*) in Fukui Prefecture but not from any other samples including 21 species of plants. These strains were used for comparative tests of F-1-1 strain.

Bacteriological properties

Strain F-1-1 consisted of Gram-negative rods was motile with peritrichous flagella (Plate I-1) and formed red-pigmented colonies (Plate I-5). It showed a positive reaction in the following tests; Voges-Proskauer test, gelatin hydrolysis, DNase activity, catalase activity, H_2S production, utilization of citrate and acetate, lysine and ornithine decarboxylase; but negative for oxidase activity, MR test, indole production, and utilization of arginate and malonate. These properties were almost the same as those of JCM 1239 of *S. marcescens* used as a control strain except for the MR test.

These data strongly suggested that F-1-1 strain belongs to *S. marcescens*. Furthermore, to confirm this assumption, the bacterial properties of F-1-1 strain were investigated by using an API 20E identification kit. As shown in Table 5, the profile index, which is expressed based on 27 biochemical properties, was 530776157 and it coincided with those of *S. marcescens*. From these results, F-1-1 strain was identified as *S. marcescens*. Other red-pigmented strains, T1–T17, showed similar results in main bacteriological properties using the API 20E kit (data not shown). These strains, therefore, also were identified as *S. marcescens*.

Fungal inhibition assay

S. marcescens F-1-1 and other *Serratia* spp. strains were assayed for *in vitro* inhibition of the germination of cystospores of *P. capsici* P-9-2. When the cystospores of *P. capsici* P-9-2 were mixed with various concentrations of F-1-1 strain, they displayed a red color 2 hr after mixing, and their germination was strongly inhibited (Plate I-3,4). The inhibition of germination by F-1-1 strain was observed even when the bacterial suspension was diluted at 1/16 (Table 2). *S. marcescens* JCM1239 and S-1-1-1 also showed a similar fungal inhibition ability, although their antagonistic ability was lower than that of F-1-1 strain. Both strains lost their antagonistic ability when diluted at 1/8, whereas, other species of *Serratia* with white colonies did not affect the germination of *P. capsici* P-9-2. However, *S. plymuthica* JCM1244 of which shows pinkish white colonies exhibited a slight inhibition ability. Thus, F-1-1 strain was the most antagonistic strain against *P. capsici* P-9-2.

Next, the effect of other red-pigmented strains of *S. marcescens* isolated from the rhizosphere of Japanese holly (*I. crenata*) on the germination of cystospores and zoosporangia of *P. capsici* P-9-2 was investigated (Table 3 and Plate I-2). To compare the strength of antifungal activity among bacterial strains including F-1-1, germination rates of cystospores and zoosporangia of *P. capsici* P-9-2 inoculated with each bacterial suspension of "1/4 dilution" were determined according to Duncan's multiple range test. As a result, the strength of antifungal activity varied with the bacterial strains according to Duncan's multiple range test. Strain F-1-1 exhibited the most powerful ability to inhibit the germination of cystospores (B rank against E rank of control) and zoosporangia (A rank against D rank of control).

Damping-off suppression assay

Effect of treatments with a bacterial suspension of *S. marcescens* F-1-1 on the suppression of damping-off of cucumber seedlings in pots was investigated. When roots of seedlings were treated with a high population of bacterial suspension (2.3×10^7 cfu/ml), the disease was

Table 2. Effect of inoculation of *Serratia marcescens* F-1-1 or *Serratia* spp. on germination of cystospores of *Phytophthora capsici* P-9-2

Bacterial strain	Colony color	Germination of cystospores of *P. capsici* P-9-2				
		1[a]	1/2	1/4	1/8	1/16
F-1-1	Red	−[b]	−	+	+	+
S. marcescens JCM[c] 1239	Red	−	−	−	3+	3+
S. marcescens S-1-1-1	Red	−	−	2+	3+	3+
S. ficalia JCM 1241	White	+	4+	4+	4+	4+
S. fonticola JCM 1242	White	2+	3+	4+	4+	4+
S. odorifera JCM 1243	White	3+	4+	4+	4+	4+
S. plymuthica JCM 1244	White[d]	+	4+	4+	4+	4+
S. orimesii JCM 5910	White	2+	3+	4+	4+	4+
S. liquefaciens JCM 5910	White	4+	4+	4+	4+	4+
Water (control)		4+				

