HrcT is a key component of the type III secretion system in *Xanthomonas* and also regulates the expression of the key *hrp* transcriptional activator HrpX

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Supplementary Materials

Files in This Data Supplement:

Supplemental file 1 - Primers used in this study (Table S1); The expression of *hrpG* in the wild-type and *hrcT* mutant of *Xoc* (Fig. S1); Phylogenetic analysis of HrcT proteins from various bacterial pathogens (Fig. S2).

13 **Table S1.** Primers used in this study.

Primer	Sequence (5'→3'; restriction sites underscored)	Description of amplified product
T-IF	ATAGGATCCAGCATGGCACCGTCATAG	Amplifies a 402-bp fragment targeting left border of *hrcT*
T-IR	CCGTCTAGATCTTTATTGAGACATTC	
T-IIF	ATATCTAGACGGGCGCCCCCATGTGC	Amplifies a 750-bp fragment targeting right border of *hrcT*
T-IIR	CGGAAGCTTCGTGTGATGAGCCAGAT	
Gm-F	CCCAAGCTTGGCGCGCCGCACCTACGCTCTCC	Amplifies a 876-bp ORF encoding Gm^r
Gm-R	CCGGAATTCGGCGCGCCGCGGCGTTGTGACAA	
Gm-IF	CGCGGATCCCCGGTGCGGACATTGGGGTGGTT	Amplifies a 401-bp fragment targeting the right border of the *hrpB1* promoter
Gm-IR	CCCAAGCTTGAGGTGCAGTGTGGAGAAGATTC	
Gm-IIF	CCGGAATTCGCTTTTAATGGCATGCGGACCTT	Amplifies 700-bp fragment at left border of *hrpB1* promoter
Gm-IIR	ACGCGTCGACTCATCGGCATGAGGGCGCGGAT	
*phrcT1*F	CGGGGTACCCGGCCGACTTCATCACCAATAACG	350-bp T1 promoter of *hrpB1*
*phrcT1*R	CCGGAATTCACTGCACCTCGCTTTTAATGGCAT	
*phrcT2*F	CGGGGTACCGAGCCTGCCTACACCTGGTCGTTG	500-bp T2 promoter of *hrcT*
*phrcT2*R	CCGGAATTCCGGGCGCCCCCATGTGCGGCGCG GT	
hrcT-F	CCGGAATTCATGAACGACGCCACCGATGCCTTG	Amplifies 831-bp fragment containing *hrcT* ORF from strain RS105
hrcT-R	CCCAAGCTTTCAGTGCGACGCGCCCGCCTTGCC A	
Txac-F	CCGGAATTCATGAACGACGCCACCGATGCCTT	831-bp *hrcT* ORF from *X. axonopodis* pv. *citri* 306
Txac-R	CCCAAGCTTTCAGTGCGACGCGGCTCTCTTGC	
TRs-F	CCGGAATTCATGGAATCGATCGATACCGTTGT	849-bp *hrcT* from *R. solanacearum* GMI1000
TRs-R	CCCAAGCTTTCACTGTGGAGTTTTCGCTTTTG	
T3000-F	CCGGAATTCATGCCTCTGGACGCGCAGAATTT	795-bp *hrcT* from *P. syringae* pv. *tomato* DC3000
T3000-R	CCCAAGCTTTCACGGCACCTGCACCACAAAGC	
*phrcT1g*F	CCGGAATTCCGGCCGACTTCATCACCAA	Amplifies 350-bp promoter of *hrpB1*
*phrcT1g*R	CGGGGTACCACTGCACCTCGCTTTTAAT	
*phrcT2g*F	CCGGAATTCGAGCCTGCCTACACCTGGTCG	Amplifies 500-bp promoter of *hrcT*
*phrcT2g*R	CTGGGTACCCGGGCGCCCCCATGTGCGGCG	
phrpXgF	CCGGAATTCCCGGATGGCATGAAATGTTCT	Amplifies 300-bp promoter region of *hrpX*
phrpXgR	CGGGGTACCCCTGCTCGTATAGGTAGGAAG	
*gus*F	GGGGTACCATGTTACGTCCTGTAGAAA	Amplifies 1,830-bp fragment containing *gusA*
*gus*R	CGGGATCCTCATTGTTTGCCTCCCTGC	
T-F	CCGGAATTCATGGACAGTCTTCCCGGAAT	A 711-bp fragment of *hrcT* fused with 6×his tag
T-hisR	CCGCTCGAGGTGGTGGTGGTGGTGGTGCGACG	

