SOP: PP030.1
Updated 5/12/15

Purification of Trehalose Monomycolate (TMM)

Materials and Reagents:

1. H37Rv γ-irradiated whole cells, 50 to 150 mg (wet weight)
2. Mettler-Toledo balance
3. Erlenmeyer flask, 1.8 L
4. Chloroform, Burdick & Jackson HPLC-grade
5. Methanol, Burdick & Jackson HPLC-grade
6. Graduated cylinder, glass, 100 ml
7. Chemical fume hood
8. Magnetic stir bar, large
9. Magnetic stir plate
10. Incubator, set at 37°C
11. Round-bottom flask, 1 L (1)
12. Whatman conical filters
13. Rotary evaporater (Rotovap)
14. Metal spatula
15. Glass Pasteur pipet
16. Rubber Pasteur pipet bulb
17. TLC reagents and equipment (note 1)
18. N_2 bath
19. Glass tubes + caps, 13 x 100 mm
20. TLC plate, silica, glass-backed preparative
21. TLC tank, large
22. Ruler
23. Pencil
24. Pipet, glass, 10 ml
25. Rubber pipet bulb
26. Vortex
27. Benchtop centrifuge

Protocol:

1._____ Freeze dry H37Rv γ-irradiated cells by lyophilization (note 2).

2._____ Weigh dried cells and transfer to a 1.8 liter Erlenmeyer flask.

3._____ Suspend cells in $CHCl_3/CH_3OH$ (2:1) at a concentration of 30 ml/g of cells (note 3).

4._____ Add a large magnetic stir bar.

5._____ Place on magnetic stir plate in a 37°C incubator and stir overnight.

6._____ Transfer extracted material to a sterile 250 ml Sorvall centrifuge bottles (note 4).

7._____ Centrifuge at 27,000 x g, 4°C for 30 minutes.

8._____ Transfer organic supernatant to 1 L round bottom flask.

9._____ Let cells air dry in a chemical fume hood; save for future use.

10._____ Dry material on a rotary evaporator and weigh.

11._____ Re-suspend the extracted material in a minimal volume of $CHCl_3/CH_3OH$ (2:1).

12._____ Apply material to preparative TLC plates (note 5).

13._____ Run preparative TLC plates in solvent system $CHCl_3/CH_3OH/H_2O$ (80:20:2).

14._____ The 2nd band closest to the origin is TMM; extract TMM from preparative TLC plates.

15._____ After 20 or more plates have been run, apply combined extracts to a fresh preparative TLC plate for cleanup. Run plate in solvent system 65:25:4 ($CHCl_3/CH_3OH/H_2O$); extract TMM from preparative TLC plates.

16._____ Assay all fractions by TLC; use solvent system $CHCl_3/CH_3OH/NH_4OH$ (80:20:2) and develop with charring spray and α-napthol spray (note 6).

17._____ Submit TMM for MALDI testing in positive electrospray mode using DHB as matrix (note 7).

Notes:

1. See Thin Layer Chromatography, SOP SP033, for a complete list of equipment and reagents.
2. See Lyophilization SOP, SP004.
3. All organic solvents should be used in a chemical fume hood.
4. Ensure that the centrifuge bottles used are compatible with the solvents being used.
5. See Preparative Thin Layer Chromatography, SOP SP032, for directions on all preparative TLC steps.
6. The TMM may also be analyzed by 2-D TLC, using the solvent system $CHCl_3/CH_3OH/H_2O$ (65:25:4) in the first dimension and $CHCl_3/CH_3OH/ H_2O$ (60:30:6) in the second dimension. The plates should then be developed with charring spray and α-naphthol spray as previously described.
7. Submit at 1 mg/ml in 2:1 chloroform/methanol. Should see cluster of peaks 1350-1550 m/z, so obtain a spectrum with this section expanded and salient peaks labelled.

References:

Slayden, RA and Barry 3rd, CE (2001). Analysis of the Lipids of *Mycobacterium tuberculosis*. *Mycobacterium tuberculosis Protocols* (Parish T and Stoker, NG ed), Humana Press Inc, Towata NJ, pp 229-246.

Besra, GS (1998). Preparation of Cell-Wall Fractions from Mycobacteria. *Methods in Molecular Biology, Volume 101: Mycobacteria Protocols* (Parish T and Stoker, NG ed), Humana Press Inc, Towata NJ, pp 91-107.