Supplementary Material

Tal1NXtc01 in *Xanthomonas translucens* pv. *cerealis* Contributes to Virulence in Bacterial Leaf Streak of Wheat

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**Running title:** Tal1NXtc01 contributes to virulence in *X. translucens* pv. *cerealis*

Supplementary Tables

Table S. Bacterial strains and plasmids used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| Strains and plasmids | | Relevant characteristics | Source |
| Strains | | | |
| *Xanthomonas translucens* pv. *cerealis* | | | |
| NXtc01 | Wild-type, isolated from *Triticum aestivum* in Xinjiang province, China, 2016 | | This study |
| Δ*tal1* | *tal1* knockout mutant of NXtc01 | | This study |
| Δ*tal2* | *tal2* knockout mutant of NXtc01 | | This study |
| TF | *tal* free mutant of NXtc01 | | This study |
| PHZW-*tal1* | Apr Spr, TF containing *tal1* *in trans* | | This study |
| PHZW-*tal2* | Apr Spr, TF containing *tal2* *in trans* | | This study |
| Δ*hrcC* | *hrcC* knockout mutant of NXtc01 | | This study |
| Δ*hrcC*/*hrcC* | Spr, *ΔhrcC* containing *hrcC* gene *in trans* | | This study |
| *Escherichia coli* | | |  |
| DH5α | F- *endA1*, *thi-1*, *recA1*, Φ80*lacZ*, ΔM15 | | This lab |
| Plasmids |  | |  |
| pKMS1 | Kmr, pUC18 polylinker, *mob*, *oriV*, *sacB* | | (Zou et al., 2011) |
| pB | Apr, pBluescript II phagemid, pUC derivative | | This lab |
| pHM1 | Spr, cosmid vector, *cos*, *parA*, *IncW* | | (Hopkins et al., 1992) |
| pZW | Apr, pBluescript II with FLAG-tag N- (831-bp) and C- (363-bp) terminus of *avrXa10.* Lacks the *Sph*I fragment containing the CRR; for expression of TAL effector genes | | (Yang et al., 2000) |
| pB-*tal1* | Apr, pB containing *tal1* at *Bam*HI site | | This study |
| pB-*tal2* | Apr, pB containing *tal2* at *Bam*HI site | | This study |
| pKMSTal1 | Kmr, pKMS1 containing sequences at the 5’ and 3’ end of *tal1*; used for generating *tal1* deletion mutant | | This study |
| pKMSTal2 | Kmr, pKMS1 containing sequences at the 5’ and 3’ end of *tal2*; used for generating *tal2* deletion mutant | | This study |
| pKMShrcC | Kmr, pKMS1 containing sequences at the 5’ and 3’ end of *hrcC*; used for generating *hrcC* deletion | | This study |
| pZW-*tal1* | Apr, CRR of *tal1* from *Sph*I digested pB-*tal1* were ligated into *Sph*I-linearized, CIP-treated pZW vector | | This study |
| pZW-*tal2* | Apr, CRR of *tal2* from *Sph*I digested pB-*tal2* were ligated into *Sph*I-linearized, CIP-treated pZW vector | | This study |
| pHZW-*tal1* | Apr Spr, pZW with *tal1* in pHM1 at *Hin*dIII site | | This study |
| pHZW-*tal2* | Apr Spr, pZW with *tal2* in pHM1 at *Hin*dIII site | | This study |
| pB-*hrcC* | Apr, pBluescript II, contains *hrcC* | | This study |
| pH1-Flag(-) | Spr, pHM1 containing MCS with FLAG-tag bacteriophage λ T0 and *E. coli* *rrnB* T1 terminators | | Yang Xiaofei, unpublished data |
| pH1-Flag(-)*hrcC* | Spr, pH1**-**Flag(-); contains *hrcC* at *Sal*I/*Kpn*I sites | | This study |

**Note**: Apr, ampicillin resistance; Kmr, kanamycin resistance; Spr, spectinomycin resistance.

Table S. Primers used in this study.

