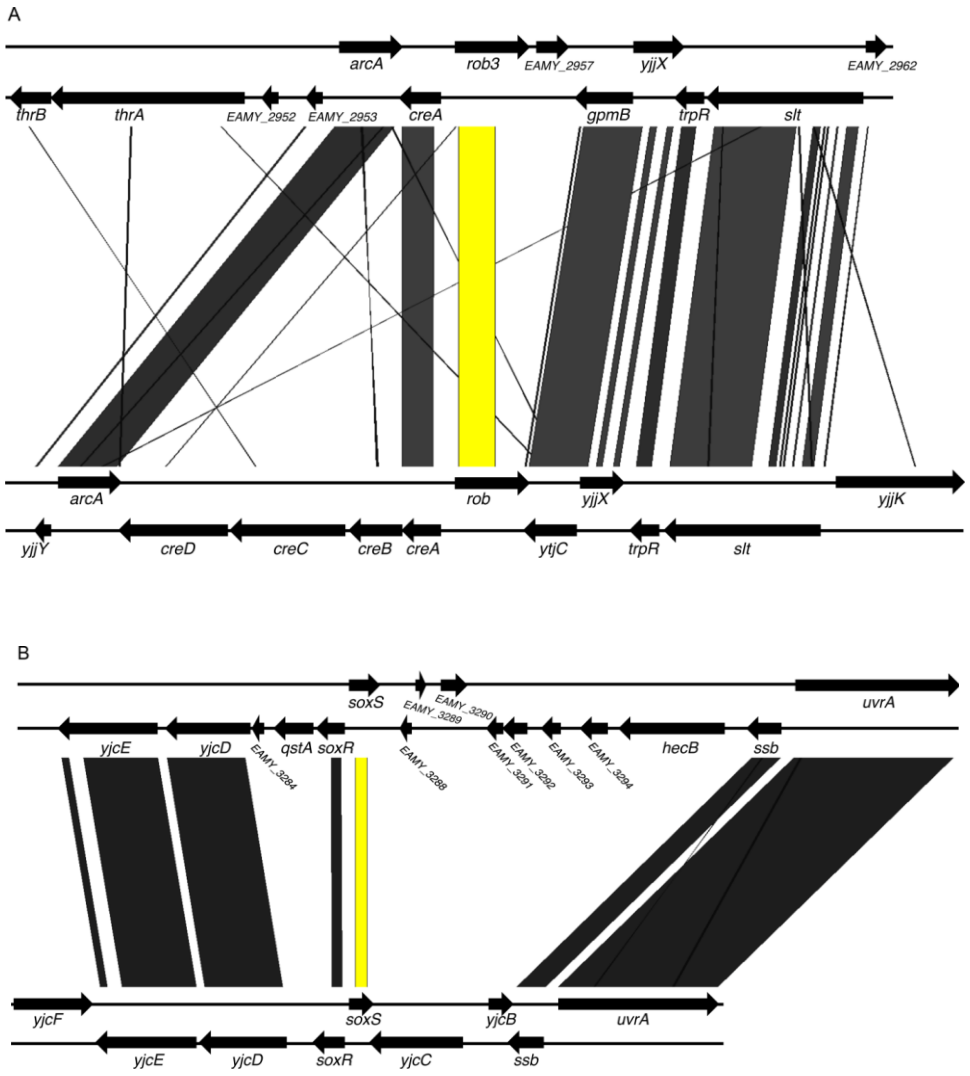
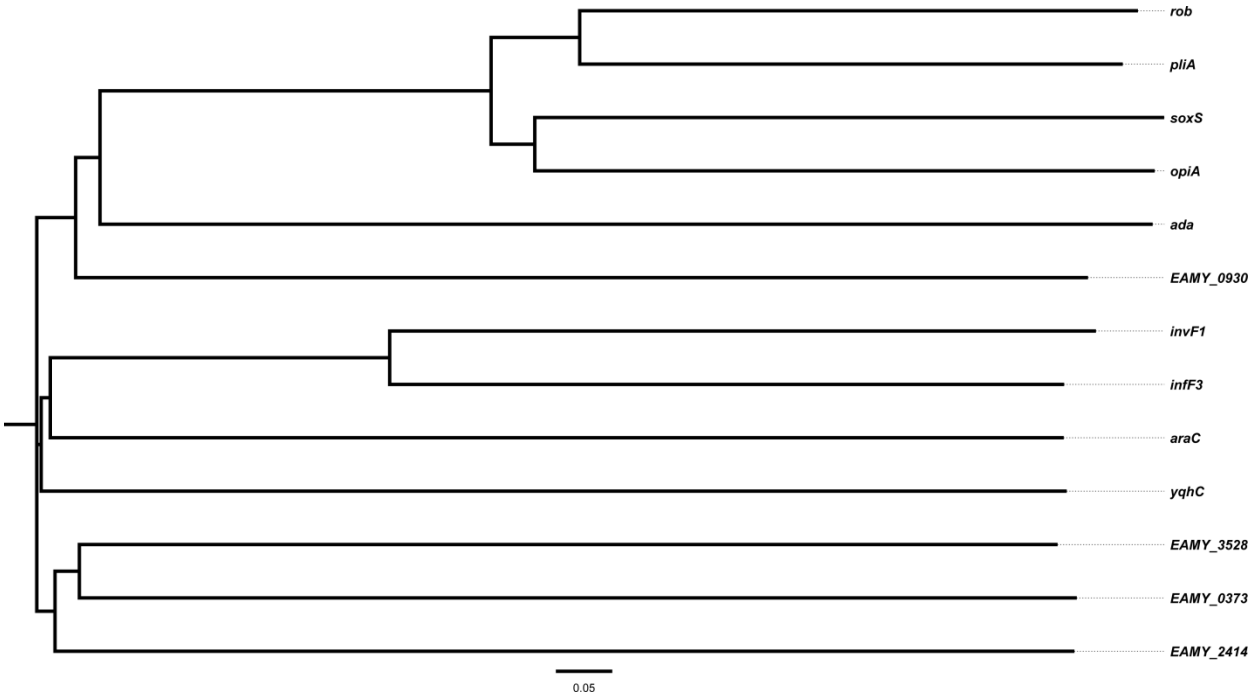
