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Survival and ecological fitness of *Pseudomonas fluorescens* genetically engineered with dual biocontrol mechanisms

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ABSTRACT

The antibiotic, 2, 4-diacetylphloroglucinol (Phl), is produced by a range of naturally occurring fluorescent pseudomonads. One isolate, *P.fluorescens* F113, protects pea plants from the pathogenic fungus, *Pythium ultimum*, by reducing the number of pathogenic lesions on plant roots but with a concurrent reduction in the emergence of plants such as pea. The genes responsible for Phl production have been shown to be functionally conserved between the wild type (wt) *P. fluorescens* strains F113 and Q2-87. In this study the genes from F113 were isolated using an optimised long PCR method and a 6.7-kb gene cluster inserted into the chromosome of the non-Phl producing *P. fluorescens* strain SBW25 *EeZY6KX*. This strain is a *lacZY*, km^R marked derivative of the wild type SBW25 which effects biological control against the plant pathogen, *Pythium ultimum* by competitive exclusion as a result of its strong rhizosphere colonising ability. We describe here the integration of the Phl antifungal and competitive exclusion mechanisms into a single strain, and the impact this has on survival and plant emergence in microcosms. The insertion of the *Phl* biosynthetic genes from the F113, into the SBW25 chromosome gave a Phl-producing transformant (strain Pa21) able to suppress *P. ultimum* through antibiotic production. The growth of Pa21 was not reduced in flask culture at

20^{o}C, compared with its parent strain. When inoculated on pea seedlings, the strain containing the Phl operon behaved similarly to the SBW25 *EeZY6KX* parent but did not show the tendency of the wt Phl producer F113 to cause lower pea seed emergence.

SBW25 *EeZY6KX* has significantly lower indigenous populations in the root than the F113 and control. This is indicative of this strain's strong colonising presence. Pa21, the Phl-modified strain is able to exclude the resident population from roots to the same degree as the SBW25 *EeZY6KX* from which it is derived. This suggests that it has maintained its competitiveness around the root systems of plants even with the introduction of the *Phl* locus. Thus, strain Pa21 possesses the qualities necessary to provide effective integrated biocontrol, through maintaining both its wild-type trait of competitive exclusion on the plant roots, whilst also expressing the genes from the F113 biocontrol strain for *Phl* production. Interestingly, however, an additional beneficial trait appears to emerge with the strain Pa21's lowered competence compared with SBW25 *EeZY6KX* in the rhizosphere soil. With fears of the spread of genetically modified organism, and persistence in the soil, this trait may be of some ecological and commercial benefit and becomes a candidate for further investigation and possible exploitation.

INTRODUCTION

Biocontrol as an economically viable addition to crop pest control is currently attracting major research interest. As the environmental, economic and functional capabilities of traditional chemical fungicides and fumigants are currently being questioned. Regulators and public alike have expressed concern on the levels of chemical protection applied to crops, as have farmers as to the economic cost of such fungicide application.

It has emerged over recent years that biological alternatives are viable alternatives to chemical pesticides for the protection of some economically important crops. In particular, fluorescent pseudomonads show potential for protective application against the fungal plant pathogen *Pythium ultimum* which causes damage to pea, sugar beet, tobacco, cucumber and tomato crops. This fungus is a major cause of damping off, a seedling disease that can greatly reduce crop yield. *Pseudomonas fluorescens* strains Q2-87, CHAO and F113 have all been shown to produce a natural fungicide 2,4-diacetylphloroglucinol (Phl) which inhibits *P. ultimum*[1,2].

This secondary metabolite has received attention as it naturally suppresses a broad range of fungal pathogens in the rhizosphere of a wide selection of economically important crop plants. In soils observed to be disease suppressive, Phl production by *P. fluorescens* is considered to be the major determinant of suppression, with 20% of pseudomonads isolated from take-all suppressive soils being able to produce the antibiotic. [3,4]

Diacetylphloroglucinol (Phl) is a phenolic metabolite with antiviral, antibacterial, antifungal antihelminthic and phytotoxic properties (figure 1) thought to be synthesised via the polyketide pathway. Molecular studies have led to the isolation of a 6-kb genomic fragment required for the biosynthesis of Phl from *Pseudomonas fluorescens* strain F113.[1]

Similar studies have identified two distinct loci involved in Phl production, in *P. fluorescens* CHAO. An 11-kb fragment from the genome of strain CHAO was shown to be able to partially complement a Phl^- Tn5 mutant, CHA625, derived from CHAO, to a Phl^+ phenotype.[2] A second distinct 22-kb genomic fragment was also found to increase Phl production when introduced into the CHAO Phl producer[5], with Phl overproduction being imparted by the *rpoD* sigma factor gene located within the fragment.[6]

Characterisation of the Phl biosynthetic loci in *P. fluorescens* sp. strain Q2-87 has shown that a 6.5-kb region is sufficient to transform 13 different Phl-non-producing *Pseudomonas* to a Phl^+ genotype.[7] The nucleotide sequence of this *Phl* biosynthetic locus revealed six genes, *phlACBD* (figure 2), organised into three transcriptional units. Flanking the biosynthetic cluster are genes *phlE* and *phlF*, which code for putative efflux protein and regulator proteins respectively.

