

Suppression of Bean Defense Responses by *Pseudomonas syringae*

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We have developed a model system to examine suppression of defense responses in bean by the compatible bacterium *Pseudomonas syringae* pv *phaseolicola*. Previously, we have shown that there is a general mechanism for the induction of the bean defense genes phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and chitinase (CHT) by incompatible, compatible, and nonpathogenic bacteria. Here, we show that bean plants infiltrated with isolates of *P. s. phaseolicola* failed to produce transcripts for PAL, CHS, or CHI up to 120 hr after infiltration and CHT transcript accumulation was significantly delayed when compared to the incompatible *P. syringae* strains. Infiltration of bean plants with 10^8 cells per mL of *P. s. phaseolicola* NPS3121 8 hr prior to infiltration with an equal concentration of incompatible *P. s. pv tabaci* Pt11528 significantly reduced the typical profile of defense transcript accumulation when compared to plants infiltrated with Pt11528 alone. A corresponding suppression of phytoalexin accumulation was also observed. NPS3121 also suppressed PAL, CHS, CHI, and CHT transcript accumulation and phytoalexin production induced by *Escherichia coli* DH5 α or the elicitor glutathione. Heat-killed NPS3121 cells or cells treated with protein synthesis inhibitors lost the suppressor activity. Taken together, these experiments suggest that NPS3121 has an active mechanism to suppress the accumulation of defense transcripts and phytoalexin biosynthesis in bean.

INTRODUCTION

The interactions of plants and bacteria have been shown to be carefully regulated, complex biological relationships. During the interactions between plants and strains of *Rhizobium* or *Agrobacterium*, plant-derived molecules function as signals that induce the expression of specific bacterial genes; the products of these bacterial genes in turn induce changes in plant gene expression resulting in morphologic and metabolic changes in the plant host (Ream, 1989; Sanchez et al., 1991). In contrast to *Agrobacterium*, very little is known about the signal exchange that occurs between other strains of phytopathogenic bacteria and their host and nonhost plants.

Previous studies have demonstrated that there are distinct changes in plant gene expression during incompatible (also known as resistant) plant–bacterium interactions. Incompatible interactions are characterized by the inhibition of bacterial multiplication, lack of symptom development, and, in many cases, the rapid plant cell necrosis commonly referred to as the hypersensitive reaction (Klement, 1982). (Note: incompatible will also be used in this manuscript to describe pathogens that induce hypersensitive reactions.) Analysis of in vitro translation products has shown that new polypeptides are produced in plants very rapidly after inoculation with incompatible bacteria (Slusarenko and Longland, 1986; Collinge et al., 1987; Ragueh et al., 1989). Studies have also shown that putative

defense protein transcripts, including phenylalanine ammonia-lyase (PAL) (Dong et al., 1991; Jakobek and Lindgren, 1993), chalcone synthase (CHS) (Dhawale et al., 1989; Jakobek and Lindgren, 1993), chalcone isomerase (CHI) (Jakobek and Lindgren, 1993), chitinase (CHT) (Godiard et al., 1990; Jakobek and Lindgren, 1993), β -1,3-glucanase (Godiard et al., 1990; Dong et al., 1991), and proteinase inhibitors I and II (Pautot et al., 1991), accumulate in plant hosts after inoculation with incompatible bacteria.

With the exception of *A. tumefaciens*, there have been few studies conducted centering upon the signal exchange that occurs during compatible (also known as susceptible) plant–bacterium interactions. In contrast with incompatible interactions, compatible interactions are characterized by bacterial growth within the host and disease development (Klement, 1982). (Note: in this manuscript compatible will be used to describe pathogens that cause disease.) Presumably, compatible pathogens must avoid activating plant defense mechanisms or inactivate those defense mechanisms that are induced. A common feature of compatible interactions is delayed defense gene transcript accumulation, when compared to defense transcript accumulation during incompatible interactions (Dixon and Harrison, 1990; Dixon and Lamb, 1990). For instance, in the bean cultivar Red Mexican, CHT transcript levels increased between 3 and 6 hr after inoculation with an incompatible isolate of *Pseudomonas syringae* pv *phaseolicola*, whereas during the interaction with a compatible isolate, CHT

