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Role of Chitinase Produced by *Chromobacterium violaceum* in the Suppression of *Rhizoctonia* Damping-off

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모잘록병(*Rhizoctonia solani*)의 억제에 있어서 *Chromobacterium violaceum*이 생산하는 Chitinase의 역할

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ABSTRACT : To determine whether chitinolytic enzymes from *Chromobacterium violaceum* C-61 play an important role in the suppression of *Rhizoctonia* damping-off, Tn*5* insertion mutants deficient in chitinolytic activity (Chi a$^-$ mutants) were selected and their chitinolytic and disease suppression were compared with those of the parental strain. Four Chi a$^-$ mutants selected from about 2,000 transconjugants did not inhibit mycelial growth of *Rhizoctonia solani* on nutrient agar-potato dextrose agar (NA-PDA) and their abilities to suppress *Rhizoctonia* damping-off were much lower than the parental strain. However, population density in the eggplant rhizosphere did not differ significantly between the parental strain and four Chi a$^-$ mutants. The crude enzyme of the parental strain inhibited growth of *R. solani* on NA-PDA and its chitinase activity was much higher than that of Chi a$^-$ mutants. But the N,N′-diacetylchitobiase activity between these isolates were not significantly different. The chitinase of Chi a$^-$ mutants was defective in 2 isoforms of 52- and 37-kDa among four isoforms of 54-, 52-, 50- and 37-kDa. A Tn*5* element was inserted into one site of 10 kb *Eco*RI fragment of chromosomal DNA in three Chi$^-$ mutants, C61-C1, -C2, and -C3. In C61-C4 mutant, a Tn*5* element was inserted into two sites of 10 kb and 4.4 kb *Eco*RI fragments. These results suggest that the chitinase of *C. violaceum* C-61 play an important role in the suppression of *Rhizoctonia* damping-off of cucumber and eggplant.

***Key words* : *Chromobacterium violaceum* C-61, Chi a$^-$ mutants, Tn*5* insertion, chitinase, suppression of *Rhizoctonia* damping-off.**

Several chitinolytic bacteria antagonistic to soilborne plant pathogens have been reported (1, 5, 11, 12, 20). Recently, we also isolated *Chromobacterium violaceum* C-61 from soil, which inhibited growth of several soilborne plant pathogens *in vitro* and suppressed damping-off of eggplant caused by *Rhizoctonia solani* (15, 16). The strain C-61 produced much more chitinase than *Serratia marcescens*, *Aeromonas caviae* and *A. hydrophila* with lower antagonistic ability (17). However, the role of chitinase in the interaction between *C. violaceum* C-61 and *R. solani* is not elucidated yet.

The role of chitinase in biological control of soilborne plant pathogens was mainly supported from studies on correlations between the bacterial ability of disease suppression and chitinase production (1, 13). Recently, Tn*5* insertion mutants have been used to know the role of antibiotic substances associated with the disease suppression (8, 14, 21). The transposon Tn*5* inserted randomly into bacterial genome results in sin-

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gle-site nonleaky polar mutations, and usually leads to inactivation of the gene (3). Therefore, Tn5 insertion mutants defective in chitinase production can be used to know the role of chitinase in the disease suppression as compared with the parental strain. The mutants can also be used to facilitate cloning of the chitinase gene which is not expressed directly in *Escherichia coli*.

The purpose of this study was to determine the role of chitinolytic enzymes produced by *C. violaceum* C-61 in the suppression of *R. solani* disease on eggplant and cucumber roots. Thus, the mutants deficient in chitinolytic activity was selected by transposon mutagenesis and compared with the parental strain for their ability to suppress damping-off and produce chitinolytic enzymes.

