Supporting Information

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SI Materials and Methods

One-milliliter samples from cultures were pelleted (16,000 × *g*, 2 min). To standardize samples, pellets were resuspended in a volume of SDS sample buffer (1) equal to OD600/10. Samples were boiled for 10 min, and equal volumes were subjected to electrophoresis. A 15% (wt/vol) SDS-polyacrylamide gel was used, and immunoblotting was performed using DegP rabbit polyclonal sera (1:30,000 dilution) (2) and mouse monoclonal clone 2D7/1 antiserum against J5 LPS core (1:5,000 dilution) (AbD Serotec), followed by donkey anti-rabbit HRP-conjugated and sheep anti-mouse HRP-conjugated antiserum, respectively (1:10,000 dilution) (GE Amersham). Immunoblots were developed with VisiGlo Plus HRP Chemiluminescent Substrate Kit (Amresco) and visualized in a ChemiDoc XRS system (Bio-Rad). Band intensity was measured using Image Lab software (Bio-Rad) and reported as ratio with respect to the band present in samples from the wild-type strain NR754, which was assigned a value equal to 1.

1. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage *T4*. *Nature* 227:680–685.

2. Ruiz N, Falcone B, Kahne D, Silhavy TJ (2005) Chemical conditionality: A genetic strategy to probe organelle assembly. *Cell* 121:307–317.

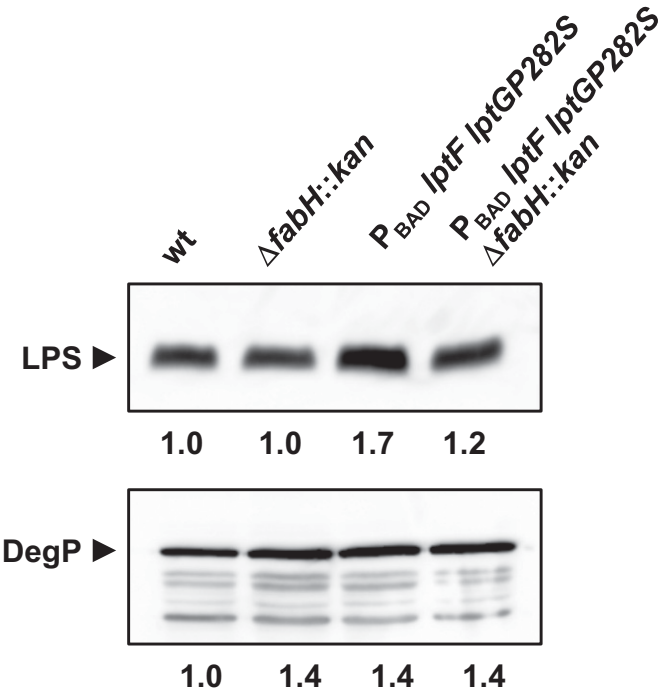

Fig. S1. Loss of FabH does not increase LPS levels or activate the σ^E and Cpx envelope stress responses. LPS and DegP levels were monitored by immunoblotting. Samples were derived from cells grown overnight in LB (wild-type and *fabH* mutants) or LB containing arabinose (NR1243). DegP levels are increased when either the σ^E or the Cpx envelope stress response is activated. Strains used are NR754 (WT), NR1769 (Δ*fabH*::*kan*), NR1243 (P_{BAD} *lptF lptGP282S*), and NR1770 (P_{BAD} *lptF lptGP282S* Δ*fabH*::*kan*). Values below each immunoblot represent the ratio of the intensity of each band with respect to that in the sample from wild-type strain NR754. These results suggest that *fabH* mutants synthesize enough LPS to cover the surface. However, this assay reports only total LPS levels. Probing the barrier quality of the outer membrane is a better way to detect defects in the levels of LPS at the surface. The *fabH* (*lpt*+) mutant is not significantly more sensitive to erythromycin and rifampin than wild type (Table 3). In addition, the *accD* (*lpt*+) mutant does not exhibit outer membrane permeability defects (Table 3). These results suggest that the levels of LPS at the cell surface of these mutants are similar to those in the wild-type strain. Nevertheless, it is interesting that the *fabH* (*lpt*+) mutant exhibits increased sensitivity to bacitracin (Table 3). The fact that this sensitivity is not seen in the *accD* mutant indicates that it is not the result of a general defect in LPS biogenesis caused by decreased fatty acid biosynthesis. Instead, it is likely that this sensitivity is related to the altered fatty acid content in the *fabH* mutant. Additional studies are needed to understand this increase in sensitivity to bacitracin.

Table S1. Profile of major fatty acids in *accD*$^+$ (NR2020) and *accDV23G* (NR2022) strains (% of total)

Fatty acid species	*accD*$^+$	*accDV23G*
12:0	3.65 ± 0.00	3.95 ± 0.26
14:0	7.10 ± 0.20	9.88 ± 0.78
14:0 3OH	8.80 ± 0.40	7.11 ± 3.28
16:1 ω7c	25.53 ± 0.10	26.85 ± 1.00
16:0	27.95 ± 0.28	26.74 ± 0.60
17:0 cyclo	1.67 ± 0.03	8.95 ± 0.52
18:1 ω 7c	20.79 ± 0.01	11.16 ± 0.06
18:0	0.78 ± 0.05	0.65 ± 0.15
Total	96.26	95.29