European Journal of Plant Pathology **108:** 449–459, 2002.

Dominant colonisation of wheat roots by *Pseudomonas fluorescens* Pf29A and selection of the indigenous microflora in the presence of the take-all fungus

Alain Chapon, Anne-Yvonne Guillerm, Laurie Delalande, Lionel Lebreton and Alain Sarniguet*
*INRA, Unité mixte de recherche INRA/ENSAR Biologie des Organismes et des Populations appliquée à la Protection des Plantes [BiO3P], Domaine de la Motte, B.P 35327, 35653 Le Rheu Cedex, France; *Author for correspondence (Phone: +33223485194; Fax: +33223485180; E-mail: alain.sarniguet@rennes.inra.fr)*

Accepted 12 March 2002

Key words: antagonism, colonisation, fluorescent pseudomonads, rhizoplane, selection, necrotic root

Abstract

Increases in populations of fluorescent pseudomonads on wheat roots are usually associated with take-all decline, natural control of take-all, a disease caused by the fungus *Gaeumannomyces graminis* var. *tritici (Ggt)*. Colonisation by *Pseudomonas fluorescens* strain Pf29A was assessed on the roots of healthy plants and of plants with take-all, and the effect of this bacterium on indigenous populations of fluorescent pseudomonads was studied. The efficacy of Pf29A as an agent for the biocontrol of take-all on five-week-old wheat seedlings was tested in non-sterile conducive soil in a growth chamber. RAPD (random amplification of polymorphic DNA) fingerprinting with a decamer primer was used to monitor strain Pf29A and culturable indigenous rhizoplane populations of fluorescent pseudomonad. Pf29A decreased disease severity and accounted for 44.6% of the culturable fluorescent pseudomonads on healthy plant rhizoplane and 75.8% on diseased plant rhizoplane. Fewer RAPD patterns were obtained when Pf29A was introduced into the soil with *Ggt*. In the presence of *Ggt* and necrotic roots, Pf29A became the dominant root coloniser and dramatically changed the diversity and the structure of indigenous fluorescent pseudomonad populations. The results show that *Ggt* and reduced lesion size on roots can trigger a specific increase in antagonist populations and that the introduction of a biocontrol agent in soil influences the structure of indigenous bacterial populations.

Introduction

Fluorescent pseudomonads are common inhabitants of the rhizosphere and are the focus of many research programmes, as a potential means of eliminating or controlling soil-borne diseases (Burr et al., 1978; Cook and Rovira, 1976; Hoefnagels and Linderman, 1999; Howell and Stipanovic, 1979; Larkin et al., 1996; Lemanceau et al., 1988; Stutz et al., 1986; Weller and Cook, 1983; Xu and Gross, 1986a,b). Fluorescent pseudomonads contribute to take-all decline, a phenomenon in which the level of this root disease naturally decreases in fields after several years of wheat monoculture (Cook and Rovira, 1976). It has been suggested that take-all decline only occurs after a severe outbreak of infection with the fungus *Gaeumannomyces graminis* var. *tritici (Ggt)*, (Gerlagh, 1968; Hornby, 1983). Thus, microbial control follows attacks on the roots by the pathogen, but is efficient only after several wheat plantings.

Some strains of fluorescent pseudomonads applied as seed treatments efficiently decreased the disease in controlled environments (Vojinovicz, 1973), but efficacy in field trials was variable (Chao et al., 1986). Differences in the colonisation abilities of the bacterium seem to account for these variable results (Mapplestone and Campbell, 1989; Xu and Gross, 1986b). The extent to which the host plant root system can be colonised is a major criterion in the selection of antagonistic micro-organisms (Chao et al., 1986; Mapplestone and Campbell, 1989; Weller, 1983; Xu and Gross, 1986b). The indigenous microflora, which may interfere with

colonisation or antagonism by the introduced antagonist, may also contribute to variation in the efficacy of biocontrol in the field (Lucas and Sarniguet, 1998).

It has been demonstrated that the presence of *Ggt* and the necrotic root lesions caused by *Ggt* influence the development of populations of certain types of pseudomonads (Sarniguet et al., 1997; Barnett et al. 1999). Some pseudomonad strains selected at random; i.e. *P. corrugata* 2140R, highly colonised the root lesions (Barnett et al. 1999). A positive correlation was shown between the presence of large populations of phloroglucinol-producing pseudomonads on roots and the occurrence of take-all decline after nine successive wheat monoculture crops (Raaijmakers and Weller, 1998). Thus, the presence of *Ggt* and necrotic roots may be involved in the development of populations of antagonistic bacteria near take-all lesions on wheat roots, a position ideal for the inhibition of further *Ggt* infection as postulated by Rovira and Wildermuth (1981). Several studies have suggested that the pathogen itself is involved in the development of antagonism. For example, field populations of *P. fluorescens* 2–79 are larger on *Ggt*-infected roots in spring and autumn, when environmental conditions favour the development of the soil microflora (Weller, 1983).

