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Authors: Flury P, Aellen N, Ruffner B, Péchy-Tarr M, Fataar S, Metla Z, Dominguez-Ferreras A, Bloemberg G, Frey J, Goesmann A, Raaijmakers JM, Duffy B, Höfte M, Blom J, Smits TH, Keel C, Maurhofer M

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Title Page

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Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and comparative genomics

Running title:

Insecticidal arsenal in plant pseudomonads

Authors and affiliations:

Pascale Flury[1], Nora Aellen[1], Beat Ruffner[1], Maria Péchy-Tarr[2], Shakira Fataar[1], Zane Metla[1,3], Ana Dominguez-Ferreras[1], Guido Bloemberg[4], Joachim Frey[5], Alexander Goesmann[6], Jos M. Raaijmakers[7], Brion Duffy[8], Monica Höfte[9], Jochen Blom[6], Theo H. M. Smits[8]*, Christoph Keel[2]* and Monika Maurhofer[1]*

1 Plant Pathology, Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland

2 Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland

3 Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, Latvia

4 Institute of Medical Microbiology, University of Zurich, Zürich, Switzerland

5 Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland

6 Bioinformatics and Systems Biology, Justus-Liebig-University Giessen, Giessen, Germany

7 Department of Microbial Ecology, Netherlands Institute of Ecology, NIOO-KNAW, 6708 PB, Wageningen, the Netherlands

8 Environmental Genomics and Systems Biology Research Group, Institute for Natural Resource Sciences, Zürich University of Applied Sciences, Wädenswil, Switzerland

9 Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, B-9000

Ghent, Belgium

*** To whom correspondence should be addressed.**

Monika Maurhofer

Plant Pathology

Institute of Integrative Biology ETH Zürich

Universitätstrasse 2, CH-8092 Zürich

Switzerland

Phone: +41 44 632 38 69

Fax: +41 44 632 15 72

E-Mail: monika.maurhofer@usys.ethz.ch

Christoph Keel

Department of Fundamental Microbiology

University of Lausanne

Biophore Building

CH-1015 Lausanne

Switzerland

Phone: +41 21 692 56 36

Fax: +41 21 692 56 05

E-Mail: christoph.keel@unil.ch

Theo HM Smits

Environmental Genomics and Systems Biology Research Group

Institute for Natural Resource Sciences

Zürich University of Applied Sciences ZHAW

Postfach, Grüental

8820 Wädenswil

Switzerland

Phone: +41 58 934 52 93

Fax: +41 43 935 52 93

E-Mail: theo.smits@zhaw.ch

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Conflict of Interest

The authors declare no conflict of interest.

Abstract

Bacteria of the genus *Pseudomonas* occupy diverse environments. The *Pseudomonas fluorescens* group is particularly well-known for its plant-beneficial properties including pathogen suppression. Recent observations that some strains of this group also cause lethal infections in insect larvae, however, point to a more versatile ecology of these bacteria. We show that 26 *P. fluorescens* group strains, isolated from three continents and covering three phylogenetically distinct sub-clades, exhibited different activities towards lepidopteran larvae, ranging from lethal to avirulent. All strains of sub-clade 1, which includes *Pseudomonas chlororaphis* and *Pseudomonas protegens*, were highly insecticidal regardless of their origin (animals, plants). Comparative genomics revealed that strains in this sub-clade possess specific traits allowing a switch between plant and insect-associated lifestyles. We identified 90 genes unique to all highly insecticidal strains (sub-clade 1) and 117 genes common to all strains of sub-clade 1 and present in some moderately insecticidal strains of sub-clade 3. Mutational analysis of selected genes revealed the importance of chitinase C and phospholipase C in insect pathogenicity. The study provides insight into the genetic basis and phylogenetic distribution of traits defining insecticidal activity in plant-beneficial pseudomonads. Strains with potent dual activity against plant pathogens and herbivorous insects have great potential for use in integrated pest management for crops.

Introduction

Bacteria of the genus *Pseudomonas* occupy diverse terrestrial, aquatic and atmospheric environments, exhibiting a wide variety of ecological behaviours. Some are feared as human or plant pathogens such as *Pseudomonas aeruginosa* or *Pseudomonas syringae;* others are welcome agents for bioremediation of pollutants such as *Pseudomonas putida.* Members of the *Pseudomonas fluorescens* group are well–known for plant-beneficial effects that improve crop health and agricultural production. Many strains of fluorescent pseudomonads isolated from the rhizosphere have been studied for their ability to suppress root diseases, to promote plant growth and to induce systemic resistance (Bakker et al 2007, Haas and Défago 2005). They harbour strain-specific arsenals of antifungal metabolites, which enable them to inhibit pathogen growth through direct antibiosis (Haas and Keel 2003, Raaijmakers et al 2010). All these features make fluorescent pseudomonads interesting organisms for use as biofertilizers and biopesticides in sustainable agriculture and several products have been commercialized (Kupferschmied et al 2013). On top of plant-beneficial activity, genomics has revealed unexpected and broader ecological versatility for these bacteria (Loper et al 2012, Paulsen et al 2005). Three of the best-characterized biocontrol strains, *Pseudomonas protegens* strains CHA0 and Pf-5 and *Pseudomonas chlororaphis* PCL1391, were shown to have potent insecticidal activity (Péchy-Tarr et al 2008, Ruffner et al 2013). When injected into the hemocoel of *Galleria mellonella* or *Manduca sexta* larvae, they rapidly multiply and cause larval death within a few hours (Péchy-Tarr et al 2008). Ecologically more relevant, these strains are also able to infect and kill insect larvae, such as *Drosophila melanogaster* and the agricultural pests *Spodoptera littoralis* or *Plutella xylostella*, after oral uptake (Olcott et al 2010, Ruffner et al 2013). Oral insecticidal activity is considered a rare trait amongst bacteria and requires specific mechanisms to cope with host immune responses and to breach the gut epithelium in order to access the hemocoel (Herren and Lemaitre 2012, Opota et al 2011, Vallet-Gely et al 2008). How *P. protegens* and *P. chlororaphis* overcome these barriers remains unclear. However, an association with insecticidal activity has been demonstrated for a set of genes termed the *fit* genes (*P. fluorescens*

52 insecticidal toxin) (Péchy-Tarr et al 2008, Péchy-Tarr et al 2013, Ruffner et al 2013). The unique
53 virulence cassette harbours the *fitD* gene encoding the proteinaceous Fit toxin as well as regulatory
54 genes and a type I secretion system (Kupferschmied et al 2014, Péchy-Tarr et al 2008, Péchy-Tarr et
55 al 2013). Nevertheless, *fitD* deletion mutants retain substantial toxicity, indicating the existence of
56 additional virulence factors (Péchy-Tarr et al 2008, Ruffner et al 2013). Mutational analyses provide
57 evidence that some of them are regulated by the global regulator GacA (Olcott et al 2010, Ruffner et
58 al 2013).
59 Insecticidal activity is not universal to the *P. fluorescens* group. A survey by Ruffner et al (2015)
60 revealed that sub-clade 2 strains (Loper et al 2012) neither harbour *fit* genes nor have ability to kill
61 *G. mellonella* larvae. In contrast, all tested *P. protegens* and *P. chlororaphis* strains, which represent
62 the sub-clade 1 (Loper et al 2012), have both the toxin and injectable activity. Accordingly,
63 *Pseudomonas* sp. strains Pf-01 and Q2-87 (formerly called *P. fluorescens* Pf-01 and Q2-87), both
64 belonging to sub-clade 2, have no oral activity against larvae of *D. melanogaster* and several
65 lepidopteran species, respectively (Olcott et al 2010, Ruffner et al 2013). Interestingly, *Pseudomonas*
66 sp. SBW25 (formerly called *P. fluorescens* SBW25) of sub-clade 3, which does not harbour the *fit*
67 genes, was shown to cause mortality and developmental delay in *D. melanogaster* larvae, but to a
68 much lower extent than *P. protegens* Pf-5 (Olcott et al 2010).
69 The discovery of insecticidal activity in fluorescent pseudomonads raises diverse ecological and
70 agronomic questions. What ecological advantage may be gained by this ability to switch from a plant
71 to an insect environment? Can we use these pseudomonads as double–agents to fight both plant
72 disease and insect pests? To date, our understanding of the interaction of plant-associated
73 pseudomonads with insects is still very poor. Although large differences in their ability to infect
74 insects were found between the strains investigated so far, no extensive data on frequency and
75 distribution of insecticidal activity throughout the whole *P. fluorescens* group is available and
76 individual strains with different phylogenetic background have never been compared directly.
77 Moreover, the precise factors beyond the Fit toxin, which enable certain fluorescent pseudomonads

to kill insects, and thereby to occupy a habitat alternative to plant roots, are still elusive. As a first step towards understanding the genomic features enabling insect pathogenicity we have taken an approach that combines bioassays with comparative genomics. We investigated twenty-six strains of fluorescent pseudomonads for their insecticidal activity and their biocontrol activity against root diseases. The strains included in our study are representative of the three phylogenetic sub-clades within the *P. fluorescens* group that harbour most plant-beneficial pseudomonads and were isolated from root but also from non-root habitats. Strong oral activity was found for all strains belonging to the phylogenetic sub-clade 1, which showed potent dual activity against insects and plant pathogens. However, we identified also a second phylogenetic group, sub-clade 3, containing strains with lower insecticidal activity. The strains were sequenced and comparative genomics revealed around 200 genes that are common and unique to the insecticidal strains and we hypothesize that this specific set of genes may represent major evolutionary events towards insect pathogenicity of *Pseudomonas* spp. Finally, we present first results from testing the involvement of some of the newly identified putative virulence factors in insecticidal activity using a mutational approach.

Materials and Methods

Bacterial strains

Strain names and origins are listed in Table 1. We use species names only for strains that cluster closely to a species type strain in the phylogenetic tree we created based on core genomes (Figure 1) and thus can clearly be assigned to a certain species. All other strains are referred to as *Pseudomonas* sp. For sequencing, strains were taken from our long-term strain storage kept at -80°C, or were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). If not otherwise stated bacterial cultures for bioassays and sequencing were grown in LB medium (Bertani 1951) overnight on a rotary shaker (180 rpm) at 24°C. For bioassays cells were washed in sterile 0.9% NaCl. OD_{600} was measured and cells diluted to the desired concentration, while assuming that a cell suspension with an OD_{600} of 0.125 contains approximately 10^8 colony forming

104 units (cfu) per ml.

105

106 **Genome sequencing, assembly and comparative genomics**

107 For sequencing the genomes, DNA was extracted from overnight cultures in LB using the Wizard
108 Genomic DNA Purification Kit (Promega AG, Dübendorf, Switzerland). All genomes apart from
109 *Pseudomonas* sp. CMR5c (for this see Supplementary Methods) were sequenced on an Illumina
110 MiSeq (2 × 300 bp shotgun sequencing) at the Quantitative Genomics Facility (QGF) of the
111 BioSystems Science and Engineering department (BSSE) of ETH Zürich located in Basel, Switzerland.
112 Subsequently, the reads were *de novo* assembled using SeqMan NGen12 (DNASTAR, Madison, WI,
113 USA) and further manually improved *in silico* using different subroutines of the Genomics Package of
114 LASERGENE 12 (DNASTAR, Madison, WI, USA).
115 All genome sequences generated in this study and several database sequences that do not contain
116 an annotation were automatically annotated in GenDB (Meyer et al 2003). The annotation of the
117 genome of *P. chlororaphis* subsp. *piscium* PCL1391 was manually improved and the whole Genome
118 Shotgun project was deposited at DDBJ/EMBL/GenBank. Genomes of all other sequenced strains
119 were deposited without annotations at DDBJ/EMBL/GenBank. Accession numbers are indicated in
120 Supplementary Table S1.
121 Comparative genomics was done using EDGAR (Blom et al 2009). Gene sets common to certain
122 strains, but absent in other strains were calculated with a cut-off of 70% amino-acid identity over
123 70% of the gene length (Smits et al 2010). For the phylogenetic tree of the core genomes, annotated
124 assemblies and genomes from the public GenBank database (NCBI) were used. However, for quality
125 reasons only genomes that consisted of less than 200 contigs and are thus classified as "high-quality
126 draft genome sequences" by the NCBI, were included.
127 The phylogeny based on the core genome of all included strains was generated in EDGAR. The
128 phylogenetic tree was created with the neighbor joining algorithm on a Kimura distance matrix as

129 implemented in the PHYLIP package version 3.57c. Due to the huge size of the core alignment and
130 the long resulting calculation time for a tree, bootstrapping was not performed.
131
132 **Insect assays**
133 Injection assays with *G. mellonella* were performed with small adaptations as described by Péchy-
134 Tarr et al (2008). More information is placed in Supplementary Methods.
135 Feeding assays: Eggs of *P. xylostella* were obtained from Syngenta Crop Protection AG (Stein,
136 Switzerland). General growth conditions for larvae before and during the experiments were 26°C,
137 60% humidity and a 16-h day, 8-h night cycle. Prior to experiments, boxes with larvae were placed at
138 18°C in the dark for 48 h. For virulence assays, 1-week-old larvae were exposed to 10 µl washed
139 bacterial cells adjusted to the desired concentration or 0.9% NaCl (controls) on a pellet of modified
140 insect diet (Gupta et al 2005, Ruffner et al 2013). To prevent injuries each larva was kept separately
141 in 128-cell bioassay trays (Frontier Agricultural Sciences, Delaware, USA). Each treatment was tested
142 on four replicates of eight larvae. Mortality was defined as the inability to react to poking.
143
144 **Construction of deletion mutants of *P. protegens* CHA0**
145 The *chiC*, *aprX* and *plcN* genes and the *rebB1-3* cluster of *P. protegens* CHA0 were deleted by an
146 allelic replacement technique using the I-SceI system with the suicide vector pEMG (Martinez-Garcia
147 and de Lorenzo 2011) as detailed in previous work (Kupferschmied et al 2014). To construct the
148 pEMG-based plasmids, the 600-700-bp upstream and downstream regions flanking the genomic
149 region to be deleted were amplified by PCR using the primer pairs specified in Supplementary Table
150 S2. The obtained fragments were digested with the relevant restriction enzymes (Supplementary
151 Table S2) and cloned into pEMG via triple ligation. Constructs were verified by sequencing. The
152 obtained suicide plasmids served then to generate the deletion mutants CHA5099 (Δ*chiC*), CHA5222
153 (Δ*aprX*), CHA5223 (Δ*plcN*), and CHA5221 (Δ*rebB1-3*) (Supplementary Table S2), using the I-SceI
154 system with the expression plasmid pSW-2.

155

156 **Chitinase activity assay**

157 Chitinase activity was measured in supernatants of cultures grown for 48 h in LB shaking with a
158 methylumbelliferone-based chitinase assay kit (Sigma, St. Louis, MO, USA) according to the
159 manufacturer's instructions.

160

161 **Statistics**

162 Data analysis was performed in R version 3.1.1. (http://www.r-project.org). Mortality rates of the
163 insect toxicity tests with wild type strains were analysed by multiple comparisons using Kruskal-
164 Wallis adjusted by Bonferroni-Holm. LT_{50} values were estimated based on the generalized linear
165 model using the MASS package in R (Venables and Ripley 2002). To test for significant differences
166 between *P. protegens* CHA0 and its mutant strains the Log-Rank test of the Survival package of R and
167 the Student's t-test were used in insect toxicity test and chitinase activity assays, respectively.

168

169 ## Results and Discussion

170 **Strain selection**

171 To obtain an extensive overview of the occurrence of insecticidal activity within the *P. fluorescens*
172 group, we selected 26 strains (Table 1). Many strains were isolated from roots and are well-known
173 for their activity against plant pathogens, others were recently isolated from completely different
174 habitats such as perch intestine and cyclops, e.g., strains *P. chlororaphis* subsp. *piscium* DSM 21509^T
175 and *P. protegens* BRIP, respectively. Type strains were included in the study when considerable
176 indications were present for close relationships of non-assigned strains to existing species.
177 The included strains, isolated on three different continents, belong to five subgroups within the *P.*
178 *fluorescens* group (Supplementary Figure S1) (Gomila et al 2015, Mulet et al 2012), that are covered
179 in the three sub-clades defined by Loper et al (2012) (Figure 1): twelve strains representing sub-

clade 1, eleven strains representing four known and five new species in sub-clade 2 (including the *P. corrugata, P. koreensis* and *P. jessenii* subgroups), and three strains in sub-clade 3 (Figure 1 and Supplementary Figure S1). A detailed overview of the phylogeny of the included strains is given in Supplementary Results, Supplementary Figure S1 and Supplementary Table S3.

Insecticidal activity and presence of the Fit toxin

Functions encoded by Fit gene cluster were demonstrated to contribute to insecticidal activity of the strains *P. protegens* strains CHA0 and Pf-5 and *P. chlororaphis* PCL1391 (Kupferschmied et al 2014, Péchy-Tarr et al 2008, Ruffner et al 2013). Searching the genomes of the selected strains revealed that the gene cluster is present in all strains of sub-clade 1, but neither in sub-clade 2 nor sub-clade 3 (Figure 2), which is in line with results obtained by Ruffner et al (2015).

All 26 strains were tested for their injectable and oral activity against insect larvae. A summary of the results is given in Figure 2. All strains of sub-clade 1 exhibited strong injectable and oral insecticidal activity whereas no strain of sub-clade 2 had an effect on larval survival in any of the test systems. However, the presence of the *fit* cluster, while indicative of strong insecticidal activity, does not seem to be the sole factor associated with the ability to kill insects, since also the tested strains of sub-clade 3, which do not contain the *fit* cluster caused some mortality, but to a much lower extent than strains of sub-clade 1. Insecticidal activity was associated with specific phylogenetic subgroups, but did not correlate with the origin of the isolate (i.e., root or non-root habitat).

To mimic a systemic infection, bacteria were injected into the hemocoel of *G. mellonella* larvae (Figure 3A). All Fit-producing strains, i.e., the entire sub-clade 1, were able to cause 100% mortality within the first 48 h, confirming and extending results of an earlier study demonstrating sub-clade 1 strains to cause 100% mortality when injected into *G. mellonella* (Ruffner et al 2015). Although all strains in sub-clade 1 are highly insecticidal, strain-specific differences for killing rate were observed. The two strains *Pseudomonas* sp. CMR5c and CMR12a, that probably represent a new species within sub-clade 1, were killing more rapidly than *P. protegens* and *P. chlororaphis* as indicated by

206 significantly shorter times to reach 50% larval mortality (LT_{50}) (Table 2). *P. chlororaphis* subsp.
207 *aureofaciens* strains CD and LMG 1245^T have higher LT_{50} values compared to strains of the *P.*
208 *chlororaphis* subspecies *piscium* and *chlororaphis* (Table 2). Thus, the kill-time reflects phylogenetic
209 relationships, which may be explained by the presumably multifactorial nature of insecticidal activity
210 of fluorescent pseudomonads. Beside a common arsenal of contributing factors harboured by all
211 insecticidal strains, some specific factors may exist, which enable certain strains or closely related
212 groups of strains to kill more efficiently than others. No injectable activity was found for strains of
213 sub-clade 2 (Figure 3A). In contrast, two strains of sub-clade 3, namely *P. fluorescens* DSM 50090^T
214 and *Pseudomonas* sp. SS101, both lacking the *fit* genes, caused lethal infections in *G. mellonella*.
215 However, mortality caused by these strains was delayed compared to infections with most strains of
216 sub-clade 1 (Table 2) and larvae lack the strong melanization response and the floppy phenotype
217 observed after infection by sub-clade 1 (Figure 3C). These symptoms might be attributed to the Fit
218 toxin as larvae injected with a *fitD* deletion mutant of *P. protegens* CHA0 lack these phenotypes
219 (Péchy-Tarr et al 2008) similarly to larvae injected with SS101 or DSM 50090^T. While injectable
220 insecticidal activity seems to be universal to strains of sub-clade 1 this is not the case for sub-clade 3.
221 The third tested strain of this sub-clade, *Pseudomonas* sp. MIACH, was not able to kill insect larvae
222 upon injection (Figure 3A), although larvae started to slightly melanize at 1-day post infection (Figure
223 3D).
224 In natural infections, a bacterium first has to breach several barriers to reach the hemocoel.
225 Therefore, the selected strains were further tested for oral activity against *P. xylostella* larvae. All
226 strains that carry the *fit* genes were able to cause high mortality within three days (Figure 3B,
227 Supplementary Table S4). In contrast to the injection assays, here *P. protegens* were the most
228 efficient insect killers in terms of extent and pace (Table 2). None of the strains of sub-clade 2 caused
229 higher mortality than observed for control larvae (Figure 3B). This result was confirmed in a second
230 oral test system where a selection of 15 strains of sub-clades 1 and 2 was fed to *S. littoralis* larvae
231 (Supplementary Methods). No sub-clade 2 strain was able to kill the larvae, whereas all Fit-

producing strains showed strong insecticidal activity (Supplementary Figure S2A). Thus, sub-clade 2 strains lack crucial traits enabling them to kill lepidopteran larvae. However, the lack of killing potential does not necessarily mean that these strains might not be able to persist in the insect gut. Persistence without killing could be a clever strategy to use the insect as a means of dispersal as a living insect will transport the bacteria further than a dead one. Monitoring bacterial cells revealed that all strains of sub-clade 1 were able to multiply within the *S. littoralis* larvae (data only shown for CHA0, Supplementary Figure S2B) and to reach about 10^8 cfu per larva while large differences were observed for strains in sub-clade 2. Several strains, namely *Pseudomonas* sp. P97.38, Q12-87 and P1.31, were indeed able to persist at levels of 10^6 to 10^7 cfu per larva, whereas others such as *P. thivervalensis* PITR2, *P. kilonensis* P12 or *Pseudomonas* sp. P1.8 underwent a 1000-fold population decline within a few days (Supplementary Figure S2B) indicating that they were cleared from the gut. Thus, although not having the ability to kill insect larvae, some strains of sub-clade 2 seem to possess features allowing certain persistence in insects.

