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In planta – complementation of *Clavibacter michiganensis* subsp. *sepedonicus* strains deficient in cellulase production or HR induction restores virulence

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Abstract

Clavibacter michiganensis subsp. *sepedonicus*, a Gram positive bacterium that causes bacterial ring rot of potato, was studied in eggplant, an alternate host, using strains that differed in phenotype. Two factors affecting virulence, the ability to induce a hypersensitive response (HR) and cellulase production, were studied. A plasmid-free isolate of *C. michiganensis* subsp. *sepedonicus* that causes HR on tobacco but is unable to produce cellulase multiplied efficiently *in planta*, but caused only weak symptoms. In contrast, a strain that is unable to induce HR on tobacco but produces cellulase was impaired in the ability to multiply in the host and caused no symptoms. When the two non-virulent strains were coinoculated into eggplants, typical disease symptoms developed. This enhancement was not due to formation of a new phenotype or significant increases in population density of either of the strains. Our results suggest that both cellulase production and the ability to induce HR are required for a successful infection process and disease induction by *C. michiganensis* subsp. *sepedonicus*. Our results additionally suggest that the ability to induce HR on non-host plants is required for multiplication in the host plant, whereas cellulase expression is necessary for induction of disease symptoms.

Abbreviations: HR – hypersensitive response; h.p.i. – hours after inoculation; d.p.i. – days after inoculation.

Introduction

Clavibacter michiganensis subspecies *sepedonicus* is a Gram positive bacterium that causes ring rot of potato (Hocker, 1981; Davis et al., 1986). This disease has occurred in major potato-growing areas in all continents, excluding Australia (Hocker, 1981). Through extensive seed certification programmes, many countries have successfully eradicated the disease, but it remains a persistent problem in potato-growing areas of northern Europe and North America, and new outbreaks of unknown source in central Europe have been reported (Anonymous, 1999). Typical ring rot symptoms consist of foliar wilt and chlorosis and characteristic rot of the vascular tissue of the tubers (Hocker, 1981). Symptom expression is strongly affected by environment, pathogen strain, inoculum dose and potato cultivar (Bishop and Slack, 1987a; Westra and Slack, 1994). Latent infections are common and can be carried over three generations in field or in potato tissue cultures (De Boer and McCann, 1990; Franc, 1999; Schuld et al., 1992). Ring rot diagnosis is routinely done by visual symptom inspection and bioassays on eggplant, an alternate host plant of *Clavibacter michiganensis* subsp. *sepedonicus* (Bishop and Slack, 1987b). These methods often fail to detect

latent infections. Thus, much research has concentrated on improving diagnostics for *C. michiganensis* subsp. *sepedonicus*, and sensitive methods based on specific antibodies (De Boer et al., 1988) and PCR amplification (Li and De Boer, 1995; Mills et al., 1997; Hu et al., 1995) are now available. In contrast to development in detection methods, progress on understanding mechanisms behind latent infections and research on pathogenicity factors of *C. michiganensis* subsp. *sepedonicus* has lagged behind.

Extracellular enzymes are known virulence factors in Gram negative plant pathogens, and enzyme production of *C. michiganensis* subsp. *sepedonicus* has also been studied. *C. michiganensis* subsp. *sepedonicus* produces cellulase (Goto and Okabe, 1958; Baer and Gudmestad, 1995) and sucrase (Baer et al., 1998) *in vitro*. Whether sucrase plays a role in pathogenicity of *C. michiganensis* subsp. *sepedonicus* is undetermined, but cellulase production seems to be necessary for virulence of this bacterium. Strains or mutants that lack cellulase production show only residual virulence in eggplant assays, but introduction of the cellulase by transformation significantly enhances virulence (Laine et al., 2000). The cellulase gene of *C. michiganensis* subsp. *sepedonicus* is carried on a large plasmid (Laine et al., 2000). The 50 kb plasmid, pCS1, is present in all studied strains of *C. michiganensis* subsp. *sepedonicus* with one exception, strain P45 (Mogen et al., 1988). This plasmidless strain lacks cellulase production also and shows only residual pathogenicity in eggplant (Nissinen et al., 1998) and in potato (Solke De Boer, pers. comm.).

