Soil enzyme activities in the rhizosphere of field-grown sugar beet inoculated with the biocontrol agent *Pseudomonas fluorescens* F113

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Abstract. *Pseudomonas fluorescens* F113, which produces the antimicrobial compound 2,4-diacetylphloroglucinol is a prospective biocontrol agent. Soil enzyme activities were used to investigate the ecological impact of strain F113 in the rhizosphere of field-grown sugar beet. There were distinct trends in rhizosphere enzyme activities in relation to soil chemistry (studied by electro-ultrafiltration). The activities of enzymes from the phosphorus cycle (acid phosphatase, alkaline phosphatase and phosphodiesterase) and of arylsulphatase were negatively correlated with the amount of readily available P, whereas urease activity was positively correlated with the latter. Significant correlations between electro-ultrafiltration nutrient levels and enzyme activity in the rhizosphere were obtained, highlighting the usefulness of enzyme assays to document variations in soil nutrient cycling. Contrary to previous microcosm studies, which did not investigate plants grown to maturity, the biocontrol inoculant had no effect on enzyme activity or on soil chemistry in the rhizosphere. The results show the importance of homogenous soil microcosm systems, used in previous work, in risk assessment studies, where inherent soil variability is minimised, and where an effect of the pseudomonad on soil enzymology could be detected.

Key words: Enzymatic activities - Biocontrol inoculant - *Pseudomonas* - Risk assessment

Introduction

Certain *Pseudomonas* spp. have been studied as promising biocontrol agents against phytopathogenic fungi (Scher and Baker 1982; Fenton *et al*. 1992; Keel *et al*. 1992; King and Parke 1993; Mathre *et al*. 1994; Weller and Thomashow 1994). In addition, genetic modifications have been proposed to improve the biocontrol performance and/or the ecological competence of wild-type biocontrol pseudomonads (Fenton *et al*. 1992; Colbert *et al*. 1993; Schnider *et al*. 1995).

The effect of biocontrol agents on soil organisms other than the target pathogen is poorly documented. A comprehensive knowledge of the ecological consequences of releasing biocontrol inoculants into the soil environment is needed before they can be safely carried out (Smit *et al*. 1992), especially when the strains have been genetically modified for improved performance (Jones *et al*. 1991; Doyle and Stotzky 1993;.Défago *et al*. 1996)

The ecological impact of *Pseudomonas* inoculants in soil has often been characterised in terms of size and composition of specific microbial groups (Jones *et al*. 1991; de Leij *et al*. 1994 and 1995; Carroll *et al*. 1995). However, these approaches do not provide a comprehensive view of the impact of an inoculant on the functioning of the soil ecosystem (Doyle and Stotzky 1993).

Enzyme activities have been used to document the ecological effect of pesticide applications to soil (Gianfreda *et al*. 1994) and the impact of soil cultivation (Jordan *et al*. 1995). They have been proposed as a tool to monitor changes in soil nutrient cycling resulting from the interactions between inoculants

and indigenous microbial populations of soil (Doyle and Stotzky 1993; Naseby and Lynch 1997a). Using soil microcosms, Doyle and Stotzky (1993) found no difference in the activity of arylsulphatase, phosphatases and dehydrogenase in bulk soil in which *Escherichia coli* had been introduced. Likewise, Jones *et al*. (1991) did not find any effect of the addition of genetically-modified pseudomonads and coliforms on transformations of fixed N in unplanted soil microcosms. In contrast, inoculation with a *Flavobacterium* species resulted in increased activity of α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase in the rhizosphere of wheat (Mawdsley and Burns 1994). Naseby and Lynch (1997b) found perturbations in several soil enzyme activities following the introduction of *P. fluorescens* into the rhizosphere of wheat in long term microcosm studies. Large perturbations were also found in short term microcosm studies using the same strain used in this field trial (Naseby and Lynch 1998). However, enzyme activities have not been used to investigate the impact of biocontrol inoculants under field conditions.

