Phylogenetically closely related pseudomonads isolated from arthropods exhibit differential insect-killing abilities and genetic variations in insecticidal factors

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Summary

Strains belonging to the *Pseudomonas protegens* and *Pseudomonas chlororaphis* species are able to control soilborne plant pathogens and to kill pest insects by producing virulence factors such as toxins, chitinases, antimicrobials or two-partner secretion systems. Most insecticidal *Pseudomonas* described so far were isolated from roots or soil. It is unknown whether these bacteria naturally occur in arthropods and how they interact with them. Therefore, we isolated *P. protegens* and *P. chlororaphis* from various healthy insects and myriapods, roots and soil collected in an agricultural field and a neighbouring grassland. The isolates were compared for insect killing, pathogen suppression and host colonization abilities. Our results indicate that neither the origin of isolation nor the phylogenetic position mirror the degree of insecticidal activity. *Pseudomonas protegens* strains appeared homogeneous regarding phylogeny, biocontrol and insecticidal capabilities, whereas *P. chlororaphis* strains were phylogenetically and phenotypically more heterogenous. A phenotypic and genomic analysis of five closely related *P. chlororaphis* isolates displaying varying levels of insecticidal activity revealed variations in genes encoding insecticidal factors that may account for the reduced insecticidal activity of certain isolates. Our findings point towards an adaption to insects within closely related pseudomonads and contribute to understand the ecology of insecticidal *Pseudomonas*.

Introduction

The *Pseudomonas* genome is complex and plastic, which allows these bacteria to colonize a wide variety of habitats such as plant roots, animals or polluted water (Silby *et al*., 2011; Rumbaugh, 2014). Different *Pseudomonas* species are either generalists or form specific interactions with certain hosts. For instance, human pathogenic multidrug-resistant *Pseudomonas aeruginosa* strains are more adapted to cystic fibrosis patients than to healthy humans (AbdulWahab *et al*., 2014; Freschi *et al*., 2018), whereas different *Pseudomonas syringae* pathovars are able to infect and cause disease in specific plant hosts (reviewed by O'Brien *et al*., 2011; Thynne *et al*., 2015). Some *Pseudomonas* are also adapted to live as endophytes which may allow the bacteria to exploit the resources of the plant host more efficiently (reviewed by Afzal *et al*., 2019). Also, *Pseudomonas stutzeri* and *Pseudomonas fluorescens* show preferences for colonizing wheat roots over cucumber roots (Tovi *et al*., 2019).

Insect microbiomes include bacteria, archaea, fungi, viruses and protists which can protect the host against invading pathogens, increase the availability of nutrients (reviewed by Dillon and Dillon, 2004; Engel and Moran, 2013; Paniagua Voirol *et al*., 2018; Gurung *et al*., 2019) or even confer resistance to pesticides (Gomes *et al*., 2020; Wang *et al*., 2020). The physiology, the diet, the behaviour and the niche of the different insect orders highly influence the composition of the microbiota and its transmission (Engel and Moran, 2013; Esposti and Romero, 2017; Paniagua Voirol *et al*., 2018; Hannula *et al*., 2019). Here, we will focus on holometabolous insects including Coleoptera, Diptera and Lepidoptera. They mostly obtain their microbial communities from their diet or the environment and the communities change throughout the different life stages of the insect (Vasanthakumar *et al*., 2008; Lauzon *et al*., 2009; Aharon *et al*., 2013; Hammer *et al*., 2014; Montagna *et al*., 2015a; Montagna *et al*., 2015b; Chen *et al*., 2016; Malacrino *et al*., 2018; González-Serrano *et al*., 2019).

Yet, Diptera and Coleoptera retain some core microbiota that is transmitted vertically through generations (Lauzon et al., 2009; Ziganshina et al., 2018; Chouaia et al., 2019; Kolasa et al., 2019). On the other hand, it has been proposed that Lepidoptera larvae do not have a core microbiota probably due to the demanding conditions of their guts such as high pH, antimicrobial peptides and the lack of pouches or cavities that can accommodate microbial communities (Engel and Moran, 2013; Hammer et al., 2017). Indeed, it has been shown that Lepidoptera larvae can obtain their microbiota from their diet and interactions with the soil (Hammer et al., 2017; Hannula et al., 2019). Other arthropods such as myriapods have not been studied as detailed as insects but one biorxiv study described Proteobacteria associated with centipedes belonging to the Geophilidae family (Geli-Cruz et al., 2019). *Pseudomonas* have been detected within the arthropod microflora in several studies (reviewed by Esposti and Romero, 2017; Paniagua Voirol et al., 2018). Although pseudomonads were detected in field and laboratory-reared Lepidoptera (Çakici et al., 2014; Chen et al., 2016; Mashtoly et al., 2019; Skowronek et al., 2020) and Coleoptera (Bahar and Demirbağ, 2007; Montagna et al., 2015a; Montagna et al., 2015b), and in laboratory-reared Diptera (Wong et al., 2015), it is still uncertain whether they have established a relationship with these insects or if they are just transient bacteria in their gut.

Some plant-beneficial pseudomonads belonging to the *Pseudomonas protegens*, *Pseudomonas chlororaphis* and *P. fluorescens* species can also be pathogens of diverse pest insect species (Péchy-Tarr et al., 2008; Jang et al., 2013; Kupferschmied et al., 2013, 2014; Péchy-Tarr et al., 2013; Ruffner et al., 2013, 2015; Flury et al., 2016, 2019; Loper et al., 2016; Vacheron et al., 2019; Vesga et al., 2020). *Pseudomonas protegens* and *P. chlororaphis* are known root colonizers and they can control soil-borne fungal pathogens (Haas and Défago, 2005). However, they can, after oral uptake, also invade the insect gut, transmigrate into the hemocoel, cause a systemic infection and kill the insect (Kupferschmied et al., 2013; Flury et al., 2019; Vacheron et al., 2019). Insecticidal activity in these two species was first related to the Fit toxin encoded by the *fitD* gene (Péchy-Tarr et al., 2008). This toxin is very similar to the Mcf (make caterpillars floppy) toxins of *Photorhabdus luminescens* and *Xenorhabdus nematophila* (Daborn et al., 2002; Waterfield et al., 2003; Ruffner et al., 2015). Subsequent work established that insecticidal activity in *P. protegens* and *P. chlororaphis* is a multifactorial trait that involves, besides the Fit toxin, various elements such as lipopolysaccharide O-antigens (Kupferschmied et al., 2014), the type VI secretion system (Vacheron

et al., 2019), the cyclic lipopeptide orfamide A (Jang et al., 2013; Flury et al., 2016), a chitinase and a phospholipase C (Flury et al., 2016), hydrogen cyanide (Flury et al., 2017), the toxins rhizoxin (Loper et al., 2016) or IPD072Aa (Schellenberger et al., 2016), and two-partner secretion (TPS) systems (Vesga et al., 2020).

Pseudomonas chlororaphis and *P. protegens* cause high levels of mortality in larvae of different Lepidoptera species such as *Spodoptera littoralis*, *Pieris brassicae*, *Manduca sexta*, *Plutella xylostella* and *Heliothis virescens* (Péchy-Tarr et al., 2008; Péchy-Tarr et al., 2013; Ruffner et al., 2013, 2015; Flury et al., 2019) and they also negatively affect pupation rates in *Delia radicum* (Flury et al., 2019). Specifically, *P. protegens* strain CHA0 is able to persist throughout different *D. radicum*, *P. xylostella* and *P. brassicae* life stages and can even be transmitted to new host plants by *D. radicum* flies (Flury et al., 2019). However, no oral effect was found for larvae of the Coleoptera *Otiorhynchus sulcatus* even though *P. protegens* CHA0 was detected in pupae and adults (Flury et al., 2019).

Most of the published *P. protegens* and *P. chlororaphis* isolates with described insecticidal activity were isolated from soil or plant environments. Thus, the first aim of this study was to isolate *P. protegens* and *P. chlororaphis* from arthropods collected in agricultural and neighbouring undisturbed grassland in order to characterize their natural association with these animals. The second aim was to investigate whether root and arthropod isolates differ in their abilities or efficacies to colonize and kill an insect host and to colonize plant roots and protect them against a soilborne pathogen. Finally, a variant calling approach was performed on known virulence traits to determine whether genetic modifications may explain the different killing ability of these *Pseudomonas*. This study indicates that *P. protegens* species is more homogeneous, whereas *P. chlororaphis* is more phylogenetically and phenotypically diverse for the studied traits, independently of their isolation origin. We identified several variations in key insecticidal genes that may be linked to an attenuation of insecticidal activity in certain *Pseudomonas* isolates, potentially reflecting an ongoing adaptation to their host.

Results and discussion

Pseudomonas protegens *and* P. chlororaphis *strains are naturally associated with arthropods, plants and soil of agricultural fields*

Pseudomonas protegens and *P. chlororaphis* strains have demonstrated insecticidal activity (Péchy-Tarr et al., 2008; Loper et al., 2012, 2016; Jang et al., 2013; Flury et al., 2016, 2017, 2019; Vacheron et al., 2019;

Vesga *et al*., 2020). However, it was not known, whether these two species are naturally associated with insects. The first aim of our study was thus to search for the presence of *P. protegens* and *P. chlororaphis* in insects and other arthropods collected from soil. We collected a total of 120 arthropods belonging to 51 different genera over 2 years at the Reckenholz facilities of Agroscope (Switzerland), i.e. in an agricultural field (cropped with wheat in 2016 and with potato in 2017) and in a patch of neighbouring grassland. A total of 660 bacteria were isolated from those arthropods after a strict surface disinfection previously validated by Flury *et al*. (2019), from roots or from soil. Ninety isolates were characterized as *Pseudomonas* spp. based on their 16S rRNA gene sequence. From those, 14 arthropod isolates were identified as *P. protegens* (~15%) and 14 as *P. chlororaphis* (~15%). Additionally, eight (~9%) *P. chlororaphis* were isolated from grassland roots, potato roots or soil. We sequenced 10 genomes of each species for this study. Surprisingly,

no *P. protegens* strains were detected in the root or soil samples collected (Table S1). The *Pseudomonas* isolates that were not assigned as *P. protegens* or *P. chlororaphis* as well as the non-*Pseudomonas* isolates were not further characterized.

