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Role of *ptsP*, *orfT*, and *sss* Recombinase Genes in Root Colonization by *Pseudomonas fluorescens* Q8r1-96[▽]

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Pseudomonas fluorescens **Q8r1-96 produces 2,4-diacetylphloroglucinol (2,4-DAPG), a polyketide antibiotic that suppresses a wide variety of soilborne fungal pathogens, including *Gaeumannomyces graminis* var. *tritici*, which causes take-all disease of wheat. Strain Q8r1-96 is representative of the D-genotype of 2,4-DAPG producers, which are exceptional because of their ability to aggressively colonize and maintain large populations on the roots of host plants, including wheat, pea, and sugar beet. In this study, three genes, an *sss* recombinase gene, *ptsP*, and *orfT*, which are important in the interaction of *Pseudomonas* spp. with various hosts, were investigated to determine their contributions to the unusual colonization properties of strain Q8r1-96. The *sss* recombinase and *ptsP* genes influence global processes, including phenotypic plasticity and organic nitrogen utilization, respectively. The *orfT* gene contributes to the pathogenicity of *Pseudomonas aeruginosa* in plants and animals and is conserved among saprophytic rhizosphere pseudomonads, but its function is unknown. Clones containing these genes were identified in a Q8r1-96 genomic library, sequenced, and used to construct gene replacement mutants of Q8r1-96. Mutants were characterized to determine their 2,4-DAPG production, motility, fluorescence, colony morphology, exoprotease and hydrogen cyanide (HCN) production, carbon and nitrogen utilization, and ability to colonize the rhizosphere of wheat grown in natural soil. The *ptsP* mutant was impaired in wheat root colonization, whereas mutants with mutations in the *sss* recombinase gene and *orfT* were not. However, all three mutants were less competitive than wild-type *P. fluorescens* Q8r1-96 in the wheat rhizosphere when they were introduced into the soil by paired inoculation with the parental strain.**

Interest in biological control continues to increase in response to public concerns about the use of chemical pesticides and recognition of the need for environmentally benign plant disease control strategies (76). Plant growth-promoting rhizobacteria (PGPR) have potential for development as biopesticides, biofertilizers, or phytostimulants, but only a few such products have been marketed, in part because the performance of introduced strains varies among fields and years. Variable root colonization can contribute significantly to this inconsistency because the introduced bacteria must attain threshold population sizes in the rhizosphere in order to be effective. Considerable research during the past 25 years has been directed toward understanding the biotic and abiotic factors that influence root colonization.

Our research has focused on colonization of the wheat rhizosphere by *Pseudomonas fluorescens* Q8r1-96, which produces the polyketide antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG). 2,4-DAPG-producing strains of *P. fluorescens* suppress root and seedling diseases of a variety of crops and play a key role in the natural biological control of take-all disease of wheat known as take-all decline (4, 19, 28, 49, 50, 65, 75). Strain Q8r1-96 is representative of D-genotype 2,4-DAPG producers as defined by restriction fragment length polymorphism analysis of the *phlD* gene (*phlD*$^+$) (39) and by repetitive-sequence-based PCR using the BOXA1R primer (42). D-genotype strains accounted for the majority of *phlD*$^+$ isolates obtained from wheat or pea plants grown in Washington state soils that naturally suppress take-all, caused by *Gaeumannomyces graminis* var. *tritici*, and *Fusarium* wilt of pea, caused by *Fusarium oxysporum* f. sp. *pisi*. Strain Q8r1-96 is exceptional because of its ability to establish and maintain large populations (up to 10^7 CFU/g of root) on the roots of wheat, pea (34, 51), and sugar beet (6) even when low doses are used. This special colonizing ability is typical of all D-genotype strains tested to date (34, 35, 51) and distinguishes these strains from typical rhizosphere pseudomonads.

Root colonization by introduced PGPR is a complex process that includes interactions among the introduced strain, the pathogen, and the indigenous rhizosphere microflora. All of these microorganisms interact with and influence each other in the context of the rhizosphere environment. Bacterial cell surface structures, such as flagella (18), fimbriae (11), and the O antigen of lipopolysaccharide (14), have been shown to influence the attachment of *Pseudomonas* cells to plant roots. Genes responsible for the biosynthesis of amino acids, vitamin B1 (67), a putrescine transport system (32), the NADH dehydrogenase NDH-1 (11), and ColR/ColS, a two-component regulatory system (16), also can influence the efficiency of root colonization. A site-specific *sss* recombinase gene, originally identified in *P. aeruginosa* 7NSK2 as an orthologue of the *Escherichia coli* site-specific recombinase gene *xerC* (26), has a role in root colonization in several strains (1, 15, 61) and even has been proposed as a potential target for improved colonization through genetic engineering (17).

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics[a]	Reference or source
Pseudomonas fluorescens strains		
Q8r1-96	DAPG$^+$ Rif^r	34
Q8r1-96Gm	Q8r1-96 tagged with mini-Tn*7-gfp2*; DAPG$^+$ Rif^r Gm^r	71
Q8r1-96*sss*	*sss*::EZ::TN<Kan2>; DAPG$^+$ Rif^r Kan^r	This study
Q8r1-96*ptsP*	*ptsP*::EZ::TN<Kan2>; DAPG$^+$ Rif^r Kan^r	This study
Q8r1-96*orfT*	*orfT*::EZ::TN<Kan2>; DAPG$^+$ Rif^r Kan^r	This study
Q2-87	DAPG$^+$ Rif^r	74
Escherichia coli strains		
S17-1(λ-pir)	*thi pro hsdM recA rpsL* RP4-2 (Tet^r::Mu) (Kan^r::Tn*7*)	Lab collection
Top 10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lac*ZΔM15 Δ*lacX74 recA1 ara*Δ*139* Δ(*ara-leu*)*7697 galU galK rpsL endA1 nupG*	Invitrogen
Plasmids		
pCPP47	Broad-host-range cosmid derived from pCPP34, tandem *cos*$^+$ par$^+$ Tet^r	3
pMOB3	Kan^r Cam^r *oriT sacB*	62
pNOT19	ColE1 *oriV* Amp^r; accessory plasmid	62
pNOT19-sss-Kan	pNOT19 containing the 2.57-kb DNA SmaI fragment with *sss* interrupted by EZ::TN<Kan2>	This study
pNOT19-ptsP-Kan	pNOT19 containing the 3.8-kb DNA SmaI fragment with *ptsP* interrupted by EZ::TN<Kan2>	This study
pNOT19-orfT-KpnI-BamHI	pNOT19 containing the 1.2-kb fragment of *orfT* flanked by KpnI and BamHI sites	This study
pNOT19-orfT-BamHI-PstI	pNOT19 containing the 1.1-kb fragment of *orfT* flanked by BamH and PstI sites	This study
pNOT19-orfT-Kan	pNOT19 containing the 2.3-kb DNA SmaI fragment with *orfT* interrupted by EZ::TN<Kan2>	This study
pNOT19-sss-Kan-MOB3	pNOT19-sss-Kan ligated with 5.8-kb NotI fragment from pMOB3	This study
pNOT19-ptsP-Kan-MOB3	pNOT19-ptsP-Kan ligated with 5.8-kb NotI fragment from pMOB3	This study
pNOT19-orfT-Kan-MOB3	pNOT19-orfT-Kan ligated with 5.8-kb NotI fragment from pMOB3	This study
pME6010	Broad-host-range plasmid; pVS1 *oriV*, p15a *oriV*, P_k Tet^r	23
pME6010-*ccdB*	Gateway destination vector derived from pME6010 with *ccdB*-Cam^r cassette flanked by *attR1* and *attR2*	This study
pMK2010	Gateway entry vector; ColE1 *oriV*, $oriT_{RP4}$ Kan^r, *ccdB*-Cam^r cassette flanked by *attP1* and *attP2*	27
pMK2010-sss	pMK2010 containing the 0.6-kb DNA fragment with *sss*	This study
pMK2010-ptsP	pMK2010 containing the 2.2-kb DNA fragment with *ptsP*	This study
pMK2010-orfT	pMK2010 containing the 1.0-kb DNA fragment with *orfT*	This study
pME6010*sss*	pME6010 containing the 0.6-kb DNA fragment with *sss*	This study
pME6010*ptsP*	pME6010 containing the 2.2-kb DNA fragment with *ptsP*	This study
pME6010*orfT*	pME6010 containing the 1.0-kb DNA fragment with *orfT*	This study

[a] DAPG$^+$, strain produces 2,4-diacetylphloroglucinol; Rif^r, rifampin resistance; Gm^r, gentamicin resistance; Amp^r, ampicillin resistance; Tet^r, tetracycline resistance; Kan^r, kanamycin resistance; Cam^r, chloramphenicol resistance.

The protein encoded by *sss*, also known as *xerC*, belongs to the λ integrase family and plays a role in DNA rearrangement and phase variation (15, 61).

As one approach to identifying determinants of the unique rhizosphere competence of Q8r1-96 and related D-genotype strains, we hypothesized that these bacteria may interact with their plant hosts more intimately than other commensal pseudomonads interact with their plant hosts and that some of the broadly conserved bacterial genes critical to pathogenicity in such varied hosts as plants and animals (36, 54) may contribute to this unusual ability. Indeed, recent evidence of the presence of type III secretion genes in the PGPR strain *P. fluorescens* SBW25 (55, 56) and the presence of related genes in many other PGPR (41, 48, 58) suggests that such commonalities may occur more frequently than was previously anticipated. In this study, we focused on *ptsP* and *orfT*, two of the genes that contribute to the pathogenicity of *P. aeruginosa* in both plant and animal systems (53, 54) and are highly conserved (levels of identity, more than 70%) in the genomes of saprophytic rhizosphere pseudomonads, and on the *sss* recombinase gene because of its known contribution to the ability of bacteria to adapt to new environments. We identified and characterized the *sss*, *orfT*, and *ptsP* orthologues in *P. fluorescens* Q8r1-96, generated mutants with mutations in each gene, and determined the contributions of the genes to rhizosphere competence and strain competitiveness in the wheat rhizosphere.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were routinely grown at 37°C in Luria-Bertani (LB) medium supplemented with standard concentrations of appropriate antibiotics (2). *P. fluorescens* strains were cultured at 28°C in King's medium B (KMB) (30) or pseudomonas agar P (PsP) (Difco Laboratories, Detroit, Mich.). In experiments with *P. fluorescens*, antibiotics were used at the following concentrations: ampicillin, 40 μg ml^{-1}; rifampin, 100 μg ml^{-1}; tetracycline, 10 μg ml^{-1}; gentamicin, 2 μg ml^{-1}; cycloheximide, 100 μg ml^{-1}, chloramphenicol, 13 μg ml^{-1}; and kanamycin, 25 μg ml^{-1}.

