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A sucrose-utilisation gene cluster contributes to colonisation of horse chestnut by *Pseudomonas syringae* pv. *aesculi*

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Abstract

Pseudomonas syringae pathovar *aesculi* (E-*Pae*) causes bleeding canker disease in the woody tissue of European horse chestnut (HC). Comparative genomic analysis of E-*Pae* with a related leaf-infecting strain (I-*Pae*) and other *P. syringae* strains identified candidate virulence genes for colonisation of woody tissue, including a sucrose uptake and utilisation system

(*scrYABCDBR* cluster) found in 162 of 206 *P. syringae* strains spanning the pangenome. Growth analysis using sucrose as sole carbon source showed that I-*Pae* (lacking the gene cluster) was unable to grow whereas E-*Pae* could grow. *P. savastanoi* pv. *phaseolicola* 1448A and *P. syringae* pv. *morsprunorum* R15244 were compromised in growth despite the presence of the gene cluster. Sucrose utilization assays using *scrB* and *scrY* mutants and complemented strains confirmed the importance of the cluster for sucrose metabolism *in vitro*. Pathogenicity assays in HC revealed the sucrose gene cluster is important for symptom development in the woody tissue. While the *scr* genes contribute to disease causation, they were not essential for pathogen fitness when compared to *hrpL* and *hopAB1* mutants. E-*Pae* caused disease symptoms in HC leaves, suggesting the strain has the potential to infect leaves as well. However, it was notable that the *scrB* mutant of E-*Pae* caused increased disease symptoms, possibly highlighting a niche adaptation strategy for I-*Pae* to cause leaf spots in HC as well as constraining E-*Pae* to predominantly infect the woody tissue.

Introduction

The bleeding canker disease of European horse chestnut (*Aesculus hippocastanum*) (HC) is widespread in Europe, including the British Isles, following a recent epidemic that has caused severe damage and death to thousands of HC trees (Green et al., 2009, Dijkshoorn-Dekker and Kuik, 2005, Green et al., 2010, Mertelik et al., 2013, Schmidt et al., 2008, Webber et al., 2008, Bultreys et al., 2008).

The disease symptoms include dark lesions or cankers on the trunk or branches of the tree that exude a dark, reddish-brown fluid. Severe infections can lead to dieback of branches, leaf discoloration, and eventually, the death of the tree if the canker girdles the stem (de Keijzer et al., 2012, Green et al., 2009, Steele et al., 2010, Webber et al., 2008). These

symptoms are caused by European (E) genotype of *Pseudomonas syringae* pathovar *aesculi* (E-*Pae*) that is related to an isolate causing leaf spots in Indian (I) HC (*Aesculus indica*) (I-*Pae*) (Durgapal and Singh, 1980). E-*Pae* causes severe symptoms and thus represents a major disease for HC. Analysis of cankers on the main stem, branches, and young shoots, showed that lesions form in the cortex and phloem and extend into the cambium (de Keijzer et al., 2012, Green et al., 2009, Steele et al., 2010), thus can severely impact water and nutrient transport and kill the tree.

Phylogenomic analysis revealed that *Pae* genomes (E-*Pae* and I-*Pae*) share the greatest sequence similarity with *P. syringae* strains from herbaceous hosts, such as *P. syringae* pv. *phaseolicola* 1448A (*Pph* 1448A) and *P. syringae* pv. *tabaci* (*Pta* 11528) infecting bean and tobacco, respectively (Green et al., 2010). *Pae* (E-*Pae* and I-*Pae*) is also phylogenomically related with pathovars that colonize woody hosts, such as *P. syringae* pathovars *cerasicola*, *savastanoi*, *morsprunorum* race 1, and *P. amygdali*, which infect ornamental cherry, olive, sweet and sour cherries, and almond, respectively (Ruinelli et al., 2019). However, 15% of the *Pae* genome is uniquely conserved, which may contribute to its adaptation to woody hosts. This suggests an evolutionary relationship where *Pae* has diverged from other pathovars to colonize and infect horse chestnut.

It has been hypothesised that some of the genomic regions conserved in *Pae* genomes are likely responsible for their association with a tree host (Green et al., 2010, Nowell et al., 2016). Moreover, there are unique genetic regions in both E-*Pae* and I-*Pae* likely indicating intimate host adaptations to enable bacterial colonisation in the tree's wood or leaf tissues (Green et al., 2010, Nowell et al., 2016). For example, there are differences in the collection of type III secretion system (T3SS) effectors (T3SE) between E-*Pae* and I-*Pae* as well as genes

related to urea metabolism, β-ketoadipate and protocatechuate-4,5-deoxygenase pathways, degradation of aromatic compounds, fatty acid biosynthesis and degradation, sucrose utilisation, and iron uptake (Green et al., 2010, Nowell et al., 2016). Some of these could explain the adaptation of E-*Pae* to the woody tissue niche, distinguishing it from the leaf coloniser I-*Pae*.

The putative sucrose uptake and utilization gene cluster present in E-*Pae* (but not I-*Pae*) is formed of 7 genes (*scrYABCDBR*) including a putative porin (*scrY*) likely involved in transport of sucrose into the cell (Hardesty et al., 1991, Reid and Abratt, 2005, Sun et al., 2016) and a sucrose invertase enzyme (*scrB*) probably involved in enzymatic digestion of sucrose to glucose and fructose (Engels et al., 2008, Gunasekaran et al., 1990, Reid et al., 1999, Reid and Abratt, 2005). In the plant, sucrose is produced by photosynthesis and distributed throughout the plant, thus having an uptake and digestion system would likely enhance the fitness of the pathogen (Braun et al., 2013, Tauzin and Giardina, 2014). We hypothesised that E-*Pae*'s ability to access and utilize sucrose enhances its capacity to efficiently colonize the phloem of stems and branches unlike I-*Pae*, which lacks the necessary genetic machinery for sucrose metabolism.

Results

Genetic analysis of a putative sucrose gene cluster in E-*Pae* 2250 and other *P. syringae* strains

E-*Pae* 2250 possesses an 8.6 kb gene cluster on the chromosome containing seven genes predicted to be involved with sucrose uptake and utilisation (see Table 1 for coordinates and Figure 1A for gene orientation). A pangenome analysis using 206 *P. syringae* and other *Pseudomonas* strains, spanning thirteen phylogroups, representing 16 species and 37

pathovars, was conducted to determine if the gene cluster is widespread (Supplementary Table 1). Of the 206 strains analysed, 162 (79%) carried the *scr* gene cluster, while 44 (21%) did not (Figure 1C). Among the 80 strains isolated from woody hosts, all carried the *scr* cluster, suggesting strong conservation of this operon in woody plant-associated strains. In contrast, among strains from non-woody hosts (n = 100), only 82 possessed the cluster, while the remaining 18 lacked it. The 25 strains with unknown host information showed a mixed distribution (Supplementary Table 1). The cluster was found in strains isolated from leaves (n = 88) and wood (n = 57), with presence generally correlating with strains infecting perennial hosts.

