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Differences in pathogenicity on adzuki bean (Vigna angularis) and virulence factors among Pseudomonas syringae strains

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Title: Differences in pathogenicity on adzuki bean (*Vigna angularis*) and virulence factors among *Pseudomonas syringae* strains

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ABSTRACT

Bacterial brown spot was first observed in Ontario on adzuki beans [*Vigna angularis* (Willd.) Ohwi & Ohashi] in Ontario in 2014, resulting in several crop losses in some fields. The disease is caused by the pathogen *Pseudomonas syringae* pv. *syringae*, which causes leaf chlorosis that leads to necrosis and defoliation as well as pod and seed damage. To elucidate putative key virulence determinants, a comparative genomics study was conducted with four *P. syringae* strains: an archived isolate, Pss27 (*P. syringae* pv. *syringae*), andMe2, Me3, and Li6, which were isolated in this study. In a phylogenetic analysis, Me2 and Me3 clustered with *P. syringae* pv. *aceris* strains, and Li6 with a *P. syringae* pv. *dysoxyli* strain. Orthologous genes, protein families, COGs, and candidate virulence factors were identified, including 173 shared putative virulence factors and the 27 genes required to assemble the type III secretion system. Pss27 had 10 unique virulence factors (ahlI, ahlR, ppkA, avrRpm1, hopAB1, hopAF1, hopAP1, hopH1, hopL1, sypA). Pathogenicity tests with two adzuki varieties, Erimo and Baoqing, indicated that

Pss27 was the most aggressive strain, based on area under the disease progress curve (AUDPC) ($p<0.0001$) and the percent diseased area ($p=0.0009$) measurements. Erimo showed higher levels of resistance than Baoqing based on AUDPC ($p<0.0001$) and percent diseased area ($p=0.0055$) evaluations.

INTRODUCTION

Pseudomonas syringae pv. *syringae* van Hall, a Gram-negative, rod-shaped bacterium, causes bacterial brown spot in beans. Bacterial brown spot, alongside common bacterial blight, caused by *Xanthomonas axonopodis* pv. *phaseoli*, and halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola,* are the three most economically important bacterial blight diseases affecting beans[1]. Yield effects of bacterial diseases in beans range from benign to entire crop loss[2,3,4,5,6]. Bacterial blights are most devastating when favourable environmental conditions persist during early growth and flowering stages[3].

Bacterial brown spot was first reported in beans grown in Ontario in 2014, with adzuki beans most severely affected[7,6]. The symptoms of all three bacterial diseases are similar, with bacterial brown spot often misdiagnosed as common bacterial blight and therefore underestimated[2]. As a result, correct strain identification is important because the *P. syringae* complex contains genetically related lineages with distinct host associations.

Control measures for bacterial brown spot include planting certified seeds, using copper-based bactericide sprays, practicing crop rotation, sanitizing farm equipment, burying plant residues, avoiding overhead irrigation, and avoiding movement through wet crop fields. The use of resistant varieties is generally considered the most effective control measure[8,9,1]. However, currently, no commercial adzuki variety grown in Ontario has complete resistance to bacterial brown spot[9]. Furthermore, no literature is available on

the molecular interactions between the pathogen and the adzuki host that confer resistance or susceptibility to the disease.

The *P. syringae* complex is made up of over 60 pathovars[10] and contains diverse members, each with a distinct narrow-range host specificity[11]. The correct identification of *P. syringae* strains has been a controversial issue for many decades[12]. Several approaches have been used to characterize *P. syringae* strains taxonomically, including pathogenicity screening, biochemical and nutritional testing, rRNA and DNA hybridization [13, 14,15,16]. These approaches all had limitations in correctly designating *P. syringae* strains, as some strains were later discovered to be synonyms or to belong to a different *Pseudomonas* species altogether[16,17]. With the advancement of genomics, multi-locus sequence typing (MLST) of housekeeping and pathogenicity-related genes has been used to reassign some *P. syringae* strains into more accurate taxonomic groups[16,18,19,20,21].

P. syringae strains employ a range of virulence factors, including extracellular polysaccharides, phytohormones, phytotoxins, and secreted effector proteins. These virulence factors are released during the epiphytic and endophytic phases of the pathogen's life cycle. These virulence factors help establish disease in the host by facilitating entry and suppressing host defense responses[22]. The repertoire of virulence factors in pathogenic strains contributes to host specificity and could also be responsible for specificity within different cultivars of the same host[23,24]. Although it has been well established that the interaction between pathogen virulence factors and host targets

68 determines compatibility or incompatibility, the mechanisms underlying the specificity of
69 virulence factors for different host targets within the same species remain unclear[23].

70 Among these virulence mechanisms, the type III secretion system (T3SS) is particularly
71 important in plant-pathogenic *Pseudomonas*, as it delivers effector proteins into host cells
72 where they can manipulate host physiology and immune responses. Nikolić et al. (2023)[24]
73 showed that the repertoire of secreted effector proteins was responsible for the degree of
74 pathogenicity expressed by different strains of *P. syringae* pv. *aptata*. The repertoire of
75 Type III Secretion System (T3SS) effectors in *P. syringae* strains was found to be the key
76 factor of pathogenicity in wheat[25,24].

77 Host selective pressures can lead to the evolution of effectors[24]. The identification of the
78 specific effectors involved in an interaction is the most promising approach to elucidate
79 host-specific mechanisms[23]. Comparative genomics of virulence factor arrays across
80 strains can be a strategy for characterizing evolutionary dynamics vital to host-specific
81 pathogenicity[18,26]. The interactions between virulence factors and host targets can serve
82 as a guide to search for the mechanisms responsible for host-specificity[23].

83 With no information available on the genetic basis of *P. syringae* pathogenicity on adzuki
84 bean, particularly differences among strains, the current work hypothesized that
85 differences in pathogenicity and aggressiveness among *P. syringae* strains used to test
86 adzuki beans for bacterial brown spot resistance are associated with differences in the
87 presence or absence of virulence-associated genes present in the strains.

88 The objectives of this study were to: (1) isolate and characterize *Pseudomonas* spp.
89 associated with adzuki bean seed and determine the genomic relationships of selected

P. syringae isolates; (2) compare the pathogenicity and aggressiveness of selected isolates on adzuki bean; (3) compare their virulence-factor, T3SS, and effector repertoires to identify candidate factors associated with differences in aggressiveness; and (4) establish a controlled-environment inoculation protocol for evaluating adzuki bean susceptibility to bacterial brown spot.

RESULTS

Candidate *P. syringae* isolates were obtained from adzuki seeds harvested in 8 of 18 locations sampled across Ontario (Figure 1A). In total, 62 candidate strains were isolated based on their morphology on milk-tween agar and luminescence. These isolates produced colonies with single cleared zones on milk-Tween agar, indicating their ability to break down casein (Figure 1B I, Supplementary Table 1), and were luminescent under UV and fluorescent lights (Figure 1B II).

The 16S rRNA gene amplicons for all candidate isolates were all of expected size (~ 200 bp (Figure 1C). NCBI BLAST queries of amplicon sequences all returned with 16S rRNA gene matches (Supplementary Table 2). Fifty-seven of the 62 query returns identified the sequence sources as *Pseudomonas* spp. Of these 57, 27 were *Pseudomonas* sp., 20 were *P. viridiflava*, five were *P. syringae*, two were *P. fluorescens,* and one each of *P. baetica*, *P. brassicacearum*, and *P. tolaasii*. All the *P. viridiflava* strains were from the Lincoln location. Both of the *P. fluorescens* strains were from Ayton. Two of the *P. syringae* strains were from Meaford (Me2, Me3), and one each from Lincoln (Li6), N. Lakes Simcoe, and Woodstock. Of the five isolates that did not return as *Pseudomonas*, three isolates from Port Perry were *Klebsiella pneumoniae*. The NCBI BLAST of the 16S rRNA sequence for the *P. syringae* pv. *syringae* strain (Pss27) obtained from Agriculture

and Agri-Food Canada (AAFC), Harrow, Ontario, returned as a match for the partial sequence of *P. syringae* strain Ma001 16S ribosomal RNA gene (Supplementary Table 2).

The whole genomes of the strains Pss27, Me2, Me3, and Li6 were sequenced to depths of 118x, 83x, 80x, and 138x, respectively. The genome sizes ranged from 6,000,811 bp for Pss27 to 5,750,434 bp for Me3 (Table 1). The number of coding sequences ranged from 5,076 to 5,357 in Pss27 to 5,076 in Me3, and the GC content was approximately 59% for all the strains. The number of RNAs (made up of rRNA and tRNA) ranged from 55 in Li6 and Me3 to 58 in Pss27. The percentage of the genomes representing coding sequences was 89% for all strains.

Phylogram of *P. syringae* strains

Core-genome phylogenetic analysis was conducted using 58 *Pseudomonas syringae* genomes, comprising 54 NCBI reference strains representing 21 pathovars and the four isolates Pss27, Me2, Me3, and Li6. The phylogeny was inferred in the zDB software using FastTree from concatenated alignments of 1,337 single-copy core orthologs. Pss27 clustered with the *P. syringae* pv. *syringae* reference strains, while Me2 and Me3 grouped with *P. syringae* pv. *aceris* strains. Li6 grouped within the closely related *P. syringae* pv. *aceris*/*dysoxyli* lineage and was positioned adjacent to the single *P. syringae* pv. *dysoxyli* strain group (Figure 2).

Based on the core-genome phylogeny, representative genomes from the most closely related pathovars were selected for pairwise whole-genome comparison in CLC Genomics Workbench. Pss27 showed 98.75–98.78% average nucleotide identity (ANI)

to the three pv. *syringae* genomes, including 98.76% ANI to strain B728a. Me2 and Me3 showed 99.20–99.29% and 99.24–99.25% ANI, respectively, to the three pv. *aceris* genomes. Li6 showed 99.13–99.21% ANI to the pv. *aceris* genomes and 99.20% ANI to the pv. *dysoxyli* genome (Figure 3).

The corresponding ANI alignment percentages ranged from 89.2–92.0% between Pss27 and the pv. *syringae* genomes, and from 92.9–93.4%, 92.2–93.9%, and 91.6–92.3% between the pv. *aceris* genomes and Me2, Me3, and Li6, respectively. Li6 showed 91.4% alignment with the pv. *dysoxyli* genome.

Cluster of orthologous genes, orthologous genes, and protein families present in the *P. syringae* strains

The four strains have highly functional gene profiles, sharing 1,992 clusters of orthologous genes (COGs) (Figure 4A – D, Figure 5A). Pss27 contained 62 unique COG annotations, compared with between 11 and 16 in Me2, Me3, and Li6. Amino acid transport and metabolism and signal transduction mechanisms were among the most abundant functional categories in all four strains, with relatively small differences in COG category abundance among strains (Figure 5B). The strains also shared 4,402 orthogroups and 2,656 protein-family/domain annotations. Pss27 had the greatest number of unique orthogroups and Protein family annotations (Figure 5C, D).

Virulence factors present in the *P. syringae* strains

Virulence factor database (VFDB) analysis identified 186,185,179 and 181 virulence-associated gene hits in Pss27, Me2, Me3, and Li6, respectively (Supplementary Table 4).

Of these, 173 were shared among all four strains. Pss27 contained 10 identified virulence factors not detected in the other strains, whereas Me2 contained 4, with no strain-specific virulence factors identified in Me3 or Li6 (Figure 5E). Pss27 and Me3 shared two additional virulence factors, Me2 and Li6 shared three, and four were shared by Me2, Me3, and Li6. The unique virulence factors were ahlI, ahlR, ppkA, avrRpm1, hopAB1, hopAF1, hopAP1, hopH1, hopL1, sypA in Pss27, and hcp1, acrB, rmlA, rmlD in Me2. There were no unique virulence factors in Me3 or Li6 (Figure 5E).

Type III secretion systems (T3SS) in *P. syringae* strains

The genomes of Pss27, Me2, Me3, and Li6 each contained the same 27 Type III secretion system (T3SS)-associated genes, indicating a highly conserved T3SS repertoire among the strains (Figure 6A). As the T3SS were identical between the four strains, Pss27 was used as a representative strain for comparison with the well-characterized reference strains *P. syringae* pv. *tomato* DC3000, pv. *phaseolicola* 1448A, and pv. *syringae* B728a. Twenty-five T3SS-associated genes were shared among Pss27 and the reference strains (Figure 6B). The 27 T3SS-associated genes were organized within an approximately 35–37 kb genomic region in all four strains, with similar overall cluster organization, although several non-T3SS genes and hypothetical proteins differed among isolates (Figure 6D).

Although the T3SS-associated gene complement was conserved among the four sequenced strains, greater variation was observed in the repertoire of type III-secreted effector proteins. Pss27 contained 20 effectors, whereas Me2, Me3, and Li6 each contained 15. Fourteen effectors were shared by all four strains. Six effectors, *avrRpm1, hopAB1, hopAF1, hopAP1, hopH1,* and *hopL1*, were detected only in Pss27, whereas

178 *hopA1* was present in Me2, Me3, and Li6 and absent from Pss27 (Figure 6C,
179 Supplementary Table 4)

180 **Pathogenicity tests of *P. syringae* strains**

181 Disease inoculation of the four isolates on detached leaves of two adzuki varieties,
182 'Erimo' and 'Baoqing', showed that all the isolates were pathogenic on both varieties,
183 with AUDPC affected by a significant isolate x variety interaction ($p=0.018$), and Pss27
184 identified as most aggressive through the highest AUDPC. Within the varieties, 'Erimo'
185 exhibited consistently lower AUDPC than 'Baoqing' for all the strains ($p<0.0001$) (Figure
186 7A-D).

187 **DISCUSSION**

188 **Identities of the *P. syringae* strains**

189 From adzuki bean seed lots collected across Ontario, 62 candidate bacterial isolates
190 were recovered, of which 57 were identified as *Pseudomonas* spp. based on 16S rRNA
191 gene sequencing. Five isolates were identified as *P. syringae*, and three of these, Me2,
192 Me3, and Li6, were selected for comparison with the previously isolated Pss27 strain,
193 which served as a reference virulent strain. Whole-genome analyses placed Pss27 with
194 *P. syringae* pv. *syringae*, whereas Me2, Me3, and Li6 were most closely related to the pv.
195 *aceris/dysoxyli* lineage. All four strains were pathogenic on the adzuki bean varieties
196 'Erimo' and 'Baoqing', with Pss27 showing the greatest aggressiveness and 'Erimo'
197 consistently developing less disease. Despite these phenotypic differences, the strains

possessed highly similar core T3SS gene complements, while greater variation was observed in their effector and other virulence-factor repertoires.

