

1 **Supplemental material**

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3 **A horizontally acquired expansin gene increases virulence of the emerging plant**
4 **pathogen *Erwinia tracheiphila***

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7 **Running title:** *An expansin increases Erwinia tracheiphila virulence*

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9 **Data Deposition Statement:** Analysis scripts and input files associated with
10 reconstruction of phylogenetic trees are available at

11 <https://github.com/lshapiro31/gh5.expansin.phylogenetics>

12 **Supplemental Tables**

13 Supplemental Table 1. Strains and plasmids used in this study.

Strains	Description	Reference
<i>Erwinia tracheiphila</i> BHKY	<i>Erwinia tracheiphila</i> parental Wild type strain. Spontaneous rifampicin resistant. Parental strain for mutants and complemented variants.	32
<i>Erwinia tracheiphila</i> BuffGH	<i>Erwinia tracheiphila</i> Wildtype isolate, used for fluorescence visualization of bacterial cells during infection.	
$\Delta exlx-gh5$	Deletion mutant strain; <i>exlx-gh5:bla</i> genetic exchange. Ampicillin resistant.	This study
$\Delta exlx$	Deletion mutant strain; clean deletion in N terminal coding region of <i>exlx</i> .	This study
Δeng	Deletion mutant strain; <i>gh5:bla</i> genetic exchange. Ampicillin resistant.	This study
$\Delta fliC$	Deletion mutant strain; <i>fliC:bla</i> genetic exchange. Ampicillin resistant.	This study
$\Delta T4P$	Deletion mutant strain; <i>T4P:bla</i> genetic exchange. Ampicillin resistant.	This study
$\Delta exlx-gh5$ (cEXLX-GH5)	Mutant strain $\Delta exlx-gh5$, complemented with wildtype promoter and <i>exp-gh5</i> coding region, inserted in a neutral chromosomic region.	This study
$\Delta exlx$ (EXLX)	Mutant strain $\Delta exlx$, complemented with wildtype promoter and <i>exlx</i> coding region, inserted in a neutral chromosomic region.	This study
Δeng (EXLX-ENG)	Mutant strain $\Delta eng5$, complemented with wildtype promoter and <i>exlx-gh5</i> coding region, inserted in a neutral chromosomic region.	This study
<i>E. coli</i> Top10	<i>E. coli</i> strain used for cloning.	
<i>E. coli</i> PIR1	<i>E. coli</i> strain for expression of R6K replication origin.	
<i>E. coli</i> S17-1 λ	<i>E. coli</i> strain used as donor for conjugation.	
Plasmids		
pDS132	Suicide plasmid for allelic replacement.	108
pMP7605	Template for the amplification of the <i>mcherry</i> gene	109
pJR74	Derived from pDS132, <i>mcherry</i> gene inserted in the <i>Xba</i> I site.	This study
pKD46	Template for <i>bla</i> gene (ampicillin resistance cassette).	97

pJR315	Integration plasmid for <i>E. tracheiphila</i> .	This study
pJR150	Plasmid carrying the construction for gene exchange between the <i>exlx-gh5</i> operon and the <i>bla</i> gene, derived from pJR74.	This study
pJR323	Plasmid carrying the construction for <i>exlx</i> deletion, derived from pJR74.	This study
pJR324	Plasmid carrying the construction for <i>gh5::bla</i> gene exchange, derived from pJR74.	This study
pJR74a	Plasmid carrying the construction for <i>fliC:bla</i> gene exchange, derived from pJR74.	This study
pJR149	Plasmid carrying the construction for Type 4 Pili operon gene exchange with <i>bla</i> gene, derived from pJR74	This study
pJR358	Plasmid carrying the construction for <i>exlx-eng</i> operon integration into the chromosome of Et-BHKY, derived from pJR315.	This study
pJR357	Plasmid carrying the construction for <i>exlx</i> gene integration into the chromosome of Et-BHKY, derived from pJR315.	This study

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16 Supplemental Table 2. Oligonucleotides used in this study.