In most studies, only a direct effect of the introduced antagonistic organism on the pathogen has been observed (Leben et al., 1987; Troxler et al., 1997; Weller, 1983; Weller and Cook 1983; Wong and Baker, 1984; Xu and Gross, 1986a). The indigenous microbial populations are assumed to be constant. This excludes the likelihood of a possible synergistic or inhibitory action with the introduced antagonistic strain. Recent studies outlined that the introduction of a bacterial inoculum into soil had some effect on the composition on the microbial communities of the rhizosphere (Schwieger and Tebbe 2001, Girlanda et al. 2001). But these works were at first dedicated to the assessment of the side-effects of genetically modified organisms.

To demonstrate that specific relationships exist between a root pathogen and a saprophytic biocontrol agent, the effect of *Ggt* and of the resultant root lesions on colonisation by Pf29A, a *P. fluorescens* strain isolated from a created suppressive soil was assessed. The RAPD fingerprinting technique was used to monitor the introduced strain. The side-effect of the introduction of a wild biocontrol agent to soil was assessed by describing the structure of populations of indigenous fluorescent pseudomonads.

Materials and methods

In vivo *take-all biocontrol assay*

Soil. Soil was collected in 1995 from a field at Le Rheu (France) that had been planted with pea in 1994 and with wheat in the autumn of 1995. Conducive soil was removed from the surface layer (5–35 cm deep), air-dried, ground, sieved and stored in sealed plastic cans at 4 °C. The soil had a high silt content (60% silt, 15.6% clay, 27.8% sand), and it was therefore mixed with sterile sand from the Loire (2 : 1 wt/wt).

Fungal innoculum. Ggt isolate 97/35 was grown for 4 weeks at 25 °C on sterilised moist barley grains. The colonised barley grains were air-dried, ground and sieved (pore diameter 1 mm) (Sarniguet et al., 1992b). The pathogenicity of the *Ggt* inoculum was assessed *in vitro*, using tubes containing perlite and the winter wheat cv. Talent.

Bacterial inoculum and seed preparation

Pf29A was isolated from a suppressive soil from Le Rheu (Sarniguet et al., 1997). Pf29A belongs to a specific REP (repetitive extragenic palindrom)-PCR cluster that mainly contains pseudomonad strains and that is linked to the presence of *Ggt* after three wheat plantings in pots. The strain was stored in 15% (v/v) glycerol nutrient broth at −80 °C. Pf29A was identified as a *P. fluorescens/putida* intermediate, using an API 20 NE test strip for bacterial identification (Biomérieux, Marcy l'Etoile, France) and additional morphological and biochemical tests (e.g. levane and tartrate tests) (Digat and Gardan, 1987; Fahy and Lloyd, 1983; Palleroni, 1984; Stanier et al., 1966). The sequence of the *oprI* gene was consistent with Pf29A belonging to the *P. fluorescens* species (De Vos et al., 1998).

For soil treatment, bacteria were grown on L-medium agar plates for 2 days at 27 °C. Bacteria were scraped from the surface of the agar with a glass rod and suspended in sterile water. Wheat seeds were surface-sterilised by immersion for 5 min in 1% (v/v) NaClO in water, rinsed three times for 5 min each in sterile distilled water and incubated for one day at 27 °C on sterile filter paper saturated with sterile deionized water, to allow the seeds to germinate before treatment.

Biocontrol test

The ability of Pf29A to control take-all was studied in multi-chambered planting containers. Each container consisted of six chambers (32 mm × 31 mm × 152 mm). *Ggt* inoculum was mixed with the conducive soil and sand mixture at a density of 500 propagules per kg of soil mixture. Each chamber was filled with 80 g of soil mixture infested with *Ggt* and was sown with a germinated seed grain of wheat (planted at a depth of 1 cm). Soil mixture not infested with Ggt was used as control. The soil was treated with Pf29A by applying 10 ml of a bacterial suspension, with the concentration adjusted to 10^9 CFU ml^{-1}, to the surface of the soil in each chamber (10^7 CFU g^{-1} of soil). Bacterial inoculation was repeated one week later, in the same conditions, when the roots reached the bottom of the soil chamber. Plants were grown for five weeks under a 14 h-day photoperiod, with a temperature of 15 °C during the day, 10 °C at night and 90% relative humidity. Soil water content was maintained at 50% of water-holding capacity. Containers were weighed weekly and water content was adjusted with non-sterile tap water. The control treatment was identical except that Pf29A was omitted, only water was applied. Two containers were used for controls and bacterial treatments with and without *Ggt*, so 12 plants were used in each case for statistical analysis. Roots were washed, dried and weighed and take-all severity assessed on the basis of root necrosis. Root disease was scored on a five-point scale where 0 = no disease, 1 = 1–10% necrosis, 2 = 11–30%, 3 = 31–60%, and 4 = 61–100% of the root system necrotic. The experiment was repeated.

