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Involvement of Nitrate Reductase and Pyoverdine in Competitiveness of *Pseudomonas fluorescens* Strain C7R12 in Soil

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Involvement of nitrate reductase and pyoverdine in the competitiveness of the biocontrol strain *Pseudomonas fluorescens* C7R12 was determined, under gnotobiotic conditions, in two soil compartments (bulk and rhizosphere soil), with the soil being kept at two different values of matric potential (−1 and −10 kPa). Three mutants affected in the synthesis of either the nitrate reductase (Nar$^-$), the pyoverdine (Pvd$^-$), or both (Nar$^-$ Pvd$^-$) were used. The Nar$^-$ and Nar$^-$ Pvd$^-$ mutants were obtained by site-directed mutagenesis of the wild-type strain and of the Pvd$^-$ mutant, respectively. The selective advantage given by nitrate reductase and pyoverdine to the wild-type strain was assessed by measuring the dynamic of each mutant-to-total-inoculant (wild-type strain plus mutant) ratio. All three mutants showed a lower competitiveness than the wild-type strain, indicating that both nitrate reductase and pyoverdine are involved in the fitness of *P. fluorescens* C7R12. The double mutant presented the lowest competitiveness. Overall, the competitive advantages given to C7R12 by nitrate reductase and pyoverdine were similar. However, the selective advantage given by nitrate reductase was more strongly expressed under conditions of lower aeration (−1 kPa). In contrast, the selective advantage given by nitrate reductase and pyoverdine did not differ in bulk and rhizosphere soil, indicating that these bacterial traits are not specifically involved in the rhizosphere competence but rather in the saprophytic ability of C7R12 in soil environments.

Fluorescent pseudomonads can suppress various soilborne diseases (40). Their efficacy has been related both to their antagonistic activities and to their rhizosphere competence (4, 6). Overall, biological control of soilborne disease achieved by fluorescent pseudomonads is often inconsistent (15, 40). This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria (35). Indeed, a clear relationship has been established between suppression of the wheat root disease take-all and that of fusarium wilts by different strains of fluorescent pseudomonads and the densities of these bacteria in the rhizosphere of the corresponding host plants (1, 33). In order to improve the efficacy and the consistency of the biological control, the use of rhizosphere-competent strains is then required. However, since the microbial inoculations would mainly be performed in soils before the plant is grown, the strains should also be able to survive in the soil and should then show a good saprophytic ability. To fulfil these requirements, progress must be made in the knowledge of bacterial traits promoting saprophytic ability under soil conditions.

Despite the abundance of iron in soils, the concentration of ferric iron available to the soilborne microflora is very low (17). Since Fe^{3+} is an essential element for most microorganisms, this ion is often a limiting factor for microbial growth and activity in soil habitats (18). Most microorganisms have developed an active strategy for iron acquisition based on the use of siderophores and of the corresponding ferrisiderophore membrane receptors (26). The major siderophores of the fluorescent pseudomonads, called pyoverdines, show a very high affinity for Fe^{3+} (23). Several studies have stressed the role played by pyoverdine-mediated iron competition in the microbial antagonism performed by biocontrol strains against some pathogens (18). Other studies have underlined the involvement of pyoverdine-mediated iron uptake in the ecological fitness of different strains of fluorescent pseudomonads (20, 25, 32). Ferric iron is indeed known to play a major role in the bacterial metabolism since it is an intermediate electron acceptor in the respiratory chain. Some fluorescent pseudomonads are able to adapt to limited oxygen conditions by using nitrogen oxides as alternative electron acceptors (38), and respiratory nitrate and nitrite reductase have been described to be implicated in the competitiveness of model strains of fluorescent pseudomonad in soil (8, 28).

The soil environment experiences major changes such as those resulting from root growth and from rainfall and/or irrigation. The growth and activity of the root system induce significant modifications in the physicochemical and biological properties of the soil surrounding the root; these modifications correspond to the so-called rhizosphere effect. This effect is partly related to the higher concentration of carbohydrates (electron donors) in the rhizosphere than in the bulk soil, due to rhizodeposition (22). This results in higher oxygen consumption due to microbial respiration in rhizosphere compared to bulk medium as reported by Højberg and Sørensen (9). This may account for the fact that the frequency of populations of fluorescent pseudomonads able to reduce nitrates in the rhizosphere is higher than that in bulk soil (2). Aeration of the soil is also affected by the hydrous pattern. Rainfall and/or irrigation leads to an increase of the soil porosity filled with

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water and to a reduction of the aeration, as demonstrated using an oxygen-sensing reporter strain of *Pseudomonas fluorescens* (10). This reduced aeration is expected to decrease the concentration of ferric iron (17), and roots were shown to influence the biological availability of ferric iron (19). Under soil conditions where the oxygen status and the ferric iron availability are subject to variations, fluorescent pseudomonads able to synthesize both nitrate reductase and pyoverdine should then have a competitive advantage.

The aim of our study was to assess the involvement of pyoverdine and nitrate reductase in the competitiveness of a biocontrol strain of *P. fluorescens*. This comparison was based on the use of isogenic mutants unable to synthesize pyoverdine, nitrate reductase, or both. The relative importance of nitrate reductase and of pyoverdine in the competitiveness of the wild-type strain was evaluated by comparing the dynamics of each mutant-to-total-inoculant (wild-type strain plus mutant) ratio. This strategy is commonly considered the most accurate in evaluating the role of a specific genotype and/or phenotype in the competence of a bacterial strain (8, 28, 36, 39). The impact of soil conditions, expected to influence the oxygen status and the ferric iron availability, was assessed by performing the competitiveness experiments in a given soil, cultivated or not with tomato and kept at two different matric potential values (−1 versus −10 kPa). The soil was sterilized prior to inoculation and the competitiveness experiments were then conducted under gnotobiotic conditions in order to make a more straightforward demonstration and to avoid any possible interference with the native microflora. Previous studies have indeed shown that the competitive advantage of different wild-type strains over their mutants was more clearly expressed under gnotobiotic than under nongnotobiotic conditions (25, 28). Therefore, the experiments presented here deal with intraspecific competition between the wild-type strain and the defective mutants.

