Increased Protein Yields from *Escherichia coli* Using PCT Extraction

The bacterium *Escherichia coli* is a widely used host for protein expression. However, the recovery of recombinant proteins hinges largely on the ability to effectively induce cell lysis. Similarly, the understanding of cellular processes is facilitated only when methods such as two-dimensional gel electrophoresis (2DGE) are capable of isolating without bias the entire protein constituency of such model organisms. An accurate representation of any organism's entire proteome can only be derived when all cells are efficiently lysed. This necessarily involves the physical disruption of cell walls or membranes and the chemical means to ensure the solubility of released proteins.

Probe sonicators and bead mills commonly are used for cell lysis. However, both sonication and bead mill oscillation can generate excessive heat, which rapidly accelerates the hydrolysis of urea and the formation of isocyanic acid, resulting in the potential carbamylation of protein amines. In addition, sonicators and bead mills present potential health risks for laboratory workers. Probe sonicators inherently aerosolize potentially pathogenic agents. Moreover, at least two researchers have been infected with West Nile Virus when tubes oscillating in a bead mill ruptured [1].

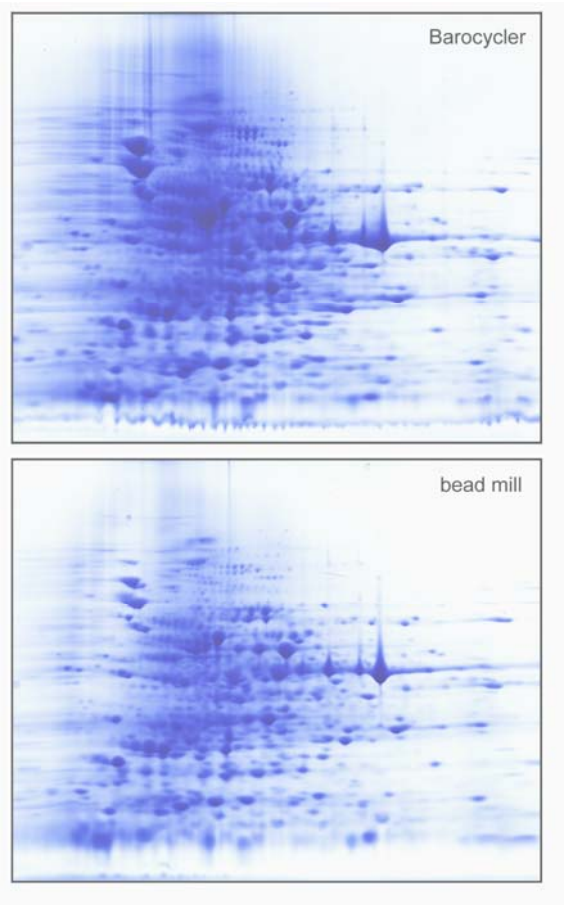

Figure 1. *E. coli lysates produced by PCT (top) or bead mill oscillation (bottom). From image analysis, the total of integrated spot volumes in the PCT lysate was 14.2% higher than the total spot volumes in the bead mill lysate. 801 proteins were revealed in the PCT lysate, compared to 760 proteins in the bead mill lysates.*

Pressure Cycling Technology (PCT)

PCT uses rapid alternating cycles of high and low pressure to induce cell lysis. Cell suspensions or tissues are placed in specially designed processing containers (PULSE Tubes) and are subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressure in a pressure-generating instrument (Barocycler Models NEP2017 or NEP3229). Pressure in the PULSE Tube increases to 35,000 PSI in less than three seconds and returns to ambient pressure in less than one second. Maximum and minimum pressures, the time sustained at each pressure level, and the number of cycles is defined using a computer or programmable logic controller interface. The Barocycler instrument reaction chambers are temperature controlled using a peripheral circulating water bath. Safety features in the design of the PCT Sample Preparation System (PCT SPS) significantly reduce risk of exposing researchers to pathogens [2].