*H*ypersensitive *r*esponse and *p*athogenicity (*hrp*) genes are fundamental pathogenicity determinants for Gram negative plant pathogenic bacteria (Lindgren, 1997). *Hrp* genes code for structural proteins of the type III secretion machinery, effector proteins secreted through the pathway (Mudgett and Staskawicz, 1998) and regulator proteins controlling expression of *hrp* genes (Lindgren, 1997). Mutants defective in the hrp secretion pathway are unable to cause a hypersensitive response (HR) on a resistant plant species or cultivar, and are not able to cause disease on a susceptible host. Several studies have shown the inability of *hrp*$^-$ mutants to multiply in host tissues (Lindgren et al., 1986; Bonas et al., 1991; Steinberger and Beer, 1988; Frey et al., 1994). Recently, Hirano and coworkers (Hirano et al., 1999) showed that *Pseudomonas syringae* pv. *syringae hrp*$^-$ mutants could not efficiently colonize host tissues under field conditions, and suggested that the hrp secretion system plays a role in pathogenicity by enabling growth and colonization of the host by bacteria.

Thus far, no homologues of *hrp* genes have been found in *Clavibacter* spp. or other Gram positive plant or animal pathogens, but two subspecies of *C. michiganensis* have been reported to elicit HR on a non-host plant (Gitaitis, 1990; Bermpohl et al., 1996; Nissinen et al., 1997). We have previously reported that *C. michiganensis* subsp. *sepedonicus* elicits HR on tobacco and that the ability of *C. michiganensis* subsp. *sepedonicus* strains to elicit HR correlates with the virulence of the strains – only the strains that induce HR on tobacco are able to cause disease in host plants (potato or eggplant). Virulent strains also secrete HR-eliciting proteins resembling harpins of Gram negative phytopathogens, whereas non-virulent strains do not (Nissinen et al., 1997).

As the essential molecular tools such as transposon mutagenesis are still under development for *Clavibacter* species, it is at the moment not possible to create marked mutants of *C. michiganensis* subsp. *sepedonicus* for virulence studies. Fortunately, there are natural isolates available that represent different phenotypes in respect to the two factors that correlate with disease induction, namely, cellulase production and elicitation of HR. In this study, we used *C. michiganensis* subsp. *sepedonicus* isolates representing three different phenotypes: fully virulent strains Cs2 and Cs7 (HR positive and cellulase positive) and two non-virulent strains, P45, which is defective in cellulase production, and Cs4, which is HR negative. We followed the behaviour of these strains in host plants in order to elucidate mechanisms affecting symptom expression and multiplication *in planta*.

Materials and methods

Bacterial strains and preparation of inocula

The *C. michiganensis* subsp. *sepedonicus* strains used in this study are all natural isolates from potato. Strains and their main characteristics are listed in Table 1. Bacteria were grown on YGM agar plates (De Boer and Copeman, 1980) at 26 °C for 6 days. Cells were suspended into sterile, ice cold 50 mM potassium phosphate buffer, pH 7.0 (P-buffer) at OD_{620} 0.6 (10^8 cfu per ml) for pathogenicity tests on eggplant and OD_{620} 0.8 for tests of hypersensitive response. Mixed inocula were prepared by mixing equal volumes of strains Cs4 and P45 adjusted to OD_{620} 0.6.