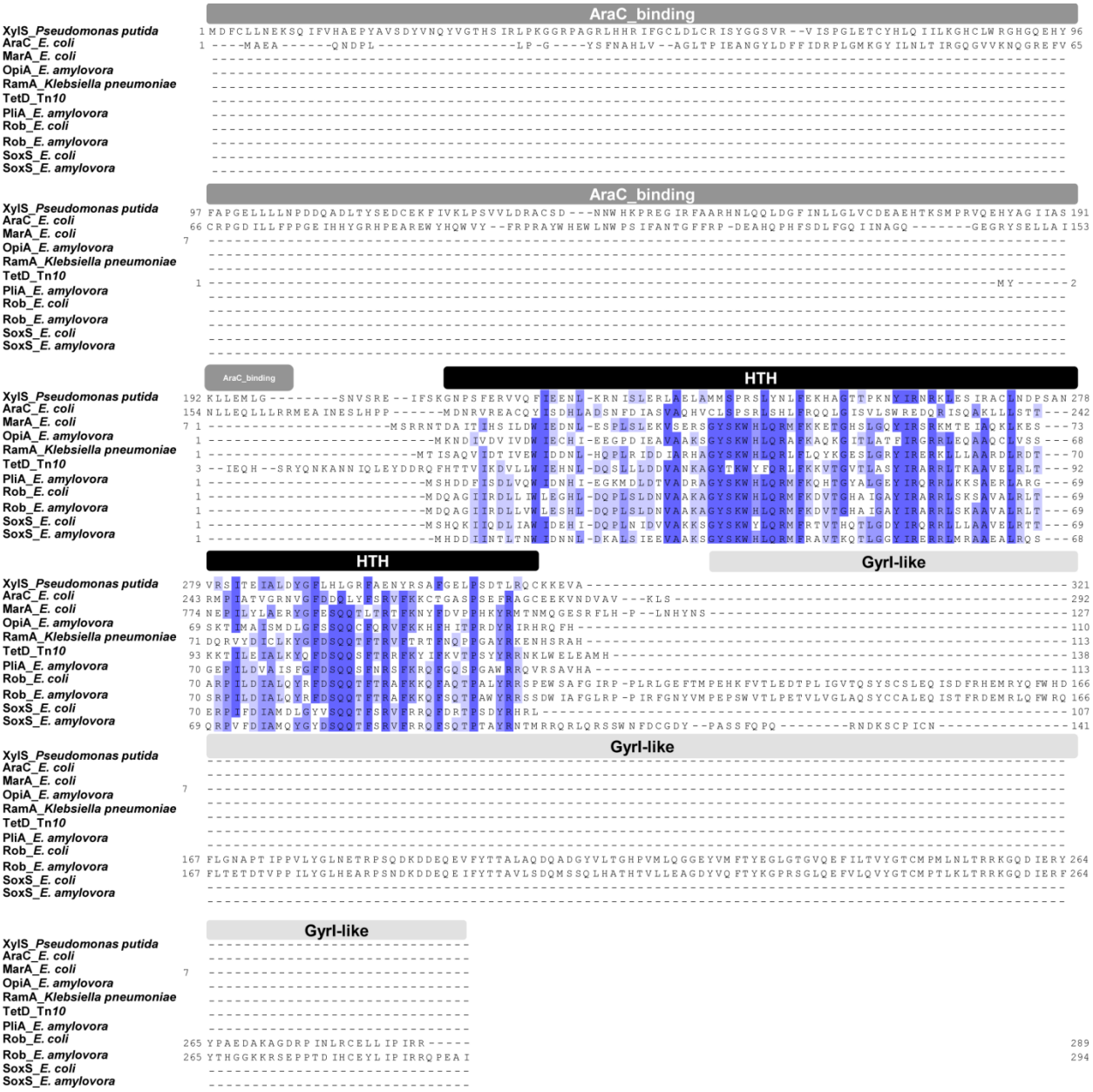


