# Supporting Material

# to

# Expression of *Vibrio harveyi* Acyl-ACP Synthetase Allows Efficient Entry of Exogenous Fatty Acids into the *Escherichia* coli Fatty Acid and Lipid A Synthetic Pathways

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Table 2S. Distribution of odd chain or deuterium-labeled fatty acids in cultures of the Δ*fabH cfa* strain YFJ305 carrying pYFJ85 determined by electrospray collision-induced mass spectrometry (*1*) (see Fig. 5). Cultures were grown overnight at 37 °C in LB in the presence of 0.02% arabinose and 100 μM fatty acid substrate. The incorporation of exogenous acids was greater in experiment 2. This seems likely to be due to suppressors of the fabH mutation present in the inoculum of experiment 1. Hence, there may have been higher levels of endogenous synthesis in experiment 1 and thus more competition with the exogenous acids for incorporation into phospholipids than in experiment 2.

| Supplement | Fatty acid products | Expected mass | Yield (% total fatty acids) | | Mean |
|---|---|---|---|---|---|
| | | | Experiment 1 | Experiment 2 | |
| C5 | 15:0 | 241.5 | 5.9 | 6.1 | 6.0 |
| | 17:1 | 267.6 | 5.9 | 8.0 | 6.95 |
| | 17:0 | 269.6 | 5.0 | 6.1 | 5.55 |
| | Total | | 16.8 | 20.1 | 18.45 |
| C7 | 15:0 | 241.5 | 2.2 | 3.6 | 2.9 |
| | 17:1 | 267.6 | 2.8 | 6.6 | 4.7 |
| | 16:0 (17:0) | 269.6 | 2.6 | 7.0 | 4.8 |
| | Total | | 7.7 | 17.2 | 12.45 |
| C9 | 15:0 | 241.5 | 2.4 | 1.6 | 2.0 |
| | 17:1 | 267.6 | 3.7 | 3.6 | 3.6 |
| | 17:0 | 269.6 | 7.3 | 7.0 | 7.2 |
| | Total | | 13.4 | 12.2 | 12.8 |
| [d-11]-C6 | [d-11]-14:0 | 238.7 | 0 | 1.9 | 0.95 |
| | [d-11]-16:1 | 264.8 | 6.1 | 15.6 | 10.8 |
| | [d-11]-16:0 | 266.8 | 4.1 | 12.9 | 8.5 |
| | [d-11]-18:1 | 292.7 | 3.9 | 10.9 | 7.4 |
| | Total | | 14.2 | 43.8 | 29.0 |
| [d-15]-C8 | [d-15]-14:0 | 242.7 | 6.0 | 1.9 | 3.9 |
| | [d-15]-16:1 | 268.6 | 6.1 | 7.9 | 7.0 |
| | [d-15]-16:0 | 270.6 | 2.6 | 12.9 | 7.8 |
| | [d-15]-18:1 | 295.7 | 10.9 | 5.8 | 8.4 |
| | Total | | 14.7 | 30.0 | 22.4 |
| d-19 C10) | [d-19]-14:0 | 246.6 | 3.5 | 9.6 | 6.6 |
| | [d-19]-16:0 | 274.7 | 26.2 | 42.8 | 34.5 |
| | [d-19]-18:0 | 302.7 | <0.5 | 2.3 | 1.2 |
| | Total | | 29.6 | 55.8 | 42.7 |