Construction of a cosmid library of *P. fluorescens* Q8r1-96. Throughout this study, standard protocols were used for plasmid DNA purification, restriction enzyme digestion, ligation, and *E. coli* transformation (2). Total DNA of wild-type strain Q8r1-96 (51) was purified by using the Marmur procedure (22),

TABLE 2. Oligonucleotides used in this study[a]

Primer	Sequence
SSS_UP	5′ CGT CTT TCG CAC CTG ATG GA 3′
SSS_LOW	5′ CCT GGG ATG GGC ACT GTC A 3′
ORFT_UP	5′ CTA CGT TTA CAA CAG CTG GA 3′
ORFT_LOW	5′ TTC ACC CAG TTC GCT CAG T 3′
ORFT_KpnI	5′ AAG TAG TAC GGG GTG ACC AG 3′
ORFT_Bam1	5′ TTT TGG ATC CAC GAA GGG TTT GCA 3′
ORFT_Bam2	5′ GCT GGA TCC TCA GGA TGC GGT C 3′
ORFT_Pst	5′ GCC CCA GCA ATA CAA ACA AC 3′
ORFT_seq1	5′ GAC GAT TTG TTC CGC GAT GC 3′
ORFT_seq2	5′ TCC AGC CAG CCG CGC ACA C 3′
PTSP3	5′ TCCAACGACCTCACCCAGTA 3′
PTSP4	5′ CGCAGCATCCACTTCACCTT 3′
PTSP5	5′ GGT GGT GAC ATT CAA GCG CGA 3′
PTSP13	5′ GAG TGT GTT CAT CGC CAG CC 3′
KAN_UP	5′ TGG CAA GAT CCT GGT ATC GGT 3′
KAN_LOW	5′ GAA ACA TGG CAA AGG TAG CGT 3′
Cm_UP	5′ ATC CCA ATG GCA TCG TAA AGA 3
Cm_LOW	5′ AAG CAT TCT GCC GAC AT 3′
sssF	5′ GTG CCC TTT GTC AGC CTG ATC CTG TTC G 3′
sssR	5′ AGC TGG GTT CTA TGA TTC GTC GCC CTT GAT GC 3′
sss2F	5′ GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT AGT GCC CTT TGT CAG C 3′
ptsPF	5′ GGA GGC TCT TCA ATG CTC AAT ACG CTG CGC AA 3′
ptsPR	5′ AGC TGG GTT CTA GAG GGT CTT GTT CGA AGC CG 3′
orfTF	5′ GGA GGC TCT TCA ATG CCC GAC CAA GAT GTA CG 3′
orfTR	5′ AGC TGG GTT CTA TGC CGT TGC TCC GGC CGA TT 3′

[a] Oligonucleotides were designed by using the Oligo 6.65 primer analysis software and were constructed in this study.

partially digested with Sau3AI, and size fractionated on a 0.3% agarose gel. The 25- to 35-kb fraction was extracted by using a QIAEX II agarose gel extraction kit (QIAGEN, Santa Clarita, Calif.) and was ligated with vector arms prepared by digesting the broad-host-range cosmid vector pCPP47 (5) with BamHI and ScaI. The ligated DNA was packaged into λ particles with a Gigapack Gold kit (Stratagene, La Jolla, Calif.), transduced into *E. coli* XL1-Blue MR, and selected on LB medium supplemented with tetracycline. Amplified and ordered copies of the genomic library were stored at −80°C in a freezing medium [2.5% (wt/vol) LB broth, 13 mM KH_2PO_4, 36 mM K_2HPO_4, 1.7 mM sodium citrate, 6.8 mM $(NH_4)_2SO_4$, 0.4 mM $MgSO_4$, 10% glycerol].

Screening of the genomic library by hybridization and PCR. The library was arrayed on BrightStar-Plus nylon membranes (7.4 by 11.4 cm; Ambion, Inc., Austin, Tex.) by replicating clones from the glycerol stocks with a 96-pin multiblot replicator (V&P Scientific, Inc., San Diego, Calif.) and a library copier that permits arraying in a 386-sample format. After arraying, the clones were grown overnight at 37°C and lysed in 0.4 M NaOH with subsequent UV cross-linking as described elsewhere (7).

The *sss* and *orfT* hybridization probes were amplified by PCR performed with *Taq* DNA polymerase (Promega, Madison, Wis.) and primers SSS_UP and SSS_LOW and primers ORFT_UP and ORFT_LOW (Table 2), respectively. The primers were developed by using the Oligo 6.65 software (Molecular Biology Insights, West Cascade, Colo.), based on the *sss* recombinase sequence from *P. fluorescens* F113 (GenBank accession number AF416734) and the *orfT* sequence from *P. fluorescens* SBW25 (http://www.sanger.ac.uk/Projects/P-fluorescens). The cycling program with the *sss* primers included a 1.5-min initial denaturation at 94°C, followed by 30 cycles of 94°C for 30 s, 61°C for 20 s, and 72°C for 1.2 min and a final extension at 68°C for 5 min. PCR amplification with the *orfT* primers was performed similarly except that the annealing step was at 62°C for 30 s and extension was at 72°C for 1 min.

The PCR products were labeled with [α-^{32}P] dATP using the *redi*prime II random primer labeling system (Amersham Pharmacia Biotech. Inc., Piscataway, N.J.) and were purified with a QIAGEN nucleotide removal kit (QIAGEN). The membranes on which the library was arrayed were prehybridized for 2 h at 60°C in a solution containing 3× SSC (60), 4× Denhardt's solution (60), 0.1% sodium dodecyl sulfate (SDS), and 300 μg per ml of denatured salmon sperm DNA (Sigma). Prehybridized membranes were incubated overnight with ~1×10^6 cpm of ^{32}P-labeled probe under the same conditions and were washed with 2× SSC–0.1% SDS at room temperature (twice), 0.2× SSC–0.1% SDS at room temperature (twice), 0.2× SSC–0.1% SDS at 60°C (twice), and 0.1× SSC–0.1% SDS at 60°C (once). The conditions used for Southern hybridization with the *orfT* probe were the conditions described above, except that 4× SSC was used for prehybridization and high-stringency washes were performed at 58°C.

Clones containing *ptsP* were identified by PCR performed with primers PTSP3 and PTSP4 (Table 2), which were developed from the genome sequence of *P. fluorescens* SBW25. The library was divided into "primary" pools (i.e., pooled clones from each 96-well plate) that were further subdivided into "secondary" pools (containing clones from each 96-well plate pooled by rows or by columns). The purified cosmid DNA from "primary" and then "secondary" pools was screened by PCR using a program that included 1 min of initial denaturation at 94°C, followed by 30 cycles of 94°C for 25 s, 60°C for 15 s, and 72°C for 20 s.

Shotgun sequencing and sequence analysis. Selected cosmid clones carrying *sss*, *ptsP*, or *orfT* were sequenced by using the EZ::TN<Kan-2> transposition system (Epicenter Technologies, Madison, Wis.). Transposition reactions were performed in vitro according to the manufacturer's recommendations, and cosmids bearing insertions in the gene of interest were shotgun sequenced with transposon-specific primers by using an ABI PRISM BigDye Terminator v.3.0 Ready Reaction cycle sequencing kit (Applied Biosystems, Foster City, Calif.). The sequence data were compiled with the Vector NTI software (Invitrogen Corp., Carlsbad, Calif.) and were analyzed with the OMIGA 2.0 software (Accelrys, San Diego, Calif.). Database searches for similar protein sequences and protein motifs and domains were performed by using NCBI's BLAST network service (http://www.ncbi.nlm.nih.gov/BLAST) and the MyHits Internet engine (46).

Allelic replacement in Q8r1-96. A spontaneous rifampin-resistant derivative of Q8r1-96 (34) was used for gene replacement mutagenesis. To construct an *sss* mutant, the *sss* gene interrupted by EZ::TN<Kan-2> was amplified with primers SSS_UP and SSS_LOW by using KOD Hot Start DNA polymerase. The cycling program included 2 min of initial denaturation at 94°C, followed by 30 cycles of 94°C for 15 s, 61°C for 30 s, and 68°C for 1.2 min and a final extension at 68°C for 5 min. The amplification product was cloned into the SmaI site of the gene replacement vector pNOT19 (62). The resultant Kan^r plasmids were digested with NotI and ligated into a 5-kb fragment carrying a pMOB3 cassette (62) linearized with NotI and containing the *sacB* and *cat* genes. The resultant plasmid was electroporated into *E. coli* S17-1(λ-pir), selected on LB medium supplemented with chloramphenicol and kanamycin, and mobilized from *E. coli* S17-(λ-pir) into *P. fluorescens* Q8r1-96 by using a biparental mating technique. Mutant clones were first selected on LB medium supplemented with rifampin, kanamycin, and 5% sucrose. Sucrose- and kanamycin-resistant clones were screened for the absence of plasmid-borne *sacB*, *bla*, and *cat* genes by PCR performed with primers SAC1 and SAC2 (38), primers BLA1 and BLA2 (38), and primers Cm_UP and Cm_LOW (Table 2), respectively. Primers KAN_UP and KAN_LOW (Table 2) were used to detect the presence of a kanamycin resistance gene in *sss* recombinase mutants, which also were screened by PCR performed with primers SSS_UP and SSS_LOW to confirm the absence of the wild-type *sss* allele.

A similar strategy was used to construct a *ptsP* mutant. Briefly, the full-length *ptsP* gene was amplified from strain Q8r1-96 with primers PTSP5 and PTSP13 (Table 2) and Expand Long PCR polymerase (Roche Applied Science, Indianapolis, Ind.). The cycling program consisted of 2 min of initial denaturation at 94°C, followed by 30 cycles of 94°C for 10 s, 60°C for 30 s, and 68°C for 3.5 min and a final extension at 68°C for 5 min. The amplification product was treated with T4 DNA polymerase, cloned into the SmaI site of pNOT19, mutagenized in vitro with EZ::TN<Kan-2>, digested with NotI, and ligated with a 5-kb fragment carrying a pMOB3 cassette (62) as described above. The resultant plasmids were electroporated into *E. coli* S17-1(λ-pir) and used for gene replacement as described above, except that the mutants were screened by PCR performed with primers PTSP5 and PTSP13 to confirm the absence of the wild-type allele.

To construct the *orfT* mutant, the 1,386-bp 5′ part of *orfT* was amplified by using KOD Hot Start DNA polymerase and oligonucleotides ORFT_KpnI and ORFT_Bam1 (Table 2). The cycling program included 2 min of initial denaturation at 94°C, followed by 35 cycles of 94°C for 15 s, 65°C for 30 s, and 68°C for 1 min and a final extension at 68°C for 5 min. The PCR product was digested with KpnI and BamHI, gel purified, and cloned into pNOT19. Next, a 1,246-bp fragment containing the 3′ part of *orfT* was amplified with primers ORFT_Pst and ORFT_Bam2 (Table 2) by using the same cycling program. The PCR product was digested with BamHI and PstI, gel purified, and cloned into pNOT19 containing the 5′ end of the *orfT* gene. These manipulations resulted in

introduction of a unique BamHI site in *orfT*. This site was then used to insert the kanamycin resistance gene from EZ::TN<Kan-2>, yielding pNOT19-orfT-Kan. The resultant plasmids were digested with NotI, ligated with a 5-kb fragment of pMOB3 containing the *sacB* and *cat* genes, and electroporated into *E. coli* S17-1(λ-pir). Gene replacement mutagenesis was carried out essentially as described above, except that the mutants were screened by PCR performed with primers ORFT_seq1 and ORFT_seq2 to confirm the absence of the wild-type *orfT* allele. All mutant clones of each of the three genes were isogenic, and only one mutant clone was used for further experiments.