Table 1. Characteristics of bacterial strains of *C. michiganensis* subsp. *sepedonicus* used in this study

Strain	Cs4	Cs2	Cs7	P45
Virulence on eggplant[1]	<2%	43–59%	56%	<2%
Plasmid status	+[2]	+[3]	+[2]	—[4]
Cellulase activity[5]	2.8 mm	3.2 mm	3.6 mm	—
HR induction on tobacco	—	+	+	+
Source	SH De Boer	RJ Copeman	B Rogers	SH De Boer
Origin	B.C., Canada	B.C., Canada	S. Idaho, USA	Quebec, Canada

[1]Mean percentage of eggplant tissue showing wilting 18 days after inoculation.
[2]Data from Nissinen et al., 1998.
[3]Unpublished observations.
[4]Data from Mogen et al., 1988.
[5]Mean cellulolytic activity on indicator plate assays expressed as mm between the edge of bacterial growth and edge of clear halo resulting from breakdown of cellulose. — = no activity.

For growth measurements, strains were grown in YGM liquid media at 26 °C in a rotary shaker using 75 ml media in 200 ml flasks and 280 rpm. Cultures (three replicates per strain) were started at OD_{620} of 0.05, and growth was monitored by measuring OD_{620} at 6 h intervals.

Assays for induction of hypersensitive response, pathogenicity and cellulolytic activity

Tobacco plants (*Nicotiana tabacum* cv. xanthi) were used for evaluating hypersensitive response. Plants were grown in pots in soil (Kekkilä OY, Kekkilä, Finland) at 23 °C and a light intensity of 200–300 μmol PAR m^{-2} s^{-1} with a 16 h photoperiod. Bacterial suspensions (prepared as described above) were infiltrated into intercellular spaces of fully expanded leaves of 6-week-old tobacco plants with a 1 ml syringe. Infiltrated areas were monitored for a necrotic response 24 and 48 h after inoculation (h.p.i.). Three inoculations were done for each sample.

Eggplants (*Solanum melongena*) cv. Black Beauty, grown under the same conditions as tobacco plants, were used to test virulence and bacterial populations *in planta*. When the first true leaf was emerging, eggplants were inoculated with bacterial suspensions in the stem immediately above the cotydelons using a 1 ml syringe with a 16 G needle. Infiltrations with sterile P-buffer were used as negative controls. Plants were screened for the presence of ring rot symptoms 18 days after inoculation (d.p.i.). The proportion of plant tissue showing wilting (0–100%) was recorded from 12 and 24 plants per sample for experiments 1 and 2, respectively. For experiment 3, 10 plants per sample were analysed, whereafter ∼1 cm stem tissue above (but not including) the inoculation point was removed for determination of bacterial population sizes.

For detection of cellulolytic activity, *C. michiganensis* subsp. *sepedonicus* strains were grown on modified M9 media supplemented with carboxymethylcellulose (Meletzus et al., 1993). For semi-quantitative determination of relative enzyme activity, 10 μl of bacterial suspension (three replicates per strain) at OD_{620} 0.8 was dropped onto plates. After 7 days, the margins of bacterial growth were marked on the plates, whereafter the plates were stained with 0.1% Congo Red for detection of cellulose hydrolysing enzymes (Teather and Wood, 1982; Baer and Gudmestad, 1995). Enzymatic activity was expressed as mm between the edge of bacterial growth and the edge of the clear halo. For characterization of bacteria isolated from plants, activity was expressed as presence or absence of haloes around colonies.

Determination of bacterial population densities in eggplants, characterization of isolated bacteria and data analysis

For determination of bacterial population densities in eggplants, stem samples were surface sterilized by dipping in 70% ethanol, weighed and homogenized (Ultra Turrax T5, IKA, Staufen, Germany) in 3 ml sterile P-buffer, of which 200 μl dilutions were spread onto YGM agar for plate counts. Bacterial numbers expressed as colony forming units (cfu) per g stem tissue fresh weight were determined for each plant and averaged to obtain a mean population density for each treatment. To confirm phenotypes of isolated bacteria, 50 (for single strains) or 250 (for mixed inoculations) isolated colonies from each sample were transferred from the dilution plates onto cellulase indicator plates with replicas prepared onto YGM plate, and cellulase activity detected as described above. Five randomly chosen colonies (for individual strains) or eight colonies showing cellulase positive phenotype and seven showing no cellulase activity (for mixed inoculations samples) were tested for induction of hypersensitive response on tobacco as described above.

