

Improved Extraction of *Rhizoctonia* and *Pythium* DNA from Wheat Roots and Soil Samples Using Pressure Cycling Technology

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Abstract:

Soilborne pathogens are important biotic factors in yield reduction in the dryland cereal production region of the Pacific Northwest. *Rhizoctonia solani* AG-8, *Rhizoctonia oryzae*, and *Pythium* spp. are causal agents of root rot, bare patch, and damping-off of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). Although these pathogens can be rapidly and specifically quantified using quantitative real-time PCR, the extraction of *Rhizoctonia* DNA from agricultural samples is often inconsistent, especially at low pathogen population densities. Using a novel extraction system that uses pressure cycling technology (PCT), we improved the extraction of *R. solani* AG-8 DNA up to 16-fold and of *P. abscissum* DNA up to 2-fold from three types of agricultural soils compared with a bead beating extraction method. PCT also yielded quantifiable amounts of *R. solani* AG-8 and *R. oryzae* DNA from lyophilized wheat roots that were otherwise recalcitrant to homogenization. Furthermore, the extractions were so consistent that pathogen quantification generally could be derived from two rather than three or four replicated extracts. Because PCT is performed in a closed system and minimizes sample shearing and heating, it confers a substantial advantage over conventional extraction systems. Here, we report for the first time the application of PCT in a laboratory setting for the improved extraction and quantification of three types of soilborne pathogens in soil samples. The effectiveness of PCT for three soils suggests that it will be beneficial for other hard-to-extract pathogen samples.

CALENDAR OF EVENTS

4TH ANNUAL U.S. HUPO
BETHESDA, MD
MARCH 16-19, 2008

SBS 14TH ANNUAL CONFERENCE & EXHIBITION
ST. LOUIS, MO
APRIL 6-10, 2008

Proteomic Profile of Differentially Regulated Proteins in Human Myocardium Before and After Cardiac Surgery Utilizing Cardioplegia and Cardiopulmonary Bypass

Poster Presented by Dr. Richard T. Clements of
*Cardiothoracic Research Lab, Department of Surgery, Beth
Israel Deaconess Medical Center and Harvard Medical
School, Boston, MA at the American Heart Association
Scientific Sessions 2007, Orlando, FL, November 4-7, 2007*

Although highly protective, cardiac surgery utilizing cardioplegia and cardiopulmonary bypass (CP/CPB) subjects myocardium to hypothermic reversible ischemic injury that can impair cardiac function. Pronounced contractile deficits in a subset of patients can result in a greatly enhanced risk of mortality. Changes in myocardial contractile activity are likely regulated via acute protein modifications. We performed the following study to determine changes in the protein profile of human myocardium following CP/CPB.

Proteins extracted using Pressure Cycling Technology (PCT)

Pre – CP/CPB

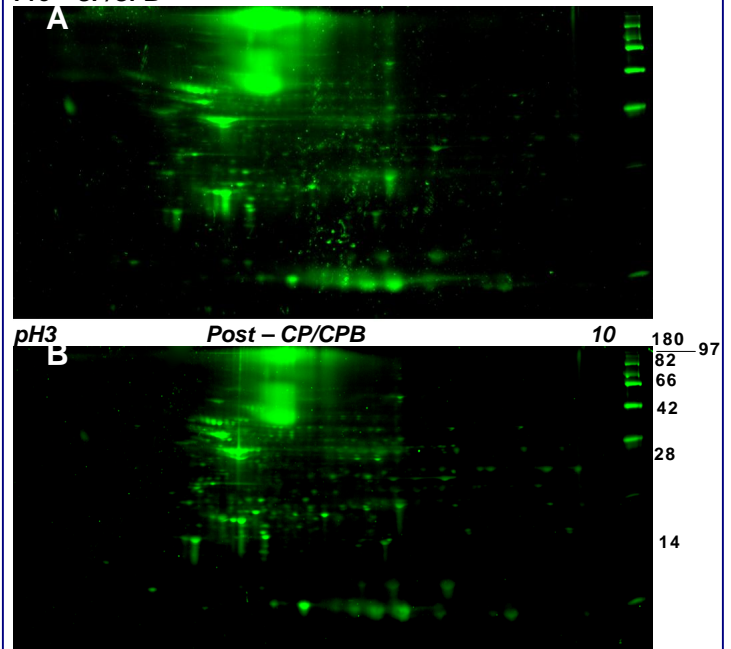


Figure 1 – Two-dimensional electrophoresis of human atrial samples before and after cardiac surgery utilizing cardioplegia and cardiopulmonary bypass. Atrial tissue lysates (100ug) were subjected to 2-D electrophoresis (8-20% SDS-PAGE, 10 -3 pH gradient) and general protein staining with SYPRO ruby. Four patient samples pre- (A) and post-CP/CPB (B) were analyzed. Representative blots from one patient are shown.

Pressure BioSciences, Inc.