The *P. protegens* isolates are closely related to each other but phylogenetically divergent from other previously described *P. protegens* root isolates (Figs 1 and S1). A proteome-wide analysis showed that the new isolates harbour similar orthologue groups compared to closely related strains (Fig. S1). In particular, these isolates harbour the same plant beneficial and insecticidal traits as other *P. protegens* strains already described, which were mainly isolated from soil or plants (Fig. 1; Table S2). This confirms that the distribution pattern of specific insecticidal and plant-beneficial traits is very conserved within this *Pseudomonas* subgroup. An exception is rhizoxin, a toxin with insecticidal activity so far only detected in a specific phylogenetic clade within the *P. protegens*
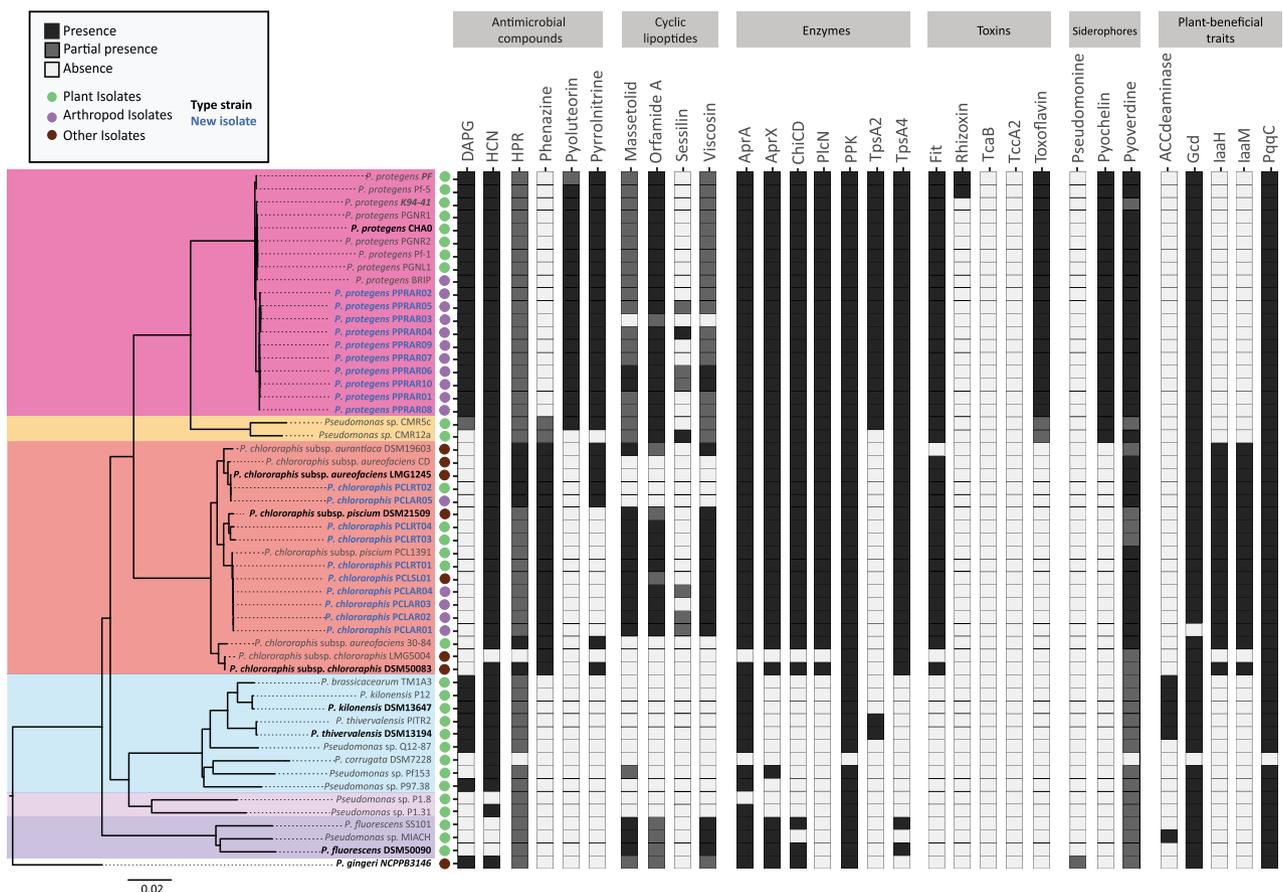

Fig. 1. Distribution of functional traits related to insecticidal and/or plant-beneficial activity among fluorescent *Pseudomonas*. The phylogenetic tree of 54 *Pseudomonas* genomes is based on the whole protein sequence extracted from the NCBI or predicted with Prokka. The squares correspond to the presence of amino acid sequences related to a functional trait. Black squares: present; grey squares: partially present; white squares: absent. Type strains are marked in bold and new isolates in blue. The origin of isolation is indicated with green dots for strains isolated from plants and with purple dots for arthropod isolates. Brown dots indicate other origins of isolation. References from all the strains are provided in Supplementary Tables S1 and S2.

Table 1. Summary of comparisons of insecticidal activity of different *Pseudomonas chlororaphis* and *Pseudomonas protegens* isolates with the reference strains *P. chlororaphis* PCL1391 and *P. protegens* CHA0.

Species	Strain	Origin of isolation	LT50 +	LT50 =	LT50 −	Log-rank +	Log-rank =	Log-rank −	=NaCl	Total experiments
P. chlororaphis	PCL1391	Tomato root								
P. chlororaphis	30.84	Wheat seed		1	1		2			2
P. chlororaphis	PCLAR01	*Aphodiinae* (Coleoptera)			3		3			3
P. chlororaphis	PCLAR02	*Aphodiinae* (Coleoptera)			2		2			2
P. chlororaphis	PCLAR03	Undefined Diptera sp.	2	3		1	4			5
P. chlororaphis	PCLAR04	*Scarabaeidae* pupa (Coleoptera)		1	5			3	3	6
P. chlororaphis	PCLAR05	*Scarabaeidae* larva (Coleoptera)	1	2	1	1	2	1		4
P. chlororaphis	PCLRT01	Grass land, roots		2		1	1			2
P. chlororaphis	PCLRT02	Potato root	3			1	2			3
P. chlororaphis	PCLRT03	Potato root		1	2	1	2			3
P. chlororaphis	PCLRT04	Potato root	1		2	1	2			3
P. chlororaphis	PCLSL01	Potato field, soil	1	1		1	1			2
P. protegens	CHA0	Tobacco root								
P. protegens	K94.41	Cucumber root	1	2		1	2			3
P. protegens	Pf-1	Tobacco root		2	1		2	1		3
P. protegens	Pf-5	Soil		2			2			2
P. protegens	PGNL1	Tobacco root		2			2			2
P. protegens	PGNR2	Tobacco root		2			2			2
P. protegens	PPRAR01	*Lithobius* (Myriapod)		2	1		2	1		3
P. protegens	PPRAR02	*Lithobius* (Myriapod)		2	1		2	1		3
P. protegens	PPRAR03	Larva (undefined Lepidoptera sp.)		3			3			3
P. protegens	PPRAR04	Agriotes (Coleoptera)		3			3			3
P. protegens	PPRAR05	*Staphylinidae* (Coleoptera)		2	1	1	1	1		3
P. protegens	PPRAR06	Agriotes (Coleoptera)		2		1	1			2
P. protegens	PPRAR07	*Lithobius* (Myriapod)		2		1	1			2
P. protegens	PPRAR08	*Geophilidae* (Myriapod)		1	1		2			2
P. protegens	PPRAR09	*Curculionidae* (Coleoptera)	1	1		2				2
P. protegens	PPRAR10	*Agrypnus murinus* (Coleoptera)		2			2			2

Numbers indicate the number of experiments in which a strain was significantly more insecticidal (+), less insecticidal (−) or not significantly different (=) compared to PCL1391 (reference for *P. chlororaphis*) or CHA0 (reference for *P. protegens*). Comparisons are based on LT50 and log-rank tests performed on Kaplan–Meyer survival curves. *Plutella xylostella* larvae were fed with pellets spiked with 4×10^6 bacterial cells and survival was recorded over time. LT50 values were calculated using the 'ecotox' package of R and considered significantly different if the 95% confidence intervals of the studied strains and references did not overlap. Insect survival curves were evaluated using the log-rank test in the 'survival' package of R and considered significantly different at $p < 0.05$. Detailed results of individual experiments are shown in Figs S2 and S3 (Kaplan–Meyer survival curves), Table S3 (LT50 values) and Table S4 (*p*-values of log-rank tests).

subgroup harbouring strains Pf-5 and PF. This toxin is absent in the new isolates, which is in line with their phylogenetic position (Fig. 1).

In contrast to *P. protegens*, *P. chlororaphis* were isolated from roots, soil and invertebrates. These isolates are closely related to the type strain *P. chlororaphis* subsp. *piscium* DSM 21509[T], *P. chlororaphis* subsp. *aureofaciens* LMG1245[T], or to *P. chlororaphis* subsp. *piscium* PCL1391 (Fig. 1, Fig. S1). Interestingly, *P. chlororaphis* isolates did not cluster according to the niche of isolation (soil, root or arthropod; Figs 1 and S1). Even though the *P. chlororaphis* subgroup was much more heterogeneous regarding the distribution of plant-beneficial/insecticidal traits, new *P. chlororaphis* isolates possess the same traits as the other strains they are clustering with (Fig. 1). Additionally, a group of strains closely related to *P. chlororaphis* subsp. *piscium* PCL1391 has more orthologue groups than the rest of the *chlororaphis* cluster (Fig. S1).

Although pseudomonads have been isolated from different laboratory-reared or field insects, none of them was fully characterized to the species level or were found to be insecticidal (Bahar and Demirbağ, 2007; Çakici et al., 2014; Bensidhoum et al., 2016; Mashtoly et al., 2019; Skowronek et al., 2020). Our results indicate that plant-beneficial *P. protegens* and *P. chlororaphis* are indeed commonly associated with apparently healthy arthropods and harbour the same traits as closely related strains isolated from soil or roots.

Pseudomonas protegens *and* P. chlororaphis *isolates show variable insecticidal activity independent of their root or arthropod origin*

Since *P. chlororaphis* and *P. protegens* isolated from arthropods and roots seem to display largely similar patterns of insecticidal traits, we wondered whether they would differ in insecticidal activity. To evaluate the insecticidal capabilities of the new isolates, second instar *P. xylostella* larvae were fed with artificial diet pellets spiked with 4×10^6 bacterial cells. Mortality was regularly

assessed which allowed to calculate the LT50 and the survival rates of the larvae. LT50 and mortality curves were compared to those of the well-studied reference strains *P. protegens* CHA0 and *P. chlororaphis* PCL1391, which are both plant-root isolates. These comparisons are summarized in Table 1 and further described in detail in Tables S3 and S4.

The performance of *P. protegens* strains was quite consistent as the killing speed and the global mortality of the larvae only differed occasionally compared to the reference *P. protegens* CHA0 (Table 1; Fig. S2A). *Pseudomonas chlororaphis* strains displayed more variability as several of them killed significantly slower or faster compared to the reference strain PCL1391 (Table 1) with particular isolates only causing partial mortality (Fig. S2B). Indeed, the LT50 values presented in Table S3 show a similar scenario with *P. protegens* strains differing much less among each other in the individual experiments than the *P. chlororaphis* strains. Kaplan–Meyer plots of individual experiments are shown in Fig. S3 and according to log-rank values in Table S4. In general, *P. protegens* killed *P. xylostella* larvae faster than *P. chlororaphis*, which was already observed by Flury *et al.* (2016). There were differences in larval susceptibility between the first sets of experiments (1–5) and the experiments performed 1 year later (6–16). In the first set, larvae died generally at a slower pace compared to the second, which was probably due to different fitness of larvae at different rearing times. LT50 values for larvae in the first set of experiments ranged from 37 to 70 h in the *P. protegens* and from 66 to 191 h in the *P. chlororaphis* treatments and in the second set from 20 and 37 h for *P. protegens* and from 20 to 57 h for *P. chlororaphis* (Table S3).

