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Evidence for Signaling between the Phytopathogenic Fungus *Pythium ultimum* and *Pseudomonas fluorescens* F113: *P. ultimum* Represses the Expression of Genes in *P. fluorescens* F113, Resulting in Altered Ecological Fitness

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There is increasing evidence that communication between members of the same species, as well as members of different species, is important for the survival of microorganisms in diverse ecological niches, such as the rhizosphere. To investigate whether the phytopathogen *Pythium ultimum* could alter gene expression in the biocontrol strain *Pseudomonas fluorescens* F113, which protects the roots of sugar beet from the fungus, a screening system was developed to detect differential expression of bacterial genes in the presence of *P. ultimum*. The transposon Tn*5*, containing a promoterless *lacZ* reporter gene, was used to generate a library of transcriptional gene fusions in *P. fluorescens* F113. By this screening procedure, five *P. fluorescens* F113 gene clusters were identified and shown to be repressed in the presence of *P. ultimum*. The ecological fitness of three of the five reporter mutants in the rhizosphere of seed-inoculated sugar beet was lower than that of the wild type. Furthermore, all five mutants were impaired in their ability to subsequently colonize the rhizosphere of uninoculated sugar beet sown repeatedly in the same soil. With the exception of reporter mutant SF10, which was impaired in nitrogen metabolism, the reporter mutants had growth requirements and biocontrol abilities similar to those of the wild type. This is the first reported case of a fungus repressing the expression of bacterial genes.

Physical and chemical characteristics of the rhizosphere environment are determined by the interaction between soil, plants, and organisms associated with the root, including bacteria, fungi, protozoa, and nematodes. Root exudates provide microorganisms with nutrients, and in return, plant growth-promoting rhizobacteria can improve crop yields and suppress disease. This effect may be direct, through the production of plant hormones (1) and nitrogen fixation (13), or indirect, through the suppression of organisms deleterious to plants including other microorganisms (24), insects, and weeds (9).

Antagonism of biocontrol strains may be important for enabling fungi to maintain a competitive life cycle in the rhizosphere. This is suggested by the observation that the population size of *Pseudomonas fluorescens* 2-79 in the wheat rhizosphere was significantly reduced in the presence of the three *Pythium* species tested (17). The mechanism by which this occurs is not understood and, as with other interactions between species, may involve cellular communication mediated by diffusible compounds. An investigation of cell-cell communication between these species is therefore important to improve our understanding of the interactions between members of such complex microbial communities.

Recent work in our laboratory has focused on the production of the antifungal compound 2,4-diacetylphloroglucinol by *P. fluorescens* F113, which protects sugar beet seedlings from damping off disease caused by *Pythium* spp. (11). To improve our understanding of the interaction between *P. ultimum* and *P. fluorescens* F113, we investigated whether diffusible factors produced by *P. ultimum* could alter gene expression in *P. fluorescens* F113. A screening system was developed to detect differential gene expression in *P. fluorescens* in the presence and absence of the fungus. With this approach, five *P. fluorescens* gene fusions which were transcriptionally down-regulated by *P. ultimum* were identified. This paper describes the isolation and characterization of these reporter mutants.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains and plasmids used in this study are listed in Table 1.

Growth of organisms. *P. fluorescens* strains were routinely grown at 28°C on sucrose asparagine medium (SA) (21). Growth of the reporter mutants was determined in minimal medium (Na_2HPO_4, 6.8 g liter^{-1}; KH_2PO_4, 3 g liter^{-1}; NaCl, 0.5 g liter^{-1}; $MgSO_4 \cdot 7H_2O$, 0.1 g liter^{-1}; $CaCl_2 \cdot 2H_2O$, 0.015 g liter^{-1} [pH 7.4]) containing one of the following nitrogen sources: NH_4Cl (M9 minimal medium), $NaNO_3$, or urea, at a final concentration of 42 mM nitrogen. Carbon sources and amino acids were added at a final concentration of 1 g liter^{-1} when used as the sole source of carbon and nitrogen; amino acids were added at a concentration of 2 mg liter^{-1} when used as the sole source of nitrogen. *Escherichia coli* strains were grown in Luria-Bertani (LB) broth at 37°C (20). *P. ultimum* was grown on SA agar (pH 6.3) at 28°C. Where appropriate, the following supplements were added to the medium at the final concentrations indicated: tetracycline, 25 μg ml^{-1} for *E. coli* and 75 μg ml^{-1} for *P. fluorescens*; kanamycin (KM), 25 μg ml^{-1} for *E. coli* and 50 μg ml^{-1} for *P. fluorescens*; ampicillin (AP), 50 μg ml^{-1} for *P. fluorescens*; agar, 15 g liter^{-1}; soft agar, 7.5 g liter^{-1}; and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 30 μg ml^{-1}.