In this paper we report the use of soil enzyme assays to evaluate the effect of the biocontrol agent *Pseudomonas fluorescens* strain F113 on ecosystem functioning, under typical farming practices. The same enzyme activities were perturbed by *P. fluorescens* inocula in short term small microcosm studies (Naseby and Lynch 1997c and 1998) and long term large microcosm studies (Naseby and Lynch 1997b). The protective effect of the strain is mediated by the production of 2,4-diacetylphloroglucinol (DAPG; Fenton *et al*. 1992; Shanahan *et al*. 1992), an antimicrobial compound that inhibits various soil microorganisms (reviewed by Keel

et al. 1992; Shanahan *et al*. 1992). The experiment was carried out in 1994, at a field site not infected by pathogens such as *Pythium* or *Aphanomyces* (Jim Powell, *pers com*), to avoid the possibility that potential negative effects of the inoculant could be masked by its disease-suppression ability.

Materials and methods

Bacterial inoculant

P. fluorescens F113Rif is a spontaneous rifampicin-resistant derivative of the wild-type F113 (Shanahan *et al*. 1992). Strain F113Rif produces the antifungal compound DAPG and like its wild type parent inhibits *Pythium ultimum in vitro* (Carroll *et al*. 1995). Sugar beet seeds (*Beta vulgaris* var. altissima) were inoculated with F113Rif at 10^6 CFU per seed, with the use of a commercial powder formulation (Germain's, King's Lynn, UK) routinely employed in Ireland to pellet sugar beet seeds.

Field site and preparation

The field site was located near Bandon (county Cork, Ireland). The soil was a Brown Podzolic soil and the A_p horizon (0-22 cm) corresponded to a loam (24% clay; 44% silt; 9.5% total organic matter). The pluviometry was 1450 mm in 1994 (118% of the yearly average). The field had been cropped with barley for 10 years prior to the experiment. The soil was prepared by ploughing in October 1993, and was limed at the equivalent rate of 2.5 t $CaCO_3$, 25 kg P and 25 kg N per ha in March 1994 (to a pH of 7.2 in water). A composite fertiliser containing 160 kg N, 49 kg P, 173 kg K, 62 kg Na, 37 kg S, and 4 kg B per ha was applied 3 days prior to sowing. The crop was sown on April 15, 1994. A further addition of N was made 45 days after sowing

(51 kg N per ha). The crop was harvested on October 13, 1994 (i.e. 181 days after sowing).

Experimental treatments and sampling of soil

Sugar beet was either seed-inoculated with *P. fluorescens* F113Rif or were not inoculated. The inoculant was found at approximately 10^6 CFU per root system several months after inoculation but was below detection limit at harvest time, when soil samples were collected in the current study.

For rhizosphere samples, a total of three plots were used for each of the two treatments. The sampling location within each plot studied was chosen at random (at least 1 m from the nearest edge of the plot) and five adjacent sugar beet plants were sampled per plot (i.e. five rhizosphere samples per plot). Rhizosphere soil (soil adhering closely to the root) was obtained from individual root systems with the help of sterile spatulas. In addition, five bulk-soil samples were obtained from random sites half-way between rows where uninoculated sugar beets were grown.

All rhizosphere and bulk-soil samples originated from the surface horizon. Samples were transferred into loosely capped 50-ml sterile vials, allowing gaseous exchange. The vials were sent overnight to the University of Surrey (England) for enzyme activity determinations. The remainder of each sample was analysed for nutrient content using electro-ultrafiltration (EUF) procedures, as follows: one

composite rhizosphere sample per plot and one composite bulk-soil sample were analysed.

Enzyme activity analyses

The soil samples were stored over night at 4^{o}C and sieved (2 mm mesh). For each of the 35 samples, the activity of key enzymes involved in the four major nutrient cycles were determined as described by Naseby and Lynch (1997a). They included β-galactosidases measured under acid and alkaline conditions (C cycle), urease (N cycle), *N*-acetylglucosaminidase (C and N cycles), acid and alkaline phosphatases and phosphodiesterase (P cycle), and arylsulphatase (S cycle).

