

Preparation of *Bacillus subtilis* Cell Lysates and Membranes

Juri Niño Bach, Marc Bramkamp*

Department of Biology I, Ludwig-Maximilians-University, Munich, Germany

*For correspondence: marc.bramkamp@uni-koeln.de

[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. MgCl₂ (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 OD₆₀₀ 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 OD₆₀₀ (here we used an OD₆₀₀ 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 MgCl₂

- 10% Glycerol (v/v)
2. CH-medium
 - 50 ml solution G (see below)
 - 0.05 ml 0.1 M CaCl₂
 - 0.02 ml 1 M MgSO₄·7H₂O
 - 0.1 ml 0.0475 M MnSO₄
 - 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 KH₂PO₄
 - 1.34 g NH₄Cl
 - 0.27 g Na₂SO₄
 - 0.24 g NH₄NO₃
 - 2.45 mg FeCl₃·6H₂O
 - ddH₂O 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.