Induction of Systemic Resistance Against Fusarium Wilt of Radish by Lipopolysaccharides of *Pseudomonas fluorescens*

M. Leeman, J. A. Van Pelt, F. M. Den Ouden, M. Heinsbroek, P. A. H. M. Bakker, and B. Schippers

Department of Plant Ecology and Evolutionary Biology, Section of Plant Pathology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, the Netherlands.

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ABSTRACT

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In commercial greenhouse trials, *Pseudomonas fluorescens* strain WCS374 suppressed Fusarium wilt and increased radish yield. In bioassays, the involvement of lipopolysaccharides (LPSs) in induction of systemic resistance was studied. Induction of systemic resistance by selected plant growth-promoting rhizobacteria (PGPR) strains of *P. fluorescens* was involved in the suppression of Fusarium wilt of radish in a special rockwool bioassay. In this bioassay, the pathogen, *Fusarium oxysporum* f. sp. *raphani*, and the PGPR strain were inoculated at spatially separate locations on the plant root and were confined to these locations throughout the experiments. PGPR strains WCS374 and WCS417 of *P. fluorescens* and their crude cell wall extracts, which contained the LPS or purified LPS (consisting of lipid A/innercore/O-antigen side chain), induced systemic resistance, whereas *P. putida* WCS358 or its crude or purified LPS did not. Neither the phage-resistant mutants of WCS374 and WCS417 lacking the O-antigenic side chain of the LPS nor the crude or purified lipid A/innercore of these mutants reduced disease incidence in this experimental design. Strain WCS374, but not its O-antigen-minus mutant, also induced systemic resistance when applied on the cotyledon of radish on an agar disk cut from a plate culture. The pathogen was delivered on the root in peat 2 days later. Thus, the resistance-inducing O-antigen of strain WCS374 was effective not only onto the root, but also on the cotyledon. In a bioassay with greenhouse soil naturally infested with the Fusarium wilt pathogen of radish, strain WCS374, but not the O-antigen-minus mutant, suppressed disease. This suggests the involvement of induced resistance in natural soil bioassays and commercial greenhouse trials.

Induced systemic resistance in plants to fungal, bacterial, and viral pathogens has been demonstrated after preinoculation with weakly aggressive strains, avirulent, and incompatible forms of the disease-causing organism (7,26). This systemic response of plants after induction is known as systemic acquired resistance (SAR) (41,42) and has been demonstrated in several plant-pathogen systems (47).

In 1983, Kempe and Sequeira (22) suggested the possible involvement of *Pseudomonas*-induced resistance in potato against bacterial wilt caused by *P. solanacearum*. Antibiosis and competition, however, cannot be excluded in their experiments. In a later study, McLaughlin and Sequeira (37) postulated that suppression of bacterial wilt by *P. solanacearum* was due to competitive exclusion. Anderson and Guerra (3) reported that roots of bean plants, which were bacterized with a pseudomonad plant growth-promoting rhizobacteria (PGPR) strain, had higher lignin content than uninoculated plants, suggesting an induction of disease resistance. Recently, evidence has suggested that plant growth-promoting *Pseudomonas* spp. induce systemic resistance to fungal root pathogens (31,51,53,62,63), fungal (58) and bacterial leaf pathogens (1,2,59), and viruses (34,36). In all of these studies, the pathogen and the resistance-inducing pseudomonad were applied at spatially separated locations on the plant, excluding direct antibiosis and competition as the mechanisms of disease suppression. For induction of systemic resistance by PGPR, the term induced systemic resistance (ISR) (23) is commonly used. ISR involves the activation of the plant's defense mechanisms, which leads to systemic protection. Whether ISR and SAR have the same characteristics is not yet clear.

Information on bacterial traits involved in PGPR-induced disease resistance is limited. Van Peer and Schippers (53) obtained evidence that lipopolysaccharides (LPSs) extracted from the outer membrane of *P. fluorescens* WCS417 induced systemic resistance in carnation to *Fusarium oxysporum* f. sp. *dianthi*. Voisard et al. (56) observed that cyanide-producing *P. fluorescens* strain CHA0 stimulated root hair formation in tobacco and suppressed root rot by *Thielaviopsis basicola* in the same plant, whereas a HCN^- mutant did not. They hypothesized that cyanide might be responsible for ISR. However, Wei et al. (58) found no evidence for the role of cyanide in ISR against *Colletotrichum orbiculare* on cucumber. Maurhofer et al. (36) showed the involvement of the pyoverdine siderophore of *P. fluorescens* CHA0 in the induction of disease resistance against tobacco necrosis virus in tobacco.

