The Proteome of *Rhodopseudomonas palustris* Revealed by Pressure Cycling Technology (PCT) and Two-Dimensional Gel Electrophoresis

Rhodopseudomonas palustris is a Gram negative, purple, non-sulfur, phototropic bacterium, and is a metabolically versatile microbe. The bacterium can grow in the presence or absence of oxygen. In response to environmental changes, it can engage in alternative metabolic processes for cellular respiration. *R. palustris* can degrade the aromatic compounds comprising lignin, the second most abundant natural polymer. As such, it is being investigated for its potential in the removal of environmental pollutants [1]. The genome of *R. palustris* has been sequenced and annotated [2]. It follows that the analysis of this microorganism's proteome has become an active area of research. Reliable proteomic analysis is contingent on the efficiency by which cells are lysed and their protein constituents released. Standard technique to efficiently lyse Gram-negative bacteria requires mechanical disruption of the cell, and either enzymatic or chemical breakdown of the cell wall.

In these studies, pressure cycling technology (PCT) was compared to a conventional sonication technique for the lysis of *R. palustris* cells. PCT was also used to compare the enzymatic hydrolysis of cell wall glycans using recombinant lysozyme and chaotropic detergent isolates to reveal protein populations by two-dimensional electrophoresis (2DGE) [3].

Pressure Cycling Technology (PCT)

PCT uses rapidly alternating cycles of high (up to 35,000 PSI) 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 and ambient pressure in a pressure-generating instrument (Barocycler™ Models NEP2017 or NEP3229). Together, the Barocycler and PULSE Tubes comprise the PCT Sample Preparation System (PCT SPS). Pressure in the PULSE Tube can increase to 35,000 PSI in less than three seconds and return 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 regulated 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 SPS significantly reduce risk of exposure to pathogens or cross contamination [4].

Methods

Cells were pelleted by centrifugation at 12,000 RCF for five minutes and suspended at a density of 0.35 g cells/mL in distilled H_2O. For each analysis, 0.57 mL of this suspension (~200 mg cells) was again pelleted by centrifugation. One sample was suspended in 1.5 mL of 7 M urea, 2 M thiourea, and 25 mM 3-(4-heptyl) phenyl 3-hydroxypropyl-dimethylammoniopropane sulfonate (C7BzO) [5]. Another sample was mixed with 0.75 mL of 2X BugBuster® Plus reagent containing recombinant lysozyme (EMD Biosciences, Madison, WI) and the volume was adjusted to 1.5 mL with dH_2O. To lyse the cells, 1.5 mL of each sample was placed in a ULSE Tube and subjected to 30 pressure cycles in a Barocycler NEP-2017. Each cycle consisted of 20 seconds at 35,000 PSI and 20 seconds at ambient pressure. The resulting lysate was immediately centrifuged at 12,000 RCF to pellet cellular debris. Each supernatant (0.1 mL) was mixed with 0.4 mL of 7M urea, 2M thiourea, 65 mM CHAPS, 40 mM Tris, 5 mM tributylphosphine, and 10 mM acrylamide and was incubated for two hours. The reaction was

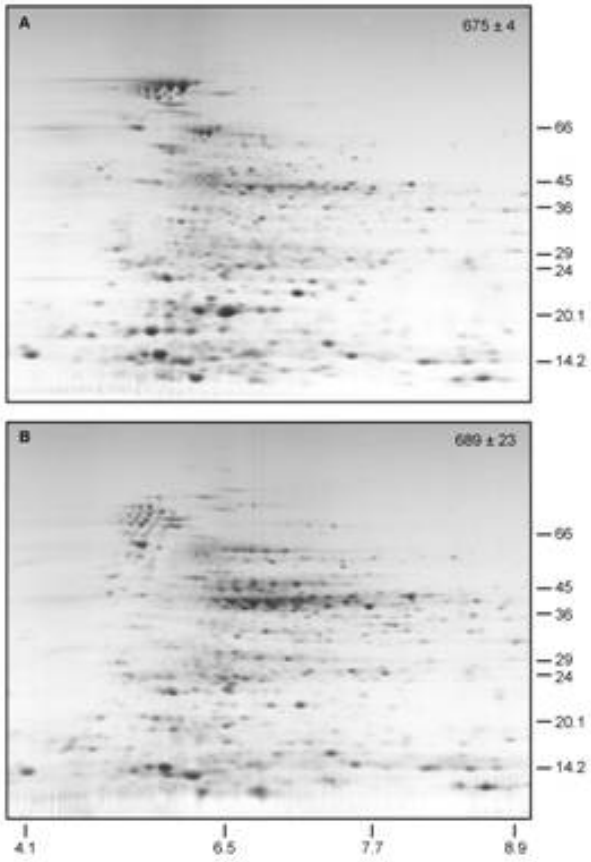

Figure 1. *2DGE showing differential protein solubilization from R. palustris lysed using the PCT SPS with either (A) the C7BzO reagent or (B) recombinant lysozyme.*

