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Rhizosphere-associated *Pseudomonas* induce systemic resistance to herbivores at the cost of susceptibility to bacterial pathogens

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Abstract

Plant-associated soil microbes are important mediators of plant defence responses to diverse above-ground pathogen and insect challengers. For example, closely related strains of beneficial rhizosphere *Pseudomonas* spp. can induce systemic resistance (ISR), systemic susceptibility (ISS) or neither against the bacterial foliar pathogen *Pseudomonas syringae* pv. tomato DC3000 (*Pto* DC3000). Using a model system composed of root-associated *Pseudomonas* spp. strains, the foliar pathogen *Pto* DC3000 and the herbivore *Trichoplusia ni* (cabbage looper), we found that rhizosphere-associated *Pseudomonas* spp. that induce either ISS and ISR against *Pto* DC3000 all increased resistance to herbivory by *T. ni*. We found that resistance to *T. ni* and resistance to *Pto* DC3000 are quantitative metrics of the jasmonic acid (JA)/salicylic acid (SA) trade-off and distinct strains of rhizosphere-associated *Pseudomonas* spp. have distinct effects on the JA/SA trade-off. Using genetic analysis and transcriptional profiling, we provide evidence that treatment of *Arabidopsis* with *Pseudomonas* sp. CH267, which induces ISS against bacterial pathogens, tips the JA/SA trade-off towards JA-dependent defences against herbivores at the cost of a subset of SA-mediated defences against bacterial pathogens. In contrast, treatment of *Arabidopsis* with the ISR strain *Pseudomonas* sp. *WCS417* disrupts JA/SA antagonism and simultaneously primes plants for both JA- and SA-mediated defences. Our findings show that ISS against the bacterial foliar pathogens triggered by *Pseudomonas* sp. CH267, which is a seemingly deleterious phenotype, may in fact be an adaptive consequence of increased resistance to herbivory. Our work shows that pleiotropic effects of microbiome modulation of plant defences are important to consider when using microbes to modify plant traits in agriculture.

KEYWORDS

Arabidopsis, *Myzus persicae*, *Pseudomonas*, rhizosphere, *Trichoplusia ni*

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1 | INTRODUCTION

Plant roots associate with complex communities of microbes that are distinct from the surrounding soil (Lundberg et al., 2012; Mendes et al., 2011). Among the hundreds to thousands of species present in the plant rhizosphere, *Pseudomonas fluorescens* and closely related *Pseudomonas* spp. are consistently enriched across diverse plant taxa in the rhizosphere compared to bulk soil (Bulgarelli et al., 2012; Coleman-Derr et al., 2016; Mendes et al., 2011; Niu, Paulson, Zheng, & Kolter, 2017). Plant interactions with nonpathogenic rhizosphere *Pseudomonas* spp. have been studied in depth because of the ability of strains in this genus to modulate diverse and beneficial agronomically important traits including plant growth, herbivore resistance and disease resistance (Couillerot, Prigent-Combaret, Caballero-Mellado, & Moënne-Loccoz, 2009; Vacheron et al., 2013). When rhizosphere *Pseudomonas* spp. strains are applied to plant roots in pure culture or enriched in the context of a complex rhizosphere community, they can enhance plant defences by several mechanisms, including directly out competing pathogens, directly killing pathogens via antimicrobial production (Couillerot et al., 2009), or modulating plant systemic defences via induced systemic resistance (ISR) to microbial pathogens and insect herbivores (Pieterse, van Wees, Hoffland, van Pelt, & van Loon, 1996; Van Oosten et al., 2008). Although many effects of rhizosphere-associated *Pseudomonas* strains on plant defences have been described, our recent discovery of a novel rhizosphere-associated *Pseudomonas*-mediated trait (Haney, Samuel, Bush, & Ausubel, 2015; described below) suggests that even within a well-studied taxa like *Pseudomonas*, there is likely unrecognized functional diversity.

Extensive molecular tools have been developed for the wild reference plant *Arabidopsis thaliana* (*Arabidopsis*) that have facilitated research on host–microbe interactions. These tools make *Arabidopsis* an ideal model to understand the genetic and hormonal mechanisms by which environmental rhizosphere bacteria modulate inducible defence signalling pathways and above-ground plantbiotic interactions (Pieterse, Van Der Does, Zamioudis, Leon-Reyes, & Van Wees, 2012). The repertoire of available *Arabidopsis* mutants, with known disruptions in pathways responsible for plant–insect and plant–microbe interactions, has enabled reverse genetics approaches to advance our understanding of systemic, multitrophic and ecologically important plant–microbe and plant–herbivore interactions (Davila Olivas et al., 2017; Groen et al., 2013; Whiteman et al., 2012). Additionally, model pathogens and herbivores that can infect or feed on *Arabidopsis* and whose ranges overlap with *Arabidopsis* have facilitated standardization of studies across laboratories and can provide information about relative activity through defined defence pathways (Gloss, Nelson Dittrich, Goldman-Huertas, & Whiteman, 2013).

Closely related strains of rhizosphere *Pseudomonas* spp. affect above-ground plant–insect and plant–microbe interactions. When associated with *Arabidopsis* roots, single isolates of *Pseudomonas* spp., including *Pseudomonas* spp. WCS417, WCS358 and SS101, can induce systemic resistance (ISR) against the foliar biotrophic bacterial pathogen, *Pseudomonas syringae*, the biotrophic fungal pathogen *Fusarium oxysporum* or the generalist herbivore *Spodoptera exigua* (van de Mortel et al., 2012; Pangesti et al., 2016; Van Oosten et al., 2008). *Pseudomonas* spp. WCS417 and SS101 strains that trigger ISR prime plants for enhanced expression of genes involved in defence signalling pathways that act against either bacteria or herbivores (Pangesti et al., 2016; Van Oosten et al., 2008; Verhagen et al., 2004). Additionally, ISR mediated by *Pseudomonas* spp. is associated with altered levels of glucosinolates, induced antiherbivory and antibacterial secondary metabolites (Clay, Adio, Denoux, Jander, & Ausubel, 2009; van de Mortel et al., 2012; Pangesti et al., 2016). Treatment of plant roots with rhizosphere ISR *Pseudomonas* spp. strains results in only slight induction of genes mediating systemic defence responses against pathogens and/or herbivores in the absence of an attack. However, association with rhizosphere *Pseudomonas* spp. primes for significantly stronger induction of both herbivore- and defence-related genes upon pest exposure (Pangesti et al., 2016; Van Oosten et al., 2008; Verhagen et al., 2004).

Arabidopsis mutants in the synthesis and downstream signalling of the defence hormones salicylic acid (SA) and jasmonic acid (JA) have been especially useful in uncovering how rhizosphere microbes modulate systemic defences (Pieterse et al., 2012). SA-dependent signalling protects plants from infection by piercing-sucking herbivores and microbial pathogens that require living plant tissue (biotrophic pathogens). JA-dependent induced defences are more effective against leaf-chewing insects and pathogens that kill plant cells (necrotrophic pathogens), and are also an important component of effective resistance against phloem-feeding aphids (Moran & Thompson, 2001). Known genes in the SA biosynthesis and signalling pathways include *ISOCHORISMATE SYNTHASE1/SALICYLIC ACID INDUCTION DEFICIENT2* (*SID2*), required for SA biosynthesis, and *PHYTOALEXIN DEFICIENT4 (PAD4)* and *NONEXPRESSOR OF PR GENES 1 (NPR1)* required for SA signalling; mutations in these genes result in enhanced susceptibility to biotrophic pathogens (Cao, Bowling, Gordon, & Dong, 1994; Dewdney et al., 2000). JA signalling genes include *JASMONATE RESISTANT1* (*JAR1)* and *CORONATINE INSENSITIVE 1* (*COI1);* mutations in these genes result in increased susceptibility to chewing herbivores (Cui et al., 2002). As a consequence of the extensive available tools and knowledge, these

Arabidopsis hormone signalling genes have been candidates for reverse genetic approaches to uncover the genetic basis of bacterial modulation of systemic defences.

Previous work has shown that ISR against foliar bacterial pathogens in *Arabidopsis* triggered by closely related rhizosphere strains of *Pseudomonas* spp. can involve distinct plant defence pathways and that the required pathways vary depending on the eliciting *Pseudomonas* spp. strain. Treatment of *Arabidopsis* hormone signalling mutants with *Pseudomonas* sp. WCS417 shows that ISR against bacterial pathogens is dependent on genes in both the SA signalling pathway via *NPR1* and the JA signalling pathway via *JAR1* (Pieterse et al., 1998), as well as the iron stress-inducible transcription factor *MYB72* (Van der Ent et al., 2008). A phenotypically indistinguishable ISR phenotype against foliar pathogens triggered by *Pseudomonas* sp. SS101 is also dependent on SA signalling via *NPR1* but independent of JA (van de Mortel et al., 2012). Together, these studies show that rhizosphere *Pseudomonas* strains that induce indistinguishable plant phenotypes—such as induction of systemic resistance to a foliar pathogen—can be mediated by induction of distinct hormonal signalling pathways.