In contrast to sub-clade 2 strains, sub-clade 3 strains were found to cause lethal oral infections in *P. xylostella*, which is to our knowledge the first report for strains of this sub-clade to orally kill lepidopteran insect larvae. However, similar to the results of the injection assay, strains of sub-clade 3 appeared to have strongly reduced oral activity compared to strains of sub-clade 1. This is in line with observations of Olcott et al (2010), who described that oral infections of *D. melanogaster* with *Pseudomonas* sp. SBW25 (sub-clade 3) were less detrimental than infections with *P. protegens* Pf-5 (sub-clade 1). Strain *Pseudomonas* sp. SS101 had significant oral insecticidal activity in all repetitions of the experiment (Figure 3B, Supplementary Table S4). However, killing occurred slower and to a lower extent than it was the case for infections with strains of sub-clade 1. *P. fluorescens* DSM 50090^T which had injectable activity against *G. mellonella* showed either no or weak insecticidal activity when fed to *P. xylostella*. We hypothesize that this strain faces difficulties to breach the gut barrier on its own, but can act as an opportunistic pathogen taking the chance when an insect gets injured or weakened by other factors. More puzzling, is the outcome for *Pseudomonas* sp. MIACH,

258 which, in spite of not killing larvae in injection experiments, seems to have slight oral activity. Strain
259 MIACH caused mortality rates of 30 - 53% though the effect was significant only in one of the two
260 experiments (Figure 3B, Supplementary Table S4). We hypothesize that this strain is able to do some
261 damage to the insect gut, without killing the insect itself, thereby promoting a secondary infection
262 by other microbes which invade the hemocoel and lead to larval death. Another explanation would
263 be that this strain is less of a generalist and causes lethal infections only in certain insect species. As
264 we tested injectable and oral activity in different insect species, we cannot exclude this possibility.
265 Although some strain-specific differences exist, we conclude that strains in sub-clade 3 mostly
266 possess some insecticidal activity but that it is by far less distinct than in Fit producing strains of sub-
267 clade 1. To date no factor contributing to pathogenicity of sub-clade 3 strains has been identified,
268 but it was suggested that so-called toxin complexes (Tc), first discovered in the entomopathogen
269 *Photorhabdus luminescens*, could play a role (Loper et al 2012). In accordance to the study of Loper
270 et al (2012) different Tc-related genes could be identified in the genomes of the strains included in
271 this study, but they were not restricted to the strains with insecticidal activity (data not shown).
272 Hence, they might play a rather subtle role in *Pseudomonas*-insect associations.
273
274 **Plant-beneficial effects are phylogenetically less predictable than insecticidal activity**
275 Although biocontrol activity against root pathogens has been demonstrated for many strains of the
276 *P. fluorescens* group, most of the species type strains have never been investigated. The lack of
277 knowledge for these strains and for the new strains from non-root habitats led us to test all 26
278 strains investigated for insecticidal activity also for their biocontrol activity against the oomycete
279 pathogen *Pythium ultimum* on cucumber roots and a subset of strains also for their in vitro inhibition
280 of *P. ultimum* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Biocontrol activity appeared to be
281 phylogenetically less predictable than insecticidal activity, as effective as well as poor biocontrol
282 strains were found throughout all the three sub-clades (Supplementary Results, Figure 2,
283 Supplementary Table S5, Supplementary Figure S3). Similar to the results on insecticidal activity, no

connection between the original habitat and the degree of plant protection was observed. Together, the bioassays with insects and pathogens identified several strains of sub-clade 1, which exhibit potent dual activity against plant pests and diseases and therefore could be of interest for implementation in integrated crop protection strategies.

Comparative genomics to identify potential factors associated to insecticidal activity

Draft genomes of all selected strains were generated with exception of the strains *P. protegens* CHA0 and *Pseudomonas* sp. SS101, for which the genomes were already available (Jousset et al 2014, Loper et al 2012), and *Pseudomonas* sp. CMR12a, for which the genome description will be released elsewhere and which was therefore not included in the comparative genomics analysis. The average number of contigs per genome was 32 (Supplementary Table S6). The obtained genome sizes range between 6.06 and 7.07 Mbp, which is in accordance to genome sizes obtained for other fluorescent pseudomonads (Loper et al 2012).

The next step was to search for genes that are common and unique to insecticidal strains, encoding candidate factors potentially involved during the infection of insect larvae. Using EDGAR (Blom et al 2009), we identified 90 genes that are present in all highly insecticidal strains (sub-clade 1), but neither in moderately insecticidal strains (sub-clade 3) nor in non-insecticidal strains (sub-clade 2) (Table 3). We further identified 117 genes that are present in all strains of sub-clade 1 as well as in one or several of the strains in sub-clade 3, but again in none of the strains of sub-clade 2 (Table 3). A full list of all identified genes can be found in Supplementary Table S7. It comprises about 28 putative transporters, 21 putative regulatory genes and over 100 enzymes and hypothetical proteins that are unique to insecticidal strains (Supplementary Table S7). Amongst the identified transporters, there are several putative amino acid transporters. Insects are very rich in amino acids (Rumpold and Schluter 2013) and thus these transporters might help to exploit the insect as a source of nutrients. The Fit toxin is specifically expressed in insects but not on plant roots (Péchy-Tarr et al 2013). This could also be the case for other virulence factors and might involve some of the

many regulatory genes that were found to be specific to insecticidal strains. However, besides the Fit toxin (Péchy-Tarr et al 2008, Péchy-Tarr et al 2013, Ruffner et al 2013), no other insecticidal toxin was identified. For most of the 207 genes unique to insecticidal strains, a prediction on the biological function of the encoded product as well as on a possible role during the infection of insects would be very speculative at the present stage. Nevertheless, the comparative genomics also revealed several genes encoding proteins with homology to known virulence factors of other bacteria and that are of interest in terms of a possible association with insecticidal activity. Presence of those genes, which are discussed below, is indicated for our selection of strains in Figure 2 and for all strains included in the phylogeny of Figure 1 in Supplementary Table S1.

Upon ingestion of pathogenic bacteria, insects produce reactive oxygen species, antimicrobial peptides (AMPs) and lysozymes to rapidly eliminate infesting bacteria (Lemaitre and Hoffmann 2007). One mechanism to counter this first line of insect immunity is to produce enzymes degrading AMPs. Exoproteases such as the Zn-dependent metallo-peptidase AprX, also called serralysin and the AprA alkaline protease were suggested to play a role during the early phase of bacterial infections (Liehl et al 2006). The gene *aprA* is present in all 25 genomes, except that of strain P1.8, whereas *aprX* was only detected in the genomes of strains belonging to sub-clade 1 (Supplementary Table S1). AprA and AprX belong to the M10 family that includes serralysin, aeruginolysin and other related exopeptidases that cause tissue damage and anaphylactic responses (Park and Ming 2002). In *Pseudomonas entomophila*, an *aprA* mutant was shown to be slightly less virulent and to have a reduced persistence in *D. melanogaster* (Liehl et al 2006). Serralysin of *Serratia marcescens* was shown to promote hemolymph bleeding in the silkworm (*Bombyx mori*) (Ishii et al 2014).

If bacteria persist within the insect gut, living cells or their toxins must breach the peritrophic membrane, a gut-delimiting chitinous matrix, to access the hemocoel (Vallet-Gely et al 2008). Chitinases affecting the peritrophic matrix are therefore potential virulence factors of entomopathogenic bacteria. For instance chitinases of *B. thuringiensis* subsp. *israelensis* IPS68 and *B. thuringiensis* subsp. *aizawai* HD133 were shown to contribute to insecticidal activity towards

336 *Culicuoides nubeculosus* and *S. littoralis,* respectively (Sampson and Gooday 1998). In insecticidal
337 strains of the *P. fluorescens* group, we identified two chitinase genes. The chitinase gene *chiC*
338 encoded next to a chitin-binding protein is present exclusively in genomes of sub-clade 1 strains,
339 whereas the second chitinase is present in nearly all *P. chlororaphis* strains and some sub-clade 3
340 strains (Supplementary Table S1).
341 PCL1391_2966 encodes for a phosphocholine-specific phospholipase C. This gene, *plcN*, was
342 detected only in sub-clade 1 strains. Phospholipases are recognized as major virulence determinants
343 in a number of bacterial species, including human, animal and several invertebrate pathogens (Farn
344 et al 2001, Songer 1997, Yang et al 2012). The *ymt* gene encoding for a phospholipase D in *Yersinia*
345 *pestis*, for example, is needed for persistence in the flea midgut (Hinnebusch et al 2002).
346 Phospholipase C produced by *Mycobacterium abscessus* is crucial for survival in amoeba and is
347 suggested to cause damage to mouse macrophages presumably by hydrolysis of membrane
348 phospholipids (N'goma et al 2015).
349 Three small genes with homology to *reb* genes were found to be present in all strains of sub-clade 1
350 and in *Pseudomonas* sp. SS101, the strain with the highest insecticidal activity of sub-clade 3. Such
351 *reb* genes have been mainly studied in *Caedibacter taenospiralis,* a *Paramecium* endosymbiont. They
352 encode R-bodies, highly insoluble protein ribbons that are typically coiled into cylindrical structures
353 but can unroll under certain conditions (Pond et al 1989) and are associated with the killing trait
354 towards sensitive *Paramecia* (Dilts and Quackenbush 1986). Orthologs of *reb* were found to be
355 present in many free-living bacteria, but their function remains unclear to date (Raymann et al
356 2013).
357 A whole cluster of genes specific to insecticidal strains (loci PCL1391_4983 to PCL1391_4994) has
358 high percentage of sequence identity to the *psl* gene cluster of *P. aeruginosa* which specifies the
359 production of the extracellular polysaccharide Psl (Franklin et al 2011). Psl was shown contributing
360 to biofilm production, tolerance to oxidizing agents and host defensive processes (Friedman and
361 Kolter 2004, Jackson et al 2004, Mishra et al 2012), i.e. traits likely useful in insect interactions.

362 Other factors, which still have to be kept in mind, are the antimicrobial compounds, such as 2,4-
363 diacetylphloroglucinol, phenazine, pyoluteorin, pyrrolnitrin and hydrogen cyanide which are crucial
364 for biocontrol activity against fungal diseases, although none is shared by all or unique to insecticidal
365 strains (Figure 2, Supplementary Table S1) (Haas and Défago 2005, Lugtenberg and Kamilova 2009,
366 Mercado-Blanco and Bakker 2007). However, some have activity against a broad spectrum of
367 organisms including plants, nematodes, arthropods and even mammalian cells (Devi and Kothamasi
368 2009, Jang et al 2013, Kwak et al 2011, Maurhofer et al 1992, Neidig et al 2011, Nisr et al 2011) and
369 thus could contribute to *Pseudomonas*-derived insecticidal activity.
370
371 **Chitinase ChiC and phospholipase PlcN contribute to oral insecticidal activity**
372 In order to verify that our combination of bioassays and comparative genomics indeed led to the
373 identification of valuable candidate genes associated with bacterial virulence towards insects, we
374 generated, in model strain *P. protegens* CHA0, in-frame deletion mutants for selected genes: *plcN*,
375 *chiC*, *aprX* and the cluster encoding homologs of *rebB*. None of the mutants differed in activity from
376 the wild type when injected directly into the hemocoel of *G. mellonella* (Figure 4A, Supplementary
377 Figure S4A and C). However, the *chiC* mutant was always significantly delayed in killing *P. xylostella*
378 larvae upon ingestion (Figure 4B, Supplementary Figure S5A). We therefore conclude that the
379 chitinase C, encoded by *chiC*, a gene common and unique to highly insecticidal strains, contributes to
380 oral insect pathogenicity of *P. protegens* CHA0. Chitinase C was found to be responsible for
381 chitobiosidase as well as endochitinase activity of *P. protegens* CHA0 as the *chiC* mutant exhibited no
382 residual chitinase activity (Figure 4C). Since also a *gacA* mutant completely lost chitinase activity
383 (Figure 4C) we believe that we identified with the *chiC* one of the hitherto unknown Gac-regulated
384 virulence factors involved in oral insecticidal activity. Furthermore, the mutant deficient for
385 phopholipase C (*plcN)* also showed reduced oral activity against *P. xylostella*. Although less distinct
386 than for the *chiC* mutant, a delay in killing was always observed for the *plcN* mutant but the effect
387 was not significant in all experiments (Supplementary Figures S4B and S5B).

388 In contrast, no difference to the wild type strain CHA0 was found for the *rebB1-3* and *aprX* mutants
389 (Supplementary Figure S4D). However, these results do not exclude a role of factors encoded by
390 these genes under different conditions or in an interaction with other insect species. Accordingly,
391 the impact of the well–characterized Fit toxin on virulence towards insects also varies between
392 insect species. Thus, a *fitD* mutant compared to the wild type *P. protegens* CHA0 is more strongly
393 reduced in virulence towards *S. littoralis* than towards *P. xylostella* (data not shown).
394 The mutational analysis performed in this study gives only a first insight into the possible
395 contribution of interesting candidate genes identified in the comparative genomics approach to
396 insecticidal activity. An in-depth analysis of the role of chitinase C and phospholipase C would
397 include the complementation of these mutants and will be subject to further studies.
398

399 Conclusions

400 We provide the first extensive overview on insecticidal activity in the *P. fluorescens* group. Whilst
401 biocontrol activity against fungal pathogens occurs throughout all studied sub-clades, insecticidal
402 activity is unique to sub-clades 1 and 3. Only strains of sub-clade 1 display strong oral insecticidal
403 activity and only they produce the Fit toxin. Intriguingly, Fit seems to contribute to the floppy and
404 melanized phenotype associated with infections by highly pathogenic strains, however, the toxin is
405 clearly not the major killing factor upon oral ingestion. Mutants of strains CHA0 and PCL1391 lacking
406 the *fit* genes cause delayed, but still substantial mortality in *S. littoralis* (Ruffner et al 2013) when
407 acquired via the oral infection route. By comparative genomics we now identified several candidate
408 genes that might contribute to insecticidal activity and we demonstrated that the absence of two of
409 these genes, encoding a specific chitinase and a phospholipase, negatively affects insecticidal
410 activity. We hypothesize that especially the chitinase C might be involved during the gut stage of the
411 infection process, causing damage to the peritrophic membrane. However, to understand the exact
412 mode of action of these pathogenicity factors during the infection process, further investigations are

413 needed. Nevertheless, the presented data highly increases the knowledge on the genetic basis of
414 insecticidal activity of fluorescent pseudomonads and points to a multifactorial nature of this trait.
415 Although we provide evidence that many strains of the *P. fluorescens* group can be insect
416 pathogenic and others might persist in insects as commensals, the ecological relevance of insects as
417 a host for these bacteria is still elusive and an intriguing field for future research. The fact that
418 certain pseudomonads, to date considered to be plant-associated, perform very well in a completely
419 different habitat such as an insect raises the question whether these bacteria are indeed mainly
420 plant-associated. Insecticidal as well as biocontrol activity against plant diseases was found to be
421 independent of the original habitat of a strain. For example, closely related strains can be isolated
422 from fish or cyclops and behave similarly well on roots as root isolates. This observation is in line
423 with other studies that found the isolation source of a bacterial strain not to be predictive for its
424 performance in another habitat (Alonso et al 1999, Grosso-Becerra et al 2014). For instance, Hilker
425 and colleagues found no correlation between original habitat and virulence in different test systems
426 for clinical and environmental clones of *P. aeruginosa* (Hilker et al 2015). In general, fluorescent
427 pseudomonads might be quite ubiquitous and probably possess an arsenal of traits allowing them to
428 easily switch niches and to conquer the habitat they encounter. Insects could be especially useful as
429 a means of dispersal, a phenomenon documented for diverse plant-pathogenic bacteria (Nadarasah
430 and Stavrinides 2011). Some *Pseudomonas syringae* strains for instance can use the pea aphid as
431 alternative primary host where they replicate to high numbers and can be deposited onto a new
432 plant host via excreted honeydew (Stavrinides et al 2009). Similarly, the rhizobacterium *P.*
433 *chlororaphis* strain L11 was found to be transmittable from one plant to another by *Diabrotica*
434 *undecimpunctata* subsp. *howardi* feeding on colonized plants (Snyder et al 1998). In contrast to
435 considering the insect as an alternative host, one could even speculate that the plant is not the
436 primary host for species like *P. protegens* and *P. chlororaphis,* but rather a transient host on which
437 they endure until they encounter the next insect host. As research to date is very much biased
438 towards plant-association, future studies especially on strains actually isolated from insects will be

required to gain a better understanding of the importance insects have as hosts for strains of the *P. fluorescens* group.

Besides its ecological relevance, insecticidal activity might be of great agronomical interest. Our bioassays revealed several strains, especially of the species *P. protegens*, that display potent dual activity, killing insect larvae and protecting plants against pathogens. Fluorescent pseudomonads are already commercially used for the biological control of plant diseases (Berg 2009, Stockwell and Stack 2007). Our discovery of strains with the capacity to control insect pests on top of fungal pathogens renders these bacteria highly interesting for a new field of application and an additional market.

Conflict of Interest

The authors declare no conflict of interest.

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References

Achouak W, Sutra L, Heulin T, Meyer JM, Fromin N, Degraeve S *et al.* (2000). *Pseudomonas brassicacearum* sp. nov. and *Pseudomonas thivervalensis* sp. nov., two root-associated bacteria isolated from *Brassica napus* and *Arabidopsis thaliana*. *Int J Syst Evol Microbiol* **50:** 9-18.

Alonso A, Rojo F, Martinez JL. (1999). Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. *Environ Microbiol* **1:** 421-430.

Bakker PAHM, Pieterse CMJ, van Loon LC. (2007). Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* **97:** 239-243.

Berg G. (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol* **84:** 11-18.

Bertani G. (1951). Studies on lysogenesis.1. the mode of phage liberation by lysogenic *Escherichia coli*. *J Bacteriol* **62:** 293-300.

Blom J, Albaum SP, Doppmeier D, Puehler A, Vorhoelter F-J, Zakrzewski M *et al.* (2009). EDGAR: A software framework for the comparative analysis of prokaryotic genomes. *BMC Bioinformatics* **10:** 154.

Burr SE, Gobeli S, Kuhnert P, Goldschmidt-Clermont E, Frey J. (2010). *Pseudomonas chlororaphis* subsp. *piscium* subsp. nov., isolated from freshwater fish. *Int J Syst Evol Microbiol* **60:** 2753-2757.

Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ, van der Drift K, Schripsema J, Kroon B *et al.* (1998). Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol Plant-Microbe Interact* **11:** 1069-1077.

D'Aes J, Hua GK, De Maeyer K, Pannecoucque J, Forrez I, Ongena M *et al.* (2011). Biological control of Rhizoctonia root rot on bean by phenazine- and cyclic lipopeptide-producing *Pseudomonas* CMR12a. *Phytopathology* **101:** 996-1004.

de Souza JT, de Boer M, de Waard P, van Beek TA, Raaijmakers JM. (2003). Biochemical, genetic, and zoosporicidal properties of cyclic lipopeptide surfactants produced by *Pseudomonas fluorescens*. *Appl Environ Microbiol* **69:** 7161-7172.

Devi KK, Kothamasi D. (2009). *Pseudomonas fluorescens* CHA0 can kill subterranean termite *Odontotermes obesus* by inhibiting cytochromecoxidase of the termite respiratory chain. *FEMS Microbiol Lett* **300:** 195-200.

Dilts JA, Quackenbush RL. (1986). A mutation in the R-body coding sequence destroys expression of the killer trait in *P. tetraurelia*. *Science* **232:** 641-643.

Farn JL, Strugnell RA, Hoyne PA, Michalski WP, Tennent JM. (2001). Molecular characterization of a secreted enzyme with phospholipase B activity from *Moraxella bovis*. *J Bacteriol* **183:** 6717-6720.

Franklin MJ, Nivens DE, Weadge JT, Howell PL. (2011). Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl. *Front Microbiol* **2:** 167.

Friedman L, Kolter R. (2004). Two genetic loci produce distinct carbohydrate-rich structural components of the *Pseudomonas aeruginosa* biofilm matrix. *J Bacteriol* **186:** 4457-4465.

Fuchs J, Defago G. (1991). Protection of cucumber plants against black root rot caused by *Phomopsis sclerotioides* with rhizobacteria. *Bulletin SROP* **14:** 57-62.

Fuchs JG, Moenne-Loccoz Y, Defago G. (2000). The laboratory medium used to grow biocontrol *Pseudomonas* sp. Pf153 influences its subsequent ability to protect cucumber from black root rot. *Soil Biol Biochem* **32:** 421-424.

