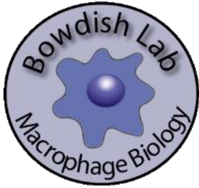

PNEUMOCOCCAL CELL WALL PURIFICATION

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BACKGROUND

- The bacterial cell wall is a structure that serves as both a protective shield for invasive pathogens and as a means of bacterial recognition by the host innate immune system. For many applications it will be desirable to obtain purified cell wall.
- The cell wall of *Streptococcus pneumoniae* is believed by our lab to contain the macrophage receptor with collagenous structure (MARCO) binding partner and we purified *S. pneumoniae* cell wall for this purpose.
- Although designed for purification of pneumococcal cell wall, this protocol can be used (with some modifications) for the purification of cell walls from other bacteria, both Gram-positive and negative.
- This protocol was adapted from (Bui et al., 2012; Desmarais, Cava, de Pedro, & Huang, 2014; Tuomanen, Liu, Hengstler, Zak, & Tomasz, 1985)

NOTES

- This protocol is designed for the purification of crude cell wall containing teichoic acids, further steps should be taken if teichoic acids are to be removed.
- The volume used herein is 100ml of initial bacterial culture, larger or smaller volumes can be used. The volume used should be dependent on the downstream application of cell wall.

EQUIPMENT

- Equipment:
  - Hotplate or heating block
  - Level II biosafety cabinet for handling live *S. pneumoniae*
- Materials:
  - RNase, DNase and proteinase K
  - PBS
  - 1M NaCl
  - SDS (5% and 2%)
  - $MgSO_4$ and $CaCl_2$ (solid)

PROTOCOL

Bacterial growth and collection:

- Preparatory Work:
  - Prepare 100ml of sterile TSB
  - Have ready 1ml stock of OD_{600} = 0.15 *S. pneumoniae*
  - Always work with live bacteria in a biosafety cabinet

1. Add entire bacteria stock to 100ml of TSB.
2. Incubate stationary at 37°C for ~5h until OD_{600} = 0.5. Check with a spectrophotometer every 30min starting at the 4h mark.
3. Before the end of the incubation period, place some PBS on ice.
4. Pellet bacteria by centrifugation at 3,200g for 30min at 4°C.
5. Dispose of supernatant. Resuspend pellet in 5ml ice-cold PBS. Keep on ice.
    - The volume can be adjusted depending on the size of the pellet. Generally, resuspend in 1x volume of pellet
    - The next step requires another 4 hours; therefore, store the suspension in a BSL-2 refrigerator at 4°C to continue purification next day
6. Transfer pellet into a 50 mL Falcon tube; add 3x volume of SDS for a final concentration of 6% v/v. Add a small stirring bar into the tube
7. Submerge the tube into a boiling water bath and stir for 4 hours at 500 rpm. This is best done in a BSL-2 cabinet. By the end, the solution should appear close to transparent, suggesting the bacteria have lysed.
8. Centrifuge at 3,200g for 30 min at room temperature to pellet any whole bacteria. Transfer the supernatant into another tube.
9. Pellet the cell wall in an ultracentrifuge for 1 hour at 250,000g. Each ultracentrifuge rotor has different specifications; therefore, consult manufacturer protocol and safety guidelines.
10. Wash 2× with 1M NaCl and repeatedly with dH_2O until no traces of SDS can be detected.
    - Presence of contaminating SDS can be assayed using a methylene green stain, detailed in **Detection of contaminating SDS using methylene green**.
11. Wash 3x with Millipore water
12. Resuspend cells in 2 pellet volumes of dH_2O.

- Concluding Work:
    - Can be stored at 4°C at this point if desired.

Removal of proteins and nucleic acids:

- Preparatory Work:
    - 100mM Tris-HCl (pH 8.0), 20mM $MgSO_4$, 10mM $CaCl_2$
    - Have a heating block at 37°C.

1. Resuspend in 1x pellet volume 100mM Tris-HCl (pH 8.0) containing 20mM $MgSO_4$ and 10mM $CaCl_2$
2. Add 10µg/ml DNase A and 50µg/ml RNase I. Incubate at 37°C for 2h.
3. Add 100µg/ml proteinase K). Incubate at 37°C overnight.

Cell wall purification

1. Have heating blocks at 95°C
2. Add 1x pellet volume 2% SDS. Incubate at 95°C for 15min.
3. Centrifuge at 250,000g for 1 hour at room temperature.
4. Wash 3x with Millipore water.
5. Lyophilize overnight

LINKS AND REFERENCES

Bui, N. K., Eberhardt, A., Vollmer, D., Kern, T., Bougault, C., Tomasz, A., … Vollmer, W. (2012). Isolation and analysis of cell wall components from Streptococcus pneumoniae. *Analytical Biochemistry*, *421*(2), 657–666. http://doi.org/10.1016/j.ab.2011.11.026

Desmarais, S. M., Cava, F., de Pedro, M. A., & Huang, K. C. (2014). Isolation and Preparation of Bacterial Cell Walls for Compositional Analysis by Ultra Performance Liquid Chromatography. *Journal of Visualized Experiments*, (83). http://doi.org/10.3791/51183

Tuomanen, E., Liu, H., Hengstler, B., Zak, O., & Tomasz, A. (1985). The induction of meningeal inflammation by components of the pneumococcal cell wall. *The Journal of Infectious Diseases*, *151*(5), 859–868.