

Figure S1



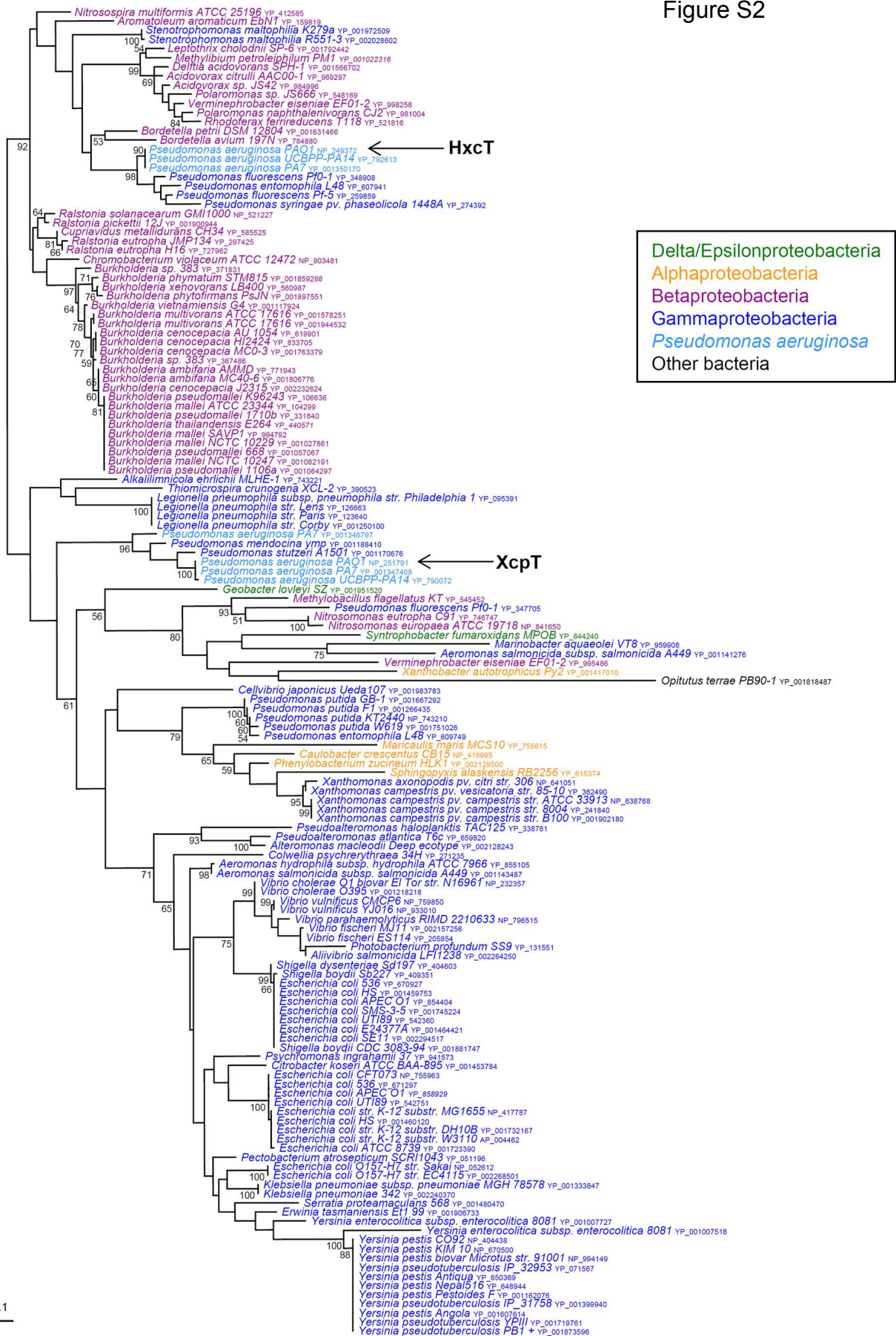


Figure S3B

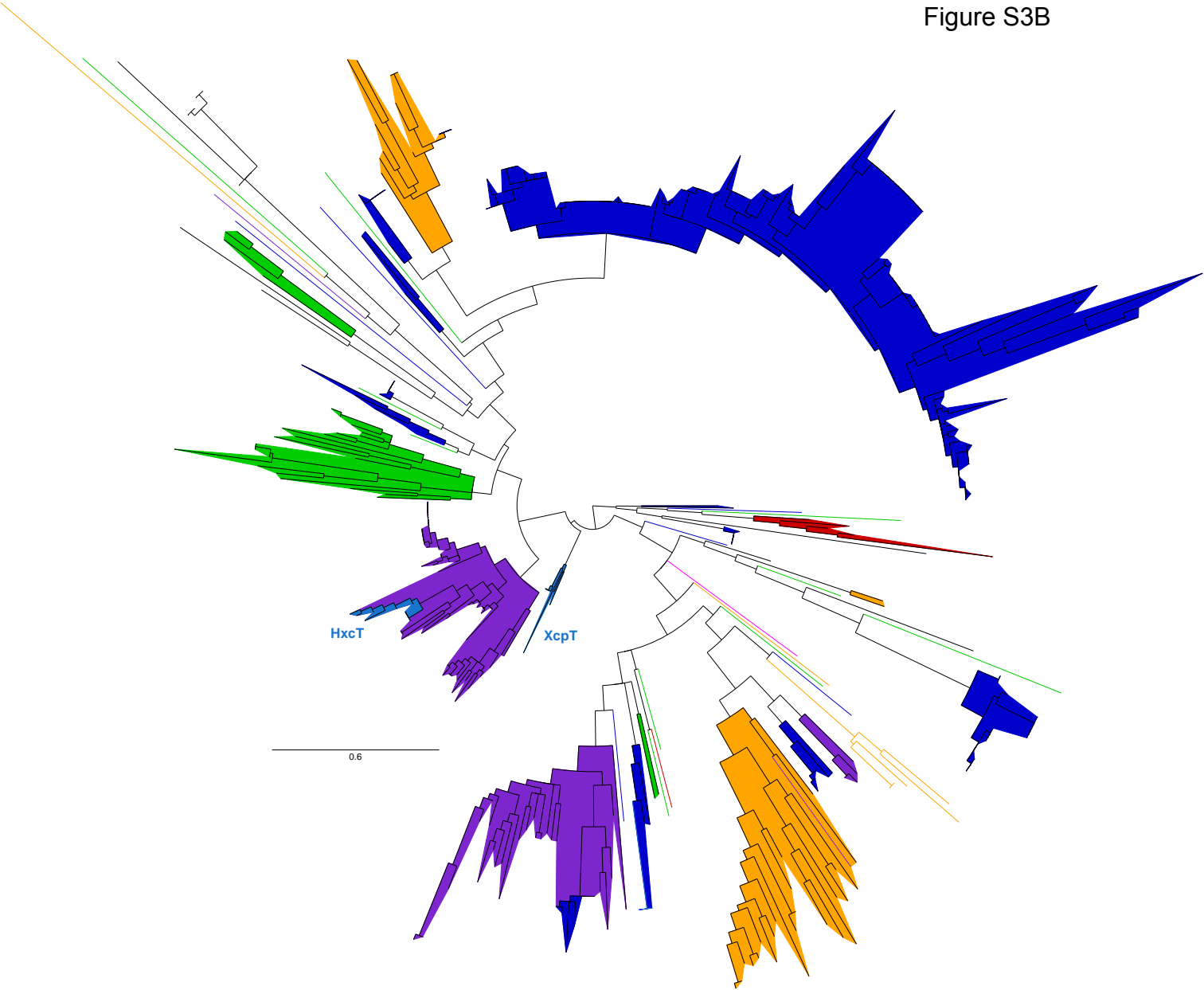


Figure S1. Maximum Likelihood phylogenetic tree based on the D1 dataset (99 sequences, 57 positions). The topology of the tree shows that T4P pilins and T2SS major pseudopilins represent two distinct homologous families. Number at nodes represent bootstrap values (only value > 50% are shown). The scale bar represents 0.1 substitutions per site. Gammaproteobacteria are in dark blue, Betaproteobacteria in purple, Alphaproteobacteria in yellow and Deltaproteobacteria in green. Sequences from *P. aeruginosa* PAO1 are underlined.

Figure S2. Maximum likelihood phylogenetic tree based on the D2 dataset (145 sequences, 114 positions). The taxonomic affiliation of each sequence is indicated by a colour code (see box for details). *Pseudomonas aeruginosa* PAO1 XcpT and HxcT are indicated by arrows. Numbers at branches represent bootstrap values (only value > 50% are shown). The scale bar represents the estimated average number of substitutions per site.