EUF analysis of soil

EUF analysis determines the amount of readily-available nutrients in soil samples, after their extraction by desorption (Németh 1979). EUF data correlate well with the amount of nutrients taken up by the sugar beet root and with beet yield (Wiklicky 1982; Britton 1988). Desorption was performed at 400 V and 80°C for 10 min. Organic nitrogen, nitrate, phosphorus, calcium, magnesium, potassium and sodium were measured with a Technicon continuous flow autoanalyser and boron, copper, zinc, manganese, molybdenum, iron and aluminium were determined using an inductively-coupled plasma mass spectrometer.

Statistical set-up and analyses

Fig. 1 shows the relevant section of the 7 x 7 Latin square used for the experiment. Only two of seven treatments (i.e. inoculation with *P. fluorescens* F113Rif and untreated control) were under investigation in this work, using three columns (i.e. I, II, III) of the Latin square. Bulk-soil samples were analysed as a control.

The two rhizosphere treatments were compared by analysis of variance and Least Square Difference ($P<0.05$). Analyses were performed using mean data from each plot for the two rhizosphere treatments. The relationships between enzyme activities and EUF soil levels in the rhizosphere at the level of plots was investigated using the Spearman Correlation Coefficient followed by a test of significance. All statistical analyses were conducted with SPSS for windows (SPSS Inc.).

Results

In this experiment, the activity of enzymes involved in nutrient cycling was monitored to evaluate whether the inoculated biocontrol agent *P. fluorescens* F113Rif had an ecological impact in the rhizosphere of sugar beet grown under typical farming conditions. Results showed that none of the eight soil enzymes measured were significantly affected by the biocontrol inoculant (Table 1). Standard errors for enzyme activities within plots were generally between 10 and 20%, which was similar to the level of data fluctuation between treatments (Table 1).

Likewise, pH and readily-available nutrients in the rhizosphere were at similar levels in the F113Rif treatment and in the control (Table 2). The variability of EUF data was generally within a 50% range. The variability of soil pH was low.

Significant negative correlations were found between EUF-P and the activities of acid phosphatase ($r = -0.85$, $P < 0.05$), alkaline phosphatase ($r = -0.95$, $P < 0.01$), phosphodiesterase ($r = -0.89$, $P < 0.05$), arylsulphatase ($r = -0.89$, $P < 0.05$) and alkaline β-galactosidase ($r = -0.87$, $P < 0.05$) in the rhizosphere of sugar beet (Table 3). In contrast, urease activity was positively correlated with EUF-P ($r = 0.93$, $P < 0.01$). However, there was no correlation between the activity of acid β-galactosidase or *N*-acetylglucosaminidase and EUF-P. The other chemical parameters measured were not correlated with any of the soil enzyme activities (Table 3) nor the EUF-P.

Discussion

Potential deleterious effects of biocontrol inoculants on the indigenous soil microbiota responsible for soil fertility may arise as a consequence of antagonism (e.g., via the production of antimicrobial compounds like DAPG) and/or competition for resources (e.g. nutrients). In the current work, the DAPG-producing biocontrol inoculant *P. fluorescens* F113Rif had no apparent effect on the activity of eight soil enzymes involved in nutrient cycling (Table 1) or on nutrient availability (Table 2) in the rhizosphere of field-grown sugar beet. These results, which suggest that the F113Rif treatment had no significant impact on the functional dynamics of the

agroecosystem, are strengthened by the fact that the inoculant had no negative effect on yield in the absence of disease pressure (data not shown).

However, pseudomonads are predominantly r-strategists and thus the inoculant was likely to be most active during the early stages of plant growth (de Leij *et al*. 1995). Possible transient effects of F113Rif were not investigated in the current work. A number of perturbations in soil enzyme activities were found in short-term microcosm experiments using a *lac*-tagged derivative of *P. fluorescens* F113 and pea (Naseby and Lynch 1998). These modifications were directly related to DAPG production as a non-producing mutant did not have the same effect. Large microcosm experiments with a different strain of *P. fluorescens* also produced significant perturbations in various soil enzyme activities (Naseby and Lynch 1997b).

