Carbon fractions in the rhizosphere of pea inoculated with 2,4 diacetylphloroglucinol producing and non-producing *Pseudomonas fluorescens* F113.

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ABSTRACT

The aim of this work was to determine the effect of wild type and functionally modified *Pseudomonas fluorescens* strains on C fractions in the rhizosphere of pea. The *lacZY* marked F113 strain produces the antibiotic 2,4 diacetylphloroglucinol (DAPG) useful in plant disease control. The modified strain of F113 was repressed in production of DAPG, creating the DAPG negative strain F113 G22. The F113 treatment resulted in a significantly lower shoot/root ratio. The F113 G22 treatment had a significantly greater indigenous and total fluorescent *Pseudomonas* population than the control and F113 (DAPG+) treatment. Both strains significantly increased the water soluble carbohydrates and the total water soluble carbon in the pea rhizosphere soil. Strain F113 significantly increased the soil protein content relative to the control but not in relation to the F113 G22 treatment. The F113 treatment had a significantly greater organic acid content than the control and F113 G22 treatments, whilst the F113 G22 treatment was also significantly greater than the control. Both inocula resulted in significantly lower phosphate contents than the control. The F113 inocula significantly increased alkaline phosphatase, sulphatase and urease activities, and reduced β glucosidase activities indicating increased carbon availability. Both inocula increased C availability, however, antibiotic production by strain F113 reduced the utilisation of organic acids released from the plant resulting in differing effects of the two strains on nutrient availability, plant growth, soil enzyme activities and *Pseudomonas* populations.

INTRODUCTION

The effect of genetically modified micro-organisms (GMMs), released as biocontrol agents, on soil nutrient cycling/soil enzyme activities and indigenous microbial populations is poorly described in the literature (Naseby and Lynch 1997a). Therefore a more comprehensive knowledge of the consequences of such releases on the rhizosphere must be provided before they can be utilised safely (Smit *et al* 1992).

De Leij *et al* (1995) reported transient perturbations in the indigenous microbiota with the introduction of wild type and genetically marked *Pseudomonas fluorescens* to the rhizosphere of wheat plants in field experiments, but did not find differences between the two respective inoculants. Other authors working on indigenous populations and ecosystem function in contained experimental systems also found such transient perturbations (Seidler 1992, Stotzky *et al* 1993 and Whipps *et al* 1996). Perturbations have been recorded by several authors with the introduction of functionally modified GMMs, including displacement of indigenous populations (Bolton *et al* 1991); suppression of fungal populations (Short *et al* 1990) reduced protozoa populations (Austin *et al* 1990) and increased carbon turnover (Wang *et al* 1991). However, methods requiring microbial growth can be hampered by the non-culturability of many micro-organisms (Colwell *et al* 1985). Molecular genetic methods are useful tools for assessing the ecology and population genetics of targeted microbial populations or communities (Mills 1994, Morgan 1991 and Van Elsas and Waalwijk, 1991), but such methods do not provide an insight into ecosystem function as a whole.

Measurement of soil enzyme activities may be useful for gaining a greater understanding of the nature of perturbations caused to ecosystem function. Soil enzyme measurements have been successfully used by Mawdsley and Burns (1995) to assess perturbations caused by the introduction of a *Flavobacterium* species, and by Naseby and Lynch (1997b) with the inoculation of a *Pseudomonas fluorescens* strain. However, Doyle and Stotzky (1993) did not find such perturbations in non-rhizosphere soil with the addition of an *Escherichia coli* strain. The work of Doyle and Stotzky (1993) did not include plants in the soil systems and thus is not ecologically relevant to soil-plant-microbe interactions. Naseby and Lynch (1998b) found evidence, using soil enzyme activities, for changes in carbon availability caused by increased C leakage from the roots with the inoculation of the same *Pseudomonas* strain used in this study.

The monitoring of soil C content is useful in the measurement of soil metabolic activity. The quantification of total organic C is in itself inadequate as a sensitive indicator of changes in soil activity (Pascual *et al* 1998a; Sparling 1992), therefore it is necessary to more specific C fractions. The total soil carbohydrate is an available source of carbon and energy to the microbial community (Pascual *et al* 1999) and is closely related to soil structural stability (Christensen, 1986). Carbohydrates of plant origin are predominately used as carbon and energy sources, whereas, microbial carbohydrates predominately serve a structural function in soil (Martens and Frankenberger, 1991).