Generation of reporter fusion mutants. A library of random transcriptional fusions, with *lacZ* as the reporter gene, was made with mini-Tn*5*::*lacZ1* (10). The pUT plasmid carrying the minitransposon was introduced into *P. fluorescens* F113 by triparental mating, with the helper plasmid pRK600. Cultures of *E. coli* HB101 carrying the helper plasmid, donor *E. coli* CC118λpir (pUTmini-Tn*5*::*lacZ1*), and recipient *P. fluorescens* F113 (10^8 cells of each) were mixed and spotted onto an LB plate. After incubation overnight at 28°C, the bacterial lawn was scraped off and resuspended in 1 ml of tryptone-yeast extract (TY) broth (20) containing 15% (vol/vol) glycerol.

Following serial dilution, individual colonies were obtained on SA medium containing KM and X-Gal for selection of *P. fluorescens* strains containing transposon insertions. Plates with and without *P. ultimum*, which had been grown for 2 days, were overlaid with SA soft agar containing KM and X-Gal. A total of

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TABLE 1. Bacterial strains, fungal strains, and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
Pseudomonad strains		
F113	Wild-type Ph1$^+$ HCN$^+$ Flu$^+$ protease$^+$	22
F113Rif	Spontaneous rifampin-resistant mutant	4
SF reporter mutants	Phl$^+$ HCN$^+$ Flu$^+$ protease$^+$	This study
Escherichia coli strains		
CC118 (λ *pir*)	Δ(*ara-leu*) *araD* Δ*lacX74 galE galK phoA20 thi-1 rpsE rpoB argE* (Am) *recA1* lysogenized with λ *pir* phage	14
HB101	*recA hsdB hsdM strA pro leu thi*	3
Bacillus subtilis A1		11
Pythium ultimum	Phytopathogenic, causal agent of damping-off	Commonwealth Mycological Institute
Plasmids		
pUTmini-Tn*5*-km	λ *pir* vector containing mini-Tn*5*-km transposon	10
pRK600	Mob$^+$ Tra$^+$ Cm^r	12

5,000 KM-resistant colonies were streaked onto the overlay to investigate differential expression of *lacZ* in the presence of the fungus.

Preparation of *P. ultimum* spent medium. *P. ultimum* was grown on SA agar (pH 6.3), which was overlaid, after growth of *P. ultimum*, with SA soft agar (pH 6.3). Following incubation for 2 to 3 days, the overlay was collected and centrifuged at 21,000 × *g* for 15 min. The supernatant was adjusted to pH 7.0 and filter sterilized. Where appropriate, *P. ultimum*-spent medium was treated with proteinase K (100 μg ml^{-1}) at 37°C for 30 min or boiled for 20 min.

β-Galactosidase assay. β-Galactosidase activity was measured by using *o*-nitrophenyl-β-D-galactopyranoside, as described by Miller (18). All assays were done in triplicate.

DNA manipulations. Plasmid DNA was prepared by the alkaline lysis method of Birnboim and Doly (2). Enzymes for restriction digestion and ligation were used according to the manufacturers' instructions. A Qiaex DNA purification kit (Qiagen) was used to purify DNA fragments isolated from agarose gels. Other DNA manipulations were carried out according to standard protocols (20).

Southern blot hybridization. Chromosomal DNA was isolated from *P. fluorescens* by the method of Chen and Kuo (8). The DNA was digested with *Bgl*II and *Bam*HI, and the resulting fragments were separated by agarose gel electrophoresis and then transferred onto a Hybond-N nylon membrane (Amersham), with the Hybaid Vacu-aid according to the manufacturer's instructions.