|  |  |  |
| --- | --- | --- |
| Primers | Sequence 5’to 3’ | Description |
| T1Nt-F | AACTGCAGTGGATGGCTTGCCCGCTC | Amplifies 640-bp fragment upstream of *tal1*; used for deletion mutagenesis |
| T1Nt-R | GCTCTAGAGCCTGTGACCAACTGTAACG |
| T1Ct-F | GCTCTAGAGCAAGCATTTGATGAAGCG | Amplifies 425-bp fragment downstream of *tal1*; used for deletion mutagenesis |
| T1Ct-R | TCCCCCGGGGCGTTTTACACCCCAGCTACT |
| T2Ct-F | TCCCCCGGGAGGCAATAGCGTCATCA | Amplifies 698-bp fragment upstream of *tal2*; used for deletion mutagenesis |
| T2Ct-R | GCTCTAGACACAAGAACCCGTAACCT |
| T2Nt-F | GCTCTAGAATTATTGCTGACGATGGC | Amplifies 413-bp fragment downstream of *tal2;* used for deletion mutagenesis |
| T2Nt-R | AACTGCAGGGAGAAGATCAAACCGAAG |
| *hrcC*-up-F | TCCCCCGGGGTGGTCAGGCAGGTATCG | Amplifies 610-bp fragment upstream of *hrcC;* used for deletion mutagenesis |
| *hrcC*-up-R | CGCGGATCCCCAACGCCACTGTCAAA |
| *hrcC*-do-F | CGCGGATCCCGGGATTGTCCAAGGTG | Amplifies 694-bp fragment downstream of *hrcC;* used for deletion mutagenesis |
| *hrcC*-do-R | CTAGTCTAGAGGAAACTTCGCTGCCTC |
| M13-R | AGCGGATAACAATTTCACACAGG | Universal primers used for confirmation and sequencing |
| M13-F | CGCCAGGGTTTTCCCAGTCACGAC |
| *hrcC*-F1 | ACGCGTCGACATGAAACAGCAACGTTCGC | Amplifies 1217-bp fragment beginning at the start codon of *hrcC;* used for complementation in pH1-Flag(-) |
| *hrcC*-R1 | TCCAGATGGCGGTTGTG |
| *hrcC*-F2 | CGCACCGTTCAGATGGA | Amplifies 1260- bp fragment extending from the middle of *hrcC* and ending before stop codon; used for complementation in pH1-Flag(-) |
| *hrcC*-R2 | CGGGGTACCGGGCGAGACCACGTGG |
| TALN18S-F | AGAGGCGACACACGAAGACA | Amplifies 255-bp fragment, used to confirm of *Sph*I-ligated *tal* gene orientation in pZW |
| TalN-R | CCGCCACTATTGCTGACGAT |

**Note**: Underlined bases indicate restriction enzyme sites.

**Table S3|**Predicted type III effectors in *X. translucens* pv. *cerealis* NXtc01 based on comparison with other *Xanthomonas* spp. included *X. translucens* pv. *cerealis* CFBP 2541, *X. translucens* pv. *undulosa* ICMP11055, *X. translucens* pv. *translucens* DSM 18974T, *X. campestris* pv. *campestris* 8004, *X. oryzae* pv. *oryzae* PX099A and *X. oryzae* pv. *oryzicola* BLS256.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| T3Es | NXtc01a | CFBP2541b | ICMP11055c | DSM18974Td | 8004e | PXO99Af | BLS256g |
| AvrBs1 | ND | + | ND | ND | + | ND | ND |
| AvrBs2 | ++ | ++ | ++ | ++ | + | + | + |
| TALEs | 2 | 2 | 7 | 8 | ND | 19 | 28 |
| AvrXccA1 | + | ND | ND | ND | + | ND | ND |
| XopAA | + | + | + | + | ND | + | + |
| XopAD | + | + | + | + | ND | + | + |
| XopAF1 | + | + | + | ++ | ND | ND | + |
| XopAH | ND | ND | + | + | + | + | + |
| XopAK | ND | ND | + | + | ND | ND | + |
| XopAM | + | + | + | + | + | ND | ND |
| XopAP | + | + | + | F | ND | ND | + |
| XopAZ | + | ND | ND | ND | + | + | + |
| T3Es | **NXtc01a** | **CFBP2541b** | **ICMP11055c** | **DSM18974Td** | **8004e** | **PXO99Af** | **BLS256g** |
| XopB | + | + | + | + | ND | ND | ND |
| XopC2 | + | + | + | + | ND | + | + |
| XopE1 | + | + | + | ND | ND | ND | ND |
| XopE2 | + | + | ND | + | + | ND | ND |
| XopE3 | ND | ND | ND | + | ND | ND | ND |
| XopE5 | + | + | + | ND | ND | ND | ND |
| XopF | ++ | ++ | ++ | ++ | + | + | + |
| XopG | + | + | + | + | ND | + | ND |
| XopJ | + | ND | ND | + | + | ND | ND |
| XopK | + | + | + | + | + | + | + |
| XopL | ++++ | ++++ | ++ | ++ | + | + | + |
| XopN | + | + | + | + | + | + | + |
| XopP | +++ | +++ | ++ | +++ | + | + | ++ |
| XopQ | + | + | + | + | + | + | + |
| T3Es | **NXtc01a** | **CFBP2541b** | **ICMP11055c** | **DSM18974Td** | **8004e** | **PXO99Af** | **BLS256g** |
| XopR | F | F | + | + | + | + | + |
| XopV | + | + | + | + | ND | + | + |
| XopX | +++ | +++ | +++ | +++ | ++ | + | + |
| XopY | + | + | + | + | ND | + | + |
| XopZ | + | + | + | + | + | ++ | + |