The *scr* gene cluster is used by E-*Pae* 2250 to utilise sucrose

E-*Pae* strain 2250 was tested for growth in minimal medium containing sucrose (10 mM or 50 mM) as the sole carbon source. Two other strains carrying putative sucrose utilisation clusters were included: a cherry pathogen *Psm* R15244 and a bean pathogen *Pph* 1448A. Additionally, the Indian *Pae* strain (I-*Pae* 3681), which lacks the sucrose gene cluster, was included for comparison. All the *Pseudomonas* strains grew well in LB medium, and none showed any significant growth in M9 minimal medium lacking a carbon source (Supplementary Figure 1). Only E-*Pae* 2250 demonstrated substantial growth in M9+ sucrose, reaching an OD600 of 0.35 and 0.6 after 24 hours in 10 mM and 50 mM sucrose, respectively (Figure 2A, 2B). *Psm* R15244 showed only slight growth in 10 mM sucrose, but after displaying an initial lag phase, it reached an OD600 of 0.5 in 50 mM sucrose. In contrast, I-*Pae* 3681 and *Pph* 1448A displayed no measurable growth in either sucrose medium. These growth phenotype differentials were supported by two-way repeated measures ANOVA, which showed significant effects of strain, time, and their interaction at both sucrose concentrations ($p < 0.001$; Supplementary Figure 2A, B). Post-hoc Tukey Kramer's tests revealed that E-*Pae*

2250 grew significantly better than all other strains at all timepoints (1000, 2000, and 3000 minutes) in 10 mM sucrose ($p < 0.001$). At 50 mM sucrose, E-*Pae* 2250 also outperformed all strains at 1000 and 2000 minutes ($p < 0.001$), although by 3000 minutes the difference with *Psm* R15244 was not statistically significant.

The different phenotypes of *Psm* R15244 and *Pph* 1448A in utilising sucrose suggested that differences in either the regulatory systems or the *scr* gene clusters in these strains were responsible for reducing the effectiveness of either uptake or breakdown of sucrose. Analysis of the nucleotide and protein sequences of the *scr* genes of E-*Pae* 2250, *Psm* R14255, and *Pph* 1448A revealed that the seven genes had conserved synteny and similar nucleotide (nt) sequence lengths of the genes (Table 2). However, several nt differences were observed in the seven genes (Table 2), with more variations observed in the *Pph* genes. The most conserved gene was the putative transcriptional regulator, with 100% identity in *Psm* R14255 and 99.70% identity in *Pph* 1448A (corresponding to five nt differences and one amino acid (aa) change). Larger variations were seen in the other six genes, whereby predicted proteins had 1 to 12 aa changes. The carbohydrate porin *scrY* was different by 3 and 4 aa, and the invertase *scrB*, exhibited 3 and 12 aa differences, in *Psm* R14255 and *Pph* 1448A, respectively. Given the putative roles of carbohydrate porin (*scrY*) in sucrose uptake and of the invertase enzyme (*scrB*) in sucrose breakdown, we focused our analysis on these as representatives of the two processes.

The invertase gene *scrB* and carbohydrate porin *scrY* are essential for E-*Pae* 2250 sucrose utilisation

To test the hypothesis that *scrB* and *scrY* genes are crucial for the sucrose growth phenotype displayed by E-*Pae*, we constructed insertional knockout mutants for *scrB* (E-*Pae* 2250::*scrB*) and *scrY* (E-*Pae* 2250::*scrY*) and created complemented strains using cloned full-length *scrB* and *scrY* genes. Growth assays with M9+50 mM sucrose demonstrated that inactivation of both *scrB* and *scrY* significantly reduced the ability of E-*Pae* 2250 to grow, with the *scrB* inactivation having a bigger impact on E-*Pae* growth (Figure 2C, 2D). The complemented strains restored growth comparable to the WT E-*Pae* 2250 strain. The doubling times of the WT, 2250::*scrB*, 2250::*scrY*, complemented strains, and empty vector controls, in M9 + 50 mM sucrose (data not shown) showed subtly different phenotypes for the mutants (WT (1639 minutes) vs 2250::*scrB* (2231 minutes) and 2250::*scrY* (1732 minutes)). Complementation of 2250::*scrB* with *scrB* increased doubling time to 1639 minutes, while complementation of 2250::*scrY* with *scrY* substantially improved doubling time to even better than the WT (1377 minutes). These phenotype differentials were supported by statistical analysis (Supplementary Figure 2C, D), where two-way repeated measures ANOVA showed significant effects of strain, time, and their interaction ($p < 0.001$). Post-hoc Tukey Kramer's tests confirmed that the *scrB* mutant exhibited significantly reduced growth at 1000 and 2000 minutes ($p < 0.001$), while the *scrY* mutant was significantly different only at 2000 minutes ($p < 0.05$). Complemented strains restored growth to near WT levels, indicating that the observed phenotypes were directly attributable to loss of *scrB* or *scrY* function.

Loss of function in *scrB* alleles encoded by *Pph* and *Psm* highlights a unique role for sucrose metabolism in E-*Pae*

To identify potential reasons for the ineffective utilisation of sucrose by *Psm* R15244 or *Pph* 1448A, we focussed on the *scrB* gene given there were more nt/aa differences compared to

scrY. The *scrB* allele from each strain was expressed in E-*Pae*2250::*scrB* (E-*Pae*::*scrB*[pBBR1-*scrB*$_{Psm}$] and E-*Pae*::*scrB*[pBBR1-*scrB*$_{Pph}$]) and growth analysis in M9 + 50 mM sucrose carried out. Neither gene was sufficient to restore full growth to the mutant compared to the WT E-*Pae scrB* allele (Figure 2E). Very little change in growth was observed using 10 mM sucrose, (Supplementary Figure 1E), whereas in 50 mM sucrose E-*Pae*::*scrB*[pBBR1-*scrB*$_{Psm}$] showed gradual increases reaching close to 0.4 OD towards the end (Figure 2E). E-*Pae*::*scrB*[pBBR1-*scrB*$_{Pph}$] did not exhibit any growth increase (Figure 2E). Statistical analysis confirmed these findings (Supplementary Figure 2E): two-way repeated measures ANOVA showed significant effects of strain, time, and interaction ($p < 0.001$), and post-hoc Tukey Kramer's tests indicated that neither heterologous strain showed a statistically significant improvement over the knockout at any timepoint ($p > 0.05$), highlighting a failure to complement. Notably, E-*Pae*::*scrB*[pBBR1-*scrB*$_{Psm}$] showed a gradual increase in growth by 3000 minutes, reaching OD600 ~0.4, but this was still significantly below WT levels ($p < 0.001$), while E-*Pae*::*scrB*[pBBR1-*scrB*$_{Pph}$] exhibited no measurable increase in OD.