a) Non-diluted bacterial suspension was applied at a concentration of 2.3×10^7 cfu/ml. 1/2-1/16 showed dilution of bacterial suspension.
b) Germination rate: −, 0 (%); +, 1-10 (%); 2+, 11-49 (%); 3+, 50-79 (%); 4+, 80-100 (%).
c) JCM: Japan Collection of Microorganisms (RIKEN).
d) Pinkish white.

Table 3. Effect of inoculation of *Serratia marcescens* strains on germination of cystospores and zoosporangia of *Phytophthora capsici* P-9-2

Bacterial strain	Germination rate (%) of *P. capsici* P-9-2	
	Cystospores	Zoosporangia
T-3[a]	41.0 AB[b]	25.8 A
T-4	58.5 CD	30.0 A
T-5	34.5 A	52.2 BC
T-6	58.4 CD	58.6 C
T-10	64.0 DE	60.4 C
T-14	65.2 DE	46.4 B
T-16	49.9 BC	23.7 A
F-1-1	43.1 B	27.7 A
Water (control)	69.7 E	72.8 D

a) Bacterial suspension was used at a dilution of 1/4 (see Table 2).
b) Values followed by the same letter are not significantly different ($p=0.01$), according to Duncan's multiple range test (inhibition-grade; A>B>C>D>E).

clearly suppressed. Even when the roots were treated with a diluted bacterial suspension (1/10 to 1/100), some degree of suppression was observed (Table 4).

Transposon mutagenesis

The relationship between the production of red pigments by a strain of *S. marcescens* F-1-1 and its antagonism was investigated. Mutants defective in red pigment biosynthesis (Red$^-$) were obtained by Tn*7* mutagenesis. Transconjugants (rifampicin 20 ppm, streptomycin 50 ppm resistant) were recovered at a frequency of 1.0×10^{-4} per recipient cell. Of these 5967 transconjugants, four Red$^-$ clones were detected in several matings.

The mutants without red pigments still displayed the original bacteriological properties of their parent strain F-1-1, when investigated using APE 20E, the identification kit of enterobacteriaceae. Moreover, they formed white colonies, with a morphology very similar to that of F-1-1 strain except for the color. These mutants acquired two new markers of streptomycin (50 ppm) and trimethoprim (500 ppm) resistance which were double markers of Tn*7*, whereas their parent strain was sensitive to both antibiotics (Table 5 and Plate I-5). It was also confirmed that the plasmid pBPW1::Tn*7* was not detected in these mutants, indicating that the plasmid acted as a suicidal vector. Since strain F-1-1 has no indigenous plasmid, a foreign plasmid introduced into the strain can be easily detected. Moreover there is no possibility of spontaneous mutation, since the mutation frequency of double markers should be less than at least 10^{-14} ($10^{-7}\times10^{-7}$). Furthermore, we could not detect any spontaneous mutants with white colonies among more than 20,000 clones. These results suggest that the mutants were induced by transposon insertion into the chromosomes of F-1-1 strain.

To confirm the insertion of the transposon into chromosomes by molecular analysis, the following experiments were conducted. Total DNA was extracted from a wild type, a rifampicin-resistant strain and two Red$^-$ mutants (W15 and W18) of strain F-1-1, respectively, and assayed for dot blot hybridization. As a result, typical colorimetric detection of the biotinylated probe was confirmed in chromosomal DNAs from two Red$^-$ mutants and pBPW1::Tn*7* DNA of a positive control (Table 5), whereas both DNAs from strains F-1-1 (wild type) and F-1-1^{rif} did not hybridize to Tn*7* DNA probe. Thus, Tn*7* insertion into the genome of strain F-1-1 was confirmed.

Antagonistic assay of transposon mutants

In vitro antagonistic assay of transposon mutants was investigated. None of the three Tn*7* mutants inhibited the germination of cystospores of *P. capsici* P-9-2. Although a slight inhibition activity was observed in W18 and W21 strains, it could be clearly distinguished from the strong activity of the F-1-1 wild type (Table 6).

