Comparative genomics identified a genetic locus in plant-associated *Pseudomonas* spp. that is necessary for induced systemic susceptibility

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Abstract

Plant root-associated microbes promote plant growth and induce systemic resistance (ISR) to foliar pathogens. In an attempt to find novel growth-promoting and ISR-inducing strains, we previously identified strains of root-associated *Pseudomonas* spp. that promote plant growth but unexpectedly induced systemic susceptibility (ISS) rather than ISR to foliar pathogens. Here we demonstrate that the ISS-inducing phenotype is common among root-associated *Pseudomonas* spp. Using comparative genomics, we identified a single *P. fluorescens* locus that is unique to ISS strains. We generated a clean deletion of the 11-gene ISS locus and found that it is necessary for the ISS phenotype. Although the functions of the predicted genes in the locus are not apparent based on similarity to genes of known function, the ISS locus is present in diverse bacteria and a subset of the genes have previously been implicated in pathogenesis in animals. Collectively these data show that a single bacterial locus contributes to modulation of systemic plant immunity.

Importance

Microbiome-associated bacteria can have diverse effects on health of their hosts, yet the genetic and molecular basis of these effects have largely remained elusive. This work demonstrates that a novel bacterial locus can modulate systemic plant immunity. Additionally, this work demonstrates that growth promoting strains may have unanticipated consequences on plant immunity and this is critical to consider when engineering the plant microbiome for agronomic improvement.

Keywords: rhizosphere, microbiome, induced systemic susceptibility, *Pseudomonas*, Arabidopsis

Introduction

Plant growth promotion by beneficial microbes has long been of interest because of the potential to improve crop yields. Individual root-associated microbial strains can promote plant growth by facilitating nutrient uptake, producing plant hormones, or improving resilience to both abiotic and biotic stresses (1). In some cases, single bacterial loci underlie beneficial effects of microbes on plants, while other traits appear to be complex and polygenic.

Pseudomonas fluorescens and related species are a model for beneficial host-associated microbes due to their genetic tractability and robust host-association across diverse eukaryotic hosts. Direct plant growth promotion (PGP) by *Pseudomonas* spp. can be mediated by bacterial production of the phytohormones auxin (2) or by the expression of 1-aminocyclopropane-1-carboxylate (ACC) deaminase that metabolizes plant-derived ethylene (1, 3). Indirect PGP through antimicrobial activity and pathogen suppression has been attributed to production of the antibiotic 2,4-diacetylphloroglucinol (DAPG) (4). However, the molecular basis of many traits such as induced systemic resistance (ISR) has remained elusive, and multiple distinct bacterial traits including production of siderophores, LPS, and salicylic acid have all been implicated (5).

We previously reported two *Pseudomonas* spp. that induce systemic susceptibility (ISS) on *Arabidopsis* and can promote growth under nutrient limiting conditions (6, 7). These same *Pseudomonas* strains suppress a subset of salicylic acid (SA)-dependent responses and promote resistance to herbivores (7). Although it is possible that ISS-inducing strains contain multiple genetic loci that affect plant growth and pathogen resistance, we hypothesized that a single bacterial trait may be responsible for both the growth and immunity phenotypes of ISS strains.

63 Growth and immunity have a reciprocal relationship in plants, leading to growth-defense
64 tradeoffs to the extent that plant stunting has been used as a proxy for autoimmunity (8). As a
65 result, we hypothesized that suppression of plant immunity by *Pseudomonas* strains that trigger
66 ISS may be a consequence of PGP activity. The genomes of ISS strains do not contain genes for
67 the ACC deaminase enzyme prevalent in other *Pseudomonas* PGP strains (3); thus, we
68 hypothesized that there may be a distinct mechanism of growth promotion in these strains.
69 Because of the high density of sampling and genome sequencing within *P. fluorescens*
70 and related species, we reasoned that if ISS is an overlooked consequence of growth promotion
71 then: 1) we should be able to identify additional ISS strains by sampling known PGP strains and
72 additional root-associated strains, and 2) assuming a single unique locus was responsible, that a
73 comparative genomics approach should reveal the underlying genetic basis of ISS.
74 Here we report that ISS is relatively common among *Pseudomonas* strains within the *P.*
75 *fluorescens* species complex. We identified new ISS isolates including previously described PGP
76 or environmental isolates and new isolates from *Arabidopsis* roots. Using comparative genomics,
77 we identified a single bacterial locus that is unique to *Pseudomonas* ISS strains. We show that
78 the putative ISS locus is necessary to elicit ISS. While the function of genes in the locus remains
79 elusive, a subset have previously been implicated in pathogenesis, and we found that the locus
80 contributes to rhizosphere growth. Collectively, these data indicate that a single microbial locus
81 contributes to a systemic immune response in a plant host.
82
83 **Results**
84 **ISS is a common feature of growth-promoting *Pseudomonas* spp.**

We previously reported that two strains of *Pseudomonas* (CH229 and CH267) induce systemic susceptibility (ISS) to the foliar pathogen *Pseudomonas syringae* pv. tomato DC3000 (*Pto*) under conditions where a well-characterized ISR strain [*P. simiae* WCS417 (9)] conferred resistance to *Pto* (6, 7). To the best of our knowledge, descriptions of *Pseudomonas*-elicited ISS against bacterial pathogens are limited to *Pseudomonas* sp. CH229 and CH267, strains that were independently isolated from the rhizospheres of wild *Arabidopsis* plants in Massachusetts, USA. We reasoned that if ISS is common among *Arabidopsis*-associated *Pseudomonas* spp., we would be able to identify additional ISS strains from *Arabidopsis* roots from plants growing at distinct sites.

We isolated 25 new fluorescent pseudomonads from wild-growing *Arabidopsis* plants from additional sites in Massachusetts and in Vancouver, Canada. We generated ~800 bp sequences of a region of the 16S rRNA gene where strains CH229 and CH267 are 99.5% identical, but each shares only <96% identity to the well-characterized ISR strain WCS417. Reasoning that new ISS strains would be closely related to CH267 and CH229, we selected 3 new isolates [1 from Massachusetts (CH235) and 2 from British Columbia (PB101 and PB106)] that were >97% identical to CH267 by 16S rRNA sequence and another 3 (from British Columbia: PB100, PB105 and PB120) that were <97% identical to CH229 and CH267 (Fig. S1). We tested these 6 new rhizosphere *Pseudomonas* isolates for their ability to trigger ISS.

Consistent with the hypothesis that ISS may be common among closely-related PGP *Pseudomonas*, we found that 2 of the 3 strains that were most closely related to CH267 (CH235 and PB101) elicited ISS (Fig. 1). Two strains with <96% identity to CH267 failed to trigger ISS: PB105 triggered ISR and PB100 had no effect on systemic defenses (Fig. 1). PB106 and PB120 consistently enhanced susceptibility in all experiments, but to a more moderate degree (*p<0.1).

Collectively, these data indicate that the ability to elicit ISS on *Arabidopsis* ecotype Col-0 may be a common feature among some, but not all, closely-related strains of *Pseudomonas* spp. isolated from the *Arabidopsis* rhizosphere.

Because ISS seemed restricted to strains that were closely related to CH267, we obtained several additional isolates with similar 16S rRNA sequences including *Pseudomonas* sp. UW4, *Pseudomonas* sp. Pf0-1, and *P. vancouverensis* DhA-51. We also tested a growth promoting strain, *Pseudomonas* sp. WCS365 that is more distantly related and to our knowledge has not been tested for ISR/ISS (Table 1). We found that UW4 and DhA-51 elicited ISS while Pf0-1 and WCS365 did not (Fig. 1). *Pseudomonas* sp. UW4 (10) and WCS365 are well-characterized growth promoting strains. *Pseudomonas* sp. Pf0-1 (11) is an environmental isolate. *Pseudomonas vancouverensis* DhA-51 is also an environmental isolate (12) and was previously shown to be closely related to Pf0-1 (13). Because DhA-51 is an environmental isolate that triggers ISS, these data show that the ability to trigger ISS is not specific to rhizosphere isolates.

To gain insights into the distinguishing features of ISS strains, we sequenced the genomes of the 6 new isolates (CH235, PB100, PB101, PB105, PB106 and PB120) from *Arabidopsis* roots as well as *P. vancouverensis* DhA-51 (UW4, WCS365, CH267 and CH229 have been sequenced previously). Whole genome sequencing was used to assemble draft genomes (Methods). We generated a phylogenetic tree using 122 conserved genes as described previously (7, 14). We found that all ISS strains are closely related to one another and fall within a monophyletic group which corresponds to the *P. koreensis*, *P. jessenii*, and *P. mandelii* subgroups of *P. fluorescens* identified in a recent phylogenomic survey of *Pseudomonas* spp. [Fig. 2B; (15)]. However, not every isolate in this clade is an ISS strain; notably Pf0-1, which has no effect on systemic immunity despite being closely related to CH229. We reasoned that the

absence of the ISS phenotype in Pf0-1 should facilitate the use of comparative genomics by allowing us to separate the phylogenetic signature from the phenotypic signature of ISS.