Isolation of root-colonising bacteria

To determine the levels of culturable aerobic rhizoplane bacteria and fluorescent pseudomonads isolated from the rhizoplane, three plants from each control and from each Pf29A-treatments with and without *Ggt*, were harvested at random. Soil adhering tightly to the roots was removed by gentle washing. Three 1-cm-long segments, taken from the region 2–6 cm below the crown, were excised from each plant. This part of the root system was used because the concentration of necrotic take-all lesions was generally highest in this region. A single replicate consisted of three root segments excised from each plant. These three root segments were placed in a sterile test tube containing 5 ml of sterile water and were subjected to sonication in a Bioblock vibracell 72405 (Fisher Bioblock Scientific, Illkirch, France) twice, for 3 s each at 40 Hz. A serial 1 in 10 dilution of the resulting bacterial suspension and 100 µl aliquots of the dilutions (three replicates per dilution) was plated on TSA 1/10 (Difco, Detroit, MI USA) for total aerobic bacteria and on CNP King B medium (Merck, Darmstadt, Germany) for fluorescent pseudomonads. CNP King B medium is a specific medium for fluorescent pseudomonads that contains 50 µg/l cycloheximide, 45 µg/l novobiocin and 75 IU/l penicillin G. Fluorescent colonies were differentiated from non-fluorescent ones by illumination with 365 nm UV light. Green and blue fluorescent colonies were apparent after 48 h of incubation. Colonies were counted after incubation at 27 °C for 2, 4 and 5 days. Petri dishes containing 50–100 single colonies were selected and the corresponding dilutions identified. Three Petri dishes per dilution prepared from healthy and diseased roots of control and Pf29A-treated plants were chosen. Each dish was divided into 32 sectors and 15 colonies were randomly picked from a different sector of each selected plate. Three populations of 45 colonies were subcultured, resulting in the survival of around 40 colonies. Thus, bacterial communities were analysed using 120 culturable strain populations (3 × 40) derived from each root type (healthy and infected with *Ggt*) of control and Pf29A-treated plants.

The isolated colonies were purified by repeated streaking on TSA 1/10 and CNP King B. Colonies were picked, mixed in 15% glycerol nutrient broth and stored at −80 °C. Bacteria isolated on TSA 1/10 and on King B CNP were used to identify group I pseudomonads and Pf29A.

Identification of rRNA group I pseudomonads and Pf29A

rRNA group I pseudomonads. rRNA group I pseudomonads are considered to correspond to the true genus *Pseudomonas*. This homologous group, defined on the basis of rRNA–DNA hybridisation (Palleroni, 1984) includes the fluorescent pseudomonads (*P. fluorescens, P. putida, P. lundensis, P. aureofaciens and P. chlororaphis*) and non-fluorescent *Pseudomonas (P. corrugata)*. The identification and counting of rRNA group I pseudomonads is facilitated by *oprI* gene amplification (De Vos et al., 1993; 1998). Each

colony isolated from TSA 1/10 was picked, suspended in 1 ml sterile deionised water, boiled for 7 min and immediately centrifuged (1150 *g*, 3 min). PCR was performed in a final volume of 12.5 µl, containing 2.5 µl of boiled bacterial suspension and 10 µl of reaction mix prepared as previously described (De Vos et al., 1993), in a Perkin-Elmer 9600 thermocycler (Roche, Indianapolis IN, USA). The resulting DNA fragments were subjected to electrophoresis in a 1.5% (wt/v) agarose gel. All PCR experiments included a negative control without DNA and a positive control with purified Pf29A DNA.

Typing of Pf29A. RAPD was used for typing strains. From 20 RAPD primers with high GC contents, a decamer, primer E7, 5′ AGATGCAGCC 3′ (Operon, Alameda CA, USA) was selected as a marker for Pf29A because this primer gave the most reproducible and specific pattern, as shown by comparison of the RAPD amplification products of Pf29A and of 21 other strains of fluorescent pseudomonads, which were identified as being close or distal to Pf29A by REP-PCR (Sarniguet et al., 1997).

The RAPD protocol was derived from several different protocols (Barbut et al., 1993; Hilton et al., 1997; Welsh and McClelland, 1990; Williams et al., 1990; Wong et al., 1996). Experiments were carried out with fluorescent pseudomonad populations isolated and stored at −80 °C. Fluorescent pseudomonads were grown overnight at 27 °C on L-agar medium. Each colony was picked, suspended in 1 ml of sterile miliQ water (Milipore, Bedford MA, USA), boiled for 7 min, and briefly centrifuged in a microcentrifuge. The resulting suspension was serially diluted (1/10) in sterile miliQ water and 2.5 µl of each 1/100 dilution was used as the DNA substrate. PCR was performed in a final volume of 15 µl containing 6.6 µl of miliQ water, 1.25 µl of 10 × PCR buffer (Roche, Indianapolis IN, USA), 1.25 µl of 25 mM $MgCl_2$ (Roche, Indianapolis IN, USA), 2 µl of 5 mM dNTPs (Boehringer, Manheim, Germany), 1 µl of 100 µM primer E7 and 0.3 µl of AmpliTaq DNA Polymerase (Roche, Indianapolis IN, USA) (5 U/µl). Amplifications were carried out in a Perkin Elmer 2400 thermocycler as follows: initial denaturation at 94 °C 30 s, followed by 45 cycles of 30 s at 92 °C, 1 min at 32 °C and 2 min at 72 °C, and a final extension for 3 min at 72 °C. A negative control without DNA and a positive control with purified Pf29A DNA were included in each RAPD run. Amplification products were separated by electrophoresis overnight at 20 V in a 1.5% agarose gel.