Whole-genome comparisons provided greater taxonomic resolution than identification based on 16S rRNA or individual housekeeping genes. Berge et al. (2014)[21] developed a phylogenetic framework for the *P. syringae* complex based on housekeeping-gene sequence divergence, using *Citrate synthase (Cts)* sequence differences of <4% for assignment to a phylogroup and <1.8% for assignment to a clade, while recommending multi-locus sequence analyses (MLSA) for greater confidence. Similarly, Marroni et al. (2023)[27] used the same *Cts* sequence similarity for initial assignment of *P. syringae* strains into phylogroups and putative pathovar designation, though MLSA using three additional housekeeping genes was often required for pathovar differentiation. More broadly, whole-genome comparisons and ANI provide greater resolution for differentiating closely related bacterial strains and are widely used for genome-based species-level taxonomic assignment[28,29,30]. The current NCBI GenBank standard for validating bacterial species assignments is based on ANI[28].

In the present study, core-genome phylogeny and ANI placed Pss27 with reference strains of *P. syringae* pv. *syringae*, with approximately 98.8% ANI to Pss9644, B48, and B728a. This genomic placement is also consistent with the origin of Pss27 from symptomatic dry bean in southern Alberta and with the established association of *P. syringae* pv. *syringae* with bacterial brown spot of *Phaseolus vulgaris*[31]. Me2 and Me3 grouped most closely with pv. *aceris*, whereas Li6 was positioned within the closely related pv. *aceris/dysoxyli* lineage. Recovery of strains closely related to pv. *aceris* and

220 pv. *dysoxyli* from adzuki bean seed is notable because these pathovars are primarily
221 associated with maple and kohekohe, respectively[32,33,34], and have not been reported as
222 established pathogens of adzuki or common bean.

223 The current detection of Me2, Me3, and Li6 that clustered with *P. syringae* pv. *aceris* and
224 *P. syringae* pv. *dysoxyli* strains and not known to be pathogens of beans in adzuki bean
225 seed samples could have been attributed to the epiphytic nature of some of these
226 bacteria. *P. syringae* strains can survive for extensive periods as epiphytes on non-host
227 plants and in environments such as snow and rain splash[35,36,37]. Epiphytic *P. syringae*
228 strains produce exopolysaccharides and surfactants to withstand the harsh conditions of
229 foliar surfaces[24]. *P. syringae* strains in phylogroup 2, including *P. syringae* pv. *aceris* and
230 *P. syringae* pv. *dysoxyli* potentially are highly epiphytic and can be associated with a wide
231 host range[24,38,39,40]. Maple, the main host of *P. syringae* pv. *aceris*[42] is abundant across
232 Ontario, with six maple species native to Ontario (https://www.ontario.ca/page/tree-atlas),
233 however, no documented occurrence of leaf spot in maple caused by *P. syringae* pv.
234 *aceris* in Ontario. Moreover, these strains will have to be isolated from maple grown in
235 Ontario before maple can be established as a host source for these strains.

236 **Pathogenicity of the *P. syringae* strains**

237 Inoculated detached leaf assays demonstrated that all four *P. syringae* isolates were
238 pathogenic, capable of causing disease on the adzuki bean varieties ‘Erimo’ and
239 ‘Baoqing’. This is particularly notable for Me2, Me3, and Li6, which were most closely
240 related to pv. *aceris* and pv. *dysoxyli*, pathovars not previously associated with disease
241 of bean. Pss27, classified as pv. *syringae* was the most virulent of the isolates tested,

measured through AUDPC, and produced greater disease severity, whereas ‘Erimo’ consistently developed less disease than ‘Baoqing’. The significant isolate × variety interaction for AUDPC further indicates that disease severity was influenced by both bacterial isolate and host genotype. Re-isolation of *P. syringae* from inoculated leaves supported the pathogenicity results, fulfilling Koch’s postulates[41], with bacterial recovery generally greater from the more susceptible ‘Baoqing’ variety. These differences in aggressiveness among isolates provided a basis for examining whether variation in their virulence-associated gene and effector repertoires could contribute to the observed phenotypes.

T3 Secretion Systems in the *P. syringae* strains

The T3SS is a major virulence mechanism in plant-pathogenic *Pseudomonas*, delivering effector proteins into host cells to modify host cellular processes and suppress immune responses[42,43,44]. Despite differences in virulence and putative pathovar among Pss27, Me2, Me3 and Li6, all four strains contained the same 27 T3SS-associated genes within similarly organized *hrp/hrc* clusters. With *hrp* (*hypersensitive response and pathogenicity*) genes being essential for T3SS-facilitated pathogenicity, while *hrc* (hypersensitive response and conserved) genes are highly conserved across the T3SSs[45]. Tegli et al. (2011)[45] also found an identical composition and organization of 27 genes in the T3SS gene clusters of different pathovars of *P. savastanoi* strains. Therefore, the greater aggressiveness of Pss27 cannot be explained simply by differences in the presence or organization of the core T3SS machinery, suggesting that differences in effector

complement, other virulence-associated genes, or their regulation may be more important.

Virulence gene repertoires of *P. syringae*

In contrast to the conserved core T3SS, the effector repertoire differed among the strains. Pss27 contained six type III effectors not detected in Me2, Me3, or Li6: *avrRpm1, hopAB1, hopAF1, hopAP1, hopH1,* and *hopL1*. Pss27 also contained four additional strain-specific virulence factors identified by VFDB: *ahlI, ahlR, ppkA,* and *sypA*. The broader effector and accessory virulence-factor repertoire of Pss27 may therefore contribute to its greater aggressiveness on adzuki bean, although the functions of these factors were not experimentally evaluated in this study.

Several of the Pss27-specific effectors have established roles in interactions between the pathogen and host plant. The AvrRpm1 effector modifies the host protein RIN4 (RPM1-INTERACTING PROTEIN4), which can promote virulence in compatible hosts but activates RPM1 (Resistance to Pseudomonas maculicola1), which mediates effector-triggered immunity in plants carrying resistance.[46,47]. HopAB1 has been associated with interference with host transcriptional regulators, including Ethylene Response Factor 8 (ERF8), and suppression of ERF8-mediated cell death, while HopAF1 targets the methionine-recycling pathway through MTN1 and MTN2, reducing pathogen-induced ethylene production and suppressing plant immunity[48,49]. Among the additional Pss27-specific virulence factors, *ahlI* and *ahlR* encode components of an AHL-mediated quorum-sensing system, with AhlI functioning as the AHL synthase and AhlR as its cognate regulator[50]. In *P. syringae* pv. *syringae*, this regulatory system influences traits

285 including alginate production, motility, epiphytic fitness, and disease development. The
286 presence of *sypA* is also notable because it encodes part of the non-ribosomal peptide
287 synthetase machinery required for syringopeptin production. Syringopeptin-deficient
288 *sypA* mutants of *P. syringae* pv. *syringae* have reduced virulence, supporting a role for
289 this phytotoxin in disease development[26,51,52].

290 For the other effectors, Cao et al. (2019)[48] showed that hopAB1 interacts with the
291 ETHYLENE RESPONSIVE FACTOR8 (ERF8) to suppress HR-induced cell death, which
292 is an important mechanism used by plants to limit pathogen spread[48]. HopAF1 was shown
293 to interfere with ethylene signalling in response to pathogen attack by interacting with
294 methylthioadenosine nucleosidase proteins MTN1 and MTN2, enzymes involved in
295 ethylene production[49]. HopH1 has been reported as a common effector in *P. syringae* pv.
296 *syringae* and *P. syringae* pv. tomato pathogenesis[53]. Although the target protein is
297 unknown, hopH1 elicited HR cell death in a nonhost, *Solanum torvum*[53]**.** The host target
298 for hopL1 is unknown, but it has been demonstrated to be involved in adherence and
299 motility during the epiphytic phase of *P. cichorii*[54,55]*.*

300 *AhlI and ahlR* encode proteins involved in alginate biosynthesis and quorum sensing in
301 P. syringae, a key signalling process that directs the expression of virulence factors during
302 pathogenesis. *AhlI,* a synthase, and one of the regulators, *ahlR,* encode the production
303 of N-acyl-homoserine lactone (AHL), the signal molecule that facilitates quorum
304 sensing[50]. The absence of the *ahlI-ahlR* gene pair in Me2, Me3, and Li6 may have
305 contributed to the lower pathogenicity observed in the strains. PpkA is a component of
306 the Type IV secretion system[56].

SypA encodes a protein that contributes to syringopeptin toxin production[51]. The ability to produce syringopeptin, syringomycin, and syringolin toxins, and the presence of synthetase genes encoding enzymes involved in toxin synthesis, are used to classify *P. syringae* strains as pathovar *syringae*[52,26]. Syringopeptin and syringomycin phytotoxins disrupt membranes and are major virulence determinants of *syringae* pathovars[51].

Taken together, the conserved core T3SS among the four strains suggests that differences in aggressiveness are more likely associated with variation in the effector repertoire and other accessory virulence factors than with the secretion apparatus itself. Pss27 contained several strain-specific factors with reported roles in host immune modulation, quorum sensing, and toxin production, providing plausible candidates that may contribute to its greater aggressiveness on adzuki bean. However, these associations do not establish causation, particularly because Pss27 belongs to a different genomic lineage from Me2, Me3, and Li6. Functional characterization of these candidate factors will therefore be required to determine their specific contributions to pathogenicity in adzuki bean

Conclusions

This study characterized *P. syringae* strains associated with adzuki bean seed and evaluated their pathogenicity on two adzuki bean varieties under controlled conditions. Whole-genome analyses placed Pss27 with *P. syringae* pv. *syringae*, whereas Me2, Me3, and Li6 were most closely related to the pv. *aceris/dysoxyli* lineage. All four strains were pathogenic on adzuki bean, although Pss27 was the most aggressive and ‘Erimo’ consistently developed less disease than ‘Baoqing’. Despite similar core T3SS gene

complements, the strains differed in their effector and broader virulence-factor repertoires, with Pss27 containing several factors not detected in the other strains. The unique virulence factors identified in this study in Pss27 represent candidates for functional testing and can be used to elucidate the plant-molecular interactions that exist between the *P. syringae* pv. *syringae* pathogen and the adzuki bean host.

MATERIALS AND METHODS

Source of bacterial strains:

Pss27, Me2, Me3, and Li6 were the bacterial strains used in this study. Pss27 was obtained from Agriculture and Agri-Food Canada (AAFC), Harrow Research Centre, Ontario, Canada, courtesy of Dr. Owen Wally. Pss27 (previously named PA1, Pseudomonas Alberta 1) was originally isolated from symptomatic common bean (*Phaseolus vulgaris*) plants near Lethbridge, Alberta, Canada, in 1994[31]. Pss27 is the strain used for bacterial brown spot screening in the disease nurseries of AAFC, Harrow, Ontario. In this study, *Pseudomonas syringae* strains Me2, Me3, and Li6 were isolated from adzuki seed lots collected in Ontario at Meaford (Me2, Me3) and Lincoln (Li6).

Adzuki seed lots collected from seventeen farm locations across Ontario were received in March 2022 (courtesy of Hensall Co-op). The seed lots were culls sorted by Hensall Co-op from adzuki crops harvested in fields in Meaford, Elmvale, Lake Simcoe, Oakwood, Kawartha Lakes, Port Perry, Rice Lake, Cobourg, Ayton, Milton, Exeter, Stratford, Alisa Craig, Tavistock, Ayra, Thamesford, and Lincoln. One leaf sample showing lesions with morphologies similar to those observed in plants with bacterial brown spot disease[1] was

collected from adzuki field plots at the University of Guelph Research Station, Woodstock, Ontario.

Isolation of bacterial strains

Cultural isolation and morphological identification were adapted from Goszczynska and Serfontein (1998)[57]. The adzuki seeds (200 g/seed lot/ location) were surface-sterilized with 600 ml of 1% v/v sodium hypochlorite and a drop of Tween 20 for 5 min and then rinsed five times with sterile distilled water. Surface-sterilized seeds were immersed in 400 ml of sterile Ringer's solution[58,59] in 1L Pyrex media bottles with screw caps and incubated for 24 h at 5 °C. The resulting cultures were well stirred, and three replicates of 0.1 ml aliquots of four ten-fold serial dilutions were plated on Milk-Tween agar.

For recovering bacteria from leaf samples, the non-necrotic margins of lesions were cut from the symptomatic leaf using a sterile scalpel blade. The leaf samples were surface-sterilized by immersing in 1% v/v sodium hypochlorite and a drop of Tween 20 for 5 min and rinsed five times with sterile distilled water. The sterilized and rinsed leaf samples were immersed in sterile Ringer's solution for 30 min, and 0.1 ml of four ten-fold serial dilutions of the suspension were plated on Milk-Tween agar in three replicates.

All plates were incubated at 28 °C for 48 h. After 48 h of incubation, creamy white colonies with a single clear zone on Milk-Tween agar were observed under UV light (BIO-RAD ChemiDoc^{TM}XRS^{+} gel doc, excitation wavelength, 302 nm) and fluorescent light for luminescence. For fluorescence observation, culture plates were viewed under a Leica S9i microscope with fluorescent light supplied by the NightSea Stereo Microscope Fluorescence Adapter (excitation wavelength, 440–460 nm) and visualized using Leica

Application Suite X. Fluorescent colonies surrounded by a clear zone on Milk-Tween agar were collected as candidate colonies (Supplementary Figure 1). Pure cultures of the 62 single-colony candidate isolates were stored as glycerol stocks at -80 °C until DNA was extracted.

DNA extraction and sequencing

Genomic identification of isolates was carried out by 16S rRNA and Illumina whole-genome sequencing. DNA of the 62 isolates was extracted with the Qiagen DNeasy® UltraClean® Microbial kit according to the manufacturer's instructions. DNA purity was estimated using the Qiagen QIAxpert equipment. DNA concentration was estimated using the Invitrogen Qubit fluorometer.