These observations suggest that the lack of *Pph* growth is due to multiple mutations in *scrB*$_{Pph}$ whereas *Psm* is experiencing degradation of function due to one or more of the three aa changes (nt 97, 803 and 1408 corresponding to residues 97, 268 and 470). InterProScan analysis of *scrB* revealed residue 268 is located in the N-terminal glycoslyhydrolase domain, while residue 470 is found in the C-terminal glycoslyhydrolase domain (Figure 1B). To examine this, site-directed mutagenesis of *scrB*$_{Pae}$ nucleotides to change residues 97 (A to G), 268 (G to A) and 470 (c to A) was attempted, but only mutations in 268 and 470 were achieved (Figure 1B). Both mutations led to reduced growth in M9 + 50 mM sucrose (Figure 2F), supported by statistical analysis (Supplementary Figure 2F): the *scrB*-803 mutant (residue 268) was

significantly impaired at all timepoints ($p < 0.001$), while the *scrB*-1408 mutant (residue 470) was only significantly different from WT at 1000 minutes ($p < 0.05$), with no significant difference from the *scrB* knockout at early timepoints ($p > 0.05$). These findings indicate both mutations impair *scrB* function, with residue 268 likely being more critical. Neither mutant supported growth above OD600 = 0.2 in 10 mM sucrose (Supplementary Figure 1F), underscoring the importance of these residues for optimal sucrose metabolism.

The *scr* genes are important for *Pae* virulence in plants

The role of *scrB* and *scrY* in supporting E-*Pae* colonisation of plants and to cause disease symptoms was investigated by inoculating the mutant and complemented mutant strains in its host (HC). The creation of E-*Pae* 2250 *hopAB1* and *hrpL* inactivation mutants served as useful controls as *hrpL* is a critical regulator and *hopAB1* was previously observed to be essential for full virulence/pathogenicity in HC (Dorati, 2011).

E-*Pae* 2250 and *scrB*, *scrY*, *hopAB1*, and *hrpL* mutant strains (and complemented strains) were inoculated into detached HC shoots. Disease assessment and pathogen growth analyses were conducted at weeks 1, 3, 4, and 5 post-inoculation (pi). The E-*Pae* 2250 caused typical disease symptoms on HC shoots characterized by rust-coloured liquid oozing on the branch, with necrotic phloem underlying the outer bark (Figure 3). No disease symptoms were observed for the *scrB*, *scrY*, *hopAB1*, and *hrpL* knockout mutants (Figure 3), while the complemented strains displayed severe necrotic lesions similar to those observed in the WT strain (Figure 3A). The *scrB* and *scrY* knockout mutants had low disease scores on HC shoots comparable to the *hopAB1* and *hrpL* knockout mutants. Bacterial growth analysis in the plant showed that E-*Pae*2250::*hrpL* and E-*Pae*2250::*hopAB1* knockout mutants were significantly impaired in growth compared to E-*Pae* 2250 strain (Figure 4). In contrast, the growth of the E-

205 *Pae*2250::*scrY* and E-*Pae*2250::*scrB* knockout mutants did not show a significant reduction
206 compared to E-*Pae* 2250 (Figure 4). Consistently, bacterial growth *in planta* was significantly
207 affected by strain type and timepoint (Supplementary Figure 3). A two-way repeated
208 measures ANOVA showed significant effects of strain ($p < 0.0001$), time ($p < 0.0001$), and their
209 interaction ($p< 0.0001$), confirming distinct colonisation dynamics across strains. Tukey–
210 Kramer post-hoc tests showed that *scrB* and *scrY* mutants had significantly lower bacterial
211 populations than E-*Pae* 2250 at multiple timepoints (weeks 3–5), similar to the *hopAB1* and
212 *hrpL* mutants. Complementation restored growth to E-*Pae* 2250 levels, confirming the
213 importance of these genes in colonisation of woody tissue.

214 Although E-*Pae* is associated with diseased woody tissue, we also tested the various
215 strains in HC leaves. Interestingly, strong necrotic symptoms appeared on HC leaves
216 inoculated with E-*Pae* 2250 two days post-inoculation (pi), suggesting that E-*Pae* 2250 can
217 cause disease symptoms in leaves at high inoculum levels (Figures 5). This was confirmed by
218 *in planta* growth analysis (Figures 6). Inoculation with the mutants and complemented strains
219 showed that the *scrY* (porin), *hrpL* and *hopAB1* mutants did not cause the same necrosis
220 phenotype as WT, but that this could be recovered by complementation with their respective
221 genes (Figures 5). Curiously, the *scrB* (invertase) mutant exhibited strong necrosis symptoms.
222 Bacterial growth analysis showed the populations of the E-*Pae*2250::*hrpL* and E-
223 *Pae*2250::*hopAB1* knockout mutants were significantly lower than that of E-*Pae* 2250 and
224 complementation restored their growth to E-*Pae* 2250 levels (Figures 6). In contrast, the
225 growth of the E-*Pae*2250::*scrY* and *Pae*2250::*scrB* knockout mutants mirrored the
226 observations seen in woody tissue and did not show a significant reduction in growth
227 compared to E-*Pae* 2250 (Figures 6). Growth analysis in leaves confirmed these findings
228 (Supplementary Figure 4). Two-way ANOVA indicated significant effects of strain and time ($p<$

0.0001), with one-way ANOVA and Tukey Kramer's post-hoc tests at days 2 and 10 post-inoculation further resolving strain-specific differences. At day 2, *scrB*, *hrpL*, and *hopAB1* complemented strains showed significantly increased populations relative to E-*Pae* 2250 ($p < 0.0001$), suggesting efficient colonisation. Meanwhile, the *scrB* mutant did not differ from WT ($p > 0.05$), in line with the observed necrosis, but *hrpL* and *hopAB1* mutants had significantly reduced populations ($p < 0.0001$). At day 10, the *scrB* mutant remained statistically indistinguishable from E-*Pae* 2250 ($p > 0.05$), but the *scrY* mutant showed significantly reduced growth ($p < 0.0001$). Complementation of *scrB* showed an unexpected significant reduction in growth compared to E-*Pae* 2250 ($p < 0.0001$), while *hrpL* and *hopAB1* mutants still exhibited significantly lower populations ($p < 0.0001$). Their complemented strains remained slightly but significantly different from E-*Pae* 2250 ($p = 0.0444$), possibly reflecting variation in expression or vector-related effects.

Discussion

Sucrose is abundant in plant tissues and can act both as a carbon source (Lemoine, 2000) and as an osmolyte (Rolland et al., 2002). It is metabolized by many bacteria through enzymes like invertases and sucrose phosphorylases (Reid and Abratt, 2005). Common features in sucrose-utilization regulons include gene architecture, uptake systems, and regulatory mechanisms. Reid and Abratt (2005) presented evidence of gene shuffling, with phylogenetic analyses suggesting that these gene clusters were acquired through horizontal gene transfer, facilitating bacterial colonization of sucrose-rich niches. Our study focussed on the analysis of a seven-gene cluster in E-*Pae* that was predicted to be involved in sucrose utilisation. One gene is a putative LacI-family sucrose transcriptional regulator, another is

predicted to be an invertase (*scrB*) and the remaining five appear to be transporters, including a porin (*scrY*).

