Protocol for Fatty Acid Analysis

(Gas Chromatography-Flame Ionization Detector (GC-FID))

A. Media and Chemicals

1. Rich broth (1 Liter) for starter

10 g Tryptone
5 g NaCl
1 g Yeast extract

2. Minimal media

i. Minimal broth (BD, #275610); 10.6 g/L
ii. Minimal agar (BD, #254410); 26.6 g/L

For any minimal medium, supplement with:
0.2% Glucose;
0.4% Glycerol;
0.4% Acetate;
1 g/L Steric acid or oleic acid ; and
4 g/L Tergitol NP-40 detergent (to aid solubilization)

3. Petroleum ether (40 - 60^{o}C)

i. Petroleum ether(40 – 60^{o}C) in ~100-200 mL MilliQ water; or
ii. Hexane

4. 6% H_2SO_4 in methanol (v/v)

i. Prepare in fume hood, using ice-cold methanol

B. Bacterial Culture

1. Pick a single colony and prepare starter culture in 5 mL Rich broth and incubate overnight with shaking at 37^{o}C.

2. Using a 1:1000 dilution, inoculate starter culture in supplemented minimal medium and incubate for 24 hours at 37^{o}C with shaking.

3. Determine the OD_{600} of the bacterial culture centrifuge at 4,000 rpm to harvest cells and collect supernatant.

C. Fatty Acid Extraction

1. Wash cell pellet thrice with 5 mL **minimal medium WITHOUT any free fatty acid** and discard the wash supernatant. In a screw-cap, glass centrifuge tube, re-

suspend the cell pellet with 1 mL minimal medium and 1 mL **6% H_2SO_4 in methanol**. In a new screw-cap, glass centrifuge tube, add 2 mL of the saved supernatant and 2 mL of 6% **H_2SO_4 in methanol**.

2. Incubate the glass centrifuge tube in a 125^{o}C oven for 2 hours or in a 100^{o}C water bath for 2 hour. Cool down samples to room temperature and add 1 mL of **petroleum ether (40-60^{o}C) in H_2O** to the glass centrifuge tube. Vortex the glass centrifuge tube for 30 seconds.

3. Centrifuge the glass centrifuge tube at 4000 rpm for 5 minutes to separate the aqueous layer and the organic layer. Using a **NEW** glass pipette, transfer the organic layer (upper layer) into a labeled sample tube. Repeat step 6-10 twice, for complete fatty acid extraction.

D. Analysis of fatty acid methyl esters (Gas Chromatography-FID)

1. The sample injector was initially held at 90^{o}C for 3 minutes, and gradually increased to 210^{o}C at 20^{o}C/min and eventually raised to and held at 230^{o}C for 10 min.
2. Helium was used as the carrier gas and the column flow rate was 1 mL/min. One µL of sample was injected and C18 FAME was used as the interior label for quantification.

E. Reference

Campbell, J.W., Morgan-Kiss, R.M. and Cronan, J.E. Jr. (2003). A new *Escherichia coli* metabolic competency: growth on fatty acids by a novel anaerobic beta-oxidation pathway. Molecular Microbiology 47(3), 793-805.