Statistical analysis

The data collected from the experiment on *in vivo* antagonism were analysed with the mean comparison test of Student–Newman–Keuls ($P = 0.05$), (SAS software, The SAS Institute Inc., Cary N.C., USA). Total aerobic bacterium populations and fluorescent pseudomonad populations were quantified as CFU per 1 cm of root (CFU cm^{-1}), log-transformed and analysed with the Student–Newman–Keuls test ($P = 0.05$). Frequencies of group I pseudomonads and Pf29A, from RAPD analysis, were used to estimate the relative sizes of the populations of these bacteria. Populations were compared using Student's *t* test and the results were expressed as described above.

RAPD profile analysis

BioNumerics software (Applied Maths, Belgium) was used to analyse RAPD profiles and the Dice index with the UPGMA clustering method to determine the degree to which profiles were related. Cophenetic correlations were calculated for each node to estimate the consistence of clusters. Clusters were collapsed on the basis of 38% similarity and consistence nodes. Only those clusters grouping at least 10 strains are described. Pf29A profile was only counted once for construction of the dendrogram and cluster analysis. Shannon–Wiener diversity index was calculated for C, C+ (derived from control plants), T and T+ strains (derived from Pf29A-treated plants) (Groth and Roelfs, 1987). The distribution of the populations was compared with the $2\hat{I}$ test from Kullback (Sokal and Rohlf 1969).

Results

Pf29A decreased take-all

The mean necrosis index of plants treated with *Ggt* and Pf29A (2.2) was significantly lower than that of control plants treated with *Ggt* alone (3.7) (Figure 1). The 2.2 index corresponded to less and smaller lesions. Control and treated plants not inoculated with *Ggt* were free of disease symptoms. The mean root dry weight of plants treated with Pf29A that were grown in soil infested with *Ggt* (43.5 mg per plant) was also significantly higher than that of control plants with only *Ggt* (16.8 mg per plant). In the absence of *Ggt*, bacterial treatment significantly increased mean root dry weight

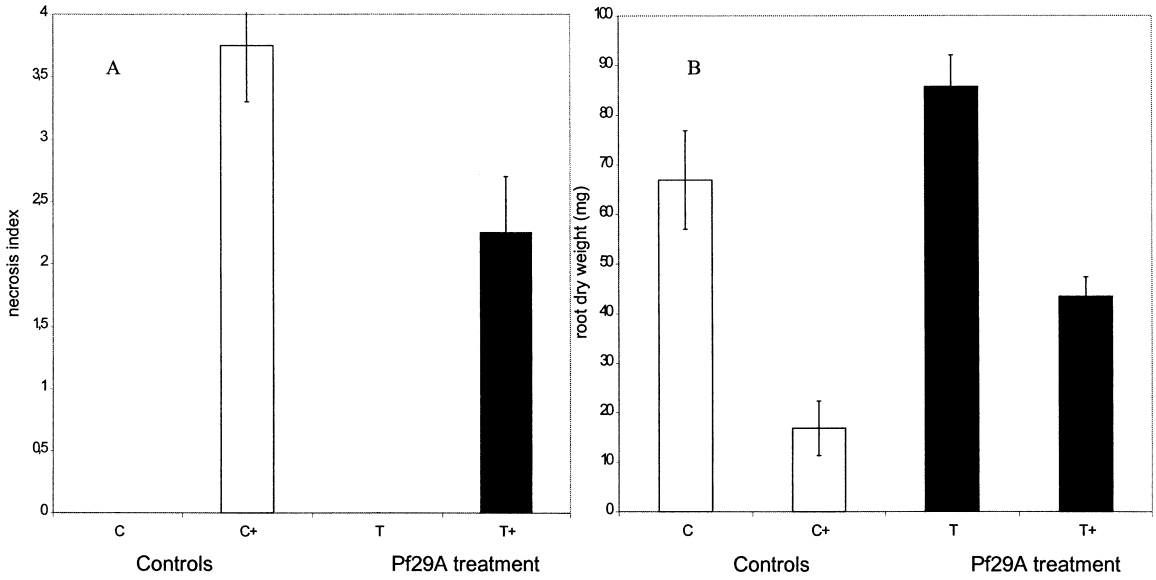


Figure 1. Effects of Pf29A on take-all severity (A) Effect on necrosis index (B) Effect on root dry weight. Plants were grown for five weeks under controlled conditions in the non-sterile soil of Le Rheu (France). C is control without *Ggt* and C+ control with *Ggt*. T is control with Pf29A treatment without *Ggt* and T+ Pf29A treatment with *Ggt*. Values are means and standard deviation of 12 plants. Treatments were analysed by a Student–Newman–Keuls test ($P = 0.05$).

