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Siderophore-mediated competition for iron and induced resistance in the suppression of fusarium wilt of carnation by fluorescent Pseudomonas spp

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Abstract

The mechanisms of suppression of fusarium wilt of carnation by two fluorescent *Pseudomonas* strains were studied.

Treatments of carnation roots with *Pseudomonas* sp. WCS417r significantly reduced fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (Fod). Mutants of WCS417r defective in siderophore biosynthesis (sid$^-$) were less effective in disease suppression compared with their wild-type. Treatments of carnation roots with *Pseudomonas putida* WCS358r tended to reduce fusarium wilt, whereas a sid$^-$ mutant of WCS358 did not.

Inhibition of conidial germination of Fod in vitro by purified siderophores (pseudobactins) of both *Pseudomonas* strains was based on competition for iron. The ferrated pseudobactins inhibited germination significantly less than the unferrated pseudobactins. Inhibition of mycelial growth of Fod by both *Pseudomonas* strains on agar plates was also based on competition for iron: with increasing iron content of the medium, inhibition of Fod by the *Pseudomonas* strains decreased. The sid$^-$ mutant of WCS358 did not inhibit Fod on agar plates, whereas the sid$^-$ mutants of WCS417r still did. This suggests that inhibition of Fod by WCS358r in vitro was only based on siderophore-mediated competition for iron, whereas also a non-siderophore antifungal factor was involved in the inhibition of Fod by strain WCS417r.

The ability of the *Pseudomonas* strains to induce resistance against Fod in carnation grown in soil was studied by spatially separating the bacteria (on the roots) and the pathogen (in the stem). Both WCS417r and its sid$^-$ mutant reduced disease incidence significantly in the moderately resistant carnation cultivar Pallas, WCS358r did not.

It is concluded that the effective and consistent suppression of fusarium wilt of carnation by strain WCS417r involves multiple mechanisms: induced resistance, siderophore-mediated competition for iron and possibly antibiosis. The less effective suppression of fusarium wilt by WCS358r only depends on siderophore-mediated competition for iron.

Additional keywords: antibiosis, biological control, disease resistance, *Fusarium oxysporum* f.sp. *dianthi*, root colonization.

Introduction

Introduction of selected antagonistic fluorescent pseudomonads into the rhizosphere can effectively suppress soil-borne plant diseases (Weller, 1988; Schippers, 1992) and plant-deleterious microbial activity (Schippers et al., 1987). Elucidation of the mechanisms involved in the interactions between pseudomonads, plant pathogens and their host-plant may improve the use of fluorescent pseudomonads as biocontrol agents. Siderophore-

mediated and antibiotic-mediated suppression of soil-borne plant diseases are the two most studied mechanisms involved in biocontrol by pseudomonads (Gutterson, 1990; Loper and Buyer, 1991; O'Sullivan and O'Gara, 1992; Schippers, 1992).

Recently, Van Peer et al. (1991) presented evidence for systemic induced resistance, a third and possibly important mechanism of biocontrol of plant pathogens by pseudomonads. By spatial separation of *Pseudomonas* sp. strain WCS417r (on the roots) and the pathogen (in the stem) it was demonstrated that WCS417r systemically induces resistance against fusarium wilt in carnation grown in rockwool. The reduced disease incidence was accompanied by an increased accumulation of phytoalexins in the *Fusarium*-infected carnation stem tissue. Van Peer et al. (1990) earlier reported that competition for iron is also involved in the suppression of fusarium wilt of carnation by WCS417r. This was shown by using iron chelators, differing in their affinity for ferric iron, in the nutrient solution of rockwool cultures. Strain WCS417r also significantly suppressed fusarium wilt of carnation grown in soil (Duijff et al., 1991). *Pseudomonas putida* WCS358r, a *Pseudomonas* strain that increased potato tuber yield in high frequency potato cropping by production of its siderophore (Bakker et al., 1986), however, was less effective (Duijff et al., 1991) .

This paper, further elucidates the mechanisms of disease suppression by strains WCS358r and WCS417r to explain differences in effectivity of biocontrol of fusarium wilt of carnation between the two strains. Special attention is paid to the role of siderophore production, by using Tn*5* mutants defective in siderophore biosynthesis, and to induced resistance, by spatially separating the pathogen in the stem and the bacteria on the roots.

