SOP: PP038

Purification of Phenolic Glycolipid from *M. canetti*

Materials:

1. Chemical fume hood
2. Rotary evaporator
3. Glass funnel
4. Support ring stand, clamps
5. Conical filter paper (VWR #28310-172)
6. Round bottom glass flasks, 250-500 ml
7. TLC plates, preparative
8. Analytical TLC sheets (VWR #5554-7)
9. TLC tanks, large and small
10. TLC aluminum racks, large and small
11. Methanol, HPLC-grade
12. Chloroform, HPLC-grade
13. Aluminum foil
14. Metal spatula
15. Ruler
16. α-naphthol
17. TLC aerosolization sprayer
18. Heat air-gun
19. Shortwave UV light box
20. Glass slides
21. Glass tubes, 13 x 100 mm
22. Teflon Oakridge tubes, 35-50 ml
23. Nitrogen bath
24. Mettler-Toledo balance

Protocol:

1._____ Obtain Folch-washed total lipid from 50-200 g wet weight (note 1).

2._____ Apply total lipid to preparative TLC plates using 20-40 mg per plate. Apply 50 µg PGL control on edge of at least one plate to visualize PGL-specific band (note 2).

3._____ Develop plates in 95:5 chloroform/methanol in large TLC tank. Allow to dry 30 min.

4._____ Score plates parallel to sides, 1 cm in from outer edge of where total lipid was applied.

5._____ Cover area between scored lines with foil, then stain outer edges with α-naphthol and charring with air-gun (note 3).

6._____ Place plates under shortwave UV light box to visualize PGL-specific band (note 4). Delineate band with pencil.

7._____ Use a glass slide to carefully scrape off silica just beneath the PGL-specific band (note 5).

8._____ Scrape silica containing the PGL band, dividing silica between glass or Teflon tubes (note 6).

9._____ Add 5-8 volumes 2:1 chloroform/methanol to 1 volume PGL-specific silica in tubes, briefly vortex, balance, and centrifuge 3K rpm, 4°C, 10 min.

10._____ Meanwhile, set up filtration system for capture of the extracts (note 7).

11._____ Filter first round of extracts into flask, then repeat extraction procedure (steps 9-11) twice for a total of three extractions.

12._____ Dry total extract down via rotary evaporation (note 8).

13._____ Evaluate purity for each batch of extracts. Apply 50-100 μg crude PGL to 10 x 10 cm analytical TLC sheets and develop with 95:5 chloroform/ methanol (note 9).

14._____ Repeat steps 2-13 for as many plates as necessary prior to running clean-up prep plates. Apply crude PGL extracts to prep plates, 500-1000 um in thickness, using no more than 15 mg per plate.

15._____ Develop plates in 95:5 chloroform/methanol as described in step 3.

16._____ Repeat steps 4-12 to obtain purified PGL extracts, and evaluate by analytical TLC as described.

17._____ Obtain final weight in a tared 13 x 100 mm tube or small glass vial.

18._____ Submit at least 10 μl of PGL at 2 μg/μl for MALDI-TOF analysis in positive mode (note 10).

19._____ Aliquot as 0.25 mg in 13 x 100 mm tubes, using as many as necessary. Excess PGL can be used as reference material for the next lot.

Notes:

1. SOP PP018. While the protocol calls for one overnight stirring in 2:1 chloroform/methanol, an additional 4 hour extraction will pull out more lipids. The combined lipids can then be Folch-washed.
2. SOP SP032. Use silica gel 60, 20 x 20 cm, fluorescent indicator F254, 500-1000 um thickness. Score plates with a spatula and ruler all the way down to the glass, 1 cm from the top to enable multiple plates to be ran evenly and consistently.
3. Apply heat evenly with heat air-gun, being careful not to dwell too long over one spot to avoid scorching and obliterating signal. This should take at least 15 seconds.
4. It should appear as a relatively white band in line with an intense purple band stained at the edges, corresponding to the PGL control.
5. Scrape an area about the width of the short side of the slide. This will ensure that no non-specific silica harboring contaminating lipids will be scraped along with PGL-containing silica in the subsequent step.
6. Teflon tubes can be used when silica volume is high. The volume of the silica should not exceed 1/8 the volume of the tube, so use an appropriate number of and appropriately sized tubes.
7. Place glass funnel in ring stand, fit with circular filter paper folded into cone, and place round-bottom flask beneath for capture of the extract. Extract from a batch of plates should not exceed ½ the volume of the flask.
8. SOP SP037. You may choose to keep track of weight of "crude" PGL from each batch of plates by taring a 13 x 100 mm tube, resuspending dry lipid in the flask in 3-5 ml 2:1 chloroform/methanol, transferring to the tube, then drying under N_2 bath. Use a Mettler-Toledo or similar digital balance to determine the weight of the lipid. This can be done to know when to proceed with clean-up of the crude PGL, considering that 30-40% crude PGL will be lost in that process.
9. SOP SP033. Stain with α-naphthol/charring. Pure PGL will appear as a single purple band approximately halfway up from the application line. Most PGL will be "crude" at this point, however, showing shadow bands above or below the main band. Run 10-20 μg *M. canetti* PGL as a control.
10. Should see a cluster of peaks at 1906 m/z with 3-4 clusters above and below this mass, each separated approximately by 14 mass units from the main 1906 peak.