Identification of Traits Implicated in the Rhizosphere Competence of Fluorescent Pseudomonads: Description of a Strategy Based on Population and Model Strain Studies

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Abstract The lack of consistency of the beneficial effects of inoculated fluorescent pseudomonads has often been related to their bad survival in the rhizosphere. In this review, we describe the strategy followed over the last decade to study traits involved in the rhizosphere competence of these bacteria. The diversity of indigenous populations associated with plant roots was first compared to that of populations associated with uncultivated soils in order to identify traits that discriminate these populations. The involvement of these bacterial traits in the rhizosphere competence was then assessed by comparing the competitiveness of a wild-type strain to that of mutants affected in the corresponding phenotypes. Finally, traits shared by populations adapted to the rhizosphere were identified by comparing both the competitiveness in the rhizosphere and the metabolism of a collection of bacterial strains. The data yielded indicated that rhizosphere competent pseudomonads show a specific metabolism especially characterized by the efficiency of the pyoverdine-mediated iron uptake and by the ability to reduce nitrogen oxides.

Keywords Diversity • Metabolism • Model strain • Mutant • Population

Résumé – Identification de caractères impliqués dans la compétence rhizosphérique des *Pseudomonas* spp. fluorescents: description d'une stratégie basée sur des études de populations et de souche modèle. Le manque de fiabilité des effets bénéfiques déterminés par les *Pseudomonas* spp. fluorescents inoculés a souvent été attribué à leur mauvaise survie dans la rhizosphère. Au cours de cette synthèse, nous décrivons la stratégie suivie lors des dix dernières années pour étudier les caractères impliqués dans la compétence rhizosphérique de ces bactéries. La diversité des populations indigènes associées aux racines a d'abord été comparée à celle des populations associées à des sols nus. L'implication de ces caractères bactériens dans la compétence rhizosphérique a ensuite été évaluée en comparant la compétitivité d'une souche modèle à celle de mutants affectés dans les phénotypes correspondants. Finalement, des caractères partagés par les populations adaptées à la rhizosphère ont été identifiés en comparant la compétitivité rhizosphérique et le métabolisme d'une collection de souches. Les données recueillies indiquent que les *Pseudomonas* spp. fluorescents adaptés à la rhizosphère présentent un métabolisme spécifique caractérisé, en particulier, par l'efficacité de leur système d'acquisition du fer basé sur les pyoverdines et par leur aptitude à réduire les oxydes d'azote.

Mots clés Population • Diversité • Souche modèle • Mutant • Métabolisme

1 Introduction

Saprophytic fluorescent *Pseudomonas* spp. constitute an oxidase-positive group, including various species *Pseudomonas fluorescens*, *P. putida*, *P. chlororaphis*

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(Bossis et al., 2000; Palleroni, 1984) and more recently described species, as for example, *P. jessenii* (Verhille et al., 1999) or *P. lini* (Delorme et al., 2002). All bacteria belonging to this group share the ability to produce soluble yellow-green pigments, pyoverdines, which act as siderophores for these bacteria (Meyer and Abdallah, 1978). These microorganisms are considered to be rhizobacteria, since their densities and activities are stimulated in the rhizosphere (Bowen and Rovira, 1976; Höfte et al., 1992; Kluepfel, 1993; Schroth et al., 1992). They are known to synthesize a variety of secondary metabolites (Budzikiezwicz, 1993; Dowling and O'Gara, 1994; Leisinger and Margraff, 1979). Some of them exert a toxic activity against various pathogenic and deleterious microorganisms, contributing to a reduction of their saprophytic growth (microbial antagonism) (Cook et al., 1995; Gutterson, 1990). Microbial antagonism against pathogenic agents decreases the frequency of root infections and of diseased plants, whereas antagonism against deleterious microorganisms enhances plant growth (Schippers et al., 1987). Plant growth promotion can also be ascribed to metabolites affecting the plant physiology such as growth substances (Glick, 1995). Specific bacterial metabolites may elicitate defense reactions of the host plant (induced systemic resistance) (Van Loon et al., 1998).

