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

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Availability:
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Publisher:

Published
DOI:10.1080/09583157.2017.1368454

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24 April 2024

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Journal:	*Biocontrol Science & Technology*
Manuscript ID	Draft
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
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Keywords:	Biological control, Rhizoctonia solani, Pythium spp., population dynamic, secondary metabolites

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

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^{-1} 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

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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, & Moënne-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,

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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^{-1}, 15 g l^{-1} 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^{-1} 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*1I 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^{-1} beef extract, 2 g l^{-1} yeast extract, 5 g l^{-1} peptone, 5 g l^{-1} sodium
133 chloride, 15 g l^{-1} 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^{-1}). 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 $MgCl_2$, 0.625 units of GoTaq Flexi DNA Polymerase (Promega, Madison,
151 WI, USA) and 1 µl of diluted bacterial DNA (5 ng μl^{-1}). 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

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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^{-1} peptone and 2 g l^{-1} 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^{-1}
181 beef extract, 2 g l^{-1} yeast extract, 5 g l^{-1} peptone, 5 g l^{-1} 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^{-1}. *R. solani* was cultured in flasks with 200 ml malt extract broth
184 (MEB, malt extract 6 g l^{-1}, maltose 1.8 g l^{-1}, dextrose 6 g l^{-1}, yeast extract 1.2 g l^{-1}) 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^{-1}.
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

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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^{-1} 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^{-1} proteose peptone, 10 ml l^{-1} glycerol, 1.5
218 g l^{-1} K_2HPO_4, 1.5 g l^{-1} $MgSO_4 \cdot 7\ H_2O$, 15 g l^{-1} 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® 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_2O. Diluted total genomic DNA (2 ng
228 μl^{-1}) 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 µl containing dNTPs 200 µM each, $MgCl_2$ 1.5 mM,
240 each primer 0.4 µM, 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/). 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

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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

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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^{T} (=DSM 19095^{T}) (AJ278812),
304 two strains (Pf6 and Pf7) to *P. fluorescens* with 99% similarity with strain ATCC 13525^{T}
305 (AF094725) and one strain (Pf9) to *P. poae* with 99% similarity with strain DSM 14936^{T}
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

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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 ± 7,59% (mean of four replicates ± SD)
347 in trial I and 89.23 ± 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 ± 5.78% and a lower degree of protection in the second
351 trial with an average disease incidence of 55.60 ± 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 x 10^5 to 1.5 x 10^7, and on untreated plants from 0 to 1
364 x 10^5. Data obtained from colony counting were then adjusted on the basis of the results of

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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 1.60×10^5
371 and 1.29×10^7 CFU g^{-1} 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 5.00×10^5 to a maximum of
373 1.29×10^7 CFU g^{-1} of root tissue; then Pf4 slowly decreased in the following weeks reaching the
374 minimum concentration of 1.60×10^5 CFU g^{-1} 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 ($CHA0^T$, 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).

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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 *rzx* gene cluster (approximately 79 kb,
417 with the highest homology 98-99% to that of Pf-5) encoding analogs of the antimitotic 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 Phl^+ Plt^+ *Pseudomonas* strain Pf4 represents a separate branch in the well-supported *P. protegens*
435 clade, which includes Phl^+ Plt^+ *Pseudomonas* strains closely related to *P. protegens* species
436 (Ramette et al., 2011) (Figure 7, Table 3) and Phl^+ 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* $CHA0^T$
439 and 96.8% with *P. saponiphila* DSM 9751^T, demonstrating that Pf4 is a member of *P. chlororaphis*
440 subgroup, most closely related to *P. protegens* strains.

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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

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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^{-1}
470 , then decline within few weeks. The lowest colonization level shown by Pf4 was 1.60 x 10^5 CFU
471 g^{-1} of lamb's lettuce root, corresponding to the threshold population density (10^5 - 10^6 CFU g^{-1} 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^T 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^T
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 b-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^T 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

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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 *rzx* 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^{T} or
536 *P. saponiphila* DSM 9751^{T} (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
562 **Funding**
563 This research was supported by a grant (L. R. 26) from Friuli Venezia Giulia Region
564 Administration (Italy) and by "Ager - Agroalimentare e Ricerca" Foundation, project "Novel
565 strategies meeting the needs of the fresh-cut vegetable sector - STAYFRESH", under Grant number
566 2010 2370.
567
568 **Disclosure statement**
569 No potential conflict of interest was reported by the authors
570

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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., & Moënne-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

For Peer Review Only

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

For Peer Review Only

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

For Peer Review Only

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

For Peer Review Only

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

For Peer Review Only

700 Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-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., & Moënne-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 Moënne-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:0.1016/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

