Analysis of *Moraxella catarrhalis* Outer Membrane Protein Profiles

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[Abstract] Phenotypes observed for certain *Moraxella catarrhalis* wild-type strains or mutants may be caused by a variety of factors including alteration of outer membrane protein composition. Examination of the outer membrane protein profiles may be a valuable tool to identify changes in outer membrane compositions of these strains. Here we describe a method to isolate and analyse *M. catarrhalis* fractions highly enriched for membrane proteins.

Materials and Reagents

1. Brain heart infusion (BHI) (Becton Dickinson and Company, catalog number: 237500) broth and BHI agar plates
2. Antibiotics
   e.g. spectinomycin or kanamycin (Merck KgaA, Calbiochem, catalog numbers: 567570-10 and 420311-5)
3. PBS
4. Bovine skin gelatin (Sigma-Aldrich, catalog number: G9382-100G)
5. ReadyPrep Protein Extraction Kit (membrane I) (Bio-Rad, catalog number: 1632088)
6. Glass beads, acid-washed (150-212 µm) (Sigma-Aldrich, catalog number: G1145)
7. 2D-Quant Kit (General Electric Company, catalog number: 80-6483-56)
8. Mini-protean TGX precast gels, 4-15% (Bio-Rad, catalog number: 456-1083)
9. Colloidal Coomassie staining (Pink *et al.*, 2012)

Equipment

1. CO_2 incubator
2. Benchtop Incubator Shaker
3. Centrifuge
4. TissueLyser LT (QIAGEN, model: 85600)

Procedure

1. *M. catarrhalis* strains were inoculated on brain heart infusion (BHI) plates (supplemented with antibiotics when appropriate), and grown overnight at 37 °C in an atmosphere containing 5% CO_2.
2. Bacteria were harvested from plate and resuspended in PBS supplemented with 0.15% gelatin (PBS-G). This suspension was used to inoculate BHI broth to an OD_{620} nm of ~ 0.05 and grown at 37 °C at 200-250 rpm until OD_{620} nm of 1.0 to 1.2 (mid-log). Use of different growth media is possible, but may affect outer membrane protein profiles. Always use the same growth media when comparing outer membrane protein profiles of different strains.
3. Subsequently, bacteria were harvested by centrifugation for 10 minutes at 3,200 *x g*.
4. Outer membranes were isolated using the ReadyPrep Membrane I kit according to manufacturer's instructions.
5. During the lysis procedure, 50 mg acid-washed glass beads (150-212 µm) were added for homogenization with the TissueLyser LT. The TissueLyser was operated 5 times for 1 minute at 50 Hz.
6. Suspensions were chilled on ice for 1 minute between every TissueLyser step.
7. Protein quantification was performed using the 2D-Quant Kit according to manufacturer's instructions.
8. Five microgram of outer membrane preparations were separated on a 4-15% TGX gel and analyzed by colloidal Coomassie staining (Pink *et al.*, 2010).

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References

1. de Vries, S. P., Burghout, P., Langereis, J. D., Zomer, A., Hermans, P. W. and Bootsma, H. J. (2013). Genetic requirements for *Moraxella catarrhalis* growth under iron-limiting conditions. *Mol Microbiol* 87(1): 14-29.
2. Pink, M., Verma, N., Rettenmeier, A. W. and Schmitz-Spanke, S. (2010). CBB staining protocol with higher sensitivity and mass spectrometric compatibility. Electrophoresis 31(4): 593-598.