Consequently, some fluorescent pseudomonads are known to improve plant health and/or growth (Gamalero et al., 2002; Lemanceau and Alabouvette, 1993; Lifshitz et al., 1987; Weller, 1988). They have been shown to play a role in the natural suppressiveness of soils to fusarium-wilts (Alabouvette and Lemanceau, 1996; Baker et al., 1986) and to take-all (Cook and Rovira, 1976; Lucas et al., 1989; Raaijmakers and Weller, 1998). Indeed, fluorescent pseudomonads are considered as potential biocontrol agents of soilborne diseases and several studies have demonstrated their efficacy as microbial inoculants (Cook et al., 1995; Haas et al., 1991; Lemanceau and Alabouvette, 1991; Moulin et al., 1996; Schippers et al., 1995; Thomashow and Weller, 1988). Despite the positive effects reported in these studies, overall biological control of soilborne diseases achieved by fluorescent pseudomonads is often inconsistent (Lemanceau and Alabouvette, 1993; Weller, 1988). This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria (Schippers et al., 1987). 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 plant (Bull et al., 1991; Raaijmakers et al., 1995). In order to improve the efficacy and the consistency of the biological control, the use of rhizosphere-competent strains is required. To fulfill this requirement, progresses must be made in our knowledge of bacterial traits promoting rhizosphere-competence of fluorescent pseudomonads.

When initiating the strategy described in the present review, most of the studies consisted in evaluating the impact of traits, expected to be important for root colonization, such as presence of flagella (De Weger et al., 1987; Howie et al., 1987; Scher et al., 1988), pili (Vesper, 1987), agglutinin (Anderson et al., 1988), membrane lipopolysaccharrides (De Weger et al., 1989), porin OprF (De Mot and Vanderleyden, 1991), protease (O'Sullivan et al., 1991) or phenazines (Mazzola et al., 1992). Evaluation of the involvement of these traits consisted in comparing the survival of a model strain and of mutant of that strain affected in the phenotype studied. Even if this strategy contributed to progress in the knowledge of the traits promoting rhizosphere competence of the strains studied (Lugtenberg and Dekkers, 1999; Lugtenberg et al., 2001), it also had limitations. Indeed, results obtained with specific strains and experimental conditions could not always be extended to other strains (De Weger et al., 1987; Howie et al., 1987; Scher et al., 1988). The differences that were recorded between strains are not surprising considering the high diversity of fluorescent pseudomonads which have been described largely after these differences were first noted (Clays-Josserand et al., 1995; Delorme et al., 2002; Keel et al., 1996; Laguerre et al., 1994; Latour et al., 1996; Lemanceau et al., 1995; Mac Spadden Gardener et al., 2000; Mavrodi et al., 2001; Meyer et al., 2002; Raaijmakers and Weller, 2001). Furthermore, the strategy described above did not reveal the importance of traits others than those expected at first to be involved in rhizosphere competence which are not necessarily the most relevant. Thus, the knowledge of the bacterial traits involved in rhizosphere competence was necessarily partial. This was already clearly stressed by De Weger et al. (1995) when comparing the phenotype of the strain *P. fluorescens* WCS365 to those of the 20 mutants, obtained by random mutagenesis, showing an impaired root colonization compared to the wild-type strain. Among these 20 mutants, 3 did not present any difference compared to the wild-type strain in the traits expected to be involved in the rhizosphere competence, clearly indicating that other unknown traits were involved in the rhizosphere competence of *P. fluorescens* WCS365. Surprisingly, at that time little attention was given to the possible involvement of carbon and energy metabolism of the bacteria in their rhizosphere competence despite the fact that the characteristics of the rhizosphere are mostly influenced by the release of photosynthetates by the host-plant (Lynch and Whipps, 1990; Whipps, 1987).

Altogether these observations led us to propose in the early 1990s a strategy based both on population and on model strain studies. We first compared the diversity of populations associated with roots and with uncultivated soils in order to discriminate traits expected to be involved in the rhizosphere competence in a non targeted way. The hypotheses, raised from the population studies, were then evaluated by comparing the competitiveness of a wild-type strain and of isogenic mutants affected in the phenotypes discriminating the rhizospheric and soil populations. Besides the emergence of hypotheses, the initial population studies enabled us to select strains representative of the diversity of populations from rhizospheric and bulk soils. The reduced number of strains considered allowed us to compare their metabolism and their competitiveness in the rhizosphere and then to identify traits shared by the rhizosphere competent populations.