Seed bacterization of radish and seed tubers of potato with *P. fluorescens* strain WCS374 resulted in significant plant growth-promotion in high-frequency radish- and potato-cropping soil (13,14), whereas *P. putida* strain WCS358 increased potato tuber yield (5,13) and root development (4) in high-frequency cropping of potato. In these studies, siderophore-mediated iron deprivation of deleterious microorganisms was considered the mode of action for the observed growth-promotion. *P. fluorescens* WCS374 also significantly suppressed Fusarium wilt of radish, caused by *F. oxysporum* Schlechtend.:Fr. f. sp. *raphani* J.B. Kendrick & W.C. Snyder, in commercial greenhouse trials (30,32). In a special rockwool bioassay developed to study ISR, evidence was ob-

Corresponding author: P. A. H. M. Bakker
E-mail address: p.bakker@boev.biol.ruu.nl

tained that strain WCS374 induces systemic resistance against Fusarium wilt of radish (31). *P. fluorescens* WCS417 suppressed take-all of wheat, caused by *Gaeumannomyces graminis* var. *tritici*, in field trials (27), induced systemic resistance in carnation and radish against Fusarium wilt (10,31,51), suppressed Fusarium wilt in carnation by competition for iron (10,54), and increased growth of tomato by internal colonization of the roots, displacing deleterious indigenous endophytic pseudomonads (52).

The objective of this investigation was to study the involvement of LPSs of PGPR *P. fluorescens* strains WCS374 and WCS417 and *P. putida* strain WCS358 in the suppression of Fusarium wilt of radish through ISR. Van Peer and Schippers (53) have already demonstrated ISR against Fusarium wilt of carnation by LPS of WCS417. In the present study, living cells, crude cell wall extract containing LPS, and purified LPS of both wild-type strains and phage-resistant mutants lacking the O-antigenic side chain of the LPS were examined in rockwool bioassays developed to study ISR. Living cells of WCS374 and a phage-resistant mutant lacking the O-antigenic side chain of the LPS were examined in a bioassay using greenhouse soil naturally infested with the pathogen.

MATERIALS AND METHODS

Radish cultivar. The radish (*Raphanus sativus* L.) cv. Saxa×Nova (moderately resistant to Fusarium wilt, seed size 2.50 to 2.75 mm) (S&G Seeds B.V., Enkhuizen, the Netherlands) was used in all experiments.

Microbial cultures and inocula. The wilt pathogen of radish, *F. oxysporum* f. sp. *raphani* (formerly *F. oxysporum* f. sp. *conglutinans* race 2) strain WCS600, was isolated from infected radishes on Komada's agar (25) modified by Gams and Van Laar (11). The fungal culture was maintained on modified Komada's agar.

The pathogen was cultured in aerated 2% malt extract. After 7 days of incubation at 22°C, 15 ml (10^7 CFU ml^{-1}) of washed microconidia was poured into 100 g of gamma-sterilized peat (neutral pH) (Agrifutur s.r.l., Alfianello, Italy). This resulted in a moisture content of approximately 50% (wt/wt). The inoculum was incubated for 4 days at 22°C before use in the bioassays. The number of CFU in the peat after incubation was determined by dilution plating on modified Komada's agar. Pathogen inoculum density in the peat was adjusted with sterile sand to 2×10^4 CFU g^{-1} of peat prior to inoculation.

P. fluorescens strain WCS374 and *P. putida* strain WCS358 originally were isolated from the rhizosphere of potato (12,13). *P. fluorescens* WCS417 was isolated from the rhizosphere of wheat grown in a field suppressive to take-all, caused by *G. graminis* var. *tritici* (27). LPS mutants LWP74-30b (374OA$^-$) (9) and WCS417B4 (417OA$^-$, isolated by W. Bitter, Department of Molecular Cell Biology, Utrecht University, Utrecht, the Netherlands) of strains WCS374 and WCS417, respectively, were used to study the role of LPSs in ISR. Both are spontaneous phage-resistant mutants, lacking the O-antigenic side chain of the LPS but with an intact innercore and lipid A. Bacteria were cultured on King's medium B (KMB) agar (24) at 27°C.

