

Supplementary Information

The phytohormone indole-3-acetic acid induces quorum sensing signal DSF turnover via a positive feedback biosynthetic loop in the phytopathogen *Xanthomonas campestris*

Stress Biology

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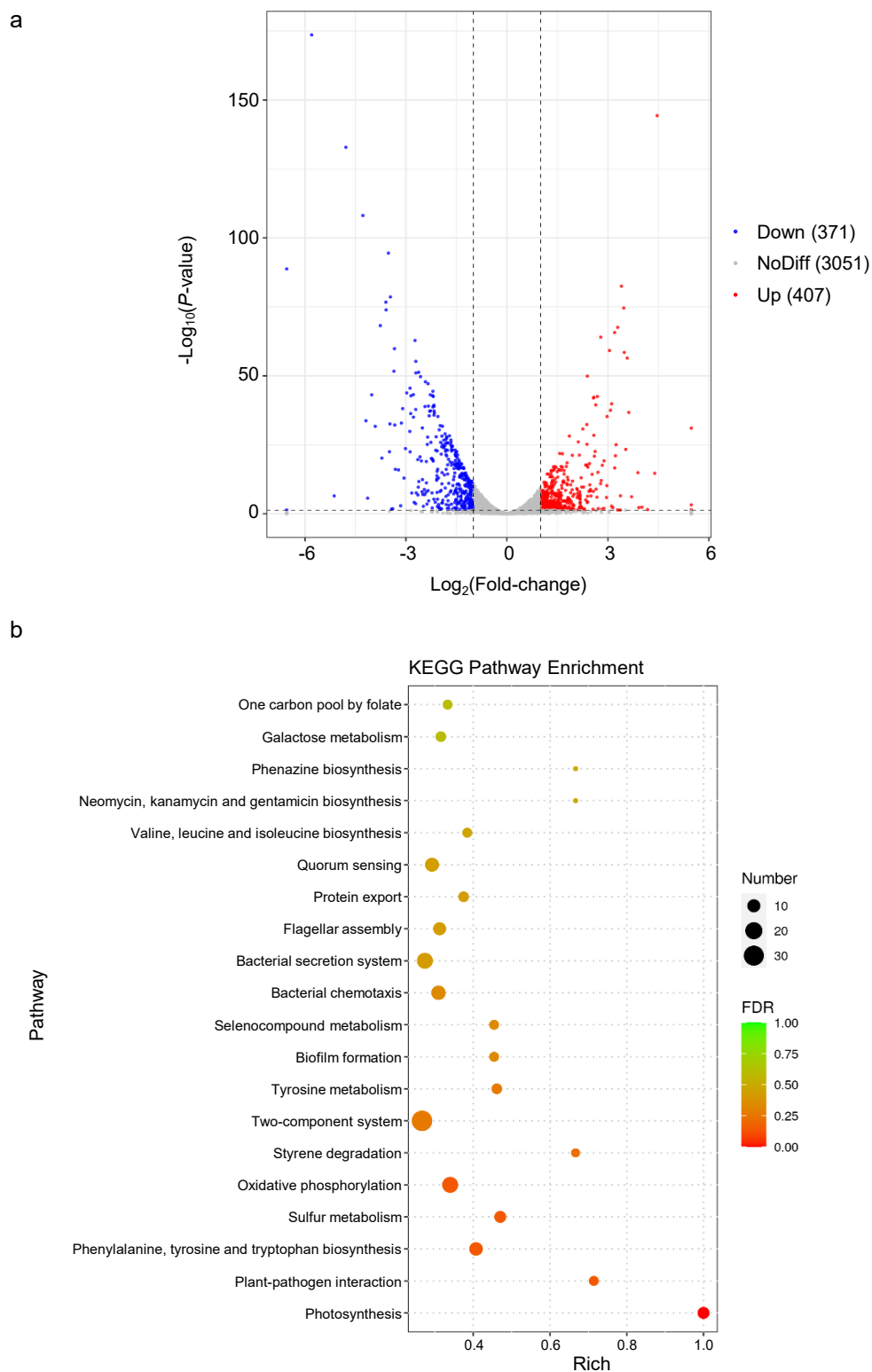


Fig. S1 The transcriptome of *Xcc* in response to indole-3-acetic acid (IAA). **a** Volcano plot of differentially transcribed genes in response to 100 μ M IAA. The $\log_2$ (Fold Change) for each gene was plotted against the statistical significance ($-\log_{10}$ of the adjusted P-value < 0.05). Vertical dashed lines represent the $\log_2$ fold change cut-off of $\geq |1.5|$. Red dots represent significant up-regulated genes, blue dots represent significant down-regulated genes. **b** KEGG enrichment analysis. The larger the Rich factor, the higher the enrichment extent. The false discovery rate (FDR) generally ranges from 0 to 1, with values closer to zero indicating more significant enrichment.