For Peer Review Only

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.
749

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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 ($CHA0^T$, 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
phlD (2,4-DAPG)	Phl2a Phl2b	GAGGACGTCGAAGACCACCA ACCGCAGCATCGTGTATGAG	62°C	745	Raaijmakers, Weller, & Thomashow, 1997
phzCD (phenazine-1-carboxylic acid)	PCA2a PCA3b	TTGCCAAGCCTCGCTCCAAC CCGCGTTGTTCCTCGTTCAT	68°C	1150	Raaijmakers *et al.*, 1997
prnD (pyrrolnitrin)	PRND1 PRND2	GGGGCGGGCCGTGGTGATGGA YCCCGCSGCCTGYCTGGTCTG	68°C	786	de Souza & Raaijmakers, 2003
prnC (pyrrolnitrin)	PrnCf PrnCr	CCACAAGCCCGGCCAGGAGC GAGAAGAGCGGGTCGATGAAGCC	64°C	720	Mavrodi *et al.*, 2001
pltC (pyoluteorin)	PLTC1 PLTC2	AACAGATCGCCCCGGTACAGAACG AGGCCCGGACACTCAAGAAACTCG	68°C	438	de Souza & Raaijmakers, 2003
pltB (pyoluteorin)	PltBf PltBr	CGGAGCATGGACCCCCAGC GTGCCCGATATTGGTCTTGACC	68°C	791	Mavrodi *et al.*, 2001
hcnBC (hydrogen cyanide)	Aca Acb	ACTGCCAGGGGCGGATGTGC ACGATGTGCTCGGCGTAC	62°C	587	Ramette, Frapolli, Défago, & Moënne-Loccoz, 2003
hcnAB (hydrogen cyanide)	PM2 PM7-26R	TGCGGCATGGGCGTGTGCCATTGCTG CCTGG CCGCTCTTGATCTGCAATTGCAGGCC	68°C	570	Svercel, Duffy, Défago, 2007

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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	*Pseudomonas protegens* CHA0^T (AJ278812)	99%
Pf2	+++	KM589021	*Pseudomonas protegens* CHA0^T (AJ278812)	100%
Pf3	+	KM589022	*Pseudomonas protegens* CHA0^T (AJ278812)	99%
Pf4	+++	KM589023	*Pseudomonas protegens* CHA0^T (AJ278812)	100%
Pf5	+	KM589024	*Pseudomonas protegens* CHA0^T (AJ278812)	99%
Pf6	++	KM589027	*Pseudomonas fluorescens* ATCC[2] 13525^T (AF094725)	99%
Pf7	+	KM589028	*Pseudomonas fluorescens* ATCC[2] 13525^T (AF094725)	99%
En8	+++	KM589029	*Enterobacter* sp. TM 1.3 (DQ279307)	99%
Pf9	++	KM589026	*Pseudomonas poae* DSM[1] 14936^T (AJ492829)	99%
En10	+	KM589030	*Enterobacter* sp. 638 (CP000653)	99%
Pf11	++	KM589025	*Pseudomonas protegens* CHA0^T (AJ278812)	99%
En12	+	KM589031	*Enterobacter aerogenes* KNUC5012 (JQ682638)	99%

767
768 * +: <1 mm inhibition zone; ++: 1 to 10 mm inhibition zone; +++: >10 mm inhibition zone.
769 [1] DSM: Deutsche Sammlung von Mikroorganismen.
770 [2] ATCC: American Type culture Collection.
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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 moslty from roots of plants
782 grown in soil.

Species	Strain	Gene cluster											
		hcn[a]	*plt*[a]	*prn*[a]	*phl*[a]	*aprA*[a]	*pvd*[a]	*pch*[a]	*has*[a]	*pfe*[a]	*ofa*[a]	*fit*[a]	*rzx*[a]
P. protegens	**CHA0**^{T}	+	+	+	+	+	+	+	+	+	+	+	-
	Cab57	+	+	+	+	+	+	+	+	+	+	+	-
	Wayne1	+	+	+	+	+	+	+	+	+	+	+	-
	Pf-5	+	+	+	+	+	+	+	+	+	+	+	+
	PF	+	+	+	+	+	+	+	+	+	+	+	+
***Pseudomonas* spp.**	**Pf4**	+	+	+	+	+	+	+	+	+	+	+	+
	Os17	+	-	-	+	+	+	+	+	+	-	+	+
	St29	+	-	-	+	+	+	+	+	+	-	+	-
	NZI7	+	-	-	+	+	+	+	+	+	-	-	-
	PH1b	+	+	-	-	+	+	+	+	+	+	+	-
	CMR5c	+	+	+	+	+	+	+	+	+	+	+	-
	CMAA1215	-	+	-	+	+	+	+	+	+	+	+	-

783
784 [a]*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; *rzx*, 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.