11 genes in a single genomic locus are unique to ISS strains and predicts ISS

To identify the potential genetic basis of the ISS phenotype, we used a previously described database of orthologous genes for *Pseudomonas* spp. (14) to identify genes that are present in ISS strains (CH229, CH235, CH267 and UW4) but are absent in the closely-related strain that has no effect on systemic defenses (Pf0-1). We used only the ISS strains with the most robust phenotypes for this analysis. We identified 29 predicted protein-coding genes absent in Pf0-1 but present in all of the other strains. Of these, 12 were small (<100 aa) hypothetical proteins. The remaining 17 predicted protein-coding genes were prioritized for further analysis and are shown in S1 Table. Intriguingly, 11 of the 17 ISS unique genes are found in a single genomic locus.

We surveyed the genomes of other *Pseudomonas* strains tested for ISS to determine if the presence of the 17 genes identified by our comparative genomics approach correlated with the ISS phenotype. We found that the 11 clustered genes were present in ISS strains (DhA-51 and PB101) and the strains with intermediate phenotypes (PB120 and PB106) but were absent in the non-ISS strain WCS365, WCS417 and PB105 (Fig. S2). The remaining 6 genes were all present in WCS365 and/or other non-ISS strains (Fig. S2). We chose to focus on the 11 ISS-unique genes ("ISS locus" hereafter) for further study.

We found that the 11 genes in the ISS locus are found at a single genomic locus in all 4 of the ISS strains (Fig. S3 and Fig. 2A). The flanking regions are conserved in the non-ISS strain Pf0-1 (Fig. 2A), indicating a recent insertion or deletion event. Within this locus, there is a single gene that is conserved in Pf0-1 in addition to two genes that are unique to each individual strain

suggesting multiple changes to this genomic region in recent evolutionary history. While all 11 genes are within the same genomic region in the ISS strains, the variability of this locus between closely related strains suggests it may be rapidly evolving.

We surveyed the genomes of sequenced isolates available in our collection for the presence of the ISS locus. We found a number of closely-related strains from various environmental sources that contained the ISS locus, as well as a more distantly related strain (Pf-5) (Fig. 2B). We tested 2 strains that contain the ISS locus (Pf-5 and GW456-L13) as well as 2 that do not (FW300-N1B4 and FW300-N2C3) and found that the presence of the ISS locus correlated with the ISS phenotype, including the distantly-related strain Pf-5 (Fig. 2C). Collectively, these data show that the presence of the 11 candidate genes in the ISS locus identified by our comparative genomics approach is predictive of the ISS phenotype.

The ISS locus is necessary for ISS

To test if the ISS locus is necessary for ISS strains to induce systemic susceptibility, we deleted the entire 15 kB locus including the region spanning the 11 genes identified in our initial comparative genomics screen in strains CH267 and UW4 (Fig. 2A). We tested these deletion mutants for their ability to induce systemic susceptibility and found that deletion of the entire 11-gene locus (ΔISSlocus), resulted in a loss of the ISS phenotype in both CH267 and UW4 (Fig. 3A and B). This indicates that the ISS locus is necessary for ISS.

The functions of the majority of the genes in the ISS locus are not apparent based on similarity to genes of known function. A predicted 2544 bp gene was annotated in the CH267 and other genomes as *speE2* due to the similarity of the predicted *C*-terminus to well-characterized spermidine synthase gene *speE1* (PputUW4_02826 and CP336_12795 in UW4 and

CH267, respectively). CH267 *speE2* has similarity to a characterized spermidine synthase gene *speE* in *P. aeruginosa* [25% predicted amino acid identity to *P. aeruginosa* PA1687 (16)]. A second *speE*-like gene in the genomes of UW4 and CH267, annotated as *speE1*, is outside of the ISS locus (PputUW4_03691 and CP336_28780 in UW4 and CH267 respectively) and is highly similar to the *P. aeruginosa speE* gene (~84.0% predicted amino acid identity) (16).

To test if the *speE2* gene is necessary for ISS, we also constructed an in-frame deletion of just the *speE2* gene in both CH267 and UW4. We found that deletion of *speE2* abolished in the ISS phenotype in both CH267 and UW4 (Fig. 2A and 2B) To determine if *speE2* is the only gene within the ISS locus that is necessary for induction of ISS, we generated a complementation plasmid where the CH267 *speE2* gene is expressed under the lac promoter (p_{lac}-*speE2*). We introduced this plasmid into the Δ*speE2* deletion and ΔISSlocus deletions in CH267. While p_{lac}-*speE2* complemented the CH267 Δ*speE2* deletion, it failed to complement the ΔISSlocus deletion (Fig. 3C) indicating that *speE2* is not the only gene within the ISS locus that is required for ISS.

Because deletion of *speE2* in CH267 and UW4 results in the specific loss of the ISS phenotype, this result indicates that the *speE1* and *speE2* genes are not functionally redundant. *SpeE1* and *speE2* differ in length and predicted structure (Fig. 4A). *SpeE1* encodes a predicted 384-amino acid protein and contains a predicted polyamine synthase domain with a predicted decarboxylated S-adenosyl methionine (dSAM) binding motif. *SpeE2* encodes a protein of a predicted 847 amino acids. Similar to *speE1,* the C-terminus of *speE2* contains a predicted dSAM-binding domain; however, *SpeE2* contains predicted transmembrane domains at its N-terminus (Fig. 4A). Spermidine synthases generate spermidine by transferring the aminopropyl group of dSAM to putrescine. Previous structural and mutagenesis analysis on human and

Thermatoga maritima SpeE1 enzymes revealed common residues important for catalysis (D276, D279, D201, and Y177 for the human SpeE1, and the corresponding D173, D176, D101, and Y76 from the *T. maritima* SpeE1) (17, 18). The catalytic mechanism was proposed to be initiated by the deprotonation of the putrescine amino group by the conserved aspartic acid D276 or D173 with the aid of the side chains of D201 or D101 and Y177 or Y76 as well as the main chain carbonyl of L277 or S174, setting up a nucleophilic attack on dcAdoMet. In addition, residue D279 or D176 is thought to play a role in substrate binding (17, 18).

To determine if SpeE2 has the potential to be a spermidine synthase, we performed an amino acid sequence alignment to see if the catalytic residues from classic spermidine synthases are conserved in SpeE2. We found that although the tyrosine residue is conserved, SpeE2 consists of different residues at the corresponding aspartic acid positions. The proposed catalytic residue D276 or D173 in the human or *T. maritima* enzymes corresponds to E624 in SpeE2 while residues D201 or D101 and D279 or D176 have been converted to T556 and P627 (Fig. 4B). Furthermore, we generated a sequence similarity network for SpeE2 with enzymes found in the PF17284 protein family and found that SpeE2 belongs to a distinct cluster away from any functionally characterized enzymes (Fig. 4C). Interestingly, the SpeE2 active site residue substitutions are almost completely conserved within and unique to the SpeE2 cluster (Fig. 4C) suggesting that while *Pseudomonas* sp. CH267 SpeE2 is unlikely to act as a spermidine synthase it may have a distinct function.

Additional roles for the ISS locus in host interactions

While *speE2* is necessary for ISS, the failure of Δ*speE2* to complement the 11-gene ISS locus deletion (Fig. 3C) indicates that at least one other gene in the ISS locus is likely required

for ISS. We tested whether *speE2* is always associated with the same larger locus across the genus *Pseudomonas*. When we analyzed our entire computational dataset of >3800 genomes from across *Pseudomonas*, we found that there was a strong correlation for the presence or absence of 9 of 11 genes ($r > 0.9$, Fig. 5A). Moreover, we also found that these 9 co-occurring genes were frequently found in the same genomic region, as there were moderate to strong correlations for 9 of the 11 genes co-occurring in the same 50-kb genomic region (Fig. 5B). From a phylogenomic standpoint, we found that these genes were broadly distributed throughout the *Pseudomonas* genus and co-occurred even in taxonomic groups far outside of the *P. fluorescens* clade (Fig. 5C). Within the *P. fluorescens* clade, the ISS locus genes are frequently found in some clades, such as the *koreensis* and *jessenii* clades, which contain most of our isolates (Fig. 5D). However, some clades are missing these genes entirely, such as the plant associated *corrugata* clade (Fig. 5D). Together, these genomic data indicate that despite their polyphyletic distribution among divergent clades of *Pseudomonas* spp., the genes in the ISS locus likely participate in conserved or similar functions.

Within the 9 genes that have a high frequency of co-occurrence, we identified a 6 gene predicted operon in the ISS locus with identical domain structure and organization that is involved in stress resistance and virulence in *Francisella tularensis* (19) (Fig. 6A). Another similar operon is associated with aerotolerance and virulence in *Bacteroides fragilis* (20). Returning to our comparative genomics database, we found that these 6 genes comprise an operon broadly conserved in the *Pseudomonas* clade that is distinctly paralogous from the 6-gene operon in the ISS locus (Fig. 6A). This raises the possibility that these six genes within the ISS locus contribute to host-bacterial interactions across diverse bacterial taxa and both plant and animal hosts (Fig. 6A).