Bacterial suspensions were prepared by scraping cultures grown overnight from KMB agar plates in 0.01 M $MgSO_4$. These cells were washed, and the suspensions were diluted and poured (1:1, vol/wt) into talcum or sterile peat (neutral pH). The number of CFU in the talcum or peat was determined by dilution plating on KMB agar. The inoculum in talcum was plated immediately after mixing the suspension with the talcum, and the inoculum in peat first was incubated overnight at 27°C. The bacterial inoculum densities were approximately 6×10^7 CFU g^{-1} of talcum and 10^9 CFU g^{-1} of peat. For bacterization of cotyledons, agar disks (4 mm diameter) were cut from a KMB plate overgrown by strain WCS374 or its O-antigen-minus mutant 374OA$^-$.

Bacterial cell wall extraction and purification of LPSs. Cell wall material of *P. fluorescens* strains WCS374 and WCS417, their O-antigen-minus mutants 374OA$^-$ and 417OA$^-$, and *P. putida* strain WCS358 were isolated from cells of cultures grown overnight on KMB agar (27°C). Cells were washed (4,000 × *g*, 5 min) in phosphate buffered saline (10 mM, pH 7.2) and subsequently lyophilized. Lyophilized cells (100 mg) were resuspended in 10 ml of 50 mM Tris-HCl and 2 mM EDTA buffer (pH 8.5) and sonicated at resonance amplitude six times for 15 s at 0 to 4°C. The sonicated suspension was centrifuged (600 × *g*, 20 min) to remove intact cells, and the supernatant was centrifuged further (8,000 × *g*, 60 min) to obtain the crude LPS in the pellet. The pellet was resuspended in 600 µl of 2 mM Tris-HCl buffer (pH 7.8) and stored at –20°C. This was the crude cell wall extract containing LPS, proteins, and RNA (crude LPS).

The crude LPS (400 µl) was purified by dialysis for 4 days at 5°C against ultrapure water, followed by lyophilization. The dry material was resuspended in 400 µl of 2 mM Tris-HCL buffer (pH 7.8) and extracted with an equal volume of phenol/chloroform (1:1, wt/vol). After vigorous shaking and centrifugation (16,000 × *g*, 5 min), the water phase containing the LPS was kept. An equal volume of fresh Tris buffer was added to the phenol-chloroform interphase mixture, and after shaking and centrifugation, this water phase was pooled with the first water phase. The water phases were shaken with an equal volume of chloroform to remove the traces of phenol from the water. The water phase was dried under vacuum (medium drying rate, Speed Vac DNA110, Savant Instruments, Inc., Farmingdale, NY), resuspended in 400 µl of Tris buffer, and stored at –20°C. This was the purified LPS.

For the bioassay, the crude and purified LPSs were diluted (15 µl of crude or 100 µl of purified LPS in 20 ml of demineralized water) to a density corresponding with the estimated density of LPS present on the inoculated living cells and added to talcum (1:1, vol/wt). Using talcum as a carrier, the LPS was localized directly on the root. The sterility of the crude and purified LPS was checked by plating on tryptic soy agar (Difco Laboratories, Detroit).

Analysis of LPSs. Patterns of LPS cell wall components in the crude and purified LPS of strains WCS374 and WCS417, of their O-antigen-minus mutants 374OA$^-$ and 417OA$^-$, respectively, and of strain WCS358, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by De Weger et al. (8). The cell envelope protein content of the crude and purified LPS was determined using bicinchoninic acid according to the method of Smith et al. (50). The 2-keto-3-deoxy-octanate (KDO) concentrations in the LPS preparations were determined according to the method of Karkhanis et al. (21) for estimating the amount of LPS present. RNAs in the LPS preparations were estimated by agarose gel electrophoresis, using ethidium bromide detection under UV light.

