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Supplementary Information for

Biosynthesis and secretion of the microbial sulfated peptide RaxX and binding to the rice XA21 immune receptor

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SI Materials and Methods

LC-SRM-MS Analysis

The SRM targeted proteomic assays were performed, as described previously (1), on an Agilent 6460 QQQ mass spectrometer system coupled with an Agilent 1290 UHPLC system (Agilent Technologies, Santa Clara, CA). Peptides were separated on an Ascentis Express Peptide C18 column [2.7-mm particle size, 160-Å pore size, 5-cm length × 2.1-mm inside diameter (ID), coupled to a 5-mm × 2.1-mm ID guard column with same particle and pore size, operating at 60°C; Sigma-Aldrich, St. Louis, MO] operated at a flow rate of 0.4 ml/min via the following gradient: initial conditions were 95% solvent A (0.1% formic acid), 5% solvent B (99.9% acetonitrile, 0.1% formic acid). Solvent B was increased to 35% over 5 min, and was then increased to 80% over 0.2 min, and held for 2 min at a flow rate of 0.6 mL/min, followed by a ramp back down to 5% B over 0.5 min where it was held for 1.5 min to re-equilibrate the column to original conditions. The eluted peptides were ionized via an Agilent Jet Stream ESI source operating in positive ion mode with the following source parameters: gas temperature = 250°C, gas flow = 13 liters/min, nebulizer pressure = 35 psi, sheath gas temperature = 250°C, sheath gas flow = 11 liters/min, capillary voltage = 3500 V, nozzle voltage = 0 V. The data were acquired using Agilent MassHunter version B.08.02. Acquired SRM data were processed using Skyline software version 3.70 (MacCoss Lab Software, Seattle, WA).