MATERIALS AND METHODS

Microorganisms, plasmids and culture conditions. The microorganisms and plasmids used in this study are listed in Table 1. Plasmid pGS9 was carried in *E. coli* WA 803. *E. coli* WA 803 was cultured at 37°C on Luria-Bertani (LB) broth or plates supplemented with kanamycin (50 μg/ml). *C. violaceum* C-61 was grown on nutrient broth supplemented with ampicillin (50 μg/ml) at 28°C. *R. solani* was grown on potato dextrose agar (PDA) at 28°C. *C. violaceum* C-61 and Tn*5* insertion mutants were stored at −70°C in nutrient broth (NB) containing 30% glycerol.

Transposon mutagenesis. The 5.7-kilobase (kb) transposon Tn*5*, which codes for kanamycin resistance (3), was transferred into *C. violaceum* C-61 by using the plasmid vector system of pGS9 (25). *E. coli* donor strain WA 803 was grown overnight at 37°C on a rotary shaking incubator in LB broth containing kanamycin. *C. violaceum* C-61 recipient cells were grown at 28°C on a rotary shaking incubator in NB containing ampicillin for 24 hrs. Approximately 10^9 donor cells and 10^9 recipient cells were sedimented and suspended in 500 μl of sterilized H_2O. The mixtures of donor and recipient cell suspensions (1 : 1) were transferred to nutrient agar (NA) plates and incubated for 12 hrs at 28°C. Transconjugants were selected on NA plates containing 50 μg/ml ampicillin and kanamycin, respectively. Mutants deficient in chitinolytic activity (Chi a$^-$ mutants) were selected from the transconjugants on chitin agar plates (20).

***In vitro* fungal inhibition assay.** Inhibition of *R. solani* by bacterial strains *in vitro* was assayed on NA-PDA plates. Samples (1 μl, containing approximately 10^9 cells) from overnight cultures of the parental strain and Chi a$^-$ mutants in NB were spotted on the edge of NA-PDA plates and dried. An agar disks (0.5 cm in diameter) of the pathogen grown on PDA for 3 days at 28°C were placed in the center of the plate, and the plates were incubated at 28°C for 3 or 4 days. The inhibition zone between the edges of the bacterial colony and the fungal mycelium was measured.

Damping-off suppression assay. Suppression of damping-off of eggplant and cucumber by bacterial strains was assayed in the Wagner pot (15×6×10 cm). The cultures of *R. solani* grown for 7 days on PDA were pulverized in a Waring blender, and then inoculated on the sterilized oatmeal-soil in 500 ml flasks. After incubation for 15 days at 28°C, the colonized oatmeal-soils were blended and sieved through 0.25 mm sieve. The inocula were added to the sterilized soils at the rate of 1% (w/w) in Wagner pots. Four-week old seedlings of eggplant and three-week old seedlings of cucumber were planted in two rows with 5 plants per row in Wagner pots filled with the infested soil. On the day of planting, 50 ml of bacterial suspension (ca. 10^9 cells/ml) was drenched onto the surface of soil in each pot. Sterile distilled water was used as a control. Damping-off incidence was measured daily until 25 days after treatment. All experiments were conducted in a greenhouse with four replicates.

Population changes of introduced bacteria. The

Table 1. Microorganisms and plasmid used in this study

Organism	Relevant characteristics[a]	Source or reference
C. violaceum C-61	Wild type, *R. solani* inhibition, Amp^r	This laboratory (16)
E. coli WA 803	*met thi supE hsdR hsdM*	Wood (25)
Plasmid pGS 9	Cm^r Km^r, p15A replicon N-tra, Tn*5* donor	Selvaraj & Iyer (19)
R. solani	Wild type, isolated from eggplant and highly virulent	This laboratory (16)

[a] Amp^r, Km^s, and Cm^r indicate resistance to ampicillin, kanamycin, and chloramphenicol, respectively.

bacterial population in soils were determined using a dilution plate method (4) on the chitin agar plates containing ampicillin, and ampicillin and kanamycin for the parental strain and Chi a$^-$ mutants, respectively.

