Ecological interaction of a biocontrol *Pseudomonas fluorescens* strain producing 2,4-diacylphloroglucinol with the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica*

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Abstract

*Erwinia carotovora* subspecies *atroseptica* is the agent of soft rot of potato and causes important crop damage in Europe. Synthetic 2,4-diacylphloroglucinol (DAPG) inhibited the growth of *E. carotovora* subsp. *atroseptica* under in vitro conditions and *Pseudomonas fluorescens* F113, which produces DAPG, was studied for biocontrol of *E. carotovora* subsp. *atroseptica*. Wild-type F113 (or the spontaneous Rifampicin-resistant mutant F113Rif) inhibited growth of *E. carotovora* subsp. *atroseptica* on solid medium, displayed bactericidal activity towards the pathogen in liquid medium, and prevented *Erwinia*-mediated rotting of wounded potato tuber under in vitro conditions. F113Rif reduced the population size of *E. carotovora* subsp. *atroseptica* in soil and on potato tuber dices in competition experiments carried out with unplanted soil and soil planted with diced potato tubers, respectively. Co-inoculation of potato tuber seeds with F113Rif and *E. carotovora* subsp. *atroseptica* reduced *Erwinia* contamination of the seed tubers compared with single inoculation with the pathogen. F113G22 is a Tn5::TetZ-induced DAPG-negative biosynthetic derivative of F113 and showed no antibiosis towards *E. carotovora* subsp. *atroseptica* in vitro. In contrast to F113Rif, F113G22 did not inhibit *Erwinia*-mediated rotting of wounded potato tuber in vitro, did not influence survival of *E. carotovora* subsp. *atroseptica* in unplanted soil or soil planted with potato tuber dices and did not reduce *Erwinia* contamination of potato seed tubers. F113G22(pCU203) is a complemented derivative with restored DAPG-producing ability. F113G22(pCU203) had similar effects against *E. carotovora* subsp. *atroseptica* as F113 (or F113Rif) under in vitro conditions and in soil microcosms. The results indicate that *P. fluorescens* F113 is a promising biocontrol agent against the potato soft rot agent *E. carotovora* subsp. *atroseptica* and suggest that the pseudomonad's ability to produce DAPG is a key factor in its inhibition of the pathogen.

Keywords: Biocontrol; *Pseudomonas*; DAPG; *Erwinia*

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1. Introduction

*Erwinia carotovora* is a broad host-range pathogen causing seed piece decay, blackleg and aerial soft rot, especially in potatoes [1]. One of the four subspecies of *E. carotovora*, i.e. subsp. *atroseptica* is considered the most important soft rot bacterium in Europe due to its ability to be pathogenic at low temperature in moist soil [2]. *E. carotovora* subsp. *atroseptica* survives better than the other three subspecies of *E. carotovora* in cool soils and causes blackleg earlier in the season. The pathogenicity of *E. carotovora* subsp. *atroseptica* (hereafter referred to as *E. carotovora*) is almost entirely limited to potato crops. Currently, control of this pathogen relies on (i) seed technology; i.e. tuber pasteurisation, sanitation and use of certified seed, (ii) application of chemical pesticides in the field and (iii) the use of potato cultivars resistant to *E. carotovora*. However, the recent development of more virulent strains of *E. carotovora*, the toxicity of chemical control towards humans and the environment, and the high cost of the control methods currently available have stimulated the search for alternative methods of control such as biological control.

The predominant group of bacteria that has been studied for biocontrol of *Erwinia*-mediated soft rot corresponds to the fluorescent pseudomonads since they are aggressive rhizosphere colonisers and produce a wide range of antimicrobial compounds [3,4]. Certain fluorescent pseudomonads can protect plants from diseases caused by root pathogens and often this biocontrol effect involves antimicrobial compounds such as siderophores [5], hydrogen cyanide [6], and antibiotics like phenazine-1-carboxylate [7], pyoluteorin [8] and 2,4-diacetylphloroglucinol (DAPG; [9–11]). Biological control of *E. carotovora* has been achieved by inoculating potato seed pieces with bacteria antagonistic to *E. carotovora* prior to planting [12,13].