The 4.0-kb *Sma*I fragment of mini-Tn*5*::*lacZ1*, containing the promoterless *trp*′-′*lacZ* fusion and the 5′ end of the KM resistance gene, was purified from an agarose gel by using the Qiaex DNA purification kit (Qiagen) and labeled by random incorporation of digoxigenin-labeled deoxyuridine triphosphate (Boehringer) according to the manufacturer's instructions. Hybridization was done at 68°C, followed by washing under stringent conditions, as described by Sambrook et al. (20). The hybridized probe was detected by using the digoxigenin nucleic acid detection kit as described by the manufacturer (Boehringer).

Characterization of biocontrol traits in *P. fluorescens* strains. Siderophore production was demonstrated by growing strains on SA agar plates and observing the production of a yellow fluorescent pigment. Strains were assayed for production of the antifungal metabolite 2,4-diacetylphloroglucinol by high-performance liquid chromatography analysis (23) and with a *Bacillus* bioassay (11). HCN production was measured as described by Castric and Castric (7). Protease production was determined by incubating colonies on skim milk agar plates (10% skim milk powder, 0.01% yeast extract, 1.5% agar) for 24 h at 28°C. Protease activity was observed as clear zones around protease-positive colonies.

Rhizosphere colonization assay on sugar beet. *P. fluorescens* F113Rif, SF2, SF3, SF5, SF9, and SF10 were assessed for colonization of the sugar beet rhizosphere. *P. fluorescens* F113Rif, a spontaneous rifampin-resistant mutant of F113, was chosen as the control strain since it was previously shown to be identical in terms of growth rate in defined and complex media to wild-type F113. It was also shown to colonize the sugar beet rhizosphere at high population levels and to produce 2,4-diacetylphloroglucinol (4). Strains used for the inoculation of sugar beet seeds (*Beta vulgaris* cv. Accord) were grown in SA medium and washed in one-fourth-strength Ringer's (Oxoid) solution. Seeds were inoculated by being soaked in cell suspensions (10^7 cells ml^{-1} in one-fourth-strength Ringer's solution) for 5 min prior to being sown. To enumerate the bacteria applied to the seed, cells were washed from seeds with one-fourth-strength Ringer's solution, serially diluted, and plated onto selective media.

Natural soil microcosms were set up by using soil collected from the sandy loam surface horizon of a brown podzolic soil naturally infested by *Pythium* spp. (>1,000 propagules/g of soil) and in which sugar beet was cropped for 2 years (Bandon, County Cork, Ireland). The soil was prepared and maintained as described by Carroll et al. (6). A total of nine sugar beet seeds were sown per pot (7.5 cm in diameter and 8 cm deep) at a depth of 1.5 cm, with the use of sterile forceps. Pots were placed in a growth chamber set at 12°C with 16 h of light; soil-water content was maintained at 32% (wt/wt) by spraying with distilled water three times a week.

The soil was sown for four successive cycles of sugar beet growth, with plants grown for 29 days in each cycle. Inoculated seeds were used only in the first cycle of sugar beet growth, and plants were sampled 1, 6, 13, 23, and 29 days after the seeds were sown. Soil and pots were discarded at each sampling, except for the soil from pots used on day 29. This soil was retained (after removal of roots) for resowing with uninoculated seeds, as previously described (6). Each of the following cycles was sampled only on day 29.

Colony counts were done for the germinating seed (i.e., seed and emerging radicle) on days 1 and 6 in the first cycle and for the whole root system at all subsequent samplings. Plants were shaken to remove loosely adhering soil. Seeds and root systems (with their closely adhering soil) were transferred to 10 ml of one-fourth-strength Ringer's solution in McCartney bottles and were vortexed for 2 min. Serial dilutions of the rhizosphere extracts were plated onto SA agar containing rifampin (100 μg ml^{-1}), AP, and cycloheximide to enumerate strain F113Rif and onto SA agar containing KM, AP, and cycloheximide to enumerate reporter mutant strains. Cycloheximide (100 μg ml^{-1}) was included in plates to inhibit fungal growth, and AP was included to improve selectivity of the media since derivatives of *P. fluorescens* F113 are naturally resistant to this antibiotic. To confirm that the *P. fluorescens* strains recovered on selective media were the same as those inoculated onto sugar beet seeds at the beginning of the colonization assay, arbitrarily primed PCR analysis was done with primer PC1 (5′ GCGCAGATCTAGCGCCTCGCCGCCGAAC 3′), as described by Moënne-Loccoz et al. (19).