(87.5 mg per plant) over that observed for control plants without *Ggt* (66.9 mg per plant). The same experiment was repeated several times and similar results were obtained in all cases (data not shown).

Pf29A was an efficient root coloniser and became dominant in the presence of Ggt

The populations of rRNA group I pseudomonads, fluorescent pseudomonads and Pf29A Ggt-infested roots and root necroses were significantly larger than those on non-infested roots (Figure 2). The differences in populations of total aerobic bacteria were statistically significant but slightly smaller than those for the three subpopulations. The derived population sizes of total aerobic bacteria and group I pseudomonads were similar, regardless of bacterial treatment, in the presence and absence of *Ggt*. The RAPD profile corresponding to Pf29A was easily identified on gels (Figure 3) and Pf29A was not detected on the roots of control plants with and without *Ggt* (Figure 2, Table 1). Pf29A accounted for 44.7% of the fluorescent pseudomonads isolated from healthy bacterium-treated wheat roots (Table 1). On the roots of plants with take-all, Pf29A accounted for as much as 77.6% of all the fluorescent pseudomonads present. The proportion of all bacteria accounted for by rRNA group I pseudomonads and fluorescent pseudomonads was 18.5 times and 67.6 times higher respectively on *Ggt*-infected roots of the diseased control plants than on healthy control plants (Table 1). In plants treated with Pf29A, the proportions of group I pseudomonads and fluorescent pseudomonads were only 3.5 and 2.6 times higher, respectively, on diseased than on healthy plants.

Pf29A selected RAPD genotypes and decreased the biodiversity of indigenous pseudomonads in the presence of Ggt

Two hundred and seventy-seven different RAPD patterns were found among the 480 strains screened with the E7 primer. Using a threshold of 38% similarity, six clusters (I, II, III, IV, V, VI) and a miscellaneous group (that contained the strains distant from the clusters at the bottom of the dendrogram) were created (Figure 4). Cluster I accounted for 37.2% of all strains and consisted of four subclusters Ia, Ib, Ic and Id. The four subclusters were not grouped because this would have required grouping with a threshold of 34% similarity and the cophenetic correlation reached only 52. Clusters I, III and V concentrated 71.9% of C strains and clusters I, II and VI, 85.2% of C+ strains. Clustering

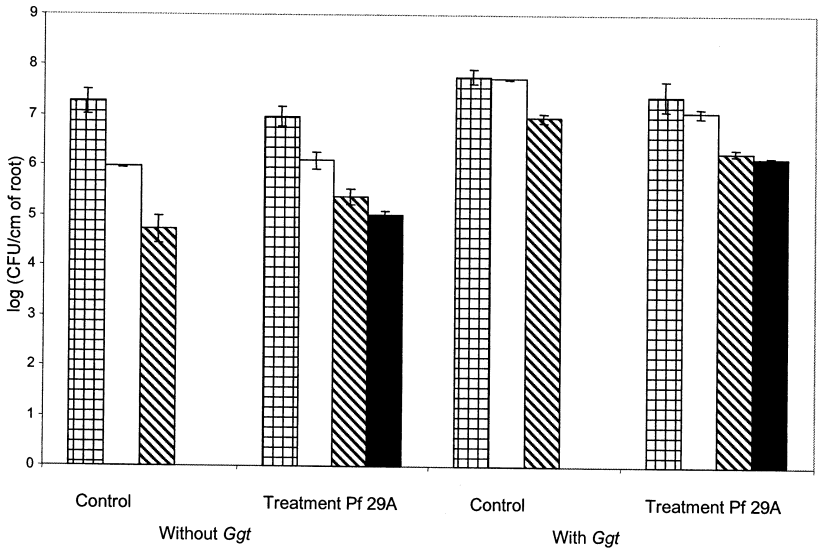


Figure 2. Effect of *Ggt* on rhizoplane colonisation by total aerobic bacteria, group I pseudomonads, fluorescent pseudomonads and Pf29A strain. Differences between populations were analysed by the Student–Newman–Keuls test ($P = 0.05$) and differences between the populations of group I pseudomonads and of Pf29A population by variance analysis. Populations are log-transformed and expressed as log CFU per cm of root: ▦ total aerobic bacteria, □ group I pseudomonads, ▨ fluorescent pseudomonads, ■ Pf29A strain.