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transcript accumulation was delayed to 20 to 24 hr (Voisey and Slusarenko, 1989). Thus, compatible bacterial pathogens may have evolved mechanisms to inhibit or suppress the induction of plant defense responses. A number of studies have indicated that fungal pathogens produce such suppressors of plant defense responses (Doke et al., 1980; Ziegler and Pontzen, 1982; Kessmann and Barz, 1986; Yamada et al., 1989). Transposon insertion mutants of *Xanthomonas campestris* pathovars have been isolated that induce hypersensitive reactions on normally susceptible plants, suggesting that bacterial plant pathogens might also produce suppressors (Daniels et al., 1984; Kamoun et al., 1992); however, to date, no suppressor has been isolated from a bacterial pathogen.

Previously, we reported that transcripts for PAL, CHS, CHI, and CHT accumulated, and phytoalexins were produced in bean after infiltration with the wild-type or hypersensitive reaction and pathogenicity-minus (*Hrp*⁻) mutants of *P. s. pv tabaci*, a compatible isolate of *P. s. pv syringae*, *P. fluorescens*, or *Escherichia coli* (Jakobek and Lindgren, 1993). In contrast, preliminary experiments demonstrated that transcript accumulation and phytoalexin production did not occur in bean after infiltration with *P. s. phaseolicola*, the causal agent of halo blight (Jakobek and Lindgren, 1993). In this paper, we present a more detailed analysis of the bean-*P. s. phaseolicola* interaction and show that transcripts for PAL, CHS, and CHI did not accumulate in bean up to 5 days after infiltration with *P. s. phaseolicola* NPS3121. We also present data indicating that this bacterium actively inhibited, or suppressed, the accumulation of defense transcripts and phytoalexin production in bean.

RESULTS

P. syringae Pathovars Induce the Accumulation of Defense Transcripts in Bean

We have previously shown that defense transcripts accumulated in bean after infiltration with *P. s. tabaci* Pt11528, an incompatible bacterium. To determine if these transcripts also accumulated in response to other *P. syringae* pathovars, bean was infiltrated with a number of strains of *P. syringae*, which are listed in Table 1, using inocula of $\sim 10^8$ cells per mL. Transcripts for PAL, CHS, CHI, and CHT were detected in leaf tissue 6 hr after infiltration with *P. syringae* pathovars *tabaci* Pt113, *angulata* Psa45 and Psa52, *glycinea* PsgR4 and PsgR0, *tomato* Pst1065 and Pst1002, *pisi* 202-12-2R and 209-21-3R, and *syringae* 655R, all of which induce a hypersensitive reaction on bean (data not shown). These transcripts were also present after infiltration with compatible *P. s. syringae* B724, B733, B728a, 4089Br, and 4076Br, or the nonpathogenic *P. syringae* isolates Cit7 and 31rif. In contrast, these transcripts were not present after infiltration with *P. s. phaseolicola* NPS3121 or PP14.

Table 1. *P. syringae* Strains Used in This Study

Pathovar	Host	Plant Reaction on Red Kidney ^a	Source
<i>phaseolicola</i>			
NPS3121	Bean	Compatible	N. Panopoulos
PP14	Bean	Compatible	P. Psallidas
19304	Bean	Compatible	A. Matthyse
<i>tabaci</i>			
Pt11528, Pt113	Tobacco	Incompatible	R. Durbin
<i>angulata</i>			
Psa45, Psa52	Tobacco	Incompatible	R. Durbin
<i>glycinea</i>			
PsgR0, PsgR4	Soybean	Incompatible	B. Staskawicz
<i>tomato</i>			
Pst1065, Pst1002	Tomato	Incompatible	J. Lindemann
<i>pisi</i>			
202-12-2R, 209-21-3R	Pea	Incompatible	M. Powelson
<i>syringae</i>			
655R	Maize	Incompatible	J. Lindemann
B724, B733, B728a	Bean	Compatible	S. Lindow
4089Br, 4076Br	Bean	Compatible	S. Hirano
<i>Unknown</i>			
Cit7, 31rif	Unknown	Incompatible	S. Lindow

^a Incompatible, development of a hypersensitive reaction by 24 hr after infiltration; compatible, development of disease symptoms by 120 hr after infiltration.