In this review, we describe this strategy developed over the last years in our group to identify traits involved in the rhizosphere competence of fluorescent pseudomonads.

2 Comparison of the Diversity of Populations from Rhizospheric and Bulk Soils

The first step of our strategy consisted in raising hypotheses on traits possibly involved in the rhizosphere competence of fluorescent pseudomonads in a nontargeted way. For that purpose, the diversity of indigenous populations of fluorescent pseudomonads associated with roots and with uncultivated soil was compared in order to possibly identify discriminating phenotypes assumed to be involved in the rhizosphere competence. Since soils are oligotrophic environments whereas the great majority of soilborne microorganisms, including the fluorescent pseudomonads, are heterotrophic, we hypothesized that nutrient competition and consequently carbon and energy metabolism of bacteria would play a major role in their adaptation to the rhizosphere. This environment is indeed characterized by the release of a significant part of the photosynthetates that would be more readily used by the microorganism having the most adapted catabolism. This catabolism is based on electron transfers between donors (organic compounds) and acceptors (coenzymes, Fe–S proteins, cytochromes, oxygen, nitrogen oxides). Rhizospheric and soil populations were then characterized for their ability to use a wide range of organic compounds and their ability to mobilize iron and to respire nitrogen oxides. More specifically, the ability of the bacterial strains to grow in the presence of 1 of the 49 sugars, 49 organic acids or 49 amino acids some of which having been described as component of the root exudates (Vancura, 1980, 1988), has been tested using API strips (BioMerieux). Ferric iron is a cofactor of proteins indispensable to the oxido-reduction processes of respiratory chain and is then essential for microbial metabolism. However, the concentration of Fe^{3+} available to the soilborne microflora is very low (Lindsay, 1979), in such way that this ion is usually a limiting factor for microbial growth and activity in soil habitats (Loper and Buyer, 1991). The ability to mobilize ferric iron of the bacteria was assessed by measuring their Minimal Inhibitory Concentration (MIC) of 8-hydroxiquinolin, a strong iron chelator (Geels and Schippers, 1983); (Lemanceau et al., 1988). Bacteria able to reduce NO_3^- (nitrate reducers) and among them those able to produce N_2O and/or N_2 (denitrifiers) were determined by biochemical methods (Clays-Josserand et al., 1995).

In order to enhance the possible rhizosphere effect, plants were continuously cultivated for 5 cycles of 8 weeks each in a given soil (Dijon, France) and the bacterial isolations were made on the last culture. The Dijon soil was kept uncultivated and was maintained in the same environmental conditions as the cultivated soil. Furthermore, in order to stress more clearly the plant effect on the soilborne populations, they were isolated along a horizontal gradient (rhizospheric soil, rhizoplane, and root tissues). All these experiments were performed with two plant species (*Linum usitatissinum* L. and *Lycopersicon esculentum* Mill.). Numerical analysis of the results enabled us to cluster isolates showing a high level of similarity (Latour, 1996).

The data yielded indicate that the plant affects both the structure and diversity of the indigenous soilborne populations of fluorescent pseudomonads. Indeed, some of the phenotypic clusters only included rhizospheric isolates whereas another only included soil isolates, clearly indicating that the rhizospheric isolates show differences in their abilities to use organic compounds compared to those of soil. More precisely, it was shown that the ability to use specific sugars (saccharose, trehalose, xylose), polyols (inositol, sorbitol) and amino acids (citrulline, trigonelline) is more frequent among rhizospheric populations than soil populations (Latour and Lemanceau, 1997); (Lemanceau et al., 1995). Overall, rhizospheric strains showed a higher similarity than those isolated from bulk soil, indicating that the diversity of the rhizospheric populations was reduced compared to that of the soil populations. This reduced diversity was even more strongly expressed for the endophytic populations. The ability to mobilize iron of the rhizospheric and soil populations was also shown to differ significantly. Indeed, the frequency distribution of the strains in the different MIC differed significantly according to their origin. The rhizospheric populations were mostly distributed in the classes corresponding to the highest concentrations of 8-hydoxiquinolin, indicating that they were more able to mobilize ferric iron in deficiency conditions than soil populations (Lemanceau et al., 1988). Finally, the proportion of strains able to respire nitrates was significantly higher in the populations associated with the roots (90 and 82% in the root tissues of flax and tomato, respectively), than in the uncultivated soil (55%). Among these strains, the proportion of denitrifiers gradually and significantly increased in the vicinity of tomato roots (44, 68, 75 and 94% in uncultivated soil, rhizosphere, rhizoplane and root tissues, respectively) and was higher in the flax rhizoplane (66%) than in the uncultivated soil.