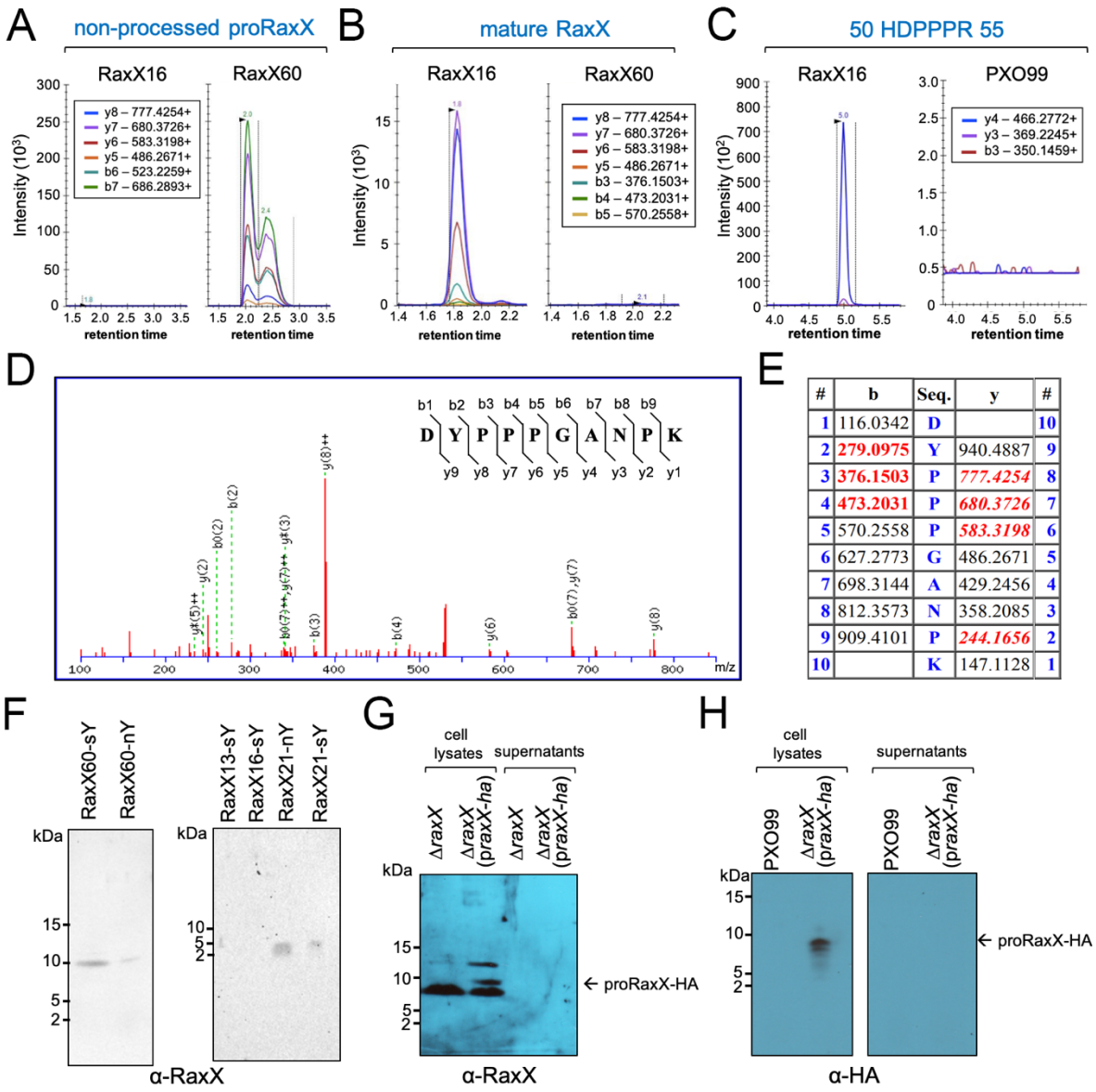
Shotgun LC-MS Analysis

Samples prepared for shotgun proteomic experiments were analyzed using an Agilent 6550 iFunnel Q-TOF mass spectrometer (Agilent Technologies) coupled to an Agilent

1290 UHPLC system as described previously (2). Peptides were separated on a Sigma–Aldrich Ascentis Peptides ES-C18 column (2.1 mm × 100 mm, 2.7 μm particle size, operated at 60°C) at a 0.400 mL/min flow rate and eluted with the following gradient: initial condition was 98% solvent A (0.1% formic acid) and 2% solvent B (99.9% acetonitrile, 0.1% formic acid). Solvent B was increased to 35% over 30 min, and then increased to 80% over 2 min, and held for 6 min at a flow rate of 0.6 mL/min, followed by a ramp back down to 2% B over 1 min where it was held for 4 min to re-equilibrate the column to original conditions. Peptides were introduced to the mass spectrometer from the LC by using a Jet Stream source (Agilent Technologies) operating in positive-ion mode (3,500 V). Source parameters employed gas temp (250°C), drying gas (14 L/min), nebulizer (35 psig), sheath gas temp (250°C), sheath gas flow (11 L/min), VCap (3,500 V), fragmentor (180 V), OCT 1 RF Vpp (750 V). The data were acquired with Agilent MassHunter Workstation Software, LC/MS Data Acquisition B.06.01 operating in Auto MS/MS mode whereby the 20 most intense ions (charge states, 2–5) within 300–1,400 m/z mass range above a threshold of 1,500 counts were selected for MS/MS analysis. MS/MS spectra (100–1,700 m/z) were collected with the quadrupole set to “Medium” resolution and were acquired until 45,000 total counts were collected or for a maximum accumulation time of 333 ms. Former parent ions were excluded for 0.1 min following MS/MS acquisition. The acquired data were exported as mgf files and searched against the latest PXO99 protein database with Mascot search engine version 2.3.02 (Matrix Science, Boston, MA).

Immunoblotting

The proteins were separated by SDS-PAGE, transferred to PVDF membranes (Bio Rad, Hercules, CA cat#162-0177) by electrophoresis and then analyzed by immunoblot using standard procedures. RaxX was detected using an anti-RaxX antibody that was raised in rabbit against RaxX11 (PPGANPKHDPP; Fig. 3*A*), derived from residues 43-53 of proRaxX (GenScript, Piscataway, NJ). Alkaline phosphatase conjugated anti-rabbit IgG (Sigma, cat#A3687) was used as secondary antibody. RaxX-HA was detected using a monoclonal anti-HA antibody produced in mouse (Sigma, cat#H3663). Alkaline phosphatase conjugated anti-mouse IgG (Sigma, cat#A3562) was used as secondary antibody. The anti-RaxX and anti-HA antibodies were used at dilutions of 1:2000. The anti-rabbit IgG and anti-mouse IgG antibodies were used at dilutions of 1:20,000. CDP-*Star* (Roche, Basel, Switzerland, cat#12041677001) was used as substrate. The membranes were exposed to X-ray films or visualized using the Gel-Doc XR$^+$ system (Bio-Rad).



#	b	Seq.	y	#
1	116.0342	D		10
2	279.0975	Y	940.4887	9
3	376.1503	P	777.4254	8
4	473.2031	P	680.3726	7
5	570.2558	P	583.3198	6
6	627.2773	G	486.2671	5
7	698.3144	A	429.2456	4
8	812.3573	N	358.2085	3
9	909.4101	P	244.1656	2
10		K	147.1128	1

