Supplemental Material

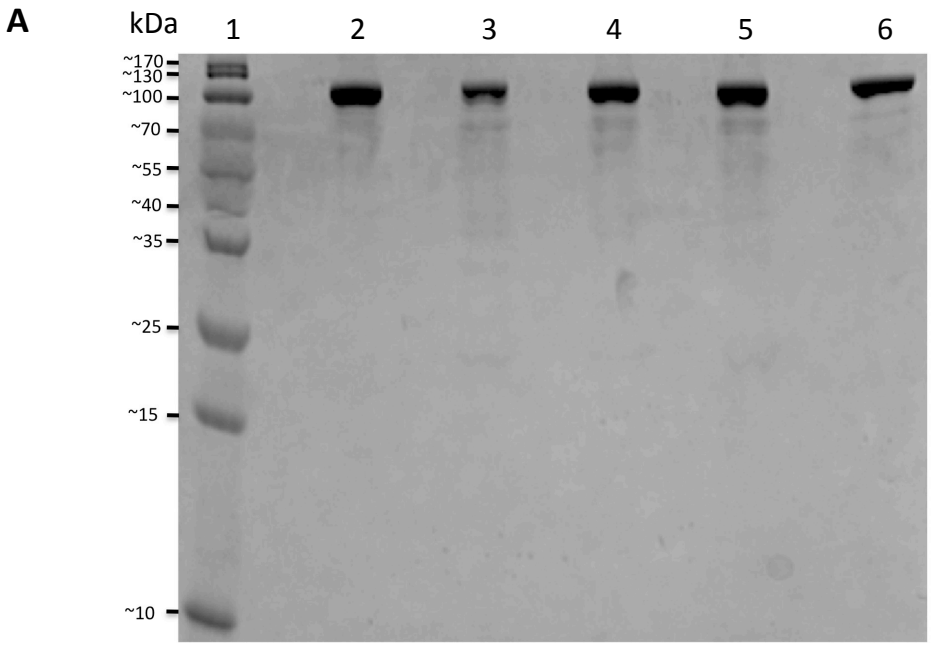
Supplemental Materials and Methods

Circular dichroism spectroscopy:

CD spectra of wild type PelA ($PelA_{\Delta 46}$) and the D528A, H600A and H604A mutants were measured using a Jasco J-810 spectrometer (Jasco Spectroscopic Company, Japan). Experiments were performed at room temperature using a quartz glass cell with a 1 mm path length. The samples were recorded from 300 – 190 nm wavelengths with a total of three scans for each. The molar elipticity was calculated based on the concentrations of protein used, which was verified using the Peirce® BCA Protein Assay Kit from ThermoScientific.

Supplemental Figure legends

Supplemental Figure 1. SDS-PAGE and CD analysis of wild type PelA and the four PelA point mutants (D528A, D530A, H600A and H604A). (A) A 14 % SDS gel was used. Lane 1, molecular weight marker in kDa; Lane 2, wild type PelA ($PelA_{\Delta 46}$) protein; Lane 3, $PelA_{\Delta 46}$ D528A mutant; Lane 4, $PelA_{\Delta 46}$ D530A mutant; Lane 5, $PelA_{\Delta 46}$ H600A mutant; Lane 6 $PelA_{\Delta 46}$ H604A mutant. Each protein was concentrated to 0.5 mg ml^{-1} and mixed 1:1 with 2X loading buffer. All proteins samples were >99% pure and the molecular weight estimated to be ~102 kDa. Proteins were purified as described in the Methods section. (B) The CD spectra for the mutants were comparable to that of the wild type. The concentration of each protein used to calculate the molar elipticity was verified using the Peirce® BCA Protein Assay Kit from ThermoScientific.