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Primer Name	Description and sequence (5' to 3')	Modification
<i>bla</i> cassette amplification		
LS23	acttttcggggaaatgtgc	
LS24	acgttaagggattttggta	
<i>mcherry</i> gene amplification		
JR72	tcttctagacgtttcttactgtacagctc	<i>Xba</i> I site
JR73	tcttctagaaattcttgacaattaatcatcg	<i>Xba</i> I site
<i>exlx-gh5::bla</i> exchange		
LS52 (Fwd 5')	acccgtaatgcaccagaac	
LS53 (reamplification)	gaggagctcttatttcgatgatggtttatgg	<i>Sac</i> I site
LS54 (Rev 5')	gcacatttccccgaaaagtagttaaacagcgcagatgg	LS23 tag
LS55 (Fwd 3')	tgacaaaatcccttaacgtacagaggatgccttgtaag	LS24 tag
LS57 (reamplification)	gaggagctccgcaaatcatcaccagtcag	<i>Sac</i> I site
LS56 (Rev 3')	gaggtatatcccggcctgac	
<i>exlx</i> clean deletion		
JR175 (Fwd 5')	Cagaactgacgcttacctcc	

JR176		
(reamplification)	<u>GAGGAGCTC</u> tcgcttcttatagagctgc	<i>SacI</i> site
JR194 (Rev 5')	gttgagctggttaaggactaagg gaaagtcagagccgctattG	JR195 tag
JR195 (Fwd 3')	<u>Caatagcggctctgactttcccttagtctctaccagctcaac</u>	JR194 tag
JR179		
(reamplification)	<u>GAGGAGCTC</u> atcgataacgctatccacac	<i>SacI</i> site
JR180 (Rev 3')	Gtatttcgtagccaatctctg	
	<i>gh5::bla</i> exchange	
JR181 (Fwd 5')	Gaatcaggcagacttggttc	
JR182		
(reamplification)	<u>GAGGAGCTC</u> gtgattacaacctgctttcg	<i>SacI</i> site
	GCACATTTCCCCGAAAAGT	
JR183 (Rev 5')	gcatgctgctacagataacc	LS23 tag
	TGACCAAAATCCCTTAACGT	
JR184 (Fwd 3')	gataaagcagaaggagcatc	LS24 tag
JR185		
(reamplification)	<u>GAGGAGCTC</u> ccccactgatgttatcgtc	<i>SacI</i> site
JR186 (Rev 3')	Catgctgttttttatattacctgc	
	Integration plasmid	
JR143 (Fwd 5')	<u>GAGGAGCTC</u> cttcaaaaatacgttcacacc	<i>SacI</i> site
JR144 (Rev 5')	gttcatacatggtaaacctg <u>AGATCTCTCGAG</u>	<i>XhoI</i> , <i>BglII</i>
	catttatcctgctgatctg	sites, JR145 tag
JR145 (Fwd 3')	<u>cagatcagcagggataaatg CTCGAGAGATCT</u>	<i>XhoI</i> , <i>BglII</i>
	caggtttgaccatgtatgaac	sites, JR144 tag
JR146 (Rev 3')	<u>GAGGAGCTC</u> aataccaatcaggaccacac	<i>SacI</i> site
	Complementation of <i>exlx-gh5</i> and <i>exlx</i>	
JR152 (Fwd)	CTCCTCGAGggaagatttcatcagcacc	<i>XhoI</i> site
JR153 (Rev <i>exlx</i>		
only)	CTCCTCGAGctgtcatgtcctgtattatatattgtg	<i>XhoI</i> site
JR154 (Rev <i>exlx-</i>		
<i>eng</i>)	CTCCTCGAGgacagtaccagtatcctgacg	<i>XhoI</i> site

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Supplemental Table 3. Species and sequences included in Figure 2.

Species	Location	Accession	Description
<i>Erwinia tracheiphila</i>	Chromosome	WP_046372116.1, WP_016193008.1	Expansin - GH5 locus
<i>Pantoea stewartii</i>	Plasmid pDSJ08	WP_044243227.1, WP_006122111.1	Expansin - GH5 locus
<i>Pectobacterium carotovorum</i>	Chromosome	WP_103860671.1	Single expansin domain
<i>Dickeya solani</i>	Chromosome	WP_022633560.1	Single expansin domain