Altogether, these data indicate that plants select specific populations of fluorescent pseudomonads and that compared to soil populations they are (i) more able to mobilize ferric iron, (ii) more frequently nitrate reducers and denitrifiers, (iii) able to use specific organic compounds as carbon and energy sources.

Since microbial inoculations would be performed on plants growing in various soils, the impact of the soil type on the plant selection towards the indigenous soilborne populations was further assessed. The two plant species were grown in two different soils and the populations were analyzed as described above. The influence of the soil type on the rhizosphere effect was then considered by comparing data collected in two different soils (Dijon and Châteaurenard, France). Although the selection recorded previously in the Dijon soil was checked in the Châteaurenard soil, this selection appeared to differ in the two soils (Latour et al., 1996). The populations associated with the roots of a given plant species cultivated in the two soils differed significantly in their ability to use the organic compounds tested. Differences in the indigenous soilborne populations only partly accounted for the variations of the populations associated with a given plant species cultivated in these two soils. Indeed the variation of the rhizosphere effect determined by a same plant species was shown to differ in the two soils even when their indigenous microflora was destroyed by γ-irradiation and replaced by the same calibrated community of fluorescent pseudomonads. The rhizosphere effect was more strongly expressed in the Châteaurenard soil and the structure of the populations re-isolated differed significantly in the rhizosphere of the plants cultivated in the Châteaurenard and in the Dijon soil (Latour et al., 1999). These data clearly indicate that it was not possible to identify common organic compounds specifically used by fluorescent pseudomonads associated with the roots of plants cultivated in different soils.

In contrast, indigenous populations of fluorescent pseudomonads selected by the two different plant species cultivated in the two soils have in common the ability to efficiently take up iron (Lemanceau et al., 1988) and to respire nitrogen oxides (Clays-Josserand et al., 1999). From these observations, we then hypothesized that these two traits might be involved in the rhizosphere competence of fluorescent pseudomonads.

3 Evaluation of the Involvement of Nitrate Reductase and Pyoverdine in the Rhizosphere Competence of a Model Strain

The second step of our strategy consisted then in assessing the above referred hypotheses, using a model strain and isogenic mutants of this strain. The *P. fluorescens* strain C7R12 is a rifampicin-resistant mutant of the strain C7 (Eparvier et al., 1991), previously isolated from the rhizosphere of flax cultivated in Châteaurenard soil (Lemanceau et al., 1988). This strain was chosen as a model strain since it was shown (i) to be a good denitrifying strain (Clays-Josserand et al., 1995), (ii) to efficiently mobilize the iron (Lemanceau et al., 1988), (iii) to be rhizosphere-competent (Eparvier et al., 1991) and (iv) to be a biocontrol agent (Lemanceau and Alabouvette, 1991). Fluorescent pseudomonads have evolved an efficient strategy of iron uptake based on the synthesis of the siderophore pyoverdine and its relevant outer membrane receptor (Meyer et al., 1987). The possible implication of pyoverdine, in the ecological competence of *P. fluorescens* C7R12 in soil and rhizosphere, was evaluated using a pyoverdine minus mutant (Pvd$^-$) obtained by random insertion of the transposon Tn*5* (Mirleau, 2000). The Tn*5* flanking DNA was amplified by inverse PCR and sequenced. The nucleotide sequence was found to show a high level of identity with *pvsB*, a pyoverdine synthetase. As expected, the mutant Pvd$^-$ was significantly more susceptible to iron starvation than the wild-type strain despite its ability to produce another unknown siderophore. As with the wild-type strain, the mutant Pvd$^-$ was able to incorporate the wild-type pyoverdine and 5 pyoverdines of foreign origin, but at a significantly lower rate despite the similarity of the outer membrane protein patterns of the two strains (Mirleau et al., 2000). The survival kinetics of the wild-type strain and of the mutant Pvd$^-$ in bulk and rhizospheric soil were compared in gnotobiotic and non-gnotobiotic conditions. In gnotobiotic conditions, when inoculated separately both strains showed a similar survival in soil and rhizosphere suggesting that iron was not a limiting factor. In contrast, when inoculated together, the bacterial competition was favorable to the pyoverdine producer C7R12. The fitness of the mutant Pvd$^-$ in the presence of the indigenous microflora, even when coinoculated with C7R12, was assumed to be related to its ability to uptake heterologous pyoverdines. Altogether, these results suggest that pyoverdine-mediated iron uptake is involved in the ecological competence of the strain *P. fluorescens* C7R12 (Mirleau et al., 2000).