A
kDa
1
2
3
4
5
6
~170
~130
~100
~70
~55
~40
~35
~25
~15
~10

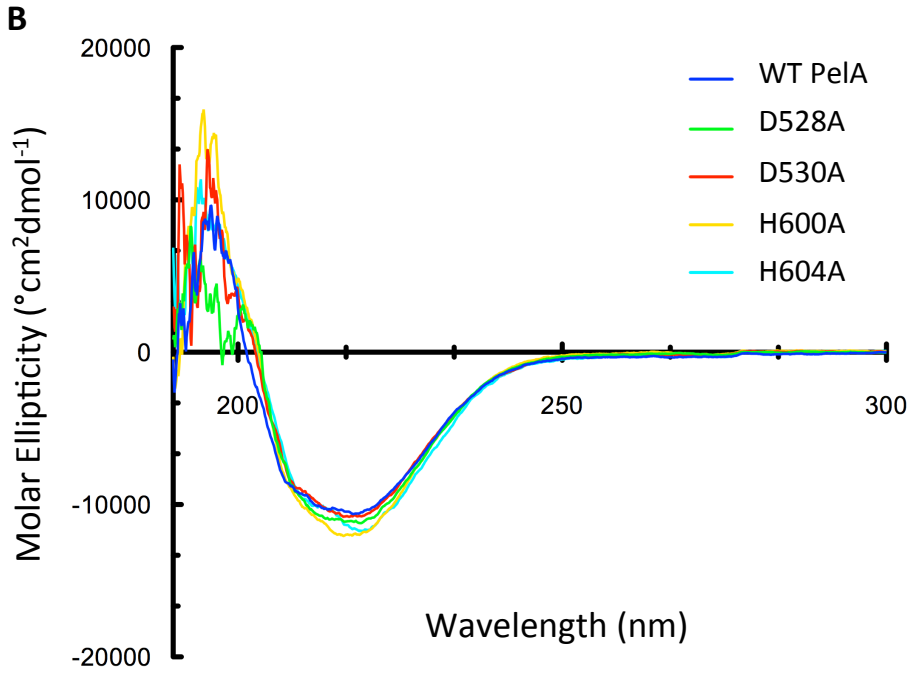

B
20000
10000
0
-10000
-20000
200
250
300
Molar Ellipticity (°cm²dmol⁻¹)
Wavelength (nm)
WT PelA
D528A
D530A
H600A
H604A

Table 1. Bacterial strains, primers and plasmids

Deletion Construct primers

PelA upstream-F HindIII	TTTAAGCTTGCTGCGCGGCGACAAATGTCC
PelA upstream-R	GCAACTCGAAGGTCTTACGGCAGGACGGCAG
PelA downstream-F	CCGTAACACCTTCGAGTTGCCGATGGAGCAG
PelA downstream-R HindIII	TTTAAGCTTCCAGTTCGGCAAAGGTCCGCG

Chromosomal PelA Point Mutation Primers

PelA upstream-F2-HindIII	TTTAAGCTTCGAGGGCGACCTGACCCTGAG
PelA downstream-R2-HindIII	TTTAAGCTTGCCTGCATCGCCGCGTAGATCTG
PelA D528A F	CCGTGCACATCGCTGGCGACGGCTTC
PelA D528A R	GAAGCCGTCGCCAGCGATGTGCACGG
PelA D530A F	CACATCGATGGCGCCGGCTTCGTCTC
PelA D530A R	GAGACGAAGCCGGCGCCATCGATGTG
PelA H600A F	GGTCGCCAGCGCTACCTTCAGCCATC
PelA H600A R	GATGGCTGAAGGTAGCGCTGGCGACC
PelA H604A F	CCATACCTTCAGCGCTCCGTTCTTCTGGC
PelA H604A R	GCCAGAAGAACGGAGCGCTGAAGGTATGG

Recombinant vector construct primers

$PelA_{\Delta 46}$ F	CTGCATATGGGCGGGCCGTCCAGCGTGGCG
$PelA_{\Delta 46}$ R	GAGCTCGAGTCAGCGGCAGACGAGTTGGCC

PelA Point mutant primers used to generate recombinant proteins

$PelA_{\Delta 46}$ D528A F	ACCGTGCACATCGCTGGCGACGGCTTC
$PelA_{\Delta 46}$ D528A R	GAAGCCGTCGCCAGCGATGTGCACGGT
$PelA_{\Delta 46}$ D530A F	GTGCACATCGATGGCGCCGGCTTCGTCTCCCGT
$PelA_{\Delta 46}$ D530A R	ACGGGAGACGAAGCCGGCGCCATCGATGTGCAC
$PelA_{\Delta 46}$ H600A F	GTCGAGGTCGCCAGCGCTACCTTCAGCCATCCG
$PelA_{\Delta 46}$ H600A R	CGGATGGCTGAAGGTAGCGCTGGCGACCTCGAC
$PelA_{\Delta 46}$ H604A F	AGCCATACCTTCAGCGCTCCGTTCTTCTGGCAG
$PelA_{\Delta 46}$ H604A R	CTGCCAGAAGAACGGAGCGCTGAAGGTATGGCT