Construction of complemented mutants. Full-length copies of *sss*, *ptsP*, and *orfT* were cloned into the stable broad-host-range plasmid vector pME6010 (23) by using Gateway Technology (Invitrogen). First, the genes were amplified by using a nested PCR protocol described by House et al. (27). Briefly, *sss*, *ptsP*, and *orfT* were amplified with gene-specific primers sssF and sssR, primers ptsPF and ptsPR, and primers orfTF and orfTR, respectively, and then *attB* sequences were introduced by reamplification with the secondary primers 2F and 2R (27) (or primers sss2F and 2R in the case of *sss*) (Table 2). All amplifications were carried out with KOD Hot Start DNA polymerase. The PCR products were then cloned into the entry plasmid pMK2010 (27) by using BP Clonase II (Invitrogen), sequenced to confirm gene integrity, and transferred with LR Clonase II (Invitrogen) to the destination vector pME6010 containing *ccdB* and *attR* cassettes (27). The resultant plasmids were electroporated (20) into Q8r1-96*sss*, Q8r1-96*ptsP*, or Q8r1-96*orfT* with a Gene Pulser II (Bio-Rad Laboratories, Hercules, Calif.).

Phenotypic characterization in vitro. Polysaccharide production was scored visually after 3 days of growth on PsP by using a scale from 0 to 5, where 0 indicated a nonmucoid isolate and 5 indicated a moderately mucoid culture. Siderophore production was determined by measuring orange halos after 2 days of growth at 28°C on CAS agar plates (63). Exoprotease activity was detected by spotting 5 μl of an exponentially growing culture whose optical density at 600 nm (OD_{600}) was adjusted to 0.1 on skim milk agar (59); a clearing zone surrounding the bacterial growth was measured after incubation for 48 and 72 h at 28°C. Production of hydrogen cyanide was monitored daily by using cyanide detection paper placed on petri dish lids for cultures grown on KMB agar amended with 0.44% glycine for 4 days at 28°C (3). All experiments described above were repeated twice with six replicates. Motility assays were performed on LB medium solidified with 0.3%, 0.5%, 1.0%, or 1.5% agar. Plates were inoculated with 5 μl of logarithmically growing bacterial cultures whose OD_{600} was adjusted to 0.1 and were incubated right side up at 28°C, and the diameter of outward expansion was measured after 24, 48, and 72 h. Experiments were repeated twice with four replicates per strain. Inhibition of *G. graminis* var. *tritici* by *P. fluorescens* Q8r1-96Gm and mutants of this strain was assayed on KMB agar as described previously (44). Carbon and nitrogen utilization profiles were generated by using Biolog SF-N2 and PM3 MicroPlates (Biolog, Inc., Hayward, Calif.), respectively, with 96 substrates each. Four independent repetitions were performed with each strain (40). Biolog assays were validated by using M9 minimal media supplemented with 0.4% D-galactose as a carbon source or with nitrogen sources at a concentration of 10 mM. Phloroglucinol compounds were extracted with ethyl acetate from bacterial cultures grown for 48 h at 27°C in KMB broth. The extracts were fractionated on a Waters NOVA-PAK C_{18} Radial-PAK cartridge (4 μm; 8 by 100 mm; Waters Corp., Milford, Mass.) as described previously (9). Two independent experiments with five replications were performed.

Rhizosphere colonization assays. Rhizosphere colonization assays were performed with the *sss* recombinase, *ptsP*, and *orfT* mutants and Q8r1-96Gm, a gentamicin-resistant derivative of the parental strain tagged with mini-Tn*7-gfp2* (71) to distinguish it from mutant strains in mixed-inoculation studies. Bacterial inocula were prepared and added to Quincy virgin soil as previously described (33) to obtain $\sim 1 \times 10^4$ CFU g^{-1} of soil and $\sim 0.5 \times 10^4$ CFU of each strain g^{-1} of soil (1:1 ratio) for single and mixed inoculations, respectively. The actual density of each strain was determined by assaying 0.5 g of inoculated soil as described by Landa et al. (33), and the control treatments consisted of soil amended with a 1% methylcellulose suspension. Experiments were repeated twice with six replicates per treatment. Spring wheat (*Triticum aestivum* L. cv. Penawawa) seeds were pregerminated on moistened sterile filter paper in petri dishes for 24 h in the dark and were sown in square pots (height, 6.5 cm; width, 7 cm) containing 200 g of Quincy virgin soil (33) inoculated with one or two bacterial strains. Wheat was grown for six successive cycles in a controlled-environment chamber at 15°C with a 12-h photoperiod. After 2 weeks of growth (one cycle), population densities of bacteria were determined as described by Mavrodi et al. (40). In order to determine the persistence of the mutants in the soil, the soil from pots that received the same treatment was decanted into plastic bags after the sixth cycle and stored at 20°C for 10 weeks before spring wheat was planted again. Plants were grown in a growth chamber for two successive 2-week cycles in the same controlled environment, processed, and analyzed as described below.

Population densities of the introduced strains were monitored by the modified dilution-endpoint method (43, 71). Briefly, individual wheat root systems were placed in 10 ml of sterile distilled water, vortexed, and sonicated. The soil suspensions were serially diluted in 96-well microtiter plates containing 1/3× KMB broth supplemented with rifampin, cycloheximide, ampicillin, and chloramphenicol and incubated for 72 h at room temperature (an OD_{600} of ≥0.07 was scored as positive) (43). After 3 days, the cultures were replicated into fresh 96-well plates containing KMB broth amended with kanamycin or gentamicin to distinguish between strains in mixed inoculations. Population densities of total culturable heterotrophic bacteria were determined by performing the same assay in 0.1× tryptic soy broth supplemented with cycloheximide (43).

Data analysis. All treatments in competitive colonization experiments were arranged in a complete randomized design. Statistical analyses were performed by using appropriate parametric and nonparametric procedures with the STATISTIX 8.0 software (Analytical Software, St. Paul, Minn.). All population data were converted to log CFU g^{-1} of soil or log CFU g^{-1} (fresh weight) of root. Differences in population densities among treatments were determined by standard analysis of variance, and mean comparisons among treatments were performed by using Fisher's protected least-significant-difference test ($P = 0.05$) or the Kruskal-Wallis all-pairwise comparison test ($P = 0.05$). The area under the colonization progress curve (AUCPC), which represented the total rhizosphere colonization for all six cycles (34), was calculated with SigmaPlot V. 8.0 (SYSTAT Software Inc., Richmond, Calif.). Data from phenotypic assays were compared by using a two-sample *t* test or the Wilcoxon rank sum test ($P = 0.05$).

Nucleotide sequence accession numbers. The *sss*, *ptsP*, and *orfT* sequences of *P. fluorescens* Q8r1-96 have been deposited in the GenBank nucleotide sequence database under accession numbers AY172655, AY816321, and AY816322, respectively.

RESULTS

Identification, cloning, and characterization of *sss*, *orfT*, and *ptsP*. Initial screening of the Q8r1-96 library by colony hybridization or, in the case of *ptsP*, by PCR yielded eight *sss*-positive clones, eight *orfT*-positive clones, and one *ptsP*-positive clone, all of which were further mapped by Southern hybridization. Briefly, purified cosmid DNA from each clone was digested with EcoRI, KpnI, and SacI, resolved on an agarose gel, blotted, and hybridized with the corresponding biotin-labeled probe. One clone containing each gene of interest then was selected for EZ::TN<Kan-2>-mediated DNA sequencing.

Included in *sss*-positive cosmid clone 7D10 was a 1,851-bp contig containing a predicted open reading frame with similarity to genes encoding numerous bacterial and phage site-specific recombinases (Fig. 1A). The *sss* gene encodes a predicted 299-amino-acid protein with a molecular mass of 33,772 Da and is flanked by two open reading frames coding for a conserved hypothetical protein and a putative hydrolase, YigB.

The deduced Sss protein is highly similar to putative recombinases from *P. fluorescens* WCS365 (NCBI accession number CAA72946; 100% identity), *P. fluorescens* Pf-5 (NCBI accession number AAY95204; 88% identity), *Pseudomonas putida* KT2440 (NCBI accession number NP_747331; 76% identity), and *P. aeruginosa* PAO1 (NCBI accession number Q51566; 71% identity). Further analyses revealed the presence of a XerC profile listed in the HAMAP database of orthologous microbial protein families (21) and two Pfam domains (residues 5 to 89 and 111 to 282) associated with phage integrases. Searches against the Cluster of Orthologous Groups of Proteins (COG) database (http://www.ncbi.nlm.nih.gov/COG) indicated that the protein belongs to COG4973 and COG0582, which contain XerC-like site-specific recombinases (E value, $3e^{-94}$). Finally, the protein structure predicted by the fold

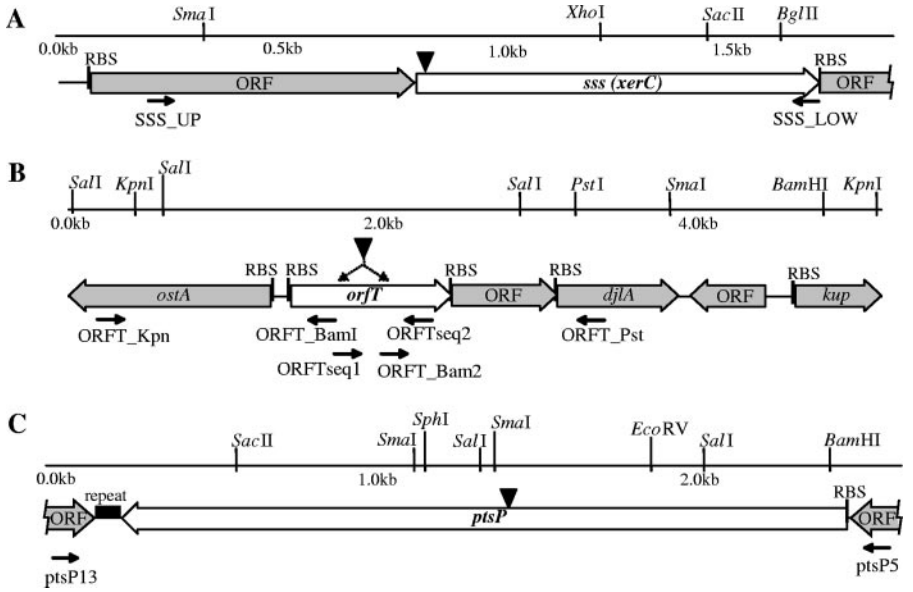

FIG. 1. Restriction maps and locations of individual genes in regions of the *P. fluorescens* Q8r1-96 genome containing *sss* (A), *orfT* (B), and *ptsP* (C). Inverted solid triangles indicate the positions of EZ::TN<Kan-2> insertions, and small horizontal arrows indicate PCR primers used in this study. The shaded arrows indicate the positions of genes and open reading frames (ORF) that were not relevant in the present study. RBS, ribosome-binding site.

recognition server 3D-PSSM (29) closely resembled that of the site-specific XerD recombinase from *E. coli* (68).

