

Supplemental data

Table S1 Proteins exhibiting homology with the RSH ppGpp synthetase domains in *Xanthomonas campestris* pv. *campestris* 8004

Table S2 Primers used for the construction and verification of the $\Delta relA$ and $\Delta relA\Delta spoT$ mutants and complementation strains

Underlined nucleotides indicate the location of restriction sites used for cloning

Table S3 Primers used for qPCR analysis in the current study

Figure S1 Predicted domains within the RelA and SpoT homologues in *Xanthomonas campestris* pv. *campestris*. Bioinformatic analysis using the InterPro online tool indicated that both proteins contained the ppGpp synthetase domain as well as other characteristic motifs of long RelA/SpoT homologue (RSH) proteins: HD, hydrolysis domain; SYNTH, synthesis domain; TGS, ThrRS (threonyl tRNA synthetase), GTPase, and SpoT; ACT, aspartokinase, chorismate mutase, and TyrA (prephenate dehydrogenase). The terms NTD and CTD indicate the amino-terminal domain and carboxy-terminal domain, respectively, while numbers indicate the amino acid location within the protein.

Figure S2 Gene expression of *relA* and *spoT* in *Xanthomonas campestris* pv. *campestris* deletion mutants and complementation strains during log-phase growth. The bar charts indicate the *relA* (A) and *spoT* (B) relative expression of mutant strains in comparison to wild-type strain. WT, $\Delta relA$, and $\Delta relA\Delta spoT$ indicate the wild-type, single mutant and double mutant, respectively, while the prefix pL3- indicates control strains used during complementation, and the suffixes (*relA*) and (*spoT*) indicate complementation with functional copies of *relA* and *spoT*, respectively. Bars correspond to one standard deviation (SD) from the mean (n = 3).

Table S1 Proteins exhibiting homology with the RSH ppGpp synthetase domains in

***Xanthomonas campestris* pv. *campestris* 8004**

Query sequence	Gene ID (NCBI)	Sequence identity (%)	Functional annotation	P value
RelA ppGpp synthetase domain	XC_RS05900	100	Bifunctional (p)ppGpp	7.00E-92
	(<i>relA</i>)			
	XC_RS04795	52.71	Synthetase/guanosine-3',5'-bis(diphosphate)	6.00E-36
	(<i>spoT</i>)			
	XC_RS07530	28.26	Ribonuclease G	2
	XC_RS15935	29.55	EAL domain-containing protein	2.6
	XC_RS08665	24.53	TonB-dependent receptor	4.9
SpoT ppGpp synthetase domain	XC_RS04795	100	Synthetase/guanosine-3',5'-bis(diphosphate)	6.00E-88
	(<i>spoT</i>)			
	XC_RS05900	53.6	Bifunctional (p)ppGpp	2.00E-35
	(<i>relA</i>)			
	XC_RS21215	45.45	Alpha-glucuronidase	2
	XC_RS04155	32.65	Membrane protein	3.2
	XC_RS00865	42.31	Amino acid ABC transporter permease	3.5
	XC_RS16350	34.69	Recombination protein RecR	3.5

Table S2 Primers used for the construction and verification of the $\Delta relA$ and $\Delta relA\Delta spoT$ mutants and complementation strains

Target gene	Primer	Sequence (5'→3')	Restriction site	Product size (bp)	Annealing temperature (°C)
<i>hrpF</i>	DLH120	CCGTAGCACTTAGTGCAATG		619	60
	DLH125	GCATTTCATCGGTCACGATTG			
<i>spoT</i>	XccSpoTF8-F	ATGACCATGATTACGACACTCCGCCCTTTCC		500	70
	XccSpoTF8-R	CAGTGATGCGCGCGGGCCGGAAGACTATGCCAG			
	XccSpoTF10-F	CCGCGCGCATCACTGCGGCGCATGTCGGCGCAG		500	70
	XccSpoTF10-R	TGCATGCCTGCAGGTGACCAGCAACCGCTGGTC			
	XccSpoTF7E-F	CGGAATTCACACTCCGCCCTTTCC	<i>EcoRI</i>	1000	70
	XccSpoTF9H-R	CCAAGCTTGACCAGCAACCGCTGGTC	<i>HindIII</i>		
	XccspoT-F	TCTGCAACTCAAGGAGATGGTC		3425	60
	XccspoT-R	GGCTGATTCCAAAGGAAACTGA			
	XccspoT1-F	AAATTCCGACGCCAGTTCTTC		142	56
	XccspoT1-R	CGAGCCCTACATCACCCATCC			
	XccspoTHB2B-F	CGGGATCCTCCAAAGGAAACTGAATAAGCG	<i>BamHI</i>	2747	62
	XccspoTHB2H-R	CCAAGCTTACATGCGCCGCAGTGAT	<i>HindIII</i>		
<i>relA</i>	XccRelAF8-F	AGTCGGTACCCGGGGTGGCAATCCACTGCGG		500	70
	XccRelAF8-R	GAAGACTGCGTGCGCAAAGGCGGCAGGGACATG			
	XccRelAF10-F	GCGCACGCAGTCTTCGCAGCCGGGGCGGCTAC		500	70
	XccRelAF10-R	ATGCCTGCAGGTGCGACTGCGCCTGCGCCACTTC			
	XccRelAF7B-F	CGGGATCCGTGGCAATCCACTGCGG	<i>BamHI</i>	1000	70
	XccRelAF9S-R	GCGTCGACCTGCGCCTGCGCCACTTC	<i>SalI</i>		
	XccrelA-F10	TCCACGTAAGTGCAGGATTGAGC		3221	56
	XccrelA-R10	CGCCGGAACACGATGACACT			