The number of culturable cells of the inoculant was below detection limit at harvest time, when samples for the current study were collected. However, introduced pseudomonads may persist as viable but nonculturable cells in the field (Défago *et al*. 1996). Furthermore, the complexity of the interactions between an inoculant and the indigenous soil microbiota necessitates that microbial inoculants be studied for potential long term effects, beyond their apparent decline in soil (Doyle and Stotzky 1993).

When dealing with early soil-borne diseases, such as *Pythium*-mediated damping-off, the most important period for the effective use of a biocontrol agent is during seed germination and the initial stages of plant growth (Paulitz 1991). In this

respect, the lack of residual ecological effect of the F113Rif treatment at harvest time in the current work is an important attribute in terms of risk assessment.

Several soil chemical characteristics were measured for each plot (Table 2) to characterise soil chemical heterogeneity. They also allowed distinctions to be made between the influence of treatments and that of soil chemistry, since nutrient availability is a major factor governing the secretion of enzymes by both the plant (Tadano *et al*. 1993) and the soil microbiota (Tarafdar and Marschner 1994; Janzen *et al*. 1995). The enzyme activities from the phosphorus cycle (acid and alkaline phosphatases, phosphodiesterase) were negatively correlated with EUF-P, which was high compared with phosphorus requirements of sugar beet (Wiklicky 1982). This indicates that the phosphorus cycle enzymes were repressed at the higher EUF-P contents. Indeed, the inverse relationship between inorganic soluble phosphate and soil phosphatase activity is well documented (Tabatabai 1982; Tadano *et al*. 1993; Tarafdar and Marschner 1994).

In contrast, urease activity was positively correlated with EUF-P in the current study, and was not affected by the any of the nitrogen measurements, which suggests that N became the most limiting nutrient at higher P levels. In addition, arylsulphatase (S cycle) and alkaline β-galactosidase (C cycle) were negatively correlated with EUF-P. This indicates that C and S are not the most limiting nutrients under higher phosphate levels. The two other enzymes from the C cycle (*N*-acetylglucosaminidase and acid β-galactosidase) were not affected by the levels of available phosphate.

In conclusion, P availability was the most important factor affecting soil enzyme activities in the rhizosphere. The differences in soil enzyme activities caused by field heterogeneity were greater than any possible effects that may have been caused by the inoculation with the biocontrol agent. However, the soil enzyme measurements proved to be a useful and sensitive indicator of soil ecosystem function, as they were strongly influenced by a relatively modest variation in soil chemistry. They will be useful in assessing the ecological impact and biosafety of derivatives of F113 genetically-modified to produce higher levels of antimicrobial phloroglucinols. The results highlight also the importance of homogenous soil microcosm systems in risk assessment work, to ensure that soil heterogeneity is low and small effects of a soil treatment can be detected.

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References

Britton J (1988) Experiences with the application of EUF soil testing in sugar beet production in Ireland. In: Proceedings Third International EUF Symposium (May 30-31, 1988) on Cost Reduction and Environmental Protection. Zuckerindustrie, Mannheim, Germany, pp 373-403

Carroll H, Moënne-Loccoz Y, Dowling DN, O'Gara F (1995) Mutational disruption of the biosynthesis genes coding for the antifungal metabolite 2,4-diacetylphloroglucinol does not influence the ecological fitness of *Pseudomonas fluorescens* F113 in the rhizosphere of sugarbeets. Appl Environ Microbiol 61:3002-3007

Colbert SF, Hendson M, Ferri M, Schroth MN (1993) Enhanced growth and activity of a biocontrol bacterium genetically engineered to utilize salicylate. Appl Environ Microbiol 59:2071-2076

Défago G, Keel C, Moënne-Loccoz Y (1996) Fate of introduced biocontrol agent *Pseudomonas fluorescens* CHA0 in soil: Biosafety considerations. In: Wenhua T, Cook RJ, Rovira A (eds) Advances in Biological Control of Plant Diseases. China Agricultural University Press, Beijing, China, pp 241-245

De Leij FAAM, Sutton EJ, Whipps JM, Lynch JM (1994) Effect of a genetically modified *Pseudomonas aureofaciens* on indigenous microbial populations of wheat. FEMS Microbiol Ecol 13:249-258

De Leij FAAM, Sutton EJ, Whipps JM, Fenlon JS, Lynch JM (1995) Impact of field release of genetically modified *Pseudomonas fluorescens* on indigenous microbial populations of wheat. Appl Environ Microbiol 61:3443-3453

Doyle JD, Stotzky G (1993) Methods for the detection of changes in the microbial ecology of soil caused by the introduction of microorganisms. Microb Releases 2:63-72

Fenton AM, Stephens PM, Crowley J, O'Callaghan M, O'Gara F (1992) Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. Appl Environ Microbiol 58:3873-3878

Gianfreda L, Sannino F, Ortega N, Nannipieri P (1994) Activity of free and immobilized urease in soil: Effects of pesticides. Soil Biol Biochem 26:777-784

Janzen RA, Cook FD, McGill WB (1995) Compost extract added to microcosms may simulate community-level controls on soil microorganisms involved in element cycling. Soil Biol Biochem 27:181-188

Jones RA, Broder MW, Stotzky G (1991) Effects of genetically engineered microorganisms on nitrogen transformations and nitrogen-transforming microbial populations in soil. Appl Environ Microbiol 57:3212-3219

Jordan D, Kremer RJ, Bergfield WA, Kim KY, Cacnio VN (1995) Evaluation of microbial methods as potential indicators of soil quality in historical agricultural fields. Biol Fertil Soils 19:297-302

Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner P, Haas D, Défago G (1992) Suppression of root diseases by *Pseudomonas fluorescens*

CHA0: Importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. Mol Plant-Microbe Interact 5:4-13

King EB, Parke JL (1993) Biocontrol of Aphanomyces root rot and Pythium damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. Plant Dis 77:1185-1188

Mathre DE, Callan NW, Johnston RH, Miller JB, Schwend A (1994) Factors influencing the control of *Pythium ultimum*-induced seed decay by seed treatment with *Pseudomonas aureofaciens* AB254. Crop Prot 13:301-307

Mawdsley JL, Burns RG (1994) Inoculation of plants with a *Flavobacterium* species results in altered rhizosphere enzyme activities. Soil Biol Biochem 26:871-882

Naseby DC, Lynch JM (1997a) Functional impact of genetically modified micro-organisms on the soil ecosystem. In: Zelikoff JT, Schepers J, Lynch JM (eds) Ecotoxicology: Responses, Biomarkers and Risk Assessment. SOS Publications, Fair Haven, NJ, USA

Naseby DC, Lynch JM (1997b) Rhizosphere soil enzymes as indicators of perturbation caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol Biochem 29:1353-1362

Naseby DC, Lynch JM (1997c) Establishment and impact of *Pseudomonas fluorescens* genetically modified for lactose utilisation and kanamycin resistance in the rhizosphere of pea. J Appl Microbiol (in press)

Naseby, DC, Lynch JM (1998) Soil enzymes and microbial population structure to determine the impact of wild type and genetically modified *Pseudomonas fluorescens* in the rhizosphere of pea. Mol Ecol (in press)

Németh K (1979) The availability of nutrients in the soil as determined by electro-ultrafiltration (EUF). Adv Agron 31:155-187

Paulitz TC (1991) Effect of *Pseudomonas putida* on the stimulation of *Pythium ultimum* by seed volatiles of pea and soybean. Phytopathol 81:1282-1287

Scher FM, Baker R (1982) Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. Phytopathol 72:1567-1573

Schnider U, Keel C, Blumer C, Troxler J, Défago G, Haas D (1995) Amplification of the house-keeping sigma factor in *Pseudomonas fluorescens* CHA0 enhances antibiotic production and improves biocontrol abilities. J Bacteriol 177:5387-5392

Shanahan P, O'Sullivan DJ, Simpson P, Glennon JD, O'Gara F (1992) Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. Appl Environ Microbiol 58:353-358

Smit E, Van Elsas JD, Van Veen JN (1992) Risks associated with the application of genetically modified microorganisms in terrestrial environments. FEMS Microbiol Rev 88:263-278.

