Supplementary information

Title: Characterization of the GntR family regulator HpaR1 of the crucifer black rot pathogen Xanthomonas campestris pathovar campestris.

Authors: Hui-Zhao Su, Liu Wu, Yan-Hua Qi, Guo-Fang Liu, Guang-Tao Lu & Ji-Liang Tang

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8004    AACGCCGTCAAAAAAATGGCGTGTTGTGCCTTGCGATGTGTTCGTTCTATGCCATAGTGC 60
B-1459  AACGCCGTCAAAAAAATGGCGTGTTGTGCCTTGCGATGTGTTCGTTCTATGCCATAGTGC 60
B100    AACGCCGTCAAAAAAATGGCGTGTTGTGCCTTGCGATGTGTTCGTTCTATGCCATAGTGC 60
33913   AACGCCGTCAAAAAAATGGCGTGTTGTGCCTTGCGATGTGTTCGTTCTATGCCATAGTGC 60
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8004    ACTGCAACACGCGATTCAATGTTGGTCCCGGCACGTGTCGGGATGCAACTTCCTGTCGTA 120
B-1459  ACTGCAACACGCGATTCAACGTTGGTCCCGGCACGCGTCGGGATGCAACTTCCTGTCGTA 120
B100    ACTGCAACACGCGATTCAACGTTGGTCCCGGCACGCGTCGGGATGCAACTTCCTGTCGTA 120
33913   ACTGCAACACGCGATTCAATGTTGGTCCCGGCACGTGTCGGGATGCAACTTCCTGTCGTA 120
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8004    CGTTCGTGCTGGCGCCTGAGCCGGTTGAATGCTGCGCGAGGTCCTGTCCCACCCAACAGA 180