Rhizosphere *Pseudomonas* spp. strains also affect systemic defences against herbivores, although the exact outcome depends on the precise interaction of both the *Pseudomonas* strain and the herbivore with plant defence signalling. Previous studies have found that ISR against *S. exigua* induced by *Pseudomonas* sp. WCS417 depends on JA signalling via *JAR1* (Van Oosten et al., 2008). ISR against *S. exigue* induced by rhizosphere *Pseudomonas* sp. SS101 is dependent on SA and glucosinolate biosynthesis (van de Mortel et al., 2012). *Pseudomonas* sp. WCS417 also affects induced defence responses against piercing-sucking, phloem-feeding herbivores. *Arabidopsis* association with *Pseudomonas* sp. WCS417 shows enhanced susceptibility to herbivory by the generalist peach aphid *Myzus persicae*, but has no effect on resistance against the specialist aphid, *Brevicoryne brassicae* (Pineda, Zheng, van Loon, & Dicke, 2012) or the specialist chewing insect *Pieris rapae* (Van Oosten et al., 2008). It is unclear whether rhizosphere bacteria that mediate enhanced resistance to foliar bacterial pathogens and chewing and/or piercing-sucking herbivores have mechanistic overlap, or whether rhizosphere bacteria are modulating distinct signalling pathways that affect these different phenotypes.

Plant resistance to pathogens and herbivores often is observed as a trade-off where the two main hormonal modulators of induced defences—SA and JA—are mutually antagonistic ("JA/SA antagonism"; Pieterse et al., 2012). As plants in natural settings are likely continually challenged with both bacterial pathogens and herbivores, it is important to understand how rhizosphere bacteria that affect one trait, like pathogen resistance, might affect other above-ground traits like responses to herbivores. It is also worth noting that a JA/SA trade-off is seemingly at odds with the observation that a single bacterial strain, such as *Pseudomonas* sp. WCS417, can simultaneously enhance resistance to both bacterial pathogens and herbivores.

We previously reported that rhizosphere *Pseudomonas* spp. strains collected from the roots of wild-growing *Arabidopsis* plants that are closely related to well-characterized *Pseudomonas* ISR strains (>97% identical by 16S rRNA) unexpectedly induce systemic susceptibility (ISS) instead of ISR to the foliar pathogen *Pto* DC3000 (Haney et al., 2015). In this study, we used the reference plant *Arabidopsis* to understand the mechanisms by which different *Pseudomonas* spp. strains in the rhizosphere induce susceptibility to foliar pathogens, and the role of ISS rhizosphere microbes in modulating JA/SA trade-offs. We predicted that rhizosphere *Pseudomonas* spp. ISS strains enhance susceptibility to a foliar bacterial pathogen by inducing JA-dependent responses and simultaneously suppressing SA-dependent responses.

We find that rhizosphere *Pseudomonas* spp. ISS strains, like ISR strains, enhance resistance to the Brassicaceae-adapted chewing herbivore, *T. ni*. This phenotype is consistent with enhancement of JA-dependent antiherbivore signalling and suppression of SA-dependent signalling. Using *Arabidopsis* hormone signalling mutants, we found that ISS and ISR against bacterial foliar pathogens are mechanistically distinct and that ISS is independent of SA signalling via *NPR1* but dependent on SA synthesis via *SID2*. Collectively, these data indicate that ISS against bacterial pathogens is a consequence of shifting the JA/SA trade-off towards JA-dependent signalling. Our work suggests that *Arabidopsis* association with *Pseudomonas* spp. strains that induce systemic susceptibility (ISS) to bacterial pathogens may be advantageous in environments where induction of JA-mediated herbivore defences is advantageous, and biotrophic pathogens do not constitute a significant selection pressure. Collectively, this work provides a mechanistic framework to understand how closely related strains of beneficial rhizosphere bacteria affect induced plant defences against multiple above-ground biotic enemies.

2 | MATERIALS AND METHODS

2.1 | Plant growth conditions

To assess the effects of rhizosphere *Pseudomonas* treatment on systemic defences, we grew *Arabidopsis* plants in Jiffy-7 peat pellets (Jiffy Products). We had previously found that this was an effective system to measure induction of systemic resistance (ISR) and susceptibility (ISS) triggered by rhizosphere Pseudomonads (Haney et al., 2015). Jiffy pellets come dehydrated and are flooded with tap water to hydrate prior to planting. The peat pellets are not sterile, and we found contain an average of $2.3 \times 10^6 \pm 5 \times 10^5$ CFU/g culturable microbes at the start of our experiments (Fig. S1) as determined by serially diluting onto King's B media and counting colonies. Jiffy Pellets are relatively low in fluorescent Pseudomonads ($7.9 \times 10^3 \pm 5 \times 10^3$; Fig. S1). For all experiments, bacterial loads in five pellets from each of three separate batches were measured. It has previously been shown that soil levels of at least 10^5 CFU *Pseudomonas* spp. per gram are required for ISR (Raaijmakers, 1995). We therefore used an amendment of 10^5 CFU/g of soil as our inoculum level for all assays involving root treatment with *Pseudomonas* spp. After addition of strains described in this study, we found ~10% of the culturable rhizosphere microbiome was *Pseudomonas* spp., on the order of 10^7 CFU/g of root (Fig. S1).

For experiments to test the effects of rhizosphere-associated *Pseudomonas* spp. on systemic defences, *Arabidopsis* plants were grown under a 12 h light/12 h dark and a 23/20°C day/night temperature regime and 75 μE cool fluorescent lights. Prior to planting, *Arabidopsis* seeds were surface-sterilized by washing with 70% ethanol for 2 min followed by 5 min in 10% bleach and three washes in sterile water. Imbibed seeds were stored at 4°C for at least 48 hr prior to use. *Arabidopsis* genotypes used in this study were selected because they were deficient in salicylic acid (SA) or jasmonic acid (JA) signalling or in induced systemic resistance. All genotypes were in the wild-type *Arabidopsis* Col-0 background and include the following: the fertile JA-insensitive mutant *coi1-16* (Ellis & Turner, 2002), SA mutants *npr1-1* (Cao et al., 1994), *sid2-2* (Dewdney et al., 2000) and *pad4-1* (Glazebrook et al., 1997), and the ISR-deficient mutant *myb72-1* (Segarra, Van der Ent, Trillas, & Pieterse, 2009).

2.2 | Bacterial strains and growth

Rhizosphere-associated *Pseudomonas* spp. and plant foliar pathogenic strains are listed in Table 1. Some *Pseudomonas* spp. strains were isolated from the roots of wild-grown *Arabidopsis* plants from eastern Massachusetts, USA, as described (Haney et al., 2015). All strains were grown at 28–30°C on LB or King's B medium.

To test the effects of rhizosphere *Pseudomonas* spp. against a naturally co-occurring pathogen, we isolated new foliar *Pseudomonas* strains from the leaves of wild-growing *Arabidopsis* plants from Cambridge, MA, at the same site where *Pseudomonas* sp. CH267 was originally isolated (Haney et al., 2015). To isolate foliar *Pseudomonas* spp., *Arabidopsis* leaves were surface-sterilized, ground in 10 mM $MgSO_4$ and plated on King's B agar medium. Fluorescent colonies that appeared 48 hr after plating were restreaked twice on King's B and then tested for infectivity in *Arabidopsis* Col-0 by infiltrating the isolates into *Arabidopsis* leaves at a final concentration of 10^3 CFU/cm^2 and then grinding and plating infected leaves 2 days later to quantify bacterial growth (see Bacterial Infection Methods below). We tested 12 putative pathogenic *Pseudomonas* isolates isolated from six plants (each isolate was from a separate leaf) spanning the range of the wild *Arabidopsis* population at the site. The isolate that grew to the highest levels, CH409, consistently grew 2–3 logs after infiltration into *Arabidopsis* Col-0 leaves, although it did not cause visible disease symptoms after 3 days, was used for further studies. To distinguish CH409 from other bacterial and fungal that might be growing in plant leaves, a rifampicin-resistant derivative of *Pseudomonas* sp. CH409 was selected. This was carried out by serially diluting overnight cultures onto King's B with rifampicin (50 μg/ml). Colonies that appeared after two days of growth were restreaked twice on media with antibiotic and retested for their ability to infect *Arabidopsis*.

TABLE 1 Strains used in this study

Strain Name	Genus and species	References
Rhizosphere-associated *Pseudomonas* spp.		
CH267	*Pseudomonas* sp. (ISS)	Haney et al. (2015)
CH229	*Pseudomonas* sp. (ISS)	Haney et al. (2015)
WCS417	*Pseudomonas* sp. (ISR)	Lamers, Schippers and Geels (1988)
WCS358	*Pseudomonas* sp. (ISR)	Geels and Schippers (1983)
Foliar Pathogens		
Pto DC3000	*Pseudomonas syringae* pv. *tomato*	Whalen, Innes, Bent and Staskawicz (1991)
Psm ES4326	*Pseudomonas syringae* pv. *maculicola*	Dong, Mindrinos, Davis and Ausubel (1991)
CH409	*Pseudomonas viridiflava*	This study
Xcr-1946	*Xanthomonas campestris* pv. *raphani*	Parker, Barber, Fan and Daniels (1993)
Xcc-BP109	*Xanthomonas campestris* pv. *campestris*	Parker et al. (1993)

Rhizosphere-associated *Pseudomonas* spp. that induce systemic resistance (ISR) or susceptibility (ISS) are indicated. All four ISS and ISR strains were used for experiments shown in Figures 1–2. For experiments corresponding to Figures 3–6, WCS417 and CH267 were used as a representative ISR and ISS strain, respectively.

