SUPPLEMENT FOR

Phosphatidylglycerol is the lipid donor for synthesis of phospholipid-linked enterobacterial common antigen

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SUPPLEMENTAL FIGURES

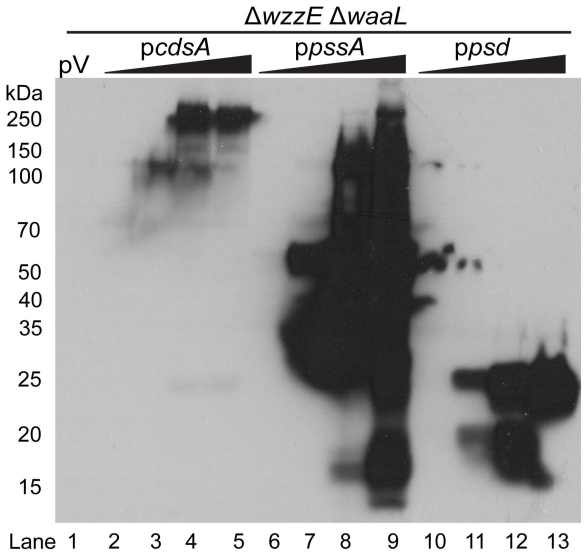

Figure S1. Protein levels resulting from overexpression of genes involved in PE synthesis. Expression of the indicated genes from ASKA plasmid constructs was assayed using the N-terminal His-tag included in the construct in the same strain background as in Figure 3A. The vector control (pV) was treated with 50 µM IPTG, while the other strains were treated with increasing concentrations of IPTG (0 µM, 10 µM, 25 µM, and 50 µM). IPTG dependent increases in protein levels are observed with some aggregation occurring at higher IPTG concentrations.

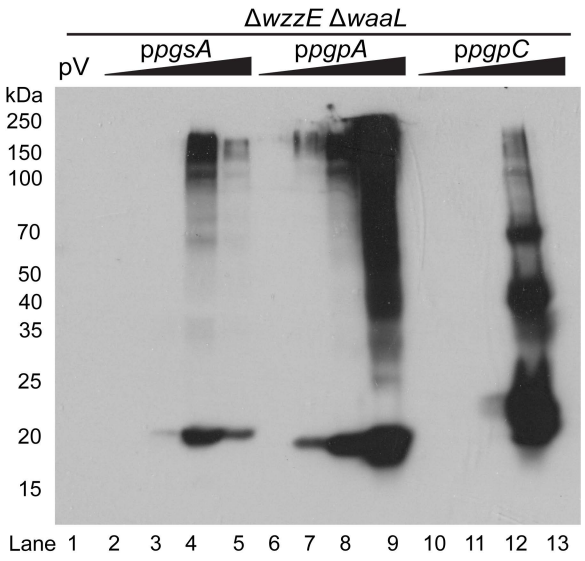

Figure S2. Protein levels resulting from overexpression of genes involved in PG synthesis. Expression from ASKA plasmid constructs was assayed using the N-terminal His-tag in the same strain background as in Figure 4A. The vector control (pV) was treated with 50 µM IPTG and the other strains were treated with increasing concentrations of IPTG (0 µM, 10 µM, 25 µM, and 50 µM). IPTG dependent increases in protein levels are observed with some aggregation occurring at higher IPTG concentrations.

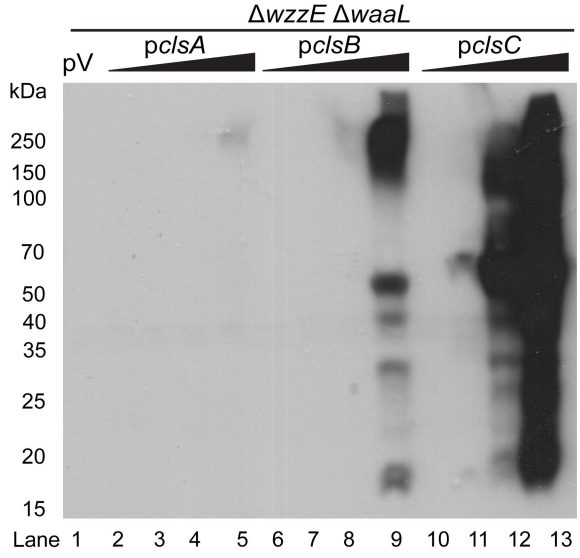

Figure S3. Protein levels resulting from overexpression of genes involved in CL synthesis. Expression of the indicated genes from ASKA plasmid constructs was assayed using the N-terminal His-tag included in the construct in the same strain background as in Figure 5A. The

vector control (pV) was treated with 50 µM IPTG, while the other strains were treated with increasing concentrations of IPTG (0 µM, 10 µM, 25 µM, and 50 µM). IPTG dependent increases in protein levels are observed for ClsB and ClsC with some aggregation occurring at higher IPTG concentrations. ClsA shows lower protein accumulation.

