World Journal of Agricultural Sciences 4 (S): 901-907, 2008
ISSN 1817-3047


# Diversity of Fluorescent Pseudomonads in Different Rhizospheres[(1)]

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**Abstract:** Root colonization by plant-growth-promoting rhizobacteria (PGPR) can be increased depending on the type of rhizosphere. The aim of the present study was to determine fluorescent Pseudomonad diversity in lettuce, parsley, arugula and chicory. Roots from these rhizospheres were sampled at different properties of small commercial producers in Campinas, state of Sao Paulo, Brazil. In the diversity analyses, Pseudomonad isolates were submitted to biochemical and physiological tests. Multivariate analysis of characteristics allowed the clustering of isolates with high levels of similarity. The parsley rhizosphere had the least fluorescent Pseudomonad diversity.

**Key words:** Specificity · PGPR · Diversity · Lettuce · Parsley · Arugula · Chicory

## INTRODUCTION

Plant productivity can be increased by plant-growth-promoting rhizobacteria (PGPR). These rhizobacteria have been found in several cultures, such as wheat [1], ornamentals plants [2], lettuce [3-5], soybean [6], citrus [7] and pine and oak [8]. The beneficial effects of PGPR can be either direct or indirect. Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth through the production of growth-regulating substances such as auxins [9], cytokinins [10] and gibberellins [11, 12] as well as through the solubilization of phosphate minerals [13]. Indirect growth promotion occurs through the elimination of pathogens by the production of β-1.3-glucanase [14], antibiotics [15], cyanide [16] and siderophores [17]. Thus, fluorescent pseudomonads can suppress a variety of soil-borne diseases, such as fusarium wilts [4].

Along with the use of rhizobacteria on plant growth promotion and biological control, many species of fluorescent pseudomonads have also been employed in environmental conservation and bioremediation of contaminated soils [18,19]. However, the use of fluorescent pseudomonads is often inconsistent. This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria. Therefore, it is necessary to study the ecology of these microorganisms in the rhizosphere as well as root colonization mechanisms, host specificity and the influence of environmental factors.

Among the factors affecting the bacterial colonization of roots, plants play a major role. Thus, the size and composition of rhizobacteria communities have been described as plant-dependent. Plant roots liberate organic compounds into the soil and the exudation of these compounds play a key role in the selective stimulation of microorganisms [20]. The aim of this study was to evaluate the phenotypic diversity of fluorescent pseudomonads in different rhizospheres. The diversity of fluorescent pseudomonads associated in four different plant species (lettuce, parsley, arugula and chicory) was compared.

## MATERIALS AND METHODS

Isolates from fluorescent pseudomonads were obtained from parsley, lettuce, arugula and chicory rhizospheres at eight small commercial farms in Campinas in the state of Sao Paulo, Brazil. Each root was vigorously shaken and placed in an Erlenmeyer flask with 0.01 M $MgSO_4 7H_2O$. Soil suspensions were obtained after 30 min of agitation. Suspensions from the rhizospheres were then spread on Petri dishes with King's B medium and incubated for 24 h at 28-30°C.

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(1)Part of the first author's master's dissertation

Fluorescent pseudomonads were chosen randomly from colonies that revealed fluorescent pigment in light near the ultraviolet wavelength on King's B medium [21]. Fifty bacterial colonies were collected. Each fluorescent pseudomonad isolated was submitted through eleven tests: production of phenazine, gelatinase, catalase and arginine dihydrolase, use of D-trehalose, L-tryptophan and citrate, reduction of nitrate, egg yolk reaction and growth at 41°C and 4°C. Almost all variables were tested by the methods described by Lelliott *et al*. [22]. The use of D-trehalose and L-tryptophan was tested by the method described by Lemanceau *et al*. [20] and the use of citrate, by the method described by Simmons [23]. Growth at 41°C and 4°C was tested according to Stanier *et al*. [24]. The results of all these tests were scored as growth occurrence (+) or growth absence (-) and it was confronted and classified according to Palleroni [25] and Stanier *et al*. [24].

Multivariate analysis was performed for the statistical analysis of phenotypic diversity. Group analyses were performed using Euclidian distance and nearest neighbor methods. The analyze of *Pseudomonas putida* and *Pseudomonas fluorescens* distribution in different rhizospheres was made by Fisher´s exact test at 5%. The statistical analyses were processed by the Genes and Statistica software programs.

## RESULTS

In order to identify the *Pseudomonas* spp. found on rhizosphere of lettuce, arugula, parsley and chicory, it were utilized eleven tests, as demostrated on Table 1, after that, it was identificated by a key of identification according to Palleroni [25].