Chitinolytic enzyme assay. The culture cells (100 μl) of the bacteria grown in NB for 1 day were inoculated in flasks (500 ml) containing 100 ml of the chitin medium (20), and then were incubated on a rotary shaker (180 rpm) at 28°C. The culture supernatants were obtained by centrifugation at 10,000 g for 20 min and added by solid ammonium sulfate at 4°C to give 80% saturation. After an incubation overnight with continuous stirring, the precipitate was collected by centrifugation at 10,000 g for 30 min, and dissolved in 1 ml of 20 mM sodium acetate buffer, pH 5.0. The solutions were used as crude enzymes after dialysis, and their protein concentrations were determined by protein assay kit (Sigma, P 5656) using purified bovine serum albumin as a reference protein. Activity of chitinase, N, N′-diacetylchitobiase and chitosanase was determined by measuring the amount of N-acetylglucosamine (NAG) released from colloidal chitin, N,N′-diacetylchitobiose (Sigma, D 1523) and glycolchitosan (Sigma, G 7753), respectively, by methods described in a previous report (17).

Detection of chitinase activity after SDS-PAGE. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (7) in 10% acrylamide gels containing 0.01% glycol chitin. After electrophoresis, protein bands were stained with silver nitrate and Calcoflour White M2R for chitinase activity by methods described in the previous report (17). The molecular weight of the protein was determined by calibration kit (Pharmacia Inc.).

Southern blot hybridization. Genomic DNA was extracted by the method of Maniatis *et al.* (9). pGS9::Tn*5* plasmid was isolated by the alkaline lysate procedure of Crosa and Falkow (2), and then purified by the polyethylene glycol treatment described by Maniatis *et al.* (9). Genomic DNA was digested with *Eco*RI and *Pst*I, and then was run in 0.8% agarose gels with Tris-borate buffer (25 v/cm for 8 hrs). pGS9::Tn*5* plasmid was digested with *Hpa*I, and then 5.4 kb internal fragments of Tn*5* (3) were purified by gene clean kit (Gene Clean Kit II, Bio 101, Inc., USA) after electrophoresis, and were used as a probe DNA. Labeling of the probe DNA and its detection were conducted by DNA labelling and detection kit (DIG DNA Labeling and Detection Kit, Boehringer Mannheim Biochemica, Germany). After electrophoresis, agarose gels were acid-depurinated in 0.25 M HCl for 20 min to facilitate transfer of large fragments. DNA fragments then transferred to nitrocellulose (Hybond™-N, Amersham, USA) by the method of Southern (9). The blotted nitrocellulose filters were prehybridized, hybridized, washed and detected according to the instructions of the manufacturers.

RESULTS

Isolation of mutants deficient in chitinolytic activity (Chi a$^-$ mutants). Ampicillin resistant (Amp^r) and kanamycin resistant (Km^r) transconjugants of *C. violaceum* C-61 were recovered at a frequency of 8.7×10^{8} per initial recipient. Spontaneous mutation into Amp^r, Km^r strains occurred at the frequency less than 10^{-9}, indicating that the transconjugants occurred by Tn*5* insertion. The chitinolytic activity of the Amp^r, Km^r transconjugants was determined visually on chitin agar plates. Four Chi a$^-$ mutants were obtained from about 2,000 transconjugants. Chitin degradation (clear zones) by the Chi a$^-$ mutants was observed after 3 days of incubation, whereas that by a parental strain was observed after 1 day incubation. The size of the clear zones in Chi a$^-$ mutants was much less than that in the parental strain after 7 days of incubation (Fig. 1).