MATERIALS AND METHODS

Organisms and growth conditions. *P. fluorescens* strain C7R12 is a spontaneous mutant of strain C7 resistant to rifampin (100 mg liter^{-1}) (7). The wild-type strain C7 was previously isolated from the rhizosphere of flax cultivated in the Châteaurenard, France, soil that suppresses fusarium wilts (16). The strain C7R12 was shown (i) to improve the suppression of fusarium wilts achieved by nonpathogenic *Fusarium oxysporum* (14) and (ii) to be rhizosphere competent (7). Pyoverdine-deficient mutant PL1 was previously obtained from strain C7R12 by Tn*5* insertion into a pyoverdine synthetase gene (25). Pseudomonad strains were grown in KB (12) or Luria broth (LB) medium (24). The antibiotics incorporated into these media were gentamicin (10 μg/ml), tetracycline (10 μg/ml), ampicillin (10 μg/ml), kanamycin (50 μg/ml), and rifampin (100 μg/ml). *Escherichia coli* host strains for plasmids were grown in LB medium at 37°C.

Construction of Nar$^-$ mutants of *P. fluorescens* C7R12. Using primers narG-TET (5′-ACG-TTG-CCA-AGG-ACT-ATG-AC-3′) and narG-END (5′-CGG-TGA-TGG-TGC-GCC-ATG-GG-3′), a 1.6-kb fragment of the *narG* gene of *P. fluorescens* C7R12 was amplified (for 3 min at 95°C; followed by 35 cycles at 95°C for 1 min, 56°C for 1 min, and 72°C for 1 min; and then 5 min at 72°C) and cloned into the pT7 plasmid according to the manufacturer's procedures (Novagen-Merck, Nogent-sur-Marne, France). To obtain the Nar$^-$ mutants, the wild-type chromosomal copy of *narG* of *P. fluorescens* C7R12 and PL1 was replaced after homologous recombination by a copy of the deleted gene with an insertion of the *apra3* gentamicin resistance gene (Fig. 1). DNA restriction, agarose gel electrophoresis, ligation, and transformation were carried out by standard methods (31). The different steps describing the construction of the cosmid vector pL3NR carrying a copy of the deleted *narG* gene with an insertion of the *apra3* gentamicin resistance gene are presented in Fig. 1. Briefly the *apra3* gene encoding resistance to gentamicin from plasmid pHP45Ω (34) was ligated into pT7NR. The Δ*narG*::*apra3* construct was then excised from pT7NR and cloned into the pLAFR3 cosmid (37), yielding pL3NR. Electrotransformation of *P. fluorescens* C7R12 and PL1 with plasmid pL3NR was achieved in a Bio-Rad pulser apparatus (with settings of 2.5 kV, 25 μF, and 200 Ω for 4 to 6 ms). Transconjugants of C7R12 and of PL1 were selected on LB medium supplemented with gentamicin and tetracycline. Recombinants showing double crossing-over were identified after several rounds of growth on LB medium containing gentamicin at 4°C to cure the pL3NR and scoring for Tc^s Gm^r colonies. Two Nar$^-$ mutants, namely, NR2 and PL1NR6 of C7R12 and PL1, respectively, were chosen. Southern blot analysis of chromosomal DNA of *P. fluorescens* C7R12 and of the NR2 and PL1NR6 mutants was performed to check the replacement of the wild-type chromosomal copy of the *narG* gene with the copy with the deletion and containing the *apra3* gentamicin resistance gene.

Growth characteristics of C7R12, NR2, and PL1NR6 in liquid medium. For preparation of inocula, strains were cultured in 10 ml of LB medium. After 24 h of incubation, bacterial cells were collected by centrifugation at 8,000 × *g* for 20 min. The cells were resuspended in LB medium to obtain an absorbance at 600 nm of 0.1.

For anaerobic treatments, 150-ml plasma flasks containing 50 ml of LB medium, supplemented with 10 mM KNO_3 or 10 mM KNO_2, were made anaerobic by evacuation and flushing three times with helium and then were aseptically inoculated through the rubber stopper with each of the different microbial inocula to obtain a final absorbance of 0.01. The denitrification rates by *P. fluorescens* C7R12 in succinate medium supplemented with 10 mM KNO_3 in the presence and in the absence of Fe^{3+} (50 μM) were compared. For these experiments, 10% acetylene was introduced in the anaerobic plasma flasks to avoid any reduction of N_2O to N_2. Nitrous oxide concentration was determined by using an MTI high-speed microgas chromatograph equipped with a catharometer detector (SRA Instruments).

For aerobic treatments, Erlenmeyer flasks containing 50 ml of LB medium were aseptically inoculated with each of the different microbial inocula to obtain a final absorbance of 0.01.

All the flasks were incubated at 25°C on an orbital shaker. Each treatment was tested in triplicate. Bacterial growth was periodically monitored by measuring the absorbance at 600 nm with a Shimadzu UV-160 spectrophotometer (Roucaire, Velizy-Villacoublay, France).