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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 (log10 CFU g^{-1} of root tissue) on lamb's lettuce roots in
834 hydroponics determined by CFU counting method. Lines A: CFU of fluorescent pseudomonads g^{-1}
835 of treated roots; B: CFU of Pf4 g^{-1} of treated roots; C: CFU of fluorescent pseudomonads g^{-1} of
836 untreated roots; D: CFU of Pf4 g^{-1} of untreated roots.

837

838 **Figure 6.** Genetic organization of the *fit* (for FitD toxin, in red) and *rzx* (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).

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

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

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

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

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

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

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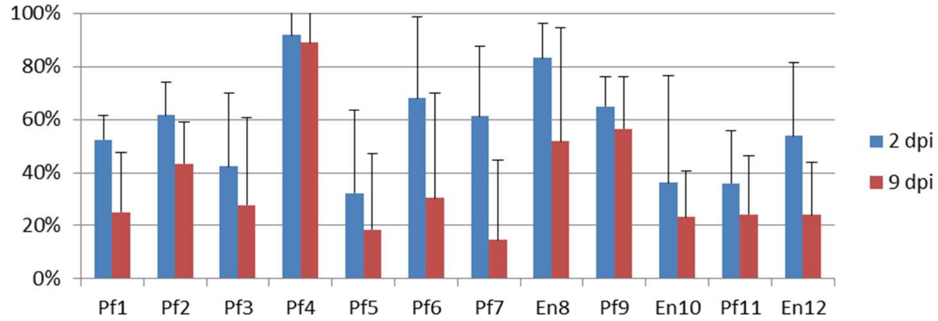

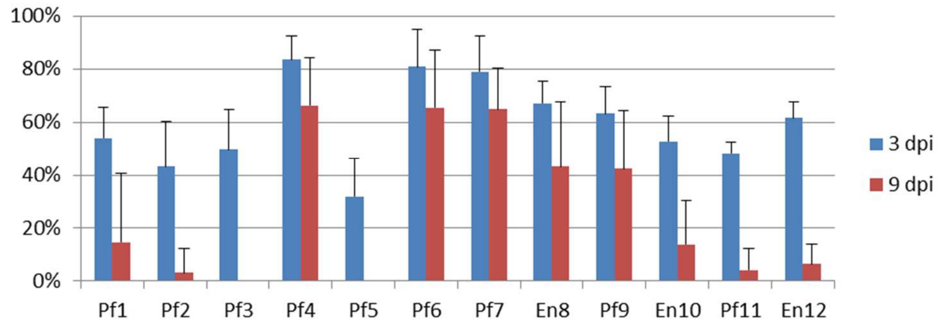

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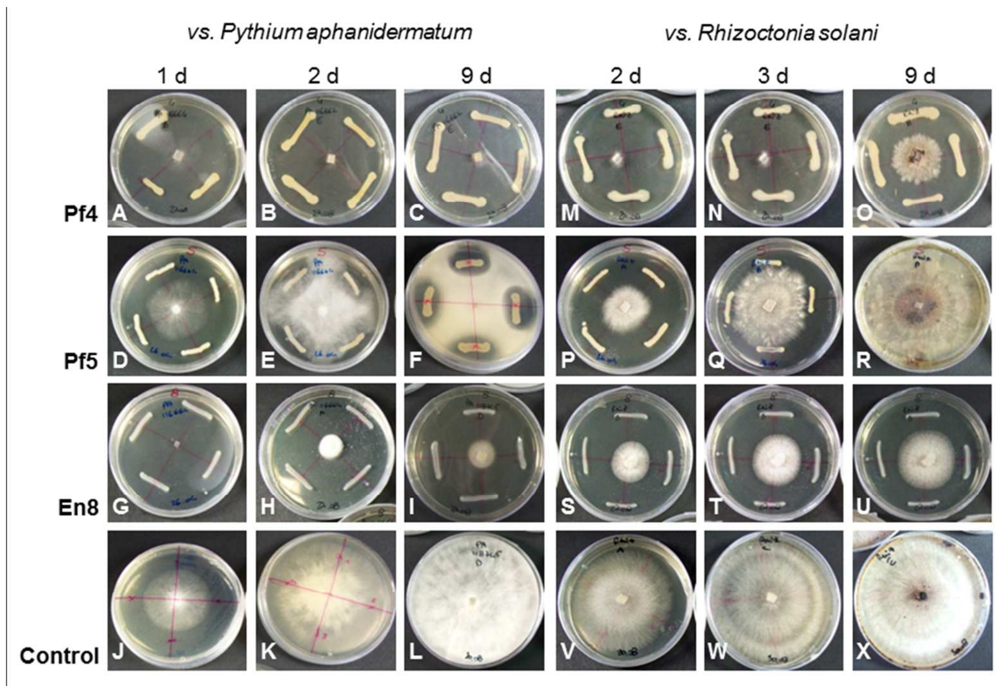

