Preparation of *Bacillus subtilis* Cell Lysates and Membranes

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[Abstract] A common feature of every eukaryotic and prokaryotic cell is that they exhibit a plasma membrane. In *Bacillus subtilis* (*B. subtilis*) roughly 25% of all proteins are putative trans- or membrane associated proteins. Here we describe a relatively simple method to separate and prepare membrane and cytosolic proteins by ultra-centrifugation.

Materials and Reagents

1. *Bacillus subtilis* (*B. subtilis*)
2. Glycerol (Carl Roth, catalog number: 7530.4)
3. Tris (J.T.Baker®, catalog number: 1414)
4. NaCl (AppliChem GmbH, catalog number: A3597.5000)
5. $MgCl_2$ (Merck KGaA, catalog number: 1.05833.1000)
6. Proteinase inhibitor (Roche Diagnostics, catalog number: 04693159001)
7. DNAse I (Roche Diagnostics, catalog number: 10104159001)
8. Lysozyme (Roche Diagnostics, catalog number: 10153516103)
9. Casein hydrolysate (Oxoid Limited, catalog number: LP0041)
10. Buffer A (see Recipes)
11. Casein Hydrolysate (CH-medium) (see Recipes)
12. Solution G (see Recipes)

Equipment

1. Glass beads (diameter 0.2-0.3 mm) (Sigma-Aldrich, catalog number: G1277)
2. French press homogenizer (Glen Mills, French press G-M™)
3. Ultra-centrifuge (Beckman Coulter, model: optima™ XPN-100)
4. Ti-70 Rotor (Beckman Coulter)
5. FastPrep tissue homogenizer (MP Biomedicals, model: 116004500)
6. Refrigerated centrifuge (*e.g.* Beckman Coulter, model: Avanti-J25)
7. Acrodisc® syringe filters (a pore size of 0.2 µm) (Pall, catalog number: 4652)

Procedure

1. Inoculate *B. subtilis* to an OD_{600} of 0.1 in appropriate medium [*e.g.* CH, lysogeny broth (LB), Spizizen minimal medium (SMM); here we used 50 ml cultures in CH-medium].
2. Grow cells to respective OD_{600} (here we used an OD_{600} of 4, cells growing at 37 °C; 150 rpm in 250 ml flasks with baffles).
3. Harvest cells by centrifugation (10,000 *x g*; 15 min; 4 °C).
4. Discard supernatant and resuspend cells in 1/5 volumes of original culture at 4 °C in pre-cooled Buffer A.
5. Centrifuge again (10,000 *x g*; 15 min; 4 °C).
6. Discard supernatant and resuspend cells in 5-8x times volume of the cell pellet in Buffer A at 4 °C supplemented with proteinase inhibitor and DNase I. Use concentrations as specified by the manufacturer.
7. Disrupt cells

   Note: If cell disruption is critical, the cell suspension can be incubated with lysozyme (50 µg/ml) on ice for 30-120 min prior to cell disruption, check microscopically.

   a. Small cell volumes (1 ml in reaction tubes) can be disrupted in a FastPrep tissue homogenizer for 30 sec at 6.5 m/s with diameter 0.2-0.3 mm glass beads and cooling for 5 min on ice between runs (minimum 5 runs).
   b. Larger cell volumes can be disrupted in a French press homogenizer at 125 MPa and 3-5 passes. Cool sample on ice for 5 min between every pass. Note that only use of a French Press system will yield inside-out vesicles.
8. Remove cell debris by centrifugation (12,000 *x g*; 15 min; 4 °C).
9. Collect the supernatant (cell lysate) and centrifuge it at ≥ 200,000 *x g*; 4 °C and 60-90 min (*e.g.* in a Beckman-Coulter® Ti-70 rotor at 45,000 rpm).

   Note: Longer centrifugation time results in a better separation of membranes at high protein concentrations.
10. The supernatant represents the cytoplasmatic fraction; the pellet contains *B. subtilis* membranes (lipids, trans-membrane proteins and membrane associated proteins).

    Note: Resuspend the pellet in an appropriate buffer, e.g. Buffer A.

Recipes

1. Buffer A

   50 mM Tris·HCl (pH 7.5)

   150 mM NaCl

   5 mM $MgCl_2$

10% Glycerol (v/v)

2. CH-medium

   50 ml solution G (see below)

   0.05 ml 0.1 M $CaCl_2$

   0.02 ml 1 M $MgSO_4 \cdot 7H_2O$

   0.1 ml 0.0475 M $MnSO_4$

   0.5 ml 2 mg x ml^{-1} tryptophan

   Sterilize by filtering using Acrodisc® syringe filters; prepare freshly

3. Solution G

   25.0 g casein hydrolysate

   9.2 g L-glutamic acid

   3.125 g L-alanine

   3.48 g L-asparagine

   3.4 g KH_2PO_4

   1.34 g NH_4Cl

   0.27 g Na_2SO_4

   0.24 g NH_4NO_3

   2.45 mg $FeCl_3 \cdot 6H_2O$

   ddH_2O 2.35 L and adjust pH to 7.0 by using 10 N NaOH

   Sterilize by autoclaving; stored at 4 °C

Acknowledgments

This protocol is adapted from Bach and Bramkamp (2013).

References

1. Bach, J. N. and Bramkamp, M. (2013). Flotillins functionally organize the bacterial membrane. *Mol Microbiol* 88(6): 1205-1217.