PelX is a UDP-*N*-acetylglucosamine C4-epimerase involved in Pel polysaccharide-dependent biofilm formation

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Table S1: Bacterial strains and plasmids used in this study

Strain/Plasmid/Primer	Genotype/Properties	Source
E. coli		
TOP10	F- *mcr*AΔ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*Z ΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(ara leu) 7697 *gal*U *gal*K *rps*L *end*A1 *nup*G, Str^R	Invitrogen
BL21CodonPlus™ (DE3)-RP	F- *ompT hsdS*(rB^- mB^-)*dcm*+ Tet^r *gal*λ (DE3) *end*AHte *met*A::Tn5(Kan^r) [*arg*U *ile*Y *leu*W Cam^r]	Stratagene
DH5α	F– Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (rK–, mK+) *pho*A *sup*E44 λ– *thi*-1 *gyr*A96 *rel*A1	Invitrogen
SM10	*thi thr leu tonA lacY supE recA::RP4-2-Tc::Mu Km λpir*, Kan^R, Tet^R	1
P. protegens		
Pf-5	Wild-type	
Pf-5 Δ*pslA*	In frame deletion of *pslA* (PFL_4208)	This study
Pf-5 Δ*pelF*	In frame deletion of *pelF* (PFL_2977)	This study
Pf-5 Δ*pslA* Δ*pelF*	In frame deletion of *pslA* and *pelF*	This study
Pf-5 Δ*pelX*	In frame deletion of *pelX* (PFL_2971)	This study
Pf-5 ΔPFL_5533	In frame deletion of PFL_5533	This study
Pf-5 Δ*pelX* ΔPFL_5533	In frame deletion of *pelX* and PFL_5533	This study
Pf-5 *pelX-V*	*pelX* with a C-terminal VSV-G (YTDIEMNRLGK) tag	This study
Pf-5 *pelF-V*	*pelF* with a C-terminal VSV-G (YTDIEMNRLGK) tag	This study
Pf-5 PFL_5533-*V*	PFL_5533 with a C-terminal VSV-G (YTDIEMNRLGK) tag	This study
Plasmids		
Protein production		
pET-28a(+)	IPTG inducible expression vector; Kan^r	Novagen
pLSM-$PelX_{Pp}^{1-309}$ $_{C232S}$	$PelX_{Pp}$ encoding the full-length protein. Expressed thrombin-cleavable fusion protein $MGSSH_6SSGLVPRGSHM$-$PelX_{Pp}^{1-309}$ in pET-28a. Mutation of cysteine 232 to serine.	This study
pLSM-$PelX_{Pp}^{1-309}$ $_{C232S/Y146F/S121A}$	$PelX_{Pp}$ encoding the full-length protein. Expressed thrombin-cleavable fusion protein $MGSSH_6SSGLVPRGSHM$-$PelX_{Pp}^{1-309}$ in pET-28a. Mutation of cysteine 232 to serine, tyrosine 146 to phenylalanine, and serine 121 to alanine.	This study
WspR overexpression		
pPSV39	Expression vector with *lacI*, lacUV5 promoter derived from pPSV35	2
pLSM21	$WspR^{R242A}$ from PAO1 encoding the full-length protein with a mutation to the allosteric inhibition site in pPSV39. IPTG inducible expression.	This study
Two Step Allelic exchange		
pEXG2	allelic exchange vector, Gen^R	2
pLSM33	pEXG2 with an in-frame deletion allele for *P. protegens* Pf-5 *pslA* (PFL_4208) Gen^R	This study
pLSM34	pEXG2 with an in-frame deletion allele for *P. protegens* Pf-5 *pelF* (PFL_2977) Gen^R	This study
pLSM35	pEXG2 with an in-frame deletion allele for *P. protegens* Pf-5 *pelX* (PFL_2971) Gen^R	This study
pLSM36	pEXG2 with an in-frame deletion allele for *P. protegens* Pf-5 PFL_5533 Gen^R	This study

pLSM37	pEXG2 with full-length *pelF* fused to a C-terminal VSV-G tag Gen^R	This study
pLSM38	pEXG2 with full-length *pelX* fused to a C-terminal VSV-G tag Gen^R	This study
pLSM39	pEXG2 with full-length PFL_5533 fused to a C-terminal VSV-G tag Gen^R	This study