Gomila M, Peña A, Mulet M, Lalucat J, García-Valdés E. (2015). Phylogenomics and systematics in *Pseudomonas*. *Front Microbiol* **6:** 214.

Grosso-Becerra MV, Santos-Medellin C, Gonzalez-Valdez A, Mendez JL, Delgado G, Morales-Espinosa R *et al.* (2014). *Pseudomonas aeruginosa* clinical and environmental isolates constitute a single population with high phenotypic diversity. *BMC Genomics* **15:** 318

Gupta GP, Rani S, Birah A, Raghuraman M. (2005). Improved artificial diet for mass rearing of the tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae). *Int J Trop Insect Sci* **25:** 55-58.

Haas D, Keel C. (2003). Regulation of antibiotic production in root-colonizing *Peudomonas* spp. and relevance for biological control of plant disease. *Annu Rev Phytopathol* **41:** 117-153.

Haas D, Défago G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* **3:** 307-319.

Herren J, Lemaitre B. (2012). Insect-microbe interactions: the good, the bad and the others. *Curr Opin Microbiol* **15:** 217-219.

Hilker R, Munder A, Klockgether J, Losada PM, Chouvarine P, Cramer N *et al.* (2015). Interclonal gradient of virulence in the *Pseudomonas aeruginosa* pangenome from disease and environment. *Environ Microbiol* **17:** 29-46.

Hinnebusch BJ, Rudolph AE, Cherepanov P, Dixon JE, Schwan TG, Forsberg A. (2002). Role of Yersinia murine toxin in survival of *Yersinia pestis* in the midgut of the flea vector. *Science* **296:** 733-735.

Ishii K, Adachi T, Hara T, Hamamoto H, Sekimizu K. (2014). Identification of a *Serratia marcescens* virulence factor that promotes hemolymph bleeding in the silkworm, *Bombyx mori*. *J Invertebr Pathol* **117:** 61-67.

Jackson KD, Starkey M, Kremer S, Parsek MR, Wozniak DJ. (2004). Identification of *psl*, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. *J Bacteriol* **186:** 4466-4475.

Jang JY, Yang SY, Kim YC, Lee CW, Park MS, Kim JC *et al.* (2013). Identification of orfamide A as an insecticidal metabolite produced by *Pseudomonas protegens* F6. *J Agric Food Chem* **61:** 6786-6791.

Jousset A, Schuldes J, Keel C, Maurhofer M, Daniel R, Scheu S *et al.* (2014). Full-genome sequence of the plant growth-promoting bacterium *Pseudomonas protegens* CHA0. *Genome Announc* **2:** e00322-00314.

Keel C, Weller DM, Natsch A, Defago G, Cook RJ, Thomashow LS. (1996). Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. *Appl Environ Microbiol* **62:** 552-563.

Kluyver AJ. (1956). *Pseudomonas aureofaciens* nov. spec. and its pigments. *J Bacteriol* **72:** 406-411.

Kruijt M, Tran H, Raaijmakers JM. (2009). Functional, genetic and chemical characterization of biosurfactants produced by plant growth-promoting *Pseudomonas putida* 267. *J Appl Microbiol* **107:** 546-556.

Kupferschmied P, Maurhofer M, Keel C. (2013). Promise for plant pest control: root-associated pseudomonads with insecticidal activities. *Front Plant Sci* **4:** 287.

Kupferschmied P, Pechy-Tarr M, Imperiali N, Maurhofer M, Keel C. (2014). Domain shuffling in a sensor protein contributed to the evolution of insect pathogenicity in plant-beneficial *Pseudomonas protegens*. *PLoS Path* **10:** e1003964.

Kwak Y-S, Han S, Thomashow LS, Rice JT, Paulitz TC, Kim D *et al.* (2011). *Saccharomyces cerevisiae* genome-wide mutant screen for sensitivity to 2,4-diacetylphloroglucinol, an antibiotic produced by *Pseudomonas fluorescens*. *Appl Environ Microbiol* **77:** 1770-1776.

Lemaitre B, Hoffmann J. (2007). The host defense of *Drosophila melanogaster*. *Annu Rev Immunol* **25:** 697-743.

Levy E, Gough FJ, Berlin KD, Guiana PW, Smith JT. (1992). Inhibition of *Septoria tritici* and other phytopathogenic fungi and bacteria by *Pseudomonas fluorescens* and its antibiotics. *Plant Pathol* **41:** 335-341.

Liehl P, Blight M, Vodovar N, Boccard F, Lemaitre B. (2006). Prevalence of local immune response against oral infection in a *Drosophila*/*Pseudomonas* infection model. *PLoS Path* **2:** e56.

Loper JE, Hassan KA, Mavrodi DV, Davis EW, 2nd, Lim CK, Shaffer BT *et al.* (2012). Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genet* **8:** e1002784.

Lugtenberg B, Kamilova F. (2009). Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* **63:** 541-556.

Martinez-Garcia E, de Lorenzo V. (2011). Engineering multiple genomic deletions in Gram-negative bacteria: analysis of the multi-resistant antibiotic profile of *Pseudomonas putida* KT2440. *Environ Microbiol* **13:** 2702-2716.

Maurhofer M, Keel C, Schnider U, Voisard C, Haas D, Defago G. (1992). Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHA0 on its disease suppressive capacity. *Phytopathology* **82:** 190-195.

Mazzola M, Zhao X, Cohen MF, Raaijmakers JM. (2007). Cyclic lipopeptide surfactant production by *Pseudomonas fluorescens* SS101 is not required for suppression of complex *Pythium* spp. populations. *Phytopathology* **97:** 1348-1355.

Mercado-Blanco J, Bakker PAHM. (2007). Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection. *Anton Leeuw Int J G* **92:** 367-389.

Meyer F, Goesmann A, McHardy AC, Bartels D, Bekel T, Clausen J *et al.* (2003). GenDB - an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res* **31:** 2187-2195.

Meyer JB, Frapolli M, Keel C, Maurhofer M. (2011). Pyrroloquinoline quinone biosynthesis gene *pqqC*, a novel molecular marker for studying the phylogeny and diversity of phosphate-solubilizing pseudomonads. *Appl Environ Microbiol* **77:** 7345-7354.

Mishra M, Byrd MS, Sergeant S, Azad AK, Parsek MR, McPhail L *et al.* (2012). *Pseudomonas aeruginosa* Psl polysaccharide reduces neutrophil phagocytosis and the oxidative response by limiting complement-mediated opsonization. *Cell Microbiol* **14:** 95-106.

Mulet M, Gomila M, Scotta C, Sanchez D, Lalucat J, Garcia-Valdes E. (2012). Concordance between whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry and multilocus sequence analysis approaches in species discrimination within the genus *Pseudomonas*. *Syst Appl Microbiol* **35:** 455-464.

N'goma JCB, Le Moigne V, Soismier N, Laencina L, Le Chevalier F, Roux AL *et al.* (2015). *Mycobacterium abscessus* phospholipase C expression is induced during coculture within Amoebae and enhances *M. abscessus* virulence in mice. *Infect Immun* **83:** 780-791.

Nadarasah G, Stavrinides J. (2011). Insects as alternative hosts for phytopathogenic bacteria. *FEMS Microbiol Rev* **35:** 555-575.

Neidig N, Paul RJ, Scheu S, Jousset A. (2011). Secondary metabolites of *Pseudomonas fluorescens* CHA0 drive complex non-trophic interactions with bacterivorous nematodes. *Microb Ecol* **61:** 853-859.

Nisr RB, Russell MA, Chrachri A, Moody AJ, Gilpin ML. (2011). Effects of the microbial secondary metabolites pyrrolnitrin, phenazine and patulin on INS-1 rat pancreatic beta-cells. *FEMS Immunol Med Microbiol* **63:** 217-227.

Olcott MH, Henkels MD, Rosen KL, Walker FL, Sneh B, Loper JE *et al.* (2010). Lethality and developmental delay in *Drosophila melanogaster* larvae after ingestion of selected *Pseudomonas fluorescens* strains. *PloS one* **5:** e12504.

Opota O, Vallet-Gely I, Vincentelli R, Kellenberger C, Iacovache I, Gonzalez MR *et al.* (2011). Monalysin, a novel ss-pore-forming toxin from the *Drosophila* pathogen *Pseudomonas entomophila*, contributes to host intestinal damage and lethality. *PLoS Path* **7:** e1002259.

Park HI, Ming LJ. (2002). Mechanistic studies of the astacin-like *Serratia* metalloendopeptidase serralysin: highly active (>2000%) Co(II) and Cu(II) derivatives for further corroboration of a "metallotriad" mechanism. *J Biol Inorg Chem* **7:** 600-610.

Paulsen IT, Press CM, Ravel J, Kobayashi DY, Myers GS, Mavrodi DV *et al.* (2005). Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat Biotechnol* **23:** 873-878.

Péchy-Tarr M, Bruck DJ, Maurhofer M, Fischer E, Vogne C, Henkels MD *et al.* (2008). Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated strains of *Pseudomonas fluorescens*. *Environ Microbiol* **10:** 2368-2386.

Péchy-Tarr M, Borel N, Kupferschmied P, Turner V, Binggeli O, Radovanovic D *et al.* (2013). Control and host-dependent activation of insect toxin expression in a root-associated biocontrol pseudomonad. *Environ Microbiol* **15:** 736-750.

Peix A, Valverde A, Rivas R, Igual JM, Ramirez-Bahena MH, Mateos PF *et al.* (2007). Reclassification of *Pseudomonas aurantiaca* as a synonym of *Pseudomonas chlororaphis* and proposal of three subspecies, *P. chlororaphis* subsp. *chlororaphis* subsp. nov., *P. chlororaphis* subsp. *aureofaciens* subsp. nov., comb. nov. and *P. chlororaphis* subsp. *aurantiaca* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **57:** 1286-1290.

Perneel M, Heyrman J, Adiobo A, De Maeyer K, Raaijmakers JM, De Vos P *et al.* (2007). Characterization of CMR5c and CMR12a, novel fluorescent *Pseudomonas* strains from the cocoyam rhizosphere with biocontrol activity. *J Appl Microbiol* **103:** 1007-1020.

Pond FR, Gibson I, Lalucat J, Quackenbush RL. (1989). R-body-producing bacteria. *Microbiol Rev* **53:** 25-67.

Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol Rev* **34:** 1037-1062.

Ramette A, Frapolli M, Fischer-Le Saux M, Gruffaz C, Meyer J-M, Défago G *et al.* (2011). *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Syst Appl Microbiol* **34:** 180-188.

Raymann K, Bobay L-M, Doak TG, Lynch M, Gribaldo S. (2013). A genomic survey of Reb homologs suggests widespread occurrence of R-bodies in proteobacteria. *G3-Genes Genomes Genetics* **3:** 505-516.

Rhodes ME. (1959). The characterization of *Pseudomonas fluorescens*. *J Gen Microbiol* **21:** 221-263.

Ruffner B, Péchy-Tarr M, Ryffel F, Hoegger P, Obrist C, Rindlisbacher A *et al.* (2013). Oral insecticidal activity of plant-associated pseudomonads. *Environ Microbiol* **15:** 751-763.

Ruffner B, Péchy-Tarr M, Höfte M, Bloemberg G, Grunder J, Keel C *et al.* (2015). Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens *Photorhabdus* and *Xenorhabdus*. *BMC Genomics* **16:** 609.

Rumpold BA, Schluter OK. (2013). Nutritional composition and safety aspects of edible insects. *Mol Nutr Food Res* **57:** 802-823.

Sampson MN, Gooday GW. (1998). Involvement of chitinases of *Bacillus thuringiensis* during pathogenesis in insects. *Microbiol-UK* **144:** 2189-2194.

Scarlett CM, Fletcher JT, Roberts P, Lelliott RA. (1978). Tomato pith necrosis caused by *Pseudomonas corrugata* n. sp. *Ann Appl Biol* **88:** 105-114.

Sikorski J, Stackebrandt E, Wackernagel W. (2001). *Pseudomonas kilonensis* sp. nov., a bacterium isolated from agricultural soil. *Int J Syst Evol Microbiol* **51:** 1549-1555.

Smits THM, Rezzonico F, Kamber T, Blom J, Goesmann A, Frey JE *et al.* (2010). Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Mol Plant-Microbe Interact* **23:** 384-393.

Snyder WE, Tonkyn DW, Kluepfel DA. (1998). Insect-mediated dispersal of the rhizobacterium *Pseudomonas chlororaphis*. *Phytopathology* **88:** 1248-1254.

Songer JG. (1997). Bacterial phospholipases and their role in virulence. *Trends Microbiol* **5:** 156-161.

Stavrinides J, McCloskey JK, Ochman H. (2009). Pea aphid as both host and vector for the phytopathogenic bacterium *Pseudomonas syringae*. *Appl Environ Microbiol* **75:** 2230-2235.

Stockwell VO, Stack JP. (2007). Using *Pseudomonas* spp. for integrated biological control. *Phytopathology* **97:** 244-249.

Stutz EW, Defago G, Kern H. (1986). Naturally-occuring fluorescent pseudomonads involved in suppression of black root-rot of tobacco. *Phytopathology* **76:** 181-185.

Tran H, Ficke A, Asiimwe T, Hofte M, Raaijmakers JM. (2007). Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. *New Phytol* **175:** 731-742.

754 Vallet-Gely I, Lemaitre B, Boccard F. (2008). Bacterial strategies to overcome insect defences. *Nat*
755 *Rev Microbiol* **6:** 302-313.
756
757 Venables WN, Ripley BD (2002). *Modern Applied Statistics with S*, Fourth edn. Springer: New York.
758
759 Wang CX, Ramette A, Punjasamarnwong P, Zala M, Natsch A, Moenne-Loccoz Y *et al.* (2001).
760 Cosmopolitan distribution of *phlD*-containing dicotyledonous crop-associated biocontrol
761 pseudomonads of worldwide origin. *FEMS Microbiol Ecol* **37:** 105-116.
762
763 Yang GW, Hernandez-Rodriguez CS, Beeton ML, Wilkinson P, Ffrench-Constant RH, Waterfield NR.
764 (2012). Pdl1 is a putative lipase that enhances *Photorhabdus* toxin complex secretion. *PLoS Path* **8:**
765 e1002692.
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767
768

Figure Legends

Figure 1. Phylogeny of the *P. fluorescens* group based on the core genome.

Genomes sequenced in this study and high quality genomes that are publicly available by February 2015 were used to generate a core genome tree in EDGAR. Strains investigated in this study are depicted in bold. Sub-clades were defined after Loper et al (2012). Sub-clade 1 corresponds to the *P. chlororaphis* subgroup, sub-clade 3 to the *P. fluorescens* subgroup, and sub-clade 2 comprises strains belonging to three different subgroups within the *P. fluorescens* group according to (Mulet et al 2012), see also Supplementary Figure 1.

Figure 2. Overview on insecticidal activity, pathogen suppression and presence of associated gene clusters in 26 strains of the *P. fluorescens* group.

Colored boxes represent activity against insects and plant pathogens as assessed within this study:

high activity, medium activity, no activity

Insecticidal activity was assessed in injection assays against *Galleria mellonella* larvae and feeding assays against *Plutella xylostella* and *Spodoptera littoralis* larvae, and depicted activities are based on the results presented in Figure 3, Table 2, Supplementary Figure S2 and Supplementary Table S4. Disease suppression was assessed in a cucumber-*Pythium ultimum* assay and activities are based on the data depicted in Supplementary Table S5. Strains indicated by an asterisk were reported to have biocontrol activity against plant diseases in earlier studies (see Table 1). *In vitro* inhibition of mycelial growth was assessed on two media against *P. ultimum* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* and activities are based on the results shown in Supplementary Figure S3. Grey boxes represent presence of selected genes/gene clusters that were found to be associated with insecticidal strains (this study) or that are required for the production of the indicated antifungal metabolites.

present, partially present, absent

794 Exact loci, which were checked for presence/absence, are indicated in Supplementary Table S1.
795 There, additional genes as well as all additional strains are presented.
796 [a] Selected genes that were identified by comparative genomics to be specific for strains that show
797 insecticidal activity. A complete list is presented in Supplementary Table S6. *P. fluorescens*
798 insecticidal toxin-cluster (*fit*), chitinase C (*chiC*), phospholipase C (*plcN*), metallopeptidase AprX
799 (*aprX*), *rebB*-cluster (*rebB*), *psl*-cluster (*psl*)
800 [b] Genes that were shown to contribute to insecticidal activity in this study (*chiC* and *plcN*) or
801 elsewhere (*fit*) (Péchy-Tarr et al 2008, Ruffner et al 2013).
802 [c] Presence/absence of gene clusters required for the production of the indicated antifungal
803 metabolites. 2,4-diacetylphloroglucinol (DAPG), phenazine (Phz), hydrogen cyanide (HCN),
804 pyrrolnitrin (Prn), pyoluteorin (Plt), 2-hexyl-5-propyl-alkylresorcinol (HPR).
805
806 **Figure 3. Oral and systemic insecticidal activity is restricted to strains of specific phylogenetic**
807 **subgroups within the *Pseudomonas fluorescens* group.**
808 A) Systemic activity against *Galleria mellonella*. Larvae were injected with 4 x 10^4 bacterial cells. Bars
809 show average mortality of three replicates with 10 larvae after 48 h. The experiment was repeated
810 twice and highly similar results were obtained. B) Oral activity against *Plutella xylostella*. Larvae were
811 exposed to artificial diet covered with 8 x 10^7 bacterial cells. Bars show average mortality of four
812 replicates with eight larvae after three days. The experiment was repeated and similar results were
813 obtained (Supplementary Table S4). Error bars show standard error of the mean. Asterisks indicate
814 strains that were significantly different from control larvae treated with 0.9% NaCl based on multiple
815 comparisons by Kruskal-Wallis adjusted by Bonferroni-Holm ($P \leq 0.05$).
816 C) Typical melanization symptoms observed after 32 h in infections with *P. protegens* CHA0^T, *P.*
817 *chlororaphis* subsp. *piscium* PCL1391, *Pseudomonas* sp. CMR5c, but not with *P. fluorescens*
818 DSM 50090^T. D) Although larvae injected with *Pseudomonas* sp. MIACH do not die, they become
819 slightly melanized compared to control larvae.

820 *P. chl.* stands for *Pseudomonas chlororaphis.*

821

822 **Figure 4. A derivative of *P. protegens* CHA0 deficient for a specific chitinase is reduced in oral, but**
823 **not in injectable activity against insect larvae.**

824 A) Systemic activity against *Galleria mellonella*. 30 larvae per treatment were injected with 2 x 10^3
825 bacterial cells and survival was recorded hourly. B) Oral activity against *Plutella xylostella*. Larvae
826 were exposed to artificial diet inoculated with 4 x 10^6 bacterial cells.
827 Significant differences according to a Log-Rank test (Survival Package in R) between treatments with
828 the wild type CHA0 and the chitinase C- negative mutant (Δ*chiC*) are indicated with ***(*P* <0.0001).
829 Each mutant was tested at least three times with similar results. A repetition of the feeding assay is
830 depicted in Supplementary Figure S5.
831 C) Chitinase activity of wild type CHA0 and its *chiC* mutant was assessed using a chitinase assay kit
832 (Sigma, St. Louis, MO, USA). Three different substrates were used to test for exo- (β-N-
833 acetylglucosaminidase and chitobiosidase) and endochitinase activity. Treatments indicated by an
834 asterisk are significantly different based on a t-test ($p \leq 0.05$).
835 CHA0, wild type; Δ*chiC*, chitinase C-negative mutant; Δ*gacA*, GacA-negative mutant; 0.9% NaCl
836 served as negative control in the virulence assay; a positive control for chitinase activity was
837 provided by the chitinase assay kit.