The identity of bacterial isolates as *C. michiganensis* subsp. *sepedonicus* (5–50 colonies per sample) was confirmed by immunofluorescence using an assay kit for *C. michiganensis* subsp. *sepedonicus* with mouse anti-Cms clone 9A1 from Agdia (Agdia Inc., Elkhart, IN, USA). Lyophilized negative and positive controls used were also from Agdia. Statistical analyses, including tests for disease induction and population densities by ANOVA, were performed using SigmaStat (SPSS Inc.).

Results

Characterization of C. michiganensis *subsp.* sepedonicus *strains*

Growth rates of the two non-virulent strains, Cs4 and P45, along with strongly virulent strain Cs7 in liquid cultures were followed for 3 days. Doubling time of all strains was approximately 6 h, and cultures reached final OD_{620} 2.8–3.5 in 2 days (data not shown).When tested for induction of HR on tobacco plants, strain Cs4 did not induce any visible response, whereas strains P45, Cs2 and Cs7 caused a confluent collapse and necrosis of the infiltrated area typical of HR 24 h.p.i. (Table 1). In indicator plate assays for cellulase production strains Cs4, Cs2 and Cs7 created clear haloes extending 2.8, 3.2 and 3.6 mm, respectively, beyond the bacterial growth. In contrast, no cellulase activity was detected in plasmid-free strain P45 (Figure 1).

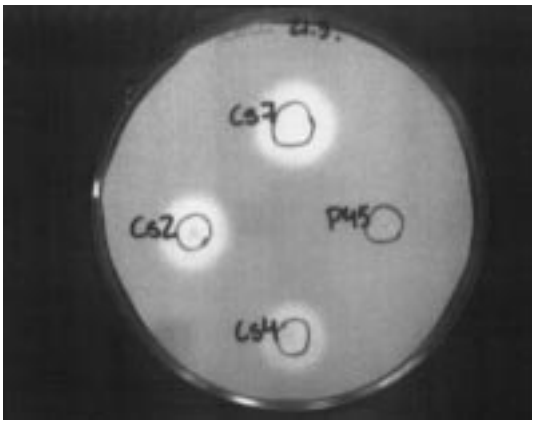

Figure 1. Cellulase activity of *C. michiganensis* subsp. *sepedonicus* strains on indicator plate. Ten μl of bacterial suspensions prepared from individual strains were pipetted onto agar plate containing carboxymethylcellulose that was stained with 0.1% Congo Red after 7 days. Margins of bacterial growth are marked on plate. Breakdown of cellulose is seen as clear haloes.

Induction of ring rot symptoms in eggplants

Three independent experiments were conducted on eggplant to assess disease severity caused by different strains and strain combinations of *C. michiganensis* subsp. *sepedonicus*. As expected, plants infected with strains Cs2 or Cs7 showed severe disease symptoms with extensive wilting typical of ring rot (Table 2). Strains Cs4 and P45 caused no symptoms or only very mild symptoms. Less than 2% of tissues in Cs4- or P45-infected plants showed any wilting (Table 2). However, co-inoculations of strain Cs4 with strain P45 induced significantly more disease symptoms than inoculations with single strains (Table 2).

In addition to wilting, severe stunting and discolouration was observed in plants infected with virulent strains Cs2 or Cs7; plants were malformed and greatly reduced in size (Figure 2). No obvious chlorosis or stunting was observed when plants were inoculated with one of the non-virulent strains, although a slight reduction in size was seen in P45-infected eggplants. However, co-inoculation of Cs4 and P45 resulted in severely stunted and chlorotic plants that resembled plants infected with highly virulent strains. The effect of co-inoculations on plant growth was more profound than the effect on wilting (Figure 2).