Amp, ampicillin; Cam, chloramphenicol; Kan, kanamycin; Gen, Gentamicin; Str, streptomycin; Tet, tetracycline

Table S2: Primers used in this study

Primers	**Sequence***
Vectors for recombinant protein production	
PelX 1F	GG **CAT ATG** TCT GCC GAA CGG ATA CTG
PelX 309R	GG **CTC GAG** CTA AAG GCT GCG GTA AAG CCG
PelX C232S F	CAG GGC CTG GAA aGC CCC GCG CCG G
PelX C232S R	C CGG CGC GGG GCt TTC CAG GCC CTG
PelX Y146F F	CC CTC ACG CCC TtC GCG GCG GAC AA
PelX Y146F R	TT GTC CGC CGC GaA GGG CGT GAG GG
PelX S121A F	G GTG TTC GCG TCC gcT GCG GCG GTC TAT GG
PelX S121A R	CC ATA GAC CGC CGC Agc GGA CGC GAA CAC C
Allelic exchange vectors	
PslA_Pf5_upF	TAG TAC AGA **GAA TTC** GGC AAT CAG CCG GGT ATG G
PslA_Pf5_upR	*G TGT GCT CAT TCA GAA GGC CTC* GCT GTC TAC AGG TTG CAA CCG
PslA_Pf5_downF	GAG GCC TTC TGA ATG AGC ACA C
PslA_Pf5_downR	T CAA TCA GTA **TCT AGA** GCA TGC AGA ACA TCC CGC CG
PelF_Pf5_upF	TAG TAC AGA **GAA TTC** CAA CTG CAT TCG CCC ACC TTC
PelF_Pf5_upR	*CAC AGC CTC CTT GTG TGG GGT* GGG GGT TGA GTC GGG GGT
PelF_Pf5_downF	ACC CCA CAC AAG GAG GCT GTG
PelF_Pf5_downR	T CAA TCA GTA **TCT AGA** CAG CGC CAG CAA CAG CCCT
PelX_Pf5_upF	TAG TAC AGA **GGT ACC** GGA ACA ACG CATAGG GAA TCGC
PelX_Pf5_upR	*GCT GCG GTA AAG CCG TTC CAG* AAC CAG TAT CCG TTC GGC AGA CAT
PelX_Pf5_downF	CTG GAA CGG CTT TAC CGC AGC

PelX_Pf5_downR	T CAA TCA GTA **AAG CTT** TCG GGG AGC AAC TGG AAA CTG
PFL_5533 upF	GTG **GAA TTC** GCT TAC TAC TTC GAC TGG TTT C
PFL_5533 upR	*GGC GAG GCC GAC GCT CAT* CAA TAC GAG GCC TTC AGC CAT
PFL_5533 downF	ATG AGC GTC GGC CTC GCC
PFL_5533 downR	GGT **AAG CTT** CAA CGC CCA CGA AGC TGG T
PelX VSVG upF	GGG **GAA TTC** ATG GCA ATA ACG GCG AGG GC
PelX VSVG upR	ttt tcc taa tct att cat ttc aat atc tgt ata AAG GCT GCG GTA AAG CCG TTC
PelX VSVG downF	tat aca gat att gaa atg aat aga tta gga aaa TAG TTC GTT GCC TTA GGG GCG
PelX VSVG downR	GGT **AAG CTT** CTG GTC CTG CAG CGC CTT G
PelF VSVG upF	GGG **GAA TTC** GGT GGT GCC GAT CAA GGA CG
PelF VSVG upR	ttt tcc taa tct att cat ttc aat atc tgt ata CAC AGC CTC CTT GTG TGG GG
PelF VSVG downF	tat aca gat att gaa atg aat aga tta gga aaa TAA ATG GCC GGC ATC GGT TTC G
PelF VSVG downR	GGG **AAG CTT** ATC ACC AGG AGG ATG CGG TTA TA
PFL_5533 VSVG upF	GGG **GAA TTC** ATG GCA ACA ACG GGG AAG GC
PFL_5533 VSVG upR	ttt tcc taa tct att cat ttc aat atc tgt ata GCG CCC CAT CAG GCG GG
PFL_5533 VSVG downF	Tat aca gat att gaa atg aat aga tta gga aaa TGA GGG AAA ACA CGC ACA TGA AA
PFL_5533 VSVG downR	GAG **AAG CTT** ACC CCG TTC GAA CAC GAC GT
Sequencing Primers	
PslA_Pf5_seqF	GGC TGG CCG GGG CGT C
PslA_Pf5_seqR	GGT GGC TCT GCT CCA GGC A
PelF_Pf5_seqF	GCG AGG CGC AGA CGT GGC
PelF_Pf5_seqR	CAC CAG CTT CTC GGC CCG
PelX_Pf5_seqF	TGC AGC AAG GAG GTG CGG G
PelX_Pf5_seqR	GGC GAC GCC ATC GAG CTC
PFL_5533 seqF	AAC TGC TCG ACG ACA CCC