Shotgun sequencing of cosmid clone 7D2, which hybridized to the *orfT* probe, revealed a 5,176-bp contig (Fig. 1B) that included a predicted open reading frame with similarity to Orf338 from *P. aeruginosa* PA14 (69). The gene, referred to here as *orfT*, is preceded by a putative ribosome-binding site, GGAGA, and encodes a predicted 341-amino-acid protein with a molecular mass of 38,655 Da. The contig contains a number of other open reading frames, two of which are located immediately downstream of *orfT* and probably are cotranscribed with it. These genes encode a conserved hypothetical protein and a putative DnaJ-like protein.

Database searches revealed that *orfT* is highly conserved in sequenced bacterial genomes, and a blastp search against the nonredundant GenBank data set returned more than 40 hits with E values less than $1e^{-50}$. The deduced OrfT protein is most similar to its counterparts from *P. fluorescens* Pf-5 (NCBI accession number AAY94838; 84% identity), *Pseudomonas syringae* pv. syringae DC3000 (NCBI accession number AAO54097; 82% identity), *P. putida* KT2440 (NCBI accession number NP_742571; 74% identity), and *P. aeruginosa* PA14 (NCBI accession number AAD22455; 67% identity). OrfT is a member of COG3178, which contains predicted phosphotransferases (E value, $1e^{-90}$), and further analyses revealed the presence of a Pfam domain associated with the aminoglycoside phosphotransferase enzyme family (residues 152 to 234). The OrfT structure predicted by 3D-PSSM (29) is related to the structures of aminoglycoside 3′-phosphotransferases from *Enterococcus faecalis* (10) and *Klebsiella pneumoniae* (45).

ptsP-positive cosmid clone 5F9 contained a 2,586-bp contig that included a predicted open reading frame with similarity to numerous bacterial *pstP* genes (Fig. 1C). The *ptsP* gene encodes a predicted 759-amino-acid protein with a molecular mass of 83,208 Da and is flanked in Q8r1-96 by a well-conserved ribosome-binding site, GGAG, and a putative transcriptional terminator comprised of a 93-bp region with imperfect dyad symmetry.

The deduced PtsP protein is highly similar to its orthologues from *P. fluorescens* Pf-5 (NCBI accession number AAY95089; 96% identity), *P. syringae* pv. syringae DC3000 (NCBI accession number AAO58710; 92% identity), *Azotobacter vinelandii* (NCBI accession number CAA74995; 87% identity), and *P. aeruginosa* PAO1 (NCBI accession number NP_249028; 86% identity). The results of domain searches revealed a conserved PROSITE phosphoenolpyruvate (PEP)-utilizing enzyme signature (residues 620 to 638) and Pfam GAF (residues 17 to 154), N-terminal (residues 178 to 302), mobile (residues 318 to 399), and TIM barrel (residues 424 to 715) domains associated with PEP-utilizing enzymes. Finally, searches against the COG database indicated that the protein belongs to COG3605 containing bacterial PtsP proteins (E value, 0.0), and the protein structure predicted by 3D-PSSM (29) was related to the structures of pyruvate phosphate dikinases from *Clostridium symbiosum* (24) and *Trypanosoma brucei* (13).

Phenotypic characteristics of the *sss*, *ptsP*, and *orfT* mutations. The motilities of wild-type Q8r1-96 and *sss*, *ptsP*, and *orfT* mutants of this strain were compared on LB medium solidified with different agar concentrations. The motility of the wild-type strain was significantly ($P = 0.05$) greater than the motilities of the *ptsP* and *orfT* mutants on 0.3% agar (Table 3). In contrast, the motility of the *sss* mutant did not differ from that of Q8r1-96. When higher concentrations of agar were used, neither the mutants nor the wild type showed the ability to swarm.

The siderophore excretion detected on CAS agar plates was significantly greater for the *ptsP* mutant, not altered for the *orfT* mutant, and significantly lower for the *sss* mutant com-

TABLE 3. Phenotypic effects of *sss*, *ptsP*, and *orfT* mutations in *P. fluorescens* Q8r1-96

Strain	Siderophore production[a]	Exoprotease production after[b]:		Motility after[c]:			MAPG production[d]	2,4-DAPG production[d]	Total production of phloroglucinal-related compounds[d]
		48 h	72 h	24 h	48 h	72 h			
Q8r1-96	8.5 a	6.7 a	9.8 a	20.2 a	32.3 a	38.7 a	2.1×10^6 a (100)	9.4×10^6 a (100)	12.0×10^6 b (100)
Q8r1-96*sss*	7.4 b	7.5 b	11.2 b	19.2 a	30.8 a	36.5 a	2.8×10^6 a (132)	12.7×10^6 b (134)	16.1×10^6 a (133)
Q8r1-96*ptsP*	11.4 b	4.5 b	8.7 b	17 b	29.6 b	35.1 b	1.1×10^6 b[e] (54)	5.7×10^6 b (60)	7.6×10^6 a[e] (63)
Q8r1-96*orfT*	8.0 a	6.8 a	10.7 a	17.2 b	27.8 b	32.8 b	2.5×10^6 a (120)	11.5×10^6 a (122)	14.6×10^6 a (121)

[a] Siderophore production was determined by measuring orange halos (in millimeters) after 2 days of growth at 28°C on CAS agar plates. The values are means for four replicate plates. Values followed by the same letter are not significantly different as determined by a two-sample *t* test in which each mutant was compared separately to the wild type.

[b] Zone of casein digestion on milk agar plates (in millimeters) after 48 and 72 h of bacterial growth. The values are means for three replicate plates. Values followed by the same letter are not significantly different as determined by a two-sample *t* test in which each mutant was compared separately to the wild type.

[c] Diameter of bacterial spread (in millimeters) on 0.3% LB agar. The values are means for six replicate plates after 24, 48, and 72 h of bacterial growth. Values followed by the same letter are not significantly different as determined by a two-sample *t* test in which each mutant was compared separately to the wild type.

[d] Production expressed as peak area/optical density. The values are means for five replicate extractions. Values followed by the same letter are not significantly different as determined by a two-sample *t* test (unless indicated otherwise) in which each mutant was compared separately to the wild type. The values in parentheses are percentages of production relative to production by Q8r1-96, which was set at 100%.

[e] Significance was determined by the Wilcoxon rank sum test ($\alpha = 0.05$).

pared with the siderophore excretion detected for the parental strain (Table 3). Differences in colony morphology were observed on some media. The *sss* and *orfT* mutants were more mucoid on PsP than the parental strain, and the *ptsP* mutant was less mucoid. Isolated colonies of the *ptsP* mutant, but not isolated colonies of the *sss* and *orfT* mutants, appeared to be more yellow than those of the parental strain when they were grown on LB medium supplemented with glucose. The extracellular protease activity was significantly greater for the *sss* mutant, not altered for the *orfT* mutant, and significantly lower

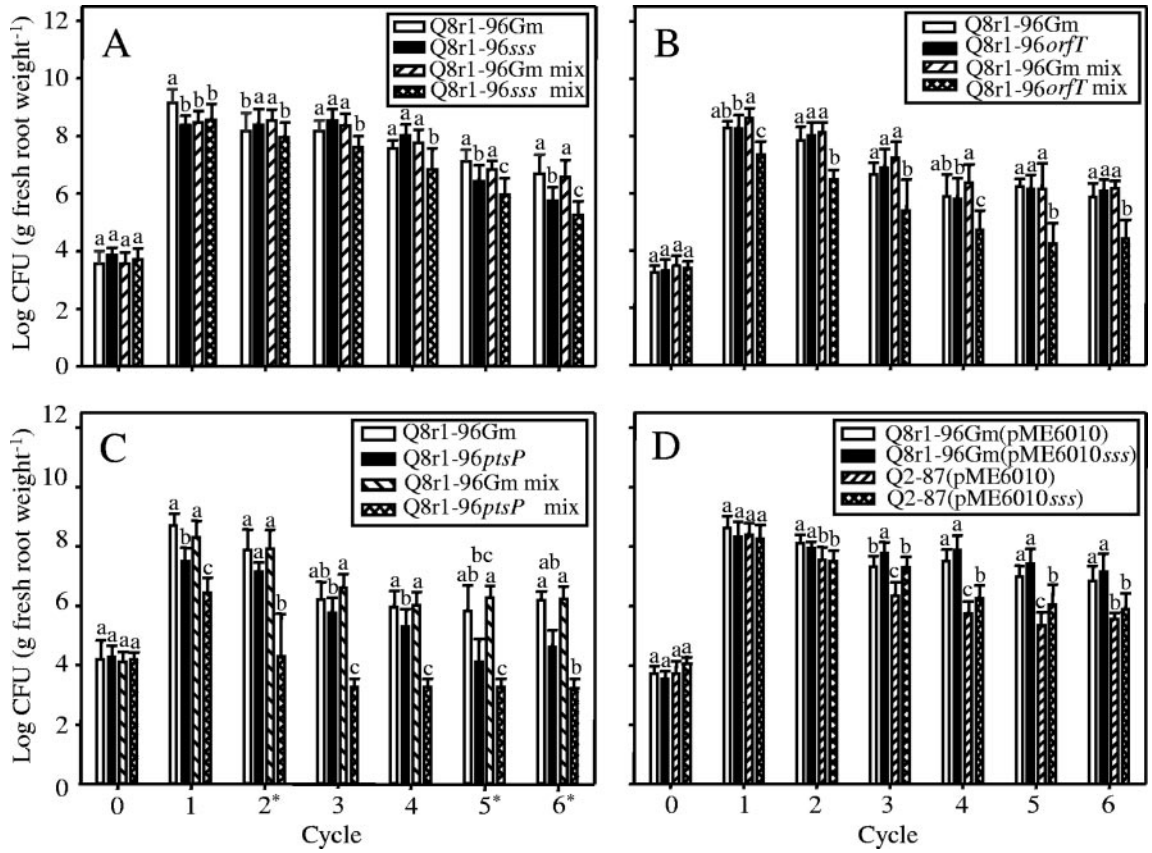

FIG. 2. Population dynamics of *Pseudomonas* strain Q8r1-96Gm, *sss* (A), *orfT* (B), and *ptsP* (C) mutants, and strains Q8r1-96Gm and Q2-87 bearing pME6010 or pME6010*sss* (D) on the roots of wheat cv. Penawawa grown in Quincy virgin soil for six consecutive 2-week cycles as described in Materials and Methods. Each strain was introduced into the soil at a final density of approximately log 4 CFU per g of soil (cycle 0) in single inoculations and approximately 0.5×10^4 CFU per g of soil in mixed inoculations. The bars indicate means, and the error bars indicate standard deviations. The same letter above bars for the same cycle indicates that the means are not significantly different ($P = 0.05$) according to a Fisher's protected least-significant-difference test (unless indicated otherwise). Cycles marked with an asterisk were analyzed by a Kruskal-Wallis test ($P = 0.05$).