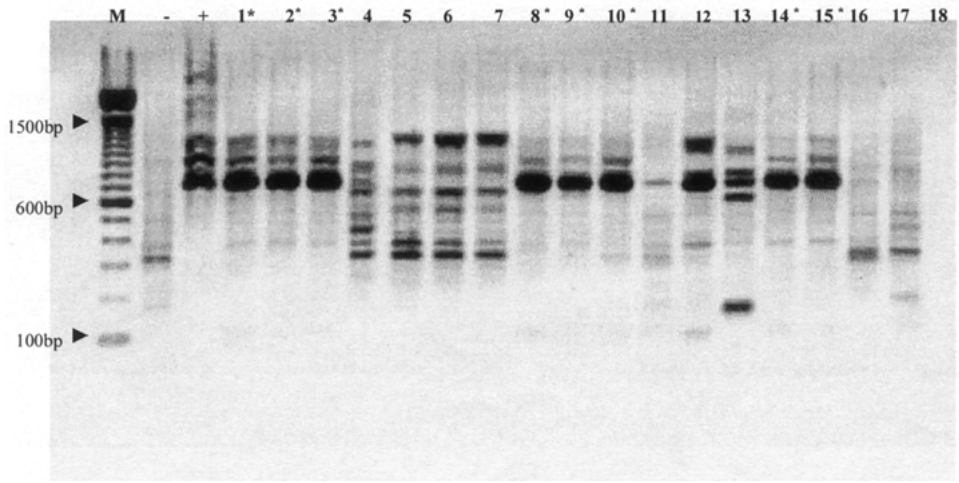


Figure 3. RAPD fingerprints with E7 primer of eighteen fluorescent pseudomonads from rhizoplane. Strains were isolated from plants treated with Pf29A in the absence of *Ggt*. Lane M is 100-bp DNA ladder (GibcoBRL). Lanes – and + are respectively negative control without template DNA and positive control with purified DNA of Pf29A. The lanes marked with ' ∗ ' correspond to strains identified as Pf29A.

differed for C and C+ strains, except for cluster I, which contained 49.1% of T0 strains and 27.8% of C+ strains. Cluster II and VI accounted for 58.8% of C+ strains. 46.1% of T strains were grouped in cluster I, with the majority of C strains. Other strains were grouped in cluster IV (23.1%) and cluster VI (15.4%), which contained 21% of C+ strains. Most of the fluorescent pseudomonads of the T+ group belonged to cluster III (24%) or the miscellaneous group (48%). Those from cluster III were related to strain Pf29A and 17 strains of group C. Cluster III contained strain Pf29A. The miscellaneous group included 48% of the T+ strains, which are the least well represented, with the 25 corresponding profiles accounting for only 9.1% of all

Table 1. Effect of the presence of *Ggt* fungus on the relative proportions of group I pseudomonads, fluorescent pseudomonads, Pf29a among total aerobic bacteria, and fluorescent pseudomonads respectively

Treatment	Group I pseudomonads	Fluorescent pseudomonads	Pf29A
	Total aerobic bacteria (%)	Total aerobic bacteria (%)	Fluorescent pseudomonads (%)
Control	4.9 d	0.25 d	0
Control with *Ggt*	90.5 a	16.9 a	0
Pf29A	13.6 c	2.7 c	44.7 b
Pf29A with *Ggt*	47.9 b	7.0 b	77.6 a

For each ratio data followed by the same letter are not significantly different at $P = 0.05$.

strains. The Shannon–Wiener index of C strains (1.50) was higher than that of C+ strains (0.98) and that of T strains (0.78) was higher than that of T+ strains (0.68). The T index was lower than the C index and the T+ index was lower than the C+ index.

Discussion

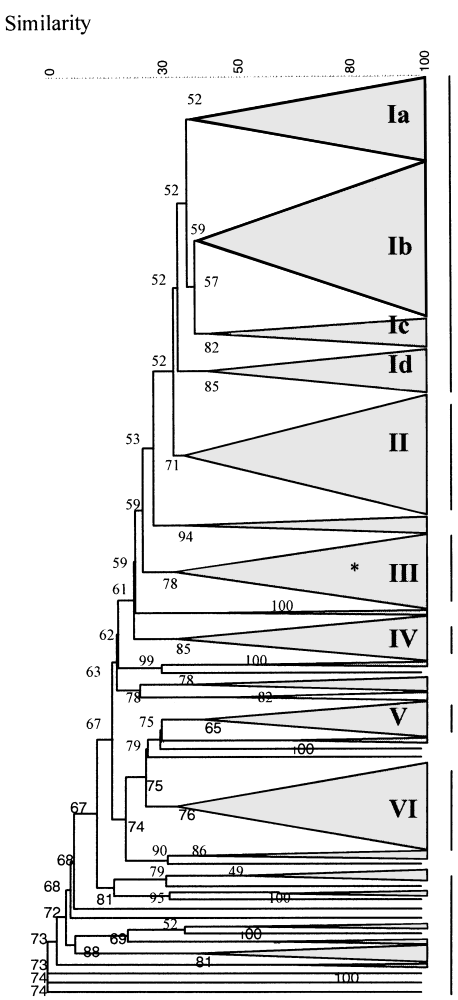
RAPD analysis was used to monitor Pf29A and to describe the structure of pseudomonad populations. A number of studies have been carried out with various primers, for which a lack of reproducibility was observed (Barbut et al., 1993; Di Cello et al., 1997). The diversity of *Burkholderia cepacia* populations in the maize rhizosphere has been analysed with two RAPD primers and 83 strains, corresponding to 68 different haplotypes obtained (Di Cello et al., 1997). However, a single primer was found to be highly discriminatory for *Salmonella* isolates, facilitating successful differentiation between strains (Hilton et al., 1997). A single discriminating and stable profile was obtained for strain Pf29A with the E7 primer.