Transcripts for PAL, CHS, and CHI Do Not Accumulate in Bean in Response to *P. s. phaseolicola*

A detailed analysis was conducted to determine if defense transcripts accumulated during the compatible interaction of *P. s. phaseolicola* and bean. Bean was infiltrated with NPS3121 at a concentration of $\sim 10^8$ cells per mL. RNA was isolated from leaf tissue harvested at various times up to 48 hr after infiltration. Transcripts for PAL, CHS, and CHI were not detected during this time period; however, CHT transcript was detected 20 to 24 hr after infiltration, as shown in Figure 1. These transcripts were not detected in control plants infiltrated with water (data not shown).

When Red Kidney plants are infiltrated with *P. s. phaseolicola* at a concentration of 10^5 cells per mL, water-soaked lesions usually appear between 72 and 96 hr after infiltration; thus, we investigated transcript accumulation in bean after infiltration with this lower inoculum dose. Total RNA was isolated from leaves at 24-hr intervals up to 120 hr after infiltration with $\sim 10^5$ cells per mL of NPS3121. Figure 2 shows that transcripts for PAL and CHS did not accumulate in leaf tissue during this period of time. However, significant levels of CHT accumulated between 48 and 72 hr after infiltration. These transcripts were not detected in control plants infiltrated with water (data not

shown). Experiments were also conducted with *P. s. phaseolicola* isolates PP14 and 19304. Transcripts for PAL and CHS were not detected in bean up to 120 hr after infiltration with either of these strains using inoculum of 10^5 cells per mL (data not shown).

NPS3121 Inhibits the Typical Defense Transcript Accumulation in Bean Elicited by *P. s. tabaci*

Bean was badly damaged due to symptom development 120 hr after infiltration with NPS3121. The lack of PAL, CHS, and CHI transcript accumulation in damaged tissue is interesting because previous studies have shown that these genes are induced in response to wounding (Lawton and Lamb, 1987; Mehdy and Lamb, 1987; Hedrick et al., 1988). Our results suggest that this bacterium may suppress the accumulation of these transcripts during infection.

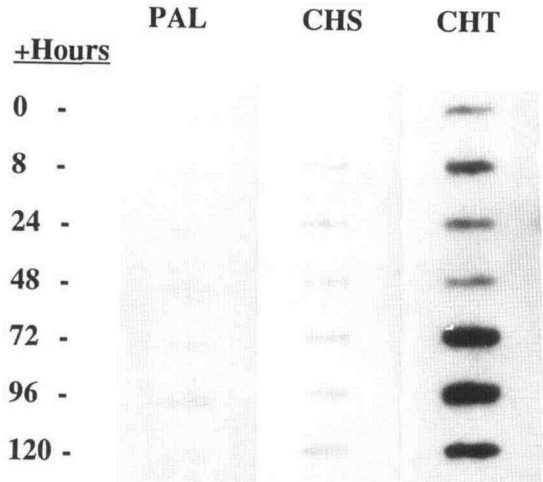


Figure 2. Defense Transcript Accumulation after Infiltration with Low Inoculum.

RNA was isolated from bean leaf tissue between 0 and 120 hr after infiltration with *P. s. phaseolicola* at a concentration of 10^5 cells per mL. Total RNA (5 μ g) from each time point was probed with PAL, CHS, and CHT.

To test this hypothesis, bean plants were infiltrated with NPS3121 at 10^5 or 10^8 cells per mL, Pt11528 at 10^8 cells per mL, or with water as a control. After an initial 8-hr incubation, the bean plants were reinfiltreated with various combinations of the same treatments. Plants were incubated for an additional 8 hr, and the infiltrated tissue was harvested and RNA was isolated as before. As shown in Figure 3, slot blot analysis revealed that plants infiltrated with NPS3121 at 10^8 cells per mL prior to infiltration with Pt11528 had reduced levels of PAL, CHS, and CHT transcripts when compared to all other treatments. Detection of suppressor activity required that NPS3121 be infiltrated 6 to 8 hr prior to infiltration with the second treatment. Transcript levels were not reduced when plants were infiltrated with NPS3121 at 10^5 cells per mL or first infiltrated with Pt11528 followed by NPS3121. Transcripts were also not detected in control plants infiltrated with H_2O .

A similar, but more detailed, study was conducted to examine the effect of NPS3121 cell concentration on suppression of transcript accumulation and phytoalexin production. Bean plants were infiltrated with NPS3121 at concentrations of 10^4 through 10^9 cells per mL, prior to infiltration with Pt11528 at 10^8 cells per mL. Slot blot analysis indicated that 10^8 or 10^9 cells per mL of NPS3121 were required to see suppression of transcript accumulation (data not shown). As shown in Figure 4, analysis of the phytoalexins by thin-layer chromatography (TLC)-*Cladosporium* bioassay demonstrated that phytoalexin levels for these plants were significantly reduced with NPS3121 at 10^7 cells per mL or greater. Concentrations of 10^8 or 10^9

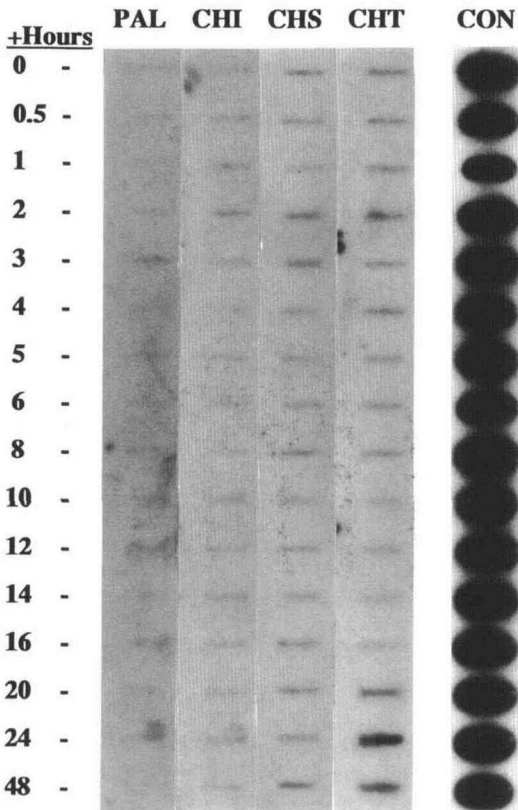


Figure 1. Defense Transcript Accumulation after Infiltration with High Inoculum.

RNA was isolated from bean leaf tissue between 0 and 48 hr after infiltration with *P. s. phaseolicola* at a concentration of 10^8 cells per mL. Total RNA (5 μ g) from each time point was probed with PAL, CHI, CHS, CHT, and H1 (labeled as CON for control).

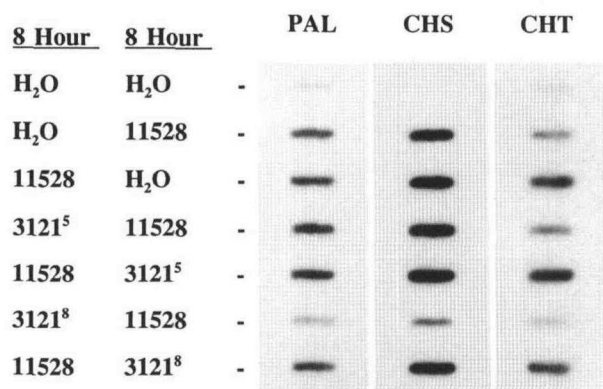


Figure 3. Suppression of Defense Transcript Accumulation by *P. s. phaseolicola* NPS3121.

Bean plants were infiltrated with *P. s. tabaci* Pt11528 (10^8 cells per mL), NPS3121 (10^5 or 10^8 cells per mL), or sterile H₂O and incubated for 8 hr. Plants were reinfected with Pt11528 (10^8 cells per mL), NPS3121 (10^5 or 10^8 cells per mL), or sterile H₂O and incubated for an additional 8 hr. RNA was isolated from leaf tissue at the conclusion of the 16-hr incubation period. Total RNA (5 μ g) from each treatment was probed with PAL, CHS, and CHT. Superscripts indicate concentration (10^5 or 10^8) of NPS3121 inoculum used for each treatment.

cells per mL suppressed phytoalexins to levels not significantly different from H₂O infiltrated plants.

NPS3121 Suppresses *E. coli* and Glutathione Elicitor Activity

Previously, it was shown that transcripts for PAL, CHS, CHI, and CHT were induced in bean after infiltration with *E. coli* DH5 α (Jakobek and Lindgren, 1993). Figure 5 shows that preinoculation with NPS3121 at 10^8 cells per mL also reduced the typical transcript accumulation that occurs in bean in response to infiltration with DH5 α .

Previous studies have also shown that the reduced form of glutathione is an elicitor of defense responses in bean (Wingate et al., 1988). We therefore wanted to determine if NPS3121 could suppress the elicitor activity of glutathione. Under our experimental conditions, transcripts for PAL, CHS, CHI, and CHT accumulated in bean leaves 8 hr following infiltration with 1, 5, or 10 mM glutathione (Figure 5). There was also a corresponding increase in phytoalexins in leaves infiltrated with glutathione (data not shown). However, if bean plants were infiltrated with NPS3121 at 10^8 cells per mL prior to infiltration with glutathione, transcript accumulation was greatly reduced (Figure 5). The TLC-*Cladosporium* bioassay demonstrated that phytoalexin levels were also suppressed by NPS3121 when compared to plants infiltrated with glutathione alone (data not shown).

Metabolically Active NPS3121 Cells Are Required for Suppressor Activity

Experiments were conducted to determine if the *P. s. phaseolicola* suppressor activity was dependent upon metabolically active cells. Figure 6 shows that when NPS3121 cells were treated with the bacterial protein synthesis inhibitors neomycin or kanamycin prior to infiltration, the suppression of transcript accumulation no longer occurred. Heat-killed NPS3121 cells also lost suppressor activity (Figure 6). These experiments suggest that *P. s. phaseolicola* has an active mechanism for suppressing bean defense responses.

DISCUSSION

All incompatible *P. syringae* pathovars, as well as compatible strains of *P. s. syringae* that we have examined thus far, induced the accumulation of putative defense transcripts in bean 6 hr after infiltration. *P. s. phaseolicola* was the only compatible *P. syringae* pathovar of those examined that did not induce transcripts for PAL, CHS, or CHI on bean, and the induction of CHT transcript was significantly delayed when compared with incompatible *P. syringae* isolates. These results with CHT accumulation are similar to those of Voisey and Slusarenko

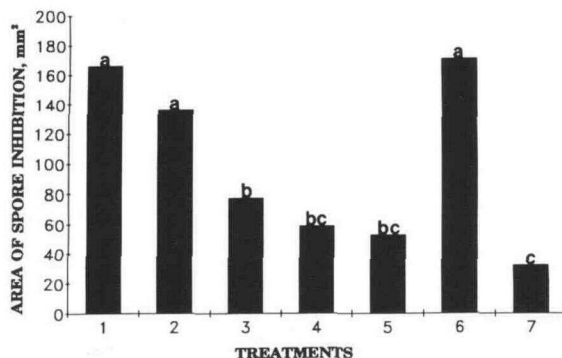


Figure 4. Suppression of Phytoalexin Accumulation by *P. s. phaseolicola* NPS3121.