TABLE 4. Population densities of indigenous, introduced wild-type, and mutant strains on the roots of wheat grown in Quincy virgin soil[a]

Expt	Strain(s)	Population densities (AUCPC)[b]:			
		Q8r1-96Gm	Mutant	Q8r1-96Gm and mutant (1:1)	Control[c]
1	Indigenous bacteria	8.75 bc	9.02 a	8.92 ab	8.59 c
	Q8r1-96Gm	7.8 a[d] (45.3 a)	ND	7.8 a[d] (45.0 a)	ND
	Q8r1-96*sss*	ND	7.6 a[d] (44.5 a)	7.0 b[d](41.4 b)	ND
2	Indigenous bacteria	8.68 b[d]	8.96 a[d]	8.79 ab[d]	8.66 ab[d]
	Q8r1-96Gm	6.8 a (39.5 b)	ND	7.1 a (41.3 a)	ND
	Q8r1-96*orfT*	ND	6.9 a (39.9 b)	5.4 b (32.0 c)	ND
3	Indigenous bacteria	8.27 b	8.51 a	8.54 a	8.46 ab
	Q8r1-96Gm	6.8 a (39.8 a)	ND	6.9 a (40.3 a)	ND
	Q8r1-96*ptsP*	ND	5.7 b (34.3 b)	4.0 c (24.3 c)	ND

[a] Raw Quincy virgin soil was treated with $\sim 10^4$ CFU per g of soil of Q8r1-96Gm and/or Q8r1-96*sss*, Q8r1-96*orfT*, or Q8r1-96*ptsP*. Mixed-inoculation treatments contained a 1:1 mixture of competing strains ($\sim 0.5 \times 10^4$ CFU per g of soil of each strain). Rhizosphere population densities of bacteria were determined by the terminal dilution endpoint assay as described in Materials and Methods.

[b] The values are mean population densities in log CFU per g (fresh weight) of root for six cycles except cycle 0. Mean population densities in each experiment were analyzed separately. Within each experiment, populations of indigenous bacteria were analyzed separately from populations of introduced wild types and mutants. The values in parentheses are the areas under the rhizosphere colonization progress curves (AUCPC) for introduced bacteria for six cycles. Different letters after values indicate that there is a statistically significant difference as determined by Fisher's protected least-significant-difference test ($P = 0.05$) (unless indicated otherwise). ND, not detected.

[c] Treatment without bacterial inoculation.

[d] The significance of differences between bacterial densities was determined by the Kruskal-Wallis all-pairwise comparison test ($P = 0.05$).

for the *ptsP* mutant compared with the extracellular protease activity of wild-type strain Q8r1-96 (Table 3). None of the mutants was impaired for hydrogen cyanide (HCN) production.

The growth kinetics of the *sss*, *ptsP*, and *orfT* mutants in 1/3× KMB and M9 media supplemented with glycerol were indistinguishable from those of wild-type strain Q8r1-96. Likewise, the carbon and nitrogen substrate utilization profiles of the *sss* and *orfT* mutants did not differ from those of Q8r1-96 on the 96 substrates contained in Biolog SF-N2 and PM3 microplates. In contrast, the *ptsP* mutant grew more slowly on D-galactose as a source of carbon and on L-cysteine as a source of nitrogen, both in the Biolog assays and in appropriately supplemented cultures grown in M9 medium.

Phloroglucinol production and fungal inhibition in vitro. *P. fluorescens* Q8r1-96 and the *sss*, *orfT*, and *ptsP* mutants all produced detectable quantities of 2,4-DAPG, as well as monoacetylphloroglucinol (MAPG) and three other uncharacterized phloroglucinol-related compounds (Table 3). The *sss* and *orfT* mutants produced larger amounts of 2,4-DAPG and total phloroglucinol-related compounds than wild-type strain Q8r1-96 produced (for the *orfT* mutant only production of total phloroglucinol-related compounds was significantly greater [$P = 0.05$]), whereas the *ptsP* mutant produced significantly less MAPG and 2,4-DAPG. The reduced phloroglucinol production by the *ptsP* mutant was correlated with the diminished ability of this mutant to inhibit growth of *G. graminis* var. *tritici* in vitro; the hyphal inhibition indices (ratios of the distance between the edge of the bacterial colony and the fungal mat to the distance between the edge of the colony and the center of the mat) for this mutant and the parental strain were 0.0 and 0.16, respectively, after 6 days. In contrast, overproduction of 2,4-DAPG by the *sss* and *orfT* mutants was not correlated with increased fungal inhibition, probably due to differences in the relative amounts of phloroglucinol compounds accumulated during the different growth regimens. The hyphal inhibition indices for the *sss* and *orfT* mutants at 6 days postinoculation were 0.12 and 0.08, respectively.

Impact of the *sss*, *ptsP*, and *orfT* mutations on rhizosphere colonization by Q8r1-96. The impact of the *sss*, *ptsP*, and *orfT* mutations on the rhizosphere competence of Q8r1-96 was assessed by performing competitive wheat root colonization assays under greenhouse conditions. The *sss*, *ptsP*, and *orfT* mutant strains were introduced into raw Quincy virgin soil either individually or, to test their competitiveness with the parental strain, in pairwise combinations (1:1 ratio) with strain Q8r1-96Gm. Figures 2A to C show the population dynamics of the strains over six 2-week growth cycles. In each case, the population densities of the introduced strains at cycle 0, the beginning of the experiments, were equivalent. In both single and mixed inoculations, the population sizes of the wild type and the *sss* recombinase and *orfT* mutant strains increased by 4 orders of magnitude by the end of cycle 1 and then slowly declined over the following six cycles.

The population densities of both Q8r1-96Gm and the *sss* mutant in the wheat rhizosphere fluctuated when the strains were introduced individually. The densities of the two strains were similar in cycles 3 and 4, and the density of the *sss* mutant was less than that of the wild-type strain in cycles 5 and 6 (Fig. 2A). However, the values for the AUCPC and mean colonization (measures of colonization across all cycles) did not differ significantly, indicating that the wild type and the *sss* mutant were equivalent in terms of rhizosphere colonization (Table 4). However, when introduced in combination with the parental strain, the *sss* mutant colonized the wheat rhizosphere significantly less than Q8r1-96Gm colonized the wheat rhizosphere in cycles 2 through 6, and the AUCPC and mean colonization

TABLE 5. Population densities of introduced wild-type and mutant strains in the rhizosphere of wheat sown in soil after 10 weeks of storage[a]

Expt	Strain	Population densities[b]		
		Q8r1-96Gm	Mutant	Q8r1-96Gm and mutant (1:1)
1	Q8r1-96Gm	6.0 a (6.8)	ND	5.8 a (6.6)
	Q8r1-96*sss*	ND	5.5 a (5.7)	4.3 b (5.2)
2	Q8r1-96Gm	6.5 a[c] (5.9)	ND	5.8 b[c] (6.2)
	Q8r1-96*orfT*	ND	5.7 b[c] (6.0)	3.5 c[c] (4.4)
3	Q8r1-96Gm	5.4 a (6.2)	ND	5.7 a (6.2)
	Q8r1-96*ptsP*	ND	3.3 b (4.6)	3.3 b (3.2)

[a] Strain recovery was determined as the mean population density on roots of wheat plants of growth following a 10-week fallow period as described in Materials and Methods.

[b] The values are mean population densities in log CFU per g (fresh weight) of root for two cycles except cycle 0. Mean population densities in each experiment were analyzed separately. The values in parentheses are population densities in log CFU per g (fresh weight) of root after cycle 6 (before soil was stored for 10 weeks). Different letters after values indicate that there is a statistically significant difference as determined by Fisher's protected least-significant-difference test ($P = 0.05$) (unless indicated otherwise). ND, not detected.

[c] The significance of differences between population densities after two cycles was determined by the Kruskal-Wallis all-pairwise comparison test ($P = 0.05$).

values of the mutant were significantly lower than those of the wild type (Table 4).

As observed for the *sss* mutant, the population densities of the *orfT* mutant in single-inoculation treatments fluctuated over the six growth cycles (Fig. 2B). The densities of the two strains did not differ significantly in any of the cycles, and the AUCPC and mean colonization values of the *orfT* mutant did not differ from those of Q8r1-96Gm (Table 4). In contrast, when the strains were introduced together into the soil, Q8r1-96Gm consistently outcompeted the *orfT* mutant. The population size of Q8r1-96Gm was significantly greater than that of the mutant in cycles 1 through 6 (Fig. 2B), and the AUCPC and mean colonization values of the mutant were significantly lower than those of the wild type in the mixed inoculations (Table 4).

Unlike the *sss* and *orfT* mutants, the *ptsP* mutant colonized the wheat rhizosphere significantly less ($P = 0.05$) than Q8r1-96Gm when the strains were introduced individually (Fig. 2C, cycles 1 and 4). The population densities of the two strains were similar in the other cycles, but the AUCPC and mean colonization values for Q8r1-96Gm were significantly higher than those for the *ptsP* mutant (Table 4). In mixed treatments, the *ptsP* mutant consistently colonized the wheat rhizosphere less than Q8r1-96Gm in cycles 1 through 6 (Fig. 2C), and the AUCPC and mean colonization values for the mutant were significantly lower ($P = 0.05$) than those for the wild type (Table 4).

The population densities for total culturable aerobic bacteria in the wheat rhizosphere for all experiments were more than log 8.4 CFU/g root. Mean colonization values of indigenous bacteria are shown in Table 4.

In order to evaluate survival of the wild type and the *sss*, *orfT*, and *ptsP* mutants in the absence of wheat roots, after the sixth cycle of the colonization experiments soils were stored at 20°C for 10 weeks, and then wheat seeds were sown again. The mean population densities recovered from the roots after two consecutive cycles are shown in Table 5. In soil into which the *sss* mutant and the wild-type strain had been introduced separately, comparable populations of the two strains were recovered from the rhizosphere, suggesting that the strains had survived equally well in the bulk soil during storage. In the soil into which the two strains had been coinoculated, a significantly larger population of the wild type than of the mutant was recovered, consistent with both the larger wild-type population at the time of storage and the tendency of the wild type to outcompete the mutant in mixed inoculations. In contrast, in the soils into which the *ptsP* or *orfT* mutant and the wild type had been introduced, whether separately or together, the recovered populations of the mutants were significantly smaller than those of the wild type (Table 5), suggesting that the mutants did not survive well in the absence of roots.

Rhizosphere colonization assays also were conducted with the *sss*, *orfT*, and *ptsP* mutants complemented with the corresponding wild-type genes carried on pME6010 (23), a stable, low-copy-number plasmid vector. The mean population densities and AUCPC values for six cycles (Table 6) indicated that

TABLE 6. Population densities of *P. fluorescens* Q8r1-96Gm and complemented mutants on the roots of wheat grown in Quincy virgin soil[a]

Expt	Strain	Population densities (AUCPC)[b]		
		Q8r1-96Gm(pME6010)	Complemented mutant	Q8r1-96Gm(pME6010) and mutant (1:1)
1	Q8r1-96Gm(pME6010)	7.6 a (43.7 ab[c])	ND	7.5 a (40.0 c[c])
	Q8r1-96*sss*(pME6010*sss*)	ND	7.6 a (44.6 a[c])	7.8 a (40.5 bc[c])
2	Q8r1-96Gm(pME6010)	7.6 a (44.2 ab)	ND	7.4 a (43.2 b)
	Q8r1-96*orfT*(pME6010*orfT*)	ND	7.7 a (44.8a)	7.4 a (42.8 b)
3	Q8r1-96Gm(pME6010)	7.6 a (43.8 ab)	ND	7.4 a (43.0 b)
	Q8r1-96*ptsP*(pME6010*ptsP*)	ND	7.7 a (45.1 a)	7.4 a (41.0 c)

[a] Raw Quincy virgin soil was treated with $\sim 10^4$ CFU g^{-1} soil of Q8r1-96Gm(pME6010) and/or Q8r1-96*sss*(pME6010*sss*), Q8r1-96*orfT*(pME6010*orfT*), or Q8r1-96*ptsP*(pME6010*ptsP*). Mixed-inoculation treatments contained a 1:1 mixture of competing strains ($\sim 0.5 \times 10^4$ CFU g^{-1} soil of each strain). Rhizosphere population densities of bacteria were determined by the terminal dilution endpoint assay described in Materials and Methods.