Soil and plant experiments. The experiments were performed in the calcic silt-clay soil from Châteaurenard. This soil is known for its natural suppressiveness to fusarium wilts, and its main characteristics have been previously described (13). The soil was air dried and sieved (for particles <2 mm in diameter). Population dynamics of the wild-type strain C7R12 and of the PL1, NR2, and PL1NR6 mutants were compared in bulk soil and in the rhizosphere of tomato grown in the Châteaurenard soil, which was kept at two matric potential values (−1 and −10 kPa). These values correspond to a proportion of the porosity filled with water equal to 51 and 35%, respectively (16). The experiments were performed under gnotobiotic conditions.

Briefly, 31 g of soil was introduced into containers (30 ml), corresponding to an apparent density of 1.06. These containers were sterilized by gamma radiation (40 kGy; Conservatome, Dagneux, France). Some containers were kept uncultivated, and others were cultivated with tomato (*Lycopersicon esculentum* Mill. cv. H63-5). Tomato seeds were sterilized in a 1.25% (vol/vol) solution of NaOCl for 20 min, washed three times with sterile distilled water, and placed on a sterile filter at 25°C for 48 h. Five tomato seedlings were transferred per container. The cultivated and uncultivated containers were placed in a flow cabinet used as a growth chamber on a cycle of 16 h of light (25°C) and 8 h of darkness (22°C). Each mutant was introduced in combination with the wild-type strain (1:1) to obtain a bacterial density of 10^5 CFU/g of dry soil.

The extraction of the bacteria for enumeration was performed as follows. Approximately 0.5 g of dry soil was collected (i) from each uncultivated container (bulk soil) and (ii) from the soil remaining at the root surface of three root systems, after a gentle brushing, in each cultivated container (rhizosphere soil). Each soil sample from the two compartments (bulk and rhizosphere soil) was suspended in 10 ml of sterile distilled water for 60 s with a Vortex shaker. The soil suspensions were diluted in sterile distilled water and plated (three plates per suspension) on KB supplemented with the antibiotics corresponding to the resistance phenotypes of the different strains. Bacterial enumeration was performed 0, 10, and 20 days after inoculation on five independent replicates (containers) per experimental treatment and date. The bacterial densities were expressed as CFU per gram of dry soil.

Statistical analyses. Since populations of bacteria approximate a log normal distribution (21), values were log transformed before analysis. Competitiveness of each mutant in the presence of the wild-type strain was represented as the mutant-to-total-inoculant (wild type plus mutant) CFU ratio. These ratios were

