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SHORT COMMUNICATION

Putative transcription antiterminator RfaH contributes to *Erwinia amylovora* virulence

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Abstract

The gram-negative bacterium *Erwinia amylovora* causes fire blight disease of apple and pear trees. The exopolysaccharide amylovoran and lipopolysaccharides are essential *E. amylovora* virulence factors. Production of amylovoran and lipopolysaccharide is specified in part by genes that are members of long operons. Here, we show that full virulence of *E. amylovora* in apple fruitlets and tree shoots depends on the predicted transcription antiterminator RfaH. RfaH reduces pausing in the production of long transcripts having an operon polarity suppressor regulatory element within their promoter region. In *E. amylovora*, only the amylovoran operon and a lipopolysaccharide operon have such regulatory elements within their promoter regions and in the correct orientation. These operons showed dramatically increased polarity in the Δ*rfaH* mutant compared to the wild type as determined by RNA sequencing. Amylovoran and lipopolysaccharide production in vitro was reduced in *rfaH* mutants compared to the wild type, which probably contributes to the *rfaH* mutant virulence phenotype. Furthermore, type VI secretion cluster 1, which contributes to *E. amylovora* virulence, showed reduced expression in Δ*rfaH* compared to the wild type, although without an increase in polarity. The data suggest that *E. amylovora* RfaH directly, specifically, and exclusively suppresses operon polarity in the amylovoran operon and a lipopolysaccharide operon.

KEYWORDS
apple, fire blight, operon, pear, type VI secretion

Fire blight is a serious necrotic disease affecting apple and pear trees that is caused by the gram-negative bacterium *Erwinia amylovora* (van der Zwet et al., 2012). *E. amylovora* uses several known virulence components to cause disease, including the type III secretion system (T3SS) to introduce bacterial proteins into the host cell, the exopolysaccharide (EPS) amylovoran, and

lipopolysaccharides (LPS) (Piqué et al., 2015). Many of the genes encoding these components are arranged in large operons (Bugert & Geider, 1995; Kim et al., 1997), yielding long, polycistronic transcriptional products.

During an ongoing screen of Tn*5* transposon mutants of wild-type *E. amylovora* strain HKN06P1 (Lee et al., 2010; Table S1) for mutants defective in virulence, two independent mutations were identified in the putative transcriptional antiterminator gene *rfaH*. The two *rfaH* mutants have transposon insertions at base positions 296 and 474 of the 498 bp *rfaH* open reading frame, disrupting codons 99 and 158 of 166 predicted codons, respectively (Figure 1a). These mutants were named *rfaH*Tn*5*-296 and *rfaH*Tn*5*-474 (Figure 1a). Subsequently, a deletion of the open reading frame of *rfaH* was constructed (Δ*rfaH*; Figure 1a). All three *rfaH* mutants displayed dramatically attenuated fire blight symptom development in an immature apple fruitlet assay (Klee et al., 2019) (Figure 1b). The *rfaH* mutants grew as well as the wild type in M9 minimal medium with sorbitol (Figure S1), indicating that *rfaH* disruption did not affect bacterial multiplication or primary metabolism. A copy of *rfaH* (Figure 1a) cloned into cosmid vector pCE (Figure S2, and Tables S1 and S2) restored the ability of the three *rfaH* mutants to cause oozing and necrosis in apple fruitlets (Figures 1c and S3). Fire blight symptoms caused by *rfaH*Tn*5*-474 in apple tree branches inoculated by shoot tip wounding (Klee et al., 2020) were significantly reduced and delayed compared to the wild type (Figure 1d). At each time point, the complementation strain caused significantly more severe disease than the *rfaH* mutant. Together, these results indicate that *rfaH* is essential for full virulence of *E. amylovora*.

In prokaryotes, the uncoupling of transcription and translation can lead to rho-dependent termination of transcripts, particularly in long polycistronic mRNAs. This causes operon polarity, in which promoter-distal genes have decreased expression compared to

