Purification of Trehalose Monomycolate (TMM)

Materials and Reagents:

1. H37Rv $\gamma$-irradiated whole cells, 50 to 150 mg (wet weight)
2. Mettler-Toledo balance
3. Erlenmeyer flask, 1.8L
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. Parafilm
10. Magnetic stir plate
11. Incubator, set at 37°C
12. Round-bottom flask, 1 L (1)
13. Rotary evaporator (Rotovap)
14. Metal spatula
15. Sorvall centrifuge bottles (1 to 6)
16. Sorvall centrifuge
17. Sorvall centrifuge rotor, GSA
18. Glass Pasteur pipet
19. Rubber Pasteur pipet bulb
20. TLC reagents and equipment (see note 1)
21. N$_2$ bath
22. Glass tubes, 16 x 100 mm (as many as pools and bands)
23. 16 mm lids, PTFE-lined (as many as tubes)
24. TLC plate, silica, glass-backed preparative
25. TLC tank, large
26. Ruler
27. Pencil
28. Pipet, glass, 10 ml
29. Rubber pipet bulb
30. Vortex
31. Benchtop centrifuge
32. Glass tubes, 13 x 100 mm (1 + number of aliquots)
33. 13 mm lids, PTFE-lined (as many as tubes)
34. CDCl$_2$, HPLC-grade (Supelco)
35. CD$_3$OD, HPLC-grade (Supelco)
36. NMR tube
37. $^1$H NMR machine (see note 2)

Protocol:

1. Freeze dry H37Rv $\gamma$-irradiated cells by lyophilization (see note 3).
2. Weigh dried cells and transfer to a 1.8 liter Erlenmeyer flask.
3. Suspend cells in CHCl$_3$/CH$_3$OH (2:1) at a concentration of 30 ml/g of cells (see note 4).
4. Add a large magnetic stir bar and cover mouth of flask with parafilm.
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.
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 CHCl₃/CH₃OH (2:1) (see note 5).
12. Apply material to preparative TLC plates (see note 6).
13. Run preparative TLC plates in solvent system CHCl₃/CH₃OH/NH₄OH (80:20:2) (see note 7).
14. The major fraction closest to the origin is TMM; extract TMM from preparative TLC plates (see note 8).
15. Dry silica under a stream of N₂.
16. Add 8 ml of CHCl₃/CH₃OH (2:1) to each tube and vortex vigorously.
17. Centrifuge at 3,000 x g, 4°C for 15 minutes.
18. Transfer the organic supernatant to new, pre-weighed 16 x 100 mm tubes (see note 9).
19. Dry under a stream of N₂.
20. Repeat steps 16 to 19 twice more.
21. Assay all fractions by TLC; use solvent system CHCl₃/CH₃OH/NH₄OH (80:20:2) and develop with charring spray and α-napthol spray (see notes 10 and 11).
22. Take 5 to 10 mg of TMM fraction and transfer to a new 13 x 100 mm tube.
23. Re-suspend TMM in 1 ml of CDCl₃/CD₃OD (2:1).
24. Completely dry under a stream of N₂.
25. Repeat steps 24 and 25 once more.
26. Re-suspend TMM in 1 ml of CDCl₃/CD₃OD (2:1).
27. Transfer the TMM suspension to a clean NMR tube and analyze by ¹H NMR (see note 12).
28. Once NMR analysis is complete, transfer TMM suspension from the NMR tube back to the 13 x 100 mm tube.
29. Completely dry under a stream of N₂.
30. Re-suspend in 1 ml of CHCl₃/CH₃OH (2:1).
31. Completely dry under a stream of N₂.
32. Repeat steps 30 and 31 once more.
33. Re-suspend in 1 ml of CHCl₃/CH₃OH (2:1).
34. Transfer from the 13 x 100 mm tube and combine with remainder of TMM.
35. Completely dry under a stream of N₂.
36. Re-suspend TMM in CHCl₃/CH₃OH (2:1) and aliquot into new 13 x 100 mm tubes.
37. Completely dry under a stream of N₂.

Notes:

1. See Thin Layer Chromatography, SOP SP033, for a complete list of equipment and reagents.
2. See NMR SOP, SP-XXX, for a complete list of equipment and reagents.
3. See Lyophilization SOP, SP004.
4. All organic solvents should be used in a chemical fume hood.
5. See Preparative Thin Layer Chromatography, SOP SP032, for directions on preparing the material for preparative TLC.
6. See Preparative Thin Layer Chromatography, SOP SP032, for directions on loading a preparative TLC plate.
7. See Preparative Thin Layer Chromatography, SOP SP032, for directions on running a preparative TLC plate.
8. See Preparative Thin Layer Chromatography, SOP SP032, for directions on extracting lipids from a preparative TLC plate.
9. The organic supernatant should be passed through a 0.2 µm PTFE syringe filter, attached to a glass 10 ml syringe, prior to placement in the pre-weighed 16 x 100 mm tube. This removes any contaminating silica from the supernatant.
10. The TMM may also be analyzed by 2-D TLC, using the solvent system CHCl₃/CH₃OH/H₂O (100:14:0.8) in the first dimension and CHCl₃/CH₃OH/NH₄OH (90:10:1) in the second dimension. The plates should then be developed with charring spray and α-napthol spray as previously described.
11. The charring spray (SOP R011) will detect any organic compound and α-napthol spray (R012) will detect any glycans.

12. See NMR SOP SP-XXX.

References:
