Phytoalexin production by wounded white bean (*Phaseolus vulgaris*) cotyledons and hypocotyls in response to inoculation with rhizobacteria

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Fifteen rhizobacteria were tested for their ability to induce a hypersensitive response and phytoalexin production by wounded white bean (*Phaseolus vulgaris*) cotyledons and hypocotyls. Of these, nine strains of the genus *Pseudomonas* and two *Serratia* strains induced a hypersensitive response and the production of three antifungal compounds by cotyledons. Two of the antifungal compounds shared uv spectra, retention time in high-performance liquid chromatography, and R_f by thin-layer chromatography that were identical to those of the phytoalexins kievitone and phaseollinisoflavan. The hypersensitive response was rarely observed and phytoalexins were not detected in extracts from cotyledons treated with water, bacterial culture fluids, *Bacillus* sp., *Bacillus subtilis*, *Corynebacterium* sp., *Azospirillum brasilense* sp. strain 7, or the pathogen *Pseudomonas syringae* pv. *phaseolicola*. The white bean root rot pathogen *Fusarium solani* f.sp. *phaseoli* induced production of five phytoalexins by cotyledons. Three were identical in their chemical properties to phytoalexins induced by the rhizobacteria, but only *F. solani* induced phaseollin production. Kievitone levels from cotyledons treated with various rhizobacteria varied from 83 to 333 μg/g fresh mass, whereas in cotyledons treated with *F. solani* the kievitone levels averaged 850 μg/g fresh mass. In similar studies with wounded hypocotyls, only the two *Serratia* spp. and *F. solani* induced production of phytoalexins other than phaseollin.

Key words: rhizobacteria, plant defense mechanisms, phytoalexins, hypersensitive response, *Phaseolus vulgaris*.

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Quinze rhizobactéries ont été testées pour leur aptitude à induire la production de phytoalexines et une réponse hypersensible chez des lésions des cotylédons et de l'hypocotyle du haricot blanc (*Phaseolus vulgaris*). Parmi celles-ci, neuf souches de genre *Pseudomonas* et deux de *Serratia* ont induit des réponses hypersensibles et la production de trois composés antifongiques les chez cotylédons. Par suite de chromatographie en couche mince, deux des antifongiques ont présenté une similarité de spectres à l'u.v., de temps de rétention en chromatographie liquide sous haute pression et de R_f, tout comme les phytoalexines kievitone et phaséollinisoflavane. La réponse hypersensible a rarement été observée et les phytoalexines n'ont pas été détectées dans les cotylédons traités avec de l'eau, des solutions de cultures bactériennes, les *Bacillus* sp., *Bacillus subtilis*, *Corynebacterium* sp., *Azospirillium brasilense* sp. souche 7, ou l'agent pathogène *Pseudomonas syringae* pv. *phaseolicola*. Le *Fusarium solani* f.sp. *phaseoli*, agent du pourridié du haricot blanc, a induit la production de cinq phytoalexines chez le cotylédons. Trois d'entre elles avaient des propriétés chimiques identiques à celles des phytoalexines induites par les rhizobactéries, mais seul le *F. solani* a induit la production de phaséolline. Les niveaux de kievitones chez les cotylédons traités avec diverses rhizobactéries ont varié de 83 à 333 μg/g de poids frais, alors que ceux résultant de traitements avec le *F. solani* ont été en moyenne de 850 μg/g de poids frais. Dans des études similaires d'hypocotyles porteurs de lésions, seuls les deux *Serratia* spp. et le *F. solani* ont induit des phytoalexines autres que de la phaséolline.

Mots clés : rhizobactéries, mécanismes de défense des plantes, phytoalexines, réponse hypersensible, *Phaseolus vulgaris*.

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Introduction

There is growing evidence that rhizobacteria, bacteria that colonize plant roots, are not just passive bystanders at the root–soil interface. Rather, these organisms actively interact with root tissues and other rhizosphere microfauna, and the results can have both negative (Schippers et al. 1987) and beneficial (Kloepper et al. 1988, 1991) impacts on plant growth and development. Beneficial effects, generally observed as improved growth, have been most commonly observed following treatment of plant tissues with bacteria belonging to the fluorescent *Pseudomonas*, *Serratia*, and *Bacillus* genera (Kloepper et al. 1988, 1991). Both direct and indirect growth promotion mechanisms were identified to be active in these bacteria. Direct effects occur from bacteria-produced compounds (Yoshikawa et al. 1993) with phytohormonal properties

or from products that facilitate uptake of nutrients such as nitrogen, phosphorus, and iron (Kloepper et al. 1991; Grimes and Mount 1987). Indirect effects occur when the activities of the rhizobacteria result in reduced populations of deleterious microbes, particularly those of plant pathogens (Bull et al. 1991; Dahiya et al. 1988; Handelsman et al. 1990; Liao 1989; Loper and Buyer 1991; Reddy et al. 1994; Voisard et al. 1989; Weller 1988).

The nature of the interactions between plant tissues and rhizobacteria is not well understood. Plant-pathogenic bacteria are differentiated from saprophytic strains by their capacity to elicit in incompatible plant tissues the hypersensitive response (HR), accumulation of phytoalexins, and other plant-dependent responses (Dixon 1986; Templeton and Lamb 1988). Rhizosphere bacteria, of which we know most about *Pseudomonas fluorescens*, do not appear to activate either the HR or the phytoalexin response (Keen et al. 1981; Lyon and Wood 1975; O'Connell et al. 1990; Smith and Mansfield 1981), although symbiotic *Rhizobium* spp. were found to induce phytoalexin accumulation in root nodules of soybeans and faba beans (Schmidt et al. 1992; Wolff and Werner 1990). The lack of any visible response by plant tissues to inoculation with rhizobacteria has been interpreted to indicate that these bacteria do not actively interact with plant cells. However, rhizosphere bacteria grow readily on dead plant tissues and produce a range of enzymes that degrade most polymers of plant cells (Wood 1984). It is highly likely that when these bacteria colonize plant tissues, particularly at wounded sites, they secrete lytic enzymes that release nutrients required for their growth. Wood (1984) suggested that in such instances bacterial growth is likely curtailed by the induction of the plants' defense responses. An interaction between rhizobacteria and wounded plant tissues, however, has not yet been definitively established.

Evidence that rhizobacteria stimulate plant defense genes is starting to emerge. For example, when carnation roots were treated with the *Pseudomonas* strain WCS417r, stems challenged with *Fusarium oxysporum* f.sp. *dianthi* accumulated more phytoalexins and had a significantly lower incidence of fusarium wilt than untreated roots (van Peer et al. 1991). Lipopolysaccharides from the bacterial cell walls were implicated in inducing the resistance response (van Peer and Schippers 1992). Leaves of cucumber plants grown from seed inoculated with various rhizobacteria strains had reduced disease when challenged with *Colletotrichum orbiculare* (Wei et al. 1991). Disease reduction was attributed to enhanced systemic resistance brought about by bacterial treatment, a response more commonly observed following challenge by incompatible pathogenic isolates (Salt and Kuc 1985). Bean cotyledons and tobacco leaves exposed to a saprophytic soil pseudomonad reacted by producing increased amounts of phenolics, including phytoalexins, by the HR, and by producing pathogenesis-related proteins (Zdor and Anderson 1992).

In a related study, we found that rhizobacteria often suppressed preemergence damping-off and postemergence root rot of infected white bean seedlings (Reddy et al. 1994). Disease reduction was not always correlated to inhibition of pathogen growth by the rhizobacteria. The present study reports on the induction by rhizobacteria of the plant defense mechanisms, the HR, and phytoalexin production, following inoculation of wounded cotyledons and hypocotyls of white bean.

Materials and methods

Cultures

Cultures of *Fusarium solani* f.sp. *phaseoli* and *Cladosporium cucumerinum* were grown on potato dextrose agar (PDA) and V-8 juice agar, respectively, at 24°C. Cultures of rhizosphere bacteria including *Bacillus* sp. strain 86-64, *Corynebacterium* sp. strain 44-9, *Pseudomonas* spp. strains 63-49, 55-14, 31-12, 61-9A, G11-32, G2-26, L-26, 36-43, 34-13, and G20-18, *Serratia* spp. strains 1-102 and 2-68, and *Azospirillum brasilense* sp. strain 7 were obtained from Allelix Crop Technologies (now Imperial Oil Chemicals Division, Ag Biologicals, Saskatoon, Sask., Canada). *Rhizobium leguminosarum* bv. *phaseoli* and *Pseudomonas syringae* pv. *phaseolicola* were obtained from the Agriculture Canada culture collection. Rhizobacteria and *R. leguminosarum* bv. *phaseoli* were grown on Difco pseudomonas agar F (PAF) and YM broth (Nelson and Child 1981), respectively, at 24°C. Cultures of *Escherichia coli* B301, *Bacillus subtilis*, and *Aquaspirillum serpens* were obtained from Dr. S. Koval, University of Western Ontario, London, Ont., Canada. Rhizobacteria inoculant was prepared from 2-day-old PAF plates, *R. leguminosarum* bv. *phaseoli* inoculant was obtained from 3-day-old YM broth, *F. solani* inoculum from 4-week-old PDA plates, and *Cladosporium cucumerinum* from 4- to 9-day-old V-8 plates. All bacterial cell and fungal spore suspensions were made in sterile distilled water and used within 10 min.

Studies of the interaction of rhizobacteria with cotyledons and hypocotyls

The white bean cultivar OAC Seaforth was used throughout this study (kindly donated by W.G. Thompson, Blenheim, Ont.). Seeds were surface sterilized in a 2% hypochlorite solution for 1 min, rinsed in tap water for 10 min, and then sown in moist vermiculite. The seeds were incubated in the dark, to suppress chlorophyll production, in a growth cabinet with a biphasic temperature cycle of 8 h at 18°C followed by 16 h at 23°C. Cotyledons were excised from etiolated hypocotyls 5 days after planting, and rinsed under running tap water, and a thin slice of tissue was cut away from the convex surface by means of a scalpel. The cotyledons were then washed for 1 h under running cold tap water, patted dry, and placed wounded side up in Petri dishes lined with moist sterile filter paper. The injured sites were inoculated with 15 μL of rhizobacterial suspensions (10^2 colony-forming units (cfu)/mL or more), suspensions of *Fusarium* conidia (10^6 conidia/mL), cell-free culture fluids, or distilled water. Tissues were incubated in the dark at 24°C for phytoalexin induction.

For assays studying rhizobacterial–hypocotyl reactions, uprooted plants were placed horizontally in Pyrex trays such that the upper part of the plant was supported above the glass surface by a Plexiglas bracket. The roots of the plants were covered with moist tissue. Starting from the area of the stem just below the cotyledons a scalpel was used to cut a 2-cm flap of tissue. The cut surfaces were inoculated with 15 μL of the inoculum preparations previously described and the trays were covered with plastic film. Incubation for phytoalexin production was as previously described.

Phytoalexin extraction

The cotyledons or the segments of treated hypocotyl tissue were placed into 50 mL of acidified methanol and heated to boiling for 2 min on a hot plate. Extraction was continued overnight on a rotary shaker at 150 rpm at 20°C. The methanol solution was then decanted and the tissues were further extracted with shaking in the same volume of fresh methanol for an additional 8 h. Pooled extracts were concentrated to the aqueous phase under reduced pressure on a rotary evaporator. The aqueous extract was partitioned twice with distilled ethyl acetate (1:2, v/v). The ethyl acetate fractions were pooled and then dried under N_2 gas. The residues were redissolved in fixed volumes of absolute ethanol.

Thin-layer chromatography and bioassay

Extracts from each treatment were spotted onto 0.2 mm thick silica-coated plastic sheets (20 × 20 cm, Merck) containing a fluorescent

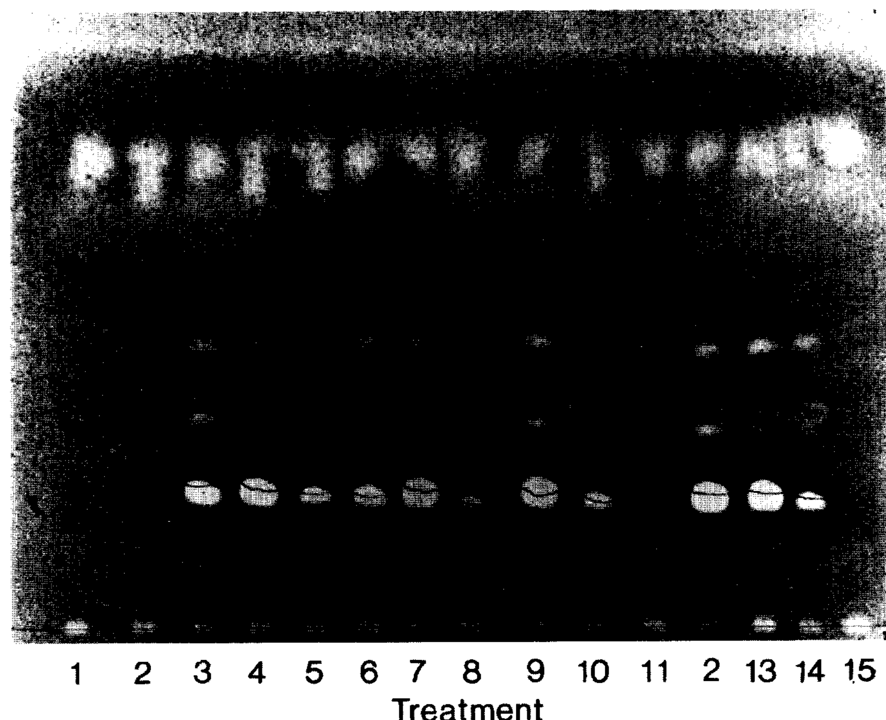

FIG. 1. TLC bioassay of methanol extracts from wounded white bean cotyledons. Lanes: 1, water; 2, *Corynebacterium* sp. strain 44-9; 3, *Pseudomonas* sp. strain 61-9A; 4, *Pseudomonas* sp. strain 31-12; 5, *Pseudomonas* sp. strain G11-32; 6, *Pseudomonas* sp. strain 63-49; 7, *Serratia* sp. strain 1-102; 8, *Pseudomonas* sp. strain G2-26; 9, *Pseudomonas* sp. strain 55-14; 10, *Pseudomonas* sp. strain L-26; 11, *Bacillus* sp. strain 86-64; 12, *Pseudomonas* sp. strain 36-43; 13, *Serratia* sp. strain 2-68; 14, *Pseudomonas* sp. strain 34-13; 15, *Pseudomonas* sp. strain G20-18. Each treatment contained 10 cotyledons inoculated with water or 10^6 microorganisms/cotyledon.

indicator. Chromatography was carried out in chloroform:ethanol (100:7, v/v). The plates were thoroughly dried and were then sprayed with *Cladosporium cucumerinum* for bioassay of phytoalexins (Homans and Fuchs 1970).

Quantitative determinations

Phytoalexins were partially purified by solid-phase extraction (SPE) on C18 Sep-Pak Plus cartridges (Waters Associates). Prior to application of the samples the cartridges were prewashed with methanol and equilibrated with methanol:water solution (10:90, v/v). Aliquots of the methanol-soluble ethyl acetate extracts were mixed with 9 volumes of water and the sample was loaded onto the C18 bed under gentle vacuum (0.5 mL/min). A series of different eluants composed of methanol:water were then applied in 5 mL volumes in the following order: 50:50, 75:25, 90:10, and 100%. Each fraction was collected, dried, and redissolved in the same pre-SPE volume of absolute ethanol. All of the antifungal activity was found to be associated with the 75:25 eluant fraction. The samples were further purified by the HPLC procedure described by Goossens and Van Laere (1983) using Waters Associates components (M510 pumps, M440 uv absorbance detector with a 280-nm filter, a U6K universal injector, the Waters interface module, and the Maxima software program for data collection and processing). A 3-cm guard column packed with Supelco silica pellicular packing preceded the 25-cm Partisil columns. Samples were loaded in absolute ethanol and eluted from the column at 25°C using a linear gradient of 100% solvent A to 100% solvent B, over a 1-h period. Solvent A consisted of hexane:chloroform (2:1, v/v) and solvent B was chloroform:methanol (100:8, v/v). The flow rate was 2 mL/min and fractions were collected at 1-min intervals. The detection limit was approximately 5 μg. Solvent from the fractions was evaporated under a stream of nitrogen gas, and the residues were redissolved in a small volume of absolute ethanol and stored at −20°C. Fractions were tested for the presence of phytoalexins as previously described. All experiments were carried out at least twice.

Results

Methanol extracts of white bean cotyledons obtained 48 h after treatment with 11 of 14 rhizobacteria were found to exhibit up to three zones of antifungal activity when visualized by thin-layer chromatogram (TLC) bioassay (Fig. 1). The zones of antifungal activity corresponded to R_f's of 0.18, 0.29, and 0.46 when the chloroform:ethanol solvent system was used. The inhibitory compound with an R_f of 0.18 had uv spectra, Gibbs test results, HPLC retention, and migration on TLC that were identical to those of a purified sample of the bean phytoalexin kievitone (Fig. 2, lane 11). The results of these four tests for the inhibitory compound with a R_f of 0.46 and the phytoalexin phaseollinisoflavan were identical (Fig. 2, lane 10). The inhibitory material that migrated to R_f 0.29 did not match any of the phytoalexins available but was suspected to be licoisoflavone A or genistein. Nonwounded cotyledons inoculated with rhizobacteria produced one phytoalexin, for which the results of the above tests were the same as those for kievitone (data not shown).

Extracts from cotyledons inoculated with *F. solani* contained the three inhibitory compounds seen with the rhizobacterial treatments and in several instances two additional inhibitory compounds were evident, one at R_f 0.72, and the other at R_f 0.50 (data not shown). These latter compounds were not obtained with any of the bacteria or control treatments. The inhibitory compound that migrated to R_f 0.72 shared results of tests carried out above with the phytoalexin phaseollin (Fig. 2, lane 9). The remaining compound or compounds were not identified.

Extracts from cotyledons that exhibited HR following treatment with rhizobacteria generally contained 5 times less

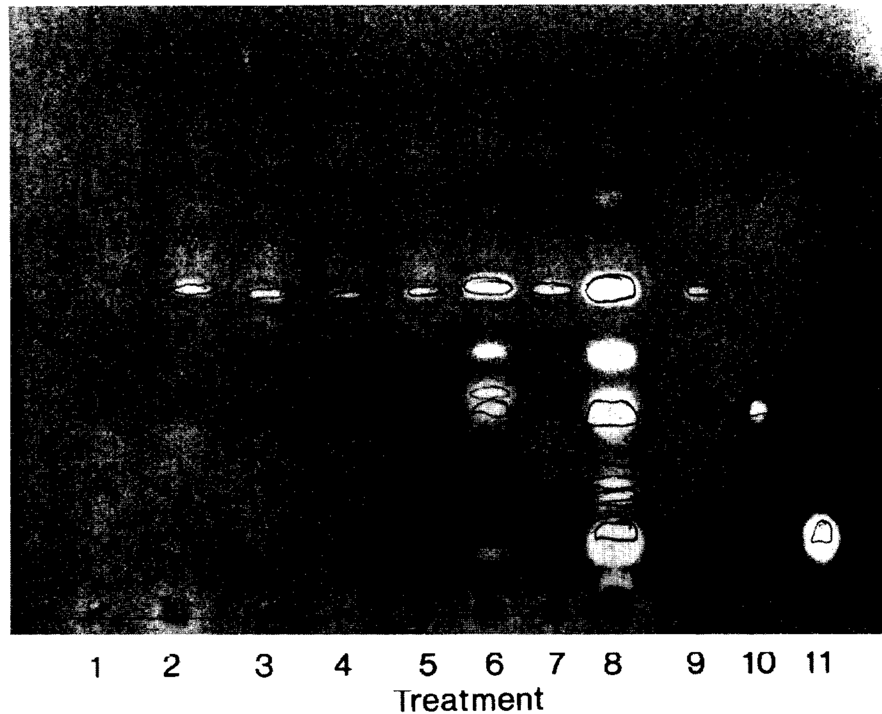

FIG. 2. TLC bioassay of methanol extracts from wounded white bean hypocotyls. Lanes: 1, water; 2, *Pseudomonas* sp. strain 55-14 cell-free extract; 3, *Pseudomonas* sp. strain 55-14; 4, *Corynebacterium* sp. strain 44-9; 5, *Bacillus* sp. strain 86-64; 6, *Serratia* sp. strain 1-102; 7, *Pseudomonas* sp. strain 63-49; 8, *F. solani*; 9, phaseollin; 10, phaseollinisoflavan; 11, kievitone. Each treatment contained 20 hypocotyls inoculated with water or 10^6 microorganisms/hypocotyl.

TABLE 1. Accumulation of kievitone on wounded cotyledons following inoculation of rhizobacteria, nonrhizophere bacteria, and pathogens

Treatment	μg kievitone/g fresh mass of cotyledons
Rhizobacteria	
Serratia sp.[a]	221.4±0.7
Pseudomonas sp.[b]	177.7±32.5
R. leguminosarum bv. *phaseoli*	99.3±0
Azospirillum brasilense	nd
Corynebacterium sp.	nd
Bacillus subtilis	nd
Bacillus sp.	nd
Nonrhizosphere bacteria	
E. coli B301	138.5±0
Aquaspirillum serpens	nd
Pathogens	
Fusarium solani f.sp. *phaseoli*	850±251
Pseudomonas syringae pv. *phaseolicola*	nd

NOTE: nd, not detected.
[a]Two species were tested.
[b]Nine species were tested.

kievitone than extracts from cotyledons treated with *F. solani*. Unlike rhizobacteria, which caused only localized surface necrosis, *F. solani* induced spreading necrotic discolouration evident throughout most of the cotyledon tissues after 48 h. The amount of kievitone produced by the cotyledons following inoculation with rhizobacteria, nonrhizobacteria, and pathogens is reported in Table 1. Extracts from cotyledons inoculated with water (Fig. 1, lane 1), cell-free culture fluids from *Pseudomonas* sp. strain 55-14 (Fig. 2, lane 2), or extracts from tissues treated with bacterial suspensions of *Corynebacterium* sp. (Fig. 1, lane 2), *Bacillus* sp. strain 86-64, *Bacillus subtilis* (Fig. 1, lane 11, Table 1, respectively), *Aquaspirillum serpens*, and *Azospirillum brasilense* sp. strain 7 (Table 1) did not contain any antifungal compounds detectable by TLC bioassay. Similarly, the bacterial pathogen *Pseudomonas syringae* pv. *phaseolicola* did not induce the HR or production of antifungal compounds in cotyledons (Table 1). Both *E. coli* and *R. leguminosarum* bv. *phaseoli* caused low levels of kievitone to accumulate (Table 1).

Pseudomonas sp. strain 63-49 was used to further characterize the effect of population size and exposure time required for phytoalexin production by cotyledons. Phytoalexins were not detected by TLC bioassay in extracts from cotyledons inoculated with less than 2×10^4 cfu/cotyledon. The zones of antifungal activity on the TLC plates increased in size with increasing numbers of strain 63-49 from 2×10^4 to 2×10^9 cfu/cotyledon (data not shown). Phytoalexins were detectable by TLC bioassay 36 and 24 h after inoculation with *Pseudomonas* sp. strain 63-49 and *F. solani*, respectively (data not shown).

Wounded hypocotyls treated with *F. solani* f.sp. *phaseoli* produced all of the phytoalexins detected in extracts from similarly treated cotyledons (Fig. 2, lane 8). However, with hypocotyls, phaseollin appeared to be the predominant phytoalexin produced. Phaseollin was detected in all rhizobacteria-inoculated hypocotyl tissues, but was also frequently found in tissues treated with distilled water only. Of the rhizobacterial treatments examined, only *Serratia* sp. strain 1-102 induced a pattern of phytoalexins similar to that seen with *F. solani* (Fig. 2, lane 6). *Serratia* sp. strain 2-68 induced a pattern of phytoalexins from hypocotyls similar to that seen with 1-102 (data not shown).

Discussion

In greenhouse experiments *Pseudomonas* sp. strain 63-49 suppressed white bean root rot caused by *F. solani* f. sp. *phaseoli* (Reddy et al. 1994). Other rhizobacteria used in this study also suppressed white bean root rot without significant reduction of fungal mycelial growth on culture medium (Reddy et al. 1994). This prompted us to investigate the ability of the rhizobacteria to induce plant defense mechanisms such as the HR and phytoalexin production.

Rhizobacteria have not been considered to be one of the many diverse stimuli of phytoalexin production in plants (Dixon 1986; Keen et al. 1981; O'Connell et al. 1990; Smith and Mansfield 1981). In this study, we demonstrated that a variety of rhizobacteria can activate phytoalexin biosynthesis in wounded bean tissues. Bacteria growing in the bathing medium of white bean roots were previously suggested to induce biosynthesis of phytoalexins by these tissues but their involvement was not clearly proven (Burden et al. 1972). More definitive results that rhizobacteria elicit phytoalexins was presented by Zdor and Anderson (1992) who showed that disease-suppressive fluorescent pseudomonads induced kievitone, phaseollinisoflavan, and phaseollin accumulation on kidney bean cotyledons.

Bean cotyledon and hypocotyl tissue produce a variety of phytoalexins (Ingham 1982) most of which are induced following exposure of the tissue to incompatible pathogenic agents or abiotic substances including heavy metals and salts (Bailey and Burden 1973; Burden et al. 1972; Dixon 1986; Smith et al. 1975; Whitehead et al. 1982). In this study, we only rarely detected phytoalexins accumulating in wounded cotyledon tissues following treatment with distilled water or with cell-free bacterial culture fluids. Several investigators, however, have reported that wounding alone induced the biosynthesis of kievitone in sterile white bean cotyledons (Whitehead et al. 1982; Goossens et al. 1987). The release of endogenous elicitors during the early stages of cell death is believed to be responsible for induction of phytoalexins (Hargreaves and Bailey 1978; Hargreaves and Selby 1978; Bowen and Heale 1987). We included an extensive washing step immediately after wounding to remove endogenous elicitors, thereby eliminating phytoalexin production in control treatments with cotyledons. This was not possible with hypocotyls, and the phaseollin detected following treatment with distilled water as well as rhizobacteria was likely stimulated by the endogenous elicitors. Since the two *Serratia* spp. induced a complement of phytoalexins in hypocotyl tissues similar to that seen with *F. solani*, elicitation here is considered to be a response to the bacteria. Interestingly, *Serratia* spp. were also among the most effective elicitors of phytoalexin biosynthesis in cotyledons, suggesting that their relationship with white bean tissues differs from that of other rhizobacteria tested.

In a number of studies, saprophytic strains of bacteria, primarily *Pseudomonas fluorescens* strains, were found to be ineffectual as elicitors of either the HR or phytoalexins when infiltrated into leaf material (Keen et al. 1981; O'Connell et al. 1990; Smith and Mansfield 1981). Studies by O'Connell et al. (1990) demonstrated that saprophytic bacteria were rapidly encapsulated into a layer of hydroxyproline-rich glycoprotein matrix secreted next to the leaf cell walls. This rapid response by the plant may eliminate any further interaction of the bacteria and the plant cell and likely precludes the full-scale induction of plant defenses. However, in tobacco cell suspension cultures, *Pseudomonas fluorescens* was found to be as capable of triggering similar early responses in plant cell reactions as pathogenic strains (Baker et al. 1991). In addition, preinfiltration of tobacco leaves with large numbers of *Pseudomonas fluorescens* cells effectively activated induced resistance mechanisms that lead to subsequent protection against virulent bacteria (Burgyán and Klement 1979). Cell-free culture fluids harvested from actively growing rhizobacteria were also shown to induce resistance mechanisms in various plant species, although the fluids by themselves had no effect on pathogen development (Schönbeck and Dehne 1986).

In this study the Gram-positive *Corynebacterium* sp. and the two *Bacillus* spp. were consistently found to be the least effective elicitors of phytoalexin biosynthesis in bean tissues. Gnanamanickam and Smith (1980) demonstrated that Gram-positive bacteria were highly sensitive to isoflavonoid phytoalexins and that as little as 2 μg of kievitone was lethal. In contrast, Gram-negative bacteria were not affected by even 200 μg of this phytoalexin (Gnanamanickam and Smith 1980). Fett and Osman (1982) also found Gram-positive bacteria to be sensitive to the phytoalexin coumestrol. In the present study phytoalexins were not detected from cotyledons inoculated with Gram-positive rhizobacteria and this may reflect a rapid inactivation of these bacteria on the tissue surface. Of the bacteria tested by Lyon and Wood (1975) and Fett and Osman (1982), *Pseudomonas fluorescens* was found to be the least sensitive to inhibition by isoflavonoid phytoalexins and as such would likely not be affected by these stress metabolites. The capacity of bacteria to colonize plant tissues, particularly the rhizosphere, is probably influenced by their sensitivity to phytoalexins.

Rhizobium leguminosarum bv. *phaseoli* and *E. coli* were both found to induce low levels of kievitone on bean cotyledons. *Rhizobium leguminosarum* bv. *viciae* and *Bradyrhizobium japonicum* were previously reported to induce phytoalexins in faba bean and soybean, respectively (Schmidt et al. 1992; Wolff and Werner 1990). Chakraborty and Chakraborty (1989) reported that bacterization of pea seed with *R. leguminosarum* bv. *viciae* was highly effective in reducing root rot caused by *F. solani* f.sp. *pisi*. They correlated this activity with detection of the phytoalexins pisatin and 4-hydroxy-2,3,9-trimethoxypterocarpan in pea epicotyls infected with *F. solani* or with both *R. leguminosarum* and *F. solani*.

Protection of *Phaseolus* sp. against attack by soilborne pathogens following induction of phytoalexin biosynthesis from rhizobacteria colonization remains to be established. It is important to note that in a field study the population of *Pseudomonas* sp. strain 63-49 on white bean cotyledons, up to 18 days after planting, was similar to that required to induce phytoalexin production on cotyledons in the laboratory (data not shown). The ability of rhizobacteria to colonize the cotyledons in sufficient numbers to induce phytoalexin production has potential implications for disease control involving the plant's defense mechanisms.

Acknowledgments

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