Supporting information for:

Interrupting biosynthesis of O-antigen or the lipopolysaccharide core produces morphological defects in *Escherichia coli* by sequestering undecaprenyl phosphate

Matthew A. Jorgenson and Kevin D. Young*

Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

*Corresponding author

E-mail: kdyoung@uams.edu

Table S1: Strains used in this study

Strain	Relevant features	Source or reference
MAJ1	MG1655 *wbbL*::IS*5*	Lab collection
MAJ330	MG1655 *frt wbbL+*	(1)
MAJ339	MAJ330 Δ*wzxB*::*kan*	This study
MAJ343	MAJ330 Δ*wecA*::*kan*	This study
MAJ344	MAJ330 Δ*wbbK*::*kan*	This study
MAJ345	MAJ330 Δ*waaL*::*kan*	This study
MAJ346	MAJ330 Δ*wbbJ*::*kan*	This study
MAJ349	MAJ330 Δ*wbbI*::*kan*	This study
MAJ350	MAJ330 Δ*wbbH*::*kan*	This study
MAJ351	MAJ330 Δ*wzzB*::*kan*	This study
MAJ356	MAJ330 Δ*wecB*::*kan*	This study
MAJ368	MAJ345 Δ*waaL*::*frt*	This study
MAJ369	MAJ343 Δ*wecA*::*frt* Δ*wzxB*::*kan*	This study
MAJ370	MAJ343 Δ*wecA*::*frt* Δ*waaL*::*kan*	This study
MAJ374	MAJ330 Δ*waaC*::*kan*	This study
MAJ375	MAJ330 Δ*waaF*::*kan*	This study
MAJ384	MAJ343 Δ*wecA*::*frt* Δ*waaC*::*kan*	This study
MAJ385	MAJ343 Δ*wecA*::*frt* Δ*waaF*::*kan*	This study
MAJ397	MAJ356 Δ*wecB*::*frt* Δ*wbbK*::*kan*	This study
MAJ398	MAJ356 Δ*wecB*::*frt* Δ*wbbJ*::*kan*	This study
MAJ411	MAJ374 Δ*waaC*::*frt*	This study
MAJ427	MAJ330/pDSW361	This study
MAJ428	MAJ330/pMAJ41	This study
MAJ429	MAJ330/pMAJ42	This study
MAJ430	MAJ330/pMAJ43	This study
MAJ431	MAJ330/pMAJ44	This study
MAJ432	MAJ330/pMAJ45	This study
MAJ433	MAJ368/pDSW361	This study
MAJ434	MAJ368/pMAJ41	This study
MAJ435	MAJ368/pMAJ43	This study
MAJ436	MAJ368/pMAJ44	This study
MAJ437	MAJ368/pMAJ45	This study
MAJ438	MAJ411/pDSW361	This study
MAJ439	MAJ411/pMAJ42	This study
MAJ440	MAJ411/pMAJ43	This study
MAJ441	MAJ411/pMAJ44	This study
MAJ442	MAJ411/pMAJ45	This study
MAJ489	MAJ339 *rmlC*::IS*5*	This study
MAJ490	MAJ339 *wbbL*::IS*5*	This study

Table S2: Plasmids used in this study

Plasmid	Relevant features	Source or reference
pDSW361	pDSW361 is a KanR derivative of pDSW204; IPTG regulation (P_{204}) *lacI*^{q} pBR ori	(2)
pMAJ41	pDSW361::*waaL*	This study
pMAJ42	pDSW361::*waaC*	This study
pMAJ43	pDSW361::*murA*	This study
pMAJ44	pDSW361::*wecG*	This study
pMAJ45	pDSW361::*uppS*	This study