Fig. S1. Mature RaxX is detected by mass spectrometry (MS) but not by immunoblot analysis. (*A-B*) The RaxX16 synthetic peptide and RaxX60 purified recombinant full-length peptide, representing the RaxX mature and non-processed precursor (proRaxX) peptides, respectively, were used to establish our selected reaction monitoring (SRM) MS methodology. SRM-MS chromatograms of the targeted RaxX tryptic peptides representing the non-processed proRaxX (*A*) and mature RaxX (*B*) peptides are shown. (*C*) SRM-MS chromatograms of the RaxX tryptic peptide HDPPPR (corresponding to proRaxX residues 50-55) in wildtype PXO99 (right) supernatant. RaxX16 (left) was used as a control. (*A-C*) The lines correspond to individual SRM transitions that were monitored for each peptide. The detected peptide b- and y-series fragment ions are indicated in the legend of each panel. (*D*) Shotgun liquid chromatography (LC) MS analysis of mature RaxX in the wildtype PXO99 supernatant. (*E*) Dominant b- and y-series ions of RaxX tryptic peptide DYPPPGANPK. The b- and y-series fragment ions

detected in *D* are indicated in red. (*F*) Anti-RaxX immunoblot of RaxX60 and synthesized peptides derived from proRaxX: RaxX21 (amino acids 35-55), RaxX16 (amino acids 40-55), and RaxX13 (amino acids 40-52). Sulfated peptides are indicated as (-sY) and non-sulfated peptides are indicated as (-nY). (*G*) Anti-RaxX immunoblot of C-terminally HA-tagged proRaxX (proRaxX-HA) expressed in the *⊿raxX* mutant. (*H*) Anti-HA immunoblot of proRaxX-HA pulled down from total cell lysates or supernatants.

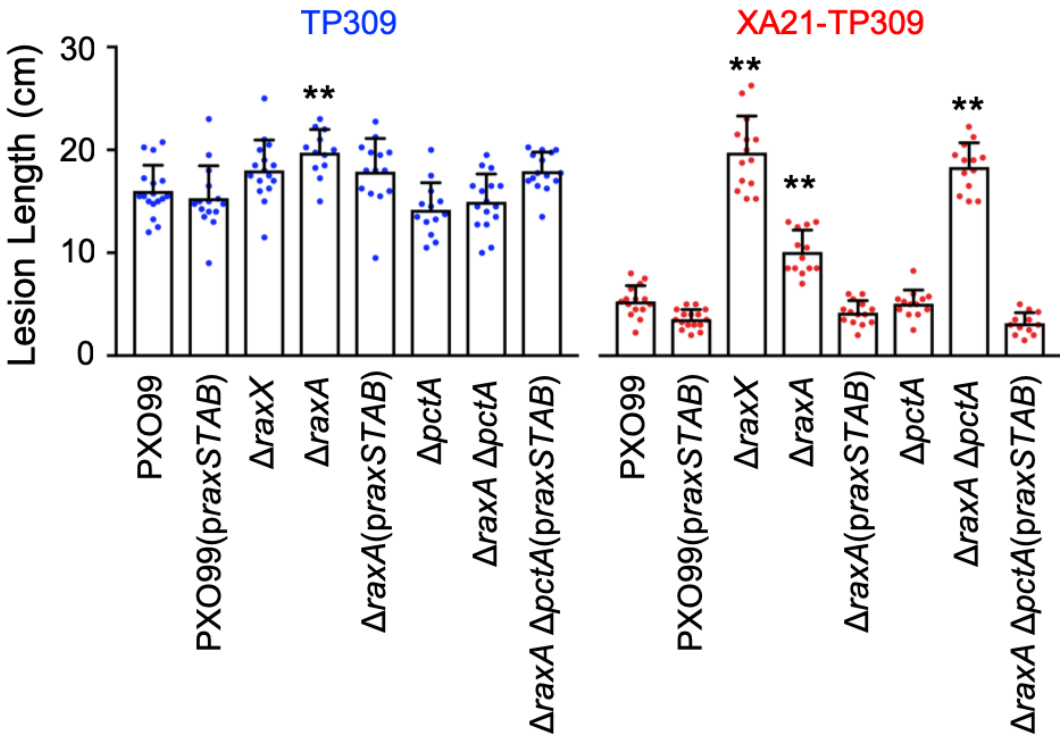


Fig. S2. PctA partially compensates for the loss of RaxA. TP309 and XA21-TP309 plants were inoculated by scissor clipping with PXO99-derived strains carrying mutant alleles of *raxX*, *raxA*, and/or *pctA* ± p*raxSTAB* plasmid. Bars represent the mean + standard deviation (SD) of lesion measurements (cm) taken 14 days post inoculation (dpi) of TP309 (blue dots) or XA21-TP309 (red dots) plants (n = 12-17). $^{**}p < 0.01$; compared to PXO99 using Dunnett's test. Similar results were observed in 2 other independent experiments.