Figure S3. Maximum likelihood phylogenetic trees (A & B) based on the D3 dataset (397 sequences, 69 positions). Taxonomic groups are indicated by colours: green = Delta/Epsilonproteobacteria, purple = Betaproteobacteria, dark blue = Gammaproteobacteria, light blue = cluster of Gammaproteobacteria containing *Pseudomonas aeruginosa* PAO1 sequences, yellow = Alphaproteobacteria, pink = Firmicutes, red = Aquificae, black = Other bacteria). The scale bar represents the inferred average number of substitution per site.

Table S1: Nomenclature of the HxcT variants

Name	Mutation (HxcT-number-XcpT)
HxcTmut2	K23Q; E84K
HxcTmut3	K23Q; E84K; S77K
HxcTmut4	K23Q; E84K; S77K; N123D
HxcTmut5	K23Q; E84K; S77K; N123D; K48D
HxcTmut6	K23Q; E84K; S77K; N123D; K48D; P117E
HxcTmut7	K23Q; E84K; S77K; N123D; K48D; P117E; K93N
HxcTmut8	K23Q; E84K; S77K; N123D; K48D; P117E; K93N; K65E
HxcTmut9	K23Q; E84K; S77K; N123D; K48D; P117E; K93N; K65E; E69K
HxcTmut6Δn5	K23Q; E84K; S77K; N123D; P117E
HxcTn5	K48D

Table S2: Mutations and corresponding primers

Name	Mutation	Primer name	Primer sequence
n1	K23Q	<i>hxcTK23Q</i> -for <i>hxcTK23Q</i> -rev	5'-gccgcatgtgtccccaggtctcgaccgtcccgaccaggcccc-3' 5'-gtcgggacggctcgagcacctgggcaccaccatcgcgggcaggatc-3'
n2	E84K	<i>hxcTE84K</i> -for <i>hxcTE84K</i> -rev	5'-aactgcgctcgtactaagcgcctccaacgacctgggcaagc-3' 5'-ggggtcgtggcaggcgcttcaggtacgagcgccagttctcgcgaggc-3'
n3	S77K	<i>hxcTS77K</i> -for <i>hxcTS77K</i> -rev	5'-cctcagatcctcggcgaagaactgcgctcgtacctgagcgcctc-3' 5'-caggtacgagcgccagttctcgcgaggcatcggcaggggcgctccg-3'
n4	N123D	<i>hxcTN123D</i> -for <i>hxcTN123D</i> -rev	5'-ccgggcgggcaggggcatcgaccgacatcggtcctcagctct-3' 5'-ccaggagccgattcggcgtcgatccctcgcgcccggctcccgtc-3'
n5	K48D	<i>hxcTK48D</i> -for <i>hxcTK48D</i> -rev	5'-gggctatcaggcgctcgacctaccgcctcgaccaggggcgc-3' 5'-ctgtcgaggcggtagaggctcgagcgctcatcagccggagattc-3'
n6	P117E	<i>hxcTP117E</i> -for <i>hxcTP117E</i> -rev	5'-ctcggcgccgacgggcaggaggggcgaggggcatcaacgcccagatcg-3' 5'-gttatccctcgcccccctcctcccgtcggcgccgagggagaaacagc-3'
n7	K93N	<i>hxcTK93N</i> -for <i>hxcTK93N</i> -rev	5'-ccaacgaccctgggcaaccctaccagtacctaatcccggc-3' 5'-attcaggtactgtaggggtccccaggggtcgtggcaggcgc-3'
n8	K65E	<i>hxcTK65E</i> -for <i>hxcTK65E</i> -rev	5'-agcaggcgaggcggcgttaggtctcgcggagcgcctccgac-3' 5'-agggcgctccgcgagcacctcaagccctgcctgctcgatagc-3'
n9	E69K	<i>hxcTE69K</i> -for <i>hxcTE69K</i> -rev	5'-ggettaaggtctcgaagcgcctccgatcctcggcgagc-3' 5'-cgaggcatcggcaggcgcttcgcgagcacctcaagccctcgc-3'