Abbreviations: The number of plus symbols indicates the copy number of T3Es in a given strain. ND, not detected; F, frameshift mutation.

a This study

b (Pesce et al., 2015)

c (Falahi Charkhabi et al., 2017)

d (Jaenicke et al., 2016)

e (Qian et al., 2005)

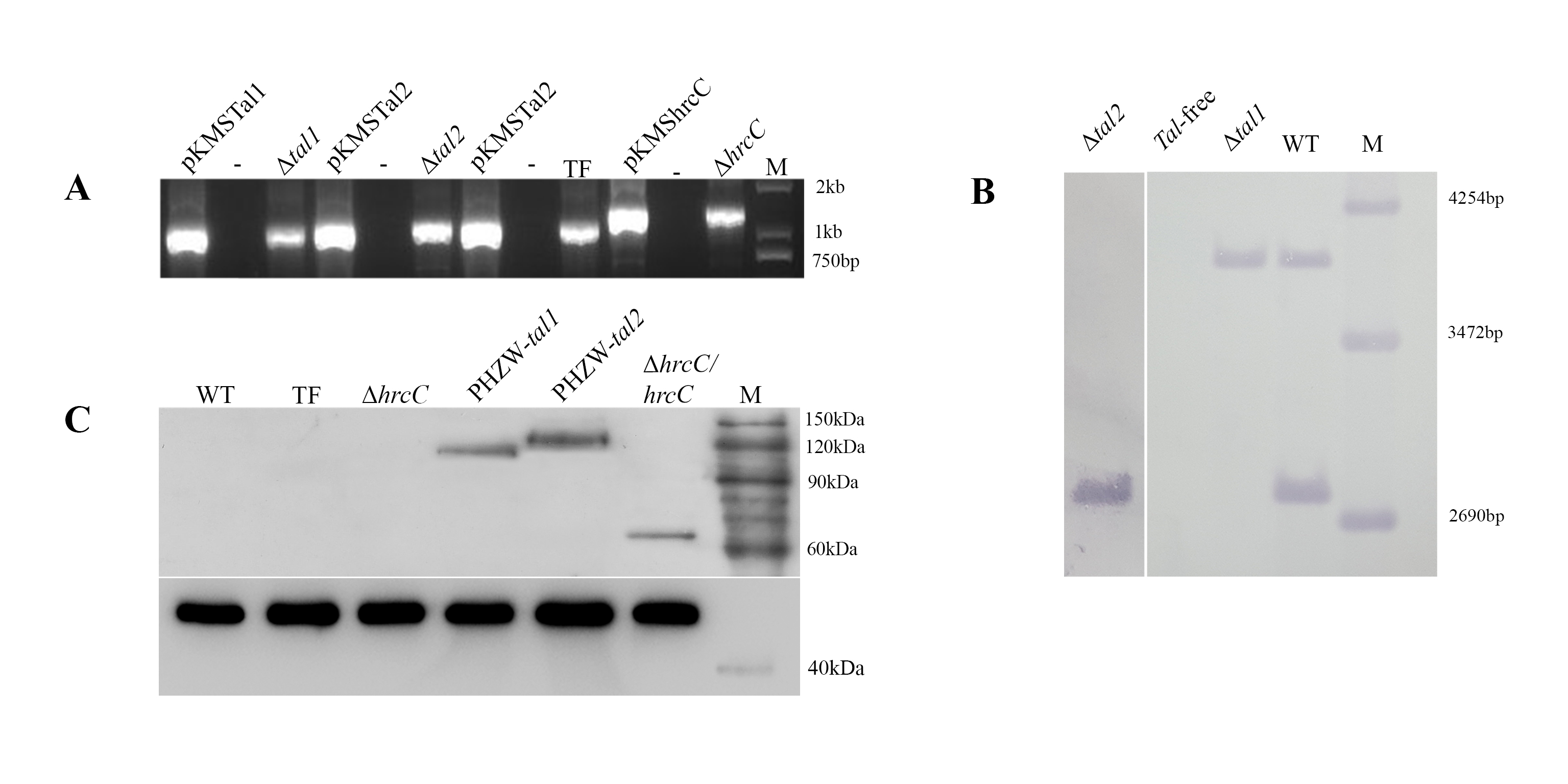
f (Salzberg et al., 2008)