Statistics. A randomized block design was used for the soil microcosm experiment. Treatments were studied in triplicate, and each replicate consisted of one plant per treatment at each sampling time. The experiment was carried out once. Since bacterial populations are log normally distributed in the rhizosphere (16), colony counts were $\log_{10}$ transformed prior to analysis. An arbitrary log value of zero was used for samples with population counts which were below the detection limit (15). Homogeneity of variances was expected based on typical fluctuations of data at each sampling time and was assessed by using Hartley's F_{max} and Cochran's *C*.

First, analysis of variance was carried out at each sampling time with the general linear model procedures of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Where appropriate, treatments were compared by using the Student-Newman-Keuls multiple-range test. Second, multivariate analyses of variance (MANOVA) with Wilks' lambda were conducted to compare each reporter mutant with F113Rif throughout several consecutive samplings, by using results from each sampling as a separate variable (6). MANOVA were done with SYSTAT (Systat Inc., Evanston, Ill.). All analyses were done at a *P* value of 0.05.

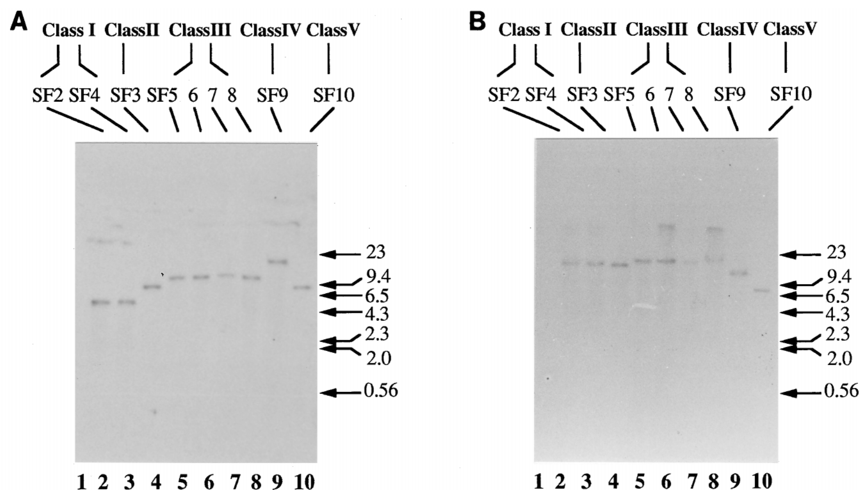

FIG. 1. Southern hybridization of genomic DNA from *P. fluorescens* F113 reporter mutants probed with the 4.0-kb *Sma*I fragment of mini-Tn*5*::*lacZ1*. (A) Genomic DNA digested with *Bam*HI; (B) genomic DNA digested with *Bgl*II. Lanes: 1, λ *Hin*dIII; 2, SF2; 3, SF4; 4, SF3; 5, SF5; 6, SF6; 7, SF7; 8, SF8; 9, SF9; 10, SF10. Size markers in kilobases are indicated on the right.

RESULTS

Identification and classification of reporter mutants. Nine reporter mutants, designated SF2 to SF10, which exhibited reduced levels of expression of the *lacZ* reporter gene in the presence of *P. ultimum* were identified. Southern hybridization analysis was used to determine how many different gene clusters with differential expression in the presence of *P. ultimum* had been isolated. Five classes of mutants were identified, each of which had mini-Tn*5*::*lacZ1* located on the same DNA fragment (Fig. 1). Class 1 contained SF2 and SF4; class 2 contained SF3; class 3 contained SF5, SF6, SF7, and SF8; class 4 contained SF9; and class 5 contained SF10. One representative from each of the mutant classes was chosen for further analysis.