Tabatabai MA (1982). Soil enzymes. In: Page AL, Miller RH, Keeney DR (eds) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9 (2nd Edn). ASA-SSSA, Madison, WI, USA, pp 903-947

Tadano T, Ozawa K, Sakai H, Osaki M, Matsui H (1993) Secretion of acid phosphatase by the roots of crop plants under phosphorus-deficient conditions and some properties of the enzyme secreted by lupin roots. Plant and Soil 155/156:95-98

Tarafdar JC, Marschner H (1994) Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol Biochem 26:387-395

Weller DM, Thomashow LS (1994) Current challenges in introducing beneficial microorganisms into the rhizosphere. In: O'Gara F, Dowling DN, Boesten B (eds) Molecular Ecology of Rhizosphere Microorganisms. VCH Publishers, Weinheim, Germany, pp 1-18

Wiklicky L (1982) Application of the EUF procedure in sugar beet cultivation. Plant and Soil 64:115-127

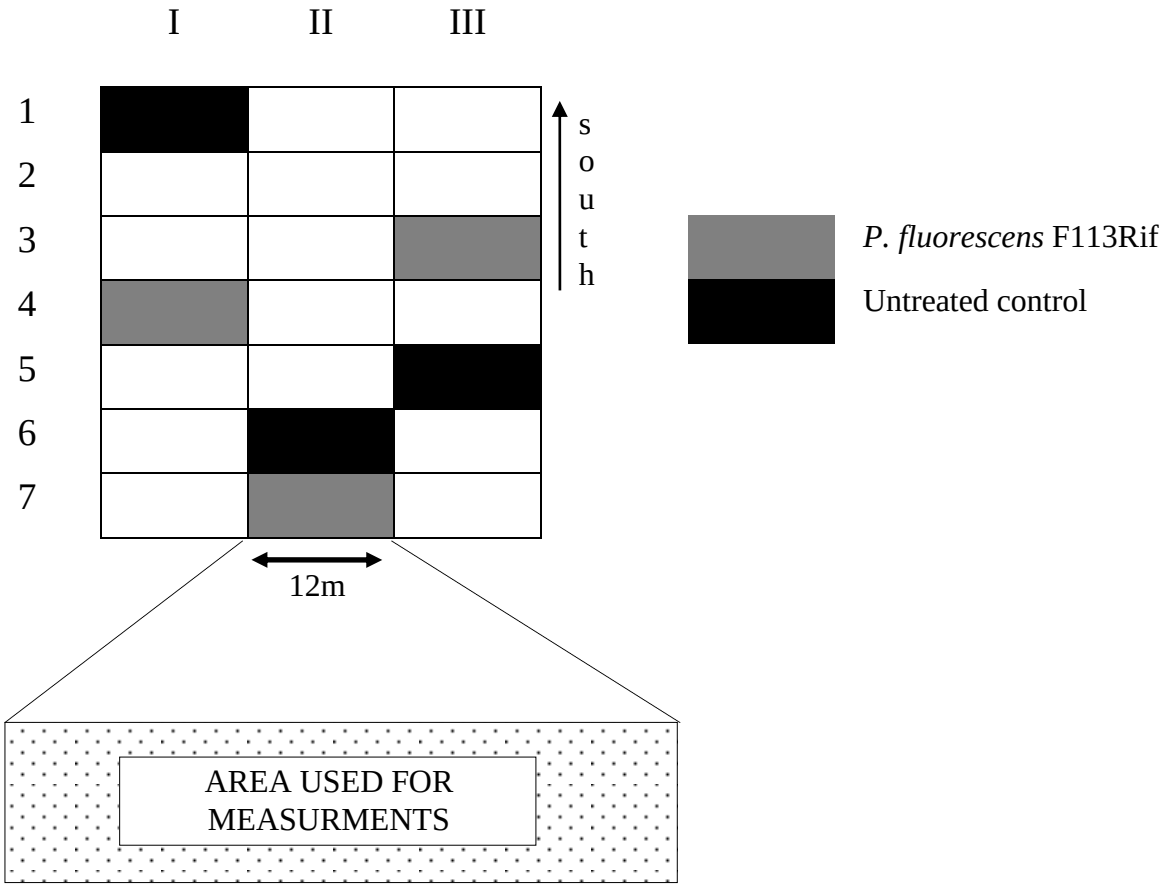

Fig. 1. Design of the experiment and plots used in the study. Three columns (i.e. I, II, III) of the original 7 x 7 Latin square were used, and two of seven treatments (i.e. inoculation with *P. fluorescens* F113Rif and untreated control) were sampled

Table 1. Soil enzyme activities in bulk soil and in rhizosphere soil from control sugar beet and sugar beets inoculated with *P. fluorescens* F113Rif (urease activity expressed as mg ammonia released/h/g dry soil and other enzyme activities as mg pNP released/h/g dry soil; Alkaline Pase, alkaline phosphatase; Acid Pase, acid phosphatase; P-diesterase, phosphodiesterase; Alkaline β-gal, alkaline β-galactosidase; Acid β-gal, acid β-galactosidase; NAGase, *N*-acetylglucosaminidase; means ± standard errors are shown). For rhizosphere samples, the results for each plot (n=5) and the means are given (there was no statistical difference between the F113Rif treatment and the untreated control)