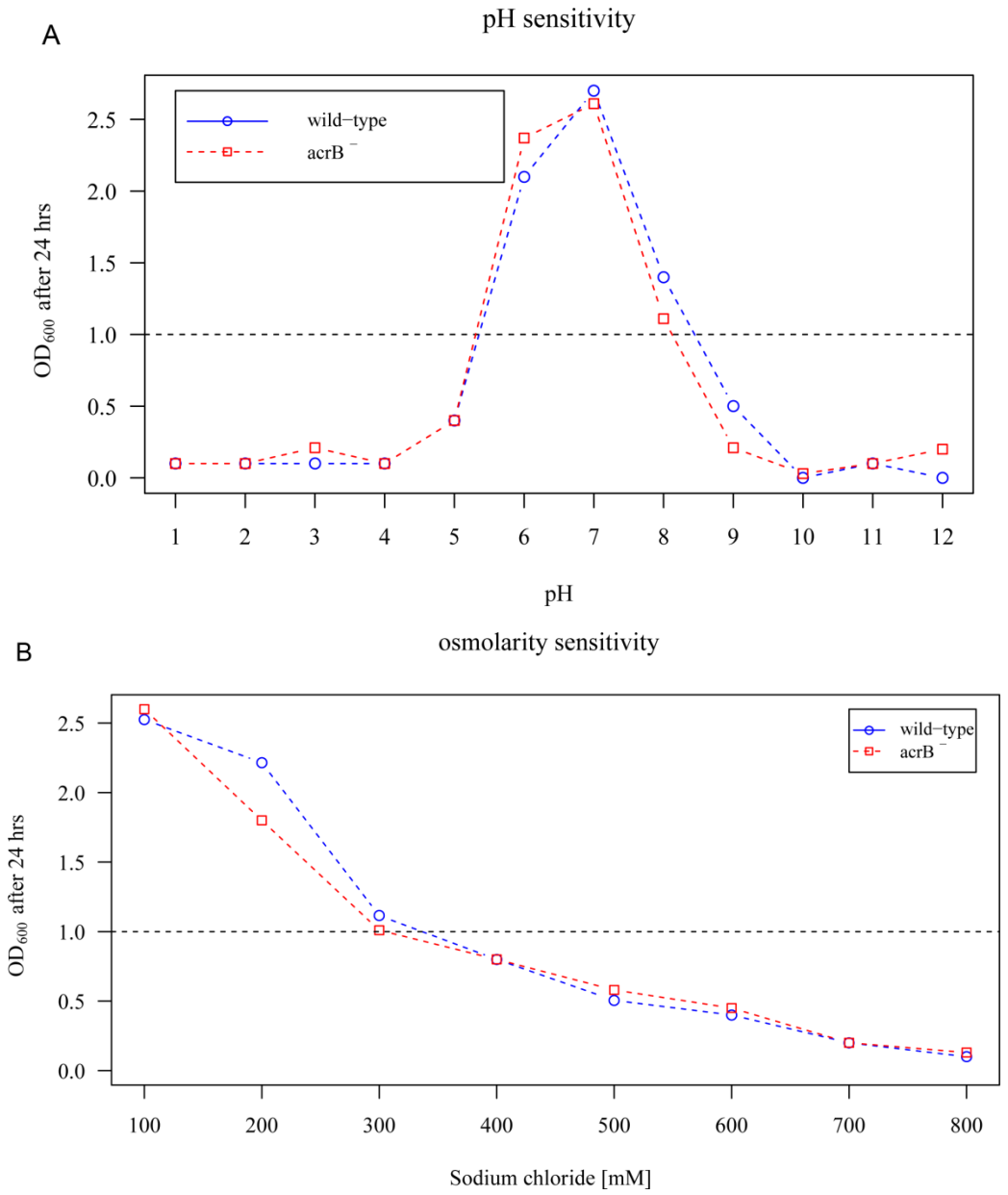
Supplementary Figure 1. Modified view of the genomic organization of the (A) *rob* locus from *E. coli* MG1655 and *E. amylovora* CFBP1430 and (B) *soxS* locus from *E. coli* MG1655 and *E. amylovora* CFBP1430 visualized by the Artemis Comparison Tool (91). The dark areas indicate homologous regions with a minimum identity cutoff score of 50% and a maximum identity cutoff score of 89%. Highlighted in yellow are the homologous areas between *rob* and *soxS*, respectively. The alignment was performed using the nucleotide search BLASTN from NCBI.