A number of DAPG-producing fluorescent pseudomonads have been studied for biocontrol of pathogenic fungi [9,14–16] and bacteria [15,16]. While the importance of DAPG has been shown for the biocontrol of certain pathogenic fungi, only circumstantial evidence is available regarding the role of DAPG in the biocontrol of *E. carotovora* by fluorescent pseudomonads. DAPG producer *P. fluorescens* F113 suppresses damping-off of sugarbeet in soil microcosms prepared with soil naturally infested by *Pythium* [14]. The objective of this study was to evaluate strain F113 for biocontrol of *E. carotovora* subsp. *atroseptica* and to investigate the role of DAPG in the interaction between the biocontrol pseudomonad and the potato soft rot pathogen.

2. Materials and methods

2.1. Strains and culture conditions

*Pseudomonas fluorescens* F113 produces the secondary metabolite DAPG [10]. A spontaneous rifampicin-resistant mutant of F113 (i.e. F113Rif) was used in some of the experiments [17]. Strain F113Rif produces DAPG and displays a similar growth rate in liquid laboratory media compared to the wildtype [17]. F113G22 is a Tn5::lacZY-induced DAPG-negative biosynthetic mutant of F113 and F113G22-(pCU203) is a complemented derivative of F113-G22 [14]. *E. carotovora* subsp. *atroseptica* strain SCR147 [12] was kindly provided by Rhodes and Logan (Queen’s University of Belfast, Northern Ireland). A spontaneous streptomycin-resistant mutant of the strain (hereafter referred to as Eca), which causes potato soft rot in vitro and grows like the wildtype in liquid culture, was used in this study.

All strains were maintained on LB agar [18] at 4°C and were grown at 28°C with shaking. Growth media included LB, sucrose asparagine (SA; [19]), SA-Fe (i.e. SA supplemented with 100 µM FeCl3) and two iron-rich minimal media derived from Shanan’s minimal medium [10]. The minimal media were composed of succinate (27 g l⁻¹; i.e. succinate/KNO₃ medium) or succrose (34.2 g l⁻¹; i.e. sucrose/KNO₃ medium), KNO₃ (4.25 g l⁻¹), Na₂HPO₄ (4.375 g l⁻¹) and KH₂PO₄ (1.875 g l⁻¹) and iron (27 mg l⁻¹). Cells from overnight cultures (approximately 10⁹ CFU ml⁻¹) were washed twice in quarter-strength Ringer’s solution (Oxoid) prior to inoculation. Antibiotics were used at the following concentrations (µg ml⁻¹): rifampicin, 100 (Rif100); kanamycin, 50 (Km50); chloramphenicol, 200 (Cm200); and streptomycin, 100 (Sm100).
2.2. *E. carotovora* subsp. *atroseptica* growth inhibition test

The effect of F113, F113G22 and F113G22p (pCU203) on growth of Eca was investigated using a growth inhibition bioassay similar to that of Fenton et al. [14]. Media used included solid LB, SA, SA-Fe, sucrose/KNO$_3$ and succinate/KNO$_3$. Three 5-µl samples of cell suspension from each pseudomonad ($2 \times 10^6$ CFU per 5-µl sample) were spotted onto solid media and the plates were incubated overnight at 28°C. An Eca cell suspension ($10^9$ CFU ml$^{-1}$) was sprayed onto the plates using an aerosol sprayer (Sigma) and the plates were further incubated at 28°C for 72 h. Three plates of each medium were used per pseudomonad studied.

The effect of DAPG on growth of Eca was investigated using a similar in vitro assay, as follows. Synthetic DAPG produced through acetylation of phloroglucinol and assayed for impurities by NMR and HPLC analysis (Chemistry Department, UCC, Cork, Ireland) was dissolved in 99% methanol (1 mM DAPG) and diluted in sterile distilled water to a concentration of 1 µM DAPG. A 15 µl aliquot was spotted onto the centre of each LB plate used. The plates were dried in a laminar-flow hood and were kept at 4°C until inoculation with Eca was performed. Eca was sprayed and the plates were incubated, as described above. The experiment was performed in triplicate.

