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Genomic-assisted characterisation of *Pseudomonas* sp. strain Pf4, a potential biocontrol agent in hydroponics

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1 **Genomic assisted characterization of *Pseudomonas sp.* strain Pf4, a potential biocontrol agent**  
2 **in hydroponics**

3

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**27 ABSTRACT**

28 In an attempt to select potential biocontrol agents against *Pythium* spp. and *Rhizoctonia* spp. root  
29 pathogens for use in soilless systems, 12 promising bacteria were selected for further investigations.  
30 Sequence analysis of the 16S rRNA gene revealed that three strains belonged to the genus  
31 *Enterobacter*, whereas nine strains belonged to the genus *Pseudomonas*. In *in vitro* assays, one  
32 strain of *Pseudomonas* sp., Pf4, closely related to *Pseudomonas protegens* (formerly *P.*  
33 *fluorescens*), showed noteworthy antagonistic activity against two strains of *Pythium*  
34 *aphanidermatum* and two strains of *Rhizoctonia solani* AG 1-IB, with average inhibition of  
35 mycelial growth >80%.  
36 Strain Pf4 was used for *in vivo* treatments on lamb's lettuce against *R. solani* root rot in small-scale  
37 hydroponics. Pf4-treated and untreated plants were daily monitored for symptoms development and  
38 after two weeks from infection, a significant protective effect of Pf4 against root rot was recorded.  
39 The survival and population density of Pf4 on roots were also checked, demonstrating a density  
40 above the threshold value of  $10^5$  CFU g<sup>-1</sup> of root required for disease suppression.  
41 PCRs having as target genes involved in the synthesis of antifungal metabolites and draft genome  
42 sequencing of Pf4 demonstrated that *Pseudomonas* sp. Pf4 has the potential to produce an arsenal of  
43 secondary metabolites (*plt*, *phl*, *ofa* and *fit-rzx* gene clusters) very similar to that of the well-known  
44 biocontrol *P. protegens* strain Pf-5.

45

**46 KEYWORDS**

47 Biological control; *Rhizoctonia solani*; *Pythium* spp.; population dynamic; secondary metabolites;  
48 draft-genome sequencing.

49

**50 1. Introduction**

51 Soilless, hydroponic systems are well suited for the cultivation of many crops, including leafy  
52 vegetables. Their main feature is the possibility to control all environmental factors, i.e. nutrient

53 solution supply, temperature, pH, dissolved oxygen concentration, electrical conductivity, light  
54 radiation, that translates into higher production, energy conservation, better control of growth,  
55 independence from soil quality (van Os, 1999).

56 Although soilless cultures have been reported as a successful alternative to the use of methyl  
57 bromide and other fumigants to avoid root-diseases caused by soil-borne pathogen microorganisms  
58 (van Os, 1999), root-diseases still occur in these systems. Sometimes disease outbreaks are even  
59 greater than in soil (McPherson, Harriman, & Pattison, 1995), promoted by suitable environmental  
60 conditions, and rapid dispersal of root-colonising agents through the cultural system (Vallance et  
61 al., 2010). The most harmful pathogenic microorganisms in hydroponic cultures are those  
62 producing zoospores, i.e. *Pythium* spp. and *Phytophthora* spp., particularly adapted to wet  
63 environment, but also *Fusarium* spp. and *Rhizoctonia solani* are of major concern (Schnitzler,  
64 2004; Paulitz & Bélanger, 2001). In particular, *R. solani* was recently detected in Italy on many  
65 leafy vegetables (Colla, Gilardi, & Gullino, 2012), including lamb's lettuce [*Valerianella locusta*  
66 (L.) Laterr.] (Garibaldi, Gilardi, & Gullino, 2006).

67 Prevention of pathogen infections, particularly in closed hydroponic systems, has become a major  
68 challenge in recent years, particularly in the light of the increasing public concern regarding the use  
69 of chemical pesticides and subsequent legislative issues (e.g., Directive 2009/128/EC). Biological  
70 control is regarded as a potentially solid alternative to the use of chemical pesticides, and can be  
71 effective also in soilless systems (Vallance et al., 2010; Postma, 2010). Since studies on  
72 suppressiveness demonstrated the potential of indigenous microflora to inhibit root diseases in  
73 hydroponic cultures (McPherson, 1998), one of the main strategies is the addition of antagonistic  
74 microorganisms to increase the level of suppressiveness (Vallance et al., 2010).

75 Rhizobacteria are the most efficient microorganisms against soil-borne pathogens, which occur in  
76 the environment at the interface of root and soil (Handelsman & Stabb, 1996). In particular,  
77 fluorescent pseudomonads can persistently colonize the rhizosphere (Couillerot, Prigent-Combaret,  
78 Caballero-Mellado, & Moënne-Loccoz, 2009), compete with root pathogens for micronutrients

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79 (especially for iron and carbon) and root surface colonization (Haas & Défago, 2005; Raaijmakers,  
80 Paulitz, Steinberg, Alabouvette, & Moëgne-Loccoz, 2009), trigger Induced Systemic Resistance  
81 (ISR) response in plants (Bakker, Pieterse, & Van Loon, 2007). A major component of biocontrol  
82 potential appears to be connected with secretion: fluorescent pseudomonads that are active  
83 biocontrol agents produce secondary metabolites that act as antimicrobial compounds, i.e. 2,4-  
84 diacetylphloroglucinol (2,4-DAPG), phenazines, pyrrolnitrin, pyoluteorin, hydrogen cyanide (HCN)  
85 (Raaijmakers, Vlami, & De Souza, 2002; Handelsman & Stabb, 1996), but also siderophores as  
86 pyoverdin, biosurfactants, extracellular lytic enzymes (Compant, Duffy, Nowak, Clément, & Barka,  
87 2005).

88 Only a limited number of studies on biological control by rhizobacteria have been carried out in  
89 soilless systems and consequently a limited number of biocontrol agents have been isolated and  
90 characterized from soilless systems. Yet it is important to understand to what extent the growing  
91 system is a relevant component in determining the potential of biological control agent. Are  
92 rhizobacteria with biological control potential isolated from hydroponics different from those  
93 isolated from soil? Are they relying on different mechanisms for the control of pathogens?

94 In this work we selected a biocontrol agent from endogenous source, the hydroponics, characterized  
95 it for both its biocontrol performances and its genomic features, with particular reference to  
96 secondary metabolites, and compared it with other known biological agents isolated from soil.  
97 Surprisingly, the strain was not dramatically different from other previously known pseudomonads  
98 biocontrol agents, indicating that the hydroponic conditions do not significantly change the  
99 mechanisms involved in biocontrol.

100

## 101 **2. Materials and methods**

### 102 **2.1. Plant pathogen strains**

103 Fungal and oomycete pathogens were obtained from culture collection and by isolation from  
104 diseased plants. Specifically, *Pythium aphanidermatum* strain CBS 118745 and strain CBS 116664,

105 were obtained from the Centraal Bureau voor de Statistiek (CBS) culture collection, and were  
106 grown on oatmeal agar (OA, oatmeal flakes boiled and filtered 30g l<sup>-1</sup>, 15 g l<sup>-1</sup> bacteriological agar).  
107 Whereas, fungal isolations were made in 2009 from diseased plants showing symptoms of root rot  
108 and wilting in an hydroponic farm in Friuli Venezia Giulia (FVG) region, north-eastern Italy. Sixty  
109 portions of lamb's lettuce or chicory roots and seedlings were washed in sterile distilled water,  
110 placed on water agar (WA, 20 g l<sup>-1</sup> bacteriological agar) plates and incubated at 24°C for 48 h. The  
111 isolates were transferred on Petri-dishes containing OA. Fungal isolates with the morphological  
112 characters of *Rhizoctonia solani* were consistently recovered and their identity confirmed by  
113 internal transcribed spacer (ITS) analysis. DNA extraction and PCR-amplification of ITS region  
114 using the universal primers ITS1/ITS4 (White, Bruns, Lee, & Taylor, 1990) and GoTaq Flexi DNA  
115 Polymerase (Promega, Madison, WI, USA) from 12 isolates of *R. solani* was carried out as  
116 previously described by Martini et al. (2009). PCR products were then digested with endonuclease  
117 *Tru*II and visualized on a 2% agarose gel, stained with GelRed™ (Biotium Inc., Hayward, CA,  
118 USA). The subsequent restriction profiles were compared, and resulted identical to each other. Two  
119 strains of *R. solani*, TR15 and TP20, were selected for sequencing and analysis of ITS region as  
120 described by Martini et al. (2009), and successively used in this work. ITS sequences (652 bp) of *R.*  
121 *solani* strains TR15 and TP20 were submitted to GenBank under accessions KM589032 and  
122 KM589033 respectively. BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) analysis allowed  
123 confirmation of their morphological identification as *R. solani* and their assignment to anastomosis  
124 group AG 1-IB (Sharon, Kuninaga, Hyakumachi, & Sneh, 2006) with 100% similarity with the  
125 GenBank sequence AJ868450 of *R. solani* (*Thanatephorus cucumeris*) strain AG1 (CBS 522.96).

126

## 127 **2.2. Isolation of potential bacterial biocontrol agents and preliminary screening**

128 Bacteria strains were isolated from the rhizosphere of healthy hydroponic lamb's lettuce plants  
129 grown in the same hydroponic farm as before. Thirty root samples were collected from healthy  
130 plants, cut in 1-1.5 cm pieces, washed in sterile distilled water and transferred on WA; plates were

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131 incubated at 24°C for 48-72 h. Each colony was re-streaked three times, and grown in pure culture  
132 on nutrient agar medium (NA, 1 g l<sup>-1</sup> beef extract, 2 g l<sup>-1</sup> yeast extract, 5 g l<sup>-1</sup> peptone, 5 g l<sup>-1</sup> sodium  
133 chloride, 15 g l<sup>-1</sup> bacteriological agar) at 24°C for 48 h.

134 Fifty-one bacterial strains were preliminarily tested by a dual culture method according to Gravel,  
135 Martinez, Antoun, and Tweddell (2005) with *P. aphanidermatum* strains CBS 118745 and CBS  
136 116664, on potato dextrose agar medium (PDA, 38 g l<sup>-1</sup>). Bacteria were inoculated at one side of a  
137 Petri dish and, after 48-h incubation, a mycelium plug was placed on the opposite site of the Petri  
138 dish, approximately 5 cm apart from the bacterial inoculation point. At the same time, positive  
139 controls of fungal pathogens were prepared by placing a mycelium plug in a Petri dish. After  
140 incubation for 7 days at room temperature (about 24°C), the presence/absence of an inhibition zone  
141 between the pathogen and each bacterium was recorded. Twelve bacterial strains that proved to  
142 inhibit the tested pathogens were selected for further investigations.

143

### 144 **2.3. Bacteria identification**

145 DNAs from the twelve selected bacterial strains were extracted according to the procedure reported  
146 on *Current protocols in Molecular Biology* (Wilson, 1997). PCR amplification of 16S rRNA gene  
147 was performed with universal primers fD1/rP1 (Weisburg, Barns, Pelletier, & Lane, 1991).  
148 Amplifications were performed with the automated One Advanced thermocycler (EuroClone,  
149 Celbio, Milan, Italy) in 25 µl reactions containing 200 µM of each of the four dNTPs, 0.4 µM of  
150 each primer, 1.5 mM MgCl<sub>2</sub>, 0.625 units of GoTaq Flexi DNA Polymerase (Promega, Madison,  
151 WI, USA) and 1 µl of diluted bacterial DNA (5 ng µl<sup>-1</sup>). The PCR program consisted of initial  
152 denaturation for 2 min at 94°C; 36 cycles of 1 min at 94°C, 1 min at 58°C, 2 min at 72°C; and a  
153 final extension for 8 min at 72°C.

154 PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System Kit (Promega,  
155 Madison, WI, USA) and sent to Genechron laboratory, (ENEA Casaccia, Rome, Italy) for  
156 sequencing. The sequences were determined with forward and reverse primers and assembled with

157 BioEdit (Hall, 1999). For bacteria identification, 16S rRNA gene sequences 1303-1409 bp long  
158 were compared with those present in GenBank using BLASTN analysis. The nucleotide sequences  
159 were deposited in GenBank.

160

#### 161 **2.4. In vitro antagonistic activity**

162 The antagonistic activity of the 12 preliminarily selected bacterial strains against *P.*  
163 *aphanidermatum* strains CBS 118745 and CBS 116664 and *R. solani* strains TR15 and TP20 was  
164 further characterized as follows. Bacterial strains were inoculated on Petri dishes containing PDA  
165 supplemented with 3 g l<sup>-1</sup> peptone and 2 g l<sup>-1</sup> yeast extract, in four diametrically opposite sites,  
166 approximately 3 cm from the centre. After a 48-h incubation at 24°C, plugs of mycelium (about 5  
167 mm in diameter) were placed in the centre of the Petri dishes. At the same time, mycelium plugs  
168 were also inoculated on Petri dishes containing only growth medium, as control reference. The  
169 plates were further incubated for 9 days, and the mycelial growth was measured daily. The assays  
170 were repeated twice, and each combination bacterial antagonist-plant pathogen was replicated at  
171 least three times. The average inhibitory effect of each strain against the two pathogens was  
172 estimated based on the percent inhibition of radial growth, calculated using the following formula  
173 (Fokkema, 1976): % inhibition =  $[(C-T) C^{-1}] \times 100$ , where C is the radial growth of the pathogen  
174 without antagonist and T is the radial growth of the pathogen in presence of the antagonist.