In summary, for both species root and arthropod isolates show similar levels of insecticidal activities. The variation observed for *P. chlororaphis* strains does not seem to be related to the phylogenetic position or the niche of isolation (plant, soil vs. arthropod). Root isolates of the *P. chlororaphis* species from crops or grassland showed similar variability in activity against *P. xylostella* larvae as did arthropod isolates (Table 1). A comparison of the clusters of orthologue genes (COG) revealed that there are no major differences in COG content according to phylogeny, origin of isolation or insecticidal capabilities (Fig. S4). This supports that the insecticidal capabilities of the strains are independent of their origin of isolation and phylogeny. Therefore, regarding the differences of killing speed and insect mortality, it could be hypothesized that these two bacterial species have the potential to modulate their virulence towards their hosts, leading to a continuum of possible interactions ranging from pathogenic to commensal under certain circumstances. Indeed, the new strains were isolated from healthy myriapods or healthy insects belonging to

different orders, mostly to Coleoptera. However, all of them were insecticidal in our feeding system with *P. xylostella*, even if they showed different progression of the infection. This might indicate species-specific adaptation. This is supported by several studies describing variable effects of *P. protegens* and *P. chlororaphis* on insects of different orders such as differences in mortality, in persistence or in causing anomalous morphologies in adults (Saravanakumar *et al.*, 2007; Olcott *et al.*, 2010; Kupferschmied *et al.*, 2013; Flury *et al.*, 2019). We speculate that the association of *P. chlororaphis* and *P. protegens* strains with Coleoptera might be predominantly commensal and only becomes pathogenic when the bacteria have access to the hemolymph. Moreover, under laboratory rearing conditions, the insects are not exposed to the nutrients and microorganisms of their natural habitat, which are relevant for the insect fitness and protection against invader microbes respectively (reviewed by Chambers and Schneider, 2012; Gurung *et al.*, 2019). The intake of different diets has been shown to affect the immune response of several insect species such as *Grammia incorrupta* and *Bombus terrestris* (Chambers and Schneider, 2012; Singer *et al.*, 2014; Roger *et al.*, 2017). Thus, further quantitative studies with field-collected Lepidoptera and Coleoptera would be necessary to confirm pathogenic or commensal species- or order-specific interactions of *P. protegens* and *P. chlororaphis* with insects.

Closely related P. chlororaphis *strains display high variability in insect colonization and killing activity but similar plant-protection abilities*

Considering the previous results, we aimed at further characterizing a subset of closely related strains that displayed divergent (i) orthologue groups, (ii) insecticidal activities towards the insect and (iii) origin of isolation (root vs. insect). We selected four *P. protegens* strains, i.e. two root isolates (CHA0 and K94.41) and two insect isolates (PPRAR03 and PPRAR04) and five *P. chlororaphis* strains including two root isolates (PCL1391 and PCLRT03) and three closely related insect isolates (PCLAR01, PCLAR03 and PCLAR04) (Fig. 1). These selected bacterial strains shared at least 96.61% of genome identity (based on ANIm values, Table S1). Strains of the same species were compared to each other for insecticidal activity, insect colonization ability, root colonization ability and efficacy in controlling root disease caused by the oomycete pathogen *Pythium ultimum*. Besides the mortality monitoring, a subset of insects was dedicated to assess the insect colonization at 5 and 30 h after infection.

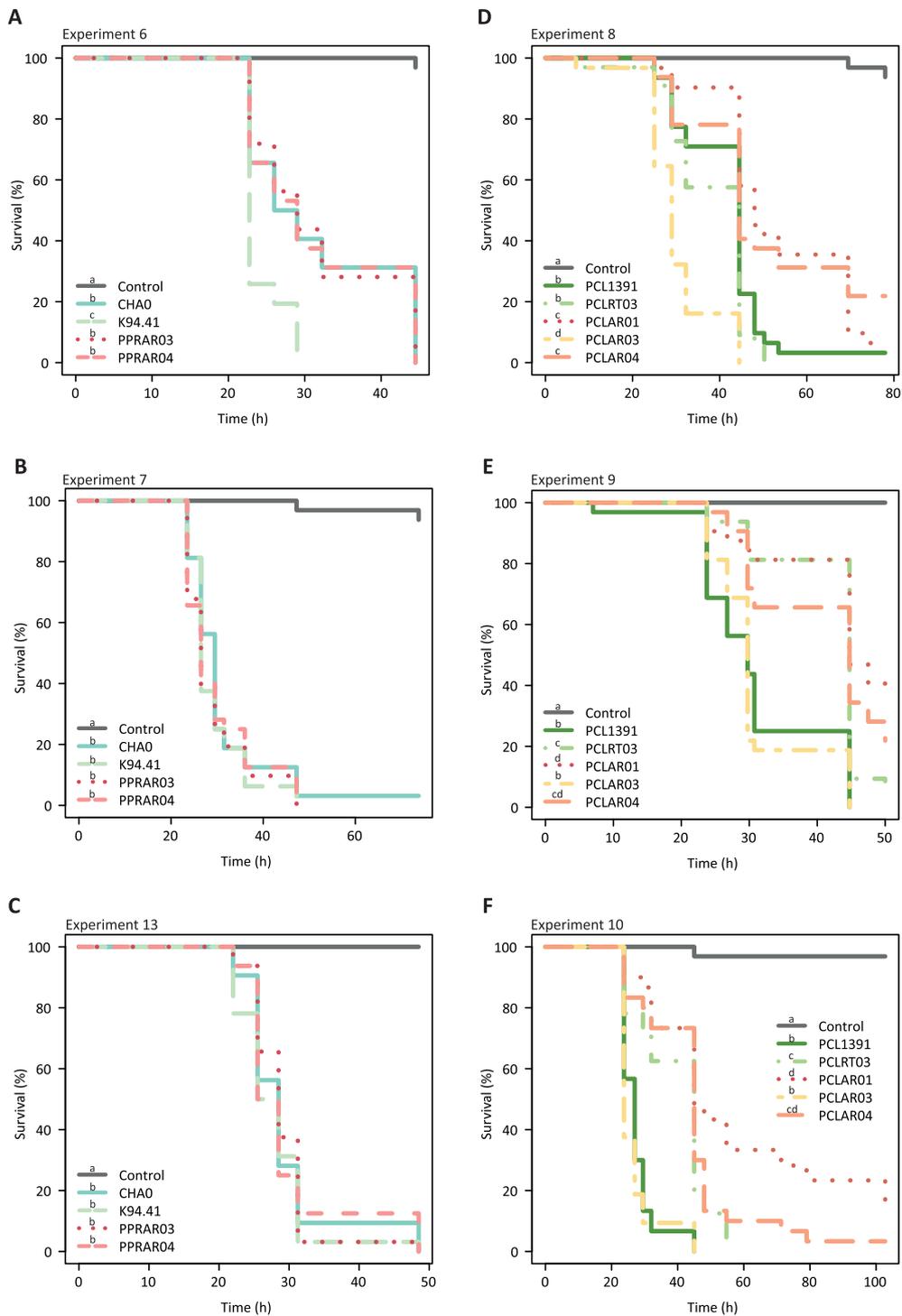*Pseudomonas protegens* isolates from roots and insects killed and colonized the *P. xylostella* larvae

Fig. 2. Survival of *Plutella xylostella* larvae after oral uptake of different *Pseudomonas protegens* (A, B, C) and *Pseudomonas chlororaphis* (D, E, F) strains. Second instar larvae were fed with artificial diet pellets spiked with 4×10^6 bacterial cells and mortality was assessed periodically by poking the insects. Strains were isolated from roots (*P. protegens* CHA0, *P. protegens* K94.41, *P. chlororaphis* PCL1391 and *P. chlororaphis* PCLRT03) or from arthropods (*P. protegens* PPRAR03, *P. protegens* PPRAR04, *P. chlororaphis* PCLAR01, *P. chlororaphis* PCLAR03 and *P. chlororaphis* PCLAR04). Statistical differences between the survival of the insects exposed to the different bacteria are depicted as different letters in the legend (Log-rank test $p < 0.05$). Thirty-two larvae were used in each experiment.

equally well in all experiments (Figs 2A–C and 3A), except the strain K94.41 that displayed a faster killing speed compared to the reference strain CHA0 in one out

of three experiments (Fig. 2A). All *P. protegens* strains were able to kill 100% of the larvae within 50 h and the insect colonization by *P. protegens* reached log 5 CFU

per larvae after 30 h (Fig. 3A). In contrast, the killing activity and the insect colonization by *P. chlororaphis* strains were more disparate at 30 h post-infection (Figs 2D–F and 3B). Indeed, the Coleoptera isolates (PCLAR01 and PCLAR04), closely related to PCL1391 in the phylogeny, displayed delayed killing speed in all three experiments compared to PCL1391. In contrast, the Diptera isolate (PCLAR03) killed similarly to PCL1391, or even faster in one of the experiments (Fig. 2D–F). Overall, we observed a higher mortality when there was a higher colonization of the insect by the *P. chlororaphis* strains (Fig. 3B).

To evaluate whether closely related strains exhibit similar root colonization and plant protection patterns, we inoculated cucumber plants with the different *Pseudomonas* strains and infected the plants with the oomycete plant-pathogen *Pythium ultimum* or left them untreated. After 9 days, the plant protection ability, assessed by measuring the disease severity (characteristic symptoms are presented in Fig. S5), the shoot fresh weight as well as the bacterial root colonization were determined. All the tested *P. protegens* and *P. chlororaphis* strains colonized roots similarly in absence of the plant pathogen (Figs S6 and S7). Moreover, the root colonization by these bacterial strains did not affect the shoot weight in the absence of the plant pathogen (Figs S8C-D and S9C-D). Regarding the plant protection ability, all *P. protegens* strains reduced disease in both experiments. Compared to infected control plants without added bacteria the number of dead plants was reduced by 50%–75% (Fig. S8A-B) and shoot weights were significantly higher (Fig. S8-D). Moreover, the root colonization levels of *P. protegens* strains slightly increased in presence of the pathogen but overall, all *P. protegens* strains colonized the roots similarly (Fig. S7). Regarding the *P. chlororaphis* strains, all of them reduced plant mortality (Fig. S8A-B), especially in the second replicate; however, their positive effect on shoot weights under *P. ultimum* attack was mostly not significant (Fig. S8A-C). Root colonization levels were similar among strains with the exception of PCLAR01 which established significantly higher population sizes on pathogen-infected roots compared to PCLRT03 and PCLAR03 in the second experiment (Fig. S9). In contrast to treatments with *P. protegens*, the root colonization by *P. chlororaphis* strains was only stimulated by the presence of the pathogen in individual cases (PCL1391, exp.1 and PCLAR03, exp. 2, Fig. S7).