[b] The values are mean population densities in log CFU per g (fresh weight) of root for six cycles except cycle 0. Mean population densities in each experiment were analyzed separately. The values in parentheses are areas under the rhizosphere colonization progress curves (AUCPC) for introduced bacteria for six cycles. Different letters after values indicate that there is a statistically significant difference as determined by Fisher's protected least-significant-difference test ($P = 0.05$) (unless indicated otherwise). ND, not detected.

[c] The significance of differences between AUCPC was determined by the Kruskal-Wallis all-pairwise comparison test ($P = 0.05$).

TABLE 7. Population densities of strains Q8r1-96Gm and Q2-87 bearing pME6010 or pME6010*sss* on the roots of wheat grown in Quincy virgin soil

Strain[a]	Mean population density[b]	AUCPC[e]
Q8r1-96Gm(pME6010)	7.6 a	43.7 ab
Q8r1-96Gm(pME6010*sss*)	7.8 a	44.6 a
Q2-87(pME6010)	6.5 b	38.9 c
Q2-87(pME6010*sss*)	6.9 ab	40.5 bc
Control[c]	ND[d]	ND

[a] Raw Quincy virgin soil was treated with $\sim 10^4$ CFU per g of soil of strain Q8r1-96Gm or Q2-87 containing the empty vector pME6010 or the pME6010 derivative with cloned *sss*. Rhizosphere population densities of bacteria were determined by the terminal dilution endpoint assay as described in Materials and Methods.

[b] The values are mean population densities in log CFU per g (fresh weight) of root for six cycles except cycle 0. Different letters after values in the same column indicate that there is a statistically significant difference as determined by Fisher's protected least-significant-difference test ($P = 0.05$).

[c] Treatment without bacterial inoculation.

[d] ND, not detected.

[e] The values are areas under the colonization progress curves for introduced bacteria for six cycles. Different letters after values in the same column indicate that there is a statistically significant difference as determined by the Kruskal-Wallis all-pairwise comparison test ($P = 0.05$).

the plasmid-borne functional gene copies did indeed complement the chromosomal mutations in *sss*, *orfT*, and *ptsP*. Only when the complemented *ptsP* mutant was present in a 1:1 mixture with the wild type was the AUCPC value less than that for the wild-type strain.

Dekkers et al. (17) reported that the ability of some strains of *P. fluorescens* to colonize root tips of tomato is improved upon introduction of a plasmid-borne *sss*-containing fragment. To determine whether *sss* from *P. fluorescens* Q8r1-96 enhanced colonization of entire wheat root systems grown in nonsterile soil under our experimental conditions, we introduced pME6010*sss* into Q8r1-96, an exceptional root colonizer, and into the closely related but less aggressive strain Q2-87. The population dynamics of the two strains harboring either the empty vector or pME6010*sss* for six cycles are shown in Fig. 2D, and the mean colonization values and AUCPC values are shown in Table 7. Only in one of six cycles did Q8r1-96Gm harboring additional copies of *sss* establish a larger (approximately threefold) population in the wheat rhizosphere than the strain carrying an empty plasmid did. In contrast, the presence of pME6010*sss* in Q2-87 significantly improved root colonization 9.1-, 3.3-, and 5.2-fold in cycles 3, 4, and 5 (Fig. 2D). However, statistical analysis of the mean population density and AUCPC values across all six cycles indicated that there were no significant differences between treatments (Table 7).

DISCUSSION

The *P. fluorescens* genes studied in this work were chosen for analysis based on the important roles that they play in other bacterial systems. Of the three genes, *sss* probably is the best known in relation to rhizosphere microbiology; it encodes a subunit of a site-specific tyrosine recombinase involved in proper segregation of circular bacterial chromosomes during cell division and in the stable maintenance of some plasmids (8). In *E. coli*, the orthologue XerC, together with XerD, forms a heterotetrameric enzyme complex that catalyzes two consecutive pairs of strand exchanges at a 28-bp *dif* site in the chromosome. In the complex, XerC specifically cleaves and exchanges the top DNA strand. However, XerC and XerD are similar to each other and act in a cooperative fashion. The Xer recombinase is highly conserved in most species of *Enterobacteriaceae*, as well as in many other bacterial genera, including *Pseudomonas* (73). In fluorescent pseudomonads, *xerC* (called *sss*) initially was identified in *P. aeruginosa* as a gene that complemented a deficiency in pyoverdin production (26), but further functional studies revealed that *sss* encodes a site-specific recombinase homologous to XerC from *E. coli* (8). Later studies established that this *sss*-encoded recombinase plays an important role in competitive rhizosphere colonization by *P. fluorescens* WCS365, where its activity is linked to phenotypic variation and high mutation frequencies in the GacA/GacS global two-component regulatory system (15, 37, 61). The role of phenotypic variation in rhizosphere *Pseudomonas* spp. has been reviewed recently (72).

The results of the sequence analysis suggest that the *sss*-like gene that we cloned from *P. fluorescens* Q8r1-96 indeed encodes a true *xerC* homologue. We included this gene in our studies of root colonization because it was shown previously to contribute to rhizosphere competence and phenotypic variation in fluorescent *Pseudomonas* spp. in a variety of crops (1, 15, 61) and because we wondered if it played a significant role in the unusual colonization properties of strain Q8r1-96. Under gnotobiotic conditions, the *sss* mutant of *P. aeruginosa* 7NSK2 colonized potato root tips 10- to 1,000-fold less than the wild type colonized potato root tips in mixed inoculations, and the corresponding mutant of *P. fluorescens* WCS365 was impaired in colonization of tomato root tips in potting soil and in colonization of radish and wheat root tips under gnotobiotic conditions (15). These gnotobiotic systems are important in identifying genes that may function in colonization, but the results obtained under such conditions must be validated in natural soil. In our studies, the bacteria were added to a natural field soil that was cropped to wheat for multiple cycles, allowing rhizosphere colonization to proceed for months under controlled conditions in the presence of indigenous microflora and percolating water. Under these conditions, the *sss* mutant of Q8r1-96 colonized the wheat rhizosphere to the same extent as its wild-type parent colonized the wheat rhizosphere, and a deficiency in rhizosphere competence of the mutant became apparent only when plants were inoculated with a mixture containing both strains (Table 4).

Inactivation of *sss* in *P. fluorescens* Q8r1-96 resulted in a phenotype similar to that of the *sss* mutant of *P. fluorescens* WCS365 (15). Q8r1-96*sss* did not differ from the wild-type parent in its carbon and nitrogen utilization profiles, growth rate, motility, or swarming behavior (Table 3). Among the minor differences observed were decreased siderophore production, also reported in *P. aeruginosa* 7NSK2 (26), and production of elevated amounts of exoprotease and phloroglucinol-related compounds (Table 3). The fact that the extracellular protease activity or HCN production of the *sss* mutant was not impaired suggests that the GacA/GacS regulatory circuitry, which coordinately regulates the production of secondary metabolites and exoprotease (77), was not disturbed in Q8r1-96*sss*. We have no immediate explanation for the phenotypic

changes observed in the *sss* mutant, and we attribute them to the pleiotropic nature of the *sss* mutation.

The second gene studied in this work, *orfT*, was selected for analysis based on its role in interactions between *P. aeruginosa* PA14 and *Arabidopsis thaliana* (53). Like the *sss* mutant of Q8r1-96, the *orfT* mutant did not differ from the wild type in the ability to colonize the rhizosphere of wheat when it was introduced into the soil alone, but it colonized significantly less than the parental strain colonized when the two strains were introduced together (Table 4). Inactivation of *orfT* resulted in reduced motility and production of elevated total amounts of phloroglucinol-related compounds (Table 3). On the other hand, the *orfT* mutant did not differ from the wild type in carbon and nitrogen utilization profiles or the production of exoprotease, hydrogen cyanide, and siderophores. Furthermore, the mutant was indistinguishable from *P. fluorescens* Q8r1-96 on KMB and LB media supplemented with 2% glucose, but it was slightly more mucoid on PsP.

The results of computer analyses of OrfT indicate that its orthologues are found in all *Pseudomonas* genomes sequenced to date. The predicted proteins share a HRDxxxN motif with eukaryotic and some prokaryotic Ser/Thr and Tyr protein kinases (66) and some aminoglycoside phosphotransferases that are responsible for bacterial resistance to aminoglycoside antibiotics, such as streptomycin and kanamycin (45). The motif represents part of an active center where the conserved aspartate residue acts as a catalytic base (45). Despite these findings, this eukaryote-like protein kinase motif is too generic to provide any clues concerning the specificity of OrfT, and the similarity to aminoglycoside phosphotransferases is partial and limited to about 40% of the polypeptide chain. Thus, at this point the exact function of *orfT* in the rhizosphere competence of *P. fluorescens* Q8r1-96 remains unclear.

Like *orfT*, the third gene studied in this work, *ptsP*, was chosen for study based on its role in interactions between *P. aeruginosa* PA14 and *A. thaliana* (53). In contrast to the *sss* and *orfT* mutants, the rhizosphere competence of Q8r1-96*ptsP* was strongly impaired, and its rhizosphere population densities were significantly lower than those of the wild type in both single and mixed inoculations (Table 4 and Fig. 2C). The mutant lost the ability to maintain a density greater than 10^6 CFU/g root during extended cycling, which is a key characteristic of Q8r1-96 and all D-genotype isolates (6, 34, 35, 51). In cycles 5 and 6, the density of the mutant was $\leq 10^3$ CFU/g root, at least 3 orders of magnitude less than that of the wild type. The *ptsP* mutant exhibited altered morphology, reduced motility and exoprotease activity, and an increased level of fluorescence. It produced significantly smaller amounts of MAPG and 2,4-DAPG (ca. 54% and 60% of the wild-type levels). Carbon and nitrogen substrate utilization profiling revealed that Q8r1-96*ptsP* grew more slowly on D-galactose and L-cysteine, respectively.

The pleiotropic phenotype exhibited by Q8r1-96*ptsP* appears to reflect the proposed global regulatory function of the *ptsP* gene, which is highly conserved in the genomes of *P. fluorescens* Pf01, SBW25, and Pf-5. The product of *ptsP* forms part of an alternative PEP:carbohydrate phosphotransferase system (PTS). PTSs are found in a wide range of gram-positive and gram-negative microorganisms and have been best studied in enteric bacteria, where they are involved in sensing, transport, and metabolism of carbohydrates, as well as in catabolite repression and inducer exclusion. The first component of a typical PTS is a carbohydrate-specific multisubunit (or multidomain) enzyme II (EII) that forms a membrane translocation channel and phosphorylates the incoming sugar (47). The second part of a PTS is involved in the phosphorylation of all PTS carbohydrates. It lacks specificity and is comprised of cytoplasmic enzyme I (EI) and histidine protein (HPr).