Table 2. Disease induction in eggplants by *C. michiganensis* subsp. *sepedonicus*

Strain(s)	Experiment number		
	1	2	3
Cs4	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.7 ± 0.8^{a}
P45	0.25 ± 0.25^{a}	0.7 ± 0.4^{a}	1.8 ± 0.6^{a}
Cs2	59.4 ± 5.1^{b}	43.3 ± 3.2^{b}	n.t.
Cs7	n.t.	n.t.	56.4 ± 9.0^{b}
Cs4+P45	9.8 ± 2.4^{c}	14.1 ± 2.1^{c}	21.6 ± 4.2^{c}

Eggplants were stem-inoculated with bacterial suspensions prepared from individual strains or a mixture of two strains. Symptoms were scored after 18 days and expressed as percentage of plant tissue showing wilting. Data presented represent the mean symptom percentage and standard error from 12, 24 and 10 plants per treatment for experiments 1, 2 and 3, respectively. Values indicated with the same superscripted letter within the same experiment do not differ significantly ($P > 0.05$). n.t. = not tested.

Figure 2. Ring rot symptoms and stunting of eggplants induced by *C. michiganensis* subsp. *sepedonicus*. Plants were stem inoculated with bacterial suspension prepared from individual strains or a mixture of two strains. Photograph was taken 18 days after inoculation.

Determination of bacterial population densities in planta

In the third experiment, bacterial population densities in infected eggplants were determined. Dilution platings of bacteria isolated from eggplant stems 18 d.p.i. revealed significant differences ($P < 0.05$) in the ability to multiply *in planta* between strains of *C. michiganensis* subsp. *sepedonicus*. The fully virulent strain Cs7 reached very high titers: the mean population densities at the time of isolation were over 10^{10} cfu per g of plant tissue fresh weight. In contrast, strain Cs4 seemed to be impaired in ability to multiply in eggplant. Two out of the ten inoculated eggplants did not contain any culturable bacteria, and the mean population density was only 7×10^7 cfu per g fresh weight (Figure 3). As the original inocula were estimated to be $\sim 10^7$ cfu per g fresh weight, no significant increase in bacterial numbers was observed. Strain P45 colonized eggplants efficiently, reaching population densities over 10^9 cfu per g plant tissue fresh weight (Figure 3). Bacterial population sizes in plants co-infected with Cs4 and P45 were comparable ($P = 0.58$) to populations isolated from P45-infected plants (Figure 3). Only occasional contaminating colonies were found on infected plants with *C. michiganensis* subsp. *sepedonicus* or control plants during the experiments.

Characterization of isolated bacteria

Bacteria isolated from eggplants were characterized to ensure that strains had retained their original phenotypes *in planta* and to estimate relative populations of strains Cs4 and P45 in the co-inoculated plants. All the colonies from plants inoculated with single strains showed original phenotypes in cellulase assays: bacteria isolated from Cs4- or Cs7-infected plants were all positive, and none the colonies from P45–infected plants showed any cellulase activity. When tested for induction of HR on tobacco, all the tested colonies behaved according to their original phenotypes: bacteria isolated from Cs7- or P45-infected plants gave confluent HR within 24 h, and no HR was observed with bacteria isolated from Cs4-infected plants.

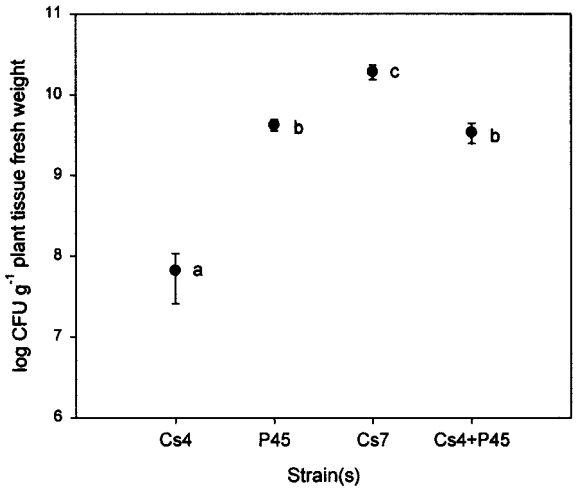

Figure 3. Bacterial population densities of *C. michiganensis* subsp. *sepedonicus* strains in eggplants. Eggplants were stem inoculated with bacterial suspension prepared from individual strains or a mixture of two strains, and bacterial numbers determined 18 days after inoculation. Data presented represents the mean log cfu per g plant tissue fresh weight and standard error from 10 plants per treatment. Values denotated with same letter do not differ significantly at $P = 0.05$.