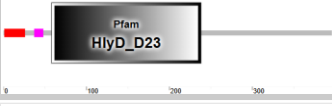
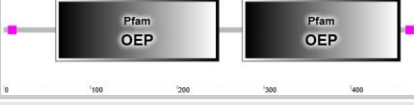
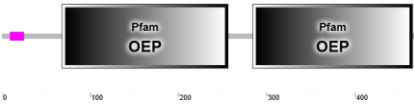
Protein	Length (AA)	Domain	Identity (%)
Xcc2541 (TrpA)	272		41.8
PA0035 (TrpA)	268		
Xcc2543 (TrpB)	405		61.9
PA0036 (TrpB)	402		
Xcc2544 (TrpI)	298		57.4
PA0037 (TrpI)	295		
Xcc1439 (IaepB)	396		45.2
PA2493 (MexE)	414		
Xcc1440 (IaepC)	1053		60.8
PA2494 (MexF)	1062		
Xcc1442 (IaepE)	474		46.8
PA2495 (OprN)	472		

Fig. S2 Domain analysis of the tryptophan synthase TrpI-TrpB-A and RND efflux pump IaepBCE in *Xanthomonas campestris* pv. *campestris* (Xcc) and TrpI-TrpB-A and MexEF-OprN in *Pseudomonas aeruginosa* PAO1.



Fig. S3 Sequence alignment of the tryptophan synthase TrpI-TrpB-A in *Xanthomonas campestris* pv. *campestris* (Xcc) and *Pseudomonas aeruginosa* PAO1.