Bean plants were infiltrated with NPS3121 at concentrations of 10^4 to 10^9 cells per mL or sterile H₂O and incubated for 8 hr. Plants were reinfected with *P. s. tabaci* Pt11528 at 10^8 cells per mL and incubated for an additional 8 hr. Extracts were prepared from this leaf tissue and analyzed for phytoalexins using TLC-*Cladosporium* bioassay. Results are represented as mean values from three replicate experiments. Means designated with the same letter are not significantly different ($P = 0.05$) according to the LSD test. Bar 1, NPS3121, 10^4 cells per mL; bar 2, NPS3121, 10^5 cells per mL; bar 3, NPS3121, 10^7 cells per mL; bar 4, NPS3121, 10^8 cells per mL; bar 5, NPS3121, 10^9 cells per mL; bar 6, infiltrated with H₂O, followed by Pt11528; bar 7, infiltrated with H₂O, followed by a second H₂O infiltration.

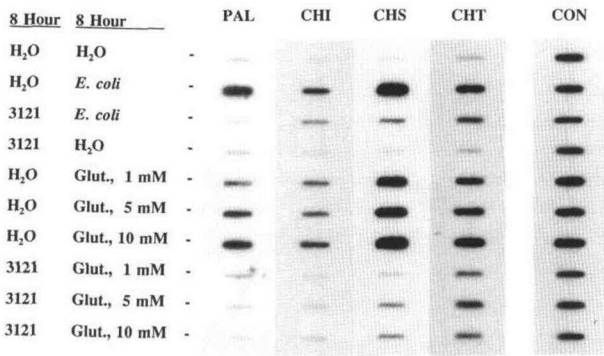


Figure 5. Suppression of *E. coli* DH5α and Glutathione Elicitor Activity by *P. s. phaseolicola* NPS3121.

Bean plants were infiltrated with NPS3121 (10⁸ cells per mL) or H₂O and incubated for 8 hr. Plants were re-infiltrated with DH5α, glutathione (Glut.), or sterile H₂O and incubated for an additional 8 hr. RNA was isolated from leaf tissue at the conclusion of the 16-hr incubation period. Total RNA (5 μg) from each treatment was probed with PAL, CHI, CHS, CHT, and H1 (labeled as CON for control).

(1989). It was interesting that the compatible *P. s. syringae* isolates induced defense transcript accumulation at 6 hr after infiltration, whereas the *P. s. phaseolicola* isolates did not. The basis for these differences has not been established, but may result from the different ways in which these two pathogens interact with bean, as demonstrated by the different disease symptoms incited by each.

Our results strongly suggest that *P. s. phaseolicola* may suppress the accumulation of transcripts for PAL, CHS, and CHI. The absence of the defense transcripts in bean after infiltration with *P. s. phaseolicola* is significant, because the corresponding enzymes are needed for phytoalexin synthesis and thus have a possible role in disease resistance. In addition, these transcripts have been shown to accumulate in bean as a response to wounding (Lawton and Lamb, 1987; Mehdy and Lamb, 1987). At 120 hr after infiltration with *P. s. phaseolicola*, bean tissue is badly damaged due to symptom development; therefore, it was interesting that these three wound-inducible transcripts did not accumulate in bean by this time. The fact that chitinase transcript accumulated during this period indicates that leaf tissue, damaged due to disease development, was still capable of RNA transcription. Thus, it would appear that *P. s. phaseolicola* may have evolved a mechanism to suppress or modify the typical defense responses of bean.

Prior infiltration with *P. s. phaseolicola* NPS3121 suppressed the typical transcript accumulation and phytoalexin production that occurs in bean after infiltration with *P. s. tabaci*, *E. coli*, or the elicitor glutathione. Significantly, the suppressor activity was lost when NPS3121 cells were heat killed or treated with protein synthesis inhibitors, indicating that active metabolism is a prerequisite for suppressor activity. The experiments

with glutathione also suggest that suppressor activity is not due to some form of interaction between bacterial strains, such as competition for plant recognition or binding sites. It is interesting that the NPS3121 suppressor inhibits the elicitor activity of various bacterial species as well as glutathione.