Strains		**Reference**
PAO1	Wild-type	Rahme et al., 1995
PAO1Δ*wspF*	*wspF;* non-polar mutation	Hickman *et al.*, 2005
PAO1Δ*wspF*Δ*pel*	*wspF;* non-polar mutation, *pelA;* polar mutant of the *pel* operon	Irie *et al.*, 2010
PAO1Δ*wspF*Δ*psl*	*wspF;* non-polar mutation, *pslBCD;* polar mutant of the *psl* operon	Irie *et al.*, 2010
PAO1Δ*wspF*Δ*psl*Δ*pel*	*wspF;* non-polar mutation, *pelA;* polar mutant of the *pel* operon, *pslBCD*; polar mutant of the *psl* operon	Borlee *et al*. 2010

PAO1Δ*wspF*Δ*psl* P_{BAD}*pel*	*wspF;* non-polar mutation, *pslBCD;* polar mutant of the *psl* operon, arabinose-inducible *pel* operon	This study
PAO1Δ*wspF*Δ*psl* P_{BAD}*pel*::*pelA*$_{D528A}$	*pelA* $_{D528A}$	This study
PA14	wild-type	Rahme *et al*., 1995
PA14Δ*pelA*	*pelA* nonpolar mutant of the *pel* operon	This study
PA14::*pelA*$_{D528A}$	*pelA* $_{D528A}$	This study
PA14::*pelA*$_{D530A}$	*pelA* $_{D530A}$	This study
PA14::*pelA*$_{H604A}$	*pelA* $_{H600A}$	This study
PA14::*pelA*$_{H600A}$	*pelA* $_{H604A}$	This study
PA14 P_{BAD}*pel*	arabinose-inducible *pel* operon	Colvin *et al*. 2011
E. coli BL21-CodonPlus(DE3)-RP	*F*$^-$ *ompT hsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r *gal l*(DE3) *endA* Hte [*argU proL* Cam^r]	Novagen

Plasmids

pEX18Gm	Suicide cloning vector, gentamicin resistance	Hoang *et al*., 1998
pEX18Gm:: Δ*pelA*	*pelA* replacement vector	This study
pEX18Gm:: Δ*pelA2*	*pelA* replacement vector for SD mutagenesis	This study
pEX18Gm:: Δ*pelA* $_{D528A}$	Site-directed *pelA* $_{D528A}$ replacement vector	This study
pEX18Gm:: Δ*pelA* $_{D530A}$	Site-directed *pelA* $_{D530A}$ replacement vector	This study
pEX18Gm:: Δ*pelA* $_{H600A}$	Site-directed *pelA* $_{H600A}$ replacement vector	This study
pEX18Gm:: Δ*pelA* $_{H604A}$	Site-directed *pelA* $_{H604A}$ replacement vector	This study
pJN105	*araC*-PBAD cassette cloned in pBBR1MCS-5, gentamicin resistance	Newman & Fuqua, 1999
pJN1120	*PA1120* cloned into pJN105	Borlee *et al*. 2010
pPSV18	shuttle vector with pBR322 origin, *bla*	Rietsch *et al*., 2004
pNA*pelA* $_{Δ46}$	pET28a based *pelA* expression vector	This study
pNA*pelA* $_{Δ46\ D528A}$	Site-directed *pelA* $_{D528A}$ expression vector	This study
pNA*pelA* $_{Δ46\ D530A}$	Site-directed *pelA* $_{D530A}$ expression vector	This study
pNA*pelA* $_{Δ46\ H600A}$	Site-directed *pelA* $_{H600A}$ expression vector	This study
pNA*pelA* $_{Δ46\ H604A}$	Site-directed *pelA* $_{H604A}$ expression vector	This study

Expression vector

pET-28a	T7 expression system inducible with IPTG	Novagen