The occurrence of the following species of fluorescent pseudomonads was observed in the rhizospheres of lettuce, arugula, parsley and chicory (Table 2): *Pseudomonas putida*, *Pseudomonas fluorescens*, phytopathogenic *Pseudomonas* (*P. syringae* and *P. cichori*), other saprophytes and intermediate *Pseudomonas* (*P. putida / P. fluorescens*). Bacteria with the same characteristics of *P. fluorescens* and *P. putida* were considered intermediate *Pseudomonas,* according to Lemanceau *et al*. [20] and Zago [26].

The distribution of *Pseudomonas fluorescens* and *Pseudomonas putida* among the different rhizospheres by Fisher´s exact test at 5% (Table 3), revealed no significant difference on the colonization by *P. putida* and *P. fluorescens.*

The tests that most contributed to the identification and to phenotypic diversity of fluorescent pseudomonads in the present study were gelatinase production and egg yolk reaction (Fig. 1). Although arginine dihydrolase

Table 1: Discrimination among Pseudomonads isolateds from rhizosphere of lettuce, arugula, salsa and chicory, throught differents tests

| | Lettuce | % total | Arugula | % total | Parsley | % total | Chicory | % total |
|---|---|---|---|---|---|---|---|---|
| Tests | 19* | 100 | 15* | 100 | 10* | 100 | 6* | 100 |
| Gelatinase | 9** | 47.0 | 8 | 53.0 | 3 | 30.0 | 4 | 67.0 |
| Grown at 41ºC | 4 | 21.0 | 2 | 13.0 | 1 | 10.0 | 0 | 0.0 |
| Grown at 4ºC | 15 | 79.0 | 14 | 93.0 | 8 | 80.0 | 4 | 67.0 |
| D-trehalose | 0 | 0.0 | 4 | 27.0 | 0 | 0.0 | 3 | 50.0 |
| L-tryptophan | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Reduction of nitrate | 19 | 100.0 | 14 | 93.3 | 10 | 100.0 | 4 | 67.0 |
| Citrate | 18 | 94.7 | 15 | 100.0 | 10 | 100.0 | 6 | 100.0 |
| Arginine dihydrolase | 19 | 100.0 | 14 | 93.0 | 10 | 100.0 | 6 | 100.0 |
| Catalase | 19 | 100.0 | 15 | 100.0 | 10 | 100.0 | 6 | 100.0 |
| Fenazine | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| Egg yolk reaction | 10 | 53.0 | 9 | 60.0 | 1 | 10.0 | 2 | 33.0 |

* Total number of isolates from each rhizosphere.

** Numbers represent positive reactions

Table 2: Species distribution of fluorescent pseudomonads among four species of plants

| | *P. syringae, P. cichori* | *Pseudomonas fluorescens* | *P.putida* | *P. putida/P. fluorescens* | Others |
|---|---|---|---|---|---|
| Plants | *N° | N° | N° | N° | N° |
| Lettuce | 1 | 0 | 6 | 8 | 4 |
| Rucula | 1 | 3 | 3 | 6 | 2 |
| Parsley | 0 | 0 | 8 | 1 | 1 |
| Chicory | 0 | 2 | 3 | 0 | 1 |
| Total | 2 | 5 | 20 | 15 | 2 |

* Number of isolates from each rhizosphere

Table 3: Distribution of *Pseudomonas fluorescens* and *Pseudomonas putida* among four species of plants

| | *Pseudomonas fluorescens* | | *P.putida* | | |
|---|---|---|---|---|---|
| Plants | *N° | % of total | N° | % of total | *p* |
| Lettuce | 0 | 0 | 6 | 30 | 0.2189 |
| Rucula | 3 | 0 | 3 | 15 | 0.0644 |
| Parsley | 0 | 60 | 8 | 40 | 0.1165 |
| Chicory | 2 | 0 | 3 | 15 | 0.2146 |
| Total | 540 | 100 | 20 | 100 | |

*P*<0.05 in Fisher's exact test is significative

Table 4: Origin and taxonomic classification of fluorescents pseudomonads isolates belonging to the phenotypic clusters defined in Fig. 2

| | | Plants | | | | |
|---|---|---|---|---|---|---|
| Clusters | | Lettuce (19)* | Rucula (15) | Parsley (10) | Chicory (6) | Species predominant in each group |
| 1 | (6)** | 3 | 2 | 1 | 0 | Saprophytic pseudomonads |
| 2 | (10) | 3 | 1 | 5 | 1 | *P. putida* |
| 3 | (6) | 3 | 2 | 1 | 0 | *P.putida/P. fluorescens* |
| 4 | (7) | 2 | 3 | 1 | 1 | *P.putida/P.fluorescens* and *P. putida* |
| 5 | (7) | 3 | 1 | 2 | 1 | *P. putida* |
| 6 | (2) | 0 | 2 | 0 | 0 | *P. fluorescens* |
| Number of isolates clustered | | 14 | 11 | 10 | 3 | |
| (% in relation to total) | | 74 % | 73% | 100% | 50% | |