ISR rockwool bioassay. Radish seeds were sown in sand and, after 5 days, were transferred to rockwool growth cubes (Rockwool/Grodan B.V., Roermond, the Netherlands). The ISR-rockwool bioassay has been described previously by Leeman et al. (31) and offers the possibility of inoculating the bacterium and pathogen at spatially separate locations on the root system. In the ISR-rockwool bioassay, the living bacterial cells, crude or purified LPS, were applied to the root tips in talcum (1 g per root system), followed 2 days later by the pathogen applied to the root base in peat (0.2 g per root). One cotyledon of 5-day-old radish seedlings was bacterized by sticking (natural adherence) an agar disk with the side overgrown with the bacterium on the underside of the cotyledon. Two days later the pathogen was inoculated onto the roots growing in a single rockwool compartment. Three weeks after inoculation of the roots with the pathogen the plants were harvested, and the percentage of plants with symptoms of Fusarium wilt (browning or blackening of the xylem tissue in root and radish and yellowing and browning of leaves) was recorded. Each treatment was replicated 12 times, and each replicate consisted of three plants. Experiments were repeated at least two times.

Bioassay in soil naturally infested with the pathogen. Sandy soil (5% organic matter) naturally infested with the Fusarium wilt pathogen of radish, was taken from a commercial greenhouse. In this greenhouse, seed treatments with strain WCS374 significantly suppressed Fusarium wilt of radish (30,32). Radish seeds were sown in 1.2-liter pots (six seeds per pot). Pots contained a 1-cm layer (100 ml) of hydrogranules (heat expanded clay particles, 8 to 15 mm diameter) (Jongkind B.V., Aalsmeer, the Netherlands) with an 8-cm layer (500 g) of infested soil on top. Alternatively, instead of the 8-cm layer of infested soil, a 2.5-cm layer (150 g) of infested soil with a 5.5-cm layer (350 g) of river sand on top was used. Ten grams of peat inoculum with either WCS374 or 374OA$^-$ (10^9 CFU g^{-1}) was mixed through the soil and sand layer. A 1.5-cm layer of uninoculated sand was used to cover the seeds.

Plants were maintained in the greenhouse at 22°C at night and 24 to 28°C during the day, at a relative humidity of approximately 70%, and supplemented with Son-t (Philips, high-pressure sodium lamp; Philips B.V., Twinhout, Belgium) lighting for 16 h day^{-1}. During the first week, pots were watered on top of the soil, and during the remaining 2 weeks, the plants received Hoagland's nutrient solution (19) modified (31) for iron nutrition by adding 10 μM Fe-EDDHA (Sequestreen, 5% ferric iron of which 80% is bound as Fe-ethylenediamine di (*o*-hydroxyphenylacetic acid)) (CIBA-GEIGY Corp., Summit, NJ) on top of the soil. Three weeks after sowing the percentage of Fusarium wilted radish plants was recorded. Each treatment was replicated 10 times. Experiments were repeated three times.

Microbial root colonization. Colonization of the root base (zone of pathogen inoculation) and root tips (zone of bacterization) of cv. Saxa×Nova in the ISR-rockwool bioassay by fluorescent pseudomonads and the introduced *Pseudomonas* strains was checked at harvest as described previously by Leeman et al. (31). In the soil bioassay, root colonization by the introduced *Pseudomonas* strains was enumerated in root washings, using the immunofluorescence colony-staining method (55) as modified by Leeman et al. (29,31).

Data analysis. Data were analyzed for significance after arcsine square root (disease data) or logarithmic (colonization data) transformations using analysis of variance followed by Fisher's least significant difference test ($\alpha = 0.05$), using SAS-software (SAS Institute, Cary, NC). Repeated experiments demonstrated the same significant differences between treatments.

RESULTS

Analysis of LPSs. Analysis of LPS patterns by SDS-PAGE confirmed the lack of the O-antigenic side chain in the O-antigen-minus mutants 374OA$^-$ and 417OA$^-$. The wild-type strains WCS358 and WCS374 possessed the characteristic LPS pattern described by De Weger et al. (8,9). The LPS patterns of WCS417 and its O-antigen-minus mutant, WCS417B4, have not been published before and are presented in Figure 1. The KDO detection method demonstrated the presence of an innercore in the wild-type and O-antigen-minus mutant strains at average concentrations of 258 and 41 ng μl^{-1}, in crude and purified LPSs, respectively. In crude LPS, the average protein concentration was 15 μg μl^{-1}. Proteins were not detectable in purified LPS. Oligonucleotide RNA concentrations were estimated to be 50 and <1 ng μl^{-1}, in crude and purified LPSs, respectively.

