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Full Length Research Paper

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

Ibrahim Mehmeti[1], Fadime Kiran[1,2] and Ozlem Osmanagaoglu[2]*

[1] Department of Chemistry, Biotechnology and Food Science, University of Life Science, Ås, Norway.
[2] Department of Biology, Faculty of Science, Ankara University, Tandogan, 06100, Ankara, Turkey.

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Finding the best method of cell lysis and extraction of protein from the lysed cells is the key step in detection and identification of extra- and intra-cellular proteins in all applications of proteomics. To develop an optimized protein extraction protocol, *Enterococcus faecalis* V583, *Lactococcus lactis* NIZO 0900 and *Pediococcus pentosaceus* OZF strains, respectively, belonging to each genus of *Enterococcus, Lactococcus* and *Pediococcus* were used as a representative cells in a study of lactic acid bacteria (LAB). This report covers the use and comparison of three different protein extraction methods including sonication, centrifugation and rupture by glass beads (FastPrep) to get a better understanding about which methods give better extract quality and higher amount of proteins when applied to one dimensional (1D) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and for subsequent analysis by two dimensional (2D)-PAGE. The results clearly showed that, all methods can be used to lyse LAB strains. However, a six fold greater amount of protein was obtained when FastPrep was applied to lyse LAB cells. Our results also indicate that, this fast and easy extraction method allows more spot-abundant polyacrylamide gels. More clear and consistent strips were detected by SDS-PAGE when proteins were extracted by FastPrep. These results testify to the suitability of FastPrep protein extraction protocols for 2D proteomic studies of representative strains of LAB.

Key words: FastPrep, sonication, centrifugation, lactic acid bacteria (LAB).

INTRODUCTION

Lactic acid bacteria (LAB) are heterogeneous group of gram-positive bacteria, which produce lactic acid as a major end-product of their fermentative metabolism. *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus* and *Carnobacterium* are the main genera of LAB. They play an important role in food and feed fermentation and preservation, either as the natural microflora or as starter cultures added under controlled conditions. Besides their technological roles, LAB make expire date of the food longer by inhibiting the growth of spoilage and pathogenic bacteria, by competing for nutrients and producing antimicrobial compounds such as organic acids, carbon dioxide, ethanol, hydrogen peroxide and bacteriocins and therefore, thought to be potential biopreservativies (Klaenhammer, 1993). By definition, bacteriocins are proteinaceous substances with bactericidal or bacteriostatic activity against sensitive bacterial species (Klaenhammer, 1988; Daeschel, 1989; Piard and Desmazeaud, 1991; Stiles and Hastings, 1991; Piard and Desmazeaud, 1992; Klaenhammer, 1993). LAB and their food products are thought to confer a variety of important nutritional and therapeutic benefits and have many documented health promoting or probiotic effects in human

*Corresponding author. E-mail: osmanaga@science.ankara.edu.tr. Tel: +905324338662. Fax: 90-312-2232395.

Abbreviations: LAB, Lactic acid bacteria; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MS, mass spectrometry; **MRS,** de Man, Rogosa and Sharpe; **DTT,** dithiothreitol; **BSA,** bovine serum albumine; **IPG,** immobilized pH gradient; **TCA,** trichloroacetic acid.

(Ljungh and Wadström, 2006). Probiotics have been defined as living microorganisms which upon ingestion in adequate numbers exert positive health effects beyond inherent basic nutrition (FAO/WHO working group, 2001). The beneficial contribution of the LAB to the food and food-related industries is considerable. However, some LAB can cause spoilage of a variety of foods, such as meats, milk and milk products, vegetables, fruit juices, sugary products, alcoholic beverages and products preserved with vinegar (Sharpe and Pettipher, 1983). Besides, it is usually accepted that with the exception of some streptococci, LAB are rarely pathogenic to man and animals (Aguirre and Collins, 1993). *Enterococcus faecalis* which is one of the most studied species among LAB has been identified as causes of nosocomial infections, causing an increasing incidence of endocarditis, bacteremia, urinary tract and neonatal infections (Schaberg et al., 1991; Moellering, 1992; Tailor et al., 1993; Dutka-Malen et al., 1995; Hancock and Gilmore, 2006). However, this common intestinal microorganism (Devriese and Pot, 1995) is also used in some traditional food industries because of its beneficial role in the development of cheese aroma and stimulation of starter LAB (Giard et al., 2001). *Lactococcus lactis* is commonly used in industrial dairy fermentations, particularly in cheese making while *Pediococcus pentosaceus* is used in meats especially for sausage making and silage inoculants (Hammes et al., 1990).

