

## Pressure BioSciences, Inc. Announces Initial Research Coverage by Zacks Investment Research and TriPoint Global Research

### Pressure BioSciences, Inc. Announces Initial Equity Research Coverage by Zacks Investment Research

SOUTH EASTON, Mass., Aug. 18, 2010 (GLOBE NEWSWIRE) -- Pressure BioSciences, Inc. (Nasdaq:[PBIO - News](#)) ("PBI" or the "Company") today announced that Zacks Investment Research ("Zacks") has initiated coverage on the Company, effective August 16, 2010. Zacks is a leading provider of independent equity research.

Copies of this research report can be obtained by contacting Mr. Grant Zeng, CFA at Zacks Equity Research, 111 North Canal Street, Chicago, IL, 60606 by telephone (312-630-9880 x9466) or email ([gzeng@zacks.com](mailto:gzeng@zacks.com)), or by contacting Zacks directly through their website ([www.zacks.com](http://www.zacks.com)).

Any opinions, judgments, estimates, or forecasts regarding the Company's historical or predicted performance or operations made by Zacks are theirs alone and do not represent opinions, judgments, estimates, or forecasts of the Company or its management. The Company does not by its reference to the research prepared by Zacks imply its endorsement or adoption of or concurrence with such information, conclusions, or recommendations.

#### About Zacks:

Founded in 1978 in Chicago, Zacks Investment Research, Inc. is one of the largest independent investment research and consulting firms in the United States. With 30 years of experience, dedicated to providing clients with the highest standards of services, Zacks has become an industry leader in providing institutional and individual investors with the analytical tools and financial information necessary to the success of their investment process.

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### Pressure Cycling Technology Applications

#### HUPO 9<sup>th</sup> Annual World Congress

September 19-23, 2010

Sydney, Australia

#### [A Proteomics Jurassic Park: The isolation of proteins from microorganisms encapsulated in amber from the Oligo-Miocene epoch 30-40 million years ago](#)

Gary B. Smejkal<sup>1,3</sup>, George O. Poinar Jr.<sup>2</sup>, Feixia Chu<sup>3</sup> and Pier Giorgio Righetti<sup>4</sup>

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#### 1. INTRODUCTION

In 1994, Woodward et al. [1] reported the isolation of DNA fragments from a Late Cretaceous dinosaur bone exhumed from bituminous strata. The following year, the cloning and sequencing of six putative dinosaur DNA fragments derived from a Cretaceous dinosaur egg fossil found in China was later dismissed as the recovered sequences were found to be more closely related to fungi rather than to reptiles or birds [2]. More recently, Asara et al. [3] identified peptides with sequence homology to avian collagen from the mineralized skeletal elements of Tyrannosaurus rex. Kaye et al. [4] challenged the finding showing that microbial biofilms formed "endocasts" in which three-dimensional structure was preserved with microscopic detail, but in which the original organic material has been totally replaced by minerals.

The most promising circumstance enabling the preservation of biomolecules over millions of years comes from amber, the fossilized resin of leguminous trees. The unfossilized resins are comprised largely of terpenoids, labdanoids, and phenolics which rapidly dehydrate the included specimen, a prerequisite for preservation, as well as possess anti-bacterial, anti-fungal, and anti-inflammatory properties that intervene with usual decomposition. When the specimen was completely engulfed in the resinous flow, having to occur within seconds, it resulted in unprecedented preservation later observed in amber fossils.

Michael Crichton's novel Jurassic Park proposed the recovery of dinosaur DNA from the alimentary tracts of hematophagous insects preserved for millions of years in amber. Though Crichton's work was purely fictional, amber inclusions have shown remarkable preservation of organisms at the tissue and cellular levels, and reptilian blood cells have been identified in partially digested blood meals from parasitic insects encapsulated in Cretaceous amber [5]. Transmission electron microscopy has revealed that subcellular components such as nuclei, endoplasmic reticulum, ribosomes, and mitochondria were still intact in 40 million year old insects preserved in amber [6].