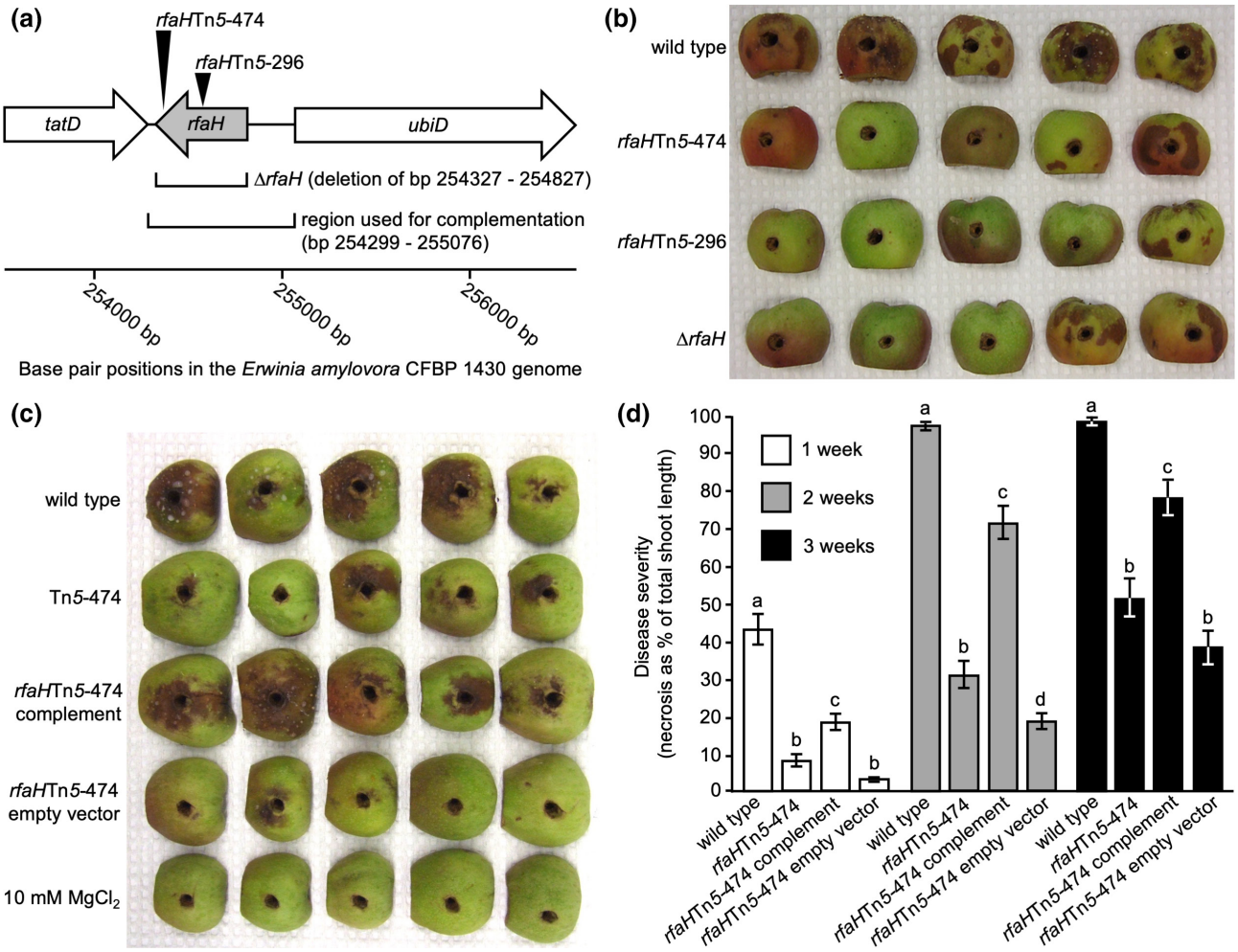

FIGURE 1 The *rfaH* gene is essential for full virulence of *Erwinia amylovora*. (a) Genomic position and context of *rfaH* in the CFBP 1430 reference genome, with Tn*5* transposon insertion locations, Δ*rfaH* deletion region, and complementation region indicated. bp, base pairs. (b) Disease symptoms on Gala apple fruitlets 1 week after inoculation with 2×10^6 colony-forming units (cfu) of the indicated *E. amylovora* strains. (c) Disease symptoms on gala apple fruitlets 1 week after inoculation with 2×10^6 cfu of the indicated strains. (d) Disease severity on greenhouse-grown Gala apple tree shoots at 1, 2 and 3 weeks postinoculation. At each time point, bars not sharing a letter have a statistically significant difference as determined by analysis of variance with post hoc Tukey tests; $p \leq 0.01$, $n = 20$ shoots per strain. For all plots, error bars indicate standard error. Each assay was performed at least twice with the same result.

promoter-proximal genes (Bailey et al., 1996). Long operons responsible for the synthesis of EPS, LPS, haemolysins and sex factors may have a short, conserved sequence called an operon polarity suppressor (*ops*) in the 5′ untranslated region (5′ UTR) (Bailey et al., 1997; Nagy et al., 2002; Nieto et al., 1996). *ops* mutations trigger increased operon polarity, and overexpression of RfaH suppresses this effect (Bailey et al., 1996). RfaH reduces pausing at both rho-dependent and rho-independent termination sites (Belogurov et al., 2009) during its interaction with the transcription elongation complex paused at *ops* sequences on the nontemplate strand of DNA (Artsimovitch & Landick, 2002).

RfaH is a paralog of the universal transcriptional regulator NusG. RfaH successfully competes with the greater abundance of NusG by binding more tightly than NusG to RNA polymerase (Wang & Artsimovitch, 2021). RfaH-modified RNA polymerases move at a rate about three times faster than unmodified polymerases and may act to overcome the natural pauses in transcribing long operons (Artsimovitch & Landick, 2002). The bulk of current knowledge about the *ops* and *rfaH* function comes from studies with animal pathogens such as *Escherichia coli* and *Salmonella enterica*.

Geneious Prime was used to search for all permutations of the *ops* consensus sequence GGCGGTAGnnTG (Artsimovitch & Landick, 2000) in a draft genome of wild-type *E. amylovora* strain HKN06P1 (unpublished data); the identical candidates were also present in the *E. amylovora* CFBP 1430 reference genome (Smits et al., 2010). Only three *ops* elements were detected that were in the correct orientation relative to gene transcription and located in a predicted 5′ UTR (Table 1). These *ops* elements were upstream of the *rfb* LPS operon, the *ams* amylovoran operon, and *yhbY*, a 294 bp stand-alone gene predicted to encode ribosome assembly protein YhbY (Table 1).

To examine effects of deletion of *rfaH* on gene expression, transcriptomes of wild-type and Δ*rfaH* bacteria were compared using RNA-sequencing (RNA-Seq; Methods S1). Three independent cultures of each strain were grown to mid-log phase in amylovoran minimal medium and RNA was isolated as previously described (Klee et al., 2020). Following rRNA depletion, single-end, 75 bp RNA-Seq was performed at the Penn State Genomics Core Facility on an Illumina NextSeq 550. Differential expression analysis was performed in Geneious Prime using the DESeq2 method (Love et al., 2014).

