

Supplementary Material

Functional characterization of VirB/VirD4 and Icm/Dot type IV secretion systems from the plant-pathogenic bacterium *Xanthomonas euvesicatoria*

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Table S1: Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics ¹	Reference or source
<i>Xanthomonas euvesicatoria</i>		
85-10	Pepper-race 2; wild type; Rif ^r	Canteros, 1990; Kousik and Ritchie, 1998
85-10Δ <i>virB4</i>	<i>virB4</i> deletion mutant of strain 85-10	This study
85-10Δ <i>virD4</i>	<i>virD4</i> deletion mutant of strain 85-10	This study
85-10Δ <i>virG</i>	<i>virG</i> deletion mutant of strain 85-10	This study
85-10Δ <i>virA</i>	<i>virA</i> deletion mutant of strain 85-10	This study
85-10Δ <i>virB11</i> Δ <i>virB1</i>	<i>virB11</i> and <i>virB1</i> deletion mutant of strain 85-10	This study
85-10Δ <i>virD4</i> Δ <i>virB11</i> Δ <i>virB1</i>	<i>virD4</i> , <i>virB11</i> and <i>virB1</i> deletion mutant of strain 85-10	This study
85-10 Δ <i>virD4</i> Δ <i>virB11</i> Δ <i>virB1</i> Δ <i>virB4</i>	Derivative of strain 85-10 deleted in <i>virD4</i> , <i>virB11</i> , <i>virB1</i> and <i>virB4</i>	This study
85-10 Δ <i>virB6</i> Δ <i>virB8</i> Δ <i>virB9</i> Δ <i>virB10</i>	Derivative of strain 85-10 deleted in <i>virB6</i> , <i>virB8</i> , <i>virB9</i> and <i>virB10</i>	This study
85-10 Δ <i>virB6</i> Δ <i>virB8</i> Δ <i>virB9</i> Δ <i>virB10</i> Δ <i>icmE</i> Δ <i>dotCB</i>	Derivative of strain 85-10 deleted in <i>virB6</i> , <i>virB8</i> , <i>virB9</i> , <i>virB10</i> , <i>icmE</i> , <i>dotC</i> and <i>dotB</i>	This study
85-10Δ <i>dotCB</i>	Derivative of strain 85-10 deleted in <i>dotC</i> and <i>dotB</i>	This study
85-10Δ <i>icmE</i>	<i>icmE</i> deletion mutant of strain 85-10	This study
85-10Δ <i>dotCB</i> Δ <i>icmE</i>	Derivative of strain 85-10 deleted in <i>dotC</i> , <i>dotB</i> and <i>icmE</i>	This study
85-10Δ <i>virB4</i> Δ <i>icmE</i>	Derivative of strain 85-10 deleted in <i>virB4</i> and <i>icmE</i>	This study
85-10Δ <i>dotCB</i> Δ <i>icmE</i> Δ <i>virB4</i>	Derivative of strain 85-10 deleted in <i>dotC</i> , <i>dotB</i> , <i>icmE</i> and <i>virB4</i>	This study
85-10:: <i>kan</i> ^R	Insertion mutant of 85-10 carrying <i>gfp</i> and the kanamycin resistance gene under control of the gentamycin resistance promoter of pBRM on pXCV183; Rif ^r , Km ^r	This study
85-10Δ <i>xopQ</i>	<i>xopQ</i> deletion mutant of strain 85-10	Adlung et al., 2016
82-8	Pepper-race 1; wild type; Rif ^r	Bonas et al., 1989
85-10:: <i>gent</i> ^R	Insertion mutant of 85-10 carrying the gentamycin resistance gene under control of the <i>lac</i> promoter in the <i>hpaFG</i> region; Rif ^r , Gm ^r	This study
85-10Δ <i>virD4</i> :: <i>gent</i> ^R	Insertion mutant of 85-10Δ <i>virD4</i> carrying the gentamycin resistance gene under control of the <i>lac</i> promoter in the <i>hpaFG</i> region; Rif ^r , Gm ^r	This study
85-10Δ <i>virB4</i> :: <i>gent</i> ^R	Insertion mutant of 85-10Δ <i>virB4</i> carrying the gentamycin resistance gene under control of the <i>lac</i> promoter in the <i>hpaFG</i> region; Rif ^r , Gm ^r	This study
<i>E. coli</i>		
OneShot®TOP10	F ⁻ <i>mcrA</i> Δ(<i>mrr</i> - <i>hsdRMS</i> - <i>mcrBC</i>) φ80 <i>lacZ</i> ΔM15 Δ <i>lacX74</i> <i>recA1</i> <i>ara</i> Δ139 Δ(<i>ara-leu</i>)7697 <i>galU</i> <i>galK</i> <i>rpsL</i> <i>endA1</i> <i>nupG</i> (Str ^r)	Invitrogen
Dh5α λpir	F ⁻ <i>recA</i> <i>hsdR17</i> (<i>r_K</i> ⁻ , <i>m_K</i> ⁺) φ80 <i>dlacZ</i> DM15 [λ <i>pir</i>]	Ménard et al., 1993
BL21(DE3)	F ⁻ <i>ompt</i> <i>hsdS20</i> (<i>r_S</i> ⁻ , <i>m_S</i> ⁻) <i>gal</i>	Stratagene
Rosetta DE3 pLysS		
JM109	F ⁻ , <i>traD36</i> <i>proA</i> + <i>B</i> + <i>lacIq</i> Δ(<i>lacZ</i>)M15/ Δ(<i>lac-proAB</i>) <i>glnV44</i> <i>e14</i> - <i>gyrA96</i> <i>recA1</i> <i>relA1</i> <i>endA1</i> <i>thi</i> <i>hsdR17</i>	Yanisch-Perron et al., 1985
BTH101	F ⁻ , <i>cya-99</i> , <i>araD139</i> , <i>galE15</i> , <i>galK16</i> , <i>rpsL1</i> (<i>StrR</i>), <i>hsdR2</i> , <i>mcrA1</i> , <i>mcrB1</i>	Euromedex; Battesti and Bouveret, 2012
Plasmids		
pRK2013	ColE1 replicon, TraRK ⁺ Mob ⁺ ; Km ^r	Figurski and Helinski, 1979
pBBR1MCS-5	Broad-host-range vector; <i>lac</i> promoter; Gm ^r	Kovach et al., 1995
pBRM	Golden Gate-compatible derivative of pBBR1MCS-5	Szczesny et al., 2010
pBRM-P	Golden Gate-compatible derivative of pBBR1MCS-5 without promoter	Szczesny et al., 2010