β-Galactosidase activity of reporter mutants. To determine the level of repression exerted by *P. ultimum* on the *lacZ* gene fusions, β-galactosidase activities were examined. Following overnight growth on sucrose asparagine medium, reporter mutants were inoculated into fresh sucrose asparagine medium containing *P. ultimum*-spent medium and grown to an optical density at 600 nm of 0.3. β-Galactosidase activities of reporter mutants grown in *P. ultimum*-spent medium compared to those in medium devoid of *Pythium* spp. ranged from 1.6-fold lower for mutant SF5 (8,568 versus 14,078 Miller units) to 5-fold lower for mutant SF10 (1,100 versus 5,250 Miller units). The chemical nature of the factor produced by *P. ultimum* was examined by protease digestion or boiling of *P. ultimum*-spent medium. In both cases repression of gene expression in reporter mutant SF2 was no longer observed, suggesting that the fungal factor is proteinaceous in nature.

Growth properties of reporter mutants. In order to determine whether the mutants were affected in any of the central metabolic pathways, their ability to utilize a range of carbon and nitrogen sources was examined. With the exception of SF10, growth of all reporter mutants on M9 minimal medium plates containing the glycolytic substrates sucrose, fructose, glucose, galactose, gluconate, and mannitol or the gluconeogenic substrates pyruvate, acetate, succinate, citrate, fumarate, malate, and α-ketoglutarate was indistinguishable from that of the wild type. SF10 was unable to grow on any of the individual carbon sources tested when NH_4Cl, $NaNO_3$, or urea was used as the nitrogen source. However, a number of biosynthetically unrelated amino acids used as both carbon and nitrogen source were able to support growth of SF10 (Table 2). Furthermore, SF10 was also able to grow on the previously mentioned glycolytic and gluconeogenic substrates when the growth medium was supplemented with amino acids at concentrations which were not sufficient to support growth on their own. These results indicate that SF10 is defective in ammonia assimilation since it is unable to grow on nitrogen sources which are metabolized to ammonia prior to assimilation, including $NaNO_3$, urea, and serine.

Antifungal properties of reporter mutants. It is possible that *P. ultimum* is capable of repressing the expression of genes involved in the biosynthesis of antifungal metabolites in order to become established in the rhizosphere. However, all reporter mutants produced HCN, protease, fluorescent siderophore, and 2,4-diacetylphloroglucinol and inhibited *P. ultimum* growth on plates.

TABLE 2. Growth of *P. fluorescens* strains on amino acids as a source of carbon and nitrogen

Growth substrate[a]	Growth of *P. fluorescens*[b]		
	F113	SF2–SF9	SF10
Alanine	+	+	−
Valine	+	+	+
Leucine	+	+	+
Isoleucine	+	+	+
Aspartate	+	+	+
Asparagine	+	+	+
Lysine	+	+	−
Threonine	+	+	−
Serine	+	+	−
Glutamate	+	+	+
Arginine	+	+	+
Proline	+	+	−
Glutamine	+	+	+
Phenylalanine	+	+	±
Histidine	+	+	−

[a] Cells were plated on agar medium containing the amino acids indicated as sole source of carbon and nitrogen.
[b] +, good growth; ±, poor growth; −, no growth.