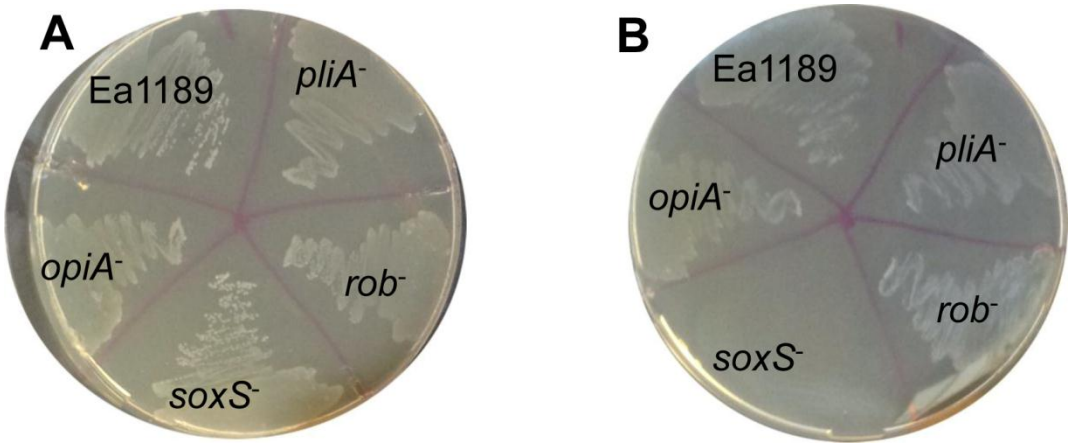
Supplementary Figure 2. Phylogenetic tree of AraC/XylS family members from the fire blight pathogen *E. amylovora*. Multiple sequence alignment was performed using ClustalO (92) and the result was visualized by FigTree (93).



