Cell Wall Fraction Analysis Adjusted from Julie Nemecek

Part I: Cell Wall Prep

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1. Place cells in weighed 2 ml tube.
2. Speed vac until dry
3. Adjust to 5mg dry cell weight
4. Wash dry weight pellet once with distilled water.
5. Suspend in 1x PBS
6. Transfer to bead beating tube and fill with same volume beads
7. Bead beat 1 min X 5, ice for 1 min in between
8. Remove sample from beads and place in a 15 ml tube.
9. Wash beads with additional 9-10 ml PBS.
10. Centrifuge 5000g to pellet cell walls.
11. Transfer to 2 ml microfuge tube.
12. Extract cells 3 times in 0.5 mL of 0.7M NaOH, 60' at 75°C.
13. Neutralize with 0.25 mL glacial acetic acid. Save extract--this is the **alkali soluble glucan** fraction (1.75mL).
14. Take remaining pellet, wash with 100mM Tris pH 7.5 (1ml).
15. Wash with 10mM Tris pH 7.5 (1ml).
16. Suspend in 1mL of 10mM Tris (pH7.5)-0.01% sodium azide and 5mg of Zymolyase 20T.
17. Incubate 16 hours at 37°C with gentle mixing.
18. Pellet insoluble material (13000 rpm, 15'). Pellet is **Zymolyase insoluble glucan.** Resuspend in 50-100 µl PBS.
19. Treat supernatant with chitinase: 1u, 25°C overnight.
20. Divide supernatant into two portions. Dialyze one portion of eluate against distilled water with pore size 8000 D-- remaining fraction is **alkali-insoluble β 1-6 glucan**.
21.The remaining eluate is analyzed for total glucan level to determine the amount of **alkali-insoluble β 1-3 glucan**.