Our analysis of the *Pseudomonas syringae* pangenome revealed that the sucrose gene cluster is present in 162 out of 206 strains, spanning diverse phylogroups, hosts, and environments. We found that strains harbouring the *scr* gene cluster were significantly enriched in plant-pathogenic lifestyles, especially those infecting woody hosts and/or multiple tissues (leaf, fruit, and stem). This association suggests that sucrose metabolism may provide a competitive advantage in complex plant tissues where nutrient availability and osmotic conditions fluctuate. Notably, 100% of strains isolated from woody hosts possessed the *scr* cluster, highlighting a potential role in adaptation to perennial plant environments, which are often more carbon-rich and osmotically variable. These findings reinforce the idea that the sucrose gene cluster contributes pathogenesis and host colonization, particularly in woody plants. However, the absence of the cluster in 44 strains suggests it is not essential for some lineages. Furthermore, variability in the nucleotide sequences of the sucrose cluster, particularly in *scrB* and *scrY*, suggests ongoing adaptation and possible degradation in certain strains. Thus, strains that colonize plants or environments with limited sucrose availability may not benefit from retaining this system, leading to its loss through genetic drift or negative selection.

E-*Pae* 2250 could efficiently metabolize sucrose, demonstrating robust growth in minimal medium supplemented with sucrose as the sole carbon source. In contrast, *Psm* R15244 and *Pph* 1448A showed limited or no growth under similar conditions, despite the presence of the sucrose gene cluster in their genomes. This disparity highlights potential differences in the functionality or regulation of the cluster among these strains. The lack of

sucrose utilization in I-*Pae* is consistent with the absence of the *scr* operon, though further testing, such as complementation would be required to confirm a causal relationship. Mutational analysis of E-*Pae*'s *scrB* and *scrY* genes further confirmed their roles in sucrose metabolism, as inactivation of either gene significantly impaired growth on sucrose. Other studies have shown the importance of *scrB* and *scrY* in sucrose metabolism across various bacterial systems. For example, in *Escherichia coli*, a frameshift mutation in *scrY* reduced sucrose uptake under growth-limiting conditions (Hardesty et al., 1991). Similarly, deletion of *scrB* in *Clostridium beijerinckii* (Reid et al., 1999) and *Corynebacterium glutamicum* (Engels et al., 2008) abolished the ability to grow on sucrose. In *Erwinia amylovora*, deletion of *scrY* and *scrA* rendered the bacterium unable to utilize sucrose, highlighting the role of these genes in sucrose metabolism (Bogs and Geider, 2000). However, further studies would be needed to determine their direct impact on pathogenicity. Collectively, these findings underscore the critical role of *scr* genes in sucrose metabolism and highlight the functional variability of the sucrose cluster among strains. The divergence observed in *Psm* and *Pph* may reflect evolutionary adaptations to specific ecological niches, where alternative metabolic pathways and carbon sources reduce the reliance on sucrose utilization.

E-*Pae* is primarily known for causing bleeding canker on European HC trees (Green et al., 2009, Green et al., 2010, McEvoy et al., 2016, Steele et al., 2010, Webber et al., 2008), whereby it infects the vascular system of the tree, leading to characteristic bleeding lesions on the trunk. While *E-Pae* affects the trunk and branches, foliar infection of *Aesculus* species has also been reported under experimental conditions (Mullett & Webber, 2013), demonstrating that leaves can serve as an additional site of infection. Our pathogenicity assays confirmed that E-*Pae* is virulent in HC leaf and woody tissue. Necrotic and dark brown lesions developed in leaves within 48 hours, along with tissue collapse. Pathogenicity assays

on HC woody tissue consistently produced the characteristic symptoms of bleeding cankers, including dark, sticky exudates and discoloured, sunken lesions, similar to symptoms observed in natural infections. The *hopAB1* effector and *hrpL* alternative sigma factor are known to be critical pathogenicity factors for many *P. syringae* strains (Jackson et al., 1999), though *hopAB1* can also be recognised by some hosts to trigger immunity (Hulin et al. 2018). Inactivation of these genes in E-*Pae* showed they are essential to cause disease in HC, showing loss of symptoms and growth. Inactivation of the *scr* genes reduced *E-Pae* symptoms in leaves and wood. The *scrY* mutant showed minimal chlorosis and necrosis in HC leaves and failed to produce bleeding lesions in woody tissue. In contrast, the *scrB* mutant caused necrotic symptoms in leaves that were more severe than E-*Pae* 2250 but showed reduced symptoms in woody tissue. These findings highlight the importance of *scrB* and *scrY* for causing full virulence symptoms in woody tissue and *scrY* in leaves, but are not essential factors for growth, like *hopAB1* and *hrpL* (Supplementary Figure 5). The observation that the *scrB* mutant displayed more severe symptoms in leaves might point to a niche-specific recognition of the pathogen via *scrB* in constraining full virulence symptoms. The reduced fitness observed in the *scrB* and *scrY* mutants on shoots, but not on leaves, suggests that these genes may be more critical for establishing infection in woody tissues. The enhanced disease symptoms of the *scrB* mutant in leaf tissue raises intriguing questions about the role of sucrose metabolism in different infection sites and reinforces recent observations that bacterial pathogens utilise distinct mechanisms to colonise different plant niches (Vadillo-Dieguez et al., 2024).

Experimental procedures

Bacterial strain, plasmids, and growth conditions

The bacterial strains and plasmids used in this study are described in Table 1. *Pseudomonas syringae* strains were grown at 27°C in lysogeny broth (LB) (Bertani, 1951) and King's medium

B (KB) (King et al., 1954). *Escherichia coli* strains were grown in LB medium at 37°C. When required, the medium was supplemented with the following: gentamycin (Gm) (25 μg mL^{-1}), kanamycin (Km) (50 μg mL^{-1}), and ampicillin (Ap) (100 μg mL^{-1}). The pCR2.1 vector was used for the insertional mutagenesis experiments, containing a gene for kanamycin resistance. The broad-host-range plasmid pBBR1MCS-5, was used for complementation of the knockout mutants. For the sucrose utilization assay, minimal medium (M9) (Stadtman, 1957) was used, with sucrose as the sole carbon source.