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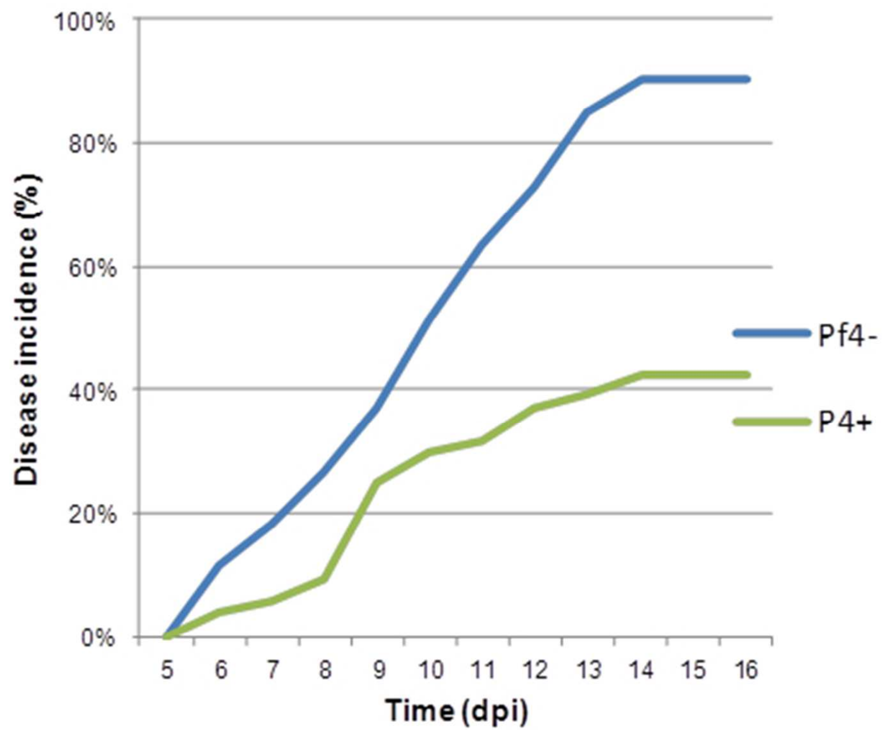

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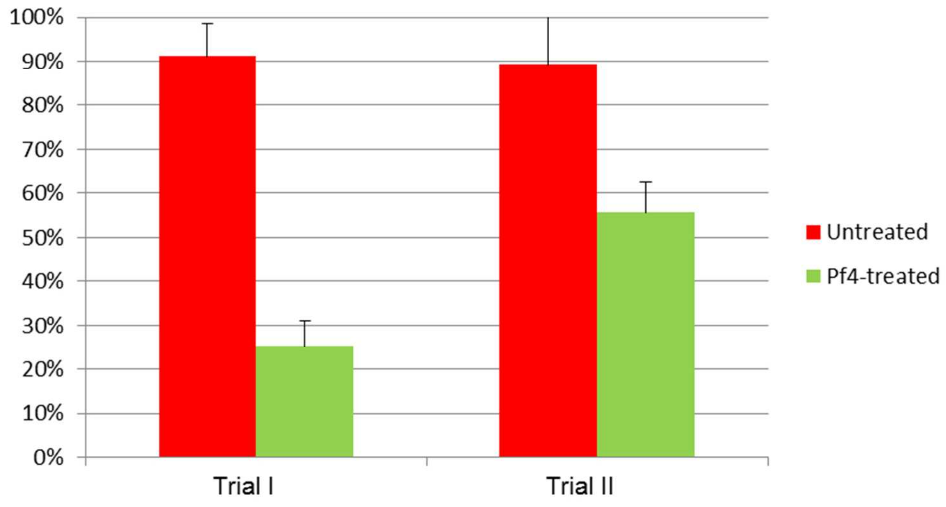

