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Membrane integration analysis for leptospiral proteins

This is a protocol describing membrane integration analysis to investigate the relationship of proteins with the membrane lipid bilayer. Membrane integration assessment is performed by fractionating leptospiral membranes by treatment with reagents designed to release peripheral membrane proteins not integrated into the lipid bilayer. Treatment of bacterial cells with lysozyme, alternating freezing and thawing, followed by centrifugation separates proteins into soluble (cytoplasmic and periplasmic) and pellet (total membrane) fractions. This protocol allows discrimination between integral (not released by the treatment) or peripheral membrane proteins (released from the membranes). However, this method does not allow discriminating between inner and outer-membrane proteins. For that purpose surface exposure should be assessed by other methods, such as surface proteolysis, surface biotinylation or surface immunofluorescence. High salt content, strong detergent and high pH are all valuable for assessing the membrane affinity. This technique requires employment of negative controls (immunoblot with antibodies for peripheral proteins, for example $P31_{LipL45}$ and integral proteins, such as OmpL37) to assess the efficiency and quality of membrane fractionation.

Method:

1. Grow *Leptospira* in EMJH medium, supplemented with 1% rabbit serum at 30° C until they reach density of ~5 x 10^8 cells/ml.

2. Harvest *Leptospira* culture by centrifugation at 6,000 x *g* for 10 min at room temperature.

3. Wash 5 x 10^9 of leptospiral cells twice with 10 mM phosphate buffered saline, pH 7.4 (PBS), containing 5 mM $MgCl_2$ and resuspend in 0.9 ml of lysis buffer (10 mM TrisHCl, pH 8.0, 5 mM EDTA, 0.5% protease inhibitor cocktail, Sigma-Aldrich) containing 1 mg/ml of lysozyme.

4. Incubate the suspension for 5 min at 4°C and subject to three cycles of freezing (-80°C) and thawing (room temperature) with vigorous vortexing.

5. Add DNase I (Sigma-Aldrich) to a final concentration of 5 μg/ml and incubate the cell suspension on ice for 20 min.

6. Recover membranes by centrifugation at 16,000 x g for 15 min at 4°C and resuspend in 0.5 ml of lysis buffer (without lysozyme).

7. Mix a 100 μl aliquot of the membrane suspension with 100 μl of either 0.2 M Na_2CO_3, 3.2 M urea, 1.2 M NaCl, or lysis buffer and incubate for 15 min at 4°C.

8. Pellet the samples at 16,000 x g for 15 min at 4°C and precipitate the supernatants with acetone.

9. Resuspend each membrane pellet (integral proteins) and its supernatant (peripheral proteins) precipitate in SDS-PAGE loading buffer and analyze by SDS-PAGE and immunoblotting.

Reference:

1. 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.