In the pot test, also, no signs of suppression against damping-off disease of cucumber seedlings by transposon mutants were observed even 15 days after the

Table 4. Suppression of damping-off of cucumber seedlings by treatment with *Serratia marcescens* F-1-1 strain

Protective treatment (Diluted bacterial suspension)	Disease incidence (%)		
	5 days[a]	10 days	15 days
P. capsici[b]+F-1-1 (1×)[c]	0.0 A[d]	20.5 A	41.3 A
P. capsici+F-1-1 (10×)	2.1 B	40.2 B	70.2 B
P. capsici+F-1-1 (100×)	2.5 B	62.4 BC	75.9 B
P. capsici+F-1-1 (1000×)	2.3 B	58.3 BC	90.5 C
P. capsici only (cont.)	3.2 C	75.1 C	91.5 C

a) Days after inoculation of F-1-1 strain to cucumber seedlings.
b) Twenty ml of cystospore suspension (10^4 spores/ml) per pot was applied.
c) Non-diluted bacterial suspension was applied at a concentration of 2.3×10^7 cfu/ml. 10×: 10 fold dilution.
d) Values followed by the same letter are not significantly different ($p=0.05$), according to Duncan's multiple range test (suppression-grade; A>B>C).

Table 5. Characterization of Tn*7*-insertion mutants of *Serratia marcescens* F-1-1

Strain	Pigment[a]	API 20E[b]	SM[r c]	TP[r d]	Tn*7*-Hybri.[e]
F-1-1 Wild Type	Red	530776157	−	−	−
F-1-1^{rif}	Red	530776157	−	−	−
F-1-1^{rif}::Tn*7* W15	White	530776157	+	+	+
F-1-1^{rif}::Tn*7* W18	White	530776157	+	+	+
F-1-1^{rif}::Tn*7* W21	White	530776157	+	+	Not tested

a) Color of colonies observed on modified LB media.
b) Profile indexes obtained by API 20E *Enterobacteriaceae*-identification kit.
c) Resistant to streptomycin (50 ppm) which is one of Tn*7* markers.
d) Resistant to trimethoprim (500 ppm) which is one of Tn*7* markers.
e) Dot blot hybridization against chromosomal DNA with Tn*7* DNA-probe.

Table 6. Effect of inoculation of Tn*7*-insertion mutants of *Serratia marcescens* F-1-1 on germination of cystospores of *Phytophthora capsici* P-9-2

Bacterial strain	Cystospores germination of *P. capsici* P-9-2					
	1[a]	1/2	1/4	1/8	1/16	1/32
F-1-1^{rif}::Tn*7* W15	4+[b]	4+	4+	4+	4+	4+
F-1-1^{rif}::Tn*7* W18	2+	4+	4+	4+	4+	4+
F-1-1^{rif}::Tn*7* W21	2+	4+	4+	4+	4+	4+
F-1-1 Wild type	−	−	+	+	+	2+
Water (control)	4+					

a) Non-diluted bacterial suspension was applied at a concentration of 2.3×10^7 cfu/ml. 1/2-1/32 showed dilution of bacterial suspension.
b) Germination rate: −, 0 (%); +, 1-10 (%); 2+, 11-49 (%); 3+, 50-79 (%); 4+, 80-100 (%).

inoculation, whereas in the case of the wild type strain, suppression was evident (Plate I-6).

Thus, red pigment-deficient mutants completely lost their antagonism against *P. capsici* P-9-2.

DISCUSSION

In this study, it was shown that *S. marcescens* F-1-1 displays an antibiotic activity *in vitro* and in pots tests and could therefore become a potential biocontrol agent for damping-off of cucumber seedlings. The finding of the *S. marcescens* acted as a biocontrol agent against this pathogen, *P. capsici*, was reported for the first time, although the bacterium had been known to be antagonistic against other pathogens such as *Sclerotium rolfsii*[8,9], *Botrytis fabae*[18], *B. cinerea*[1,5], *Fusarium oxysporum* f. sp. *cyclaminis*[6], and so on.