The application of Phl-producing strains is not entirely without adverse effects. Naseby and Lynch[8] describe the impact on pea plants were inoculated with wt Phl producer F113, and the genetically modified over-producer derivative F113OP. The deleterious effects were believed to be due to the high levels of Phl present during early seed germination. Fears have been alleviated that the Phl was intrinsically toxic to the pea plants through examination of the effect of varying concentrations of the synthetic Phl on plant emergence. Concentrations of Phl of up to 1000x the physiological concentrations detected from disease-suppressive soils showed no effect on the emergence rate of pea plants. It was concluded that Phl production was contributing to the reduced plant emergence, but only in conjunction with the F113 as a delivery inoculum. An alternative Phl delivery systems was therefore sought.

In contrast to these strains that effect biocontrol through metabolite production *P. fluorescens* strain SBW25 isolated from the phylosphere of sugar beet also protects pea plants from the pathogen *P. ultimum*, although it does not produce Phl or any secondary metabolites that can be attributed to this effect.[9,10] Protection is attributed to the strong colonising ability of this organism on the roots of the plants, so successfully competitively excluding the pathogenic fungus. This bacterium with the genetic markers xylE, lacZY and KmR on its chromosome was the first free-living genetically modified bacterium to be released into the field to investigate the bio safety of genetically modified bacteria in the environment. [11,12]

Integration of genetic material has been previously shown to reduce the fitness of modified organisms through increasing genetic load and insertion-disruption of functional genes.[12,13] This has been recorded in the *P. fluorescens* SBW25 strain carrying chromosomal genetic markers. Even with this reduction in fitness, SBW25 *EeZY6KX*, still maintains its competitive colonisation advantage with respect to the natural Phl producers described earlier.[14]

One of the most promising approaches to delivering effective biocontrol agents is to enhance the natural biocontrol capabilities of a good strain with a genetically introduced method of protection. *P. fluorescens* strains F113, Q2-87 and CHOA, all effect their biocontrol capability through production of the secondary metabolite, Phl. *P. fluorescens* SBW25, effects its biocontrol behavior through competitive exclusion of the

pathogen *in planta*[15]. This study investigates the effect of integrating these two distinct methods of biocontrol through the chromosomal insertion of the genes for Phl production into the SBW25 *EeZY6KX*.

As part of a responsible research strategy following the creation of genetically modified SBW25 derivatives, the ecological fitness and perturbations *in planta* to both the plant and resident microbial communities were investigated before contemplating field studies.

Results

Construction of strains with integrated Phl genes. A high fidelity long PCR technique was used to amplify the 6.7 kb region containing the entire Phl biosynthetic operon using genomic DNA from strain F113 as a template and three pairs of primers designed using the sequence for the *Phl* genes from *P. fluorescens* Q2-87 (GenBank accession no. U41818). The PCR product was ligated in to plasmid pMC1871 (table 1) to give the integrating suicide vector, pMCPhl9 (table 1). After confirmation of plasmid size, the presence of the Phl operon was confirmed using PCR primers homologous to the Phl cluster.

Strain SBW25 *EeZY6KX* strain marked with *xylE, lacZY*, Km^R (table1) was electroporated with the suicide plasmid, pMCPhl9 to deliver the Phl operon into the chromosome and transformants that exhibited the anticipated Tc^R, $LacZY^+$, Km^R phenotype were isolated. Probing of these colonies with PCR primers designed to anneal to the *Phl* genes resulted in positive bands for all the transformants tested. One was selected, given the designation Pa21, and used for subsequent experimental investigations.

Measurement of Phl by HPLC and bioassay. *P. ultimum* plate bioassay of strain Pa21, showed increased inhibition of the fungal pathogen compared to SBW25 *EeZY6KX*, the Phl non-producing parent. Following the positive bioassay observed for strain Pa21, Phl production was analysed by HPLC in comparison with F113 (wt) and the Phl over-producing strain, F113OP. Synthetic standards revealed a retention time of 11mins. Strain Pa21 and F113 produce approximately equal quantities of Phl, whilst F113OP (containing Phl operon in a multicopy plasmid) produced the greatest levels of Phl. Actual amounts?

Impact on fitness and survival of Pa21. Growth of strain Pa21 in shake flask culture at 20°C was similar to its SBW25 *EeZY6KX* parent strain. Pa21 was tested further in microcosm studies to assess any impact on ecological fitness of integrating the Phl operon. As described in the experimental protocol, after treatment with different strains plants were harvested after 21 days and several parameters measured.

Measurements of microbial populations. The total bacterial counts on the roots of plants treated with F113 were significantly lower than plants treated with SBW25 EeZY6KX(parent strain), Pa21 (SBW25 EeZY6KXmodified Phl producer) and the control (fig. 3a). Bacterial populations in the surrounding soil were not significantly different between any of the treatments. There appears to be no impact by Pa21 on total bacterial counts on the root or rhizosphere soil.