246 To test if the ISS locus is required for *Pseudomonas* to grow in the Arabidopsis
247 rhizosphere, we tested the UW4 and CH267 ΔISSlocus and Δ*speE2* mutants for rhizosphere
248 growth. We transformed the wildtype and mutant CH267 and UW4 strains with a GFP plasmid
249 and used a previously described 48-well plate assay to quantify bacterial growth in the
250 rhizosphere (6). Under these conditions, we observed a significant decrease in rhizosphere
251 growth of ΔISScluster deletion mutants in both the UW4 and CH267 backgrounds (Fig. 6B). We
252 found no decrease in rhizosphere colonization by Δ*speE2* mutants in either the CH267 or UW4
253 genetic background (Fig. 6B). Together these data indicate that the ISS locus contributes growth
254 in the rhizosphere; however, the Δ*speE2* mutant has a loss of ISS while retaining normal
255 rhizosphere growth indicating a dual role in both rhizosphere colonization and ISS for this
256 genetic locus.
257
258 **Discussion**
259 Plant root-associated ("rhizosphere") microbes perform a diversity of functions that
260 benefit their plant hosts including nutrient uptake and defense. Functional characterization of
261 individual plant-associated bacterial and fungal strains of potential agronomic importance (i.e.
262 growth promoters or nitrogen fixers) is widespread (5). However, closely-related strains of
263 bacteria can have very distinct effects on plant growth and defense (13), and these effects can be
264 dependent on environmental context (1). Lack of known correlations between microbial
265 genotype and potential effects on plant hosts present a challenge when attempting to infer the
266 effect that a microbe may have on its plant host from sequence identity alone.
267 Our use of comparative genomics and isolate phenotyping to identify the genetic basis of
268 a complex microbial-derived trait indicates that this is an effective approach to identifying

important microbial traits to improve plant health. For comparative genomics to be effective, traits should be controlled by single or limited genomic loci, and phylogeny should not be predictive of function. In this case, a close relative of ISS strains, *Pseudomonas* sp. Pf0-1 (>99% identical by full length 16S rRNA to the ISS strains) does not affect systemic defenses (Fig. 1), which allowed us to use comparative genomic to identify the underlying basis. We previously used this approach to find the genomic basis of a pathogenic phenotype within a clade of commensals (14). It has been previously observed that phylogeny is not predictive of function for ISR strains (13) suggesting that comparative genomics may be appropriate to find the basis of additional plant-associated traits.

We found that the ISS locus encodes genes involved in both triggering ISS and promoting rhizosphere colonization. Loss of the entire locus results in a loss of ISS and a decrease in growth in the rhizosphere; however, loss of *speE2* impairs ISS but not rhizosphere growth suggesting that there may be multiple plant-association functions encoded in this locus. The function of the *speE2* gene and other genes within the ISS locus are not readily apparent from similarity to previously characterized enzymes. As spermidine and other polyamines should directly enhance plant resistance through generation of ROS (21), it is possible that the *speE2* enzyme converts spermidine or another polyamine to a non-defense inducing molecule. The highly conserved nature of the active-site residues within *speE2*-like genes suggests a novel function in this enzyme.

While enhancement of systemic susceptibility is not an obviously agronomically useful plant trait, several ISS strains promote growth and enhance resistance to insect pests (6, 7). Using ISS strains might be beneficial for crops where insects are the primary pressure on crop productivity. However, the ubiquity of ISS by plant growth-promoting strains illustrates the

292 complexity of host-microbe interactions and should be considered when engineering the
293 microbiome.
294
295 **Materials and Methods**
296 **Plant growth conditions**
297 For all experiments, plants were grown in Jiffy-7 peat pellets (Jiffy Products) under a 12 h
298 light/12 h dark at 22 °C temperature regime. Seeds were surface sterilized by washing with 70%
299 ethanol for 2 minutes followed by 5 minutes in 10% bleach and 3 washes in sterile water. Seeds
300 were stored at 4^{o} C until use. Unless otherwise indicated, seeds were sowed in Peat pellets (Jiffy
301 7) and placed in a growth chamber under 12-hour days and 75 μM cool white fluorescent lights
302 at 23^{o} C.
303
304 **Bacterial growth and 16S rRNA sequencing**
305 *Pseudomonas* strains were cultured in LB or King's B at 28 °C. New *Pseudomonas* strains were
306 isolated from the roots of wild-grown *Arabidopsis* plants around eastern Massachusetts, USA
307 and British Columbia, Canada as described (6). New *Pseudomonas* isolates were preliminary
308 identified based on fluorescence on King's B and confirmed by 16S rRNA sequencing.

Strain	Genus and species	Isolated From	Location	Reference
CH267	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Cambridge, MA USA	(6)
CH235	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Carlisle, MA USA	(6)
CH229	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Carlisle, MA USA	(6)
PB100	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Vancouver, BC Canada	This study
PB101	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Vancouver, BC Canada	This study
PB105	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Vancouver, BC Canada	This study
PB106	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Vancouver, BC Canada	This study

PB120	*Pseudomonas* sp.	*Arabidopsis* rhizosphere	Eastham, MA USA	This study
WCS417	*P. simiae*	Wheat rhizosphere	Netherlands	(22)
UW4	*Pseudomonas* sp.	Reeds	Waterloo, ON Canada	(10)
Pf0-1	*Pseudomonas* sp.	Environmental soil		(11)
DhA-51	*P. vancouverensis*	Environmental soil	Vancouver, BC Canada	(12)
WCS365	*Pseudomonas* sp.	Tomato rhizosphere	Netherlands	(23)
Pf-5	*Pseudomonas* sp.	Cotton rhizosphere	College Station, TX USA	(24)
GW456-L13	*P. fluorescens*	Groundwater	Oakridge, TN USA	(25)
FW300-N1B4	*P. fluorescens*	Groundwater	Oakridge, TN USA	(25)
FW300-N2C3	*P. fluorescens*	Groundwater	Oakridge, TN USA	(25)

Table 1. Bacterial strains used in this study

ISS assays

ISS and ISR assays were performed as described (7, 26). Briefly, *Pseudomonas* rhizosphere isolates were grown at 28 °C in LB medium. For inoculation of plant roots for ISR and ISS assays, overnight cultures were pelleted, washed with 10 mM $MgSO_4$ and resuspended to a final OD_{600} of 0.02. Jiffy pellets were inoculated 9 days after seed germination with 2 mls of the indicated bacterial strains at a final OD_{600} of 0.02 ($5x10^5$ CFU g^{-1} Jiffy pellet). For infections, the leaves of 5-week old plants were infiltrated with *Pto* DC3000 at an OD_{600} = 0.0002 (starting inoculum ~10^3 CFU/cm^2 leaf tissue). Plants were maintained under low light (<75 µM) and high humidity for 48 hours. Leaf punches were harvested, ground, and plated to determine CFU counts.

16S rRNA sequencing, bacterial genome sequencing, assembly and phylogenomics

Bacterial DNA preps were performed using Qiagen Purgene Kit A. 16S rRNA was amplified using 8F and 1391R and sequenced using 907R. Bacterial genomic library prep and genome

sequence was performed as described (7). Briefly, bacterial DNA was isolated using Qiagen Purgene Kit A and sonicated into ~500 bp fragments. Library construction was performed as described (7), individually indexed and sequenced using MiSeq V3 paired end 300 bp reads. After barcode splitting, approximately 500,000 to 1 million reads were used for each sample to assemble draft genomes of the strains *Pseudomonas* sp. CH235, PB100, PB101, PB105, PB106, PB120 and *P. vancouverensis* DhA-51. Genome assembly was carried out as previously described (7) and draft genomes are available from NCBI (see below).

Phylogenomic tree building

To generate the 29-taxon species tree used in Figs. 2B and 4E, we made use of an alignment of 122 single-copy genes we previously found to be conserved in all *Pseudomonas* strains (14). From this amino acid alignment, we extracted 40,000 positions ignoring sites where >20% of the taxa had gaps. Using RAxMLv8.2.9, we inferred 20 independent trees under the JTT substitution model using empirical amino acid frequencies and selected the one with the highest likelihood. Support values were calculated through 100 independent bootstrap replicates under the same parameters.