All the red-pigmented strains of *S. marcescens* isolated from the rhizosphere of two plants and supplied from stock cultures of the institutes displayed an antifungal activity against *P. capsici* P-9-2, whereas none of the strains with non-pigmented (white) colonies of *Serratia* spp. showed any antifungal activity against this pathogen. Therefore, we assumed that the red pigments produced by this bacterium might be antibiotic substances. Then, we attempted to induce transposon-insertion mutants with a red pigment-producing ability.

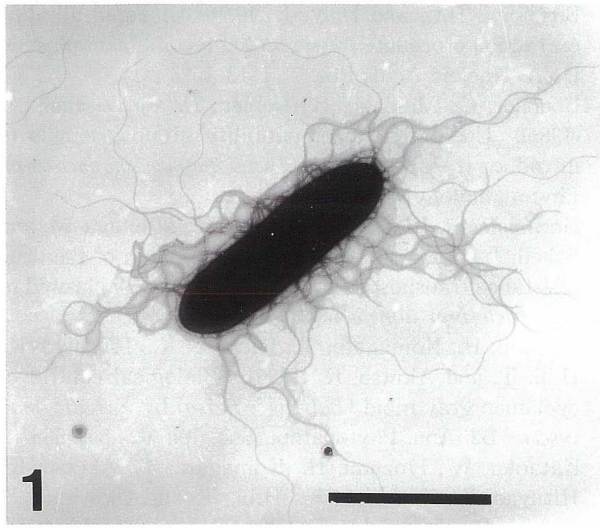
The vector (carrier) for the introduction of the transposon was selected among many vectors. The plasmid pBPW1::Tn*7* which had been used as a suicidal vector of Tn*7* in some bacteria was employed in this experiment[12]. Tn*7* had been introduced into plant pathogenic bacteria such as *Agrobacterium*[4], *Pseudomonas syringae*[11,12], *Ralstonia solanacearum*[3] and so on. In the present study, four transposon mutants lacking a red pigment-producing ability were obtained. These mutants, which were unable to produce red pigments, did no longer hold an inhibitory effect on *P. capsici* P-9-2 *in vitro* and in pot tests. These results strongly suggest that the red pigments produced by the strain are antibiotics and play an important role in biological control.

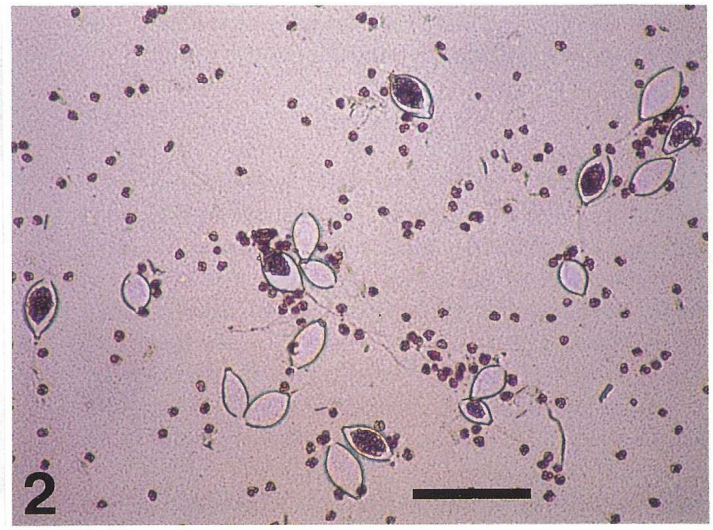
As biocontrol mechanisms of *S. marcescens* in other plant diseases, the inhibition of growth of the pathogen by a chitinase produced from this bacterium has been reported[1,5,14,18]. The biocontrol mechanism of *S. marcescens* F-1-1 is not based on the chitinase activity, since in all the four Tn*7* mutants which had lost their antagonistic activity the chitinase activity was still present (data not shown). It is, therefore, suggested that the red pigments display an antibiotic activity and play an important role in the biocontrol of damping-off of cucumber seedlings.