Table S3: Vectors and Plasmids

Name	Description	Reference
pCR2.1	ColE1, fl <i>ori</i> , Ap ^R , Km ^R	Invitrogen
pMMB190	Ap ^R , pMM66EH, <i>tac</i> promoter, <i>lacZ</i> α	(1)
pJN105	Gm ^R , <i>araC</i> -pBAD, (broad-host-range vector)	(2)
pKNG101	Sm ^R , <i>mobRK2</i> , <i>sacBR</i> ⁺ (suicide vector)	(3)
pRK2013	Km ^R , ColE1, Tra+ Mob+ (RK2)	(4)
pET22b	<i>pelB</i> , T7 lac, Ap ^R , pBR322 <i>ori</i>	Novagen
pETG-22b	<i>pelB</i> , T7 lac, Ap ^R , pBR322 <i>ori</i> , C-ter his6-tag, gateway site added for recombination cloning	(5)
pKNG Δ <i>hxcT</i>	<i>Bam</i> HI/ <i>Apa</i> I 1000bp DNA fragment carrying <i>hxcV'</i> - <i>HxcX'</i> into the pKNG84, Sm ^R	This work
pCR2.1- <i>hxcT</i> wt	0.6 kb DNA fragment containing <i>hxcT</i> wild type cloned into pCR2.1	(6)
pMMB-XcpT	<i>Eco</i> RI DNA fragment carrying the <i>xcpT</i> gene cloned into the pMMB190, under the <i>tac</i> promoter	(7)
pMMB-XcpT ^H	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>xcpT(His6)</i> gene cloned into the pMMB190	(8)
pMMB-HxcT	<i>Eco</i> RI DNA fragment containing <i>hxcT</i> wild type cloned in pMMB190	This work
pMMB-HxcT ^H	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)</i> wild type gene cloned into the pMMB190	This work
pMMB-HxcTmut2	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut2</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut2	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut2</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut3	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut3</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut3	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut3</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut4	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut4</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut4	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut4</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut5	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut5</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut5	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut5</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut6	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut6</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut6	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut6</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut7	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut7</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut7	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut7</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut8	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut8</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut8	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut8</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut9	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut9</i> gene cloned into the pMMB190	This work

pMMB-HxcT ^H mut9	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut9</i> gene cloned into the pMMB190	This work
pMMB-HxcTmut6Δn5	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTmut6Δn5</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H mut6Δn5	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)mut6Δn5</i> gene cloned into the pMMB190	This work
pMMB-HxcTn5	<i>Eco</i> RV- <i>Bam</i> HI DNA fragment carrying the <i>hxcTn5</i> gene cloned into the pMMB190	This work
pMMB-HxcT ^H n5	<i>Bam</i> HI/ <i>Hind</i> III DNA fragment carrying the <i>hxcT(His6)n5</i> gene cloned into the pMMB190	This work
pMMB-HxcTPf	<i>Eco</i> RI DNA fragment carrying the <i>P. fluorescens</i> PF-5 <i>hxcT</i> gene cloned into pMMB190	This work
pJN-XcpT	<i>Xba</i> I/ <i>Sac</i> I DNA fragment carrying the <i>xcpT</i> gene cloned into the pJN105	This work
pJN-HxcT	<i>Xba</i> I/ <i>Sac</i> I DNA fragment carrying the <i>hxcT</i> wild type gene cloned into the pJN105	This work
pJN-HxcTmut5	<i>Xba</i> I/ <i>Sac</i> I DNA fragment carrying the <i>hxcTmut5</i> gene cloned into the pJN105	This work
pET-XcpTp _{NH}	<i>Bam</i> HI/ <i>Hind</i> III 500bp DNA fragment coding for the soluble domain of XcpT with a N-ter his6 tag, fused to the PelB leader peptide, cloned into the pET22b	(8)
pET-22b-HxcTp	attR1/attR2 987bp coding for the soluble domain of HxcT with a N-ter TEV protease cleavage site and a his6-tag, fused to the pelB cloned into the pET-22b	This work

Sm, streptomycin Ap, ampicilin; Gm, gentamicin; Km, kanamycin

Table S4: Strains

Name	Description	Reference
<i>E. coli</i>		
TG1	<i>SupEΔ(lac-proAB)thi hsdRΔ5 (F' :traD36 rpoA⁺B⁺lacI^qZAM15)</i>	Laboratory Collection
BL21(DE3)	<i>hsdS gal (1 cIts857 ind1 Sam7 nin5 lacUV5-T7 gene1)</i>	Invitrogen
CC118λpir	<i>Δ(ara-leu) araD ΔlacX74 galE galK phoA20 thi-1 rpsE rpoB argE(Am) recA1 Rf^R(λpir)</i>	(9)
<i>P. aeruginosa</i>		
PAO1	Prototrophe, chl-2	B. Holloway
<i>ΔxcpT</i>	Chromosomal <i>xcpT</i> deletion in PAO1	Laboratory collection
<i>ΔxcpTΔhxcT</i>	Chromosomal <i>xcpT</i> and <i>hxcT</i> deletion in PAO1	This work

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