838

839

Table 1. Strain information

Strain	Former Name	Geographic Origin	Habitat/ Host[a]	Biocontrol ability	Genome sequenced	References
***P. protegens* CHA0^T**	*P. fluorescens* CHA0^T	Switzerland	Tobacco	Cucumber-Pu, Tobacco-Tb, Wheat-Ggt, Tomato-Forl	(Jousset et al 2014)	(Haas and Défago 2005, Keel et al 1996, Ramette et al 2011, Stutz et al 1986)
***P. protegens* PGNR1**	*P. fluorescens* PGNR1	Ghana	Tobacco	Cucumber-Pu Tomato-Forl	this study	(Keel et al 1996)
***P. protegens* BRIP**		Switzerland	Cyclops	ND	this study	(Ruffner et al 2015)
***P. protegens* K94.41**	*P. fluorescens* K94.41	Slovakia	Cucumber	Cucumber-Pu Tomato-Forl	this study	(Wang et al 2001)
***P. protegens* PF**	*P. fluorescens* PF	Oklahoma, USA	Wheat leaves	Wheat-St	this study	(Keel et al 1996, Levy et al 1992)
***Pseudomonas* sp. CMR5c**		Cameroon	Red cocoyam	Cocoyam-Pm	this study	(Perneel et al 2007)
***Pseudomonas* sp. CMR12a**		Cameroon	Red cocoyam	Cocoyam-Pm Bean-Rs	this study	(D'Aes et al 2011, Perneel et al 2007)
***P. chlororaphis* subsp. *piscium* DSM 21509^T**		Lake of Neuchâtel, Switzerland	Intestine of European perch	ND	this study	(Burr et al 2010)
***P. chlororaphis* subsp. *piscium* PCL1391**		Spain	Tomato	Tomato-Forl	this study	(Chin-A-Woeng et al 1998)
***P. chlororaphis* subsp. *aureofaciens* LMG 1245^T**		Netherlands	River Clay	ND	this study	(Kluyver 1956, Peix et al 2007)
***P. chlororaphis* subsp. *aureofaciens* CD**		Switzerland	Cyclops (water)	ND	this study	(Ruffner et al 2015)
***P. chlororaphis* subsp. *chlororaphis* LMG 5004^T**		-	Contaminated plate	ND	this study	(Peix et al 2007)
***P. brassicacearum* TM1A3**	*P. fluorescens* TM1A3	Switzerland	Tomato	Cucumber-Pu, Cotton-Rs	this study	(Fuchs and Defago 1991, Keel et al 1996)

***P. thivervalensis* DSM 13194^T**		France	Rapeseed	ND	this study	(Achouak et al 2000)
***P. thivervalensis* PITR2**	*P. fluorescens* PITR2	Albenga,Italy	Wheat	Cucumber-Pu, Tomato-Forl	this study	(Keel et al 1996)
***P. kilonensis* P12**	*P. fluorescens* P12	Switzerland	Tobacco	Tobacco-Tb	this study	(Keel et al 1996)
***P. kilonensis* DSM 13647^T**		Germany	Agricultural soil	ND	this study	(Sikorski et al 2001)
***Pseudomonas* sp. Q12-87**	*P. fluorescens* Q12-87	Washington, USA	Wheat	Wheat-Ggt	this study	(Keel et al 1996)
***Pseudomonas* sp. P97.38**	*P. fluorescens* P97.38	Switzerland	Cucumber	Cucumber-Pu Tomato-Forl	this study	(Wang et al 2001)
***P. corrugata* LMG 2172^T**		United Kingdom	Tomato stem	ND	this study	(Scarlett et al 1978)
***Pseudomonas* sp. Pf153**	*P. fluorescens* Pf153	Switzerland	Tobacco	Cucumber-Pu	this study	(Fuchs et al 2000)
***Pseudomonas* sp. P1.8**		Switzerland	Earthworm	ND	this study	(Ruffner et al 2015)
***Pseudomonas* sp. P1.31**		Switzerland	Woodlouse (dead)	ND	this study	(Ruffner et al 2015)
***P. fluorescens* DSM 50090^T**		United Kingdom	pre-filter tanks	ND	this study	(Rhodes 1959)
***Pseudomonas* sp. MIACH**	*P. fluorescens* MIACH	Switzerland	Wheat	ND	this study	(Meyer et al 2011)
***Pseudomonas* sp. SS101**	*P. fluorescens* SS101	The Netherlands	Wheat	Cucumber-Pc Tomato-Pi	(Loper et al 2012)	(de Souza et al 2003, Kruijt et al 2009, Mazzola et al 2007, Tran et al 2007)

Ggt, *Gaeumannomyces graminis* var. *tritici*; Forl, *Fusarium oxysporum* f. sp. *radicis-lycopersici*; Ps, *Phomopsis sclerotioides*; Pc, *Phytophthora capsici*; Pi, *Phytophthora infestans*; Pu, *Pythium ultimum*; Pm, *Pythium myriotylum;* Rs, *Rhizoctonia solani*; Tb, *Thielaviopsis basicola*; St, *Septoria tritici*.
ND, not documented
[a] Plant hosts: If not otherwise stated strains were isolated from roots or rhizosphere.

Table 2. Lethal time 50 (LT_{50}) values for *Galleria mellonella* and *Plutella xylostella* larvae upon injection or oral uptake, respectively, of *Pseudomonas* strains

Sub-clade	Strain	Injection, LT_{50} (h)	Oral, LT_{50} (d)
Sub-clade 1	*P. protegens* CHA0^T	26.3 (25.9; 26.6)^b	1.65 (1.44; 1.86)^a
	P. protegens CHA0^T	26.9 (26.5; 27.4)$^{\dagger\,\gamma}$	
	P. protegens PGNR1	29.3 (28.9; 29.8)^{de}	1.74 (1.56; 1.92)^{ab}
	P. protegens BRIP	29.0 (28.6; 29.4)^d	1.58 (1.40; 1.76)^a
	P. protegens K94.41	26.3 (25.9; 26.7)^b	1.58 (1.40; 1.76)^a
	P. protegens PF	34.4 (33.3; 35.6)^g	1.63 (1.43; 1.83)^a
	Pseudomonas sp. CMR5c	24.5 (24.2; 24.9)$^{\dagger\,\beta}$	2.24 (1.98; 2.49)^c
	Pseudomonas sp. CMR12a	22.0 (21.5; 22.6)$^{\dagger\,\alpha}$	2.63 (2.31; 2.95)^c
	P. chl. piscium DSM 21509^T	27.2 (26.7; 27.6)^c	1.66 (1.46; 1.86)^a
	P. chl. piscium PCL1391	24.9 (24.5; 25.3)^a	2.19 (1.87; 2.51)^{bc}
	P. chl. aureofaciens LMG 1245^T	30.1 (29.6; 30.6)^e	2.56 (2.24; 2.89)^c
	P. chl. aureofaciens CD	33.7 (32.8; 34.7)^g	2.08 (1.76; 2.39)^{abc}
	P. chl. chlororaphis LMG 5004^T	26.7 (26.2; 27.1)^{bc}	2.33 (1.97; 2.68)^c
Sub-clade 2	*P. brassicacearum* TM1A3	NA	NA
	P. kilonensis DSM 13647^T	NA	NA
	P. kilonensis P12	NA	NA
	P. thivervalensis DSM 13194^T	NA	NA
	P. thivervalensis PITR2	NA	NA
	Pseudomonas sp. Q12-87	NA	NA
	Pseudomonas sp. P97.38	NA	NA
	P. corrugata DSM 7228^T	NA	NA
	Pseudomonas sp. Pf153	NA	NA
	Pseudomonas sp. P1.8	NA	NA
	Pseudomonas sp. P1.31	NA	NA
Sub-clade 3	*P. fluorescens* DSM 50090^T	32.0 (31.4; 32.7)^f	NA
	Pseudomonas sp. MIACH	NA	NA
	Pseudomonas sp. SS101	37.9 (36.9; 38.8)$^{\dagger\,\delta}$	NA
control	0.9 % NaCl	NA	NA

Galleria mellonella larvae were injected with 4 x 10^4 washed bacterial cells of the indicated strains. *Plutella xylostella* larvae were exposed to food pellets inoculated with 8 x 10^7 bacterial cells. LT_{50} values are estimates based on the generalized linear model using the MASS package in R (Venables and Ripley 2002). LT_{50} estimates were calculated from three replicates with ten larvae per replicate for *G. mellonella* and from four replicates with eight larvae per replicate for *P. xylostella*. Numbers in brackets depict 95% confidence intervals for LT_{50} and significantly different values within the same column are followed by different letters.

† These strains were tested in a separate experiment.

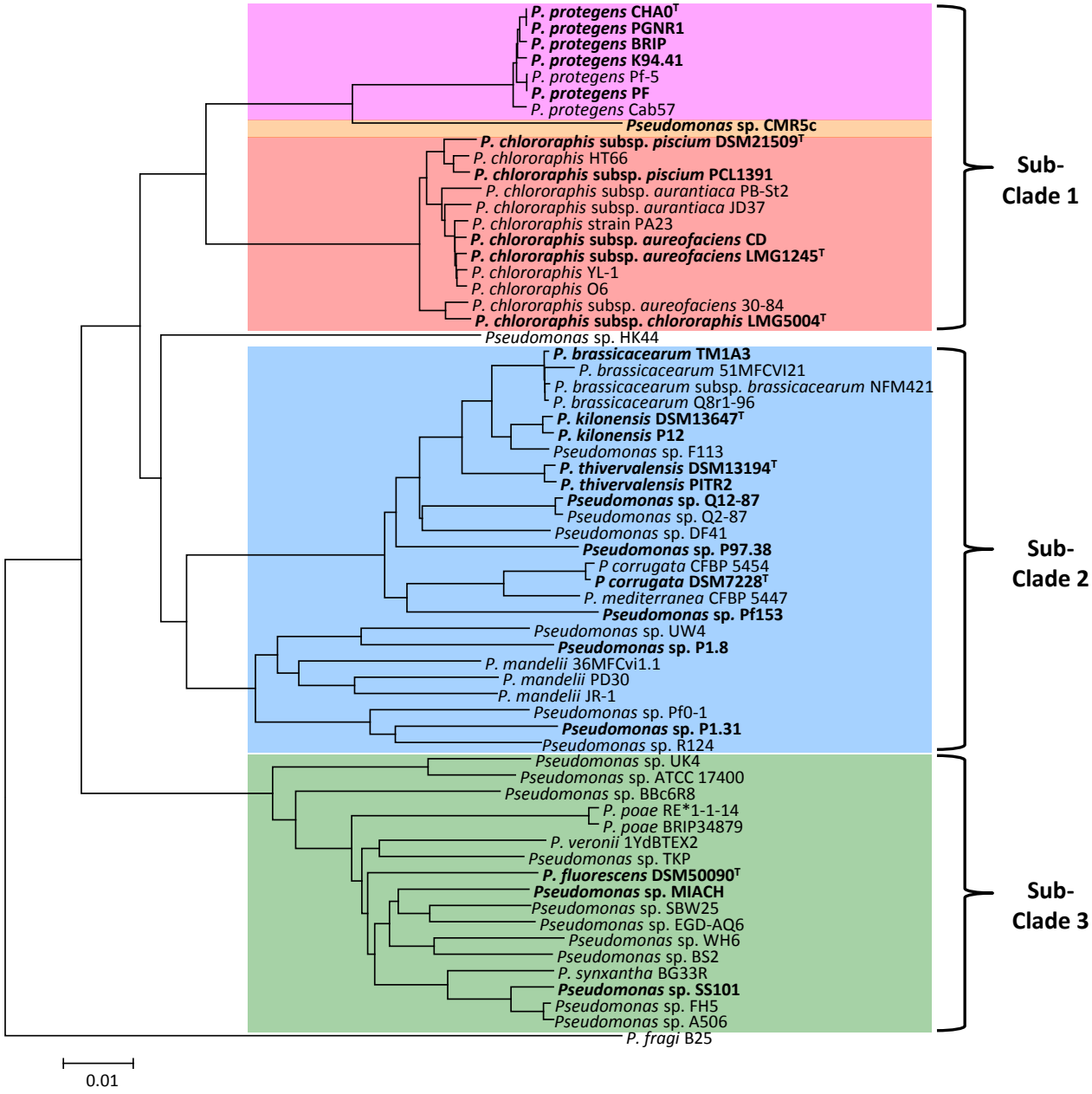
NA = no LT_{50} value was calculated, because end mortality was less than 50%.

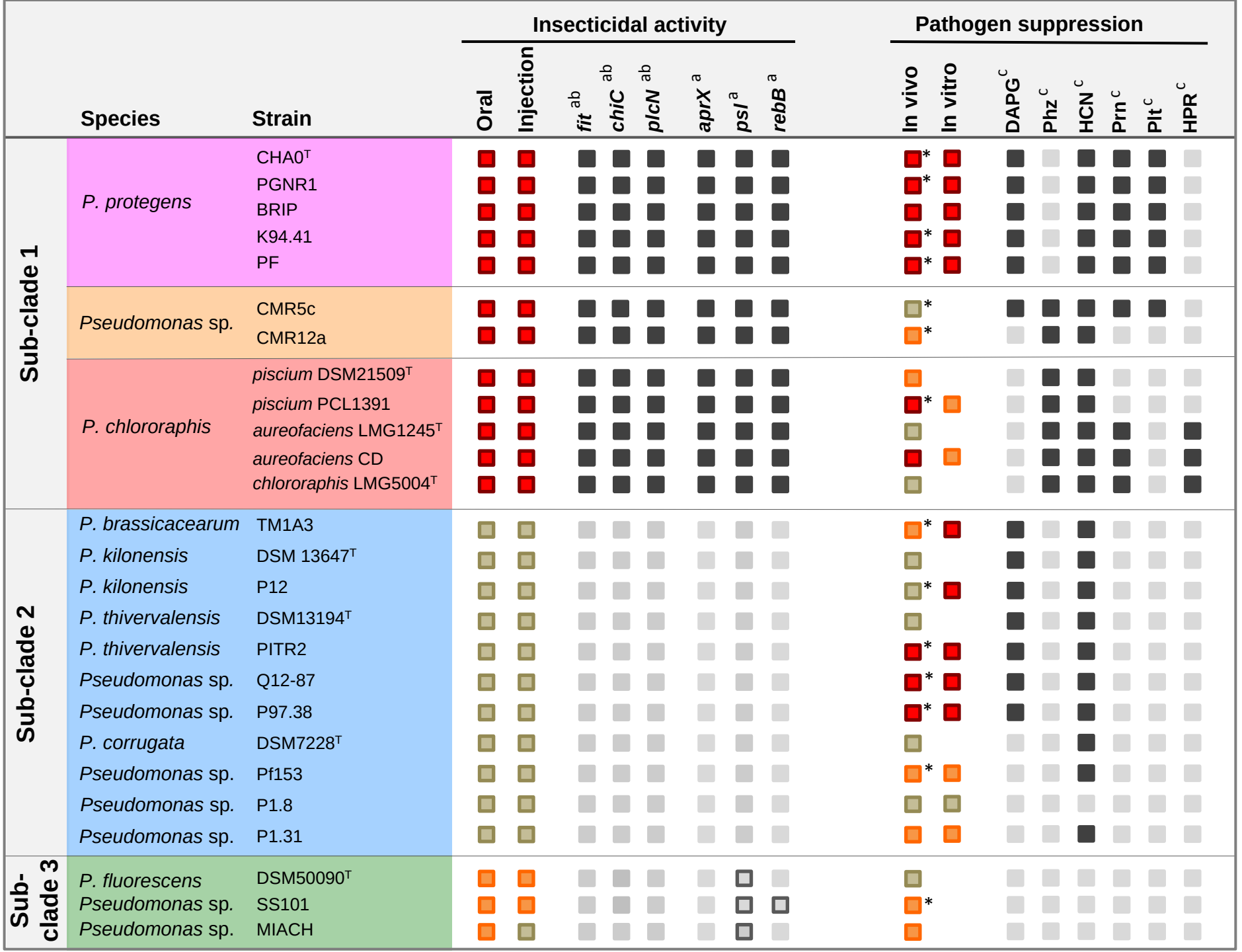
P. chl. = Pseudomonas chlororaphis

Table 3. Genes associated with insecticidal activity.

Sub-clade 1	Sub-clade 2	Sub-clade 3			Number of Genes
		SS101	DSM 50090^T	MIACH	
+	-	-	-	-	**90**
+	-	+	+	+	**57**
+	-	+	+	-	**20**
+	-	+	-	+	**7**
+	-	+	-	-	**20**
+	-	-	+	+	**13**
+	-	-	+	-	**0**
+	-	-	-	+	**0**

Numbers of genes that are specific to insecticidal strains.
Presence of genes was defined as 70% amino acid pairwise identity over at least 70% of gene length for the pairwise comparisons.
Only genes that were common to all strains of sub-clade 1 (highly-insecticidal strains), but not found in any strain of sub-clade 2 (non-insecticidal strains) were considered.
+ indicates genes present in all these strains
- indicates genes absent in all these strains


P. protegens CHA0T
P. protegens PGNR1
P. protegens BRIP
P. protegens K94.41
P. protegens Pf-5
P. protegens PF
P. protegens Cab57
Pseudomonas sp. CMR5c
P. chlororaphis subsp. piscium DSM21509T
P. chlororaphis HT66
P. chlororaphis subsp. piscium PCL1391
P. chlororaphis subsp. aurantiaca PB-St2
P. chlororaphis subsp. aurantiaca JD37
P. chlororaphis strain PA23
P. chlororaphis subsp. aureofaciens CD
P. chlororaphis subsp. aureofaciens LMG1245T
P. chlororaphis YL-1
P. chlororaphis O6
P. chlororaphis subsp. aureofaciens 30-84
P. chlororaphis subsp. chlororaphis LMG5004T
Pseudomonas sp. HK44
P. brassicacearum TM1A3
P. brassicacearum 51MFCVI21
P. brassicacearum subsp. brassicacearum NFM421
P. brassicacearum Q8r1-96
P. kilonensis DSM13647T
P. kilonensis P12
Pseudomonas sp. F113
P. thivervalensis DSM13194T
P. thivervalensis PITR2
Pseudomonas sp. Q12-87
Pseudomonas sp. Q2-87
Pseudomonas sp. DF41
Pseudomonas sp. P97.38
P corrugata CFBP 5454
P corrugata DSM7228T
P. mediterranea CFBP 5447
Pseudomonas sp. Pf153
Pseudomonas sp. UW4
Pseudomonas sp. P1.8
P. mandelii 36MFCvi1.1
P. mandelii PD30
P. mandelii JR-1
Pseudomonas sp. Pf0-1
Pseudomonas sp. P1.31
Pseudomonas sp. R124
Pseudomonas sp. UK4
Pseudomonas sp. ATCC 17400
Pseudomonas sp. BBc6R8
P. poae RE*1-1-14
P. poae BRIP34879
P. veronii 1YdBTEX2
Pseudomonas sp. TKP
P. fluorescens DSM50090T
Pseudomonas sp. MIACH
Pseudomonas sp. SBW25
Pseudomonas sp. EGD-AQ6
Pseudomonas sp. WH6
Pseudomonas sp. BS2
P. synxantha BG33R
Pseudomonas sp. SS101
Pseudomonas sp. FH5
Pseudomonas sp. A506
P. fragi B25
0.01
Sub-Clade 1
Sub-Clade 2
Sub-Clade 3


Insecticidal activity
Pathogen suppression
Species
Strain
Oral
Injection
fit ab
chiC ab
plcN ab
aprX a
psl a
rebB a
In vivo
In vitro
DAPG c
Phz c
HCN c
Prn c
Plt c
HPR c
Sub-clade 1
P. protegens
CHA0T
PGNR1
BRIP
K94.41
PF
Pseudomonas sp.
CMR5c
CMR12a
P. chlororaphis
piscium DSM21509T
piscium PCL1391
aureofaciens LMG1245T
aureofaciens CD
chlororaphis LMG5004T
Sub-clade 2
P. brassicacearum
TM1A3
P. kilonensis
DSM 13647T
P. kilonensis
P12
P. thivervalensis
DSM13194T
P. thivervalensis
PITR2
Pseudomonas sp.
Q12-87
Pseudomonas sp.
P97.38
P. corrugata
DSM7228T
Pseudomonas sp.
Pf153
Pseudomonas sp.
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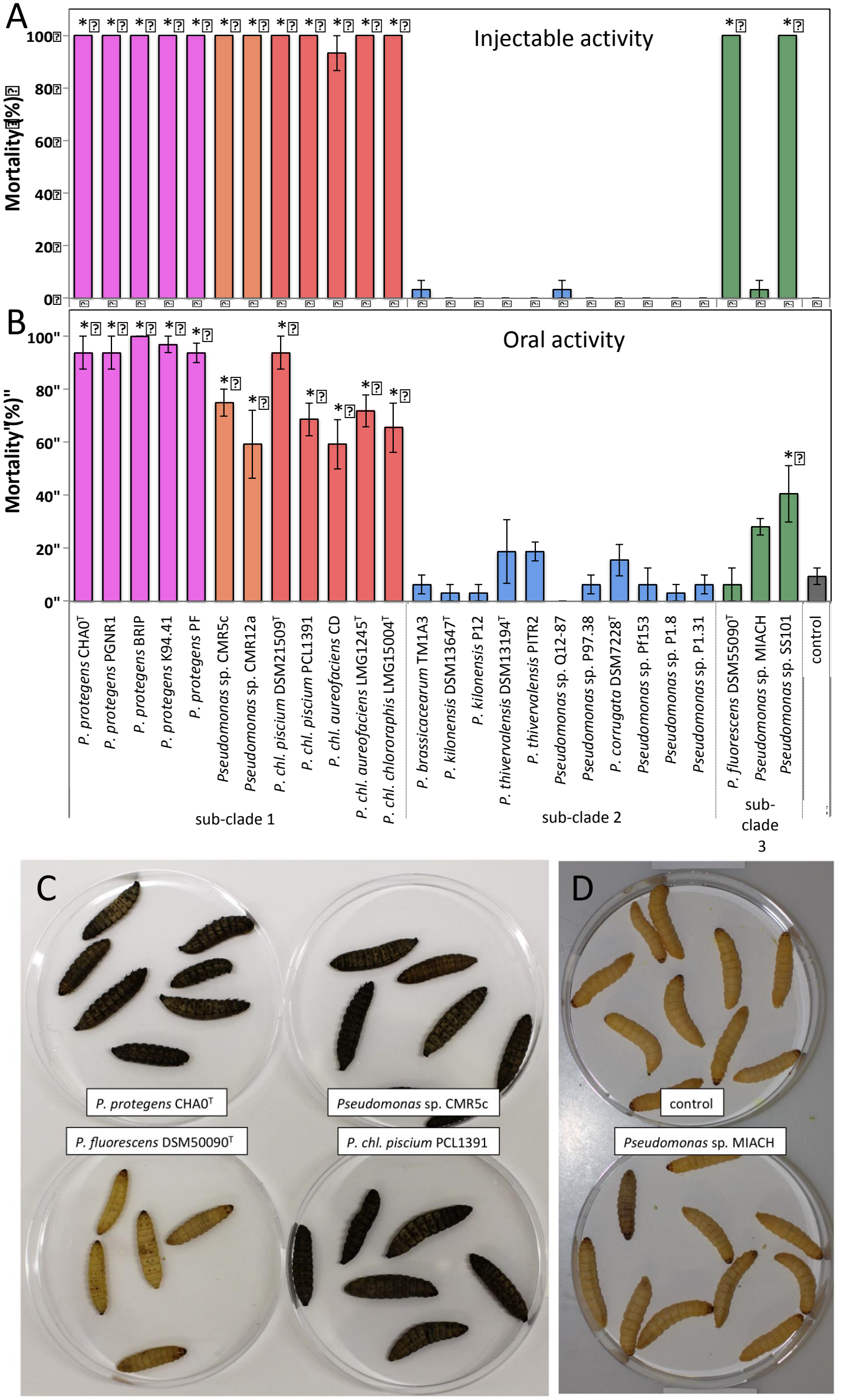
Pseudomonas sp.
SS101
Pseudomonas sp.
MIACH