Materials and methods

Microorganisms and preparation of bacterial and conidial suspensions. Rifampicin resistant derivatives of *Pseudomonas putida* WCS358: WCS358r (Geels and Schippers, 1983; Glandorf et al., 1992) and of *Pseudomonas* sp. WCS417: WCS417r (Lamers et al., 1988; Van Peer et al., 1990) were used. The role of siderophore production in disease suppression was investigated by using the siderophore biosynthesis defective (sid^-) Tn*5* mutant of WCS358: JM218 and the sid^- Tn*5* mutants of WCS417r: S680 and M634. JM218 was obtained and characterized by Marugg et al. (1985) and used in several studies (Bakker et al., 1987; Lemanceau et al., 1992). The sid^- mutant S680 was obtained by Tn*5*-mediated mutagenesis according to the method of Marugg et al. (1985), M634 was obtained by Tn*5*-mediated mutagenesis according to the method of Whitta et al. (1985). S680 and M634 do not fluoresce, and have a single Tn*5* transposon insertion in the chromosomal DNA and similar outer membrane protein patterns (D. Gurdian, unpublished results). On chrome azurol S agar plates (Schwyn and Neilands, 1987) there was no siderophore production by the sid^- mutants detected. Their growth rate did not differ from the wild-type strains in RS-medium (RSM) (Buyer et al., 1989) and in King's medium B (KB) (King et al., 1954).

The fusarium wilt pathogen used is *Fusarium oxysporum* f. sp. *dianthi* (Fod) race 2 isolate WCS816 (Baayen, 1986).

Suspensions of *Pseudomonas* strains and their derivatives were prepared from cultures grown for 2 days at 27 °C on KB agar. Cells were harvested and centrifuged (20 min, 4000 rpm) and washed twice in tap water.

Conidial suspensions of Fod were prepared from potato dextrose agar cultures, grown for 7 days at 23 °C. Conidia were harvested by scrubbing them of the agar plates in tap water and filtering the suspension through glasswool to remove mycelial fragments.

Influence of pseudobactin 358 (PSB358) and pseudobactin 417 (PSB417) on conidial germination. Erlenmeyer flasks (1L) containing 400 ml succinate medium (Meyer and Abdallah, 1978) adjusted to pH 7 (0.1 M NaOH) were inoculated with 1 ml of a stationary phase culture of WCS358r or WCS417r grown in KB medium. After incubation for 4 days at 24 °C under mechanical agitation, unferrated pseudobactins were isolated from the cultures according to the method of Van der Hofstad et al. (1986). The isolated pseudobactins were lyophilized, dissolved in small volumes of deionized water, eluted over Sephadex G-25 (PD-10 columns, Pharmacia) and again lyophilized. The purity of the resulting PSB358 preparation was 95–100% as was determined spectrophotometrically. The molecular weight (1313 D) and extinction coefficient (13670 mol^{-1} cm^{-1} at 400 nm and pH 7) (G.A.J.M. van der Hofstad, unpublished results) derived from at least 95% pure PSB358, checked by HPLC gel filtration (Van der Hofstad et al., 1986), were used as standards. The concentration of the purified PSB417 was determined by ferrating the pseudobactin with small quantities of 0.1 mM $FeCl_3$ until fluorescence of the pseudobactin solution was maximally quenched, assuming a 1:1 stoicheiometry (Meijer and Abdallah, 1978) of the FePSB417-complex. Fluorescence was measured with a fluorescence spectrophotometer (Optica, model 115) at an extinction wavelength of 412 nm and an emission wavelength of 461 nm.

Glass test tubes containing 3 ml RS-medium (RSM), buffered with 0.1 M HEPES and adjusted to pH 7 (0.1 M NaOH), were inoculated with 330 µl of a suspension of 10^6 conidia of Fod ml^{-1}. Either filter-sterilized PSB358 or PSB417 was added to the RSM, both unferrated (0.2, 2, 10, 20 µM) as well as ferrated (2 or 20 µM). After incubation for 10 h at 22 °C under mechanical agitation, the percentage of germinated conidia was determined microscopically. Each treatment was replicated three times.