To build the 3,886-taxon phylogeny of the *Pseudomonas* genus in Figs. 5C and S1, the same 122-gene alignment was used. For computational feasibility, the alignment was randomly subsampled to 10,000 amino acid positions, again ignoring sites that were highly gapped (>20%). FastTree v2.1.9 was used to build the phylogeny using default parameters. The phylogeny was rooted to a clade of *Pseudomonas* identified as an outgroup to all other *Pseudomonas* spp. as previously described (14). To more easily visualize this tree, we collapsed

347 monophyletic clades with strong support (as determined by FastTree's local Shimodaira-
348 Hasegawa test) that correspond with major taxonomic divisions identified by Hesse et al. (2018).
349 To build the tree for the *Pseudomonas fluorescens* (*Pfl*) subclade seen in Figs. 5D and S2,
350 we identified 1,873 orthologs specific to the *Pfl* clade found in >99% of all strains in the clade
351 and then aligned them all to the hidden Markov models generated by PyParanoid using
352 hmmalign, prior to concatenation. This alignment had 581,023 amino acid positions, which we
353 trimmed to 575,629 positions after masking sites with >10% of taxa with gaps. From this
354 alignment, we randomly subsampled 120,000 sites for our final phylogenomic dataset. Using
355 RAxMLv8.2.9, we inferred 20 independent trees under the JTT substitution model using
356 empirical amino acid frequencies and selected the one with the highest likelihood. Support
357 values were calculated through 100 independent bootstrap replicates under the same parameters.
358
359 **Comparative Genomics**
360 Comparative genomics analyses were performed by using a previously described framework for
361 identifying PyParanoid pipeline and the database we built for over 3800 genomes of
362 *Pseudomonas* spp. Briefly, we had previously used PyParanoid to identify 24,066 discrete
363 groups of homologous proteins which covered >94% of the genes in the original database. Using
364 these homolog groups, we annotated each protein-coding sequence in the newly sequenced and
365 merged the resulting data with the existing database, generating presence-absence data for each
366 of the 24,066 groups for 3,886 total *Pseudomonas* genomes.
367 To identify the groups associated with induction of systemic susceptibility, we compared
368 the presence-absence data for 4 strains with ISS activity (*Pseudomonas* spp. CH229, CH235,
369 CH267, and UW-4) and 1 strain with no activity (*Pseudomonas* sp. Pf0-1). We initially

suspected that ISS activity was due to the presence of a gene or pathway (i.e. not the absence of a gene) and thus initially focused on genes present only in Pf0-1. We identified 29 groups that were present in the 4 ISS strains but not in Pf0-1.

To obtain the correlation coefficients in Figs. 4D and 5A, we coded group presence or absence as a binary variable and calculated Pearson coefficients across all 3,886 genomes. To calculate the correlation coefficients in Fig. 5B, we split the genomic database into 50-kb contiguous regions and assessed group presence or absence within each region. Because this dataset is heavily zero-inflated, we ignored regions that had none of the 11 groups, taking the Pearson coefficient of the 11 genes over the remaining regions.

Initial annotation of the ISS groups was based on generic annotations from GenBank Further annotation of the 11 groups specific to the ISS locus was carried out using the TMHMM v2.0 server, the SignalP 4.1 server and a local Pfam search using the Pfam-A database from Pfam v31.0. To identify homologous genes in the genomes of *Francisella tularensis* subsp. *holarctica* and *Bacteroides fragilis* YCH46, we relied on locus tags reported in the literature which we confirmed using annotation based on another Pfam-A domain search.

Deletion of the *speE2* gene and 11-gene ISS locus

Deletions in the CH267 and UW4 strains were constructed by a two-step allelic exchange as described (27). The flanking regions directly upstream and downstream of the 11-gene ISS locus or the *speE2* gene were amplified and joined by overlapping polymerase chain reaction (PCR) using genomic DNA as template and primers listed in Table 2. Following digest, the product was ligated into the pEXG2 suicide vector that contains the *sacB* gene for counter-selection on sucrose (28). The recombinant plasmid was then transformed into calcium-competent *E. coli*

393 DH5α by heat shock. After confirmation of correct insert by PCR and sequencing, the plasmid
394 was transformed into WM3064 (29). Conjugation of plasmid into CH267 and UW4 from
395 WM3064 was performed by biparental mating on King's B media supplemented with
396 diaminopimelic acid, and transconjugants were selected using 10 µg/mL gentamicin and 15
397 µg/mL nalidixic acid. The second recombination leading to plasmid and target DNA excision
398 was selected for by using sucrose counter-selection. Gene deletions in CH267 and UW4 were
399 confirmed by PCR amplification of the flanking regions with primers listed in Table 2, agarose
400 gel electrophoresis and Sanger sequencing.
401

402 **Table 2. Primers used to generate the mutant *Pseudomonas* strains analyzed in this study.**

Strain	Primer type	Primer name	Restriction site	Sequence (5'→3')
CH267	Upstream forward	CH409	HindIII	AAAAAGCTTAGTCGCAACCTCGCCTCGACTGAC
ΔISSlocus	Upstream reverse	CH410	—	AAACGGGCGGGAGCAGCACTTGG
	Downstream forward	CH411	—	CACTGACTCCGCTTATTGTTTTGTGTC
	Downstream reverse	CH412	EcoRI	AAAGAATTCTTCACGCCGCCGCAGGATGTC
	Upstream confirmation	PB401	—	CGCTATGACCTGGGCCGCAACGAA
	Downstream confirmation	PB402	—	CCGACGCCGACCATGAGCGAAA
CH267	Upstream forward	CH413a	HindIII	AAAAAGCTTGCTCCAGCAAAACCGTCGCTCCA
Δ*speE*	Upstream reverse	CH414a	—	CTCTCGTCATCCGATCATTCCCACGCGG
	Downstream forward	CH415	—	GAATGATTGTTCCCATGCATAGCGTGG
	Downstream reverse	CH416a	EcoRI	AAAGAATTCCCGGGCTCGACTGGTTCCCGA
	Upstream confirmation	PB403	—	CTACAGCCAACTCAAGGAGGCCAA
	Downstream confirmation	PB404	—	CGGGTGAGGTCTCGAACGAGATGT
UW4	Upstream forward	CH401	HindIII	AAAAAGCTTACGCCTCGGCCATCGGTGTACC
ΔISSlocus	Upstream reverse	CH402	—	GAAAGGCTCCTGCAGAAGATCGAAC
	Downstream forward	CH403	—	GTAACACCTCCAAACGTTCCGGGAT
	Downstream reverse	CH404	EcoRI	AAAGAATTCAACGCACCTGCACATCGGCTGCG
	Upstream confirmation	PB405	—	GGGTCATGTCCCTGACCAGCA

	Downstream confirmation	PB406	—	GGGTCGAATTCCGTGTCGCCAA
UW4	Upstream forward	CH405	HindIII	AAAAAGCTTGAGCCGATTGAGCTGGATGCGG
Δ*speE*	Upstream reverse	CH406	—	TACGACTTCCATGGTCCAGGTGCG
	Downstream forward	CH407	—	TCGGGGGGCTGGCTCAAAGG
	Downstream reverse	CH408	EcoRI	AAAGAATTCACGAGTCGGCGCTCAAACGCG
	Upstream confirmation	PB407	—	CGCGAACCTGTGGACCAGCGAGTT
	Downstream confirmation	PB408	—	CGCGAACCGCGCTGCAAGAA
p_{lac}-*speE2*	Upstream forward	speE_up2	HindIII	AAAAAGCTTCCACGCTATGCATGGGAACAA
	Downstream reverse	speE_down1	BamHI	AAAGGATCCGGATGACGAGAGTCACTGC
	Confirmation primer 1	PB409	—	GGGCGTGTCGAATACCGGCGA
	Confirmation primer 2	PB410	—	GCGCGGCTCGCCGTT
	Confirmation primer 3	PB411	—	CGCCGCCGGCGATGGA

Complementation of the *speE2* gene

The *speE2* gene was amplified by PCR using CH267 genomic DNA as template, as well as the primers listed in Table 2. Following restriction digestion, the ~2.6 kb insert was ligated into the pBBR1MCS-2 vector into the multiple cloning site located downstream of a lac promoter. Ligation mixture was then introduced into *E. coli* DH5α by heat shock, and transformants were selected using LB media supplemented with 25-50 µg/mL kanamycin. Presence of correct insert was confirmed by PCR, restriction digest and Sanger sequencing. pBBR1-MCS2::p_{lacZ}-speE2$_{CDS}$ plasmids were maintained in *E. coli* DH5α λpir with 25 µg/mL of Kanamycin. To construct a conjugating strain, Calcium-competent *E. coli* WM3064 was first transformed with pBBR1-MCS2::p_{lacZ}-speE2$_{CDS}$ or pBBR1-MCS2 by heat shock. To conjugate *Pseudomonas* sp. CH267, 1 mL of overnight cultures of *Pseudomonas* sp. CH267 and *E. coli* WM3064 carrying the appropriate plasmids were washed twice and resuspended with 0.5 mL of 100 mM $MgCl_2$. The resuspended *Pseudomonas* sp. CH267 was mixed with *E. coli* WM3064 strains at 1:2 ratio. Six 25 µL mating spots were placed on LB plates supplemented with 0.3 mM of Diaminopimelic

418 acid (DAP). The mating spots were allowed to dry before incubating at 28°C for 4 hr. The
419 mating spots were then scraped off and resuspended in 1 mL of 100 mM $MgCl_2$. 100 µL of the
420 suspension was plated on LB-Kanamycin. Colonies were restreaked to confirm antibiotic
421 resistance.
422
423 **Multiple sequence alignment and Sequence Similarity Network (SSN) Generation**
424 Multiple sequence alignment was performed with Clustal Omega (30). The SSN was created
425 using the enzyme function initiative (EFI-EST) web tool (31) by inputting the SpeE2 amino acid
426 sequence with the amino acid sequences from the spermidine synthase tetramerization domain
427 with the code PF17284 using UniRef90 seed sequences instead of the whole family. Sequences
428 will less than 100 amino acids were also excluded resulting in a total of 6523 sequences. An
429 alignment score threshold or E-value cutoff of 10^{-100} was used to generate the SSN which was
430 visualized using Cytoscape (32).
431
432 **Rhizosphere colonization assay.**
433 Arabidopsis seedlings were grown in 48-well plates and rhizosphere growth of bacteria was
434 quantified as previously described (6). Briefly, *Arabidopsis* seeds were placed individually in 48-
435 well clear-bottom plates with the roots submerged in hydroponic media (300 µl 0.5× MS media
436 plus 2% sucrose). The medium was replaced with 270 µl 0.5× MS media with no sucrose on day
437 10, and plants were inoculated with 30 µl bacteria at an OD_{600} of 0.0002 (final OD_{600}, 0.00002;
438 ~1,000 cells per well) on day 12. Plants were inoculated with wild-type *Pseudomonas* CH267 or
439 UW4 strains containing plasmid pSMC21 (*pTac-GFP*) (33). Fluorescence was measured with a
440 SpectraMax i3x fluorescence plate reader (Molecular Devices) (481/515 excitation/emission) 5

441 days post inoculation. A standard curve related fluorescence to OD was generated to estimate
442 CFU/wells (OD_{600} = 1 = 5 × 10^8 CFU/mL).
443
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449 Graduate Scholarship Council Award to Y.L., and an NSERC CGS-M award to Z. L.
450
451 **Author Contributions**
452 C.H., R.A.M., and P.B. designed experiments. P.B. Y.S. Y.L. and C.H.H. performed
453 experiments. C.H., R.A.M., Z.L. analyzed data and R.A.M. performed genome assembly,
454 annotation, phylogenetic analysis and comparative genomics. M.H. and K.R. performed
455 bioinformatic analyses of *speE2* function. C.H.H., P.B. and R.A.M. wrote the manuscript with
456 input from all.
457
458 **Data Availability**
459 Data for the Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank
460 under the accessions RRZJ00000000 (CH235), RRZK00000000 (DhA-51), RWIM00000000
461 (PB106), RWIN00000000 (PB120), RWIO0000000 (PB105), RWIQ00000000 (PB100), and
462 RWIR00000000 (PB101). The versions described in this paper
463 are versions RRZJ01000000 (CH235), RRZK01000000 (DhA-51), RWIM01000000 (PB106),

RWIN01000000 (PB120), RWIO0100000 (PB105), RWIQ01000000 (PB100), and RWIR01000000 (PB101).

Declaration of interests: The authors declare no competing interests.

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568 **Figure Legends**

569

570 **Figure 1. Induced Systemic Susceptibility (ISS) is common among closely-related strains of**
571 ***Pseudomonas* spp.** Isolates of *Pseudomonas* were tested for their ability to modulate systemic
572 defenses; bars are colored to indicate % relatedness to CH267 by partial 16S rRNA sequence as
573 indicated in the key. Data are the average of 3-5 biological replicates with 2 leaves from each of
574 6 plants (n=12) per experiment. Means +/- SEM are shown. Letters designate levels of
575 significance ($p<0.05$) by ANOVA and Tukey's HSD tests.

576

577 **Figure 2. The presence of a genomic island is predictive of the ISS phenotype. (A)** A
578 genomic island identified through comparative genomics is present in the ISS strains CH229,
579 CH235, CH267 and UW4 and absent in Pf0-1 (no effect on systemic defense) and WCS417 (ISR
580 strain). **(B)** Phylogenetic tree based on 122 core *Pseudomonas* genes. Genome sequencing of
581 new strains shows the island is present in strains that enhance susceptibility but not in those that
582 trigger ISR or have no effect. **(C)** Two strains with the island (GW456-L13 and Pf-5) and two
583 without (N1B4 and N2C3) were tested for ISS/ISR. Only those with the island significantly
584 enhanced susceptibility. Data are the average of 3 biological replicates with 2 leaves from each
585 of 6 plants (n=12) per experiment. Means +/- SEM are shown. *$p<0.05$ by ANOVA and Tukey's
586 HSD.

587

588 **Figure 3. The ISS locus and *speE2* gene are necessary for ISS. (A-B)** The *speE2* gene and the
589 entire 11-gene locus were deleted from CH267 (A) and UW4 (B). **(C)** Expression of *speE2* from
590 a plasmid is sufficient to complement the CH267 Δ*speE2* mutant but not the ΔISSlocus mutant.

591 Data are the average of 3 biological replicates with 2 leaves from each of 6 plants (n=12) per
592 experiment. Means +/- SEM are shown. *p<0.05 by ANOVA and Tukey's HSD.
593
594 **Figure 4. *speE2* is different from characterized spermidine synthases. (A)** The genome of
595 CH267 contains two *speE* homologues. Both contain predicted d-SAM binding domains and a
596 spermidine synthase domain. Only SpeE2 contains predicted N-terminal transmembrane
597 domains. **(B)** Multiple sequence alignment of predicted amino acid sequence of CH267 SpeE2
598 and the relatively distantly related Pf-5 SpeE2 gene along with SpeE1-like proteins from CH267,
599 *E. coli*, *Homo sapiens,* and *Arabidopsis thaliana.* Although the catalytic (blue) and binding-site
600 (yellow) are conserved in all SpeE1 homologues, both SpeE2 genes have changes in these
601 regions (gray). (**C**) Sequence Similarity Network (SSN) of SpeE2 and protein sequences found
602 with the PFAM domain code PF17284. Sequences that have the conserved residues D201/D101,
603 D276/D173, and D279/D176 similar to the human and *T. maritima* SpeE1 are colored blue while
604 sequences that had conserved residues T556, E624, P627 similar to SpeE2 are colored red.
605 Clusters with only 1 sequence were removed for simplicity.
606
607 **Figure 5. 9 genes in the ISS locus nearly always co-occur and are present across the**
608 ***Pseudomonas* genus. (A)** Correlation coefficient matrix for 9 genes in the ISS locus across all
609 3,886 *Pseudomonas* genomes in the comparative genomics database. **(B)** Correlation coefficient
610 matrix for the 9 ISS genes across every 50-kb genomic region that contains at least one of the 9
611 genes. **(C)** Distribution of the 9 ISS genes across subclades of the *Pseudomonas* genus. (**D**)
612 Distribution of the 9 ISS genes within subclades of the *P. fluorescens* group.
613