In summary, the studied *P. protegens* isolates performed equally well on plants and in insects. In contrast, *P. chlororaphis* strains were quite homogenous in colonizing roots and suppressing the root pathogen but showed markedly higher variability regarding insect colonization and oral activity. Our findings show that, among

three phylogenetically closely related insect isolates, the two beetle isolates (PCLAR01 and PCLAR04) were less efficient *P. xylostella* colonizers/killers than the Diptera isolate. This might point to a possible adaptation to different insect species/orders that should be explored in future research, especially, since the isolated strains were not tested in the same arthropod species where they were found. These phenotypic differences could be related to small genetic and regulatory variations as shown for antimicrobial resistance in *P. aeruginosa* (Freschi *et al.*, 2018), or to other genomic plasticity mechanisms of importance for bacterial adaptation (reviewed by Silby *et al.*, 2011; Darmon and Leach, 2014; Li *et al.*, 2021).

Genetic differences between closely related Pseudomonas *may reflect recent host adaptation*

Insecticidal activity of *Pseudomonas* is a multifactorial trait, relying mainly on the production of the entomotoxin Fit, chitinases, antimicrobials and phospholipases (Péchy-Tarr *et al.*, 2008; Loper *et al.*, 2012; Kupferschmied *et al.*, 2013, 2016; Péchy-Tarr *et al.*, 2013; Flury *et al.*, 2016, 2017, 2019). As we highlighted a differential behaviour of *P. chlororaphis* isolates during insect assays, we analysed the COG terms associated to the strains PCLAR01, PCLAR03 and PCLAR04 and we performed a single nucleotide polymorphism (SNP) analysis comparing these insect isolates with the closely related root isolate *P. chlororaphis* subsp. *piscium* PCL1391.

The COG analysis revealed that the Coleoptera isolates PCLAR01 and PCLAR04 have more specific genes related to the 'replication, recombination and repair', 'mobilome, prophages and transposons', 'lipid transport and metabolism' and 'signal transductions mechanisms' terms than PCLAR03. On the other hand, the Diptera isolate PCLAR03 harbours more genes related to 'amino acid transport and metabolism', 'cell wall/membrane/envelope biogenesis', 'coenzyme transport and metabolism', 'energy production and conversion', 'nucleotide transport and metabolism' and 'translation, ribosomal structure and biogenesis' (Fig. S10). The extra metabolism and transduction-related genes in the Coleoptera isolates could be important to establish a commensal relationship with the insect. The higher number of genes related to the 'mobilome and recombination' COG category attests a higher genome plasticity that might be important to orient the bacteria to non-pathogenic lifestyles on insects.

Although these four strains shared at least 99% of genomic identity (Table S1), we identified more than 25 000 SNPs between the three insect isolates and PCL1391 (Table S5). PCLAR01 and PCLAR04 have ~25 000 variations and PCLAR03 ~29 000 compared to
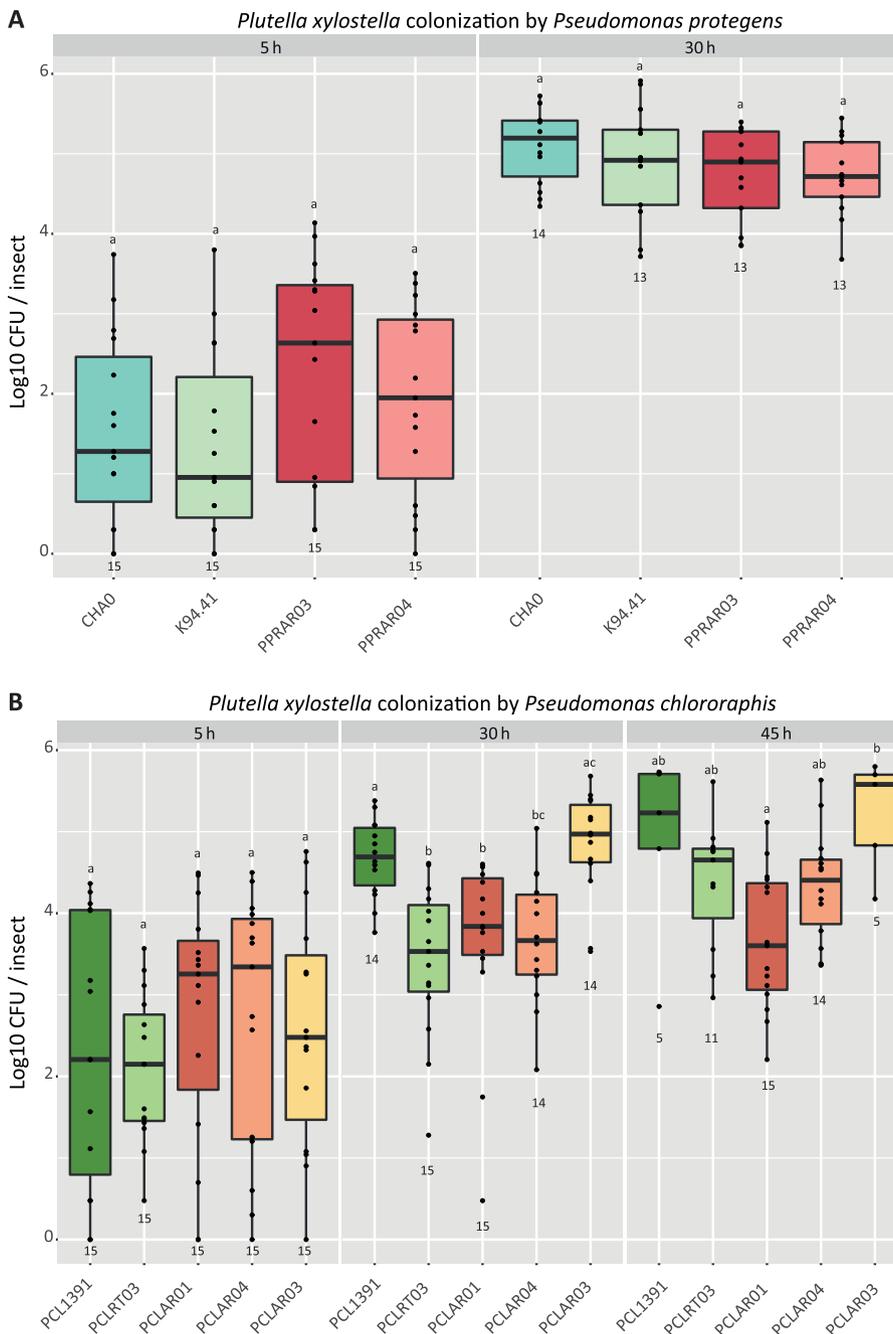
Fig. 3. Colonization of *Plutella xylostella* by different strains of *Pseudomonas protegens* (A) and *Pseudomonas chlororaphis* (B) after oral uptake. Second instar larvae were fed with pellets of artificial diet spiked with 4×10^6 bacterial cells. Larvae were surface sterilized and homogenated after 5, 30 or 45 h and serial dilutions of homogenates plated on selective medium. Strains were isolated from roots (*P. protegens* CHA0, *P. protegens* K94.41, *P. chlororaphis* PCL1391 and *P. chlororaphis* PCLRT03) or from arthropods (*P. protegens* PPRAR03, *P. protegens* PPRAR04, *P. chlororaphis* PCLAR01, *P. chlororaphis* PCLAR03 and *P. chlororaphis* PCLAR04). For each time point, significant differences between strains are indicated with different letters on top of the boxplots (Dunn's test $p < 0.05$). Numbers at the bottom of boxplots indicate numbers of extracted larvae.

the PCL1391 genome (NZ_CP027736.1) but only between 15% and 19% of the total changes were intragenic non-synonymous variations, i.e. cause an amino acid or structural change in the resulting protein (Table S5). The presence of additional genes in PCLAR01 and PCLAR04 (Figs S1 and S10) together with small genetic changes in specific regions (Table S5) might be another reason why the Coleoptera strains are delayed in insect killing compared to the Diptera isolate PCLAR03. It has been shown in *Yersinia pestis* and

Salmonella enterica serovar Typhi that, in order to adapt to a specific host, it is necessary to inactivate certain coding regions of the genome (Chain *et al.*, 2004; Klemm and Dougan, 2016). On the other hand, intergenic modifications could result in important changes in regulatory sequences such as small RNAs, riboswitches, promoters, terminators and regulator binding sites (Waters and Storz, 2009). These non-coding regions were shown to be under selective pressure in several pathogenic bacterial species, e.g. *Escherichia coli*, *Klebsiella*

pneumoniae, *S*. *enterica*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Thorpe *et al*., 2017).