Influence of Chi a$^-$ mutants of *C. violaceum* on *R.*

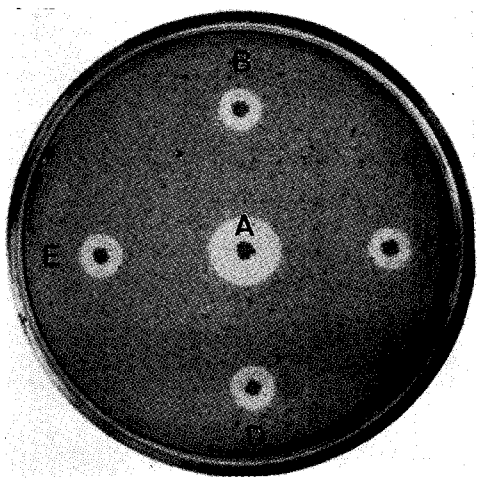

Fig. 1. Clear zones of *C. violaceum* C-61 (A) and Chi a$^-$ mutants (B, C, D, E) formed on chitin agar plates (20) after incubation at 28°C for 5 days.

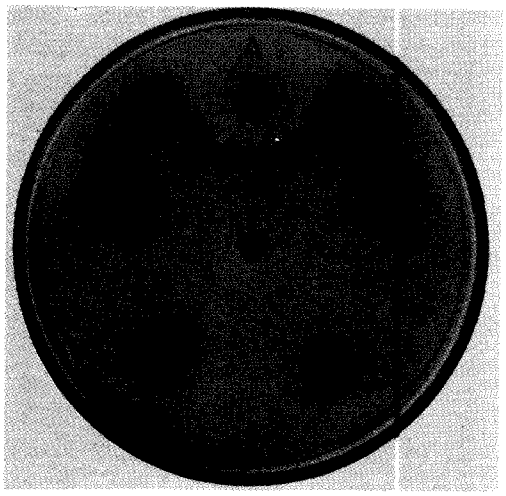

Fig. 2. Influence of *C. violaceum* C-61 (A) and Chi a⁻ mutants (B, C, D, E) on mycelial growth of *R. solani* (F). The bacteria and the fungus were simultaneously inoculated on NA-PDA, and then incubated at 28°C for 4 days.

Table 2. Influence of the parental strain and Chi a⁻ mutants of *C. violaceum* C-61 on damping-off incidence of eggplant and cucumber caused by *R. solani*

Strain	Damping-off incidence (%)[a]	
	Eggplant	Cucumber
Control (no bacteria)	53.3 a[b]	48.3 a
Parental strain C-61	16.7 b	6.7 c
Mutant C61-A1	46.3 a	41.7 ab
Mutant C61-A2	57.3 a	43.3 a
Mutant C61-A3	57.3 a	50.0 a
Mutant C61-A4	54.7 a	38.3 b

[a] The disease incidence was investigated 25 days after the bacterial treatment in sterilized soil infested with *R. solani* inocula, and the values are means of two experiments with three replicates per experiment.

[b] Means within a column followed by the same letters are not significantly different at 5% level by Duncan's multiple range test.

***solani* and damping-off.** To determine whether Chi a⁻ mutants can inhibit *R. solani* growth and suppress damping-off, a parental strain and four Chi a⁻ mutants were compared for their ability on NA-PDA and in soil infested with *R. solani*. Chi a⁻ mutants did not inhibit growth of *R. solani in vitro*, although the parental strain did (Fig. 2). Treatment of Chi a⁻ mutants produced significantly more incidence of damping-off of eggplants than that of the parental strain. The incidence