Figure 4a shows numerical changes in the introduced pseudomonad population living on roots. Compared with the SBW25 *EeZY6KX* parent strain and the Phl-producing strain F113, numbers of Pa21 recovered were statistically greater. Both SBW25-derived strains showed higher recoveries than the natural Phl-producer, F113.

In contrast, the highest recoveries of inocula from rhizosphere soil samples were observed with strain SBW25 *EeZY6KX*; significantly greater than both Pa21 and F113 (Figure 4b).

The impact of treatments on indigenous pseudomonads is shown in figure 3b with significant differences between treatments. Following treatment with parent strain SBW25 *EeZY6KX* the indigenous population of pseudomonads was substantially lowered compared with F113and control treatments, with Pa21 displaying an intermediate level of impact.

The SBW25 *EeZY6KX* and Pa21 SBW25 derivatives show a significant increase in the population of non-pathogenic root fungi compared to the control and F113 treatments (fig. 3c). The latter showed no difference with respect to the control treatment. The fungal population in the soil was significantly increased by the SBW25 *EeZY6KX* treatment compared with all others. There was no difference in soil fungal populations between any of the other treatments including Pa21

Plant growth studies. As seen previously, seed emergence was significantly reduced in the F113 (wt)-treated plants compared with the SBW25 *EeZY6KX*, Pa21 and control treatments (Figure 5). There were, however, no significant differences between shoot or root weights between the treatments resulting in the calculated shoot – to – root ratio being unchanged between the different treatment groups.

Treatment with Pa21, compared with controls showed a significant increase in root length. There were no significant differences between the other treatments. Lateral root development and nodulation was significantly increased by the SBW25 *EeZY6KX* strain with respect to the control. Nodulation was also increased following treatment with Pa21. There was no significant difference in nodulation between the other treatment groups.

Discussion

The goal of this work was to look at the potential for combining the proven biocontrol capabilities of different *Pseudomonas* strains through the application of genetic modification. This is a novel approach to crop protection, combining established biocontrol mechanisms through the simultaneous production of natural antibiotics with competitive exclusion of the pathogen by the host bacterium.

Having modified an appropriate strain it is desirable, therefore, to assess the impact on survival of combining these two very different biocontrol mechanisms in a single strain of proven ecological fitness. If the high colonisation ability unique to the SBW25 derived strains has been significantly reduced, then an important potential weapon against plant pathogens may have been lost.

Strains Pa21 and SBW25 *EeZY6KX*, were both present on roots in greater numbers than the F113 from which the Phl operon was obtained. This is due to the competitive nature of these organisms during root colonisation. Strain SBW25 *EeZY6KX* has previously been shown to colonise more strongly than Phl-producing pseudomonads such as Q2-87, F113 and CHOA and this is believed to account for the biocontrol ability of the strain against the fungal plant pathogen, *Pythium ultimum*. This aggressive root colonisation appears to have been maintained in the Phl-producing transformant Pa21, which exhibits an identical colony morphology, growth rate in liquid culture and can maintain a comparable population to its parent on roots. The insertion into strain Pa21, in itself, does not appear, therefore, to exert sufficient load to reduce fitness on nutrient-rich roots with respect to the parent.

In soil the highest population recovery is observed for SBW25 *EeZY6KX* with recovery significantly lower for populations recovered of F113, with the biggest change from root to soil population seen in the Phl-modified strain Pa21. Manipulation of this strain appears to have altered its ability to survive the nutrient deficient conditions often found in soil, whilst maintaining the strong colonising characteristics of the SBW25 EeZY6KXparent on nutrient rich roots.

Impact on the indigenous pseudomonad population also showed characteristics that would suggest no change in behavior following modification. SBW25 *EeZY6KX* has significantly lower indigenous populations in the root than F113 and Control. This is indicative of a strong colonising presence of this strain being able to dominate and competitively exclude the resident populations. Pa21 is also able to exclude the resident population to the same degree as its parent strain. This trend suggests that it has, in-part, maintained

its competitiveness around the root systems of plants even with the introduction of the *Phl* locus, and as seen previously, does not impart pathogenic effects on the pea plant, as does the F113 strain.

For this work this strain was successfully developed as a host for the expression of the *Phl* biosynthetic operon; offering the potential for both delivering antifungal metabolite to the plant seed without the deleterious effects of the F113 wt and also controlling pathogens by competitive exclusion.

Most notably on root systems, Pa21 shows no significant changes in colonising ability compared to its SBW25-derived parent. The new SBW25-derived strain, Pa21, demonstrates the ability to produce Phl and inhibit the fungal pathogen in *P. ultimum* bioassays. In conclusion, Phl is produced, and biocontrol is achieved, but without some of the negative impact on plant emergence as seen with the wild type Phl producer, F113.

In all strain, Pa21, may possess the necessary qualities of for effective biocontrol through integrating its wild-type trait of competitive exclusion on the plant roots, whilst concurrently expressing the genes from strain F113 for *Phl* biosynthesis.

An additional beneficial trait also appears to emerge with the lowered competence of Pa21 in surrounding soil. With fears of the spread of genetically modified organism, and persistence in the soil, both real and imagined, this trait may be of some ecological and commercial benefit and is a candidate for further investigation with a view to exploitation.