Methods

To illustrate PCT performance, 60 mg of lyophilized *E. coli* K12 was reconstituted in 10 mL of 7M urea, 2M thiourea, and 25 mM C7BzO (3-(4-heptyl) phenyl 3-hydroxypropyl dimethylammonio propanesulfonate) [3]. Twenty-five microliters of 200 mM tributylphosphine were added and 1.5 mL of this cell suspension was transferred to each of two PULSE Tubes.

The PULSE Tubes were subjected to five pressure cycles in the Barocycler NEP2017 instrument. Each cycle consisted of 20 seconds at 35,000 PSI followed by 20 seconds at ambient pressure. The contents of the two PULSE Tubes were combined and centrifuged at 24,000 RCF for 10 minutes to remove cellular debris.

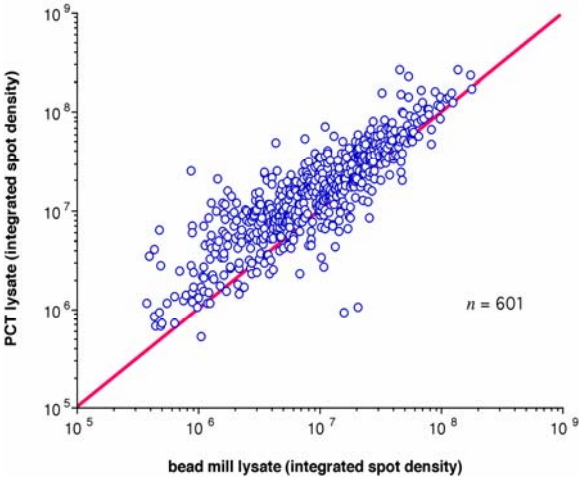

Figure 2. *Logarithmic plot comparing integrated spot density of 601 matched protein spots in two-dimensional gels of PCT (ordinate) and bead mill (abscissa) lysates. The theoretical slope of 1.00 is indicated (solid red line).*

Alternatively, 1.5 mL of the cell suspension was transferred to each of two 2 mL polypropylene tubes. For the bead mill procedure, samples were placed in tubes and subsequently loaded into the adapter rack of a Retsch MM 301 mixer mill with tungsten carbide grinding balls provided by the manufacturer (Retsch GmbH, Haan, Germany). The tubes were cycled three times for one minute at 1,800 oscillations per minute. After each cycle, the temperature of the samples reached 40º C requiring that the tubes be removed from the adapter and placed on ice for several minutes between cycles. (A potentially precarious practice since the solubility of urea, thiourea, and the C7BzO detergent is compromised below 18º C.) The contents of the two tubes were combined and centrifuged at 24,000 RCF for 10 minutes to remove cellular debris. Three milliliters of each lysate were alkylated for two hours following the addition of 10 mM acrylamide and 40 mM Tris.

Proteins were precipitated with 80% acetone at room temperature for 30 minutes. The flocculent was pelleted by centrifugation at 24,000 RCF for 10 minutes. Pellets were dissolved in 3 mL of ion-exchanged 7M urea, 2M thiourea, and 65 mM CHAPS. Dried immobilized gradient strips, pH range 3-10, (Proteome Systems, Woburn, MA, USA) were hydrated with 0.2 mL of each lysate for six hours. 2DGE was performed as described [4]. Image analysis was performed using Progenesis Discovery and Editor (Nonlinear Dynamics, Newcastle Upon Tyne, UK).

Results and Discussion

PCT extracted 14.2% more total protein from *E. coli* than was extracted using a bead mill. Image analysis of the 2D gels in Figure 1 revealed several low abundance proteins in the PCT lysate that were not detected in the bead mill lysate. The graph in Figure 2 provides numerical data to support the visual impression in Figure 1. The preponderance of points in the ordinal quadrant shows the improved efficiency of cell lysis by PCT as compared to bead mill oscillation since the integrated spot volumes measured in 2D gels deviate from the theoretical slope of 1.00. A slope of 1.00 is expected only if the methods are equivalent.

Acknowledgements

PBI gratefully acknowledges the contributions of Alexander Lazarev of Proteome Systems, Inc. and Sunny Tam and Douglas Hinerfeld of UMMS Proteomics Consortium to this Application Note.

References

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