Then, we focused our attention on the SNPs affecting genes known as keystones for insecticidal activity like those of the *fit* cluster (encoding entomotoxin Fit along with its type I secretion system, Péchy-Tarr *et al*., 2008), *plcN* (encoding a phospholipase, Flury *et al*., 2016; Vesga *et al*., 2020), *chiD* (encoding a chitinase, Flury *et al*., 2016) and also genes encoding effector proteins of TPS systems (Vesga *et al*., 2020). The *fit* cluster has
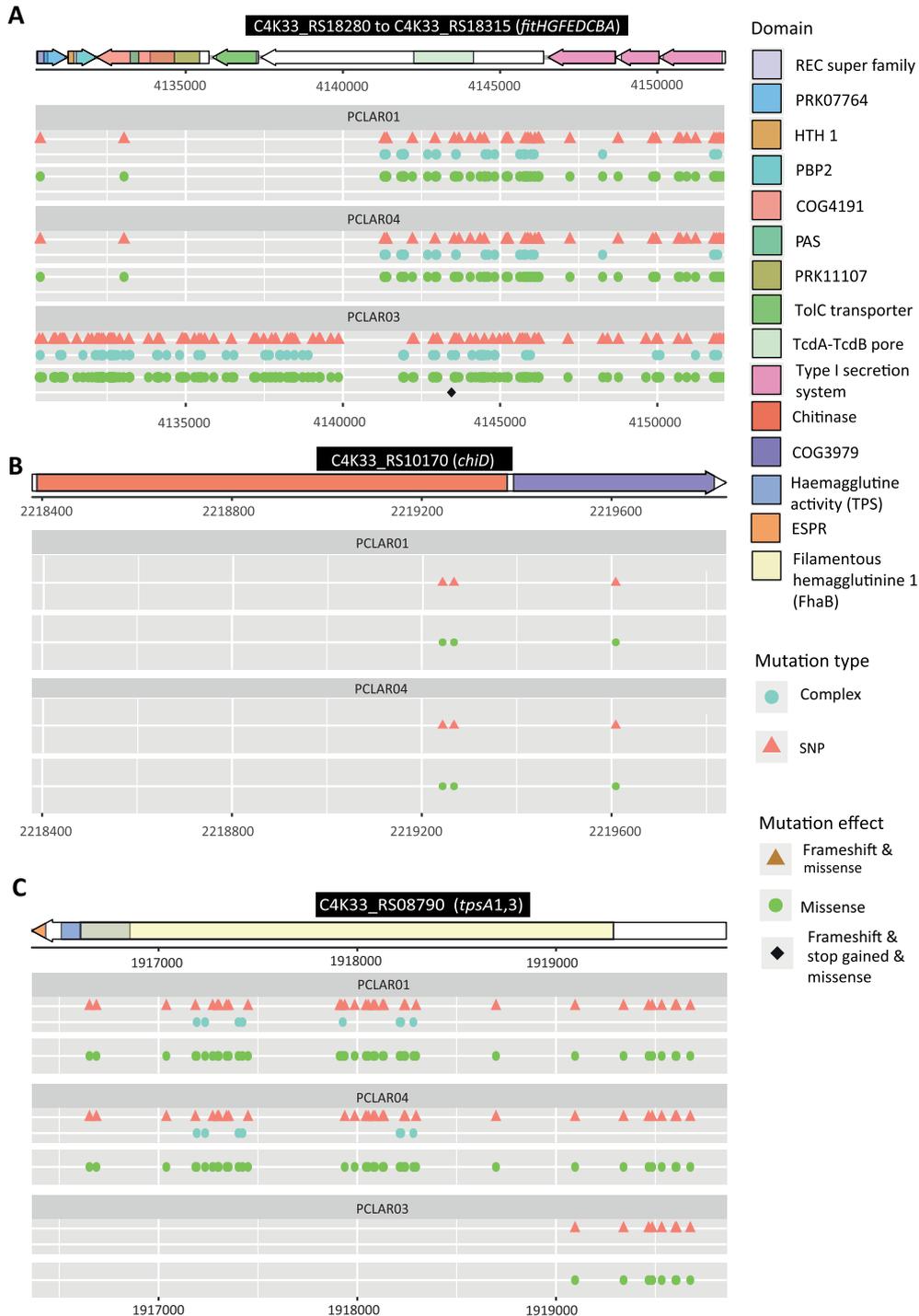

Fig. 4. Legend on next page.

numerous SNPs in the three strains but the regions of the Coleoptera isolates PCLAR01 and PCLAR04 related to the transport and regulation of the production of the Fit toxin had fewer SNPs than the corresponding region of the Diptera isolate PCLAR03 (Fig. 4A). The *fitD* gene showed numerous SNPs in all three strains, several of them affected the TcdA-TcdB pore-forming domain, which is the active domain of the protein (Ruffner *et al*., 2015). PCLAR01 and PCLAR04 have nine changes in amino acids in this active domain while PCLAR03 has 13. Additionally, PCLAR03 has a SNP that could lead to a stop codon inside the TcdA-TcdB domain but the insecticidal activity of this strain was even enhanced in one experiment (Fig. 4A). This change does not affect the virulence of the strain which supports the already known involvement of other mechanisms in insect pathogenesis in pseudomonads (Ruffner *et al*., 2013; Flury *et al*., 2016; Keel, 2016; Vesga *et al*., 2020).

Only the Coleoptera isolates had SNPs in the *chiD* gene while the Diptera isolate showed neither synonymous nor non-synonymous changes compared to PCL1391 (Fig. 4B; Table S6). Two out of three detected SNPs in the Coleoptera isolates affected the chitinase domain, one of them leading to a change of an amino acid (Fig.4B). Finally, the *plcN* gene in all three isolates had only one change from serine to proline that might not have a functional effect since these two amino acids are polar non-charged amino acids (Fig. S11A).

In addition to the insecticidal factors described above, we took a closer look at SNPs in three genes homologous to the *tpsA* genes encoding effector proteins of TPS systems involved in macrophage pyroptosis (Basso *et al*., 2017). C4K33_RS19905 and C4K33_RS21280 of PCL1391 have 77.97% and 80.08% nucleotide identity with the *tpsA2* and *tpsA4* of *P. protegens* CHA0 respectively. CK33_ RSA08790 has 80.13% nucleotide identity with *tpsA1* and 80.6% with *tpsA3* of CHA0. We, therefore, adopt the CHA0 annotation for these genes and will further refer to C4K33_RS19905 as *tpsA2*, to C4K33_RS21280 as *tpsA4* and to CK33_ RSA08790 as *tpsA1/A3*. We observed that compared to PCL1391, *tpsA4* did not have SNPs in any of the analysed strains and PCLAR03 did not show any changes in the *tpsA2* either (Table S6). On the other hand, the two Coleoptera isolates PCLAR01 and PCLAR04 displayed many missense variations in *tpsA2* encoding the TpsA2 effector, i.e. SNPs that lead to a predicted amino acid change in the protein (Fig. S11). In *P. protegens* CHA0, *tpsA2* is suggested to be involved in insect gut colonization/transmigration from gut to hemolymph (Reboud *et al*., 2017a; Reboud *et al*., 2017b; Vesga *et al*., 2020). It is tempting to speculate that the SNPs detected in the *tpsA2* gene of the two Coleoptera isolates could lead to reduced respectively, slowed down oral activity observed for these isolates in the *Plutella* feeding assays (Table 1; Fig. S3; Tables S3 and S4).

Compared to PCL1391, the three *P. chlororaphis* strains show more synonymous than non-synonymous variations for all these insecticidal factors, i.e. FitD, ChiD, PlcN and TpsA2. (Table S6). This is not the case for the third analysed TpsA encoding gene *tpsA1/A3*, which exhibits more non-synonymous than synonymous variations (Table S6). This gene shows a high concentration of missense variations throughout the coding sequence in the two Coleoptera isolates but only at the 5′ end of the gene for the Diptera isolates (Fig. 4C). TpsA encoding genes have been related to insecticidal activity in *P. protegens* CHA0 (Vesga *et al*., 2020) and macrophage killing and pathogenesis in *P. aeruginosa* PA7 and *Serratia marcescens* (Reboud *et al*., 2017a; Reboud *et al*., 2017b). The diversification of TpsA proteins might be important in the establishment of the diverse functional host–pathogen interactions. Therefore, the TpsA produced by PCLAR01 and PCLAR04 might be defective due to the different SNPs in the nucleotide sequence which leads to a delayed insecticidal activity.

Fig. 4. Non-synonymous variations in the hemagglutinin-like coding sequences of the gene cluster encoding the insecticidal toxin FitD of *P. chlororaphis* strains (A), the chitinase ChiD (B) and the two-partner secretion system TpsA1/3 (A). Genes are depicted by arrows on top of the panels A, B and C, and the protein domain coding sequences are indicated within each gene. Lower panels show the variations harboured by strains PCLAR01 (Coleoptera isolate), PCLAR04 (Coleoptera isolate) and PCLAR03 (Diptera isolate) compared to the genome of the reference strain *P. chlororaphis* PCL1391. *fitHGF* are the regulatory genes of the fit cluster. REC: response regulator domain; HTH 1 and PBP2: typical domains of signal transducers; PRK11107 and COG4191: part of a sensor histidine-kinase; PAS: is a structural domain. The TcdA-TcdB domain of the FitD toxin is predicted to form a pore in the target cell (Daborn *et al*., 2002; Waterfield *et al*., 2003; Ruffner *et al*., 2015). The *fitABCE* genes harbour the domains of a type I secretion system predicted to secrete the FitD toxin to the extracellular space. The chitinase domain has the active function of degrading chitin; COG3979: chitodextrinase domain that catalyses the hydrolysis of chitin oligosaccharides. The hemagglutinin activity (TPS) domain is used to interact with the transporter protein for membrane translocation; the filamentous hemagglutinins are repeats used to attach to the host cells and translocate into the host; Filamentous hemagglutinin 1 (FhaB): filamentous-hemagglutinin domain of Bordetella pertussis; ESPR: signal domain related to type V secretion systems. Mutation type: SNP = single nucleotide polymorphisms; complex = insertions, deletions or larger sequence changes. Mutation effect: modifications that the mutations will cause in the protein, i.e. missense when they cause a change of amino acid and frame-shift when the translation frame is affected.

Both COG and SNP analyses of the present study highlight the genomic diversity in the insecticidal *Pseudomonas* isolates. High genetic variation allows a bacterial population to rapidly expand and fill a new niche or colonize a new host. The easy switch of hosts, as it seems to be the case for *P. protegens* and *P. chlororaphis*, indicates that these bacteria have developed as generalists rather than specialists. *Pseudomonas* are well known to undergo numerous genetic re-arrangements during host colonization, which increases the variability within the population and allows it to adapt to and survive in a new environment (Broek *et al*., 2005).

Conclusion

Pseudomonas are present in very different environments as free bacteria and also associated to other organisms in pathogenic, commensal or beneficial relationships. Our study shows that the two species *P. protegens* and *P. chlororaphis* naturally occur in several insect and myriapod classes. Interestingly all *P. protegens* strains isolated in this study were always associated with insects, whereas we found insecticidal *P. chlororaphis* associated with insects, myriapods, roots and soil which is an indication for the variability and adaptability of this latter species. All *P. protegens* and *P. chlororaphis* strains we have characterized so far have the ability to colonize plant and insect hosts, exercise a certain insect-killing activity and control root pathogens. The findings presented here provide evidence that *P. protegens* isolates are much more consistent in insecticidal abilities while *P. chlororaphis* are more variable which might reflect a certain degree of adaptation to different hosts. However, this remains purely speculative since also all *P. protegens* isolates obtained in this study, although highly lethal to *P. xylostella*, were isolated from healthy arthropods, plant roots or soil. Altogether, our observations raise the question of whether these species have evolved towards commensal, pathogenic and symbiotic interactions with arthropods as a new niche or if they are just very versatile colonizers able to adapt to any new environment. This highlights how far we actually are from fully understanding their ecology and the particular mechanisms allowing them to colonize such contrasting environments. We observed that the isolation site or the phylogeny does not always reflect the insecticidal capabilities of fluorescent *Pseudomonas* as closely related strains exhibit differential insecticidal activities. In two *P. chlororaphis* insect isolates displaying lower insecticidal activity we have identified non-synonymous variations in some insecticidal factors which potentially could explain their reduced or slowed down insect-killing capacities. Our findings corroborate that these bacteria are multi-talented and able to conquer very different niches and exploit cross-kingdom

hosts. The ability of *P. protegens* and *P. chlororaphis* to successfully colonize plant roots, to enhance plant growth and control pests and pathogens, makes them promising candidates for future biocontrol applications.

Experimental procedures

Isolation of Pseudomonas *from different habitats*

To assess the natural occurrence of *P. protegens* and *P. chlororaphis*, arthropods, soil and plant roots were collected in spring 2016 and spring 2017 in an agricultural field (wheat in 2016, potato in 2017) and a neighbouring undisturbed grassland area. The field is located at Agroscope Reckenholz, Zurich, Switzerland (47° 25′ 32″ N; 8° 30′ 57″ E). Arthropod, root and soil samples were collected up to 50 cm of soil depth (soil horizon characteristics are included in Table S7). The collected arthropods were visually classified as detailed as possible.