terminated by ultrafiltration in an Ultrafree 0.5 mL centrifugal filtration device (Millipore, Danvers, MA, USA) and exchanged to a solution containing 7 M urea, 2 M thiourea, and 65 mM CHAPS. A duplicate set of samples was treated by sonication for six (6) repetitions, each for 30 seconds. The samples were placed on ice for one (1) minute after every two sonication intervals to cool the sample. Dried, immobilized pH gradient strips (IPG) were hydrated with 0.2 mL of each lysate for six hours. Isoelectric Focusing (IEF) and 2DGE was performed [6]. Gels were stained with ProteomIQ® Blue (Proteome Systems, Woburn, MA, USA). Image analysis was performed using Progenesis Discovery® and Editor (Nonlinear Dynamics, Newcastle Upon Tyne, UK).

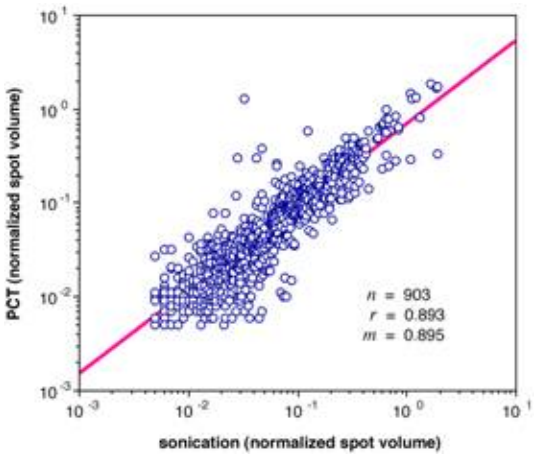

Figure 2. *Plot of the integrated densities of 903 matched protein spots from two-dimensional gels comparing PCT (ordinate) and sonication (abscissa) for the lysis of R. palustris. The C7BZO reagent was used for both samples.*

Results and Discussion

Based on the Bradford assay, PCT yielded 5.3% more total protein from *R. palustris* than probe sonication when using the C7BzO reagent for lysis. PCT yielded 1.8% more protein than sonication when lysozyme was used. Although the differences in yield are not vastly different, we propose that PCT SPS is a more reproducible method than sonication. Sonication is known to produce aerosols and to cause foaming of detergent solutions. However, with the PCT SPS, samples are fully contained in PULSE Tubes, and no severe aerosols are produced, and foaming is minimal.

Using PCT in combination with the chaotrope C7BzO reagent yielded 17.1% more protein than recombinant lysozyme and sonication. The 2DGE (Figure 1) compares extracts derived either by C7BzO or lysozyme reagent, and shows the differential solubilization of proteins. We hypothesize that more cells are lysed with the BugBuster reagent, which weakens the cell wall prior to PCT, but that more proteins are solubilized when the C7BzO reagent is used. It would be advantageous to use the enzymatic approaches when downstream affinity purification is the goal, since this method uses physiological buffers that help preserve biological activity. When 2DGE is the chosen method of analysis, stringent chaotropic detergent solutions should be used in combination with PCT, since hydrophobic proteins will remain soluble for IEF analysis. Additionally, PCT provides a convenient means for buffer optimization, as multiple samples can be prepared simultaneously using different buffers.

Figure 2 graphically illustrates the integrated densities of over 900 matched protein spots from two-dimensional gels comparing PCT to sonication lysates. The slightly elevated Y intercept (Y_0) where $Y_0 > X_0$ suggests that PCT releases more detectable low abundance proteins.

Acknowledgements

PBI gratefully acknowledges the contributions of Oak Ridge National Laboratories, Myra Robinson and Alexander Lazarev of Proteome Systems, and Elena Chernokalskaya of Millipore Corporation to this Application Note.

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