2.3 | Bacterial infection and *Trichoplusia ni* herbivory assays

For assays measuring the effects of rhizosphere *Pseudomonas* spp. on bacteria pathogens or herbivores, Jiffy pellets were inoculated 9 days after seed germination with 2 ml of the indicated bacterial strain applied to the soil, near the base of the seedling, at a final OD_{600} of 0.02 (10^5 CFU/g Jiffy pellet).

Assays to quantify the effects of root-associated *Pseudomonas* spp. on systemic bacterial infection including *Pto* DC3000 and other *Pseudomonas syringae* and *Xanthomonas campestris* strains were performed as described (Daudi et al., 2012) using 30- to 37-day-old *Arabidopsis* plants. Briefly, an overnight culture of *Pto* DC3000 grown in King's B was spun down, washed and resuspended in 10 mM $MgSO_4$. Bacteria were diluted to a final OD_{600} of 0.0002, and 0.1 ml was infiltrated into the abaxial sides of leaves of ~5-week-old *Arabidopsis* using a 1-ml syringe without a needle. Plants were left covered for 2 days, and 7-mm punches (two leaves from each of six plants; $n = 12$) were extracted, ground and serially diluted to determine CFUs. Data shown are the average of at least three biological replicates. Student's t tests were used to compare each treatment to buffer of the same genotype.

Trichoplusia ni assays to quantify the effects of root-associated *Pseudomonas* spp. on systemic herbivory were performed as described previously (Groen et al., 2013) on plants pretreated with rhizosphere isolates of *Pseudomonas* spp.; *T. ni* eggs (Benzon Research or Natural Resources Canada) were incubated at 30°C for 36 hr with 12-hr days. One newly hatched caterpillar from the batch was randomly chosen and placed per 30-day-old *Arabidopsis* rosette. Plants were covered with a mesh bag, and caterpillars were allowed to feed for 7 days before weighing. The weight of newly hatched caterpillars is negligible, and so final caterpillar weight correlates with

host plant quality (Cui et al., 2002). At least 25 larvae were weighed per treatment, per experiment. We noticed a significant replicate effect in the final weight of larvae fed on buffer-treated Col-0 plants. As a result, all data from a single experiment were normalized to the buffer-treated Col-0 control for that experiment prior to averaging the data from at least 3 experiments. Student's t tests were used to compare each treatment to buffer of the same genotype.

To correlate *T. ni* larval weight with foliar growth of *Pto* DC3000, experimental sets of plants were split to use for both assays. Only experiments that included herbivory and pathogen growth measurements were used to generate Figure 3, which represented three biological replicates. Regression analysis was performed with RStudio on log-transformed or log–log-transformed data. For a logarithmic fit, we found an intercept of 8.3 ± 0.2 ($p = 6.12 \times 10^{-14}$) and a slope of 0.81 ± 0.18 ($p < .001$) and an $R^2 = 0.61$ ($F_{1,13} = 20.33$, $p = .0005875$). For an inverse power law (on log–log-transformed data), we found an intercept of 7.2 ± 0.12 ($p < 2 \times 10^{-16}$) and intercept of -2.53 ± 0.37 ($p = 1.27 \times 10^{-5}$) and an $R^2 = 0.78$ ($F_{1,13} = 46.16$, $p = 1 \times 10^{-5}$). For Figure 3c, the inverse power law relation and 95% confidence interval was plotted on log-transformed data.

To detect any interaction among foliar pathogen genotype and soil rhizosphere treatment (Figure 5), we performed a mixed model ANOVA in SAS, across six experimental blocks (three experiments included *Pto* DC3000, *Psm* ES4326 and *Pv* CH409; the other three included *Pto* DC3000 and both *Xanthomonas* strains). Within the model, we included foliar pathogen genotype and rhizosphere treatment and their interaction as fixed effects and block nested within treatment as a random effect.

2.4 | Bacterial genome sequencing and phylogenomic analysis

To determine phylogenetic placement with the fluorescent Pseudomonads, we sequenced the genomes of rhizosphere *Pseudomonas* spp. CH229 and CH267 and the pathogenic isolate *Pseudomonas* sp. CH409. DNA was isolated using a Qiagen Purgene Kit A and sonicated into ~500-bp fragments. Library construction was performed as described for an Illumina-based next-generation sequencing platform (Zhang, Millet, Ausubel, & Borowsky, 2014), individually indexed and sequenced using MiSeq V3 paired-end 300-bp reads. Nine bacterial genomes were multiplexed in the same run (the remaining six genomes are not described in this manuscript).

After barcode splitting and joining 3.02, 3.61 and 3.97 million reads for CH229, CH267 and CH409, respectively, were used to assemble a draft of each genome. Adapters were trimmed from the reads using Scythe (https://github.com/vsbuffalo/scythe) prior to quality trimming using Sickle (https://github.com/najoshi/sickle). Overlapping read pairs were merged into single reads using PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2014). Both merged reads and unmerged read pairs were used as input for genome assembly using SPAdes using the "—careful" parameter (Bankevich et al., 2012). The raw assembly contig was processed using an in-house python script to filter out contigs with low coverage or short length. The final curated assembly of CH409 consisted of 80 contigs comprising a 5.92-Mb genome. The genome was annotated by NCBI after uploading to GenBank using prokka, and 5,159 predicted coding sequences were found. The final assembly of CH229 consisted of 89 contigs comprising a 6.05-Mb genome and 5,350 coding sequences. The final assembly of CH267 consisted of 48 contigs comprising a 6.52-Mb genome and 5,831 predicted coding sequences. Code for the filtering script and assembly pipeline can be found at https://github.com/ryanmelnyk/GenomeAssembly. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accessions NXDO00000000 (CH409), NXHE00000000 (CH229) and NXNJ00000000 (CH267). The versions described in this study are version NXDO00100000, NXHE01000000 and NXNJ01000000, respectively.

To determine the placement of *Pseudomonas* spp. CH409, CH229 and CH267 within the fluorescent Pseudomonads (Figure 2), PhyloPhlAn was used to generate a species tree based on ~400 highly conserved housekeeping genes (Segata, Börnigen, Morgan, & Huttenhower, 2013). The input to PhyloPhlAn consisted of finished and draft representative genomes of *Pseudomonas* spp.

2.5 | Aphid proliferation assays

Myzus persicae were from a laboratory-maintained colony, originally collected from lettuce plants from the horticultural greenhouse at UBC (March 2017) and maintained on a mix of *Arabidopsis* genotypes. *Myzus persicae* colony growth assays were performed by placing wingless female aphids of similar size on 30-day-old *Arabidopsis* rosettes (five aphids per plant), enclosed in a breathable mesh bag. Aphids were allowed to feed and reproduce for 8 days, at which point the bags were removed and the aphids were counted. At least 25 plants were used per treatment per experiment. All experiments were repeated at least two times with similar results; significance was assessed by ANOVA and Tukey's HSD.

2.6 | Quantitative RT-PCR

Systemic gene expression was monitored in response to *Pto* DC3000 infection or *T. ni* herbivory after root treatment with *Pseudomonas* spp. that induce ISS or ISR. To monitor gene expression in response to *Pto* DC3000 infection, whole leaves were collected 24 hr after infiltration with 10 mM $MgCl_2$ or *Pto* DC3000. For qRT-PCR experiments with *T. ni*, plants were covered with a mesh net after placement of two larvae on each rosette. For mock treatment, plants were netted but with no caterpillar. Entire leaves were collected after 24 hr and leaves with visible damage were selected. For all experiments, one infected or herbivore damaged leaf from each of five plants was collected for each time point and treatment. qPCR data are an average of 4–5 biological replicates. The experiments used for qPCR were distinct from those for Figures 1–3; *T. ni* and *Pto* DC3000 phenotypes were measured for each experiment to confirm the treatments were effective.

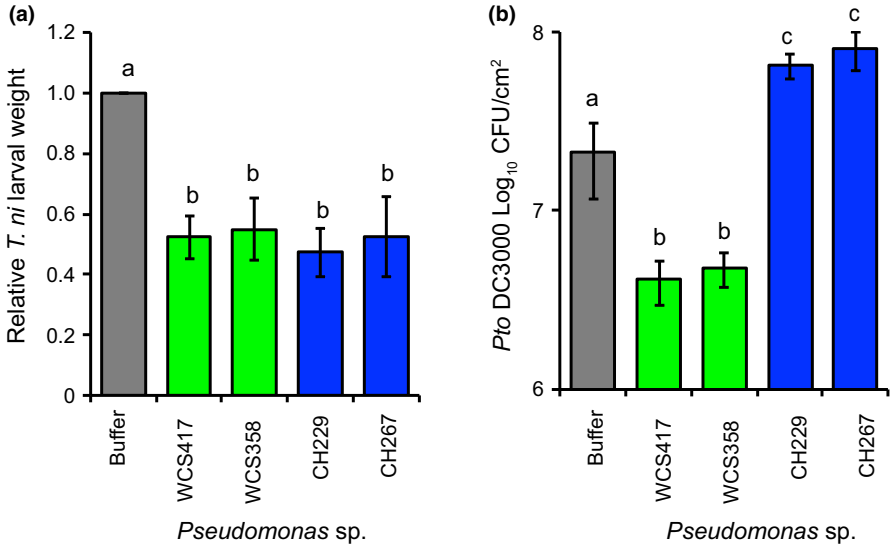

FIGURE 1 *Pseudomonas* spp. strains that trigger ISS and ISR to *P. syringae* pv. tomato DC3000 (*Pto* DC3000) all induce systemic resistance to the herbivore *Trichoplusia ni*. (a, b) Col-0 roots were treated with *Pseudomonas* spp. strains known to trigger ISS (CH229 and CH267) or ISR (WCS417 and WCS358) and challenged with *T. ni*. (a) or *Pto* DC3000 (b). Data are the average of four biological replicates with $n = 25$ larvae for (a) and $n = 12$ leaves (two from each of six plants) for (b). Letters designate significance by ANOVA and Student's *t* tests $p < .01$ [Colour figure can be viewed at wileyonlinelibrary.com]