A
Injectable activity
Mortality (%)
100
80
60
40
20
0
B
Oral activity
Mortality (%)
100
80
60
40
20
0
P. protegens CHA0T
P. protegens PGNR1
P. protegens BRIP
P. protegens K94.41
P. protegens PF
Pseudomonas sp. CMR5c
Pseudomonas sp. CMR12a
P. chl. piscium DSM21509T
P. chl. piscium PCL1391
P. chl. aureofaciens CD
P. chl. aureofaciens LMG1245T
P. chl. chlororaphis LMG15004T
P. brassicacearum TM1A3
P. kilonensis DSM13647T
P. kilonensis P12
P. thivervalensis DSM13194T
P. thivervalensis PITR2
Pseudomonas sp. Q12-87
Pseudomonas sp. P97.38
P. corrugata DSM7228T
Pseudomonas sp. Pf153
Pseudomonas sp. P1.8
Pseudomonas sp. P1.31
P. fluorescens DSM55090T
Pseudomonas sp. MIACH
Pseudomonas sp. SS101
control
sub-clade 1
sub-clade 2
sub-clade 3
C
P. protegens CHA0T
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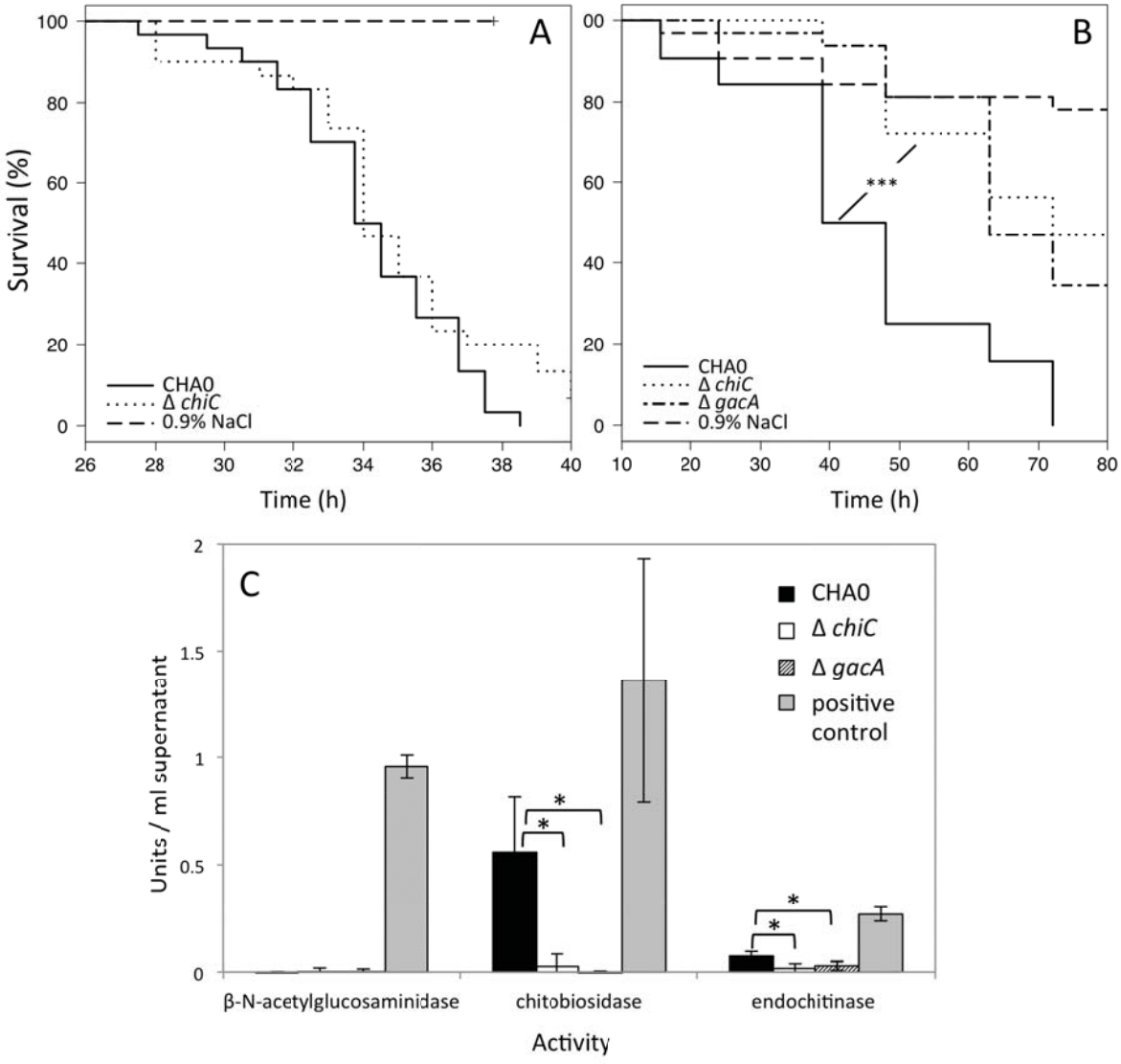
P. fluorescens DSM50090T
P. chl. piscium PCL1391
D
control
Pseudomonas sp. MIACH


A
B
C
Survival (%)
Time (h)
CHA0
Δ chiC
Δ gacA
0.9% NaCl
***
Units / ml supernatant
positive control
β-N-acetylglucosaminidase
chitobiosidase
endochitinase
Activity

Supplementary Information

CONTENT

References

Supplementary Materials and Methods

Sequencing of strain *Pseudomonas* sp. CMR5c

Paired-end sequence reads of genomic DNA of *Pseudomonas* CMR5c were generated using the Illumina HiSeq2500 system. The *de novo* assembly analysis was performed using the "*de novo* assembly" option of the CLC Genomics Workbench version 7.0.4. The scaffolding analysis was performed using the SSPACE Premium scaffolder version 2.3 (Boetzer et al 2011). Automated gap closure analysis was done using GapFiller version 1.10 (Boetzer and Pirovano 2012). No further manual assembly was performed.

Bioinformatics

Housekeeping genes of sequenced strains were collected from the annotated genomes, cropped to the size of the fragments used for phylogeny and concatenated according to Mulet et al (2012). Alignments were done using Muscle in MEGA v6.0, and a phylogenetic analysis was done with the maximum likelihood method.

Pairwise average amino acid identities (AAI) were calculated in EDGAR (Blom et al 2009). GGDC values are calculated using the Genome-to-Genome Distance Calculator Version 2 and reported according to formula 2, best suited when including draft genomes (Auch et al 2010, Meier-Kolthoff et al 2013).

Injection assay with *Galleria mellonella*

Washed bacterial cells (10 µl) suspended in 0.9% sterile NaCl solution and adjusted to the desired concentration were injected into the hemolymph of ultimate-instar *Galleria mellonella* larvae (Hebeisen Fishing, Zürich, Switzerland) using a 1-ml syringe with a 27-gauge needle in a repetitive dispensing Tridak Stepper (Intertronic, Oxfordshire, UK). Sterile 0.9% NaCl solution served as control. Three times ten larvae were injected per treatment and kept in Petri dishes at 24°C in the dark. Larvae were scored as live or dead regularly over two days. Mortality was defined as the inability of larvae to react to poking.

Feeding assay with *Spodoptera littoralis*

Food pellet assays with *Spodoptera littoralis* were performed as described by Ruffner et al. (2013). Briefly, third instar larvae of *S. littoralis* (Syngenta Crop Protection AG, Stein) were exposed to modified insect diet (Gupta et al 2005, Ruffner et al 2013) inoculated with 4×10^7 colony forming units per food pellet. For control treatments, pellets were treated with 10 µl sterile 0.9% NaCl solution. Instead of using petri dishes (Ruffner et al 2013), pellets were

placed into Greiner six-well plates and presented to one larva per well. Five plates were prepared per bacterial strain (30 larvae per treatment). Larvae were incubated in the dark at room temperature and were fed with fresh, bacteria free diet when necessary. Survival rates were recorded daily. Larvae were considered to be dead when they did not react to repeated poking.

Bacterial colonization of *Spodoptera littoralis* larvae

Bacteria were extracted from surviving larvae at the end of the experiment. Larvae were surface-disinfested for 30 s in 70% ethanol, rinsed with sterile water and homogenized in 10 ml sterile 0.9% saline solution with a Polytron PT-DA 2112 blender (Kinematica, Littau, Switzerland). Serial dilutions of the resulting homogenate were then plated onto King's B agar (King et al 1954) supplemented with ampicillin (40 µg ml^{-1}), chloramphenicol (13 µg ml^{-1}), and cycloheximide (100 µg ml^{-1}) to select for the bacterial strains fed to the larvae. Plates for bacterial quantification were incubated for two days at 27°C before colony counting.

Identification of biocontrol activity

Biocontrol of *Pythium* damping-off of cucumber was assessed for each strain adapted after Sharifi-Tehrani et al (1998). Aliquots of 200 µl of over-night cultures of bacteria grown in LB were plated on King's B agar (King et al 1954) and incubated for 24 h at 24°C. Bacteria were then scraped off the plate and washed in sterile distilled H_2O. Each bacterial strain was added to five pots filled with 120 g of TREF go PP7000 plant substrate (Gvz Rossat AG, Otelfingen, Switzerland) to a final concentration of 5×10^7 cfu per g soil. Each pot was inoculated with 0.3 g of *Pythium* inoculum grown on millet seeds and planted with three pre-germinated cucumber seeds. Pots were incubated at 70% humidity for 16 h with light (15 klux) at 22°C, followed by an 8-h dark period at 20°C. After 12 d, shoot weight per pot was recorded. Biocontrol activity was calculated after Rezzonico et al (2007) as:

$(1 - ((W_C - W_I)/(W_C - W_P))) \times 100$

using shoot weight obtained in the control with neither bacterial nor pathogen inoculum (W_C), in the unprotected control with the pathogen alone (W_P) and in presence of the tested bacterial strain and the pathogen (W_I). A total of seven experiments was conducted and each strain was tested at least twice. The model strain *P. protegens* CHA0 was included as a reference in each experiment.

In vitro inhibition of plant pathogens

F. oxysporum Schlecht. f. sp. *radicis-lycopersici* strain Forl22 (Forl) and *P. ultimum* Trow strain 67-1 (Pu) were cultivated on malt agar as described by Sharifi-Tehrani et al (1998). In vitro inhibition of Pu and Forl was assessed on malt agar (MA) and GCY (Tambong and Höfte 2001) plates. Mycelial plugs were placed at the center of the plates either one day before (for Pu) or one day after (for Forl) adding the bacteria. Bacterial strains were grown overnight in LB. Cells were washed with sterile distilled H_2O and a suspension of an OD_{600} of 1.0 was prepared. The suspension was streaked out in a square around the mycelial plug using an inoculation loop. Plates were inoculated at 24°C. The mycelial diameter was measured after 2 d for Pu and after 8 d for Forl. Each strain was tested twice on each medium with four replicates.

Statistics

Data analysis was performed in R version 3.1.1. (http://www.r-project.org). Mortality rates of the insect toxicity tests with wild-type strains and data on in-vitro inhibition of plant pathogens were analysed by multiple comparisons using Kruskal-Wallis adjusted by Bonferroni-Holm. LT_{50} values were estimated based on the generalized linear model using the MASS package in R (Venables and Ripley 2002). To test for significant differences in insect toxicity tests between *P. protegens* CHA0 and its mutant strains the Log-Rank test of the Survival package of R was used. To identify strains with significant biocontrol activity, a t-test was performed testing each strain against the respective unprotected control with pathogen alone.

Supplementary Results

Phylogeny of sequenced isolates

Many strains of the *Pseudomonas fluorescens* group were classified years ago, and their taxonomic status was not updated since then. We performed a comparative systematic study to correctly assign the isolates that were sequenced in this study to the phylogeny within the genus *Pseudomonas* (Mulet et al 2012). A recent study (Gomila et al 2015) has performed similar work with other published genomes, some of which were also included in our study.

The core genome tree (Figure 1), practically a core genome Multilocus Sequence Analysis (MLSA) (Blom et al 2009), confirmed the phylogenetic position of a range of isolates that we included in sequencing. For publicly available genomes, the position is corresponding to the study of Gomila et al (2015), whereas we confirmed the position of some isolates that already had a unconfirmed status in phylogeny. The phylogenetic position of the isolates could now be confirmed using digital DNA-DNA hybridization (Supplementary Table S3)(Meier-Kolthoff et al 2014), average amino acid identities (Supplementary Table S3) of the core genome (Konstantinidis et al 2006) and the four-gene MLSA (Supplementary Figure S1) (Gomila et al 2015, Mulet et al 2012).

Five strains that were sequenced in this study are now included in *P. protegens* (Ramette et al 2011): strains CHA0^T, PGNR1, BRIP, K94.41 and PF, while two isolates (PCL1391 and CD), already included in *P. chlororaphis* (Chin-A-Woeng et al 1998, Ruffner et al 2015), can now be assigned to the respective subspecies as we also included three of the subspecies type strains in the genome analysis. Strain PCL1391 belongs to *P. chlororaphis* subsp. *piscium*, whereas strain CD is a *P. chlororaphis* subsp. *aureofaciens*. By searching the annotations for genes coding for the differential phenotypes as described in literature (Burr et al 2010, Peix et al 2007), we could confirm these designations.

Using the data generated in this study, strain TM1A3 is confirmed as member of the species *P. brassicacearum* by its relationship to type strain *P. brassicacearum* subsp. *brassicacearum* NFM421^T, but this strain cannot yet be assigned to a subspecies, as MLSA or genomic data for the other subspecies are missing. Based on AAI and DNA-DNA hybridization strains P12 and PITR2 are closely related to the type strains of *P. kilonensis and P. thivervalensis*, respectively, which were also sequenced in this study and can thus be assigned to these species (Supplementary Table S3).

We cannot assign a species name to the other species. Three strains (Q12-87, P97.38 and Pf153) belong to the *P. corrugata* subgroup, while *Pseudomonas* sp. MIACH is included in

the *P. fluorescens* subgroup (Supplementary Figure 1). This is in agreement with the core genome tree (Figure 1). *Pseudomonas* sp. P1.8 is, based on the MLSA, belonging to the *P. jessenii* subgroup, while *Pseudomonas* sp. P1.31 is a member of the *P. koreensis* subgroup. The closest related genome-sequenced strains included in Figure 1 were also assigned to the corresponding subgroups (Gomila et al 2015). However, none of these can be assigned to a known *Pseudomonas* species, indicating that these strains represent novel species within the genus.

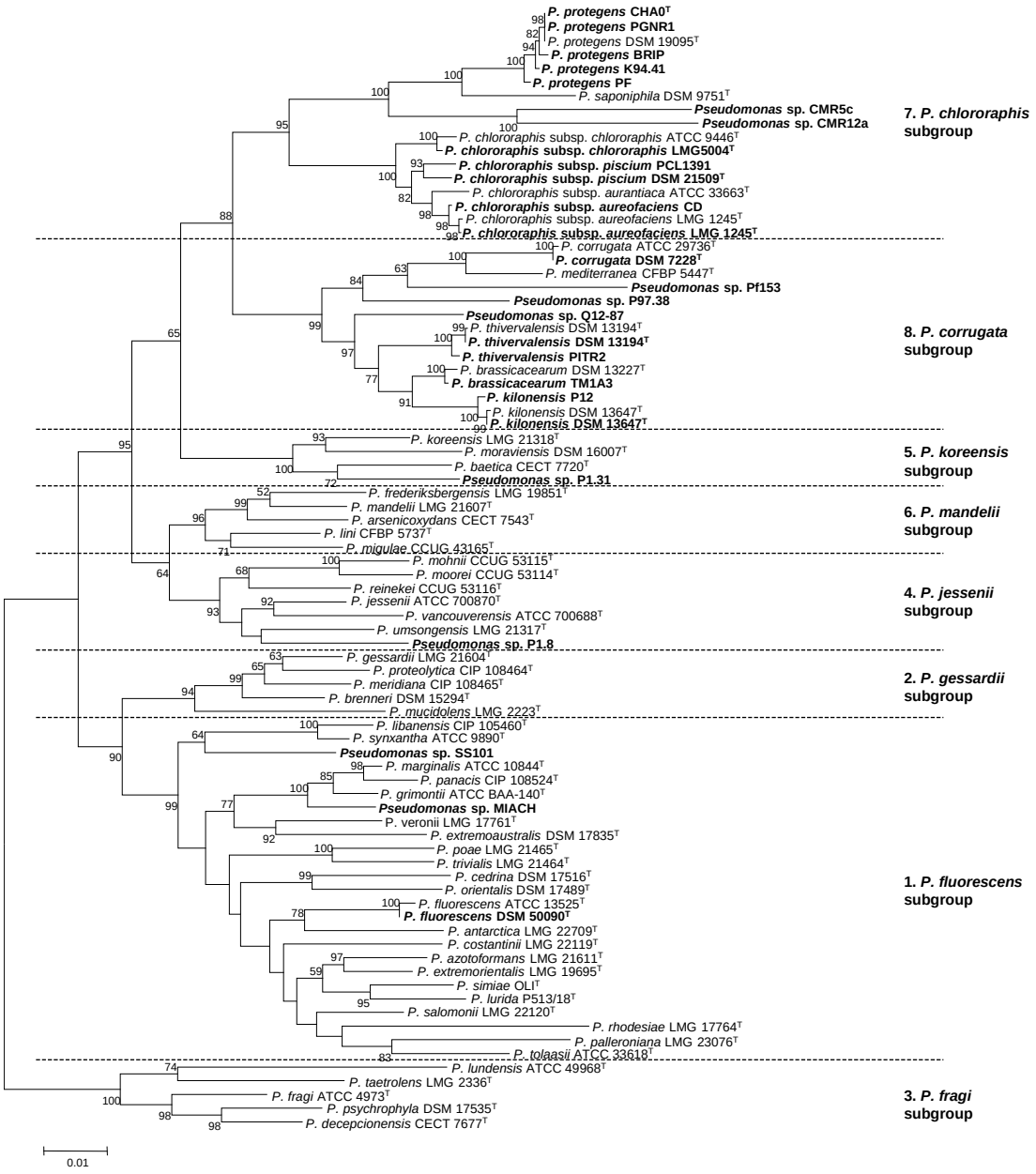
Plant-beneficial effects and antifungal compounds

In a pot experiment all strains were tested for their biocontrol activity against the oomycete pathogen *Pythium ultimum* on cucumber roots. In all sub-clades, several strains were found to display effective plant protection whereas others had no significant biocontrol activity. Thus, biocontrol activity seems to be phylogenetically less predictable than insecticidal activity. The presence of biosynthetic genes for the two important antifungal metabolites 2,4-diacetylphloroglucinol (DAPG) (*phl*) and phenazine (Phz) (*phz*) was not necessarily linked to *P. ultimum* biocontrol since strains *P. chlororaphis* subsp. *aureofaciens* LMG 5004 (*phz*$^+$), *P. kilonensis* DSM 13647^T (*phl*$^+$), and *Pseudomonas* sp. CMR5c (*phz*$^+$, *phl*$^+$) did not provide any protection against the root pathogen (Figure 2). In contrast, all *P. protegens* strains displayed significant biocontrol ability in repeated experiments (Supplementary Table S5). The fact that the strains *Pseudomonas* sp. CMR5c and *P. kilonensis* P12 that were previously shown to have strong biocontrol activity (Keel et al 1996, Perneel et al 2007) did not protect cucumber plants against *P. ultimum* might be explained by the different experimental conditions used in this study, such as the plant as well as the pathogen species or the substrate.