* Total number of isolates ** Number of isolates in each group

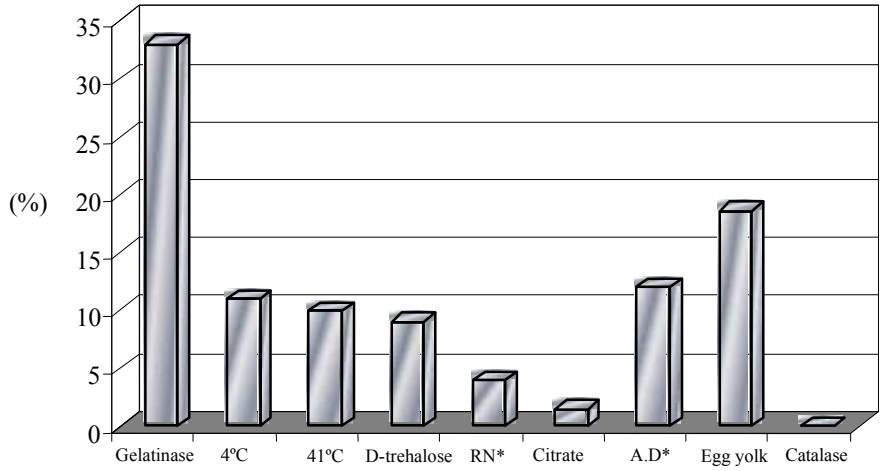


Fig. 1: Relative contribution of each test for the identification of fluorescent pseudomonads. Descriptive statistics informing the coefficients of phenotypic variation (CV) obtained of 9 biochemical descriptors *RN: Nitrate redutase; AD: Arginine dihidrolase

production, growth at 4°C and at 41°C and the use of D-trehalose contributed less than gelatinase production and egg yolk reaction, these tests were also very important for discrimination among the isolates of bacteria. The reduction of nitrate and the use of citrate also contributed, though in a smaller proportion. Catalase production did not contribute to the identification analysis or phenotypic diversity (Fig. 1).

The evaluation of fluorescent pseudomonad diversity in the lettuce, arugula, parsley and chicory rhizospheres was accomplished through the construction of a dendrogram as well as Euclidian distance and nearest neighbor methods (Fig. 2).

Clusters of fluorescent pseudomonad isolates were formed with basis on similarity and isolates with more than 80% similarity were clustered (Table 4). Thirty