2.3. In vitro competition between *E. carotovora* subsp. *atroseptica* and *P. fluorescens*

Competition experiments were conducted by co-inoculating SA-Fe broth with Eca and F113Rf, F113G22, or F113G22(pCU203) in a 1:1 ratio. Each strain was inoculated at the level of about $10^6$ CFU ml$^{-1}$. The experiment was performed in 250 ml Erlenmeyer flasks containing 100 ml of medium and the flasks were incubated at 28°C with shaking (rotary shaker; 280 rpm). Flasks inoculated with single strains were used as controls. Colony counts were carried out periodically using 1 ml samples from the flasks. Three 20 µl volumes were spotted onto SA+Rif100 (for F113Rf), SA+Km50 (for F113G22), SA+Km50+Cm200 (for F113G22(pCU203)), and SA+Sm100 (for Eca). The plates were incubated at room temperature (approximately 20°C) for 72 h and the colonies were counted. The experiment was performed in duplicate.

2.4. In vitro pathogenicity bioassay

The effect of *P. fluorescens* on the pathogenicity of Eca was investigated using an in vitro bioassay in which pseudomonads were each co-inoculated with Eca into wounded potato tuber slices. Tubers (about 5 cm in diameter) of potato cv. 'Kerr's Pink' were washed free of soil in sterile distilled water and were soaked for three min in 2% sodium hypochlorite solution to disinfect the tuber surface. The tubers were rinsed in sterile distilled water and cut in slices (about 1 cm thick). Three wells were aseptically punched in each slice using a 5 mm diameter metal rod. The tuber slices were dried with sterile filter paper (Whatman No. 541). Each well was co-inoculated with 10 µl cell suspensions (i.e. $10^7$ CFU of each strain) of Eca and of either F113Rf, F113G22, or F113G22(pCU203). The experiment was also carried out with each strain inoculated singly. The tuber slices were placed into Petri dishes perforated for aeration and the latter were put into anaerobic jars (BBL Gas Pak Systems) lined with moist tissue paper. The jars were incubated at 12°C for 96 h and the surface area of rotted potato tissue was measured. The experiment was performed in duplicate.

2.5. Survival of Eca in unplanted soil microcosms

Soil microcosms consisted of 100 g of natural soil sampled from the top horizon (sandy loam texture; pH$_{H_2O}$ 6.9) of a brown podzolic soil. Sampling took place in a field located near Ovens (County Cork, Ireland) that was under sugarbeet cultivation. The soil was sieved through a 5-mm mesh screen and soil microcosms were prepared as described elsewhere [14,20]. Cell suspensions of Eca were added to the soil (about $10^6$ CFU g$^{-1}$ of soil) and the soil was mixed throughout. Pseudomonads were then inoculated into the soil at cell concentrations of approximately $10^9$, $10^6$ or $10^5$ CFU g$^{-1}$ of soil and the soil was mixed uniformly. The water content of the soil was adjusted to 25% w/w (i.e. 60% saturation of the soil porosity) using distilled water and
the soil was watered, by spraying, every three days. The pots were placed in a growth chamber (12°C; 16-h photoperiod) in a randomized block design. Pseudomonads tested included F113Rif, F113G22 and F113G22(pCU203). Pseudomonads and Eca were also inoculated singly, as controls. At weekly intervals, a 1-g soil sample was taken from each pot and extracted in quarter-strength Ringer’s solution for three min. A serial dilution was performed in the same solution and 100 µl volumes were spread-plated in duplicate onto SA+Rif100, SA+Km50, SA+Km50+Cm200 and SA+Sm100 to enumerate F113Rif, F113G22, F113G22(pCU203) and Eca, respectively. The experiment was performed in duplicate.

2.6. Survival of Eca in soil microcosms planted with potato tuber pieces

Soil microcosms were prepared using natural soil sampled from the sandy-loam surface horizon of a brown podzolic soil located near Bandon (County Cork, Ireland) and cropped with sugarbeet. The soil for this experiment was not collected at the Ovens site because sugarbeet was not grown at the time of sampling. Both field sites correspond to the same pedon and the soil materials sampled displayed essentially similar physico-chemical characteristics (data not shown). Soil microcosms and incubation conditions were as described above, except that 150 g of soil was used per pot. Eca was inoculated into soil before planting, at the concentration of about 2 x 10^6 CFU g^-1 of soil. Tuber pieces from potato cv. ‘Kerr’s Pink’ were soaked into a cell suspension of F113Rif, F113G22, or F113G22(pCU203) for 5 min. The experiment was also run with single inoculation of Eca or pseudomonad. The tuber pieces were planted at a depth of 3.5 cm (one tuber piece per pot) and the soil water content was adjusted to 26% w/w (about 60% saturation of the soil porosity) using distilled water. The pots were placed in the growth chamber for six weeks, as described above. At the end of the experiment, the tuber pieces were removed from the pots and colonisation of the tuber surface was determined. The tuber pieces were extracted in quarter-strength Ringer’s solution for 3 min. Dilution, plating, and colony counts were done as described above. The experiment was first performed using one-cm³ tuber dice (pseudomonads: 8 x 10^5 CFU tuber dice^-1) and was run in triplicate. The same experiment was then carried out using pre-sprouted potato seed tubers (pseudomonads: 8 x 10^5 CFU seed tuber^-1) and four replications were studied per treatment.