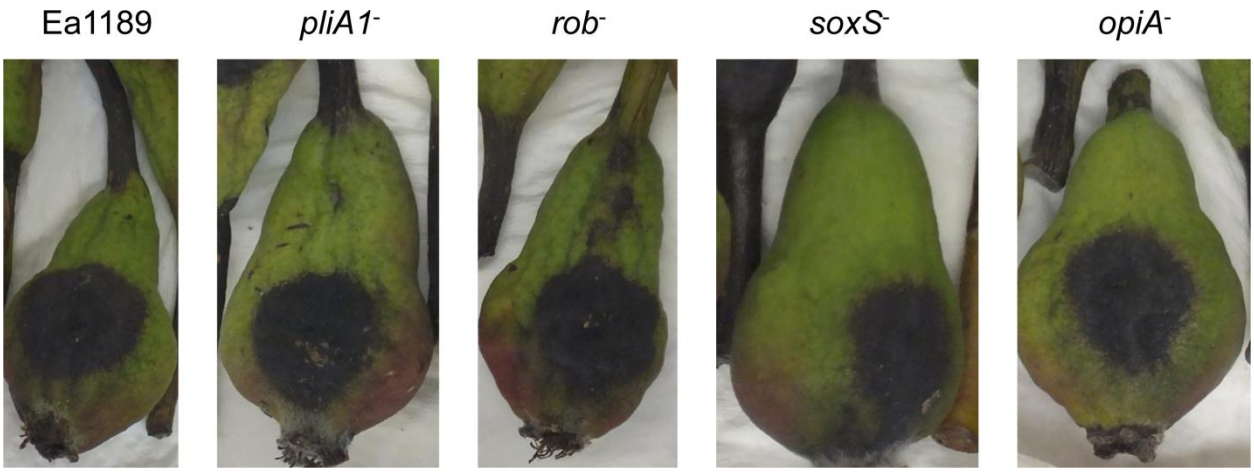
Supplementary Figure 3. Multiple sequence alignment (MSA) of the amino acid sequences of members of the AraC/XylS family of transcriptional regulators. The MSA is based on following proteins: XylS from *Pseudomonas putida* (P07859), AraC from *E. coli* (P0A9E1), MarA from *E. coli* (P0ACH6.2), RamA from *Klebsiella pneumonia* (Q48413), TetD from transposon Tn*10* (P28816), Rob from *E. coli* (P0ACl1.1), SoxS from *E. coli* (P0A9E4.2), PliA from *E. amylovora* (YP_003531153.1), Rob from *E. amylovora* (YP_003532311.1), SoxS from *E. amylovora* (YP_003532640.1) and OpiA from *E. amylovora* (YP_003532913.1). Computational analysis was performed using ClustalO (92). Data presentation was done with Jalview (94). Additionally, the AraC binding domain (dark gray bar) in conjunction with the helix-turn-helix (HTH) DNA-binding domain (black bar) as well as the Rob-specific GyrI-like binding domain (light gray) were determined using Pfam (95) and manually added to the graph. Conserved amino acid residues are shown in blue.