The water-soluble organic matter fraction is important as it is the most labile part of the organic matter and therefore the most susceptible to mineralisation (Cook and Allan, 1992). Many authors describe this as an important process in pedogenesis (Duchaufour, 1991), along with other processes such as metal complexation and buffering effects in soil solutions (Kuiters

and Mulder, 1993). As this fraction is the most labile, it is the first to be utilised by the soil microorganisms as a carbon and energy source. It comprises of a heterogeneous mixture of varying molecular weight components, such as mono- and polysaccharides, polyphenols, proteins and low molecular weight organic acids (Kuiters and Denneman, 1987; Fahey and Yavitt, 1988). Barber and Gunn (1974) found that the major components of cereal root leakage under mechanical stress were organic acids and it is therefore, possible that organic acids were the major component of the increase in C availability found by Naseby and Lynch (1998b).

The deletion of 2,4-diacetylphloroglucinol (DAPG) production in the *Ps. fluorescens* F113 G22 strain is a functional modification, and allows the assessment of the impact of an antimicrobial-producing inoculum in comparison with a non-producer. These genetic differences are designed to have an effect on the ecosystem and indeed Shanahan *et al* (1992b) has shown that the wild type has an inhibitory effect *in vitro* on both bacteria and fungi and has isolated DAPG from soil (Shanahan *et al* 1992a). DAPG production has also been shown in the rhizosphere of microcosm grown plants (Keel *et al*, 1992 and Maurhofer *et al*, 1995) and is useful in the control of damping off diseases (Fenton *et al* 1992).

The aim of the experiment reported here was to investigate the effect of inoculation of the pea rhizosphere with a *Ps fluorescens* strain with a functional modification. This strain was compared to its *Tn5* mutated non-antibiotic producing derivative in large numbers in the pea rhizosphere to assess the impact of the inoculum and antibiotic production on rhizosphere organic acids, other carbon fractions and available nutrients.

MATERIALS AND METHODS

Soil description

The soil used was a sandy loam of the Holiday Hills series, taken from Merrist Wood Agricultural College (Surrey), and had been under permanent pasture for at least 15 years. The analysis of the soil, conducted at the University of Surrey, was pH 5.4, particle ratio 10:9:81 clay: silt: sand respectively, and organic matter content 1.6% by weight.

Microcosm

Coarsely sieved (6 mm) loose soil (250 g) was placed in experimental microcosms, as used by Naseby and Lynch (1998a), consisting of 210 mm high acetate cylinders, slotted between the top and base of plastic 90 mm diameter Petri dishes creating semi-enclosed microcosms.

Bacterial strains and treatments

Two strains of *Pseudomonas fluorescens* were used with different modifications. Strain F113 that produces the antibiotic 2,4 diacetylphloroglucinol (DAPG), and is marked with a *lac*ZY gene cassette, and a DAPG negative derivative (strain F113 G22) produced by Tn*5* mutagenisis (Shanahan *et al* 1992b).

The bacteria were grown on full strength tryptone soya agar (Oxoid) on petri dishes for 4 days at 30°C. The bacteria were suspended in 10 ml^{-1} of sterile quarter strength Ringer's

solution using disposable plastic plate spreaders to scrape off the bacterial mat and the colony forming units (c.f.u.) were determined. Control plates (without bacteria) were also flooded with quarter strength Ringers solution and surface scraped with spreaders. The resulting suspensions containing 6×10^9 c.f.u. ml^{-1} were subsequently used to imbibe pea seeds (*Pisium sativum* var. Montana), at a ratio of one seed per ml, for 8 hours (stirred every 30 minutes) resulting in between 2 and 4×10^8 c.f.u. per pea seed.

Experimental design

Each treatment was replicated eight times. Each microcosm consisted of eight imbibed seeds, planted at a depth of approximately 1 cm below the soil surface. Thirty ml^{-1} of water was added to each microcosm before they were placed in a random design into a growth chamber (Vindon Scientific) set at a 16 hour photoperiod with a day/night temperature regime of $21^{o}C/15^{o}C$ respectively. The relative humidity was maintained at 70% and the light intensity was 10,000 lux at shelf level.

Sampling and analysis

After 17 days growth the microcosms were harvested, after which soil closely associated with the plant roots (rhizosphere soil) was collected by shaking soil closely associated with the roots over a 2 mm sieve and stored over-night at $4^{o}C$. Subsequently each sample was assayed for soil alkaline phosphatase, aryl sulphatase, β glucosidase and urease to assess the effect upon P, S, C and N cycle enzymes respectively, by the methods of Naseby and Lynch (1997b).

The TOC content, humic substances, soluble and precipitated carbon and water-soluble carbon were determined by the potassium dichromate oxidation in sulphuric acid method of Yeomans and Bremner (1989). Total carbohydrates were digested with 72% sulphuric acid and determined using the antrone reaction of Brink *et al*., (1960). Water-soluble protein was estimated from the water-soluble fraction using the method of Lowry *et al*., (1951). Phenolic compounds were measured by a modification of Folin method (Kuwatsuda and Shindo, 1973) and water-soluble carbohydrates were determined according to Brink *et al*., (1960).

Rhizosphere soil was analysed for water soluble anion, cation and organic acid contents by adding 5ml of water to 1g of rhizosphere soil in a 10ml centrifuge tube. The soil suspensions were mixed for 1h on a carousel rotor before being centrifuged at 4000 rev min^{-1} for 15 minutes. The supernatant was decanted off into clean test tubes and kept at 4°C until required on the same day. A DIONEX^{TM} DX100 ion chromatograph with a conductivity detector was used to determine the concentration of ions and organic acids in samples of the supernatant using the manufacturers recommended conditions and procedures.

Shoot and root fresh weights were measured and a 1g-root sample from each replicate was macerated in 9 ml^{-1} of sterile quarter strength Ringers solution using a pestle and mortar. P1 medium (Katoh and Itoh, 1983) was used for the enumeration of indigenous, fluorescent *Pseudomonas*. To enable quantification of introduced *Ps. fluorescens* strains; this medium was amended with 50 ppm X-Gal upon which recovered *lac*ZY modified *Pseudomonas* could be identified as blue colonies. P1 plates were incubated at 25°C and enumerated after 5 days growth. The sum of the indigenous and introduced *Pseudomonas* populations were calculated and described as total *Pseudomonas* populations.

Statistical analysis

Data were analysed using SPSS for Windows (SPSS inc.) by means of a one way ANOVA and subsequently differences between treatments (multiple comparisons) were determined using least significant differences (LSD) between means as the post-hoc test.

RESULTS

Plant growth measurements (Table 1) were used to assess the potential impact of the different inocula on crop productivity. As neither shoot nor root weights showed significant differences between treatments, yet the F113 (DAPG+) inocula resulted in a greater root weight, the results were converted into shoot to root ratio, to take both into account together.

The F113 G22 treatment had a significantly greater indigenous and total (the sum of the introduced and indigenous populations) fluorescent *Pseudomonas* population (Table 2) than the control and F113 (DAPG+) treatment. However, the intoduced populations of F113 and F113 G22 were not significantly different (Table 2).

Both bacterial inocula significantly affected a number of carbon fractions (table 3). Both strains significantly increased the water-soluble carbohydrates and the total water-soluble carbon. However, neither of the strains significantly affected the total carbohydrates or the total organic carbon content of the rhizosphere soil. Strain F113 significantly increased the protein content relative to the control but not in relation to the F113 G22 treatment, which was intermediate in rhizosphere soil protein content.

The inocula significantly affected a variety of rhizosphere soil organic acid contents (Table 4). Malic and glycolic acids were not detected in rhizosphere soil under any of the treatments (data not shown) whilst tartaric acid was detected with the inoculation of both strains of F113 but was not detected in the control. Lactic, acetic and succinic acids along with the total organic acids were all found in significantly greater quantities in rhizosphere soil inoculated with strain

F113 than with the F113 G22 inocula which was in turn significantly greater than the control treatment. Oxalic and formic acids were found in significantly greater quantities with the F113 treatment than with both the control and F113 G22 treatments.

