SUPPLEMENTAL DATA

Supplemental Data Table 1. Bacterial strains and plasmids used in the experiment.

Strains or Plasmids	Description	Reference
Strains		
Francisella tularensis LVS	Pm ^{r}, Live vaccine strain	USAMRIID, Frederick, MD
Esherichia coli DH5α	F^{-} (*φ80dlacZ vM15*) *recA1 endA1 gyrA96 thi-1 hsdR17(r^{-}k M^{+}k) supE44 relA1 deoR Δ(lacZYA-argF) U169*	63
Shigella dysenteriae sd197	*S. dysenteriae* serotype 1	MGH[a], Boston, MA
*Ft.*LVS::*wbtA*	Pm ^{r,} Km^{r}, Live vaccine strain ; *ΔwbtA*	31
*Ft.*LVS::*wzy*	Pm ^{r}, Live vaccine strain ; *Δwzy*	This study
*Ft.*LVS::*wzy*/pTH21	Pm ^{r}, Live vaccine strain ; *Δwzy* harboring pTH21	This study
*Ft.*LVS::*wzy*/pTH33	Pm ^{r}, Live vaccine strain ; *Δwzy* harboring pTH33	This study
Plasmids		
pEX18Tc	6.3-kb; Tc^{r}; *oriT*$^{+}$ *sacB*$^{+}$, gene replacement vector with MCS from pUC18	64
pEX18Km	6.8-kb; Km^{r}; *oriT*$^{+}$ *sacB*$^{+}$, gene replacement vector with MCS from pUC18	This study
pPV	Ap ^{r}, Cm^{r}, *sacB*, mob	38
pFNLTP6	6.9-kb; pFNLTP1 derivative with *Nde*I, *Eco*RI, *Sma*I, *Not*I, *Nhe*I, and *Xho*I restriction enzyme sites (MCS2) cloned between the *Kpn*I and *Bam*HI sites; km ^{r}Ap^{r}	65
pCR2.1 TOPO	3.9-kb; plasmid for cloning PCR product, km ^{r}Ap^{r}	Invitrogen, Carlsbad, CA
pTH30	11.4-kb; pEX18Km harboring *sacB* (from pPV) and upstream and downstream of *wzy*	This study
pTH17	7.1-kb; pFNLTP6 harboring P$_{groEL}$	This study
pTH21	8.3-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*	This study
pTH33	8.3-kb; pFNLTP6 harboring P$_{groEL}$::*wzy$_{shigella}$*	This study
pE25A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{E25A}$	This study
pK27A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{K27A}$	This study
pD52A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D52A}$	This study
pD144A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D144A}$	This study
pK152A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{K152A}$	This study
pK153A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{K153A}$	This study
pE157A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{E157A}$	This study
pE163A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{E163A}$	This study
pK165A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{K165A}$	This study
pR167A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{R167A}$	This study
pD177A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D177A}$	This study
pE277A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{E277A}$	This study
pD287A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D287A}$	This study
pH290A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{H290A}$	This study
pK399A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{K399A}$	This study
pE400A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{E400A}$	This study
pR406A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{R406A}$	This study
pK399A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{K399A}$	This study
pG176A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G176A}$	This study
pG176E	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G176E}$	This study
pG176S	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G176S}$	This study
pG176K	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G176K}$	This study
pD177S	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D177S}$	This study
pD177E	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D177E}$	This study
pD177K	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{D177K}$	This study
pG178A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G178A}$	This study
pG178S	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G178S}$	This study
pG178E	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G178E}$	This study
pG323A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G323A}$	This study
pG323S	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G323S}$	This study
pG323E	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G323E}$	This study
pG323R	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G323R}$	This study
pG323A	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G324A}$	This study
pG323E	8.1-kb; pFNLTP6 harboring P$_{groEL}$::*wzy*$_{G324E}$	This study
wzy-gfp	8.9-kb; pFNLTP6 harboring *wzy$_{::}$gfp*	This study
P-gfp	7.8-kb; pFNLTP6 harboring P$_{groEL}$*::gfp*	This study
P-wzy-gfp	9.1-kb; pFNLTP6 harboring P$_{groEL}$*::wzy::gfp*	This study
P-wzy (G_{176}E)-gfp	9.1-kb; pFNLTP6 harboring P$_{groEL}$*::wzy(G_{176}E)::gfp*	This study
P-wzy (D_{177}A)-gfp	9.1-kb; pFNLTP6 harboring P$_{groEL}$*::wzy(D_{177}A)::gfp*	This study
P-wzy (G_{323}E)-gfp	9.1-kb; pFNLTP6 harboring P$_{groEL}$*::wzy(G_{323}E)::gfp*	This study
P-wzy (Y_{324}E)-gfp	9.1-kb; pFNLTP6 harboring P$_{groEL}$*::wzy(Y_{324}E)::gfp*	This study
P-wzy (K_{152}A)-gfp	9.1-kb; pFNLTP6 harboring P$_{groEL}$*::wzy(K_{352}E)::gfp*	This study