Supplementary Figure 4. Effect of pH (A) and salt concentrations (B) on growth of *E. amylovora* Ea1189 (blue line) and of the *acrB*-deficient mutant Ea1189 3 (red line) in AMM2 medium.



Supplementary Figure 5. Paraquat resistance of *E. amylovora* Ea1189 wild-type, *pliA*-, *rob*-, *soxS*- and *opiA*-deficient mutants, respectively, on MHB II agar plates without paraquat (A) and with 10 µg/ml paraquat (B). Plates were analyzed for growth after 2 days of incubation at 28ºC.



Supplementary Figure 6. Disease symptoms developed on immature pear fruits inoculated with *E. amylovora* Ea1189 and corresponding *pliA*-, *rob*-, *soxS*- and *opiA*-deficient mutants, respectively, two weeks after incubation at 18°C. Pictures represent one out of ten pear fruits per infection.

Supplementary Table 1 Plasmids used in this study

Plasmid	Relevant characteristics[a]	Reference or source
pBlueScript II SK(+)	Ap^r, ColE1 origin	Stratagene
pBlueScript II KS(+)	Ap^r, ColE1 origin	Stratagene
pBBR1MCS	Cm^r, ColE1 origin	(46)
pCAM-MCS	Ap^r, pCAM140-derivative without mini-Tn5, contains the MCS of pBluescript II SK (+)	(8)
pFCm1	Ap^r, Cm^r, source of Cm^r cassette flanked by FRT sequences	(96)
pCAM-Km	Km^r, variant of the gene replacement vector pCAM-MCS, Ap^r replaced by Km^r	This study
pCAM-Km.pliA-ko.Cm	Km^r, Cm^r, contains a 1-kb fragment of *pliA* from *E. amylovora* Ea1189, insertion of 1.1-kb Cm-FRT cassette from pFCm1 in *Pst*I site	This study
pCAM-Km.rob-ko.Cm	Km^r, Cm^r, contains a 1.1-kb fragment of *rob* from *E. amylovora* Ea1189, insertion of 1.1-kb Cm-FRT cassette from pFCm1 in blunt-ended *Aat*II site	This study
pCAM-Km.soxS-ko.Cm	Km^r, Cm^r, contains a 1.3-kb fragment of *soxS* from *E. amylovora* Ea1189, insertion of 1.1-kb Cm-FRT cassette from pFCm1 in *Eco*RV site	This study
pCAM-Km.opiA-ko.Cm	Km^r, Cm^r, contains a 2-kb fusion fragment of the 5' and 3' region of *opiA* from *E. amylovora* Ea1189, insertion of 1.1-kb Cm-FRT cassette from pFCm1 in *Kpn*I site of the fusion	This study
pBlueSK.pliA	Ap^r, contains a 594-bp fragment carrying *pliA* of *E. amylovora* Ea1189 including the upstream promoter region	This study
pBlueKS.pliA	Ap^r, contains a 353-bp fragment carrying *pliA* of *E. amylovora* Ea1189	This study
pBlueSK.rob	Ap^r, contains a 1160-bp fragment carrying *rob* of *E. amylovora* Ea1189 including the upstream promoter region	This study
pBlueKS.rob	Ap^r, contains a 896-bp fragment carrying *rob* of *E. amylovora* Ea1189	This study
pBlueSK.soxS	Ap^r, contains a 606-bp fragment carrying *soxS* of *E. amylovora* Ea1189 including the upstream promoter region	This study
pBlueKS.soxS	Ap^r, contains a 448-bp fragment carrying *soxS* of *E. amylovora* Ea1189	This study
pBlueSK.opiA	Ap^r, contains a 720-bp fragment carrying *opiA* of *E. amylovora* Ea1189 including the upstream promoter region	This study
pBlueKS.opiA	Ap^r, contains a 343-bp fragment carrying *opiA* of *E. amylovora* Ea1189	This study
pBBR.egfp.TIR	Cm^r, contains the TIR-*egfp*-T_0 cassette in pBBR1MCS in opposite orientation with respect to *lac* promoter	(8)
pBBR.acrA-Pro.egfp	Cm^r, contains a 133-bp fragment carrying the promoter region of *acrA*, transcriptional fusion of *acrA* with *egfp*	(45)
pET-28a(+)	*Km^r*, ColE1 origin, expression vector with T7lac promoter, N- and C-terminal His tag	Novagen
pET28a.acrR	*Km^r*, contains a 0.6-kb fragment carrying *acrR* of *E. amylovora* Ea1189, C-terminal translational fusion with His-tag	This study
pET28a.rob	*Km^r*, contains a 0.9-kb fragment carrying *rob* of *E. amylovora* Ea1189, C-terminal translational fusion with His-tag	This study

[a] Antibiotic resistance: Ap^r, ampicillin; Cm^r, chloramphenicol; Km^r, kanamycin.