DNA techniques for mutagenesis and analysis

Chromosomal DNA of E-*Pae* 2250 was isolated using a PureLink Genomic DNA Kit (Invitrogen, UK) according to the manufacturer's instructions. The concentration and the purity of the genomic DNA were assessed using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, UK), with further quantification performed using a Qubit fluorometer (Invitrogen, UK), for greater accuracy. PCR reactions were prepared using 2X Q5 high-fidelity master mix (NEB, UK), with 10 μM forward primer, 10 μM reverse primer, 100 ng template DNA, nuclease-free water. The primers used for gene deletion and complementation are listed in Table 2. The PCR amplification program included denaturation at 98 °C for 30 sec, followed by 35 cycles of 98 °C for 10 sec (denaturation step), *50–72°C (annealing, optimized using the NEB Tm Calculator), and 72 °C for 20–30 seconds per 1kb (elongation step) and final elongation at 72 °C for 2 min. After verifying the PCR product size by gel electrophoresis, the products were purified using either the Qiaquick PCR purification kit (Qiagen, UK) or the Monarch® Genomic DNA Purification Kit (NEB, UK). Colony PCR, performed with 2X GoTaq Green Master Mix (Promega, UK), was used to confirm the presence or absence of insert DNA in the plasmid constructs and knockout and complemented strains. The PCR conditions for this master mix included denaturation at 95 °C for 5 min, followed by 35 cycles of, 95 °C for 30 sec

(denaturation step), *50–72°C (annealing step), and 72 °C for 1 minute/kb (elongation step) and final elongation at 72 °C for 5 min.

Phylogenomic analysis of *Pseudomonas syringae* sucrose-gene cluster

For the phylogenomic analysis of the *P. syringae* sucrose-gene cluster, a pangenome-based approach was used to infer phylogenetic relationships among 206 strains within the *P. syringae* species complex. Whole-genome data for these strains were retrieved from NCBI, and non-annotated genomes were processed using Prokka with default parameters (Seemann, 2014). The annotated GFF files were then used as input for Panaroo, applying the strict stringency mode to identify core genes across isolates (Tonkin-Hill et al., 2020). Subsequently, IQ-TREE (Nguyen et al., 2015) was used to estimate maximum-likelihood phylogenies based on the core gene alignment obtained from Panaroo. The tree was constructed using ModelFinder to determine the best-fit substitution model, with 1,000 ultrafast bootstrap replicates (-m MFP -b 1000 -alrt 100) to ensure robustness. The resulting tree was visualized using iTOL v6 (Letunic and Bork, 2024), where multiple annotation features were applied to highlight the presence and absence of the sucrose-gene cluster across phylogroups. Our initial hypothesis was that the presence of the *scr* gene cluster might correlate with specific ecological or host associations, particularly among strains infecting woody hosts. To explore this, we annotated each strain in the dataset with metadata on isolation source, including host type (woody vs. non-woody), host tissue (leaf, stem, fruit), and environmental reservoirs (water, soil). These metadata are provided in Supplementary Table S1, and the species tree was correspondingly annotated in Figure 1C to indicate the presence or absence of the *scr* cluster alongside host type.

To assess the similarity of the sucrose gene cluster in E-*Pae* 2250 with other *Pseudomonas* strains, a MEGABLAST analysis was performed using the NCBI database, followed by multiple nucleotide and protein sequence alignments using MUSCLE (Geneious). Heatmaps and similarity matrices were automatically generated to compare nucleotide sequence identities across different strains. The E-*Pae* sucrose gene cluster was used to search for homologous gene sequences across the pangenome using Blastn, with a minimum coverage threshold of 70% and identity threshold of 80% to identify positive matches. For a strain to be classified as possessing the sucrose gene cluster, all seven genes were required to be present and located within a single genomic region, maintaining their predicted orientation and proximity (Figure 1A).

Construction of gene knockout vectors for E-*Pae* 2250

To construct *scrB*, *scrY*, *hopAB1* and *hrpL* mutants, an insertional inactivation strategy based on the principle of homologous recombination was used (Rozhdestvenskaya et al., 2010). All strains, vectors, and plasmid constructs used in this study are detailed in Table 3. Knockout mutants of E-*Pae* 2250 were constructed as follows: for each knockout vector, two primers (Table 4) were designed to amplify most of the gene length using the whole genome sequence of E-*Pae* 2250. Two external restriction enzyme sequences matching multiple cloning sites of pCR2.1 vector were also added to the primer sequences (Table 4). Prior to ligation, each of the insert DNA (amplified and purified as described above) and the vector were digested separately using the following recipe: 1µg DNA, 10X compatible NEB buffer, 20 Units each enzyme. The reaction mix was made to 50 µL with nuclease free water and incubated for 1 hour at 37°C. Products from the digest releasing a fragment at the expected size were purified with the Monarch PCR & DNA Cleanup Kit (NEB, UK). The pre-digested and purified insert and

vector were mixed and ligated with T4 ligase according to the following recipe: 100 ng vector, required mass insert (ng) for molar 3:1 ratio, 5X NEB ligase buffer 5U/µL T4 DNA Ligase (NEB, UK). The required mass insert was determined using the formula: required mass insert (ng) = desired insert/vector molar ratio x mass of vector (ng) x ratio of insert to vector lengths. The control reaction was prepared the same as the ligation reaction but with no insert DNA in the ligation mix. After incubation overnight at 4°C, the control and ligation reactions were purified using the Monarch PCR & DNA Cleanup Kit (NEB, UK).

Preparation of E-*Pae* 2250 competent cells

Pae electrocompetent cells were prepared following (Chuanchuen et al., 2002) protocol developed for *P. aeruginosa* and *E. coli* with minor modifications. Briefly, E-*Pae* 2250 cells were grown in 10 mL LB broth at 27°C overnight until they reached saturation (OD_{600}=1.5). Aliquots of 1 mL of the stationary phase E-*Pae* culture were transferred to pre-chilled microcentrifuge tubes sitting on ice. Cells were then harvested at room temperature by centrifugation for 30 s at approximately 13 000× g. The cell pellets were resuspended with 750 µL of 0.5M sterile ice-cold sucrose. The supernatant was removed, and the cells were washed 2 more times with 750 ml of 0.5M sterile ice-cold sucrose. The cell suspensions were kept on ice for 10 min and then centrifuged at approximately 13 000× g for 30 s at room temperature. After decanting the supernatant, the pellets were then resuspended in 200 µL 0.5M sterile ice-cold sucrose and freeze stocked until use.

Transformation of ligation reaction

The purified controls and ligation reaction were transformed into *E. coli* DH5α, a strain compatible with blue and white screening, using chemically competent cells via heat-shock

transformation following standard protocols (SAMBROCK, 1990). After transformation, a 1:10 dilution of the reaction mixture was plated, and the remaining concentrated sample was spread onto LB Km plates containing Km (50 µg/mL) and 40 mg/mL X-gal (Merck, Germany). Plates were incubated overnight at 37°C. Three to five km-resistant white colonies, indicating successful insertion, were selected from each transformation plate and grown overnight in LB broth supplemented with Km (50 µg/mL) at 37°C. Plasmid DNA was extracted from these cultures using the PureLink™ Quick Plasmid Miniprep Kit (Invitrogen). To verify the presence of correct insert, 1 µg of plasmid DNA was subjected to restriction digestion in a 20 µL reaction volume containing 10 Units of each of the appropriate restriction enzymes and a 10X NEB buffer compatible with both enzymes. The digested products were analysed by agarose gel electrophoresis to confirm the release of a fragment corresponding to the expected insert size. Candidate clones were sent for sequencing using M13F/R plasmid-specific primers (Table 4) to check for any point mutations in the insert.