The alkaline phosphatase, urease and aryl sulphatase activities (Table 5) were significantly greater with the inoculation of the F113 DAPG+ strain than in the control ($p < 0.05$), whilst the F113 G22 treatment was intermediate in activity. The β glucosidase activity (Table 5) was significantly lower with the inoculation of the F113 DAPG+ strain than the control whilst the F113 G22 treatment was intermediate in activity.

Both inocula significantly increased the potassium, magnesium, calcium and chloride levels in the rhizosphere soil with respect to the control (table 6). The F113 inocula also resulted in significantly greater levels of magnesium and calcium than the F113 G22 treatment. However, both inocula resulted in significantly lower phosphate contents than the control.

DISCUSSION

Plant growth

The conversion into shoot/root ratio has been used extensively in the past (Clark and Reinhard, 1991) and has been suggested to be an indicator of plant stress, whereby the lower the shoot/root ratio (or higher the root/shoot ratio) the more stressed the plant. It should be recognised however, that such stressed plants may be more effective in acquiring water and nutrients as a result of the expanded root system and thus this is a positive adaptive response to such stresses.

As the F113 (DAPG+) strain produced a significantly smaller shoot/root ratio than the control and the F113 G22, it can be deduced that this strain has attributes that cause stress in pea plants, that have been deleted in the F113 G22 strain, and thus the plant stress is a result of the DAPG production. Either the antibiotic affected the plant directly or the indigenous microbial community structure in the rhizosphere, which in turn caused plant stress by an increase in detrimental micro-organisms and/or a reduction in beneficial populations (protective or stimulatory organisms).

Pseudomonas populations

It is conceivable that the inoculation of a micro-organism into the environment will cause the largest effects upon populations of a similar nature to the released strain, i.e. the indigenous *Pseudomonas* populations. Therefore, it was necessary to enumerate not only the introduced strains but also the indigenous population.

The greater indigenous fluorescent *Pseudomonas* with the F113 G22 treatment suggests that this strain promoted the *Pseudomonas* indigenous population. However as the F113 inocula resulted in a smaller indigenous *Pseudomonas* population than its DAPG mutated derivative (F113 G22) it is therefore directly attributable to the DAPG production and caused by repression of the indigenous population by the antibiotic.

From the total *Pseudomonas* populations and indigenous *Pseudomonas* populations, it is evident that neither strain displaced the indigenous populations. It is, therefore, plausible that the F113 G22 inocula had an additive effect on the total *Pseudomonas* population by occupying a niche distinct from that of the indigenous *Pseudomonas* populations. Evidence for this hypothesis comes from the smaller indigenous *Pseudomonas* population with introduction of F113 (DAPG+), where the inocula did not form a larger population to compensate for the reduced indigenous *Pseudomonas* population. This was shown with the resulting total *Pseudomonas* population which was similar to the control with the F113 (DAPG+), and significantly ($p<0.05$) smaller than the F113 G22 treatment.

Rhizosphere soil carbon fractions

The only C fractions affected by the inocula were the water-soluble carbohydrates and the total water-soluble carbon, whilst strain F113 significantly increased the protein content relative to the control. This indicates that the effects of the strains are mainly on easily available water soluble C fractions. The soluble fractions are more easily available to the microbial community and therefore where immediate effects of the microbial community are most evident (Van Veen *et al* 1985; Pascual *et al* 1998b). The total organic C content in soil comprises of all the sub

fractions measured (carbohydrates, proteins and organic acids etc.) and is therefore less sensitive to perturbation. The inoculation of the *Pseudomonas* strains may change the quality of the TOC, however this would not be indicated by the quantitative measurement of TOC (Pascual *et al* 1999). The total carbohydrate content as with the TOC did not show a significant effect with the inoculation of the *Pseudomonas.*

Organic acid contents

The organic acid contents indicate significant increases in the available carbon in the rhizosphere (by stimulation of root exudation or leakage) with both inocula, but with quantitative differences between the two. The data also corroborates previous work with the same *Pseudomonas* strains where a variety of soil enzyme activities indicated an increase in available C with the inoculation of strain F113 (Naseby and Lynch, 1998b). Other studies have highlighted changes in root exudation caused by biocontrol *Pseudomonas fluorescens* strains, for example Mozafar *et al* (1992) showed changes in the organic and amino acids exuded by tomato plants with the inoculation of *Ps. fluorescens*.

Ps. fluorescens can utilise most simple organic acids as carbon sources via the Krebs cycle which helps provide a wide metabolic range for the colonisation of diverse niches such as soil or the rhizosphere (Latour and Lemanceau, 1997). Therefore, organic acids would not be expected to accumulate in the rhizosphere. Illmer *et al* (1995) showed that a *Pseudomonas* species did not release organic acids in detectable amounts and the mutated strain, F113 G22, did not cause such a large increase in organic acids in this study, therefore, the increase in organic acids found can-not be directly attributed to the inocula. This implies that the increase in the release of these organic acids is advantageous to the *Pseudomonas* inocula and the

indigenous *Pseudomonas* population. The indigenous *Pseudomonas* population was significantly greater with the F113 G22 treatment which also had a significantly greater organic acid content than the control, but was much lower than the F113 treatment. This suggests that the F113 (DAPG+) had an inhibitory effect on the indigenous *Pseudomonas* population which lead to a build up in the organic acids released from the plant root. The non-DAPG producing strain F113 G22 did not cause such an inhibitory effect and therefore a greater indigenous population was formed which caused a depletion in the organic acid pool released from the plant.

Enzyme activities

The greater alkaline phosphatase and aryl sulphatase activities with the F113 DAPG+ inocula infers that the effect is caused by the production of DAPG, as the effect was not found with the inoculation F113 G22. Increased available inorganic soluble phosphate is known to have an inverse effect on soil phosphatase activity (Tabatabai, 1982 and Tadano *et al*, 1993) and similar tends occur in sulphatase activity in relation to sulphate availability. If this theory is correct, the F113 (DAPG$^+$) strain must have caused a decrease in the available phosphate, thus causing an overall increase in activity. This decrease in available P and S may have taken the form of an increase in the amount of available carbon in the rhizosphere as shown by the increase in organic acids, increasing the ratio of C to available P and S which increased the microbial P and S demand. However, soil available P levels were similar with both inocula and both were significantly lower than the control. This suggests that there was an increased demand for P with both inocula. Urease activity was greater with the F113 inocula showing a similar trend to

the phosphatase and sulphatase activities, and was related to a decrease in the available ammonium (table 6).

An inverse trend was found with the β glucosidase (C cycle) activity in comparison to the general trend found in the P and S cycle enzymes (as was found in Naseby and Lynch, 1996b). The activity was lowest with the inoculation of the F113 (DAPG$^+$) strain whilst the other treatments resulted in comparable higher activities. Again the mechanisms of the perturbations are uncertain, but may be the result of a change in community structure towards populations that produce less of the enzymes. A more likely explanation, as indicated by the P and S cycle enzymes, is a physiological effect upon the ecosystem and the indigenous populations, where P and S are the limiting nutrients and C is not, as shown by the increase in available carbon.

Ion contents

The increases in potassium, magnesium, calcium and chloride with the inocula are likely to be related to the release of organic acids by plant roots. Organic acids have been implicated in the solubilisation of nutrients in soil, for example, Otani *et al* (1996) found that organic acids released by pigeonpea were important in the release of P from feric phosphates and aluminium phosphates. Kraffczyk *et al* (1984) showed that organic acids are important in the release of cations, specifically potassium, from soil which explains the increase in available cations with the inocula. Therefore, the increase in organic acid release from the plant root with the F113 inocula may be of advantage to the plant in terms of nutrient uptake. The depletion of the organic acid pool caused by the increase in the *Pseudomonas* population in the F113 G22

treatment in comparison to the F113 treatment therefore resulted in less ions being mobilised or a greater proportion of the ion pools being utilised.

The slight decrease in available P with the inoculation of both *Pseudomonas* strains is in spite of the increase in organic acids released, which would increase the solubilisation of P from soil. However, this is a large increase in available C and lead to P being relatively a more limiting nutrient in soil and thus there was a greater demand for P. Indeed, an increase in phosphatase activity with the inoculation of strain F113 in the pea rhizosphere, which is an indication of P limitation.

Both F113 and F113 G22 caused an increase in the organic acids released from the plant root (by leakage or exudation). The overall effect of the antibiotic production by strain F113 was therefore not directly on the plant, but was a reduction in the utilisation of organic acids released from the plant resulting in differing effects of the two strains on nutrient availability, plant growth and *Pseudomonas* populations. Therefore the inoculation of *Pseudomonas fluorescens* caused significant changes in the chemistry and biochemistry of the pea rhizosphere and the production of DAPG had an even greater effect than the impact of inoculation alone. The attributes of such inocula that affect nutrient release from plant growth should therefore be assessed in future GMM studies with the possibility of tailering inocula to stimulate the release of nutrients that the inocula can utilise to promote its population and efficacy. The possibility of detrimental effects on nutrient cycling and on the plant should also be evaluated. This therefore indicates that functional GMM studies should assess the effect upon the plant and nutrient cycles/enzyme activities.

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Table 1: Mean pea plant shoot and root weights and the ratio between the two, as affected by DAPG producing and non producing *Ps. fluorescens* inoculation.

Plant#	Control*	F113 G22*	F113*
Shoot wt (g)	1.11 ± 0.07	1.09 ± 0.07	1.11 ± 0.05
Root wt (g)	0.62 ± 0.08	0.60 ± 0.1	0.68 ± 0.09
S/R Ratio	1.79 ± 0.04^{b}	1.82 ± 0.04^{b}	1.63 ± 0.04^{a}

*Treatments; control, no inocula; F113 G22, inoculated with *lac*ZY marked DAPG- (Tn*5* mutated) *Ps. fluorescens* F113 G22; F113, inoculated with *lac*ZY marked DAPG+ *Ps. fluorescens* F113.

S/R ratio: ratio of shoot weight to root weight. Standard errors for means (n=8) indicated. Significant differences between treatments at p=0.05 level indicated by different letters.

Table 2: Log fluorescent *Pseudomonas* populations in the rhizosphere of pea plants inoculated with DAPG producing and non producing *Ps. fluorescens* strains.

Population#	Control*	F113 G22*	F113*
Ind Pseu	6.25 ±0.014[a]	6.59 ±0.042[b]	6.15 ±0.045[a]
Int Pseu	N/A	6.16 ±0.054	6.16 ±0.032
Tot Pseu	6.25 ±0.014[a]	6.72 ±0.034[b]	6.45 ±0.033[a]

*Treatments; control, no inocula; F113 G22, inoculated with *lac*ZY marked DAPG- (Tn*5* mutated) *Ps. fluorescens* F113 G22; F113, inoculated with *lac*ZY marked DAPG+ *Ps. fluorescens* F113.

c.f.u./g fresh root. Ind pseu, indigenous *Pseudomonas;* int pseu, introduced *Pseudomonas;* tot pseu, total *Pseudomonas.* Standard errors for means (n=8) indicated. Significant differences between treatments at p=0.05 level indicated by different letters.

N/A: not applicable as no genetically marked strains were introduced

Table 3: Carbon fractions of pea rhizosphere soil inoculated with DAPG producing and non producing *Pseudomonas fluorescens* strain F113.

C fraction#	Control*	F113 G22*	F113*
Total carbohydrates	918.32 ± 60.73	1006.64 ± 67.73	962.32 ± 69.14
Total organic C	1.02 ± 0.063	0.93 ± 0.069	0.95 ± 0.044
Carbohydrates (H$_2$O)	139.44 ± 8.57 [a]	200.04 ± 8.36 [b]	209.41 ± 8.29 [b]
Water soluble C	1855.32 ± 139.74[a]	2731.78 ± 77.73 [b]	2669.73 ± 54.92 [b]
Protein	56.16 ± 4.78 [a]	69.56 ± 4.66 [ab]	78.38 ± 5.88 [b]

Expressed as ppm/g dry soil. Standard errors for means (n=8) indicated. Significant differences between treatments at p=0.05 level indicated by different letters.

*Treatments; control, no inocula; F113 G22, inoculated with *lac*ZY marked DAPG- (Tn*5* mutated) *Ps. fluorescens* F113 G22; F113, inoculated with *lac*ZY marked DAPG+ *Ps. fluorescens* F113.

Table 4: Organic acids extracted from pea rhizosphere soil inoculated with DAPG producing and non producing *Pseudomonas fluorescens* strain F113.