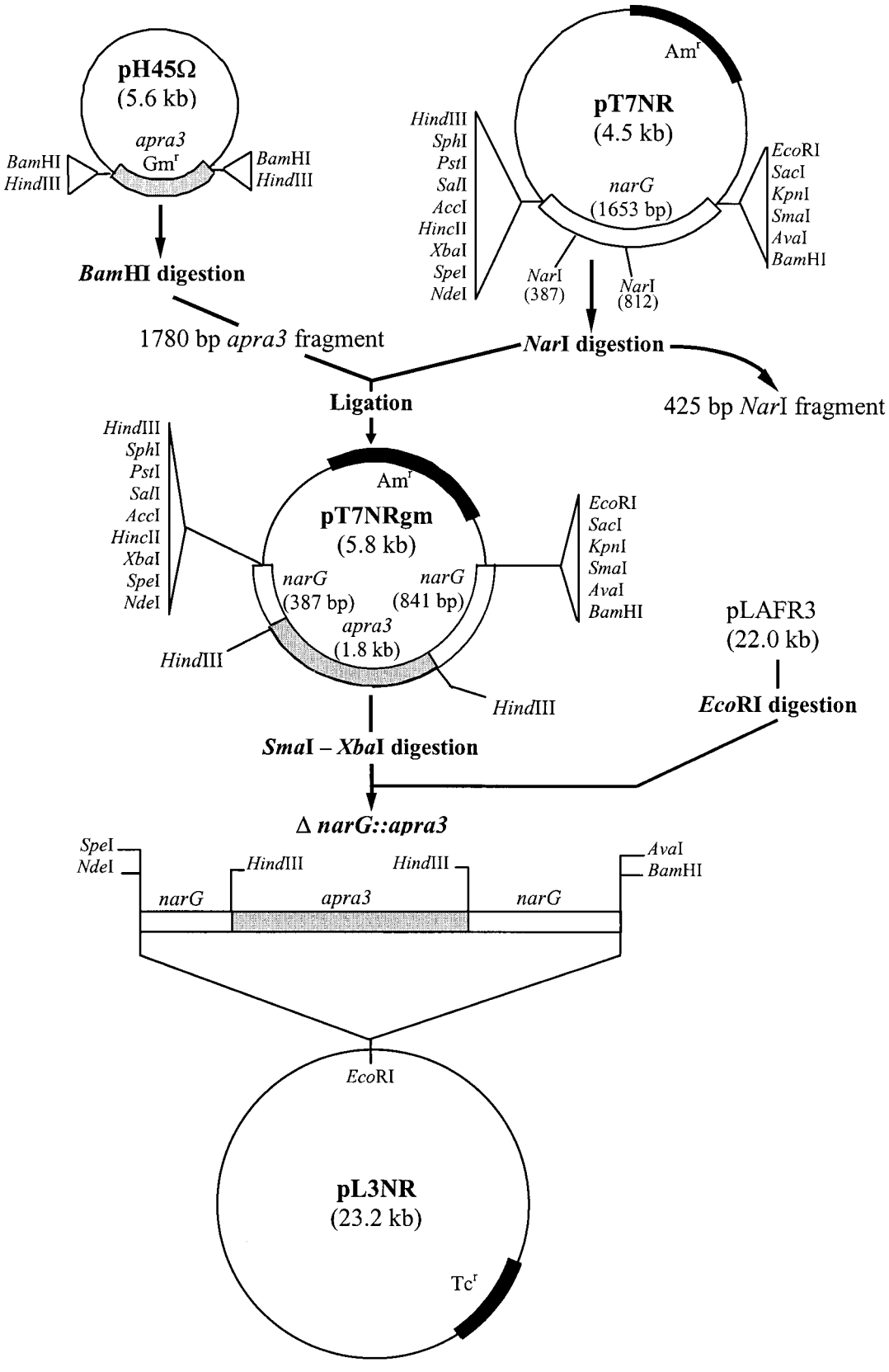

FIG. 1. Construction of a cosmid vector containing the deleted *narG* gene with an insertion consisting of a gentamicin resistance (Gm^r) gene. A 1.8-kb *Bam*HI fragment containing the *apra3* gene from pHP45Ω was ligated into the *Nar*I sites of pT7NR after filling in to generate blunt ends. The resulting deleted *narG* gene with an insertion of the *apra3* gentamicin resistance gene was then cut from the pT7NRgm plasmid with *Sma*I-*Xba*I and cloned into the *Eco*RI site of pLAFR3 (37).

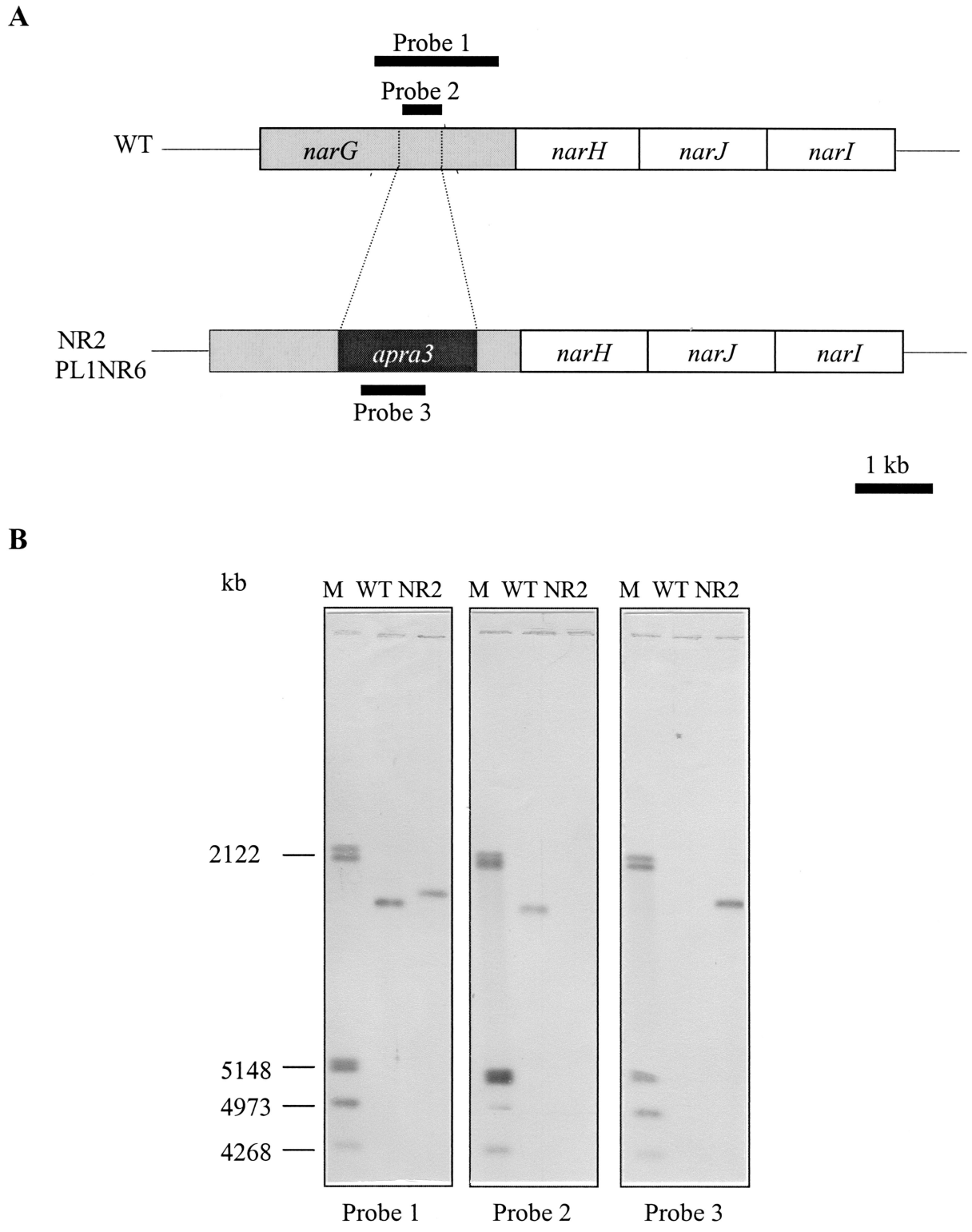

FIG. 2. Deletion-insertion of the *nar*G gene coding for the catalytic subunit of the membrane-bound nitrate reductase of the wild-type strain C7R12 (WT) and of the PL1 mutant. (A) The 1.6-kb fragment of the *narG* gene (probe 1), the deleted fragment of the *narG* gene (probe 2), and an internal fragment of *apra3* gene (probe 3) were used as probes; their sizes and localizations are indicated. (B) Southern analysis of *Xba*I-digested chromosomal DNA from the wild-type strain and NR2 mutant with probes 1, 2, and 3, labeled with digoxigenin-11-dUTP (Boehringer, Mannheim, Germany). As identical hybridization profiles were obtained with both NR2 and PL1NR6, only those of NR2 are presented. Probe 1 lanes show the presence of a higher band for the NR2 mutant than for C7R12 resulting from the 425-bp *Nar*I deletion and newly introduced 1.8-kb *apra3* gene in the *narG* gene. Probe 2 lanes show the presence of a hybridization signal only for the wild-type strain C7R12, indicating replacement of the *narG* gene with the deleted one in the NR2 mutant. Probe 3 lanes show the presence of a band similar in size to the one obtained with probe 1 only in the NR2 mutant, indicating the presence of *apra3* in the *narG* deleted gene.