175

#### 176 **2.5. In vivo activity of *Pseudomonas sp.* strain Pf4 against *Rhizoctonia solani***

177 The bacterial strain that showed the best *in vitro* antagonistic activity, i.e. *Pseudomonas sp.* strain  
178 Pf4, was chosen for *in vivo* application with the aim to evaluate its protective effect against *R.*  
179 *solani* root rot and its persistence and concentration on the rhizosphere of lamb's lettuce plants  
180 growing in a soilless system. Pf4 was cultured in flasks with 50 ml of nutrient broth (NB, 1 g l<sup>-1</sup>  
181 beef extract, 2 g l<sup>-1</sup> yeast extract, 5 g l<sup>-1</sup> peptone, 5 g l<sup>-1</sup> sodium chloride) at 24°C for 36 h, pelleted  
182 with centrifugation at 6500 rpm for 10 min at 4°C and suspended in sterile distilled water to a final

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183 concentration of  $10^9$  CFU ml<sup>-1</sup>. *R. solani* was cultured in flasks with 200 ml malt extract broth  
184 (MEB, malt extract 6 g l<sup>-1</sup>, maltose 1.8 g l<sup>-1</sup>, dextrose 6 g l<sup>-1</sup>, yeast extract 1.2 g l<sup>-1</sup>) at 24°C for 14-  
185 18 d; the mycelium was rinsed with sterile distilled water and thoroughly grinded to obtain an  
186 homogeneous suspension. Lamb's lettuce plants were grown in a plant growth room, with the  
187 following conditions: temperature 26°C, photoperiod of 11 h light/13 h dark, in small scale floating  
188 systems (15 l tanks) with a standard solution widely used by horticultural farms in north-eastern  
189 Italy, as reported by Iacuzzo et al. (2011). Specifically, eight tanks were prepared, in each tank  
190 about 50 lamb's lettuce plants were grown. Bacterial treatments were carried out on four of the  
191 eight tanks (4 replicates for Pf4 treatment) and successively infected with the pathogen, the other  
192 four tanks were only infected with the pathogen (4 replicates for untreated plants). Eight additional  
193 tanks, prepared as above and not inoculated with the pathogen, served as negative controls.

194 Pf4 bacterial suspensions were used for three treatments: the first was applied on seeds by  
195 immersion in the bacterial suspension for 10 min, the second was applied on seedlings  
196 (approximately  $10^7$  CFU/seedling) about 7 days after seeding; whereas the third one was applied 18  
197 days after seeding directly into the nutrient solution at a final concentration of  $10^6$  CFU ml<sup>-1</sup>.  
198 Successively, Pf4-treated and untreated plants were artificially infected with the fungal pathogen.  
199 For fungal infection, a bunch of lamb's lettuce plants growing in miniaturized floating system were  
200 infected through root immersion for 2 h in the suspension of *R. solani* mycelium. Three days after  
201 the third bacterial treatment, six infected plants were put in each of the eight tanks, and used as  
202 source of inoculum. Disease development was scored daily for up to three weeks. The number of  
203 plants with *R. solani* symptoms (limping, wilting, and/or complete withering) was scored.

204 The experiment was repeated twice (trial I and trial II). Statistical analysis was performed  
205 separately on data obtained from each experiment. The data of disease incidence in percentage were  
206 subjected to arcsine transformation and to unpaired T-test with Welch correction using the software  
207 GraphPad InStat version 3.00 (GraphPad Software Inc., San Diego, CA, USA).

208

209 *2.5.1. Survival and population density of Pseudomonas sp. strain Pf4 on lamb's lettuce roots in*  
210 *hydroponics*

211 In order to determine the survival and population density of the inoculated bacteria, root samples  
212 (30-300 mg) were weekly collected from two plants randomly selected from each negative control  
213 tank of trial I for a period of four weeks, starting 18 days after seeding, just before the application of  
214 bacterial suspension into the nutrient solution. Roots from Pf4-treated and untreated plants were  
215 weighed, placed in sterile distilled water (1 ml 10 mg<sup>-1</sup> root tissue) and kept on a rotary shaker for 2  
216 h. Aliquots (100 µl) of the obtained suspensions and of tenfold serial dilutions were plated in  
217 duplicate, using a spreader, onto King's B medium (20 g l<sup>-1</sup> proteose peptone, 10 ml l<sup>-1</sup> glycerol, 1.5  
218 g l<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 1.5 g l<sup>-1</sup> MgSO<sub>4</sub>·7 H<sub>2</sub>O, 15 g l<sup>-1</sup> agar, pH 7.2) (King, Ward, & Raney, 1954) plates.  
219 Colonies were counted (CFU counting method) after 48 h incubation at 25°C, using UV-light.

220 Molecular identity of 15 colonies from each of the four weekly samplings, for a total of 60 colonies  
221 from treated plants and 60 colonies from untreated plants, was assessed by a strain-specific  
222 EvaGreen<sup>®</sup> real-time PCR method, the development of which will be described in a separate paper  
223 (Martini & Moruzzi, unpublished). Bacterial suspensions were prepared with 100 µl of sterile PCR  
224 water and bacteria scraped from the agar surface with a sterile plastic loop, successively boiled for  
225 10 min at 99°C. 1 µl of boiled bacterial suspensions was used as a template in 20 µl-PCR reactions  
226 including 0.3 µM each primer Pfluor4GyrBF3 and Pfluor4GyrBR2, 1X Sso Fast EvaGreen  
227 SuperMix (Bio-Rad Inc., Hercules, CA, USA), and sterile H<sub>2</sub>O. Diluted total genomic DNA (2 ng  
228 µl<sup>-1</sup>) of Pf4 was used as positive control in real-time PCRs. Cycling conditions in a 96-well Bio-Rad  
229 CFX96 RealTime PCR System (Bio-Rad Inc., Hercules, CA, USA) were as follows: initial  
230 denaturation at 98 °C for 2 min; 45 cycles of 5 s at 98 °C; 5 s at 64 °C. A low resolution melting  
231 curve (ramp from 65°C to 95°C with 0.5°C increments and holding times of 5 s) was programmed  
232 at the end of the cycling reaction.

233

234 **2.6. In vitro screening for genes associated with antibiotic production in *Pseudomonas sp.* Pf4**

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235 Bacterial strain Pf4 was examined by PCR for the presence of genes involved in antibiotic  
236 production using gene-specific primers. Table 1 lists the target genes and PCR primer sets used for  
237 the detection of genes encoding the selected antibiotics: 2,4-diacetylphloroglucinol (2,4-DAPG),  
238 phenazine-1-carboxylic acid, pyrrolnitrin, pyoluteorin, hydrogen cyanide. All primers sets were  
239 used in PCR mixtures with a total volume of 25  $\mu$ l containing dNTPs 200  $\mu$ M each, MgCl<sub>2</sub> 1.5 mM,  
240 each primer 0.4  $\mu$ M, 0.625U GoTaq Flexi (Promega, Madison, WI, USA). The PCR cycling  
241 conditions were: initial denaturation for 2 min at 94°C; 34 cycles of 1 min at 94°C, 40 s at 68°C (or  
242 62/64°C) (Table 1), 1 min at 72°C; and a final extension for 8 min at 72°C. PCR products were  
243 separated by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and captured with  
244 a DigiDoc-It imaging system (UVP, Cambridge, United Kingdom).

245

#### 246 **2.7. Library preparation, draft genome sequencing, assembly and annotation.**

247 Genomic DNA was prepared for sequencing by the Nextera DNA sample preparation kit (Illumina),  
248 according to the manufacturer's instructions. Sequencing was performed on an Illumina MiSeq  
249 platform using indexed paired-end 300-nucleotide v2 chemistry at the Istituto di Genomica  
250 Applicata (Udine, Italy). Paired reads were assembled into contigs using the A5-miseq pipeline  
251 (Tritt, Eisen, Facciotti, & Darling, 2012).

252 Automated annotation of *Pseudomonas sp.* Pf4 draft genome sequence was performed using the  
253 RAST server (Aziz et al., 2008) and the NCBI Prokaryotic Genome Annotation Pipeline  
254 ([http://www.ncbi.nlm.nih.gov/genome/annotation\\_prok/](http://www.ncbi.nlm.nih.gov/genome/annotation_prok/)). Orthologs inference and comparison with  
255 *P. protegens* Pf-5 was achieved with the standalone OMA program  
256 (<http://omabrowser.org/standalone/>).

257 Secondary metabolite production clusters were examined using the antiSMASH program (Medema  
258 et al., 2011). Sequence (BLAST) analysis of gene clusters for the synthesis of antibiotics,  
259 exoenzyme, cyclic lipopeptide, siderophores, toxin, and of Gac/Rsm homologues in *Pseudomonas*  
260 sp. Pf4 was conducted and similarities to those in *P. protegens* and other closely related

10

261 *Pseudomonas* spp. strains was recorded (Loper et al., 2012; Takeuchi et al., 2015; Flury et al., 2016;  
262 Garrido-Sanz et al., 2016).

263 Contig 8 sequence of *Pseudomonas* sp. Pf4 containing the *fit-rzx* cluster was scanned for regions of  
264 genomic islands, putative signatures of HGT, using the IslandViewer3 website (Dhillon et al., 2015)  
265 with the algorithms IslandPick (Langille, Hsiao, & Brinkman, 2008), SIGI-HMM (Waack et al.,  
266 2006) and IslandPath-DIMOB (Hsiao, Wan, Jones, & Brinkman, 2003).

267

### 268 **2.8. Phylogenetic analysis based on MLSA**

269 For the MLSA-based phylogenies a total of 28 *Pseudomonas* strains of *P. chlororaphis* (including  
270 *P. protegens*- and *P. saponiphila*-related strains) and *P. corrugata* subgroups in the *P. fluorescens*  
271 group according to Mulet, Lalucat, and García-Valdés (2010) and Mulet et al. (2012) were  
272 analysed, comprising Pf4, 10 type strains (Gomila, Peña, Mulet, Lalucat, & García-Valdés, 2015)  
273 and 17 *Pseudomonas* strains whose complete or draft genome are available in the databases. The  
274 sequences of *gyrB*, *rpoD* and *rpoB* housekeeping genes along with the 16S rDNA gene sequence  
275 were retrieved from the genomic annotation, if available, and by performing BLASTN on the  
276 genomic sequence if otherwise. Genes for the type strains were retrieved from the PseudoMLSA  
277 database (<http://www.uib.es/microbiologiaBD/Welcome.php>).

278 The sequences of four genes were cut and concatenated as described by Mulet et al. (2010), and  
279 successively aligned with CLUSTAL W from the Molecular Evolutionary Genetics Analysis  
280 program-MEGA7 (Kumar, Stecher, & Tamura, 2016). The maximum parsimony (MP) tree was  
281 obtained using the Tree-Bisection-Regrafting (TBR) algorithm, implemented in the MEGA7, with  
282 search level 3 in which the initial trees were obtained by the random addition of sequences (10  
283 replicates). *P. syringae* ATCC19310 type strain was used as an outgroup taxon to root the tree.  
284 Bootstrapping (500 replicates) was performed to estimate the stability and support for the inferred  
285 clades.

286

### 287 3. Results

#### 288 3.1. Isolations and preliminary screenings

289 Bacterial colonies isolated from thirty lamb's lettuce root samples were used in preliminary dual  
290 culture tests with two *P. aphanidermatum* strains (CBS 118745 and 116664). Among the 51  
291 bacterial strains tested, 12 strains showed growth limiting activity, as summarized in Table 2. After  
292 4 days of incubation, three of the 12 bacteria showed an inhibition zone of more than 10 mm, while  
293 four showed an inhibition zone ranging from 1 to 10 mm. The remaining five bacteria showed a  
294 reduced inhibition zone, although no physical contact was observed between the bacterial and the  
295 oomycete growth.

296 The identification of the 12 bacterial strains was preliminary carried out by sequence analysis using  
297 BLASTN of PCR amplified ribosomal DNAs, that resulted about 1303-1409 bp in length  
298 (accession numbers listed in Table 2). According to the sequence analysis, three bacterial strains  
299 (En8, En10, En12) with 16S rDNA gene sequence similarities of 99.2-99.3% among them belonged  
300 to *Enterobacter* spp., showing sequence identities of about 99% with three different *Enterobacter*  
301 sp. strain sequences deposited in GenBank, while the other nine strains belonged to *Pseudomonas*  
302 *fluorescens* group. Specifically, six strains (Pf1, Pf2, Pf3, Pf4, Pf5, Pf11) were closely related to *P.*  
303 *protegens* showing a 99-100% sequence similarity with strain CHA0<sup>T</sup> (=DSM 19095<sup>T</sup>) (AJ278812),  
304 two strains (Pf6 and Pf7) to *P. fluorescens* with 99% similarity with strain ATCC 13525<sup>T</sup>  
305 (AF094725) and one strain (Pf9) to *P. poae* with 99% similarity with strain DSM 14936<sup>T</sup>  
306 (AJ492829).

307

#### 308 3.2. In vitro antagonistic activity

309 The results of *in vitro* antagonism tests of each of the 12 bacterial strains towards the plant  
310 pathogens *P. aphanidermatum* and *R. solani* are shown in Figures 1A and 1B respectively. Since *P.*  
311 *aphanidermatum* strains CBS 118745 and CBS 116664, and the *R. solani* strains TR15 and TP20  
312 showed a nearly identical behaviour, combined data for each species are shown. The data from all

313 replicates of the two experiments were also combined (Figure 1). Examples of the recorded  
314 bacterial antagonisms are given in Figure 2. All bacterial strains demonstrated the ability to inhibit  
315 the growth of both fungal pathogens, at least in the first 2-3 days of incubation, however bacterial  
316 strain Pf4 exhibited the highest inhibitory activity against both pathogens *P. aphanidermatum* and  
317 *R. solani* with 91.78% and 83.70% inhibition, after 2 and 3 days of incubation respectively. After 9  
318 days of incubation, its inhibitory activity was still very high showing 88.89% and 66.17% of  
319 inhibition against *P. aphanidermatum* and *R. solani*, respectively (Figure 1). Interestingly, *P.*  
320 *aphanidermatum* could not be recovered from plates where it was incubated together with Pf4,  
321 suggesting that Pf4 had a fungicidal activity against it.