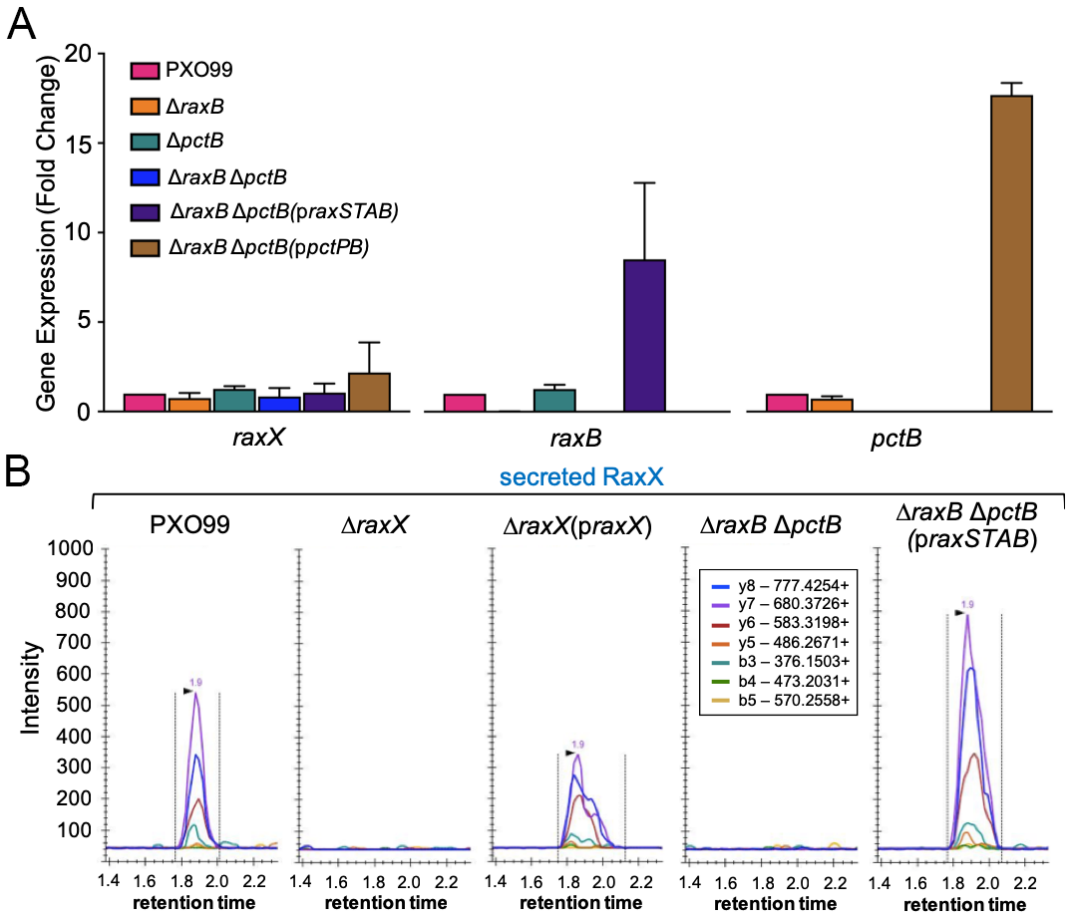


Fig. S3. The Δ*raxB* Δ*pct* double mutant phenotype is complemented with p*raxSTAB*. (*A*) RNA was extracted from TP309 leaf samples collected 6 days after scissor clipping inoculation with the PXO99-derived strains carrying mutant alleles of *raxB* and/or *pctB* ± p*raxSTAB* or p*pctPB* plasmid. *raxX*, *raxB*, and *pctB* gene expression were analyzed by RT-qPCR, normalized with the *ampC2* reference gene and expressed as fold change + SD compared to the PXO99 wildtype parental strain (n=3). Similar results were observed in 2 other independent experiments. (*B*) SRM-MS chromatograms of mature RaxX detected in supernatants of the indicated strains.