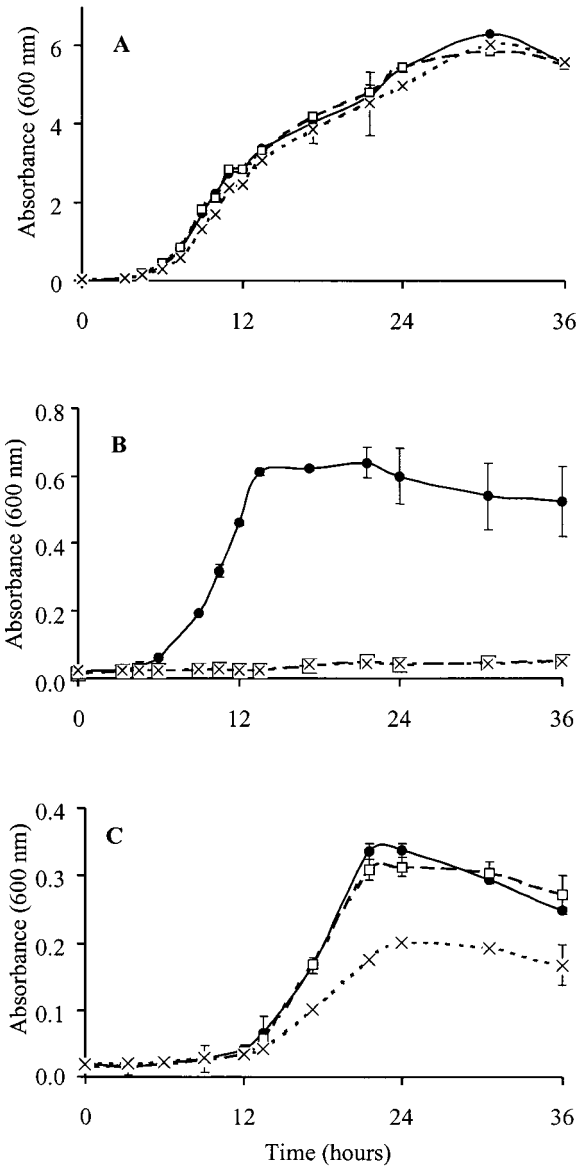

FIG. 3. Growth kinetics of C7R12 (●), NR2 (□), and PL1NR6 (×) under aerobic conditions in LB medium (A) and under anaerobic conditions in LB medium supplemented either with KNO_3 (20 mM) (B) or with KNO_2 (10 mM) (C). Error bars, standard deviations.

angular transformed before statistical analysis. Transformed values of microbial enumeration and of mutant-to-total-inoculant ratio were submitted to repeated-measure analysis using Statview software (1996 release; Abacus Concepts, Inc., Berkeley, Calif.). The significant threshold value was fixed at $P = 0.05$.

Nucleotide sequence accession number. The sequence of the 1.6-kb *narG* fragment has been deposited in the GenBank database under accession number AF 197465.

RESULTS AND DISCUSSION

Construction of the Nar$^-$ mutants of *P. fluorescens* C7R12. The two Nar$^-$ mutants, NR2 and PL1NR6, were constructed in the present study by disrupting the *narG* gene in the wild-type strain (C7R12) and in the Pvd$^-$ mutant PL1, respectively. The deduced amino acid sequence of the 1.6-kb amplified fragment shows 83% identity to the corresponding NarG subunit from *P. fluorescens* YT101 (29). Southern blot analysis on chromosomal DNA digested with *Xba*I from C7R12, NR2, and PLINR6 confirmed the allelic exchange of the *narG* gene in the NR2 and PL1NR6 mutants as described in Fig. 2.

Growth characteristics of the Nar$^-$ mutants of *P. fluorescens* C7R12. Growth characteristics of the Nar$^-$ mutants and wild-type strain under conditions of aerobiosis and anaerobiosis with nitrate or nitrite as the electron acceptor were compared.

Since similar growth kinetics were recorded with the wild-type strain and the Nar$^-$ mutants under aerobic conditions, the mutants appeared to be not affected in their ability to use oxygen as the sole electron acceptor (Fig. 3A). As expected the Nar$^-$ mutants were unable to use nitrate as the sole electron acceptor to sustain growth, while the wild-type strain reached an absorbance of 0.6 after 12 h (Fig. 3B). Altogether, these data validate the use of the Nar$^-$ mutants obtained for further ecological studies, in the same way that the use of the Pvd$^-$ mutant PL1 was previously validated (25).