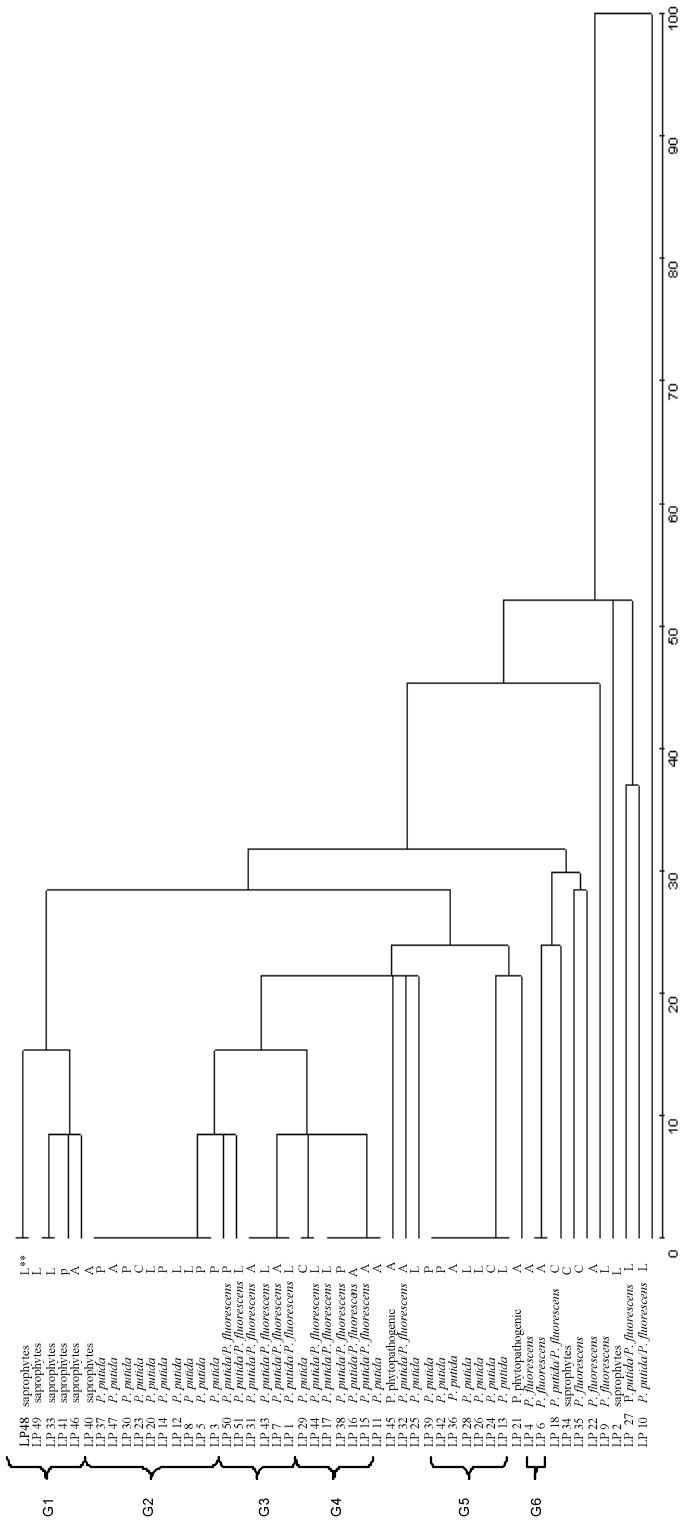


Fig. 2: Dendrogram of phenotypic characteristics of fluorescent pseudomonad from rhizospheres of lettuce * (L), arugula (A), parsley (P) and chicory (C). G1: Group 1; G2: Group 2; G3: Group 3; G4: Group 4, G5: Group 5 and G6: Group 6 (All descriptors)

eight of the 50 fluorescent pseudomonad isolates were included in 6 clusters. Only the isolates classified as a phytopathogenic pseudomonad (*P. syringae* and *P. cichori*) were not clustered. Fluorescent pseudomonad isolates from the parsley rhizosphere (100%) were clustered more often than isolates from lettuce (74%), arugula (73%) and chicory (50%) (Table 4).

## DISCUSSION

The ability of bacteria to survive in soil determines their success in colonizing rhizospheres. In this study, the distribution of *P. fluorescens* and *P. putida* among the different rhizospheres, by Fisher´s exact test at 5% (Table 3). It could be concluded that there is no significant difference on the colonization by *P. putida* and *P. fluorescens* according to the type of rhizosphere. However, Latour *et al.* [27] observed a greater occurrence of *P. fluorescens* than *P. putida* in the rhizosphere of flax and tomato plants. The preference for a particular type of fluorescent Pseudomonas depends on the characteristics of the soil and type of plant. Clays-Josserand *et al.* [28] observed a greater number of *P. fluorescens* in the tomato rhizosphere than in uncultivated soils, whereas the opposite occurred with *P. putida*. The tomato rhizosphere may have released a substance that favored the development of *P. fluorescens* over *P. putida.* Thus, the colonization of bacteria on the rhizosphere could be influenced by the type of host.

Fluorescent pseudomonad isolates from the parsley rhizosphere (100%) were clustered more often than isolates from lettuce (74%), arugula (73%) and chicory (50%) (table 4). These results indicate that diversity among parsley isolates was lower than lettuce, arugula and chicory isolates, suggesting that the parsley is under stronger selective pressure. In the present study, fluorescent pseudomonad diversity in the parsley rhizosphere was lesser than in the other plants. Lemanceau *et al.* [20] found lesser diversity of fluorescent pseudomonas in the tomato rhizosphere when compared to flax. Thus, different plant species may influence microorganisms in the rhizosphere in different ways. Smalla *et al.* [29] observed that microorganisms in the rhizosphere of potato and colza were more similar to one another than ones in the strawberry rhizosphere. Analysis of the distribution of these isolates in different groups with regard to their origin provides evidence that plant species has selective influence on the community of fluorescent pseudomonads in its own rhizosphere, but this influence varies from one species to another.

Regarding root colonization, PGPR can either be specific to the plant species, specific to the cultivar or not plant-specific. Miller *et al.* [30] believed that these differences are due to the quantity and composition of the root exudates, which may vary from one plant species to another and even from a cultivar to another. Thus, differences between isolates in the use of substrates may be related to differences in the composition of the respective root exudates.

It is possible to conclude that the ability of the rhizobacteria to use a specific carbon source is an important characteristic in the selection of microorganisms, which can contribute to functional diversity, defined as the number, type, activities and rates at which an assembly of substrates is used by the microorganism community [31]. This is very important for to production of bacterial inoculants. If a particular isolate introduced in the rhizosphere is able to use a carbon source from the root exudates or otherwise has some mechanism that favors its establishment in the rhizosphere, it has a competitive advantage over bacteria from the soil that do not have this ability. Thus, the development of bacteria present in inoculants would be beneficial [20].

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