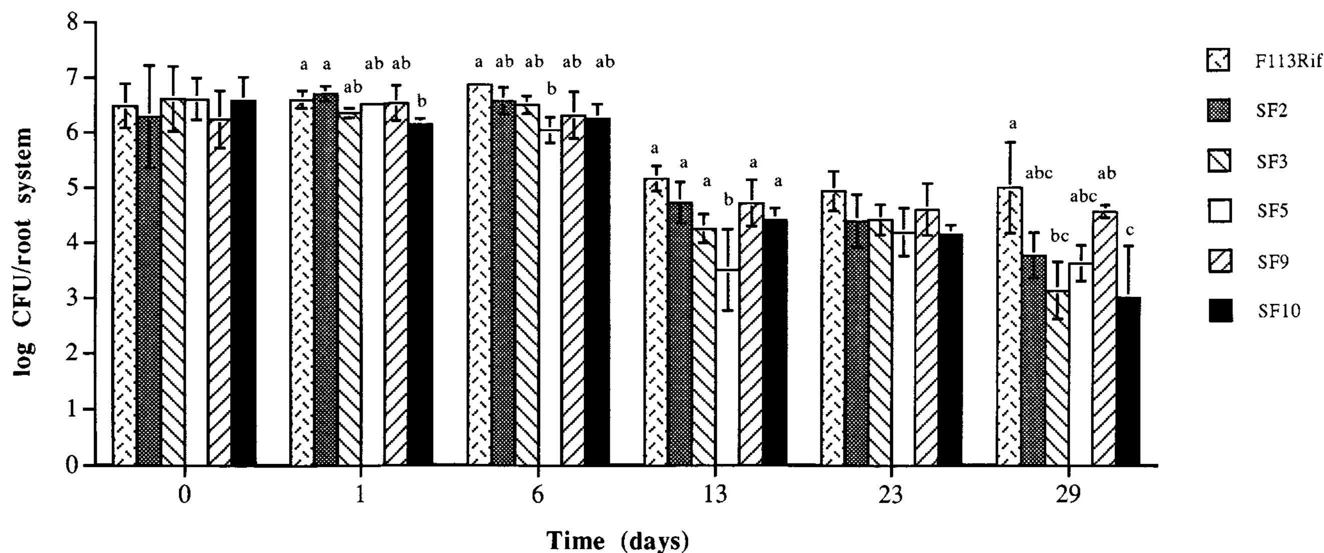

FIG. 2. Population counts of *P. fluorescens* F113Rif and reporter mutants of F113 in the rhizosphere of seed-inoculated sugar beet. Letters are used to indicate statistical relationships where statistical differences were found. Error bars indicate standard deviations.

Colonization of the rhizosphere of sugar beet by F113Rif and reporter mutants introduced as seed inoculants. Strain F113Rif and the reporter mutants were evaluated for their abilities to colonize the rhizosphere of sugar beet, an environment in which interactions between fluorescent pseudomonads and the phytopathogen *P. ultimum* would normally take place. Colony counts of F113Rif stabilized at about 5 log CFU/root system once the seed coat was lost (Fig. 2). Population counts of SF2 and SF9 were identical to those of F113Rif on the basis of statistical analyses carried out at each sampling time and MANOVA performed with data obtained on days 1 to 29 (Table 3). In contrast, colony counts of SF3, SF5, and SF10 were either similar to or lower than those of F113Rif, depending on the sampling time considered (Fig. 2). The results of MANOVA indicated that SF5 and SF10 had population dynamics different from that of F113Rif (Table 3). For reporter mutant SF3 the probability associated with the MANOVA (i.e., 0.079) was only marginally higher than the threshold of 0.05, and a second MANOVA using data for days 13, 23, and 29 indicated that colony counts of SF3 were statistically different from those of F113Rif throughout that part of the experiment (F ratio of 15.6; $P = 0.001$). Arbitrarily primed PCR analysis of the pseudomonad strains recovered from the rhizosphere, on selective medium, showed that they were identical to those inoculated onto the sugar beet seeds at the beginning of the colonization experiment (data not shown). In conclusion, the ability of the reporter mutants to colonize the rhizosphere of seed-inoculated sugar beet was similar to (SF2 and SF9) or lower than (SF3, SF5, and SF10) that of F113Rif.

Colonization of the rhizosphere of uninoculated sugar beet by F113Rif and reporter mutants of F113 present in soil. After plants were harvested on day 29, roots were removed from the soil before it was resown with uninoculated sugar beet seeds. Untreated sugar beet seeds were resown three times in the same soil to study the effect of competition from the resident soil microbiota on reporter mutant colonization of the sugar beet rhizosphere.