Most plant colonisation studies are based on the recovery of an introduced marked antagonistic bacterium (Bloemberg et al., 1997; Bull et al., 1991; Etchebar et al., 1998; Troxler et al., 1997). These techniques require additional studies to ensure that the phenotype of the bacterium, its antagonistic properties and ecology are not affected by the marker. No genetic modification of strain Pf29A was required for the RAPD technique, making it possible to study the ecology of the wild-type strain in the rhizosphere. Unlike other monitoring methods, RAPD typing can be performed without prior knowledge of the target DNA and, with an optimised primer, it can be applied to the study of any micro-organism.

Some limitation to the RAPD method used is that bacteria had to be spread on medium, so the threshold for detection could be high: 10^2–10^3 bacteria per cm of root or per g of soil. But in our case, the abundance of Pf29A strain was higher (so largely above this limit) in treated soils. The limit allowed us to concentrate on the most abundant genotypes outside Pf29A and not to give a too high weight to rare genotypes. With the RAPD method, each CFU had to be checked. One other limit is that our purpose was dedicated to culturable bacteria. But our analysis was made about comparisons between populations isolated with the same method. The used method was time consuming, but we characterised the genotype of each strain and obtained results about the validity of Pf29A identification and about the population structure.

The ability to colonise plant roots is an essential characteristic of many plant growth promoting, antagonistic and pathogenic bacteria (Chin-A-Woeng et al., 1998; Etchebar et al., 1998; Leben et al., 1987; Simons et al., 1996; Suslow and Schroth, 1982; Weller, 1983). A positive correlation between the root population of *P. fluorescens* 2-79RN10 (or bacterial dose applied per seed) and the biocontrol of take-all has been demonstrated (Bull et al., 1991). In this work, Pf29A efficiently colonised wheat roots regardless of the presence of *Ggt* and accounted for 44.7% of the culturable fluorescent pseudomonads in the absence of *Ggt*.

The presence of *Ggt* and lesioned roots increased the colonisation of wheat roots by group I pseudomonads, fluorescent pseudomonads and Pf29A. The major effect of root necrosis is probably related to the greater availability of nutrients for soil microbial communities. The increase in population size of group I pseudomonads and fluorescent pseudomonads was lower with Pf29A treatment (factors 3.5 and 2.6 respectively) than without the same treatment (factors of 18.6 and 67.6). If the increase in pseudomonad populations is related to the size of the lesions, the biocontrol activity of Pf29A which leads to a smaller increase in these populations can be explained by the reduction in lesion size. Despite the disease reduction, the proportion of pseudomonads remained high. But the main conclusion is that Pf29A became the dominant fluorescent pseudomonad in the rhizoplane in the presence of *Ggt* and smaller lesions. The Pf29A population accounted for 77.6% of fluorescent pseudomonads. Despite a reduction in the quantity of necrotic lesions, the Pf29A



Clusters	Groups (% of strains)			
	C	C+	T	T+
I (Ia, Ib, Ic, Id)	49.1	27.4	46.1	4
II	4.2	37.8	0	0
III (* Pf29*A*)	14.4	0	2.6	24
IV	0.8	4.2	23.1	4
V	8.4	1.1	0	0
VI	3.3	21	15.4	0
Miscellaneous	7.6	4.2	2.6	48
Other strains	12.2	4.3	10.2	20

Figure 4. Dendrogram showing genetic relationship among 480 fluorescent pseudomonads strains, based on the 277 RAPD patterns produced with E7 primer. Genetic distances were calculated by using Dice indice and the dendrogram was built with the UPGMA method. Clusters were collapsed on the base of 38% of similarity. Cophenetic correlations were determined for each node. Pseudomonad strains were named according to their origin: strains isolated from *Ggt* infested and *Ggt* non infested control plants were respectively designed as C and C+ (for control) respectively. Those from *Ggt* infested and *Ggt* non infested Pf29A treated plants were T and T+ (for treatment). The compositions of principal clusters and of the miscellaneaous group were calculated in relative percent of the total number of profile for each group. The populations differ in their distribution as tested with $2\hat{I}$ test ($P = 0.05$).

root population doubled and limited the extension of take-all lesions. The development of *P. fluorescens* Pf29A antagonism is probably related to the selective stimulation of root colonisation by strain Pf29A induced by lesions or the presence of *Ggt*. So this trait is not limited to *P. corrugata* species (Barnett et al. 1999).