Role of siderophore-mediated competition for iron in inhibition of fungal growth. Suspensions of *Pseudomonas* strains WCS358r, WCS417r and their sid^- derivatives were diluted in sterile tap water to a concentration of 10^8 cells ml^{-1} and spot-inoculated on modified RSM agar plates amended with 0, 2, 20 or 200 µM $FeCl_3$. After incubation for two days at 27 °C, a suspension of 10^7 conidia of Fod ml^{-1} in sterile tap water was atomized over the plates. After incubation for two days at 23 °C, antagonistic activity was measured as the width of the zone without fungal growth (mm), surrounding the *Pseudomonas* colony, divided by the diameter of the inhibiting *Pseudomonas* colony (mm). Each treatment consisted of four replicate agar plates.

Role of siderophore production in disease reduction. Two cultivars of carnation (*Dianthus caryophyllus* L.) were used: Lena and Pallas, susceptible and moderately resistant, respectively, to race 2 of *Fusarium oxysporum* f. sp. *dianthi* (Niemann and Baayen, 1988). Carnation cuttings, rooted in perlite, were obtained from van Staaveren B.V. (Aalsmeer, the Netherlands). Roots of the cuttings were washed free from perlite with tap water, excess water was removed from the roots with filter paper and roots were immersed in a suspension of 10^9 *Pseudomonas* cells ml^{-1} tap water, or tap water only (control) for 20 minutes. Excess bacterial suspension or water was removed from the roots with filter paper and each cutting was planted in a cone-shaped container (Teku) filled with 600 g of a sandy loam with $pH(H_2O) = 7.2$. Ten days later, plants and soil were bacterized for a second time by pouring 10 ml of a suspension of 10^8 *Pseudomonas* cells ml^{-1} tap water on top of the soil.

Two weeks after planting, roots and soil were inoculated with Fod by pouring 10 ml of a suspension of 2×10^6 conidia ml^{-1} tap water on top of the soil followed by watering the plants with 30 ml of tap water.

Plants were watered two times a week with tap water and once a week with a half strength Hoagland nutrient solution without micro-nutrients and adjusted to pH 6.8. Plants were grown in a glasshouse with a photoperiod of 16 h at 22 °C and at 18 °C during the dark period.

The numbers of diseased plants of cvs Lena and Pallas were recorded at 14 and 16 weeks, respectively, after inoculation with Fod. For each treatment 6 replicates of 6 plants each were used for disease assessment.

Spatially separated inoculation of Fod and pseudomonads. Plants were treated as described above, however, Fod was spatially separated from the introduced bacteria by inoculating 20 µl of a suspension of 10^7 conidia of Fod ml^{-1} sterile tap water directly into the stem as described by Baayen and Elgersma (1985), 3 weeks after planting of the rooted cuttings. From 3 days before inoculation, the plants did not receive any water or nutrient solution to stimulate the uptake of the conidia in the xylem. Three hours after inoculation, plants were watered once more. The numbers of diseased plants were recorded 14 weeks after inoculation with Fod. For each treatment 5 replicates of 5 plants each were used for disease assessment.

The entire carnation stem, starting from 2 cm below the site of inoculation with Fod, was examined for external and internal colonization by the introduced bacteria. Four plants per treatment were examined at the time of inoculation and at 5 and 13 weeks after inoculation with Fod. Stem surface colonization by the introduced bacteria was examined by pressing 5 cm stem segments into KB^+ agar (Geels and Schippers, 1983) supplemented with 150 ppm rifampicin (KB^+ rif). Internal colonization was examined by pressing slices of the surface-disinfected stem (flamed after dipping in 70% ethanol) into the KB^+ rif agar. Slices were cut at the point of inoculation with Fod, at 1 cm below, and at 3 and 5 cm above this point and also further upward at each 5 cm interval. Bacterial growth was checked after incubation of the agar plates for 72 h at 27 °C.