322 In addition to Pf4, *P. aphanidermatum* was strongly inhibited also by bacterial strain Pf9 (*P. poae*)  
323 and En8 (*Enterobacter sp.*) that showed 56.39% and 51.81% inhibition of growth after 9 days,  
324 respectively, and moderately inhibited by Pf2 (*P. protegens*) with 43.47% inhibition. In presence of  
325 the other strains, *P. aphanidermatum* was only slightly inhibited (between 14.68% and 30.56%).  
326 Furthermore, *R. solani* was strongly inhibited also by bacterial strains Pf6 (*P. fluorescens*) and Pf7  
327 (*P. fluorescens*), that showed respectively 65.42% and 64.89% inhibition of growth after 9 days;  
328 these bacteria were effective as Pf4 at the end of the assay, but less effective than it after 2, 3 and 7  
329 days of incubation. *R. solani* was moderately inhibited by En8 and Pf9 (with 43.09% and 42.35%  
330 inhibition, respectively), and slightly or not inhibited (between 0% and 14.81%) in presence of the  
331 other strains.

332

### 333 **3.3. In vivo activity of *Pseudomonas sp.* strain Pf4 against *Rhizoctonia solani***

334 Pf4-treated and untreated lamb's lettuce plants were artificially infected with the fungal pathogen *R.*  
335 *solani* in order to test the protective effect of Pf4. In both groups of plants the first symptoms of  
336 disease appeared at 6 days after fungal infection (dpi) and developed very fast, especially on  
337 untreated plants (Figure 3). In fact, on untreated plants there was a sudden rise at 7 dpi, and then the  
338 number of symptomatic plants increased constantly; on Pf4-treated plants, there was a sudden rise

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339 at 8-9 dpi, and a slow progression of the disease until 14 dpi. After 14 days, no new infections were  
340 observed, neither on untreated or treated plants. In any case, plants infected by *R. solani* showed a  
341 sudden shrivelling of leaves, and withered completely in 1-2 days; roots and crown became  
342 yellowish-brown and rotted.

343 Figure 4 with data of disease incidence from the two trials (four replicates each), shows the effects  
344 of Pf4 inoculation on lamb's lettuce plants infected with *R. solani* at 14 dpi, when the maximum  
345 number of wilted plants was reported. Untreated plants showed a very high disease incidence in  
346 both trials with an average disease incidence equal to  $91.10 \pm 7.59\%$  (mean of four replicates  $\pm$  SD)  
347 in trial I and  $89.23 \pm 15.05\%$  in trial II; whereas plants treated with Pf4 showed a much lower  
348 disease incidence, even though the protection effect in the two trials showed some difference.  
349 Namely, Pf4-treated plants exhibited a very high protection against *R. solani* in the first trial with an  
350 average disease incidence equal to  $25.17 \pm 5.78\%$  and a lower degree of protection in the second  
351 trial with an average disease incidence of  $55.60 \pm 6.97\%$ . Nevertheless, statistical analysis showed  
352 that Pf4 displayed an extremely significant (P value is 0.0006, Welch's approximate  $t = 9.757$  with 4  
353 degrees of freedom) and significant (p value is 0.0313, Welch's approximate  $t = 3.832$  with 3  
354 degrees of freedom) biocontrol activity in trial I and II respectively, against the unprotected control  
355 with pathogen alone.

356

357 *3.3.1. Survival and population density of Pseudomonas sp. strain Pf4 on lamb's lettuce roots in*  
358 *hydroponics*

359 The survival and population density of Pf4 on the rhizosphere of lamb's lettuce plants growing in  
360 small scale floating systems, as determined by CFU counting method, is reported in Figure 5. Lines  
361 A and C show the overall CFU counts on King's B agar of fluorescent pseudomonads on the roots  
362 of Pf4-treated and untreated plants, respectively.

363 On treated plants, CFU counts ranged from  $2 \times 10^5$  to  $1.5 \times 10^7$ , and on untreated plants from 0 to  $1$   
364  $\times 10^5$ . Data obtained from colony counting were then adjusted on the basis of the results of

365 molecular analysis (Figure 5; lines B and D) carried out on randomly sampled fluorescent colonies.  
366 In each sample taken from treated roots, 80% to 100% of the colonies gave a positive reaction  
367 (Figure 5, line B) with specific primers Pfluor4gyrB F3/R2, displaying a Ct range between 9 and 17  
368 and a unique melting peak at 86.0°C; whilst in samples collected from untreated roots none of the  
369 fluorescent colonies gave a positive reaction (Figure 5, line D). CFU counts of Pf4, over a time span  
370 longer than the average growing cycle of lamb's lettuce in hydroponics, ranged between  $1.60 \times 10^5$   
371 and  $1.29 \times 10^7$  CFU g<sup>-1</sup> of root tissue. In particular, Pf4 went across a quick increase in the first  
372 week after its inoculation in the tanks, rising the initial concentration of  $5.00 \times 10^5$  to a maximum of  
373  $1.29 \times 10^7$  CFU g<sup>-1</sup> of root tissue; then Pf4 slowly decreased in the following weeks reaching the  
374 minimum concentration of  $1.60 \times 10^5$  CFU g<sup>-1</sup> of root tissue after four weeks.

375

#### 376 **3.4. In vitro screening for genes associated with antibiotic production in *Pseudomonas* strain**

##### 377 ***Pf4***

378 PCR primers sets for conserved sequences of genes involved in the biosynthesis of five antibiotics  
379 were targeted against Pf4 strain. Of the five genes investigated, those involved in the synthesis of  
380 2,4 DAPG (*phlD*), pyrrolnitrin (in both loci *prnD* and *prnC*), pyoluteorin (in both loci *pltC* and  
381 *pltB*) and in cyanide production (in both loci *hcnBC* and *hcnAB*) were detected in *Pseudomonas sp.*  
382 Pf4, although in locus *hcnAB* a faint PCR signal was obtained even with less stringent PCR  
383 conditions. Whereas, gene sequence for phenazine-1-carboxylic acid wasn't detected in Pf4. In all  
384 cases where a positive signal was obtained, the PCR products were of the expected size.

385

#### 386 **3.5. Genome-wide sequence data**

387 We conducted draft-genome sequencing to obtain information on strain Pf4. The Illumina  
388 sequencing provided 1,149,353,940 nts of 300 nts reads that passed the quality check. Sequencing  
389 of the Pf4 library provided 3,828,938 reads which were assembled into 36 contigs (N50 = 688,889;

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390 largest contig: 1,018,138) for a total of 6,832,152 nts (a coverage of 100.9X). The G+C content was  
391 62.5%, which is similar to that of other sequenced *Pseudomonas* sp. genomes.

392 Automated annotation of the *Pseudomonas* sp. Pf4 draft genome sequence using the NCBI pipeline  
393 assigned a total of 5,907 candidate protein coding-genes, with 1,324 (22.41%) annotated as  
394 hypothetical proteins. The assembly predicted a total of 62 tRNA and 11 (6 5S, 3 16S, 2 23S) rRNA  
395 sequences. The draft genome sequence of *Pseudomonas* sp. Pf4 has been deposited in the  
396 DDBJ/EMBL/GenBank database under the accession no. LUUD00000000 The BioProject  
397 designation for this project is PRJNA315258 and the BioSample accession no. is SAMN04554942.

398 Four gene clusters (*hcn*, *plt*, *prn*, and *phl*) encoding the enzymes for the synthesis of the typical  
399 antibiotics of *P. protegens* were found in the genomic sequence of strain Pf4 (Tables 3 and S1),  
400 which supported the results obtained by PCR analyses for all four antibiotic biosynthetic genes  
401 described above. The *hcn* and *phl* gene clusters showed high homology (91-99% and 92-99%  
402 respectively) with those of *P. protegens* strains (CHA0<sup>T</sup>, Pf-5 and Cab57) (Gross & Loper, 2009;  
403 Takeuchi, Noda, & Someya, 2014) and closely related *Pseudomonas* sp. Os17 and St29 (Takeuchi  
404 et al., 2015). The *plt* gene cluster showed very high homology (98-100%) only with that of *P.*  
405 *protegens* strains; and the *prn* gene cluster showed high homology (92-98%) with those of *P.*  
406 *protegens* strains and *P. chlororaphis* strains (Table S1).

407 Other typical gene clusters encoding factors associated to biocontrol found in the Pf4 genome and  
408 highly similar to their homologs in *P. protegens* and/or *Pseudomonas* sp. Os17 and St29 (Tables 3  
409 and S1) include the *aprA* gene cluster (for the major extracellular protease AprA); the genes  
410 associated with the Gac/Rsm signal transduction pathway; the gene clusters for pyoverdine, found  
411 in the Pf4 genome at four different loci (Gene ID 17855-17860, 29340-29435, 04660-04610, and  
412 04555-04545) as reported in Pf-5 (Gross & Loper, 2009) and Cab57 (Takeuchi et al., 2014); and the  
413 genes associated with the synthesis of other siderophores (i.e. enantio-pyochelin, hemophore  
414 biosynthesis and ferric-enterobactin receptor) (Tables 3 and S1).

415 Among more uncommon genes encoded in the Pf4 genome we found the gene cluster for orfamides  
416 (82-85% similar to that of *P. protegens*), and the complete *rxz* gene cluster (approximately 79 kb,  
417 with the highest homology 98-99% to that of Pf-5) encoding analogs of the antimetabolic macrolide  
418 rhizoxin in *P. protegens* Pf-5 (Loper, Henkels, Shaffer, Valeriote, & Gross, 2008), just upstream the  
419 *fit* cluster (with the highest homology 89-97% to that of *P. protegens* strains) (Figure 6, Table S1)  
420 encoding a functional insect toxin reported in *P. protegens* Pf-5 (Péchy-Tarr et al., 2008).

421 The homology search of the gene cluster over the entire genome suggested that the known pathways  
422 for the synthesis of phenazine may not be present in the Pf4 strain, confirming PCR results  
423 described above.

424

### 425 **3.6. Phylogenetic analysis based on MLSA**

426 A phylogenetic tree (Figure 7) was generated based on the concatenated sequences with a total  
427 length of 3712 nucleotides in the following order: 16S rRNA (1288 nt), *gyrB* (798 nt), *rpoD* (711  
428 nt), and *rpoB* (915 nt).

429 In the phylogenetic tree, three well-supported clades can be distinguished, two of them including *P.*  
430 *protegens*-/*P. saponiphila*-related strains (*P. protegens* clade) and *P. chlororaphis*-related strains  
431 (*P. chlororaphis* clade) respectively, both belonging to *P. chlororaphis* subgroup according to  
432 Mulet et al. (2010; 2012), and the third clade (*P. corrugata* clade) corresponding to *P. corrugata*  
433 subgroup (Mulet et al., 2010; 2012).

434  $\text{Phl}^+ \text{Plt}^+$  *Pseudomonas* strain Pf4 represents a separate branch in the well-supported *P. protegens*  
435 clade, which includes  $\text{Phl}^+ \text{Plt}^+$  *Pseudomonas* strains closely related to *P. protegens* species  
436 (Ramette et al., 2011) (Figure 7, Table 3) and  $\text{Phl}^+ \text{Plt}^-$  *Pseudomonas* strains closely related to *P.*  
437 *saponiphila* (Takeuchi et al., 2015; Wu et al., 2016).

438 In the MLSA of these four genes, sequence similarity of Pf4 was 97.28% with *P. protegens* CHA0<sup>T</sup>  
439 and 96.8% with *P. saponiphila* DSM 9751<sup>T</sup>, demonstrating that Pf4 is a member of *P. chlororaphis*  
440 subgroup, most closely related to *P. protegens* strains.

441

442 **4. Discussion**

443 A pool of bacterial microorganisms was isolated from roots of healthy lamb's lettuce plants  
444 growing in floating system in a farm in which a *R. solani* root rot outbreak occurred in 2009, with  
445 the aim to select microorganisms well adapted to soilless environment and synchronized with the  
446 pathogen in time and space (Postma, 2010). Molecular identification based on 16S rRNA gene  
447 sequences revealed that nine of the 12 selected bacteria belonged to genus *Pseudomonas* (six strains  
448 most closely related to *P. protegens*, two to *P. fluorescens* and one to *P. poae*), and three to  
449 *Enterobacter*. Bacteria from these genera are common inhabitants of rhizosphere, both in soil and in  
450 soilless system, and are well known as biocontrol agents against diseases caused by soil-borne  
451 fungal pathogens (Couillerot et al., 2009; Haas & Défago, 2005; Pliego, Ramos, de Vicente, &  
452 Cazorla, 2011).

453 Pf4, the isolate showing the strongest antagonistic *in vitro* activity was further characterized. It was  
454 able to clearly inhibit the growth of both pathogens *Pythium aphanidermatum* and *Rhizoctonia*  
455 *solani in vitro*; it was then shown in *in vivo* tests with pre-treatment of lamb's lettuce plants  
456 growing in hydroponics to reduce significantly *R. solani* disease incidence, despite some  
457 inconsistency in the degree of the suppressive activity in the two trials. Whether the variability in  
458 the efficacy could be ascribed to the growing system (soilless) or due to factors not associated to the  
459 growing system, such as poor host colonization by the biocontrol agent or variable expression of  
460 genes involved in disease suppression, as reported for experiments carried out in soil (Raaijmakers  
461 et al., 2002) could not be ascertained and deserves further investigations.

462 During *in vivo* test (trial I), the persistence and concentration of Pf4 on the rhizosphere were  
463 monitored by a conventional culturing method and molecular analysis, that demonstrated that the  
464 totality or majority of the fluorescent pseudomonads from treated roots corresponded to Pf4, while  
465 in the case of untreated ones none of the fluorescent pseudomonads resembled Pf4. Hence, Pf4 was  
466 capable of surviving at high level of population in the rhizosphere for a period of 4 weeks starting

18

467 18 days after seeding, therefore exceeding the entire lamb's lettuce growing cycle in floating  
468 system. The population dynamics were consistent with those reported in literature for soil (Haas &  
469 Défago, 2005), i. e. artificially inoculated biocontrol agent initially colonize roots at  $10^7$ - $10^8$  CFU g<sup>-1</sup>,  
470 then decline within few weeks. The lowest colonization level shown by Pf4 was  $1.60 \times 10^5$  CFU  
471 g<sup>-1</sup> of lamb's lettuce root, corresponding to the threshold population density ( $10^5$  -  $10^6$  CFU g<sup>-1</sup> of  
472 root) that must be reached by *Pseudomonas* spp. strains for effective disease suppression in soil  
473 (Haas & Défago, 2005).