Two hundred and fifty colonies from the co-inoculated plants were tested for cellulase production to estimate relative proportions of Cs4 and P45. Thirteen colonies gave positive results on indicator plates, suggesting that the relative percentage of Cs4 in the mixed populations was approximately 5%. Colonies were tested for HR-induction, and only the colonies negative in cellulase assays gave HR, indicating that the strains had retained their original phenotypes (in cellulase production and HR induction) *in planta*. Moreover, co-inoculation with P45 did not markedly affect the population densities of Cs4 *in planta*: based on plate counts, the mean population density of Cs4 in co-infected plants was estimated to be $\sim 10^8$ cfu per g plant tissue fresh weight. The identity of the isolated bacteria was tested by immunofluorescence using *C. michiganensis* subsp. *sepedonicus*-specific antibody. All the 200 tested colonies reacted positively in immunofluorescence assays, confirming their identity as *C. michiganensis* subsp. *sepedonicus*.

Discussion

The causal agent of potato ring rot, *C. michiganensis* subsp. *sepedonicus* is a poorly characterized bacterium, and the virulence factors and mechanisms leading to latent infections are virtually unknown. In this study, we investigated the behaviour of four strains with different phenotypes in order to understand mechanisms affecting the survival in host plant as well as the factors that affect symptom expression by this bacterium.

Strains Cs2 and Cs7 were positive for both cellulase activity and HR induction. These strains also caused severe disease symptoms when inoculated into eggplants. In contrast, strains P45, which is HR positive but cellulase negative, and Cs4, which is HR negative but produces cellulase, were virtually non-virulent in plant assays. There were no differences in growth rates in standard culture media among the four strains, indicating that the inability of strains Cs4 or P45 to cause disease was not due to disturbances in basic metabolic functions necessary for bacterial multiplication.

Cellulase activity correlated with plasmid status of strains; all but P45 harbour a 51 kb plasmid, pCS1. This is not surprising, since it has been previously determined that the cellulase gene of *C. michiganensis* subsp. *sepedonicus* is carried on pCS1 (Laine et al., 2000). A role for plasmid-borne cellulase in disease induction has also been reported for *C. michiganensis* subsp. *michiganensis*, where the plasmid-free isolate CMM100, which lacks both cellulase production and pathogenic phenotype on the host (tomato), is restored in virulence by restoring cellulase production (Meletzus et al., 1993). Cellulolytic activity by *C. michiganensis* subsp. *sepedonicus in planta* has been reported (Larson, 1944; Christie, 1990). Electomicrographs reveal thinning and disruption of xylem cell walls in *C. michiganensis* subsp. *sepedonicus*-infected vessels, which could lead to vessel plugging and inhibition of water transport (Christie, 1990). This could explain the wilting caused by the ring rot bacterium. Although we cannot rule out the possibility of genes for other virulence factors potentially present in pCS1, the significant enhancement of virulence in P45 by introduction of the cellulase gene (Laine et al., 2000) suggests that cellulase is the major virulence determinant in pCS1.

However, cellulase activity is not sufficient for virulence, as exemplified by strain Cs4, which produces normal amounts of cellulase on indicator plates, but does not induce HR on tobacco plants, and does not cause significant disease symptoms on eggplant. The correlation between the ability to induce HR and virulence is well established in Gram negative phytopathogens (Lindgren, 1997) and has also been reported for two subspecies of *C. michiganensis*

(Bermpohl et al., 1996; Nissinen et al., 1997). For *C. michiganensis* subsp. *sepedonicus*, five strains (strains Cs4, 3M, 3NM, R5 and R8) were found to be consistently negative in both HR induction and disease induction (Nissinen et al., 1997).