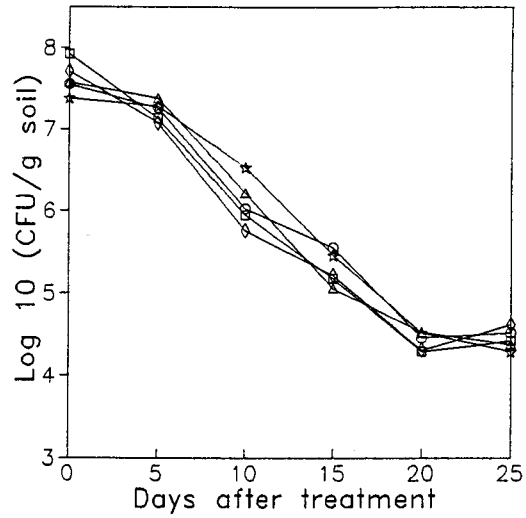

Fig. 3. Changes of population density of *C. violaceum* C-61 and Chi a⁻ mutants in eggplant soil infested with *R. solani* inocula; parental strain C-61 (◇), and Chi a⁻ mutants, C61-C1 (☆), C61-C2 (△), C61-C3 (o) and C 61-C4 (□). The bacterial populations were determined using a dilution plate method (4) on the chitin agar plates after 50 ml bacterial suspension (ca. 10^9 cells/ml) was drenched onto the surface of soil in each pot.

of damping-off of cucumber was also similar in tendency with those of eggplant, although disease severity varied among experiments (Table 2).

Root colonization by Chi a⁻ mutants of *C. violaceum*. Since the reduced suppression of Chi a⁻ mutants could be due to their inability to maintain significant populations around the roots, the density of the parental strain and four Chi a⁻ mutants treated in the rhizosphere was investigated. Both the parental strain and the four Chi a⁻ mutants were continuously decreased until 20 days after treatment, and maintained approximately 5×10^4 CFU per g of soil in 20~25 days. However, no significant differences were observed between the parental strain and Chi a⁻ mutants in their density colonizing in the rhizosphere (Fig. 3).

***R. solani* inhibition by crude enzymes.** Since Chi a⁻ mutants showed very low protein concentration in a 3-day culture, *R. solani* inhibition was assayed by the crude enzymes from a 3-day culture in the parental strain and a 7-day culture in Chi a⁻ mutants. The crude enzyme of the parental strain inhibited *R. solani* growth *in vitro*, but that of Chi a⁻ mutants did not (Fig. 4). When boiling for 30 min, however, the crude enzyme of the parental strain did not inhibit *R. solani*

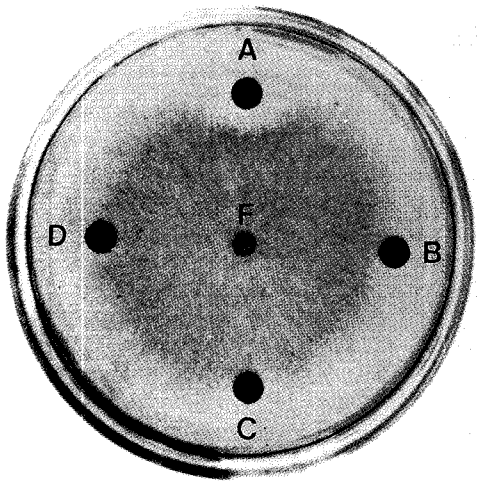

Fig. 4. Influence of crude enzymes extracted from culture supernatants of *C. violaceum* C-61 (A) and Chi a⁻ mutants (B, C, D) on mycelial growth of *R. solani* (F). Supernatants (100 ml) cultured for 3 days in parental strain and for 7 days in Chi a⁻ mutants were precipitated by 80% saturation of ammonium sulfate, and dissolved in 1 ml of 20 mM sodium acetate, pH 5.0. A hundred μl of the dissolved solution (crude enzymes) and 0.5 cm PDA disk of *R. solani* were loaded on NA-PDA and were incubated at 28°C for 3 days.

growth (data not shown).

Activity and characteristics of chitinolytic enzymes. In the case of a 3-day culture of the parental strain, protein concentration, chitinase activity and N, N′ - diacetylchitobiase activity were 4,088 μg/ml, 115.4 U/μl and 7.5 U/μl, respectively, which were much higher than those of Chi a⁻ mutants with ranges of 78~95 μg/ml, 1.8~2.9 U/μl and 0.3~0.9 U/μl, respectively. In the case of a 7-day culture, the parental strain was about 2 times higher in protein concentration and chitinase activity than Chi a⁻ mutants, although not significantly in their N,N′-diacetylchitobiase activity (Table 3). The cell growth of the parental strain and Chi a⁻ mutants in the chitin medium did not differ significantly (data not shown).

SDS-PAGE was carried out with the parental strain and Chi a⁻ mutants at the same protein concentrations. Protein bands by silver nitrate staining showed similar tendency between the parental strain and Chi a⁻ mutants, although they differed in the patterns of some bands (Fig. 5A). Among them, chitinase activity of the parental strain was detected in four bands with molecular weight of 54-, 52-, 50- and 37-kDa. However, chitinase activity of Chi a⁻ mutants were detected only in two bands of 54- and 50-kDa and was defective in two bands of 52- and 37-kDa (Fig. 5B).

Table 3. Protein concentration and chitinolytic activity of crude enzymes secreted by *C. violaceum* C-61 and Chi a⁻ mutants in chitin medium[a]

Culture period	Strain	Protein (μg/ml)[b]	Enzyme activity (U/μl)[c]	
			Chitinase	Chitobiase[d]
3 days	Strain C-61	4,088	115.4	7.5
	C 61-C1	78	1.8	0.5
	C 61-C2	82	2.2	0.3
	C 61-C3	88	2.5	0.9
	C 61-C4	95	2.9	0.7
7 days	Strain C-61	3,483	91.8	7.3
	C 61-C1	1,702	43.7	6.5
	C 61-C2	1,681	42.5	7.7
	C 61-C3	1,695	41.8	6.9
	C 61-C4	1,711	43.2	7.2

[a] Crude enzymes represent the solution dissolved in 1 ml of 20 mM sodium acetate buffer, pH 5.0, after 100 ml of culture supernatant was precipitated by 80% saturation of ammonium sulfate.

[b] Determined by protein assay kit (Sigma, P 5656) using purified bovine serum albumin as reference protein.

[c] A unit represents the amount of enzyme required to produce 1 μmole of NAG, when the reaction mixtures of a crude enzyme and a substrate are incubated at 37°C, pH 5.0 for 30 min. Each value represents the mean of three separate determinations.

[d] N,N′ -diacetylchitobiase.

Physical characterization of Tn*5* insertion mutants. Since the parental strain and Chi a⁻ mutants did not have an indigenous plasmid and pGS9 plasmid, total DNA was used in Southern blot analysis. The DNAs were digested with *Eco*RI, and then hybridized with digoxigenin-labeled 5.4 kb *Hpa*I fragment of Tn*5*. The DNA from a parental strain was not hybridized, but three mutants were hybridized to one fragment of about 10 kb and one mutant hybridized to two fragments of 10 kb and 4.4 kb (Fig. 6A).

To further clarify the insertion or the sites of Tn*5* in Chi a⁻ mutants, the DNAs were hybridized after *Pst*I digestion. Because *Pst*I cleaves four sites of Tn*5*, three fragments of set size in Tn*5* and two boundary fragments adjacent to Tn*5* should be hybridized by 5.4 kb *Hpa*I fragment of Tn*5*. All Chi a⁻ mutants were hybridized in fragments of about 0.8, 1.0 and 2.5 kb, set sizes in Tn*5*. In addition, three mutants were hy-