g (Bogdanove et al., 2011)

**Table S4.** COG functional classifications and colors for Figure 1.

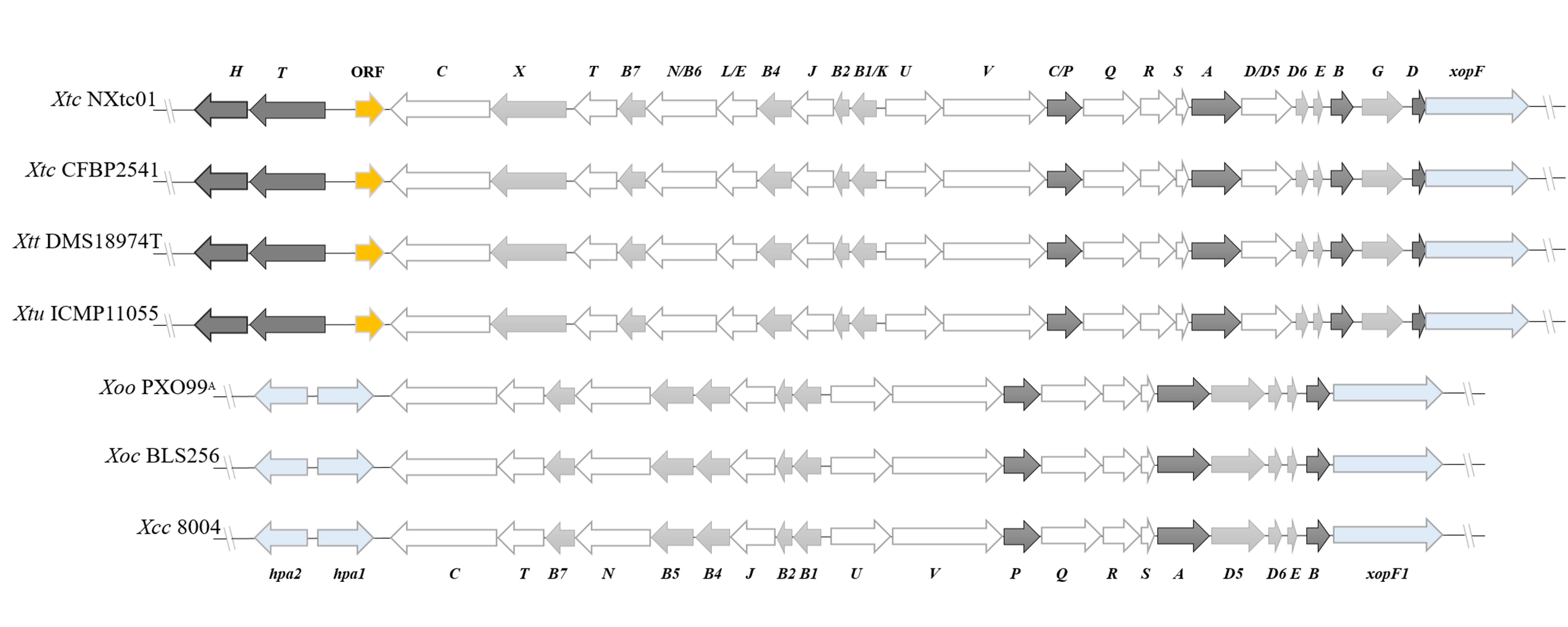
|  |  |  |
| --- | --- | --- |
| **Single letter COG class** | **Functional description** | **Color in Figure 1** |
| A | RNA processing and modification | Orange3 |
| B | Chromatin structure | Black |
| C | Energy production/conversion | RoyalBlue4 |
| D | Cell division/chromosome partitioning | AntiqueWhite1 |
| E | Amino acid transport/metabolism | DodgerBlue1 |
| F | Nucleotide transport/metabolism | SkyBlue3 |
| G | Carbohydrate transport/metabolism | Blue1 |
| H | Coenzyme transport/metabolism | LightBlue1 |
| I | Lipid transport/metabolism | Cyan3 |
| J | Translation/ribosomal structure | Gold1 |
| K | Transcription | DarkOrange1 |
| L | Replication/recombination/repair | DarkOrange3 |
| M | Cell wall/membrane/envelope biogenesis | PeachPuff3 |
| N | Cell motility | MediumPurple1 |
| O | Chaperones | PaleGreen1 |
| P | Inorganic ion metabolism | MediumPurple4 |
| Q | Secondary metabolite biosynthesis/transport/catabolism | Aquamarine4 |
| R | General function prediction | Gray90 |
| S | Function unknown | Gray70 |
| T | Signal transduction | Tomato1 |
| U | Intracellular trafficking/secretion | DeepPink |
| V | Defense mechanisms | Pink |
| W | Extracellular structures | Green |
| Y | Nuclear structure | Yellow |
| Z | Cytoskeleton | Red |
| None | No COG category | Gray50 |