Experimental protocol

Bacterial Strains, plasmids and growth conditions. Bacterial strains and plasmids used in this study are shown in table 1. *Escherichia coli* DH5a was used as the host strain for all plasmids and cloning procedures. All strains were maintained on Luria broth and grown at 30^{o}C for pseudomonads and 37^{o}C for *E. coli*, and supplemented with kanamycin (50 μg ml^{-1}) and tetracycline (25 μg ml^{-1}), where appropriate. *Pythium ultimum* (IMI 308273) was used as the fungal pathogen for *in vivo* bioassays.

PCR. *Taq2000*™ and *TaqPlus Long*™ (Stratagene) were used for long PCR amplifications with *Expand High Fidelity*™ (Roche) and optimised using Opti-prime™ PCR buffer optimisation (Stratagene), addition of 15 % glycerol and *Taq* extender™ PCR additive (Stratagene) with reactions performed in a Perkin-Elmer GeneAmp PCR System 2400™, using microAmp™ tubes.

Construction of integrating plasmid. Plasmid pMC1871 (Pharmacia) was used as a suicide vector for the *Phl* genes. pMC1871 was linearised with *Ecl136II* (Fermentas) and ligated to the 6.7 kb *Phl* fragment.

Transformation of SBW25. Cells were grown at 30°C in shake flask to mid log phase on a rotary shaker at 30°C, with subsequent manipulations carried out at 4°C. After washing and resuspension in 15% glycerol (V/V), pure plasmid DNA was added to 50 μl of the suspension on ice for 1 min, before transfer to an electroporation cuvette (0.2-cm gap, BioRad), electroporation (BioRad GenePulsar (2.5 kV, 200 Ω and 25 μFD)) and resuspension in 0.5 ml SOB broth at 30°C for 4 hours before plating on LB (Tc^{+}) plates incubated for 48 hours at 30^{o}C. Tet^{r}, Kan^{r} and $LacZY^{+}$ colonies were sub-cultured and their DNA probed with PCR primers homologous to the *Phl* biosynthetic genes.

Microcosm studies. *Pythium* inoculated or uninoculated soil (150 g) was placed in experimental microcosms constructed from 210-mm high polyacetate cylinders. Each treatment was replicated 5 times and each microcosm consisted of five imbibed seeds. Twenty-five ml 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.

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, was pH 5.4, particle ratio 10:9:81 clay: silt: sand respectively, and organic matter content 1.6 % by weight. The total NPK contents by weight were 0.124%, 0.033% and 0.861% respectively.

Microbial strains and treatments. Suspensions of *Pseudomonas fluorescens* containing $6x10^{9}$ c.f.u/ml were used to imbibe pea seeds (*Pisum sativum* var. Montana) to give $2.8 \times 10^{8} \pm 0.4 \times 10^{8}$ cfu per pea seed (no significant differences in inoculation potential between strains were observed)

Sampling and analysis. After 21 days the numbers of emerged plants was counted along with measurements of plant shoot and root weights, numbers of nodules, lateral roots, lesions and root lengths for each plant per microcosm. Closely associated rhizosphere soil for bacterial estimates extraction of Phl.

Estimates of filamentous fungi were made from pooled fresh root samples (1g) taken from each microcosm and macerated in 9 ml Ringers solution. Rhizosphere soil (1g) from each microcosm was also suspended in 9 ml Ringers solution. Filamentous fungal populations were then quantified by plating a ten fold dilution series onto 10% malt extract agar (100 ppm streptomycin and 50 ppm rose bengal) plates following

incubation at 20°C for 5 days before enumeration. P1 medium [16] (amended with 50 ppm X-Gal as appropriate) incubated at 25°C for 5 days was used for the enumeration of fluorescent *Pseudomonas* species. Total culturable bacteria were enumerated after 7 days growth at 25°C on tryptone soya agar (10% w/v).

Extraction of Phl. Phl was isolated from the rhizosphere of the pea plants on the principle of the method described by Bonsall *et al.*[17].

Plate Bioassays. Plate bioassays were performed against a pathogenic fungus, *Pythium ultimum* grown on potato dextrose agar (PDA). Test bacterial colonies were spotted onto the plates 2 cm from the Petri dish edge. After placing the fungal plug at the centre of the dish, the plates were incubated at 20°C, for 4 days, until the fungus had grown to the edge of the dish. Evidence of fungal inhibition by the test bacteria was recorded.

HPLC analysis. A 100 x 4.8-mm (for soil and root extract) or 200 x 4.8 mm (for broth extract) reverse phase, Spherisorb C[18] column was eluted with solvent (1 ml/min) using an isocratic mobile phase (78% ACN, 0.1% TFA) with absorbance of eluant measured at λ_{270nm} (data recorded on a PeakNet Chromatography Workstation (Dionex)).

Tolerance to synthetic Phl. Pea seeds were imbibed with 500 ng, 5 ug and 50 ug per seed of synthetic Phl (100 seeds per treatment). Seed growth conditions were as described previously, and seed emergence was recorded after five days.