The DESeq2 analysis of the transcriptome data identified 58 genes with a $\log_2$ fold change (LFC) >1 or <−1 and an adjusted *p* value <0.01 (Figure S4). Of these 58 genes, 52 were expressed at a lower level in Δ*rfaH* relative to the wild type, while only six were expressed at a higher level (Figure S4 and Table S3). The 58 differentially expressed genes identified in our analysis represent c.1.5% of genes in *E. amylovora*, which is in line with *rfaH*'s regulatory action on a small subset of genes in animal pathogens (Wang & Artsimovitch, 2021). Principal component analysis (PCA) of the expression data showed clear clustering of the wild-type and Δ*rfaH* samples along the first principal component (PC1; Figure S5), supporting the validity of the results. Analysis of RNA-Seq reads showed that the *rfaH* promoter was still active in Δ*rfaH* and produced a short transcript without a coding region (Figure S6).

Genes in the *ams* operon, which is involved in amylovoran production (Bugert & Geider, 1995), had reduced transcript abundance in Δ*rfaH* compared to the wild type, with LFCs ranging from −1 to −4 (Figure 2a and Table S3). Genes in the *rfb* operon, which is involved in LPS and EPS production (Klee et al., 2020), also had reduced transcript abundance in Δ*rfaH* relative to the wild type, with LFCs from −1.8 to −5.3 (Figure 2b and Table S3). In both the *rfb* and *ams* clusters, *ops*-distal genes tended to be more down-regulated than *ops*-proximal genes (Figure 2a,b). These gene expression results and the presence of *ops* elements in the promoter regions of both operons (Figure 2a,b and Table 1) are consistent with the operons' dependence on putative transcription antiterminator activity of RfaH for normal expression. Although *yhbY* has a predicted *ops* (Table 1), *yhbY* was not differentially expressed in Δ*rfaH* compared to the wild type (Table S3). Therefore, we conclude that *yhbY* expression is not directly or indirectly influenced by RfaH.

Although type VI secretion system gene cluster 1 (T6SS-1; De Maayer et al., 2011) does not have a predicted *ops*, genes in this cluster had reduced transcript abundance in Δ*rfaH* compared to the wild type across the cluster, with LFCs ranging between −1.5 and −4.8, there was no consistent pattern of increased down-regulation of distal genes relative to the genes proximate to the start of the gene cluster (Figure 2c and Table S3). T6SS-1 does not have an *ops* sequence upstream, suggesting that RfaH is not involved in polarity suppression in the T6SS-1 gene cluster. No differential gene expression between Δ*rfaH* and the wild type was detected in the other two T6SS clusters (T6SS-2 and T6SS-3; Table S3).

TABLE 1 Locations of predicted *ops* elements in relation to potential regulatory target genes

Ops location	*Ops* sequence (5′–3′)	Potential *ops* regulatory target gene or gene cluster	Gene cluster or single gene open reading frame span	Number of genes
2,296,419 to 2,296,430 [complement]	GGCGGTAGcgTG	*ams* gene cluster (amylovoran)	2,280,397 to 2,296,193 [complement]	12
2,277,972 to 2,277,983 [complement]	GGCGGTAGcgTG	*rfb* gene cluster (LPS or EPS)	2,268,192 to 2,277,963 [complement]	9
395,731 to 395,742 [complement]	GGCGGTAGaaTG	*yhbY* gene (ribosome assembly RNA-binding protein YhbY)	395,402 to 395,695 [complement]	1

Note: Nucleotide positions are in the *Erwinia amylovora* CFBP 1430 reference genome, GenBank accession FN434113.1 (Smits et al., 2010).