With nitrite as the sole electron acceptor, the wild-type strain and NR2 mutant had similar growth rates, reaching an absorbance of 0.3 after 20 h (Fig. 3C). Unexpectedly, the mutant PL1NR6, affected in the synthesis of both pyoverdine and nitrate reductase, showed a lower growth rate than that of the wild-type strain and of the Nar$^-$ mutant NR2 when cultivated with nitrite as the sole electron acceptor (Fig. 3C). A reduced growth of the Pvd$^-$ mutant PL1 was also recorded under these experimental conditions (data not shown). Altogether, these data suggest a possible interaction between the pyoverdine-mediated iron uptake and the nitrite reduction. Expression of respiratory enzymes containing heme molecules, such as the denitrifying nitrate reductase, was previously showed to be iron dependent (5). The heme d_1 was also described in the nitrite reductase encoded by the *nirS* gene (41), and thus, the expression of this denitrifying enzyme would also be expected to be iron dependent. The presence of the *nirS* gene, recently described in *P. fluorescens* C7R12 (EMBL accession number AF197467) (30), is then in favor of our hypothesis on the possible relation between the pyoverdine-mediated iron uptake and the nitrite reduction in C7R12. This hypothesis is further supported by the lower denitrification rate by *P. fluorescens* C7R12 recorded under iron-limiting conditions as in-

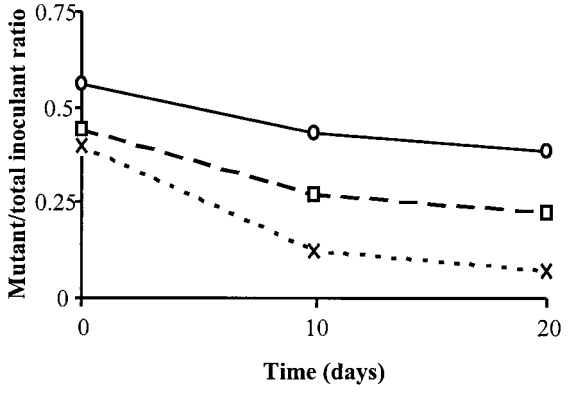

FIG. 4. Dynamics of the PL1 (○), NR2 (□), and PL1NR6 (×) mutant-to-total-inoculant ratios, with all experiments conditions (soil compartments and values of matric potential) being combined.

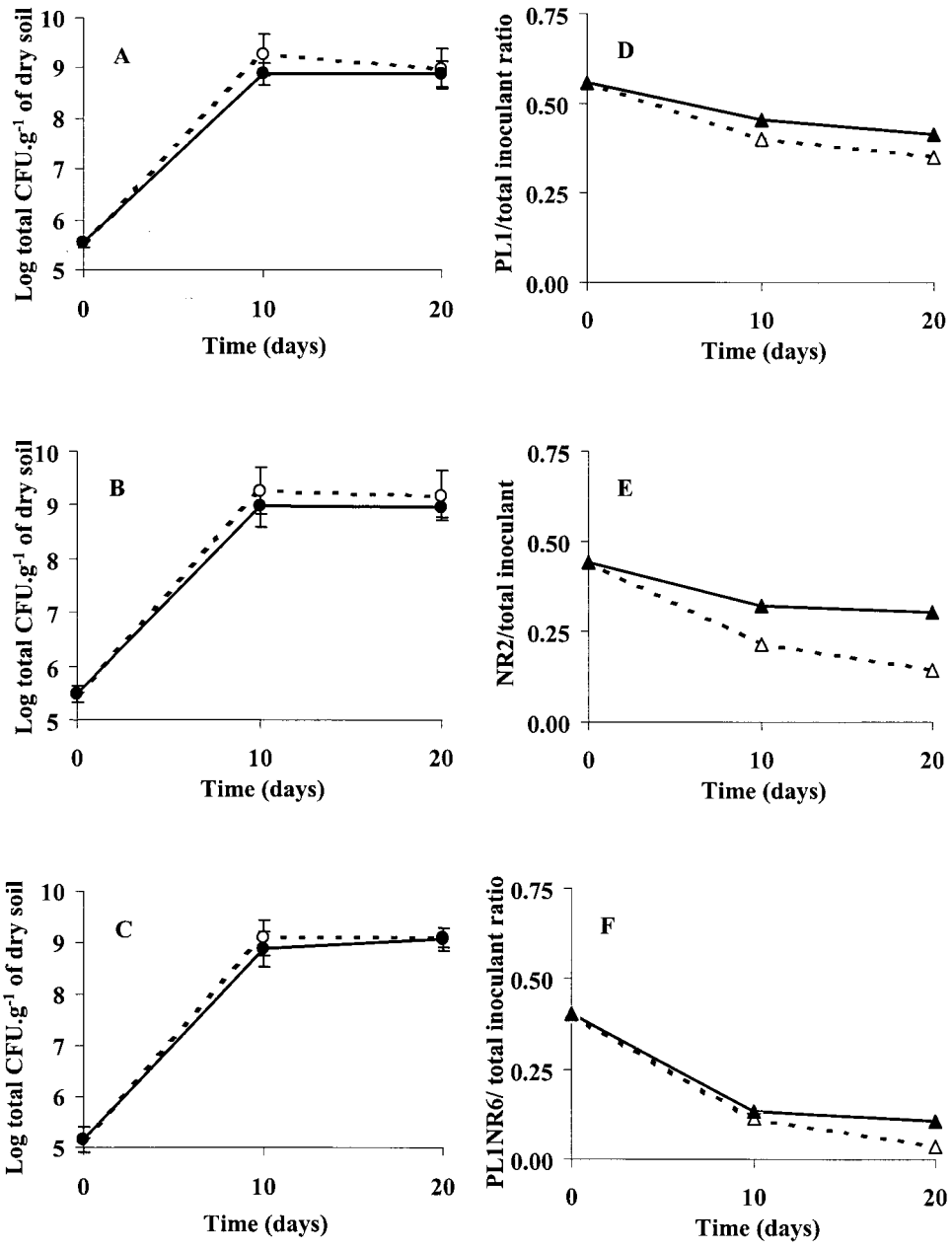

FIG. 5. Dynamics of the combinations of the wild-type strain C7R12 with the mutant PL1 (A), NR2 (B), or PL1NR6 (C) and of the ratios of the mutants PL1 (D), NR2 (E), and PL1NR6 (F) to total inoculant in soil kept at −1 kPa (open symbols) and −10 kPa (closed symbols). Error bars, standard deviations.

dicated by the level of N_2O production by C7R12, which was 4.3 times lower in the absence than in the presence of ferric iron after 29 h of growth in succinate medium.