F113Rif was capable of recolonizing the rhizosphere at levels higher than 4 log CFU/root system, even after three cycles of growth of sugar beet without reinoculation. In contrast, population levels of reporter mutants SF2 and SF9 in the last two and three cycles, respectively, were lower than those of F113Rif (Fig. 3). The reduced ecological fitness of the two mutants was also obvious from MANOVA of data from all samplings on day 29 (Table 3). Reporter mutants SF3, SF5, and SF10 were more severely compromised in their abilities to recolonize the sugar beet rhizosphere. The levels of SF3 and SF10 were below the detection limit by the end of the first growth cycle, and SF5 was below the detection limit by the end of the second growth cycle of uninoculated sugar beet (Fig. 3). In conclusion, these data demonstrate that all five reporter mutants of F113 compared with F113Rif had lower levels of ecological competence in the sugar beet rhizosphere, indicating that the affected genes play an important role in rhizosphere survival.

DISCUSSION

In order to investigate whether *P. ultimum* was able to affect gene expression in *P. fluorescens* F113, a screening procedure was developed to detect pseudomonad genes responsive to signals produced by this fungus. With this screening procedure, nine *P. ultimum*-responsive genes were identified and were

TABLE 3. Results of MANOVA (Wilks' lambda) for colony counts of *P. fluorescens* strains in the sugar beet rhizosphere[a]

Mutant strain	First cycle of sugar beet growth		Day-29 samplings	
	F ratio	$P > F$	F ratio	$P > F$
SF2	1.39	0.249	18.9	<0.001*
SF3	3.32	0.079	74.3	<0.001*
SF5	4.88	0.036*	33.8	<0.001*
SF9	1.11	0.302	11.7	<0.002*
SF10	4.69	0.039*	77.0	<0.001*

[a] Results for F113Rif versus the indicated mutant are given. Colony counts of inoculated strains on days 1, 6, 13, 23 and 29 with seed-inoculated sugar beet (i.e., first cycle of sugar beet growth) were used as variables in the MANOVA. A four-variable MANOVA was done with data from the last sampling (day 29) in each cycle of sugar beet growth (one cycle of seed-inoculated sugar beet and three cycles of uninoculated sugar beet). Asterisks indicate F ratios which were statistically significant at P of 0.05.

