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Cell surface proteolysis of intact *Leptospira* cells

This is a proteinase-K treatment protocol specifically developed for assessing surface-exposure of leptospiral proteins. Proteinase-K is non-specific protease that cleaves any accessible protein. Care should be taken to avoid disruption of the fragile outer membrane of leptospiral cells and subsequent exposure of subsurface proteins. This technique requires employment of several negative controls (immunoblot with antibodies for subsurface, preferably periplasmic proteins) to assess the integrity of the outer membrane.

Method:

1. Grow *Leptospira* in EMJH medium, supplemented with 1% rabbit serum at 30° C until they reach mid- to late-log phase (density of 5 x 10^7 to 5 x 10^8 cells/ml).

2. Harvest *Leptospira* culture by low-speed centrifugation at 2,000 x *g* for 7 min at room temperature

3. Gently resuspend in PBS-5 mM $MgCl_2$ to a final concentration of 2 x 10^9 cells/ml

4. Add Proteinase K (Sigma-Aldrich) in proteolysis buffer (10 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$) was added to a final concentration of 12.5 to 100 μg/ml. For a negative control, dd proteolysis buffer alone to the cell suspension

5. After incubation for 1 h at 37°C, stop the reactions by adding 5 μl of the peptidase inhibitor, phenylmethylsulfonyl fluoride (Sigma-Aldrich) (50 mM in isopropanol)

6. Centrifuge the suspensions at 9,000 x g for 5 min and wash twice with PBS-5 mM $MgCl_2$

7. Analyze the proteolysis of surface proteins by SDS-PAGE and immunoblotting

Reference:

Pinne, M. and Haake, D. A. "A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of *Leptospira interrogans*," PLoS ONE 4:e6071 (2009). PMID: 19562037.