MP Biomedicals

Case study - *Lactic Acid Bacteria (LAB)*

Food

Comparaison of three methods for determination of protein concentration in lactic acid bacteria for proteomics studies.

Ibrahim Mehmeti, Fadime Kiran, and Ozlem Osmanagaoglu

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Overview

- **Keywords:** FastPrep®, Sonication, centrifugation, lactic acid bacteria (LAB)
- **Aim of the study:** Development of an optimized protein extraction protocol
- **Application:** One dimensional (1D) SDS-PAGE. Two dimensional (2D)-PAGE
- **Sample type:** Bacteria (*E. faecalis, P. pentosaceus* and *L. lactis*)
- **Material:** Sonicator, centrifuge, FastPrep® 120 instrument
- **Buffers:** 8M Urea, 2M Thiourea, 0,5% CHAPS, 10 mM DTT and 0,1% immobilized pH gradient (IPG)

Protocol and Parameters

1. Pellets were resuspended in 400 µL rehydration buffer containing 9M Urea, 2M Thiourea, 0,5% CHAPS, 10 mM DTT and 0,1% immobilized pH gradient (IPG).
2. Cells were lysed with acid washed glass beads (diameter of 212 to 300 µm) using a FastPrep® 120 at speed 6 m/s for 3 x 45 sec. at 4°C. After each cycle the solution was kept on ice for 1 min.
3. Cell debris were removed by centrifugation at 14,000 x g for 10 min at 4°C.
4. Before analysis, the supernatant was kept at -20 °C.

Results

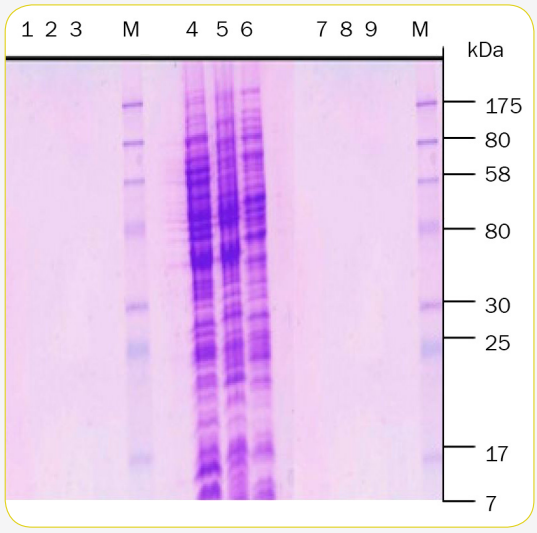
Six fold greater amount of protein was obtained with FastPrep® bead beater:

Method	Sonication	Centrifugation	FastPrep
E. faecalis V583	1.33 ± 0.01	1.25 ± 0.01	4.85 ± 0.05
L. lactis NIZO 0900	1.25 ± 0.02	1.32 ± 0.01	6.23 ± 0.06
P. pentosaceus OZF	1.32 ± 0.01	1,16 ± 0.02	5.66 ± 0.04

Mean±SD of protein concentrations (µg/µl) *of each strain obtained by three different methods. Values are mean ± S.D.(standard deviation) of results of three experiments.*

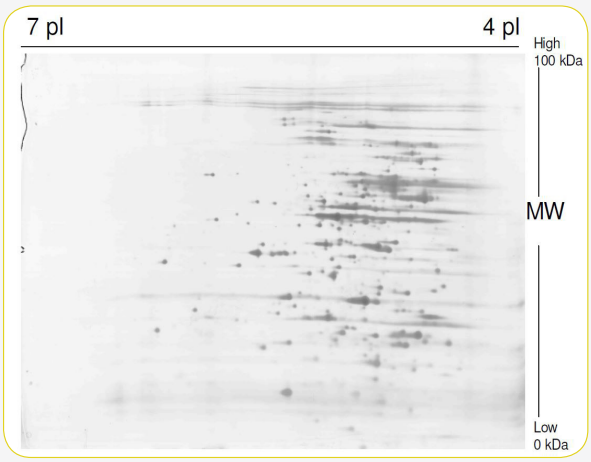
Results

Higher efficiency and higher quality in extracting proteins with FastPrep® method:



Representative Coomassie brilliant blue stained SDSPAGE *illustrating the intracellular proteins of three representative strains of LAB. The lanes (1 to 9) contains extracts of P.pentosaceus OZF (lanes 1, 4, 7); E. faecalis V583 (lanes 2, 5, 8) and L. lactis NIZO 0900 (lanes 3, 6, 9) obtained by centrifugation (lanes 1 to 3), FastPrep® (lanes 4 to 6) and sonication (lanes 7 to 9); M, prestained broad range protein marker (Bio Labs).*

FastPrep® method showed higher protein resolution and spot intensity of all proteins:



Representative 2D-PAGE *illustrating the intracellular proteins of E. faecalis V583 at pI 4 to 7 after lysing cells by FastPrep®. See higher protein resolution and spot intensity.*

Conclusion

- The results show that higher amount of proteins were obtained when the cells were lysed with a FastPrep®. **Six times higher protein concentration**, which is important for proteomics, was obtained with extraction by FastPrep®.
- SDS-PAGE gel images show that **higher amounts of proteins are obtained only when proteins extracted by FastPrep® method**.
- **More than 400 protein, spots**, with isoelectric points (pI) ranging from 4.0 to 7.0 and molecular weights (MW) from 0 to 100 kDa, were observed with 2D-PAGE analysis. In addition, proteins with less abundance and high molecular weights were **resolved clearly and detected strongly on 2D gel when FastPrep® method was used**.
- FastPrep® extraction method was **an efficient and reliable method for lysing and/or extracting proteins of *LAB*** for proteomic approach and **reproducible amounts** of bacterial proteins can successfully be extracted.
- Pictures were excellent enough to be used in alignment for statistical analyses and spots well-resolved for MALDI TOF analyses.

Successful sample preparation using the MP Biomedicals FastPrep® product line has been highlighted in thousands of scientific articles. To access articles and other materials, visit www.mpbio.com/FastPrepLibrary.

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