Table S3: Primers used in this study

Primer	Sequence[a]	Purpose
P21	CAGGAATTCTTGTCTGCTACTCAACCACTTAG	pMAJ45
P23	ATACTTCTGCTAATAATTTTCTCTGAGAGCATGCATTGTG TGTAGGCTGGAGCTGCTTCG	Δ*wecA*::*kan*
P24	TGTGTCATCACATCCTCATTTATTTGGTTAAATTGGGGCT ATTCCGGGGATCCGTCGACC	
P27	CGCAAAGGCGCTCGCCGCTTATTCGAAGAGAATCGATGT GTGTAGGCTGGAGCTGCTTCG	Δ*wecB*::*kan*
P28	ACAGAAATGGTCGCAAAACTCATAGTGATATCCGATTATT ATTCCGGGGATCCGTCGACC	
P118	ATAATCGTACATAAAATCCTCAGCAAACCAGTAATTTATTA TTCCGGGGATCCGTCGACC	Δ*wzxB*::*kan*
P119	TTACGTTAGATGAGCTTATCAGATTAAAATTAATTGCATGT GTAGGCTGGAGCTGCTTCG	
P180	CAAAAATCATAAAGAAATACAATCATGAGACCAAATTATG TGTAGGCTGGAGCTGCTTCG	Δ*wbbK*::*kan*
P181	GATTATTATATATACCATTTCAATGTTCTTCAGTAATAAAA TTCCGGGGATCCGTCGACC	
P184	GGAAGTTACTTCAGGGATGTTCTTGAAGAGGTGATCGAT GTGTAGGCTGGAGCTGCTTCG	Δ*wbbJ*::*kan*
P185	TTGACCGCAGAAACAACGACTATGCTTTTTCCCATAATTT ATTCCGGGGATCCGTCGACC	
P192	AACTATGCGGACTTGGAAATTTCCGTCAGTTAGGGTAAT GTGTAGGCTGGAGCTGCTTCG	Δ*wzzB*::*kan*
P193	TTCTTTAAAACCGAAAAGATTACTTCGCGTTGTAATTGCG ATTCCGGGGATCCGTCGACC	
P196	CTCTTTATCAAGTGAAAAATATAATGAGTACGGATTAATG TGTAGGCTGGAGCTGCTTCG	Δ*wbbH*::*kan*
P197	TCATTCAAAAAATACATTTTCACTTTATTTTCTGGGCCTTA TTCCGGGGATCCGTCGACC	
P208	CATGGCCTGGCTGAATCGCGACGCATAAGAGCTCTGCAT GTGTAGGCTGGAGCTGCTTCG	Δ*waaF*::*kan*
P209	CGATCAAAACCCGCATCCGTCAGGCTTCCTCTTGTAACA AATTCCGGGGATCCGTCGACC	
P212	ACTCAACGCGCTATTGTTACAAGAGGAAGCCTGACGGAT GTGTAGGCTGGAGCTGCTTCG	Δ*waaC*::*kan*
P213	ATGTTAGCATGTTTTACCTTTATAATGATGATAACTTTTCA TTCCGGGGATCCGTCGACC	
P229	CAGGAATTCCTAACATCCTTTAAACTTCATTC	pMAJ41
P231	CAAGAATTCCGGGTTTTGATCGTTAAAAC	pMAJ42
P233	CAGGAATTCAATAACAACACCACGGCACC	pMAJ44
P251	TTGCTGCAGTTAATTAATTGTATTGTTACG	pMAJ41
P252	TTGCTGCAGTTATAATGATGATAACTTTTC	pMAJ42
P253	TTGCTGCAGTCATAGGTTGCCGGTGTAGTG	pMAJ44

P254	TTGCTGCAGTCAGGCTGTTTCATCACCGGG	pMAJ45
P278	AGCAGTTTTGGAAAAGTTATCATCATTATAAAGGTAAAAC ATTCCGGGGATCCGTCGACC	Δ*waaL*::*kan*
P279	TAACTCACTTCTTAAACTTGTTTATTCTTAATTAATTGTATG TAGGCTGGAGCTGCTTCG	

[a]All primer sequences are written 5'→ 3'. Restriction sites are underlined.

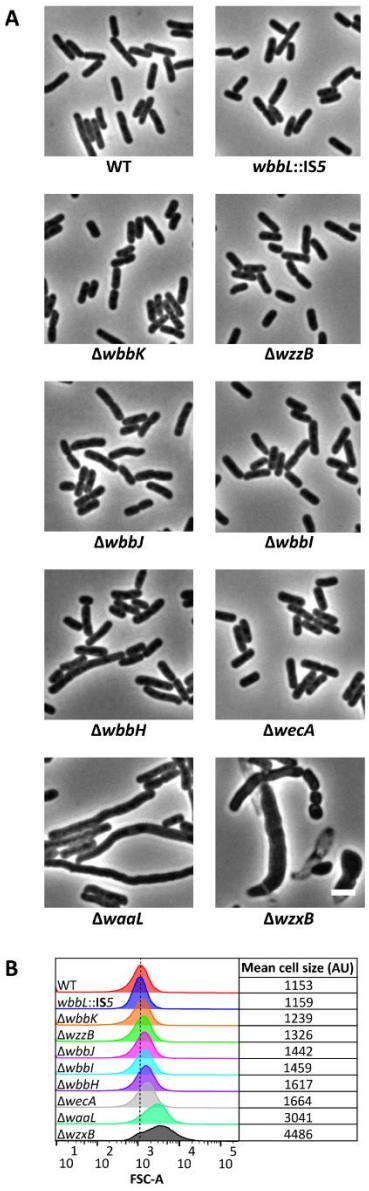

Figure S1. Disrupting O-antigen biosynthesis induces cell shape defects. (A) Micrographs of cells from which the indicated genes were deleted. Cells were grown at 37°C in LB until they reached an OD_{600} = 0.5-0.6. The cells were then fixed and photographed by phase-contrast microscopy. Micrographs of Δ*wzxB* cells are from overnight cultures because the strain readily develops suppressing mutations that correct the shape defect. The white bar represents 3 μm. (B) Flow cytometry data from