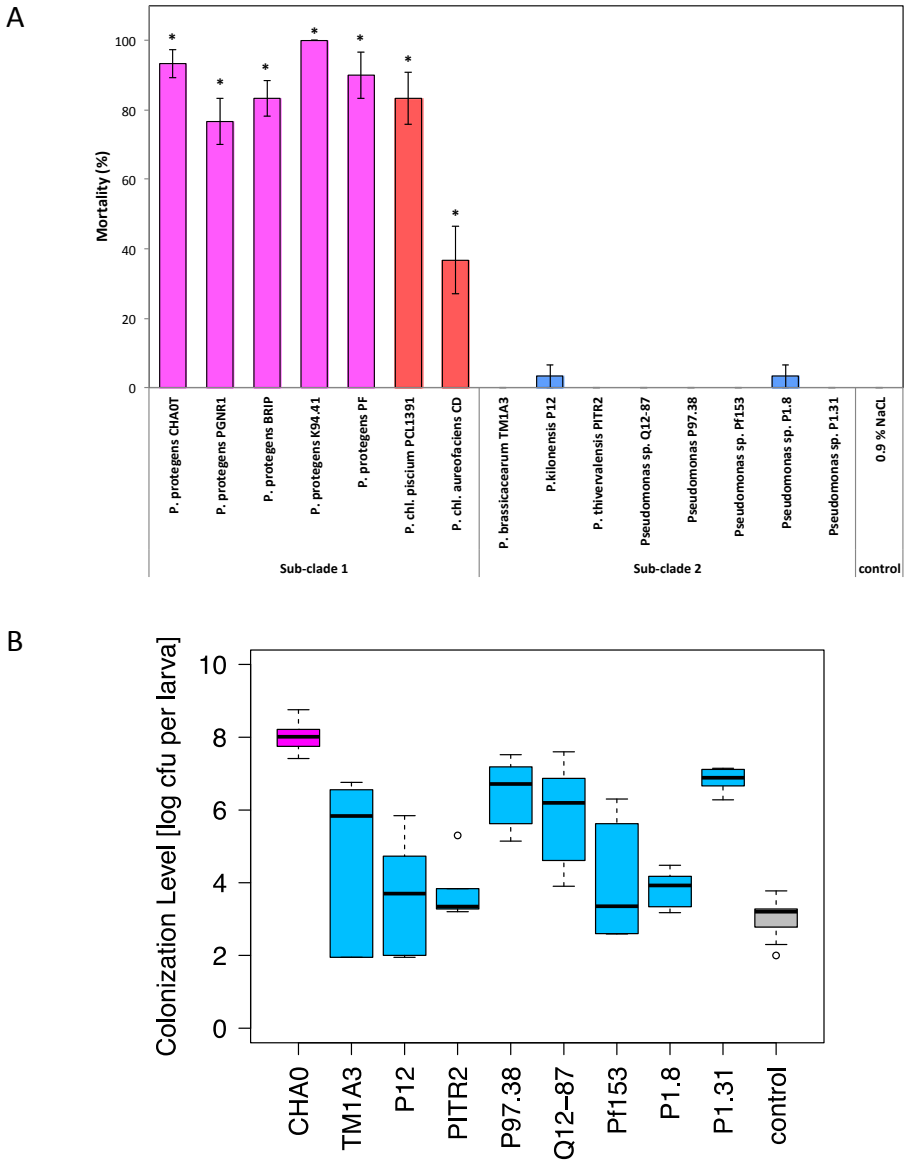
Similar to the results on insecticidal activity, no connection between the original habitat and the degree of plant protection was observed. Thus, also the strains *P. chlororaphis* subsp. *piscium* DSM 21509^T or *P. protegens* BRIP recently isolated from fish and cyclops, respectively, provided significant biocontrol activity (Supplementary Table S5). In general, antifungal metabolite production appears to be less an adaptation to life on roots than a universal defence mechanism against microbial competitors. For instance the fish isolate *P. chlororaphis* subsp. *piscium* DSM 21509^T was also found to have *in vitro* activity towards the oomycete fish pathogen *Saprolegnia parasitica,* which causes significant losses in fish hatcheries and breeding units (data not shown). Thus, an isolate from a certain habitat could also be used as biocontrol agent in a completely different ecological context.

A subset of 15 strains of sub-clade 1 and 2 was further tested for *in vitro* inhibition of mycelial growth of *P. ultimum* and a second plant pathogen, *Fusarium oxysporum*, on MA and GCY medium, favouring the production of DAPG or Phz, respectively (Figure 2, Supplementary Figure S3). Throughout both phylogenetic groups, all strains except P1.8 were found to exhibit *in vitro* pathogen inhibition with DAPG and Phz producing strains performing best on media conducive to metabolite biosynthesis.

Supplementary Figures



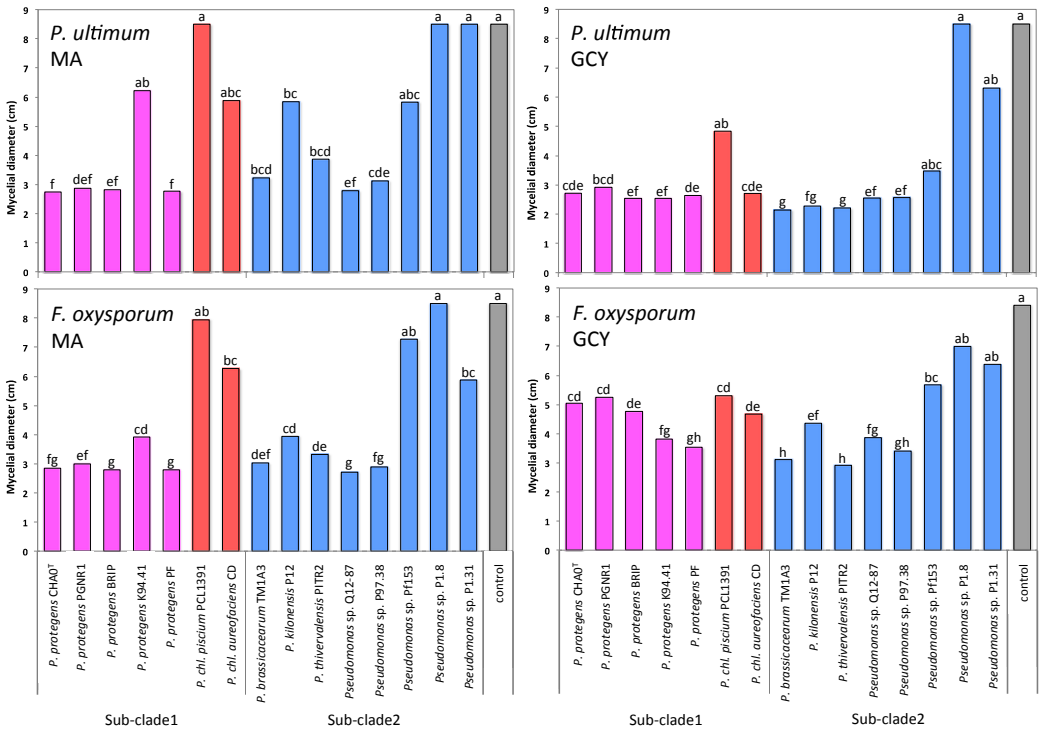
Supplementary Figure S1: Maximum Likelihood phylogenetic tree of strains belonging to the *P. fluorescens* group based on the four-gene (16S rRNA, *gyrB*, *rpoB* and *rpoD*) MLSA scheme of Mulet et al (2012). Strains investigated in this study are indicated in bold. Bootstrap values over 50% are indicated in the tree.



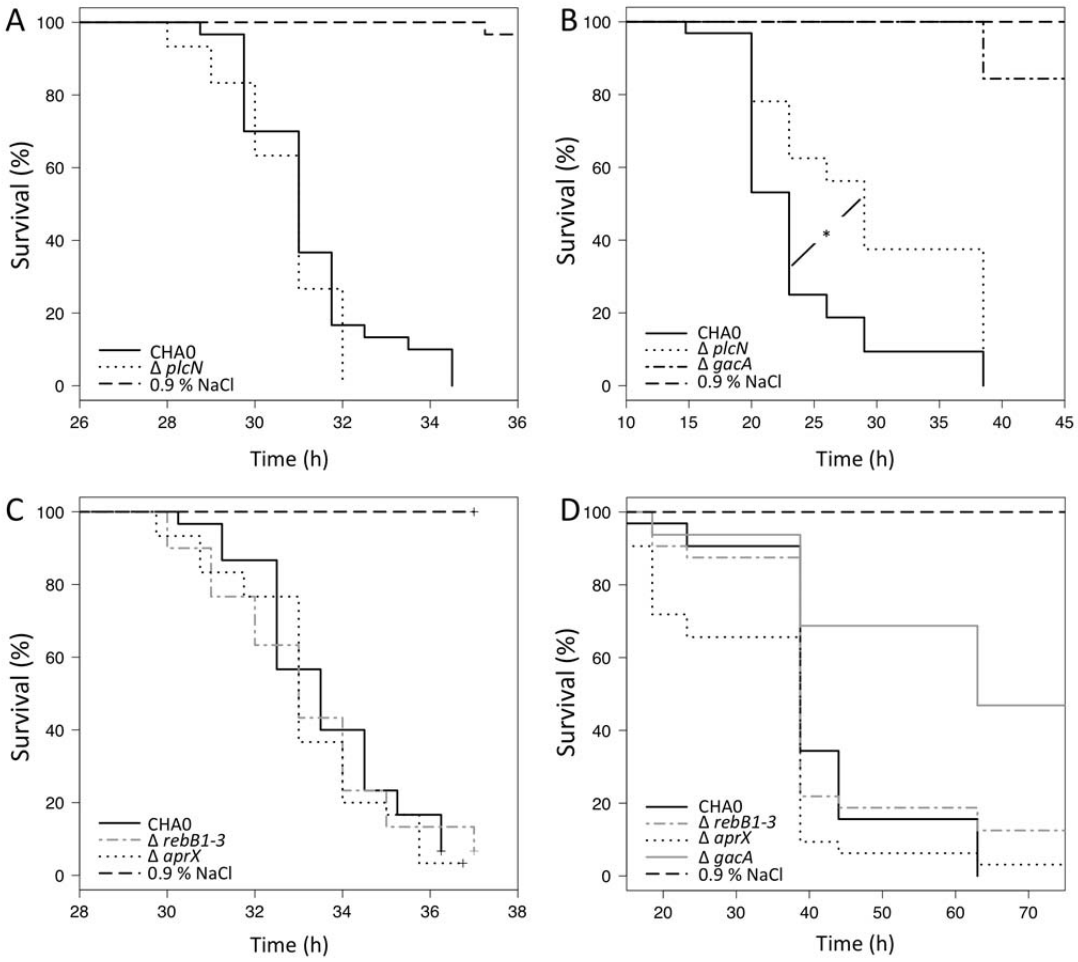
Supplementary Figure S2. Sub-clade 1 strains cause lethal oral infections in *Spodoptera littoralis* larvae while sub-clade 2 strains, although some of them are able to persist in the insect, do not kill the larvae.

Survival (A) and colonization (B) rates of *S. littoralis* larvae upon feeding on artificial diet inoculated with either 4 x 10^7 cells of the indicated *Pseudomonas* strains or 0.9% NaCl (control). A) Survival of larvae after 5 d. Bars show means (± se) of five replicates with six larvae each. Asterisks indicate bacterial treatments that were significantly different from the control based on multiple comparisons by Kruskal-Wallis adjusted by Bonferroni-Holm ($p \leq 0.05$). Each strain was tested in an independent second experiment with highly similar results.

B) Some strains of sub-clade 2 are able to persist in *S. littoralis* larvae whereas numbers of others strongly decline within a few days. To get an estimate of the capacity of inoculants to persist and multiply within *S. littoralis* larvae upon ingestion, six surviving larvae were extracted and colonization levels were assessed by plating serial dilutions on selective medium at the end of the experiment. Data derived from two independent experiments. Strains of sub-clade 1, here represented by *P. protegens* CHA0, generally multiply to levels of about 10^8 cfu/larva. Colonization levels in control larvae represent bacterial background levels on King's B agar (King et al 1954) supplemented with ampicillin (40 µg ml^{-1}), chloramphenicol (13 µg ml^{-1}), and cycloheximide (100 µg ml^{-1}).



Supplementary Figure S3. Inhibition of mycelial growth of *Pythium ultimum* and *Fusarium oxysporum* on MA and GCY medium. Bacteria were streaked out in a square around a plug of oomycete/fungal mycelium and mycelial diameter was measured after 2 days for *P. ultimum* A) and 8 days for *F. oxysporum* B). Strains with different letters were significantly different from each other based on multiple comparisons by Kruskal-Wallis adjusted by Bonferroni-Holm ($p \leq 0.05$). Each strain was tested in an independent second experiment with highly similar results.



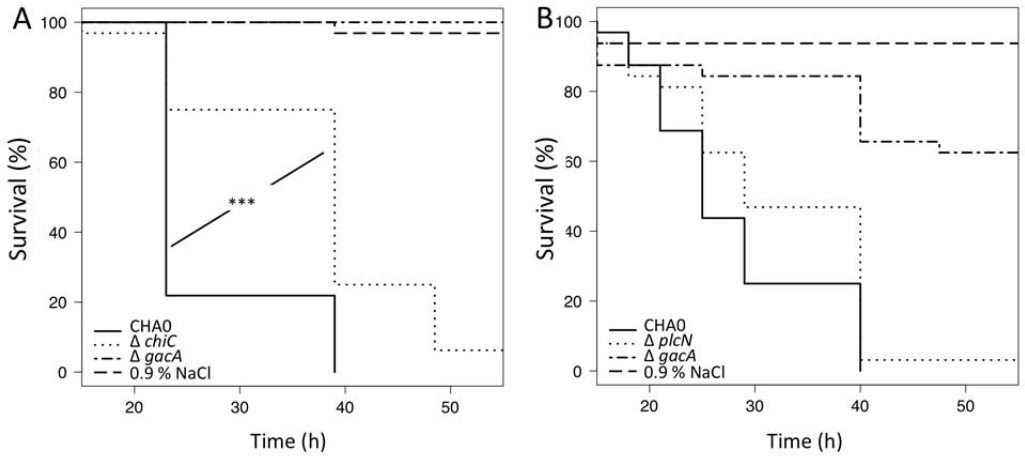
Supplementary Figure S4. Deletion of *plcN* (encoding phospholipase C), but not of *aprX* or the *reb* cluster, reduces oral activity of *P. protegens* CHA0 against insect larvae.

A, C) Systemic activity against *Galleria mellonella*. 30 larvae per treatment were injected with 2 x 10^3 bacterial cells and survival was recorded every hour.

B, D) Oral activity against *Plutella xylostella*. Larvae were fed on artificial diet inoculated with 4 x 10^6 bacterial cells.

B) The virulence of the phospholipase C-negative (Δ*plcN*) mutant was slightly reduced compared to the wild type strain (p-value<0.01, Log-Rank test, Survival Package in R). Although the effect was not significant in all experiments, the tendency of slower killing was always observed. Each mutant was tested at least three times with similar results. One repetition is depicted in Supplementary Figure S5.

CHA0, wild type; Δ*plcN*, phospholipase *C*-negative mutant; Δ*gacA*, GacA-negative mutant; Δ*rebB1-3*, mutant for the *rebB*-cluster; Δ*aprX*, metallopeptidase AprX-negative mutant; 0.9% NaCl served as negative control.



Supplementary Figure S5. Repetition of experiments depicted in Figure 4 and Supplementary Figure S4.

Oral activity against *Plutella xylostella*. Larvae were exposed to artificial diet inoculated with 4 x 10^6 bacterial cells.

A) Significant differences according to a Log-Rank test (Survival Package in R) between treatments with the wild type CHA0 and the chitinase C-negative mutant are indicated with ***(p-value <0.0001). CHA0, wild type; Δ*chiC*, chitinase C-negative mutant; Δ*gacA*, GacA-negative mutant; Δ*plcN*, phospholipase C-negative mutant; 0.9% NaCl served as negative control.

Supplementary Tables

Supplementary Table S1. Full list of gene clusters associated with biocontrol or insecticidal activity for all strains shown in Figure 1. Supplementary to overview Figure 2.

		Gene or metabolite name (Locus tag)																					
Accession number	Strain	*fit* PFL_2980-PFL_2987	*chiC* PCL1391_1854-PCL1391_1855	chitinase PCL1391_3057-PCL1391_3058	*plcN* PCL1391_2966	*aprX* PCL1391_2141	*aprA* PCL1391_3021	*psl* PCL1391_4983,4985-4994	*rebB* PCL1391_0072,-0073,_0075,_0076		DAPG PFL_5951-PFL_5958	PCA PCL1391_4880-PCL1391_4888	PCN PCL1391_4889	2-OH-PCA PchO6_5227	HCN PFL_2577-PFL_2579	PRN PFL_3604-PFL_3607	PLT PFL_2784-PFL_2800	HPR PchO6_4242-PchO6_4244	rhizoxin PFL_2989-PFL_2997	CLP PFL_2145-PFL_2147
CP003190.1	*P. protegens* CHA0T	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	-	+
LHUV00000000	*P. protegens* PGNR1	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	-	+
LHUW00000000	*P. protegens* BRIP	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	-	+
LHUU00000000	*P. protegens* K94.41	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	-	+
CP000076.1	*P. protegens* Pf-5	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	+	+
LHUX00000000	*P. protegens* PF	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	+	+
AP014522.1	*P. protegens* Cab57	+	+	-	+	+	+	+	+		+	-	-	-	+	+	+	-	-	+
LHUY00000000	*Pseudomonas* sp. CMR5c	+	+	-	+	+	+	+	+		+	+	+	-	+	+	+	-	-	+
LHUZ00000000	*P. chlororaphis* subsp. *piscium* JF3835T	+	+	+	+	+	+	+	+		+	+	+	-	+	+	-	-	-	+
ATBG00000000	*P. chlororaphis* HT66	+	+	+	+	+	+	+	+		-	+	+	-	+	-	-	-	-	+
LFUT00000000	*P. chlororaphis* subsp. *piscium* PCL1391	+	+	+	+	+	+	+	+		-	+	+	-	+	-	-	-	-	+
AYUD00000000.1	*P. chlororaphis* subsp. *aurantiaca* PB-St2	±	+	+	+	-	+	+	±		-	+	-	+	+	+	-	+	-	-
CP009290.1	*P. chlororaphis* subsp. *aurantiaca* JD37	±	+	+	+	+	+	+	+		-	+	-	+	+	+	-	+	-	-
CP008696.1	*P. chlororaphis* PA23	+	+	+	+	+	+	+	+		-	+	-	+	+	+	-	+	-	-
LHVB00000000	*P. chlororaphis* CD	+	+	+	+	+	+	+	+		-	+	-	+	+	+	-	+	-	-
LHVA00000000	*P. chlororaphis* subsp. *aureofaciens* LMG 1245T	+	+	±	+	+	+	+	+		-	+	-	+	+	+	-	+	-	-
AWWJ00000000	*P. chlororaphis* YL-1	+	+	+	+	+	+	+	±		-	+	-	+	+	+	-	+	-	-
CM001490.1	*P. chlororaphis* O6	+	+	+	+	+	+	+	+		-	+	-	+	+	+	-	+	-	-
CM001559.1	*P. chlororaphis* subsp. *aureofaciens* 30-84	+	+	+	+	+	+	+	+		-	+	-	+	+	+	-	+	-	-
LHVC00000000	*P. chlororaphis* subsp. *chlororaphis* LMG 5004T	+	+	+	+	+	+	+	+		-	+	+	-	+	+	-	+	-	-
AFOY00000000	*Pseudomonas* sp. HK44	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
LHVD00000000	*P. brassicacearum* TM1A3	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
AZOC00000000.1	*P. brassicacearum* 51MFCVI2.1	-	-	-	-	-	-	-	-		-	-	-	-	+	-	-	-	-	-
CP002585.1	*P. brassicacearum* subsp. *brassicacearum* NFM421	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
AHPO00000000	*P. brassicacearum* Q8r1-96	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
LHVH00000000	*P. kilonensis* DSM 13647T	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
LHVG00000000	*P. kilonensis* P12	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
CP003150.1	*Pseudomonas* sp. F113	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
LHVE00000000	*P. thivervalensis* DSM13194T	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
LHVF00000000	*P. thivervalensis* PITR2	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
LHVI00000000	*Pseudomonas* sp. Q12-87	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
AGBM00000000	*Pseudomonas* sp. Q2-87	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
CP007410.1	*Pseudomonas* sp. DF41	-	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
LHVJ00000000	*Pseudomonas* sp. P97.38	-	-	-	-	-	+	-	-		+	-	-	-	+	-	-	-	-	-
ATKI00000000	*P. corrugata* CFBP5454	-	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
LHVK00000000	*P corrugata* DSM7228 T	-	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
AUPB00000000.1	*P. mediterranea* CFBP 5447	-	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
LHVL00000000	*Pseudomonas* sp. Pf153	-	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
CP003880.1	*Pseudomonas* sp. UW4	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
LHVM00000000	*Pseudomonas* sp. P1.8	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
ARLP00000000	*P. mandelii* 36MFCvi1.1	±	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
AZQQ00000000	*P. mandelii* PD30	-	-	-	-	-	+	-	-		-	-	-	-	-	-	-	-	-	-
CP005960.1, CP005961.1	*P. mandelii* JR-1	-	-	+	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
CP000094.2	*Pseudomonas* sp. Pf0-1	-	-	+	-	+	+	-	-		-	-	-	-	+	-	-	-	-	+
LHVN00000000	*Pseudomonas* sp. P1.31	-	-	-	-	-	+	-	-		-	-	-	-	+	-	-	-	-	-
ALYL00000000	*Pseudomonas* sp. R124	-	-	+	-	+	+	-	-		-	-	-	-	+	-	-	-	-	+
CP008896.1	*Pseudomonas* sp. UK4	-	-	-	-	-	+	+	-		-	-	-	-	-	-	-	-	-	-
JENC00000000.1	*Pseudomonas* sp. ATCC 17400	-	-	-	-	-	+	+	-		-	-	-	-	-	-	-	-	-	-
AKXH00000000	*Pseudomonas* sp. BBc6R8	-	-	-	+	-	+	±	-		-	-	-	-	-	-	-	-	-	-
CP004045.1	*P. poae* RE*1-1-14	-	-	-	-	-	+	±	-		-	-	-	-	-	-	-	-	-	+
AMZW01000000	*P. poae* BRIP34879	-	-	-	-	-	+	±	-		-	-	-	-	-	-	-	-	-	+
AOUH00000000	*P. veronii* 1YdBTEX2	±	-	-	-	-	+	-	-		-	-	-	-	-	-	-	-	-	-
CP006852.1	*Pseudomonas* sp. TKP	-	-	-	-	-	+	±	-		-	-	-	-	-	-	-	-	-	-
LHVP00000000	*P. fluorescens* DSM50090T	-	-	+	-	-	+	±	-		-	-	-	-	-	-	-	-	-	+
LHVO00000000	*Pseudomonas* sp. MIACH	-	-	-	-	-	+	±	-		-	-	-	-	-	-	-	-	-	+
NC_012660.1	*Pseudomonas* sp. SBW25	-	-	-	-	-	+	±	-		-	-	-	-	-	-	-	-	-	+
AVQG00000000	*Pseudomonas* sp. EGD-AQ6	-	-	-	-	-	+	±	-		-	-	-	-	-	-	-	-	-	-
CM001025.1	*Pseudomonas* sp. WH6	-	-	-	-	-	+	-	-		-	-	-	-	-	-	-	-	-	-
AMZG00000000.1	*Pseudomonas* sp. BS2	-	-	-	-	-	+	-	-		-	-	+	-	-	-	-	-	-	-
CM001514.1	*P. synxantha* BG33R	-	-	+	-	-	+	±	±		-	-	-	-	-	-	-	-	-	+
CM001513.1	*Pseudomonas* sp. SS101	-	-	+	-	-	+	±	±		-	-	-	-	-	-	-	-	-	+
AOJA00000000	*Pseudomonas* sp. FH5	-	-	+	-	-	+	±	±		-	-	-	-	-	-	-	-	-	-
NC_017911.1; NC_021361.1	*Pseudomonas* sp. A506	-	-	+	-	-	+	±	±		-	-	-	-	-	-	-	-	-	-
AHZX00000000.1	*P. fragi* B25	-	-	-	-	+	-	-	-		-	-	-	-	±	-	-	-	-	-