Since LAB have a couple of interesting properties of great economic value, the growth of interest in LAB shows an increase in the concern of their rational use. The characterization of these organisms using the tools of proteomics as well as other approaches in systems biology (genomics, transcriptomics, metabolomics) will generate the knowledgebase necessary to an understanding of their biology. Compared with genomic studies, investigations at the proteome level provide detailed information such as protein abundance and posttranslational modifications. Proteomics is defined as the analysis of the entire protein complement expressed in a cell or any biological sample at a given time under specific conditions (Dierick et al., 2002). Proteomic technologies are powerful tools to study the physiological response of bacteria to various environmental stress conditions (Renzone et al., 2005). A better understanding of the mechanisms of stress resistance should permit an understanding of the bases of the adaptive responses and cross protection and to rationalize their exploitation in order to prepare LAB for industrial processes (Van de Guchte et al., 2002). A proteomic study includes sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), higher resolutions of two dimensional (2D)-PAGE, tryptic digestion of proteins from gel and protein identification by mass spectrometry (MS) by comparing the results with a data base (Haynes et al., 1998; Giard et al., 2001; Angelika et al., 2004). Extraction of proteins is challenging and inconsistent and has long been an issue for scientists (Natarajan et al., 2005). An efficient protein extraction methodology is quite important for sample preparation and for subsequent 2D-PAGE and MS analysis. Cell lysis is the first step in protein extraction and purification protocol. In any proteomic experiment, finding the best method to lyse cells and/or extract proteins from cells and the reliability of the methods is the most important step, as a reliable and comprehensive protein extraction is the closest proteomic equivalent to a fully sequenced and annotated genome (Cilia et al., 2009). Any biological conclusions that are obtained from a proteomic study are only as strong as the data indicating that, the extracts are reproducible and rich in protein diversity (Cilia et al., 2009). Many techniques including physical and detergent-based methods are available for cell disruption and protein extraction and have been used by several researchers in their own works for different purposes (Grabskia, 2009). However, there is only a few studies regarding comparison of these protein extraction methods (Bhaduri and Demchick, 1983; De Mey et al., 2008; Abram et al., 2009). These techniques can vary widely in reproducibility and in representation of the total proteome. Currently, although many methods have been developed and reported, there is no developed single protein extraction method. Besides, these methods have to be adapted, often two or more methods have to be combined and further optimized to obtain the best possible yield and purity for different species of organisms, for the type of sample (microbial cells or mammalian tissue) to be analyzed and for the interested proteins (soluble cytosolic or highly insoluble membrane proteins). Therefore, the protein extraction method remains a challenge for scientists in the accurate analysis of proteins (Natarajan et al., 2005).

In this study, we compared three different methods for the extraction of proteins from LAB including sonication, centrifugation and mechanic rupture by glass beads (FastPrep) to determine their efficiency in separating proteins by SDS-PAGE and for subsequent analysis by 2D-PAGE.

MATERIALS AND METHODS

Bacterial strains, media and growth conditions

Three representative strains belonging to LAB genera were selected: *E. faecalis* V583, *P. pentosaceus* OZF (on which proteome studies have been carrying out) and *L. lactis* NIZO 0900. *E. faecalis* V583 and *P. pentosaceus* OZF were grown in de Man, Rogosa and Sharpe (MRS) broth at 37°C, while GM 17 medium (M17 broth, pH 6.5, supplemented with 1.0% w/v glucose) was used for growth of *L. lactis* NIZO 0900 at 30°C. Cultures were maintained in their appropriate broths as frozen stocks with 15% (v/v) sterile glycerol and stored at -80°C. Before experimental use, they were subcultured twice in their appropriate medium and temperatures. Three biological replicates (independent cultures) for each protocol were made and samples in the late exponential phase were used for each strain.