Transformation of E-*Pae* 2250 cells by electroporation

Recombinant plasmid DNA was used to be electroporated into the cells of the wildtype strain E-*Pae* 2250. Electrocompetent cells of E-*Pae* 2250 were thawed on ice and 50 µL were aliquoted in sterile 1.5 mL tubes and suspended well by carefully flicking the tubes. For each gene knockout, 50 µg of recombinant plasmid DNA was added to electrocompetent cells, and the mix was placed on ice for 30 min. The transformation was then added to pre-chilled electroporation cuvettes (2 mm) (Bio-Rad, UK). The electroporation of E-*Pae* 2250 was performed on a Gene Pulser (Bio-Rad) using the following parameters: capacitance 25 µFD, voltage 2.5 kV, resistance 400 Ω as adapted by (Kámán-Tóth et al., 2018). Following electroporation, bacteria were suspended immediately with 1 mL KB medium and incubated

at 27 °C and 250 rpm for 4 h. The cells were then plated in a dilution series on KB supplemented with Km (50 µg mL^{-1}). Knockout mutants were then confirmed by PCR analysis using the conditions described for the GoTaq Green Master Mix (see above). This confirmation involved a combination of vector primers and gene-specific primers, as detailed in Table 4.

Construction of complemented strains

To complement the knockout mutants, the wildtype gene of interest was reintroduced into the mutant strain on the broad-host range pBBR1MCS-5 (Table 3). Genes were PCR amplified using primers designed to amplify the full-length gene and to facilitate its ligation into the pBBR1MCS-5 vector (Table 4). The gene of interest was PCR amplified using primers designed to amplify the full-length gene and to facilitate its ligation into the pBBR1MCS-5 vector (Table 4). PCR conditions, ligation and transformation protocols followed those used for constructing the knockout vectors. To verify the correct plasmid construct, which should harbour the full-length gene, electrocompetent knockout mutant strains were prepared as described previously. Following electroporation of the plasmid into these strains, cells were plated on KB medium supplemented with gentamicin (Gm 25 µg mL^{-1}).

Heterologous expression and site-directed mutagenesis

To assess the functional significance of mutations in the *scrB* gene on sucrose utilization, we used both heterologous expression and site-directed mutagenesis (SDM) approaches. These targeted substitutions were based on alignment data with *Psm* R15244, which highlighted polymorphisms that could influence the protein's function (data not shown). For heterologous expression we introduced *scrB* alleles from *Psm* R15244 and *Pph* 1448A into a *scrB* knockout mutant of E-*Pae*2250 (E-*Pae*2250::*scrB*) to generate the strains E-

460 *Pae*::*scrB*[pBBR1-*scrB*$_{Psm}$] and E-*Pae*::*scrB*[pBBR1-*scrB*$_{Pph}$], respectively (Table 3).
461 Complementation and SDM were performed using the broad-host-range plasmid pBBR1MCS-
462 5 (Gm^{R}). Specific primers were designed to amplify the *scrB* gene from each strain (Table 4).

463 For SDM, specific nucleotide substitutions were introduced into the *scrB* gene at positions
464 803 and 1408, corresponding to amino acid changes at the active site, N-terminal, and C-
465 terminal regions, respectively. To introduce specific nucleotide substitutions in the *scrB* gene
466 for SDM, primers were designed using the NEBaseChanger™ tool. For each mutation, the
467 forward primer included the desired nucleotide change(s) at the centre, with at least 10
468 complementary nucleotides on the 3´ side of the mutation to ensure proper binding. The
469 reverse primer was designed as the reverse complement of the region surrounding the
470 mutation site, allowing the 5´ ends of the two primers to anneal back-to-back (Table 4). For
471 SDM, we used the Q5® Site-Directed Mutagenesis Kit (E0554) (New England BioLabs), where
472 the Kinase, Ligase and DpnI (KLD) treatment was followed according to the manufacturer's
473 instructions (New England BioLabs). This involved PCR amplification of the target gene with
474 mutagenic primers, phosphorylation, ligation, and DpnI digestion to degrade template DNA,
475 facilitating the creation of the desired mutations within the *scrB* gene. After confirming the
476 SDM plasmid constructs (pBBR1 MCS-5) with single mutations, we introduced these plasmids
477 into the *scrB* knockout mutant strain E-*Pae*2250::*scrB*. This resulted in the generation of the
478 strains E-*Pae*::*scrB*[pBBR1-*scrB*$_{\text{E-}Pae2250(803)}$] and E-*Pae*::*scrB*[pBBR1-*scrB*$_{\text{E-}Pae2250(1408)}$] (Table 3)
479 each harbouring a specific nucleotide substitution at one of the targeted positions within the
480 *scrB* gene. PCR amplification, ligation, transformation, and electroporation into the E-
481 *Pae*2250::*scrB* knockout mutant followed the protocols described above. All generated
482 strains, including those for heterologous expression and SDM, were assessed for sucrose
483 utilization as described above for other strains.

Sucrose assay

For the functional analysis of *scrB*, and *scrY* genes, wildtype, knockout mutants, complemented strains, and empty vectors controls were grown overnight in LB medium. Km (50 mg mL^{-1}) and/or Gm (25 mg mL^{-1}) were added to the medium when appropriate. After 24h of incubation at 28°C supernatant, the bacterial cultures were centrifuged, and the supernatant was removed. The cells were washed two to three times with M9 medium and resuspended in M9 minimal medium to an optical density (OD_{600}) of 0.2, corresponding to $2x10^8$ CFU mL^{-1}. Growth experiments were conducted in liquid LB medium or liquid M9 minimal medium, supplemented with either 10 mM or 50 mM sucrose as carbon and energy sources. One hundred microliter of bacterial culture and 100 µl of each medium were aliquoted into wells of a Greiner 96-well flat-bottomed plate (Thermo Fisher Scientific). Growth was monitored by measuring OD600 every 30 min for 48h, with 10 seconds shaking period before each measurement, using the TECAN SPARK® Multimode Microplate Reader (Thermo Fisher Scientific). Each sample was replicated three times. To assess bacterial growth kinetics, doubling times were calculated using GraphPad Prism 10. Growth data were collected at regular intervals and plotted against time. The logarithm of the population was used as the Y variable, and time was used as the X variable. Nonlinear regression was performed using the "Log of exponential growth" model from the growth equations panel in GraphPad Prism. This model fits the equation: $Y=\log Y_0+k*X$, where $\log Y_0$ is the starting population in logarithmic units, and k is the rate constant. Doubling time (td) was calculated as $\ln(2)/k$.