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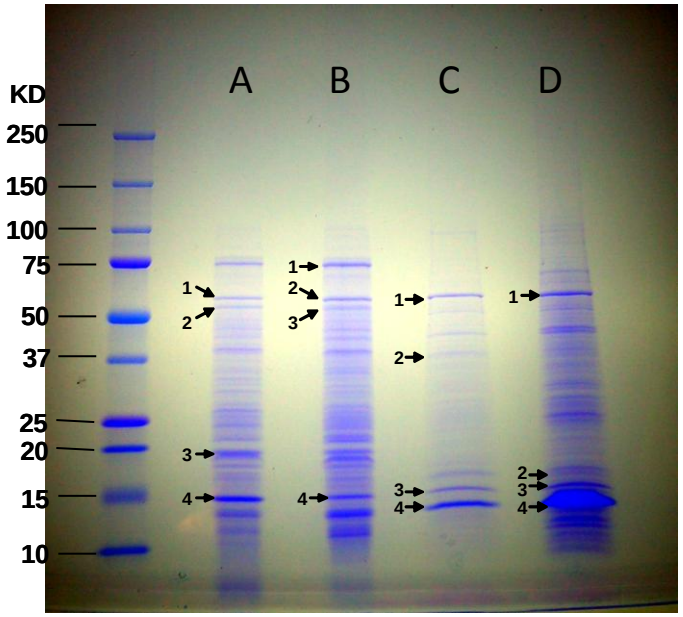
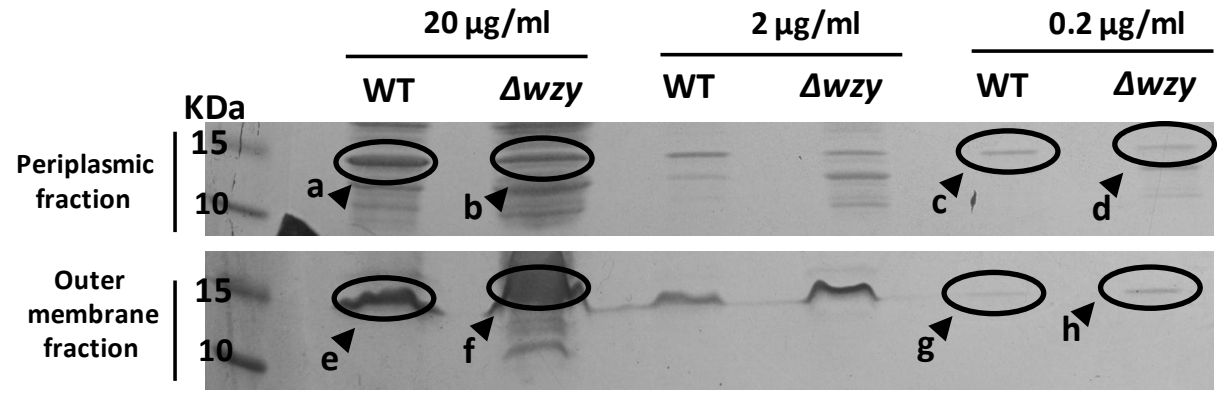