To our knowledge, no other reports of specific bacterial suppression of phytoalexin biosynthesis have been published. A number of suppressors have been isolated from fungal pathogens. Glucan suppressors were isolated from *Phytophthora infestans* mycelia and zoospores that inhibited the hypersensitive reaction and phytoalexin accumulation that occurred in potato tuber tissue in response to an incompatible race of the same fungus (Doke et al., 1980). Glycoprotein suppressors were isolated from *P. megasperma* f sp *glycinea* that inhibited the accumulation of the phytoalexin glyceollin in soybean cotyledons that had been treated with a glucan elicitor isolated from the cell wall of the same pathogen (Ziegler and Pontzen, 1982). A glycoprotein suppressor was also isolated from *Ascochyta rabiei* that inhibited phytoalexin accumulation in chickpea (Kessmann and Barz, 1986). Spores of *Mycosphaerella pinodes* produce a suppressor that inhibits the accumulation of transcripts for PAL and CHS, the corresponding PAL enzyme activity, and the production of pisatin in pea (Yamada et al., 1989). Thus, the production of suppressors may be a common strategy employed by fungal as well as bacterial phytopathogens for overcoming host defense responses.

Previous studies have shown that phaseolotoxin, commonly produced by *P. s. phaseolicola*, inhibits the production of the hypersensitive response and phytoalexin production in resistant bean cultivars (Gnanamanickam and Patil, 1977). Studies conducted in our laboratory indicated that phaseolotoxin was

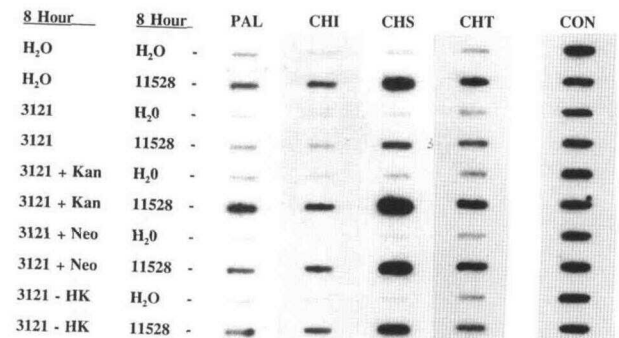


Figure 6. Effects of Antibiotics and Heat Treatment on *P. s. phaseolicola* NPS3121 Suppressor Activity.

Bean plants were infiltrated with NPS3121 (10⁸ cells per mL), NPS3121 treated with kanamycin (Kan) or neomycin (Neo), heat-killed NPS3121 cells (HK), or sterile H₂O and incubated for 8 hr. Plants were re-infiltrated with Pt11528 (10⁸ cells per mL) or H₂O and incubated for an additional 8 hr. RNA was isolated from leaf tissue at the conclusion of the 16-hr incubation period. Total RNA (5 μg) from each treatment was probed with PAL, CHI, CHS, CHT, and H1 (labeled as CON for control).

not responsible for the suppression that we detected. NPS4336 and NPS4347 (Peet et al., 1986), which are toxin-minus mutants of NPS3121, suppressed transcript accumulation in an identical manner to the wild-type strain (data not shown).

Previously, we described a general mechanism for the induction of defense transcripts and phytoalexin production by bacteria in bean, which is distinct from the specific mechanism associated with the hypersensitive response (Jakobek and Lindgren, 1993). The experiments described in this manuscript suggest that *P. s. phaseolicola* has evolved a mechanism to actively suppress defense transcript accumulation and phytoalexin biosynthesis in bean. Currently, we have not determined if the suppressor activity is an essential pathogenicity factor for NPS3121 or if it is encoded by a *hrp* gene. Our data support the hypothesis that there are different signal transduction pathways for the activation and suppression of plant defense responses during the interactions of bean and *P. syringae* pathovars. Currently, experiments are underway to determine if bacteria-mediated suppression of plant defense responses is a common feature of other plant bacterial interactions. We are also attempting to isolate Tn5 mutants of NPS3121 that lack suppressor activity, thereby facilitating the cloning of suppressor gene(s).