474 Since the fluorescent pseudomonads population level of untreated plants was quite similar at the  
475 end of the monitoring period, we could confirm previous works (Vallance et al., 2010) indicating  
476 that also in soilless cultures a bacterial population could naturally and quickly develop without  
477 artificial inoculation, even though starting with a "microbiological vacuum" (Postma, 2010).

478 In order to shed light on the mechanisms underlying the biocontrol properties of *Pseudomonas sp.*  
479 Pf4, PCRs having as target genes encoding antibiotic synthesis and draft genome sequencing were  
480 undertaken. Indeed, both methods showed the presence in Pf4 of genes involved in the biosynthesis  
481 of typical *P. protegens* secondary metabolites, such as genes clusters *hcn*, *plt*, *prn*, and *phl*, involved  
482 in the production of hydrogen cyanide, pyoluteorin, pyrrolnitrin and 2,4-DAPG, respectively. The  
483 biosynthesis of pyoluteorin was claimed (Garrido-Sanz et al., 2016) to be specific of *P. protegens*  
484 within the *P. fluorescens* group; however the results of this study and of that of Flury et al. (2016)  
485 demonstrated that also other *Pseudomonas* spp. strains (i.e. Pf4, PH1b, CMR5c and CMAA1215,  
486 Table 3 and Fig. 7) in the *P. chlororaphis* subgroup harbour *plt* gene cluster.

487 In addition to the above, also other gene clusters coding for extracellular enzymes as *apr* gene  
488 cluster and siderophores as *pch*, *has* and *pfe* gene clusters, besides Gac/Rsm homologues and small  
489 regulatory RNAs, showed high homology with *P. protegens* strains, as well as with *Pseudomonas*  
490 sp. Os17 and St29, supporting the notion of a close relatedness of Pf4 to both groups of fluorescent  
491 pseudomonads. Interestingly, Pf4 also has the biosynthetic potential for metabolites that are less  
492 universally spread among the fluorescent pseudomonads; in particular, with our genomic drafting

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493 we discovered in Pf4 the gene clusters for the cyclic lipopeptide orfamide A, for the insect toxin  
494 FitD and for rhizoxin analogs, recently identified natural products discovered through genomics-  
495 guided approaches. Orfamide A, a biosurfactant influencing swarming motility of Pf-5, was shown  
496 to function as an antifungal agent, to lyse oomycete zoospores, and to act as an insecticidal agent  
497 (Gross & Loper, 2009; Ma et al., 2016). The gene cluster for orfamides, which has been identified  
498 in strain Pf-5 mining *Pseudomonas* genomes (Gross et al., 2007) was also found in the genomes of  
499 other *P. protegens* strains, CHA0<sup>T</sup> and Cab57 (Takeuchi et al., 2014), and of *P. protegens*-related  
500 strains (i.e. *Pseudomonas* spp. CMR5c, CMR12a, CMAA1215, PH1b) (Ma et al., 2016). The Fit  
501 insect toxin cluster was first identified in *P. protegens* Pf-5, in which the production of this toxin  
502 has been associated with the lethality of this strain for the tobacco hornworm *Manduca sexta*  
503 (Péchy-Tarr et al., 2008). The complete gene cluster has also been identified in *P. protegens* CHA0<sup>T</sup>  
504 and several other *P. protegens* strains, in closely related *Pseudomonas* spp. Os17, St29 and CMR5c,  
505 in *P. chlororaphis* strains O6, 30–84 and many others, suggesting that Fit toxin is consistently and  
506 exclusively shared by strains belonging to the *P. chlororaphis* subgroup [corresponding to sub-  
507 clade 1 after Loper et al. (2012)] (Loper et al., 2012; Péchy-Tarr et al., 2013; Takeuchi et al., 2015;  
508 Garrido-Sanz et al., 2016; Flury et al., 2016).

509 Rhizoxins are 16-membered polyketide macrolides that exhibit significant phytotoxic, antifungal  
510 and antitumoral properties by binding to  $\beta$ -tubulin, thereby interfering with microtubule dynamics  
511 during mitosis. The complete *rxz* cluster has been initially reported in *P. protegens* Pf-5 (Loper et  
512 al., 2008). This cluster has been found to be absent from two other fully sequenced *P. protegens*  
513 strains, CHA0<sup>T</sup> and Cab57 (Takeuchi et al., 2014), but present in *P. protegens* PF and closely  
514 related *Pseudomonas* sp. Os17 (Takeuchi et al., 2015; Loper et al., 2016) in the *P. fluorescens*  
515 group.

516 In Pf4 the rhizoxin biosynthesis gene cluster is adjacent to the gene cluster encoding for the  
517 production of the FitD insect toxin. To date only few other closely related *Pseudomonas* spp.  
518 strains, *P. protegens* strains Pf-5 and PF and the related strain *Pseudomonas* sp. Os17, are known to

519 have the *Fit* and *rhizoxin* gene clusters linked (i.e. the *fit-rzx* cluster) in their genomes. As in *P.*  
520 *protegens* Pf-5 and *Pseudomonas* sp. Os17, the genomic region with the *fit-rzx* gene clusters of Pf4  
521 did not showed the characteristics of a genomic island, although Loper et al. (2016) suggested that  
522 the *fit-rzx* clusters of Pf-5 and closely related strains have a complex evolutionary history that  
523 includes HGT. Loper et al. (2016) demonstrated that the *fit-rxz* cluster confers oral and injectable  
524 toxicity to a broader set of insects than either the *fit* or *rxz* clusters alone, therefore Pf4 represents a  
525 potential bacteria that may exhibit oral toxicity towards agriculturally relevant insect pests as Pf-5.  
526 Testing *in vivo* insecticidal activity would be an interesting address for future research on Pf4.  
527 Draft genome of Pf4 allowed also to obtain the sequence of the housekeeping *rpoD*, *gyrB* and *rpoB*  
528 genes, which represent the three genes besides the 16S rRNA gene used in the multilocus sequence  
529 analysis (MLSA) developed by Mulet et al. (2010) and proved to be a useful tool for *Pseudomonas*  
530 spp. identification at the species level (Gomila et al., 2015). MLSA is a major contribution to  
531 accurate identification, needed since a large number of strains with disease suppression potential are  
532 reported as *P. fluorescens*, but only some of them are presently retained within this species (Bossis,  
533 Lemanceau, Latour, & Gardan, 2000; Mulet et al., 2010). Mulet et al. (2010) established a similarity  
534 of 97.0% in the MLSA of these four genes as the threshold value for strains in the same species in  
535 the genus *Pseudomonas*. The sequence similarity obtained between Pf4 and *P. protegens* CHA0<sup>T</sup> or  
536 *P. saponiphila* DSM 9751<sup>T</sup> (97.28 and 96.80% respectively) and the phylogenetic analysis indicated  
537 that Pf4 potentially belong to a novel *Pseudomonas* species, as it forms a clearly distinct lineage  
538 within the *P. protegens* clade (Figure 7) in the *P. chlororaphis* subgroup defined according to Mulet  
539 et al. (2010; 2012).

540

## 541 **5. Conclusions**

542 Pf4 displayed the ability to inhibit the growth of *R. solani* and *P. aphanidermatum* *in vitro*, and the  
543 capacity to suppress root rot caused by *R. solani* *in vivo*, on lamb's lettuce plants grown in  
544 hydroponics. Despite the fact that it was isolated from the roots of plants in hydroponic culture, Pf4

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545 was not only at the taxonomic level, but also at the genomic level, rather similar to other strains of  
546 *Pseudomonas spp.* that have been isolated from soil and shown to be active biocontrol agent in soil.  
547 In particular, it could be inferred from the drafted genome sequence that Pf4 has the potential to  
548 produce an arsenal of secondary metabolites very similar to that of the well-known biocontrol *P.*  
549 *protegens* strain Pf-5. Actually, Pf4 is the only not-*P. protegens* strain among those analysed of  
550 closely related *Pseudomonas spp.*, which is more like Pf-5 in the type of secondary metabolites  
551 produced. Moreover, Pf4 can colonize lamb's lettuce roots for the entire growth cycle of this crop in  
552 floating system at a density of  $10^5$ - $10^7$  CFU  $g^{-1}$  of root, therefore above the threshold required for  
553 suppression of root diseases in soil. This work support the notion that key factors conferring the  
554 ability to suppress root diseases in soil are also of paramount relevance in hydroponics. After the  
555 recent discovery that certain pseudomonads cannot only suppress fungal plant diseases but also  
556 have the potential to control insect pests, the results of this work further widen the application  
557 targets of the so called *P. chlororaphis* subgroup, adding value to their use as biocontrol agents and  
558 opening up new industrial opportunities toward the development of unique biopesticides for  
559 biological control of plant diseases and pests using the same product in different growth  
560 environments.

561

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567

#### 568 **Disclosure statement**

569 No potential conflict of interest was reported by the authors

570

571 **References**

- 572 Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., ... & Meyer, F. (2008).  
573 The RAST Server: rapid annotations using subsystems technology. *BMC Genomics*, *9*, 75.  
574 doi:10.1186/1471-2164-9-75
- 575 Bakker, P. A., Pieterse, C. M., & Van Loon, L. C. (2007). Induced systemic resistance by  
576 fluorescent *Pseudomonas* spp. *Phytopathology*, *97*, 239-243. doi:10.1094/PHYTO-97-2-0239
- 577 Bossis, E., Lemanceau, P., Latour, X., & Gardan, L. (2000). The taxonomy of *Pseudomonas*  
578 *fluorescens* and *Pseudomonas putida*: current status and need for revision. *Agronomie*, *20*, 51-  
579 63. doi:10.1051/agro:2000112
- 580 Colla, P., Gilardi, G., & Gullino, M. L. (2012). A review and critical analysis of the European  
581 situation of soilborne disease management in the vegetable sector. *Phytoparasitica*, *40*, 515-523.  
582 doi:10.1007/s12600-012-0252-2
- 583 Compant, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-  
584 promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future  
585 prospects. *Applied and Environmental Microbiology*, *71*, 4951-4959.  
586 doi:10.1128/AEM.71.9.4951-4959.2005
- 587 Couillerot, O., Prigent-Combaret, C., Caballero-Mellado, J., & Moëgne-Loccoz, Y. (2009).  
588 *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of  
589 soil-borne phytopathogens. *Letters in Applied Microbiology*, *48*, 505-512. doi:10.1111/j.1472-  
590 765X.2009.02566.x
- 591 de Souza, J. T., & Raaijmakers, J. M. (2003). Polymorphisms within the *prnD* and *pltC* genes from  
592 pyrrolnitrin and pyoluteorin-producing *Pseudomonas* and *Burkholderia* spp. *FEMS microbiology*  
593 *ecology*, *43*, 21-34. doi:10.1111/j.1574-6941.2003.tb01042.x
- 594 Dhillon, B. K., Laird, M. R., Shay, J. A., Winsor, G. L., Lo, R., Nizam, F., ... & Brinkman, F. S.  
595 (2015). IslandViewer 3: more flexible, interactive genomic island discovery, visualization and  
596 analysis. *Nucleic Acids Research*. doi:10.1093/nar/gkv401

Characterization of the biocontrol agent *Pseudomonas sp.* Pf4

- 597 Flury, P., Aellen, N., Ruffner, B., Péchy-Tarr, M., Fataar, S., Metla, Z., ... & Maurhofer, M. (2016).  
598 Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and  
599 comparative genomics. *The ISME Journal*. doi:10.1038/ismej.2016.5
- 600 Fokkema, N. J. (1976). Antagonism between fungal saprophytes and pathogens on aerial plant  
601 surfaces. *Microbiology of Aerial Plant Surfaces*, 487-505.
- 602 Garibaldi, A., Gilardi, G., & Gullino, M. L. (2006). First report of *Rhizoctonia solani* AG 4 on  
603 lamb's lettuce in Italy. *Plant Disease*, 90, 1109. doi:10.1094/PD-90-1109C
- 604 Garrido-Sanz, D., Meier-Kolthoff, J. P., Göker, M., Martín, M., Rivilla, R., & Redondo-Nieto, M.  
605 (2016). Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. *PloS one*,  
606 11, e0150183. doi:10.1371/journal.pone.0150183
- 607 Gomila, M., Peña, A., Mulet, M., Lalucat, J., & García-Valdés, E. (2015). Phylogenomics and  
608 systematics in *Pseudomonas*. *Frontiers in Microbiology*, 6, 214. doi:10.3389/fmicb.2015.00214
- 609 Gravel, V., Martinez, C., Antoun, H., & Tweddell, R. J. (2005). Antagonist microorganisms with  
610 the ability to control *Pythium* damping-off of tomato seeds in rockwool. *BioControl*, 50, 771-  
611 786. doi:10.1007/s10526-005-1312-z
- 612 Gross, H., Stockwell, V. O., Henkels, M. D., Nowak-Thompson, B., Loper, J. E., & Gerwick, W. H.  
613 (2007). The genomisotopic approach: a systematic method to isolate products of orphan  
614 biosynthetic gene clusters. *Chemistry & Biology*, 14, 53-63. doi:10.1016/j.chembiol.2006.11.007
- 615 Gross, H., & Loper, J. E. (2009). Genomics of secondary metabolite production by *Pseudomonas*  
616 spp. *Natural Product Reports*, 26, 1408-1446. doi:10.1039/b817075b
- 617 Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent  
618 pseudomonads. *Nature Reviews Microbiology*, 3, 307-319. doi:10.1038/nrmicro1129
- 619 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis  
620 program for Windows 95/98/NT. In *Nucleic Acids Symposium Series* (Vol. 41, pp. 95-98).
- 621 Handelsman, J., & Stabb, E. V. (1996). Biocontrol of soilborne plant pathogens. *The Plant Cell*, 8,  
622 1855-1869. doi:10.1105/tpc.8.10.1855