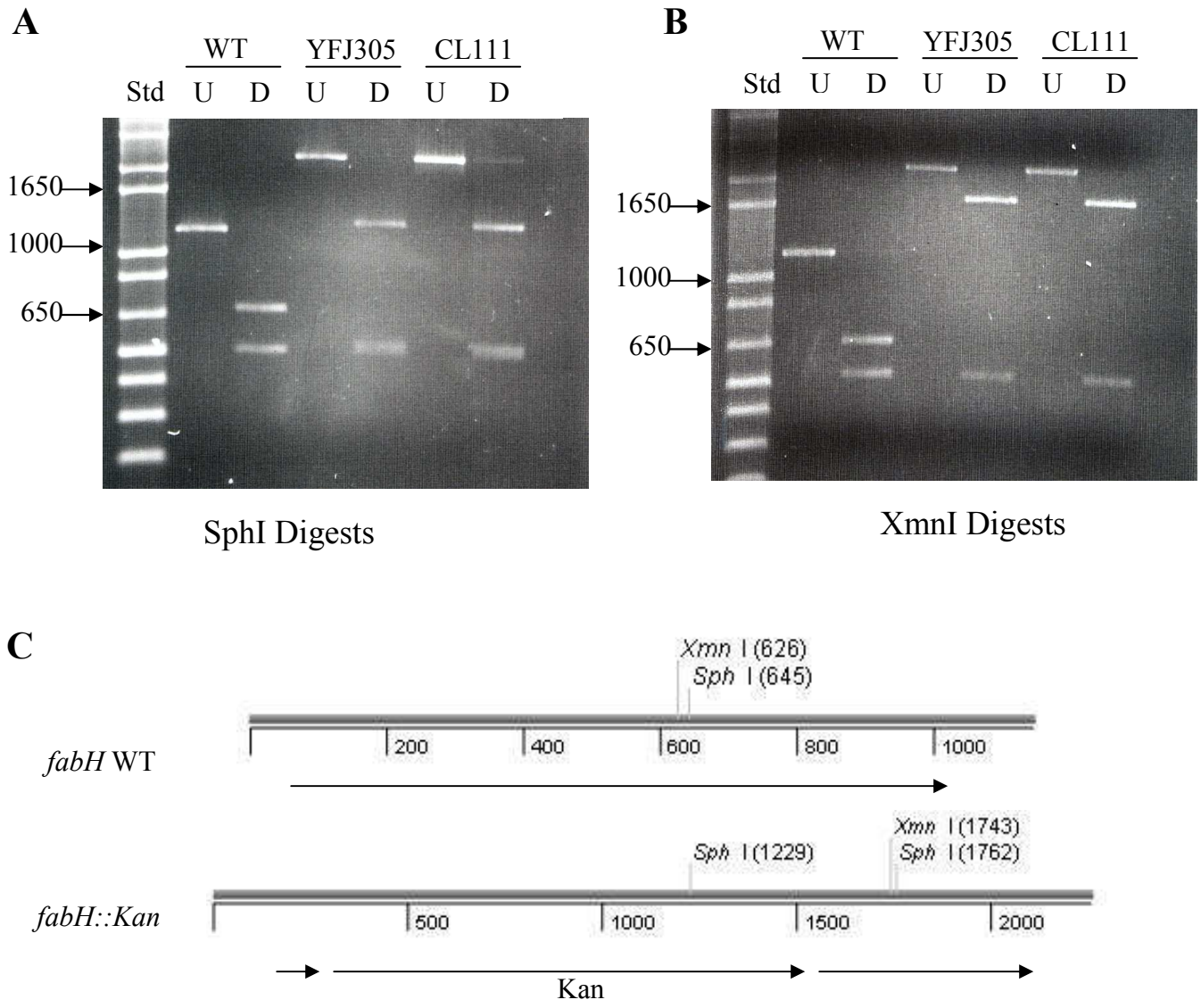


Fig. 1S. Confirmation of the *fabH*::*Kan* disruption in the Δ*fabH* deletion strain YFJ305. The chromosomal *fabH* region was PCR amplified with primers flanking the *fabH* coding sequence (Experimental Methods) from strains UB1005 (wild type), CL111 (*2*) (from which the *fabH*:Kan was P1 transduced) and the *fabH* deletion strain YFJ305. The PCR products were analyzed by restriction digestions with either SphI (Panel A) or XmnI (Panel B). U: undigested PCR product; D: digested PCR product; Std: 1 Kb Plus DNA ladder (Invitrogen). The wild type gave the expected 1149 bp PCR product and both YFJ305 and CL111 gave the expected 2266 bp PCR product. With SphI digestion the wild type PCR product gave two fragments (644 and 505 bp). whereas the YFJ305 and CL111 PCR products were expected to give three fragments (505, 533, and 1038 bp). However, in both digests the 1038 bp band was present and the 505 and 533 bp

bands could not be resolved such that a thick band of ~500 bp was present (Panel A). XmnI digestion of the wild type PCR product gave two fragments (625 and 524 bp) whereas both YFJ305 and CL111 PCR products gave the expected two fragments (1742 and 524 bp) (Panel B). Panel C shows the maps of the expected PCR products and their digestion products.