PFL_5533 seqR	GCA CGA ACG ATG ATG TCA C
WspR overexpression	
WspR-F	GC **GAA TTC** AGG AGG ATA TTC ATG CAC AAC CCT CAT GAG AGC
WspR-R	GC **AAG CTT** TCA GCC CGC CGG GGC

*****Restriction site sequences are in **bold**; regions of complementary to the target amplicon are underlined; lower case letters denote a nucleotide substitution or mismatch to sequence (in the case of VSV-G tag); regions of complementarity to facilitate splicing of PCR products are in *italics*

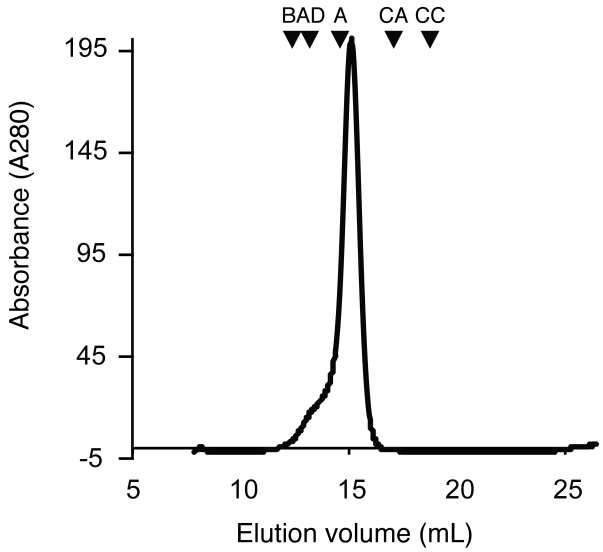

Figure S1: PelX^{C232S} forms a dimer in solution. Analytical gel filtration demonstrates that PelX^{C232S} exists as a dimer in solution, eluting at a molecular weight of approximately 63 kDa. Expected molecular weight: 34.6 kDa. Protein standards used to calibrate the column are indicated by inverted triangles; BA, β-amylase; AD, alcohol dehydrogenase; A, albumin; CA, carbonic anhydrase; CC, cytochrome C. The molecular weights of β-amylase, alcohol dehydrogenase, albumin, carbonic anhydrase, and cytochrome C are 200 kDa, 150 kDa, 66 kDa, 29 kDa, and 12.4 kDa, respectively.

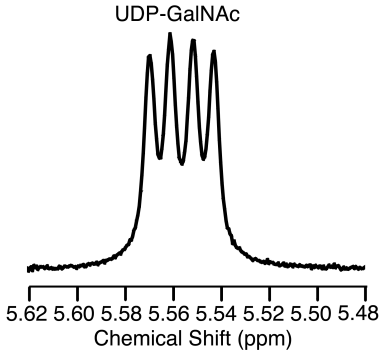

Figure S2: PelX$^{C232S/Y146F/S121A}$ is catalytically inactive towards UDP-GalNAc. ^{1}H NMR spectrum from the reaction of PelX$^{C232S\ S121A\ Y146F}$ with UDP-GalNAc.