Root colonization. At regular intervals, roots (approximately 300 mg fresh weight) were sampled from four plants per treatment. The root samples were shaken for 30 sec (Vortex) in glass test tubes containing 5 ml 0.1 M $MgSO_4$ and 1 g of glass beads (0.18 mm diameter). The suspensions were diluted and plated on KB^+ supplemented with 150 ppm rifampicin for estimating the number of colony forming units (CFU) of strains WCS358r and WCS417r, or on KB^+ supplemented with 200 ppm kanamycin sulphate and 200 ppm streptomycin sulphate for estimating the number of CFU of the sid^- Tn5 mutants. Samples were also plated on KB^+ agar for estimating the total number of CFU of pseudomonads, on tryptic soy agar supplemented with 100 ppm cycloheximide for estimating the total number of CFU of aerobic bacteria and on Komada agar (Komada, 1975) modified as described by Gams and Van Laar (1982) for estimating the total number of CFU of *Fusarium oxysporum*. Root colonization was expressed as log (CFU per gram of root fresh weight).

Data analysis. Data were analyzed by analysis of variance, followed by mean separation with least significant difference (LSD) at $P = 0.05$ (Sokal and Rohlf, 1981). Alternatively, 95%-confidence intervals of the means were calculated (Sokal and Rohlf, 1981). All experiments were performed at least twice.

Results

Influence of pseudobactin 358 (PSB358) and pseudobactin 417 (PSB417) on conidial germination. The purified pseudobactins PSB358 and PSB417 inhibited conidial germination of Fod in RS medium significantly at concentrations of 0.2 μM and higher (Fig. 1).

The ferric pseudobactins inhibited the conidial germination significantly less than the unferrated pseudobactins. However, the ferrated PSB417 still inhibited conidial germination significantly compared with the control.

Role of siderophore-mediated competition for iron in inhibition of fungal growth. On RSM agar plates, both WCS417r and its sid^- mutants (S680 and M634) inhibited growth of Fod stronger than WCS358r (Fig. 2). The sid^- mutant of WCS358 (JM218) was not inhibitory at all. With increasing iron concentration in the medium, inhibition of growth of Fod by the pseudomonads decreased. When the $FeCl_3$ concentration in the medium was raised to 200 μM, WCS358r failed to inhibit Fod, whereas WCS417r and its sid^- mutants still did.

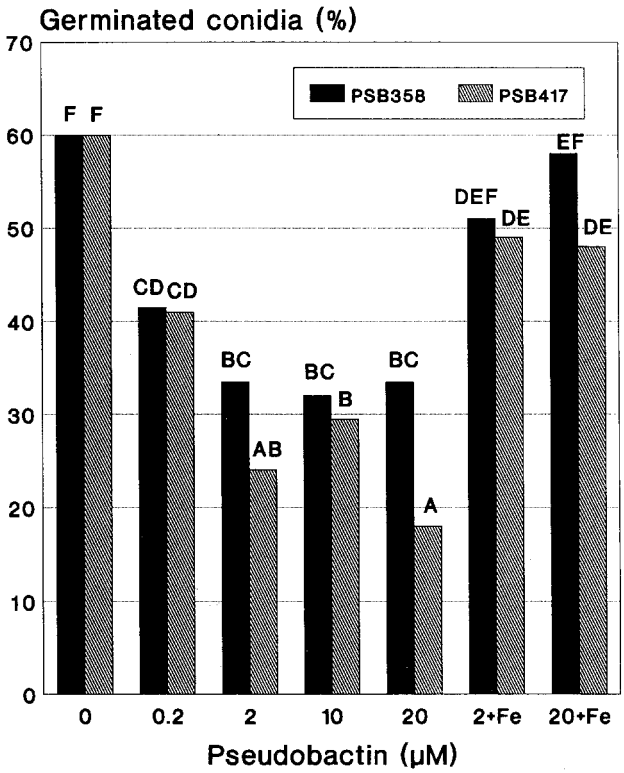

Fig. 1. Germination of conidia of *Fusarium oxysporum* f. sp. *dianthi*, as influenced by the purified unferrated –, or ferrated pseudobactins (+Fe) of *Pseudomonas* strains WCS358r (PSB358) or WCS417r (PSB417) after incubation for 10 h at 22 °C in RS-medium. Bars with no corresponding letter differ significantly ($P = 0.05$).