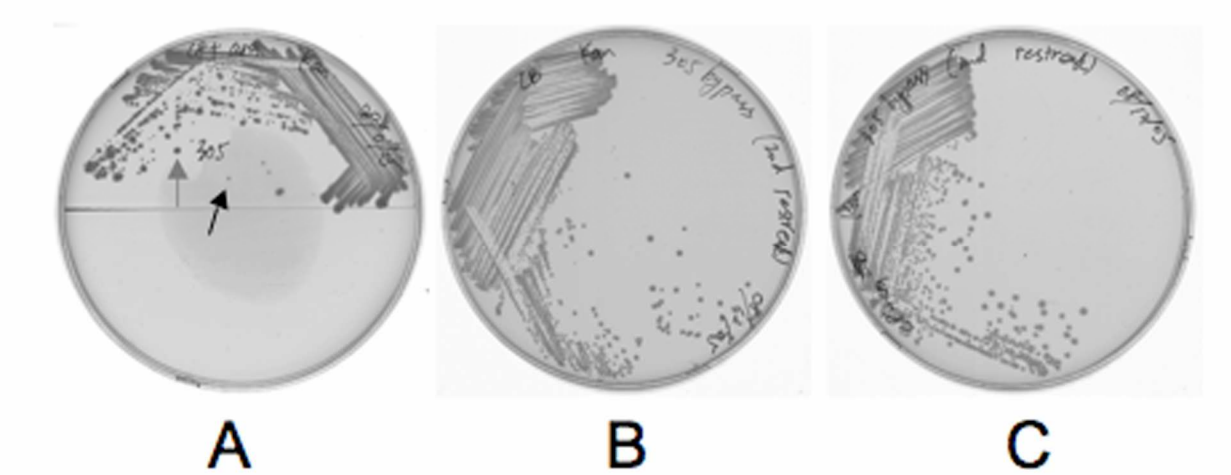


Fig. 2S. Extragenic suppressors of the Δ*fabH* mutation of strain YFJ305. Plate A. Strain YFJ305 streaked on LB plates supplemented with octanoate and arabinose was incubated at 37°C for two days. The black arrow shows the tiny colonies characteristic of Δ*fabH* strains whereas the gray arrow shows an extragenic suppressor colony. This suppressor colony was streaked on an LB plate (Plate B) or (Plate C) a plate of LB supplemented as was plate A. Incubation was at 37°C overnight