Compared competitiveness of Pvd$^-$ mutant and Nar$^-$ mutants. Data for all environmental conditions (matric potential and soil compartments) were combined for each mutant (Fig. 4). All three mutants showed a reduced competitiveness compared to the wild-type strain as indicated by the decrease of the different mutant-to-total-inoculant ratios. These data indicate that nitrate reductase and pyoverdine are involved in the fitness of the wild-type strain *P. fluorescens* C7R12. This conclusion is in agreement with previous studies showing the involvement of nitrate reductase and of pyoverdine in the competitiveness of *P. fluorescens* YT101 and *P. fluorescens* C7R12, respectively (8, 25).

Combination of all data from the different environmental conditions further showed that overall the competitive advantages given by nitrate reductase and by pyoverdine are similar, as indicated by the lack of interaction between the mutant-to-total-inoculant ratio and time ($P = 0.11$) (Fig. 4). As expected, the PL1NR6 curve differed significantly ($P < 0.05$) from those of PL1 and NR2, indicating that the double mutation of genes encoding nitrate reductase and pyoverdine synthesis resulted in a lower competitiveness of the double mutant compared to the single mutants NR2 and PL1.

Nitrate reductase and pyoverdine were shown to give a com-

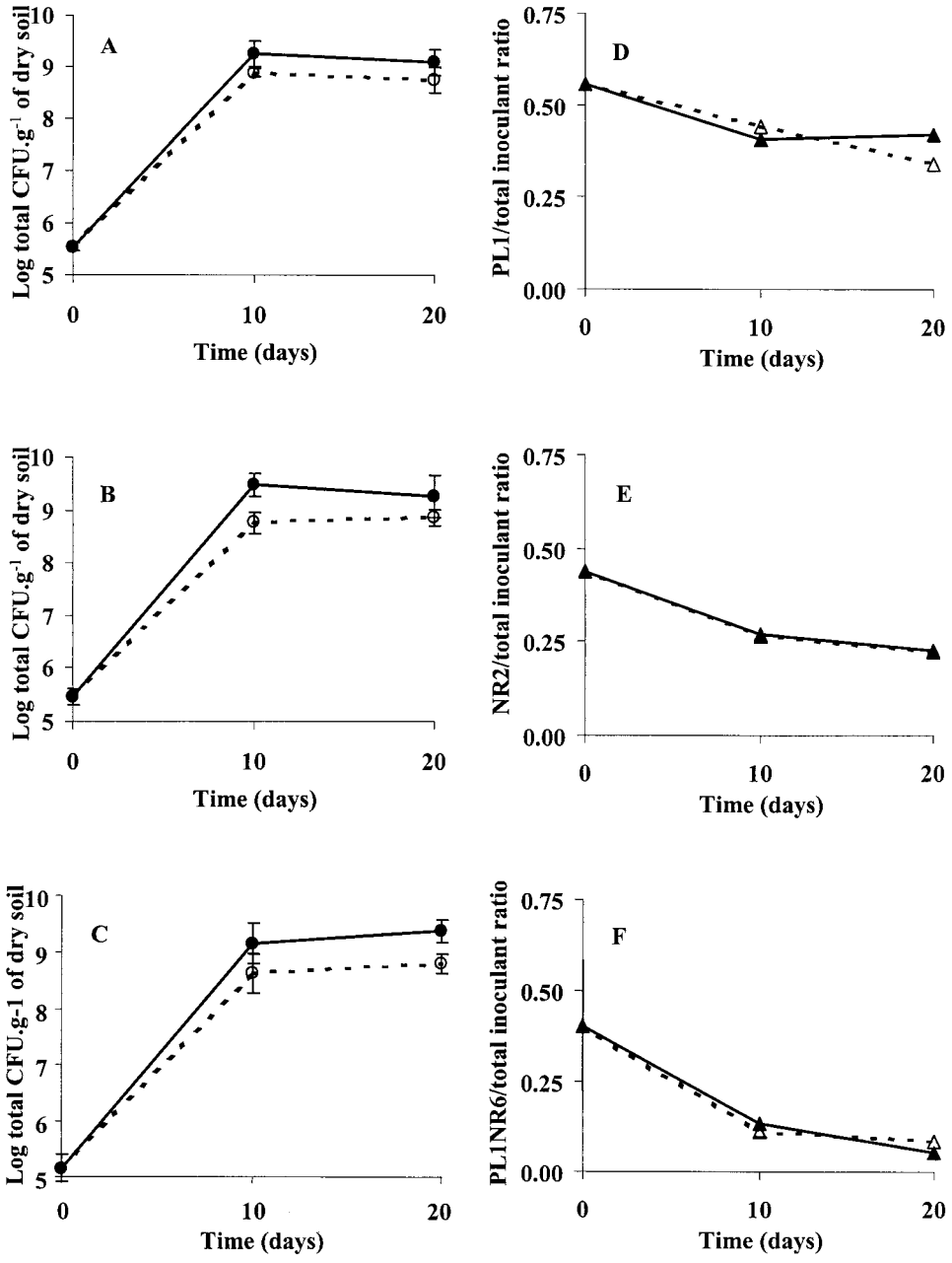

FIG. 6. Dynamics of the combinations of the wild-type strain C7R12 with the mutant PL1 (A), NR2 (B), or PL1NR6 (C) and of the ratios of the mutants PL1 (D), NR2 (E), and PL1NR6 (F) to total inoculant in bulk soil (open symbols) and rhizosphere soil (closed symbols). Error bars, standard deviations.

petitive advantage over the wild-type strain, although the intensity of this advantage varied according to the environmental conditions and to the mutants as described below.