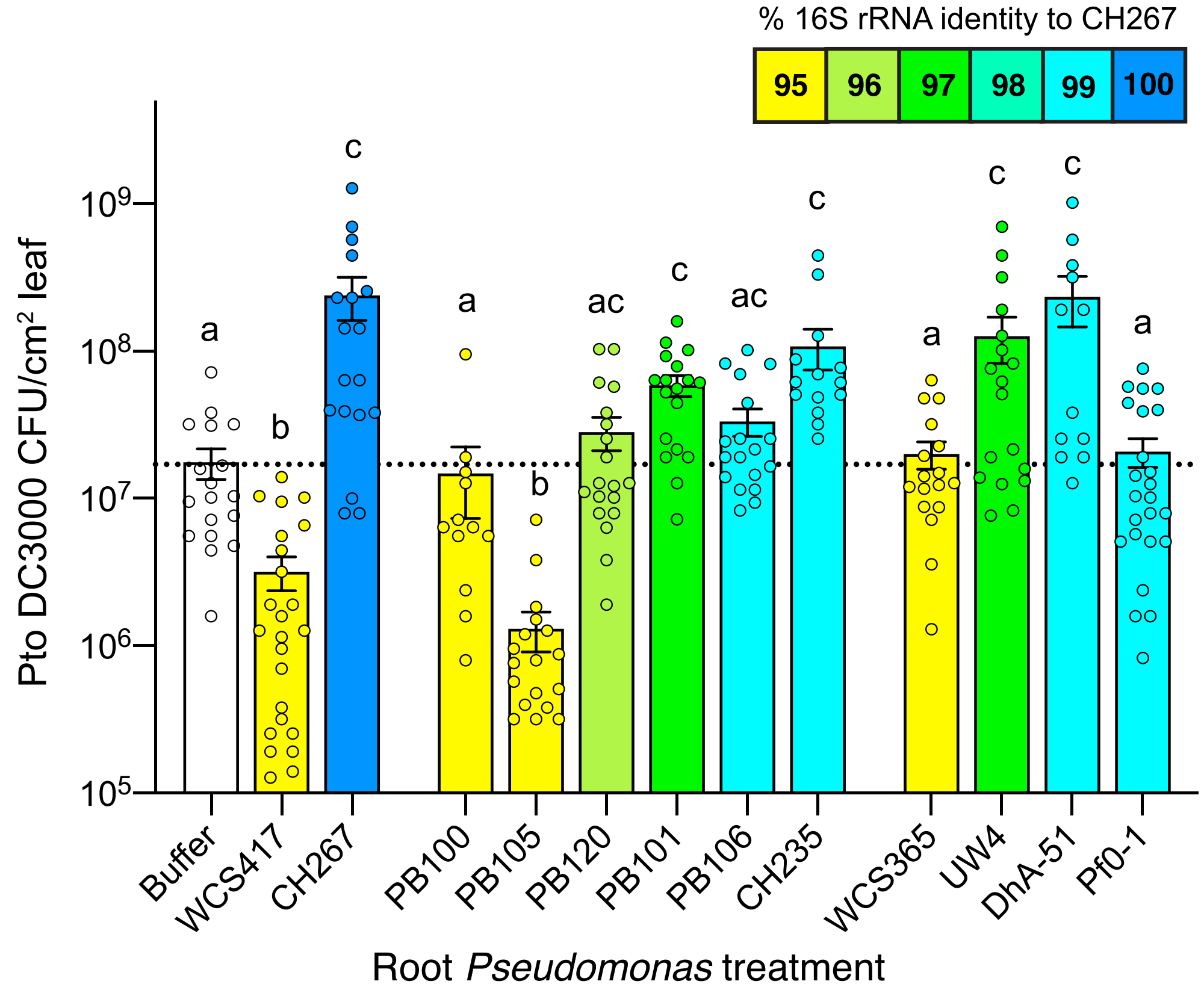
Figure 6. A conserved subset of genes in the ISS locus contribute to virulence and host association in mammalian pathogens and in *Pseudomonas* spp. (A) Of the 11 genes in the ISS locus, 6 are contained within a paralogous operon that is present in CH267 and most other *Pseudomonas* spp. An operon with a similar configuration is also present in mammalian pathogens and has been implicated in virulence. (B) The ISS locus, but not the *speE2* gene, promotes rhizosphere colonization. We tested the ΔISSlocus and Δ*speE2* mutant in CH267 and UW4 using a 48-well plate-based rhizosphere colonization assay. Data shown are from 5 days post inoculation. *p<0.05 between mutants in a genetic background by ANOVA and Tukey's HSD.

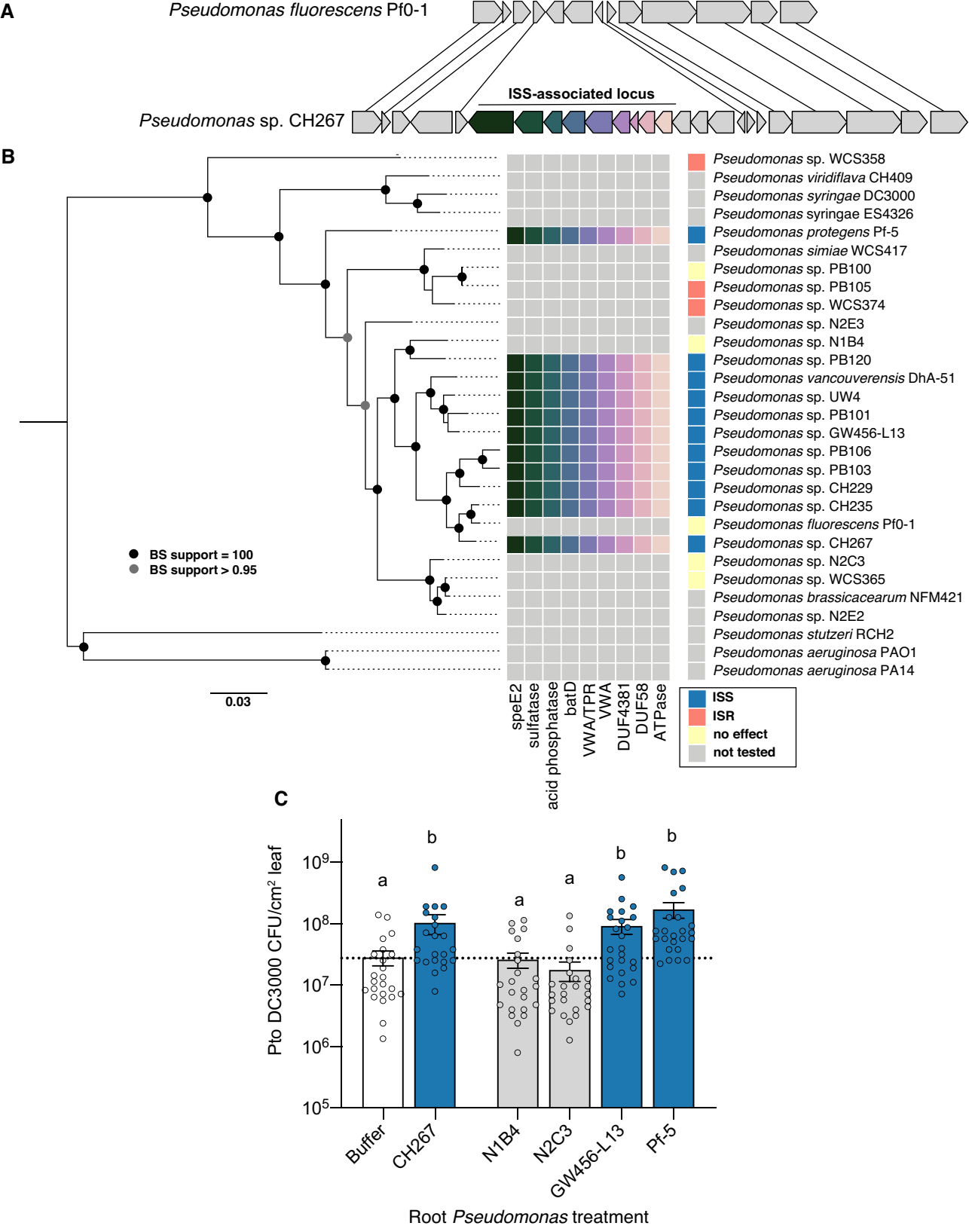
S1 Table. Unique loci identified in comparative genomics. The genome content of 4 ISS strains (CH267, CH235, UW4 and CH229) was compared with the closely-related non-ISS strain Pf0-1. 17 predicted protein-coding genes were identified.

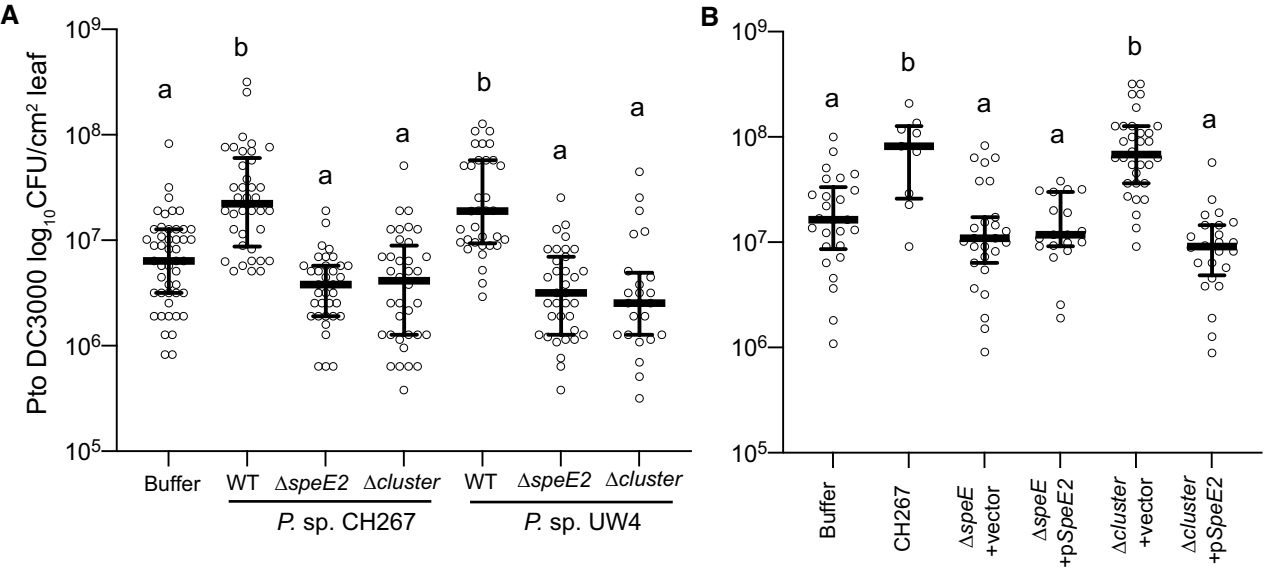
Figure S1. Correlation matrix of 16S rRNA similarity of new *Pseudomonas* isolates from the Arabidopsis rhizosphere. Isolates were selected based on similarity (>97% identical by partial 16S rRNA) to CH267 (CH235, PB101 and PB106) or distance (<97% identity by partial 16S rRNA) to CH267 (PB120, PB100, PB105). Isolates from the rhizosphere of Arabidopsis growing in *Massachusetts, USA or #British Columbia, Canada.