- 623 Hsiao, W., Wan, I., Jones, S. J., & Brinkman, F. S. (2003). IslandPath: aiding detection of genomic  
624 islands in prokaryotes. *Bioinformatics*, *19*, 418-420. doi:10.1093/bioinformatics/btg004
- 625 Iacuzzo, F., Gottardi, S., Tomasi, N., Savoia, E., Tommasi, R., Cortella, G., ... & Cesco, S. (2011).  
626 Corn salad (*Valerianella locusta* (L.) Laterr.) growth in a water-saving floating system as  
627 affected by iron and sulfate availability. *Journal of the Science of Food and Agriculture*, *91*, 344-  
628 354. doi:10.1002/jsfa.4192
- 629 King, E. O., Ward, M. K., & Raney, D. E. (1954). Two simple media for the demonstration of  
630 pyocyanin and fluorescin. *The Journal of Laboratory and Clinical Medicine*, *44*, 301-307.
- 631 Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis  
632 version 7.0 for bigger datasets. *Molecular Biology and Evolution*, msw054.  
633 doi:10.1093/molbev/msw054
- 634 Langille, M. G., Hsiao, W. W., & Brinkman, F. S. (2008). Evaluation of genomic island predictors  
635 using a comparative genomics approach. *BMC Bioinformatics*, *9*, 1. doi:10.1186/1471-2105-9-  
636 329
- 637 Loper, J. E., Henkels, M. D., Shaffer, B. T., Valeriote, F. A., & Gross, H. (2008). Isolation and  
638 identification of rhizoxin analogs from *Pseudomonas fluorescens* Pf-5 by using a genomic  
639 mining strategy. *Applied and Environmental Microbiology*, *74*, 3085-3093.  
640 doi:10.1128/AEM.02848-07
- 641 Loper, J. E., Hassan, K. A., Mavrodi, D. V., Davis II, E. W., Lim, C. K., Shaffer, B. T., ... &  
642 Henkels, M. D. (2012). Comparative genomics of plant-associated *Pseudomonas* spp.: insights  
643 into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genetics*, *8*,  
644 e1002784. doi:10.1371/journal.pgen.1002784
- 645 Loper, J. E., Henkels, M. D., Rangel, L. I., Olcott, M. H., Walker, F. L., Bond, K. L., ... & Taylor,  
646 B. J. (2016). Rhizoxin, orfamide a, and chitinase production contribute to the toxicity of  
647 *Pseudomonas protegens* strain Pf-5 to *Drosophila melanogaster*. *Environmental Microbiology*,  
648 *18*, 3509–3521. doi:10.1111/1462-2920.13369

Characterization of the biocontrol agent *Pseudomonas sp.* Pf4

- 649 Ma, Z., Geudens, N., Kieu, N. P., Sinnaeve, D., Ongena, M., Martins, J. C., & Höfte, M. (2016).  
650 Biosynthesis, chemical structure, and structure-activity relationship of orfamide lipopeptides  
651 produced by *Pseudomonas protegens* and related species. *Frontiers in Microbiology*, 7, 382.  
652 doi:10.3389/fmicb.2016.00382
- 653 Martini, M., Musetti, R., Grisan, S., Polizzotto, R., Borselli, S., Pavan, F., & Osler, R. (2009).  
654 DNA-dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and  
655 *Epicoccum nigrum*. *Plant Disease*, 93, 993-998. doi:10.1094/PDIS-93-10-0993
- 656 Martini, M., & Moruzzi, S. (2017). *Specific detection and quantification of the biocontrol agent*  
657 *Pseudomonas sp. strain Pf4 by real-time PCR and high-resolution melting (HRM) analysis*.  
658 Unpublished manuscript.
- 659 Mavrodi, O. V., McSpadden Gardener, B. B., Mavrodi, D. V., Bonsall, R. F., Weller, D. M., &  
660 Thomashow, L. S. (2001). Genetic diversity of phlD from 2, 4-diacetylphloroglucinol-producing  
661 fluorescent *Pseudomonas* spp. *Phytopathology*, 91, 35-43. doi:10.1094/PHYTO.2001.91.1.35
- 662 McPherson, G. M. (1998). Root diseases in hydroponics—their control by disinfection and evidence  
663 for suppression in closed systems. *Int. Congr. Plant Pathology*, Edinburgh, Scotland.
- 664 McPherson, G. M., Harriman, M. R., & Pattison, D. (1995). The potential for spread of root  
665 diseases in recirculating hydroponic systems and their control with disinfection. *Mededelingen-*  
666 *Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent*  
667 *(Belgium)*.
- 668 Medema, M. H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M. A., ... &  
669 Breitling, R. (2011). antiSMASH: rapid identification, annotation and analysis of secondary  
670 metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic acids*  
671 *research*, 39(suppl 2), W339-W346. doi:10.1093/nar/gkr466
- 672 Mulet, M., Lalucat, J., & García-Valdés, E. (2010). DNA sequence-based analysis of the  
673 *Pseudomonas* species. *Environmental Microbiology*, 12, 1513-1530. doi:10.1111/j.1462-  
674 2920.2010.02181.x

- 675 Mulet, M., Gomila, M., Scotta, C., Sánchez, D., Lalucat, J., & García-Valdés, E. (2012).  
676 Concordance between whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass  
677 spectrometry and multilocus sequence analysis approaches in species discrimination within the  
678 genus *Pseudomonas*. *Systematic and Applied Microbiology*, 35, 455-464.  
679 doi:10.1016/j.syapm.2012.08.007
- 680 Paulitz, T. C., & Bélanger, R. R. (2001). Biological control in greenhouse systems. *Annual Review*  
681 *of Phytopathology*, 39, 103-133. doi:10.1146/annurev.phyto.39.1.103
- 682 Péchy-Tarr, M., Bruck, D. J., Maurhofer, M., Fischer, E., Vogne, C., Henkels, M. D., ... & Keel, C.  
683 (2008). Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated  
684 strains of *Pseudomonas fluorescens*. *Environmental Microbiology*, 10, 2368-2386.  
685 doi:10.1111/j.1462-2920.2008.01662.x
- 686 Péchy-Tarr, M., Borel, N., Kupferschmied, P., Turner, V., Binggeli, O., Radovanovic, D., ... &  
687 Keel, C. (2013). Control and host-dependent activation of insect toxin expression in a root-  
688 associated biocontrol pseudomonad. *Environmental Microbiology*, 15, 736-750.  
689 doi:10.1111/1462-2920.12050
- 690 Pliego, C., Ramos, C., de Vicente, A., & Cazorla, F. M. (2011). Screening for candidate bacterial  
691 biocontrol agents against soilborne fungal plant pathogens. *Plant and Soil*, 340, 505-520.  
692 doi:10.1007/s11104-010-0615-8
- 693 Postma, J. (2010). The status of biological control of plant diseases in soilless cultivation. In *Recent*  
694 *Developments in Management of Plant Diseases* (pp. 133-146). Springer Netherlands.
- 695 Raaijmakers, J. M., Weller, D. M., & Thomashow, L. S. (1997). Frequency of antibiotic-producing  
696 *Pseudomonas* spp. in natural environments. *Applied and Environmental Microbiology*, 63, 881-  
697 887.
- 698 Raaijmakers, J. M., Vlami, M., & De Souza, J. T. (2002). Antibiotic production by bacterial  
699 biocontrol agents. *Antonie van Leeuwenhoek*, 81, 537-547. doi:10.1023/A:1020501420831

Characterization of the biocontrol agent *Pseudomonas* sp. Pf4

- 700 Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moëgne-Loccoz, Y. (2009).  
701 The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial  
702 microorganisms. *Plant and Soil*, *321*, 341-361. doi:10.1007/s11104-008-9568-6
- 703 Ramette, A., Frapolli, M., Défago, G., & Moëgne-Loccoz, Y. (2003). Phylogeny of HCN synthase-  
704 encoding *hcnBC* genes in biocontrol fluorescent pseudomonads and its relationship with host  
705 plant species and HCN synthesis ability. *Molecular Plant-Microbe Interactions*, *16*, 525-535.  
706 doi:10.1094/MPMI.2003.16.6.525
- 707 Ramette, A., Frapolli, M., Fischer-Le Saux, M., Gruffaz, C., Meyer, J. M., Défago, G., ... &  
708 Moëgne-Loccoz, Y. (2011). *Pseudomonas protegens* sp. nov., widespread plant-protecting  
709 bacteria producing the biocontrol compounds 2, 4-diacetylphloroglucinol and pyoluteorin.  
710 *Systematic and Applied Microbiology*, *34*, 180-188. doi:10.1016/j.syapm.2010.10.005
- 711 Schnitzler, W. H. (2003). Pest and disease management of soilless culture. In *South Pacific Soilless*  
712 *Culture Conference-SPSCC 648* (pp. 191-203). doi:10.17660/ActaHortic.2004.648.23
- 713 Sharon, M., Kuninaga, S., Hyakumachi, M., & Sneh, B. (2006). The advancing identification and  
714 classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with  
715 the classical anastomosis grouping. *Mycoscience*, *47*, 299-316. doi:10.1007/s10267-006-0320-x
- 716 Svercel, M., Duffy, B., & Défago, G. (2007). PCR amplification of hydrogen cyanide biosynthetic  
717 locus *hcnAB* in *Pseudomonas* spp. *Journal of Microbiological Methods*, *70*, 209-213.  
718 doi:10.1016/j.mimet.2007.03.018
- 719 Takeuchi, K., Noda, N., & Someya, N. (2014). Complete genome sequence of the biocontrol strain  
720 *Pseudomonas protegens* Cab57 discovered in Japan reveals strain-specific diversity of this  
721 species. *PLoS one*, *9*, e93683. doi:10.1371/journal.pone.0093683
- 722 Takeuchi, K., Noda, N., Katayose, Y., Mukai, Y., Numa, H., Yamada, K., & Someya, N. (2015).  
723 Rhizoxin analogs contribute to the biocontrol activity of a newly isolated *Pseudomonas* strain.  
724 *Molecular Plant-Microbe Interactions*, *28*, 333-342. doi:10.1094/MPMI-09-14-0294-FI

- 725 Tritt, A., Eisen, J. A., Facciotti, M. T., & Darling, A. E. (2012). An integrated pipeline for de novo  
726 assembly of microbial genomes. *PloS one*, 7, e42304. doi:10.1371/journal.pone.0042304
- 727 Vallance, J., Déniel, F., Le Floch, G., Guérin-Dubrana, L., Blancard, D., & Rey, P. (2010).  
728 Pathogenic and beneficial microorganisms in soilless cultures. *Agronomy for Sustainable*  
729 *Development*. doi:10.1051/agro/2010018
- 730 Van Os, E. A. (1999). Closed soilless growing systems: a sustainable solution for Dutch greenhouse  
731 horticulture. *Water Science and Technology*, 39, 105-112.
- 732 Waack, S., Keller, O., Asper, R., Brodag, T., Damm, C., Fricke, W. F., ... & Merkl, R. (2006).  
733 Score-based prediction of genomic islands in prokaryotic genomes using hidden Markov models.  
734 *BMC Bioinformatics*, 7, 142. doi:10.1186/1471-2105-7-142
- 735 Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA  
736 amplification for phylogenetic study. *Journal of Bacteriology*, 173, 697-703.  
737 doi:10.1128/jb.173.2.697-703.1991
- 738 Weller, D. M. (2007). *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30  
739 years. *Phytopathology*, 97, 250-256. doi:10.1094/PHYTO-97-2-0250
- 740 White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. W. (1990). Amplification and direct  
741 sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to*  
742 *Methods and Applications*, 18, 315-322.
- 743 Wilson, K. (1997). Preparation of genomic DNA from bacteria. *Current Protocols in Molecular*  
744 *Biology* 2.4.1-2.4.5. doi:10.1002/0471142727.mb0204s56
- 745 Wu, L., Shang, H., Wang, Q., Gu, H., Liu, G., & Yang, S. (2016). Isolation and characterization of  
746 antagonistic endophytes from *Dendrobium candidum* Wall ex Lindl., and the biofertilizing  
747 potential of a novel *Pseudomonas saponiphila* strain. *Applied Soil Ecology*, 105, 101-108.  
748 doi:10.1016/j.apsoil.2016.04.008.
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Characterization of the biocontrol agent *Pseudomonas sp.* Pf4

## 750 Supplemental online material

751 **Table S1.** Sequence analysis of gene clusters for the synthesis of antibiotics, exoenzyme, cyclic  
752 lipopeptide, siderophores, and toxin, and of Gac/Rsm homologues in *Pseudomonas sp.* Pf4 and  
753 similarities to those in *P. protegens* strains (CHA0<sup>T</sup>, Pf-5, Cab57) and in other most closely related  
754 *Pseudomonas sp.* strains (Os17, St29). Similarity to *P. chlororaphis* strains was also verified in the  
755 case of *prn* and *fit* gene clusters.

756

## 757 Tables

758 **Table 1.** Target genes encoding enzymes involved in the biosynthesis of several antibiotics and  
759 primer sets used for their amplification in *Pseudomonas sp.* Pf4 strain from this study.

