Chitin extraction protocol (Hiram Sanchez 2-28-18)

1- Harvest cells by centrifugation and dry in lyophilizer overnight.
2- Measure 10 mg of dry cell material and wash in 2 ml of cold water 2x
3- Collect cell material by centrifugation 4000g.
4- Resuspend the pellet in 1 ml of KOH 6% and transfer to a glass tube.
5- Place the tube in a water bath at 80C for 90 min.
6- Remove tubes from water bath, add 0.5 ml glacial acetic acid and cool the tubes on ice.
7- Centrifuge solution at 4,000 g for 3 min. Remove and discard supernatant.
8- Wash the pellet 2X with cold water and centrifuge as before.
9- Resuspend the pellet in 1.0 ml of 50 mM buffer KH_2PO_4 , pH 6.5
10- Add 0.5 U of chitinase and 5 U of Zymolyase 20T
11- Incubate at 37 C for 6 -24 hrs under slow shaking. Centrifuge at 3,000 g for 5 min and collect supernatant.
12- To 1 ml of sample, add 0.5 ml of solution A incubate at 100 C for 20 min.
13- Add 3.5 ml of 96% ethanol followed by 0.5 ml of solution B. Pipette gently to mix.
14- Incubate at RT for 45 min. Read abs at 520 nm

Solution A = 1.5N Na_2CO_3 in 4% acetyl-acetone.

Solution B = 1.6 gm of 4 dimethylaminobenzaldehyde in 30 ml of concentrated HCl and 30 ml of ethanol

Use glucosamine as standard curve from 1000 ug-3ug/ml, treat std curve samples as in steps 12-14

For publication;

The chitin extraction was prepared as previously described by Popolo et all (1) with some modifications. Ten (10 mg) of dry cell walls were washed twice in 2ml of ice cold water and centrifuged for 10 min at 4000g. The pellet was suspended in 1 ml of 6 % KOH transferred to a glass tube and placed in the water bath for 90 min at 80 C. Tubes were remove from the water bath, cooled on ice and 0.5 ml of Acetic Acid added to each tube, centrifuged for 3 min at 4000g. The pellet was wash twice with 2 ml of cold water and centrifuge. The pellet was re- suspended in 1 ml of 50 mM KH_2PO_4 buffer pH 6.5. To each tube, 0.5 units of chitinase and 5 units of Zymolase 20 T added. Incubate at 37C for 24 hrs with slow shaking, centrifuge as before, keep supernatant. In a fresh tube add 1 ml of sample supernatant, 0.5 ml of Solution A, (1.5N Na_2CO_3 in 4% acetyl-acetone) incubate at 100 C for 20 min. Add 3.5 ml of 96% ethanol followed by 0.5 ml of solution B

(1.6 gm of 4 dimethylaminobenzaldehyde in 30 ml of concentrated HCl and 30 ml of ethanol). Incubate at RT for 45 min. read absorbance at 520 nm. A standard curve can be prepare using Glucosamine in a range from 1000-2 ug/ml. To 1ml of standard curve, add 0.50 ml of solution A, heat at 100C for 20 min in water bath. Add 3.5ml of 96% ethanol and 0.5 ml of solution B. Mix with pipette and incubate for 45 min at RT. Place 200 ul of each sample and std curve in tripliquets in a 96 well plate and read absorbance at 520.

Reference.

1- Journal of Bacteriology, Jan.1997, Vol 179, No.2 p463-469
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