Figure S2. Distribution of loci identified by comparative genomics ISS loci across *Pseudomonas* strains. Comparative genomics between ISS strains UW4, CH229, CH235 and CH267 (black arrows) and non-ISS strain Pf0-1 (red arrow) identified 17 predicted protein-

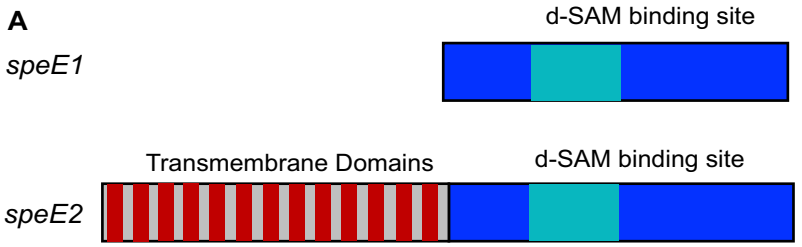
637 coding genes >100 aa that were absent in Pf0-1 and present in strains that induce ISS. 11 of these
638 genes were found in a single genomic locus (box) and were absent in the non-ISS strain
639 WCS365.
640
641 **Figure S3. The ISS locus is highly variable between closely-related strains**
642 The 11 genes in the ISS locus are present in the ISS strains Pf0-1, CH235, CH267 and CH299
643 but absent in Pf0-1. Genes in the ISS locus are colored as in the key at the bottom of the figure
644 and in Fig. 2. Conserved genes not unique to the ISS strains are colored similarly among strains;
645 genes in gray are not conserved between strains at this locus. In CH229, Pf0-1 and CH267 the
646 genes flanking the ISS locus are conserved in the same orientation suggesting a recent insertion
647 or deletion event.


% 16S rRNA identity to CH267
95
96
97
98
99
100
Pto DC3000 CFU/cm² leaf
10⁹
10⁸
10⁷
10⁶
10⁵
a
b
c
a
b
ac
c
ac
c
a
c
c
a
Buffer
WCS417
CH267
PB100
PB105
PB120
PB101
PB106
CH235
WCS365
UW4
DhA-51
Pf0-1
Root Pseudomonas treatment


A
Pseudomonas fluorescens Pf0-1
ISS-associated locus
Pseudomonas sp. CH267
B
Pseudomonas sp. WCS358
Pseudomonas viridiflava CH409
Pseudomonas syringae DC3000
Pseudomonas syringae ES4326
Pseudomonas protegens Pf-5
Pseudomonas simiae WCS417
Pseudomonas sp. PB100
Pseudomonas sp. PB105
Pseudomonas sp. WCS374
Pseudomonas sp. N2E3
Pseudomonas sp. N1B4
Pseudomonas sp. PB120
Pseudomonas vancouverensis DhA-51
Pseudomonas sp. UW4
Pseudomonas sp. PB101
Pseudomonas sp. GW456-L13
Pseudomonas sp. PB106
Pseudomonas sp. PB103
Pseudomonas sp. CH229
Pseudomonas sp. CH235
Pseudomonas fluorescens Pf0-1
Pseudomonas sp. CH267
Pseudomonas sp. N2C3
Pseudomonas sp. WCS365
Pseudomonas brassicacearum NFM421
Pseudomonas sp. N2E2
Pseudomonas stutzeri RCH2
Pseudomonas aeruginosa PAO1
Pseudomonas aeruginosa PA14
BS support = 100
BS support > 0.95
0.03
speE2
sulfatase
acid phosphatase
batD
VWA/TPR
VWA
DUF4381
DUF58
ATPase
ISS
ISR
no effect
not tested
C
Pto DC3000 CFU/cm² leaf
10⁹
10⁸
10⁷
10⁶
10⁵
a
b
a
a
b
b
Buffer
CH267
N1B4
N2C3
GW456-L13
Pf-5
Root Pseudomonas treatment


A
B
Pto DC3000 log10 CFU/cm2 leaf
10^9
10^8
10^7
10^6
10^5
a
b
a
a
b
a
a
Buffer
WT
ΔspeE2
Δcluster
WT
ΔspeE2
Δcluster
P. sp. CH267
P. sp. UW4
Buffer
CH267
ΔspeE +vector
ΔspeE +pSpeE2
Δcluster +vector
Δcluster +pSpeE2

A

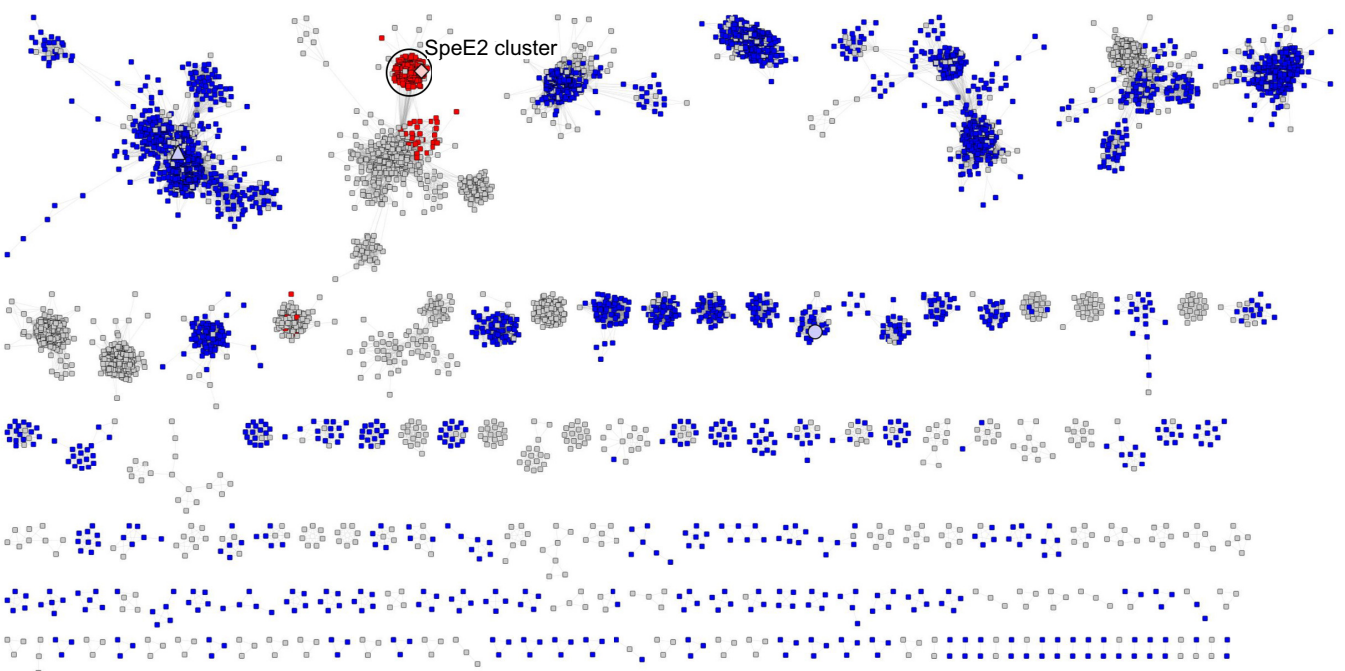


B

```
CH267SpeE2   GDAMPSLRYMRIQALLPLLIHNGEPRSALVIGFGTGITAGALLRYPGLEHRVVAELLPSV   581
Pf5SpeE2     GDAMPSLRYMRIQALLPLLIHNGEPRSALVIGFGTGITAGALLRYPGLEQRVVAELLPAV   569
CH267SpeE1   TE-ADEFIYHEMLTHVPILAH-GTAKRVLIIGGGDGGMLREVTKHASVEHITMVEIDGTV   115
EcSpeE       TE-RDEFIYHEMMTHVPLLAH-GHAKHVLIIGGGDGAMLREVTRHKNVESITMVEIDAGV   113
HsSpeE       TE-RDEFSYQEMIANLPLCSH-PNPRKVLIIGGGDGGVLREVVKHPSVESVVQCEIDEDV   129
AtSpeE       TE-RDECAYQEMITHLPLCSI-SNPKKVLVIGGGDGGVLREVARHSSVEQIDICEIDKMV   125
              :   .  * .: : :*:       : .*:** * *     : :: .:*     *:   *

CH267SpeE2   IKAAPLFKGNFNAA--SDPGVDVRLRDGRQELLRS-PQTYDLITLEPPPPSAAGVVNLYS   638
Pf5SpeE2     LDAAPLFQGNFNAA--SDPGIQIRLQDGRQELLRS-PQNYDLITLEPPPPSAAGVVNLYS   626
CH267SpeE1   VDMCKEFLPNHSKGAYDDPRLNLVIDDGMRFVATT-TEKFDVIISDSTDPIGPG-EVLFS   173
EcSpeE       VSFCRQYLPNHNAGSYDDPRFKLVIDDGVNFVNQT-SQTFDVIISDCTDPIGPG-ESLFT   171
HsSpeE       IQVSKKFLPGMAIG-YSSSKLTLHVGDGFEFMKQN-QDAFDVIITDSSDPMGPA-ESLFK   186
AtSpeE       VDVAKQYFPNVAVG-YEDPRVNLIIGDGVAFLKNAAEGTYDAVIVDSSDPIGPA-KELFE   183
             :. .  :  .   .  .. . : : **   :       :* :  :   *  . .   *:
```