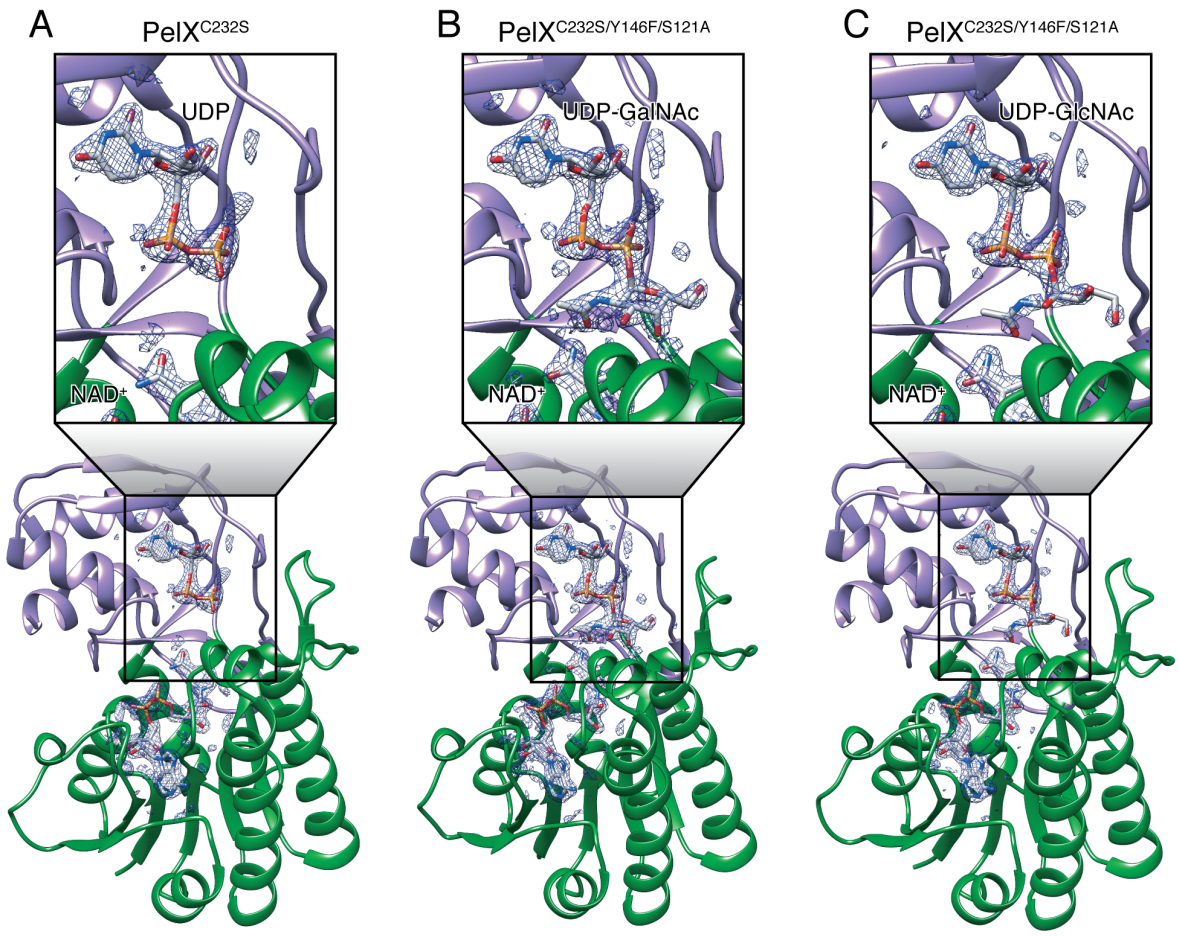

Figure S3: PelX$^{C232S/Y146F/S121A}$ density of the ligands. PelX is displayed with its N-terminal Rossmann-fold domain shown in green, and its C-terminal substrate-binding α/β-domain in purple as in Figure 5. (A) PelX^{C232S} with density shown for UDP and NAD$^+$ (B) PelX$^{C232S/Y146F/S121A}$ in complex with UDP-GalNAc and NAD$^+$ and (C) PelX$^{C232S/Y146F/S121A}$ in complex with UDP-GlcNAc and NAD$^+$. All three structures were modeled with NAD$^+$ and nucleotide or sugar-nucleotide shown in stick representation, with the corresponding |2mFo-DFc| map displayed as black mesh contoured at 2.0 σ.

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