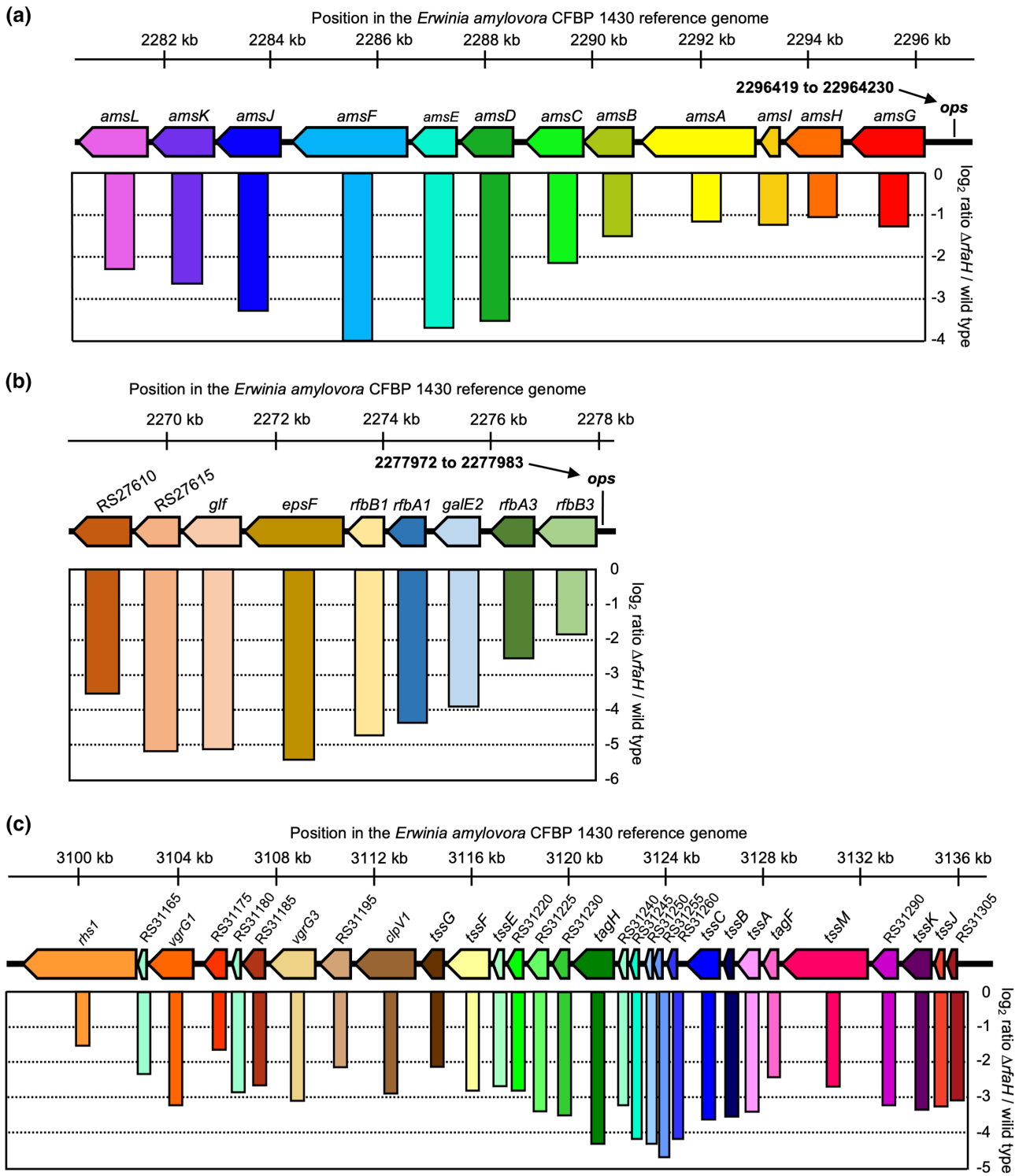

FIGURE 2 Effect of deletion of *rfaH* on expression of the *Erwinia amylovora* amylovoran *ams* and lipopolysaccharide *rfb* operons and type VI secretion system cluster 1 (T6SS-1), as determined by RNA sequencing. (a) Relative transcript abundance for *ams* operon genes in Δ*rfaH* compared to the wild type. The *ops* element position is indicated. (b) Relative transcript abundance for *rfb* operon genes in Δ*rfaH* compared to the wild type. The *ops* element position is indicated. (c) Relative transcript abundance for T6SS-1 genes in Δ*rfaH* compared to the wild type. For clarity, the commonly used *E. amylovora* locus names as given in GenBank annotation FN434113.1 are used wherever available; locus names or numbers from NCBI reference annotation NC_013961.1 are used for all other loci.

RNA-Seq coverage maps illustrate the sharp increase in polarity in the *rfb* and *ams* gene clusters in Δ*rfaH* compared to the wild type (Figure 3a). The method of Johnson et al. (2020) was used to quantify operon polarity for these as well as several other gene clusters and monocistronic transcripts for comparison (Figure 3b). Polarity is represented as a ratio of the mRNA read density at the