Supplementary Figures



**Figure S1. Knockout and complementation analysis of *tal* and *hrcC* genes in NXtc01.** The *tal1*, *tal2*, *tal*-free and *hrcC* mutants were generated using suicide vector pKMS1. **(A)** Lanes show the constructs (pKMSTal1, pKMSTal2 and pKMShrcC) that were used as positive controls and mutants (Δ*tal1*, Δ*tal2*, TF and Δ*hrcC*) that were screened via PCR. Primers were as follows: T1Nt-F/T1Ct-R, amplifies a 1071-bp fragment; T2Ct-F/T2Nt-R, amplifies a 1124-bp fragment; and hrcC-up-F/hrcC-do-R, amplifies a 1317-bp fragment. **(B)** Southern blot of *Bam*HI-digested genomic DNA of WT and mutants, probed with a DIG-labeled *Sph*I fragment of the NXtc01 *tal1* gene. **(C)** Immunodetection of flag-tagged Tal1 (104.8 kDa) and Tal2 (119.3 kDa) in the *tal*-free mutant, and flag-tagged HrcC (66.8 kDa) in the Δ*hrcC* mutant. WT, *tal*-free and the Δ*hrcC* mutant were included as controls. Flag-tagged proteins were detected by autoradiography. An anti-E. coli RNA polymerase α antibody was used as loading control shown in lower panel. Marker sizes are indicated on the right side of each panel.

**Abbreviations:** M, marker; positive control, construct used for homologous recombination; (-) negative control, PCR without template DNA; WT, wild-type *Xtc* NXtc01; Δ*tal1*, NXtc01 *tal1* deletion mutant; Δ*tal2*, NXtc01 *tal2* deletion mutant; TF, NXtc01 *tal*-free mutant; PHZW-*tal1*, NXtc01 TF with *tal1* *in trans*; PHZW-*tal2*, NXtc01 TF with *tal2 in trans*; Δ*hrcC*, NXtc01 *hrcC* deletion mutant; and Δ*hrcC*/*hrcC*, NXtc01 Δ*hrcC* containing *hrcC in trans*.



**Figure S2.** Comparison of the T3SS gene clusters in *X. translucens* pv. *cerealis* Nxtc01, *X. translucens* pv. *cerealis* CFBP 2541 (Pesce et al., 2015)*,* *X. translucens* pv. *undulosa* ICMP11055 (Falahi Charkhabi et al., 2017), *X. translucens* pv. *translucens* DSM 18974T (Jaenicke et al., 2016), *X. campestris* pv. *campestris* 8004 (Qian et al., 2005), *X. oryzae* pv. *oryzae* PX099A (Salzberg et al., 2008) and *X. oryzae* pv. *oryzicola* BLS256 (Bogdanove et al., 2011). The *hrc*, *hrp*, *hpa*, T3Es and hypothetical genes are represented by white, light gray, dark gray, light blue and gold arrows, respectively; arrowheads show the transcriptional orientation. Distance between operons are not drawn to scale.

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