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Isolation and identification of membrane vesicle-associated proteins in Gram-positive bacteria and mycobacteria

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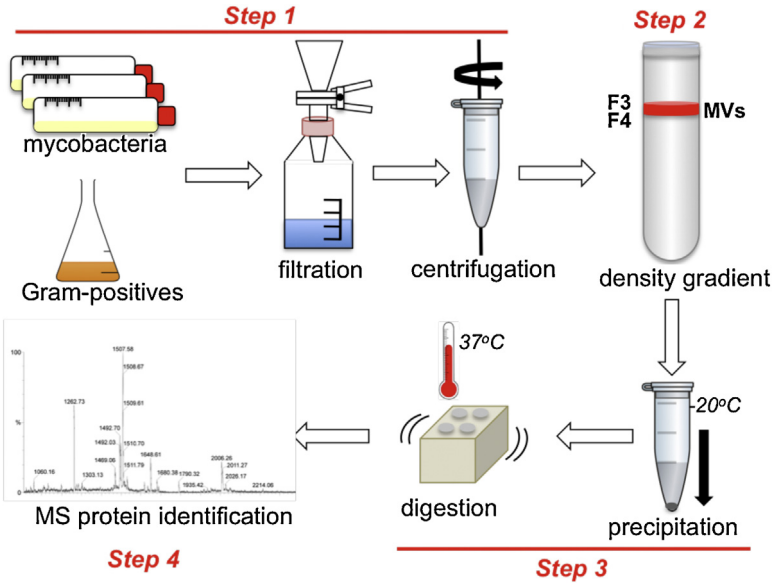
GRAPHICAL ABSTRACT



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A B S T R A C T

Many intracellular bacterial pathogens naturally release membrane vesicles (MVs) under a variety of growth environments. For pathogenic bacteria there are strong evidences that released MVs are a delivery mechanism for the release of immunologically active molecules that contribute to virulence. Identification of membrane vesicle-associated proteins that can act as immunological modulators is crucial for opening up new horizons for understanding the pathogenesis of certain bacteria and for developing novel vaccines. In this protocol, we provide all the details for isolating MVs secreted by either mycobacteria or Gram-positive bacteria and for the subsequent identification of the protein content of the MVs by mass spectrometry. The protocol is adapted from Gram-negative bacteria and involves four main steps: (1) isolation of MVs from the culture media; (2) purification of MVs by density gradient ultrucentrifugation; (3) acetone precipitation of the MVs protein content and in-solution trypsin digestion and (4) mass spectrometry analysis of the generated peptides and protein identification. Our modifications are:

- Growing Mycobacteria in a chemically defined media to reduce the number of unrelated bacterial components in the supernatant.
- The use of an ultrafiltration system, which allows concentrating larger volumes.
- In solution digestion of proteins followed by peptides purification by ziptip.

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A R T I C L E I N F O

Method: Protein content of bacteria membrane vesicles

Keywords: Gram positive bacteria, Membrane vesicles, Protein content, Vesicles isolation

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Method details

Isolation of pure mycobacterial and Gram-positive bacteria (*Bacillus subtillis*) membrane vesicles involves growing the bacteria in liquid media to mid-logarithmic phase, followed by clarification of the cell supernatant by sequential filtration and concentration. The concentrate is then ultracentrifuged and the pellet is submitted to density gradient ultracentrifugation to obtain the pure membrane vesicle fractions [1–3]. Proteins in the purified fractions are then acetone precipitated and in-solution digested with trypsin. Generated peptides are separated by nano-LC and analyzed by mass spectrometry for protein identification [4,5].

Step 1: isolation of membrane vesicles

Materials

- Minimal media (MM): Minimal media or chemically defined media is used to reduce the number of components in the culture supernatant. To approximately 3.9 L of double distilled water in a 4 L beaker, add 4 g KH_2PO_4, 10 g Na_2HPO_4 and 2 g asparagine. Adjust the pH to 7.0 using HCl. Filter-sterilize (0.22 mm) the solution. Add 56 ml of filter-sterilized 50% (v/v) glycerol, and 0.4 ml of filter-sterilized 500 mg/l ferric ammonium citrate, and 0.4 ml of 5 mg/l $CaCl_2$, and 0.4 ml of filter-sterilized 1 mg/l $ZnSO_4$.
- 7H9 media: To 450 ml of double distilled water in a 500 ml beaker, add 2.35 g of 7H9 Middlebrook broth. When dissolved, filter-sterilize (0.22 μm) the solution and add 50 ml of the sterile supplement OADC.
- Brain Heart Infusion (BHI) broth and agar (Difco).
- Luria Bertani (LB).
- Plastic squared inkbottles (30 ml).
- Orbital shaker 90 rpm at 37 °C.
- Roller bottles 850 cm^2 (Corning) equipped with a membrane on the cap.
- Rack with a horizontal rolling motion (30 rpm) at 37 °C.
- Vacuum filtration units (1000 ml, low protein binding) equipped with 0.45 μm and 0.22 μm PVDF membrane.

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