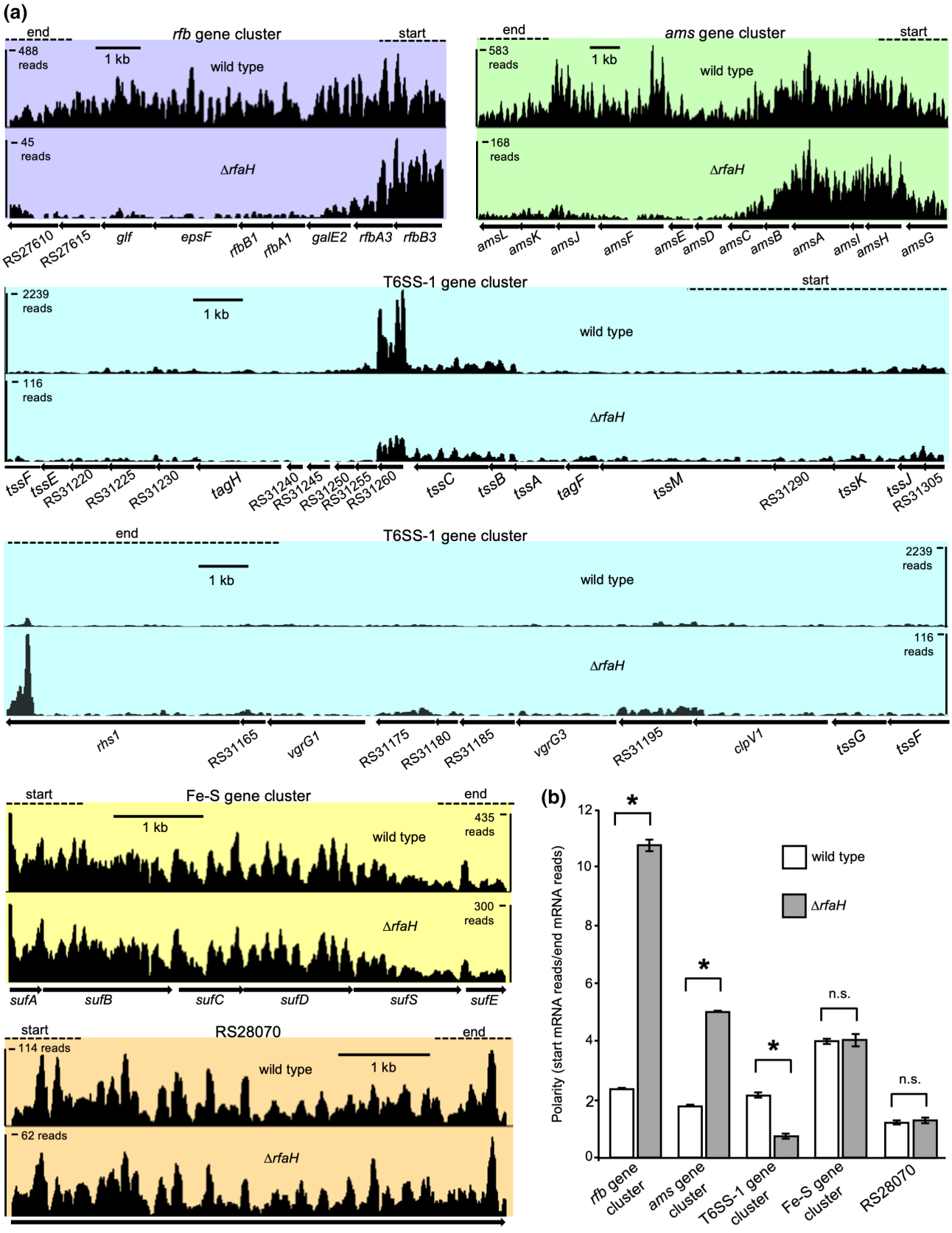

FIGURE 3 Deletion of *rfaH* exacerbates 5′ operon polarity in the *Erwinia amylovora ams* and *rfb* gene clusters. (a) RNA-Seq coverage (reads per base) maps of the indicated gene clusters in wild-type and Δ*rfaH E. amylovora*. (b) 5′ polarity of the indicated genes and gene clusters in wild-type and Δ*rfaH E. amylovora*. Operon polarity was calculated as the ratio of mRNA reads mapping to the 5′-most 15% ("start") of the gene or gene cluster to reads mapping to the 3′-most 15% ("end") gene regions indicated in panel (a). Asterisks indicate statistically significant differences by *t* test ($p \leq 0.01$, $n = 3$) comparison. n.s., not significant; kb, kilobase pairs.

beginning and end of the gene cluster, where values greater than 1 indicate 5′ polarity. While the *rfb* and *ams* gene clusters in the wild type displayed some degree of 5′ polarity, the polarity of both clusters was significantly higher in the Δ*rfaH* mutant compared to the wild type. These trends are consistent with an antiterminator role for RfaH in *rfb* and *ams* gene cluster transcription.

While the T6SS-1 gene cluster also exhibited some degree of 5′ polarity in the wild type, 5′ polarity was significantly decreased in Δ*rfaH* compared to the wild type (Figure 3b). Thus, RfaH does not appear to play an antitermination role for the T6SS-1 gene cluster, which is consistent with the lack of an *ops* element upstream of this gene cluster. The RNA-Seq coverage map for T6SS-1 showed a generally similar pattern in the wild type and Δ*rfaH*, with dramatically reduced numbers of reads across the entire T6SS-1 gene cluster in Δ*rfaH* (Figure 3a). The decreased polarity of the T6SS-1 gene cluster in Δ*rfaH* compared to the wild type is explained by the peak in RNA-Seq reads in the "end" region of the T6SS-1 cluster, which results in a substantially greater number of RNA-Seq reads in the end region compared to the "start" region (Figure 3a).

To put the polarity analysis into a wider context, polarity was analysed for one additional presumptive operon and two representative genes. The iron sulphur cluster assembly presumptive 5.7 kb operon (*sufA–sufE*) had no difference in 5′ polarity in Δ*rfaH* compared to the wild type (Figure 3). The same was true for putative helicase-encoding gene RS28070 (Figure 3) and the virulence gene *hrpN* (Wei et al., 1992; Figure S7). The *hrpN* gene was selected because of its strong baseline expression and average gene length for *E. amylovora*, while RS28070 was selected because it has a long single open reading frame of 5.5 kb. The polarity analysis quantifies the antitermination effect of RfaH on the *rfb* and *ams* gene clusters in *E. amylovora* and emphasizes the specificity of this activity.

The gene expression changes in the *ams* operon in the Δ*rfaH* mutant compared to the wild type (Figure 2a) suggest that amylovoran production might be affected by loss of *rfaH*. Amylovoran levels were indeed significantly lower in the Δ*rfaH* mutant compared to the wild type (Figure 4a). Because amylovoran is important for *E. amylovora* virulence (Bellemann & Geider, 1992; Bennett & Billing, 1978),

