Adhesion of *Moraxella catarrhalis* to Respiratory Tract Epithelial Cells

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[Abstract] *Moraxella catarrhalis* is a human-restricted pathogen that is responsible for respiratory tract infections such as childhood otitis media (OM) and exacerbation of chronic obstructive pulmonary disease (COPD) in adults. Successful colonization and infection by *M. catarrhalis* depends on its ability to attach to the respiratory tract mucosal epithelium. This protocol describes a method to measure adherence of *M. catarrhalis* to epithelial cell lines *in vitro*.

Materials and Reagents

1. Human pharyngeal epithelial cell line Detroit 562 (ATCC, catalog number: CCL-138)
2. Type II alveolar epithelial cell line A549 (ATCC, catalog number: CCL-185)
3. DMEM + GlutaMAX™-I (Life Technologies, Invitrogen™, catalog number: 31966-047)
4. Fetal calf serum (FCS) (Greiner Bio-One GmbH, catalog number: 758093S5403)
5. Trypsin-EDTA (0.25%-1 mM) (Life Technologies, catalog number: 25300-054)
6. Brain heart infusion (BHI) (Becton Dickinson and Company, catalog number: 237500) broth and BHI agar plates
7. Antibiotics: spectinomycin or kanamycin (Merck KgaA, Calbiochem, catalog numbers: 567570-10 and 420311-5)
8. Bovine skin gelatin (Sigma-Aldrich, catalog number: G9382-100G)
9. Glycerol (Merck KgaA, catalog number: 1.04092.1000)
10. PBS without Ca^{2+} and Mg^{2+} (Westburg BV, catalog number: BE17-516F/12)
11. Saponin (Sigma-Aldrich, catalog number: 47036-50G-F)

Equipment

1. 24-well tissue culture plate (Falcon®, catalog number: 353407)
2. 75 cm^2 culture flask
3. Centrifuge
4. CO_2 incubator
5. Benchtop Incubator Shaker

Procedure

1. The human pharyngeal epithelial cell line Detroit 562 and the type II alveolar epithelial cell line A549 were routinely grown in DMEM with GlutaMAX™-I and 10% FCS at 37 °C and 5% CO_2.
2. For passage or seeding purposes epithelial cells were washed once in 10 ml PBS and detached using trypsin-EDTA as follows: add 3 ml Trypsin-EDTA per 75 cm^2 culture flask, incubate 5 min at 37 °C and 5% CO_2 until cells have detached from the bottom, collect cells with 7 ml culture medium, count cells, spin required amount for 5 min at 400 *x g*, resuspend pellet in required volume of culture medium.
3. Two days prior to the adherence assay, 2 x 10^5 Detroit 562 cells per well were seeded into a 24-well tissue culture plate, and after one day, the growth medium was refreshed.
4. A549 cells were seeded into 24-well tissue culture plates one day prior to the assay at 4 x 10^5 cells.
5. For both cell lines, monolayers of approximately 1 x 10^6 cells per well were used for adherence assays.
6. *M. catarrhalis* strains were inoculated on brain heart infusion (BHI) plates (supplemented with antibiotics when required) and grown overnight at 37 °C in an atmosphere containing 5% CO_2.
7. Bacteria were harvested from plate and resuspended in PBS supplemented with 0.15% gelatin (PBS-G).
8. 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. Subsequently, glycerol was added to a final concentration of 20%, and 1-ml aliquots were stored at -80 °C.
9. Before each assay, bacteria were thawed on ice, washed once in 1 ml DMEM with GlutaMAX™-I and 1% FCS (infection medium) and resuspended in the infection medium to 1 x 10^7 CFU ml^{-1}.
10. Epithelial cells were washed twice with 1 ml PBS, infected with 1 ml of the *M. catarrhalis* suspension (multiplicity of infection, 10 bacteria per cell), centrifuged for 5 minutes at 200 *x g* to facilitate contact between bacteria and cells, and incubated 1 h at 37 °C in a 5% CO_2 environment.
11. Non-adherent bacteria were removed by 3 washes with 1-ml PBS (PBS was added and subsequently carefully aspirated, no agitation). Detroit 562 or A549 cells were detached and lysed by addition of 1 ml 1% saponin in PBS-G followed by incubation at 37 °C and 5% CO_2 for 10 min.

12. CFUs were enumerated by plating 10-fold serial dilutions on BHI plates supplemented with the appropriate antibiotics. The percentage adherence of the mutants was calculated as the fraction of the inoculum that bound to the Detroit 562 cells.

Acknowledgments

This protocol was published in: de Vries *et al.* (2013). This study was financially supported by Vienna Spot of Excellence (VSOE) grant (ID337956).

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.