Identified proteins from MASS Spec. analysis	Predicted subcellular location	Presence of identified proteins in each lane			
		A (periplasm)	B (periplasm)	C (outer membrane)	D (outer membrane)
peroxidase/catalase	Periplasm[66]	○ (A-1)	○ (B-2)	× (C-1)	× (D-1)
peroxiredoxin,AhpC-TSA family protein	Periplasm[67]	○ (A-4)	○ (B-4)	× (C-3)	× (D-3)
outer membrane protein OmpH	Outer Membrane[68]	× (A-4)	× (B-4)	○ (C-3)	○ (D-3)
outer membrane lipoprotein blc	Outer Membrane[69]	× (A-4)	× (B-4)	○ (C-3)	○ (D-3)
17KDa outer membrane protein	Outer membrane[70]	× (A-4)	× (B-4)	○ (C-3)	○ (D-3)

O - *presence (of the protein in lane),* X-*no presence (of the protein in lane) A: Ft.LVS.wt (periplasm); B: Ft.LVS::wzy (periplasm); C: Ft.LVS.wt (outer membrane) ; D: Ft.LVS::wzy (outer membrane) ;*

Supplemental Data Figure 1. Mass spectrometry analysis of proteins located in subcellular fractions. The isolated subcellular fractions were analyzed by SDS-PAGE gel (4-20% gradient gel) and 4 randomly chosen protein bands in each fraction was analyzed by Mass spectrometry to identify the proteins and predict their subcellular locations. The table shows the fraction-specific protein distribution in SDS-PAGE gel bands from the periplasmic and outer membrane fraction. Although they have the same MW, different proteins are found in each fraction indicating the purity of the fractions. For example, peroxidase/catalase, which is a periplasmic protein, was only detected in A-1 and B-2 (periplasmic fraction bands), but not in the C-1 and D-1 band which are collected from the outer membrane fractions even though these four bands are the same MW).

	Predicted Subcellular Location	Presence in the lane after MS spec analysis							
		Periplasmic fraction				Outer membrane fraction			
		a	b	c	d	e	f	g	h
		20 µg/ml	20 µg/ml	0.2 µg/ml	0.2 µg/ml	20 µg/ml	20 µg/ml	0.2 µg/ml	0.2 µg/ml
peroxiredoxin, AhpC-TSA family protein	Periplasm	○	○	○	○	×	×	×	×
17kDa outer membrane protein	Outer membrane	×	×	×	×	○	○	○	○



○ ***:*** *presence of the protein (after Mass spec. analysis)*
×***:*** *no presence of the protein (after Mass spec. analysis)*
a-h: *~15 KDa size of protein band in each fraction (All samples were ran in 4-20% gradient gell and stained with Coomasie).*
20 µg/ml: *original protein concentration in sample fractions (calculated by Bradford assay)*
0.2 µg/ml : *1/100 diluted concentration of sample fractions*

Supplemental Data Figure 2. Mass spectrometry analysis for purity estimation. The concentrations of periplasmic or outer membrane fraction from wild type or mutant were normalized to 20 µg/ml and 1/100 dilution of normalized fractions (0.2 µg/ml) were prepared. In order to determine whether the specific proteins in the diluted samples can still be identified, both the diluted and undiluted samples were analyzed by SDS-PAGE and a 15KDa band in each fraction was further analyzed by Mass spectrometry. Based on this analysis, the fractions we are investigating have at least 99% purity because a fraction specific protein can still be detected in a 1/100 dilution of the membrane fraction yet not be seen in the periplasmic fraction and vice versa. For example, a periplasmic protein 'peroxiredoxin' was detected in the periplasmic fractions from wild type and mutant organisms even at a 1/100 dilution. This protein was not detected in the outer membrane fraction even in the undiluted sample. Therefore the periplasmic fraction is over 99% free of outer membrane.

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