	CGCCC GCCTT GCCAC	
O-F	CATGGCTACATGCTGACAGCCTA	Nested primers for
I-F	CGCGGATCCACAGCCTACTGATGATGATCAGTAC ATG	5'-RACE
hrcTO-R	ATCGTGCAATTGCTGCTGGAAATGC	Nested gene-specific
hrcTI-R	CGATCGCAAGATCCACCAGCACAA	primers for 5'-RACE of *hrcT*
1-2-F	GAGGTTGAAGCCGACCATGGTCAGCTCGT	Amplified 800-bp fragment
1-2-R	TGTCCCGGGTCGGTCGTGAGTGGGCTCAT	between *hrpB1* and *hrpB2*
2-J-F	AAACACAACGTCACCACACACCCGACC	Amplified 872-bp fragment
2-J-R	TCGATGTGCAGAACGATGGCGTGCGCA	between *hrpB2* and *hrcJ*
J-4-F	ATCAGGACATTGGCCAATCGCGCGGGCA	Amplified 909-bp fragment
J-4-R	GACGCGTCCAAAGTCACGCCAGACGAC	between *hrcJ* and *hrpB4*
4-5-F	CGCAGACTGCGCAGCTGATCGCGCAGAT	Amplified 1100-bp
4-5-R	GGGTAATCCAGGCGTGGTCGGGATGGTC	fragment between *hrpB4* and *hrpB5*
5-N-F	TCATCAGGCCATCCACGATGCGCACGCCGGTC	Amplified 583-bp fragment
5-N-R	GCGTGTGCGAATGGGATACGGGCGTGTTCGAG	between *hrpB5* and *hrcN*
N-7-F	ACGGTGCGGAAGTGTTCATGCTGCAGGATCGT	Amplified 610-bp fragment
N-7-R	CGATCCGATTGCCGAGGAAGTGCGCGGCATCC	between *hrcN* and *hrpB7*
7-T-F	TCAGTGCGACGCGCCCGCCTTGCCACCCAGGT	Amplified 1337-bp
7-T-R	ATGCGTGAGCCTGCCTACACCTGGTCGTTGCT	fragment between *hrpB7* and *hrcT*
T-C-F	TGTGTTGGAGGAGCCACCGCCGATTGGCTGTA	Amplified 1014-bp
T-C-R	GACAAGCTCGAACCGTCGAACTTGAGTCAGCC	fragment between *hrcT* and *hrcC*
N-T-F	ATCGTGCAATTGCTGCTGGAAATGCAGGAA	Amplified 2717-bp
N-T-R	ATGGGATACGGGCGTGTTCGAGACCGATCT	fragment between *hrcN* and *hrcT*
B7-T	ATGCGTGAGCCTGCCTACACCTGGTCGTTGCT	*hrpB7* gene, 510-bp
B7-R	CAGCGCGACCAGTGTCTCGATCGCGTCTTCTT	
T-F	ATGAACGACGCCACCGATGCCTTGCTGGCCCT	*hrcT* gene, 831-bp
T-R	TCAGTGCGACGCGCCCGCCTTGCCACCCAGGT	
hpa2-F	AGCAGAATCGAATGAATGGCATGCG	*hpa2* gene fragment,
hpa2-R	CGTGCGTCAGTGTGTATGTAGCAGC	145bp
hpa1-F	CCAGGGTATCTCGGAAAAGCAACTG	*hpa1* gene fragment,187bp
hpa1-R	ATTCATCAGCATCTGGGTGAGTGGC	
hrpB1-F	TGCTGACGATTCAACCGGTACGTCC	*hrpB1* gene fragment,
hrpB1-R	GGCTGATCGAAGAAGACGCCGATGC	110bp
hrcU-F	GAGACACCGCTTGATATTGCC	*hrcU* gene fragment,180
hrcU-R	GCCCTCGCTTTCCTTGTATTC	bp
hrpE-F	CTTCGAACAGGGTATGGATGG	*hrpE* gene fragment,162
hrpE-R	TTGAGCTGCGTGATCTTGTTG	bp
hpa4-F	CTGGCTTGAGCAACAAGGTATTGAG	*hpa4* gene fragment, 187