<i>Dickeya zae</i>	Chromosome	WP_026358096.1	Single expansin domain
<i>Dickeya dianthicola</i>	Chromosome	WP_024104808.1, WP_103415919.1	Expansin - GH5 locus
<i>Lonsdalea quercina</i>	Chromosome	WP_026740116.1	Single expansin domain
<i>Lonsdalea iberica</i>		WP_094110348.1	Single expansin domain
<i>Lonsdalea britannica</i>	Chromosome	WP_094118757.1	Single expansin domain
<i>Brenneria salicis</i>		WP_113865163.1	Single expansin domain
<i>Pectobacterium wasabiae</i>	Chromosome	WP_005968506.1	Single expansin domain
<i>Pectobacterium betavasculorum</i>	Chromosome	WP_010275007.1	Single expansin domain
<i>Pectobacterium peruviane</i>	Chromosome	WP_113627469.1	Single expansin domain
<i>Brenneria salicis</i>	Chromosome	WP_113865163.1	Single expansin domain
<i>Xanthomonas sacchari</i>	Chromosome	WP_043094747.1	Single expansin domain
<i>Xanthomonas albilineans</i>	Chromosome	WP_045769814.1	Single expansin domain
<i>Xanthomonas translucens</i>	Chromosome	WP_003477706.1	Single expansin domain
<i>Xanthomonas arboricola</i>	Chromosome	SUZ34847.1	Single expansin domain
<i>Xanthomonas maliensis</i>	Chromosome	WP_031340658.1	Fused 1,4-beta-cellobiosidase-expansin
<i>Xylella fastidiosa</i>	Chromosome	WP_020851755.1	Fused 1,4-beta-cellobiosidase-expansin
<i>Xylella taiwanensis</i>	Chromosome	WP_038269744.1	Fused 1,4-beta-cellobiosidase-expansin
<i>Xanthomonas oryzae</i>	Chromosome	WP_131078740.1	Fused 1,4-beta-cellobiosidase-expansin
<i>Xanthomonas citri</i>	Chromosome	WP_096035367.1	Fused 1,4-beta-cellobiosidase-expansin
<i>Xanthomonas campestris</i>	Chromosome	KFA09681.1	Fused 1,4-beta-cellobiosidase-

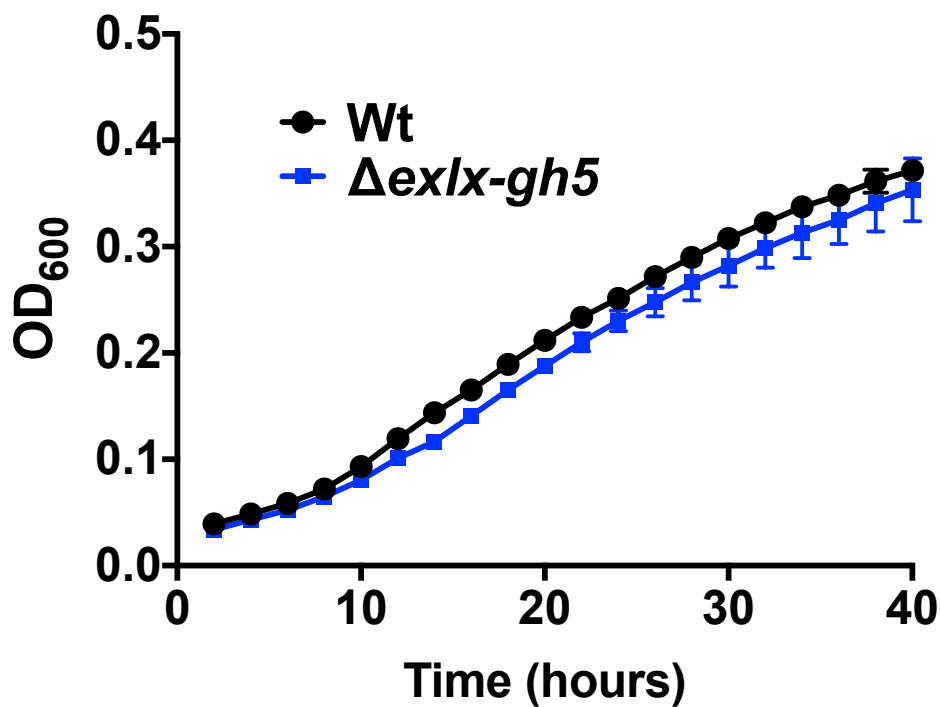
			expansin
<i>Xanthomonas phaseoli</i>	Chromosome	WP_017161821.1	Fusioned 1,4-beta-cellobiosidase-expansin
<i>Ralstonia pseudosolanacearum</i>	Chromosome	WP_120452416.1	Single expansin domain
<i>Ralstonia solanacearum</i>	Chromosome	WP_134927564.1	Single expansin domain
<i>Myxococcus virescens</i>	Chromosome	WP_090491481.1	Single expansin domain
<i>Myxococcus fulvus</i>	Chromosome	WP_046714021.1	Single expansin domain
<i>Clavibacter michiganensis</i>	Plasmid pCM1	PRJNA19643, AM711865.1	Multidomain GH5-CBMII-expansin,
	Chromosome	4JCW_A	Single expansin domain
<i>Streptomyces scabiei</i>	Chromosome	FN554889.1, WP_013005169.1	Single expansin domain
	Chromosome	CBG75940.1	Single expansin domain
<i>Streptomyces cellulosa</i>	Chromosome	WP_030660953.1	SSL4- expansin fusion
<i>Streptomyces griseus</i>	Chromosome	WP_051866281.1	Single expansin domain
<i>Streptomyces ipomoeae</i>	Chromosome	WP_009327266.1	Single expansin domain
<i>Paenibacillus xylanexedens</i>	Chromosome	WP_124115839.1	Single expansin domain
<i>Bacillus licheniformis</i>	Chromosome	WP_073425687.1	Single expansin domain
<i>Bacillus glycinifermentans</i>	Chromosome	WP_048355312.1	Single expansin domain
<i>Bacillus pumilus</i>	Chromosome	WP_024718945.1	Single expansin domain
<i>Bacillus cellulosensis</i>	Chromosome	APP16610.1	Single expansin domain
<i>Bacillus halotolerans</i>	Chromosome	WP_106021706.1	Single expansin domain
<i>Bacillus subtilis</i>	Chromosome	WP_128737925.1	Single expansin domain
<i>Acidovorax avenae</i>	Chromosome	WP_107177641.1	Fusioned CelA1 cellulase - expansin
<i>Acidovorax radialis</i>	Chromosome	WP_010459029.1	Single expansin