The involvement of nitrogen oxide respiration in the rhizosphere competence of *P. fluorescens* C7R12 was also assessed by comparing the competitiveness of the wild-type strain to that of a mutant affected in nitrate reductase synthesis (Nar$^-$). Nitrate reductase catalyses the first step of denitrification. The corresponding experiments were performed under gnotobiotic conditions. The Nar$^-$ mutant was obtained by site-directed mutagenesis (Mirleau et al., 2001). The selective advantage given by nitrate reductase over the wild-type strain was assessed by measuring the dynamic of the mutant-to-total-inoculant (wild-type strain plus mutant) ratio. The Nar$^-$ mutant clearly showed a lower competitiveness than the wild-type strain, indicating that nitrate reductase is important. However, the selective advantage given by nitrate reductase was more strongly expressed under conditions of lower aeration (Mirleau et al., 2001). Comparison of the competitiveness of the Pvd$^-$ and Nar$^-$ mutants indicated that the competitive advantages given to C7R12 by nitrate reductase and pyoverdine were similar. A double mutant (Pvd$^-$Nar$^-$), obtained by site-directed mutagenesis of the Pvd$^-$ mutant, presented the lowest competitiveness (Mirleau et al., 2001).

Altogether, these data indicate the importance of pyoverdine-mediated iron uptake and nitrate respiration on the fitness of the biocontrol agent *P. fluorescens* C7R12. The competitive advantage given to the wild-type strain by pyoverdine and nitrate reductase 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.

4 Identification of Bacterial Traits Shared by Rhizosphere Competent Populations

The conclusions made following the model strain approach described above are obviously only valid for the model strain studied and only concerned two traits.

In order to extent our conclusions to populations of fluorescent pseudomonads and to assess the possible involvement of other metabolic activities, we came back to a population approach for the next step of our strategy. In contrast with the first step of the strategy aiming at the identification of traits shared by the populations selected by the plant, we now assessed metabolic characteristics shared by populations adapted to the rhizosphere.

The adaptation to the rhizosphere was evaluated by measuring the competitiveness of the studied populations in the rhizosphere of tomato cultivated in soil, in the presence of the indigenous microflora and microfauna (non-gnotobiotic conditions). The corresponding experiments are very labour intensive and could then only be performed on a limited number of strains (23 strains). Twenty-one strains came from the selection of one strain from each of the clusters previously defined (Latour et al., 1996; Lemanceau et al., 1995) with the 340 strains tested. These 21 strains are then expected to be representative of the diversity of the larger bacterial collection from which they are issued (Delorme, 2001). Two reference strains were added: (a) *P. fluorescens* C7R12 studied in the model strain approach (see Section "Evaluation of the Involvement of Nitrate Reductase and Pyoverdine in the Rhizosphere Competence of a Model Strain") and (b) *P. fluorescens* A6 known for its ability to promote plant growth (Gamalero et al., 2002) and to suppress soilborne diseases (Lemanceau and Samson, 1983; Berta et al., unpublished data). The results obtained with these 23 strains clearly indicate the high diversity of the strains for their competitiveness in the tomato rhizosphere. Indeed, the survival rate of the strains varied from 0.11% for the less competitive strain to 61.4% for the most competitive (Delorme et al., unpublished data).

Relations between these observations and metabolic characteristics already described above – such as the ability to assimilate a wide range of organic compounds and the ability to reduce NO_3^- and/or N_2O, and additional traits such as the ability to synthesize extracellular enzymes (gelatin liquefaction, levan production) (Lelliot et al., 1966), to synthesize *N*-acyl-homoserine-lactones (NAHL) involved in quorum sensing (Elasri et al., 2001), to synthesize phenazines (antibiotic compounds) (Raaijmakers et al., 1997), the characterization of the pyoverdine-mediated iron uptake (siderotype, ability to incorporate heterologous pyoverdines) (Meyer et al., 1997) – were evaluated by appropriated statistical methods. Multiple correspondence analyses were first applied to identify possible traits explaining the bacterial competitivity, and were then followed by mean multiple comparisons and variance analyses to determine if these traits were indeed involved in the rhizosphere competence of the fluorescent pseudomonads (Delorme, 2001).