Partial amplification of the 16S rRNA genes in the extracted DNA samples was carried out using the Invitrogen™ Platinum™ II Taq Hot-Start DNA Polymerase kit according to the manufacturer's instructions. Universal primers (forward 5´-ATT GAC GGG GRC CCG CAC and reverse 5´-CGA GCT GAC GAC AGC CAT GCA) (Invitrogen™) that target conserved regions of bacterial 16S rRNA genes were used for PCRs with the DNA samples from the isolates. . Reaction conditions for PCR were according to the kit master mix manufacturer's instructions (ThermoFisher Scientific). The protocol for polymerase chain reaction (PCR) was set at one cycle of initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 15 s, and extension at 68 °C for 15 s followed by holding at 4 °C. A negative control was included in the PCR. The PCR products alongside a 1-kilobase ladder (Invitrogen 1Kb Plus DNA Ladder) were run through a 1% agarose gel in 1x TBE buffer in an electrophoresis unit (BIORAD Sub-

Cell® GT) for 45 min and visualized on the gel doc (BIO-RAD ChemiDoc^{TM}XRS^{+}) to confirm the presence and expected sizes of the amplicons.

The PCR products were sequenced by Sanger sequencing at the University of Guelph Agriculture and Food Laboratory. NCBI BLAST was used to match the sequence similarity of the 16S rRNA partial gene sequences. Whole-genome sequencing was conducted on the five isolates returned as *Pseudomonas syringae* from NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). DNA samples were sequenced on the Illumina NextSeq platform at the University of Guelph Advanced Analytics Centre Genomic Facility. The 20 ng DNA /µL samples were sequenced to give 1M 300 bp paired-end reads, which resulted in approximately 100x sequencing depth per bacterial genome. To validate the identity of the *Pseudomonas syringae* pv. *syringae* strain (Pss27) received from AAFC Harrow, 16S rRNA sequencing and Illumina whole-genome sequencing were carried out as described above (Supplementary Figure 1).

Genome assembly and annotation

Following Illumina NextSeq whole-genome sequencing, quality control, and adaptor trimming, *de novo* assembly of the Illumina paired-end FASTq raw reads into contigs was conducted on the CLC Genomics Workbench 24 (QIAGEN). NCBI BLAST was used to check for the similarities of contig sequences to the NCBI GenBank database (https://www.ncbi.nlm.nih.gov). Contigs of bacterial isolates returned as *P. syringae* were assembled into whole-genome scaffolds using the software Ragtag[60]. Assembly of contigs into whole-genome scaffolds was carried out by aligning the bacterial contigs against the *P. syringae* pv. *syringae* reference sequence, B48 (accession number:

PRJNA971231), downloaded from NCBI GenBank. Genome annotation of the whole-genome assemblies was done by the Rapid Annotations using Subsystems Technology (RAST) server[61] and DFAST[62].

Comparative genomics of *P. syringae* strains

Fifty-four *Pseudomonas syringae* strains from 21 pathovars downloaded from NCBI GenBank were matched to the whole-genome sequences of Pss27, Me2, Me3, and Li6. There were three representative strains per pathovar, except for the following, for which the other available strains were tagged “low-quality” by NCBI GenBank: pv. *castaneae* (two strains), pv. *spinaceae* (two strains), pv. *avellanae* (two strains), pv. *daphniphylli* (two strains), pv. *raphphiolepidis* (two strains), pv. *dysoxyli* (one strain), and pv. *japonica* (one strain). NCBI GenBank classifies submissions as low-quality sequences where long stretches of assemblies have a high proportion of ambiguous bases and low-complexity sequences or other indications of low quality (https://www.ncbi.nlm.nih.gov/datasets/docs/v2/data-processing/policies-annotation/genome-processing/genome_notes/).

A phylogram of the strains was generated using the zDB software[63]. The phylogeny was built with FastTree (default settings) using 1,337 concatenated single-copy orthologs[63]. CheckM was used to estimate the completeness and contamination based on 107 marker genes[64]. The heatmap and phylogram of whole-genome average nucleotide identity (ANI) comparison between Pss27, Me2, Me3, Li6, and *P. syringae* strains from NCBI GenBank were generated with CLC Genomics (QIAGEN).

The virulence factors in the annotated whole-genome assemblies of Pss27, Me2, Me3, and Li6 were identified with the VFanalyzer tool of the Virulence Factor Database (VFDB)[65,66]. The virulence factors, Type III Secretion Systems (T3SSs), and T3SS effectors present in Pss27, Me2, Me3, and Li6 were compared. In addition, the T3SSs of three other pathogenic *Pseudomonas syringae* pathovar strains available on the VFDB: P*. syringae pv. syringae* (B728a), *P. syringae* pv. *tomato* (DC3000), *P. syringae* pv. *phaseolicola* (1448A) were compared to those in Pss27, Me2, Me3, and Li6.

Comparative genomics of the orthologous groups, the clusters of orthologous genes (COGs), and protein families present in the genomes of Pss27, Me2, Me3, and Li6 was conducted using the zDB software[63]. Circular maps of the COGs in the Pss27, Me2, Me3, and Li6 genomes were generated using the GenoVi software[67]. GenoVi uses the Eggnog mapper[68,69] to identify COGs.

Protocol for inoculating adzuki with *P. syringae* pv*. syringae*

Since no publication on screening adzuki beans for their susceptibility to bacterial brown spot (caused by *P. syringae pv. syringae*) exists, a protocol was developed by adapting the screening protocol for common bacterial blight in common bean (*Phaseolus vulgaris*) used by Emily Morneau of the AAFC, Harrow Research Centre (personal communication). In the current study, the control of growth conditions for adzuki beans was found to be critical for success. In general, adzuki beans in controlled growth conditions require cooler temperatures than the common bean. For optimum screening conditions, the adzuki bean plants were grown in growth rooms with 22/18 °C day/night temperatures and a 16-h photoperiod at 435 µmol m^{-2} s^{-1}.

For planting, 150 x 180 mm pots were filled with BM6 potting soil mix (Berger), and the pots were saturated with water. A vermiculite soil topping to control insect pests was not used, as vermiculite was observed to damage emerging seedlings. Bug Scan ® sticky traps (Biobest) were used to control insect pests. Adzuki seeds were planted 1.5 cm deep in the potting soil. Five adzuki seeds were planted per pot, and the stand was thinned to two seedlings after two weeks post-emergence. To ensure the soil was well-drained for the germinating seeds, watering was paused for the first two weeks after initial soil saturation. Two weeks after planting, pots with young seedlings were saturated with 1 g/L of NPK 15 15 15 (Plant-Prod). Subsequently, watering was done after the soil surface had dried out but before plants showed any signs of drought stress. Watering per plant was varied and was dependent on plant architecture. Larger plants required more frequent watering than smaller genotypes. At the pod formation stage, the plants with larger architectures (approximately 100 cm in height) required daily watering, while smaller plants (approximately 30 cm) required watering every 3 to 4 days. Under growth room conditions, indeterminate plant types could grow up to 100 cm in height.

For inoculation, a liquid culture of a *P. syringae pv. syringae* strain was made by diluting a 24-hour-old culture grown on King's B agar media in sterile Ringer's solution to an optical density of 0.3 (equivalent to 10^8 CFU/ml)[70]. Six-week-old plants were transferred into a misting tent at relative humidity greater than 95% for 48 h. Plants were inoculated using the multiple-needle technique[71]. Mock inoculation was sterile Ringer's solution. After 48 h of misting, two fully expanded trifoliate leaves per plant were inoculated by piercing leaves placed on an inoculum-saturated foam with a floral frog. The floral frog provided a means to make multiple simultaneous piercings in the leaves. After

inoculation, the plants were left in the tent without misting for 48 h, and then the plants were transferred back to the growth room.

Both visual and digital disease evaluations were performed on the inoculated trifoliate leaves. Visual disease ratings were conducted weekly, for up to four weeks post-inoculation. A 1 – 9 disease rating scale was used with the following classifications: 1 – 3, resistant; 4 – 6, intermediate; 7 – 9, susceptible[72].

In the fourth week post-inoculation, inoculated trifoliate leaves were removed from the plants and photographed. The images of the leaves were digitally quantified for the percentage of leaf area with disease lesions, termed the percentage of infection, using the APS ASSESS 2.0 software[73]. The AUDPC values were computed using weekly rating scores. AUDPC was calculated using the formula:

$$\sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where: y is the disease rating of the i^{th} observation at week (t) post-inoculation. n is the number of observations (Supplementary Figure 2).

Plant materials

Erimo and Baoqing adzuki varieties used in this study were obtained originally from Hensall Co-op. Erimo and Baoqing were the parents of an existing RIL population at the University of Guelph dry bean breeding program. The RILs were developed to select for seed quality traits (colour and size)[74]. Erimo and Baoqing had not previously been screened for resistance to bacterial brown spot. In order to exploit the available adzuki resources, Erimo and Baoqing were selected for this study. Erimo is a medium-maturing

variety[74], with erect architecture, and grows to about 30 cm in height. Baoqing is a later-maturing variety, indeterminate, and grows to about 100 cm in height.

Pathogenicity test

Comparisons of the pathogenicity and aggressiveness of the bacterial strains were done by inoculating Erimo and Baoqing with Pss27, Me2, Me3, and Li6. The plants were grown in a growth room in the Crop Science building under conditions described above. The experiment was a completely randomized design with 8 treatments, 2 controls, and three replicates. Five adzuki seeds were planted per 150 x 180 mm pot and thinned to two plants per pot, two weeks post-planting. Six-week-old plants (the onset of flowering) were transferred to a misting tent for inoculation. After 48 h of misting in a tent at a relative humidity of 100%, two trifoliate leaves per plant were inoculated as described above. Only one of the two inoculated plants was rated for disease. The plant rated for disease per pot served as a replicate. There were three completely randomized replicates in the misting tent. To avoid cross-contamination, the controls and plants inoculated with different strains were separated by about 30 cm in the misting tent.

After inoculation, the plants were left in the tent without misting for 48 h and then transferred back to the growth room. Both visual and digital disease estimation were carried out on the inoculated trifoliate leaves as described above.

Re-isolation of strains used for inoculation

Pss27, Me2, Me3, and Li6 were reisolated from inoculated leaves 3 weeks post-inoculation. For re-isolation, 0.2 g samples of the non-necrotic margins of lesions excised from inoculated trifoliate leaves were surface-sterilized for 5 min in 1% v/v sodium

hypochlorite solution with a drop of Tween 20. The surface-sterilized leaf explants were rinsed three times with distilled water and immersed in an isotonic solution (quarter-strength Ringer's solution)[57] for 15 min. Four ten-fold serial dilutions of the stock solutions were spread-plated on Milk-Tween agar[57] in three replicates. Creamy white colonies with a single clear zone on Milk-Tween agar that were luminescent under UV light were counted as *P. syringae* colonies

Statistics

Analysis of variance for AUDPC, percentage of infection, and colony counts was conducted using SAS 9.4. GLIMMIX was used, with *P. syringae* strains, adzuki genotypes, and the interactions between the strains and adzuki genotypes considered as fixed effects. The least-squares means were separated using the Tukey test ($\alpha = 0.05$). Weekly disease ratings were computed into an AUDPC value and statistically analyzed. The Gaussian distribution was used to analyze the AUDPC and the percentage of infection values, and the negative binomial distribution was used to analyze bacterial colony counts. The percentage of infection values were log-transformed to fit the Gaussian model. For all distributions, the assumptions that the residuals (errors) were random, normally distributed, independent of treatment and design effect, had a mean of zero, and were homogeneous were checked. The W-statistics (Shapiro-Wilk), Q-Q plots, and distribution of the residuals were used to check for the normality of the residuals. Boxplots of residuals were used to check for homogeneity and the independence of treatment and design effects. Plots of predicted versus residuals were used to check for randomness of errors. Where the assumptions were not met, a non-

parametric test was used as in the case of the negative binomial distribution used for colony counts. The means ± standard error (SE) were presented as results.

Author contributions: KPP conceived, planned, and supervised the study. UEO developed the study, conducted laboratory and controlled experiments, carried out statistical and bioinformatics analyses, and wrote the manuscript. OW provided the Pss27 strain and the protocol for isolation of *P. syringae* from culture, and served as advisor for the study. All authors reviewed and edited the manuscript.