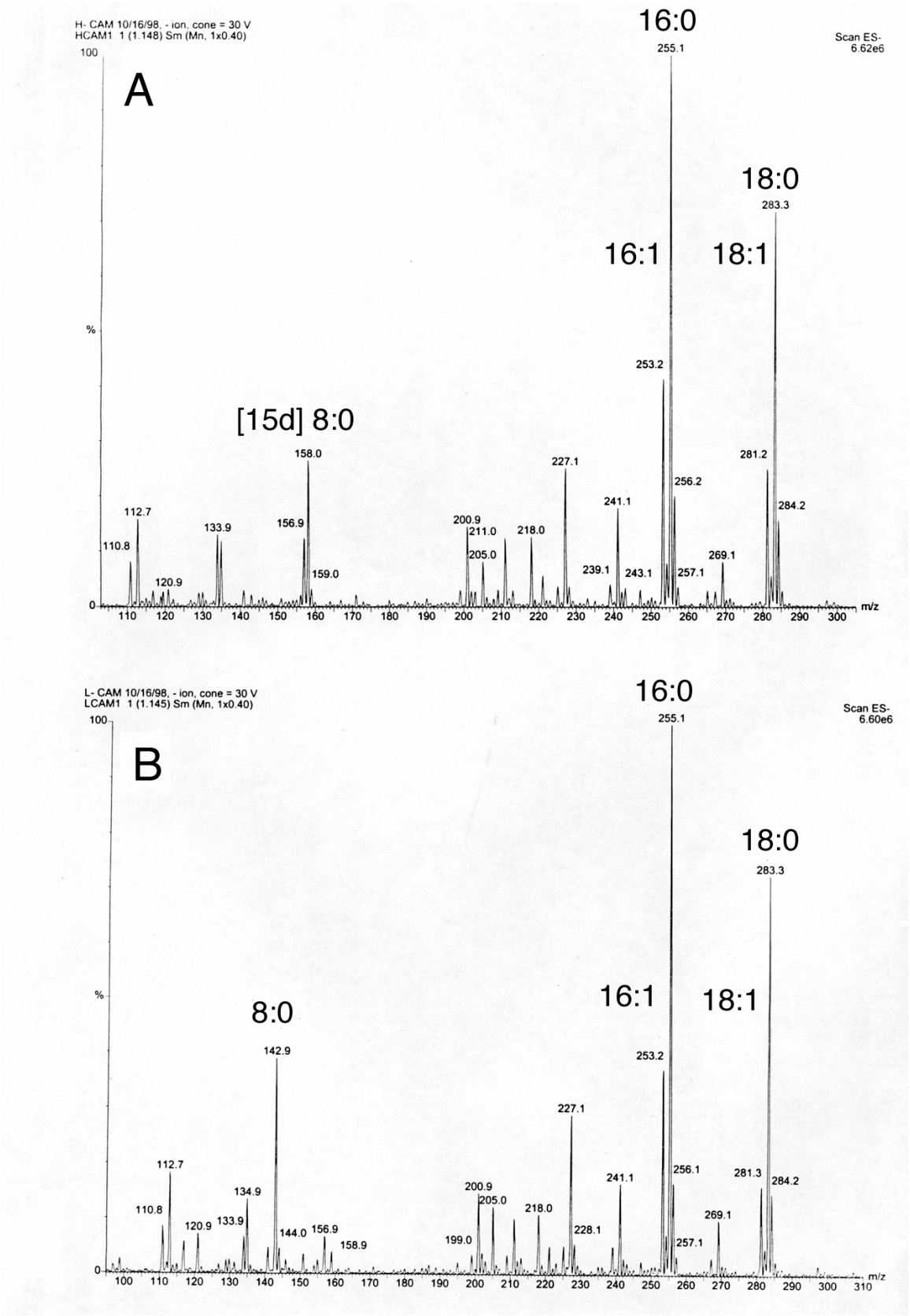
H- CAM 10/16/98, - ion, cone = 30 V
HCAM1 1 (1.148) Sm (Mn, 1x0.40)
Scan ES-
6.62e6
A
16:0
255.1
18:0
283.3
16:1
18:1
[15d] 8:0
253.2
158.0
227.1
281.2
256.2
241.1
284.2
112.7
133.9
156.9
200.9
211.0
218.0
110.8
205.0
269.1
120.9
159.0
239.1
243.1
257.1
m/z
L- CAM 10/16/98, - ion, cone = 30 V
LCAM1 1 (1.145) Sm (Mn, 1x0.40)
Scan ES-
6.60e6
B
16:0
255.1
18:0
283.3
16:1
18:1
8:0
142.9
253.2
227.1
112.7
256.1
241.1
281.3
284.2
134.9
200.9
205.0
218.0
110.8
120.9
133.9
144.0
156.9
269.1
228.1
158.9
199.0
257.1
m/z

Fig. 3S. Growth with deuterated octanoate fails to alter the fatty acid profile of *E. coli*.

The phospholipids from two cultures of strain JWC255 (*fadE62 cfa*::kan) grown overnight on rich broth containing with either 18 mM [d15]octanoate (panel A) or 18 mM octanoate (panel B) were extracted and analyzed by electrospray collision-induced mass spectrometry (*1*). Residual octanoate supplements carried along in the extraction are labeled as well as the major fatty acids of the phospholipids