B-1459  CGTTCGTGCTGGCGCCTGAGCCGGTTGAATGCTGCGCGAGGTCCTGTCCCACCCAACAGA 180
B100    CGTTCGTGCTGGCGCCTGAGCCGGTTGAATGCTGCGCGAGGTCCTGTCCCACCCAACAGA 180
33913   CGTTCGTGCTGGCGCCTGAGCCGGTTGAATGCTGCGCGAGGTCCTGTCCCACCCAACAGA 180
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8004    GGCAGCCAGCTACACGCATGAAGAAACTGATCGGACGACTCTGC-CAAGGCCTCAGCCTG 239
B-1459  GGCAGCCAGCTACACGCATGAAGAAACTGATCGGACGACTCGTCGCAAGGCCTCAGCCTG 240
B100    GGCAGCCAGCTACACGCATGAAGAAACTGATCGGACGACTCTGC-CAAGGCCTCAGCCTG 239
33913   GGCAGCCAGCTACACGCATGAAGAAACTGATCGGACGACTCTGC-CAAGGCCTCAGCCTG 239
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8004    GCTCTGCTCTGCTCGATG 257
B-1459  GCTCTGCTCTGCTCGATG 258
B100    GCTCTGCTCTGCTCGATG 257
33913   GCTCTGCTCTGCTCGATG 257
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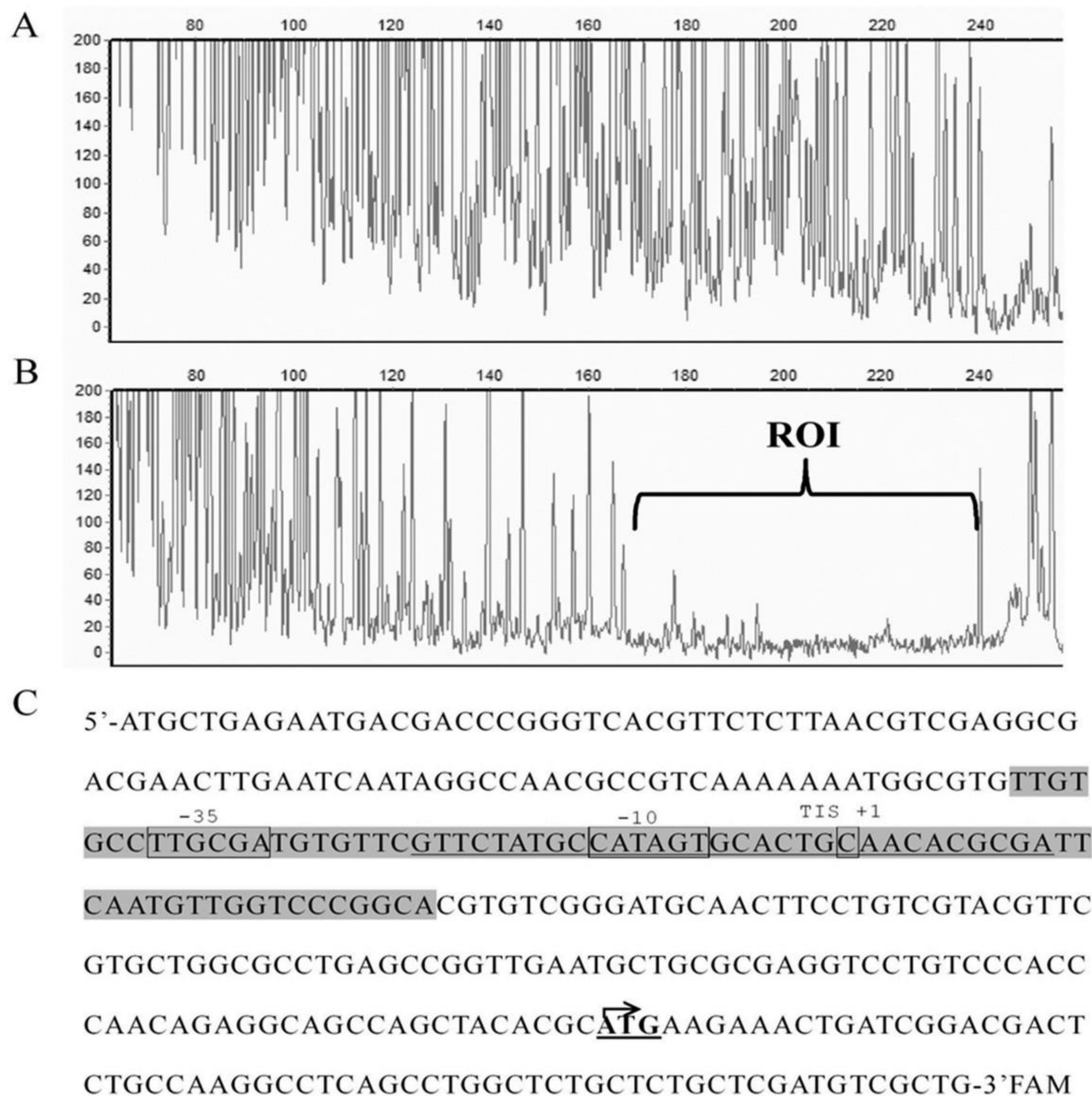
Supplementary Figure 1. Comparison of the 255-bp *gumB* promoter sequences of *Xcc* strains 8004, ATCC33913, B100 and B-1459. Rectangular squares indicate the bases in the strains differ from one another. The predicted translation start site ATG in strain B-1459 is underlined.