RNA was extracted using a Qiagen RNAeasy isolation kit. 1–3 μg of RNA was DNAse treated with Turbo DNA-free (Ambion) in a 30 μl reaction (33–100 ng/μl RNA), and single stranded cDNA was generated using Superscript III (Invitrogen) and Oligo dT primers using 8 μl of the DNAse-treated reaction in 20 μl volume (14–40 ng/μl RNA). Quantitative PCR was run on a 7,500 Fast Real-Time PCR machine (Applied Biosystems) using 0.5 μl template cDNA (made from ~7 to 20 ng total RNA), 1 μl of 5 mM primer mix and 2X PowerUp™ SYBR™ Green Master Mix (ThermoFisher). qPCR primers are included in Table S1. Induction of specific hormone-inducible pathways was monitored via expression of SA-inducible genes (*PR1*, *PR2*, *GST6*), JA-inducible genes (*MYC2*, *VSP1* and *VSP2*), JA/Ethylene (ET)-inducible (*ERF1*, *ORA59* and *PR4*) and microbe-associated molecular pattern (MAMP)-inducible (*MYB51* and *CYP71A13*). Transcript abundance was normalized to *EIF4A* levels (Millet et al., 2010), and relative fold change was calculated using the $2^{-\Delta\Delta C_T}$ method (Livak & Schmittgen, 2001). The heatmap (Figure 6) was generated using RStudio heatmap.2 function using $\log_2$ fold change in relative gene expression. Statistical significance was determined by ANOVA and Tukey's HSD.

3 | RESULTS

3.1 | ISS strains elicit resistance to the insect herbivore, *Trichoplusia ni*

Pseudomonas spp. CH267 and CH229, isolated from the roots of *Arabidopsis* plants growing wild in Cambridge and Carlisle Massachusetts, USA, in 2014, were previously shown to induce systemic susceptibility (ISS) to the pathogenic bacterium *Pseudomonas syringae* DC3000 (*Pto DC3000*) (Haney et al., 2015). As discussed in the introduction, the JA/SA trade-off predicts that resistance against *Pto* DC3000 should come at a cost of JA-mediated defences against chewing insects such as caterpillars (Cui et al., 2002; Pieterse et al., 2012). To determine whether rhizosphere *Pseudomonas* spp. ISS strains elicit systemic effects on herbivory, we tested whether inoculation of *Arabidopsis* roots with ISS strains affects the feeding of the cabbage looper, *Trichoplusia ni*, which is an herbivore of Brassicaceae spp., including *Arabidopsis* (Cui et al., 2002; Jander, Cui, Nhan, Pierce, & Ausubel, 2001) and has a range that overlaps with the site of *Pseudomonas* spp. CH267 and CH229 collection.

We found that the ISS strains *Pseudomonas* spp. CH267 and CH229 conferred resistance to herbivory by *T. ni* as measured by reduced larval weight gain, consistent with upregulation of JA-mediated defences and suppression of SA-mediated defences (Figure 1a). The ISR strains *Pseudomonas* spp. WCS417 and WCS358 also induced resistance to *T. ni*. Under conditions where ISR strains *Pseudomonas* spp. WCS417 and WCS358 both confer resistance to bacterial pathogens, *Pseudomonas* spp. CH229 and CH267 confer susceptibility (Figure 1b). These data suggest that the enhanced resistance to herbivores triggered by *Pseudomonas* spp. CH229 and CH267 comes at a cost of defences against a bacterial pathogen.

3.2 | ISS strains *Pseudomonas* spp. CH229 and CH267 fall within the *fluorescens* group of *Pseudomonas*

By full length 16S rRNA sequence, we previously reported that *Pseudomonas* spp. CH229 and CH267 are ~97% identical to *Pseudomonas* sp. WCS417 and 99% identical to one another (Haney et al., 2015; GenBank Accession nos. # KP253040 and KP253044). To determine placement of these isolates within *Pseudomonas* spp., we sequenced the genomes of *Pseudomonas* spp. CH229 and CH267. Draft genomes were assembled (Methods). Phylogenomic analysis places CH229 and CH267 within the *P. koreensis* subgroup previously described (Berendsen et al., 2015) and as close relatives of the environmental isolate *Pseudomonas* sp. PfO-1 and the plant growth promoting isolate *Pseudomonas* sp. UW4 (Figure 2).

Some virulent pathogens like *P. syringae* exploit JA/SA antagonism by producing the JA-Ile mimic Coronatine (COR), which dampens local and systemic SA-dependent defences by enhancing JA

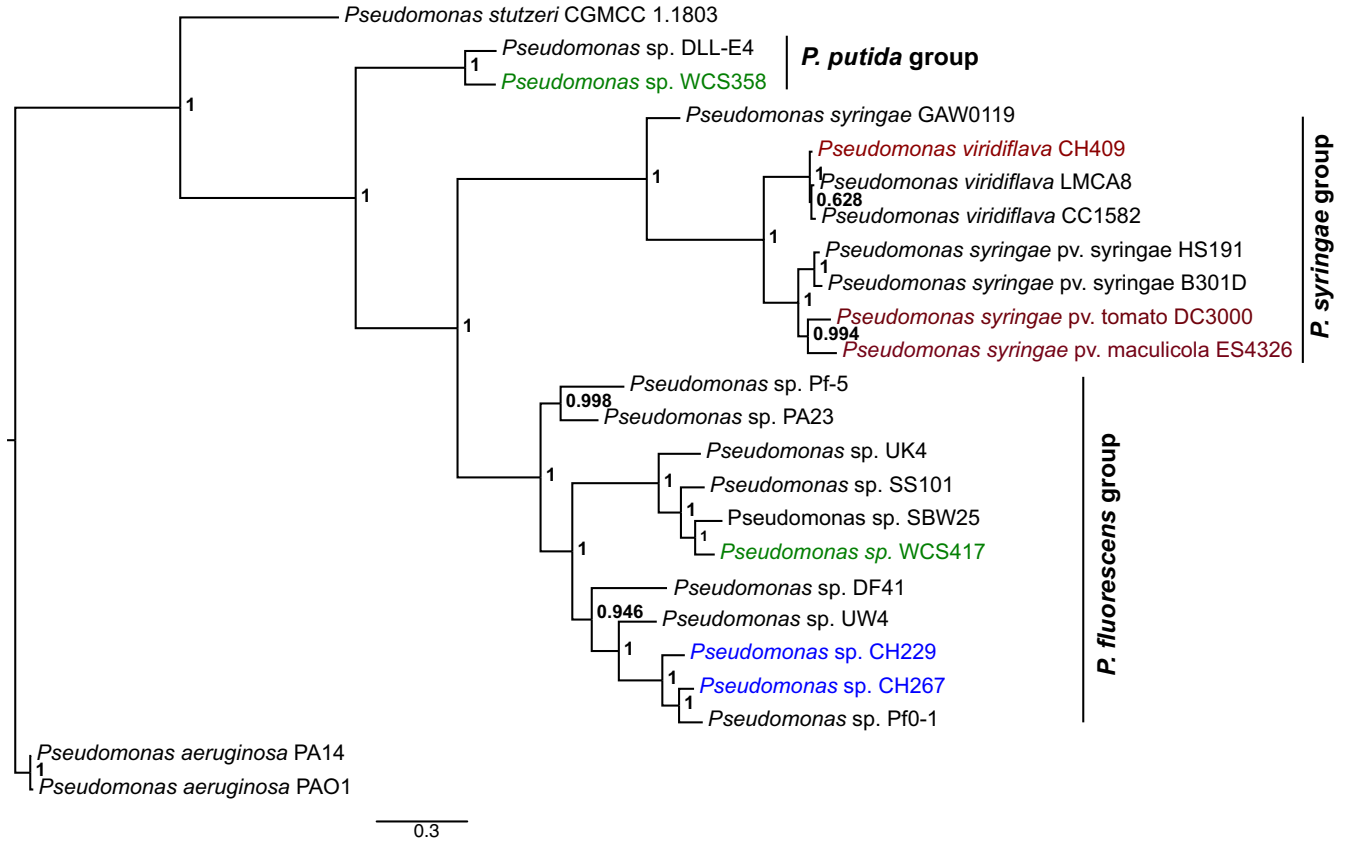

FIGURE 2 Phylogenetic analysis places *Pseudomonas* sp. CH229 and CH267 within the *P. fluorescens* group of fluorescent Pseudomonads. The genomes of *Pseudomonas* sp. CH229 and CH267 were sequenced and were used to generate a species tree based on ~400 highly conserved housekeeping genes (Segata et al., 2013). Strains used in this study that induce systemic susceptibility (ISS) are shown in blue; strains that induce systemic resistance (ISR) are shown in green, and pathogenic strains of *Pseudomonas* used in this study are shown in red [Colour figure can be viewed at wileyonlinelibrary.com]

signalling (Zheng et al., 2012). However, we were unable to identify genes with significant similarity to genes involved in coronatine biosynthesis in the genomes of CH229 and CH267. It is therefore unlikely that coronatine production is the mechanism by which CH229 and CH267 suppress SA-related defences.