Treatment[a]	Alkaline Pase	Acid Pase	P-diesterase	Arylsulphatase	Alkaline β-gal	Acid β-gal	NAGase	Urease
Bulk soil	1.66 ± 0.19	4.96 ± 0.25	0.47 ± 0.02	0.26 ± 0.03	0.33 ± 0.04	0.18 ± 0.02	0.23 ± 0.04	0.80 ± 0.31
Rhizosphere soil:								
Control (I1)	0.67 ± 0.24	5.52 ± 0.15	0.14 ± 0.03	0.07 ± 0.03	0.13 ± 0.03	0.22 ± 0.02	0.25 ± 0.02	0.93 ± 0.09
Control (II6)	2.26 ± 0.22	6.76 ± 0.46	0.63 ± 0.08	0.37 ± 0.04	0.40 ± 0.05	0.30 ± 0.04	0.32 ± 0.10	0.59 ± 0.13
Control (III5)	1.62 ± 0.35	5.47 ± 1.05	0.47 ± 0.10	0.28 ± 0.07	0.32 ± 0.07	0.28 ± 0.04	0.31 ± 0.03	0.86 ± 0.14
Average control	1.52 ± 0.22	5.92 ± 0.39	0.41 ± 0.07	0.24 ± 0.04	0.28 ± 0.04	0.27 ± 0.02	0.29 ± 0.03	0.79 ± 0.08
F113Rif (I4)	1.24 ± 0.28	5.93 ± 0.19	0.29 ± 0.05	0.15 ± 0.05	0.18 ± 0.05	0.23 ± 0.02	0.29 ± 0.02	0.81 ± 0.12
F113Rif (II7)	2.53 ± 0.29	7.42 ± 1.12	0.47 ± 0.05	0.35 ± 0.04	0.40 ± 0.06	0.33 ± 0.04	0.24 ± 0.01	0.68 ± 0.13
F113Rif (III3)	1.88 ± 0.19	7.61 ± 0.56	0.49 ± 0.04	0.25 ± 0.04	0.28 ± 0.03	0.28 ± 0.03	0.36 ± 0.08	0.63 ± 0.13
Average F113Rif	1.88 ± 0.21	6.99 ± 0.44	0.42 ± 0.03	0.25 ± 0.03	0.29 ± 0.04	0.33 ± 0.02	0.30 ± 0.03	0.71 ± 0.07

[a] The plot from which each rhizosphere sample was taken is indicated in brackets

Table 2. EUF levels and pH in bulk soil and in rhizosphere soil from control sugar beet and sugar beets inoculated with *P. fluorescens* F113Rif (EUF levels expressed as ppm[a] or ppb[b]). For rhizosphere samples, the results for each plot and the means ± standard errors for each treatment are given (there was no statistical difference between the F113Rif treatment and the untreated control)