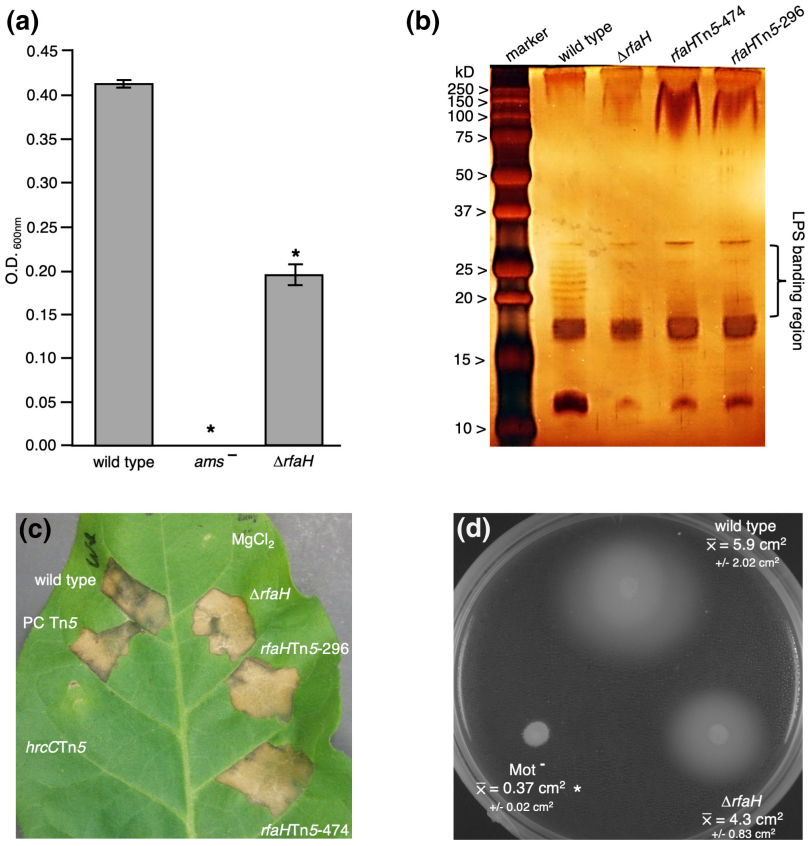

FIGURE 4 Virulence-related phenotypes of *Erwinia amylovora rfaH* mutants. (a) Amylovoran production of indicated strains after 24 h growth in amylovoran minimal medium. Asterisks indicate a statistically significant difference from the wild type by pairwise *t* tests ($p \leq 0.01$, $n = 3$). (b) Lipopolysaccharide (LPS) production by indicated strains as visualized by polyacrylamide gel electrophoresis and silver staining. (c) Hypersensitive response elicitation by indicated strains infiltrated at 1×10^8 cfu/ml into *Nicotiana tabacum* 'Glurk', photographed 48 h after infiltration. Four leaves were inoculated for this experiment, with all four leaves showing identical results. (d) Swimming motility in 0.3% Luria Bertani agar at 36 h postinoculation with indicated strains; the Mot⁻ negative control strain is a nonmotile Tn*5* mutant of HNK06P1. Average diameter of swimming area indicated with standard deviations; the asterisk indicates the statistically significant difference from the wild type by pairwise *t* test ($p \leq 0.01$, $n = 5$) comparison. Each assay was performed at least twice with the same result.

lower production of this EPS probably contributes to *rfaH* mutants' virulence defect. In contrast, the stand-alone *lsc* gene, encoding the levansucrase enzyme responsible for synthesis of the EPS levan, was not differentially expressed in the *rfaH* mutant compared to the wild type (Table S3), and levansucrase enzymatic activity was unaffected by mutation of *rfaH* (Figure S8).

LPS production was also altered in the *rfaH* mutants (Figure 4b), consistent with reduced expression of the *rfb* operon (Figure 2b). LPS contributes to bacterial survival in hosts (Kutschera & Ranf, 2019) and virulence is reduced in *E. amylovora* strains lacking O-antigen (Berry et al., 2009; Ray et al., 1986). Our results show that *rfaH* is required by *E. amylovora* for normal LPS and EPS production, as reported for gram-negative animal pathogens (Bittner et al., 2002; Garrett et al., 2016; Leskinen et al., 2015; Nagy et al., 2002).

Several genes in the T6SS-1 gene cluster result in reduced pathogenicity when deleted in *E. amylovora* (Tian et al., 2017). The significantly reduced expression of these genes in Δ*rfaH* might contribute to the virulence defect of Δ*rfaH*. In addition, altered amylovoran levels have been reported in T6SS gene deletion mutants of *E. amylovora* (Tian et al., 2017). The significantly decreased expression of these T6SS-1 genes in Δ*rfaH* compared to the wild type could potentially contribute to the reduced amylovoran production in Δ*rfaH* compared to the wild type. Deletion analysis of T6SS genes has also demonstrated their importance in *E. amylovora* competition with other bacteria (Kamber et al., 2017; Tian et al., 2017). However, we found no consistent difference in Δ*rfaH* and the wild type's ability to compete with *E. coli* during in vitro qualitative (Figure S9) and quantitative (Figure S10) competition assays (Decoin et al., 2015; Tian et al., 2017). It might be expected that T6SS gene deletions would result in stronger phenotypic changes than the relatively reduced gene expression across the T6SS-1 cluster we detected in Δ*rfaH*.

E. amylovora causes a T3SS-dependent hypersensitive response (HR) when infiltrated into leaves of nonhost tobacco (*Nicotiana tabacum*). While several long presumptive operons are involved in *E. amylovora* T3SS production (Kim et al., 1997; Oh et al., 2005), no *ops* elements reside upstream of these operons, predicting that *rfaH* is not involved in their regulation. This was confirmed by the RNA-Seq data, which showed that transcript abundance of T3SS genes did not differ between Δ*rfaH* and the wild type (Table S3). Consistent with this finding, *rfaH* mutants triggered an HR in tobacco leaves, as did the wild type (Figure 4c).

Swimming motility contributes to *E. amylovora* virulence in natural infections (Bayot & Ries, 1986), and reduced swimming motility due to lack of antiterminator activity has been observed in other pathogens, such as *S. enterica* (Kong et al., 2009). While several predicted swimming motility-associated genes, including *flgB1*, *flgC1* and *flgD1*, had lower transcript abundance in Δ*rfaH* compared to the wild type, the data did not meet our fold-change cut-off (Table S3). Δ*rfaH* consistently showed a slight, though not statistically significant, reduction in swimming motility (Figure 4d). Thus, decreased expression of certain flagellin genes in Δ*rfaH* was insufficient to have a major impact on swimming motility. Because *ops* elements were not found upstream of these motility-associated genes, the relatively minor effects on motility gene expression and phenotype appear to be indirect results of *rfaH* deletion.