hpa4-R	GAGCCAGATCCACCACCGGTCATTC	bp
hpa3-F	TTGGGTTACCTGGTGAGCTTG	*hpa3* gene fragment, 215
hpa3-R	CCAGCCATACTCGGTCAGCAG	bp
gyrB-F	CGGCACTTACGACTCCAGCAAG	*gyrB* gene fragment,
gyrB-R	CGACCAGGATTTTCACCACGATG	191bp
pXF	CCCAAGCTTCCGGATGGCATGAAATGTTCTGT	Amplifies 300-bp promoter
pXR	CTAGTCTAGACCTGCTCGTATAGGTAGGAAGA	region of *hrpX*
TF	CCCAAGCTTATGAACGACGCCACCGATGCCTT	*hrcT* gene, 831-bp
TR	CTAGTCTAGATCACAGATCTTCTTCAGAAATAA	

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Fig. S1 The expression of *hrpG* in the wild-type and *hrcT* mutant of *Xoc*. A. pGGUS-mediated glucuronidase activity in RS105 and R∆*hrcT*. Strains were incubated in XOM3 medium for 12 h at 28°C. GUS activity was determined by measuring the OD_{600} at 415 nm using 4-MUG as a substrate. B. Expression analysis of *hrpG* by real-time qRT-PCR. RNAs were isolated from cultures of RS105 and R∆*hrcT.* All strains were grown in XOM3 for 12 h at 28°C, and relative mRNA levels were calculated with respect to the expression level of the corresponding transcript in RS105. The different columns above were measured by a paired, two-tailed Student *t*-test ($P \leqslant 0.05$) relative to the wild-type. The experiments were repeated three times and similar results were obtained.

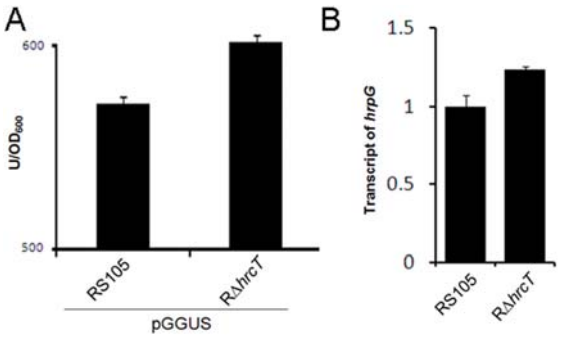

Fig. S2 Phylogenetic analysis of HrcT proteins from various bacterial pathogens. HrcT from *Xoc* RS105 was compared with related HrcT proteins from *Xoc* BLS256, *X. campestris* pv. *vesicatoria* Xcv85-10, *X. axonopodis* pv. *citri* Xac 306, *X. oryzae* pv. *oryzae* PXO99^{A}, *X. campestris* pv. *campestris* Xcc8004, *A. citrulli*, *R*. *solanacearum* GMI1000, *Burkholderia* sp., *P*. *syringae* pv. *tomato* DC3000, *E. amylovora, D. dadantii* 3937, *S. boydii*, *S. enterica* and *Y. pseudotuberculosis.* The protein IDs were provided before the pathogen names. The analysis was used performed using MEGA 4 software.