Role of LPSs in induction of resistance. Strains WCS374 and WCS417 and their crude or purified LPSs containing lipid A/innercore/O-antigen side chain delivered in talcum significantly reduced Fusarium wilt of radish cv. Saxa×Nova (Fig. 2). Strain WCS358, its crude or purified LPS, the O-antigen-minus mutants 374OA$^-$ and 417OA$^-$, and their crude or purified lipid A/innercore did not suppress the disease.

Among the treatments at harvest, no significant differences in root colonization were observed with respect to the total aerobic bacteria populations (2.8 to 4.7 × 10^8 CFU g^{-1} of root fresh weight), the total fluorescent pseudomonad populations (4.3 to 5.3 × 10^7 CFU g^{-1} of root fresh weight), and the introduced fluorescent *Pseudomonas* strains and their mutants (5.8 to 8.1 × 10^6 CFU g^{-1} of root fresh weight). Introduced bacteria were never detected (detection limit for applied fluorescent *Pseudomonas* strains was 1.3 × 10^2 CFU g^{-1}) on the roots at the root base (zone of pathogen inoculation).

Delivery of bacteria on cotyledons. Delivery of bacteria by sticking agar disks overgrown with WCS374 at the underside of radish seedling cotyledons also induced systemic resistance when the pathogen was applied 2 days later onto the roots in rockwool cubes in peat (Fig. 3). The O-antigen-minus mutant 374OA$^-$ did not induce resistance. The introduced bacteria were not present on or in the roots of radish at harvest.

Bioassay in soil naturally infested with the pathogen. In the bioassay with greenhouse soil naturally infested with *F. oxysporum* f. sp. *raphani*, WCS374 significantly reduced disease when the pathogen was present only in the soil layer at the bottom of the pot, whereas the O-antigen-minus mutant 374OA$^-$ had no effect (Fig. 4). When the pathogen was present throughout the soil in the pot, no disease suppression was observed for both WCS374 and 374OA$^-$. At harvest, the wild-type strain and the O-antigen-minus mutant colonized the roots to an average density of 2.2 and 1.9 × 10^6 CFU g^{-1} of root fresh weight, respectively, in both systems. The total population of fluorescent pseudomonads was 3.0 × 10^7 CFU g^{-1} of root. The percentage of diseased plants in the control treatments did not differ between the two set ups, although the inoculum density of the pathogen was less in the set up with the soil and sand layers.

DISCUSSION

In the ISR-rockwool bioassay, *F. oxysporum* f. sp. *raphani* and the introduced *Pseudomonas* strain remained at spatially separate

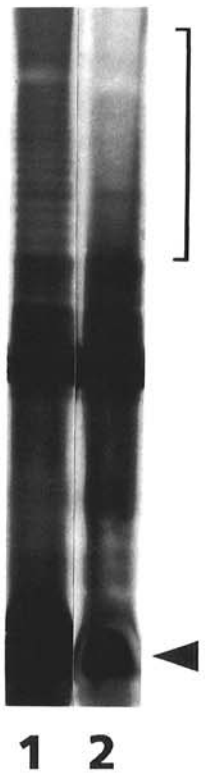

Fig. 1. Silver-stained lipopolysaccharide (LPS) patterns of proteinase K-treated LPS preparations of *Pseudomonas fluorescens* strain WCS417 (lane 1) and its O-antigen-minus mutant strain WCS417B4 (lane 2), obtained after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The bracket indicates the O-antigen side chain region, and the arrow indicates the position of the innercore.

locations on the root system of radish throughout the experiments. Relocation of these microorganisms, either through the root interior or over the root surface, can be excluded, because their populations could not be detected in root macerates of the uninoculated root parts. This confirms observations by Leeman et al. (31). Antibiosis and competition between the two organisms, therefore, can be excluded. The observed disease suppression can be explained best by the ISR against Fusarium wilt disease in radish by *P. fluorescens* strains WCS374 and WCS417.