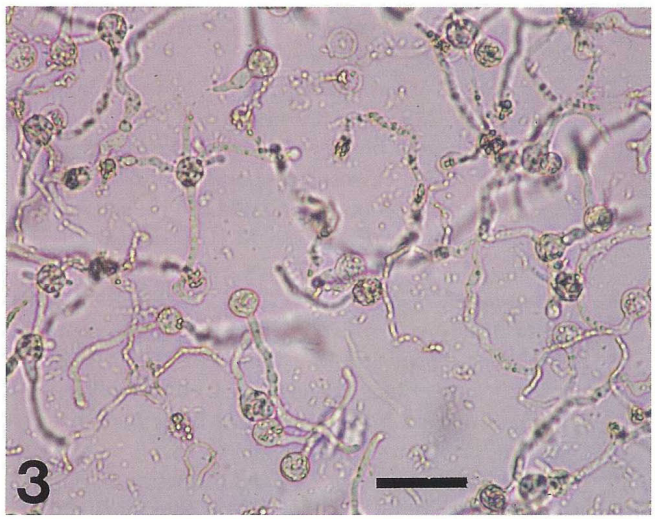
Literature cited

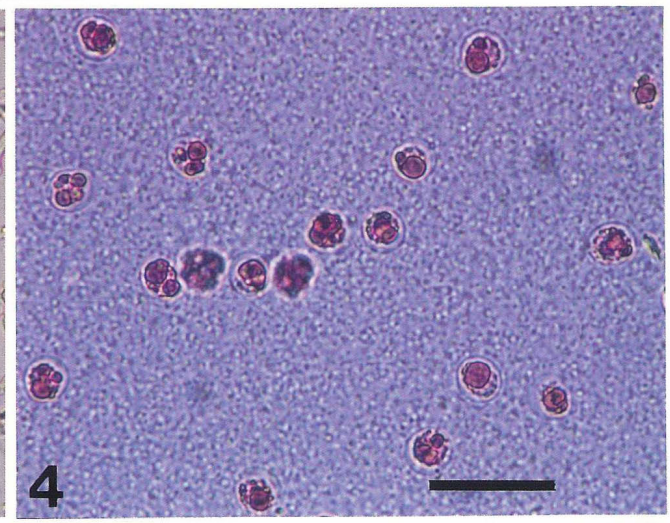
1. Akutsu, K., Hirano, A., Yamamoto, M., Hirayae, K., Okayama, S. and Hibi, T. (1993). Growth inhibition of *Botrytis* spp. by *Serratia marcescens* B2 isolated from tomato phylloplane. Ann. Phytopathol. Soc. Jpn. 59 : 18-25.
2. Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7 : 1513-1523.
3. Boucher, C., Message, B., Debieu, D. and Zischek, C. (1981). Use of P-1 incompatibility group plasmids to introduce transposons into *Pseudomonas solanacearum*. Phytopathology 71 : 639-642.
4. Hernalsteens, J.P., De Greve, H., Van Montagu, M. and Schell, J. (1978). Mutagenesis by insertion of the drug resistance transposon Tn*7* applied to the Ti plasmid of *Agrobacterium tumefaciens*. Plasmid 1 : 218-225.
5. Iyozumi, H., Komagata, T., Hirayae, K., Tsuchiya, K., Hibi, T. and Akutsu, K. (1996). Biological control of cyclamen gray mold (*Botrytis cinerea*) by *Serratia marcescens* B2. Ann. Phytopathol. Soc. Jpn. 62 : 559-565.
6. Kataoka, W., Iyozumi, H., Komagata, T., Akutsu, K., Hirayae, K., Tsuchiya, K., Hibi, T. and Okuyama, S. (1994). Biocontrol by *Serratia marcescens* B2 against *Fusarium* wilt of cyclamen. Ann. Phytopath. Soc. Jpn. 60 : 743 (abstract in Japanese).
7. Loper, J.E., Corbell, N., Kraus, J., Nowak-Thompson, B., Henkels, M.D. and Carnegie, S. (1994). Contributions of molecular biology towards understanding mechanisms by which rhizosphere pseudomonads effect biological control. *In* Improving Plant Productivity with Rhizosphere Bacteria (Ryder, M.H. *et al.* eds.), CSIRO Division of Soil, Adelaide, Australia, pp. 89-96.
8. Ordentlich, A., Elad, Y. and Chet, I. (1987). Rhizosphere colonization by *Serratia marcescens* for the control of *Sclerotium rolfsii*. Soil Biol. Biochem. 19 : 747-751.
9. Ordentlich, A., Elad, Y. and Chet, I. (1988). The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phytopathology 78 : 84-88.
10. Poplawsky, A.R., Peng, Y.F. and Ellingboe, A.H. (1988). Genetics of antibiosis in bacterial strains suppressive to take-all. Phytopathology 78 : 426-432.
11. Sato, M., Staskawicz, B.J., Panopoulos, N.J., Petters, S. and Honma, M. (1981). A host-dependent hybrid plasmid suitable as a suicidal carrier for transposable elements. Plasmid 6 : 325-331.
12. Sato, M., Nishiyama, K. and Panopoulos, N.J. (1985). Conjugal transmission and replicative properties of plasmid pBPW1, and antigen alteration induced by the plasmid in pathovars of *Pseudomonas syringae*. Ann. Phytopathol. Soc. Jpn. 51 : 426-434.
13. Staskawicz, B.J., Dahlbeck, D. and Keen, W.T. (1984). Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. Proc. Natl. Acad. Sci. USA 81 : 6024-6028.
14. Sundheim, L., Poplawsky, A.R. and Ellingboe, A.H. (1988). Molecular cloning of two chitinase genes from *Serratia marcescens* and their expression in *Pseudomonas* species. Physiol. Mol. Plant Pathol. 33 : 483-491.
15. Thomashow, L.S. and Weller, D.M. (1996). Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. *In* Plant-Microbe Interactions, Volume 1 (Stacey, G. and Keen, N. eds.), Chapman and Hall, New