Our results support the conclusion that the *rfaH* gene in *E. amylovora* encodes a transcriptional antiterminator, as predicted by the genome annotation. We also conclude that *E. amylovora* RfaH directly and specifically influences the transcription of the *ams* and *rfb* operons in *E. amylovora* to facilitate normal amylovoran and LPS production and full virulence. We surmise that RfaH effects on T6SS-1 expression are indirect, possibly resulting from the alterations in amylovoran and LPS in Δ*rfaH*. Interestingly, while other long operons and gene clusters are required for *E. amylovora* virulence, including T3SS gene clusters, these do not appear to require the antiterminator activity of RfaH for normal expression. Our data indicate that RfaH transcription antitermination influence is restricted to the *ams* and *rfb* operons in *E. amylovora*. The substantially attenuated virulence of *E. amylovora rfaH* mutants is consistent with similar findings for *rfaH* mutants in animal pathogens including *S. enterica*, *K. pneumoniae*, and *E. coli* (Bachman et al., 2015; Gao et al., 2013; Nagy et al., 2006). While an *ops* element was noted upstream of a putative EPS operon in *Dickeya dadantii* (*Erwinia chrysanthemi*; Condemine et al., 1999), we have not discovered any other examinations of RfaH function in plant pathogens.

AUTHOR CONTRIBUTIONS

S.M.K., J.P.S., J.H., C.V., A.C.H., B.L.L. and K.A.P. planned and performed experiments and collected data. T.W.M. designed and coordinated the study. S.M.K., J.P.S., J.H. and T.W.M. wrote the manuscript. All the co-authors reviewed, edited and approved the manuscript.

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DATA AVAILABILITY STATEMENT

The RNA-seq data will be deposited in a MIAME-compliant database upon acceptance for publication.

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REFERENCES

Artsimovitch, I. & Landick, R. (2000) Pausing by bacterial RNA polymerase is mediated by mechanistically distinct classes of signals. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 7090–7095.

Artsimovitch, I. & Landick, R. (2002) The transcriptional regulator RfaH stimulates RNA chain synthesis after recruitment to elongation complexes by the exposed nontemplate DNA strand. *Cell*, 109, 193–203.

Bachman, M.A., Breen, P., Deornellas, V., Mu, Q., Zhao, L., Wu, W. et al. (2015) Genome-wide identification of *Klebsiella pneumoniae* fitness genes during lung infection. *mBio*, 6, e00775.

Bailey, M.J.A., Hughes, C. & Koronakis, V. (1996) Increased distal gene transcription by the elongation factor RfaH, a specialized homologue of NusG. *Molecular Microbiology*, 22, 729–737.

Bailey, M.J.A., Hughes, C. & Koronakis, V. (1997) RfaH and the *ops* element, components of a novel system controlling bacterial transcription elongation. *Molecular Microbiology*, 26, 845–851.

Bauer, D.W. (1990) *Molecular genetics of pathogenicity of Erwinia amylovora: techniques, tools and their application*. PhD thesis. Ithaca, NY: Cornell University.

Bayot, R.G. & Ries, S.M. (1986) Role of motility in apple blossom infection by *Erwinia amylovora* and studies of fire blight control with attractant and repellent compounds. *Phytopathology*, 76, 441–445.

Bellemann, P. & Geider, K. (1992) Localization of transposon insertions in pathogenicity mutants of *Erwinia amylovora* and their biochemical characterization. *Microbiology*, 138, 931–940.

Belogurov, G.A., Mooney, R.A., Svetlov, V., Landick, R. & Artsimovitch, I. (2009) Functional specialization of transcription elongation factors. *EMBO Journal*, 28, 112–122.

Bennett, R.A. & Billing, E. (1978) Capsulation and virulence in *Erwinia amylovora*. *Annals of Applied Biology*, 89, 41–45.

Berry, M.C., McGhee, G.C., Zhao, Y. & Sundin, G.W. (2009) Effect of a *waaL* mutation on lipopolysaccharide composition, oxidative stress survival, and virulence in *Erwinia amylovora*. *FEMS Microbiology Letters*, 291, 80–87.

Bittner, M., Saldías, S., Estévez, C., Zaldívar, M., Marolda, C.L., Valvano, M.A. et al. (2002) O-antigen expression in *Salmonella enterica* serovar typhi is regulated by nitrogen availability through RpoN-mediated transcriptional control of the *rfaH* gene. *Microbiology*, 148, 3789–3799.

Bugert, P. & Geider, K. (1995) Molecular analysis of the *ams* operon required for exopolysaccharide synthesis of *Erwinia amylovora*. *Molecular Microbiology*, 15, 917–933.

Condemine, G., Castillo, A., Passeri, F. & Enard, C. (1999) The PecT repressor coregulates synthesis of exopolysaccharides and virulence factors in *Erwinia chrysanthemi*. *Molecular Plant-Microbe Interactions*, 12, 45–52.

De Maayer, P., Venter, S.N., Kamber, T., Duffy, B., Coutinho, T.A. & Smits, T.H.M. (2011) Comparative genomics of the type VI secretion systems of *Pantoea* and *Erwinia* species reveals the presence of putative effector islands that may be translocated by the VgrG and Hcp proteins. *BMC Genomics*, 12, 576.

Decoin, V., Gallique, M., Barbey, C., Le Mauff, F., Duclairoir-Poc, C., Feuilloley, M.G.J. et al. (2015) A *Pseudomonas fluorescens* type 6 secretion system is related to mucoidy, motility and bacterial competition. *BMC Microbiology*, 15, 72.