For the arthropod samples, all animals were surface disinfected first with 96% ethanol and then with 70% ethanol (20 s each). After each disinfection step, the animals were rinsed with distilled H_2O amended with 0.05% SDS. The animals were homogenized in 1 ml or 15 ml 0.9% NaCl depending on their size with a Polytron PT-DA 2112 blender (Kinematica, Littau, Switzerland). For root samples, the soil attached to the roots was removed with tap water. From 0.5–2 g of roots were incubated in 50 ml of sterile 0.9% NaCl solution overnight at 3 °C without shaking. Roots were shaken for 30 min at 3 °C and 350 rpm to detach the bacteria from the root surface. For the soil samples, 20 g of sieved soil was suspended in 100 ml of 0.9% NaCl and solution shaken for 3 h at 150 rpm and 3 °C.

Undiluted and serially diluted suspensions derived from arthropods, roots and soil were plated on the *Pseudomonas* isolation medium King's B supplemented with ampicillin, 40 μl ml^{-1}; chloramphenicol, 13 μl ml^{-1}; and cycloheximide, 100 μl ml^{-1} (KB+++) and kept at 24°C (King *et al*., 1954; Landa *et al*., 2002). Growing colonies were purified twice successively in KB+++. All strains were stored in 43% glycerol at −80°C.

Identification of P. chlororaphis *and* P. protegens

A single colony from each isolate was picked and cultured overnight at 24°C in lysogeny broth (LB, Bertani, 1951), diluted and used for PCR with the DreamTaq polymerase (ThermoFisher Scientific, MA, USA). To confirm that the bacteria isolated belong to the *Pseudomonas* genus, a PCR was performed on all isolated strains using a *Pseudomonas*-specific 16S rRNA primer (forward Pse435F 5′-<u>ACT TTA AGT TGG GAG GAA GGG</u>-3′; reverse Pse686R 5′-<u>ACA CAG GAA ATT</u>

CCA CCA CCC-3′; annealing temperature 60°C, expected fragment of 252 bp; Bergmark *et al*., 2012). Then to detect specifically the isolates assigned to *P. protegens* and *P. chlororaphis* subgroups, positive strains were then screened using a *fitD*-specific primer (forward FitD66F 5′-CTA TCG GGT SCA GTT CAT CA-3′; reverse FitD308R 5′-TTC TTG TCG GSA AAC CAC T-3′; annealing temperature 60°C; expected fragment of 242 bp; Flury *et al*., 2017). So far, the presence of the Fit toxin was exclusively found in strains belonging to *P. protegens* and *P. chlororaphis* species (Flury *et al*., 2016). Finally, to confirm the assignation of the isolates to either *P. protegens* or *P. chlororaphis* species, DNA from *fitD*-positive *Pseudomonas* strains was extracted with Wizard Genomic DNA purification Kit (Promega, WI, USA) and a larger fragment of the 16S rRNA (1465 bp) was amplified using the following primers: forward 16F27 5′-AGA GTT TGA TCM TGG CTC AG-3′; reverse 16R1492 5′-TAC GGY TAC CTT GTT ACG ACT T-3′ and annealing temperature of 55°C (Lane, 1991). The PCR product was purified with the FastAP Thermosensitive Alkaline phosphatase and the Exonuclease I (ThermoFisher Scientific). The sequencing reaction was set up with the purified PCR product using the Big Dye v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) and further cleaned with Sephadex GM-50 (GE-Healthcare, MA USA). PCR fragment sequencing was performed by Sanger sequencing following manufacturer's instructions (3130xl DNA analyser, Applied Biosystems) to identify *P. chlororaphis* and *P. protegens*. The data generated for bacterial phylogenetic identification were generated in collaboration with the Genetic Diversity Center (GDC), ETH Zurich.

Pseudomonas protegens *and* P. chlororaphis *genome sequencing*

The DNA from 10 *P. chlororaphis* strains and 13 *P. protegens* strains was extracted with Wizard Genomic DNA purification Kit (Promega) and the genome sequenced by MiSeq (2 × 300 paired-end v3 with Nextera XT library kit, Illumina, CA, USA). Alternatively, DNA was extracted with the MagAttract high-molecular-weight DNA kit (Qiagen, Germany) and sequenced by PacBio (SMRT v3, SMRTbell template preparation kit v1.0, Pacific Biosciences, CA, USA). The *de novo* genome assembly was performed using MIRA v4.0.2 with standard settings in accurate mode (MiSeq-sequenced strains) or RS_HGAP_Assembly.4 protocol in SMRT Link v6.0 for (PacBio sequenced strains). Genomes were deposited in NCBI's genome repository under the Bioproject ID PRJNA637204.

The GC content, the average nucleotide identity and the tetranucleotide analysis indexes were assessed with JSpecies v1.2.1 and are listed in Table S2, which also indicates the DNA extraction method and the sequencing technology for each strain.

Insect assays

To assess the insecticidal activities of the new isolates, a *P. xylostella* feeding assay was performed as described in detail in Flury *et al*. (2019). Briefly, *P. xylostella* eggs obtained from Syngenta Crop Protection AG (Stein, Switzerland) were reared in a growth chamber at 25°C, 60% of relative humidity and a cycle of 16 h day (162 μmol m^{-2} s^{-1}) and 8 h of darkness. Second instar larvae (about 5 days old) were fed with artificial diet pellets spiked with 10 μl of sterile 0.9% NaCl solution or of cell suspensions of the different bacterial strains (4 × 10^8 colony-forming units (CFU)/pellet). Cell suspensions were prepared with washed cells from overnight LB cultures. To avoid cannibalism, larvae were kept individually in 128-well bioassay trays (Frontier Agricultural Sciences, Delaware, USA) at the same conditions used for rearing. Survival was regularly assessed by poking the larvae with a tip. Survival curves of different treatments were compared using the log-rank test (*p*-value <0.05) from the 'survival 3.1.8' package (https://github.com/therneau/survival, Therneau and Grambsch, 2000) of R 3.6.0 (www.r-project.org). Statistical differences were calculated for the individual experiments but pooled experiments were plotted in a Kaplan–Meier graph as well to show an overview of all the experiments together. LT50 values were estimated using the 'ecotox 1.4.1' R package (https://www.rdocumentation.org/packages/ecotox/versions/1.4.1) using LT_probit option and considered significantly different when the 95% intervals were not overlapping between the strains. All LT50 values are included in Table S3 and *p*-values of the log-rank test in Table S4. All strains were tested at least twice in independent experiments. In total, 16 experiments were performed. In every experiment, reference strains *P. chlororaphis* PCL1391 and *P. protegens* CHA0 were included as an internal standard.

A subset of *P. xylostella* larvae in the feeding assays was used to assess the ability of the strains to colonize insects. Five larvae per treatment were collected at different time points (5 and 30 h for *P. protegens* strains and 5, 30 and 45 h for *P. chlororaphis* strains) and washed twice in 70% EtOH for 20 s with subsequent rinsing with sterile H$_2$O amended with 0.05% SDS. Insects were homogenized in 1 ml of sterile 0.9% NaCl solution with a Polytron PT-DA 2112 blender (Kinematica). Homogenates were serially diluted and plated onto KB+++ medium. CFUs were assessed after incubating plates for 2 days at 27°C. There were no statistical differences between the experiments (Kruskal–Wallis *p*-value <0.05),

therefore, the data were pooled. Statistical differences in colonization numbers among strains were assessed using a Dunns's test (*p*-value <0.05) from the 'FSA' R package (https://github.com/droglenc/FSA). In the boxplot graphs, boxes correspond to the 25th and 75th percentiles, lines inside the boxes indicate the median and whiskers correspond to 1.5 times the interquartile range.

Plant assays

The ability of isolated strains to colonize roots and to protect plants against a root pathogen was tested in a cucumber pot assay with natural soil and the oomycete pathogen *Pythium ultimum*. The soil collected at the sampling site at Agroscope Reckenholz, Switzerland (see above) was used because we wanted to test the root colonization ability and disease suppressive capacity of the new isolates under competitive conditions, i.e. in the presence of the native soil microorganisms from where the isolates were obtained.

Cultivation of pathogen, bacteria and plants. Pythium ultimum strain Pu-11 was grown on a malt agar plate at 18°C for 7 days. Three plugs with fungal mycelium were transferred to twice-autoclaved millet (organic millet, Migros, Switzerland) and the pathogen was cultivated at 18°C for another 7 days. *Pseudomonas* isolates were grown overnight in LB at 180 rpm and 24°C. From these cultures, aliquots of 200 µl were spread onto KB+++ plates and incubated at 27°C for 24 h. Bacteria were scraped from plates and washed with sterile 0.9% NaCl solution. Suspensions used for pot inoculation were adjusted to an OD_{600} of 4.0 (approx. 3.2×10^9 cells ml^{-1}). Cucumber seeds of the variety 'Chinese Snake' (Bigler Samen AG, Thun, Switzerland) were sterilized in 1.4% NaClO for 30 min, washed thoroughly with sterile water and left to germinate on moistened filter paper for 1.5 days at 24°C in the dark.

Set up of pot experiments. 250 ml pots were filled with 320 g of a 4:1 soil/quartz sand (1.5–2.2 mm diameter) mixture. Each pot received 1 g of the *P. ultimum*-millet inoculum. Control treatments without pathogen received 1 g of autoclaved *P. ultimum*–millet mixture. The pathogen inoculum was thoroughly mixed into the soil. Then, 5 ml of a *Pseudomonas* cell suspension was added per pot resulting in a final concentration of 10^7 cells per gram of soil. Control treatments were amended with the same volume of sterile 0.9% NaCl solution. Finally, pots received 45 ml of sterile water and three cucumber seedlings were planted per pot. Plants were grown for 10 days in a growth chamber set at 70% of relative humidity with a cycle of 16 h of light (210 mmol m^2 s^{-1}) at 22°C, followed by an 8 h dark period at 20°C. Six replicate pots

with pathogen and six without pathogen were prepared per bacterial strain. Two independent experiments were performed over time.

Evaluation of pot experiments. Nine days after inoculation, plant mortality and disease severity were assessed (see Fig. S5 for disease classification) and shoots fresh weight was assessed. Roots were washed, weighted and incubated overnight at 3 °C in 50 ml of autoclaved sterile 1% NaCl solution. The next day, roots were shaken at 400 rpm and 3 °C for 30 min to detach the bacteria from the root surface. Serial dilutions of the obtained root suspensions were plated onto KB+++ and CFU numbers were assessed after 2 days of incubation at 27°C.