These statistical analyses indicated that the populations selected by the plant were more competitive than the populations isolated from bulk soils. However, some strains isolated from the rhizosphere were not necessarily competitive in this environment, confirming then the relevance of the competitivity experiments developed by Delorme et al. (unpublished data).

Excepted one strain, the most competitive strains all belonged to the same siderotype, which was different from that of the less competitive strains showing various other siderotypes. The only strain differing by its siderotype from the other competitive strains also differed from these strains by its ability to synthesize NAHL and phenazine. The most competitive strains were all able to reduce nitrogen oxides to dinitrogen gas. In contrast, the most competitive strains did not belong to the same phenotypic clusters indicating that these strains could not be differentiated from the less competitive on the basis on their auxanogram. The only electron donors used in common by the most competitive strains was trehalose. Altogether, these data clearly show the importance of the carbon and energy metabolism and more especially of specific electron acceptors (ferric iron, nitrogen oxides) in the rhizosphere competence of the fluorescent pseudomonads.

All the most competitive strains had the gelatinase. Half of them produced levansucrase whereas none of the less competitive showed this ability. These enzymes contribute to modifications of the root environment which may be favorable for bacterial survival in the rhizosphere. Indeed, the activity of the gelatinase, a protease with a broad spectrum, leads to the release of essential amino acids and then contribute to their increased availability for the bacteria (Curl and Truelove, 1986; Simons et al., 1997). In the same way, the synthesis of the levan, a polymer of the fructose known to be present in the rhizosphere, contribute to the aggregation of soil adhering to roots and then favor a more porous structure in rhizosphere soil (Bezzate et al., 2000).

Concerning bacterial secondary metabolism, statistical analyses of the data indicated that the ability to produce NAHL explains rhizosphere competence of the fluorescent pseudomonads tested. The only strain producing phenazine, showing also the ability to synthesize NAHL, appeared to be competitive in the rhizosphere, however this was not significant since there was only that strain having this ability among those tested.

A major conclusion of this study is that the rhizosphere competence has a multifactorial determinism that confirms the limitations of the research made only on specific traits. As an example, it appeared that (a) the most competitive strains have the ability to use trehalose, (b) however some non-competitive strains show also this ability, (c) but these non-competitive strains do not have the ability to denitrify and then (d) the competitive strains present both the ability to use trehalose and to denitrify (Delorme, 2001).

Another major conclusion is that depending on the strains, the strategy of adaptation to the rhizosphere differs. The data yielded allowed us to stress two types of behavior. In the first one, the strains show a specific carbon and energy metabolism. They share the same siderotype, they are able to fully reduce nitrogen oxides and they have the ability to assimilate trehalose and to produce gelatinase. The competitivity of the second type would rather be ascribed to its ability to antagonize the indigenous microflora through the synthesis of antibiotic (phenazine) and its probable regulation through NAHL production as described in the strain *P. aureofaciens* 30–84 (Wood et al., 1997).

5 Discussion

This review describes the strategy that we developed over the last decade in order to identify bacterial traits involved in the rhizosphere competence of fluorescent pseudomonads. The originality of this strategy is to have associated both population and model strain approaches (Fig. 1).

Our study strategy first consisted in comparing indigenous populations associated with roots and bulk soils in order to identify traits allowing the discrimination of these two types of populations, these traits being then expected to be involved in the rhizosphere competence. During these diversity studies, our attention was focused on the bacterial carbon and energy metabolism because of the importance of trophic relations among microorganisms, mostly heterotrophic, in soils (Lemanceau et al., 1988; Lockwood, 1964) and rhizospheres (Curl and Truelove, 1986; Duijff et al., 1999). The diversity studies were conducted in the rhizosphere of two plant species cultivated in two different soils, and in these same soils kept uncultivated. Comparison of the diversity of bacterial populations associated with a given soil and with roots of a given plant species was also performed by Mavingui et al. (1992) and Frey et al. (1997). And the possible crop specificity towards fluorescent pseudomonads was previously showed by Glandorf et al. (1993). However, to our knowledge, our studies were among the very first to include both the effects of the plant species and of the soil-type on the selection achieved by the host-plant towards bacterial soil populations. These studies allowed us to identify traits shared by pseudomonads populations selected by two different plant species cultivated in two different soils.

