Supplementary Information for:

Chaperone OsmY facilitates the biogenesis of a major family of autotransporters

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Table S1. Strains and plasmids used in this study

Bacterial strain	Genotype and relevant features	Reference
MC4100	*F- araD139 Δ(argF-lac)U169 rpsL150 relA1 flb5301 deoC1 ptsF25 thi*	
ZY001	MC4100 *ΔosmY*	This study
ZY002	MC4100 *Δflu*	This study
ZY003	MC4100 *ΔfluΔosmY*	This study
ZY004	Transform pBADmyc-his into MC4100	This study
ZY005	Transform pBADmyc-his into ZY001	This study
ZY006	Transform pBAD*ytjA* into ZY001	This study
ZY007	Transform pBAD*osmY* into ZY001	This study
ZY008	Transform pBAD*osmY/ytjA* into ZY001	This study
ZY009	Transform pTrc into ZY002	This study
ZY010	Transform pTrc*flu* into ZY002	This study
ZY011	Transform pTrc into ZY003	This study
ZY012	Transform pTrc*flu* into ZY003	This study
ZY013	Transform pBADmyc-his into ZY002	This study
ZY014	Transform pBAD*ehaA* into ZY002	This study
ZY015	Transform pBAD*tibA* into ZY002	This study
ZY016	Transform pBADmyc-his into ZY003	This study
ZY017	Transform pBAD*ehaA* into ZY003	This study
ZY018	Transform pBAD*tibA* into ZY003	This study
ZY019	Transform pTrcEspP into MC4100	This study
ZY020	Transform pTrcEspP into ZY001	This study
ZY021	Transform pTrcEspP into ZY002	This study
ZY022	Transform pTrcEspP into ZY003	This study
Plasmid		
pBADmyc-his	Expression vector; P_{BAD}-dependent expression	
pBAD*osmY*	Cloning *osmY* through primer 1 and 2 into pBADmyc-his vector	This study
pBAD*ytjA*	Cloning *ytjA* through primer 3 and 4 into pBADmyc-his vector	This study
pBAD*osmY/ytjA*	Cloning *osmY* and *ytjA* through primer 1&4 into pBADmyc-his vector	This study
pBAD*ehaA*	Cloning *ehaA* through primer 5 and 6 into pBADmyc-his vector	This study
pBAD*tibA*	Cloning *tibA* through primer 7 and 8 into pBADmyc-his vector	This study
pTrc	Expression vector; P_{lac}-dependent expression	
pTrcflu	Cloning flu through primer 9&10 into pTrc vector	This study
pTrcEspP	Cloning EspP into pTrc vector	Ieva et al., 2008

Table S2. Oligo primers used in this study

Oligo primer	Sequence (5′–3′)
1	GAGGAATTAACCATGACTATGACAAGACTGAAGATTTC
2	GACCGTTTAAACTCACTTAGTTTTCAGATCATTTTTAACGC
3	GAGGAATTAACCATGTTTCGTTGGGGCATCATATTTC
4	GACCGTTTAAACTCAGGGTCGTTTTCGGCCCATG
5	GAGGAATTAACCATGGCATTTAATGCGTTGCTGTTTATG
6	GATGAGTTTTTGTTCGAATTGCCACTTAATGCCAACC
7	GAGGAATTAACCATGAATAAGGTCTATAACACTGT
8	GATGAGTTTTTGTTCGAAGTTGATTCGGAAACCAACGC
9	GAGGAATAAACCATGAAACGACATCTGAATACC
10	TTTAATCTGTATCAGAAGGTCACATTCAGTGTG
11	CGGACACACAGCCACTAATAC
12	AGCAGTACACCGCCATTTC
13	GTGGGTACAGCCTTCTGTTATC
14	GTCCAGTGATGTGCATTCT
15	TCGTCTGGAAAAAGCTGCAACT
16	TACGTCATCTTCGGTGTAGCCC

Table S3. Spectral counts of several proteins identified by LC-MS/MS

Identified protein	Spectral counts in WT	Spectral counts in *ΔosmY*
OxyR	14	11
Dam	18	20
SurA	11	14
SecA	44	43
Skp	27	23
DegP	9	7
OmpA	126	139
OmpC	124	286
OmpF	65	44
OmpT	65	86
OmpX	56	64
BamA	50	54
BamB	13	21
BamC	25	23
BamD	34	34
BamE	3	0
TamA	2	0
TamB	3	0