Target gene (antibiotic)	Primer	Sequence (5'-3')	Annealing T°	Expected size of PCR product	Reference
<i>phlD</i> (2,4-DAPG)	Phl2a	GAGGACGTCGAAGACCACCA	62°C	745	Raaijmakers, Weller, & Thomashow, 1997
	Phl2b	ACCGCAGCATCGTGTATGAG			
<i>phzCD</i> (phenazine-1-carboxylic acid)	PCA2a	TTGCCAAGCCTCGTCCAAC	68°C	1150	Raaijmakers <i>et al.</i> , 1997
	PCA3b	CCGCGTTGTTCCCTCGTTCAT			
<i>prnD</i> (pyrrolnitrin)	PRND1	GGGGCGGGCGTGGTATGGA	68°C	786	de Souza & Raaijmakers, 2003
	PRND2	YCCCGCSGCCTGYCTGGTCTG			
<i>prnC</i> (pyrrolnitrin)	PrnCf	CCACAAGCCCGCCAGGAGC	64°C	720	Mavrodi <i>et al.</i> , 2001
	PrnCr	GAGAAGAGCGGGTCGATGAAGCC			
<i>pltC</i> (pyoluteorin)	PLTC1	AACAGATCGCCCCGGTACAGAACG	68°C	438	de Souza & Raaijmakers, 2003
	PLTC2	AGGCCCGGACACTCAAGAACTCG			
<i>pltB</i> (pyoluteorin)	PltBf	CGGAGCATGGACCCCAAGC	68°C	791	Mavrodi <i>et al.</i> , 2001
	PltBr	GTGCCCGATATTGGTCTTGACC			
<i>hcnBC</i> (hydrogen cyanide)	Aca	ACTGCCAGGGCGGATGTGC	62°C	587	Ramette, Frapolli, Défago, & Moënne- Loccoz, 2003
	Acb	ACGATGTGCTCGGCGTAC			
<i>hcnAB</i> (hydrogen cyanide)	PM2	TGCGGCATGGGCGTGTGCCATTGCTG CCTGG	68°C	570	Svercel, Duffy, Défago, 2007
	PM7-26R	CCGCTCTGATCTGCAATTGCAGGCC			

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763 **Table 2.** Preliminary data of antagonistic activity against *P. aphanidermatum* after 4 days of  
 764 incubation and molecular identification based on BLASTn analysis of 16S rRNA gene sequences  
 765 with corresponding GenBank accession numbers of 12 selected bacterial strains. Abbreviation: Pf,  
 766 bacteria belonging to *P. fluorescens* group; En, bacteria belonging to *Enterobacter* spp.

Bacterial strain ID	Antagonistic activity*	Accession No.	GenBank closest relative (accession no.)	% similarity
Pf1	++	KM589020	<i>Pseudomonas protegens</i> CHA0 <sup>T</sup> (AJ278812)	99%
Pf2	+++	KM589021	<i>Pseudomonas protegens</i> CHA0 <sup>T</sup> (AJ278812)	100%
Pf3	+	KM589022	<i>Pseudomonas protegens</i> CHA0 <sup>T</sup> (AJ278812)	99%
Pf4	+++	KM589023	<i>Pseudomonas protegens</i> CHA0 <sup>T</sup> (AJ278812)	100%
Pf5	+	KM589024	<i>Pseudomonas protegens</i> CHA0 <sup>T</sup> (AJ278812)	99%
Pf6	++	KM589027	<i>Pseudomonas fluorescens</i> ATCC <sup>2</sup> 13525 <sup>T</sup> (AF094725)	99%
Pf7	+	KM589028	<i>Pseudomonas fluorescens</i> ATCC <sup>2</sup> 13525 <sup>T</sup> (AF094725)	99%
En8	+++	KM589029	<i>Enterobacter</i> sp. TM 1.3 (DQ279307)	99%
Pf9	++	KM589026	<i>Pseudomonas poae</i> DSM <sup>1</sup> 14936 <sup>T</sup> (AJ492829)	99%
En10	+	KM589030	<i>Enterobacter</i> sp. 638 (CP000653)	99%
Pf11	++	KM589025	<i>Pseudomonas protegens</i> CHA0 <sup>T</sup> (AJ278812)	99%
En12	+	KM589031	<i>Enterobacter aerogenes</i> KNUC5012 (JQ682638)	99%

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768 \* +: &lt;1 mm inhibition zone; ++: 1 to 10 mm inhibition zone; +++: &gt;10 mm inhibition zone.

769 <sup>1</sup> DSM: Deutsche Sammlung von Mikroorganismen.770 <sup>2</sup> ATCC: American Type culture Collection.

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Characterization of the biocontrol agent *Pseudomonas* sp. Pf4

779 **Table 3.** Overview on presence (+)/absence (-) of secondary metabolites biosynthetic gene clusters  
 780 in *P. protegens* and closely related *Pseudomonas* spp. strains. Except Pf4 isolated in the present  
 781 work from roots in hydroponics, all the other strains were isolated mostly from roots of plants  
 782 grown in soil.

Species	Strain	Gene cluster											
		<i>hcn</i> <sup>a</sup>	<i>plt</i> <sup>a</sup>	<i>prn</i> <sup>a</sup>	<i>phl</i> <sup>a</sup>	<i>aprA</i> <sup>a</sup>	<i>pvd</i> <sup>a</sup>	<i>pch</i> <sup>a</sup>	<i>has</i> <sup>a</sup>	<i>pfe</i> <sup>a</sup>	<i>ofa</i> <sup>a</sup>	<i>fit</i> <sup>a</sup>	<i>rxz</i> <sup>a</sup>
<i>P. protegens</i>	CHA0 <sup>T</sup>	+	+	+	+	+	+	+	+	+	+	+	-
	Cab57	+	+	+	+	+	+	+	+	+	+	+	-
	Wayne1	+	+	+	+	+	+	+	+	+	+	+	-
	Pf-5	+	+	+	+	+	+	+	+	+	+	+	+
	PF	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pseudomonas</i> spp.	Pf4	+	+	+	+	+	+	+	+	+	+	+	+
	Os17	+	-	-	+	+	+	+	+	+	-	+	+
	St29	+	-	-	+	+	+	+	+	+	-	+	-
	NZI7	+	-	-	+	+	+	+	+	+	-	-	-
	PH1b	+	+	-	-	+	+	+	+	+	+	+	-
	CMR5c	+	+	+	+	+	+	+	+	+	+	+	-
	CMAA1215	-	+	-	+	+	+	+	+	+	+	+	-

783  
 784 <sup>a</sup>*hcn*, for hydrogen cyanide; *plt*, for pyoluteorin; *prn*, for pyrrolnitrin; *phl*, for 2,4-diacetylphloroglucinol; *aprA*, for  
 785 major extracellular protease AprA; *pvd*, for pyoverdine; *pch*, for enantio-pyochelin; *has*, for hemophore biosynthesis;  
 786 *pfe*, for ferric-enterobactin receptor; *ofa*, for orfamide; *fit*, for FitD toxin; *rxz*, for rhizoxin.  
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804 **Figure legends**

805 **Figure 1.** Antagonistic activity (% inhibition of fungal growth, y axis) of 12 potential antagonistic  
806 bacterial strains (x axis) against *P. aphanidermatum* CBS 118745 and CBS 116664 (A), and *R.*  
807 *solani* TR15 and TP20 (B), under *in vitro* conditions after 2 or 3 days of incubation respectively,  
808 and at the end of the experiments (9 days of incubation). Error bars indicate standard deviations.

809

810 **Figure 2.** (A-L) Growth of *P. aphanidermatum* cultures at 1, 2 and 9 days of incubation with  
811 different bacterial antagonists: A-C, Pf4 (strain with maximum antagonistic activity); D-F, Pf5  
812 (strain with minimum antagonistic activity); G-I, En8 (strain with strong antagonistic activity); J-L,  
813 pure culture of *P. aphanidermatum*. Control colony reached the maximum diameter in 2 days (K);  
814 at that time even the less efficient strains showed a quite high inhibition activity, ranging between  
815 32.41% and 68.13% (E). No physical contact was observed for the entire duration of the assay  
816 between all the bacteria tested, including those showing low inhibition activity (F), and the  
817 mycelium of *P. aphanidermatum*.

818 (M-X) Growth of *R. solani* cultures at 2, 3 and 9 days of incubation with different bacterial  
819 antagonists: M-O, Pf4; P-R, Pf5; S-U, En8; V-X, pure culture of *R. solani*. Control colony reached  
820 the maximum diameter in 3 days (W), and even the less efficient strains showed at that time a  
821 significant inhibition, ranging between 31.94% and 61.67% (Q). In some cases, a change in *R.*  
822 *solani* mycelium colour becoming darker brown (R), or a change in the shape of the colony edges  
823 becoming uneven and jagged (O), were observed.

824

825 **Figure 3.** Incidence (% of symptomatic plants per total number of plants observed) dynamics of  
826 root rot caused by *R. solani* on lamb's lettuce plants, Pf4-treated (Pf4+) or untreated (Pf4-), from 5  
827 to 16 dpi.

828

829 **Figure 4.** Data of disease incidence (% of symptomatic plants per total number of plants observed)  
830 of root rot caused by *R. solani* in the two trials at 14 dpi on Pf4-treated or untreated lamb's lettuce  
831 plants. Error bars indicate standard deviations.

832

833 **Figure 5.** Population density of Pf4 (log<sub>10</sub> CFU g<sup>-1</sup> of root tissue) on lamb's lettuce roots in  
834 hydroponics determined by CFU counting method. Lines A: CFU of fluorescent pseudomonads g<sup>-1</sup>  
835 of treated roots; B: CFU of Pf4 g<sup>-1</sup> of treated roots; C: CFU of fluorescent pseudomonads g<sup>-1</sup> of  
836 untreated roots; D: CFU of Pf4 g<sup>-1</sup> of untreated roots.

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838 **Figure 6.** Genetic organization of the *fit* (for FitD toxin, in red) and *rxz* (for rhizoxin analogs, in  
839 blu) gene clusters in the genome of Pf4, obtained using SnapGene software (from GSL Biotech;  
840 available at [snapgene.com](http://snapgene.com)).

841

842 **Figure 7.** MP phylogenetic tree of strains belonging to *P. chlororaphis* and *P. corrugata* subgroups  
843 based on four-gene (16S rRNA, *gyrB*, *rpoD* and *rpoB*) MLSA scheme of Mulet et al. (2010; 2012).  
844 Bootstrap values over 50% are indicated in the tree.

845

846

1 **Table S1.**

2 Sequence analysis of gene clusters for the synthesis of antibiotics, exoenzyme, cyclic lipopeptide,  
 3 siderophores, and toxin, and of Gac/Rsm homologues in *Pseudomonas* sp. Pf4 and similarities to  
 4 those in *P. protegens* strains (CHA0<sup>T</sup>, Pf-5, Cab57) and other most closely related *Pseudomonas* sp.  
 5 strains (Os17, St29). Similarity to *P. chlororaphis* strains was also verified in the case of *prn* and *fit*  
 6 gene clusters.

7

Gene ID (NCBI)	Gene name (ID for PFL)	Position (NCBI)	Size of product (amino acids)	% amino acid homology	<i>Pseudomonas</i> sp.
<b><i>hcn</i> gene cluster (for hydrogen cyanide)</b>					
A1348_23065	<i>hcnA</i> (2577)	6: 391003–391320 (+)	105	98 97	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_23070	<i>hcnB</i>	6: 391317–392726 (+)	469	95 91	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_23075	<i>hcnC</i> (2579)	6: 392719–393972 (+)	417	99 96	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
<b><i>plt</i> gene cluster (for pyoluteorin)</b>					
A1348_17270	<i>pltM</i> (2784)	4: 360091–361599 (–)	502	99	<i>P. protegens</i> strains
A1348_17275	<i>pltR</i>	4: 361596–362627 (–)	343	98	<i>P. protegens</i> strains
A1348_17280	<i>pltL</i>	4: 363114–363380 (+)	88	100	<i>P. protegens</i> strains
A1348_17285	<i>pltA</i>	4: 363394–364743 (+)	449	100	<i>P. protegens</i> strains
A1348_17290	<i>pltB</i>	4: 364776–372152 (+)	2458	98	<i>P. protegens</i> strains
A1348_17295	<i>pltC</i>	4: 372201–377525 (+)	1774	99	<i>P. protegens</i> strains
A1348_17300	<i>pltD</i>	4: 377576–379210 (+)	544	98-99	<i>P. protegens</i> strains
A1348_17305	<i>pltE</i>	4: 379212–380354 (+)	380	99	<i>P. protegens</i> strains
A1348_17310	<i>pltF</i>	4: 380351–381844 (+)	497	99	<i>P. protegens</i> strains
A1348_17315	<i>pltG</i>	4: 381848–382630 (+)	260	99	<i>P. protegens</i> strains
A1348_17320	<i>pltZ</i>	4: 382636–383307 (–)	223	99	<i>P. protegens</i> strains
A1348_17325	<i>pltI</i>	4: 383383–384396 (+)	337	99	<i>P. protegens</i> strains
A1348_17330	<i>pltJ</i>	4: 384393–386162 (+)	589	99	<i>P. protegens</i> strains
A1348_17335	<i>pltK</i>	4: 386172–387314 (+)	380	99	<i>P. protegens</i> strains
A1348_17340	<i>pltN</i>	4: 387331–388437 (+)	368	99	<i>P. protegens</i> strains
A1348_17345	<i>pltO</i>	4: 388449–389945 (+)	498	98-99	<i>P. protegens</i> strains
A1348_17350	<i>pltP</i> (2800)	4: 390011–390616 (+)	201	99	<i>P. protegens</i> strains
<b><i>prn</i> gene cluster (for pyrrolnitrin)</b>					
A1348_27080	<i>prnA</i> (3604)	8: 330759–332375 (–)	538	96 94-96	<i>P. protegens</i> strains <i>P. chlororaphis</i> strains
A1348_27075	<i>prnB</i>	8: 329674–330759 (–)	361	92-95 92	<i>P. chlororaphis</i> strains <i>P. protegens</i> strains
A1348_27070	<i>prnC</i>	8: 327929–329632 (–)	567	97-98 95-97	<i>P. protegens</i> strains <i>P. chlororaphis</i> strains
A1348_27065	<i>prnD</i> (3607)	8: 326813–327904 (–)	363	94-96 94	<i>P. chlororaphis</i> strains <i>P. protegens</i> strains
<b><i>phl</i> gene cluster (for 2,4-diacetylphloroglucinol)</b>					