pBRM-P(stop)	Golden Gate-compatible derivative of pBRM-P with a stop codon in the 3' region	Hausner et al., 2013
pBRNM	Golden Gate-compatible derivative of pBBR1MCS-5 with 3 x c-Myc-encoding sequence in the 5' region of the inserted gene and a stop codon in the 3' region; Gm ^r	This study
pBRM-P+T	Golden Gate-compatible derivative of pBBR1MCS-5 without promoter upstream of the cloning sites and an <i>rrnB T1</i> terminator	Helm and Schmidtke, unpublished
pDSK602	Broad-host-range vector containing a <i>lacUV5</i> promoter; Sm ^r	Murillo et al., 1994
pICH41021	Derivative of pUC19 with mutated <i>BsaI</i> site; Ap ^r	Hausner et al., 2019
pUC57	Classical cloning vector with mutated <i>BsaI</i> site; Ap ^r	Thermo Fisher Scientific
pLAND	Derivative of pOK1 containing fragments of the <i>hpaFG</i> region, the <i>lac</i> promoter and a triple <i>c-Myc</i> epitope-encoding sequence; Sm ^r	Lorenz et al., 2012
pLAND-P	Derivative of pOK1 containing fragments of the <i>hpaFG</i> region, a triple <i>c-Myc</i> epitope-encoding sequence and lacking a promoter upstream of the <i>BsaI</i> sites; Sm ^r	Lorenz et al., 2012
pOGG2	Golden Gate-compatible derivative of suicide vector pOK1, <i>sacB sacQ mobRK2 oriR6K</i> ; Sm ^r	Schulze et al., 2012
pAGM9121	pUC19-derived vector, <i>lacZα</i> fragment flanked by <i>BpiI</i> sites (CTCA/CGAG); GGAG/CGCT <i>BsaI</i> fusion sites; Sm ^r	Addgene #51833; Weber et al., 2011
pICH47732	Derived from pUC19, level 1 for promoters; <i>lacZα</i> fragment flanked by <i>BsaI</i> sites (GGAG/CGCT); TGCC/GCAA <i>BpiI</i> fusion sites; Ap ^r	Addgene #48000; Weber et al., 2011
pICH79631	Derived from pICH50251, containing dTALE-2 with stop-codon for position 2 in level M; GCAA/ACTA <i>BpiI</i> fusion sites; Ap ^r	Gift from S. Marillonnet
pICH50122	Derived from pUC19, end linker for position 3 contains a terminator sequence; ACTA/ GGGA <i>BpiI</i> fusion sites; Ap ^r	Gift from S. Marillonnet
pICH77739	Level 2 vector derived from pBIN19, RK2 ori, <i>lacZα</i> fragment flanked by <i>BpiI</i> sites; Km ^r	Weber et al., 2011
pKT25 _{GG}	Golden Gate-compatible derivative of pKT25 encoding the T25 fragment downstream of a <i>lac</i> promoter and in frame with a C-terminal FLAG epitope-encoding sequence; contains <i>lacP-eforRed</i> flanked by <i>BsaI</i> sites downstream of the <i>T25-FLAG</i> fragment; Km ^r	Otten and Büttner, 2021
pKNT25 _{GG}	Golden Gate-compatible derivative of pKNT25, encodes the T25 fragment in frame with an N-terminal FLAG epitope-encoding sequence downstream of the <i>lac</i> promoter, contains <i>lacP-eforRed</i> flanked by <i>BsaI</i> sites upstream of the <i>T25-FLAG</i> fragment; Km ^r	Otten and Büttner, 2021
pUT18 _{GG}	Golden Gate-compatible derivative of pUT18 containing <i>lacP-eforRed</i> flanked by <i>BsaI</i> sites upstream of the <i>FLAG-T18</i> fragment; Gm ^R	Otten and Büttner, 2021
pUT18 _{C_{GG}}	Golden Gate-compatible derivative of pUT18C containing <i>lacP-eforRed</i> flanked by <i>BsaI</i> sites downstream of the <i>T18-FLAG</i> fragment; Gm ^R	Otten and Büttner, 2021
pICH41021-virD4 ₁₋₉₅₀	Derivative of pICH41021 containing bp 1 - 950 of <i>virD4</i> flanked by <i>BsaI</i> sites with TATG and GCCT overhangs	This study
pICH41021-virD4 ₉₅₁₋₁₄₅₀	Derivative of pICH41021 containing bp 951 - 1450 of <i>virD4</i> flanked by <i>BsaI</i> sites with GCCT and GTCA overhangs	This study
pICH41021-virD4 ₁₄₅₁₋₁₆₇₁	Derivative of pICH41021 containing bp 1451 – 1671 of <i>virD4</i> flanked by <i>BsaI</i> sites with GTCA and GGTG overhangs	This study

pUC57-XCV4361 _{prom}	Derivative of pUC57 containing the putative promoter of XCV4361 flanked by <i>Bsal</i> sites with ATTC and TATG overhangs	This study
pICH41021-traI	Derivative of pICH41021 containing <i>traI</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pUC57-XCV0332	Derivative of pUC57 containing XCV0332 flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pICH41021-XCV3751	Derivative of pICH41021 containing <i>XCV3751</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pUC57-XCV1120	Derivative of pUC57 containing <i>XCV1120</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pICH41021-virB10	Derivative of pICH41021 containing <i>virB10</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pICH41021-virB11	Derivative of pICH41021 containing <i>virB11</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pICH41021- virB7	Derivative of pICH41021 containing <i>virB7</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pICH41021-dotB	Derivative of pICH41021 containing <i>dotB</i> flanked by <i>Bsal</i> sites with TATG and GGTG fusion sites	This study
pICH41021-dotO ₁₋₁₀₉	Derivative of pICH41021 containing bp 1 - 109 of <i>dotO</i> flanked by <i>Bsal</i> sites with TATG and AGAC overhangs	This study
pICH41021-dotO ₁₁₀₋₃₀₄₈	Derivative of pICH41021 containing bp 110 - 3048 of <i>dotO</i> flanked by <i>Bsal</i> sites with AGAC and GTG overhangs	This study
pICH41021-icmE ₁₋₆₄₅	Derivative of pICH41021 containing bp 1 - 645 of <i>icmE</i> flanked by <i>Bsal</i> sites with TATG and ACGA overhangs	This study
pICH41021-icmE ₆₄₆₋₇₉₆	Derivative of pICH41021 containing bp 646 - 796 of <i>icmE</i> flanked by <i>Bsal</i> sites with ACGA and GACG overhangs	This study
pICH41021-icmE ₇₉₇₋₁₂₄₈	Derivative of pICH41021 containing bp 797 - 1248 of <i>icmE</i> flanked by <i>Bsal</i> sites with GACG and GGTG overhangs	This study
pICH41021-dotL ₁₋₁₄₆₀	Derivative of pICH41021 containing bp 1 - 1460 of <i>dotL</i> flanked by <i>Bsal</i> sites with TATG and ACCG overhangs	This study
pICH41021-dotL ₁₄₆₁₋₂₀₀₁	Derivative of pICH41021 containing bp 1461 - 2001 of <i>dotL</i> flanked by <i>Bsal</i> sites with ACCG and GCGG overhangs	This study
pICH41021-dotL ₂₀₀₂₋₂₅₆₈	Derivative of pICH41021 containing bp 2002 - 2568 of <i>dotL</i> flanked by <i>Bsal</i> sites with GCGG and GGTG overhangs	This study
pXCV38 _{82-8::spec^R}	Derivative of the native plasmid pXCV38 from <i>X. euvesicatoria</i> strain 82-8 containing a spectinomycin resistance gene inserted in <i>avrBs3</i>	Bonas et al., 1989
Expression constructs and suicide vectors		
pDSF300	Derivative of pDSK602 encoding AvrBs3	Van den Ackerveken et al., 1996
pBRNM-XCV3751	Derivative of pBRNM encoding c-Myc-XCV3751 under control of the <i>lac</i> promoter	This study
pBRNM-XCV1120	Derivative of pBRNM encoding c-Myc-XCV1120 under control of the <i>lac</i> promoter	This study
pBRNM-XCV0332	Derivative of pBRNM encoding c-Myc-XCV0332 under control of the <i>lac</i> promoter	This study
pBRM-P(stop)-XCV4361 _{prom} -c-Myc-traI	Derivative of pBRM-P(stop) encoding c-Myc-TraI under control of the putative <i>XCV4361</i> promoter	This study
pLAND-virB4	Derivative of pLAND containing <i>virB4</i>	This study
pLAND-P-XCV0160 _{prom} -icmE	Derivative of pLAND-P containing <i>icmE</i> under the control of the XCV0160 promoter	This study
pLAND-gent ^R	Derivative of pLAND containing the gentamycin resistance gene cassette from vector pBRM	This study