Treatment[c]	Organic N[a]	NO_3[a]	P[a]	Ca[a]	Mg[a]	K[a]	Na[a]	B[a]	Cu[b]	Zn[b]	Mn[a]	Mo[a]	Fe[b]	Al[b]	$pH_{(H20)}$
Bulk soil	17	4.0	38	519	19	118	32	1.3	161	165	0.5	13	3.3	4.8	7.0
Rhizosphere soil:															
Control (I1)	17	7.2	53	549	27	388	59	1.5	144	183	1.2	16	3.2	3.9	7.2
Control (II6)	12	4.7	30	480	19	208	27	1.1	73	170	1.2	14	1.8	2.2	6.9
Control (III5)	17	5.4	41	396	19	210	24	0.9	92	160	0.6	12	2.8	4.3	6.9
Average control	15 ± 1.7	5.8 ±0.7	41 ± 6.6	475 ±44	22 ± 3	269 ±60	37 ± 11	1.2 ±0.2	103 ±21	171 ±7	1 ±0.2	14 ± 1	2.6 ±0.4	3.5 ±0.6	7 ±0.1
F113Rif (I4)	15	6.1	44	665	27	211	15	0.8	72	193	1.6	16	1.6	2.3	6.9
F113Rif (II7)	14	5.8	31	593	25	197	23	0.7	100	254	1.5	15	2.5	3.2	7.0
F113Rif (III3)	21	9.0	31	548	25	321	45	0.9	110	126	1.4	14	2.4	2.8	7.0
Average F113Rif	17 ± 2	7.0 ± 1	35 ± 4	602 ±34	26 ±1	243 ±39	28 ± 9	0.8 ±0.1	94 ± 11	191 ±37	1.5 ±0.1	15 ±0.6	2.2 ±0.3	2.8 ±0.3	7.0 ±0.0

[c] The plot from which each rhizosphere sample was taken is indicated in brackets

Table 3. Coefficients of correlation between enzyme activities and EUF levels in the rhizophere of sugar beet (* significant at *P*=0.05; ** significant at *P*=0.01; n=7)

Soil enzyme	Organic N	NO_3	P	Ca	Mg	K	Na	B	Cu	Zn	Mn	Mo	Fe	Al	$pH_{(H20)}$
Alkaline phosphatase	-0.35	-0.24	-0.95 **	-0.14	-0.40	-0.51	-0.48	-0.60	-0.37	0.27	0.11	-0.37	-0.32	-0.29	-0.53
Acid phosphatase	-0.001	0.52	-0.85 *	0.26	0.3	0.23	-0.04	-0.59	-0.45	0.15	0.70	0.18	-0.49	-0.67	-0.13
Phosphodiesterase	-0.31	-0.43	-0.89 *	-0.45	-0.75	-0.62	-0.5	-0.38	-0.35	-0.11	-0.24	-0.68	-0.28	-0.17	-0.69
Arylsulphatase	-0.41	-0.42	-0.89 *	-0.39	-0.64	-0.59	-0.52	-0.48	-0.37	0.15	-0.13	-0.57	-0.25	-0.16	-0.61
Alkaline β-galactosidase	-0.39	-0.47	-0.87 *	-0.39	-0.66	-0.63	-0.46	-0.39	-0.23	0.19	-0.20	-0.57	-0.12	-0.05	-0.51
Acid β-galactosidase	-0.34	0.17	-0.62	-0.12	-0.003	0.05	-0.29	-0.66	-0.68	0.34	0.46	-0.04	-0.48	-0.53	-0.34
N-acetylglucosaminidase	0.32	0.52	-0.34	-0.24	-0.05	0.29	-0.02	-0.37	-0.57	-0.64	0.23	-0.26	-0.52	-0.52	-0.44
Urease	0.21	-0.07	0.93 **	-0.04	0.19	0.20	0.24	0.44	0.42	0.05	-0.36	0.16	0.54	0.62	0.43