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### CALENDAR OF PBI EVENTS

<u><a href="#">THE 21ST INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION</a></u>	<u><a href="#">ASCB- AMERICAN SOCIETY FOR CELL BIOLOGY 2010</a></u>
OCTOBER 11-14, 2010	DECEMBER 11-15, 2010
SAN ANTONIO, TEXAS	PHILADELPHIA, PA

**A Proteomics Jurassic Park: The isolation of proteins from microorganisms encapsulated in amber from the Oligo-Miocene epoch 30-40 million years ago: Continued from Page 1**

This report describes the isolation of high molecular mass protein aggregates from Oligo-Miocene amber from the Dominican Republic. LC-MS/MS identified several proteins with sequence homology to *Saccharomyces cerevisiae* proteins. While mass spectra does not confirm the source of these peptides, the high degree of protein crosslinking suggests that they are not of contemporary origin.

## 2. Methods

### 2.1 Sample Preparation

All procedures were performed under sterile conditions in a laminar flow hood. Amber pieces containing *Hymenaea protera* leaves were first scrubbed in 5% SDS with a dental brush, then heated to 90°C in 2% SDS and copiously rinsed in water. The amber was rinsed with 100% ethanol just prior to fracture. **The amber was fractured and the fragments were ground to a fine triturate using in a sterile Shredder PULSE Tube with serrated metal ram insert (Pressure BioSciences, South Easton, MA). Triturates were** extracted in 125 mM Tris-HCl pH 6.8 containing 2% SDS, 5 mM tributylphosphine, 20 mM AEBSF, 10 mM EDTA and 25 mM phenylacetylthiazolium bromide for 100 X 100 seconds at **35,000 psi maximum pressure in a Barocycler NEP 3229 (Pressure BioSciences, South Easton, MA)**. Samples were filtered in a Ultrafree CL centrifugal filter (Millipore Corporation, Danvers, MA) the filtrates were applied directly to 8-16% polyacrylamide gradient gels (BioRad, Hercules, CA). Gels were stained using the mass spectrophotometry-compatible SilverQuest Silver Stain Kit (Invitrogen, Carlsbad, CA).

## 3. RESULTS AND DISCUSSION

From mass spectra of trypsin digests, 86 peptides with sequence homology to 20 *Saccharomyces cerevisiae* proteins were identified in amber isolates (Table 1). The yeast is likely to be associated with plants and insects embedded in the amber. Experimental procedures for protein extraction, gel electrophoresis and mass spectrometric analysis were tightly controlled with blank samples. The fact that *S. cerevisiae* proteins were identified only in amber samples eliminates the possibility of contemporary contamination. More importantly, all these proteins were identified from a band at the interface of stacking and resolving gel, suggesting an extreme high degree of cross-linking for these proteins during amber formation. Mass spectrometric analysis of another gel band of amber sample did not lead to the identification of any protein. Exclusion of these proteins from 4% polyacrylamide gels indicated molecular masses of several million Daltons, and failure of the aggregates to penetrate these gels proved to be an effective means for concentrating trace proteins from paleontological samples while concomitantly removing interfering substances such as SDS prior to trypsin digestion and LC-MS/MS.

Identifying multiple proteins of *S. cerevisiae* origin was initially surprising. Therefore, peptide sequences of top five proteins were further interrogated to verify the species assignment. For example, enolase 1 (Figure 2) sequences from *S. cerevisiae* and the rubber tree *Hevea brasiliensis* were compared. Although seven of the eleven identified peptides hit conserved regions with high sequence similarity, none of the peptides had identical sequence between these two proteins. Blast NCBI protein database on the other four peptides in divergent regions only returned the saprophyte protein.