ISR in the Fusarium wilt of radish model appears to be different from the classic SAR. In SAR, a set of genes, including those that encode for pathogenesis-related proteins, are expressed (33,57). Hoffland et al. (20), however, demonstrated that suppression of Fusarium wilt of radish in the ISR-rockwool bioassay is not associated with an accumulation of pathogenesis-related proteins. Furthermore, it is generally accepted that SAR is induced by necrosis-causing organisms (7,26,57), and the fluorescent PGPR pseudomonad strains used in this study are nonpathogenic bacteria that do not cause necrosis.

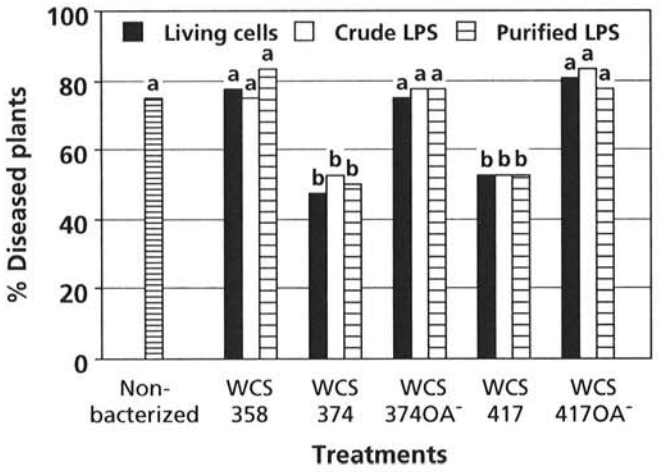

Fig. 2. Percentage of radish plants with Fusarium wilt in the induced systemic resistance (ISR)-rockwool bioassay. The root tips were treated with living cells of *Pseudomonas fluorescens* strain WCS374 or WCS417 or their O-antigen-minus mutants 374OA⁻ and 417OA⁻, *P. putida* strain WCS358, or the corresponding crude or purified lipopolysaccharide from each strain in talcum. Two days later, the pathogen in peat was inoculated onto the root base. Bars with the same letter are not significantly different at $P \leq 0.05$, Fisher's least significant difference test, based on unpooled data from a single experiment.

To obtain purified LPSs of the *Pseudomonas* strains, the hot-phenol method of Westphal and Jann (60) was applied first. However, this method could not be used successfully, because the LPS partitioned in the interphase and not, as expected, in the water phase. An explanation for this phenomenon can be found in the pH and salt content of the extractable cell wall suspension, which may have caused the LPS to be less hydrophilic. Another explanation is that the pseudomonads tested have LPSs with slightly different physiochemical properties than the *Enterobacteriaceae* for which the hot-phenol method was developed. The original protocol for this method (60) is not clear on the possible interference of the salt content and pH of the suspension in the purification of LPS. After unsuccessful attempts to use other methods to remove proteins from the crude LPSs, such as trichloroacetic acid, acetone, and ammonium sulfate, the phenol-chloroform purification method (described earlier) was developed successfully.

Living cells of strains WCS374 or WCS417 of *P. fluorescens*, their crude or purified LPSs containing lipid A/innercore/O-antigen side chain, all applied in talcum on the root tips of radish, induced systemic resistance in the ISR-rockwool bioassay. Neither living cells of the O-antigen-minus mutants of these strains nor their crude or purified LPSs containing lipid A/innercore reduced disease incidence. *Pseudomonas*-mediated ISR appears to differ among the strains used, since neither the living cells nor the crude or purified LPS of strain WCS358 induced systemic resistance in radish in this bioassay. The purified LPSs contained no detectable amounts of proteins and only small amounts of oligonucleotide RNAs (<2.5%). No differences were found between the strains concerning the amount of innercore present in the LPS preparations.