live cells in panel A. Histograms of the FSC-A from 100,000 events (cells). The mean cell size of the wild type (red graph) is represented by the vertical dashed line and is expressed in arbitrary units (AU). Data is representative of two independent experiments. Strains: MAJ330 (WT), MAJ1 (*wbbL*::IS*5*), MAJ344 (Δ*wbbK*), MAJ351 (Δ*wzzB*), MAJ346 (Δ*wbbJ*), MAJ349 (Δ*wbbI*), MAJ350 (Δ*wbbH*), MAJ343 (Δ*wecA*), MAJ345 (Δ*waaL*) and MAJ339 (Δ*wzxB*).

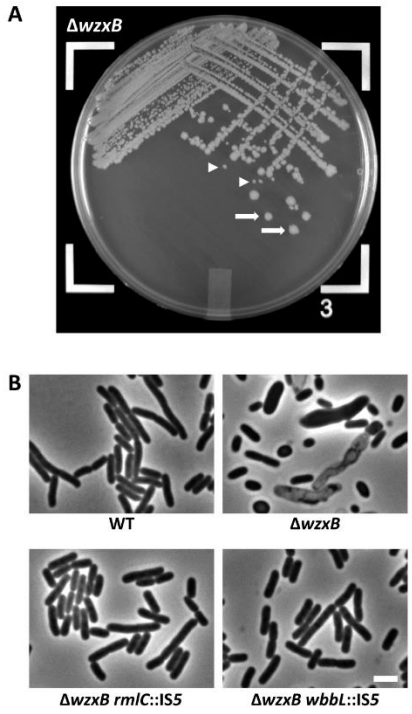

Figure S2. Δ*wzxB* cells readily acquire suppressor mutations. (A) Streak plate of Δ*wzxB* cells grown overnight on LB medium at 37°C. Δ*wzxB* cells normally give rise to small colonies (arrowheads), but they readily develop suppressing mutations that result in bigger colonies (arrows). (B) Micrographs of Δ*wzxB* and Δ*wzxB* suppressing cells. Suppressing mutations were mapped to *rmlC* and *wbbL*, whose disruption prevents the formation of O-antigen intermediates. Cells were grown and imaged as described in the legend to Figure S1. Micrographs of Δ*wzxB* cells are from overnight cultures because the strain readily develops suppressor mutations that correct the shape defect. The white bar represent 3 μm. Strains: MAJ330 (WT), MAJ339 (Δ*wzxB*), MAJ489 (Δ*wzxB* *rmlC*::IS*5*) and MAJ490 (Δ*wzxB* *wbbL*::IS*5*).

