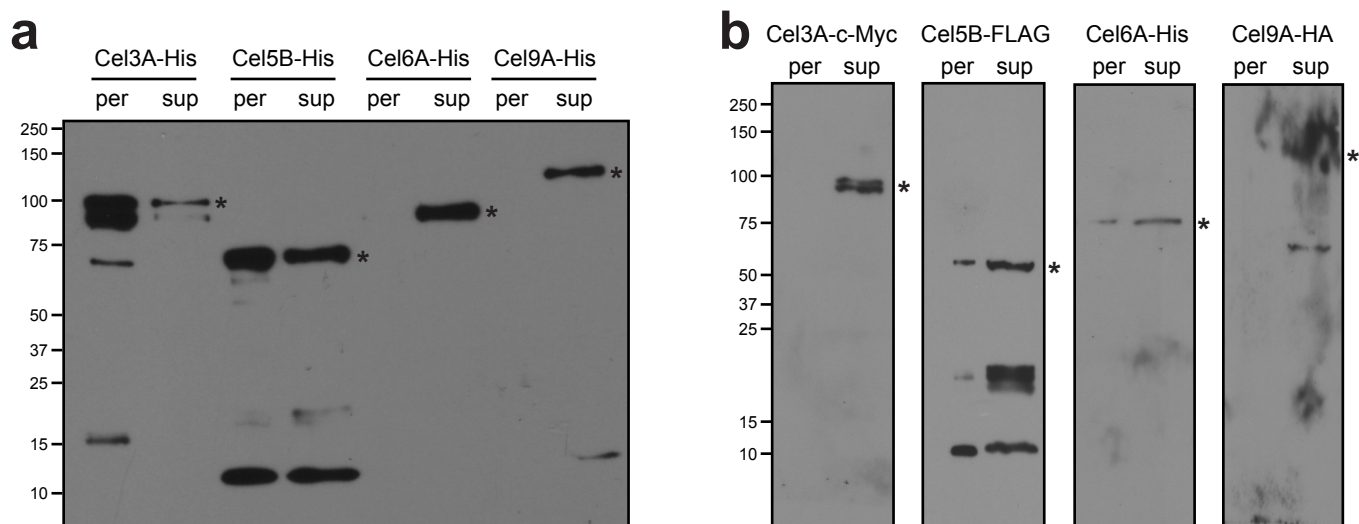


Supplemental Figure S1. Expression and purification of YebF. (a) Western blot analysis of cell-free supernatant fractions from wt BW25113 cells or mutant strains expressing YebF-FT. Blot was probed using anti-His antibodies. (b) Growth curves of the same strains in (a). Cells were grown aerobically at 37°C in liquid LB medium in shake flasks. (c) Purification of YebF from culture supernatants from various wt and knockout mutants of *E. coli* BW25113 and BL21(DE3) as indicated. SDS-PAGE analysis was performed using 5 μ g of each purified protein followed by staining with Coomassie stain. Total protein was determined following IMAC purification using a BCA protein assay kit. Titer of YebF in mg of protein per L supernatant are indicated for each case.



Supplemental Figure S2. Extracellular secretion of cellulases fused to YebF. (a) Western blot analysis of periplasmic (per) and supernatant (sup) fractions from wt BW25113 cells separately expressing YebF-Cel3A, YebF-Cel5B, YebF-Cel6A, and YebF-Cel9A. Proteins were detected using anti-His antibodies. (b) Western blot analysis of periplasmic (per) and supernatant (sup) fractions from wt BW25113 cells co-secreting all four YebF-cellulase fusion proteins. Blots were probed using (from left to right) anti-c-Myc, anti-FLAG, anti-His, and anti-HA antibodies. Asterisks denote the full-length target protein in each case.