2.7. Statistical analyses

Two to four replications were used per treatment. Colony counts were log₁₀-transformed before analysis. All results were analysed by analysis of variance, using procedures of the Statistical Analysis System (SAS; The SAS Institute, Cary, NC, USA). When appropriate, treatments were compared using Fisher’s least significant difference (LSD) test. All analyses were conducted at P = 0.05 level.

3. Results

3.1. Effect of F113, F113G22 and F113G22(pCU203) on growth of E. carotovora subsp. atroseptica in vitro

The effect of DAPG producer P. fluorescens F113 on growth of Eca in vitro was studied by growing the pseudomonad as a single colony in the center of plates and monitoring the growth of Eca subsequently inoculated onto the plates by spraying of a cell suspension. Strain F113 inhibited growth of Eca on LB, SA, SA-Fe, and sucrose/KNO₃ plates (Table 1) but had no effect on succinate/KNO₃ plates, where F113 does not produce DAPG (data not shown). Similar results were obtained when plates were incubated at 12°C instead of 28°C prior to inoculation with Eca. The experiment was also carried out by spraying plates with the DAPG-sensitive indicator strain Bacillus subtilis A1 [14] and similar results were obtained regarding the ability of F113 to inhibit the growth of strain A1 on the five media studied.

DAPG-negative biosynthetic mutant F113G22 did not inhibit growth of Eca on LB, SA-Fe, sucrose/KNO₃ and succinate/KNO₃ plates (Table 1). However, F113G22 inhibited growth of Eca on SA plates, a medium that is low in iron and consequently fa-
Table 1
Inhibition of growth of *E. carotovora* subsp. *atroseptica* by *P. fluorescens* F113, F113G22 and F113G22(pCU203) under in vitro conditions at 28°C

<table>
<thead>
<tr>
<th></th>
<th>Media conducive to DAPG production</th>
<th>Media not conducive to DAPG production</th>
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<tbody>
<tr>
<td></td>
<td>LB</td>
<td>SA-Fe</td>
</tr>
<tr>
<td>No pseudomonad</td>
<td>none a¹</td>
<td>none a</td>
</tr>
<tr>
<td>F113</td>
<td>+++² b</td>
<td>+++ b</td>
</tr>
<tr>
<td>F113G22</td>
<td>none a</td>
<td>none a</td>
</tr>
<tr>
<td>F113G22(pCU203)</td>
<td>++ c</td>
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</table>

Pseudomonads were spotted and grown on the plates prior to inoculation with Eca.
¹For each medium, letters in bold are used to indicate statistical differences between treatments assessed by LSD.
²Diameter of inhibition zone between 2.4 and 3.0 cm are indicated by +++ and those between 1.5 and 2.3 cm are indicated by ++.

vours siderophore production and in which F113 produces low amounts of DAPG [10]. Similar results were obtained when the pseudomonad was grown at 12°C instead of 28°C. F113G22 failed to inhibit DAPG-sensitive *B. subtilis* strain Al on all media with the exception of solid SA. F113G22(pCU203), a complemented mutant of F113G22 with restored DAPG-producing ability inhibited growth of Eca like F113 on SA and sucrose/KNO₃ plates (Table 1). F113G22(pCU203) also inhibited growth of Eca on LB and SA-Fe plates, albeit to a lesser extent compared to F113. F113G22(pCU203), like F113, had no effect on Eca on succinate/KNO₃ plates. Similar results were obtained when F113G22-(pCU203) was grown at 12°C instead of 28°C. F113G22(pCU203) also inhibited DAPG-sensitive *B. subtilis* strain Al on all media tested except on solid succinate/KNO₃.