Loci were defined as being present when showing 70% similarity over 70% of gene length to the loci indicated in the table. As none of the strains harbors all loci, three different reference strains (PCL1391, Pf-5, O6) were used.

+, gene/s present; -, gene/s absent; ±, gene cluster partially present

P. fluorescens insecticidal toxin-cluster (*fit*); chitinase C (*chiC*); phospholipase C (*plcN*); metallopeptidase *aprX* (*aprX*); alkaline metalloprotease *aprA* (*aprA*); *rebB*-cluster (*rebB*); *psl*-cluster (*psl*); 2,4-diacetylphloroglucinol (DAPG); phenazines: phenazine-1-carboxamide (PCN), phenazine-1-carboxylic acid (PCA), 2-hydroxy-PCA (2-OH-PCA); hydrogen cyanide (HCN); pyrrolnitrin (Prn), pyoluteorin (Plt); 2-hexyl-5-propyl-alkylresorcinol (HPR); cyclic lipopeptide (CLP)

As most genomes consist of several contigs, genes might be found to be absent in a certain strain although they are in fact present, but are located at the border of contigs.

Supplementary Table S2. **Strains, plasmids and primers used in this study**

Name	Relevant characteristics [1] or sequence (5′ → 3′) [2]	Reference or comment
Pseudomonas protegens		
CHA0	Wild type	(Jousset et al 2014, Stutz et al 1986)
CHA5099	Δ*chiC* (deletion of PFLCHA0_c21380)	This study
CHA5221	Δ*rebB1-3* (deletion of PFLCHA0_c01820 through PFLCHA0_c01860)	This study
CHA5222	Δ*aprX* (deletion of PFLCHA0_c25470)	This study
CHA5223	Δ*plcN* (deletion of PFLCHA0_c31570)	This study
Escherichia coli		
DH5α, DH5α λpir	Laboratory strains	(Sambrook and Russel 2001)
Plasmids		
pEMG	pSEVA212S; *ori*R6K, *lacZα* MCS flanked by two I-SceI sites; Km^r, Ap^r	(Martinez-Garcia and de Lorenzo 2011)
pME8327	pEMG-Δ*chiC*; suicide plasmid for the in-frame deletion of PFLCHA0_c21380 (*chiC*) in CHA0; Km^r	This study
pME11026	pEMG-Δ*rebB1-3*; suicide plasmid for the deletion of the PFLCHA0_c01820 to PFLCHA0_c01860 region (*rebB1-3* cluster) in CHA0; Km^r	This study
pME11027	pEMG-Δ*aprX*; suicide plasmid for the in-frame deletion of PFLCHA0_c25470 (*aprX*) in CHA0; Km^r	This study
pME11028	pEMG-Δ*plcN*; suicide plasmid for the in-frame deletion of PFLCHA0_c31570 (*plcN*) in CHA0; Km^r	This study
pSW-2	*ori*RK2, *xylS*, P_m::*I-sce*I; Gm^r	(Martinez-Garcia and de Lorenzo 2011)
Primers		
aprX-del-1	GGAATTCGATGGGCCTGTTCTGAGAGG, EcoRI	Deletion of CHA0 *aprX*
aprX-del-2	CCCAAGCTTTGCTTCCGAGAGTGCTTTTGAC, HindIII	Deletion of CHA0 *aprX*
aprX-del-3	CCCAAGCTTAGCCTGATGATCGACCTGAC, HindIII	Deletion of CHA0 *aprX*
aprX-del-4	CGGGATCCTACCAGCAGTTCTGCAACCAG, BamHI	Deletion of CHA0 *aprX*
chiD-1	CGGAATTCGCCACAGGCTCAACTAAAACAT, EcoRI	Deletion of CHA0 *chiC*
chiD-2	GGGGTACCAATGCTCGGCATCAGGGAAGCA, KpnI	Deletion of CHA0 *chiC*
chiD-3	GGGGTACCCATGGCTGAGTTGTGACGGCCA, KpnI	Deletion of CHA0 *chiC*
chiD-4	CGGGATCCCGCTTACCAATGATTACAACTG, BamHI	Deletion of CHA0 *chiC*
plcC-del-1	GGAATTCATAACGCCACCCATTTCAGC, EcoRI	Deletion of CHA0 *plcN*
plcC-del-2	CCCAAGCTTACTGGGCATGGGTTATTGAGTC, HindIII	Deletion of CHA0 *plcN*
plcC-del-3	CCCAAGCTTGCATGAAGACCTTGGCAAAAATG, HindIII	Deletion of CHA0 *plcN*
plcC-del-4	CGGGATCCGGCCTATGCACGAAAGTTGT, BamHI	Deletion of CHA0 *plcN*
reb-del-1	GGAATTCGTATTGCCCGGTTTGCAGC, EcoRI	Deletion of CHA0 *reb* cluster
reb-del-2	CCCAAGCTTACTGGGCATGGGTTATTGAGTC, HindIII	Deletion of CHA0 *reb* cluster
reb-del-3	CCCAAGCTTGCATGAAGACCTTGGCAAAAATG, HindIII	Deletion of CHA0 *reb* cluster
reb-del-4	CGGGATCCCGCTTACCAATGATTACAACTG, BamHI	Deletion of CHA0 *reb* cluster

[1] Ap^r, ampicillin; Gm^r, gentamicin; and Km^r, kanamycin resistance, respectively.
[2] Specified restriction sites are underlined.

Supplementary Table S3. Mean amino acid identities (AAI) and Genome-to-Genome Distance Calculator (GGDC) values for all genomes related to *P. brassicacearum*, *P. kilonensis* and *P. thivervalensis*.

A AAI values

		1	2	3	4	5	6	7	8	9	10	11	12
1	*P. brassicacearum* subsp. *brassicacearum* NFM 421^T		99.82	99.81	99.85	97.74	97.74	97.82	96.44	96.39	94.67	94.67	94.87
2	*P. brassicacearum* TM1A3	99.82		99.79	99.84	97.73	97.73	97.82	96.44	96.38	94.66	94.67	94.85
3	*P. brassicacearum* 51MCFVI21	99.81	99.79		99.81	97.72	97.72	97.78	96.42	96.37	94.66	94.65	94.84
4	*Pseudomonas* sp. Q8r1-96	99.85	99.84	99.81		97.73	97.73	97.82	96.43	96.38	94.66	94.65	94.86
5	*P. kilonensis* DSM 13647^T	97.74	97.73	97.72	97.73		99.49	98.33	96.61	96.59	94.54	94.54	94.73
6	*P. kilonensis* P12	97.74	97.73	97.72	97.73	99.49		98.33	96.61	96.57	94.50	94.50	94.72
7	*Pseudomonas* sp. F113	97.82	97.82	97.78	97.82	98.33	98.33		96.62	96.58	94.58	94.58	94.71
8	*P. thivervalensis* DSM 13194^T	96.44	96.44	96.42	96.43	96.61	96.61	96.62		99.48	94.43	94.43	94.41
9	*P. thivervalensis* PITR2	96.39	96.38	96.37	96.38	96.59	96.57	96.58	99.48		94.40	94.40	94.37
10	*Pseudomonas* sp. Q12-87	94.67	94.66	94.66	94.66	94.54	94.50	94.58	94.43	94.40		99.79	94.48
11	*Pseudomonas* sp. Q2-87	94.67	94.67	94.65	94.65	94.54	94.50	94.58	94.44	94.40	99.79		94.47
12	*Pseudomonas* sp. DF41	94.87	94.85	94.84	94.86	94.73	94.72	94.71	94.41	94.37	94.48	94.47	

B GGDC values

		1	2	3	4	5	6	7	8	9	10	11	12
1	*P. brassicacearum* subsp. *brassicacearum* NFM 421^T		96.0	96.9	96.6	59.1	59.1	61.0	46.2	45.9	36.5	36.7	38.6
2	*P. brassicacearum* TM1A3	96.0		96.3	96.1	59.2	59.1	60.9	46.1	46.0	36.3	36.4	38.4
3	*P. brassicacearum* 51MCFVI21	96.8	96.3		97.0	59.1	59.2	61.1	46.2	46.0	36.5	36.5	38.4
4	*Pseudomonas* sp. Q8r1-96	96.6	96.1	97.0		59.0	58.9	60.9	46.1	46.0	36.6	36.7	38.5
5	*P. kilonensis* DSM 13647^T	59.1	59.2	59.1	59.0		88.5	64.5	47.4	47.0	36.4	36.5	38.8
6	*P. kilonensis* P12	59.1	59.1	59.2	58.9	88.5		64.5	47.2	46.9	36.5	36.5	38.3
7	*Pseudomonas* sp. F113	61.0	60.9	61.1	60.9	64.5	64.5		46.7	46.3	36.2	36.3	38.4
8	*P. thivervalensis* DSM 13194^T	46.2	46.1	46.2	46.1	47.4	47.2	46.7		88.0	35.4	35.5	37.2
9	*P. thivervalensis* PITR2	45.9	46.0	46.0	46.0	47.0	46.9	46.3	88.0		35.3	35.3	37.0
10	*Pseudomonas* sp. Q12-87	36.5	36.3	36.5	36.6	36.4	36.5	36.2	35.4	35.3		96.0	37.1
11	*Pseudomonas* sp. Q2-87	36.7	36.4	36.5	36.7	36.5	36.5	36.3	35.5	35.3	96.0		37.2
12	*Pseudomonas* sp. DF41	38.6	38.4	38.4	38.5	38.8	38.3	38.4	37.2	37.0	37.1	37.2	

Supplementary Table S4. Lethal time (LT_{50}) and survival of *Plutella xylostella* larvae upon oral uptake of *Pseudomonas* strains.

Sub-clade	Strain	LT50 (d)		survival (%) at 3 dpi ±sdev			
Sub-clade 1	*P. protegens* CHA0^T	1.6	(1.5; 1.8) ^{abc}	3.1	±	6.3	*
	P. protegens PGNR1	1.6	(1.4; 1.8) ^{abc}	0.0	±	0.0	*
	P. protegens BRIP	1.3	(1.1; 1.5) ^{a}	0.0	±	0.0	*
	P. protegens K94.41	1.0	(-19.1; 21.2) ^{abcdef}	0.0	±	0.0	*
	P. protegens PF	1.4	(1.2; 1.6) ^{ab}	0.0	±	0.0	*
	Pseudomonas sp. CMR12a	1.9	(1.7; 2.2) ^{cde}	6.3	±	7.2	*
	P. chl. piscium DSM 21509^T	1.6	(1.4; 1.8) ^{abc}	3.1	±	6.3	*
	P. chl. piscium PCL1391	1.7	(1.5; 1.9) ^{bcd}	0.0	±	0.0	*
	P. chl. aureofaciens CD	1.5	(1.3; 1.7) ^{ab}	3.1	±	6.3	*
	P. chl. aureofaciens LMG 1245^T	2.1	(1.9; 2.4) ^{def}	21.9	±	12.0	*
	P. chl. chlororaphis LMG 5004^T	2.4	(2.1; 2.8) ^{ef}	31.3	±	12.5	*
Sub-clade 2	*P. brassicacearum* TM1A3	NA		81.3	±	21.7	
	P. kilonensis DSM 13647^T	NA		93.8	±	7.2	
	P. kilonensis P12	NA		87.5	±	10.2	
	P. thivervalensis DSM 13194^T	NA		84.4	±	12.0	
	P. thivervalensis PITR2	NA		90.6	±	12.0	
	Pseudomonas sp. Q12-87	NA		81.3	±	7.2	
	Pseudomonas sp. P97.38	NA		87.5	±	17.7	
	P. corrugata DSM 7228^T	NA		75.0	±	21.7	
	Pseudomonas sp. Pf153	NA		93.8	±	7.2	
	Pseudomonas sp. P1.8	NA		87.5	±	10.2	
	Pseudomonas sp. P1.31	NA		84.4	±	12.0	
Sub-clade 3	*P. fluorescens* DSM 50090^T	NA		65.6	±	12.0	*
	Pseudomonas sp. MIACH	2.7	(1.8; 3.5) ^{def}	45.8	±	26.0	*
	Pseudomonas sp. SS101	2.8	(2.3; 3.3) ^{f}	46.9	±	27.7	*
control	0.9% NaCL	NA		96.9	±	6.3	

Repetition of the experiment depicted in Figure 3 and Table 2. *Plutella xylostella* larvae were exposed to food pellets inoculated with 8 x 10^7 bacterial cells. LT_{50} values are estimates based on the generalized linear model using the MASS package in R (Venables and Ripley 2002). Numbers in brackets depict 95% confidence intervals for LT_{50} and significantly different values within the same column are followed by different letters.

NA = no LT_{50} value was calculated, because end mortality was less than 50%.

Asterisks indicate significant differences compared to control larvae treated with 0.9% NaCl based on multiple comparisons by Kruskal-Wallis adjusted by Bonferroni-Holm ($p \leq 0.05$).

Supplementary Table S5. Biocontrol activity against *Pythium ultimum* on cucumber plants

Sub-clade	Strain	Biocontrol Activity relative to *P. protegens* CHA0					
		repetition 1			repetition 2		
Sub-clade 1	*P. protegens* CHA0^T	1.00		*	1.00		*
	P. protegens PGNR1	1.02	± 0.14	*	0.76	± 0.05	*
	P. protegens BRIP	0.94	± 0.24	*	1.07	± 0.09	*
	P. protegens K94.41	0.43	± 0.21	*	0.50	± 0.23	*
	P. protegens PF	0.35	± 0.16	*	0.49	± 0.33	*
	Pseudomonas sp. CMR5c	0.02	± 0.04		0.00	± 0.00	
	Pseudomonas sp. CMR12a	0.51	± 0.25	*	0.00	± 0.00	
	P. chl. piscium DSM 21509^T	0.54	± 0.28	*	0.21	± 0.30	
	P. chl. piscium PCL1391	0.72	± 0.15	*	0.55	± 0.30	*
	P. chl. aureofaciens LMG 1245^T	0.15	± 0.23		0.39	± 0.56	
	P. chl. aureofaciens CD	0.89	± 0.11	*	0.51	± 0.11	*
	P. chl. chlororaphis LMG 5004^T	0.00	± 0.00		0.00	± 0.00	
Sub-clade 2	*P. brassicacearum* TM1A3	0.72	± 0.22	*	0.31	± 0.31	
	P. kilonensis DSM 13647^T	0.00	± 0.00		0.00	± 0.00	
	P. kilonensis P12	0.23	± 0.29		0.18	± 0.20	
	P. thivervalensis DSM 13194^T	0.31	± 0.30		0.28	± 0.28	
	P. thivervalensis PITR2	0.70	± 0.15	*	0.74	± 0.07	*
	Pseudomonas sp. Q12-87	0.67	± 0.21	*	0.58	± 0.32	*
	Pseudomonas sp. P97.38	0.61	± 0.12	*	0.51	± 0.17	*
	P. corrugata DSM 7228^T	0.00	± 0.00		0.06	± 0.14	
	Pseudomonas sp. Pf153	0.52	± 0.12	*	0.25	± 0.38	
	Pseudomonas sp. P1.8	0.05	± 0.12		0.33	± 0.34	
	Pseudomonas sp. P1.31	0.61	± 0.11	*	0.54	± 0.21	*
Sub-clade 3	*P. fluorescens* DSM 50090^T	0.00	± 0.00		0.00	± 0.00	
	Pseudomonas sp. MIACH	0.39	± 0.47		1.02	± 0.47	*
	Pseudomonas sp. SS101	0.82	± 0.25	*	0.00	± 0.00	

Biocontrol activity was calculated after Rezzonico et al (2007) as:
$(1 - ((W_C - W_I)/(W_C - W_P))) \times 100$
using shoot weight obtained in the control with neither bacterial nor pathogen inoculum (W_C), in the unprotected control with the pathogen alone (W_P) and in presence of the tested bacterial strain and the pathogen (W_I). Due to the large number of strains, not all strains could be tested in the same experiment. Therefore, biocontrol activity is shown relative to the biocontrol activity of our model strain *P. protegens* CHA0, which was included as a reference in all experiments. A total of seven experiments was performed and each strain was tested at least twice (repetition 1 and repetition 2). Biocontrol activity for *P. protegens* CHA0 ranged between 63% and 100%.
Means of five replicates ± sdev are shown. Statistics was performed for each experiment separately on absolute biocontrol activity values. Asterisks indicate that strains displayed significant biocontrol activity based on a t-test ($p = 0.05$) against the unprotected control with pathogen alone (W_P).

Supplementary Table S6. Genomic features

Sub-clade	Strain	# Reads * 10^6	# Contigs	Genome size (Mbp)	N50 (kb)	Coverage
Sub-clade 1	*P. protegens* PGNR1	1.96	15	6.86	871	75
	P. protegens BRIP	1.12	19	6.89	701	44
	P. protegens K94.41	1.28	17	6.99	582	50
	P. protegens PF	1.41	14	7.07	1051	52
	Pseudomonas sp. CMR5c	22.35	44	6.76	502	37
	P. chlororaphis subsp. *piscium* DSM21509^T	1.35	36	7.04	414	51
	P. chlororaphis subsp. *piscium* PCL1391	1.29	17	6.86	820	51
	P. chlororaphis subsp. *aureofaciens* LMG 1245^T	1.43	45	7.02	311	54
	P. chlororaphis subsp. *aureofaciens* CD	1.92	32	6.8	388	75
	P. chlororaphis subsp. *chlororaphis* LMG 5004^T	1.13	15	6.79	875	44
Sub-clade 2	*P. brassicacearum* TM1A3	2.16	29	6.69	552	86
	P. kilonensis DSM 13647^T	1.60	44	6.39	281	66
	P. kilonensis P12	1.98	44	6.39	277	81
	P. thivervalensis DSM 13194^T	1.65	25	6.58	445	67
	P. thivervalensis PITR2	1.75	26	6.77	661	68
	Pseudomonas sp. Q12-87	1.33	45	6.30	261	56
	Pseudomonas sp. P97.38	2.12	36	6.06	278	92
	P. corrugata DSM 7228^T	1.63	31	6.13	374	71
	Pseudomonas sp. Pf153	1.70	30	5.98	577	75
	Pseudomonas sp. P1.8	1.87	43	6.36	325	79
	Pseudomonas sp. P1.31	2.18	48	6.27	262	92
Sub-clade 3	*P. fluorescens* DSM 50090^T	1.43	17	6.39	973	59
	Pseudomonas sp. MIACH	1.37	73	6.82	236	54

Supplementary Table S7. Genes specific to insecticidal strains. Locus tags (prefix PCL1391_) and gene names are indicated for *Pseudomonas chlororaphis* subsp. *piscium* PCL1391.