pOGG2-kan ^R gfp(pXCV183)	Derivative of pOGG2 carrying the flanking sequences of nucleotide position 2831 in pXCV183 and containing the kanamycin resistance and the <i>gfp</i> gene	This study
pOGG2-ΔvirB4	Derivative of pOGG2 carrying flanking sequences of <i>virB4</i>	This study
pOGG2-ΔvirD4	Derivative of pOGG2 carrying flanking sequences of <i>virD4</i>	This study
pOGG2-ΔvirG	Derivative of pOGG2 carrying flanking sequences of <i>virG</i>	This study
pOGG2-ΔvirA	Derivative of pOGG2 carrying flanking sequences of <i>virA</i>	This study
pOGG2-ΔvirB11ΔvirB1	Derivative of pOGG2 carrying flanking sequences of <i>virB11</i> and <i>virB1</i>	This study
pOGG2-ΔvirB6ΔvirB8ΔvirB9ΔvirB10	Derivative of pOGG2 carrying flanking sequences of <i>virB6</i> and <i>virB10</i>	This study
pOGG2-ΔicmE	Derivative of pOGG2 carrying flanking sequences of <i>icmE</i>	This study
pOGG2-ΔdotCB	Derivative of pOGG2 carrying flanking sequences of <i>dotC</i> and <i>dotB</i>	This study
BACTH constructs		
pUT18 _{GG} -virD4	Derivative of pUT18 _{GG} encoding VirD4-FLAG-T18	This study
pUT18 _{CGG} -virD4	Derivative of pUT18 _{CGG} encoding T18-FLAG-VirD4	This study
pKNT25 _{GG} -virD4	Derivative of pKNT25- _{GG} encoding VirD4-FLAG-T25	This study
pKT25 _{GG} -virD4	Derivative of pKT25 _{GG} encoding T25-FLAG-VirD4	This study
pUT18 _{GG} -dotL	Derivative of pUT18 _{GG} encoding DotL-FLAG-T18	This study
pUT18 _{CGG} -dotL	Derivative of pUT18 _{CGG} encoding T18-FLAG-DotL	This study
pKNT25 _{GG} -dotL	Derivative of pKNT25- _{GG} encoding DotL-FLAG-T25	This study
pKT25 _{GG} -dotL	Derivative of pKT25 _{GG} encoding T25-FLAG-DotL	This study
pUT18 _{GG} -XCV3751	Derivative of pUT18 _{GG} encoding XCV3751-FLAG-T18	This study
pUT18 _{CGG} -XCV3751	Derivative of pUT18 _{CGG} encoding T18-FLAG-XCV3751	This study
pKNT25 _{GG} -XCV3751	Derivative of pKNT25- _{GG} encoding XCV3751-FLAG-T25	This study
pKT25 _{GG} -XCV3751	Derivative of pKT25 _{GG} encoding T25-FLAG-XCV3751	This study
pUT18 _{GG} -XCV0332	Derivative of pUT18 _{GG} encoding XCV0332-FLAG-T18	This study
pUT18 _{CGG} -XCV0332	Derivative of pUT18 _{CGG} encoding T18-FLAG-XCV0332	This study
pKNT25 _{GG} -XCV0332	Derivative of pKNT25- _{GG} encoding XCV0332-FLAG-T25	This study
pKT25 _{GG} -XCV0332	Derivative of pKT25 _{GG} encoding T25-FLAG-XCV0332	This study
pUT18 _{GG} -XCV1120	Derivative of pUT18 _{GG} encoding XCV1120-FLAG-T18	This study
pUT18 _{CGG} -XCV1120	Derivative of pUT18 _{CGG} encoding T18-FLAG-XCV1120	This study
pKNT25 _{GG} -XCV1120	Derivative of pKNT25- _{GG} encoding XCV1120-FLAG-T25	This study
pKT25 _{GG} -XCV1120	Derivative of pKT25 _{GG} encoding T25-FLAG-XCV1120	This study
pUT18 _{GG} -traI	Derivative of pUT18 _{GG} encoding TraI-FLAG-T18	This study
pUT18 _{CGG} -traI	Derivative of pUT18 _{CGG} encoding T18-FLAG-TraI	This study
pKNT25 _{GG} -traI	Derivative of pKNT25- _{GG} encoding TraI-FLAG-T25	This study
pKT25 _{GG} -traI	Derivative of pKT25 _{GG} encoding T25-FLAG-TraI	This study
pUT18 _{GG} -virB10	Derivative of pUT18 _{GG} encoding VirB10-FLAG-T18	This study
pUT18 _{CGG} -virB10	Derivative of pUT18 _{CGG} encoding T18-FLAG-VirB10	This study
pKNT25 _{GG} -virB10	Derivative of pKNT25- _{GG} encoding VirB10-FLAG-T25	This study
pKT25 _{GG} -virB10	Derivative of pKT25 _{GG} encoding T25-FLAG-VirB10	This study
pUT18 _{GG} -virB11	Derivative of pUT18 _{GG} encoding VirB11-FLAG-T18	This study
pUT18 _{CGG} -virB11	Derivative of pUT18 _{CGG} encoding T18-FLAG-VirB11	This study
pKNT25 _{GG} -virB11	Derivative of pKNT25- _{GG} encoding VirB11-FLAG-T25	This study
pKT25 _{GG} -virB11	Derivative of pKT25 _{GG} encoding T25-FLAG-VirB11	This study
pUT18 _{GG} -virB7	Derivative of pUT18 _{GG} encoding VirB7-FLAG-T18	This study

pUT18 _{CGG} -virB7	Derivative of pUT18 _{CGG} encoding T18-FLAG-VirB7	This study
pKNT25 _{GG} -virB7	Derivative of pKNT25- _{GG} encoding VirB7-FLAG-T25	This study
pKT25 _{GG} -virB7	Derivative of pKT25 _{GG} encoding T25-FLAG-VirB7	This study
pUT18 _{GG} -dotB	Derivative of pUT18 _{GG} encoding DotB-FLAG-T18	This study
pUT18 _{CGG} -dotB	Derivative of pUT18 _{CGG} encoding T18-FLAG-DotB	This study
pKNT25 _{GG} -dotB	Derivative of pKNT25- _{GG} encoding DotB-FLAG-T25	This study
pKT25 _{GG} -dotB	Derivative of pKT25 _{GG} encoding T25-FLAG-DotB	This study
pUT18 _{GG} -dotO	Derivative of pUT18 _{GG} encoding DotO-FLAG-T18	This study
pKNT25 _{GG} -dotO	Derivative of pKNT25- _{GG} encoding DotO-FLAG-T25	This study
pKT25 _{GG} -dotO	Derivative of pKT25 _{GG} encoding T25-FLAG-DotO	This study
pUT18 _{GG} -icmE	Derivative of pUT18 _{GG} encoding IcmE-FLAG-T18	This study
pUT18 _{CGG} -icmE	Derivative of pUT18 _{CGG} encoding T18-FLAG-IcmE	This study
pKNT25 _{GG} -icmE	Derivative of pKNT25- _{GG} encoding IcmE-FLAG-T25	This study
pKT25 _{GG} -icmE	Derivative of pKT25 _{GG} encoding T25-FLAG-IcmE	This study
Constructs for promoter-reporter assays		
pICH41021-virB2 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virB2</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-virB5 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virB5</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-virD4 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virD4</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-virG _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virG</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-dotA _{prom}	Derivative of pICH41021 containing the putative promoter of <i>dotA</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-dotD _{prom}	Derivative of pICH41021 containing the putative promoter of <i>dotD</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-icmL _{prom(229)}	Derivative of pICH41021 containing 229 bp upstream of the annotated start site of <i>icmL</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-icmL _{prom(129)}	Derivative of pICH41021 containing 129 bp downstream of the annotated start site of <i>icmL</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	This study
pICH41021-hrpB1 _{prom}	Derivative of pICH41021 containing the promoter of <i>hrpB1</i> flanked by <i>Bsal</i> sites with ATTC and TATG overhangs for insertion into pBRM-P	Hausner et al., 2019
pICH41021-sfgfp	Derivative of pICH41021 containing <i>sfgfp</i> flanked by <i>Bsal</i> sites with TATG and GGTG overhangs for insertion into pBRM	Hausner and Büttner, unpublished
pBRM-P+T-virB2 _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>virB2</i> promoter	This study
pBRM-P+T-virB5 _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>virB5</i> promoter	This study
pBRM-P+T-virD4 _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>virD4</i> promoter	This study
pBRM-P+T-virG _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>virG</i> promoter	This study
pBRM-P+T-dotA _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>dotA</i> promoter	This study
pBRM-P+T-dotD _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>dotD</i> promoter	This study

pBRM-P+T-icmL _{prom(229)} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>icmL</i> promoter upstream of the annotated start codon	This study
pBRM-P+T-icmL _{prom(129)} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the putative <i>icmL</i> promoter downstream of the annotated start codon	This study
pBRM-P+T-hrpB1 _{prom} -sfgfp	Derivative of pBRM-P+T encoding sfGFP under control of the <i>hrpB1</i> promoter	This study
pBRM-sfgfp _{plac}	Derivative of pBRM encoding sfGFP under control of the <i>lac</i> promoter	Hausner and Büttner, unpublished
pICH41021-virB2 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virB2</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-virB5 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virB5</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-virD4 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virD4</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-virG _{prom}	Derivative of pICH41021 containing the putative promoter of <i>virG</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-dotA _{prom}	Derivative of pICH41021 containing the putative promoter of <i>dotA</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-dotD _{prom}	Derivative of pICH41021 containing the putative promoter of <i>dotD</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-icmL _{prom(229)}	Derivative of pICH41021 containing a putative promoter 229 bp upstream of the annotated start codon of <i>icmL</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-icmL _{prom(129)}	Derivative of pICH41021 containing a putative promoter 129 bp downstream of the annotated start codon of <i>icmL</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pICH41021-hrpB1 _{prom}	Derivative of pICH41021 containing the putative promoter of <i>hrpB1</i> flanked by <i>Bpil</i> sites with CTCA and CGAG overhangs for fusion with <i>dTALE-2</i>	This study
pAGM9121-virB2 _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>virB2</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-virB5 _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>virB5</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-virD4 _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>virD4</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-virG _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>virG</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-dotA _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>dotA</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-dotD _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>dotD</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-icmL _{prom(229)}	Derivative of pAGM9121 containing the putative promoter 229 bp upstream of the annotated start codon of <i>icmL</i> flanked by <i>Bsal</i> sites with GGAG and CGCT overhangs	This study