Since the factor experiment had a significant impact on root colonization and shoot weight data (Kruskal–Wallis *p*-value <0.05), the data of individual experiments could not be pooled. Data were first subjected to Shapiro–Wilk normality test (*p*-value <0.05) to check normal distribution. Differences in root colonization were assessed with one-way ANOVA (*p*-value <0.05) for experiments with normally distributed data (both *P. protegens* experiments and the second *P. chlororaphis* experiment) or Kruskal–Wallis (*p*-value <0.05) if data were not normally distributed (first *P. chlororaphis* experiment). Shoot weight data of all experiments were not normally distributed. Therefore, a Dunn's test (*p*-value <0.05) was used to assess the differences between the shoot weights of plants treated with the different *Pseudomonas* strains. Kruskal–Wallis and ANOVA test are part of the 'stats' package built-in within R. In the boxplot graphs, boxes correspond to the 25th and 75th percentiles, lines inside the boxes indicate the median and whiskers correspond to 1.5 times the interquartile range.

Genome analyses

Orthologue analysis. Genomes from 54 *Pseudomonas* strains were annotated with Prokka 1.14.6 (Seemann, 2014) using the standard settings. Further information about the strains is displayed in Table S1. The resulting 54 amino acid sequences were analysed with Orthofinder 2.3.12 (Emms and Kelly, 2015) to obtain a presence–absence matrix of orthologue groups across the different strains. The phylogenetic information was processed with FigTree 1.4.4 to generate the tree (https://github.com/rambaut/figtree/releases/tag/v1.4.4).

Secondary metabolites genome mining. The distribution of insecticidal and plant-beneficial traits within the *Pseudomonas fluorescens* species complex was done by screening a total of 126 amino acid sequences by BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A function was considered present inside a genome when

showing at least 65% of identity over 70% of the length of the amino acid sequences, a lower than 50 bit-score and an *e*-value of less than 0.01. Gene clusters were considered present only when all the coding sequences fulfilled the previously mentioned requirements and partial when there were matches for some of the coding sequences of the cluster. The amino acid sequences of the studied traits are included in Supplementary File S2.

Clusters of orthologue genes counting. The 54 *Pseudomonas* genomes were compared using Roary 3.13.0 (Page *et al*., 2015) to assess the gene presence. Prokka (Seemann, 2014) annotations were used to assess the frequency of different COG in different strain categories, namely, phylogeny, insecticidal activity and origin of isolation. The same analysis was performed on the Coleoptera and Diptera interesting strains using only the specific genes of each of these strains.

SNPs in Coleoptera and Diptera isolates. To detect the genetic differences between the characterized isolates and their reference (*P. chlororaphis* subsp. *piscium* PCL1391, accession number NZ_CP027736.1), Snippy was used indicating that the inputs were contigs and not raw reads. Non-synonymous variations were selected to identify which genes had the highest content of changes among the strains. Specific regions corresponding to the genes C4K33_RS18280 to C4K33_18315 (*fitHGFEDCBA* cluster), C4K33_RS15780 (*plcN*), C4K33_RS10170 (*chiD*), C4K33_RSA19905 (*tpsA2*), C4K33_RS08790 (*tpsA1/3*) and C4K33_RS21280 (*tpsA4*) in PCL1391 (NZ_CP027736.1) were analysed more in detail. To check if any of the variations were affecting the predicted protein domains, the nucleotide sequence of the genes was used to find conserved domains in the Conserved Domain database of the NCBI (www.ncbi.nlm.nih.gov/cdd/).

Acknowledgements

Data Availability Statement

The genome sequences were deposited on the NCBI under the BioProject ID PRJNA637204.

References

AbdulWahab, A., Taj-Aldeen, S.J., Ibrahim, E., Abdulla, S. H., Muhammed, R., Ahmed, I., *et al*. (2014) Genetic relatedness and host specificity of *Pseudomonas aeruginosa* isolates from cystic fibrosis and non-cystic fibrosis patients. *Infect Drug Resist* **7**: 309–316.

Afzal, I., Shinwari, Z.K., Sikandar, S., and Shahzad, S. (2019) Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. *Microbiol Res* **221**: 36–49.

Aharon, Y., Pasternak, Z., Ben Yosef, M., Behar, A., Lauzon, C., Yuval, B., and Jurkevitch, E. (2013) Phylogenetic, metabolic, and taxonomic diversities shape mediterranean fruit fly microbiotas during ontogeny. *Appl Environ Microbiol* **79**: 303–313.

Bahar, A., and Demirbağ, Z. (2007) Isolation of pathogenic bacteria from *Oberea linearis* (Coleptera: *Cerambycidae*). *Biologia* **62**: 13–18.

Basso, P., Wallet, P., Elsen, S., Soleilhac, E., Henry, T., Faudry, E., and Attrée, I. (2017) Multiple *Pseudomonas* species secrete exolysin-like toxins and provoke Caspase-1-dependent macrophage death. *Environ Microbiol* **19**: 4045–4064.

Bensidhoum, L., Nabti, E., Tabli, N., Kupferschmied, P., Weiss, A., Rothballer, M., *et al*. (2016) Heavy metal tolerant *Pseudomonas protegens* isolates from agricultural well water in northeastern Algeria with plant growth promoting, insecticidal and antifungal activities. *Eur J Soil Biol* **75**: 38–46.

Bergmark, L., Poulsen, P.H.B., Al-Soud, W.A., Norman, A., Hansen, L.H., and Sørensen, S.J. (2012) Assessment of the specificity of *Burkholderia* and *Pseudomonas* qPCR assays for detection of these genera in soil using 454 pyrosequencing. *FEMS Microbiol Lett* **333**: 77–84.

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

Broek, D.V.D., Bloemberg, G.V., and Lugtenberg, B. (2005) The role of phenotypic variation in rhizosphere *Pseudomonas* bacteria. *Environ Microbiol* **7**: 1686–1697.

Çakici, F.Ö., Sevií, M.,.A., Demiírbağ, Z., and Demií, R.,.İ. (2014) Investigating internal bacteria of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae and some *Bacillus* strains as biocontrol agents. *Turk J Agric For* **38**: 12.

Chain, P.S.G., Carniel, E., Larimer, F.W., Lamerdin, J., Stoutland, P.O., Regala, W.M., *et al*. (2004) Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. *Proc Natl Acad Sci U S A* **101**: 13826–13831.

Chambers, M.C., and Schneider, D.S. (2012) Pioneering immunology: insect style. *Curr Opin Immunol* **24**: 10–14.

Chen, B., Teh, B.-S., Sun, C., Hu, S., Lu, X., Boland, W., and Shao, Y. (2016) Biodiversity and activity of the gut

microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci Rep* **6**: 29505.

Chouaia, B., Goda, N., Mazza, G., Alali, S., Florian, F., Gionechetti, F., *et al*. (2019) Developmental stages and gut microenvironments influence gut microbiota dynamics in the invasive beetle *Popillia japonica* Newman (Coleoptera: *Scarabaeidae*). *Environ Microbiol* **21**: 4343–4359.

Daborn, P.J., Waterfield, N., Silva, C.P., Au, C.P.Y., Sharma, S., and ffrench-Constant, R.H. (2002) A single *Photorhabdus* gene, makes caterpillars floppy (*mcf*), allows *Escherichia coli* to persist within and kill insects. *Proc Natl Acad Sci U S A* **99**: 10742–10747.

Darmon, E., and Leach, D.R.F. (2014) Bacterial genome instability. *Microbiol Mol Biol Rev* **78**: 1–39.

Dillon, R.J., and Dillon, V.M. (2004) The gut bacteria of insects: nonpathogenic interactions. *Annu Rev Entomol* **49**: 71–92.

Emms, D.M., and Kelly, S. (2015) OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol* **16**: 157–171.

Engel, P., and Moran, N.A. (2013) The gut microbiota of insects – diversity in structure and function. *FEMS Microbiol Rev* **37**: 699–735.

Esposti, M.D., and Romero, E.M. (2017) The functional microbiome of arthropods. *PLoS One* **12**: e0176573.

Flury, P., Aellen, N., Ruffner, B., Péchy-Tarr, M., Fataar, S., Metla, Z., *et al*. (2016) Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and comparative genomics. *ISME J* **10**: 2527–2542.

Flury, P., Vesga, P., Dominguez-Ferreras, A., Tinguely, C., Ullrich, C.I., Kleespies, R.G., *et al*. (2019) Persistence of root-colonizing *Pseudomonas protegens* in herbivorous insects throughout different developmental stages and dispersal to new host plants. *ISME J* **13**: 860–872.

Flury, P., Vesga, P., Péchy-Tarr, M., Aellen, N., Dennert, F., Hofer, N., *et al*. (2017) Antimicrobial and insecticidal: cyclic lipopeptides and hydrogen cyanide produced by plant-beneficial *Pseudomonas* strains CHA0, CMR12a, and PCL1391 contribute to insect killing. *Front Microbiol* **8**: 1–15.

Freschi, L., Bertelli, C., Jeukens, J., Moore, M.P., Kukavica-Ibrulj, I., Emond-Rheault, J.-G., *et al*. (2018) Genomic characterisation of an international *Pseudomonas aeruginosa* reference panel indicates that the two major groups draw upon distinct mobile gene pools. *FEMS Microbiol Lett* **365**: 1–11.

Geli-Cruz, O.J., Cafaro, M.J., Santos-Flores, C.J., Ropelewski, A.J., and Van Dam, A.R. (2019) Taxonomic survey of *Anadenobolus monilicornis* gut microbiota via shotgun nanopore sequencing. *BioRxiv*.

Gomes, A.F.F., Omoto, C., and Cônsoli, F.L. (2020) Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. *J Pest Sci* **93**: 833–851.

González-Serrano, F., Pérez-Cobas, A.E., Rosas, T., Baixeras, J., Latorre, A., and Moya, A. (2019) The gut microbiota composition of the moth *Brithys crini* reflects insect metamorphosis. *Microb Ecol* **79**: 960–970.

Gurung, K., Wertheim, B., and Salles, J.F. (2019) The microbiome of pest insects: it is not just bacteria. *Entomol Exp Appl* **167**: 156–170.

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

Hammer, T.J., Janzen, D.H., Hallwachs, W., Jaffe, S.L., and Fierer, N. (2017) Caterpillars lack a resident gut microbiome. **114**: 9641–9646.

Hammer, T.J., McMillan, W.O., and Fierer, N. (2014) Metamorphosis of a butterfly-associated bacterial community. *PLoS One* **9**: e86995.

Hannula, S.E., Zhu, F., Heinen, R., and Bezemer, T.M. (2019) Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. *Nat Commun* **10**: 1254.

Jang, J.Y., Yang, S.Y., Kim, Y.C., Lee, C.W., Park, M.S., Kim, J.C., and Kim, I.S. (2013) Identification of orfamide A as an insecticidal metabolite produced by *Pseudomonas protegens* F6. *J Agric Food Chem* **61**: 6786–6791.

Keel, C. (2016) A look into the toolbox of multi-talents: insect pathogenicity determinants of plant-beneficial pseudomonads. *Environ Microbiol* **18**: 3207–3209.