	XccrelA3-F	CCGCGATGTGGACGAAACC		142	56
	XccrelA3-R	CTTCCGCCAGATGCTGTAGATG			
	XccrelAHB1E-F	CGGAATTCACCGTCCGCTTGCTGC	<i>EcoRI</i>	2800	68
	XccrelAHB1B-R	CGGGATCCATGCGGTGGCGTCCGTC	<i>BamHI</i>		
pLAFR3	pR3-conF1	TGCCGTGCTCGTGTTTCGGGGG		~300+insert	58
	pR3-conR1	GAGTTAGCTCACTCATTAGG			

Underlined nucleotides indicate the location of restriction sites used for cloning

Table S3 Primers used for qPCR analysis in the current study

Target gene	Primer	Sequence (5'→3')	Final Conc. (nM)	Product size (bp)	R ²	Efficiency (%)
<i>pbpA</i>	<i>pbpA</i> -F	GACGGGCCACTCCACTTCTG	500	199	0.9995	95
	<i>pbpA</i> -R	GCAACAACGGCGTGCTCAAC				
<i>ugpC</i>	<i>ugpC</i> -F	AAGGTGTCCGCAAGGTCTAC GA	500	159	0.9983	103
	<i>ugpC</i> -R	TGCCC GCACTGATGTCCTCC				
<i>relA</i>	<i>XccrelA3</i> -F	CCGCGATGTGGACGAAACC	500	142	0.9996	100
	<i>XccrelA3</i> -R	CTTCCGCCAGATGCTGTAGAT G				
<i>spoT</i>	<i>XccspoT1</i> -F	AAATCCGACGCCAGTTCTTC	500	142	0.9955	93
	<i>XccspoT1</i> -R	CGAGCCCTACATCACCCATCC				

Figure S1

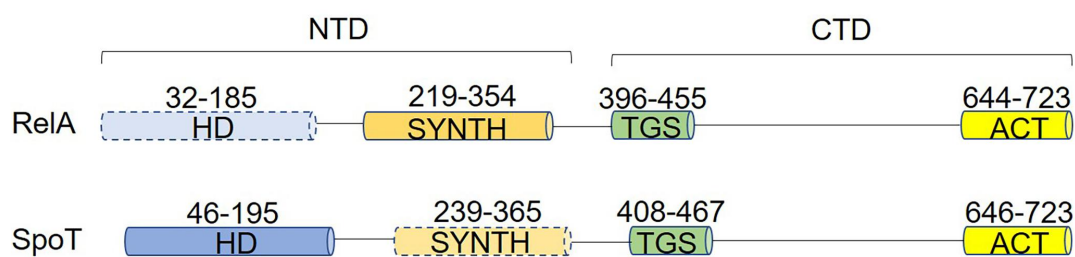


Figure S1 Predicted domains within the RelA and SpoT homologues in *Xanthomonas campestris* pv. *campestris*. Bioinformatic analysis using the InterPro online tool indicated that both proteins contained the ppGpp synthetase domain as well as other characteristic motifs of long RelA/SpoT homologue (RSH) proteins: HD, hydrolysis domain; SYNTH, synthesis domain; TGS, ThrRS (threonyl tRNA synthetase), GTPase, and SpoT; ACT, aspartokinase, chorismate mutase, and TyrA (prephenate dehydrogenase). The terms NTD and CTD indicate the amino-terminal domain and carboxy-terminal domain, respectively, while numbers indicate the amino acid location within the protein.

Figure S2

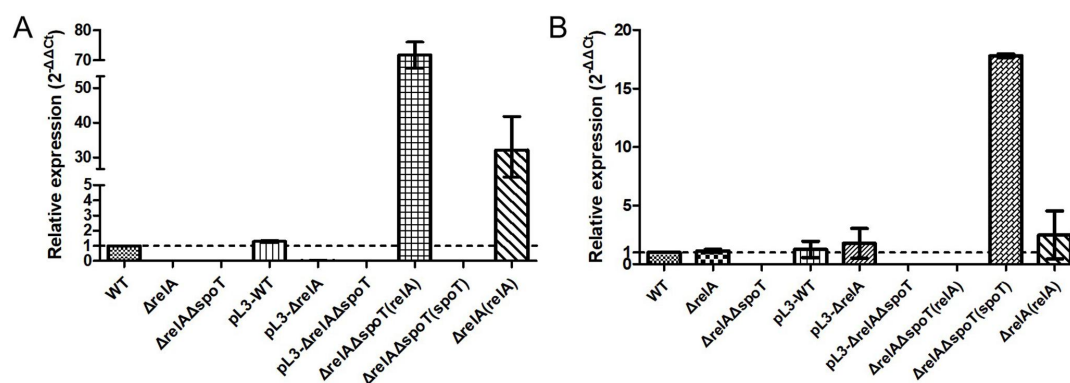


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