Preparation of Peptidoglycan

Materials and Reagents

1. AGP (mAGP without mycolates), 50 to 150 mg
2. Sulfuric acid, 0.05M
3. Water, HPLC-grade (VWR BJ365-4)
4. Reagents for alditol acetates (note 4)
5. Glass tubes with PTFE-lids, 16 x 100 mm
6. Magnetic stir bar, small
7. Magnetic stir plate
8. Warm room or reach-in incubator, 37°C
9. Magnetic stir bar remover
10. Benchtop centrifuge
11. Vortex
12. Pasteur pipet, glass
13. Pasteur pipet bulb, rubber
14. Savant speed-vac
15. Glass tubes, 13 x 100 mm
16. Glass capillary pipet, 10 µl
17. Glass capillary pipetor, 10 µl
18. Gas Chromatograph

Protocol

1. _____ Transfer AGP into a new 16 x 100 mm glass tube (note 1).
2. _____ Add 5 ml of 0.05M sulfuric acid and a small magnetic stir bar.
3. _____ Cap tube and place on a magnetic stir plate at 37°C for four days.
4. _____ Remove the small magnetic stir bar and centrifuge at 3,000 x g, 25°C for 15 minutes.
5. _____ Transfer the supernatant to a new 16 x 100 mm tube (note 2).
6. _____ Add 5 ml of HPLC-grade water to the pellet.
7. _____ Cap tube and vortex vigorously.
8. _____ Centrifuge at 3,000 x g, 25°C for 15 minutes.
9. _____ Decant the supernatant into a separate 16 x 100 mm tube and completely dry on savant speed-vac. Repeat wash steps 6-9 to get rid of residual AG (note 3).
10. _____ Dry and weigh the pellet. Re-suspend in 5 ml of HPLC-grade water with 30 min waterbath sonication.
11. _____ Transfer two 25 µl aliquots to two new 13 x 100 mm glass tubes.
12. _____ Completely dry on savant and prepare alditol-acetate derivatives (note 4).
13. _____ Analyze derivatives by GC to ensure the purified PG does not contain arabinose and galactose (note 5).
14. _____ Submit sample in triplicate for amino acid analysis (note 6).
15. _____ Make 0.25 mg aliquots based on the dry weight, dry and store at -80°C.
Notes

1. AGP is obtained from SOP PP014.

2. At this point the supernatant is soluble arabinogalactan, and should be further purified using SOP PP012.

3. See SOP SP005 for use of savant. The dry washes need to be tested for traces of AG.

4. See SP022 for derivative preparation and GC operation.

5. If the GC analysis shows contaminating arabinose and/or galactose, then the protocol needs to be repeated, until the peptidoglycan is devoid of arabinogalactan.

6. See SPO47.1

Reference

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