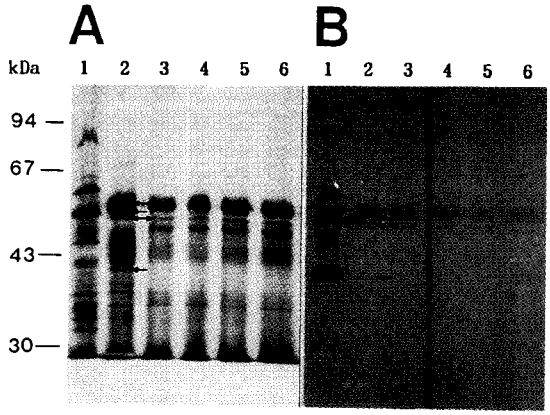

Fig. 5. SDS-PAGE of (A) protein and (B) chitinase secreted by *C. violaceum* C-61 and Chi a⁻ mutants; commercial chitinase (Sigma, C 1650) (lane 1), parental strain (lane 2), and Chi a⁻ mutants, C61-C1 (lane 3), C 61-C2 (lane 4), C61-C3 (lane 5) and C61-C4 (lane 6). The crude enzymes of supernatant cultured for 3 days for the parental strain and for 7 days for Chi a⁻ mutants were loaded on gels containing 0.01% glycol chitin. (A) protein bands were detected after silver nitrate staining and (B) bands with chitinase activities were detected under UV illumination after staining with Calcofluor White M2R. The arrows indicate chitinase isoforms of 54-, 52-, 50- and 37-kDa.

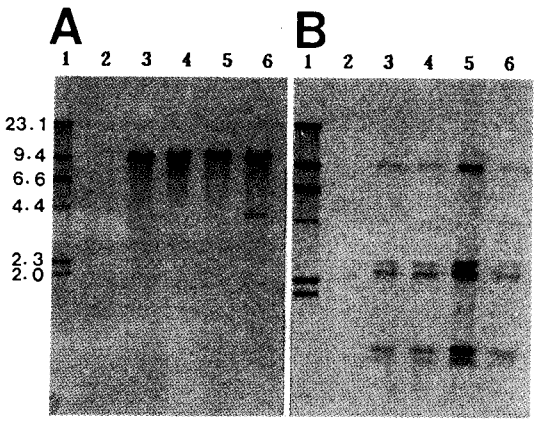

Fig. 6. Southern blot analysis of *Eco*RI-digested (A) and *Pst*I-digested (B) total DNA from *C. violaceum* C-61 and Chi a⁻ mutants; DIG-labeled *Hin*dIII DNA (lane 1), parental strain (lane 2), and Chi a⁻ mutants, C61-C1 (lane 3), C61-C2 (lane 4), C61-C3 (lane 5) and C61-C4 (lane 6). Blots of digested DNA were hybridized with digoxigenin-labeled 5.4 kb *Hpa*I fragment of Tn*5*.

bridized in the boundary fragments of about 2.3 and 9.0 kb, and one mutants were hybridized in about 2.3, 9.0 kb and about 4.4, 4.0 kb (Fig. 6B). The Tn*5* was found to be inserted into one site in three mutants and two sites in one mutant, and also be inserted into the same or the similar sites in all mutants except for one mutant with additional Tn*5* insertion.

Discussion

Tn*5* mutants of *C. violaceum* C-61 deficient in the chitinolytic activity (Chi a⁻ mutants) did not suppress the growth of *R. solani* on NA-PDA and damping-off incidences of eggplant and cucumber. The less suppression of Chi a⁻ mutants might be not due to their inability to maintain effective populations in the rhizosphere, because comparable numbers of the parental strain and the mutants were isolated from the rhizosphere. Moreover, the crude enzymes of the parental strain inhibited *R. solani* growth *in vitro* but those of Chi a⁻ mutants did not. These results support the role of the chitinolytic enzymes in the suppression of damping-off incidences by *C. violaceum* C-61.

Several reports suggested that a chitinase and/or a β-1,3-glucanase play an important role in suppression of plant diseases (1, 10, 13, 22, 23). However, *C. violaceum* C-61 did not produce a β-1,3-glucanase (16), indicating that β-1,3-glucanase is not associated with the disease suppression of *C. violaceum*. On the other hand, the parental strain of *C. violaceum* C-61 produced much higher chitinolytic enzymes, especially chitinase, than Chi a⁻ mutants. In a previous report (17), *C. violaceum* C-61 also produced much more chitinase than *S. marcescens*, *A. hydrophila* and *A. caviae* with lower disease suppression, although its N,N′-diacetylchitobiase activity was lower than that of *S. marcescens*. These results suggest that the chitinase of *C. violaceum* C-61 may play an important role in the inhibition of *R. solani*. In addition, the ability of *C. violaceum* C-61 to inhibit *R. solani* could be associated with ability of its chitinase production.