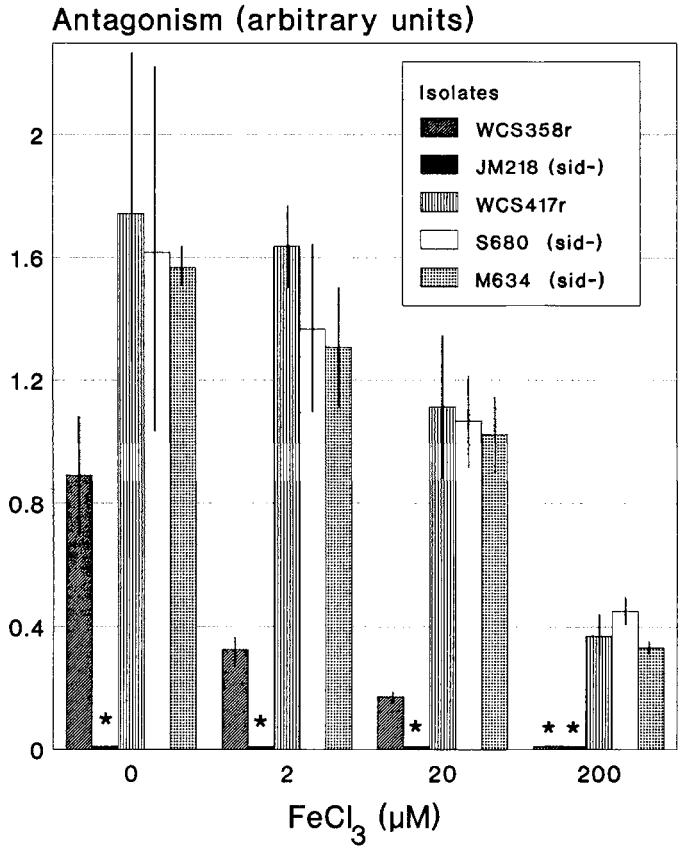

Fig. 2. Antagonism by *Pseudomonas* strains WCS358r, WCS417r and sid⁻ mutants (sid⁻) to *Fusarium oxysporum* f. sp. *dianthi* on RSM agar plates. Antagonism is expressed as the width of the zone without fungal growth (mm), surrounding the *Pseudomonas* colony, divided by the diameter of the inhibiting *Pseudomonas* colony (mm). JM218 is the sid⁻ mutant of WCS358. S680 and M634 are the sid⁻ mutants of WCS417r. The 95%-confidence intervals of the means are indicated. Antagonism did not occur at means marked with an asterisk.

Role of siderophore production in disease reduction. Strain WCS417r significantly reduced disease incidence in both the susceptible cv. Lena and the moderately resistant cv. Pallas when the roots were treated with the pseudomonads and Fod was soil-inoculated (Table 1). Its sid⁻ mutants M634 and S680 only reduced disease incidence significantly in experiment I in cv. Lena and in cv. Pallas, respectively.

The disease suppression by WCS358r and its sid⁻ mutant JM218 in both cultivars was never significant. However, disease incidence in the WCS358r treated plants was consistently lower compared with that in the JM218 treated plants.

Spatially separated inoculation of Fod and pseudomonads. Fusarium wilt in Fod stem-inoculated plants was significantly reduced in the moderately resistant cv. Pallas by treatment of the roots with WCS417r or its sid⁻ mutant S680 (Table 2). These isolates failed to

Table 1. Incidence of fusarium wilt in susceptible carnation cv. Lena and moderately resistant cv. Pallas after root treatment of carnation cuttings with *Pseudomonas* strains WCS358r, WCS417r or Tn*5*-insertion sid$^-$ mutants of these strains (sid$^-$), 14 (cv. Lena) or 16 weeks (cv. Pallas) after soil-inoculation with conidia of *Fusarium oxysporum* f. sp. *dianthi*.

Treatment	Diseased plants (%)			
	cv. Lena		cv. Pallas	
	exp I	exp II	exp I	exp II
Control[1]	68 b	92 b	37 cd	44 b
WCS358r	52 ab	80 ab	33 bcd	30 ab
JM218 (sid$^-$)	60 ab	86 b	40 d	35 ab
WCS417r	48 a	61 a	17 a	18 a
S680 (sid$^-$)	52 ab	78 ab	23 ab	31 ab
M634 (sid$^-$)	48 a	80 ab	27 abc	27 ab
LSD[3] ($P = 0.05$)	19	20	12	20

[1] Control: water treatment.
[2] Values within the same column and with no corresponding letter differ significantly ($P = 0.05$).
[3] LSD = least significant difference.

reduce disease incidence significantly in the susceptible cv. Lena. WCS358r did not reduce disease incidence in either stem-inoculated cultivar.