3.2. Effect of synthetic DAPG on growth of *E. carotovora* subsp. *atroseptica* in vitro

The addition of synthetic DAPG as a 15 µl spot of DAPG solution onto LB agar inhibited the growth of Eca on the plates when DAPG was used at concentrations greater than the threshold value of 23 µM DAPG (Fig. 1). Growth of Eca was not inhibited on plates receiving a 15 µl spot of methanol in which no DAPG had been dissolved. For concentrations higher than 23 µM DAPG, the increase in diameter of the zone of inhibition was linear with increasing log values of DAPG concentration.

![Graph](image)

Fig. 1. Inhibition of Eca by synthetic 2,4-diacyltetralinol on LB agar.
3.3. In vitro competition between *E. carotovora* subsp. *atroseptica* and *P. fluorescens*

Competition experiments were conducted by co-inoculating SA-Fe broth with Eca and F113Rif, F113G22, or F113G22(pCU203) in a 1:1 ratio. Co-inoculation with Eca and F113Rif resulted in lower cell numbers of Eca compared with those obtained with single inoculation of Eca (Fig. 2A,B). In contrast, F113G22 had no effect on Eca cell numbers and cell numbers of F113G22 were actually lower when the strain was co-inoculated with Eca compared with F113G22 inoculated singly (Fig. 2C,D). DAPG producer F113G22(pCU203) reduced cell numbers of Eca in competition experiments (Fig. 2E,F) and the effect of the pseudomonad on Eca was similar to that exerted by F113Rif (Fig. 2B,F), although occurring 30 instead of 12 h after co-inoculation.

3.4. Effect of *P. fluorescens* on Eca pathogenicity in vitro

*P. fluorescens* F113Rif reduced the extent of Eca-mediated soft rot of the potato tuber slices in vitro when both bacteria were co-inoculated into wells punched into the tuber slices (Fig. 3). Strain F113Rif failed to affect Eca-mediated soft-rotting of potato tissue when co-inoculation was performed using 10 μl cell suspensions containing 10^5 instead of 10^7 CFU of F113Rif. In contrast to F113Rif, DAPG-negative derivative F113G22 did not prevent Eca-mediated soft rot compared to situations where only Eca was inoculated. The complemented strain, F113G22(pCU203) also reduced Eca-mediated soft rotting of the tuber slice and the effect of the pseudomonad was similar to that of F113Rif. No soft rotting took place when any of the
three pseudomonads were inoculated in the absence of Eca.

3.5. Effect of *P. fluorescens* on survival of Eca in unplanted soil microcosms

Co-inoculation of soil with F113Rif (at 10^4, 10^6 or 10^8 CFU g^-1 soil) and 10^6 CFU Eca g^-1 soil resulted in lower cell numbers of Eca compared to those in soil inoculated with Eca alone (Fig. 4A). The effect of F113Rif, F113G22 and F113G22-(pCU203) introduced in soil at 10^8 CFU g^-1 soil on cell numbers of Eca co-inoculated at 10^6 CFU g^-1 soil was also investigated. Unlike F113Rif, F113G22 had no effect on Eca cell numbers in soil during the course of the six-week experiment (Fig. 4B). The complemented derivative F113G22-(pCU203) reduced the survival of Eca in soil, although to a lesser extent compared with F113Rif.

3.6. Effect of *P. fluorescens* on colonisation of tuber dice by Eca in soil microcosms

There was no difference in survival on tuber dice between strains F113Rif, F113G22 and F113G22 (pCU203) in soil microcosms in the absence of Eca (Fig. 5A,C,E). Inoculation of one cm^3 tuber dice with F113Rif prior to planting in soil previously inoculated with Eca (2×10^6 CFU g^-1 soil) reduced the extent of colonisation of tuber dice by Eca in the six-week experiment (Fig. 5A,B). Inoculation of tuber dice with the DAPG-negative mutant F113G22 did not affect their colonisation by Eca compared to that of uninoculated tuber dice (Fig. 5C,D). However, inoculation of the tuber dice with the complemented
pseudomonad F113G22(pCU203), with restored ability to produce DAPG, reduced Eca colonisation of the tuber dice, in a similar fashion as when F113Rif was used (Fig. 5E,F). In this experiment, no soft rotting of the potato tissue was found, even in the treatment where unoinoculated tuber dice were planted in Eca-inoculated soil.