Although most of the chitinolytic bacteria were reported to have several chitinase isoforms or isozymes (6, 17, 18, 24, 26), the roles of chitinase isoforms in the chitinase production is not known. However, our results showed that the ability of *C. violaceum* C-61 to produce chitinase and suppress damping-off was dramatically decreased when two isoforms of 52- and 37-kDa were defective among four chitinase isoforms of 54-, 52-, 50- and 37-kDa. This result suggests that each chitinase isoform may play a role in the chitinase

production and the disease suppression. In this study, however, the roles of individual isoform of 54-, 52-, 50- and 37-kDa in the chitinase production and the disease suppression were not elucidated, because only Chi a$^-$ mutants with the same characteristics were obtained. Thus, we are now selecting Tn*5* mutants defective in other chitinase isoforms, 54- and 50-kDa, to further clarify the roles of each isoform.

C. violaceum C-61 did not contain an indigenous plasmid, which indicates that all genes are in a chromosomal location. Southern blot analysis also showed that Tn*5* element was inserted into a 10 kb *Eco*RI fragment in three Chi a$^-$ mutants and in 10 kb and 4.4 kb *Eco*RI fragments in a mutant. Since the size of Tn*5* is 5.7 kb (3), Tn*5* element in a 4.4 kb *Eco*RI fragment is considered to be deleted. In addition, these results suggest that genes essential for 52- and 37-kDa chitinase isoform production are around 10 kb *Eco*RI fragment of the chromosomal DNA. Numbers and sites of Tn*5* inserted in the chromosomal DNA were also identified in *Pst*I fragments. Tn*5* mutants reported here can be used in the isolation of genes encoding 52- and 37-kDa chitinase production.

요 약

Chromobacterium violaceum C-61이 생산하는 chitin 분해효소가 *R. solani*에 의한 가지, 오이 모잘록병 억제에 중요한 역할을 하는지를 알아보기 위하여 chitin 분해력이 낮은 Tn*5* 삽입 돌연변이주를 선발, 병 억제력 및 chitin 분해효소의 특성을 모균주와 비교하였다. 약 2,000개의 변이체 중에서 chitin 분해력이 낮은 4 돌연변이주가 선발되었는데, 이들 균주는 NA-PDA 배지 위에서 *R. solani*를 억제하지 못하였고, 모잘록병 억제력도 모균주에서 보다 훨씬 더 낮았다. 그러나, 토양에서의 서식 밀도는 모균주와 돌연변이주들 간에 큰 차이가 없었다. 한편 모균주의 조효소액은 *R. solani*의 생장을 억제하였을 뿐만 아니라 돌연변이주들의 조효소액보다 훨씬 더 높은 chitinase 활성을 나타냈다. 그러나, N,N′-diacetylchitobiase 활성은 모균주와 돌연변이주간에 큰 차이가 없었다. 아울러, 4 돌연변이주 모두는 54-, 52-, 50-, 37-kDa의 chitinase 동위효소 중에서 52-와 37-kDa의 동위효소에 결함이 있었는데, 3 돌연변이주는 약 10 kb *Eco*RI 염색체 절편에 Tn*5*가 하나 삽입되었고, 1 돌연변이주는 10 kb와 4.4 kb *Eco*RI 절편에 둘 삽입되어 있었다. 이러한 결과들은 *C. violaceum* C-61이 생산하는 chitinase가 *R. solani*에 의한 모잘록병 억제에 중요한 역할을 한다는 것을 나타낸다.

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