None of the introduced *Pseudomonas* strains could be reisolated from the stem surface or stem tissue of carnation.

Table 2. Incidence of fusarium wilt in susceptible carnation cv. Lena and moderately resistant cv. Pallas after root treatment of carnation cuttings with *Pseudomonas* strains WCS358r, WCS417r or a Tn*5*-insertion sid$^-$ mutant (S680) of WCS417r, 14 weeks after stem-inoculation with conidia of *Fusarium oxysporum* f. sp. *dianthi*.

Treatment	Diseased plants (%)			
	cv. Lena		cv. Pallas	
	exp I	exp II	exp I	exp II
Control[1]	96 a[2]	96 a	56 b	60 b
WCS358	88 a	92 a	52 b	52 ab
WCS417r	84 a	92 a	20 a	36 a
S680 (sid$^-$)	nd[4]	92 a	nd	36 a
LSD[3] ($P = 0.05$)	17	17	19	24

[1] Control: water treatment.
[2] Values within the same column and with no corresponding letter differ significantly ($P = 0.05$).
[3] LSD = least significant difference.
[4] nd = not determined.

Root colonization. The average number of colony forming units (CFU) of the introduced *Pseudomonas* strains per gram of root fresh weight decreased from log 5.7 ± 0.1 CFU at two weeks after planting to log 3.9 ± 0.5 CFU at the end of the experiment (Table 3, exp. 2). Expressed as relative percentages, the average number of introduced pseudomonads on the roots decreased from 19% to 8% of the total *Pseudomonas* population and from 1.5% to 0.02% of the total aerobic bacteria population. On most of the sampling dates, WCS358r colonized roots in higher densities than WCS417r (Table 3).

In the first experiment, JM218 colonized roots of cv. Lena significantly less than WCS358r. In the second experiment, they colonized roots equally well, with two exceptions: for cv. Lena at 8 weeks after planting and for cv. Pallas at the end of the experiment, JM218 showed significantly lower numbers of CFU than WCS358r.

The sid$^-$ mutants of WCS417r showed significantly lower numbers of CFU only for M634 in experiment I for cv. Pallas at 4 weeks after planting and for S680 in experiment II for cv. Lena at 8 weeks after planting, if compared with their wild-type. Occasionally, also significantly higher numbers of CFU of these sid$^-$ mutants did occur.

The numbers of CFU of Fod on the roots were not affected by the introduced *Pseudomonas* strains and approximated log 4.2 during both experiments.

Table 3. Root colonization of susceptible carnation cv. Lena and moderately resistant cv. Pallas following root treatment with *Pseudomonas* strains WCS358r, WCS417r or Tn*5*-insertion sid$^-$ mutants of these strains.

Treatment	log(CFU/g root fresh wt.)							
	cv. Lena				cv. Pallas			
	Weeks after planting				Weeks after planting			
	2	4	8	16	2	4	8	18
Exp I								
WCS358r	nd[4]	5.8 c[2]	5.7 d	nd	nd	5.8 b	5.0 a	nd
JM218 (sid$^-$)	nd	3.2 a	3.4 a	nd	nd	nd	4.8 a	nd
WCS417r	nd	4.5 b	4.2 bc	nd	nd	5.4 b	4.8 a	nd
S680 (sid$^-$)	nd	4.8 b	4.6 c	nd	nd	5.7 b	4.5 a	nd
M634 (sid$^-$)	nd	4.6 b	3.8 ab	nd	nd	4.5 a	4.5 a	nd
LSD[3] ($P = 0.05$)		0.8	0.6			0.7	0.6	
Exp II								
WCS358r	5.6 a	5.7 b	6.2 c	4.6 a	5.7 a	5.5 b	5.7 c	4.6 d
JM218 (sid$^-$)	5.7 a	5.9 b	5.5 b	4.1 a	5.6 a	5.2 b	5.3 bc	3.2 a
WCS417r	5.9 a	4.4 a	5.5 b	4.8 a	5.3 a	4.0 a	5.0 b	3.7 ab
S680 (sid$^-$)	5.7 a	5.5 b	5.4 b	4.2 a	5.7 a	5.2 b	4.1 a	4.2 c
M634 (sid$^-$)	5.8 a	4.7 a	4.6 a	4.2 a	5.5 a	5.5 b	5.3 bc	4.0 bc
LSD ($P = 0.05$)	0.6	0.6	0.7	0.8	0.4	0.7	0.8	0.4