Increases in root colonisation by a saprophytic bacterium can be induced artificially, using transgenic

plants overproducing rare substrates along with transformed bacteria able to catabolise these substrates. Transgenic *Lotus corniculatus*, producing opines, selected and increased specific target populations of root-associated bacteria, especially those of opine-using bacteria (Oger et al., 1997). The observed increases were continuous, so the resulting populations of the introduced transformed bacterium became enormous. A similar selective effect was observed by introducing strain Pf29A into soil in the presence of *Ggt*, without making use of genetically modified organisms. So, a similar mechanism for a selective induction of colonisation that is based on a specific bacterial catabolism could be involved after root infection by *Ggt*.

The introduction of *Ggt* into the soil from Le Rheu led to structural changes in fluorescent pseudomonad populations after wheat cultivation. This study, based on RAPD markers, demonstrated the involvement of *Ggt* in the selectivity of root colonisation by pseudomonads. The selection of *in vitro* antagonistic and antibiotic-producing pseudomonad strains in the presence of *Ggt* after several successive wheat cultures is now well established (Brown, 1981; Charigkapkorn and Sivasithamparam, 1987; Raaijmakers and Weller, 1998; Vojinovicz, 1973). *Gaeumannomyces g.* var. *avenae*, a pathogen closely related to *Ggt*, selected antagonistic fluorescent pseudomonads in patches of turf grass infected with take-all, the proportion of antagonist being inversely correlated to the distance to the centre of the patch (Sarniguet et al., 1992a). In the present study, the main change in the structure of the fluorescent pseudomonad community was observed when Pf29A was introduced into soil, in the presence of *Ggt*. The diversity was strongly decreased compared to all the other treatments. Fourty-eight per cent of the pseudomonad strains differed from Pf29A and formed a separate group after distance analysis. Conversely, no effect of strain Pf29A on indigenous fluorescent pseudomonads was observed in the absence of *Ggt*: 69.2% of the strains were the same as those isolated in the absence of Pf29A treatment and the total communities had a high Shannon diversity index. The absence of strain in type III (Pf29A type) cluster with *Ggt* treatment and without Pf29A treatment whereas the percentage of such strains increased with double *Ggt* and Pf29A treatment could be surprising. One explanation could be that type III cluster was not a marker correlated to the presence of *Ggt*. Besides, the abundance in the type I cluster was inversely correlated with the presence of *Ggt*. Another explanation is that the Pf29A treatment have changed the impact of *Ggt* presence. The lesion size was reduced by Pf29A treatment. Each treatment, as considered alone (with *Ggt*, with Pf29A), already led to small shifts in the population distribution when compared to the the non-treated roots. But the root–*Ggt*–Pf29A interactions could create another biochemical environment in the rhizosphere. One hypothesis could be that Pf29A has a specific catabolism of root exudates after root colonisation by *Ggt* and that proximal (i.e. from the same cluster) strains could share or compete in the new created niche.

The effect on indigenous pseudomonad communities of introducing *Ggt* into soil has been studied by many researchers (Charigkapakorn and Sivasithamparam, 1987; Raaijmakers and Weller, 1998; Sarniguet et al., 1997; Vojinovicz, 1973). In contrast, much less attention has been paid to the effect of antagonist release on related communities. Thus, in the presence of *Ggt*, Pf29A highly influenced the development of indigenous fluorescent pseudomonad communities. The synergy between *Ggt* in microflora selection could be another possible mechanism for the development of antagonism to be examined in addition to the described activities of soil introduced biocontrol agents (antibiosis, competition for nutrients, hyperparasitism, induction of plant resistance). For that, the biocontrol activity of the indigenous microflora selected by Pf29A and *Ggt* remains to be assessed to demonstrate this hypothesis.

This study demonstrates the value of the E7 primer as a marker of Pf29A. The results confirm the selective effects of *Ggt* and root lesions on fluorescent pseudomonad populations and show that *Ggt* and root lesions were responsible for the dominance of Pf29A in the wheat rhizoplane. *Ggt* and Pf29A had synergistic effects on the development of populations of indigenous fluorescent pseudomonads. Similar changes occured on wheat roots in the presence of *Ggt* after several continuous croppings of wheat, when take-all decline is established (Sarniguet et al., 1997). The use, in fields, of such strains that probably do not eradicate the disease at the first planting, could accelerate the establishment of take-all decline.

Acknowledgements

We thank Micheline Leray for technical assistance and Dr Philippe Fravalo from Faculté d'Odontologie de Rennes for suggestions in the development of our RAPD technique. We thank Virginia Stockwell and

Axel Edelman for critical reading the manuscript and improving the English grammar.

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