pAGM9121-icmL _{prom(129)}	Derivative of pAGM9121 containing the putative promoter 129 bp downstream of the annotated start codon of <i>icmL</i> flanked by <i>BsaI</i> sites with GGAG and CGCT overhangs	This study
pAGM9121-hrpB1 _{prom}	Derivative of pAGM9121 containing the putative promoter of <i>hrpB1</i> flanked by <i>BsaI</i> sites with GGAG and CGCT overhangs	This study
pICH47732-virB2 _{prom}	Derivative of pICH47732 containing the putative promoter of <i>virB2</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-virB5 _{prom}	Derivative of pICH47732 containing the putative promoter of <i>virB5</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-virD4 _{prom}	Derivative of pICH47732 containing the putative promoter of <i>virD4</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-virG _{prom}	Derivative of pICH47732 containing the putative promoter of <i>virG</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-dotA _{prom}	Derivative of pICH47732 containing the putative promoter of <i>dotA</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-dotD _{prom}	Derivative of pICH47732 containing the putative promoter of <i>dotD</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-icmL _{prom(229)}	Derivative of pICH47732 containing the putative promoter 229 bp upstream of the annotated start codon of <i>icmL</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-icmL _{prom(129)}	Derivative of pICH47732 containing the putative promoter 129 bp downstream of the annotated start codon of <i>icmL</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH47732-hrpB1 _{prom}	Derivative of pICH47732 containing the putative promoter of <i>hrpB1</i> flanked by <i>BpiI</i> sites with TGCC and GCAA overhangs	This study
pICH77739-virB2 _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>virB2</i> promoter	This study
pICH77739-virB5 _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>virB5</i> promoter	This study
pICH77739-virD4 _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>virD4</i> promoter	This study
pICH77739-virG _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>virG</i> promoter	This study
pICH77739-dotA _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>dotA</i> promoter	This study
pICH77739-dotD _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>dotD</i> promoter	This study
pICH77739-icmL _{prom(229)} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>icmL</i> promoter	This study
pICH77739-icmL _{prom(129)} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the putative <i>icmL_{new}</i> promoter	This study
pICH77739-hrpB1 _{prom} -dTALE2	Derivative of pICH77739 encoding dTALE-2 under control of the <i>hrpB1</i> promoter	This study

¹ Ap, ampicillin; Gm, gentamycin; Km, kanamycin; R, resistant; Rif, rifampicin; Sm, spectinomycin; Str, streptomycin.

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Table S2: Primers used in this study.

Name	Sequence ¹
Primers for the generation of expression constructs	
virD4-Bsa-for	TTT GGTCTCT <i>TATG</i> TCCAAACCCAAAATCATCG
virD4-950-rev	TTT GGTCTCT <i>AGGC</i> GAAGGTTTCATCCGCCTG
virD4-950-for	TTT GGTCTCT <i>GCCT</i> CGATCATGGGCACCTTG
virD4-1450-rev	TTT GGTCTCT <i>TGAC</i> GTTCTTCTTCTTGATGG
virD4-1450-for	TTT GGTCTCT <i>GTCA</i> CCCGAGGGCGGGAAACCAC
virD4-Bsa-rev	TTT GGTCTCT <i>CACC</i> CACTTTGATGGTAAGC
traI-pB-for	TTT GGTCTCT <i>TATG</i> ATGCTCAATGTGACGCCGATTCCG
traI-pB-rev	TTT GGTCTCT <i>CACC</i> GCCAAAGCGTCCGTACTCGGACG
3751-Bsa-for	TTT GGTCTCT <i>TATG</i> CCGACGAACTACTCAC
3751-Bsa-rev	TTT GGTCTCT <i>CACC</i> ACCGACGCTGCGCCCCTGGC
XCV0332-for	TTT GGTCTCT <i>TATG</i> ACCGACGCACCGGTCATC
XCV0332-rev	TTT GGTCTCT <i>CACC</i> GCGCGGCGGCAAACGCAG
XCV1120-for	TTT GGTCTCT <i>TATG</i> GTGGAAGTCCGGCAAATC
XCV1120-rev	TTT GGTCTCT <i>CACC</i> CATTGTCATCCTTG
icmE-F1_for	TTT GGTCTCT <i>TATG</i> ATGGCTCAGCAGGAAAGCGATATC
icmE-F1-rev	TTT GGTCTCT <i>TCGT</i> CGGAGGCCGTACTTGCGAC
icmE-F2-for	TTT GGTCTCT <i>ACGA</i> CATGGCGGAAACCACCAG
icmE-F2-rev	TTT GGTCTCT <i>CGTC</i> CCGCGCATGACGCTTC
icmE-F3-for	TTT GGTCTCT <i>GACG</i> ATCGAAACCAGCGAGTTCG
icmE-F3-rev	TTT GGTCTCT <i>CACC</i> TTTGCCGAATGCGGATGC
dotB-for	TTT GGTCTCT <i>TATG</i> AACGCGGTGCTCTCCGACCAG
dotB-rev	TTT GGTCTCT <i>CACC</i> CTCACCCTCCCCGGCCAG
dotO-F1-for	TTT GGTCTCT <i>TATG</i> AAGATCGTCGAGCAAATCGCAGATATTG
dotO-F1-rev	TTT GGTCTCT <i>GTCT</i> TCGGTGTATTTCGATGTCCAGGTAG
dotO-F2-for	TTT GGTCTCT <i>AGAC</i> GAAACCACCTTCGTGCTCAAG
dotO-F2-rev	TTT GGTCTCT <i>CACC</i> TGCACGGCGGTCACCTTG
dotL-F1-for	TTT GGTCTCT <i>TATG</i> AGCATCCAGGGCGTCGACAAGC
dotL-F1-rev	TTT GGTCTCT <i>CGGT</i> GGGGTCCTCAACCTTCCCAATG
dotL-F2-for	TTT GGTCTCT <i>ACCG</i> AAACCTGGGAGATGATCCAAGCAAT GGGCGGCGAAG
dotL-F2-rev	TTT GGTCTCT <i>CCGC</i> CGGCGCTGCCGCCACCAGATACAG
dotL-F3-for	TTT GGTCTCT <i>GCGG</i> TGGACTCAATGCTCAGCCGCAG
dotL-F3-rev	TTT GGTCTCT <i>CACC</i> AAGCTCATTCGGCGCTCCTGAACTG
virB10-for	TTT GGTCTCT <i>TATG</i> AGCGATCAGACCAACAAGCCGCAG
virB10-rev	TTT GGTCTCT <i>CACC</i> CGGGATGAACGTCACGCCGTCG
virB11-for	TTT GGTCTCT <i>TATG</i> TCGCAGATTGCCTCCTTC
virB11-rev	TTT GGTCTCT <i>CACC</i> CGCACCTGCATAGATG
XCVc0034-for	TTT GGTCTCT <i>TATG</i> CCTAAGGCCCTCTGGACATG
XCVc0034-rev	TTT GGTCTCT <i>CACC</i> GTGTCCGATTGGTTGTCATCC
prom4361-for	TTT GGTCTCT <i>ATTC</i> AGGACAACGCGGTTTCGTG
prom4361-rev	TTT GGTCTCT <i>CATA</i> GCGCGTGGCCTATGCG
Bsa-prom0160-for	TTT GGTCTCT <i>ATTC</i> TGATCGAAGATGCTGCTG
Bsa-prom0160-rev	TTT GGTCTCT <i>GATG</i> TGGACAGCGGCGCAG
Bsa-icmE-for-pLAND	TTT GGTCTCT <i>CATC</i> ATGATGGCTCAGCAGGAAAGC
Gent-Bsa-for	TTT GGTCTCT <i>TATG</i> TTACGCAGCAGCAAC
Gent-Bsa-Stop-rev	TTT GGTCTCT <i>CACC</i> TTAGGTGGCGGTAATTG