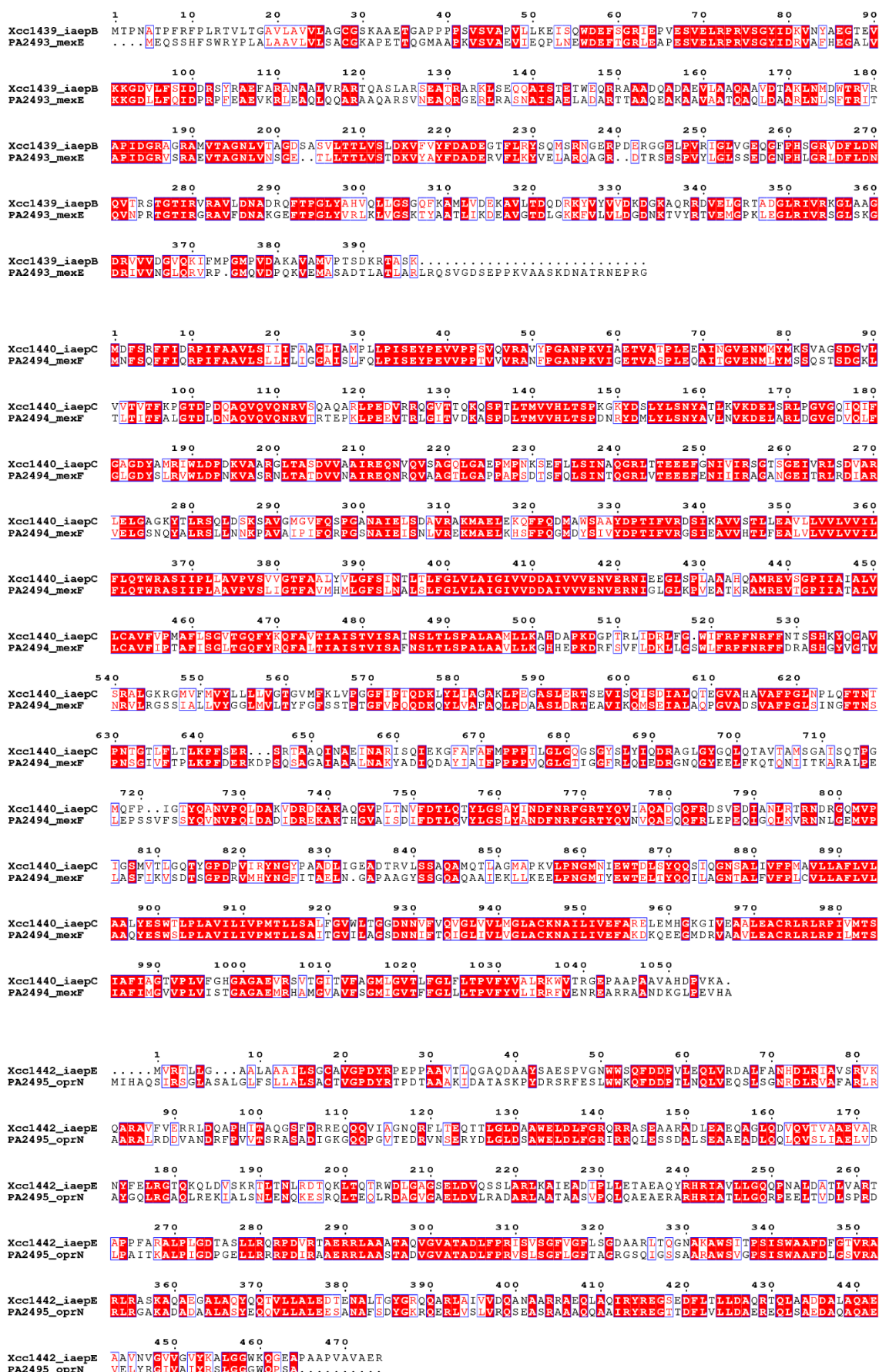


Fig. S4 Sequence alignment of the RND efflux pump laepBCE in *Xanthomonas campestris* pv. *campestris* (Xcc) and MexEF-OprN *Pseudomonas aeruginosa* PAO1.

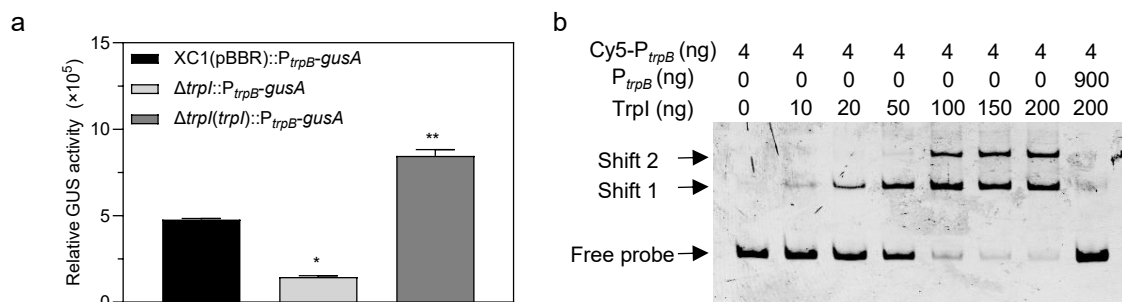


Fig. S5 TrpI positively regulates gene *trpBA* transcript expression in Xcc. **a** The relative GUS activity of the reporter strain XC1(pBBR)::P_{trpB}-gusA, Δ trpI::P_{trpB}-gusA and Δ trpI(trpI)::P_{trpB}-gusA in the XYS at 24 hpi. **b** EMSA was performed using a Cy5-labeled DNA probe, P_{trpB} and 10–200 ng TrpI protein; 900 ng unlabeled P_{trpB} was added as a cold probe. Shown are the averages for three technical repeats with standard deviation. Statistically significant differences are denoted by one asterisk ($p \leq 0.05$) or two asterisks ($p \leq 0.01$)

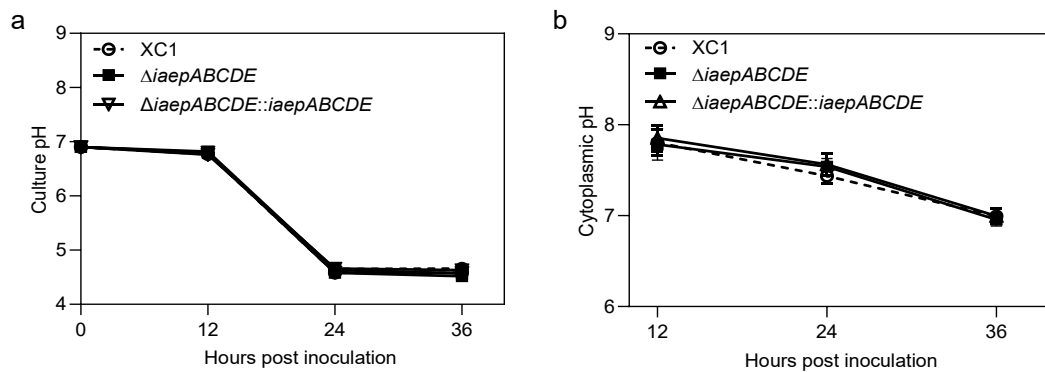


Fig. S6 Deletion of *iaepABCDE* had no significant effect on the culture pH or cytoplasmic pH in XC1. **a** The culture pH of XC1, $\Delta iaepABCDE$, and $\Delta iaepABCDE::iaepABCDE$ in YYS medium. **b** The cytoplasmic pH of XC1, $\Delta iaepABCDE$, and $\Delta iaepABCDE::iaepABCDE$ in YYS medium. Shown are the averages for three technical repeats with standard deviation

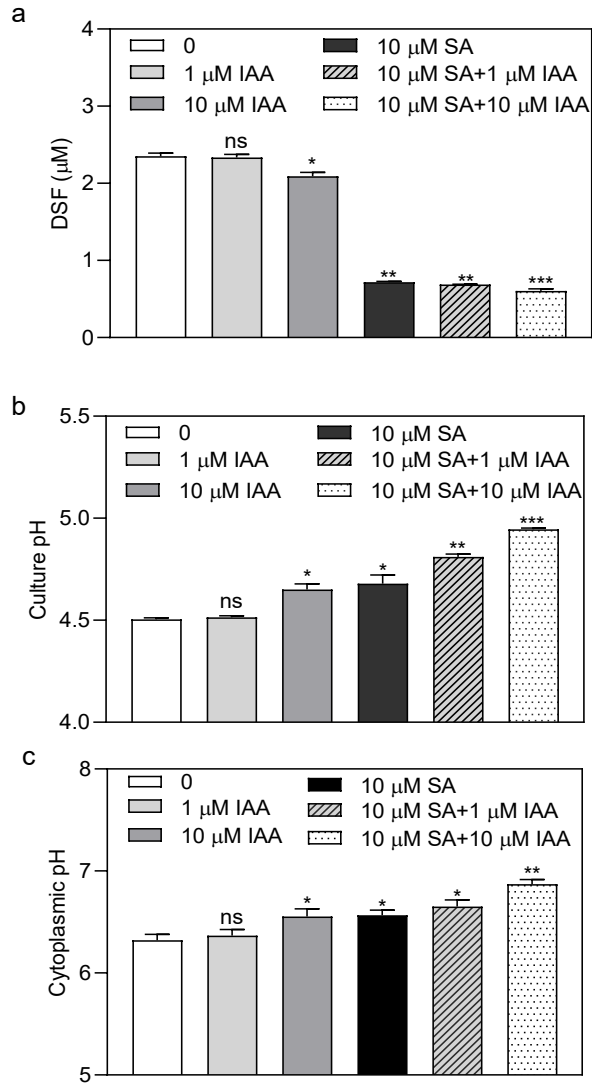


Fig. S7 Cumulative effect between IAA and SA on DSF turnover, culture pH and cytoplasmic pH
a DSF levels in $\Delta rpfC$ in YYS medium supplemented with individual 1 μM IAA, 10 μM IAA, 10 μM SA, and their combinations. **b** and **c** Culture pH and cytoplasmic pH in XC1 in YYS medium supplemented with individual 1 μM IAA, 10 μM IAA, 10 μM SA, and their combinations. Results are presented as the averages of three technical repeats with standard deviations. * $p \leq 0.05$, ** $p \leq 0.01$, ns: not significant

Table S1 Bacterial strains and plasmids used in this study

	Properties / characteristics	Reference / source
Xcc Strains		
XC1	Xcc wild-type strain, Rif ^R	Lab stock
$\Delta rpfC$	The <i>rpfC</i> in-frame deletion mutant, Rif ^R	He et al., 2006
$\Delta rpfB$	The <i>rpfB</i> in-frame deletion mutant, Rif ^R	Zhou et al., 2015
$\Delta rpfBC$	The <i>rpfB rpfC</i> double deletion mutant, Rif ^R	Zhou et al., 2015
$\Delta rpfBC::rpfB$	The <i>rpfB rpfC</i> double deletion mutant complemented with a single copy of <i>rpfB</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	Zhou et al., 2015
$\Delta rpfB::rpfB$	The <i>rpfB</i> in-frame deletion mutant complemented with a single copy of <i>rpfB</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	Zhou et al., 2015
$\Delta trpA$	The <i>trpA</i> in-frame deletion mutant, Rif ^R	This study
$\Delta trpA::trpA$	The <i>trpA</i> deletion mutant complemented with a single copy of <i>trpA</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$\Delta Xcc2542$	The <i>Xcc2542</i> in-frame deletion mutant, Rif ^R	This study
$\Delta trpB$	The <i>trpB</i> in-frame deletion mutant, Rif ^R	This study
$\Delta trpB::trpB$	The <i>trpB</i> deletion mutant complemented with a single copy of <i>trpB</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$\Delta rpfC\Delta trpB$	The <i>rpfC trpB</i> double deletion mutant, Rif ^R	This study
$\Delta rpfC\Delta hepABCD$	The <i>rpfC hepABCD</i> double deletion mutant, Rif ^R	Lab stock
$\Delta iaepABCDE$	The <i>iaepABCDE</i> in-frame deletion mutant, Rif ^R	This study
$\Delta iaepABCDE::iaepABCDE$	The <i>iaepABCDE</i> mutant complemented with a single copy of <i>iaepABCDE</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$\Delta rpfC\Delta iaepABCDE$	The <i>rpfC iaepABCDE</i> double deletion mutant, Rif ^R	This study
$\Delta rpfC\Delta iaepABCDE::iaepA BCDE$	The <i>rpfC iaepABCDE</i> double deletion mutant complemented with a single copy of <i>iaepABCDE</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$\Delta rpfB\Delta iaepABCDE$	The <i>rpfB iaepABCDE</i> double deletion mutant, Rif ^R	This study
$\Delta rpfB\Delta iaepABCDE::rpfB$	The <i>rpfB iaepABCDE</i> double deletion mutant complemented with a single copy of <i>rpfB</i> inserted at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$XC1::iaaHM$	The XC1 harboring a single copy of P _{groES} - <i>iaaHM</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$\Delta rpfC::iaaHM$	The $\Delta rpfC$ harboring a single copy of P _{groES} - <i>iaaHM</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$XC1::trpB-A$	The XC1 harboring a single copy of P _{groES} - <i>trpB-A</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$\Delta rpfC::trpB-A$	The $\Delta rpfC$ harboring a single copy of P _{groES} - <i>trpB-A</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
$XC1::P_{rpfF}-gusA$	The XC1 harboring a single copy of T0T1-P _{rpfF} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	Song et al. 2022
$XC1::P_{rpfB}-gusA$	The XC1 harboring a single copy of T0T1-P _{rpfB} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	Song et al. 2022
$XC1(pBBR)::P_{trpB}-gusA$	The XC1(pBBR) harboring a single copy of T0T1-P _{trpB} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R Kan ^R	This study
$\Delta trpI::P_{trpB}-gusA$	The <i>trpI</i> in-frame deletion mutant in strain $XC1::P_{trpB}-gusA$ (pBBR), Rif ^R Kan ^R	This study
$\Delta trpI(trpI)::P_{trpB}-gusA$	The strain $\Delta trpI::P_{trpB}-gusA$ harboring the expression plasmid pBBR- <i>trpI</i> , Rif ^R Kan ^R	This study
$XC1(pBBR-mCherry-pHluorin)$	XC1 wide type harboring the expression plasmid pBBR- <i>mCherry</i> -pHluorin, Rif ^R Kan ^R	Song et al. 2022
$\Delta iaepABCDE$ (pBBR- <i>mCherry</i> -pHluorin)	The <i>iaepABCDE</i> harboring the expression plasmid pBBR- <i>mCherry</i> -pHluorin, Rif ^R Kan ^R	This study
$\Delta iaepABCDE::iaepABCDE$ (pBBR- <i>mCherry</i> -pHluorin)	The <i>iaepABCDE::iaepABCDE</i> harboring the expression plasmid pBBR- <i>mCherry</i> -pHluorin, Rif ^R Kan ^R	This study

Plasmids	Properties / characteristics	Reference / source
Xcc Strains		
XC1:: <i>gusA</i>	The XC1 harboring a single copy of T0T1- <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	Lab stock
XC1::P _{trpB} - <i>gusA</i>	The XC1 harboring a single copy of T0T1-P _{trpB} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	This study
XC1::P _{hep} - <i>gusA</i>	The XC1 harboring a single copy of T0T1-P _{hep} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	Lab stock
XC1::P _{atpB} - <i>gusA</i>	The XC1 harboring a single copy of T0T1-P _{atpB} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	Lab stock
XC1::P _{iaepA} - <i>gusA</i>	The XC1 harboring a single copy of T0T1-P _{iaepA} - <i>gusA</i> at the <i>attTn7</i> site on its chromosome, Rif ^R	Lab stock
<i>Escherichia coli</i> strains		
DH5α	<i>E. coli</i> F– Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1	Lab stock
S17-1	res [–] pro mod ⁺ integrated copy of RP4, mob ⁺	Lab stock
RK2013	Triparental mating helper strain, Kan ^R	Lab stock
BL21(DE3)	<i>E. coli</i> B F [–] dcm ompT hsdS(r _B – m _B –) gal [malB ⁺] _{K-12} (λ ^S)	Lab stock
BL21(AI)	F [–] ompT hsdS _B (r _B ·m _B) gal dcm araB::T7RNAP-tetA	Lab stock
Plasmids		
pK18mobsacB	A mobilizable vector, allows for selection of double crossover in <i>Xcc</i> , Kan ^R	Schäfer <i>et al.</i> , 1994
pK18- <i>trpA</i>	<i>Xcc trpA</i> deletion cassette in pK18mobscaB, Kan ^R	This study
pK18- <i>Xcc2542</i>	<i>Xcc Xcc2542</i> deletion cassette in pK18mobscaB, Kan ^R	This study
pK18- <i>trpB</i>	<i>Xcc trpB</i> deletion cassette in pK18mobscaB, Kan ^R	This study
pK18- <i>trpI</i>	<i>Xcc trpI</i> deletion cassette in pK18mobscaB, Kan ^R	This study
pK18- <i>iaepABCDE</i>	<i>Xcc iaepABCDE</i> deletion cassette in pK18mobscaB, Kan ^R	This study
mini-Tn7T-Gm	a versatile mini-Tn7 delivery vector mini-Tn7T-Gm, Gm ^R	Choi and Schweizer, 2006
mini-Tn7-P _{groES} - <i>iaaHM</i>	promoter region of <i>groES</i> and gene cluster <i>iaaHM</i> cloned in mini-Tn7T-Gm, Gm ^R	This study
mini-Tn7-P _{groES} - <i>trpB-A</i>	promoter region of <i>groES</i> and gene cluster <i>trpB-A</i> cloned in mini-Tn7T-Gm, Gm ^R	This study
mini-Tn7- <i>trpA</i>	promoter region and gene cluster of <i>trpA</i> cloned in mini-Tn7T-Gm, Gm ^R	This study
mini-Tn7- <i>trpB</i>	promoter region and gene cluster of <i>trpB</i> cloned in mini-Tn7T-Gm, Gm ^R	This study
mini-Tn7- <i>iaepABCDE</i>	promoter region and gene cluster of <i>iaepABCDE</i> cloned in mini-Tn7T-Gm, Gm ^R	This study
pBBR1MCS-2	Plasmid for gene complementation, Kan ^R	Kovach et al. 1995
pBBR1MCS-2- <i>mCherry-pHluorin</i>	<i>mCherry-pHluorin</i> cloned in pBBR1MCS-2, Kan ^R	Song et al. 2022
pBBR1MCS-2- <i>trpI</i>	<i>trpI</i> cloned in pBBR1MCS-2, Kan ^R	This study
mini-Tn7-T0T1- <i>gusA</i>	mini-Tn7T-Gm containing a T0T1 terminator fused to the coding region for <i>gusA</i> , Gm ^R	Chen et al. 2020
mini-Tn7-T0T1-P _{trpB} - <i>gusA</i>	mini-Tn7T-Gm containing a T0T1 terminator and 516-bp promoter region of <i>trpB</i> fused to the coding region for <i>gusA</i> , Gm ^R	This study
pET-28a- <i>rpfB</i>	pET-28a containing <i>rpfB</i> , Kan ^R	Zhou et al., 2015
pET14b	His-tag protein expression vector, Amp ^R	Lab stock
pET14b- <i>trpI</i>	pET-14b containing <i>trpI</i> , Amp ^R	This study

Table S2 Oligonucleotide primers used in this study

Application	Primer and application	Sequence (5' to 3')
Amplification of Xcc <i>trpA</i> deletion cassette	<i>trpA_del_F1</i>	ctatgacatgattacgaattcGCGGGCGTGGCGATGATGGT
	<i>trpA_del_R1</i>	GCCAGACCGACCTCGGGCACGCCGAGCTCGATGATGTT
	<i>trpA_del_F2</i>	CGAGGTCGGTCTGGCGCTGATC
	<i>trpA_del_R2</i>	caggtcgactctagaggatccCAATCCATGCAAGGCGGCCG
Amplification of Xcc <i>Xcc2542</i> deletion cassette	<i>Xcc2542_del_F1</i>	ctatgacatgattacgaattcGCGGGCGGTGATGGCATTGC
	<i>Xcc252_del_R1</i>	CACCGCCATAGTGATCCGCCGCCAGTGCAGACAGG
	<i>Xcc2542_del_F2</i>	ATCACTATGGCGGTGACGACGGC
	<i>Xcc2542_del_R2</i>	caggtcgactctagaggatccTGCACGGCTTCGATCACATAGGC
Amplification of Xcc <i>trpB</i> deletion cassette	<i>trpB_del_F1</i>	ctatgacatgattacgaattcGCTACGTGCGCTGCGTTAGAGTGC
	<i>trpB_del_R1</i>	CGCAGGCGCTGATCGTCGCGCCCATGTAGACCACG
	<i>trpB_del_F2</i>	CGATCAGCGCTGCGTGGG
	<i>trpB_del_R2</i>	caggtcgactctagaggatccGCGATGGTGTGCACGTCCTTGTC
Amplification of Xcc <i>trpI</i> deletion cassette	<i>trpI_del_F1</i>	ctatgacatgattacgaattcGCCTGGCCGATGGTGTGTTGATC
	<i>trpI_del_R1</i>	CACTCCACGCCCAGCGTCACGTGCAGGGCGTCGGC
	<i>trpI_del_F2</i>	GCTGGGCGTGGAGTGGATCGG
	<i>trpI_del_R2</i>	caggtcgactctagaggatccTCAGACGCTTCGGGCAGCTGAG
Amplification of Xcc <i>iaepABCDE</i> deletion cassette	<i>iaepABCDE_del_F1</i>	ctatgacatgattacgaattcTGCGGCATCCCCTCCACG
	<i>iaepABCDE_del_R1</i>	GCGTTGCGCCAGCTCGG
	<i>iaepABCDE_del_F2</i>	GAGCTGGCGCAACGCCGCGAGGGTTCGGAAGACTTCCTG
	<i>iaepABCDE_del_R2</i>	caggtcgactctagaggatccCGGCCACCGAGCGCTTGC
<i>trpA</i> complementation by mini-Tn7T-Gm	P _{trpB} (Tn7)_F	catgagctcactagtggatccCCCGGGCGGTTGCTCGTTTG
	P _{trpB} (Tn7)_R	GGGAGCTATTCTCCGCAACACTGC
	<i>trpA</i> (Tn7)_F	GGAGGAATAGCTCCCATGAGCCGCGCGCCCGACC
	<i>trpA</i> (Tn7)_R	cgaggtaccggggccaagcttGTTGGGCACCTGCATGCAACCG
<i>trpB</i> complementation by mini-Tn7T-Gm	<i>trpB_rec_F</i>	catgagctcactagtggatccCCCGGGCGGTTGCTCGTTTG
	<i>trpB_rec_R</i>	cgaggtaccggggccaagcttCATGCCACCGCCGAGGTTGC
<i>iaepABCDE</i> complementation by mini-Tn7T-Gm	<i>iaepABCDE_rec_F</i>	catgagctcactagtggatccGTGACTGGGATGGTCCAATCCCAGACG
	<i>iaepABCDE_rec_R</i>	cgaggtaccggggccaagcttCCCTGCCATCTCAGCGCTCGG
P _{groES} - <i>iaaHM</i> expression by mini-Tn7T-Gm	P _{groES} (Tn7)_F	catgagctcactagtggatccGCCGTTGTAGAGATAGATGCGGTAGC
	P _{groES} (Tn7)_R	AGAGAAAGACCCTCTTAAGTGATTGATTGG
	<i>iaaHM</i> (Tn7)_F	AGAGGGTCTTTCTCTGTGAACCTCTCCCACTCCGGTTTCG
	<i>iaaHM</i> (Tn7)_R	cgaggtaccggggccaagcttCACCCGGGCGGCGATTAG
P _{groES} - <i>trpB-A</i> expression by mini-Tn7T-Gm	P _{groES} (Tn7)_F	catgagctcactagtggatccGCCGTTGTAGAGATAGATGCGGTAGC
	P _{groES} (Tn7)_R	AGAGAAAGACCCTCTTAAGTGATTGATTGG
	<i>trpB-A</i> (Tn7)_F	AGAGGGTCTTTCTCTATGTCGGCCAGCCCATCAGTG
	<i>trpB-A</i> (Tn7)_R	cgaggtaccggggccaagcttGTTGGGCACCTGCATGCAACCG