Protein extraction

In this investigation, three different extraction methods including sonication, centrifugation and mechanical rupture by glass beads (FastPrep) were used to extract proteins or lyse cells from 3 representative strains of LAB. Before the extraction of cells by use of each method, samples of 50 ml were taken from the late exponential phase. Pellets were obtained by centrifugation at 6000×g for 10 min at 4°C (SIGMA 3K30) and used in each method.

Sonication method

Pellets were resuspended in 1 ml 20 mM TRIS buffer (pH 7.5) containing 5 mM EDTA and 5 mM $MgCl_2$. The cells were lysed by using transonic T460/H sonicator (John Morris Scientific Pty Ltd.) for 3 times, each 2 min in between which the cell suspension was kept on ice for 3 min. The cell debris was removed by centrifugation at 14000×g for 10 min at 4°C (Duché et al., 2002). The supernatant containing extracted proteins was stored at -20°C until further analysis.

Centrifugation method

Pellets were resuspended in 500 µl of buffer containing 0.3% SDS, 200 mM dithiothreitol (DTT), 28 mM Tris HCl, 20 mM Tris. Cell suspension containing microfuge tubes were stirred gently for 10 min at 4°C on a shaker (IKA-Werke KS260B), followed by removing of cells by centrifugation at 14000×g for 10 min at 4°C. Supernatant was incubated at 100°C for 5 min and then, chilled on ice. 24 µl of buffer containing 24 mM Tris, 476 mM Tris-HCl, 50 mM $MgCl_2$, 1 mg/ml DNaseI and 0.25 mg/ml RNaseA was then, added and incubated again on ice for 15 min. The reaction was stopped by addition of four volumes of ice-cold acetone and precipitation of proteins was allowed for 20 min on ice. The cell debris was removed by centrifugation at 14000×g for 10 min at 4°C (Giard et al., 2001). The supernatant containing protein extracts was stored at -20°C until further analysis.

FastPrep method

Proteins were extracted by using the FP120 FastPrep bead-beater (BIO101/Savent) by alkaline-lysis protocol. In brief, the pellets resuspended in 400 µl rehydration buffer including 8 M Urea, 2 M Thiourea, 0.5% CHAPS, 10 mM DTT and 0.1% immobilized pH gradient (IPG). Cells were lysed by vortexing them with acid washed glass beads with a diameter of 212 to 300 µm (Biospec Products, Inc.) by use of FastPrep FP120 device at 6 m/s for 3×45 s at 4°C, each 3 min in between which the cell suspension was kept on ice for 1 min. The cell debris was removed by centrifugation at 14000×g for 10 min at 4°C (Larsen et al., 2006). The supernatant containing protein extracts was stored at -20°C until further analysis.

Protein determination

Total cellular protein concentrations were determined by use of a commercially colorimetric DC protein assay kit according to the manufacturer protocol (Bio-Rad) with bovine serum albumine (BSA) as a standard and absorbance measurement at 750 nm. The kit is reported not to interfere with any chemicals used throughout extraction protocols and therefore, is compatible with isoelectric focusing (IEF) (Bradford, 1976; Angelika et al., 2004). Samples and standards were replicated three times.

SDS-PAGE

15% acrylamide separating gel and 4% stacking gel were used for SDS-PAGE and prestained protein marker-brand range (Bio-Labs) was used as a molecular weight standard. SDS-PAGE gels were run at 80 V for 15 min and then, at 120 V for 2 h (Laemmli, 1970). At the end of electrophoresis, gels were visualized by staining with Coomassie brilliant blue according to Sambrook et al. (1992) and destained for 2 h in a solution containing 7.5% methanol and 5% glacial acetic acid.