Organic Acid#	Control*	F113 G22*	F113*
Oxalic	1.191 ± 0.056^a	1.245 ± 0.048^a	1.416 ± 0.069^b
Tartaric	N/D	0.145 ± 0.038^b	0.158 ± 0.044^b
Citric	4.435 ± 0.218	4.275 ± 0.123	4.447 ± 0.146
Formic	0.024 ± 0.008^a	0.056 ± 0.014^a	0.159 ± 0.024^b
Lactic	0.362 ± 0.055^a	0.900 ± 0.094^b	1.628 ± 0.134^c
Acetic	0.049 ± 0.017^a	0.391 ± 0.051^b	1.522 ± 0.167^c
Succinic	0.025 ± 0.009^a	0.169 ± 0.019^b	0.588 ± 0.076^c
Total	6.086 ± 0.363^a	7.18 ± 0.387^b	9.918 ± 0.66^c

Expressed as ppm./g dry soil. Standard errors for means (n=8) indicated. Significant differences between treatments at p=0.05 level indicated by different letters.
N/D Not detected.

*Treatments; control, no inocula; F113 G22, inoculated with *lac*ZY marked DAPG- (Tn*5* mutated) *Ps. fluorescens* F113 G22; F113, inoculated with *lac*ZY marked DAPG+ *Ps. fluorescens* F113.

Total; sum of the organic acids detected, nb. Malic and glycolic acids were not detected in any treatment.

Table 5: Soil enzyme activities in the rhizosphere of pea plants inoculated with DAPG producing and non producing *Pseudomonas fluorescens* strain F113.

Enzyme#	Control*	F113 G22*	F113*
Alkaline phosphatase	0.69 ± 0.037^a	0.71 ± 0.062^{ab}	0.78 ± 0.031^b
Sulphatase	0.031 ± 0.01^a	0.04 ± 0.006^{ab}	0.048 ± 0.005^b
β glucosidase	0.56 ± 0.085^b	0.53 ± 0.081^{ab}	0.44 ± 0.041^a
Urease	39.78 ± 1.98^a	41.22 ± 2.91^{ab}	44.62 ± 2.32^b

Expressed as mg pNP released/g dry soil. Urease expressed as mg NH_3 released/g dry soil. Standard errors for means (n=8) indicated. Significant differences between treatments at p=0.05 level indicated by different letters.

*Treatments; control, no inocula; F113 G22, inoculated with *lac*ZY marked DAPG- (Tn*5* mutated) *Ps. fluorescens* F113 G22; F113, inoculated with *lac*ZY marked DAPG+ *Ps. fluorescens* F113.

Table 6: Ions extracted from pea rhizosphere soil inoculated with DAPG producing and non producing *Pseudomonas fluorescens* strain F113.

Ion#	Control*	F113 G22*	F113*
Sodium	29.86 ± 0.83	29.99 ± 1.01	30.52 ± 1.18
Ammonium	5.95 ± 0.37^b	5.79 ± 0.51^b	4.67 ± 0.53^a
Potassium	10.54 ± 0.31^a	12.08 ± 0.25^b	12.86 ± 0.31^b
Magnesium	12.74 ± 0.26^a	14.48 ± 0.08^b	15.12 ± 0.07^c
Calcium	75.08 ± 1.62^a	86.45 ± 0.3^b	88.48 ± 0.28^c
Chloride	22.47 ± 0.52^a	25.40 ± 0.76^b	26.19 ± 0.81^b
Nitrite	2.17 ± 0.01	2.18 ± 0.02	2.29 ± 0.01
Nitrate	22.94 ± 0.37	24.50 ± 0.43	24.31 ± 0.28
Phosphate	19.19 ± 0.42^b	18.07 ± 0.18^a	18.22 ± 0.16^a
Sulphate	14.26 ± 0.37	14.25 ± 0.38	13.75 ± 0.35

Expressed as ppm./g dry soil. Standard errors for means (n=8) indicated. Significant differences between treatments at p=0.05 level indicated by different letters.

*Treatments; control, no inocula; F113 G22, inoculated with *lac*ZY marked DAPG- (Tn*5* mutated) *Ps. fluorescens* F113 G22; F113, inoculated with *lac*ZY marked DAPG+ *Ps. fluorescens* F113.