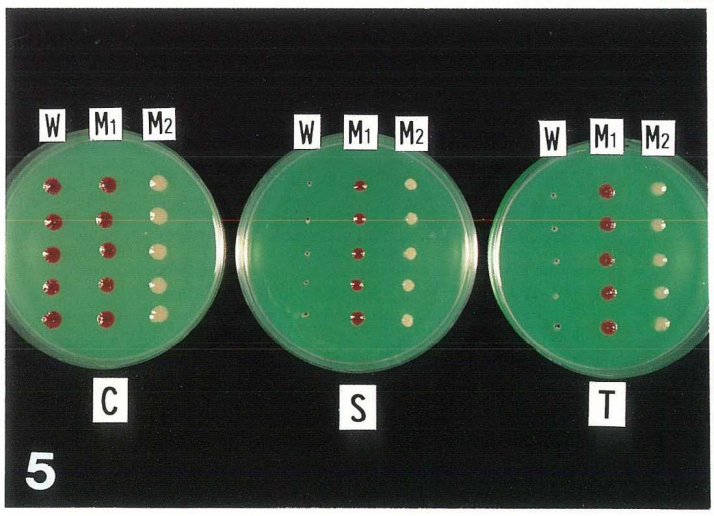
Plate I

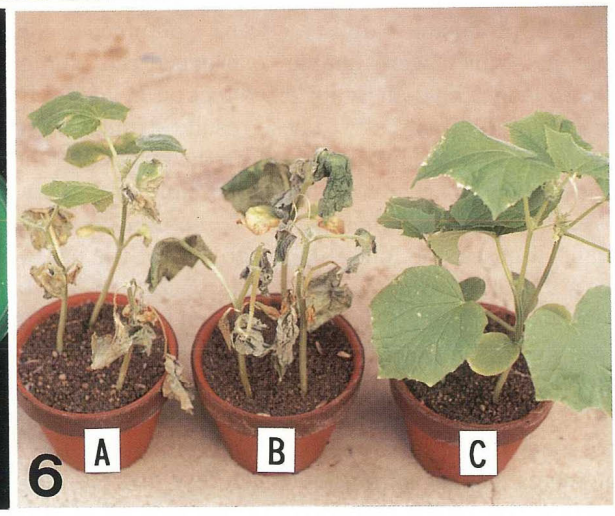












York, USA, pp. 187-235.
16. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982). Molecular Cloning : A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. Nishiyama, K. (1981). Studies on the pathogenicity-related substances in *Pseudomonas coronafaciens* var. *atropurpurea*. Bull. Natl. Inst. Agric. Sci. C35 : 1-55.
18. Yamamoto, M., Iyozumi, H., Akutsu, K., Hirayae, K., Hibi, T. and Okuyama, S. (1992). Suppressive effect by *Serratia* sp. B2 against *Botrytis fabae*-induced chocolate spot disease of broad bean. Ann. Phytopathol. Soc. Jpn. 58 : 582-583 (abstract in Japanese).

和 文 摘 要

岡本 博・佐藤 守・佐藤善司・伊阪実人：*Serratia marcescens* F-1-1 によるキュウリ灰色疫病菌 *Phytophthora capsici* の生物的防除およびトランスポゾン挿入変異株によるその防除機構の解析

キュウリ灰色疫病菌 *Phytophthora capsici* P-9-2 に拮抗作用を有する赤色色素産生菌 F-1-1 株が，福井県のサルビア根圏から分離された。本菌株は各種細菌学的性質の調査により *Serratia marcescens* と同定された。本菌株は，*in vitro* 試験で *P. capsici* P-9-2 の遊走子嚢および被嚢胞子の発芽を強く抑制した。また，キュウリ苗の根圏土壌にあらかじめ F-1-1 株を接種しておくことにより，その後の病原菌の感染を強く抑制した。この拮抗作用は，F-1-1 株の他，イヌツゲ根圏等から分離された赤色色素産生性 *S. marcescens* のすべての株で観察されたが，白色コロニーの *Serratia* 属細菌（6 種）では認められなかった。拮抗作用機構を知るため，F-1-1 株のトランスポゾン（Tn*7*）挿入変異株の作出を行った結果，4 株の赤色色素産生喪失株を得た。これら変異株は，前述の *in vitro* 試験およびキュウリ苗のポット試験のいずれにおいても，拮抗作用を失っていた。以上のように，F-1-1 株は，キュウリ灰色疫病の生物的防除に十分利用可能なこと，およびその防除機構は本菌株の赤色色素産生能に基づくことが明らかになった。

Explanation of plates

Plate I