[1] CFU = colony forming units.
[2] Values within the same column and with no corresponding letter differ significantly ($P = 0.05$).
[3] LSD = least significant difference.
[4] nd = not determined..

Discussion

The purified pseudobactins of both fluorescent *Pseudomonas* strains WCS358r and WCS417r inhibited conidial germination of Fod at a concentration as low as 0.2 μM (Fig. 1). If siderophores are produced by pseudomonads in the rhizosphere indeed, germination and subsequent active penetration of the pathogen into the roots (Pegg, 1985) may be reduced. No reports on quantitative estimates of pseudobactin concentrations in the rhizosphere are available, although Reid et al. (1984) extracted hydroxamate siderophores in concentrations of 10 μM from rhizosphere soil. However, indirect evidence for in situ production of pseudobactins exists. Bakker et al. (1988) demonstrated the production of pseudobactin 358 (PSB358) in the rhizosphere of potato by an increase of population density of sid$^-$ mutant JM218 after roots were treated with the pseudobactin producing parental strain WCS358. A different sid$^-$ *Pseudomonas* strain, unable to utilize FePSB358, was not stimulated in population density in the rhizosphere by WCS358. Also Loper and Lindow (1991) provided indirect evidence for siderophore production by pseudomonads in situ. They demonstrated expression of siderophore biosynthesis genes in the rhizosphere by ice-nucleating activity of a *Pseudomonas fluorescens* strain that was constructed by cloning a promoter from a siderophore biosynthesis gene upstream of a promoterless ice nucleation gene.

We showed that the ferrated pseudobactins of both strain WCS358r and WCS417r inhibited germination of Fod significantly less than the unferrated pseudobactins (Fig. 1). This suggests that the in vitro siderophore-mediated inhibition of conidial germination of Fod is based on competition for iron. However, the ferrated PSB417r still inhibited germination significantly. Purity, and consequently the concentration of PSB417 could not be determined as exactly as for PSB358, because the molecular weight of PSB417 has not yet been determined. So, either the concentration of the PSB417 was higher than that of PSB358 and PSB417 was not ferrated completely, or the purified PSB417 still contained some non-siderophore fractions, inhibitory to conidial germination of Fod. A non-iron related inhibitory effect of either ferrated or unferrated PSB417 can also not be excluded.

Also the antagonism on agar plates suggests that competition for iron is involved in the suppression of Fod, because increase of iron content of the medium decreased the antagonistic activity of the *Pseudomonas* strains tested (Fig. 2). The sid$^-$ mutant JM218 did not antagonize Fod. Apparently, siderophore production is the only factor responsible for inhibition of Fod by strain WCS358r on RSM-agar. The sid$^-$ mutants of WCS417r, however, were still inhibitory. Apparently, WCS417r and its sid$^-$ mutants produce a non-siderophore antifungal factor on RSM-agar, inhibitory to Fod. The synthesis or inhibiting activity of this putative antifungal factor is negatively influenced by iron, because the antagonistic activity of the sid$^-$ mutants of WCS417r decreased with increasing iron content of the medium. Production by pseudomonads of negatively iron-regulated antibiotics has earlier been reported by Gill and Warren (1988) and Thomashow and Weller (1990).

In the biocontrol experiments, carnations were grown in a soil that can be considered favourable for siderophore-mediated competition for iron. The soil is well aerated and its pH(H_2O) is 7.2. At these conditions, solubility of iron is low and Fe^{3+} concentration, mainly controlled by low soluble ferric hydroxides, is approximately 10^{-19} M (Lindsay and Schwab, 1982). This corresponds with the critical Fe^{3+} concentration below which chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* was suppressed by *Pseudomonas putida* strain A12 (Simeoni et al., 1987).