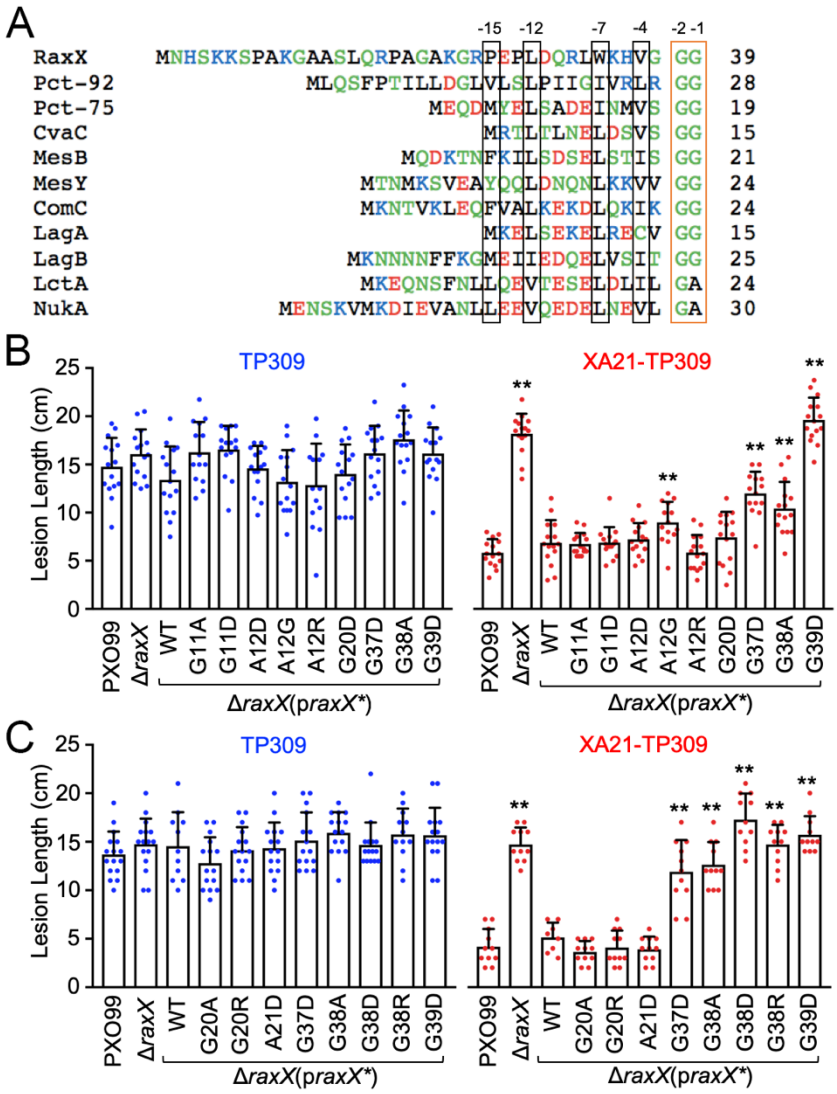


Fig. S4. RaxX immunogenicity is not compromised by mutation of residues distal from the cleavage site. (*A*) Alignment of the leader peptide from proRaxX and putative PctB substrates, Pct-92 and Pct-75, to experimentally verified leader sequences of propeptides processed and secreted through the PCATs listed in Fig. 6*A*. Polar residues indicated in green, basic residues in blue, acidic residues in red, and hydrophobic residues in black. Conserved Gly-Gly preceding the cleavage site boxed in orange, hydrophobic residues in conserved positions -4, -7, -12, and -15 from the cleavage site are boxed in black. (*B*-*C*) TP309 and XA21-TP309 plants were inoculated with the indicated strains using the scissor clip method. Bars represent the mean + SD of lesion measurements (cm) on TP309 (blue dots) or XA21-TP309 (red dots) plants 14 dpi; n = 14-15 (*B*), 8-16 (*C*). $^{**}p < 0.01$; compared to PXO99 using Dunnett's test. Similar results were observed in 2 other independent experiments except A12G formed short lesions comparable to PXO99 in the other experiments.

Table S1. Bacterial strains used in this study.

strain	relevant characteristics*	reference
Escherichia coli		
E. coli DH5α	used for general cloning	
Xanthomonas oryzae pv. *oryzae*		
PXO99	Philippine race 6 wildtype strain PXO99^{A}, Cp^{R}	(3)
PXO99(p*raxSTAB*)	wildtype strain with p*raxSTAB*, Cp^{R}, Km^{R}	This study
Δ*raxX*	PXO_RS06000 marker-free deletion mutant, Cp^{R}	(4)
Δ*raxX*(p*raxX*)	Δ*raxX* mutant with p*raxX*, Cp^{R}, Km^{R}	(4)
Δ*raxX* (p*raxX-His*)	Δ*raxX* mutant with p*raxX-His*, Cp^{R}, Km^{R}	This study
Δ*raxX*(p*raxX-ha*)	Δ*raxX* mutant with p*raxX-ha*, Cp^{R}, Gm^{R}	This study
Δ*raxB*	PXO_RS06015 marker-free deletion mutant, Cp^{R}	This study
Δ*pctB*	PXO_RS14825 marker-free deletion mutant, Cp^{R}	This study
Δ*raxB* Δ*pctB*	PXO_RS06015 and PXO_RS14825 marker-free double deletion mutant, Cp^{R}	This study
Δ*raxB* Δ*pctB*(p*raxSTAB*)	Δ*raxB* Δ*pctB* double mutant with p*raxSTAB*, Cp^{R}, Km^{R}	This study
Δ*raxB* Δ*pctB*(p*pctPB*)	Δ*raxB* Δ*pctB* double mutant with p*pctPB*, Cp^{R}, Km^{R}	This study
Δ*raxA*	PXO_RS06010 marker-free deletion mutant, Cp^{R}	This study
Δ*pctA*	PXO_RS14840 marker-free deletion mutant, Cp^{R}	This study
Δ*raxA* Δ*pctA*	PXO_RS06010 and PXO_RS14840 marker-free double deletion mutant, Cp^{R}	This study
Δ*raxA*(p*raxSTAB*)	Δ*raxA* mutant with p*raxSTAB*, Cp^{R}, Km^{R}	This study
Δ*raxA* Δ*pctA*(p*raxSTAB*)	Δ*raxA* Δ*pctA* double mutant with p*raxSTAB*, Cp^{R}, Km^{R}	This study
Δ*raxB* Δ*pctB*(p*raxSTAB-C28S*)	Δ*raxB* Δ*pctB* double mutant with p*raxSTAB-C28S*, Cp^{R}, Km^{R}	This study
Δ*raxB* Δ*pctB*(p*raxSTAB-H101D*)	Δ*raxB* Δ*pctB* double mutant with p*raxSTAB-H101D*, Cp^{R}, Km^{R}	This study
Δ*raxX*(p*raxX-G11A*)	Δ*raxX* mutant with p*raxX-G11A*, Cp^{R}, Km^{R}	This study
Δ*raxX*(p*raxX-G11D*)	Δ*raxX* mutant with p*raxX-G11D*, Cp^{R}, Km^{R}	This study
Δ*raxX*(p*raxX-A12D*)	Δ*raxX* mutant with p*raxX-A12D*, Cp^{R}, Km^{R}	This study

Δ*raxX*(p*raxX-A12G*)	Δ*raxX* mutant with p*raxX-A12G*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-A12R*)	Δ*raxX* mutant with p*raxX-A12R*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G20A*)	Δ*raxX* mutant with p*raxX-G20A*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G20D*)	Δ*raxX* mutant with p*raxX-G20D*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G20R*)	Δ*raxX* mutant with p*raxX-G20R*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-A21D*)	Δ*raxX* mutant with p*raxX-A21D*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G37D*)	Δ*raxX* mutant with p*raxX-G37D*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G38A*)	Δ*raxX* mutant with p*raxX-G38A*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G38D*)	Δ*raxX* mutant with p*raxX-G38D*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G38R*)	Δ*raxX* mutant with p*raxX-G38R*, Cp^R, Km^R	This study
Δ*raxX*(p*raxX-G39D*)	Δ*raxX* mutant with p*raxX-G39D*, Cp^R, Km^R	This study
Δ*raxST*	PXO_RS06005 marker-free deletion mutant, Cp^R	(4)

* Cp^R-cephalexin resistance, Km^R-kanamycin resistance, Gm^R-gentamicin resistance

Table S2. Primers used in this study.

primer name	sequence (5'-3')*
primers for cloning XA21^{ECD}	
XA21_mel-F	ggtcgtatacatttcttacatctatgcgAGTGACGACGATGGTGATGCTG
XA21_CTSH-R	gcaccctggaagtacaggttctcTTTTCTGTTCTCTAGTAATGGACAACATCG
primers for constructing gene knockouts	
raxB-EcoRI-A	caccGAATTCGTTACCGCACCGCCTTGACG
raxB-B	AGTGCCAAGCGCCACACCGATCATCGCAGCGTGCCGTGCA
raxB-C	TGCACGGCACGCTGCGATGATCGGTGTGGCGCTTGGCACT
raxB-HindIII-D	gaccAAGCTTCAGGATCCAGCCGCCGATGA
pctB-EcoRI-A	caccGAATTCGCTGCCTCCCATTTGCTTTG
pctB-B	AACGAGTGTGGCCTGCCCCGTTCAGTTCGTTCCAGTTG
pctB-C	CAACTGGAACGAACTGAACGGGGCAGGCCACACTCGTT
pctB-HindIII-D	gaccAAGCTTCAAAAGCCTCGGGCAAACC
raxA-BamHI-A	caccGGATCCCATCCCTCCAACGTGCAGAT
raxA-B	AACCAGCGGCTCATCGCAGCGTCAAGCGCGTCATCGGCC
raxA-C	GGCCGATGACGCGCTTGACGCTGCGATGAGCCGCTGGTT
raxA-HindIII-D	gaccAAGCTTATGCCGCCGAGGTGACGCTT
pctA-BamHI-A	caccGGATCCTAGGAGGAGCTATCGGCGCT
pctA-B	CACCGATGCATCCAAACCAGGGCGTGTGTTCTGGACCGGT
pctA-C	ACCGGTCCAGAACACACGCCCTGGTTTGGATGCATCGGTG
pctA-HindIII-D	gaccAAGCTTCATGCCAACCAAGAATCGCC
primers for complementation	
raxX-F	cACCTGCGAGGACGTCAGAATGAAC
raxX-R	ATGGTGCCCGGGGTTGCGCGGCGGCGGATC
raxSTAB-BamHI-F	caccGGATCCGTCACAGCGCCTGAAGGGACG
raxB-HindIII-R	gaccAAGCTTGGAGGTGGACACTTCACGCC