Bacterial fitness in liquid culture. Relative growth was investigated in shake flask culture at 20°C with OD (λ_{550}) measured concurrently with counts made on LB agar.

Bacterial fitness in planta. The overnight culture was used to inoculate full strength TSA for 3 days at 30°C. The bacteria was suspended in 10 ml of sterile quarter strength Ringer's solution using disposable plastic plate spreaders to scrape off the bacterial mat and the colony forming units were determined. Control plates (without bacteria) were also flooded with quarter strength Ringer's solution and the surface scraped with spreaders. The resulting suspensions containing 6×10^9 c.f.u / ml were subsequently used to imbibe pea seeds (*Pisum sativum* var. Montana), at a ratio of one seed per ml, for 4 hours (stirred every 30 minutes), resulting in between 2 and 4×10^8 c.f.u. per seed.

The experimental microcosms were constructed from 210 mm high acetate cylinders. Each treatment comprised 5 replicates and each microcosm consisted of five or eight imbibed seeds, planted at a depth of approximately 1 cm below the soil surface. 25 ml of water was added to the soil in each microcosm which

were harvested after 21 days with the following measurements made; the plant shoot and root (wet and dry) weight, number of nodules, length of each root and the number of lateral roots. Rhizosphere soil (closely associated with the plant roots) samples were collected and stored at 4^{o}C. The samples were subsequently used for microbial populations' counts. One gram of soil from each replicate was suspended in 9 ml Ringers solution, as was one gram of mashed root from each replicate. Filamentous fungal populations were quantified by plating a ten fold dilution series of each root macerate or soil suspension onto 10% malt extract agar containing 100 ppm streptomycin and 50 ppm rose bengal. Plates were incubated at 20 ^{o}C for 5 days before enumeration. P1 medium [11] was used for the enumeration of root indigenous, fluorescent *Pseudomonas*, plates were incubated at 25^{o}C and enumerated after 5 days growth. To enable quantification of the introduced *P.fluorescens* the media was amended with X-gal (50 ug/ml). Tryptone soya agar (10%) was used for the enumeration of the culturable bacteria.

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.

Acknowledgements

We are grateful for the financial support of EU on this project. We also wish to thank Professor Fergal O'Gara (UCC, Cork, Ireland) and Dr. Mark Bailey (CHE, Oxford) for the supply of some of the materials used in this project.

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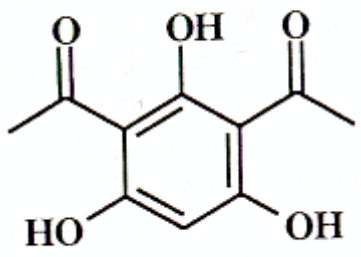
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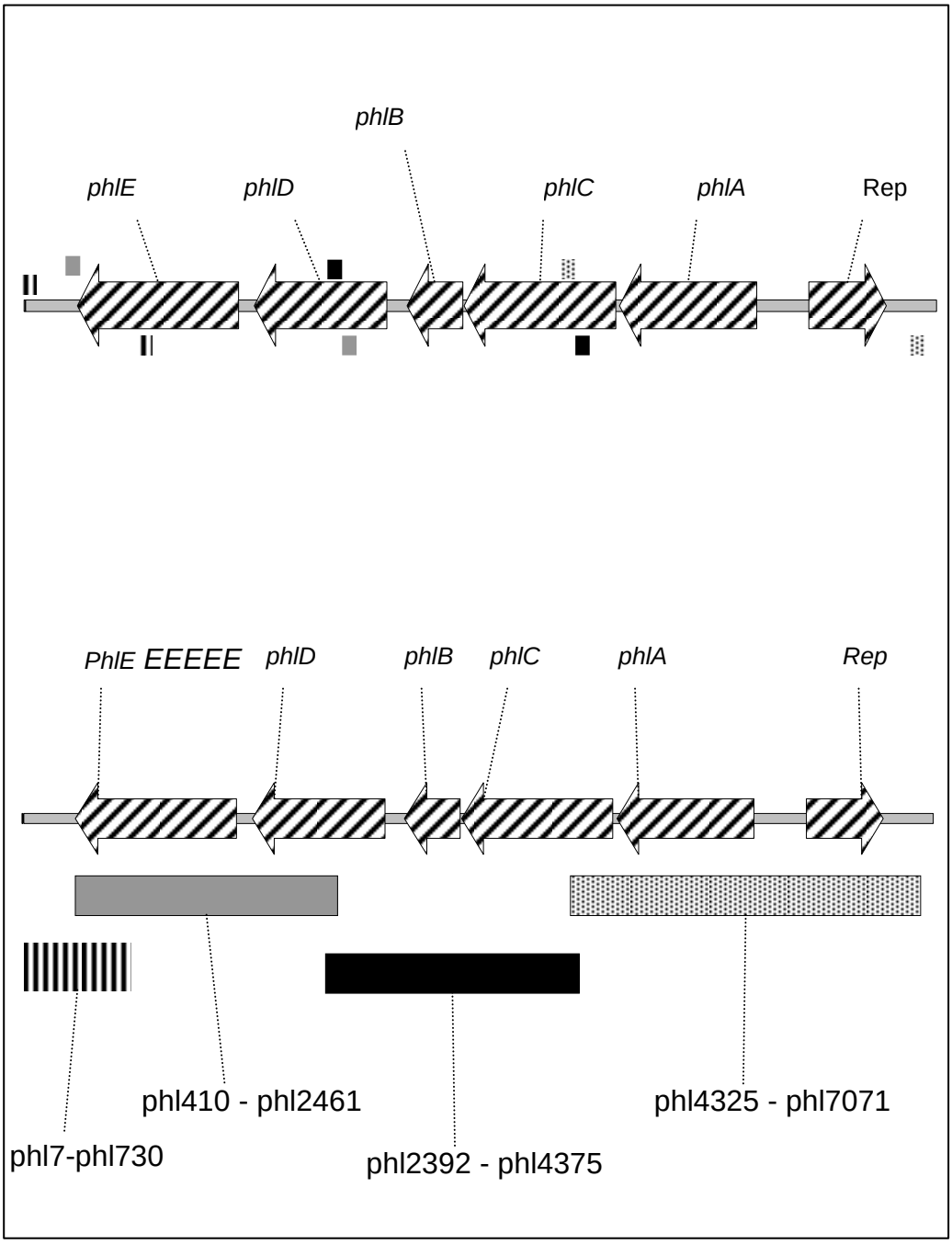
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PhlE EEEEE
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phlB
phlC
phlA
Rep
phl410 - phl2461
phl4325 - phl7071
phl7-phl730
phl2392 - phl4375