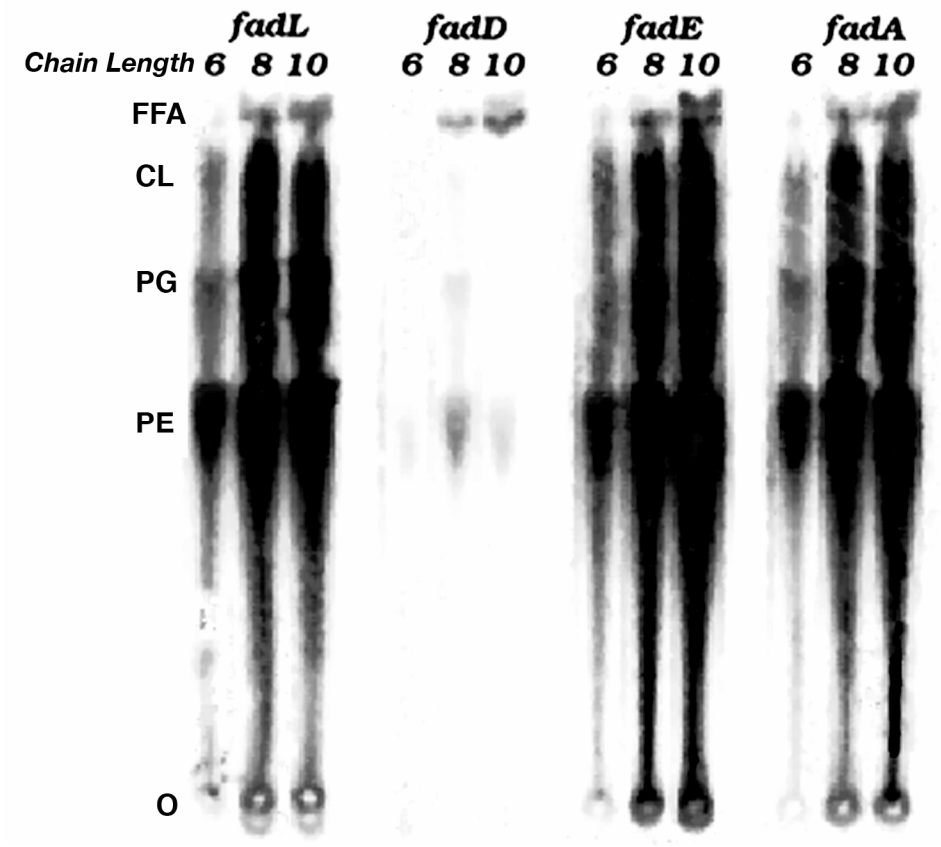


Fig. 4S. Fluorogram of total cellular phospholipids separated by silica gel Gthin layer chromatography. The samples were extracted (*3*) from the cells of overnight rich broth (*4*) cultures of the various ß-oxidation defective strains (given above each set of three lanes) grown the presence of 1 μCi/ml (50-55 mCi/mmol, approximately 18 μM) of the [1-$^{14}$C] acid denoted above the lane. These short chain acids are not components of the cellular phospholipids and thus elongation of the acids to at least fourteen carbon atoms is required prior to incorporation into phospholipid. The strains (all from the coli Genetic Stock Center, Yale University) were CAG18493 (*fadL*::Tn*10*), K27 (*fadD88*), K19 (*fadE62*) and CAG18486 (*fadA*::Tn*10*) blocked in the outer membrane transport protein, acyl-CoA synthetase, acyl-CoA dehydrogenase and thiolase of ß-oxidation, respectively(*5*). The plates were activated, spotted and first developed in ether/petroleum ether/acetic acid (70/30/2) to move any free fatty acids to the front. A second development in chloroform/methanol/acetic acid (65/25/8) separated the phospholipid species. The phospholipids are PE, phosphatidylethanolamine; PG, phosphatidylglycerol and CL,

cardiolipin identified by their characteristic motilities in this chromatographic system. FFA denotes free fatty acids (the lack of any residual hexanoic acid can be attributed to its volatility).

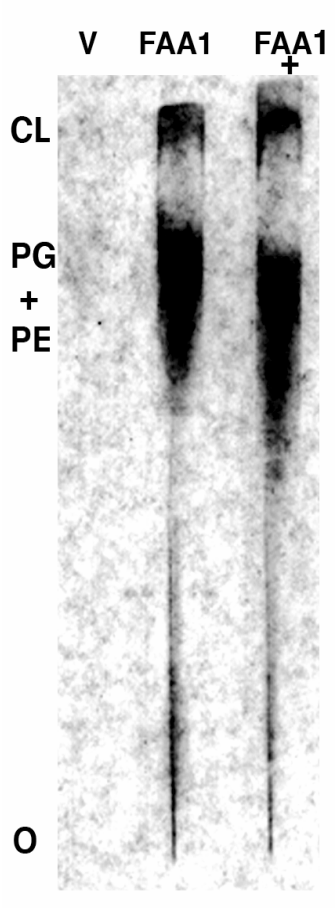


Fig. 5S. *E. coli* FadD function can be replaced by *Saccharomyces cerevisiae* FAA1. The experiment was done with [1-$^{14}$C]octanoate as in Fig. 3S and the designations are the same The host strain was K27 (*fad88*) carrying either the vector plasmid (V) or pBB277 which encodes the *S. cerevisiae* FAA1 expressed from the vector *tac* promoter (*6*). In the right hand lane IPTG was added (denoted by the + sign) to 1 mM. Separation of PE and PG failed in this experiment probably due to high atmospheric humidity.

References


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