Primers for colony PCR	
dotC-detect-for	TTGATTTTCGGCTCCTTGCTC
dotC-detect-rev	ATCTGGTCTGCCTGCTGCTC
dotB-detect-for	CTTCTGGTCCATGCCAAAGC
dotB_detect_rev	CGCGGAGGATGATCGAGATA
icmE-F1-for	TTT GGTCTCT <i>TATG</i> ATGGCTCAGCAGGAAAGCGATATC
icmE-F3-rev	TTT GGTCTCT <i>CACC</i> TTTGCCGAATGCGGATGC
virB11-detect-for	GAATCTCGATGACGGCGTTC
virB11-detect-rev	GAATCTCGCCCATGATGAC
virB4-detect-for	GTTCCAGCGCTCCCACGGTC
virB4-detect-rev	GTGCGCGTTGTGCAACACG
virB10-for	TTT GGTCTCT <i>TATG</i> AGCGATCAGACCAACAAGCCGCAG
virB10-rev	TTT GGTCTCT <i>CACC</i> CGGGATGAACGTCACGCCGTCG
virD4-detect-for	GGAGAACTTCGACCTGACCA
virD4-detect-rev	GTCCGTGAGCAGGAAGTCAT
avrBs3-Bsa-for	TTT GGTCTCT <i>TATG</i> GATCCCATTCGTTT
avrBs3-Bsa-50 rev	TTT GGTCTCT <i>GATC</i> GGACATCGTCCGCCGAGC
hpaF-seq-for	CTTCAATTGACGATGGTC
hpaG-seq-rev	CTGCATGAGGCCGATGTTTTTCAG
pKT25/pUT18-for	ACTGGAAAGCGGGCAGTGAG
pKT25-rev	GCTGCGCAACTGTTGGGAAG
pUT18-rev	GGTGTCGGGGCTGGCTTAAC
pBRM-1180-for	ATGCATGCGCCAATACGCAAACCG
pBRM-endlacZ-rev	CACTCATCGCAGTCGGCCTATTG
M13-for	GTA AAAACGACGGCCAGTG
M13-rev	GGAAACAGCTATGACCATG
pOK-mcs-for	CAAACCACGTCAAATAATCAATTATG
pOK-mcs-rev	CTTGTCCTTTAACGAGGATTGTTAC
Primers for the generation of deletion constructs	
pXCV183-F1-for	TTT GGTCTCT <i>CGAC</i> CCGGACAGCTCGGTTCCGGCCTTG
pXCV183-F1-rev	TTT GGTCTCT <i>ACTG</i> ATTGCACCATGCAACTAGCCCCG AGCTGAAG
pXCV183-F2-prom-for	TTT GGTCTCT <i>CAGT</i> TGACATAAGCCTGTTCGGTTTCG
pXCV183-F2-prom-rev	TTT GGTCTCT <i>CGTT</i> GCTGCTCCATAACATCAAACATCG
pXCV183-F3-kan-for	TTT GGTCTCT <i>AACG</i> ATGATTGAACAAGATGGATTGCA CGCAG
pXCV183-F3-kan-rev	TTT GGTCTCT <i>GAAT</i> TCAGAAGA AACTCGTCAAGAAGGC GATAGAAG
pXCV183-F4-promlacZ-for	TTT GGTCTCT <i>ATTG</i> GCAGCTGGCAGCAGACAG
pXCV183-F4-GFP-rev/	AAA GGTCTCT <i>CACC</i> TCATCATTTGTACAG
pXCV183-F5-for	TTT GGTCTCT <i>GGTG</i> TCAGGGCGCATCGGTATCGCCACGAG
pXCV183-F5-rev	TTT GGTCTCT <i>ATGG</i> GCCGCAGCCGATACTAAGCACCTAGAG
virG-pOGG2-1-for	TTT GGTCTCT <i>CGAC</i> CCTGGTGGGACACTCCTGGGG CGGCAC
virG-pOGG2-1-rev	TTT GGTCTCT <i>GGAA</i> TGCAGCTTGATCATCGACTCTCTATC
virG-pOGG2-2-for	TTT GGTCTCT <i>TTCC</i> GAGCACGCTCGATGACACAGCCGCC GGCGCGAGC
virG-pOGG2-2-rev	TTT GGTCTCT <i>ATGG</i> AGCAGCACGGCGCTGAAGATCGTC
pOGG2-virA/C-for	TTT GGTCTCT <i>CGAC</i> TGGAACCAGCAGTTCCAGCACG
pOGG2-virA/C-rev	TTT GGTCTCT <i>GTGC</i> CTAGAAGTTACAACGCAGGATG
pOGG2-virA/N-for	TTT GGTCTCT <i>GCAC</i> AGGCTGAAGAAGATCAGC
pOGG2-virA/N-rev	TTT GGTCTCT <i>ATGG</i> CGAGCCAGGCCAGACCATCG
virB6-10-pOGG2-1-for	TTT GGTCTCT <i>ATGG</i> TTGCAACAGCAGTTTCAGCAG
SD-virB6-10-pOGG2-1-rev	TTT GGTCTCT <i>TGGT</i> TGTCATCCCCATAAACGTGCTTG

virB6-10-pOGG2-2-for	TTT GGTCTCT <i>ACCA</i> CGTGACGTTTCATCCC GTGAG
virB6-10-pOGG2-2-rev	TTT GGTCTCT <i>CGAC</i> GGCCAACCTTCTCAAACGGCAGC
virB4-pOGG2-Bsa-FIfor	TTT GGTCTCT <i>CGAC</i> CGCTTGGACATAGCTGTG
virB4-pOGG2-Bsa-FIrev	TTT GGTCTCT <i>GTCA</i> GTCGAACTTCTCTTTTAAAACCTC
virB4-pOGG2-Bsa-FIIfor	TTT GGTCTCT <i>TGAC</i> CATGAAAAAGACCC
virB4-pOGG2-Bsa-FIIrev	TTT GGTCTCT <i>ATGG</i> CTATACCGCGAGCGCAAG
virD4-FII-BsaI-rev	TTT GGTCTCT <i>CGAC</i> CGTCGCGGGTACTTGAG
virD4-FII-BsaI-for	TTT GGTCTCT <i>TGAT</i> TGATGTCACATTTGCAG
virD4-FI-BsaI-for	TTT GGTCTCT <i>ATCA</i> GCGCCCTTCTCCTTAGG
virD4-FI-BsaI-rev	TTT GGTCTCT <i>ATGG</i> TGGCATGTGTCAGTTCCG
virB11-pOGG2-Bsa-FII-for	TTT GGTCTCT <i>CGAC</i> CATGAAGTCGGCCATCATC
virB11-pOGG2-Bsa-FII-rev	TTT GGTCTCT <i>GTCA</i> GGCTCGGCCCTCACGGGATG
virB1-pOGG2-Bsa-FI-for	TTT GGTCTCT <i>TGAC</i> CGGCTTTTCTCGCATGC
virB1-pOGG2-Bsa-FI-rev	TTT GGTCTCT <i>ATGG</i> GCCATCGTGTTGCGAACCTT
pOGG2-icmE-F1-for	TTT GGTCTCT <i>CGAC</i> CCTGCCGGTACCACTGTGC
pOGG2-icmE-FI-rev	TTT GGTCTCT <i>TTTC</i> CTGCTGAGCCATCACG
pOGG2-icmE-FII-for	TTT GGTCTCT <i>GAAA</i> TAAAGGCTCCCCATGAAC
pOGG2-icmE-FII-rev	TTT GGTCTCT <i>ATGG</i> CGCTTTCAGTTGGATAC
pOGG2-dotCB-F1-for	TTT GGTCTCT <i>CGAC</i> ATGTTTGCGCCCGATAGC
pOGG2-dotCB-FI-rev	TTT GGTCTCT <i>TCAC</i> AAACCGCCATTGCTG
pOGG2-dotCB-FII-for	TTT GGTCTCT <i>GTGA</i> GAGGGTGAGTGATGTCAG
pOGG2-dotCB-FII-rev	TTT GGTCTCT <i>ATGG</i> GCGATCTGCGGTTACCAG
Primers for the amplification of predicted promoter regions	
promvirB2-for	TTT GGTCTCT <i>ATTC</i> ACTATGCGGTTTATCTGGTTG
promvirB2-rev	TTT GGTCTCT <i>CATA</i> TCTGAAACCTCTCAGATTGTTAG
promvirB5-for	TTT GGTCTCT <i>ATTC</i> ATTGCCGACTGACGCACATCTATCG
promvirB5-rev	TTT GGTCTCT <i>CATA</i> ATCGAAGGTCGGGATGGGGGTTCCG
promvirD4-for	TTT GGTCTCT <i>ATTC</i> GATATCGTGTTGTGGCGATTACG
promvirD4-rev	TTT GGTCTCT <i>CATA</i> GCGCCCTTCTCCTTAGGCTTG
promvirG-for	TTT GGTCTCT <i>ATTC</i> GTCGGATCGGGCTGGCGACG
promvirG-rev	TTT GGTCTCT <i>CATA</i> CGACTCTCTATCCCGGCATG
promdotA-for	TTT GGTCTCT <i>ATTC</i> GCCTGATCTGGCCCCGGTCATC
promdotA-rev	TTT GGTCTCT <i>CATA</i> ATTGACGAGCGCGCCACAAACG
promdotD-for	TTT GGTCTCT <i>ATTC</i> TAGCGACACATATACGTGACCGTG
promdotD-rev	TTT GGTCTCT <i>CATA</i> CGGGGCACGTTGCCTTTCGTTG
promicmL-for	TTT GGTCTCT <i>ATTC</i> CCGGCATAGGTTGGACACAGG
promicmL-rev	TTT GGTCTCT <i>CATA</i> GAACTCTGCCGGTCACTCG
promicmL(129)-for	TTT GGTCTCT <i>ATTC</i> GTGCAATCGGTGTCCAACCTATGC
promicmL(129)-rev	TTT GGTCTCT <i>CATA</i> TCGTCGCGCCACAACCTGTTG
dTALE-pvirB2-for	GAAGAC AA <i>CTCA</i> GGAG ACTATGCGGTTTATCTGGTTG
dTALE-pvirB2-rev	GAAGAC AA <i>CTCG</i> AGCG TCTGAAACCTCTCAGATTGTTAG
dTALE-pvirB5-for	GAAGAC AA <i>CTCA</i> GGAG ATTGCCGACTGACGCACATCTATCG
dTALE-pvirB5-rev	GAAGAC AA <i>CTCG</i> AGCG ATCGAAGGTCGGGATGGGGGTTCCG
dTALE-virD4-for	GAAGAC AA <i>CTCA</i> GGAG GATATCGTGTTGTGGCGATTACG
dTALE_virD4_rev	GAAGAC AA <i>CTCG</i> AGCG GCGCCCTTCTCCTTAGGCTTG
dTALE-pvirG-for	GAAGAC AA <i>CTCA</i> GGAG GTCGGATCGGGCTGGCGACG
dTALE-pvirG-rev	GAAGAC AA <i>CTCG</i> AGCG CGACTCTCTATCCCGGCATG
dTALE-pdotA-for	GAAGAC AA <i>CTCA</i> GGAG GCCTGATCTGGCCCCGGTCATC
dTALE-pdotA-rev	GAAGAC AA <i>CTCG</i> AGCG ATTGACGAGCGCGCCACAAACG
dTALE-pdotD-for	GAAGAC AA <i>CTCA</i> GGAG TAGCGACACATATACGTGACCGTG
dTALE-pdotD-rev	GAAGAC AA <i>CTCG</i> AGCG CGGGGCACGTTGCCTTTCGTTG