At harvest, all strains had colonized the root to the same extent. Therefore, we concluded that the O-antigenic side chain of the lipopolysaccharide, present on the outer membranes of strains WCS374 and WCS417 of *P. fluorescens*, may be involved in the induction of resistance in radish. This agrees with Van Peer and Schippers (53), who reported that extracted LPS of *P. fluorescens* WCS417 induced systemic resistance in carnation against Fusarium wilt. The application of WCS374 on the cotyledon also was effective in inducing systemic resistance. The O-antigen-minus mutant 374OA⁻, however, did not reduce disease. So the resistance-inducing factor, most probably located on the O-anti-

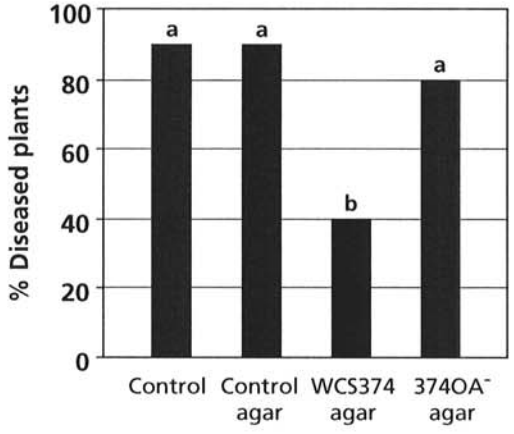

Fig. 3. Percentage of radish plants with Fusarium wilt in the induced systemic resistance (ISR)-rockwool bioassay. The underside of a cotyledon of 5-day-old radish seedlings was untreated or treated with King's medium B agar disks without bacteria, with *Pseudomonas fluorescens* strain WCS374, or with its O-antigen-minus mutant 374OA⁻. Two days later, the pathogen in peat was inoculated onto the root. Bars with the same letter are not significantly different at $P \leq 0.05$, Fisher's least significant difference test, based on unpooled data from a single experiment.

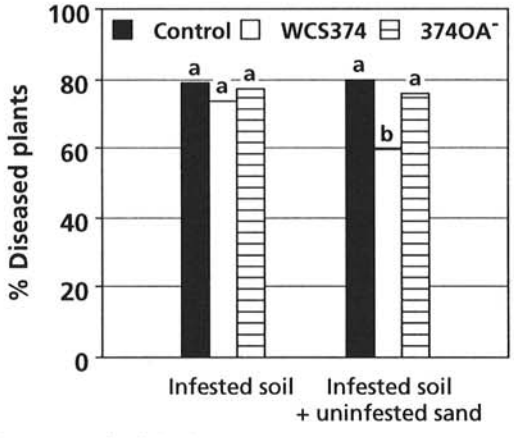

Fig. 4. Percentage of radish plants with Fusarium wilt in a pot bioassay with soil naturally infested with *Fusarium oxysporum* f. sp. *raphani*. Peat untreated or treated with *Pseudomonas fluorescens* strain WCS374 or with its O-antigen-minus mutant 374OA⁻ was mixed through the soil (and sand layer) before sowing. Pots contained a 1-cm layer of hydrogranules at the bottom and an 8-cm layer of infested soil, or a 2.5-cm layer of infested soil with a 5.5-cm layer of river sand on top. Bars with the same letter are not significantly different at $P \leq 0.05$, Fisher's least significant difference test, based on unpooled data from a single experiment.

gen side chain of strain WCS374 is not only effective on the root, but also on the cotyledon.

The involvement of bacterial LPS in bacterium-plant interactions was described earlier. Tobacco leaves infiltrated with LPS of the pathogen *P. solanacearum* showed systemic disease resistance and accumulated a protein band, which did not comigrate with the pathogenesis-related proteins induced by tobacco necrosis virus or tobacco mosaic virus (16,28). Electron microscopy demonstrated that infiltrated LPS attached to tobacco mesophyll cell walls and induced ultrastructural changes in the host cell wall (16,48). LPS mutants of *Rhizobiaceae* with an altered, a little, or no O-antigen side chain, were unable to nodulate leguminous plants (15,17,38,39). In this field of research, signaling functions, protecting against toxic molecules derived from the plant, masking elicitors of host defense responses, or providing an appropriate surface for interactions during endocytosis, are thought to be the possible function of the LPS in symbiosis between plants and rhizobia (17,38).

In the ISR-rockwool bioassay with radish, the O-antigen side chain of the LPS may have served as a signal or trigger in the induction of defense mechanisms in the plant. Van Peer et al. (51) and Van Peer and Schippers (53) demonstrated a correlation between disease suppression and accelerated accumulation of phytoalexins in carnation when *P. fluorescens* WCS417 or its LPS and the pathogen, *F. oxysporum* f. sp. *dianthi*, were inoculated at spatially separate locations on the plant.