3.3 | ISS strains enhance JA-dependent signalling and depress SA-dependent signalling

Previous work has shown a reciprocal relationship between resistance to *T. ni* and to *P. syringae* (Cui et al., 2002). To further study this reciprocal relationship, and to determine whether *T. ni* resistance and *Pto* DC3000 infection are a quantitative metric of JA/SA trade-offs (whether knowing the degree of resistance to one challenger should predict the level of resistance to the other), we compared resistance versus susceptibility to infection by *Pto* DC3000 and herbivory by *T. ni* in a panel of *Arabidopsis* mutants that have altered JA or SA synthesis, perception or signalling. We observed that the mutant *coi1-16*, required for JA perception, had increased resistance to *Pto* DC3000 and increased sensitivity to *T. ni* herbivory (Figure 3a,b). We observed the opposite responses in SA mutants: the SA signalling mutant *npr1-1* and the SA synthesis mutant *sid2-2* had increased sensitivity to *Pto* DC3000 and increased resistance to *T. ni* herbivory.

When the resistance or sensitivity of *Arabidopsis* JA and SA mutants to *Pto* DC3000 or *T. ni is* plotted against one another, a reciprocal relationship is observed (Figure 3c). An inverse power law ($R^2 = 0.78$; $y = 164.7x^{-0.3}$) had the best fit to the data where x = *Pto* DC3000 CFU/cm^2 and y = *T. ni* weight gain. This indicates that the *Pto* DC3000 infection and *T. ni* herbivory are quantitative metrics for JA/SA trade-offs and that resistance to one should be predictive of resistance to the other.

When the phenotypes of wild-type Col-0 plants treated with rhizosphere *Pseudomonas* spp. ISR strains are plotted onto the same graph as the *Arabidopsis* mutants, a predicted disruption of JA/SA antagonism is observed for *Pseudomonas* spp. WCS417 and WCS358 (Figure 3c). The level of resistance to *Pto* DC3000 predicted by *T. ni* resistance falls outside of the 95% confidence interval for this reciprocal relationship. In contrast to the ISR strains, *Pseudomonas* sp. ISS strains CH267 and CH229 fell within the 95% confidence interval for a reciprocal relationship between *T. ni* herbivory and *Pto* DC3000 resistance (Figure 3c), suggesting that these strains are shifting the JA/SA balance by enhancing JA-dependent signalling and suppressing SA-dependent signalling.

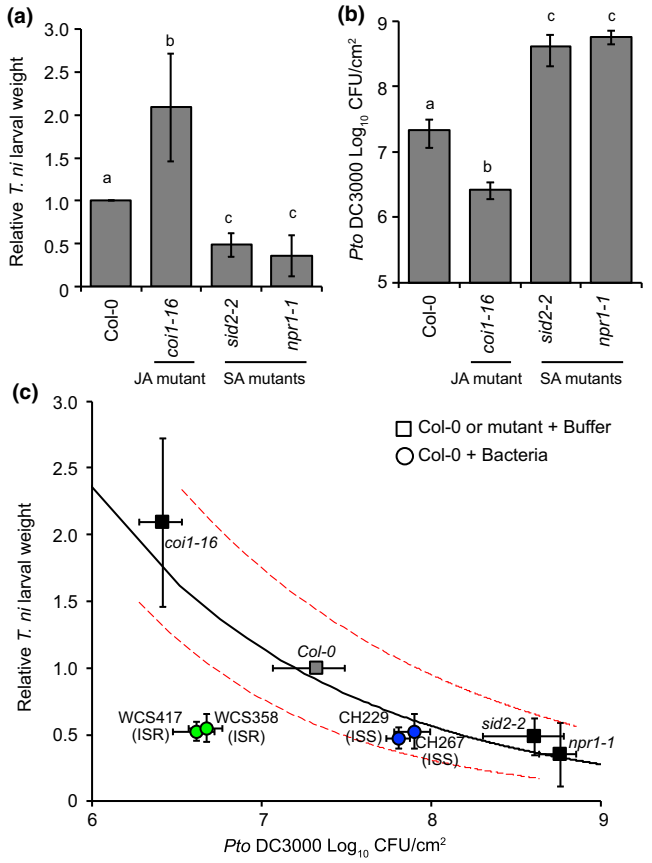

FIGURE 3 *Arabidopsis* resistance or susceptibility to caterpillars of *T. ni* or the bacterial strain *Pto* DC3000 provides a quantitative metric for JA-SA antagonism. (a, b) Col-0 plants or mutants impaired in JA signalling (*coi1-16*) and SA synthesis or signalling (*sid2-2* or *npr1-1*, respectively) were challenged with either *T. ni* (a) or *Pto* DC3000 (b); letters indicate significance levels ($p < .01$) by ANOVA and *t* tests. (c) Plotting *T. ni* weight against *Pto* DC3000 growth in *Arabidopsis* JA and SA mutants shows that this is a quantitative metric for the JA/SA trade-off. Linear regression analysis of log–log-transformed data found that an inverse power law ($R^2 = 0.78$; $y = 164.7x^{-0.3}$; $F_{1,13} = 46.16$, $p = 1 \times 10^{-5}$) had the best fit to the data where x = *Pto* DC3000 CFU/cm^2 and *y* = *T. ni* weight gain. 95% confidence intervals are indicated with dotted red lines. *T. ni* and DC3000 data for (c) are the same data shown in Figures 1 and 3a–c. Data shown are the average of at least three biological replicates with $n > 25$ larvae for *T. ni* experiments; $n = 12$ for *Pto* DC3000 experiments (two infected leaves from each of six plants) [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | ISS modulation of the JA/SA trade-off requires SA synthesis and JA perception but is *NPR1* independent

Because the ISS strains *Pseudomonas* spp. CH267 and CH229 promote resistance to herbivores, apparently at the cost of defence to *Pto* DC3000, we hypothesized that both herbivore resistance and *Pto* DC3000 susceptibility should be dependent on JA and SA signalling. ISR induced by *Pseudomonas* sp. WCS417 is independent of SA biosynthesis but requires SA signalling via *NPR1*, JA signalling via *JAR1* (Figure 3 and Pieterse et al., 1998), and the iron stress-inducible transcription factor *MYB72* (Van der Ent et al., 2008). We found that similarly to ISR against *Pto* DC3000 induced by rhizosphere treatment by *Pseudomonas* sp. WCS417, ISS against *Pto* DC3000 triggered by *Pseudomonas* sp. CH267 depends on JA perception and signalling via *COI1* (Figure 4a). However, unlike ISR induced by *Pseudomonas* spp., ISS against *Pto* DC3000 triggered by *Pseudomonas* sp. CH267 does not depend on *NPR1*, *PAD4* or *MYB72*. Instead, we found that ISS via *Pseudomonas* sp. CH267 depends on SA synthesis via *SID2*. These data indicate that ISS against *Pto* DC3000 elicited by CH267 is mechanistically distinct from ISR elicited by *Pseudomonas* sp. WCS417 and is dependent on SA biosynthesis and JA perception.

Induce systemic resistance triggered by *Pseudomonas* sp. WCS417 against the caterpillars of the generalist chewing moth, *S. exigua*, is dependent on JA signalling via *JAR1* (Van Oosten et al., 2008). We found that caterpillar resistance triggered by both *Pseudomonas* spp. WCS417 and CH267 was dependent on JA perception via the F-box protein *COI1* and SA synthesis via *SID2* and independent of *NPR1* and *PAD4* (Figure 4b). These data are consistent with the conclusion that ISS strain *Pseudomonas* sp. CH267 induces resistance to herbivores and susceptibility to pathogens through increasing JA-mediated defences via COI1 at the expense of SA-mediated defences via SID2.

3.5 | *Pseudomonas* spp. strains that trigger ISS against bacterial pathogens have no effect on defence against aphids

While treatment of *Arabidopsis* roots with *Pseudomonas* sp. WCS417 has been shown to inhibit the performance of chewing insects, it was shown to trigger ISS against the generalist peach aphid *Myzus persicae* (Pineda et al., 2012). *Arabidopsis* resistance to *M. persicae* is *PAD4* and *NPR1* dependent and *SID2* independent (Moran & Thompson, 2001; Pegadaraju, Knepper, Reese, & Shah, 2005). As a result, we hypothesized that *Pseudomonas* sp. CH267 would not affect *M. persicae* herbivory as aphid herbivory is not affected by the pathways that our genetic data (Figure 4) suggest are suppressed by rhizosphere treatment with *Pseudomonas* sp. CH267. As predicted, there was no change in aphid performance in response to rhizosphere treatment with *Pseudomonas* sp. CH267 or on the SA biosynthesis mutant *sid2-2* (Fig. S2). In contrast, we found increased aphid numbers on the JA perception mutant *coi1-16* and in response to rhizosphere treatment with *Pseudomonas* sp. WCS417. These data suggest that although ISS elicited by *Pseudomonas* sp. CH267 is dependent on SA signalling, this strain specifically affects outcomes related to *NPR1*-independent herbivore and pathogen responses.