Supplemental Table S1. Characterization of outer membrane integrity and permeability

Assay	wt	$\Delta entC$	$\Delta entE$	$\Delta envZ$	$\Delta m zrA$	$\Delta nlpD$	$\Delta ompR$	$\Delta tnaA$	$\Delta yihF$
RNAseI leakage ^a	-	-	-	+	-	-	++	-	-
Detergent sensitivity ^b	-	+	-	++	-	+	++	-	-
MBP leakage ^c	-	-	-	++	+	-	+	-	-
DsbA leakage ^c	-	-	-	++	+	-	+	-	-

a. - no clearing; + moderate clearing; ++ significant clearing

b. - resistant; + mildly sensitive; ++ sensitive

c. - none detectable; + low amount; ++ high amount

Supplemental Table S2. Plasmids used in this study

Plasmid	Description*	Source
pTrc99A	P _{trc} promoter; ColE1 <i>ori</i> , Amp ^r	(Amann et al., 1988)
pBAD33	P _{BAD} promoter; ColE1 <i>ori</i> , Cm ^r	(Guzman et al., 1995)
pET-21a	T7 promoter; pBR322 <i>ori</i> , Amp ^r	Novagen
pTrc-YebF	<i>yebF</i> cloned in pTrc99A	This study
pTrc-YebF-FT	Tetracycline “FLAsH tag” (FT) with C-terminal 6x-His epitope cloned into pTrc-YebF	This study
pTrc-ΔspYebF-FT	YebF-FT lacking 21-residue N-terminal Sec export signal in pTrc99A	This study
pTrc-spTorA-ΔYebF-FT	YebF-FT modified with N-terminal TorA signal peptide in pTrc-ΔspYebF-FT	This study
pET-21a-YebF	<i>yebF</i> cloned in pET-21a with C-terminal 6x-His epitope	This study
pTrc-YebF-Cel3A	<i>C. japonicus cel3A</i> gene from in pTrc-YebF with C-terminal 6x-His tag	This study
pTrc-YebF-Cel5B	<i>C. japonicus cel5B</i> gene in pTrc-YebF with C-terminal 6x-His tag	This study
pTrc-YebF-Cel6A	<i>C. japonicus cel6A</i> gene in pTrc-YebF with C-terminal 6x-His tag	This study
pTrc-YebF-Cel9A	<i>C. japonicus cel9A</i> gene in pTrc-YebF with C-terminal 6x-His tag	This study
pYebF-CEL4	Polycistronic expression of YebF-Cel6A-6x-His, YebF-Cel9A-HA, YebF-Cel5B-FLAG, YebF-Cel3B-c-Myc	This study
pBAD33-EntC	<i>entC</i> cloned into pBAD33	This study
pBAD33-EntE	<i>entE</i> cloned into pBAD33	This study
pBAD33-EnvZ	<i>envZ</i> cloned into pBAD33	This study
pBAD33-MzrA	<i>mzrA</i> cloned into pBAD33	This study
pBAD33-NlpD	<i>nlpD</i> cloned into pBAD33	This study
pBAD33-OmpR	<i>ompR</i> cloned into pBAD33	This study
pBAD33-TnaA	<i>tnaA</i> cloned into pBAD33	This study
pBAD33-YihF	<i>yihF</i> cloned into pBAD33	This study
pTrc-OmpA-FT	<i>ompA</i> with C-terminal FT and 6x-His in pTrc99A	This study
pTrc-OmpF-FT	<i>ompF</i> with C-terminal FT and 6x-His in pTrc99A	
pTrc-OsmY-FT	<i>osmY</i> with C-terminal FT and 6x-His in pTrc99A	
pTrc-ChiA-FT	<i>chiA</i> with C-terminal FT and 6x-His in pTrc99A	
pTrc-PelB-FT	<i>D. dadantii pelB</i> gene with C-terminal FT and 6x-His in pTrc99A	
pTrc-AvrPto-FT	<i>P. syringae avrPto</i> gene with C-terminal FT and 6x-His in pTrc99A	This study
pCHAP4278	Entire <i>gsp</i> locus in pACYC184	(Francetic et al., 2000)

pCPP2006	Entire <i>D. dadantii</i> <i>out</i> locus in pCPP19	(He et al., 1991)
pCPP2156	Entire <i>D. dadantii</i> <i>hrp</i> locus in pCPP19	(Ham et al., 1998)

*genes are from *E. coli* unless indicated otherwise

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