Bacterial Strain or Plasmid	Relevant Characteristics	Source or reference
E. coli		
DH5a	HsdR17(r^- m^+) supE44 thi-1 recA1 GyrA96 (nal^r) relA1 $(lacZYA\text{-}argF)_{U196}$ (lacZ M15)	Laboratory stocks
P. fluorescens		
SBW25	Sugar beet leaf isolate	De Leij *et al*., 1995
SBW25 *EeZY6KX*	Sugar beet leaf isolate with markers $LacZY^+$, Km^r and XylE	Bailey *et al.*1995
Pa21	Transformants containing Phl Locus, Tc^+, $LacZY^+$, Km^r	This study
F113	Natural Phl producer isolated in Ireland	Shanahan *et al,* 1992
F113OP	F113 containing plasmid pCUGP, extrachromosomal copies of Phl genes	O'Gara, personal communication
Plasmids		
pMC1871	Tc^+, $LacZY^+$, oriC	Pharmacia
pMCPhl9	Tc^+, Phl^+, oriC	This study

Figure 1 Structure of the antifungal compound 2,4-diacetylphloroglucinol.

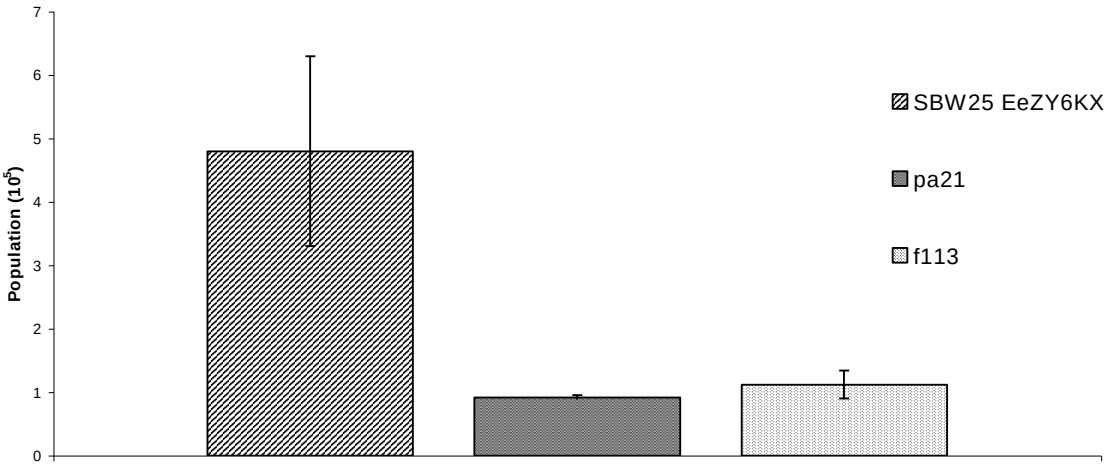
Figure 2 Schematic diagram of the Phl biosynthetic locus as revealed by sequence data from *P. fluorescens* Q2-87 (Acc. no. U41818). Coloured blocks show position of primer pairs and corresponnding PCR fragments produced using using *P.fluorescens* F113 DNA as template. Key needed?