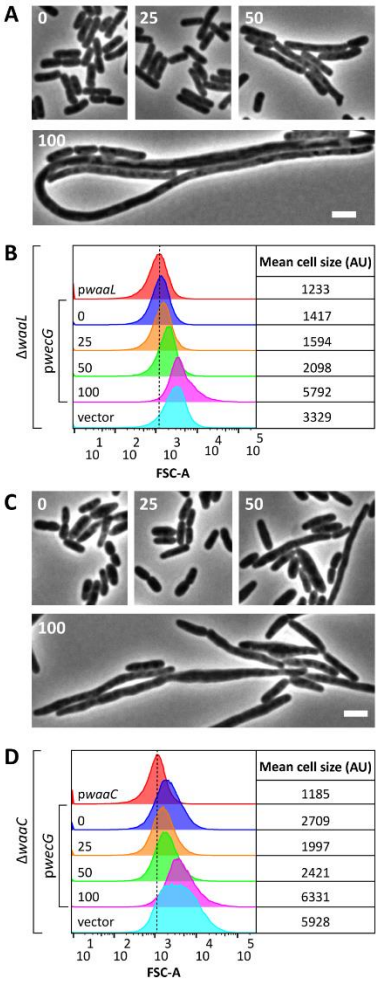

Figure S3. Overproducing ECA in cells that also accumulate O-antigen intermediates causes filamentation. (A) Micrographs of Δ*waaL* cells expressing various amounts of *wecG* in *trans*. Cells were grown at 37°C in LB containing various concentrations (μM) of IPTG (indicated on the micrographs) until the cells reached an OD_{600} = 0.5-0.6. The cells were then fixed and photographed by phase-contrast microscopy. The white bar represents 3 μm. (B) Flow cytometry data from live cells in panel A. Histograms of the forward scatter area from 100,000 events (cells). The mean cell size of Δ*waaL* cells expressing *waaL* in *trans* (red graph) is represented by the vertical dashed line and is expressed in arbitrary units (AU). Note that IPTG was added

to 100 uM for Δ*waaL* cells harboring p*waaL* or the empty vector. Data is representative of two independent experiments. Strains: MAJ434 (Δ*waaL*/p*waaL*), MAJ436 (Δ*waaL*/p*wecG*) and MAJ433 (Δ*waaL*/vector). (C and D). Data for Δ*waaC* cells expressing various amounts of *wecG in trans*. The experimental conditions were the same as described in panels A and B. Strains: MAJ439 (Δ*waaC*/p*waaC*), MAJ441 (Δ*waaC*/p*wecG*) and MAJ438 (Δ*waaC*/vector).

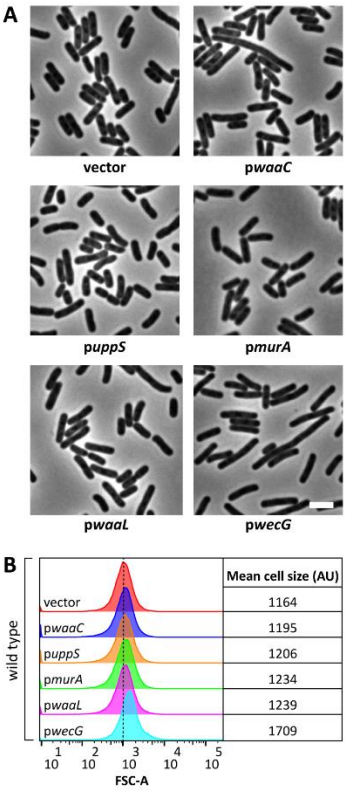

Figure S4. Overexpressing other genes has little or no effect on cell shape in wild type *E. coli*. (A) Micrographs of wild type cells containing derivatives of pDSW361 that express the indicated genes. Cells were grown at 37°C in LB containing 100 μM IPTG until the cells reached an OD_{600} = 0.5-0.6. The cells were then fixed and photographed by phase-contrast microscopy. The white bar represents 3 μm. (B) Flow cytometry data from live cells in panel A. Histograms of the forward scatter area from 100,000 events (cells). The mean cell size of the wild type with the empty vector (red graph) is represented by the vertical dashed line and is expressed in arbitrary units (AU). Data is representative of two independent experiments. Strains: MAJ427 (vector), MAJ429 (p*waaC*), MAJ432 (p*uppS*), MAJ430 (p*murA*), MAJ428 (p*waaL*) and MAJ431 (p*wecG*).

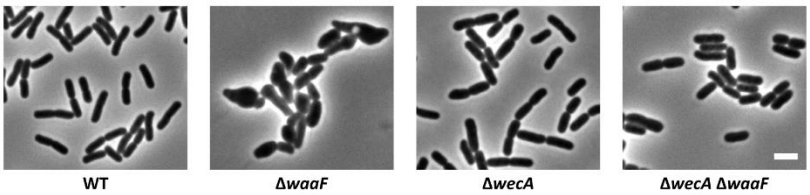

Figure S5. Morphological defects of LPS core mutants depend on O-antigen biosynthesis. Micrographs of cells with the indicated genotypes. Cells were grown and imaged as described in the legend to Figure S1. The white bar represents 3 μm. Strain: MAJ330 (WT), MAJ375 (Δ*waaF*), MAJ343 (Δ*wecA*) and MAJ385 (Δ*wecA* Δ*waaF*).

Supplemental References

1. **Rendueles O, Beloin C, Latour-Lambert P, Ghigo JM.** 2014. A new biofilm-associated colicin with increased efficiency against biofilm bacteria. ISME J **8:**1275-1288.
2. **Weiss DS, Chen JC, Ghigo JM, Boyd D, Beckwith J.** 1999. Localization of FtsI (PBP3) to the septal ring requires its membrane anchor, the Z ring, FtsA, FtsQ, and FtsL. J Bacteriol **181:**508-520.