3.6 | ISS strains enhance the virulence of diverse pathogens

As increased susceptibility to bacterial pathogens appears to be a detrimental consequence of *Arabidopsis* association with rhizosphere *Pseudomonas* spp., we tested whether *Pseudomonas* sp. CH267 would enhance susceptibility to bacterial pathogens that naturally

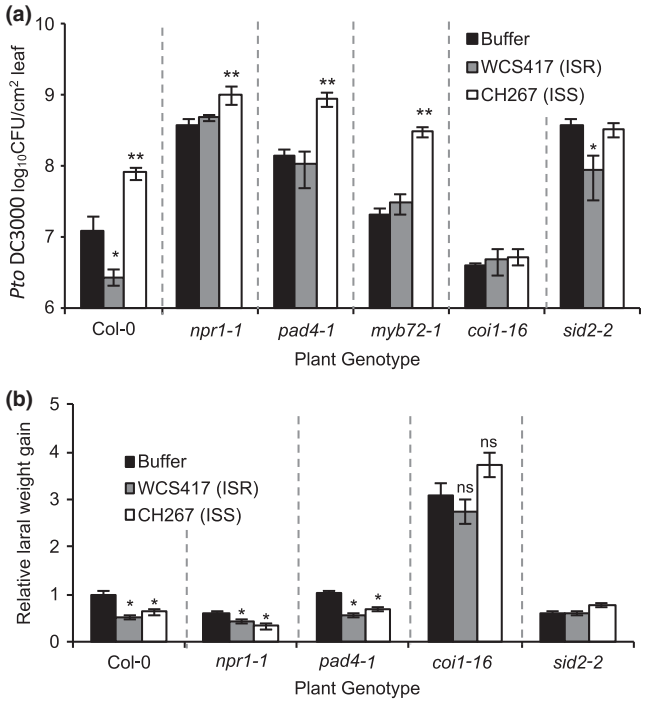

FIGURE 4 Induced systemic resistance (ISR) and susceptibility (ISS) against *Pto* DC3000 are mechanistically distinct. (a) Treatment of *Arabidopsis* roots with *Pseudomonas* sp. CH267 triggers ISS to a bacterial pathogen under conditions where *Pseudomonas* sp. WCS417 triggers ISR. ISS is not dependent on *NPR1*, *PAD4* and *MYB72* but is dependent on *COI1* and *SID2*. * and ** significantly decreased or increased respectively; $p < .05$ by Student's *t* test relative to buffer-treated plants. (b) Resistance against *T. ni* induced by both *Pseudomonas* spp. WCS417 and CH267 is still intact in *npr1-1* and *pad4-1* mutants, which are deficient in ISR. Induced resistance against *T. ni* depends on JA signalling via *COI1* and SA biosynthesis via *SID2*. $n > 25$ larval per treatment; within each experiment, each larval weight average was normalized to Col-0 buffer treated. Data shown are the averages of three biological replicates. *$p < .05$ by Student's *t* test relative to buffer- or mock-treated controls; ns—not significant

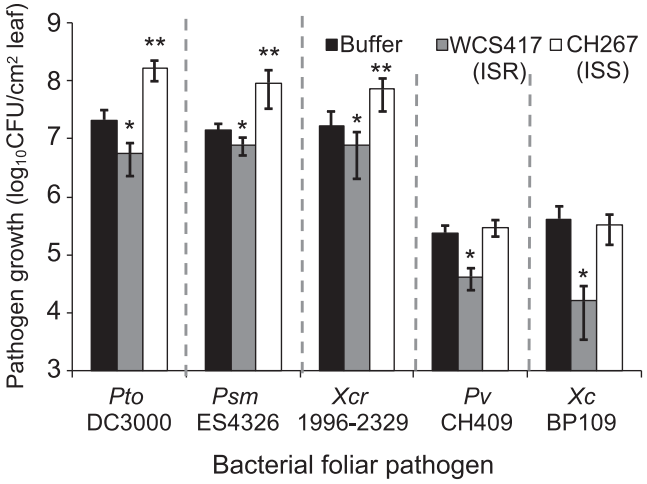

FIGURE 5 *Pseudomonas* spp. strains that induce systemic susceptibility (ISS) cause a greater enhancement of susceptibility against virulent bacterial pathogens. ISS strain *Pseudomonas* sp. CH267 enhances susceptibility to virulent pathogens while ISR strain *Pseudomonas* sp. WCS417 enhances resistance to less virulent *Pseudomonas syringae* and *Xanthomonas campestris* pathogens (* or **$p < .001$ by Student's *t* test relative to buffer-treated plants infected with the same pathogen). We found a significant rhizosphere treatment by pathogen interaction using a mixed model ANOVA ($F_{8,35} = 2.37$, $p = .04$). *Pseudomonas viridiflava* CH409 was isolated from the leaves of wild-growing *Arabidopsis* plants at the same site as *Pseudomonas* sp. CH267. Data are the average of three independent experiments with pathogen level quantified in at least six leaves per experiment

co-occur with wild *Arabidopsis* and *Pseudomonas* sp. CH267. We went back to the site where *Pseudomonas* spp. CH267 and CH229 were isolated (Haney et al., 2015) and collected leaves from wild-growing Arabidopsis plants and isolated new *Pseudomonas* strains (Methods). We found an isolate, *Pseudomonas* sp. CH409, which could consistently grow 2–3 logs when infiltrated into *Arabidopsis* leaves. We sequenced the genome of *Pseudomonas* sp. CH409 and generated a species tree using ~400 housekeeping genes, which placed this strain basal to the *P. syringae* clade within the species *Pseudomonas viriflavida* (Figure 2). The genome of *Pseudomonas* sp. CH409 is ~97% identical to the *P. viridiflava* type strain ATCC 13223 and so according to proposed methods in Standards in Genomic Sciences (Federhen et al., 2016) was named *P. viridiflava* CH409.

We tested whether rhizosphere treatment with *Pseudomonas* sp. CH267 could enhance the virulence of the natural isolate *P. viridiflava* CH409 and found no effect on virulence (Figure 5), although rhizosphere treatment with *Pseudomonas* sp. WCS417 enhanced resistance to CH409. Because CH267 does not result in enhanced susceptibility to a natural *P. viridiflava* strain isolated from the same *Arabidopsis* population, these data suggest that ISS strains like *Pseudomonas* sp. CH267 may not be detrimental to natural populations.

Some virulent pathogens like *P. syringae* exploit JA/SA antagonism by producing the JA-Ile mimic coronatine (COR), which dampens local and systemic SA-dependent defences by enhancing JA signalling (Zheng et al., 2012). We reasoned that on ISS-treated *Arabidopsis*, virulent pathogenic bacterial strains, including COR-producing strains of *P. syringae*, might exhibit greater enhancement of their virulence than weak pathogens; COR suppression of SA signalling might act synergistically with *Pseudomonas* sp. CH267-mediated suppression of SA signalling. Efficacy of ISR triggered by *Pseudomonas* spp., on the other hand, has been shown to negatively correlate with the virulence of the pathogen that is used to monitor ISR (Ton, Davison, Van Wees, Van Loon, & Pieterse, 2001). We therefore tested whether ISS elicited by strain *Pseudomonas* sp. CH267 against several *P. syringae* and *Xanthomonas campestris* strains spanning a range of virulence (as measured by relative growth of the pathogen in *Arabidopsis* leaves) and whether the level of enhanced pathogen growth was correlated with the virulence of the pathogens.

Consistent with our prediction, we found that while ISS strain *Pseudomonas* sp. CH267 enhanced susceptibility to relatively virulent *P. syringae* (COR-producing *Pto*DC3000 and *Psm*ES4326) and

X. campestris pathogens ($p < .001$ by Student's *t* test relative to buffer treatment of the same genotype), it had no significant effect on systemic defences against pathogens with lower virulence (Figure 5). Conversely, rhizosphere treatment with the ISR strain *Pseudomonas* sp. WCS417 conferred resistance to pathogens with both low and high virulence ($p < .001$). To detect any interaction among foliar pathogen genotype and soil rhizosphere treatment, we performed a mixed model ANOVA. We found a significant difference between the effect of rhizosphere treatment on pathogens of different virulence levels ($F_{8,35} = 2.37$, $p = .04$) indicating that the magnitude of the effect of rhizosphere treatment with *Pseudomonas* spp. CH267 and WCS417 is dependent on the specific challenging pathogen.

3.7 | *Pseudomonas* strains that trigger ISS against bacterial pathogens suppress induction of a subset of SA responses and prime plants for JA-dependent responses

Induce systemic resistance triggered on *Arabidopsis* by multiple distinct *Pseudomonas* spp. strains is associated with slight changes in systemic transcriptional responses in the absence of pathogen or herbivore challenge (Pangesti et al., 2016; Verhagen et al., 2004) and no detectible changes in foliar hormone levels (Pieterse et al., 2012). The most prominent known molecular feature of ISR treated plants is so-called priming for a more robust transcriptional response after pathogen or herbivore challenge (van de Mortel et al., 2012; Pangesti et al., 2016; Verhagen et al., 2004). To determine whether the enhanced susceptibility to *Pto* DC3000 and resistance to *T. ni* caused by rhizosphere treatment with the ISS strain *Pseudomonas* sp. CH267 is also associated with priming *Arabidopsis* for a more robust or a diminished response, we used qRT-PCR to measure expression of known pathogen- and herbivore-inducible genes. *Arabidopsis* roots were pretreated with *Pseudomonas* spp. WCS417 or CH267 and challenged with *Pto* DC3000, *T. ni*, or mock challenged, and tissue was harvested 24 hr later. We monitored expression of the SA-inducible genes (*PR1*, *PR2*, *GST6*), JA-inducible genes (*MYC2*, *VSP1* and *VSP2*), JA/ethylene (ET)-inducible (*ERF1*, *ORA59* and *PR4*), and microbe-associated molecular pattern (MAMP)-inducible (*MYB51* and *CYP71A13*) genes in *Arabidopsis* plants pretreated with *Pseudomonas* spp. CH267 or WCS417 ± DC3000 and ± *T. ni*.