King, E.O., Ward, M.K., and Raney, D.E. (1954) Two simple media for the demonstration of pyocyanin and fluorescin. *J Lab Clin Med* **44**: 301–307.

Klemm, E., and Dougan, G. (2016) Advances in understanding bacterial pathogenesis gained from whole-genome sequencing and phylogenetics. *Cell Host Microbe* **19**: 599–610.

Kolasa, M., Ścibior, R., Mazur, M.A., Kubisz, D., Dudek, K., and Kajtoch, Ł. (2019) How hosts taxonomy, trophy, and endosymbionts shape microbiome diversity in beetles. *Microb Ecol* **78**: 995–1013.

Kupferschmied, P., Chai, T., Flury, P., Blom, J., Smits, T.H. M., Maurhofer, M., and Keel, C. (2016) Specific surface glycan decorations enable antimicrobial peptide resistance in plant-beneficial pseudomonads with insect-pathogenic properties. *Environ Microbiol* **18**: 4265–4281.

Kupferschmied, P., Maurhofer, M., and Keel, C. (2013) Promise for plant pest control: root-associated pseudomonads with insecticidal activities. *Front Plant Sci* **4**: 1–17.

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

Landa, B.B., de Werd, H.A.E., McSpadden Gardener, B.B., and Weller, D.M. (2002) Comparison of three methods for monitoring populations of different genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere. *Phytopathology* **92**: 129–137.

Lane, D. (1991) 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*. Chichester, England: John Wiley and Sons, E. Stackebrandt and M. Goodfellow, pp. 115–147.

Lauzon, C.R., McCombs, S.D., Potter, S.E., and Peabody, N.C. (2009) Establishment and vertical passage of *Enterobacter* (*Pantoea*) *agglomerans* and *Klebsiella pneumoniae* through all life stages of the mediterranean fruit fly (Diptera: *Tephritidae*). *Ann Entomol Soc Am* **102**: 85–95.

Li, E., Zhang, H., Jiang, H., Pieterse, C.M.J., Jousset, A., Bakker, P.A.H.M., and de Jonge, R. (2021) Experimental evolution-driven identification of Arabidopsis rhizosphere competence genes in *Pseudomonas protegens*. *mBio* **12**(3).

Loper, J.E., Hassan, K.A., Mavrodi, D.V., Davis, E.W., Lim, C.K., Shaffer, B.T., *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.

Loper, J.E., Henkels, M.D., Rangel, L.I., Olcott, M.H., Walker, F.L., Bond, K.L., *et al*. (2016) Rhizoxin analogs, orfamide A and chitinase production contribute to the toxicity of *Pseudomonas protegens* strain Pf-5 to *Drosophila melanogaster*. *Environ Microbiol* **18**: 3509–3521.

Malacrino, A., Campolo, O., Medina, R.F., and Palmeri, V. (2018) Instar- and host-associated differentiation of bacterial communities in the Mediterranean fruit fly *Ceratitis capitata*. *PLoS One* **13**: e0194131.

Mashtoly, T.A., El-Zemaity, M.S., Abolmaaty, A., Abdelatef, G.M., Marzouk, A.A., and Reda, M. (2019) Phylogenetic characteristics of novel *Bacillus weihenstephanensis* and *Pseudomonas* sp. to desert locust, *Schistocerca gregaria* Forskål (Orthoptera: Acrididae). *Egypt J Biol Pest Control* **29**: 85.

Montagna, M., Chouaia, B., Mazza, G., Prosdocimi, E.M., Crotti, E., Mereghetti, V., *et al*. (2015a) Effects of the diet on the microbiota of the red palm weevil (Coleoptera: Dryophthoridae). *PLoS One* **10**: e0117439.

Montagna, M., Gómez-Zurita, J., Giorgi, A., Epis, S., Lozzia, G., and Bandi, C. (2015b) Metamicrobiomics in herbivore beetles of the genus *Cryptocephalus* (Chrysomelidae): toward the understanding of ecological determinants in insect symbiosis. *Insect Sci* **22**: 340–352.

O'Brien, H.E., Thakur, S., and Guttman, D.S. (2011) Evolution of plant pathogenesis in *Pseudomonas syringae*: a genomics perspective. *Annu Rev Phytopathol* **49**: 269–289.

Olcott, M.H., Henkels, M.D., Rosen, K.L., Walker, F.L., Sneh, B., Loper, J.E., and Taylor, B.J. (2010) Lethality and developmental delay in *Drosophila melanogaster* larvae after ingestion of selected *Pseudomonas fluorescens* strains. *PLoS One* **5**: e12504.

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., *et al*. (2015) Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* **31**: 3691–3693.

Paniagua Voirol, L.R., Frago, E., Kaltenpoth, M., Hilker, M., and Fatouros, N.E. (2018) Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. *Front Microbiol* **9**: 1–14.

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.

Péchy-Tarr, M., Bruck, D.J., Maurhofer, M., Fischer, E., Vogne, C., Henkels, M.D., *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.

Reboud, E., Basso, P., Maillard, A., Huber, P., and Attrée, I. (2017a) Exolysin shapes the virulence of *Pseudomonas aeruginosa* clonal outliers. *Toxins* **9**: 364–376.

Reboud, E., Bouillot, S., Patot, S., Béganton, B., Attrée, I., and Huber, P. (2017b) *Pseudomonas aeruginosa* ExlA and *Serratia marcescens* ShlA trigger cadherin cleavage by promoting calcium influx and ADAM10 activation. *PLoS Pathog* **13**: e1006579.

Roger, N., Michez, D., Wattiez, R., Sheridan, C., and Vanderplanck, M. (2017) Diet effects on bumblebee health. *J Insect Physiol* **96**: 128–133.

Ruffner, B., Péchy-Tarr, M., Höfte, M., Bloemberg, G., Grunder, J., Keel, C., and Maurhofer, M. (2015) Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens *Photorhabdus* and *Xenorhabdus*. *BMC Genomics* **16**: 609–623.

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.

Rumbaugh, K.P. (2014) Genomic complexity and plasticity ensure *Pseudomonas* success. *FEMS Microbiol Lett* **356**: 141–143.

Saravanakumar, D., Muthumeena, K., Lavanya, N., Suresh, S., Rajendran, L., Raguchander, T., and Samiyappan, R. (2007) *Pseudomonas*-induced defence molecules in rice plants against leaffolder (*Cnaphalocrocis medinalis*) pest. *Pest Manag Sci* **63**: 714–721.

Schellenberger, U., Oral, J., Rosen, B.A., Wei, J.-Z., Zhu, G., Xie, W., *et al*. (2016) A selective insecticidal protein from *Pseudomonas* for controlling corn rootworms. **354**: 634–637.

Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**: 2068–2069.

Silby, M.W., Winstanley, C., Godfrey, S.A.C., Levy, S.B., and Jackson, R.W. (2011) *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiol Rev* **35**: 652–680.

Singer, M.S., Mason, P.A., and Smilanich, A.M. (2014) Ecological immunology mediated by diet in herbivorous insects. *Integr Comp Biol* **54**: 913–921.

Skowronek, M., Sajnaga, E., Pleszczyńska, M., Kazimierczak, W., Lis, M., and Wiater, A. (2020) Bacteria from the midgut of common cockchafer (*Melolontha melolontha* L.) larvae exhibiting antagonistic activity against bacterial symbionts of entomopathogenic nematodes: isolation and molecular identification. *Int J Mol Sci* **21**: 1–18.

Therneau, T.M., and Grambsch, P.M. (2000) *Modeling Survival Data: Extending the Cox Model*. New York: Springer-Verlag.

Thorpe, H.A., Bayliss, S.C., Hurst, L.D., and Feil, E.J. (2017) Comparative analyses of selection operating on non-translated intergenic regions of diverse bacterial species. *Genetics* **206**: 363–376.

Thynne, E., McDonald, M.C., and Solomon, P.S. (2015) Phytopathogen emergence in the genomics era. *Trends Plant Sci* **20**: 246–255.

Tovi, N., Frenk, S., Hadar, Y., and Minz, D. (2019) Host specificity and spatial distribution preference of three *Pseudomonas* isolates. *Front Microbiol* **9**: 1–11.

Vacheron, J., Péchy-Tarr, M., Brochet, S., Heiman, C.M., Stojiljkovic, M., Maurhofer, M., and Keel, C. (2019) T6SS contributes to gut microbiome invasion and killing of an herbivorous pest insect by plant-beneficial *Pseudomonas protegens*. *ISME J* **13**: 1318–1329.

Vasanthakumar, A., Handelsman, J., Schloss, P.D., Bauer, L.S., and Raffa, K.F. (2008) Gut microbiota of an invasive subcortical beetle, *Agrilus planipennis* fairmaire, across various life stages. *Mol Ecol Evol* **37**: 1344–1353.

Vesga, P., Flury, P., Vacheron, J., Keel, C., Croll, D., and Maurhofer, M. (2020) Transcriptome plasticity underlying plant root colonization and insect invasion by Pseudomonas protegens. *ISME J* **14**: 2766–2782.

Wang, G.-H., Berdy, B.M., Velasquez, O., Jovanovic, N., Alkhalifa, S., Minbiole, K.P.C., and Brucker, R.M. (2020) Changes in microbiome confer multigenerational host resistance after sub-toxic pesticide exposure. *Cell Host Microbe* **27**: 213–224.

Waterfield, N.R., Daborn, P.J., Dowling, A.J., Yang, G., Hares, M., and Ffrench-Constant, R.H. (2003) The insecticidal toxin makes caterpillars floppy 2 (Mcf2) shows similarity to HrmA, an avirulence protein from a plant pathogen. *FEMS Microbiol Lett* **229**: 265–270.

Waters, L.S., and Storz, G. (2009) Regulatory RNAs in bacteria. *Cell* **136**: 615–628.

Wong, A.C.-N., Luo, Y., Jing, X., Franzenburg, S., Bost, A., and Douglas, A.E. (2015) The host as the driver of the microbiota in the gut and external environment of *Drosophila melanogaster*. *Appl Environ Microbiol* **81**: 6232–6240.

Ziganshina, E.E., Mohammed, W.S., Shagimardanova, E.I., Vankov, P.Y., Gogoleva, N.E., and Ziganshin, A.M. (2018) Fungal, bacterial, and archaeal diversity in the digestive tract of several beetle larvae (Coleoptera). *Biomed Res Int* **2018**: 1–15.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

File S1. Supplementary figures.
File S2. Coding sequences used in the BLAST analysis.
Table S1. Genome information of the sequenced strains of this study.
Table S2. List of used genomes.
Table S3. LT50 raw data.
Table S4. Pairwise p-values of the survival log-rank test.
Table S5. Summary of the SNPs found in the PCLAR01, PCLAR03 and PCLAR04 strains.
Table S6. Summary of the SNPs found in the genes of interest of PCLAR01, PCLAR03, PCLAR04.
Table S7. Soil characteristics.
Appendix S1. Supporting information.