2D-PAGE

2D gel electrophoresis was run to examine the quality of the extracted proteins and was performed according to the method of O'Farrell (1975) with some modifications. Briefly, 75 µg crude protein extracts were solubilized in 450 µl of a rehydration buffer and applied on 24 cm IPG strips with a linear pH range of 4 to 7 gradients (Bio-Rad) for the first dimension. IEF was carried out on a Multiphor II electrophoresis unit (Pharmacia) at 50°C with the following program: 50 V in 30 min, 500 V for 1 h, 1000 V in 1 h and 10 000 V for 7 h (total 70 000 V h). In the first step, the IPGs were equilibrated for 15 min each in SDS equilibration buffer (6 M urea, 50 mM Tris-HCl, 30% Glycerol, 2% SDS and 0.01% bromophenol blue) containing 1% DTT followed by 15 min in an equilibration buffer containing 5% iodoacetamide prior to second dimension PAGE in 12% polyacrylamide gel in 375 mM Tris-HCl, 0.1% SDS, 0.1% APS and 0.031% TEMED. Following electrophoresis, the gels were fixed for two hours in 7% acetic acid / 10% methanol and stained with silver nitrate overnight; followed by destaining with 7% acetic acid / 10% methanol and imaged.

Silver staining of 1D and 2D gels

Protein spots were visualized by staining with silver nitrate as described previously (Lauber et al., 2001) and run in triplicate for each biological replicate to confirm the reproducibility of the protein patterns. Gels were fixed overnight in 40% ethanol and 10% acetic acid, washed two times for 20 min in 30% ethanol followed by 1 min in thiosulphate reagent and incubated 20 min in 0.2% silver nitrate. The gels were rinsed three times for 20 s with milli-Q water and for 10 to 30 s with 3% sodium carbonate, 0.05% p-formaldehyde. Gels were developed by incubation in sodium carbonate solution and the reaction was stopped in 0.5% glycine.

Statistical analysis

Statistical analysis was made using the Statistical Package for the Social Sciences (SPSS) 15.0 software (SPSS Inc, Chicago, IL, USA). Data were analyzed by oneway analysis of variance (ANOVA) followed by Tukey's posthoc test (GraphPad Prism v.3.0, GraphPad Software, San Diego, CA, USA) and shown as mean and standard deviation (S.D.). In all statistical analyses, $p < 0.05$ was taken as the level of significance.

RESULTS AND DISCUSSION

For the one (1D) and two-dimensional (2D) polyacrylamide gel electrophoresis techniques used in the initial experimental process of proteomics, the sample preparation and/or high quality resolution of proteins are the most important point for quantification of proteins to optimize

Table 1. Mean±SD of protein concentrations (µg/µl) of each strain obtained by three different methods.

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

Values are mean ± S.D.(standard deviation) of results of three experiments.

over-expressed as well as under-expressed proteins between strains. It is required under the same experimental conditions, to compare different proteins that were identified from spots showing consistent differences in intensity and/or abundance. Because of the differences between physical and chemical properties of proteins, it is important to select an appropriate standard assay of choice for a particular sample since different extraction protocols may favor proteins from different strains.

Due to the increase in application of LAB in industries, the exploration of the stress resistance mechanisms should let us to discover the fundamentals of the adaptive responses and cross protection and to modernize their application to prepare LAB for industrial processing. Among these strains, *E. faecalis* and *L. lactis* are the LAB most commonly used in dairy fermentation, while *P. pentosaceus* is being used in meat and vegetables fermentation (Hammes et al., 1990). In this study we compared three different protein extraction methods (sonication, centrifugation and FastPrep) for their compatibility with 1D- and 2D-PAGE analysis by use of 3 representative strains of LAB. In proteomics approaches, evaluation, standardization and selection of efficient methods are quite important for proteins analysis since the presence of nonprotein impurities can critically affect the quality of 2D-PAGE separation by formation of artifactual spots and what is reported in this work is the relative efficiency or degree of extraction of the various methods