dTALE-picmL-for	GAAGAC AA <i>CTCA</i> GGAG CCGGCATAGGTTGGACACAGG
dTALE-picmL-rev	GAAGAC AA <i>CTCG</i> AGCG GAACTCTGCCGGTCACTCG
dTALE-picmL(129)_for	GAAGAC AA <i>CTCA</i> GGAG GTGCAATCGGTGTCCAACATGC
dTALE-picmL(129)-rev	GAAGAC AA <i>CTCG</i> AGCG TCGTCGCGCCACAACATGTTG
dTALE-phrpB1-for	GAAGAC AA <i>CTCA</i> GGAG GAATGTGTGGATGGCCT GGCGAAG
dTALE_phrpB1-rev	GAAGAC AA <i>CTCG</i> AGCG GCGGACCGTACACGTGGGGAACG
¹ <i>Bsa</i> I and <i>Bpi</i> I recognition sites are shown in bold, resulting overhangs in italics.	

Table S3: Accession numbers and predicted functions of VirB/VirD4 proteins from *A. tumefaciens* and *x. axonopodis* pv. *citri* strain 306.

Protein	<i>A. tumefaciens</i> strain GV3101 [NZ_KY000036.1]		<i>X. axonopodis</i> pv. <i>citri</i> strain 306 Chromosome [AE008923]		<i>X. axonopodis</i> pv. <i>citri</i> strain 306 pXAC64 [AE008925]		Putative function/ localization ²
	accession number ¹	aa	accession number ¹	aa	accession number ¹	aa	
VirB1	WP_010974915.1	245	AAM37466.1	280	AAM39282.1	292	lytic transglycosylase
VirB2	WP_010891502.1	121	AAM37465.1	136	AAM39293.1	125	pilin subunit
VirB3	WP_010891501.1	108	AAM37464.1	103	AAM39292.1	99	inner membrane platform
VirB4	WP_010974916.1	789	AAM37463.1	817	AAM39291.1	877	ATPase
VirB5	WP_010891499.1	220	AAM37462.1	275	AAM39290.1	220	pilus-tip protein
VirB6	WP_010974917.1	295	AAM37461.1	350	AAM39287.1	288	inner membrane platform
VirB7	WP_010891497.1	168	AAM37471.1	139	AAM39288.1	131	lipoprotein, outer membrane core complex
VirB8	WP_010891496.1	237	AAM37470.1	348	AAM39286.1	224	inner membrane platform
VirB9	WP_010891495.1	393	AAM37469.1	255	AAM39285.1	260	outer membrane core complex
VirB10	WP_010891494.1	377	AAM37468.1	389	AAM39284.1	406	inner/outer membrane core complex
VirB11	WP_010974918.1	344	AAM37467.1	346	AAM39283.1	340	ATPase
VirD4	WP_010974920.1	668	AAM37472.1	557	-	-	ATPase
VirG	WP_010891492.1	253	AAM36537.1	124	-	-	two-component system response regulator
VirA	WP_010974914.1	833	AAM36536.1	676	-	-	two-component system sensor kinase
TraG	-	-	-	-	AAM39276.1	524	conjugative coupling protein TrwB
TraI	-	-	-	-	AAM39277.1	991	conjugative relaxase TrwC

¹ These proteins were used for the pairwise sequence alignments with corresponding proteins from *X. euvesicatoria* strain 85-10. The corresponding amino acid identities between homologous proteins are given in Fig. 1.

² See main text for details and references.

Table S4: Sequences of predicted *vir* and *icm/dot* promoters from *X. euvesicatoria*.

Predicted promoter of:	Sequence (5' – 3') ¹
<i>virB2</i>	actatgCGGTTatctggttgaggCGgagcacgagagcagccGGTggtgCGgatgacggtgaagtaccacgcacaacaag cttatgaggtcgttaccgCGcttgtaaaccaagctctggaacgcttgacatagctgtggaattcatcaaggagagctgcccgc acacaaagaccgtgctgtagttttgcccgtagcacacctaacaatctgagaggttcaga
<i>virB5</i>	attgCGgactgacgcacatctatcGctactttgcctcacatcgcaattggcaaccaaggCGctttacgcagacaaggctctaag attgggagaaatgatcagtgcccatgtggcagtgggCGgaagtacaagcattgctgtctaccagcGctccgacattcattgatac CGcttaactgacttccctgttcattgctccccgaacccccatccgaccttcgat
<i>virD4</i>	gatatcgtgttgCGgattacgcgtgacgaggtgatgacccgacgCGgctggtgcatagcCGctcgtgacggatgCGggtagc CGgatcagccgGatttGctctgGaccagGcaaggctgGatcGatcGctggtgGagagcGagagcGgCGgacGcaagtc gagctgtcGaccataagcCGgctgctgctgctgctgacccGcatcGtatgcaagcctaaggagaaggCGc
<i>virG</i>	gCGgatcGGgctGGcagCGggtcCGctccctaccCGtGtGtcGcagagcGagtgGctGgtcacaagatGaacaaaagtcGt catcGaccacGGcagCGgagcGtgaccaccCGctcGtcGacGtgGcaagCGacacaaaacacCGtagcatcGtGctGgaagat gCGtccccCGacGGctatcGtgGacCGcGcatgGacGcacttccccaacCGgctGattcGgtcaccCGagCGgatcG acactCGgaatcGtccCGaacCGgtcGatcCGggatagagagtcG
<i>dotA</i>	gcctgatctgcccGgtatccGGCGgCGgagcCGaccagCGatcGGgctaagagtgGctcccGatccacagtcGagttctcG gCctatccatgacGctgaccaagtcCCcagttccGGgGcataactacGccagaactcGacgagctgggggGtCGgaggtcta gCGcagccatGtcacatccGtcaatatagttatGccttagtttaacatcGtttGtggCGcGctGtcaat
<i>dotD</i>	tagcGacacatacGtgaccGtgacagagcCGcctGtcacatcGttcGcatatagcGacGcGagtacacGGtggaagtcG gGtGccgagtgGtgatGtgctGtgGGggGtgattGcagagatacGGCGgataacctGcGccacaaatGtGtGtaatccatG ccacGtattcaaccaacGaaaggcaacGtgccccG
<i>icmL229</i>	ccGGcataggttgacacagCGcCGcaacagctgatttctagtgatttGcGCGgtGcttCGcGacGtccatcacttGgtGCGgt aagtcagtgaaGtgatgaccGctgtagcactcGcGgataagtagCGcCGcGctacatcGtggaatggacGtccagcagctGct gatGtGCGcCGgtGtgaccGcataaaGGcCGcCGcGagtgaccGGcagagttc
<i>icmL129</i>	gtGcaatcGgtGtccaactatcCGgttctccccagCGcaaaGGccacattcGttGgcatatcGttgacCGcctGttatGgtGctGt atcctcGtgaacgatttccaaacagttGtgCGcGacga

¹ Predicted *vir* boxes are indicated in yellow.