Pathogenicity assays:

To study the implications of the *hopAB1*, *hrpL*, *scrB*, and *ScrY* sucrose genes on E-*Pae* 2250 virulence, pathogenicity, and colonization, assays were performed on leaves and shoots of HC. Infection of detached HC leaves was followed using the protocol of (Ruinelli et al., 2019). Leaves from wild European HC trees were freshly collected, surface-disinfected, and air-dried under a sterile flow bench. Dormant one-year HC shoots were collected and cut to approximately 15 cm in length. These were stored overnight in a cold, moist environment until inoculation. Five shoots per strain per time point were thoroughly disinfected with 70% ethanol for 5 minutes and allowed to air dry. For inoculation, bacteria were cultured overnight (18 hours) on LB medium (supplemented with antibiotics when necessary) at 28°C with shaking at 200 rpm. The bacterial culture was then centrifuged at 3500 x g for 5 minutes, and the resulting pellet was washed twice with sterile PBS. The pellet was resuspended in sterile PBS and adjusted to an optical density (OD600) of 0.2, corresponding to approximately 10^8 CFU mL^{-1}. HC leaves were infiltrated with 50 µL of bacterial suspension using a 1 mL needle-less hypodermic syringe (Fisher Scientific, UK), injecting into the intracellular spaces of the primary leaves. Sterile PBS was used as a negative control. Inoculated HC leaves were placed into self-sealed clear plastic boxes lined with Parafilm and incubated at 25°C for one week under daylight conditions. Symptoms on leaves were assessed and recorded every 2 days post-inoculation and scored as follows: 0 = no symptom, 1 = yellowing, 2 = increased yellowing/chlorosis, and 3 = strong tissue necrosis. A strain was considered pathogenic if it caused a clear brownish necrotic spot at the site of infiltration. For the HC shoot bioassay, inoculation was performed by making a cut wound and applying 50 µL of the bacterial suspension to the expanding leading shoot, approximately 1–2 cm above the previous year's terminal bud scar. The wounds were sealed with Parafilm, and the shoots were placed into sterile glass test tubes and incubated at 17°C for five weeks. Lesion development and disease

severity were scored weekly based on observed lesion characteristics. Disease severity on inoculated HC shoots was scored on a scale from 0 to 5, where 0 = no symptoms, 1 = dry wood (no disease), 2 = browning liquid (onset of disease), 3 = necrotic lesion (intermediate lesion), 4 = browning and necrosis (advanced disease), and 5 = red oozing liquid (progressed and severe disease).

For pathogen fitness assessment, 7-mm leaf disks were cut from the infection area every 2 days post-inoculation using Eppendorf snap caps. Four HC leaf disks were collected per strain. The disks were placed into 2 mL of sterile PBS and homogenized using a FastPrep Tissue Homogenizer (MP Biomedicals). Additionally, from inoculated HC shoots, a 1-cm section of shoot tissue was removed from the inoculated site, placed in 2 mL sterile PBS, and shaken for one hour at 180 rpm at 27°C. Twenty microliters of the homogenized leaf tissue and the shoot shake samples were diluted in 180 µL sterile PBS. Five microliters of each of the 10^{-6}, 10^{-7}, and 10^{-8} dilutions were plated onto selective media. Plates were incubated at 27°C, and colony-forming units (CFU/mL) were counted after 24 to 48 hours. Data analysis was performed using GraphPad Prism v 10.1.0 (San Diego, California USA, www.graphpad.com).

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Author Conflict of Interest Statement

The authors declare no conflicts of interest.

Compliance with Ethical Standards

This article does not involve studies with human and/or animal participants.

Data Availability statement

The whole-genome sequence of *Pseudomonas syringae* pv. *aesculi* is available in GenBank under the following accession numbers: BioProject PRJNA957756 and BioSample SAMN34263333. Whole-genome sequence (WGS) accessions, strain origins, and all related data for the phylogenomic analysis are provided in Supplementary Table 1.

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Figure legends:

Figure 1: Comparative analysis of sucrose metabolism gene clusters across *P. syringae* strains. **A**: Genomic organization of sucrose metabolism gene clusters in *E-Pae* 2250 with annotations for the specific genes and their functions including carbohydrate porin, ABC transporter substrate-binding protein, sugar ABC transporter permease, carbohydrate ABC transporter permease, phosphate ABC transporter ATP-binding protein, sucrose-6-phosphate hydrolase, LacI-family sucrose transcriptional regulator. B: InterProScan visualization of *scrB* protein with its annotated functional domains and conserved residues, where residues 268 and 470 are located in the N-terminal and the C-terminal domains, respectively. C: Phylogenetic tree of *Pseudomonas* phylogroups based on core genome alignment. The phylogenetic tree was generated from a pangenome analysis using Panaroo, focusing on core gene alignment. The maximum-likelihood phylogeny was constructed using IQ-TREE, employing the ModelFinder Plus (MFP) and AUTO commands to identify the best-fit substitution model and optimize CPU usage, respectively. Bootstrap support values were calculated with 1,000 replicates to assess

the robustness of the tree. The visualization, produced with iTOL v6, highlights the distribution of *Pseudomonas* phylogroups (PG1-PG13) with sucrose gene cluster presence (blue squares) or absence (white squares), along with bootstrap values shown by varying circle sizes. A strain without a square indicates the source of isolation is unknown.

Figure 2: Growth comparison of E-*Pae* 2250 and mutant strains on sucrose medium. **A, B**: Growth of *Pseudomonas* wildtype strains in M9 minimal media supplemented with sucrose at 10 mM and 50 mM, respectively. **C, D**: Growth of E-*Pae*2250 *scrB* (invertase) and *scrY* (sucrose porin) knockout mutants in 50 mM sucrose-supplemented media, respectively. **E**: Functional complementation of E-*Pae*2250::*scrB* knockout mutant with heterologous *scrB* alleles from *Psm* R15244 and *Pph* 1448A. **F**: Functional complementation of E-*Pae*2250::*scrB* knockout mutant with site-directed mutations introduced at the 803 bp and 1408 bp positions in the native *scrB* gene. All growth curves represent data from individual growth assays. While experiments were repeated independently with comparable trends, the data shown are from a single representative experiment. Optical Density (OD_{600}) was measured at regular 30-minutes interval, and error bars represent standard deviation from technical triplicates. A more detailed statistical analysis is provided in Supplementary Figure 2.

Figure 3. Disease progression of E-*Pae* 2250, knockout mutants, and complemented strains on HC shoots. Symptoms were recorded at weeks 1, 3, 4, and 5 post-inoculation (10^8 CFU mL^{-1}) for E-*Pae* 2250, *scrB*, *scrY*, *hrpL*, and *hopAB1* mutants, complemented strains, and empty vectors. Columns show time points, and rows correspond to bacterial strains. Symptoms included necrosis, tissue discoloration, and lesion development. This experiment was performed once with five biological replicates per strain, with each replicate corresponding to a different shoot.