Locus Tags	Gene	*P. protegens*	*Pseudomonas* sp. CMR	*P. chlororaphis*	*Pseudomonas* sp. SS101	*P. fluorescens* DSM50090^T	*Pseudomnas* sp. MIACH	sub-clade 2
PCL1391_0010	Thioesterase	+	+	+	-	-	-	-
PCL1391_0029	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_0030	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_0072	RebB like protein	+	+	+	+	-	-	-
PCL1391_0073	Hypothetical protein	+	+	+	+	-	-	-
PCL1391_0075	RebB protein	+	+	+	+	-	-	-
PCL1391_0076	RebB protein	+	+	+	+	-	-	-
PCL1391_0101	Hypothetical protein	+	+	+	+	+	-	-
PCL1391_0108	Cyanate transport protein CynX	+	+	+	+	+	+	-
PCL1391_0109	CMP deaminase	+	+	+	+	+	-	-
PCL1391_0110	Putative ankyrin-containing lipoprotein	+	+	+	-	-	-	-
PCL1391_0111	LysR family transcriptional regulator	+	+	+	+	+	+	-
PCL1391_0170	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_0171	Polysaccharide deacetylase	+	+	+	-	-	-	-
PCL1391_0195	Ketosteroid isomerase	+	+	+	-	-	-	-
PCL1391_0279	Histidine-specific permease	+	+	+	+	+	+	-
PCL1391_0332	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_0603	Kynurenine formamidase	+	+	+	+	+	+	-
PCL1391_0604	Tryptophan 2,3-dioxygenase	+	+	+	+	+	+	-
PCL1391_0605	Aromatic amino acid transport protein AroP	+	+	+	+	+	+	-
PCL1391_0610	AsnC family transcriptional regulator	+	+	+	+	+	+	-
PCL1391_0611	Kynureninase	+	+	+	+	+	+	-
PCL1391_0612	Amino acid permease	+	+	+	+	+	+	-
PCL1391_0639	Alpha/beta hydrolase	+	+	+	-	-	-	-
PCL1391_0640	membrane protein	+	+	+	+	+	+	-
PCL1391_0733	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_0734	Phosphatidylcholine hydrolyzing phospholipase	+	+	+	+	+	+	-
PCL1391_0828	Serine transporter	+	+	+	+	+	-	-
PCL1391_0938	Signal transduction histidine kinase	+	+	+	+	-	-	-
PCL1391_0939	Hisitdine kinase	+	+	+	+	-	-	-
PCL1391_0940	LuxR family transcriptional regulator	+	+	+	+	+	+	-
PCL1391_0949	GNAT family acetyltransferase	+	+	+	-	-	-	-

PCL1391_1183	Membrane protein	+	+	+	-	-	-	-
PCL1391_1217	TonB-denpendent receptor	+	+	+	+	+	+	-
PCL1391_1218	Iron dicitrate transporter FecR	+	+	+	+	+	+	-
PCL1391_1219	RNA polymerase sigma factor	+	+	+	+	+	+	-
PCL1391_1245	Oxidoreductase	+	+	+	-	-	-	-
PCL1391_1247	GNAT family acetyltransferase	+	+	+	-	-	-	-
PCL1391_1251	Cyclic diguanylate phosphodiesterase	+	+	+	-	-	-	-
PCL1391_1352	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_1354	LysR family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_1370	AraC family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_1510	Endoribonuclease L-PSP	+	+	+	+	-	-	-
PCL1391_1588	Probable sugar efflux transporter	+	+	+	+	+	+	-
PCL1391_1733	Extradiol dioxygenase	+	+	+	-	+	+	-
PCL1391_1817	MFS transporter	+	+	+	-	-	-	-
PCL1391_1854	Chitin-binding protein	+	+	+	+	+	-	-
PCL1391_1855	Chitinase	+	+	+	+	+	-	-
PCL1391_1901	HxlR family transcriptional regulator	+	+	+	+	-	+	-
PCL1391_1903	Heme transporter CcmD	+	+	+	-	-	-	-
PCL1391_1904	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_1905	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_1906	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_1910	Haloacid dehalogenase	+	+	+	+	+	+	-
PCL1391_1978	IclR family transcriptional regulator	+	+	+	+	-	-	-
PCL1391_1979	ABC transporter substrate-binding protein	+	+	+	+	-	-	-
PCL1391_1980	Amino acid ABC transporter permease	+	+	+	+	-	-	-
PCL1391_1982	FAD-dependent oxidoreductase	+	+	+	+	-	-	-
PCL1391_2008	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_2011	RNA-binding protein	+	+	+	-	-	-	-
PCL1391_2016	RNA 3'-terminal phosphate cyclase	+	+	+	-	-	-	-
PCL1391_2021	Diaminopimelate decarboxylase	+	+	+	-	-	-	-
PCL1391_2037	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2051	RNA polymerase subunit sigma-70	+	+	+	+	-	+	-
PCL1391_2053	Putative TonB-dependent receptor	+	+	+	+	-	-	-
PCL1391_2076	Methyl-accepting chemotaxis protein	+	+	+	-	-	-	-
PCL1391_2141	Serralysin	+	+	+	-	-	-	-
PCL1391_2164	Alpha/beta hydrolase	+	+	+	-	+	+	-
PCL1391_2185	GNAT family acetyltransferase	+	+	+	+	+	-	-
PCL1391_2193	ABC transporter permease	+	+	+	-	+	+	-
PCL1391_2194	ABC transporter substrate-binding protein	+	+	+	-	+	+	-
PCL1391_2195	Methionine sulfoxide reductase A	+	+	+	-	-	-	-
PCL1391_2197	RND transporter	+	+	+	+	+	+	-

PCL1391_2199	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_2220	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2221	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2281	Acyl-CoA dehydrogenase	+	+	+	-	+	+	-
PCL1391_2405	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2433	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2479	Hypothetical protein	+	+	+	+	+	-	-
PCL1391_2481	Na/Pi cotransporter	+	+	+	-	-	-	-
PCL1391_2482	Hypothetical membrane protein	+	+	+	-	-	-	-
PCL1391_2483	Magnesium-transporting ATPase, P-type 1	+	+	+	+	+	+	-
PCL1391_2484	Conserved hypothetical protein	+	+	+	+	+	-	-
PCL1391_2556	MFS transporter	+	+	+	+	-	-	-
PCL1391_2557	L-2-hydroxyglutarate oxidase LhgO	+	+	+	+	-	-	-
PCL1391_2558	GntR family transcriptional regulator	+	+	+	+	-	-	-
PCL1391_2605	AraC family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_2609	Sulfite reductase	+	+	+	-	-	-	-
PCL1391_2610	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2645	Polyketide cyclase	+	+	+	+	+	+	-
PCL1391_2659	MFS transporter	+	+	+	+	-	+	-
PCL1391_2660	Oxidoreductase	+	+	+	-	-	-	-
PCL1391_2673	Transcriptional activator protein CzcR	+	+	+	+	+	+	-
PCL1391_2790	Glycosyltransferase	+	+	+	+	+	+	-
PCL1391_2887	Biopolymer transporter ExbD	+	+	+	+	+	-	-
PCL1391_2966	Non-hemolytic phospholipase C	+	+	+	-	-	-	-
PCL1391_2967	Membrane protein	+	+	+	-	-	-	-
PCL1391_2972	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_2973	Cyclic diguanylate phosphodiesterase	+	+	+	-	-	-	-
PCL1391_2987	Putative ABC transporter, permease subunit	+	+	+	+	+	-	-
PCL1391_2988	Putative ABC transporter, substrate-binding protein	+	+	+	+	+	-	-
PCL1391_2989	Putative ABC transporter, ATP-binding protein	+	+	+	+	+	-	-
PCL1391_2990	Acyl-CoA dehydrogenase	+	+	+	+	+	-	-
PCL1391_2992	AraC family transcriptional regulator	+	+	+	+	+	-	-
PCL1391_3032	Aminotransferase	+	+	+	-	+	+	-
PCL1391_3062	Amino acid transporter	+	+	+	-	-	-	-
PCL1391_3089	MFS transporter	+	+	+	-	-	-	-
PCL1391_3117	4-Hydroxyphenylacetate 3-monooxygenase oxygenase component	+	+	+	-	-	-	-
PCL1391_3126	(R,R)-Butanediol dehydrogenase	+	+	+	-	-	-	-
PCL1391_3130	Hypothetical protein	+	+	+	-	+	+	-
PCL1391_3144	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_3145	LysR family transcriptional regulator	+	+	+	+	+	+	-

PCL1391_3234	Transporter	+	+	+	-	-	-	-
PCL1391_3422	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_3423	Conserved hypothetical protein	+	+	+	+	+	+	-
PCL1391_3454	Response regulator FitH	+	+	+	-	-	-	-
PCL1391_3455	Transcriptional regulator FitG	+	+	+	-	-	-	-
PCL1391_3456	Sensor histidine kinase FitF	+	+	+	-	-	-	-
PCL1391_3457	Channel protein FitE	+	+	+	-	-	-	-
PCL1391_3458	Cytotoxin FitD	+	+	+	-	-	-	-
PCL1391_3459	Type I secretion system ATPase FitC	+	+	+	-	-	-	-
PCL1391_3479	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_3513	Putative glucosidase	+	+	+	+	+	+	-
PCL1391_3514	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_3515	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_3565	Molybdenum cofactor biosynthesis protein MoaA	+	+	+	-	-	-	-
PCL1391_3566	Molybdenum cofactor biosynthesis protein B	+	+	+	-	-	-	-
PCL1391_3569	Molybdopterin synthase catalytic subunit	+	+	+	-	-	-	-
PCL1391_3570	Molybdenum cofactor biosynthesis protein MoaD	+	+	+	-	-	-	-
PCL1391_3571	Molybdenum cofactor biosynthesis protein MoaC	+	+	+	-	-	-	-
PCL1391_3574	Molybdopterin-dependent oxidoreductase alpha subunit	+	+	+	-	-	-	-
PCL1391_3575	Cytochrome D ubiquinol oxidase subunit I	+	+	+	-	-	-	-
PCL1391_3576	Ubiquinol oxidase subunit II, cyanide insensitive	+	+	+	-	-	-	-
PCL1391_3600	TonB-denpendent receptor	+	+	+	+	+	+	-
PCL1391_3671	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_3751	Nucleoside 2-deoxyribosyltransferase	+	+	+	-	-	-	-
PCL1391_3843	Conserved hypothetical protein	+	+	+	+	+	-	-
PCL1391_3855	DNA polymerase subunit beta	+	+	+	+	+	+	-
PCL1391_3876	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_3932	HAD family hydrolase	+	+	+	-	-	-	-
PCL1391_3935	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_3937	Amidohydrolase	+	+	+	-	-	-	-
PCL1391_3989	Methyltransferase	+	+	+	-	-	-	-
PCL1391_4028	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_4037	Hemolysin secretion/activation protein, ShlB family	+	+	+	+	+	-	-
PCL1391_4083	LuxR family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_4176	Glutathione S-transferase	+	+	+	+	+	+	-
PCL1391_4307	Hypothetical protein	+	+	+	+	+	-	-
PCL1391_4350	AraC family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_4351	Fatty acid hydroxylase	+	+	+	-	-	-	-

PCL1391_4367	TetR family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_4386	Lysine transporter LysE	+	+	+	-	-	-	-
PCL1391_4387	LysR family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_4607	Putative arginase	+	+	+	+	-	+	-
PCL1391_4608	Transporter	+	+	+	+	-	+	-
PCL1391_4609	Transporter	+	+	+	+	-	+	-
PCL1391_4610	Fatty acid desaturase	+	+	+	-	-	-	-
PCL1391_4611	Structural protein MipA	+	+	+	+	+	+	-
PCL1391_4612	2,3-Diketo-5-methylthio-1-phosphopentane phosphatase	+	+	+	+	+	+	-
PCL1391_4613	Adenosylmethionine-8-amino-7-oxo-nanoate aminotransferase	+	+	+	+	+	+	-
PCL1391_4614	Esterase	+	+	+	+	+	+	-
PCL1391_4615	Hypothetical protein	+	+	+	+	+	+	-
PCL1391_4616	Sensor histidine kinase	+	+	+	+	+	+	-
PCL1391_4617	Fis family transcriptional regulator	+	+	+	+	+	+	-
PCL1391_4626	50S ribosomal protein L31	+	+	+	+	+	-	-
PCL1391_4642	TonB-denpendent receptor	+	+	+	+	+	+	-
PCL1391_4646	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_4723	HIT family hydrolase	+	+	+	-	-	-	-
PCL1391_4735	Serine hydroxymethyltransferase	+	+	+	-	+	+	-
PCL1391_4798	Hypothetical membrane protein	+	+	+	+	-	-	-
PCL1391_4800	XRE family transcriptional regulator	+	+	+	+	-	-	-
PCL1391_4801	Histidine kinase	+	+	+	+	-	-	-
PCL1391_4891	GNAT family acetyltransferase	+	+	+	-	-	-	-
PCL1391_4896	D-alanyl-alanine synthetase	+	+	+	-	+	+	-
PCL1391_4904	MFS transporter	+	+	+	-	-	-	-
PCL1391_4905	LysR family transcriptional regulator	+	+	+	-	-	-	-
PCL1391_4907	Short-chain dehydrogenase	+	+	+	+	-	-	-
PCL1391_4917	Cobalt-zinc-cadmium resistance protein CzcD	+	+	+	+	+	-	-
PCL1391_4925	Acid phosphatase	+	+	+	+	+	+	-
PCL1391_4982	Hypothetical protein	+	+	+	-	+	+	-
PCL1391_4983	Glycosyl transferase PslA	+	+	+	+	+	+	-
PCL1391_4985	Glycosyl transferase PslC	+	+	+	+	+	+	-
PCL1391_4986	Polysaccharide biosynthesis/export protein PslD	+	+	+	+	+	+	-
PCL1391_4987	Polysaccharide biosynthesis/export protein PslE	+	+	+	+	+	+	-
PCL1391_4988	glycosyl transferase PslF	+	+	+	+	+	+	-
PCL1391_4989	Glycosyl hydrolase PslG	+	+	+	+	+	+	-
PCL1391_4990	Glycosyl transferase PslH	+	+	+	+	+	+	-
PCL1391_4991	Glycosyl transferase PslI	+	+	+	+	+	+	-
PCL1391_4992	Membrane protein PslJ	+	+	+	+	+	+	-
PCL1391_4993	Acetyltransferase	+	+	+	+	-	-	-

PCL1391_4994	Membrane protein PslK	+	+	+	+	+	+	-
PCL1391_5052	Hypothetical protein	+	+	+	-	+	+	-
PCL1391_5077	Benzoate transporter	+	+	+	+	-	+	-
PCL1391_5099	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_5179	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_5182	Copper-containing nitrite reductase	+	+	+	-	-	-	-
PCL1391_5360	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_5397	Aminoglycoside N(6')-acetyltransferase	+	+	+	-	-	-	-
PCL1391_5511	Putative membrane protein	+	+	+	-	-	-	-
PCL1391_5577	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate ami-transferase	+	+	+	+	+	+	-
PCL1391_5659	Membrane protein	+	+	+	-	+	+	-
PCL1391_5765	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_5770	Hypothetical protein	+	+	+	-	-	-	-
PCL1391_5773	Membrane protein	+	+	+	+	+	+	-
PCL1391_5799	TraR family zinc finger protein	+	+	+	+	+	-	-
PCL1391_5806	Phosphoribosyl-AMP cyclohydrolase 2	+	+	+	-	+	+	-

+, gene present; -, gene absent

Loci shaded in grey are discussed in the text.

References

Auch AF, von Jan M, Klenk H-P, Goeker M. (2010). Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* **2:** 117-134.

Blom J, Albaum SP, Doppmeier D, Pühler A, Vorhölter F-J, Zakrzewski M *et al.* (2009). EDGAR: a software framework for the comparative analysis of prokaryotic genomes. *BMC Bioinformatics* **10:** 154.

Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. (2011). Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* **27:** 578-579.

Boetzer M, Pirovano W. (2012). Toward almost closed genomes with GapFiller. *Genome Biol* **13:** R56.

Burr SE, Gobeli S, Kuhnert P, Goldschmidt-Clermont E, Frey J. (2010). *Pseudomonas chlororaphis* subsp. *piscium* subsp. nov., isolated from freshwater fish. *Int J Syst Evol Microbiol* **60:** 2753-2757.

Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ, van der Drift K, Schripsema J, Kroon B *et al.* (1998). Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol Plant-Microbe Interact* **11:** 1069-1077.

Gomila M, Peña A, Mulet M, Lalucat J, García-Valdés E. (2015). Phylogenomics and systematics in *Pseudomonas*. *Front Microbiol* **6:** 214.

Gupta GP, Rani S, Birah A, Raghuraman M. (2005). Improved artificial diet for mass rearing of the tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae). *Int J Trop Insect Sci* **25:** 55-58.

Jousset A, Schuldes J, Keel C, Maurhofer M, Daniel R, Scheu S *et al.* (2014). Full-genome sequence of the plant growth-promoting bacterium *Pseudomonas protegens* CHA0. *Genome Announc* **2:** e00322-00314.

Keel C, Weller DM, Natsch A, Defago G, Cook RJ, Thomashow LS. (1996). Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. *Appl Environ Microbiol* **62:** 552-563.

King EO, Ward MK, Raney DE. (1954). 2 simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44:** 301-307.

Konstantinidis KT, Ramette A, Tiedje JM. (2006). The bacterial species definition in the genomic era. *Phil Trans R Soc B* **361:** 1929-1940.

Martinez-Garcia E, de Lorenzo V. (2011). Engineering multiple genomic deletions in Gram-negative bacteria: analysis of the multi-resistant antibiotic profile of *Pseudomonas putida* KT2440. *Environ Microbiol* **13:** 2702-2716.

Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* **14:** 60.

Meier-Kolthoff JP, Klenk H-P, Göker M. (2014). Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. *Int J Syst Evol Microbiol* **64:** 352-356.

Mulet M, Gomila M, Scotta C, Sánchez D, Lalucat J, García-Valdéz E. (2012). Concordance between whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry and multilocus sequence analysis approaches in species discrimination within the genus *Pseudomonas*. *Syst Appl Microbiol* **35:** 455-464.

Peix A, Valverde A, Rivas R, Igual JM, Ramírez-Bahena M-H, Mateos PF *et al.* (2007). Reclassification of *Pseudomonas aurantiaca* as a synonym of *Pseudomonas chlororaphis* and proposal of three subspecies, *P. chlororaphis* subsp. *chlororaphis* subsp. nov., *P. chlororaphis* subsp. *aureofaciens* subsp. nov., comb. nov. and *P. chlororaphis* subsp. *aurantiaca* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **57:** 1286-1290.

Perneel M, Heyrman J, Adiobo A, De Maeyer K, Raaijmakers JM, De Vos P *et al.* (2007). Characterization of CMR5c and CMR12a, novel fluorescent *Pseudomonas* strains from the cocoyam rhizosphere with biocontrol activity. *J Appl Microbiol* **103:** 1007-1020.

Ramette A, Frapolli M, Fischer-Le Saux M, Gruffaz C, Meyer J-M, Défago G *et al.* (2011). *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Int J Syst Evol Microbiol* **34:** 180-188.

Rezzonico F, Zala M, Keel C, Duffy B, Moenne-Loccoz Y, Defago G. (2007). Is the ability of biocontrol fluorescent pseudomonads to produce the antifungal metabolite 2,4-diacetylphloroglucinol really synonymous with higher plant protection? *New Phytol* **173:** 861-872.

Ruffner B, Péchy-Tarr M, Ryffel F, Hoegger P, Obrist C, Rindlisbacher A *et al.* (2013). Oral insecticidal activity of plant-associated pseudomonads. *Environ Microbiol* **15:** 751-763.

Ruffner B, Péchy-Tarr M, Höfte M, Bloemberg G, Grunder J, Keel C *et al.* (2015). Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens *Photorhabdus* and *Xenorhabdus*. *BMC Genomics* **16:** 609.

Sambrook J, Russel DW (2001). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press: New York.

Sharifi-Tehrani A, Zala M, Natsch A, Moenne-Loccoz Y, Defago G. (1998). Biocontrol of soil-borne fungal plant diseases by 2,4-diacetylphloroglucinol-producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA. *Eur J Plant Pathol* **104:** 631-643.

Stutz EW, Defago G, Kern H. (1986). Naturally-occuring fluorescent pseudomonads involved in suppression of black root-rot of tobacco. *Phytopathology* **76:** 181-185.

Tambong JT, Höfte M. (2001). Phenazines are involved in biocontrol of *Pythium myriotylum* on cocoyam by *Pseudomonas aeruginosa* PNA1. *Eur J Plant Pathol* **107:** 511-521.

Venables WN, Ripley BD (2002). *Modern Applied Statistics with S*, Fourth edn. Springer: New York.