Table S5: Accession numbers and predicted functions of Icm/Dot proteins from *L. pneumophila*.

Protein	<i>L. pneumophila</i> strain <i>Philadelphia1</i> [NC002942.5]		Putative function/ localization ²
	accession number ¹	aa	
DotA	WP_010948386.1	1048	inner membrane protein
DotB	WP_011947587.1	369	ATPase
DotC	WP_010948375.1	303	lipoprotein, outer membrane core complex
DotD	WP_010948374.1	163	lipoprotein, outer membrane core complex
IcmT	WP_010946190.1	86	inner membrane protein
IcmW	WP_010948388.1	151	cytoplasmic chaperon
IcmP/DotM	WP_011214699.1	376	inner membrane protein
IcmO/DotL	WP_010946195.1	783	ATPase
IcmL/DotI	WP_010946198.1	212	inner membrane protein
IcmK/DotH	WP_015444826.1	360	periplasmic
IcmE/DotG	WP_010946200.1	1048	inner membrane protein
IcmG/DotF	WP_010946201.1	269	inner/outer membrane core complex
IcmC/DotE	WP_010946202.1	194	inner membrane protein
IcmD/DotP	WP_011214707.1	132	inner membrane protein
IcmJ/DotN	WP_011214708.1	208	cytoplasmic
IcmB/DotO	WP_010946205.1	1009	cytoplasmic

¹ These proteins were used for the pairwise sequence alignments with corresponding proteins from *X. euvesicatoria* strain 85-10. The corresponding amino acid identities between homologous proteins are given in Fig. 2.

² See main text for details and references.

Table S6: Transfer of plasmid pXCV38₈₂₋₈::*spec*^R from strain 82-8 into the recipient strain 85-10::*gent*^R.

Donor		Recipient		Transconjugant¹		
Strain	CFU	Strain	CFU	Strain	CFU	% of recipient
82-8 pXCV38 ₈₂₋₈ :: <i>spec</i> ^R	5 × 10 ⁸	85-10:: <i>gent</i> ^R	5 × 10 ⁸	85-10:: <i>gent</i> ^R pXCV38 ₈₂₋₈ :: <i>spec</i> ^R	3.59 × 10 ⁵	0.072
	6.45 × 10 ⁸		6.45 × 10 ⁸		1.343 × 10 ⁷	2.08
	7.05 × 10 ⁸		7.05 × 10 ⁸		8.23 × 10 ⁶	1.17
85-10:: <i>gent</i> ^R pXCV38 ₈₂₋₈ :: <i>spec</i> ^R	5.55 × 10 ⁸	85-10:: <i>kan</i> ^R	5.55 × 10 ⁸	85-10:: <i>kan</i> ^R pXCV38 ₈₂₋₈ :: <i>spec</i> ^R	44.000	0.008
	5.52 × 10 ⁸		5.52 × 10 ⁸		41.500	0.008
	4.56 × 10 ⁸		4.56 × 10 ⁸		35.600	0.008

¹ Transconjugants were selected on NYG plates containing rifampicin, gentamycin and spectinomycin. The presence of plasmid pXCV38₈₂₋₈::*spec*^R was confirmed by agarose gel electrophoresis and PCR (see text). Numbers represent the numbers of transconjugants obtained in three independent conjugation experiments using the listed donor and recipient strains.

Table S7: XVIPCDs in T4S candidate substrates from *X. euvesicatoria*.

Protein	Amino acid sequence ¹
XCV0332	MTDAPVIHQGPVGPDPHPEHPDHYLFAQIREAVSVLDAELGKPVDAQSERMAARLLPLA KQHGFQVDYVVLRSRHLGVEGENVFLVVRGELS ¹ DPAHLRAH ¹ ITTQEAMETSV ¹ DASLAQ LEEINRRLMLRLPPR
XCV1120	MEVRQIGKGRAGQPDIEPQAVASNEAVLADNPAHPDHSTYQQIHSWVRGTGNWNEEE SKNVTASLYKQQTEDPLLKRVDQVTGGLGRDGAHNVFAVYAPHGMGVAPMFHAHV ¹ DGRQAAQEPAAQNLQQAQAEAIQQTQVROQVLEQTQQQNVQQEQGPRMTM
XCV3751	MPTNYSRAQVLDIIEREA ¹ AERNIPRDDFLRFAHVETGGAFDELASRGGGAKGLFQFTP PTAQQYGIAGRELDVANTDAAARLYLDNRTSLVNRHERDGRPYLSGKPEPDGLDMY LAHQQGAGGYRSLQTAIATGSFGLESTRANILNNVGEKELKSLTGVSATFRRMSDKD MAQTFVQYWDTKMDRIRIPEKGIPISTTQQAPSHAAPAHSAPAQPEKPAAQGI ¹ ALHAA YDLTNKYDHVKYGLGAKDPDKGRVDCSGWV ¹ VEMQ ¹ NATMDEINKGAGKAI ¹ FTKDEKF SPGFDSASELLRKAELRSGVLIQ ¹ GKDVTAQTLKEGMIIGEDNGPQSWDKGRYK ¹ GIDHIT MVVRDPKDGQLKISQSRGGEGVELSSLD ¹ SYLERK ¹ HAKGVKLYASDPLSEARDLLQERS QNKQ ¹ QSHAATEHKPSQA ¹ HAAADAPGVLENARGA ¹ EVRTLQQT ¹ LQQLGYK ¹ DAGGNEL KADGAYGQRTSEAVKAFQRAHGLQDDGVVGRDTQAALKQAEKTPLLSEKTNPDHPLF NQAVNKLEQLGPNAFANRQQLVNAAGHMTFEAKVSGMQRIDMV ¹ AQSKDGNGLFAV ¹ QGQPTDPAHQRIYTDKATAAERPLEQSSNAVRQEAQTQTQVVDQHEQQR ¹ SRQGRSVG
TraI	MLNVTPIRGNNQYAAAHYFSAADDYYAKEHPGEWQGGQAQALGLTG ¹ PVEQAQLSRL LDGQLP ¹ NGERIQTTFDPTDNKKRMGLDLTFSAPKSVSMQALVAGDKDV ¹ TAAHDRAVT RALEQVERLAEARKKVKGSYRERTANMVIGKFRHEMSRAKDPQLH ¹ THAVVLNMTQ RADGAWRALSNEDIFRVQHEVDALYKAELARGLQALGYAIRLVDDQGNFELDHISRD QIEAFSARSRVIEEALANEGKTRATATTLEKQIISLATRPRKDESREL ¹ VKQYWVEKSRE LGIDYGLRSQLDGRTYEAGDSFGTRGRERGDAGDRIAATSLPASLTPAQAVVQYAINH LTEREAVVRETALTATALRRAVGLAGPDEVRAEIKRLAGQGALIEAVPAYRMADRKD GPALSSAGWKS ¹ YLQELKGWSDKQAQ ¹ QYVGN ¹ AIKQGS ¹ LVPAEKRYTTQKALAREKAIL AIERTGRGAIEPIMTAAAVKTALEGTALSAGQ ¹ RS ¹ AVETIVSTKNRFVGIQGDAGTGKTY TVNQAV ¹ ALIKQASAVSEGYRTVALAPYGNQVKALKNEGLEAHTLASFLRTKDKPIDGK TIIVLDES ¹ GVGARQMEQVMRVVEKAGARMVLLGDTKQTEAIEAGKPF ¹ AQLQDGM QTARISEIQRQKDH ¹ ELKTAVEHAAEGRVTPSLAHIKHVEELKEPIERHRAIVNDYIQLTE PERRETLIVAGTNEARREINRMVRQSLDLTGKGR ¹ EFETLTRVDMTQAQRRFAPS ¹ YQPG MVIQPEKDDQKAGLTRGETYRVKDALPGNALVLQRQDGT ¹ TTINPRKATQLSVYRLER AELSVGDTV ¹ RINRNDPGRDLTNGDRMRVAGVIGGVKLESVEQRDGRPARALELPTN RPLHLEHAYASTVHSAQGLTNDRALIALD ¹ TNSRTTSMNLYYVAISRACHEARVYTNSV KELPAAIARRFDKTTALAIQRERQLQRDAGM ¹ PKGAADGKQALQRQFQQQRKQPA SGKKPSEYGRFG

¹Conserved amino acid sequences of the XVIPCD are indicated as follows: GLxRIDHV in yeqlflow, FAVQGxxDPAHxRAHV in green and the glutamine-rich C-terminal tail in purple.