pctPB-SacI-F	gaccGAGCTCACAGACCGATTTCAGCACCA
pctB-XbaI-R	gaccTCTAGATCACGCACTCAAGACGTGAT
primers for site-directed mutagenesis	
raxB-C28S-F	GATGATCTTGCAGGGGCAGGTCGGCGAAaGCGGATTGG
raxB-C28S-R	ATCATGGCCATGGCCGCCAATCCGCtTTCGCCGA
raxB-H101D-F	CTGCATCGTGCATTGGGATCTGAATgATTTCGTGGT
raxB-H101D-R	TGCCCACCCGCTTGAGCACCACGAAATcATTCAGA
raxX-G11A-F	CGAAAAAATCGCCCGCCAAGGcTGCGGCATCGCTGCAGCGGCCCG
raxX-G11A-R	CGGGCCGCTGCAGCGATGCCGCAgCCTTGGCGGGCGATTTTTTTCG
raxX-G11D-F	CGAAAAAATCGCCCGCCAAGGaTGCGGCATCGCTGCAGCGGCCCG
raxX-G11D-R	CGGGCCGCTGCAGCGATGCCGCAtCCTTGGCGGGCGATTTTTTTCG
raxX-A12D-F	CGAAAAAATCGCCCGCCAAGGGTGacGCATCGCTGCAGCGGCCCG
raxX-A12D-R	CGGGCCGCTGCAGCGATGCgtCACCCTTGGCGGGCGATTTTTTCG
raxX-A12G-F	CGAAAAAATCGCCCGCCAAGGGTGgGGCATCGCTGCAGCGGCCCG
raxX-A12G-R	CGGGCCGCTGCAGCGATGCCcCACCCTTGGCGGGCGATTTTTTTCG
raxX-A12R-F	CGAAAAAATCGCCCGCCAAGGGTagGGCATCGCTGCAGCGGCCCG
raxX-A12-R-R	CGGGCCGCTGCAGCGATGCCctACCCTTGGCGGGCGATTTTTTCG
raxX-G20A-F	GCATCGCTGCAGCGGCCCGCCGcgGCCAAGGGCCGACCGGAGCCGCTCGA
raxX-G20A-R	TCGAGCGGCTCCGGTCGGCCCTTGGCcgCGGCGGGCCGCTGCAGCGATGC
raxX-G20D-F	GCTGCAGCGGCCCGCCGacGCCAAGGGCCGACCGGAGCCG
raxX-G20D-R	CGGCTCCGGTCGGCCCTTGGCgtCGGCGGGCCGCTGCAGC
raxX-G20R-F	GCATCGCTGCAGCGGCCCGCCaggGCCAAGGGCCGACCGGAGCCGCTCGA
raxX-G20R-R	TCGAGCGGCTCCGGTCGGCCCTTGGCcctGGCGGGCCGCTGCAGCGATGC
raxX-A21D-F	GCATCGCTGCAGCGGCCCGCCGGGGACAAGGGCCGACCGGAGCCGCTCGA

raxX-A21D-R	TCGAGCGGCTCCGGTCGGCCCTTGTCCCCGGCGGGCCGCTGCAGCGATGC
raxX-G37D-F	GTCTGTGGAAGCATGTCGaCGGTGGGGACTATCCCCCGCCGGGCGCG
raxX-G37D-R	CGCGCCCGGCGGGGGATAGTCCCCACCGtCGACATGCTTCCACAGAC
raxX-G38A-F	GTCTGTGGAAGCATGTCGGCGcTGGGGACTATCCCCCGCCGGGCGCG
raxX-G38A-R	CGCGCCCGGCGGGGGATAGTCCCCAgCGCCGACATGCTTCCACAGAC
raxX-G38D-F	CAGCGTCTGTGGAAGCATGTCGGCGacGGGGACTATCCCCCGCCGGGCGCG
raxX-G38D-R	CGCGCCCGGCGGGGGATAGTCCCCgtCGCCGACATGCTTCCACAGACGCTG
raxX-G38R-F	CAGCGTCTGTGGAAGCATGTCGGCcgtGGGGACTATCCCCCGCCGGGCGCG
raxX-G38R-R	CGCGCCCGGCGGGGGATAGTCCCCacgGCCGACATGCTTCCACAGACGCTG
raxX-G39D-F	GTCTGTGGAAGCATGTCGGCGGTGacGACTATCCCCCGCCGGGCGCG
raxX-G39D-R	CGCGCCCGGCGGGGGATAGTCgtCACCGCCGACATGCTTCCACAGAC
primers for RT-qPCR	
ampC2-qF	GACTCGTAATGCCTACGACC
ampC2-qR	AATTGCTCGTAGAAGCTGCC
raxX-qF	AAAATCGCCCGCCAAGGGT
raxX-qR	TCAATGGTGCCCGGGGTTG
raxB-qF	AGTTGGGCTTTCAATGGAT
raxB-qR	GACCAACGTCTGGGTGAATC
pctB-qF	GACGCATCTGTTGGAATCGC
pctB-qR	AACTGGCTGGCCGTATTGAA

* Restriction sites are underlined.