Influence of matric potential on competitiveness of the Pvd$^-$ mutant and Nar$^-$ mutants. Figure 5A to C show, at matric potentials of −1 and −10 kPa, the population dynamic of the combinations of the wild-type strain C7R12 together with the mutant PL1, NR2, or PL1NR6, respectively. For all microbial combinations, the total bacterial density (wild-type strain plus mutant), initially at 10^5 to 10^6 CFU · g of dry soil^{-1}, increased to roughly 10^8 to 10^9 CFU · g of dry soil^{-1} 10 days after inoculation and remained constant at day 20. The densities of microbial combinations, including mutants PL1 and PL1NR6, were nonsignificantly different ($P = 0.1$ and $P = 0.9$, respectively) at both values of matric potential (Fig. 5A and C), while a significant difference ($P < 0.05$) was recorded for the combination including NR2 (Fig. 5B).

For all microbial combinations, the mutant-to-total-inoculant ratio decreased with time (Fig. 5D to F), indicating a reduced competitiveness of the mutants compared to the wild-type strain. However, the influence of the matric potential varied according to the mutants. For the combination including the Pvd$^-$ mutant PL1, the decrease of the mutant-to-total-inoculant ratio did not differ significantly ($P = 0.07$) at either

matric potential (Fig. 5D). In contrast, this ratio decreased significantly ($P < 0.05$) more at −1 than at −10 kPa for the combinations including the Nar$^-$ mutants NR2 and PL1NR6 (Fig. 5E and F). Altogether, these data indicate that the competitiveness of the Pvd$^-$ mutant PL1 did not differ with regard to the matric potential and that the one of the Nar$^-$ mutants NR2 and PL1NR6 was significantly lower at a potential of −1 kPa than at a potential of −10 kPa. These observations confirm our initial hypothesis suggesting that the selective advantage given to the wild-type strain C7R12 by the nitrate reductase would be expressed under conditions of lower aeration. Several studies have stressed the impact of matric potential on soil-borne fluorescent pseudomonads (3, 10, 11, 27). However, to our knowledge, the present study is the first to demonstrate the implication of an enzyme in the bacterial adaptation to variations of matric potential.

Influence of plant roots on competitiveness of Pvd$^-$ mutant and Nar$^-$ mutants. Figure 6A to C show, in bulk and rhizosphere soil, the population dynamic of the combinations of the wild-type strain C7R12 together with the mutants PL1, NR2, and PL1NR6, respectively. For all microbial combinations, the total bacterial density (wild-type strain plus mutant), initially at 10^5 to 10^6 CFU · g of dry soil^{-1}, increased to 10^8 to 10^9 CFU · g of dry soil^{-1} 10 days after inoculation and remained constant at day 20. Total bacterial densities were always significantly higher ($P < 0.05$) in the rhizosphere than in bulk soil (Fig. 6A to C). These data show that a rhizosphere effect was expressed towards the strains introduced even if this expression was not as high as the one recorded under nongnotobiotic conditions as previously described (25). The observation of a rhizosphere effect under the present experimental conditions warrants the comparison of the competitiveness of the different mutants made in soil and rhizosphere when combined with the wild-type strain.

For all microbial combinations, the mutant-to-total-inoculant ratios decreased with time (Fig. 6D to F), indicating a reduced competitiveness of the mutants compared to the wild-type strain. In contrast, the competitiveness of the mutants did not differ significantly in the bulk and rhizosphere soil, as shown by the lack of significant differences in the decrease of the mutant-to-total-inoculant ratio ($P = 0.23$, $P = 0.82$, and $P = 0.48$, for PL1, NR2, and PL1NR6, respectively) in these two environments (Fig. 6D to F). Altogether, these data indicate that nitrate reductase and pyoverdine were implicated in both soil and rhizosphere competence, and then more generally in the saprophytic competence of the wild-type strain. This conclusion supports the data of Mirleau et al. (25) showing that the higher competitiveness of the wild-type C7R12 over the Pvd$^-$ mutant PL1 was expressed both in bulk and rhizosphere soil.

Conclusion. In this study, nitrate reductase and pyoverdine were shown to be involved in the intraspecific competitiveness of the biocontrol agent *P. fluorescens* C7R12 under soil conditions. The competitive advantage given to the wild-type strain by nitrate reductase and pyoverdine over the defective mutants was expressed not only in the rhizosphere but also in bulk soil, indicating that these two bacterial traits are implicated in the bacterial saprophytic competence in soil environments. The selective advantage given to C7R12 by the nitrate reductase was more strongly expressed under conditions of lower aeration, in such a way that the ability of *P. fluorescens* C7R12 to switch from one metabolic pathway (respiratory chain) to the other (nitrogen respiration) would account for its ability to remain competent under various soil environment conditions. Further research should now be performed to determine if the bacterial traits shown in the present study to be involved in the intraspecific competitiveness of the biocontrol agent *P. fluorescens* C7R12 also play a role in this agent's interspecific competitive ability in the presence of indigenous microflora.

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