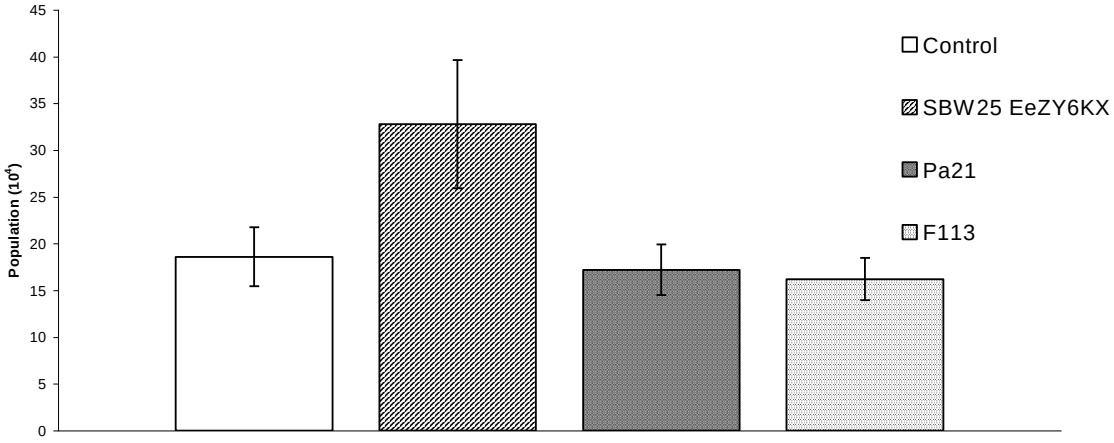
Figure 3. Impact of treatments on indigenous microbial populations. a) Total bacterial counts isolated from 1 gram of roots of 21-Day pea plants. Figures taken from the mean of 5 microcosms from each treatment. (b) Number of indigenous pseudomonads isolated per gram of root from 21-day old pea plants. Samples are the mean of 5 microcosms per treatment sampled. (c) Total fungal population from 21-day old pea soil. Samples are the mean of 5 microcosms per treatment sampled.

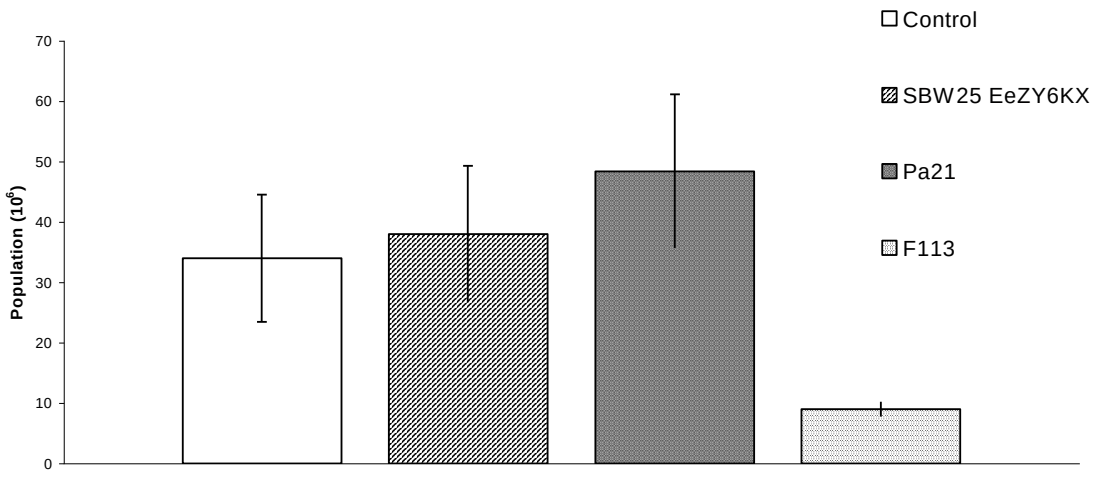
Figure 4a Introduced pseudomonad population in the rhizosphere (root) of 21-day old pea plants. (n = 5).
Figure 4b Introduced pseudomonad population in the rhizosphere (soil) of 21-day old pea plants. (n = 5).

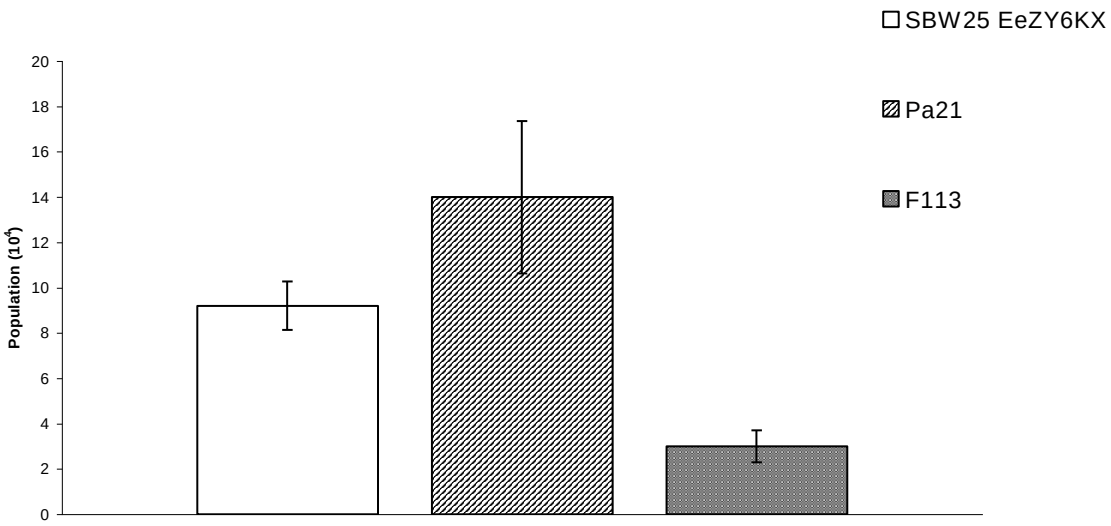
Figure 5 Mean emergence of Pea plants after 5-days of the 21-day microcosm experiment. Five microcosms per treatment, each contain 8 plants per microcosm.

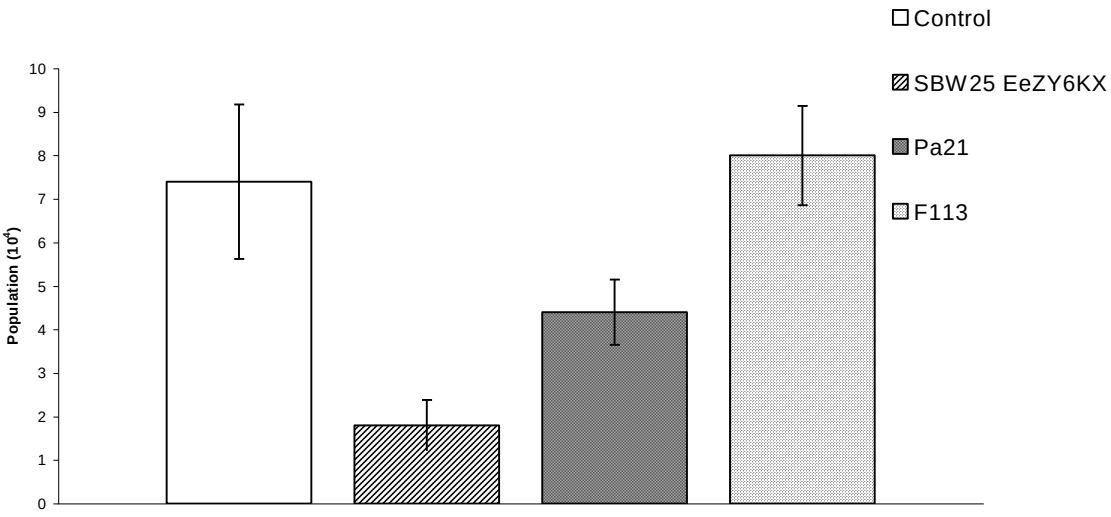
Table 1 Bacterial strains and plasmids used in this experiment

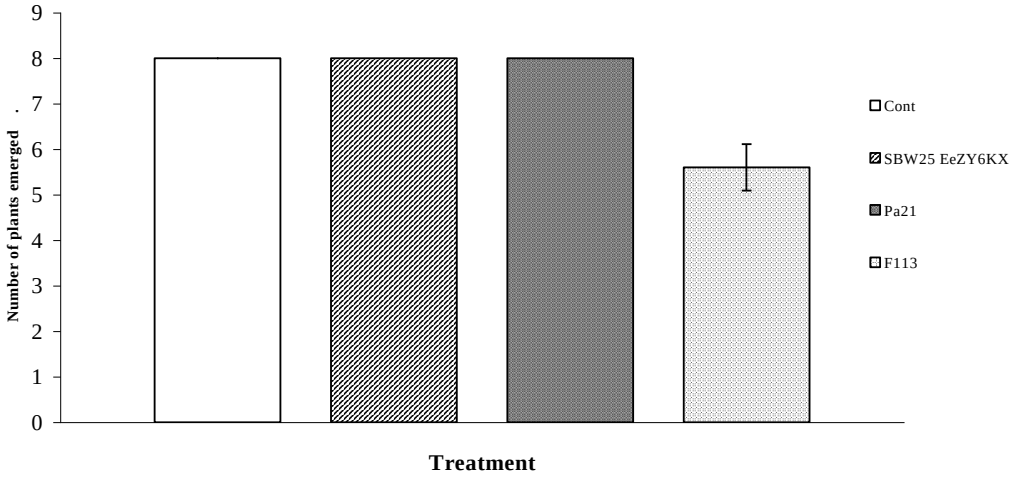

Population (10^5)
0
1
2
3
4
5
6
7
SBW25 EeZY6KX
pa21
f113


45
40
35
30
25
20
15
10
5
0
Population (10^6)
Control
SBW25 EeZY6KX
Pa21
F113


Control
SBW25 EeZY6KX
Pa21
F113
Population (10^6)
70
60
50
40
30
20
10
0


SBW25 EeZY6KX
Pa21
F113
Population (10^4)
0
2
4
6
8
10
12
14
16
18
20


Control
SBW25 EeZY6KX
Pa21
F113
Population (10^4)
0
1
2
3
4
5
6
7
8
9
10


9
8
7
6
5
4
3
2
1
0
Number of plants emerged
Cont
SBW25 EeZY6KX
Pa21
F113
Treatment