C



E-value = 1 x 10^{-100}

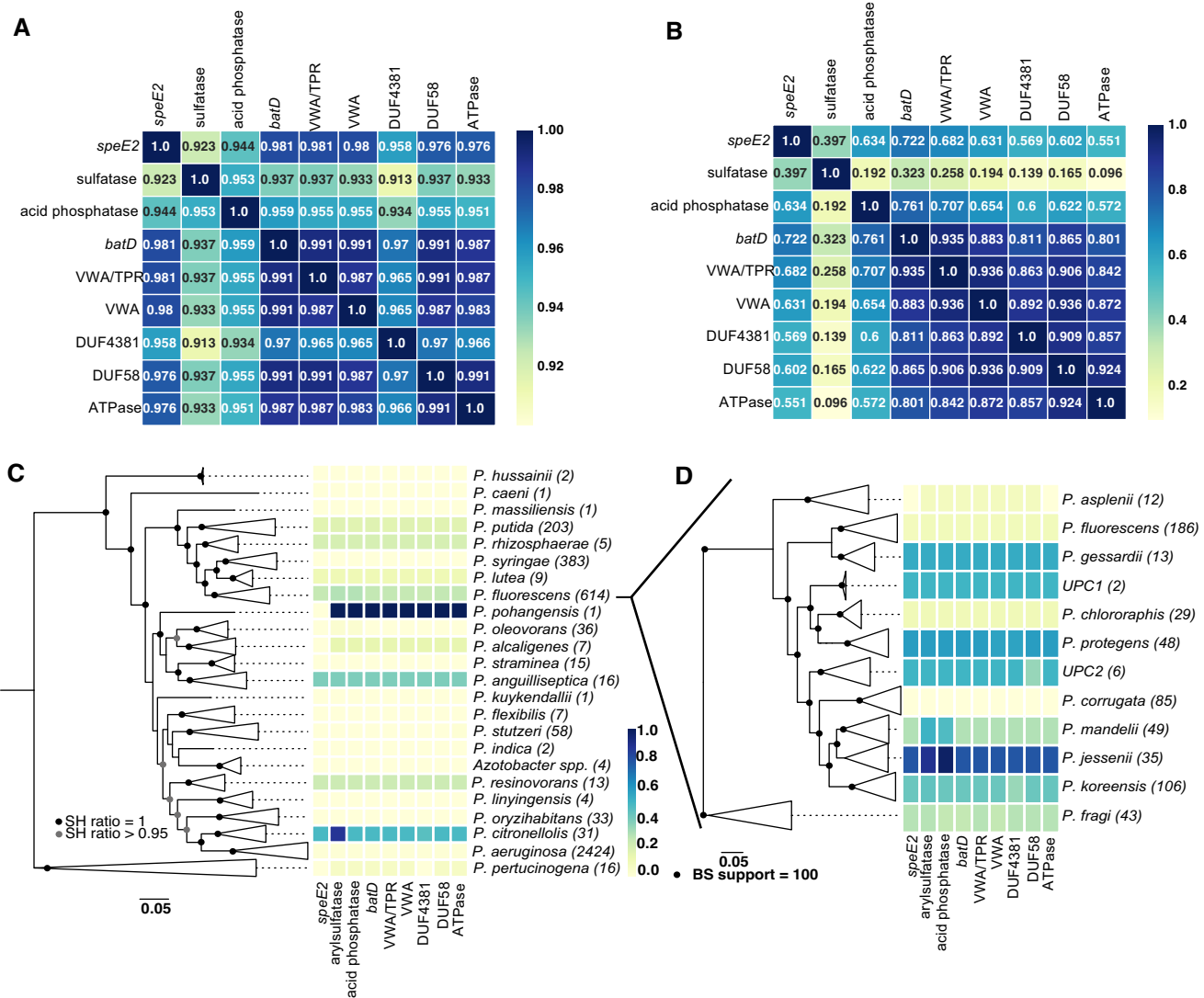
SpeE2 cluster

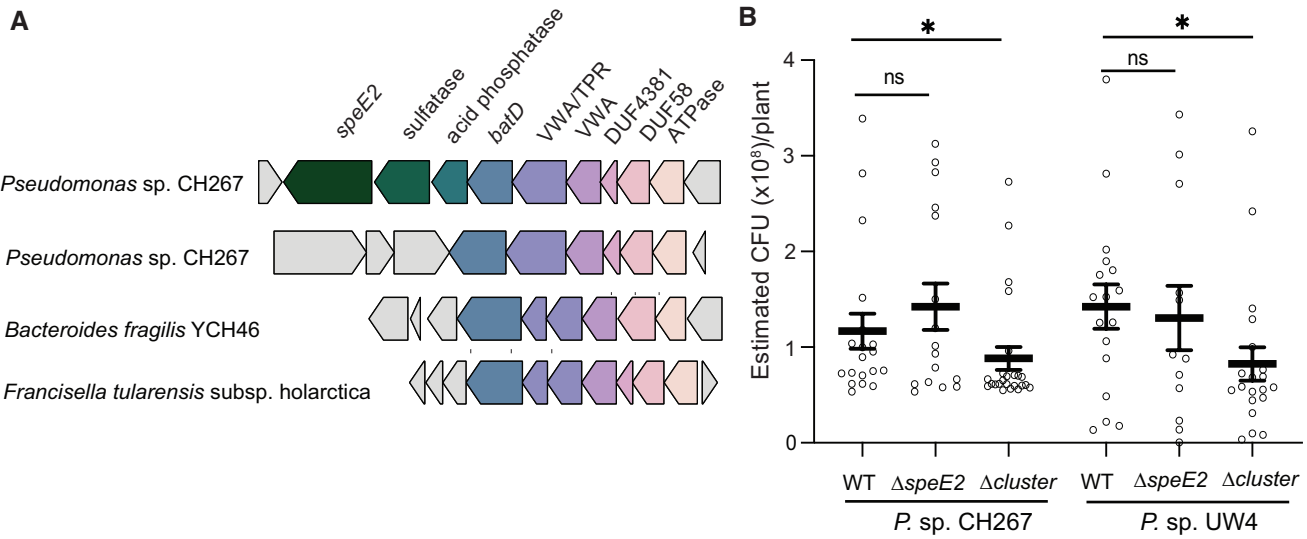
Human SpeE1

T. maritima SpeE1

Active-site residues conserved with SpeE2

Acitive-site residues conserved with Human and *T. maritima* SpeE1


A
speE2
sulfatase
acid phosphatase
batD
VWA/TPR
VWA
DUF4381
DUF58
ATPase
1.00
0.98
0.96
0.94
0.92
B
1.0
0.8
0.6
0.4
0.2
C
P. hussainii (2)
P. caeni (1)
P. massiliensis (1)
P. putida (203)
P. rhizosphaerae (5)
P. syringae (383)
P. lutea (9)
P. fluorescens (614)
P. pohangensis (1)
P. oleovorans (36)
P. alcaligenes (7)
P. straminea (15)
P. anguilliseptica (16)
P. kuykendallii (1)
P. flexibilis (7)
P. stutzeri (58)
P. indica (2)
Azotobacter spp. (4)
P. resinovorans (13)
P. linyingensis (4)
P. oryzihabitans (33)
P. citronellolis (31)
P. aeruginosa (2424)
P. pertucinogena (16)
SH ratio = 1
SH ratio > 0.95
0.05
arylsulfatase
D
P. asplenii (12)
P. fluorescens (186)
P. gessardii (13)
UPC1 (2)
P. chlororaphis (29)
P. protegens (48)
UPC2 (6)
P. corrugata (85)
P. mandelii (49)
P. jessenii (35)
P. koreensis (106)
P. fragi (43)
BS support = 100


A
speE2
sulfatase
acid phosphatase
batD
VWA/TPR
VWA
DUF4381
DUF58
ATPase
Pseudomonas sp. CH267
Pseudomonas sp. CH267
Bacteroides fragilis YCH46
Francisella tularensis subsp. holarctica
B
Estimated CFU (x10^8)/plant
ns
ns
*
*
WT
ΔspeE2
Δcluster
WT
ΔspeE2
Δcluster
P. sp. CH267
P. sp. UW4