The siderophore producing *Pseudomonas* strains always reduced fusarium wilt of carnation more effectively than their sid$^-$ mutants (Table 1), but the variation within treat-

ments prevented statistically significant differences between treatments with wild-type and sid⁻ mutant.

The more effective disease suppression by the wild-type WCS417r compared to its sid⁻ mutants can not be explained by impaired root colonization of the latter, because at most of the sampling dates they colonized equally well (Table 3). Therefore, it is most likely that siderophore production is involved in the reduction of fusarium wilt by strain WCS417r. Siderophore production does not seem to play an important role in colonization of carnation roots by WCS417r at the experimental conditions.

Root colonization of cv. Lena by sid⁻ mutant JM218 was significantly less compared with that by the wild-type WCS358r in one of the experiments (Table 3). However, in a second experiment JM218 colonized roots of both cv. Lena and cv. Pallas equally well as WCS358r at nearly all sampling dates. Colonization of potato roots by JM218 was also not impaired compared to a siderophore producing Tn*5*-mutant of WCS358 (Bakker et al., 1987). This was explained by the ability of WCS358 and its sid⁻ mutants to utilize a broad spectrum of siderophores (Bakker et al., 1990). For WCS358r, also its siderophore production does not seem to play an important role in root colonization.

The sid⁻ mutants of WCS417r were effective enough to reduce disease incidence significantly in two cases (Table 1). Probably, induction of resistance or the production of the unidentified antifungal factor, alone or in combination, were already effective in controlling fusarium wilt of carnation. Only wild-type WCS417r was able to consistently reduce disease incidence.

Van Peer et al. (1991) obtained evidence for induced resistance by strain WCS417r in moderately resistant carnation cv. Pallas grown on rockwool and, although exceptionally, also in susceptible cv. Lena. Our study confirms the results of Van Peer et al. (1991): after treatment of the roots of carnation with WCS417r, this strain does not colonize the stem of carnation and still significantly reduces fusarium wilt in cv. Pallas when Fod is stem-inoculated (Table 2). However, in our study WCS417r did not significantly reduce fusarium wilt in the susceptible cv. Lena, if *Fusarium* and *Pseudomonas* were spatially separated. Our results also demonstrate that induction of resistance by strain WCS417r is not restricted to rockwool-substrate, but can also be triggered in carnation cv. Pallas grown in soil.

The sid⁻ mutant of WCS417r (S680) reduced disease incidence of stem-inoculated carnation cv. Pallas equally well as its wild-type (Table 2). This indicates that siderophore production by strain WCS417r is not involved in induced resistance. According to Van Peer and Schippers (1992) the lipopolysaccharides of the cell envelope of WCS417r seem to be the trigger for induction of resistance in carnation against fusarium wilt.

In conclusion, induced resistance, siderophore-mediated competition for iron and probably also antibiosis are involved in the suppression of fusarium wilt by *Pseudomonas* sp. WCS417r. The non-significant suppression by *Pseudomonas putida* WCS358r in our experiments seems only to depend on siderophore-mediated competition for iron.

Preliminary experiments showed that WCS358r significantly reduced fusarium wilt of carnation in raw soil, but not in steamed soil (Duijff et al., 1991). In the steamed soil, disease incidence was higher, most likely because of a reduced antagonistic soil microflora. Strain WCS358r on its own, seems to be not or less effective in reducing fusarium wilt of carnation if disease incidence is high. Lemanceau and Alabouvette (1991) demonstrated that a selection of fluorescent pseudomonads, not effective on their own, significantly improved the control of fusarium diseases attributed to a non-pathogenic *Fusarium oxysporum* strain Fo47. Recently, Lemanceau et al. (1992) showed that WCS358r significantly increased the suppression of fusarium wilt in carnation if co-inoculated with Fo47, but was not effective on its own. *Pseudomonas* sp. WCS417r, however, is effective

on its own in reducing fusarium wilt of carnation. The more effective and more consistent suppression of fusarium wilt by strain WCS417r compared with that by strain WCS358r can be explained by its multiple biocontrol mechanisms.

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