3.7. Effect of *P. fluorescens* on colonisation of seed tubers by *Eca* in soil microcosms

Colonisation of pre-sprouted potato seed tubers by Eca was reduced when F113Rif was inoculated onto the seed tubers prior to planting them in Eca-enriched soil. F113G22 colonised the potato tubers to a lesser extent than F113Rif in uninoculated soil (Fig. 6A) and in soil inoculated with Eca (Fig. 6B). F113G22 was unable to influence colonisation of the seed tubers by Eca, whereas F113G22(pCU203) reduced seed tuber colonisation by Eca to the same extent as F113Rif did. No soft rotting of the seed tubers was found in any of the treatments.

4. Discussion

Certain fluorescent pseudomonads can improve plant growth through the control of harmful microorganisms and this often involves the production of enzymes and/or secondary metabolites such as antibiotics and siderophores [7,9,10,21,22]. In this work, the effect of DAPG-producing *P. fluorescens* F113 on the potato soft rot pathogen *E. carotovora* subsp. *atroseptica* was investigated.

F113 inhibited growth of Eca on a number of solid media that are conducive to DAPG production.
but had no effect on succinate/KNO₃ plates, where F113 does not produce DAPG (Table 1). These results suggest that DAPG production may be involved in Eca growth inhibition. The effect of DAPG on Eca was confirmed by the results of experiments in which F113G22, a transposon-induced DAPG-negative biosynthetic mutant of F113 and F113G22(pCU203), a DAPG-producing complemented derivative of F113G22 were compared with F113. Interestingly, F113G22 inhibited growth of Eca on SA plates, a low-iron medium that favours siderophore production in F113 and F113G22, suggesting that siderophore production also can contribute to biological control of Eca under these labora-
tory conditions. Strains F113 and F113G22 produce minute amounts of siderophore on iron-rich media SA-Fe, LB and sucrose/KNO₃, which indicate that Eca growth inhibition on these media resulted from the strain’s ability to produce DAPG. These results show that siderophore production by F113 may play a role in biocontrol of Eca under iron limiting conditions, but that DAPG is the metabolite primarily responsible for the control of Eca by F113 in vitro. The involvement of several secondary metabolites in biocontrol of black root rot of tobacco and damping-off of cress by pseudomonads has been demonstrated by Laville et al. [23] and Maurhofer et al. [24], respectively. On all three media where F113 produces DAPG (i.e. LB, SA-Fe and sucrose/KNO₃), F113G22(pCU203) inhibited growth of Eca to a lesser extent compared to F113. F113G22 (pCU203) produces less DAPG than F113 under certain laboratory conditions [14], which further indicates the role of DAPG in Eca growth inhibition. Vincent et al. [11] also failed to fully complement a Tn5-induced DAPG-negative mutant of *Pseudomonas* for DAPG production. Previous work has shown the prevalence of DAPG production in strains of *P. fluorescens* inhibitory to *E. carotovora* subsp. *atroseptica* [15] but the current study is the first one to demonstrate specifically the role of DAPG production by pseudomonads in their inhibition of *E. carotovora* subsp. *atroseptica* in vitro. Supplementation of LB plates with synthetic DAPG indicated that a concentration threshold of 23 μM DAPG was required to inhibit Eca growth at 28°C (Fig. 1). The level of Eca inhibition implemented by F113 and F113G22(pCU203) on LB plates was also achieved using synthetic DAPG at concentrations of 230 μM and 63 μM, respectively. Previous work has shown that *E. carotovora* subsp. *atroseptica* was inhibited by DAPG used at the concentration of 50 μg (i.e. 20 μmoles) of DAPG per disc [16].