The concentrations of proteins extracted by three methods are shown in Table 1. The results show that higher amount of proteins were obtained when the cells were lysed with mechanic rupture using glass beads (FastPrep). There were no significant differences between the sonication and centrifugation methods of these three strains ($p > 0.05$) when compared with FastPrep method which was found significantly different when compared with the other two methods ($p < 0.05$). As can be seen in Table 1, although, all the methods had given suitable amount of proteins, six times higher protein concentration, which is important for proteomics, especially before proceeding IEF part was obtained with extraction by FastPrep. While applying the samples for IEF, getting successful result depends on staining strategies as well as different protein concentrations rather than protein amount (Angelika et al., 2004). Extraction of proteins by FastPrep is also described for LAB in many proteomic research reports to obtain total cellular protein extracts from relatively small amounts of biomass (Wang et al., 2005; Dahl et al., 2007; Koskenniemi et al., 2009). There appear to be no reports in proteomics making a comparison of the various protein extraction methods using industrially important strains of LAB. Some studies focus on comparing protocols for the extraction of proteins from a large variety of organisms which are not LAB. For example, in insects, a TCA-acetone extraction method certainly performed well for the purpose of preparing quantitative 2D gel electrophoresis-based separations when compared with the use of phenol- and detergent-based methods and these experiments were able to assess the differences between the two aphid genotypes (Cilia et al., 2009). In plants, protein extraction has been problematic as standard protocols must contend with high concentrations of salt ions in plant tissues. Wang et al. (2007) have developed an improved method for protein extraction from *Salicornia europaea* using borax, polyvinylpolypyrrolidone and phenol and this allowed removal of interfering compounds and salt ions. The comparative study of this method with several other protocols using NaCl-treated *S. europaea* shoots demonstrated that, this method gave the best distinction of proteins on 2-DE gels (Wang et al., 2007). In another study, three different protein extraction methods were compared for proteomic analysis; the sucrose, Tris-HCl and trichloroacetic acid (TCA)/acetone methods were all compared and the sucrose extraction buffer was found to be the most efficient and reliable method for extracting proteins from pine needles (Cai-yun et al., 2005). Sheoran et al. (2009) evaluated four protein extraction methods including TCA/acetone, phenol, direct IEF buffer and Tris-HCl buffer, using tomato pollen for a proteome analysis. Their results showed that the TCA-acetone and phenol protein extraction methods were better than the two tested methods for tomato pollen proteome analysis (Sheoran et al., 2009). For preparation of protein extracts from yeasts, there are many methods of extraction from lysis with glass beads, to boiling in SDS-PAGE buffer or extraction with NaOH and β-mercaptoethanol. Kushnirov has reported that a mild alkali treatment followed by boiling in a standard electrophoresis loading buffer was efficient, easy and reliable for the electrophoretic analysis of different strains of *Saccharomyces cerevisiae* and of the yeast *Hansenula polymorpha* DL-1 (Kushnirov, 2000).

For determination of protein fraction of microbial cells,

the use of many different extraction and analysis methods is known. For *Escherichia coli* six different protein extraction protocols were tested and compared. Comparison was based on the reliability of the methods and it was found that cell lysis using the BugBuster protein extraction reagent (Bugbuster) gave the best results (De Mey et al., 2008).

Methods employing sonication have been used in many studies involving the extraction of proteins from LAB. While there seem to be as many different lysis buffer preparations as their are reports in the literature, a consistent application of sonication has been shown in which samples are generally sonicated three times and the cycle involves 2 min of sonication with 3 min rest intervals with sample kept on ice. In our study we used TrisHCl buffer, pH 7.5, containing 5 mM EDTA and 5 mM $MgCl_2$. In studying the proteome of intestinal probiotic *Lactobacillus reuteri* strain grown under acid stress conditions, Lee and Pi (2010) used a Tris-based lysis buffer (62.5 mM Tris-HCl, pH 6.8) combined with mechanical disruption by ultrasonication, much as we have done in our study. In contrast, others have used different lysis buffers such as denaturing buffers for 2-DE (7 M urea, 2 M thiourea, 4% (w/v) CHAPS and 50 mM DTT) containing complete protease inhibitors (Yuan et al., 2006; Wang et al., 2009). In some studies, the cells were directly sonicated without any use of a special lysis buffer (De Angelis et al. 2001; Di Cagno et al. 2007).