Supplementary Table 2 Primers used in this study

Primer	Sequence (5' - 3')	Characteristic(s)
***pliA, rob, soxS, opiA* knockout**		
pliA_ko_fwd	TTCTTCAGCACGGCTCCG	used to clone *pliA* knockout vector
pliA_ko_rev	ACTGCGTTGAACTGGCGC	used to clone pliA knockout vector
rob_ko_fwd	TCTTCAGCCAGTCCCAGC	used to clone *rob* knockout vector
rob_ko_rev	TTCCAGCAGCACCGTGTG	used to clone *rob* knockout vector
soxS_ko_fwd	TTCATCACGCCACTGCTG	used to clone *soxS* knockout vector
soxS_ko_rev	AGCAATCACCACCTGAGC	used to clone *soxS* knockout vector
opiA-A5-1	TTTCCACGGTTGGGTGGC	used to clone *opiA* knockout vector
opiA-A6 (rev)	CCCTATAGTGAGTCGGTACCTGAACGGCACTGCTGGT	used to clone *opiA* knockout vector
opiA-B3 (fwd)	GGTACCGACTCACTATAGGGCACCGCCAGTTTCACTG	used to clone *opiA* knockout vector
opiA-B4	ATAGCGCCTGATGATGCG	used to clone *opiA* knockout vector
pliA, rob, soxS, opiA knockout verification		
pliA_out1 (fwd)	AGTGCTAATGCCTGCTGG	Primer flanking *pliA* knockout fragment (used to confirm insertion of Cm cassette)
pliA_out2 (rev)	AACGATGTGCCAACGGTG	Primer flanking *pliA* knockout fragment (used to confirm insertion of Cm cassette)
rob_out1 (fwd)	GACCAACTTGCTGACAGG	Primer flanking *rob* knockout fragment (used to confirm insertion of Cm cassette)
rob_out2 (rev)	ATATCCTGCCCCTTACGC	Primer flanking *rob* knockout fragment (used to confirm insertion of Cm cassette)
soxS_out1 (fwd)	TTCGCTCAGGCGGTCATC	Primer flanking *soxS* knockout fragment (used to confirm insertion of Cm cassette)
soxS_out2 (rev)	ACGCCCTGGTTTGACCTG	Primer flanking *soxS* knockout fragment (used to confirm insertion of Cm cassette)
opiA_out1 (fwd)	ACCTCATCAATCGCCAGC	Primer flanking *opiA* knockout fragment (used to confirm insertion of Cm cassette)
opiA_out8 (rev)	TATGCCGAACTGCCAGCG	Primer flanking *opiA* knockout fragment (used to confirm insertion of Cm cassette)
cat_out2	CTTACGTGCCGATCAACG	reverse primer used to confirm insertion of Cm cassette
cat_out3	AGCATTCATCAGGCGGGC	reverse primer used to confirm insertion of Cm cassette
cat_out4	ACAAGGTGCTGATGCCGC	forward primer used to confirm insertion of Cm cassette
cat_out5	GTGATGGCTTCCATGTCG	forward primer used to confirm insertion of Cm cassette
***pliA, rob, soxS, opiA* overexpression**		
pliA-P-fwd-KpnI	TATGGTACCATGGCTTCTCCCTGTTTTCCG	used to clone *pliA* overexpression vector
pliA_fwd_KpnI	ACCGATGAGTCATGATGAT	
pliA_rev_BamHI	GTGCATGCCTGAAATCTGG	used to clone *pliA* overexpression vector
rob-P-fwd-ApaI	ATAGGGCCCTGCAACGCCGCAGAGCAAAA	used to clone *rob* overexpression vector
rob_fwd_KpnI	TATGGACCAAGCCGGTATC	
rob_rev_SacII	GCAATCTGATAGCACCCGC	used to clone *rob* overexpression vector
soxS-P-fwd-KpnI	TATGGTACCGCCACATCACCAGGAGTC	used to clone *soxS* overexpression vector
soxS_fwd_KpnI	TATGCATGACGACATCATC	
soxS_rev_BamHI	GCCCGTGCGGGGATCCGCA	used to clone *soxS* overexpression vector
opiA-P-fwd-ApaI	ATAGGGCCCTTACGGCTGCGTTTAAACAG	used to clone *opiA* overexpression vector
opiA_fwd_KpnI	GACAATGTCGTTTTTCATT	
opiA_rev_BamHI	CCGGCTAACTCAGTGAAACTG	used to clone *opiA* overexpression vector
Electrophoretic mobility shift assay		
acrA-P-fwd2	TGTTTGGTATTTCGTGCC	used to amplify *acrAB* promoter region
acrA-P-rev2-Cy5	CTGAAAGCATCAGAACGG	used to amplify *acrAB* promoter region, Cy5 labeled
acrR-NcoI	ATACCATGGCACGAAATACCAAACAAC	used to clone *acrR* into C-terminal His-tag protein expression vector
acrR-EcoRI	TAAGAATTCGGGGCCGGTTTGCGCAAGGTTACG	used to clone *acrR* into C-terminal His-tag protein expression vector
Quantitative RT-PCR		
pliA_RT_fwd	AAGCGATCTTGTGCAGTGG	
pliA_RT_rev	GAATGTATTCGCCCAGTGC	
rob_RT_fwd	CACTTGATAACGTCGCAGC	
rob_RT_rev	AACGTAAAGCCACTGCTGC	
soxS_RT_fwd	CAACACCCTGACCAACTGG	
soxS_RT_rev	GCATTAAACGGCGTTCCCG	
opiA_RT_fwd	CGATGTCATCGTAGACTGG	
opiA_RT_rev	AAAGGTCGCCAGAGTGATG	
recA_RT_fwd	TAAGGGCTCCATCATGCGC	
recA_RT_rev	ACCTGCAAAGTCAGGGTGG	

Supplementary Table 3 BLASTP results for PliA, Rob, SoxS and OpiA homologues from *E. amylovora* Ea1189 and *E. coli* MG1655 (Ec)[a,b]