The implication of these traits in the rhizosphere adaptation was then evaluated by a model strain approach that consisted in comparing the rhizospheric competitiveness of a wild-type strain to that of mutants. This approach based on the comparison of the survival kinetics of wild-type strains to that of mutants, affected in specific phenotypes, has been commonly used to assess the involvement of these phenotypes in the rhizosphere competence of various bacterial strains (Carroll et al., 1995; De Weger et al., 1995; Duijff et al., 1997; Höfte et al., 1992; Mazzola et al., 1992). However, an originality of our work was to evaluate the relative importance of two bacterial traits thanks to the use of single and double mutants. Another originality was to assess the impact of a specific environmental parameter (soil aeration) on the involvement of the studied traits in the rhizosphere competence of the wild-type strain. The choice of this parameter was made since aeration is known (a) to vary a lot in the rhizosphere and (b) to affect bacterial fitness in the rhizosphere (Höjberg and Sörensen, 1993; Höjberg et al., 1999; Meikle et al., 1995).

Besides the identification of traits expected to be involved in rhizosphere competence, our early diversity studies enabled us to select strains representative of the populations associated with soils and roots. This reduced number of strains made possible the evaluation of their competitiveness in the rhizosphere.

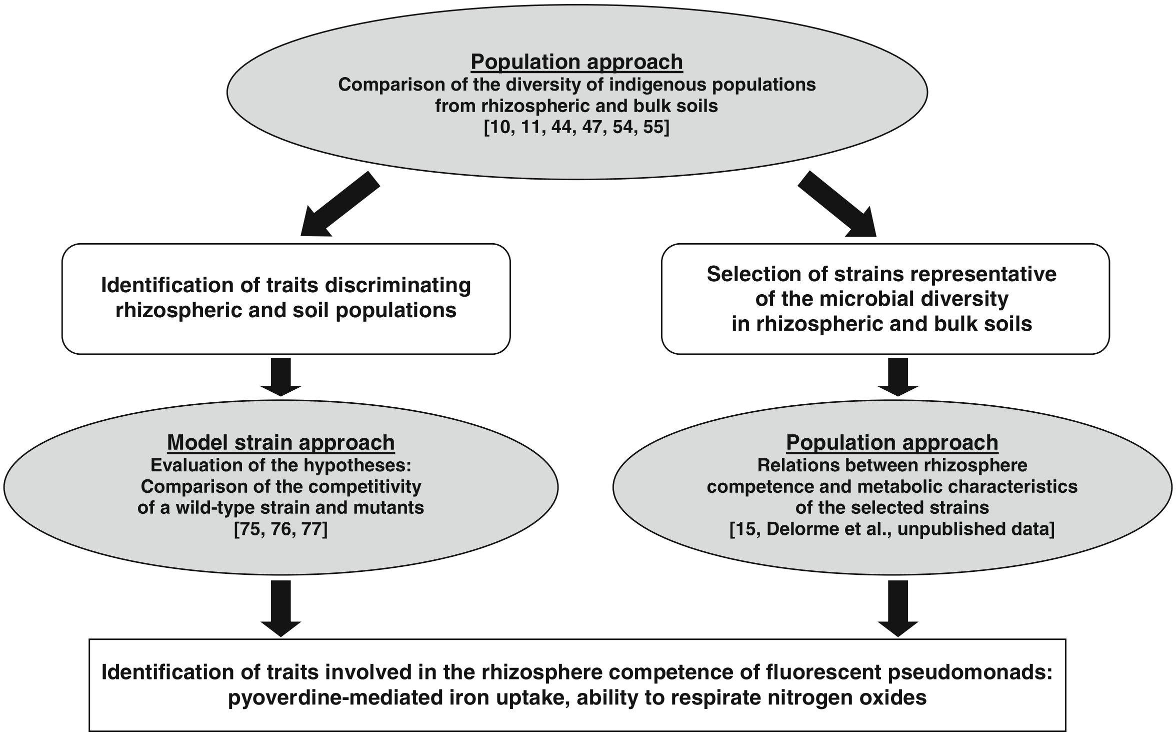

Fig. 1 Schematic representation of the approaches followed to identify bacterial traits involved in the rhizosphere competence of fluorescent pseudomonads