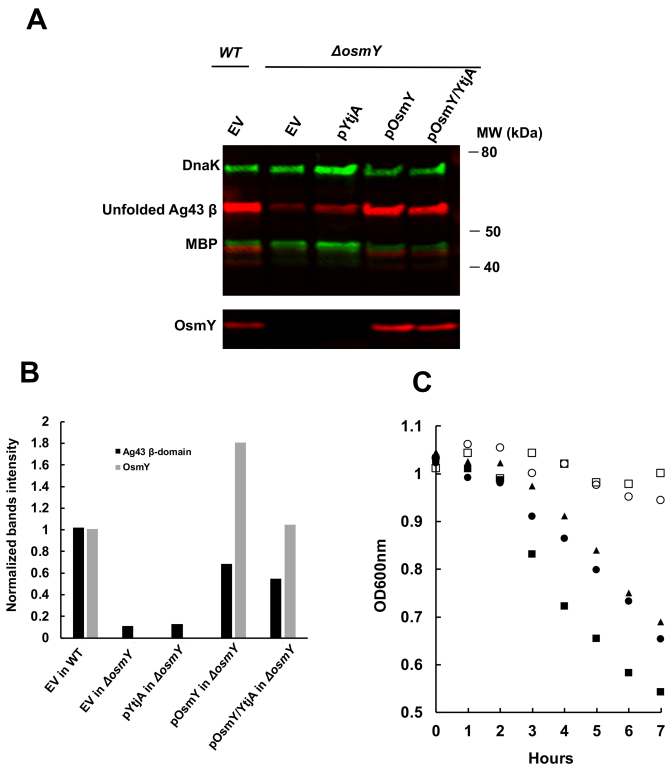

Fig. S1. Expression of the gene downstream from *osmY* does not promote Ag43-mediated autoaggregation in an *osmY*-null strain. WT harboring the pBAD empty vector (EV) and *ΔosmY* harboring EV, pBAD*ytjA* (abbreviated pYtjA on the figure), pBAD*osmY* (abbreviated pOsmY on the figure), and pBAD*osmY/ytjA* (pOsmY/YtjA), respectively, were cultured in LB media with 100 μg/ml ampicillin and 0.002% L-arabinose until they reached an OD_{600} of 1.0. Cells were centrifuged and washed with PBS buffer.

A. 1 ml of cell pellets was resuspended in SDS-reducing sample buffer followed by boiling at 95°C for 5 min, then analyzed by SDS-PAGE and western blot using antiserum raised against the indicated proteins.

B. Ag43 β domain and OsmY bands were quantified by Image J, the intensities were normalized to DnaK bands intensities, the level of Ag43 and OsmY present in the empty vector strain were set to one, to allow for easy comparison.

C. Autoaggregation of cells was assayed by taking samples 1 cm below the liquid surface for optical density readings at 600 nm. ■, WT harboring EV. □, *ΔosmY* harboring EV. ○, *ΔosmY* harboring pBAD*ytjA*. ●, *ΔosmY* harboring pBAD*osmY*. ▲, *ΔosmY* harboring pBAD*osmY/ytjA*.