In *E. coli*, *ptsP* encodes a nitrogen-specific EI paralogue called EI^{Ntr} that, together with EII^{Ntr} and NPr (paralogues of EII^{Fru} and HPr, respectively), forms a regulatory PTS phosphoryl transfer chain (52). Functional analyses have shown that this chain is involved in sugar-dependent utilization of certain amino acids and somehow links the metabolism of carbon and nitrogen. In *P. putida*, EII^{Ntr} either positively or negatively (and largely in a glucose-independent fashion) controls the expression of over 100 genes, some of which are members of the σ^{54} regulon (12). Based on the results of this study, it was proposed that EII^{Ntr}, which together with EI^{Ntr} and NPr presumably forms an alternative PTS in *P. putida*, functions as a global regulatory factor rather than as a promoter-specific regulatory factor. Genes coding for EI^{Ntr} also were described for *A. vinelandii* (64), *Bradyrhizobium japonicum* (31), *P. aeruginosa* (69), and *Legionella pneumophila* (25). In these microorganisms, as in *E. coli*, *ptsP* primarily plays a regulatory role and does not directly participate in phosphorylation or utilization of carbohydrates. Similarly, the *ptsP* mutant of Q8r1-96 did not differ considerably from the wild type in utilization of the sugars, amino acids, and organic acids included among the substrates present in Biolog MicroPlates. Recently, it was reported that *ptsP* plays an important role in the regulation of pyocyanin production but did not influence the quorum-sensing system in *P. aeruginosa* (78).

In conclusion, the data presented in this paper suggest novel functions for two genes, *ptsP* and *orfT*, that previously were linked with pathogenesis in *P. aeruginosa*. The *ptsP* and *orfT* mutants of Q8r1-96 did not have nonspecific growth defects in vitro, and the effects of the mutations became apparent only when the mutants were tested in the rhizosphere, either individually or in competition with the parental strain. In this respect, both genes fulfill the criteria for "true" rhizosphere colonization determinants, as described by Lugtenberg et al. (36). To our knowledge, this is the first report to provide evidence that *ptsP* is involved in rhizosphere colonization by fluorescent pseudomonads. The results of sequence analyses suggest that in *P. fluorescens* Q8r1-96, as in *P. aeruginosa* (57) and *P. putida* (12), PtsP (EI^{Ntr}) forms part of the regulatory phosphoryl transfer chain that may coordinate the metabolism of carbon and nitrogen. Unfortunately, despite the fact that EI^{Ntr}, EII^{Ntr}, and NPr have been purified and characterized biochemically, their exact functions remain poorly understood even in *E. coli* (70). However, if PtsP has a role in integrating carbon and nitrogen utilization, as it is thought to have in some bacteria, then the mutant phenotype might be manifested differently in the synthetic media used in our growth studies than in the complex mixture of sugars, organic acids, and amino acids present in root exudates. More comprehensive growth studies are necessary to determine whether such differences

may account for the limited effects of the mutation on growth in vitro compared to the profound impact observed in the wheat rhizosphere.

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REFERENCES

1. **Achouak, W., S. Conrod, V. Cohen, and T. Heulin.** 2004. Phenotypic variation of *Pseudomonas brassicacearum* as a plant root-colonization strategy. Mol. Plant-Microbe Interact. **17:**872–879.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 2002. Short protocols in molecular biology, 5th ed. John Wiley and Sons, New York, N.Y.
3. **Bakker, A. W., and B. Schippers.** 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biol. Biochem. **19:**451–457.
4. **Bangera, M. G., and L. S. Thomashow.** 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. J. Bacteriol. **181:**3155–3163.
5. **Bauer, D. W., and A. Collmer.** 1997. Molecular cloning, characterization, and mutagenesis of a *pel* gene from *Pseudomonas syringae* pv. *lachrymans* encoding a member of the *Erwinia chrysanthemi* PelADE family of pectate lyases. Mol. Plant-Microbe Interact. **10:**369–379.
6. **Bergsma-Vlami, M., M. E. Prins, M. Staats, and J. M. Raaijmakers.** 2005. Assessment of genotypic diversity of antibiotic-producing *Pseudomonas* spp. in the rhizosphere by denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **71:**993–1003.
7. **Birren, B., E. D. Green, S. Klapholz, M. R. Myers, H. Riethman, and J. Roskams.** 1999. Genome analysis: a laboratory manual, vol. 3. Cloning systems. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
8. **Blakely, G. W., A. O. Davidson, and D. J. Sherratt.** 2000. Sequential strand exchange by XerC and XerD during site-specific recombination at *dif**. J. Biol. Chem. **275:**9930–9936.
9. **Bonsall, R. F., D. M. Weller, and L. S. Thomashow.** 1997. Quantification of 2.4-diacetylphloroglucinol produced by fluorescent *Pseudomonas* spp. in vitro and in the rhizosphere of wheat. Appl. Environ. Microbiol. **63:**951–955.
10. **Burk, D. L., W. C. Hon, A. K.-W. Leung, and A. M. Berghuis.** 2001. Structural analyses of nucleotide binding to an aminoglycoside phosphotransferase. Biochemistry **40:**8756–8764.
11. **Camacho Carvajal, M. M.** 2000. Molecular characterization of the role of type 4 pili, NADH-I and PyrR in rhizosphere colonization of *Pseudomonas fluorescens* WCS365. Ph.D. thesis. Utrecht University, Utrecht, The Netherlands.
12. **Cases, I., J.-A. Lopez, J.-P. Albar, and V. De Lorenzo.** 2001. Evidence of multiple regulatory functions for the PtsN (IIA^{Ntr}) protein of *Pseudomonas putida*. J. Bacteriol. **183:**1032–1037.
13. **Cosenza, L. W., F. Bringaud, T. Baltz, and F. M. D. Vellieux.** 2001. The 3.0 Å resolution crystal structure of glycosomal pyruvate phosphate dikinase from *Trypanosoma brucei*. J. Mol. Biol. **318:**1417–1432.
14. **Dekkers, L. C., A. J. van der Bij, I. H. M. Mulders, C. C. Phoelich, R. A. R. Wentwoord, D. C. M. Glandorf, C. A. Wijffelman, and B. J. J. Lugtenberg.** 1998. Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (*nuo*) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol. Plant-Microbe Interact. **11:**763–771.
15. **Dekkers, L. C., C. C. Phoelich, L. van der Fits, and B. J. J. Lugtenberg.** 1998. A site-specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens* WCS365. Proc. Natl. Acad. Sci. USA **95:**7051–7056.
16. **Dekkers, L. C., C. J. P. Bloemendaal, L. A. de Weger, C. A. Wijffelman, H. P. Spaink, and B. J. J. Lugtenberg.** 1998. A two-component system plays an important role in the root-colonizing ability of *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. **11:**45–56.
17. **Dekkers, L. C., I. H. M. Mulders, C. C. Phoelich, T. F. C. Chin-a-Woeng, A. H. M. Wijfjes, and B. J. J. Lugtenberg.** 2000. The *sss* colonization gene of the tomato-*Fusarium oxysporum* f. sp. *radicis-lycopersici* biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild-type *Pseudomonas* spp. bacteria. Mol. Plant-Microbe Interact. **13:**1177–1183.
18. **de Weger, L. A., C. I. M. Van der Vlugt, A. H. M. Wijfjes, P. A. H. B. M. Bakker, B. Schippers, and B. Lugtenberg.** 1987. Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J. Bacteriol. **169:**2769–2773.
19. **Duffy, B. K., and G. Défago.** 1997. Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology **87:**1250–2157.
20. **Enderle, P. J., and M. A. Farwell.** 1998. Electroporation of freshly plated *Escherichia coli* and *Pseudomonas aeruginosa* cells. BioTechniques **25:**954–958.
21. **Gattiker, A., K. Michoud, C. Rivoire, A. H. Auchincloss, E. Coudert, T. Lima, P. Kersey, M. Pagni, C. J. A. Sigrist, C. Lachaize, A.-L. Veuthey, E. Gasteiger, and A. Bairoch.** 2003. Automatic annotation of microbial proteomes in Swiss-Prot. Comput. Biol. Chem. **27:**49–58.
22. **Gerhardt, P., R. G. E. Murray, W. Wood, and N. R. Krieg (ed.).** 1994. Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
23. **Heeb, S., Y. Itoh, T. Nishijyo, U. Schnider, C. Keel, J. Wade, U. Walsh, F. O'Gara, and D. Haas.** 2000. Small, stable shuttle vectors based on the minimal pVS1 replicon for use in gram-negative, plant-associated bacteria. Mol. Plant-Microbe Interact. **13:**232–237.
24. **Herzberg, O., C. C. Chen, S. Liu, A. Tempczyk, A. Howard, M. Wei, D. Ye, and D. Dunaway-Mariano.** 2002. Pyruvate site of pyruvate phosphate dikinase: crystal structure of the enzyme-phosphonopyruvate complex, and mutant analysis. Biochemistry **41:**780–787.
25. **Higa, F., and P. H. Edelstein.** 2001. Potential virulence role of the *Legionella pneumophila ptsP* ortholog. Infect. Immun. **69:**4782–4789.
26. **Hofte, M., Q. Dong, S. Kourambas, V. Krishapillai, D. Sharratt, and M. Mergeay.** 1994. The *sss* gene product, which affects pyoverdin production in *Pseudomonas aeruginosa* 7NSK2, is a site-specific recombinase. Mol. Microbiol. **14:**1011–1020.
27. **House, B. L., M. W. Mortimer, and Kahn, M. L.** 2004. New recombination methods for *Sinorhizobium meliloti* genetics. Appl. Environ. Microbiol. **70:**2806–2815.
28. **Keel, C., U. Schnider, M. Maurhofer, C. Voisard, J. Laville, U. Burger, P. Wirthner, D. Haas, and G. Défago.** 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of the bacterial secondary metabolite 2,4-diacetylphlorogluciol. Mol. Plant-Microbe Interact. **5:**4–13.
29. **Kelley, L. A., R. M. MacCallum, and M. J. E. Sternberg.** 2000. Enhanced genome annotation using structural profiles in the program 3D-PSSM. J. Mol. Biol. **299:**499–520.
30. **King, E. O., M. K. Ward, and D. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44:**301–307.
31. **King, N. D., and M. R. O'Brian.** 2001. Evidence for direct interaction between enzyme I^{Ntr} and aspartokinase to regulate bacterial oligopeptide transport. J. Biol. Chem. **276:**21311–21316.
32. **Kuiper, I., G. V. Bloemberg, S. Noreen, J. E. Thomas-Oates, and B. J. J. Lugtenberg.** 2001. Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. **14:**1096–1104.
33. **Landa, B. B., H. A. E. de Werd, B. B. McSpadden Gardener, and D. M. Weller.** 2002a. Comparison of three methods for monitoring populations of different genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere. Phytopathology **92:**129–137.
34. **Landa, B. B., O. V. Mavrodi, J. Raaijmakers, B. B. McSpadden Gardener, L. S. Thomashow, and D. M. Weller.** 2002b. Differential ability of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. Appl. Environ. Microbiol. **68:**3226–3237.
35. **Landa, B. B., D. V. Mavrodi, J., Raaijmakers, L. S. Thomashow, and D. M. Weller.** 2003. Interactions between strains of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains in the rhizosphere of wheat. Phytopathology **93:**982–994.
36. **Lugtenberg, B. J. J., L. C. Dekkers, and G. V. Bloemberg.** 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu. Rev. Phytopathol. **39:**461–490.
37. **Martinez-Granero, F., S. Capdevila, M. Sanchez-Contreras, M. Martin, and R. Rivilla.** 2005. Two site-specific recombinases are implicated in phenotypic variation and competitive rhizosphere colonization in *Pseudomonas fluorescens*. Microbiology **151:**975–983.
38. **Mavrodi, D. V., R. F. Bonsall, S. M. Delaney, M. J. Soule, G. Phillips, and L. S. Thomashow.** 2001. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. J. Bacteriol. **183:**6454–6465.
39. **Mavrodi, O. V., B. B. McSpadden Gardener, D. V. Mavrodi, D. M. Weller, and L. S. Thomashow.** 2001. Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. Phytopathology **91:**35–43.
40. **Mavrodi, O. V., D. V. Mavrodi, D. M. Weller, and L. S. Thomashow.** 2006. The role of *dsbA* in root colonization by *Pseudomonas fluorescens* Q8r1-96. Microbiology **152:**863–872.
41. **Mazurier, S., M. Lemunier, S. Siblot, C. Mougel, and P. Lemanceau.** 2004. Distribution and diversity of type III secretion system-like genes in saprophytic and phytopathogenic fluorescent pseudomonas. FEMS Microbiol. Ecol. **49:**455–467.
42. **McSpadden Gardener, B. B., K. L. Schroeder, S. E. Kalloger, J. M. Raaij-**