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Figures, figure legends, and tables

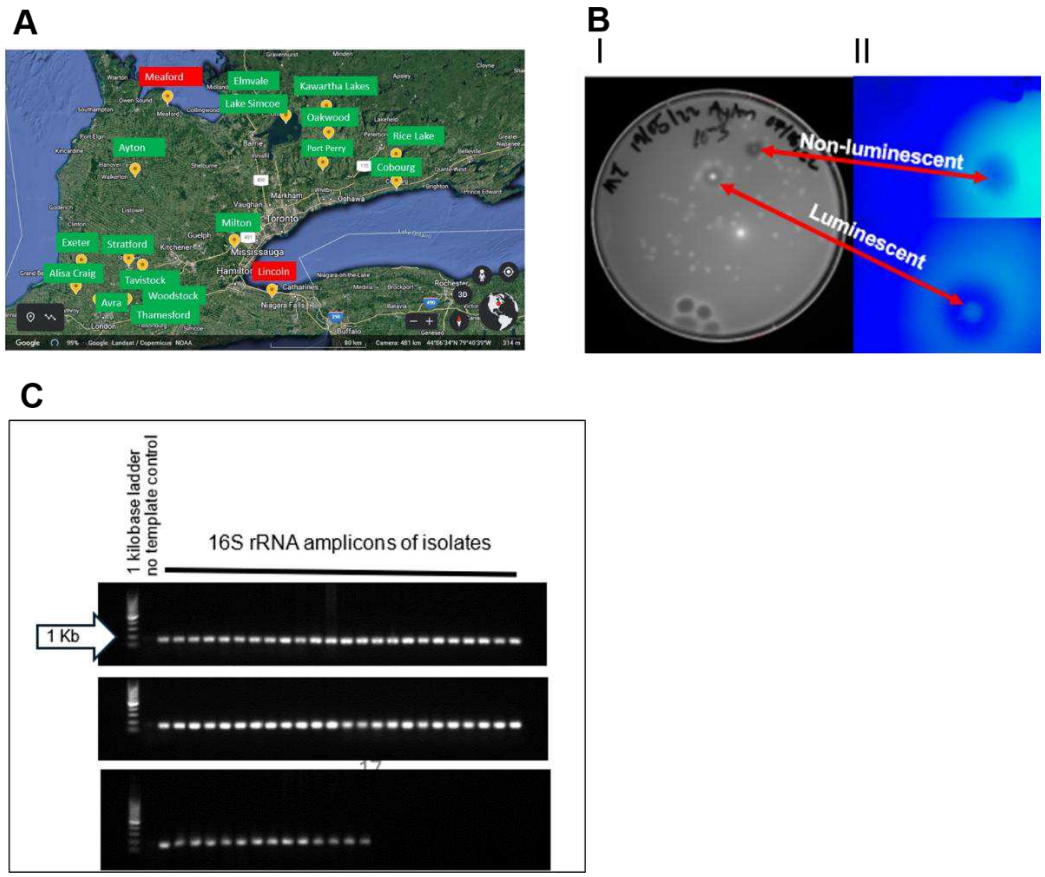
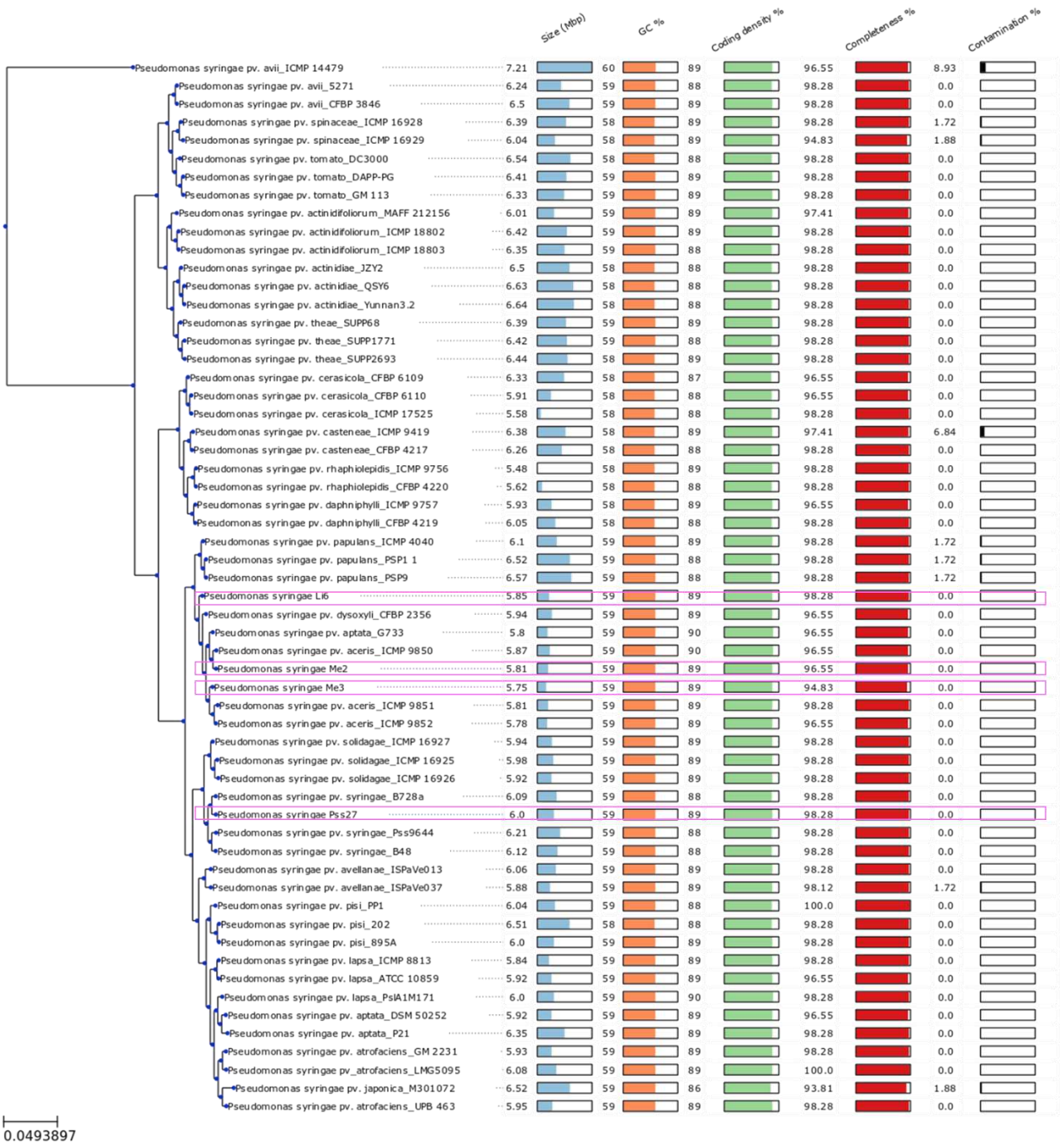


Figure 1A – C: Isolation and identification of Pss27, Me2, Me3, and Li6

A, Provincial locations of adzuki crops from which seed lots were culled that were used to isolate bacterial strains. Locations that were positive for *P. syringae* strains are colour-coded red. **B,** Luminescent and non-luminescent colonies with clear zones on milk-tween

844 agar under **I,** UV, and **II,** fluorescent lights. Candidate *P. syringae* isolates were those
845 colonies that had a single clear zone on milk-tween agar and that were luminescent.
846 **C,**16S rRNA partial gene amplicons obtained from candidate *P. syringae* isolates. The
847 amplicons were the expected size of approximately 200 bp.



848

849 **Figure 2:** Phylogram based on whole-genome sequences of 54 *P. syringae* strains from

850 21 pathovars downloaded from NCBI and Pss27, Me2, Me3, and Li6. Pss27 clustered

851 with all three *P. syringae* pv. *syringae* strains. Me2 and Me3 clustered with the *P.*

syringae pv. *aceris* strains, and Li6 clustered with the single *P. syringae* pv. *dysoxyli* strain. The phylogeny was built with FastTree (default parameters) using 1,337 concatenated single-copy orthologs. Figure generated on the zDB webapp.

A

		1	2	3	4	5	6	7	8	9	10	11
P. syringae pv. syringae_B48	1		98.76	98.75	94.43	94.43	94.42	94.49	94.48	94.46	94.35	98.94
P. syringae pv. syringae_B728a	2	90.35		98.76	94.47	94.44	94.46	94.44	94.52	94.47	94.42	98.74
Pss27	3	90.80	92.00		94.47	94.43	94.45	94.46	94.51	94.48	94.44	98.78
Me2	4	83.96	84.51	85.88		99.24	99.16	99.29	99.20	99.27	99.18	94.43
Me3	5	84.79	85.66	87.18	93.62		99.20	99.25	99.24	99.25	99.21	94.44
Li6	6	83.53	85.44	85.59	91.41	92.69		99.13	99.16	99.21	99.20	94.41
P. syringae pv. aceris_ICMP 9850	7	84.13	85.31	86.26	92.85	93.94	92.29		99.24	99.26	99.22	94.46
P. syringae pv. aceris_ICMP_9851	8	83.43	84.59	85.39	93.02	92.21	91.59	92.94		99.96	99.18	94.48
P. syringae pv. aceris_ICMP_9852	9	84.38	85.41	86.21	93.36	93.58	92.23	93.85	97.74		99.22	94.46
P. syringae pv. dysoxyli_CFBP_2356	10	84.67	84.44	85.40	91.80	91.88	91.39	91.40	91.53	92.40		94.37
P. syringae pv. syringae_Pss9644	11	92.17	89.44	89.92	83.33	84.60	84.11	83.55	83.18	83.84	83.54	

B

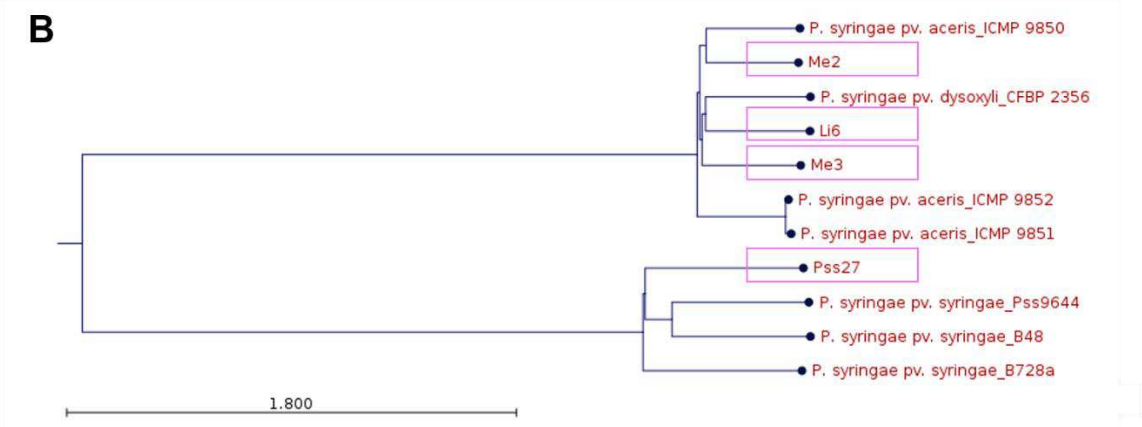


Figure 3A and B: A, Heatmaps of ANI (top) and alignment percentages (bottom) among Pss27, Me2, Me3, and Li6 and pathovars *syringae*, *aceris,* and *dysoxyli*. **D,** Phylogram based on ANI comparisons between Pss27, Me2, Me3, and Li6 and pathovars of *syringae*, *aceris,* and *dysoxyli* obtained from NCBI. The phylogram was generated using CLC Genomics Workbench 24 (QIAGEN). The whole-genome sequence assemblies of the strains isolated in this work have been deposited at NCBI GenBank under the BioProject PRJNA1263496 and BioSample accession numbers SAMN48523524,

863 SAMN48523525, SAMN48523526, and SAMN48523527 for Pss27, Me2, Me3, and Li6,

864 respectively.

865

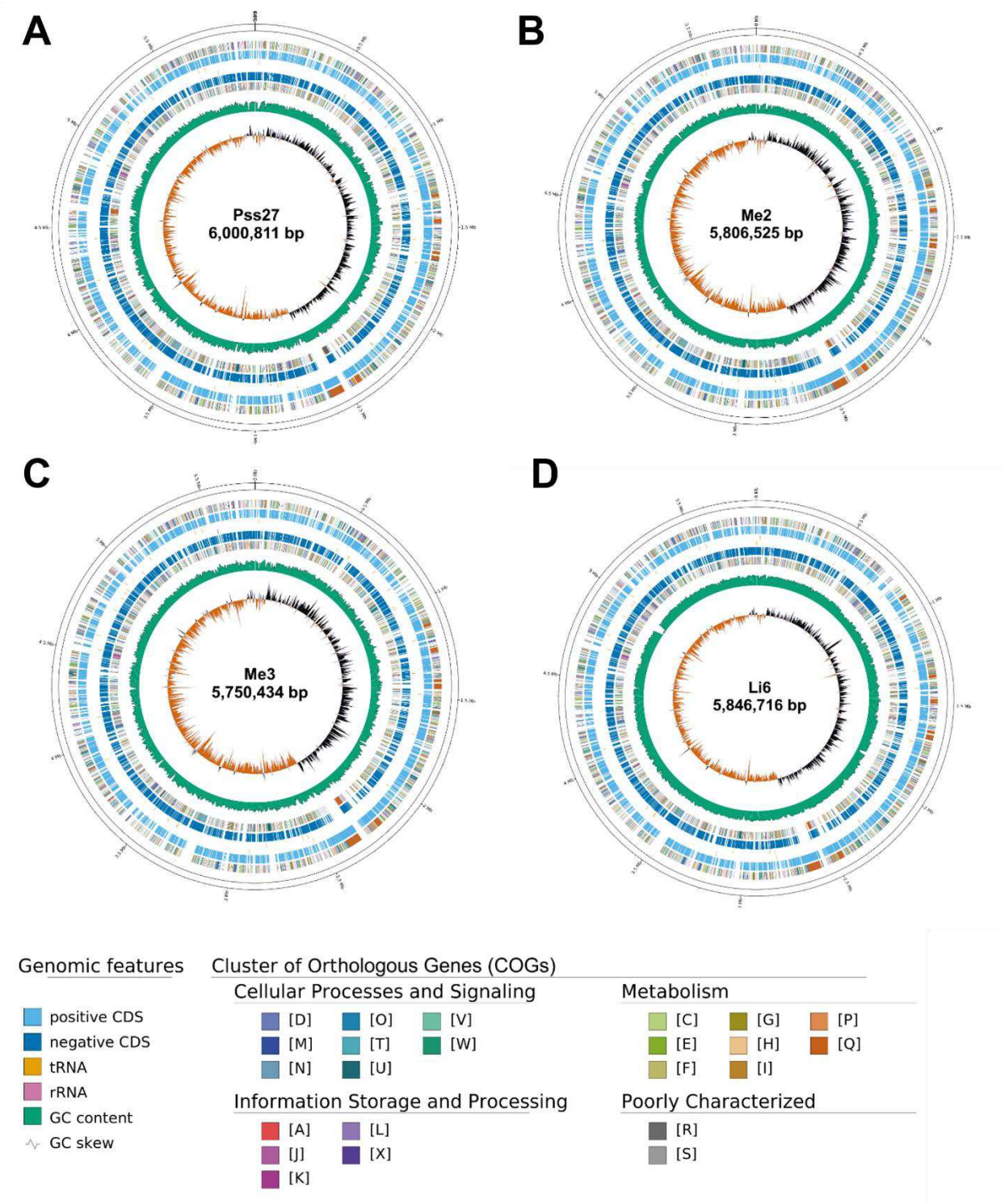


Figure 4A – D: Circular maps of the bacterial chromosomes for Pss27, Me2, Me3, and Li6. From outside to inside: Track 1, physical map of the genome (~6.0 Mb); Track

2, Locations and functions of Clusters of Orthologous Genes (COGs) in the forward DNA strand; Track 3, Gene locations in the forward strand; Track 4, tRNA and rRNA gene locations in the forward strand; Track 5, tRNA and rRNA gene locations in the reverse strand. Track 6, Gene locations in the reverse DNA strand; Track 7, Locations and functions of COGs in the reverse DNA strand; Track 8, GC content; Track 9, GC skew. Map generated with GenoVi with Eggnog mapper for COG identification. COG Categories: A - RNA processing and modification, C - Energy production and conversion, D - Cell cycle control, chromosome division and chromosome partitioning, E - Amino acid transport and metabolism. F, Nucleotide transport and metabolism, G - Carbohydrate transport and metabolism, H - Coenzyme transport and metabolism, I - Lipid transport and metabolism, J – Translation, ribosomal structure and biogenesis, K – Transcription, L - Replication, recombination and repair, M - Cell wall/membrane/envelope biogenesis, N - Cell motility, O - Posttranslational modification, protein turnover, chaperones, P - Inorganic ion transport and metabolism, Q - Secondary metabolites biosynthesis, transport and catabolism, R - General function prediction only, S - Function unknown, T - Signal transduction mechanisms, U - Intracellular trafficking, secretion, and vesicular transport, V - Defense mechanisms, W - Extracellular structures, X – nan.