1. Electron micrograph of the antagonistic bacterium *S. marcescens* F-1-1 (bar represents 1 μm).
2. Suppression of germination of zoosporangia and cystospores of *P. capsici* P-9-2 by treatment with *S. marcescens* F-1-1 (bar represent 50 μm).
3. Normal germination of cystospores of *P. capsici* P-9-2 (bar represent 30 μm).
4. Suppression of germination of cystospores of *P. capsici* P-9-2 by treatment with *S. marcescens* F-1-1. Cystospores with red staining are observed (bar represent 30 μm).
5. Colonies of *S. marcescens* F-1-1 (W) and its transposon (Tn*7*) mutants (M_1 : F-1-1^{rif}::Tn*7*, Red$^+$ and M_2 : F-1-1^{rif}::Tn*7*, Red$^-$) observed on modified LB medium with or without antibiotics, streptomycin (50 ppm) or trimethoprim (500 ppm). C : LB medium (All strains grow well but Tn*7*-mutant, M_2, can not produce red pigments.). S : LB medium supplemented with streptomycin (only Tn*7*-mutants, M_1 and M_2, can grow). T : LB medium supplemented with trimethoprim (Only Tn*7*-mutants, M_1 and M_2, can grow).
6. Suppression of damping-off disease of cucumber seedlings by treatment with *S. marcescens* F-1-1 or its transposon mutant W18 at 15 days after inoculation. A : Treatment with red pigment-deficient mutant W18 induced by Tn*7* (severe damping-off symptoms are observed). B : Treatment with water (control) (severe damping-off symptoms are observed). C : Treatment with F-1-1 wild type (no symptoms are observed).