makers, L. S. Thomashow, and D. M. Weller. 2000. Genotypic and phenotypic diversity of *phl*D-containing *Pseudomonas* isolated from the rhizosphere of wheat. Appl. Environ. Microbiol. **66:**1939–1946.
43. **McSpadden Gardener, B. B., D. V. Mavrodi, L. S. Thomashow, and D. M. Weller.** 2001. A rapid PCR-based assay characterizing rhizosphere populations of 2,4-DAPG-producing bacteria. Phytopathology **91:**44–54.
44. **McSpadden Gardener, B. B., and D. M. Weller.** 2001. Changes in populations of rhizosphere bacteria associated with take-all disease of wheat. Appl. Environ. Microbiol. **67:**4414–4425.
45. **Nurizzo, D., S. C. Shewry, M. H. Perlin, S. A. Brown, J. N. Dholakia, R. L. Fuchs, T. Deva, E. N. Baker, and C. A. Smith.** 2003. The crystal structure of aminoglycoside-3′-phosphotransferase-IIa, an enzyme responsible for antibiotic resistance. J. Mol. Biol. **327:**491–506.
46. **Pagni, M., V. Ionnidis, L. Cerutti, M. Zahn-Zabal, C. V. Jongeneel, and L. Falquet.** 2004. Myhits: a new interactive resource for protein annotation and domain identification. Nucleic Acids Res. **32:**W332–W335.
47. **Postma, P. W., J. W. Lengeler, and G. R. Jacobson.** 1996. Phosphoenolpyruvate:carbohydrate phosphotransferase systems, p. 1149–1174. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
48. **Preston, G. M., N. Bertrand, and P. B. Rainey.** 2001. Type III secretion in plant growth-promoting *Pseudomonas fluorescens* SBW25. Mol. Microbiol. **41:**999–1014.
49. **Raaijmakers, J. M., D. M. Weller, and L. S. Thomashow.** 1997. Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. Appl. Environ. Microbiol. **63:**881–887.
50. **Raaijmakers, J. M., and D. M. Weller.** 1998. Natural plant protection by 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in take-all decline soils. Mol. Plant-Microbe Interact. **11:**144–152.
51. **Raaijmakers, J. M., and D. M. Weller.** 2001. Exploiting the genetic diversity of *Pseudomonas* spp: characterization of superior colonizing *P. fluorescens* strain Q8r1-96. Appl. Environ. Microbiol. **67:**2545–2554.
52. **Rabus, R., J. Reizer, I. Paulsen, and M. H. Saier, Jr.** 1999. Enzyme I^{Ntr} from *Escherichia coli*. J. Biol. Chem. **274:**26185–26191.
53. **Rahme, L. G., M.-W. Tan, L. Le, S. M. Wong, R. G. Tompkins, S. B. Calderwood, and F. M. Ausubel.** 1997. Use of model plant hosts to identify *Pseudomonas aeruginosa* virulence factors. Proc. Natl. Acad. Sci. USA **94:**13245–13250.
54. **Rahme, L. G., F. M. Ausubel, H. Cao, E. Drenkard, B. C. Goumerov, G. W. Lau, S. Mahajan-Miklos, J. Plotnikova, M.-W. Tan, J. Tsongalis, C. L. Walendziewicz, and R. G. Tompkins.** 2000. Plants and animals share functionally common bacterial virulence factors. Proc. Natl. Acad. Sci. USA **97:**8815–8821.
55. **Rainey, P. B.** 1999. Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere. Environ. Microbiol. **1:**243–257.
56. **Rainey, P. B., and G. M. Preston.** 2000. *In vivo* expression technology strategies: valuable tools for biotechnology. Curr. Opin. Biotechnol. **11:**440–444.
57. **Reizer, J., A. Reizer, M. J. Lagrou, K. R. Folger, C. K. Stover, and M. H. Saier, Jr.** 1999. Novel phosphotransferase systems revealed by bacterial genome analysis: the complete repertoire of *pts* genes in *Pseudomonas aeruginosa*. J. Mol. Microbiol. Biotechnol. **1:**289–293.
58. **Rezzonico, F., G. Defago, and Y. Moënne-Loccoz.** 2004. Comparison of ATP-encoding type III secretion system *hrcN* genes in biocontrol fluorescent pseudomonas and in phytopathogenic proteobacteria. Appl. Environ. Microbiol. **70:**5119–5131.
59. **Sacherer, P., G. Defago, and D. Haas.** 1994. Extracellular protease and phospholipase C are controlled by the global regulatory gene *gacA* in the biocontrol strain *Pseudomonas fluorescens* CHA0. FEMS Microbiol. Lett. **116:**115–160.
60. **Sambrook, J., E. F. Fritisch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
61. **Sanchez-Contreras, M., M. Martin, M. Villacieros, F. O'Gara, I. Bonilla, and R. Rivilla.** 2002. Phenotypic selection and phase variation occur during alfalfa root colonization by *Pseudomonas fluorescens* F113. J. Bacteriol. **184:**1587–1596.
62. **Schweizer, H. P.** 1992. Allelic exchange in *Pseudomonas aeruginosa* using novel ColE1-type vectors and a family of cassettes containing portable *oriT* and the counter-selectable *Bacillus subtilis sacB* marker. Mol. Microbiol. **6:**1195–1204.
63. **Schwyn, B., and J. B. Neilands.** 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. **160:**47–56.
64. **Segura, D., and G. Espin.** 1998. Mutational inactivation of a gene homologous to *Escherichia coli ptsP* affects poly-β-hydroxybutyrate accumulation and nitrogen fixation in *Azotobacter vinelandii*. J. Bacteriol. **180:**4790–4798.
65. **Shanahan, P., D. J. O'Sullivan, P. Simpson, J. D. Glennon, and F. O'Gara.** 1992. Isolation of 2,4-diacetylphlorogluciol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. Appl. Environ. Microbiol. **58:**353–358.
66. **Shi, L., M. Potts, and P. J. Kennelly.** 1998. The serine, threonine, and/or tyrosine-specific protein kinases and protein phosphatases of prokaryotic organisms: a family portrait. FEMS Microbiol. Rev. **22:**229–253.
67. **Simons, M., H. P. Permentier, L. A. de Weger, C. A. Wijffelman, and B. J. J. Lugtenberg.** 1997. Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. **10:**102–106.
68. **Subramanya, H. S., L. K. Arciszewska, R. A. Baker, L. E. Bird, D. J. Sherratt, and D. B. Wigley.** 1997. Crystal structure of the site-specific recombinase, XerD. EMBO J. **16:**5178–5187.
69. **Tan, M.-W., L. G. Rahme, J. A. Sternberg, R. G. Tompkins, and F. M. Ausubel.** 1999. *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. Proc. Natl. Acad. Sci. USA **96:**2408–2413.
70. **Tchieu, J. H., V. Norris, J. S. Edwards, and M. H. Saier, Jr.** 2001. The complete phosphotransferase system in *Escherichia coli*. J. Mol. Microbiol. Biotechnol. **3:**329–346.
71. **Validov, S., O. Mavrodi, L. De La Fuente, A. Boronin, D. Weller, L. S. Thomashow, and D. Mavrodi.** 2005. Antagonistic activity among *phlD*-containing fluorescent *Pseudomonas* spp. FEMS Microbiol. Lett. **242:**249–256.
72. **van den Broek, D., G. V. Bloemberg, and B. Lugtenberg.** 2005. The role of phenotypic variation in rhizosphere *Pseudomonas* bacteria. Environ. Microbiol. **7:**1686–1697.
73. **Villion, M., and G. Szatmari.** 1998. Cloning and characterization of the *Proteus mirabilis xerD* gene. FEMS Microbiol. Lett. **164:**83–90.
74. **Vincent, M. N., L. A. Harrison, J. M. Brackin, P. A. Kovacevich, P. Murkerji, D. M. Weller, and E. A. Pierson.** 1991. Genetic analysis of the antifunfgal activity of a soilborne *Pseudomonas aureofaciens* strain. Appl. Environ. Microbiol. **57:**2928–2934.
75. **Weller, D. M., J. M. Raijmakers, and L. S. Thomashow.** 1997. The rhizosphere ecology of antibiotic-producing pseudomonas and their role in take-all decline, p. 58–64. *In* A. Ogoshi, K. Koboyashi, Y. Homma, N. Kondo, and S. Akino (ed.), Plant growth-promoting rhizobacteria: present status and future prospects. Kakanishi Printing, Sapporo, Japan.
76. **Whipps, J. M.** 2001. Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. **52:**487–511.
77. **Whistler, C. A., N. A. Corbell, A. Sarniguet, W. Ream, and J. E. Loper.** 1998. The two-component regulators GacS and GacA influence accumulation of the stationary-phase sigma factor σ^{54} and the stress response in *Pseudomonas fluorescens* Pf-5. J. Bacteriol. **180:**6635–6641.
78. **Xu, H., W. Lin, H. Xia, S. Xu, Y. Li, H. Yao, F. Bai, X. Zhang, Y. Bai, P. Saris, and M. Qiao.** 2005. Influence of *ptsP* gene on pyocyanin production in *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **253:**103–109.