Supplementary Figure 2. Determination of the RNA polymerase binding region in the *gumB* promoter of *Xcc* strain 8004 by dye primer-based DNase I footprint assay. Electropherograms show the protection pattern of the *gumB* promoter after digestion with DNase I following incubation in the absence (**A**) or presence (**B**) of 1 U *E. coli* RNA polymerase (RNAP). ROI, region of interest. (**C**) DNA sequence of 330-bp fragment (labelled with FAM at the 3' end) used in this assay. The protected region by RNAP, i.e. the RNAP-binding region, is shown in the grey background (-41 to +29 relative to the transcription initiation site). The HpaR1 binding site is underlined. The transcription initiation site is indicated by a square, and the putative translation start codon ATG is underlined. The -35 and -10 elements are boxed.

Supplementary Table 1. Primers used in this study

Primer	Nucleotide sequence (5′→3′)	The amplified segment or the primer location
Pgum-1F Pgum-1R	ACAGTTGAATTCGGCCCATGTCGCAAGACGTG ACAGTTGGATCCCATCGAGCAGAGCAGAGCCAG	546-bp DNA sequence upstream of the start codon of *gumB* of *Xcc* strain B-1459 (include ATG), used for construction of reporter plasmids and site-directed mutagenesis.
Pgum-2F Pgum-2R	AACAACAGGCCCATGTCGCA CATCGAGCAGAGCAGAGCCAG	529-bp DNA sequence upstream of the start codon of *gumB* of *Xcc* strain B-1459 (include ATG), used for EMSA, FAM-labeled.
Pgum-3F Pgum-3R	ATGCTGAGAATGACGACCCGGG CAGCGACATCGAGCAGAGCAGA	330-bp DNA fragments spanning nucleotides -164 to +166 from the transcription initiation site of the *gumB* promoter region of *Xcc* strain B-1459, used for Dye primer-based DNase I footprint assay, FAM-labelled.
Pgum-4F Pgum-4R	GTCGAGGCGACGAACTTGAA CATCGAGCAGAGCAGAGCCAG	287-bp DNA sequence upstream of the start codon of *gumB* of *Xcc* strain B-1459 (include ATG), used for EMSA, FAM-labelled.
gumivtF gumivtR	AACAACAGGCCCATGTCGCAAG CGCTCCAGATCGTCGATCTGAAAC	678-bp DNA fragments including the promoter region and 209-bp coding region downstream of the start codon of *gumB* of *Xcc* strain 8004, used for *in vitro* transcription assay.
gumivt-1F gumivt-1R	AATGTTGGTCCCGGCACGTG CGCTCCAGATCGTCGATCTGAAAC	329-bp DNA fragments including 209-bp coding region downstream of the start codon of *gumB* of *Xcc* strain 8004, without the -35 and -10 promoter elements, used as a control in *in vitro* transcription assay.
gumNF gumNR	GATCTGTTGCTGGTGAAGGTGTTTC CTGAAGGTAGCCAGCGCGATA	171-bp internal fragment of *gumB*, used for qRT-PCR.
RTPgum1 RTPgum2 RTPgum3	TCGTCCACTGCACCAGTGACCGTGA TGACCGTTCTGGTCGATGCGGACCT CATCGAGCAGAGCAGAGCCAG	located in *gumB* ORF, used for 5′-RACE
PM1F PM1R	CCTTGCGATGTGTTCTTTCTATGCCATAGTGC GCACTATGGCATAGAAAGAACACATCGCAAGG	Used for creating M1 DNA fragment with point mutation.
PM2F PM2R	CCTTGCGATGTGTTCGCTCTATGCCATAGTGCAC GTGCACTATGGCATAGAGCGAACACATCGCAAGG	Used for creating M2 DNA fragment with point mutation.
PM3F PM3R	GCGATGTGTTCGTTCTATGCCACAGTGCACTGCAA CACGCG CGCGTGTTGCAGTGCACTGTGGCATAGAACGAAC ACATCGC	Used for creating M3 DNA fragment with point mutation.
PM4F PM4R	GATGTGTTCGTTCTATGCCATCGTGCACTGCAACA CGCG CGCGTGTTGCAGTGCACGATGGCATAGAACGAAC ACATC	Used for creating M4 DNA fragment with point mutation.
PM5F PM5R	CTATGCCATAGTGCACTTCAACACGCGATTCAATG CATTGAATCGCGTGTTGAAGTGCACTATGGCATAG	Used for creating M5 DNA fragment with point mutation.
PM6F PM6R	CTATGCCATAGTGCACTGCCACACGCGATTCAATG TTGG CCAACATTGAATCGCGTGTGGCAGTGCACTATGGC ATAG	Used for creating M6 DNA fragment with point mutation.