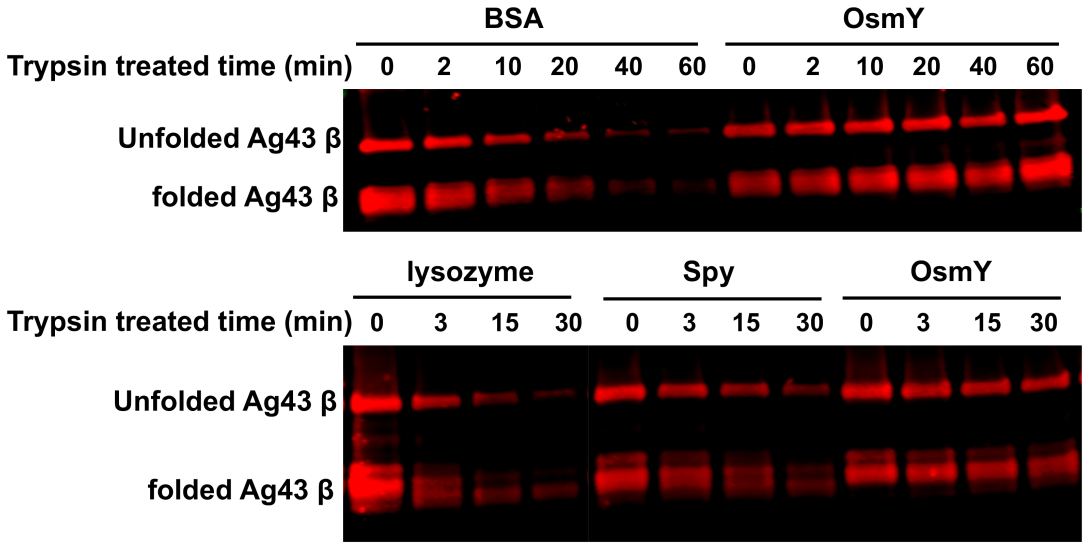

Fig. S2. Trypsin digestion of the Ag43 β domain in LDAO micelles. 5 μM samples of purified Ag43 β domain were refolded in 0.5% LDAO preincubated with 25 μM BSA, lysozyme, Spy, or OsmY, respectively, for 20 min at 37°C. Trypsin was added to the reaction mixture, and equal volumes of sample were removed into 10 mM PMSF at the indicated time points. Samples were analyzed by SDS-PAGE and western blot using antiserum raised against the β domain. In all experiments, the molar ratio of the Ag43 β domain to trypsin was 2000:1.

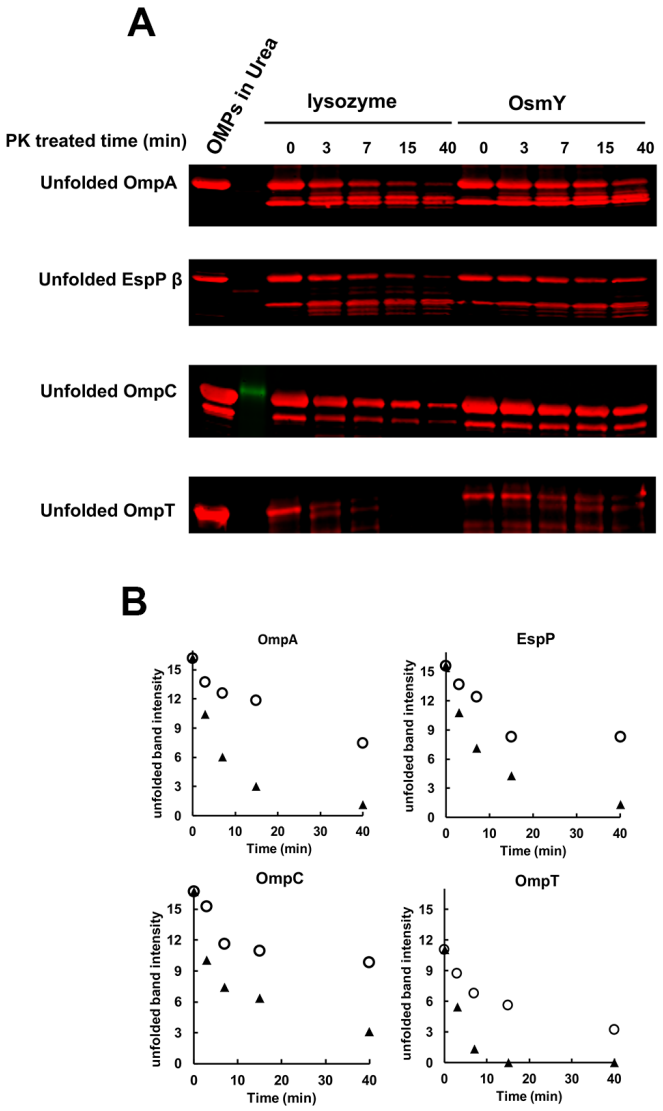

Fig. S3. PK digestions of OMPs in LDAO micelles.

A. 5 μM samples of purified OmpA, EspP β domain, OmpC, or OmpT were refolded in 0.5% LDAO for 20 min, at 37°C. PK was added to the reaction mixture, and equal volumes of sample were removed into 10 mM PMSF at the indicated time points. Samples were analyzed by SDS-PAGE and western blot using antiserum raised against the indicated OMPs. In all experiments, the molar ratio of OMP to PK was 1000:1.

B. The unfolded bands for each of the OMPs were quantified by Image J and plotted against time. ○, digestion with OsmY. ▲, digestion with lysozyme.