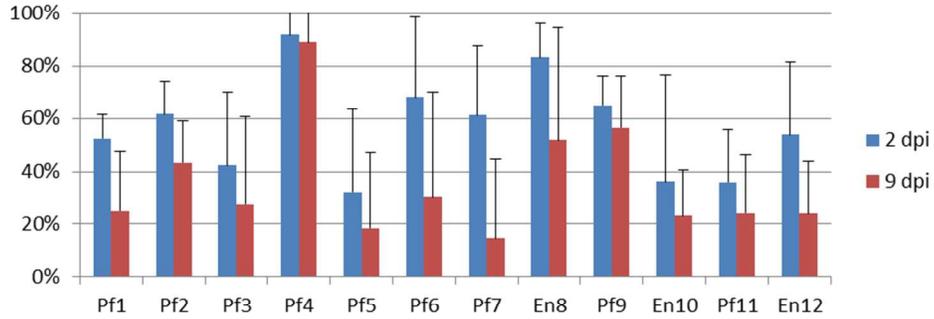
A1348_10485	<i>phlH</i> (5951)	2: 363678–364352 (-)	224	93-94 90	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_10490	<i>phlG</i>	2: 364495–365379 (+)	294	96 93	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_10495	<i>phlF</i>	2: 365432–366034 (-)	200	97	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_10500	<i>phlA</i>	2: 366497–367585 (+)	362	96 94-95	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_10505	<i>phlC</i>	2: 367615–368811 (+)	398	99	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_10510	<i>phlB</i>	2: 368824–369264 (+)	146	99 96-99	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_10515	<i>phlD</i>	2: 369473–370522 (+)	349	99	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_10520	<i>phlE</i> (5958)	2: 370633–371910 (+)	425	98 92	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
<b><i>apr</i> gene cluster</b>					
A1348_26990	<i>aprA</i> (3210)	8: 308831–310279 (-)	482	96 93	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_26985	<i>Inh</i> (PFL_3209)	8:308354..308737 (-)	128	84 96	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_26980	<i>aprD</i>	8: 306344–308137 (-)	597	95 94	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_26975	<i>aprE</i>	8: 305013–306347 (-)	444	97-98 96-97	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_26970	<i>aprF</i> (3206)	8: 303649–305010 (-)	453	98 94	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
<b>Gac/Rsm homologues in <i>Pf4</i></b>					
A1348_03275	<i>gacS</i> (4451)	0: 690217–692970 (-)	917	97	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_25980	<i>gacA</i> (3563)	7: 486282–486866 (+)	194	100	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_03020	<i>rsmA</i> (4504)	0: 641626–641814 (+)	62	100	<i>Pseudomonas</i> spp.
A1348_09780	<i>rsmE</i> (2095)	2: 219078–219797 (+)	239	96 92	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_15270	<i>retS</i> (0664)	3: 607391–610177 (-)	928	97	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_28385	<i>ladS</i> (5426)	9: 172345–174711 (+)	788	93 91	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
<b>small regulatory RNAs</b>					
—	<i>rsmZ</i> (6285)	1: 506535–506661 (+)	127 nt	99 98	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
—	<i>rsmY</i> (6291)	2: 73788–73906 (+)	118 nt	100 99	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
—	<i>rsmX</i> (6289)	10:86797–86915 (+)	119 nt	98 97-98	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
<b><i>pvd</i> gene cluster (for pyoverdine)</b>					
A1348_17855	<i>pvdQ</i> (2902)	4: 506592–508925 (+)	777	91 85	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_17860	<i>fpvR</i> (2903)	4: 508978–509979 (-)	333	91 90	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_29340	<i>pvdA</i> (4079)	10: 26184–27521 (-)	445	88	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_29345	<i>fpvI</i>	10: 27719–28201 (-)	160	85 84	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_29350	RND efflux	10: 28524–29696 (+)	390	96	<i>Pseudomonas</i> sp. Os17, St29

	Transporter (4081)					<i>P. protegens</i> strains
A1348_29355	ABC efflux Transporter (4082)	10: 29697–31670 (+)	657	97 91		<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_29360	RND efflux Transporter (4083)	10: 31678–33069 (+)	463	95 76-77		<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_29365	PFL_4084	10: 33186–33485 (+)	99	94 90 47-49		<i>Pseudomonas</i> sp. St29 <i>Pseudomonas</i> sp. Os17 <i>P. protegens</i> strains
A1348_29370	PFL_4085	10: 33514–33951 (+)	145	62-63		<i>P. protegens</i> strains
A1348_29375	<i>pvdP</i> (4086)	10: 34004–35632 (-)	542	95 59		<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_29380	<i>pvdM</i>	10: 35806–37155 (+)	449	99 95		<i>Pseudomonas</i> sp. Os17 <i>Pseudomonas</i> sp. St29
A1348_29385	<i>pvdN</i>	10: 37188–38474 (+)	428	71-74 99		<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17
A1348_29390	<i>pvdO</i>	10: 38522–39412 (+)	296	91 68-69 100		<i>Pseudomonas</i> sp. St29 <i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17
A1348_29395	<i>pvdF</i>	10: 39445–40464 (+)	339	76 66		<i>Pseudomonas</i> sp. St29 <i>P. protegens</i> strains
A1348_29400	<i>pvdE</i>	10: 40789–42444 (+)	551	100 79		<i>Pseudomonas</i> sp. Os17 <i>Pseudomonas</i> sp. St29
A1348_29405	<i>fpvA</i>	10: 42552–45035 (+)	827	74-75 100		<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17
A1348_29410	<i>pvdD</i>	10: 45701–56242 (-)	3513	42 39-41 99		<i>Pseudomonas</i> sp. St29 <i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17
A1348_29415	<i>pvdJ</i> (4094)	10: 56263–59334 (-)	1023	53-54 45 99		<i>Pseudomonas</i> sp. St29 <i>Pseudomonas</i> sp. Os17 <i>Pseudomonas</i> sp. St29
A1348_29425	<i>pvdI</i> (4095)	10: 60472–69768 (-)	3098	37 35-36 97		<i>Pseudomonas</i> sp. St29 <i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17
A1348_29430	Siderophore-interacting protein (4096)	10: 69943–70911 (+)	322	63 48		<i>Pseudomonas</i> sp. St29 <i>P. protegens</i> strains
A1348_29435	PFL_4097	10: 71090–71830 (-)	246	91 85		<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_04660	PFL_4169	0: 999307–1000530 (-)	407	98 97 91		<i>Pseudomonas</i> sp. St29 <i>Pseudomonas</i> sp. Os17 <i>P. protegens</i> strains
A1348_04655	PFL_4170	0: 998771–999310 (-)	179	99 93-94 90		<i>Pseudomonas</i> sp. Os17 <i>P. protegens</i> strains <i>Pseudomonas</i> sp. St29
A1348_04650	PFL_4171	0: 998433–998771 (-)	112	99 94-96 88		<i>Pseudomonas</i> sp. Os17 <i>P. protegens</i> strains <i>Pseudomonas</i> sp. St29
A1348_04645	PFL_4172	0: 997864–998436 (-)	190	97 93-95 94		<i>Pseudomonas</i> sp. Os17 <i>P. protegens</i> strains <i>Pseudomonas</i> sp. St29
A1348_04640	PFL_4173	0: 996899–997828 (-)	309	100 98 84-85		<i>Pseudomonas</i> sp. St29 <i>Pseudomonas</i> sp. Os17 <i>P. protegens</i> strains
				98 98		<i>P. protegens</i> strains <i>Pseudomonas</i> sp. St29

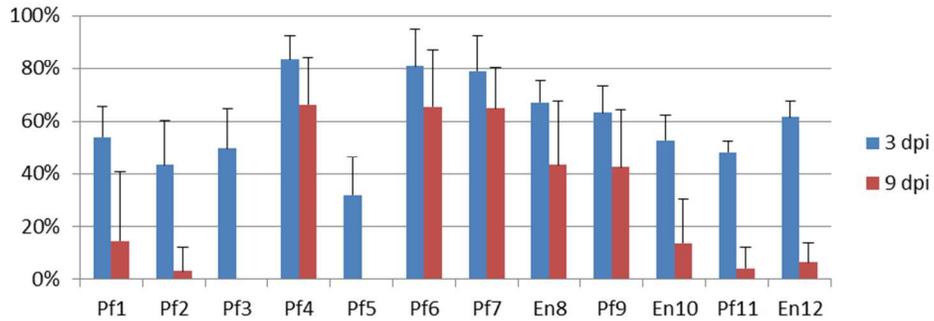
				96	<i>Pseudomonas</i> sp. Os17
				98	<i>P. protegens</i> strains
A1348_04635	PFL_4174	0: 996159–996902 (-)	247	98	<i>Pseudomonas</i> sp. St29
				97	<i>Pseudomonas</i> sp. Os17
A1348_04630	PFL_4175	0: 995246–996145 (-)	299	99	<i>P. protegens</i> strains
				99	<i>Pseudomonas</i> sp. Os17, St29
A1348_04625	PFL_4176	0: 994262–995245 (-)	327	97	<i>Pseudomonas</i> sp. Os17, St29
				93	<i>P. protegens</i> strains
A1348_04620	PFL_4177	0: 993202–994029 (-)	275	94-95	<i>Pseudomonas</i> sp. Os17, St29
				88-90	<i>P. protegens</i> strains
A1348_04615	PFL_4178	0: 992415–992639 (+)	74	99	<i>P. protegens</i> strains
				99	<i>Pseudomonas</i> sp. Os17, St29
A1348_04610	<i>pvdH</i> (4179)	0: 990920–992332 (+)	470	97	<i>Pseudomonas</i> sp. Os17, St29
				95-96	<i>P. protegens</i> strains
A1348_04555	<i>pvdL</i> (4189)	0: 963956–976972 (+)	4338	97	<i>Pseudomonas</i> sp. Os17, St29
				95-96	<i>P. protegens</i> strains
A1348_04550	<i>pvdS</i>	0: 963033–963581 (-)	182	100	<i>P. protegens</i> strains
				99	<i>Pseudomonas</i> sp. Os17, St29
A1348_04545	<i>pvdY</i> (4191)	0: 962639–962992 (+)	117	70-71	<i>P. protegens</i> strains
				67	<i>Pseudomonas</i> sp. Os17, St29
<b><i>pch</i> cluster (for enantio-pyochelin)</b>					
A1348_15840	<i>pchR</i> (3497)	4: 49492–50394 (-)	300	97	<i>Pseudomonas</i> sp. Os17, St29
				95	<i>P. protegens</i> strains
A1348_15845	<i>pchD</i>	4: 50770–52437 (+)	555	90	<i>P. protegens</i> strains
				88	<i>Pseudomonas</i> sp. Os17, St29
A1348_15850	<i>pchH</i>	4: 52421–54175 (+)	584	90	<i>Pseudomonas</i> sp. Os17, St29
				89	<i>P. protegens</i> strains
A1348_15855	<i>pchI</i>	4: 54172–55935 (+)	587	87	<i>Pseudomonas</i> sp. Os17, St29
				86-87	<i>P. protegens</i> strains
A1348_15860	<i>pchE</i>	4: 55928–59398 (+)	1156	88	<i>Pseudomonas</i> sp. Os17
				88	<i>P. protegens</i> strains
A1348_15865	<i>pchF</i>	4: 59395–64815 (+)	1806	87	<i>Pseudomonas</i> sp. St29
				94	<i>P. protegens</i> strains
A1348_15870	<i>pchK</i>	4: 64827–65927 (+)	366	93-94	<i>Pseudomonas</i> sp. Os17, St29
				85-86	<i>P. protegens</i> strains
A1348_15875	<i>pchC</i>	4: 65924–66703 (+)	259	84	<i>Pseudomonas</i> sp. Os17, St29
				93-94	<i>Pseudomonas</i> sp. Os17, St29
A1348_15880	<i>pchB</i>	4: 66727–67050 (+)	107	90	<i>P. protegens</i> strains
				85	<i>Pseudomonas</i> sp. Os17, St29
A1348_15885	<i>pchA</i> (3488)	4: 67043–68476 (+)	477	84	<i>P. protegens</i> strains
				89	<i>P. protegens</i> strains
				86	<i>Pseudomonas</i> sp. Os17, St29
<b><i>has</i> gene cluster (for hemophore biosynthesis)</b>					
A1348_28615	<i>hasI</i> (5380)	9: 223960–224481 (+)	173	96-97	<i>P. protegens</i> strains
				95	<i>Pseudomonas</i> sp. Os17, St29
A1348_28620	<i>hasS</i>	9: 224545–225558 (+)	337	93	<i>P. protegens</i> strains
				87	<i>Pseudomonas</i> sp. Os17, St29
A1348_28625	<i>hasR</i>	9: 225690–228395 (+)	901	95-96	<i>P. protegens</i> strains
				95	<i>Pseudomonas</i> sp. Os17, St29
A1348_28630	<i>hasA</i>	9: 228479–229096 (+)	205	97	<i>P. protegens</i> strains
				92	<i>Pseudomonas</i> sp. Os17, St29
A1348_28635	<i>hasD</i>	9: 229315–231099 (+)	594	97-98	<i>P. protegens</i> strains
A1348_28640	<i>hasE</i>	9: 231096–232445 (+)	449	96	<i>P. protegens</i> strains
A1348_28645	<i>hasF</i> (5374)	9: 232442–233779 (+)	445	94-95	<i>P. protegens</i> strains
<b><i>pfe</i> gene cluster (for ferric-enterobactin receptor)</b>					
A1348_23430	<i>pfeR</i> (2665)	6: 473816–474508 (-)	230	93-94	<i>Pseudomonas</i> sp. Os17, St29
				92-93	<i>P. protegens</i> strains