Uses PCT to Study *Fagus grandifolia*
Tree Bark Proteins

Poster: Pressure Cycling Technology (PCT)
Mediated Sample Preparation Schemes
Enabling High Quality Two-Dimensional Gel
Electrophoresis of *Fagus Grandifolia* Tree
Bark Proteins
Presented at the

Plant and Animal Genome XVI Conference
San Diego, CA, January 12-16, 2008.

Poster No. P888

ABSTRACT:

Pressure Cycling Technology (PCT) was used to extract proteins from *Fagus grandifolia* tree bark samples. PCT in combination with newly developed extraction reagents yielded 5-10 times more protein than homogenization with conventional buffers. However, tannins from the tree bark interfered with two-dimensional gel electrophoresis (2DGE) of proteins. The inclusion of polyvinylpyrrolidone (PVP) in the extraction media resulted in the removal of tannins, but with concomitant loss of proteins. A scheme using ultrafiltration to concentrate the protein samples, followed by the use of polyvinylpolypyrrolidone (PVPP) spin columns to remove interfering polyphenols, enabled high quality 2DGE. Alternatively, high molecular weight tannins were effectively removed by ultrafiltration using 100,000 Da nominal molecular weight limit (NMWL) membranes where proteins were partitioned to the filtrate rather than the retentate. Likewise, such filtrates produced high quality 2D gels with minimal tannin interference.

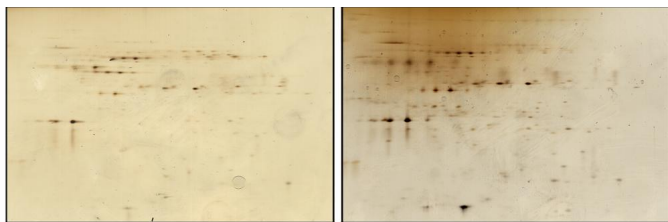


Figure 1. 2D gels comparing proteins isolated from *F. grandifolia* phloem by homogenization in standard buffer (left) and by PCT using the ProteoSOLVE CE Lysis Reagent (right). The homogenized sample was boiled for 10 minutes in 100 mM Tris, 5% SDS, 1% BME. The PCT sample was not boiled. Protein yields from duplicate phloem samples were 1.13 ± 0.10 mg/mL by PCT compared to 0.24 ± 0.02 mg/mL by homogenization. Gel loads were normalized to initial tissue mass. IPGs were pH 3-10.

Application Focus

Increased Protein Yields from Murine and Rat
Liver Tissues Using PCT

Sequencing of the human genome revealed approximately 30,000 genes. However, the number of genes is significantly lower than the prior estimate of 80,000-140,000. The actual number of genes, is too small to account for the complexity of humans. The explanation for Human complexity is in the dynamic nature of proteins that make up the proteome. This is attributable to the alternate splicing of RNA and post-transcriptional modifications of proteins, like glycosylation or phosphorylation. Researchers are now focusing their efforts on developing a better understanding of the human proteome. It is expected that the elucidation of the proteome will lead to better diagnosis and treatment of disease through the identification of clinically important biomarkers. The quality of proteins extracted during initial sample preparation is critical to the success of downstream separation techniques and detection technologies. Here, we present a comparison of Pressure Cycling Technology (PCT) to conventional physical methods (sonication, homogenization, and pulverization) for the extraction of proteins from murine liver tissue. In addition, to illustrate the reproducibility of the PCT method and to demonstrate efficient processing of extremely small amounts of tissue, submilligram quantities of rat liver tissue were processed. Following extraction, total protein concentrations were determined by quantitative protein assays, and samples were analyzed by one- and two-dimensional gel electrophoresis (1DGE and 2DGE, respectively).

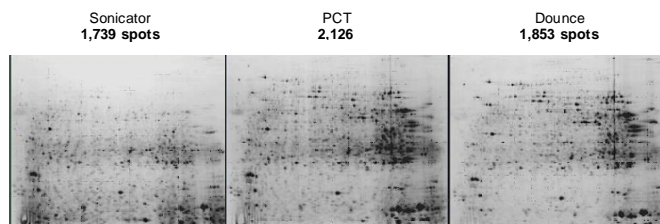


Figure 1. 2DGE of mouse liver lysates illustrating the loss of higher MW proteins that occurs with sonication. Overall protein spot detection was significantly higher in PCT processed samples. PCT was performed for 10 pressure cycles; each cycle consisted of 20 seconds at 35,000 PSI followed by 20 seconds at ambient pressure. IPGs were pH 4.5-6.5.

Results

As shown in Figure 1, high molecular weight proteins were significantly diminished in lysates produced by probe sonication. This was commensurate with an increase in low molecular weight proteins in the sonication lysates. We hypothesize either that the gradual deterioration of sonicator probes and the release of metal ions into the sample could facilitate reactivation of proteolytic enzymes or that the oxidation of proteins causes degradation of the high molecular weight proteins. While there does not appear to be a similar detectable loss of high molecular proteins derived from the ground glass dounce method, fewer total spots were detected compared to PCT-extracted samples. PCT released more protein from mouse liver, including unique proteins, which were not detectable in sonication or dounce homogenates.