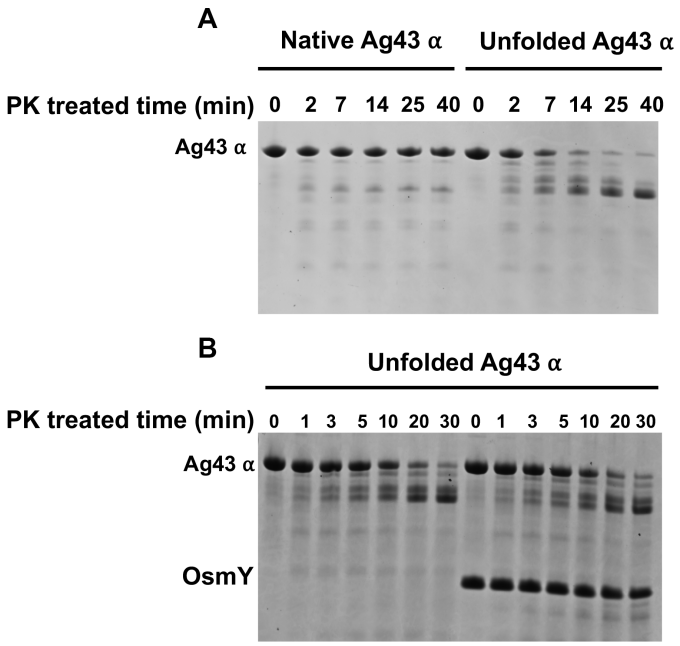

Fig. S4. PK digestion of the Ag43 α domain.

A. 10 μM samples of purified Ag43 α domain (native form) and unfolded Ag43 α domain were treated with PK at 37°C. The unfolded proteins were obtained by diluting the 8 M urea-denatured form 50 times into 50 mM Tris pH 8.0 without urea. Equal volumes of sample were removed from the reaction mixture into 10 mM PMSF to stop protease activity, and then analyzed by SDS-PAGE and Coomassie blue staining.

B. 10 μM samples of unfolded Ag43 α domain were treated with PK in the presence or absence of OsmY. In both experiments, the molar ratio of the Ag43 α domain to PK was 5000:1.

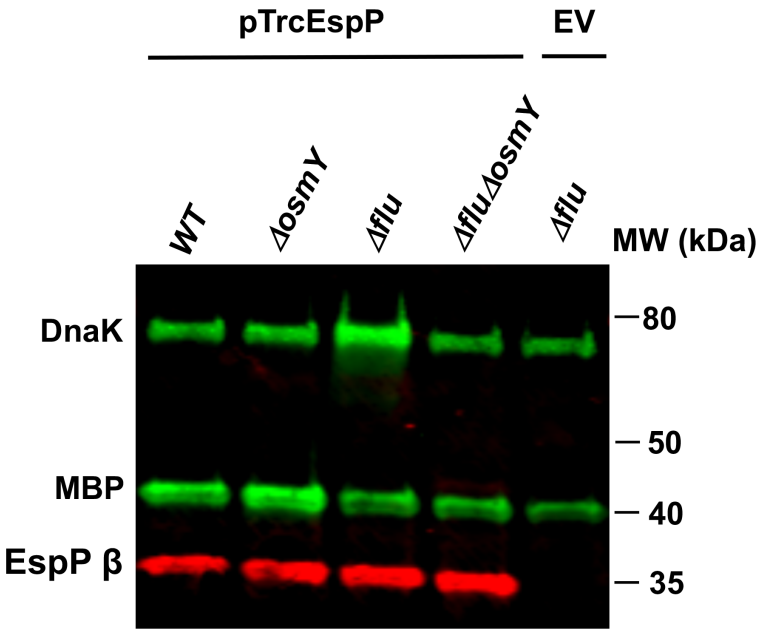

Fig. S5. The deletion of the *osmY* gene does not affect the steady-state level of EspP.
WT, *ΔosmY, Δflu* and *ΔfluΔosmY* harboring pTrcEspP, and *Δflu* harboring empty vector (EV) were cultured in LB media with 100 μg/ml ampicillin at 37 degrees C shaking at until they reached an OD_{600} of 0.6. 0.5 mM IPTG was added to induce protein expression and the cells were incubated at 37 degrees C with shaking for 3 h. Cells were centrifuged at 3000 × *g* for 10 min and then washed by resuspending in PBS buffer. Whole cell lysates were analyzed by SDS-PAGE followed by western blotting using antiserum raised against DnaK, MBP and EspP β domain.