Figure 4: Bacterial growth dynamics of E-*Pae*2250, knockout mutants, and complemented strains over five weeks on HC shoots. Shoot inoculations were performed with 10^8 CFU mL^{-1} bacterial suspensions. Samples were taken at weekly intervals for five weeks. Bacterial populations (CFU mL^{-1}) were plotted over time, each data point represents the mean of five biological replicates; and error bars indicate the standard error of the mean (SEM). A more detailed statistical analysis is provided in Supplementary Figure 3.

Figure 5: Pathogenicity of E-*Pae* 2250 mutant strains on HC leaves. HC leaves inoculated (10^8 CFU mL^{-1}) with E-*Pae* 2250, *scrY*, *hrpL*, *hopAB1*, and *scrB* mutants, complemented strains, empty vectors and PBS control. Symptoms were assessed every 2 days post-inoculation (dpi), for up to 10 days. E-*Pae* 2250strain caused strong necrotic symptoms, which were absent in PBS controls. Mutants *scrY*, *hrpL*, and *hopAB1* did not cause necrosis, but complementation restored symptoms similar to E-*Pae* 2250. The *scrB* mutant induced necrosis similar to E-*Pae* 2250. Photos are representative of three independent biological replicates per treatment, with each strain inoculated on three separate HC leaves.

Figure 6: Bacterial growth dynamics of E-*Pae* 2250, mutants, and complemented strains on HC leaves over 10 days. Populations (CFU mL^{-1}) were plotted over time. Mutants *scrY*, *hrpL*, and *hopAB1* showed reduced growth, while *scrB* growth was comparable to E-*Pae* 2250. Each data point represents the mean of three independent biological replicates, with error bars indicating the SEM. A more detailed statistical analysis is provided in Supplementary Figure 4.

Supporting Information legends:

Supplementary Table 1: Metadata for *Pseudomonas syringae* strains used in phylogenomic analysis, including host, phylogroup, accession number, geographic origin, collection date, tissue type, lifestyle, and host woodiness.

Supplementary Figure 1: **Growth comparison of *Pseudomonas syringae* wildtype strains under different nutritional conditions**. **A, B**: Growth of *Pseudomonas* wildtype strains in Luria-Bertani (LB) broth and M9 minimal medium, respectively. *Pph* 1448A strain was used as negative control for sucrose utilization. KB broth was used as positive control. **C, D**: Growth of *scrB* (invertase) and *scrY* (sucrose porin) mutant strains in Luria-Bertani (LB) broth, respectively. **E:** Functional complementation of *scrB* in E-*Pae*2250::*scrB* knockout mutant with heterologous *scrB* alleles from *Psm* R15244 and *Pph* 1448A, on sucrose-supplemented media at 10 mM. **F**: Functional complementation of *scrB* in E-*Pae*2250::*scrB* knockout mutant with site-directed mutations introduced at the 803 bp and 1408 bp positions in *scrB* gene, on sucrose-supplemented media at 10 mM. Bacterial growth was measured at optical density $(OD)_{600\ nm}$ and monitored every 30 min for 48 hours. Values are the mean of 3 replicates.

Supplementary Figure 2: Statistical analysis of sucrose-dependent growth in *Pseudomonas* strains and *scr* mutants in support of data in Figure 2. A, B: Growth comparisons of *Pseudomonas* wildtype strains in M9 minimal medium supplemented with 10 mM (A) and 50 mM (B) sucrose. Two-way repeated measures ANOVA revealed significant effects of strain, time, and strain × time interaction (all $p < 0.001$). Tukey's Kramer post-hoc tests at selected timepoints (1000, 2000, and 3000 min) showed that E-*Pae* 2250 grew significantly better than all other strains at 10 mM sucrose across all three timepoints ($p < 0.001$). At 50 mM sucrose, E-*Pae* 2250 also outperformed all other strains at 1000 and 2000 minutes ($p < 0.001$); at 3000 minutes, however, the difference with *Psm* R15244 was not statistically significant. C: Growth

of the *scrB* deletion mutant was significantly reduced compared to E-*Pae* 2250 at 1000 and 2000 minutes, but not at 3000 minutes. D: *scrY* mutant had significant impact only at 2000 minutes. E: Heterologous *scrB* alleles from *Pph* and *Psm* failed to restore growth significantly compared to the deletion mutant, indicating no statistically significant improvement. F: Site-directed *scrB* mutant at position 803 displayed significantly impaired growth at all timepoints compared to E-*Pae* 2250, whereas the *scrB*-1408 mutant showed a significant difference only at 1000 minutes. At 1000 and 2000 minutes, these mutants did not significantly differ from the *scrB* knockout, indicating failure to complement.

Supplementary Figure 3: Statistical analysis of growth kinetics of E-*Pae*2250 and mutant strains in inoculated HC shoots at weeks 1, 3, and 5 post-inoculation in support of data in Figure 4. Two-way repeated measures ANOVA revealed significant effects of strain type, time, and a significant strain × time interaction (all $p < 0.0001$), indicating differential colonization dynamics among E-*Pae* 2250, mutants, complemented strains, and empty vector controls. Tukey Kramer post-hoc test was used to compare growth between strains at each timepoint. One-way ANOVA also confirmed overall differences in strain performance across all timepoints.

Supplementary Figure 4: Two-way ANOVA of E-*Pae*2250 and mutant strains growth in inoculated HC leaves in support of data in Figure 6. In addition, a one-way ANOVA with Tukey Kramer's post-hoc test was used to compare each mutant and complemented strain directly to E-*Pae* 2250 at days 2 and 10 post-inoculation. At day 2, significant increases in bacterial population were observed for complemented strains of *scrB*, *hrpL*, and *hopAB1* (all $p < 0.0001$), relative to E-*Pae* 2250. *scrB* mutant did not differ significantly, while *hrpL* and *hopAB1* mutants showed significantly lower populations ($p < 0.0001$). Empty vector controls also

showed lower populations. At day 10, the *scrB* mutant is not different than the WT, while the *scrY* mutant showed significantly lower populations. Complementation of *scrB* showed a significant decrease in growth ($p < 0.0001$). The *hrpL* and *hopAB1* mutants still displayed significantly lower growth ($p < 0.0001$), and complemented strains were slightly different from E-*Pae* 2250 ($p = 0.0444$).

Supplementary Figure 5: Comparative fitness of E-*Pae* 2250, mutants, and complemented strains in shoot and leaf tissues over time. Colony-forming units (CFU) of E-*Pae* 2250, *hrpL*, *hopAB1*, *scrB*, and *scrY* mutants were quantified in HC shoot and leaf tissues over multiple time points to assess competitive fitness. A, B display CFU counts in shoots (left) and leaves (right) for *hrpL* and *hopAB1* mutants relative to the wt. C, D show CFU counts for *scrB* and s*crY* mutants compared to E-*Pae* 2250 in shoots (left) and leaves (right).