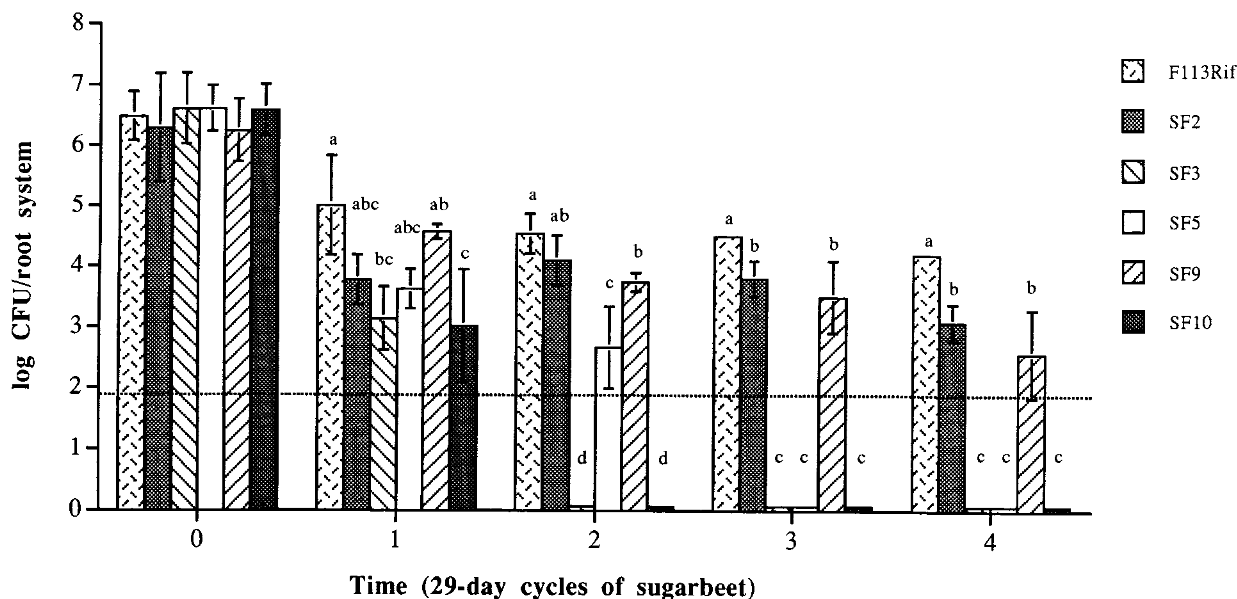

FIG. 3. Population counts of *P. fluorescens* F113Rif and reporter mutants of F113 in the rhizosphere at 29 days after the sowing of inoculated (cycle 1) and uninoculated (cycles 2 to 4) sugar beet. For visual clarity, population counts below the detection limit (indicated with a dotted line) are arbitrarily shown as 0.1 log CFU/root system. Letters are used to indicate statistical relationships where statistical differences were found. Error bars represent standard deviations.

assigned to five classes on the basis of Southern blot analysis. The heat-labile and proteinase-sensitive nature of the response suggests the involvement of a protein or peptide in repression of the *P. fluorescens* gene(s). Current work is aimed at identifying both the proteinaceous compound and the stage of the fungal life cycle at which it is produced.

As a mechanism for survival it may be expected that *P. ultimum* would repress the expression of *P. fluorescens* genes involved in the production of antifungal metabolites; however, the biocontrol traits in each of the mutants were unaltered. This observation may simply reflect the fact that the relevant gene fusions were not picked up in this screen, and repression of biocontrol functions by *P. ultimum* cannot be ruled out until reporter genes fused to promoters that regulate biocontrol functions are examined.

Since the reporter mutants were unaffected in their biocontrol abilities in vitro, they were further characterized by determining their growth requirements. All were identical to the wild type with the exception of SF10, which was unable to grow on a range of carbon sources when nitrogen was supplied as ammonia, nitrate, or urea. However, SF10 was able to grow on these carbon sources when the medium was supplemented with low levels of those amino acids which supported growth as the sole source of carbon and nitrogen. This is unlikely to be due to providing an auxotroph with the appropriate amino acid since SF10 could grow on amino acids belonging to a number of unrelated biosynthetic families as the sole source of carbon and nitrogen. The observation that SF10 could grow on amino acids which are metabolized via tricarboxylic acid cycle intermediates (e.g., glutamine or glutamate) but not on the corresponding carbon skeleton (i.e., α-ketoglutarate) indicates that this mutant is impaired in nitrogen assimilation rather than carbon metabolism. This is also indicated by the inability of SF10 to utilize nitrogen sources which are metabolized via ammonia. Since assimilation of nitrogen is essential for growth, repression by *P. ultimum* of a gene(s) involved in this process is an effective means of inhibiting *P. fluorescens.*

With the exception of SF10, reporter mutants were identical to the wild type with respect to their growth requirements and biocontrol traits in vitro. This raises the possibility that some of the *P. fluorescens* genes repressed by *P. ultimum* are important for adaptation of the pseudomonad to the particular environment in which *P. fluorescens* is detrimental to the fungus. Results from the microcosm experiments showed that all five mutants were compromised in their abilities to colonize the sugar beet rhizosphere. For three of them (SF3, SF5, and SF10), this reduced ecological fitness was already obvious within the first cycle of sugar beet growth. It is of particular interest to note that defects in genes (those repressed in SF3 and SF5) with no apparent involvement in central metabolism or biocontrol traits of *P. fluorescens* F113 in vitro had the same effect on the rhizosphere ecology of *P. fluorescens* as a defect in nitrogen assimilation.

Efficient colonization of the rhizosphere is a prerequisite for effective suppression of fungal root diseases by biocontrol pseudomonads (5, 24). Therefore, the simultaneous repression of genes essential for the ecological competence of *P. fluorescens* in the rhizosphere may be a more effective strategy to overcome the biocontrol activity of this antagonistic rhizobacterium than to repress the expression of individual biocontrol traits. These findings have important implications in terms of biocontrol, and further work in our laboratory will address this issue.

In conclusion, the results from this study demonstrate that a pathogenic fungus can alter gene expression in *P. fluorescens*. This phenomenon may be of considerable importance for fungal bacterial interactions in general and has major implications for the control of gene expression in complex microbial communities. The information gained now allows a detailed characterization of this interspecies signaling to be undertaken.

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