De Weger et al. (8) demonstrated that the composition of pseudomonad LPSs are strain-specific and useful for characterizing and identifying strains of fluorescent *Pseudomonas* spp. Strains WCS358, WCS374, and WCS417 differ in their LPS patterns. Neither *P. putida* WCS358 nor its LPS was able to induce resistance in this bioassay; the LPS composition of WCS358 obviously is unable to sensitize the defense mechanisms of radish plants, whereas the O-antigenic side chain of the LPSs of strains WCS374 and WCS417 are able to do so. This suggests that the induction of resistance depends on the composition of the O-antigenic side chain. Graham et al. (16) presented evidence that the lipid A/innercore part, but not the O-antigen, cell wall proteins, free phospholipids, or nucleic acids of the LPS preparations of the pathogen *P. solanacearum*, induced disease resistance in tobacco leaves. This inducer activity was apparently nonspecific for two reasons: i) a variety of other gram-negative bacteria also induced resistance (16), and ii) the structure of the lipid A/innercore is fairly constant among many gram-negative bacteria (35), unlike the strain specific O-antigen part (8).

However, Whatley et al. (61) demonstrated that two strains of *P. solanacearum* differing in their LPS composition also caused different resistance responses in tobacco leaves. The results presented here suggest that in the PGPR-mediated ISR the strain-specific O-antigen side chain polysaccharide, instead of the nonspecific lipid A/innercore, acts as the inducer of resistance. Neither the living cells and crude or purified LPS of strain WCS358 nor the living cells and crude or purified lipid A/innercore of the O-antigen-minus mutants of strains WCS374 and WCS417 induced resistance in radish.

Poly- and oligosaccharides of plant (6) and pathogen cell walls (43) have been implicated as signal molecules in the induction of resistance. They can act as phytoalexin elicitors in soybean (18, 46), induce protease inhibitors, and trigger the hypersensitive response in plants (44). Sequeira and Graham (49) presented evidence that potato lectin-binding sites are present in the polysaccharide O-antigen side chain portion of the *Agrobacterium* LPS. The interaction between the O-antigen polysaccharides of bacteria and the receptors of the plant on the plasma membrane may trigger disease resistance. The *Pseudomonas* spp. strain-specific ISR presented in this paper may be explained by the composition of the strain-specific O-antigen side chain. Among other constituents, the LPS of the resistance-inducing strain WCS374 contains rhamnose and fucose, whereas the LPS of strain WCS358, which does not induce resistance, does not contain these sugars (8). Similarly, a polysaccharide signal released from injured tomato leaves (PIIF) that induced proteinase inhibitor accumulation contained, among others, both rhamnose and fucose (45). Therefore, rhamnose and fucose also could play a central role in ISR in radish against Fusarium wilt by *P. fluorescens* strains WCS374 and WCS417.

Strain WCS374 and other root-colonizing pseudomonads when applied on seed are capable of colonizing the cotyledons (40). The observed suppression of Fusarium wilt of radish after seed treatment with WCS374 in commercial greenhouse trials (30,32), therefore, also may have been the result of induction of resistance in the cotyledons as well as in the roots. The pot bioassay with greenhouse soil naturally infested with *F. oxysporum* f. sp. *raphani* also demonstrated the ineffectiveness of the O-antigen-minus mutant, whereas the wild-type WCS374 reduced disease significantly. This suggests the involvement of ISR in that system and, most likely, also in the commercial greenhouse trials (30,32).

In the pot experiments, the disease was only suppressed by WCS374 when the pathogen was present only in a small layer of soil at the bottom of the pot, whereas WCS374 was present throughout the pot. Apparently, *Pseudomonas*-mediated ISR requires a certain period of time before the radish plant is systemically protected. This also was demonstrated in the ISR-rockwool bioassay, in which a time interval of at least 1 day between bacterization and pathogen inoculation was required for significant disease suppression (31).

Future research should focus on the identification of the components of the O-antigenic side chain of the LPSs of *P. fluorescens* WCS374 and WCS417 that function as the trigger or signal for the sensitization of radish root cells, on in planta signal translocation, and on the nature of the induced defense mechanisms of radish.

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