Gao, Q., Xu, H., Wang, X., Zhang, D., Ye, Z., Gao, S. et al. (2013) RfaH promotes the ability of the avian pathogenic *Escherichia coli* O2 strain E058 to cause avian colibacillosis. *Journal of Bacteriology*, 195, 2474–2480.

Garrett, S.B., Garrison-Schilling, K.L., Cooke, J.T. & Pettis, G.S. (2016) Capsular polysaccharide production and serum survival of *Vibrio vulnificus* are dependent on antitermination control by RfaH. *FEBS Letters*, 590, 4564–4572.

Johnson, G.E., Lalanne, J.-B., Peters, M.L. & Li, G.-W. (2020) Functionally uncoupled transcription-translation in *Bacillus subtilis*. *Nature*, 585, 124–128.

Kamber, T., Pothier, J.F., Pelludat, C., Rezzonico, F., Duffy, B. & Smits, T.H.M. (2017) Role of the type VI secretion systems during disease interactions of *Erwinia amylovora* with its plant host. *BMC Genomics*, 18, 628.

Kim, J.F., Wei, Z.M. & Beer, S.V. (1997) The *hrpA* and *hrpC* operons of *Erwinia amylovora* encode components of a type III pathway that secretes harpin. *Journal of Bacteriology*, 179, 1690–1697.

Klee, S.M., Sinn, J.P. & McNellis, T.W. (2019) The apple fruitlet model system for fire blight disease. In: Gassmann, W. (Ed.) *Methods in molecular biology, 1991*. New York, N.Y: Humana, pp. 187–198.

Klee, S.M., Sinn, J.P., Christian, E., Holmes, A.C., Zhao, K., Lehman, B.L. et al. (2020) Virulence genetics of an *Erwinia amylovora* putative polysaccharide transporter family member. *Journal of Bacteriology*, 202, e00390-20.

Kong, Q., Liu, Q., Roland, K.L. & Curtiss, R., 3rd. (2009) Regulated delayed expression of *rfaH* in an attenuated *Salmonella enterica* serovar typhimurium vaccine enhances immunogenicity of outer membrane proteins and a heterologous antigen. *Infection and Immunity*, 77, 5572–5582.

Kutschera, A. & Ranf, S. (2019) The multifaceted functions of lipopolysaccharide in plant-bacteria interactions. *Biochimie*, 159, 93–98.

Lee, S.A., Ngugi, H.K., Halbrendt, N.O., O'Keefe, G., Lehman, B., Travis, J.W. et al. (2010) Virulence characteristics accounting for fire blight disease severity in apple trees and seedlings. *Phytopathology*, 100, 539–550.

Leskinen, K., Varjosalo, M., Li, Z., Li, C.-M. & Skurnik, M. (2015) Expression of the *Yersinia enterocolitica* O:3 LPS O-antigen and outer core gene clusters is RfaH-dependent. *Microbiology*, 161, 1282–1294.

Love, M.I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 550.

Nagy, G., Dobrindt, U., Schneider, G., Khan, A.S., Hacker, J. & Emödy, L. (2002) Loss of regulatory protein RfaH attenuates virulence of uropathogenic *Escherichia coli*. *Infection and Immunity*, 70, 4406–4413.

Nagy, G., Danino, V., Dobrindt, U., Pallen, M., Chaudhuri, R., Emödy, L. et al. (2006) Down-regulation of key virulence factors makes the *Salmonella enterica* serovar typhimurium *rfaH* mutant a promising live-attenuated vaccine candidate. *Infection and Immunity*, 74, 5914–5925.

Nieto, J.M., Bailey, M.J.A., Hughes, C. & Koronakis, V. (1996) Suppression of transcription polarity in the *Escherichia coli* haemolysin operon by a short upstream element shared by polysaccharide and DNA transfer determinants. *Molecular Microbiology*, 19, 705–713.

Oh, C.-S., Kim, J.F. & Beer, S.V. (2005) The Hrp pathogenicity Island of *Erwinia amylovora* and identification of three novel genes required for systemic infection. *Molecular Plant Pathology*, 6, 125–138.

Piqué, N., Miñana-Galbis, D., Merino, S. & Tomás, J.M. (2015) Virulence factors of *Erwinia amylovora*: a review. *International Journal of Molecular Sciences*, 16, 12836–12854.

Ray, T.C., Smith, A.R.W., Carter, K.J. & Hignett, R.C. (1986) Composition of lipopolysaccharides from four strains of *Erwinia amylovora*. *Journal of General Microbiology*, 132, 3159–3167.

Smits, T.H., Rezzonico, F., Kamber, T., Blom, J., Goesmann, A., Frey, J.E. et al. (2010) Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Molecular Plant-Microbe Interactions*, 23, 384–393.

Tian, Y., Zhao, Y., Shi, L., Cui, Z., Hu, B. & Zhao, Y. (2017) Type VI secretion systems of *Erwinia amylovora* contribute to bacterial competition, virulence, and exopolysaccharide production. *Phytopathology*, 107, 654–661.

Wang, B. & Artsimovitch, I. (2021) NusG, an ancient yet rapidly evolving transcription factor. *Frontiers in Microbiology*, 11, 619618.

Wei, Z.-M., Laby, R.J., Zumoff, C.H., Bauer, D.W., He, S.Y., Collmer, A. et al. (1992) Harpin, elicitor of the hypersensitive response produced by the plant pathogen Erwinia amylovora. *Science*, 257, 85–88.

van der Zwet, T., Orolaza-Halbrendt, N. & Zeller, W. (2012) *Fire blight. History, biology, and management*. St. Paul, MN: The American Phytopathological Society Press.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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