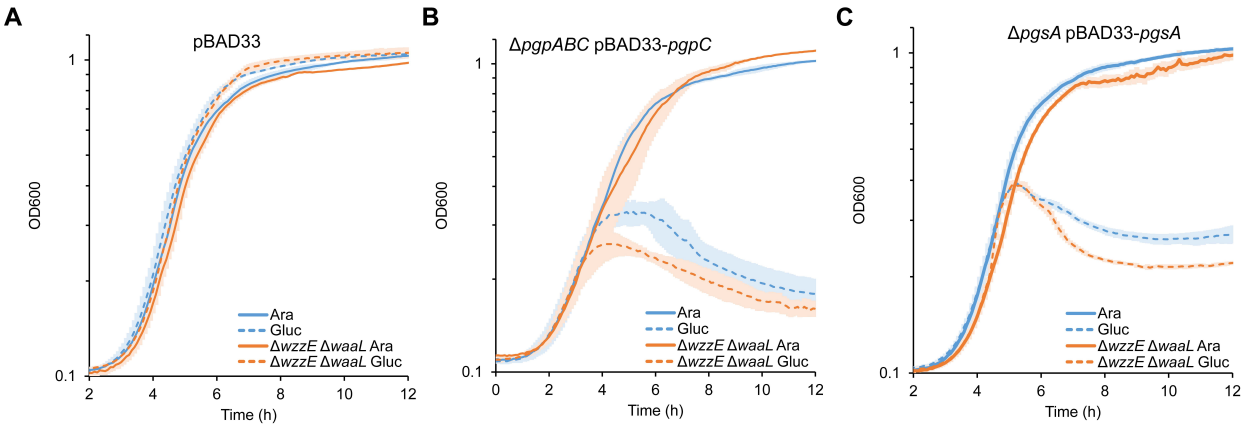

Figure S4. PG depletion causes additional growth defects in an ECA$_{PG}$ only background. Growth curves were performed strains with the indicated genotype alone (blue) or with Δ*wzzE* Δ*waaL* (orange) carrying a plasmid with the P$_{BAD}$ promoter. Cultures were treated with arabinose to induce expression from the P$_{BAD}$ promoter (solid lines) or glucose to repress expression (hashed lines). Growth curves were started with a 1:10,000 dilution of cultures grown with arabinose unless otherwise noted. Data are the mean of three biological replicates ± the SEM. **(A)** Strains carrying an empty plasmid showed very similar growth when treated with arabinose or glucose. **(B)** Cultures grown in arabinose were diluted 1:500 for growth curves. Depletion of PgpC in strains with deletions of *pgpA*, *pgpB*, and *pgpC* caused lysis. However, strains that can make only ECA$_{PG}$ from ECA precursor (Δ*wzzE* Δ*waaL*) lysed earlier in growth than strains that can make all three forms of ECA. **(C)** Depletion of PgsA causes more lysis in the Δ*wzzE* Δ*waaL* strain background than in the wild type background.

SUPPLEMENTAL TABLE

Table S1: Primers used in this study

Primer	**Sequence 5' (overlap/ANNEAL) 3'**
Del-*pgsA* F (*pgsA*::kan recombineering)	AGCAACATAGGGGTAATCTTACTGACAACAGATAGTTACCCGTCATTA TGATTCCGGGGATCCGTCGACC
Del-*pgsA* R (*pgsA*::kan recombineering)	ATCGTTTGCTGAAAATTACGCCGAAACGATCACTGATCAAGCAAATCT GCGTGTAGGCTGGAGCTGCTTCG
pBAD33 F	CCGGGGATCCTCTAGAGTC
pBAD33 R	GTACCGAGCTCGAATTCG
pgpC (o/l pBAD33) F	agcgaattcgagctcggtacTCAGGATGTTTCAGTCCAG
pgpC (o/l pBAD33) R	cgactctagaggatccccggCTATTCCAGTTGCTGGAG
pgsA (o/l pBAD33) F	agcgaattcgagctcggtacCTTACTGACAACAGATAGTTAC
pgsA (o/l pBAD33) R	cgactctagaggatccccggTCACTGATCAAGCAAATC
clsA (o/l pBAD33) F	agcgaattcgagctcggtacACAATGCGCTTTCAAAAG
clsA (o/l pBAD33) R	cgactctagaggatccccggTTACAGCAACGGACTGAAG