Centrifugation is another technique used during a protein extraction method. Of course before the use of a centrifugation technique, the membrane or cell wall must be broken. The details of the membrane lysis step can be different in each protocol as different chemical means (lysozyme, DTT, etc.) are employed. The details of the centrifugation step also vary, but the treatment with cold acetone on ice as the last step is generally same in many protocols. In our study, two different buffers were used and the final step of the protocol was an ice-cold acetone precipitation for 20 min on ice. Koistinen et al. (2007) and Plumed-Ferrer et al., (2008) used centrifiguation steps in their proteomic research of the *Lactobacillus plantarum* strain; they wash frozen bacterial pellets, treat with lysozyme at 37°C for 45 min, followed by DNase treatment, with a final centrifugation step after which the supernatant is mixed ice-cold acetone to precipitate the proteins. This protocol is quite useful for laboratories that may not be equipped with a sonicator, FastPrep cell disruptor or a pressure cell disrupter.

The use of the FastPrep method requires a special instrument. In our study, cells were lysed with glass beads in a FastPrep cell disruptor. This method has been used in many proteomics studies involving LAB (Wang et al., 2005; Dahl et al., 2007; Koskenniemi et al., 2009). What often varies in the FastPrep method is the type of buffer used, the ratio of the volume (as an amount) of glass beads to the volume of the pelleted cell mass and the disruption time for homogenization. In our study, we used an extraction buffer that is directly used as a rehydration buffer for polyacrylamide strips used IEF (8 M urea, 2 M thiourea, 0.5% CHAPS, 10 mM DTT and 0.1% pH 3-10 ampholytes). Koskenniemi et al. (2009) and McLeod et al. (2010) used only 10 mM Tris-HCl buffer, pH 7.5 and their disruption time was different from ours. If there is no FastPrep cell disrupter in laboratory, a method combining the use of centrifugation with glass beads has been described (Giard et al., 2001) and is more efficient than employing just centrifugation alone.

Although, the extraction method by FastPrep showed a higher efficiency and higher quality in extracting proteins compared with the two other methods to check these recoveries and to clarify the results of protein concentrations, proteins have been applied to SDS-PAGE. It can be clearly seen from the Coomassie brilliant blue (Figure 1) and silver nitrate (data not shown) stained SDS-PAGE gel images, that higher amounts of proteins are obtained only when proteins extracted by FastPrep method were used (lanes 4, 5 and 6 in Figure 1) as compared to the other lines in which proteins extracted by two other methods were loaded (lanes 1, 2 and 3, in which the proteins extracted by centrifugation is loaded while lanes 7, 8 and 9 are the lanes of proteins extracted by sonication). However, the lack of protein bands in Figure 1 does not mean that the other two methods did not work. It means that protein concentration applied to SDS-PAGE is very low because when pooled, supernatants of the samples were concentrated by using speed vacuum centrifuge to a final volume of 10 µl and applied to SDS-PAGE, visible protein bands were obtained (data not shown). Therefore, when the FastPrep method is unavailable and one needs to use either sonication or centrifugation for extracting proteins for proteomics studies, it is recommended to concentrate cells by use of vacuum centrifuge following extraction protocols. Proteins extracted by FastPrep method have also been applied to 2D-PAGE for further confirmation (Figure 2). In this application, only *E. faecalis* V583 and *P. pentosaceus* OZF strains were used. When the proteins of *E. faecalis* V583 and *P. pentosaceous* OZF extracted by FastPrep were separated by 2D-PAGE, 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. The little disturbance of vertical and horizontal streaks as well as regular and reproducible protein spots were seen in the figure (2D gel illustrating the intracellular proteins of *P. pentosaceus* OZF is not given here).

The FastPrep method showed higher protein resolution and spot intensity of all proteins. In addition, proteins with less abundance and high molecular weights were resolved clearly and detected strongly on 2D gel when FastPrep method was used. Besides, differentially expressed proteins were detected by presence of clear and well resolved protein spots on 2DE (unpublished data). This confirmed that, the method with FastPrep extraction was an efficient and reliable method for lysing

