Surface Polysaccharide Extraction and Quantification

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[Abstract] Gram-negative bacterial cells possess two membranes - the inner cytoplasmic membrane and the outer membrane. The two membranes are distinct in their composition; the inner membrane is composed of a phospholipid bilayer, whereas the outer membrane (OM) is composed of an asymmetrical bilayer, with the outer leaflet containing lipopolysaccharide (LPS) (Raetz and Whitfield, 2002). Surface polysaccharides, such as LPS O-antigen, or capsular polysaccharide, are often tightly associated with the OM (Whitfield, 2006). This tight association can be used to generate a rough quantification of surface polysaccharides of Gram-negative bacterial cells, as the OM can easily be dissociated from cells without associated cell lysis (Brimacombe *et al*., 2013). The following method describes how to quickly extract and quantify OM-associated polysaccharides.

Materials and Reagents

1. Culture of bacterial cells (This procedure works only for Gram-negative bacteria, for example *Escherichia coli*, *Pseudomonas aeurginosa*, or *Rhodobacter capsulatus*. The outer membrane, specifically LPS, is essential for this procedure to work)
2. 50 mM sodium chloride (NaCl) dissolved in deionized H_2O
3. 50 mM ethylenediaminetetraacetic acid (EDTA) (EMD Millipore, catalog number: 324503)
4. Phenol (Fisher Scientific, catalog number: A92-100)
5. 93% sulfuric acid (Avantor Performance Materials, catalog number: 2900-10)
6. Carbohydrate stock solution (see Recipes)

Equipment

1. Microcentrifuge
2. Microfuge tubes (ESBE, catalog number: ESB-ES00507C)
3. Spectrophotometer
4. Cuvettes
5. Glass test tubes
6. Glass pipettes

Procedure

A. Extraction of surface polysaccharides from Gram-negative bacteria

1. Grow bacteria to desired growth phase (generally stationary phase) in desired growth medium.

   Note: Growth medium may affect surface polysaccharide levels, so the same media should be used for all experiments if possible.
2. Measure OD_{650} of culture; dilute to < 1 OD if necessary to get an accurate measurement.
3. Normalize cultures to OD_{650} of 2.0 (or to maximum OD that bacterial culture will grow to if it is less than 2.0).
4. Harvest 1 ml of each normalized culture by centrifugation at 14,500 *x g* for 5 minutes in a microcentrifuge.
5. Carefully remove supernatant with a pipette, discard tip.
6. Wash cells by re-suspending in 1 ml of 50 mM NaCl, pellet by centrifugation at 14, 500 *x g* for 5 minutes, remove supernatant.
7. Repeat step A6 four additional times (5 total washes).
8. Re-suspend cells in 1 ml of 50 mM EDTA, and incubate at 37 °C for 60 minutes (EDTA causes LPS to dissociate, thus releasing the OM from cells).
9. Pellet cells by centrifugation at 14,500 *x g* for 5 minutes, carefully remove supernatant and transfer to fresh microfuge tube (supernatant contains all surface polysaccharides, including LPS, capsule *etc*.).

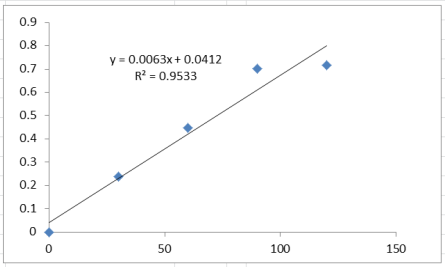
B. Quantification of surface polysaccharides

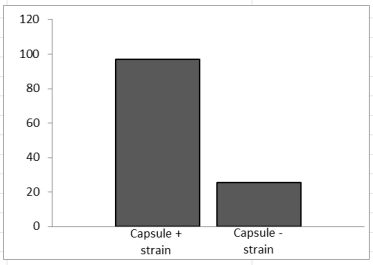
1. Prepare carbohydrate standards by diluting carbohydrate stock solution into 1 ml aliquots of: 0, 30, 60, 90, and 120 µg/ml of carbohydrate (*e.g.* 970 µl of dH_2O + 30 µl of 1 mg/ml stock solution to generate a 30 µg/ml standard).
2. Prepare clean, acid washed glass test tubes (for a suggested protocol, see Reference 4). Pipette 200 µl of standards, a 200 µl control of 50 mM EDTA, and all test samples into separate tubes.
3. Move to fume hood.
4. Add 200 µl of 5% phenol to all tubes, mix well by shaking.
5. Add 1 ml of 93% sulfuric acid; mix well by swirling (use caution).
6. Allow colour to develop for 10 minutes at room temperature (reaction should turn yellow; intensity depends on carbohydrate concentration). Additional mixing by gentle swirling every 2-3 minutes may help reaction proceed faster.
7. Measure OD_{490} of all reactions in a spectrophotometer; concentration of carbohydrates can then be calculated from the standard curve.

Note: If necessary, dilute reactions in dH_2O to get accurate spectrophotometer readings.

Representative Data

Carbohydrate [ ] (µg/ml)	OD650				
0	0	Sample	Rhodobacter capsulatus B10 (capsule +)	Rhodobacter sphaeroides 2.4.1 (capsule -)	EDTA
30	0.237	OD650	0.758	0.309	0.107
60	0.449	Zeroed to EDTA	0.651	0.202	0
90	0.701	Calculated carb concentration (ug/ml)	96.79365079	25.52380952	
120	0.717				





Recipes

1. Carbohydrate stock solution
   50:50 mixture of 0.5 mg/ml each of sucrose and fructose
   Final concentration of 1 mg/ml carbohydrate (molecular biology grade recommended)

Acknowledgments

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References

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