The *MYC2*-dependent branch of the JA signalling pathway is required for defence against herbivores; the *ORA59/ERF1*-dependent branch of the JA/Ethylene (ET) pathway favours resistance to necrotrophic pathogens and is antagonistic to the *MYC2* pathway [reviewed in (Broekgaarden, Caarls, Vos, Pieterse, & Van Wees, 2015)]. We found treatment of *Arabidopsis* roots with *Pseudomonas* sp. CH267 favoured induction of the JA signalling pathway regulated by *MYC2* including downstream target *VSP2* at the cost of the JA/ET pathway regulated by *ORA59/ERF1* and the downstream target *PR4* (Figure 6a–c). In contrast, consistent with previous reports, the *Pseudomonas* sp. WCS417 ISR strain favoured expression of the JA/ET pathway including *MYC2* and *PR4* over the JA pathway (Figure 6a–c) (Pangesti, Pineda, Dicke, & van Loon, 2015; Pangesti et al., 2016; Van Oosten et al., 2008). Collectively, these data indicate that rhizosphere treatment with the ISS strain *Pseudomonas* sp. CH267 treatment suppresses induction of the JA/ET pathway regulated *ORA59/ERF1* and favours *MYC2*-dependent induction of antiherbivore responses.

Surprisingly, in *Pseudomonas* sp. CH267-treated *Arabidopsis*, we observed normal induction of the SA-dependent genes *PR1* and *GST6* and the MAMP/SA-inducible genes *CYP71A13* and *MYB51* after pathogen challenge in distal leaves (Figure 6a,d,e). However, the SA-inducible gene *PR2* failed to be induced in response to pathogen challenge indicating that *Pseudomonas* sp. CH267 treatment might suppress induction of a subset of SA-dependent genes (Figure 6e). These data suggest that although ISS is dependent on a diminution of SA signalling, not all SA-mediated responses are abolished.

4 | DISCUSSION

Here, we show that closely related strains of rhizosphere *Pseudomonas* spp. (Figure 2) can differentially modulate above-ground inducible host defences against foliar microbial pathogens and different classes of insect herbivores (Figure 7). We characterized the genetic basis by which distinct but closely related strains of rhizosphere *Pseudomonas* spp. induce systemic resistance (ISR) or susceptibility (ISS) to the bacterial pathogen *Pto* DC3000 and the herbivore *Trichoplusia ni*. We find that ISS against the bacterial foliar pathogens triggered by *Pseudomonas* sp. CH267, which is a seemingly deleterious phenotype, may in fact be an adaptive consequence of increased resistance to herbivory. These results suggest that *Arabidopsis* association with rhizosphere *Pseudomonas* spp. that induce ISS to bacterial pathogens may be beneficial in environments where induction of JA-mediated herbivore defences is advantageous and where biotrophic pathogens do not constitute a significant selection pressure.

We found that two strains of *Pseudomonas* spp. that induce systemic susceptibility, CH229 and CH267, are closely related to one another and close relatives of well-characterized environmental isolate PfO-1 (Compeau, Al-Achi, Platsouka, & Levy, 1988) and the growth promoting strain UW4 (Shah, Li, Moffatt, & Glick, 1998). While phylogeny has been shown to not be predictive of function within well-studied ISR strains (Berendsen et al., 2015), we are not aware of studies that look at induced systemic defences after treatment with UW4 and PfO-1. Further studies may reveal whether ISS is a shared feature of closely related bacteria within the *P. fluorescens* group.

Using *Arabidopsis* hormone mutants, we found that resistance to *T. ni* and *Pto* DC3000 is quantitative metrics for the JA/SA trade-offs and that rhizosphere *Pseudomonas* spp. have distinct interactions with JA/SA antagonism (Figure 1c). Further, we found that degree of resistance to one biotic challenge is predictive of resistance to the other, where induced resistance to a bacterial pathogen inversely correlates with susceptibility to an herbivore. We found that this rule is intact after rhizosphere treatment with the ISS strain

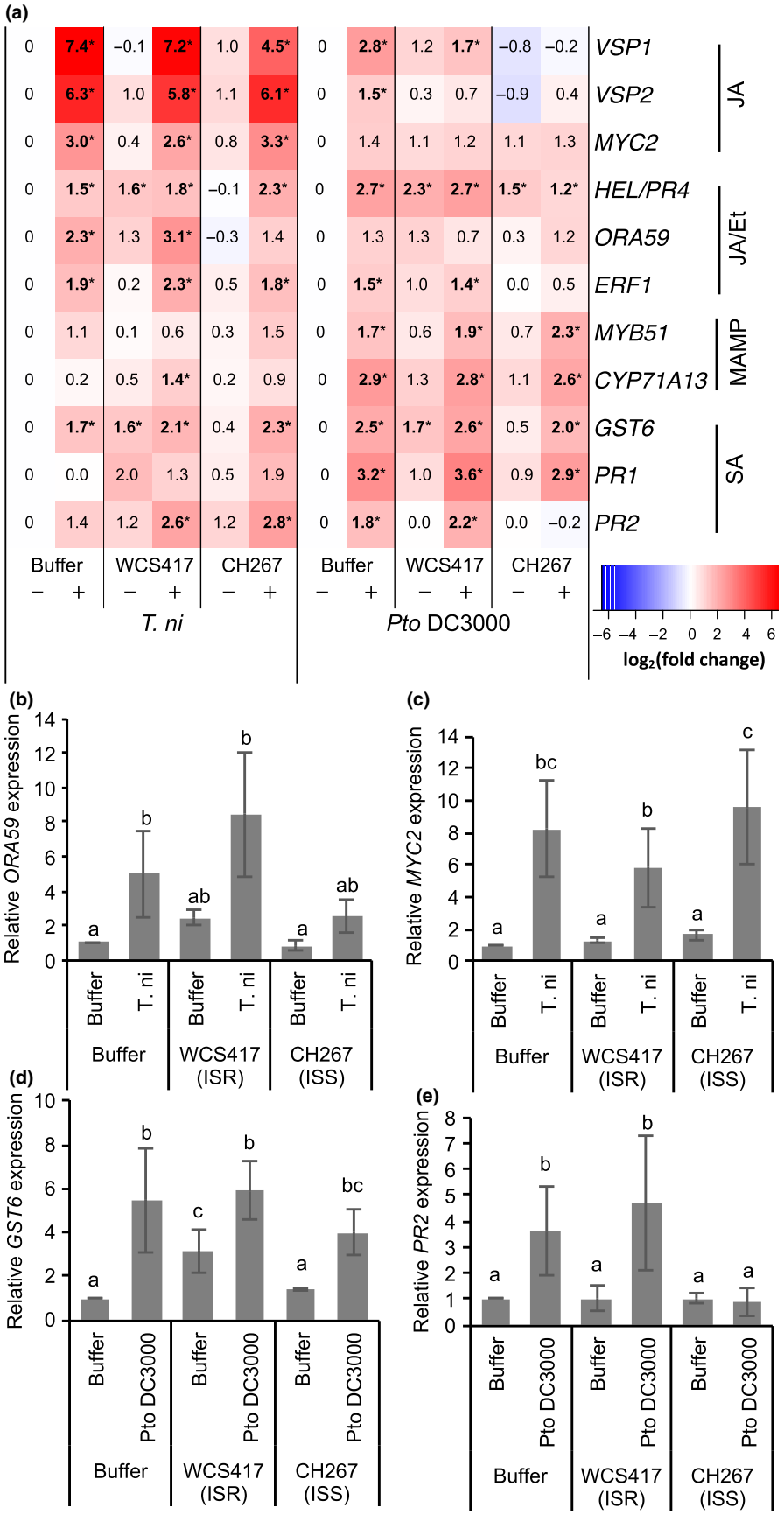

FIGURE 6 Systemic transcriptional responses after rhizosphere treatment with *Pseudomonas* spp. strains that trigger ISS or ISR. (a) qRT-PCR was used to monitor expression level in systemic leaves in *Arabidopsis* plants after rhizosphere inoculation with *Pseudomonas* spp. WCS417 or CH267 24 hr after challenge with *T. ni* or *Pto*DC3000. The heatmap includes values of the $\log_2$fold change in expression value normalized to the individual mock treated or buffer control for each treatment. Expression changes that are significantly different ($p < .1$) from buffer- and mock-treated samples by ANOVA and Tukey's HSD are shown in bold with an *. Data are the average of at least four biological replicates. (b–e) Relative expression after root treatment with buffer, ISR strain *Pseudomonas* sp. WCS417, and ISS strain *Pseudomonas* sp. CH267 of (b) *ORA59* with and with *T. ni* challenge, (c) *MYC2* with and without *T. ni* challenge, (d) *GST6* with and without *Pto* DC3000 challenge and (e) *PR2* with and without before and after challenge with *Pto* DC3000. Letters indicate levels of significance $p < .1$ by ANOVA and Tukey's HSD [Colour figure can be viewed at wileyonlinelibrary.com]

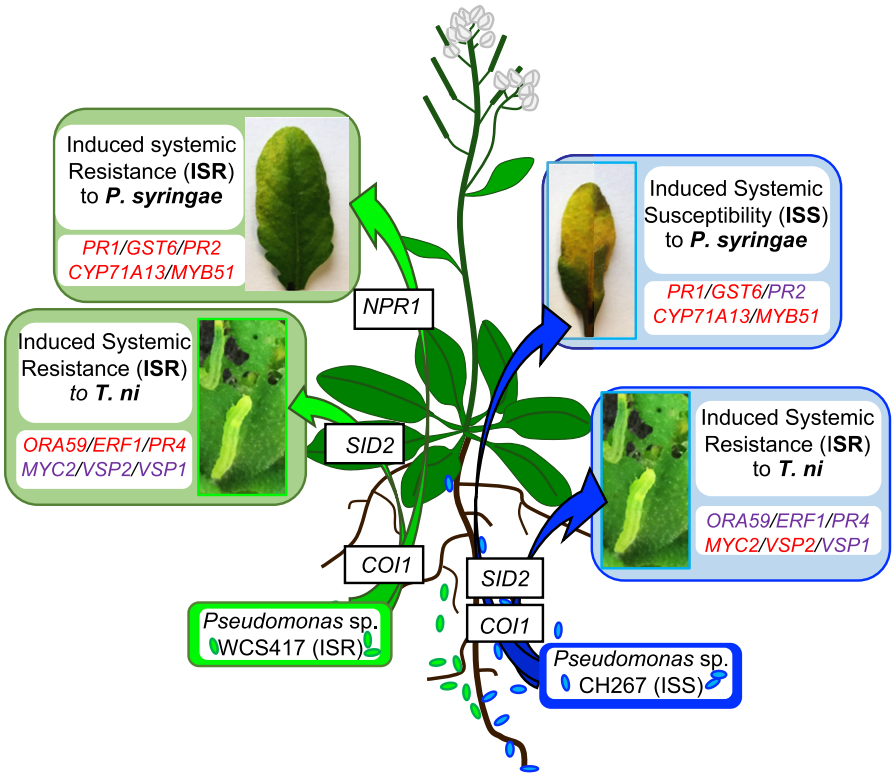

FIGURE 7 Summary of effects of rhizosphere *Pseudomonas* spp. on systemic signalling and transcriptional responses. Genetic evidence is shown in black text; transcriptional upregulation is shown in red text and downregulation relative to wild-type *Pto* DC3000 or *T. ni* challenged tissue is shown in purple. Both *Pseudomonas* spp. WCS417 and CH267 induce systemic resistance to herbivores via *COI1* and *SID2*. Systemic transcriptional responses favour *MYC2*-dependent gene expression after *Pseudomonas* sp. CH267 treatment and *T. ni* challenge, while the *ORA59/ERF1* pathway is favoured after rhizosphere treatment with WCS417. ISR against *Pto* DC3000 induced by *Pseudomonas* sp. WCS417 is dependent on *NPR1* while ISS against *Pto* DC3000 is dependent on *SID2*. With the exception of *PR2*, systemic induction of SA and pathogen-inducible gene expression is intact in systemic leaf tissue after treatment with ISS strain *Pseudomonas* sp. CH267 [Colour figure can be viewed at wileyonlinelibrary.com]

Pseudomonas sp. CH267, which promotes JA-dependent signalling at the cost of SA signalling, resulting in enhanced herbivore resistance at the cost of resistance to bacterial pathogens. In contrast, consistent with previous reports, the rhizosphere strain *Pseudomonas* sp. WCS417 induces resistance to both a foliar bacterial pathogen and a chewing herbivore through simultaneous induction of the SA and JA signalling pathways (van de Mortel et al., 2012; Pineda et al., 2012; Van Oosten et al., 2008). Together, these results demonstrate that strain-level differences in the composition of the rhizosphere microbiome can result in dramatically different outcomes to above-ground biotic interactions.

Rhizosphere treatment of *Arabidopsis* with *Pseudomonas* sp. CH267 results in stronger presentation of JA-dependent defences reminiscent of the phenotype observed in an *Arabidopsis* SA mutant impaired in antibiotrophic pathogen defences (Figure 1c). However, we provide evidence that only a subset of NPR1-independent, SA responses are affected by *Pseudomonas* CH267 treatment. Our herbivory experiments with the *npr1-1 Arabidopsis* mutant showed that ISS strain *Pseudomonas* sp. CH267 could still promote JA-dependent antiherbivory defences, at the cost of SA-dependent defences, in an *npr1-1* mutant background but not in a *sid2-2* mutant (Figure 4) indicating the *Pseudomonas* sp. CH267 modulates the JA/SA trade-off independently of *NPR1*. Additionally, with the exception of *PR2*, we observed normal induction of SA-dependent genes after exposure to the foliar pathogen *Pto* DC3000 (Figure 6), which also supports that only a subset of SA-dependent processes are suppressed by rhizosphere treatment with *Pseudomonas* sp. CH267. *PR2* encodes a ß-1,3-glucanase whose expression is both SA and abscisic acid (ABA) dependent and is required for callose deposition in response to bacterial pathogens (Oide et al., 2013). Induction of *PR2* in response to a biotrophic pathogen is only partially dependent on SA signalling via *NPR1* (Reuber et al., 1998). In contrast, *PR1* is nearly completely dependent on SA signalling via *NPR1* (Cao et al., 1994; Reuber et al., 1998). Importantly, whereas *PR2* is highly upregulated when the SA-dependent, partially *NPR1*-independent transcription factor *WRKY70* is overexpressed, *PR1* is not (Li, Brader, & Palva, 2004) indicating that *PR1* and *PR2* are subject to distinct regulation. Collectively, these findings point to *Pseudomonas* sp. CH267-induced modulation of the JA/SA trade-off through an *NPR1*-independent mechanism to promote resistance to herbivores at the cost of resistance to pathogens.

We use transcriptional profiling to monitor the effects of distinct strains of rhizosphere *Pseudomonas* spp. on systemic JA- and SA-dependent defence gene induction. The JA signalling pathway has two distinct branches that are mutually antagonistic (Verhage et al., 2011). The first requires the *MYC2* transcription factor, controls resistance to herbivores and is solely regulated by JA (Lorenzo, Chico, Sánchez-Serrano, & Solano, 2004). The second, regulated by the *ORA59/ERF1* transcription factors, controls resistance to necrotrophic pathogens and is coregulated by JA and ET (Pré et al., 2008). We found that treatment of wild-type *Arabidopsis* roots with *Pseudomonas* sp. CH267 induces the *MYC2* branch of JA signalling and represses the *ORA59/ERF1* branch after herbivore challenge (Figure 6). This study and previous work has shown that treatment of *Arabidopsis* with the ISR strain *Pseudomonas* sp. WCS417 favours induction of the *ORA59/ERF* branch over the *MYC2* branch (Pangesti et al., 2016; Figure 6). Induction of the *MYC2* branch of JA signalling (Figure 6c) is sufficient to explain increased foliar resistance to *T. ni* after rhizosphere treatment with *Pseudomonas* sp. CH267 (Figure 1a).

Variation in microbial communities between individuals can be a driver of phenotypic variation in many eukaryotic species. For instance, the presence *Helicobacter pylori* in the human gut can explain much of the variance in susceptibility to stomach ulcers and cancer. Current microbiome sequencing methods using 16S rRNA sequencing do not resolve strain and functional level differences in rhizosphere *Pseudomonas* spp. (*Pseudomonas* spp. CH267 and WCS417 are 97% identical by 16S rRNA and would be called the same OTU by current methods; www.earthmicrobiome.org). Even so, many microbiome studies identify more than one OTU within the *P. fluorescens* and *P. putida* groups in the rhizosphere of a single population of *Arabidopsis* (Bulgarelli et al., 2012; Haney et al., 2015) indicating that it is possible that multiple strains of *Pseudomonas* with diverse functional potential coexist within the rhizosphere of an individual plant, or within a single population. We propose that bacterial strain diversity in the plant microbiome may be a driver of phenotypic diversity and plasticity within a population.

By leveraging a naturally occurring tri-trophic system, we describe a novel mechanism of modulation of induced plant defence pathways by rhizosphere microbes. Our work shows that there is likely unrecognized functional diversity in the vast inventory of plant-associated microbes and that a substantial amount of phenotypic plasticity can be introduced into plant–microbe and plant–insect defences through rhizosphere bacteria. That closely related rhizosphere strains can induce such dramatically different phenotypes—such as ISS and ISR—suggests plants may associate with microbial symbionts that are adaptive to specific local biotic stresses. A much more nuanced understanding of the molecular mechanisms by which closely related rhizosphere strains induce different plant defence phenotypes has important implications for guiding development of beneficial strains for agriculture. This work will facilitate precise plant rhizosphere microbiome manipulation to protect plants against pests of the greatest agronomic importance.

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DATA ACCESSIBILITY

The described Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the Accession nos. NXDO00000000 (CH409), NXHE00000000 (CH229) and NXNJ00000000 (CH267). The versions described in this manuscript are version NXDO00100000, NXHE01000000 and NXNJ01000000, respectively.

AUTHOR CONTRIBUTIONS

C.H.H., L.R.S., J.C., N.E.P. and F.M.A. conceived experiments and discussed results. C.H.H., C.L.W., L.R.S., L.R.O., S.K., L.X. and J.H. performed experiments. C.H.H., C.L.W., L.R.S. and R.A.M. analysed data. C.H.H. and L.R.S. wrote the manuscript with input from all.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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