Table S3. Plasmids used in this study.

plasmid	relevant characteristics*	reference
suicide plasmids for mutant generation		
pUFR80	suicide vector, Km^R, *sacB*$^+$	(5)
pUFR-Δ*raxB*	pUFR80 derivative for marker free deletion of *raxB* (*PXO_RS06015*), contains flanking regions 1284753-1285462 and 1287619-1288354, Km^R, *sacB*$^+$	This study
pUFR-Δ*pctB*	pUFR80 derivative for marker free deletion of *pctB* (*PXO_RS14825*), contains flanking regions 3259290-3260114 and 3262230-3263109, Km^R, *sacB*$^+$	This study
pUFR-Δ*raxA*	pUFR80 derivative for marker free deletion of *raxA* (*PXO_RS06010*), contains flanking regions 1283506-1284235 and 1285451-1286264, Km^R, *sacB*$^+$	This study
pUFR-Δ*pctA*	pUFR80 derivative for marker free deletion of *pctA* (*PXO_RS14840*), contains flanking regions 3265677-3266576 and 3267822-3268702, Km^R, *sacB*$^+$	This study
complementation plasmids		
pVSP61	broad host range vector used for complementation, Km^R	(6)
pLN615	pML123 derivative gateway destination vector for C-terminal fusion of HA tag, Gm^R	(7)
p*raxX*	pVSP61 derivative, contains *raxX* with native promoter (c1282744-1283226), Km^R	(4)
p*raxX-His*	p*raxX* derivative with C-terminal 6x His tag, Km^R	(4)
p*raxX-ha*	pLN615 derivative, contains *raxX*, Gm^R	This study
p*raxSTAB*	pVSP61 derivative, contains *raxSTAB* with native promoter (1282966-1287798), Km^R	This study
p*pctPB*	pVSP61 derivative, contains *pctP* and *pctB* with native promoter (c3260115-3263496), Km^R	This study
RaxB peptidase mutant plasmids		
p*raxSTAB-C28S*	p*raxSTAB* derivative, cysteine 28 of RaxB mutated to serine, Km^R	This study
p*raxSTAB-H101D*	p*raxSTAB* derivative, histidine 101 of RaxB mutated to aspartate, Km^R	This study
RaxX leader mutant plasmids		
p*raxX-G11A*	p*raxX* derivative, proRaxX glycine 11 mutated to alanine, Km^R	This study
p*raxX-G11D*	p*raxX* derivative, proRaxX glycine 11 mutated to aspartate, Km^R	This study
p*raxX-A12D*	p*raxX* derivative, proRaxX alanine 12 mutated to aspartate, Km^R	This study

p*raxX-A12G*	p*raxX* derivative, proRaxX alanine 12 mutated to glycine, Km^R	This study
p*raxX-A12R*	p*raxX* derivative, proRaxX alanine 12 mutated to arginine, Km^R	This study
p*raxX-G20A*	p*raxX* derivative, proRaxX glycine 20 mutated to alanine, Km^R	This study
p*raxX-G20D*	p*raxX* derivative, proRaxX glycine 20 mutated to aspartate, Km^R	This study
p*raxX-G20R*	p*raxX* derivative, proRaxX glycine 20 mutated to arginine, Km^R	This study
p*raxX-A21D*	p*raxX* derivative, proRaxX alanine 21 mutated to aspartate, Km^R	This study
p*raxX-G37D*	p*raxX* derivative, proRaxX glycine 37 mutated to aspartate, Km^R	This study
p*raxX-G38A*	p*raxX* derivative, proRaxX glycine 38 mutated to alanine, Km^R	This study
p*raxX-G38D*	p*raxX* derivative, proRaxX glycine 38 mutated to aspartate, Km^R	This study
p*raxX-G38R*	p*raxX* derivative, proRaxX glycine 38 mutated to arginine, Km^R	This study
p*raxX-G39D*	p*raxX* derivative, proRaxX glycine 39 mutated to asparate, Km^R	This study

* Cp^R-cephalexin resistance, Km^R-kanamycin resistance, Gm^R-gentamicin resistance

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