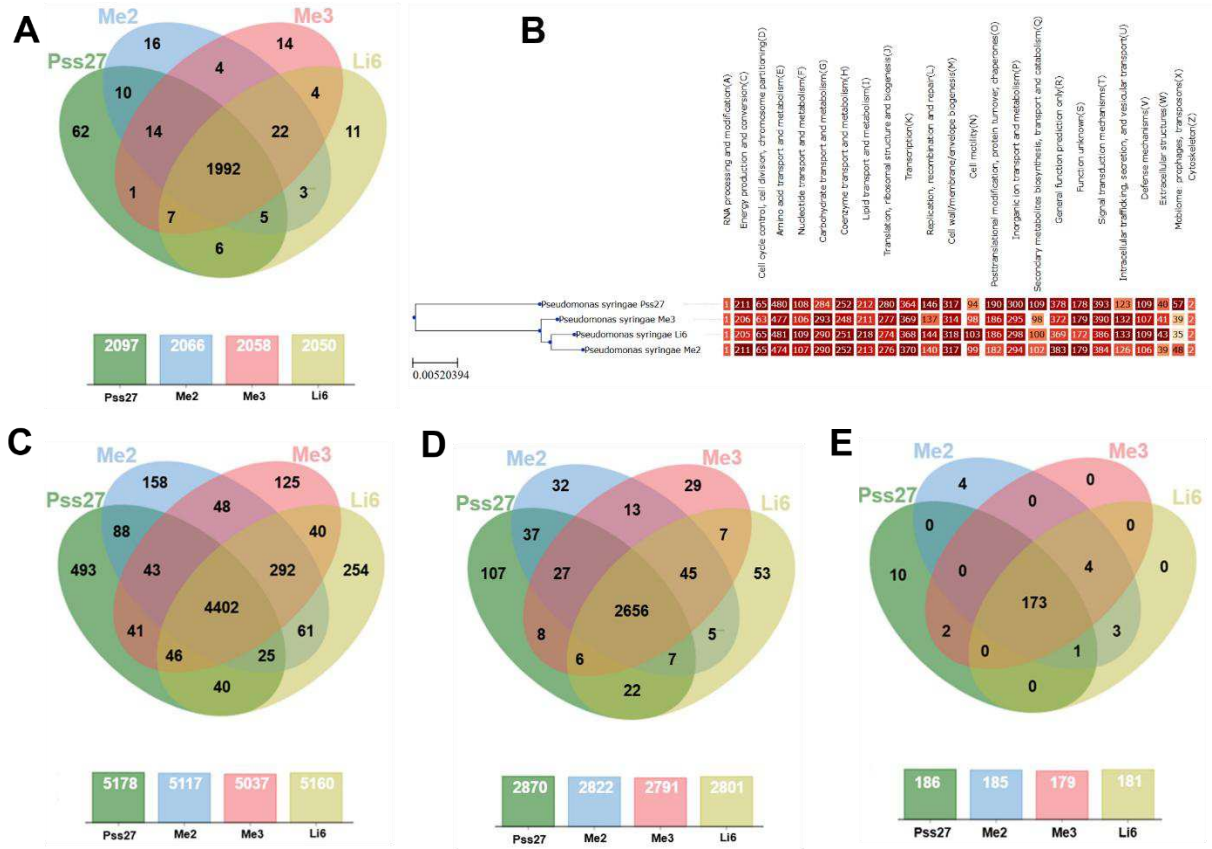


Figure 5: A – E: Comparative genomics of Pss27, Me2, Me3, and Li6.

A, Number of shared and unique COGs. **B**, Phylogeny and Heatmap based on the number of COGs present in each COG category. **C,** Number of shared and unique orthologous group genes. **D,** Number of shared and unique protein families. **E,** Number of shared and unique virulence factors. The unique virulence factors were ahlI, ahlR, ppkA, avrRpm1, hopAB1, hopAF1, hopAP1, hopH1, hopL1, sypA in Pss27, and hcp1, acrB, rmlA, rmlD in Me2. Numbers in bars represent the total for each strain in each category. Figures were generated on the zDB webapp.

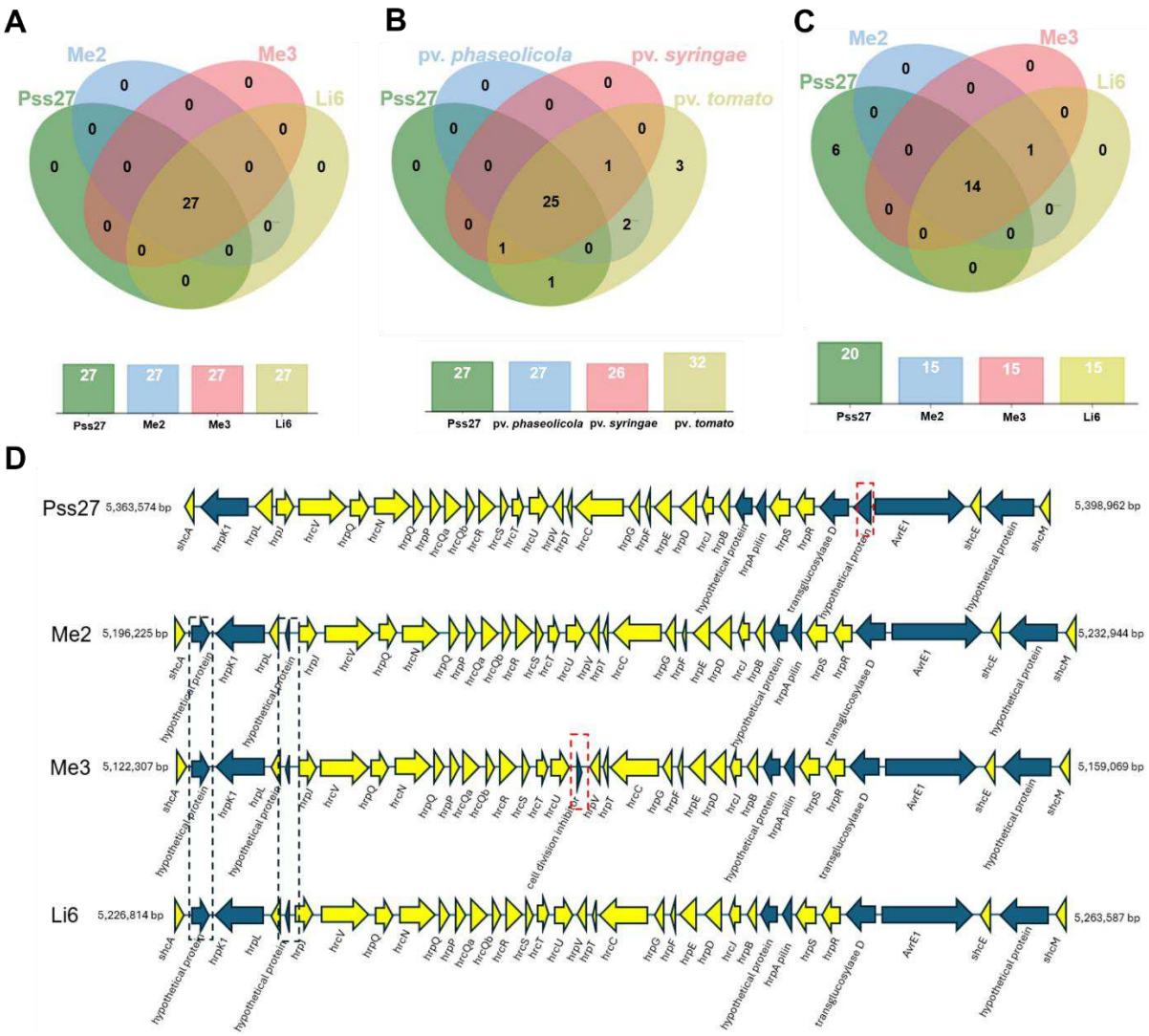


Figure 6A – D: Shared and unique genes of T3SS and effectors in *P. syringae* strains. **A,** Number of T3SS genes in Pss27, Me2, Me3, and Li6. **B,** Number of T3SS genes in Pss27, *P. syringae* pv. syringae (B728a), *P. syringae* pv. *tomato* (DC3000), and *P. syringae* pv. *phaseolicola* (1448A). **C,** Number of effectors in Pss27, Me2, Me3, and Li6. The unique factors in different isolates include: Pss27 (avrRpm1, hopAB1, hopAF1, hopAP1, hopH1, hopL1) ; and Me2, Me3, and Li6 (hopA1). **D,** Physical maps of ORFs of the T3SS clusters in Pss27, Me2, Me3, and Li6. Genes are shown in sequence in the

T3SS clusters. All T3SS genes are located within a 35 – 37 kb window in Pss27, Me2, Me3, and Li6. Yellow arrows are the 27 genes involved in the T3SS in the strains. Blue arrows are genes not part of the T3SS, present within the T3SS gene clusters. Black dotted boxes show genes unique to Me2, Me3, and Li6. Red dotted boxes show genes present only in Pss27 or Me3.

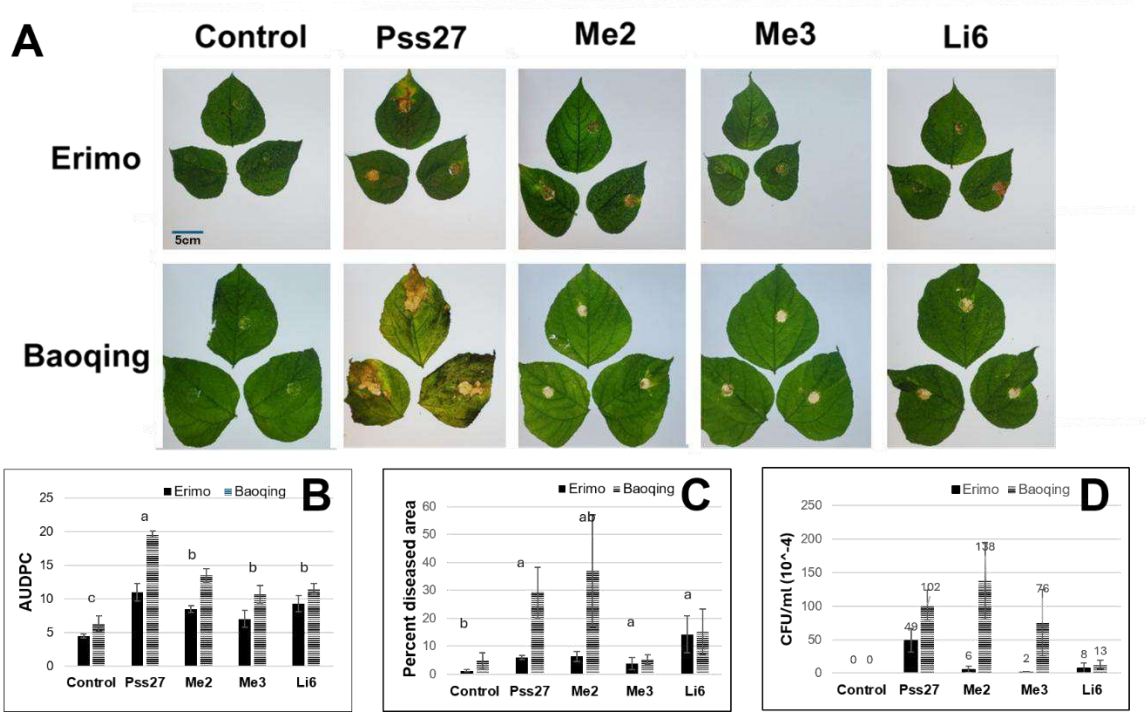


Figure 7A – D: Pathogenicity tests and colony counts in adzuki varieties inoculated with Pss27, Me2, Me3, and Li6. **A,** Erimo and Baoqing trifoliate leaves inoculated with Pss27, Me2, Me3, and Li6 at four weeks post-inoculation. Control plants were mock-inoculated with sterile Ringer's solution. **B,** AUDPC of adzuki varieties inoculated with Pss27, Me2, Me3, and Li6. **C,** Percentage of infection of adzuki varieties inoculated with Pss27, Me2, Me3, and Li6. **D,** *P. syringae* colony counts after 21 days in Erimo and Baoqing plants inoculated with Pss27, Me2, Me3, and Li6 at 10^{-4} dilution. Control

plants were mock-inoculated with sterile Ringer's solution, n=3 ± SE. (Means with differing superscripts are significantly different at $p < 0.05$ in AUDPC and the percentage of infection).