1045F 1045R	CGACGACGTTTGAGCACGCCGTCCTAT AAGCCGCTGTTCATCTGGTCCGCCA	311-bp DNA sequence including the promoter region and 122-bp coding region of the gene *XC_1045*, used for EMSA, FAM-labelled.
3077F 3077R	TCTCACTCTGTCTTGCAAACTGCGA AATGGGCATCGAAAACCAGAAGC	890-bp DNA fragment including the promoter region and coding region of *hrpG* of *Xcc* strain 8004, used as a control in *in vitro* transcription assay.
16SF 16SR	GCCTAACACATGCAAGTCGAACGGC AATATTCCCCACTGCTGCCTCCCG	325-bp DNA fragment of the 16S rDNA sequence, used for qRT-PCR.
PD1F PD1R	TGTGCCTTGCGATGTGTTGTTCTATGCCATAGTGCACT AGTGCACTATGGCATAGAACAACACATCGCAAGGCACA	Used for creating *gumB* mutant promoter with one nucleotide deletion.
PD2F PD2R	TGTGCCTTGCGATGTGTGTTCTATGCCATAGTGCACT AGTGCACTATGGCATAGAACACACATCGCAAGGCACA	Used for creating *gumB* mutant promoter with two nucleotides deletion.
PIN1F PIN1R	TGTGCCTTGCGATGTGTTCCGTTCTATGCCATAGTGCACT AGTGCACTATGGCATAGAACGGAACACATCGCAAGGCACA	Used for creating *gumB* mutant promoter with one nucleotide insertion.
PIN2F PIN2R	TGTGCCTTGCGATGTGTTCCAGTTCTATGCCATAGTGCACT AGTGCACTATGGCATAGAACTGGAACACATCGCAAGGCACA	Used for creating *gumB* mutant promoter with two nucleotides insertion.
PIN3F PIN3R	TGTGCCTTGCGATGTGTTCTCAGTTCTATGCCATAGTGCACT AGTGCACTATGGCATAGAACTGAGAACACATCGCAAGGCACA	Used for creating *gumB* mutant promoter with three nucleotides insertion.

Supplementary Table 2. Strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Reference or source
E. coli		
JM109	*RecA*1, *endA*1, *gyrA*96, *thi*, *supE*44, *relA*1△ (*lac-proAB*)/F' [*traD36, lacI*^{q}, *lacZ* △M15]	(1)
DH5α	Φ80△*lacZM*15 *recA1 endA1 deoR*	Gibco BRL, Life Technologies
JM109/pQE-30-2736	JM109 harbouring pQE-30-2736	(2)
X. campestris pv. *campestris*		
8004	Wild type, Rif^r	(3)
2736nk	As 8004, but *XC_2736*::pK18*mob*, non-polar effect. Rif^r, Kan^r	(2)
8004/pGUSgum	8004 harbouring pGUS*gum*, Rif^r Tet^r	This work
2736nk/pGUSgum	2736nk harbouring pGUS*gum*, Rif^r Kan^r Tet^r	This work
Plasmids		
pLAFR6	A promoterless derivative of pLAFR3, Tet^r	(4)
pRK2073	Helper plasmid, Tra+, Mob+, ColE1, Spc^r.	(5)
pQE-30	Expression vector, allowing the production of fusion proteins containing amino terminal 6xHis-tagged sequences. Amp^r	Qiagen, Germany
pQE-30-2736	pQE-30 containing a 360-bp fragment of *hpaR1* gene coding region.	(2)
pL6*gus*	pLAFR6 containing a 1,832-bp *gusA* ORF (excluding ATG), Tet^r	(6)
pGUSgum	pLAFR6 containing 546-bp promoter region of the gene *gumB* fused to the coding region of *gusA*, Tet^r	This work
pK18*mob*	pUC18 derivative, *lacZα* Kan^r, *mob* site. Suicide plasmid in *Xcc*.	(7)
pK*gum*	The suicide plasmid pK18mob containing 546-bp *gumB* promoter.	This work
$pGUSgum_{M1-6}$	pLAFR6 containing 546-bp *gumB* promoter with 6 different single mutated nucleotide, respectively, fused to the coding region of g*usA*, Tet^r	This work
$pGUSgum_{15}$	pLAFR6 containing the mutant *gumB* promoter with 1 nucleotide deletion in the spacer between -35 and -10 elements, fused to the coding region of *gusA*, Tet^r	This work
$pGUSgum_{14}$	pLAFR6 containing the mutant *gumB* promoter with 2 nucleotides deletion in the spacer between -35 and -10 elements, fused to the coding region of *gusA*, Tet^r	This work
$pGUSgum_{17}$	pLAFR6 containing the mutant *gumB* promoter with 1 nucleotide insertion in the spacer between -35 and -10 elements, fused to the coding region of *gusA*, Tet^r	This work
$pGUSgum_{18}$	pLAFR6 containing the mutant *gumB* promoter with 2 nucleotides insertion in the spacer between -35 and -10 elements, fused to the	This work

	coding region of *gusA*, Tet^r	
$pGUSgum_{19}$	pLAFR6 containing the mutant *gumB* promoter with 3 nucleotides insertion in the spacer between -35 and -10 elements, fused to the coding region of *gusA*, Tet^r	This work

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