			domain
<i>Acidovorax citruli</i>	Chromosome	ABM33954.1	Fused CelA1 cellulase - expansin

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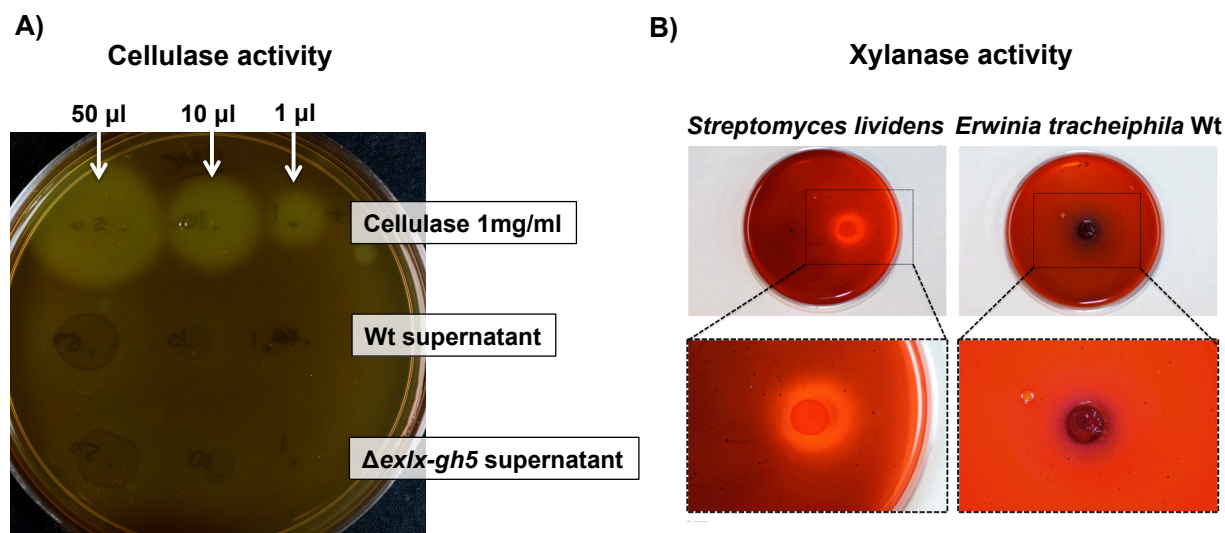
25 Supplemental Figures and Legends

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27 **Supplemental Figure 1. *In vitro* growth of Wt and Δexlx-gh5.** A single colony of Wt or Δexlx-
28 gh5 strains were picked into 3 ml of liquid Kb media and grown for 48 h at 25°C with shaking.
29 Then, 1 ml of each culture was washed once with 1 volume of PBS and diluted with fresh KB
30 media to OD₆₀₀ of 0.05. Four replicates of 300 μl were placed in a clear 96-well microplate and
31 growth was followed in these standing liquid cultures using a microplate reader, by measuring
32 absorbance at 600 nm every 2 h for 40 h at a constant temperature of 25°C. Average ± SD is
33 shown.

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37 **Supplemental Figure 2. Tests to detect *E. tracheiphila* cellulase and xylanase activity *in***38 ***vitro*. A)** Commercial cellulase and supernatants of Wt and the Δ exlx-gh5 mutant were spotted in

39 media containing agar and 1% CMC, incubated at 30°C for 48 h, and then flooded with Gram's

40 Iodine. Halos were imaged after 24 h at RT. **B)** *E. tracheiphila* and the xylan degrading species41 *Streptomyces lividens* were grown in KB agar. An overlay of 1% xylan and 1% agar was spread

42 on top of the colonies. Plates were incubated at 30°C for 48 h, and flooded with 1% Congo Red.

43 Halos were imaged and measured after 24 h at room temperature.

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