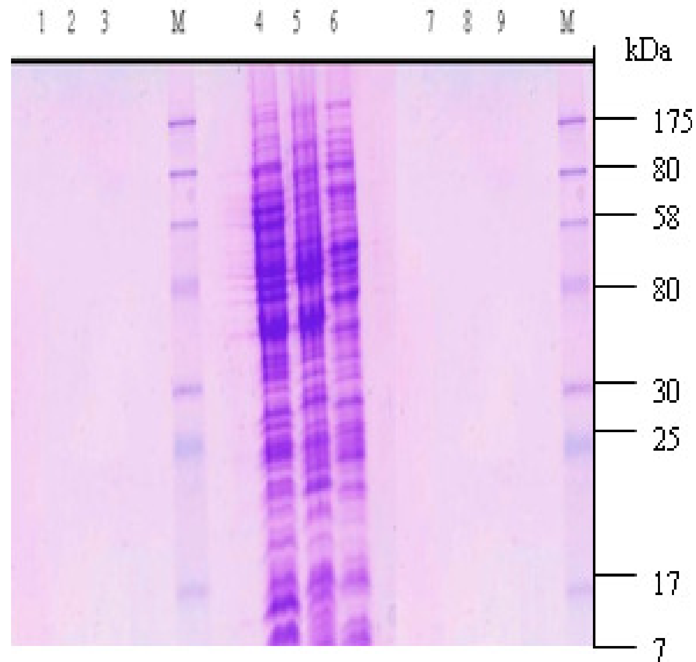

Figure 1. Representative Coomassie brilliant blue stained SDS-PAGE illustrating the intracellular proteins of three representative strains of LAB. The lanes (1 to 9) contains extracts of *P. pentosaceus* OZF (lanes 1, 4, 7); *E. faecalis* V583 (lanes 2, 5, 8) and *L. lactis* NIZO 0900 (lanes 3, 6, 9) 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).

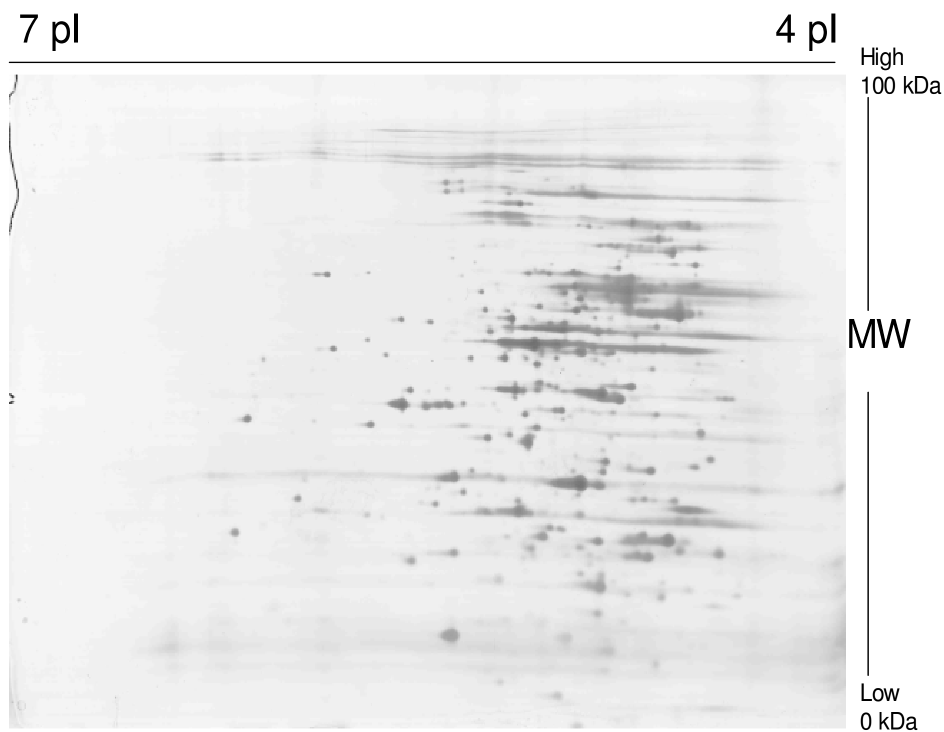

Figure 2. Representative 2D-PAGE illustrating the intracellular proteins of *E. faecalis* V583 at pI 4 to 7 after lysing cells by FastPrep. See higher protein resolution and spot intensity.

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 (Figure 2).

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