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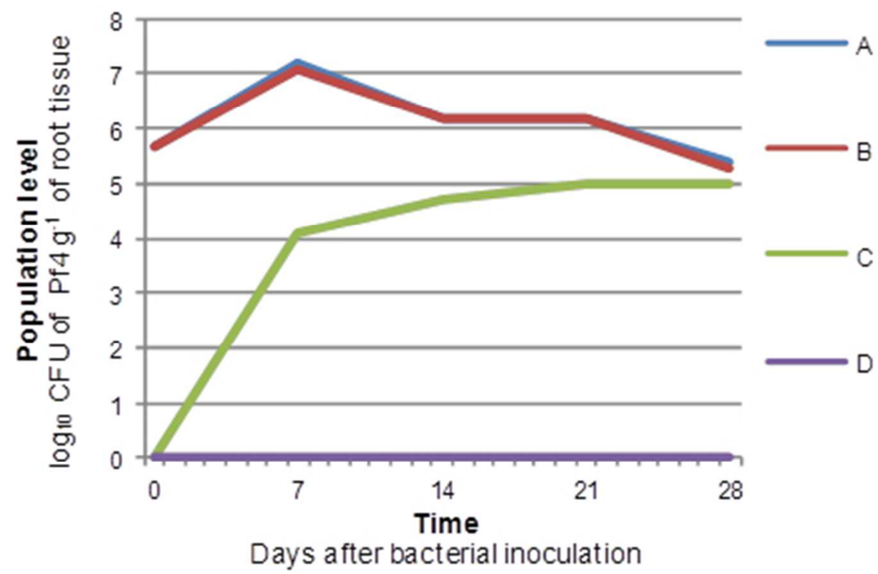

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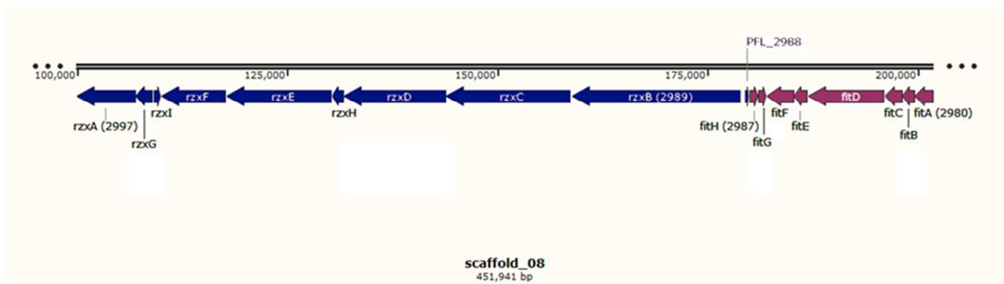

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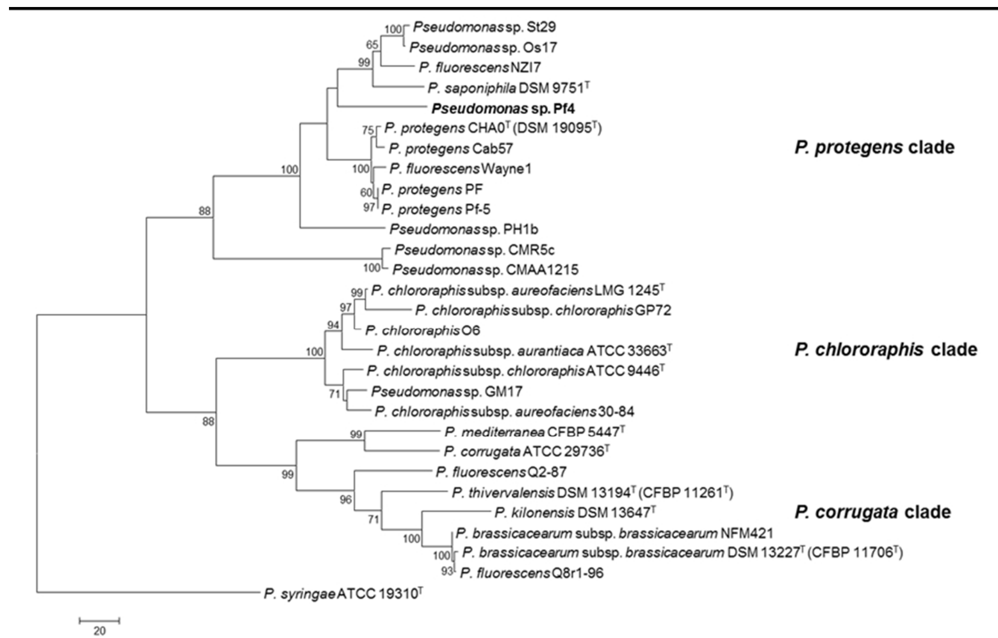

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