METHODS

Bacterial Strains and Growth Conditions

Pseudomonas syringae strains used in this study are given in Table 1. *Pseudomonas* strains were cultured at 28°C in King's medium B (King et al., 1954). *Escherichia coli* DH5 α (Bethesda Research Laboratories) was grown at 37°C in Luria-Bertani medium (Sambrook et al., 1989). Bacto agar (Difco) at 1.5% (w/v) was added to media for plate cultures. Antibiotics (Sigma) were used for selection at the following concentrations (μ g per mL): rifampicin, 100; tetracycline, 15 or 20.

Growth and Inoculation of Plants

Phaseolus vulgaris cv Red Kidney was grown as described previously (Jakobek and Lindgren, 1993). Bacterial strains were grown overnight, harvested by centrifugation, and washed in sterile distilled H₂O. Cell suspensions were adjusted with sterile distilled H₂O to the appropriate concentration. Standard plate count procedures were used to verify inoculum concentrations. Primary leaves of 6- to 8-day-old Red Kidney plants were inoculated using vacuum infiltration. Infiltrated plants were placed in a growth chamber and incubated at 22°C with a 14-hr light/10-hr dark cycle until leaf tissue was harvested. The reduced form of glutathione (Sigma) was used in experiments at 1, 5, or 10 mM concentrations, as described in Results.

To test the effects of bacterial protein synthesis inhibitors on suppressor activity, cultures of NPS3121 grown overnight in King's medium B supplemented with rifampicin were harvested, washed with sterile distilled H₂O, and resuspended at a concentration of $\sim 10^9$ colony-forming units per mL in fresh King's B supplemented with 1 mg/mL streptomycin, neomycin, or kanamycin. The cultures were incubated

with shaking at 28°C for 1 hr. The cells were then pelleted by centrifugation, washed, resuspended in sterile H₂O, and infiltrated into plants as described above. When testing heat treatment on suppressor activity, NPS3121 cells at an initial concentration of 10^9 cells per mL were incubated for 1 hr at 60°C prior to infiltration. After antibiotic or heat treatment, cells were plated for viable counts; no colonies appeared after such treatments in any experiment.

For each treatment or time point, three to six plants were sampled to minimize experimental variation. All experiments were repeated at least twice.

RNA Isolation and Blot Analysis

RNA isolation and slot blot analyses were conducted as described previously (Jakobek and Lindgren, 1993). Hybridization probes included the 1.7-kb PstI fragment from pPAL5 (cDNA clone for bean phenylalanine ammonia-lyase [PAL]) (Edwards et al., 1985), the 1.4-kb EcoRI fragment from pCHS1 (cDNA clone for bean chalcone synthase [CHS]) (Ryder et al., 1984), the 865-bp EcoRI fragment from pCHI1 (cDNA clone for bean chalcone isomerase [CHI]) (Mehdy and Lamb, 1987), and the 650-bp BamHI-KpnI fragment from pCHT12 (cDNA clone for bean chitinase [CHT]) (Hedrick et al., 1988). Hybridization conditions were as described previously (Jakobek and Lindgren, 1993). All blots were also hybridized with a bean cDNA clone, designated H1, which is complementary to a constitutively expressed gene with unknown function (Lawton and Lamb, 1987). These control hybridizations verified that equal amounts of all RNA samples were loaded onto each filter.

Phytoalexin Isolation and Analysis

Phytoalexins were isolated and their activity was examined using the thin-layer chromatography (TLC)-*Cladopsorium* bioassay as described previously (Jakobek and Lindgren, 1993).

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