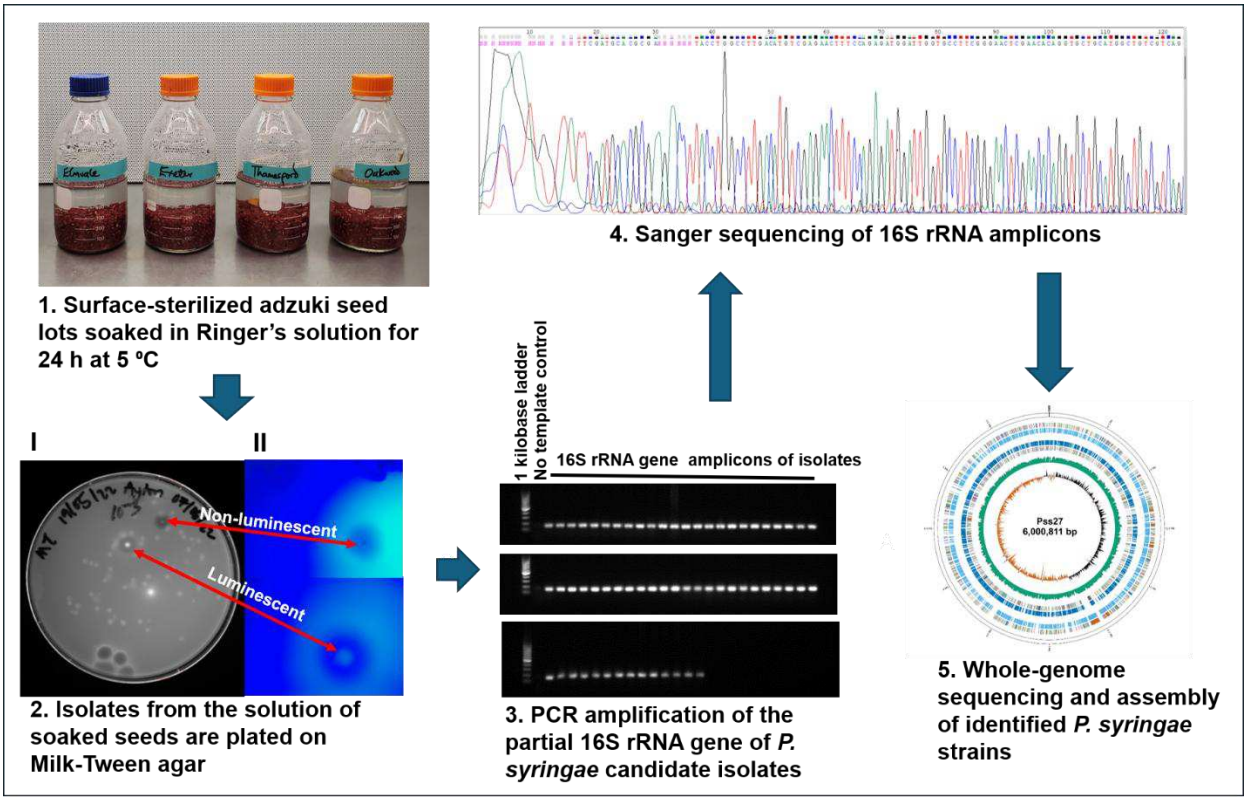
Table 1: Genome summary of Pss27, Me2, Me3, and Li6

Strains	Genome size (bp)	# of coding sequences	GC Content	# of RNAs	% of coding density
Pss27	6,000,811	5,347	59.4	58	89
Me2	5,806,525	5,184	59.4	57	89
Me3	5,750,434	5,076	59.5	55	89
Li6	5,846,916	5,217	59.1	55	89

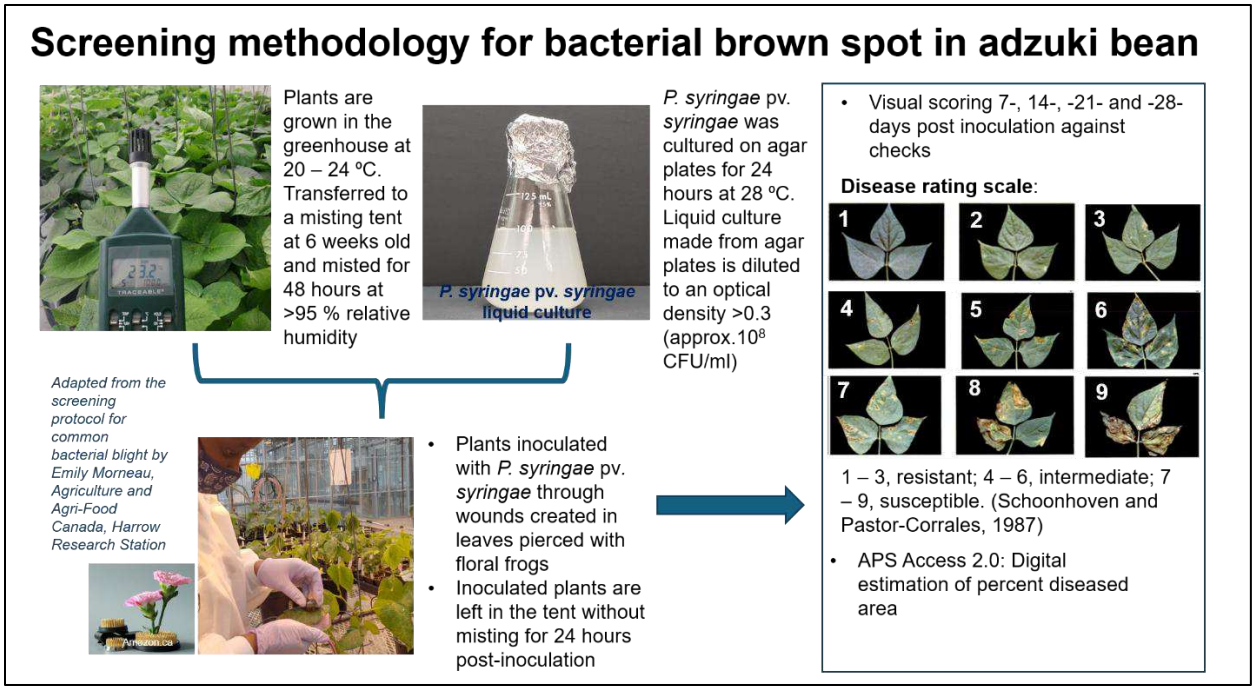
Table 2: Number of virulence keyword search gene products in the orthologous groups, COGs, and protein families in Pss27, Me2, Me3, and Li6.

Strain	Orthologous groups	COGs	Protein families
Pss27	5	7	13
Me2	3	3	6
Me3	3	2	8
Li6	3	3	6

SUPPLEMENTAL



Supplementary Figure 1: Workflow for morphological and genomic methods used to test *P. syringae* candidate isolates. Some *P. syringae* pathovars form colonies with a single clear zone on Milk-Tween agar and are luminescent under UV (I) and fluorescent light (II). Candidate *P. syringae* isolates were those colonies that had a single clear zone on Milk-Tween agar and that were luminescent. 16S rRNA partial gene amplicons obtained from candidate *P. syringae* isolates had the expected ~200 bp length.



Supplementary Figure 2: Protocol for screening bacterial brown spot in adzuki bean. Adzuki plants were grown in the growth room at 20 – 24 °C and transferred to the misting tent at six weeks post-emergence. After 48 h of misting, the plants were inoculated with a 24-hour-old *P. syringae* pv. *syringae* culture with 10^8 CFU/ ml. The inoculated plants were left in the tent with the mist turned off for another 48 hours and returned to the growth room. Weekly ratings of the extent of the disease symptoms were conducted for four weeks, using a 1 – 9 disease rating scale. The percentage of inoculated leaves affected by disease lesions was estimated from photographs of the excised trifoliate leaves four weeks post-inoculation.

Supplementary Table 1: Adzuki seed lots from farm locations across Southern Ontario (Courtesy of Hensall Co-op, March, 2022)

S/N from Hensall Co-op	Origin	Morphology of cultures of adzuki seed lots aliquot on Milk-Tween (MT) agar
44779	Lincoln (Li)	Isolates 5, 6, and 11 have golden-yellow colonies. Other isolates have dark brown colonies.
44780	Tavistock	No colonies that break down casein on MT.
44781	Stratford (St)	Yellow colonies.
44782	Oakwood	No colonies that break down casein on MT.
44783	Rice Lake (RL)	Yellow colonies.
44784	Elmvale	No colonies on MT with surface sterilization of seeds.
44785	Ayton (Ay)	Yellow colonies.
44786	Kawatha Lakes	No colonies that break down casein on MT.
44787	N. Lakes Simcoe (NLS)	Only one yellow colony.
44788	Avra	No colonies on MT with surface sterilization of seeds.
44789	Coburg (Co)	Dark brown colonies.
44790	Milton	No colonies on MT with surface sterilization of seeds.
44791	Meaford (Me)	Yellow colonies.
44792	Exeter (Ex)	No colonies that break down casein on MT.
44793	Ailsa Craig	No colonies that break down casein on MT.
44794	Thamesford	No colonies that break down casein on MT.
44795	Port Perry (PP)	Pale yellow colonies.

Supplementary Table 2: Sanger sequencing of partial 16S rRNA amplicons and BLAST search hit of sequence in bacterial isolates

S/N	Isolate	Sanger sequence of partial 16S rRNA target amplicon	BLAST search hit
1	Ex1	Forward: NNNNNNNNNNNNNNNNTTCGATGCACGCGANNNNNNTACCTGGCCTTGACATGTCGAGAACTTTCCAGAG ATGGATTGGTCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNCNNNNNNNNNNNNGNNGNACCAATCNNTCTCTGGAAGTTCTCGACATGTCAAGGCCAGGTAAGGTTCT TCGCGTTGCATCGAATTAAACCACATACTCCACCGCTTGTGCGGGCCCCCGTCNNNNN	*Stenotrophomonas maltophilia* strain B20 16S ribosomal RNA gene, partial sequence
2	Ay1	Forward:NNNNNNNNNNNNNNNNNNCGAAGCACGCGAAGANCCTTACCNGGCCTTGACATCCAATGAACTTT CTAGAGATAGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA	*Pseudomonas* sp. strain LJ-A13 16S ribosomal RNA

		Reverse: NNNNNNNNNNNNNNNNANGNACCAATCTATCTCTAGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNN	gene, partial sequence
3	Ay2	Forward: NNNNNNNNNNNNNNNNATTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAGAGAT AGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNN Reverse: NNNNCNNNNNNNNNNNNNNANGNACCAATCTATCTCTAGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNN	*Pseudomonas fluorescens* strain CCTCC AB92001 16S ribosomal RNA gene, partial sequence
4	Ay3	Forward: NNNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAACTTTCTAGAGA TAGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNN Reverse: GNNNCNNNNNNNNNNNNNNANNNNCCAATCTATCTCTAGAAAGTTCATTGGNTGTCAAGGCCTGGTAAGG TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATAN	*Pseudomonas fluorescens* strain CCTCC AB92001 16S ribosomal RNA gene, partial sequence
5	Co1	Forward: NNNNNNNNNNNNNNNNTCGAGCACGCGAAGANCCTTACCAGGCCTTGACATCCAATGAACTTTCCNGAGATG GATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNN Reverse: GNNNNNNNNNNNNNNNNNNNNNNNNNNCCAATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAA GGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATAN	*Pseudomonas* sp. N19.27 16S ribosomal RNA gene, partial sequence
6	Co2	Forward: NNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCNNATGAACTTTCCAGAGATGG ATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNN Reverse: GNNNNNNNNNNNNNNNNNNNNNNNNNCCAATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAAGG TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATAN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
7	Co3	Forward: NNNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCNAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGATG GATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
8	Co4	Forward: NNNNNNNNNNNNNNNNATTCNAGCACGCGAAGANCCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN	*Pseudomonas* sp. N19.27 16S ribosomal RNA

		Reverse: GGNNNNNNNNNNNNNNTCGAGCACGCGNAGAACCTCTACCNGGCCTTGACATCCAATGAATCCTTTAGAGA TAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN	gene, partial sequence
9	Co5	Forward: NNNNNNNNNNNNNNNNATTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAG ATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNCNNNNNGCACCAATCNNTCTCTGGNNGNTCATTGGNTGTCAAGGCCTGGTAAGGTTCTT CGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
10	PP1	Forward: GNNNNNNNNNNNNNNTCGATGCACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGAT GGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNANNNNNNNTCNNTCTCTGGNAANNTCTNGTGNNTGTCANGACCANGGTAA GGTTCTTCGCGTTGCATCNAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNATAN	*Klebsiella pneumoniae* strain AV3 16S ribosomal RNA gene, partial sequence
11	PP2	Forward: GNNNNNNNNNNNNNNNNNCGATGCACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAG ATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNGNNNNNNNNNNANGCACCNATCNNTCTCTGGAANNTCTGTGGATGTCAAGACCAGGTAAGGTTCTTC GCGTTGCAT CGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNNNN	*Klebsiella pneumoniae* strain AV3 16S ribosomal RNA gene, partial sequence
12	PP3	Forward: NNNNNNNNNNNNNNTTCGATGCACGCGNAGANCCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGAT GGATTGGTG CCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNCNNNGNCACCAATCNNTCTCTGGNNAGTTCTGTGGATGTCAAGACCAGGTAAGGT TCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNNN	*Klebsiella pneumoniae* strain AV3 16S ribosomal RNA gene, partial sequence
13	Me1	Forward: NNNNNNNNNNNNNNNTCGAGCACGCNNGANCCTTACNNGGCCTTGACATCCAATGAACTTTCCAGAGANG GATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: GGNNNNNNNNNNNNCNNNNGNACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNN	*Pseudomonas baetica* strain NTCC5 16S ribosomal RNA gene, partial sequence

14	Me2	Forward: NNNNNNNNNNNNNTNTTCGAGCACGCGNAGAACCTTACCNNGGCCTTGACATCNNATGAACTTTCCAGAGA TGGATGGGTGCCTTCGGGAACATNTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: GNNNNNNNNNNNNNNNNNNNNNANNATCNNTCTCTGGAAGTTCATCTNGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas syringae* strain LH-y3 16S ribosomal RNA gene, partial sequence
15	Me3	Forward: NNNNNNNNNNNNNNNNATTCGAGCACGCGAAGANCCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAG ATGGATGGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNGNACCCATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTT CGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCANNNNN	*Pseudomonas syringae* strain LH-y3 16S ribosomal RNA gene, partial sequence
16	Me4	Forward: NNNNNNNNNNNNNNATTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAACTTTCCAGAGATG GATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGNNNN Reverse: NNNNNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCANNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
17	Li1	Forward: GNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGATA GAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNNNGNANTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
18	Li2	Forward: NNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGAT AGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNN Reverse: NNNNGNNNNNNNNNNNNNNGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
19	Li3	Forward: NNNNNNNNNNNNNNNNNNNNAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAG ATAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNNN Reverse: NNNNNNNNNNNNNNNNNNNNNNNNNNNNTCCTCTNTCTCTAAAGGANTCATTGGATGTCANGGNCTGNTAA GGTTCTTCGCGTTGCTTCCAATTAAACCNNATGCTCCACCGCTTGTGCCGGCCCCCCNCNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence

20	Li4	Forward: GGNNNNNNNNNNNNNNTCGAGCACGCGNAGAACCTCTACCNGGCCTTGACATCCAATGAATCCTTTAGAGA TAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNGNANTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain EW3_F06 16S ribosomal RNA gene, partial sequence
21	Li5	Forward: NNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGAT AGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNCNNNNNNNNNNNNNNNGCANTCCTCTATCTCTAAAGGANTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
22	Li6	Forward: NNNNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAACTTTCCAGAG ATGGATGGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNNGNACCCATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAAGG TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNN	*Pseudomonas syringae* strain LH-y3 16S ribosomal RNA gene, partial sequence
23	Li7	Forward: NNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGATAGA GGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNANGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
24	Li8	Forward: NNNNNNNNNNNNNNNTTCGANGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGA TAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
25	Li9	Forward: NNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGATA GAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNNNNNTCCTCTATCTCTAAAGGANTCATTGGNTGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence

26	Li10	Forward: NNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAATCCTTTAGAGAT AGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGNNNNNN Reverse: NNNNNNNNNNNNNNNNNNNNGCANTCCTCTATCTCTAAAGGANTCATTGGATGTCANGGCCTNGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
27	Li11	Forward: GNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGATA GAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNGCANTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
28	Li12	Forward: NNNNNNNNNNNNGNNNTNNNCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAATCCTTTAGA GATAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCNTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNNNNNANTCCTCTATCTCTAAAGGATTCATTGGNTGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
29	Li13	Forward: NNNNNNNNNNNNNNNNNNNTCNAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAG AGATAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNCNNNNNNNGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
30	Li14	Forward: NNNNNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAATCCTTTAGA GATAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNN Reverse: NNNNNNNNNNNCNNNNNNNGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
31	Li15	Forward: NNNNNNNNNNNNNNNTTCGANGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAATCCTTTAGAGA TAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGNNNNN Reverse: NNNNNNNNNNNNNNNNNNNNNNNGNANTCCTCNATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGG TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATAN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence

32	Li16	Forward: NNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGAT AGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGNNNNN Reverse: NNNNNNNNNNNNNNNNNNNGNNNTCCTCTATCTCTAAAGGANTCATTGGATGTCAAGGCCTGGTAAGGTTC TTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
33	Li17	Forward: GNNNNNNNNNNNNNNNNNNCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGA GATAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNN Reverse: NNNNNNNNNNNNNNNNNNNNANGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
34	Li18	Forward: NNNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGA TAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNGNNNTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
35	Li19	Forward: NNNNNNNNNNNNNNNNNNNNNNNACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAATCCTTTAGAGA TAGAGGAGTGCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNGNNNNNNNNNNNNNNGCANTCCTCTATCTCTAAAGGANTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
36	Li20	Forward: NNNNNNNNNNNNNNNNATTCGAGCACGCGNAGAACCTTACCNGGCCTTGACATCCNATGAATCCTTTAGAGA TAGAGGAGTNCCTTCGGGAGCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNNNNGNANTCCTCTATCTCTAAAGGANTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
37	RL1	Forward: NNNNNNNNNNNNNNNNNNNNNNGNANTCCTCTATCTCTAAAGGANTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN Reverse: GNNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTT CGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence

38	RL2	Forward: NNNNNNNNNNNNNNNATTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: GGNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGNNNNTCATTGGATGTCAAGGCCTGGTAAGGTTC TTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
39	RL3	Forward: NNNNNNNNNNNNNNTTCGAGCNNGCGAAGAACCTTACCNGGCCTTGACATCCAATGAACTTTCCAGAGAN GGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNNGNACNNNTCNNTCTCTGNNNNNTCATTGGATGTCANGGNCTGNTAAGG TTCTTCGCGTTGCTTCCAATTAAACCANNTGCTCCACCGCTTGTGCCGNCCCCCCNCNATNN	*Pseudomonas* sp. strain BR1 16S ribosomal RNA gene, partial sequence
40	RL4	Forward: NNNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTT CGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
41	RL5	Forward: NNNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNN Reverse: NNNNNNNNNNNNNNNNNNNNGNACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
42	RL6	Forward: GNNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAACTTTCCAGAG ANGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNNNN Reverse: NNNNNNNNNNNNNNNNNNNNNNGNANCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
43	RL7	Forward: NNNNNNNNNNNNNNNANTNNNNNNNCGCGAAGAACCTTACNAGGCCTTGACATCCAATGAACTTTCCAGA GATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNCNNNNNNNNNNNNNNNGNACCAATCNNTCTCTGGNNNNTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. strain BR1 16S ribosomal RNA gene, partial sequence

44	St1	Forward: GNNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACNAGGCCTTGACATCCAATGAACTTTCCAGAGA NGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTC TTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
45	St2	Forward: GNNNNNNNNNNNNNNNNTCGNNNNNCGCGNNGAACCTTACCAGGCCTTGACATCCNATGAACTTTCCNG AGANGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCNTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNNGCNNCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTC TTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. strain BR1 16S ribosomal RNA gene, partial sequence
46	St3	Forward: GNNNNNNNNNNNNNNNNNNTCNNNNCACGCGNNGNACCTTACCANGGCCTNTGACATCCNATGAACNNT TCCANGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGGNGCTGCATGGCTGTCNTCNNCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNNNGGNACCAATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAAG GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
47	St4	Forward: NNNNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCNAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGA Reverse: NNNNNNNNNNNNNNNNNNNAAGGCACCAATCNNTCTCTGGNNNNTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
48	St5	Forward: NNNNNNNNNNNNNNNNNNTCNNNNCACGCGNANNANCTNNNNNNGCCTTGACATCCNATGAACTTTCCN GAGATGGATTGGTGCCTTCGGGAACATTGANACAGGTGCTGCATGGCTGTCCTCNGCTCGA Reverse: NNNNNNNNNNNNNNCNNNNNGGNACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTC TTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNNNNN	*Pseudomonas brassicacearum* strain SLr13 16S ribosomal RNA gene, partial sequence
49	St6	Forward: NNNNNNNNNNNNNNNTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAACTTTCCAGAGAN GGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNNAGGCACCAATCNNTCTCTGGAAGTTCATTGGNTGTCAAGGCCTGGTAAG GTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence

50	St7	Forward: NNNNNNNNNNNNNNNTCGAAGCACGCGAAGAACCTTACCAGGCCTTGACATCNAATGAACTTTCCNGNNG ATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNAGGCACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGT TCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
51	St8	Forward: GNNNNNNNNNNNNNNNNTNTTCGANCACGCGNANNNCCTTACCNGGCCTNNGACATCCNATGAACTTTCC NGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCCTCCNCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNGGCACCAATCNNTCTCTGGANAGTTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. N19.27 16S ribosomal RNA gene, partial sequence
52	St9	Forward: NNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCNGGCCTTGACATCCAATGAACTTTCCAGAGAT GGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCNN Reverse: NNNNNNNNNNNNNNNNNNNNNNGGCACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGG TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
53	St10	Forward: NNNNNNNNNNNNNNNTNNNTCNNNCACGCNNNNNANCTTACNNNGGCCTNTGACATCNNATGAACTTTC CNGAGATGGATTGGTGCCTTCGGNAACATTGANACAGGNGCTGCNTGGCTGTCCTCCNCTCGA Reverse: GNNNNNNNNNNNNNNNNNNNGNACCAATCNNTCTCTGGAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATNNA	*Pseudomonas* sp. strain GR45 16S ribosomal RNA gene, partial sequence
54	St11	Forward: GNNNNNNNNNNNNNNNNTNTTCNNGCACGCGNNNNNCCTTACNANNGCCTTGACATCNNATGAACTTTCC NGAGATGGATTGGTGCCTTCGGGAACATTGANACAGGNGCTGCATGGCTGTCNTCNGCTCGA Reverse: GNNNNNNNNNNNNNNNNANGCACCAATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTT CGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas tolaasii* strain PT11 16S ribosomal RNA gene, partial sequence
55	St12	Forward: NNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGATG GATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: GNNNNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGNNNNTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence

56	St13	Forward: NNNNNNNNNNNNNNNTCGAAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAACTTTCCAGNNG ATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTN Reverse: NNNNNNNNNNNNNNNNNNNNGCACCAATCNNTCTCTGGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
57	NLS1	Forward: NNNNNNNNNNNNNNNNTTCGAGCACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGA TGGATTGGTGCCTTCGGGANCATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNNNNNNGNACCAATCNNTCTCTGNNNNNTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCANNNNN	*Pseudomonas syringae* strain Ma001 16S ribosomal RNA gene, partial sequence
58	WS1	Forward: NNNNNNNNNNNNNNTANTCNNNNNNCGCNNNNNANNNTACCNNNGNCTTGACANCNNATGAATCCNTTA GAGATAGAGGAGTGNCTTCNGGAGCATTGANACNGGTGCTGNNTGGCTGTCCTCNNCTCGA Reverse: NNNNNNNNNNNNNNNNNNNNGCANTCCTCTATCTCTAAAGGATTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNTNNN	*Pseudomonas viridiflava* strain XJ-4 16S ribosomal RNA gene, partial sequence
59	WS2	Forward: NNNNNNNNNNNNNNNTTCGAAGCACGCGAAGAACCTTACNNGGCCTTGACATCCAATGAACTTTCCAGNAG ATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNGNNNNNNNNNNNNANGCACCAATCNNTCTCTGGNNNNTCATTGGATGTCAAGGCCTGGTAAGGTTCT TCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. WXGRY8 strain SJN8 16S ribosomal RNA gene, partial sequence
60	WS3	Forward: GNNNNNNNNNNNNNNTTNGAAGCACGCGAAGAACCTTNACCNGGCCTTGACATCNAATGAACTTTCCAGNA GATGGATGGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGN Reverse: NNNNNNNNNNNNNNNNCCNNNGGCANNATCNNTCTCTGGNAAGTTCATTGGATGTCAAGGCCTGGTAAGGTT CTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas syringae* pv. *syringae* strain JCK-CSHB55 16S ribosomal RNA gene, partial sequence
61	WS4	Forward: NNNNNNNNNNNNNNNNNNNNTCGATGCACGCNNNNNNCNNTACCTACCCNNTGACATGTACGGAAGGCC GAAGAGATTTGNCTGTGCTCNAAAGAGAACCGTAACACAGGNGCTGCNTGGCTGTCCTCNNCTCGA Reverse:	*Massilia* sp. strain B2P089 16S ribosomal RNA gene, partial sequence

		NNNNNNNNNNNNNNNNNNTTTCGAGCACNGCCAATCTCTTCGGCCTTCCGTACATGTCAAGGGTAGGTAAGG TTTTTCGCGTTGCATCGAATTAATCCACATCATCCACCGCTTGTGCGGGCCCCCGTCNNNNN	
62	WS5	Forward: NNNNNNNNNNNNNNNATTCGNNNCACGCGNNGNACCNTACCTGNCCNTGACATGCTGAGAACTTTCCNGA GANGGATTGGTGCCTTCGGGAACTCNNACNCNGGTGCTGCNTGGCTGTCCTCCNCTCGA Reverse: NNNNNNNNNNNNNNNCNNNNGGCACCAATCNNTCTCTGGANAGTTCTCAGCATGTCAAGGCCAGGTAAGG TTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCNNNNNN	*Pseudomonas* sp. strain NT-4 16S ribosomal RNA gene, partial sequence
1	*Pss27	Forward: NNNNNNNNNNNNNNNNNTCGAGCACGCGAAGANCCTTACNNGGCCTTGACATCCAATGAACTTTCCAGAGA TGGANNNNNNNNNNCGGGAGCATTGAGACAGGTGCTGCATGGCTGNCNNCANCNNNN	*Pseudomonas syringae* strain Ma001 16S ribosomal RNA gene, partial sequence

**P. syringae* pv. *syringae* strain received from AAFC, Harrow (August 4, 2021)

Supplementary Table 3

"Virulence" keyword search gene products in the orthologous groups, COGs, and protein families in Pss27, Me3, Me3, and Li6

Cluster of orthologous groups (COGs)					
Pss27					
COG	**Function**	**Description**	**Presence in selection (%)**	**Presence in database (%)**	
COG2761	O (Posttranslational modification, protein turnover, chaperones)	Predicted dithiol-disulfide isomerase, DsbA/YjbH family (virulence, stress resistance)	100	100	
COG2996	R (General function prediction only)	Predicted RNA-binding protein YitL, contains S1 domains, virulence factor B family	100	100	
COG3943	S (Function unknown)	Uncharacterized protein RhuM, Salmonella virulence factor	100	25	
COG3950	R (General function prediction only)	Predicted ATP-binding protein involved in virulence	100	75	
COG4456	S (Function unknown)	Virulence-associated protein VagC (function unknown)	100	25	
COG4457	Q (Secondary metabolites biosynthesis, transport and catabolism)	Virulence factor SrfB-related protein	100	25	
COG4458	Q (Secondary metabolites biosynthesis, transport and catabolism)	Virulence factor SrfC-related protein	100	25	
Me2					
COG	**Function**	**Description**	**Presence in selection (%)**	**Presence in database (%)**	
COG2761	O (Posttranslational modification, protein turnover, chaperones)	Predicted dithiol-disulfide isomerase, DsbA/YjbH family (virulence, stress resistance)	100	100	
COG2996	R (General function prediction only)	Predicted RNA-binding protein YitL, contains S1 domains, virulence factor B family	100	100	
COG3950	R (General function prediction only)	Predicted ATP-binding protein involved in virulence	100	75	
Me3					
COG	**Function**	**Description**	**Presence in selection (%)**	**Presence in database (%)**	

COG2761	O (Posttranslational modification, protein turnover, chaperones)	Predicted dithiol-disulfide isomerase, DsbA/YjbH family (virulence, stress resistance)	100	100	
COG2996	R (General function prediction only)	Predicted RNA-binding protein YitL, contains S1 domains, virulence factor B family	100	100	
Li6					
COG	**Function**	**Description**	**Presence in selection (%)**	**Presence in database (%)**	
COG2761	O (Posttranslational modification, protein turnover, chaperones)	Predicted dithiol-disulfide isomerase, DsbA/YjbH family (virulence, stress resistance)	100	100	
COG2996	R (General function prediction only)	Predicted RNA-binding protein YitL, contains S1 domains, virulence factor B family	100	100	
COG3950	R (General function prediction only)	Predicted ATP-binding protein involved in virulence	100	75	
Orthologous groups					
Pss27					
Orthogroup	**Description**	**COG**	**Presence in selection (%)**	**Presence in database (%)**	
group_1185	virulence protein, putative (1)	-	100	100	
group_2051	Virulence factor mviM (1)	COG3132 (1)	100	100	
group_4017	avirulence protein AvrF (1)	-	100	100	
group_5150	Putative virulence factor (1)	COG3266 (1)	100	25	
group_5815	Putative virulence factor (1)	COG4458 (1)	100	25	
Me2					
Orthogroup	**Products**	**COG**	**Presence in selection (%)**	**Presence in database (%)**	
group_1185	virulence protein, putative (1)	-	100	100	
group_2051	Virulence factor mviM (1)	COG3132 (1)	100	100	
group_4017	avirulence protein AvrF (1)	-	100	100	

Me3					
Orthogroup	**Description**	**COG**	**Presence in selection (%)**	**Presence in database (%)**	
group_1185	virulence protein, putative (1)	-	100	100	
group_2051	Virulence factor mviM (1)	COG3132 (1)	100	100	
group_4017	avirulence protein AvrF (1)	-	100	100	
Li6					
Orthogroup	**Description**	**COG**	**Presence in selection (%)**	**Presence in database (%)**	
group_1185	virulence protein, putative (1)	-	100	100	
group_2051	Virulence factor mviM (1)	COG3132 (1)	100	100	
group_4017	avirulence protein AvrF (1)	-	100	100	
Protein families (pfam)					
Pss27					
Domain	**Description**	**Presence in selection (%)**	**Presence in database (%)**		
PF03534	Salmonella virulence plasmid 65kDa B protein	100	50		
PF03536	Salmonella virulence-associated 28kDa protein	100	100		
PF03538	Salmonella virulence plasmid 28.1kDa A protein	100	50		
PF03543	Yersinia/Haemophilus virulence surface antigen	100	25		
PF03631	Virulence factor BrkB	100	100		
PF06057	Bacterial virulence protein (VirJ)	100	100		
PF07520	Virulence factor SrfB	100	25		
PF10139	Putative bacterial virulence factor	100	25		
PF11160	Hypervirulence associated proteins TUDOR domain	100	100		

PF12843	Putative quorum-sensing-regulated virulence factor	100	100		
PF13310	Virulence protein RhuM family	100	25		
PF13652	Putative quorum-sensing-regulated virulence factor	100	100		
PF16847	Avirulence AvrPtoB, BAK1-binding domain	100	25		
Me2					
Domain	**Description**	**Presence in selection (%)**	**Presence in database (%)**		
PF03536	Salmonella virulence-associated 28kDa protein	100	100		
PF03631	Virulence factor BrkB	100	100		
PF06057	Bacterial virulence protein (VirJ)	100	100		
PF11160	Hypervirulence associated proteins TUDOR domain	100	100		
PF12843	Putative quorum-sensing-regulated virulence factor	100	100		
PF13652	Putative quorum-sensing-regulated virulence factor	100	100		
Me3					
Domain	**Description**	**Presence in selection (%)**	**Presence in database (%)**		
PF03534	Salmonella virulence plasmid 65kDa B protein	100	50		
PF03536	Salmonella virulence-associated 28kDa protein	100	100		
PF03538	Salmonella virulence plasmid 28.1kDa A protein	100	50		
PF03631	Virulence factor BrkB	100	100		
PF06057	Bacterial virulence protein (VirJ)	100	100		
PF11160	Hypervirulence associated proteins TUDOR domain	100	100		
PF12843	Putative quorum-sensing-regulated virulence factor	100	100		

PF13652	Putative quorum-sensing-regulated virulence factor	100	100		
Li6					
Domain	**Description**	**Presence in selection (%)**	**Presence in database (%)**		
PF03536	Salmonella virulence-associated 28kDa protein	100	100		
PF03631	Virulence factor BrkB	100	100		
PF06057	Bacterial virulence protein (VirJ)	100	100		
PF11160	Hypervirulence associated proteins TUDOR domain	100	100		
PF12843	Putative quorum-sensing-regulated virulence factor	100	100		
PF13652	Putative quorum-sensing-regulated virulence factor	100	100		

Supplementary Table 4: Virulence factors present in *P. syringae* strains

Virulence class	**Virulence factors**	**Pss27**	**Me2**	**Me3**	**Li6**
Adherence	Flagella	flaG	flaG	flaG	flaG
		fleN	fleN	fleN	fleN
		fleQ	fleQ	fleQ	fleQ
		fleR	fleR	fleR	fleR
		fleS	fleS	fleS	fleS
		flgA	flgA	flgA	flgA
		flgB	flgB	flgB	flgB
		flgC	flgC	flgC	flgC
		flgD	flgD	flgD	flgD
		flgE	flgE	flgE	flgE
		flgF	flgF	flgF	flgF
		flgG	flgG	flgG	flgG

		flgH	flgH	flgH	flgH
		flgI	flgI	flgI	flgI
		flgJ	flgJ	flgJ	flgJ
		flgK	flgK	flgK	flgK
		flgL	flgL	flgL	flgL
		flgM	flgM	flgM	flgM
		flgN	flgN	flgN	flgN
		flhA	flhA	flhA	flhA
		flhB	flhB	flhB	flhB
		flhF	flhF	flhF	flhF
		fliA	fliA	fliA	fliA
		fliC	fliC	fliC	fliC
		fliD	fliD	fliD	fliD
		fliE	fliE	fliE	fliE
		fliF	fliF	fliF	fliF
		fliG	fliG	fliG	fliG
		fliH	fliH	fliH	fliH
		fliI	fliI	fliI	fliI
		fliJ	fliJ	fliJ	fliJ
		fliK	fliK	fliK	fliK
		fliL	fliL	fliL	fliL
		fliM	fliM	fliM	fliM
		fliN	fliN	fliN	fliN
		fliO	fliO	fliO	fliO
		fliP	fliP	fliP	fliP
		fliQ	fliQ	fliQ	fliQ
		fliR	fliR	fliR	fliR
		fliS	fliS	fliS	fliS
		fliT	fliT	fliT	fliT
		motA	motA	motA	motA
		motB	motB	motB	motB
		motC	motC	motC	motC
		motD	motD	motD	motD

		motY	motY	motY	motY
	Type IV pili biosynthesis	fimU	fimU	fimU	fimU
		fimV	fimV	fimV	fimV
		pilB	pilB	pilB	pilB
		pilC	pilC	pilC	pilC
		pilD	pilD	pilD	pilD
		pilE	pilE	pilE	pilE
		pilF	pilF	pilF	pilF
		pilM	pilM	pilM	pilM
		pilN	pilN	pilN	pilN
		pilO	pilO	pilO	pilO
		pilP	pilP	pilP	pilP
		pilQ	pilQ	pilQ	pilQ
		pilR	pilR	pilR	pilR
		pilS	pilS	pilS	pilS
		pilT	pilT	pilT	pilT
		pilU	pilU	pilU	pilU
			pilX	pilX	pilX
		pilY1	pilY1	pilY1	pilY1
		pilZ	pilZ	pilZ	pilZ
	Type IV pili twitching motility-related proteins	chpA	chpA	chpA	chpA
		chpC	chpC	chpC	chpC
		pilG	pilG	pilG	pilG
		pilH	pilH	pilH	pilH
		pilI	pilI	pilI	pilI
		pilJ	pilJ	pilJ	pilJ
Antiphagocytosis	Alginate biosynthesis	alg44	alg44	alg44	alg44
		alg8	alg8	alg8	alg8
		algA	algA	algA	algA
		algC	algC	algC	algC
		algD	algD	algD	algD
		algE	algE	algE	algE
		algF	algF	algF	algF

		algG	algG	algG	algG
		algI	algI	algI	algI
		algJ	algJ	algJ	algJ
		algK	algK	algK	algK
		algL	algL	algL	algL
		algX	algX	algX	algX
	Alginate regulation	algP/algR3	algP/algR3	algP/algR3	algP/algR3
		algQ	algQ	algQ	algQ
		algR	algR	algR	algR
		algU	algU	algU	algU
		algW	algW	algW	algW
		algZ	algZ	algZ	algZ
		mucA	mucA	mucA	mucA
		mucB	mucB	mucB	mucB
		mucD	mucD	mucD	mucD
		mucP	mucP	mucP	mucP
Iron uptake	Achromobactin biosynthesis and transport	acsA	acsA	acsA	acsA
		acsB	acsB	acsB	acsB
		acsC	acsC	acsC	acsC
		acsD	acsD	acsD	acsD
		cbrA	cbrA	cbrA	cbrA
		cbrB	cbrB	cbrB	cbrB
		cbrC	cbrC	cbrC	cbrC
		cbrD	cbrD	cbrD	cbrD
	Pyochelin		pchF		pchF
	Pyoverdine receptors	fpvA	fpvA	fpvA	fpvA
	Pyoverdine	pvdA	pvdA	pvdA	pvdA
		pvdD	pvdD	pvdD	pvdD
		pvdE	pvdE	pvdE	pvdE
		pvdG	pvdG	pvdG	pvdG
		pvdH	pvdH	pvdH	pvdH
			pvdI		pvdI
			pvdJ		pvdJ

		pvdL	pvdL	pvdL	pvdL
		pvdM	pvdM	pvdM	pvdM
		pvdN	pvdN	pvdN	pvdN
		pvdO	pvdO	pvdO	pvdO
		pvdP	pvdP	pvdP	pvdP
		pvdQ	pvdQ	pvdQ	pvdQ
		pvdS	pvdS	pvdS	pvdS
Protease	Alkaline protease	aprA	aprA	aprA	aprA
Quorum sensing	Acylhomoserine lactone synthase	hdtS	hdtS	hdtS	hdtS
	N-(3-oxo-hexanoyl)-Lhomoserine lactone QS system	ahlI			
		ahlR			
Regulation	GacS/GacA two-component system	gacA	gacA	gacA	gacA
		gacS	gacS	gacS	gacS
Secretion system	Harpins	hrpA2	hrpA2	hrpA2	hrpA2
		hrpK1	hrpK1	hrpK1	hrpK1
		hrpW1	hrpW1	hrpW1	hrpW1
		hrpZ1	hrpZ1	hrpZ1	hrpZ1
	Hcp secretion island-1 encoded type VI secretion system (H-T6SS)	clpV1	clpV1	clpV1	clpV1
			hcp1		
		icmF1	icmF1	icmF1	icmF1
		ppkA			
		vgrG1	vgrG1	vgrG1	vgrG1
	P. aeruginosa TTSS		pcrD	pcrD	pcrD
			pscN	pscN	pscN
	P. syringae T3SS effectors	avrE1	avrE1	avrE1	avrE1
		avrRpm1			
			hopA1	hopA1	hopA1
		hopAA1	hopAA1	hopAA1	hopAA1
		hopAB1			
		hopAC1	hopAC1	hopAC1	hopAC1
		hopAE1	hopAE1	hopAE1	hopAE1
		hopAF1			
		hopAG1	hopAG1	hopAG1	hopAG1

		hopAH1	hopAH1	hopAH1	hopAH1
		hopAH2	hopAH2	hopAH2	hopAH2
		hopAI1	hopAI1	hopAI1	hopAI1
		hopAJ2	hopAJ2	hopAJ2	hopAJ2
		hopAK1	hopAK1	hopAK1	hopAK1
		hopAN1	hopAN1	hopAN1	hopAN1
		hopAP1			
		hopH1			
		hopI1	hopI1	hopI1	hopI1
		hopJ1	hopJ1	hopJ1	hopJ1
		hopL1			
		hopM1	hopM1	hopM1	hopM1
	P. syringae T3SS	hrcC	hrcC	hrcC	hrcC
		hrcJ	hrcJ	hrcJ	hrcJ
		hrcN	hrcN	hrcN	hrcN
		hrcQa	hrcQa	hrcQa	hrcQa
		hrcQb	hrcQb	hrcQb	hrcQb
		hrcR	hrcR	hrcR	hrcR
		hrcS	hrcS	hrcS	hrcS
		hrcT	hrcT	hrcT	hrcT
		hrcU	hrcU	hrcU	hrcU
		hrcV	hrcV	hrcV	hrcV
		hrpB	hrpB	hrpB	hrpB
		hrpD	hrpD	hrpD	hrpD
		hrpE	hrpE	hrpE	hrpE
		hrpF	hrpF	hrpF	hrpF
		hrpG	hrpG	hrpG	hrpG
		hrpJ	hrpJ	hrpJ	hrpJ
		hrpL	hrpL	hrpL	hrpL
		hrpO	hrpO	hrpO	hrpO
		hrpP	hrpP	hrpP	hrpP
		hrpQ	hrpQ	hrpQ	hrpQ
		hrpR	hrpR	hrpR	hrpR

		hrpS	hrpS	hrpS	hrpS
		hrpT	hrpT	hrpT	hrpT
		hrpV	hrpV	hrpV	hrpV
		shcA	shcA	shcA	shcA
		shcE	shcE	shcE	shcE
		shcM	shcM	shcM	shcM
	ACE T6SS(Escherichia)	aec16	aec16	aec16	aec16
Toxin	Phytotoxin syringomycin	syrB1	syrB1	syrB1	syrB1
		syrB2	syrB2	syrB2	syrB2
		syrC	syrC	syrC	syrC
		syrD	syrD	syrD	syrD
		syrE	syrE		syrE
		syrF	syrF	syrF	syrF
		syrP	syrP	syrP	syrP
	Phytotoxin syringopeptin	sypA			
		sypB		sypB	
		sypC		sypC	
Efflux pump	AcrAB(Klebsiella)		acrB		
Serum resistance	LPS rfb locus(Klebsiella)		rmlA		

1