	Length (aa)	**PliA**	**Rob**	**SoxS**	**OpiA**
PliA	113	100%	53%	50%	43%
Rob	294	53%	100%	59%	47%
SoxS	141	50%	59%	100%	48%
OpiA	110	43%	47%	48%	100%
MarA Ec	127	46%	49%	46%	42%
Rob Ec	289	54%	**72%**	57%	44%
SoxS Ec	107	50%	57%	**64%**	49%

[a] Accession numbers: PliA (YP_003531153), Rob (YP_003532311), SoxS (YP_003532640), OpiA (YP_003532913), MarA Ec (P0ACH5), Rob Ec (AAA97292), SoxS Ec (AAC77032)

[b] Bold numbers indicate the highest sequence identity values

Supplementary Table 4 Antimicrobial susceptibility profiles of *E. amylovora* Ea1189 and of the *pliA-*, *rob-, soxS-* and *opiA*-deficient mutant

Drug	MIC (µg/ml)[a]				
	Ea1189	**Ea1189.pliA$^-$**	**Ea1189.rob$^-$**	**Ea1189.soxS$^-$**	**Ea1189.opiA$^-$**
Acriflavine	125	125	125	125	125
Amikacin	6.25	6.25	6.25	6.25	6.25
Azithromycin	0.63	0.63	0.63	0.63	0.63
Bile	5000	5000	> 5000	5000	5000
Cadmium acetate	50	50	50	50	50
Cefepime	12.5	12.5	12.5	12.5	12.5
Chloramphenicol	3.13	> 100	> 100	> 100	> 100
Clotrimazole	> 5000	> 5000	> 5000	> 5000	> 5000
Copper sulphate	1250	1250	1250	1250	1250
Crystal violet	12.5	12.5	12.5	12.5	12.5
Daidzein	> 5000	> 5000	> 5000	> 5000	> 5000
Erythromycin	1.25	1.25	1.25	1.25	1.25
Ethidium Bromide	500	500	500	500	500
Fusidic acid	250	250	500	250	250
Genistein	> 1000	> 1000	> 1000	> 1000	> 1000
Naladixic acid	2.5	2.5	2.5	2.5	2.5
Naringenin	1000	1000	1000	1000	1000
Norfloxacin	0.16	0.16	0.16	0.16	0.16
Novobiocin	250	250	250	500	250
Phloretin	5000	5000	5000	5000	5000
Rhodamine 6G	125	125	125	125	125
Silver nitrate	3.13	3.13	3.13	3.13	3.13
Tannin	2500	2500	2500	2500	2500
Tobramycin	1.56	1.56	1.56	1.56	1.56
Zinc sulphate	500	500	500	500	500

[a] MIC values were determined by the 2-fold dilution assay in three or more independent experiments.

Supplementary Table 5 Virulence assay on apple rootstock MM 106

Strain	Re-isolated bacterial cells[a]		
	1 dpi	**3 dpi**	**7 dpi**
Ea1189	$3.5 \times 10^7 \pm 2.5 \times 10^7$	$9.6 \times 10^7 \pm 6.2 \times 10^6$	$4.6 \times 10^8 \pm 2.0 \times 10^8$
Ea1189.pliA$^-$	$1.6 \times 10^7 \pm 6.3 \times 10^6$	$8.8 \times 10^7 \pm 6.5 \times 10^7$	$1.9 \times 10^8 \pm 2.8 \times 10^7$
Ea1189.rob$^-$	$1.3 \times 10^7 \pm 9.9 \times 10^6$	$1.7 \times 10^8 \pm 1.6 \times 10^8$	$8.0 \times 10^8 \pm 2.9 \times 10^7$
Ea1189.soxS$^-$	$3.6 \times 10^7 \pm 3.3 \times 10^7$	$1.2 \times 10^8 \pm 9.1 \times 10^6$	$5.1 \times 10^7 \pm 2.1 \times 10^7$
Ea1189.opiA$^-$	$1.8 \times 10^7 \pm 5.4 \times 10^6$	$8.9 \times 10^7 \pm 6.9 \times 10^7$	$7.4 \times 10^8 \pm 6.0 \times 10^8$

[a] Bacteria were inoculated by prick technique in the shoot tips with an inoculum of 5×10^6 CFU/shoot. Establishment of a population of *E. amylovora* Ea1189, *pliA, rob, soxS* and *opiA* mutant (CFU/shoot) was determined 1, 3 and 7 days post inoculation (dpi), respectively.