A similar approach based on the selection of strains representative of the diversity, assessed by PCR-RAPD, of the 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. enabled Raaijmakers and Weller (2001) to select the superior root-colonizing *P. fluorescens* Q8r1-96. In our case, the level of competitiveness in the rhizosphere of the selected strains was compared to several characteristics of their metabolism in order to identify traits shared by populations adapted to the rhizosphere. This type of relation was applied by Ellis et al. (2000) to identify conserved traits in fluorescent pseudomonads with antifungal activity.

The main conclusion obtained following the strategy developed is that the carbon and energy metabolism of fluorescent pseudomonads plays a major role in their rhizosphere competence.

More specifically our data show the importance of the ability to use ferric iron and nitrogen oxides as electron acceptors in the rhizosphere competence of fluorescent pseudomonads. This conclusion agrees with the fact that (a) fluorescent pseudomonads require oxygen or nitrogen oxides for oxidization of all the substrates, excepted arginine (Latour and Lemanceau, 1997), and that (b) the ferric iron and oxygen content is usually low in the rhizosphere (Höjberg and Sörensen, 1993; Höjberg et al., 1999; Loper and Henkels, 1997). The ability to use efficiently both types of electron acceptors is favorable to the respiratory potential of fluorescent pseudomonads. This potential directly affects the assimilation of nutrients by *Pseudomonas* and consecutively their multiplication. Indeed, aerobic respiration and denitrification allow both the production of ATP by oxidative phosphorylation and the reoxidation of NADH and $FADH_2$ coenzymes. This reoxidation is required for the cycle of Krebs that appears to play a major role for pseudomonads metabolism and more specifically for the nutrient assimilation by these bacteria (Latour and Lemanceau, 1997). Ecological observations made by the group of Lugtenberg on the rhizosphere competence of the model strain *P. fluorescens* WCS365 are in agreement with these metabolic characteristics. Indeed, the ability to use organic acids in the rhizosphere, oxidized by the cycle of Krebs, are involved in the rhizosphere competence of this model strain (Lugtenberg et al., 2001), whereas the ability to use sugars by Entner-Doudoroff pathway does not confer a competitive advantage to this bacterial strain (Lugtenberg et al., 1999).

In fact, from our data it was not possible to identify a common auxanogram shared by the populations being the most competitive in the rhizosphere. This observation could be related to the ability of the pseudomonads to use a wide range of organic compounds which would then allow them to adapt their metabolism to various environments and more specifically to the evolution of composition of the root exudates according to the plant species, the plant development and the soil type.

Despite the progresses made in the knowledge of the bacterial traits involved in the rhizosphere competence of fluorescent pseudomonads, our study strategy presents limitations. The first one is that only metabolic traits, mostly related to carbon and energy metabolism of bacteria, were taken in account. Other relations than trophic between the microflora and the host-plant may probably also play a role in the rhizosphere competence of the fluorescent pseudomonads: molecular recognition through signal molecules and/or membrane characteristics (Dénarié et al., 1996; Duijff et al., 1999), ability to stand or detoxify toxic compounds, etc… A second limitation is that even if the number of traits tested were important during the population studies, they still remain quite low and moreover were chosen a priori. Other strategies than the one described in the present review were aimed at searching untargeted traits involved in the rhizosphere competence of model strain, following an approach based on random mutagenesis to identify genes targeted by the insertion sequence in mutants showing an impaired root colonization (Lam et al., 1990; Lugtenberg and Dekkers, 1999) or to identify genes preferentially expressed in the rhizosphere (Rainey, 1999).

Development of methods of functional genomic and proteomic should allow to provide new insights on the microbial determinism of the rhizosphere competence. Further research should also take more in consideration the effect of the host-plant in the interaction plants – microorganisms, and plant traits affecting the rhizosphere microflora should also be determined. The final goal would be to identify plant/microbe couples the most favorable for the survival and activity of the beneficial introduced organisms.

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