Application	Primer and application	Sequence (5' to 3')
Digital PCR analysis	<i>Xcc0548</i> -dPCR-Forward	ACCTGCAGAACCTGGTTTATC
	<i>Xcc0548</i> -dPCR-Probe	TTCTGGACCATCCACGTCGACAC
	<i>Xcc0548</i> -dPCR-Reverse	CCAGAATGCGAAGACCATCA
	<i>Xcc2543</i> -dPCR-Forward	GACATCGAACGGCAGAAGAT
	<i>Xcc2543</i> -dPCR-Probe	AGTTGCTCGGTGCGAAGGTCATC
	<i>Xcc2543</i> -dPCR-Reverse	TCGCGCATCGCTTCATT
	<i>Xcc4169</i> -dPCR-Forward	GTGAAGAAGCTGATCAAGACG
	<i>Xcc4169</i> -dPCR-Probe	CACCATTGCCAAGGTCAGCAGC
	<i>Xcc4169</i> -dPCR-Reverse	CACGGTGATCCATGTAGTA
	<i>Xcc1438</i> -dPCR-Forward	ACGGCGATATCTCCATCG
	<i>Xcc1438</i> -dPCR-Probe	CAACGGCTTGGCTTCTTCGTGC
	<i>Xcc1438</i> -dPCR-Reverse	GACCGACAGCAGGTGTA
Promoter of <i>trpB</i> for GUS activity	P _{trpB} -F	taggacaaatccgcc <u>aagctt</u> CCCGGGCGGTTGCTCGTTTG
	P _{trpB} -R	gtagttaggcccgggatccGGGAGCTATTCTCCGCAACACTGC
TrpI protein expression by pBBR	<i>trpI</i> -F	gataagctgatatcgaattcATGCCACCTCCCTGCCCC
	<i>trpI</i> -R	cgctctagaactagtggatccGACGCCGCTGCTCGATGTAGC
TrpI protein expression	<i>trpI</i> (pET)_F	gtgccgcgcggcagccatgATGCCACCTCCCTGCCCC
	<i>trpI</i> _R	gctttgttagcagccgatccGACGCCGCTGCTCGATGTAGC
Promoter of <i>trpB</i> for EMSA	Cy5-P _{trpB} -F	agccagtggcgataagCAGAGAACGCACATGCCGGCTG
	Cy5-P _{trpB} -R	agccagtggcgataagGGCATCCGGGTACGCATGAAAGTC

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