The antagonistic effect of F113 on Eca may be bacteriostatic — preventing the growth of Eca, or bactericidal — affecting Eca survival. This point was investigated in competition experiments in vitro, where F113Rif, F113G22 and F113G22(pCU203) were co-inoculated each with Eca. Strain F113Rif was bactericidal to Eca as cell numbers of the latter decreased sharply before the time where Eca usually reaches the highest cell density in pure culture (Fig. 2). The effects of F113G22 and F113G22(pCU203) on Eca indicate that the bactericidal effect of F113Rif on Eca resulted from the strain’s ability to produce DAPG. In this work, no inhibition of Eca growth on plates took place at DAPG concentrations below 23 μM, which implies that DAPG produced by F113 becomes toxic to Eca only when the concentration of DAPG exceeds a threshold level. This is in accordance with the facts that F113Rif had no effect on Eca in liquid LB until 12 h after inoculation and that F113G22(pCU203), which produces DAPG to a lesser extent compared to F113, required 30 h to affect Eca cell numbers. Interestingly, F113G22 had no effect on cell numbers of Eca, but then Eca appeared to be detrimental to the pseudomonad. This experiment indicated that (i) F113Rif exhibits a bactericidal mode of control against Eca, (ii) a build-up of DAPG may be necessary in the medium for biocontrol of Eca by F113, and (iii) the partially complemented strain F113G22(pCU203) takes longer to exert the same effect on Eca as F113Rif, possibly due to a slower accumulation of DAPG or the inability to produce DAPG in concentrations as high as those achieved by F113Rif.

DAPG producer F113Rif also inhibited Eca-mediated soft rotting of potato tuber slices when both bacteria were co-inoculated into wounded tissue in vitro (Fig. 3). Results obtained with F113G22 and F113G22(pCU203) showed that biocontrol of Eca on potato tuber slices by F113Rif involved the strain’s ability to produce DAPG.

Further investigation was aimed at determining how soil factors would interfere with the ability of F113Rif to control Eca in soil and in the rhizosphere of potato. F113Rif exhibited a bactericidal effect towards Eca (introduced at 10⁶ CFU g⁻¹ soil) in unplanted soil when F113Rif was co-inoculated with Eca at cell densities of between 10⁴ and 10⁵ CFU g⁻¹ soil (Fig. 4A). The time period required for F113Rif to reduce Eca cell numbers decreased when higher inoculum sizes of F113Rif were used in the soil. Potato tissue colonisation by Eca was reduced when potato tuber dice or seed tubers were inoculated with F113Rif at the time of planting (Figs. 5 and 6). The ability of F113Rif to control the growth of Eca on potato tuber dice and seed tubers in soil suggests that the environ-
mental conditions at the surface of these potato tuber pieces are conducive to DAPG production by F113Rif.

Unlike F113Rif, F113G22 did not affect Eca survival in unplanted soil (Fig. 4B), although Eca had no effect on cell numbers of F113G22 in soil (data not shown). F113G22 had no effect either on the colonisation of potato tuber dice or seed tubers by Eca (Figs. 5 and 6). Interestingly, F113G22 did not colonise the seed tubers to the same extent as F113Rif in the absence of Eca. This is the first report where the loss of DAPG-producing ability affects colonisation of the pseudomonad in soil microcosms. In contrast to F113G22, F113G22(pCU203) reduced the cell numbers of Eca in unplanted soil microcosms, confirming that DAPG was involved in the control of Eca in soil. The pseudomonad also reduced colonisation of potato tuber dice and seed tubers by Eca. Collectively, these results demonstrate the importance of DAPG production in the effect of F113 and F113Rif on Eca.

In the current work, the soil microcosm experiments were designed with the objective to assess the nature of the interactions between DAPG-producing pseudomonads and E. carotovora subsp. atroseptica. Eca did not cause soft rotting of the potato tuber pieces under the experimental conditions chosen despite colonising the tuber dice and seed tubers. E. carotovora subsp. atroseptica can survive in soil for up to 10 weeks when the soil is cool and moist and enters the potato via the roots or wounds in the tuber [25]. E. carotovora subsp. atroseptica is an opportunistic pathogen and can reside in the potato tuber at low levels for some time before starting to proliferate and becoming pathogenic [2]. This suggests that the current soil microcosm experiments were too short to observe soft rot symptoms.

The results of the study indicate that DAPG-producing P. fluorescens F113 is a promising biocontrol agent against the potato soft rot pathogen E. carotovora subsp. atroseptica and suggest that the strain’s biocontrol ability results from its capacity to produce the secondary metabolite DAPG. Further work will evaluate the ability of F113 to suppress Eca-mediated soft rot of potato in soil microcosms, under experimental conditions that favour development of the disease.

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