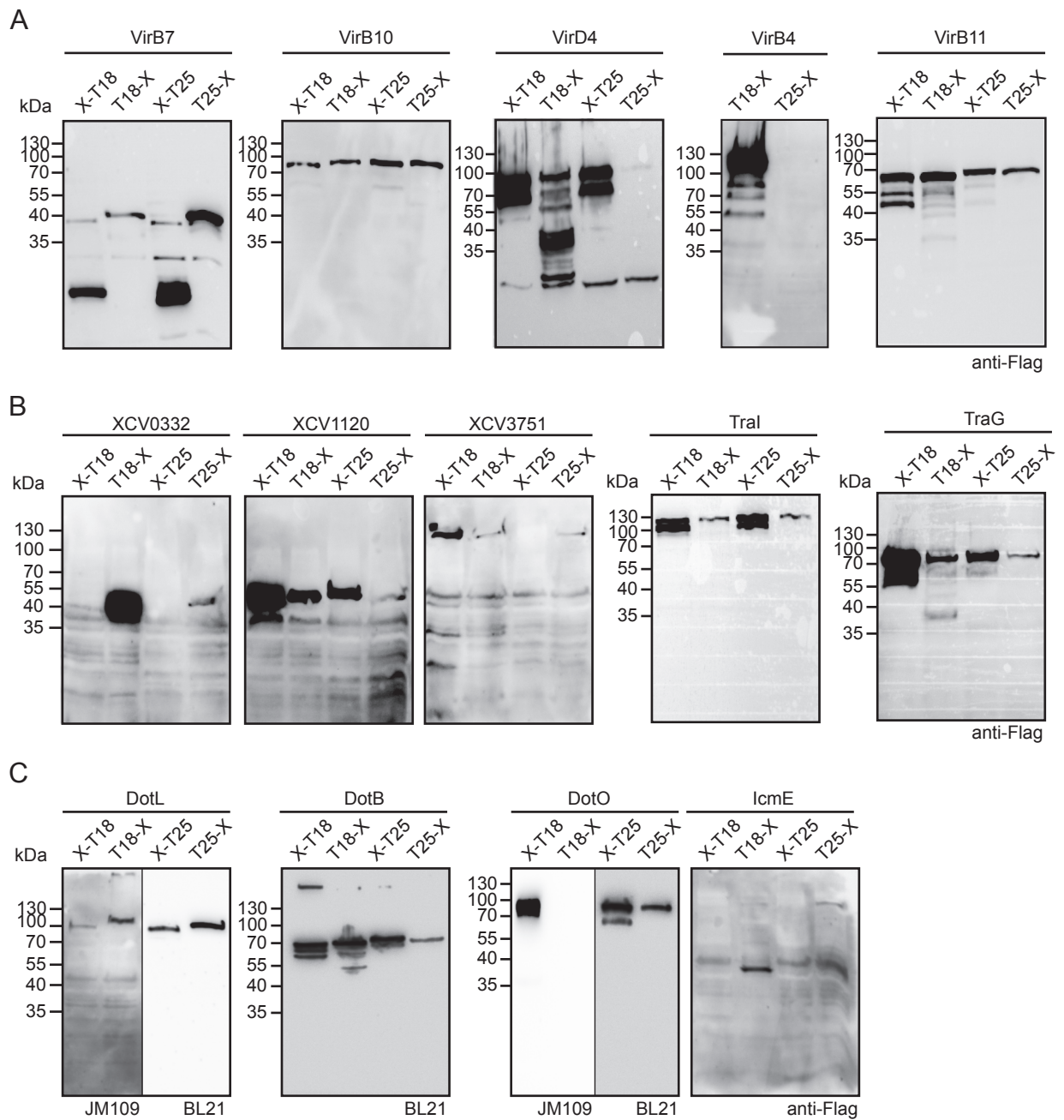


Figure S1
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Figure S1: Synthesis and immunological detection of T18 and T25 fusion proteins in *E. coli* strain JM109.

A) Immunological detection of VirB fusion proteins. Cell extracts from *E. coli* strain JM109 with expression constructs encoding T18 and T25 fusions of VirB7, VirB10, VirB4, VirB11 and VirD4 as indicated were analysed by immunoblotting, using a FLAG epitope-specific antibody. Asterisks mark signals at the expected size of the fusion proteins. Additional signals likely correspond to degradation products or result from unspecific binding of the antibody to proteins in the cell extracts.

B) Analysis of T18 and T25 fusions of candidate T4S substrates and TraG. Cell extracts of *E. coli* strain JM109 containing expression constructs for the synthesis of T18 and T25 fusions of XCV0332, XCV1120, XCV3571, TraI and TraG as indicated were analysed by immunoblotting as described in (A). Proteins at the expected size are indicated by asterisks. Note that XCV0332-T18, XCV0332-T25 and XCV3751-T25 were not detectable.

C) Immunological detection of T18 and T25 fusions of Icm and Dot proteins. JM109 cell extracts containing expression constructs for the synthesis of T18 and T25 fusions of DotL, DotB, DotO and IcmE were analysed by immunoblotting as described in (A). Proteins at the expected size of the fusions are indicated with asterisks. Additional signals likely correspond to degradation products or protein complexes in case of DotB, which were not dissolved during SDS-PAGE. Note that T18-DotO and IcmE-T18 were not detectable.

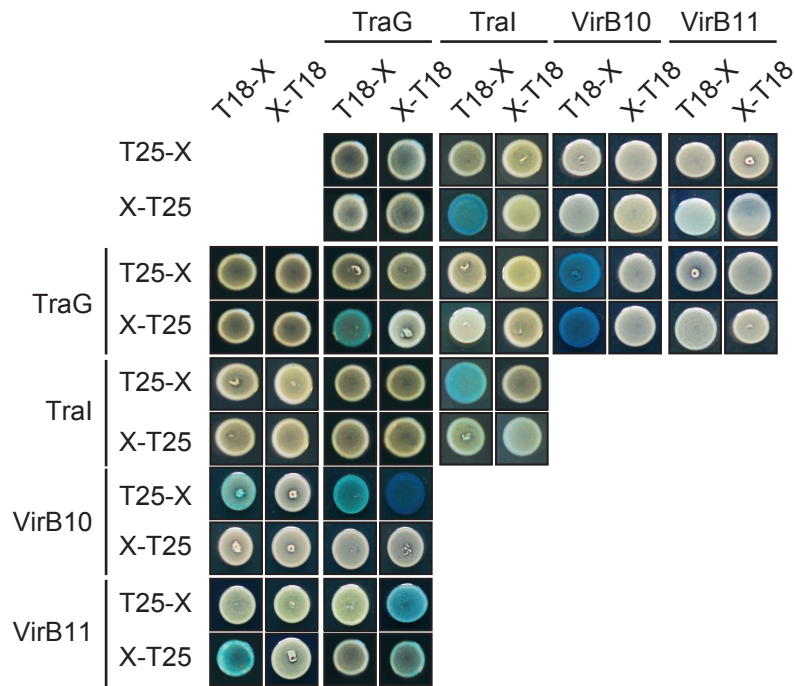


Figure S2
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Figure S2: Results of BACTH assays with TraG from *X. euvesicatoria*.

T18 and T25 fusions of TraG, the predicted ATPase VirB11, the structural component VirB10 and the putative relaxase TraI were analysed in *E. coli* BTH101 cells as indicated and bacterial cultures were grown on indicator plates containing X-gal and IPTG. As control, fusion proteins were tested against the T18 or T25 domain alone as indicated. All proteins were stably synthesized as was shown by immunoblot analysis of bacterial cell extracts, using a FLAG epitope-specific antibody (see Fig. S1). Interaction studies were performed at least three times with similar results. One representative colony is shown. Results are summarized in Table 2.