Colonization studies revealed that the HR-negative strain Cs4 was unable to multiply efficiently *in planta*; population densities were more than 100-fold lower than those of P45 or Cs7. However, 80% of analysed eggplants contained living bacteria, indicating that in spite of the inability to multiply efficiently, Cs4 was still able to survive in eggplant xylem tissues, at least for the duration of the experiment. In addition to Cs4, another non-virulent and HR$^-$ strain of *C. michiganensis* subsp. *sepedonicus*, 3M has been found to be severely impaired in host colonization (R. Nissinen, unpublished results). The correlation between ability to induce HR on non-host and ability to multiply in host has also been reported for *C. michiganensis* subsp. *michiganensis* (Bermpohl et al., 1996). The mechanism behind the inability of HR$^-$ *Clavibacter* strains to multiply in host plants is unknown. However, the inability of HR$^-$ *C. michiganensis* subsp. *sepedonicus* strains to secrete HR inducing proteins and their ability to grow normally in culture media, but not *in planta*, resembles intriguingly the phenotype observed in *hrp*$^-$ mutants of Gram negative pathogens (Lindgren et al., 1986; Bonas et al., 1991; Hirano et al., 1999).

Unlike Cs4, the plasmid-free strain P45 colonized plants efficiently, reaching population levels comparable to the virulent strain. Despite high bacterial titers, almost no symptoms were visible on P45-infected eggplants. Similarly, the plasmid-free cellulase deficient isolate of *C. michiganensis* subsp. *michiganensis*, CMM100, multiplies efficiently *in planta*, but causes no disease symptoms (Meletzus et al., 1993). This would suggest that cellulase activity is not required for host colonization by *Clavibacter* spp., but is essential for symptom expression. The tendency of *C. michiganensis* subsp. *sepedonicus* to form latent infections in potato, where no symptoms are seen in spite of high bacterial titers, suggests that some strains may down-regulate cellulase expression *in planta*. Further investigations of this phenomenon, whose understanding is important for controlling spread of the disease, may require further development of molecular methods that do not yet exist for this bacterium.

When the two strains that were unable to cause wilting when inoculated alone, P45 and Cs4, were co-inoculated into eggplants, typical disease symptoms developed. A similar phenomenon has been reported for *Erwinia amylovora*, where HR$^-$ mutants and mutants defective in extracellular polysaccharides complement each other for symptoms formation (Belleman and Geider, 1992), and for *Pseudomonas syringae* pv. *syringae*, where co-inoculation of an *hrp*$^-$ mutant with a *gacS*$^-$ mutant results in disease symptoms (Hirano et al., 1999). For *Pseudomonas syringae* pv. *syringae*, co-inoculation of an *hrp*$^-$ mutant with a *gacS*$^-$ mutant significantly increased population sizes of the HR$^-$ strain and the authors suggested that the increase in population sizes of *hrp*$^-$ mutants led to symptom expression (Hirano et al., 1999). In contrast, in our study, Cs4 was estimated to form only 5% of mixed populations, giving estimated population densities only two times higher than in plants inoculated with Cs4 alone. Thus, it seems that the mechanism involved in symptom enhancement would not require an increase in bacterial numbers. Further characterization of bacteria isolated from eggplants revealed that the populations in co-inoculated eggplants consisted of bacteria with original phenotypes of P45 and Cs4; all the tested colonies were either cellulase or HR negative. This indicates that the observed enhancement in disease expression was not due to a new virulent phenotype resulting from transfer of pCS1, and thus the cellulase gene, to P45. Rather, we hypothesize that the strains were able to compensate each other's defects by each providing factors necessary for successful invasion and disease induction.

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