A1348_23425	<i>pfeS</i>	6: 472479–473816 (–)	445	96-97 94-95	<i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_23420	<i>pfeA</i> (2663)	6: 470135–472375 (–)	746	95-97 96	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29
<b><i>ofa</i> gene cluster (for orfamide A)</b>					
A1348_18430	<i>ofaA</i> (2145)	5: 35808–42188 (–)	2126	82	<i>P. protegens</i> strains
A1348_18425	<i>ofaB</i>	5: 22429–35544 (–)	4371	85	<i>P. protegens</i> strains
A1348_18420	<i>ofaC</i> (2147)	5: 7709–22432 (–)	4907	84	<i>P. protegens</i> strains
<b><i>fit</i> gene cluster (for FitD toxin)</b>					
A1348_26560	<i>fitA</i> (2980)	8: 199520–201661 (–)	713	96 93 88-91 96-97	<i>P. protegens</i> strains <i>Pseudomonas</i> sp. Os17, St29 <i>P. chlororaphis</i> strains <i>P. protegens</i> strains
A1348_26555	<i>fitB</i>	8: 198135–199523 (–)	462	93 88-92 97	<i>Pseudomonas</i> sp. Os17, St29 <i>P. chlororaphis</i> strains <i>P. protegens</i> strains
A1348_26550	<i>fitC</i>	8: 195973–198132 (–)	719	88-92 90 93-94	<i>P. chlororaphis</i> strains <i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_26545	<i>fitD</i>	8: 186846–195857 (–)	3003	77-83 80	<i>P. chlororaphis</i> strains <i>Pseudomonas</i> sp. Os17, St29
A1348_26540	<i>fitE</i>	8: 185262–186767 (–)	501	93-96 85-87 86 89	<i>P. protegens</i> strains <i>P. chlororaphis</i> strains <i>Pseudomonas</i> sp. Os17, St29 <i>P. protegens</i> strains
A1348_26535	<i>fitF</i>	8: 181945–185181 (–)	1078	77 67-75 95-96	<i>Pseudomonas</i> sp. Os17, St29 <i>P. chlororaphis</i> strains <i>P. protegens</i> strains
A1348_26530	<i>fitG</i>	8: 181031–181948 (+)	305	88 82-88 90-91	<i>Pseudomonas</i> sp. Os17, St29 <i>P. chlororaphis</i> strains <i>P. protegens</i> strains
A1348_26525	<i>fitH</i> (2987)	8: 180030–181010 (+)	326	75-81 80	<i>P. chlororaphis</i> strains <i>Pseudomonas</i> sp. Os17, St29
<b><i>rxz</i> gene cluster (for rhizoxin)</b>					
A1348_26520	hypothetical protein PFL_2988	8: 179502–179906 (+)	134	98 84	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26515	<i>rxzB</i> (2989)	8: 158807–178849 (–)	6680	98 79	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26510	<i>rxzC</i>	8: 143811–158636 (–)	4941	98 81	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26505	<i>rxzD</i>	8: 131692–143814 (–)	4040	98 80	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26500	<i>rxzH</i>	8: 130286–131695 (–)	469	99 90	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26495	<i>rxzE</i>	8: 117720–130220 (–)	4166	98 80	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26490	<i>rxzF</i>	8: 110029–117654 (–)	2541	98 78	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26485	<i>rxzI</i>	8: 109125–109991 (+)	288	99 88	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26480	<i>rxzG</i>	8: 106937–108964 (–)	675	98 84	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17
A1348_26475	<i>rxzA</i> (2997)	8: 99945–107012 (–)	2355	98 74	<i>P. protegens</i> Pf-5 <i>Pseudomonas</i> sp. Os17

**A Inhibition of *P. aphanidermatum* growth**

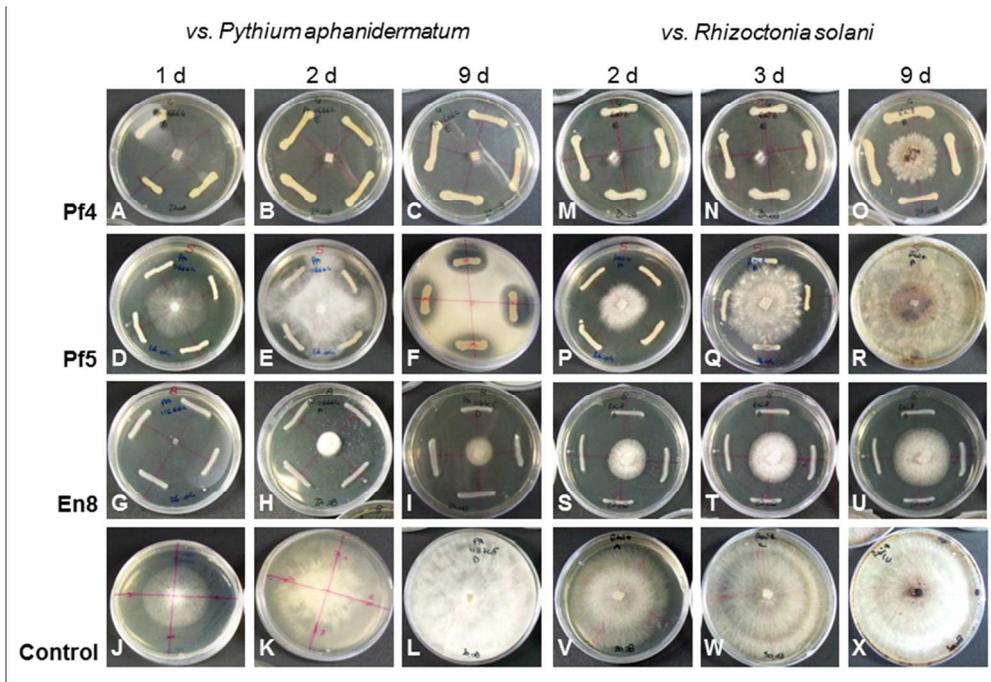


**B Inhibition of *R. solani* growth**



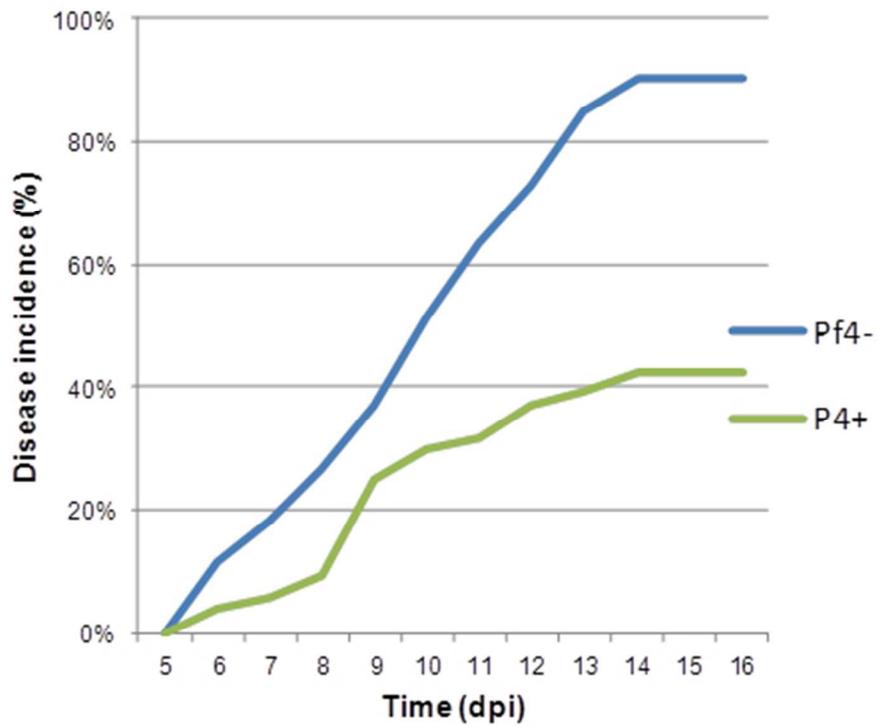
155x138mm (150 x 150 DPI)

Only



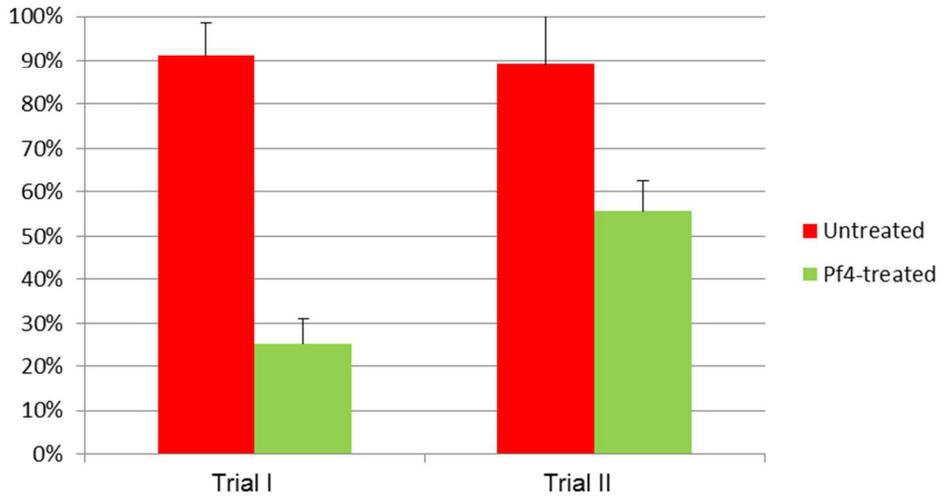
146x99mm (150 x 150 DPI)

view Only



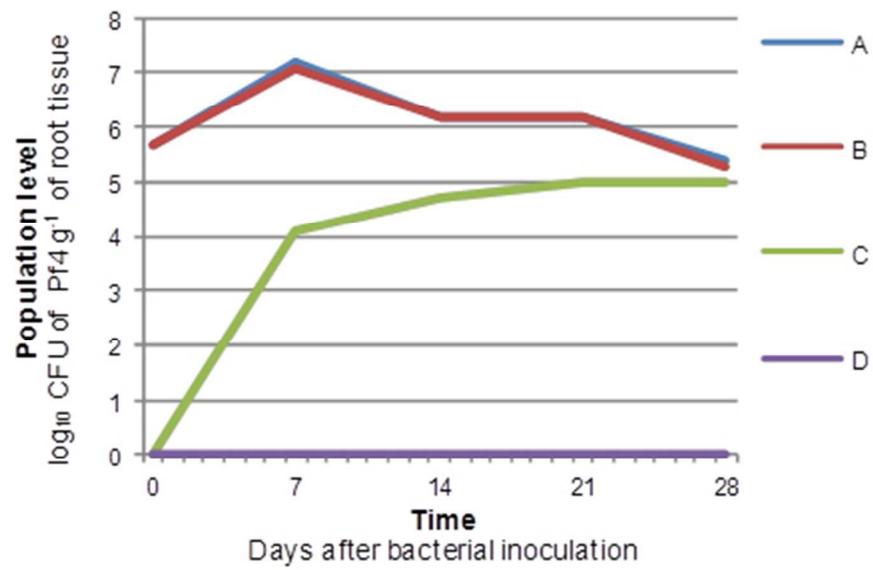
79x62mm (150 x 150 DPI)

View Only

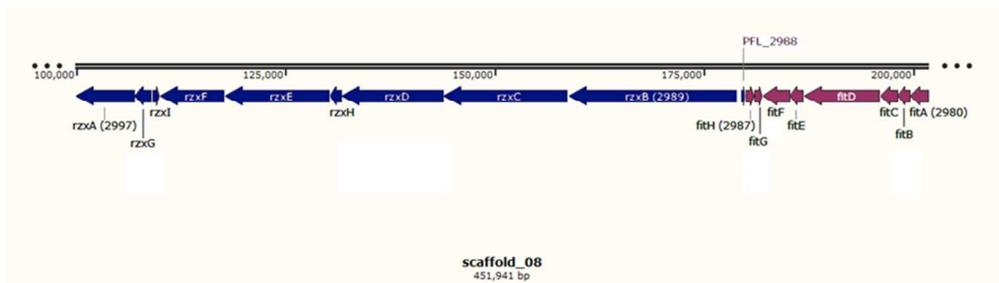


143x80mm (150 x 150 DPI)

Review Only

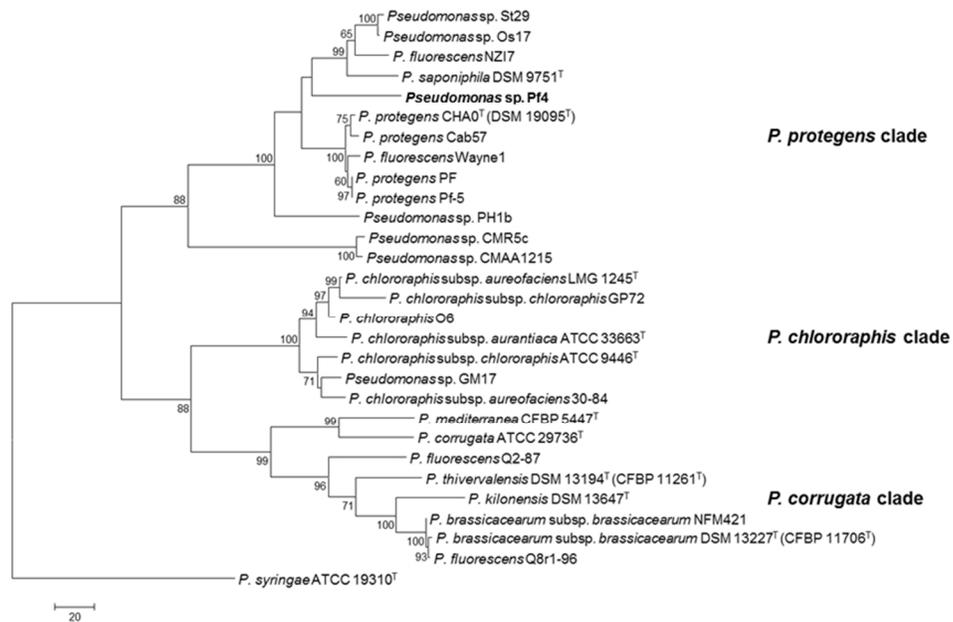


75x49mm (150 x 150 DPI)



150x42mm (150 x 150 DPI)

Peer Review Only



153x98mm (150 x 150 DPI)