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**Pressure BioSciences, Inc. Announces Initial Equity Research Coverage by TriPoint Global Research: Continued from Page 1**

SOUTH EASTON, Mass., Sept. 22, 2010 (GLOBE NEWSWIRE) -- Pressure BioSciences, Inc. (Nasdaq:[PBI](#) - [News](#)) ("PBI" or the "Company") today announced that TriPoint Global Research ("TriPoint") has initiated coverage on the Company, effective September 21, 2010. TriPoint provides independent investment research and innovative, objective analysis for publicly traded companies.

The 21-page initial research report, written by TriPoint Senior Analyst Denise Resnik, MS., can be obtained directly from TriPoint through their website ([www.tripointglobalresearch.com](http://www.tripointglobalresearch.com)).

Any opinions, judgments, estimates, or forecasts regarding the Company's historical or predicted performance or operations made by TriPoint are theirs alone and do not represent opinions, judgments, estimates, or forecasts of the Company or its management. The Company does not by its reference to the research prepared by TriPoint imply its endorsement or adoption of or concurrence with such information, conclusions, or recommendations.

### About TriPoint Global Research:

TriPoint Global Research was established to meet the growing need for independent research and innovative, objective analysis in a rapidly changing marketplace. TriPoint Global Research strives to bring a consultative/strategic approach to equity research, using comprehensive industry expertise and extensive relationships to analyze the core components of a company.

**Dr. Nate Lawrence  
(V.P. of Marketing for PBI)**

**Presented a Talk at  
The Second Annual Current and Future  
Advances in Human Identification  
Conference**

September 19-21, 2010 Hampton, VA

**[Pressure Cycling Technology \(PCT\):  
Potential Applications in Forensic Science](#)**

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## Pressure Cycling Technology Applications

HUPO 9<sup>th</sup> Annual World Congress  
September 19-23, 2010  
Sydney, Australia  
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### A Comparison of Sample Preparation Methods Based on Enzymatic Processes for Efficient Proteomic Analysis

Choi H 1, Lee S 1, Kwon K 1, Yoo J 1, Kim J 1, Ji K2, 1 Korea Basic Science Institute, O-chang Campus, Chungcheongbukdo, Korea, 2 CM Corporation Ltd., Seoul, Korea

#### Background

In proteomic workflows, trypsin digestion is critical and one of the most time consuming steps in the analytical process. Traditional proteomic sample preparation methods typically utilize urea for protein denaturation followed by overnight trypsin digestion. *In order to look for more effective method for proteome analysis, we demonstrated the high pressure cycling technology (PCT) during enzyme digestion and compared to the conventional method.* We have examined the effectiveness of pressurization and use of urea in digestion at low and high temperature condition [Sic].

#### Methods

In this study, the PCT sample preparation system was used to accelerate enzymatic reactions, such as protein digestion with proteolytic enzymes, to prepare samples for analysis by mass spectrometry. The standard protein mixture or protein extracts from human mesenchymal stem cells were digested with trypsin using PCT system at 25, 37 and 50°C for 1hr. The resulted peptides were analyzed by NanoLC/ESI-MS/MS with LTQ/FT mass spectrometer.

#### Results and Conclusion

From the standard protein mixture analysis, even though rapid digestion for 1hr, *PCT method with urea at 50°C showed the best results in number of peptides identified and sequence coverage.* Considering carbamylated peptide by heat and urea, however, we selected the PCT method with urea at 25° C as the optimized method for rapid enzyme digestion. The optimized method was applied for stem cell proteome analysis. 262 and 237 proteins were identified by the PCT method with urea at 25° C for 1hr and the conventional method without urea at 37° C for 16hr, respectively. In comparisons of two methods, there were relatively the large numbers of miss cleaved peptides in PCT method. *It is considered the high pressure cycling technology helps to denature proteins. Then, it would provide the enzyme better access for digestion.*

**Abstract Only Available at this Time**

**Mr. Richard T. Schumacher**  
**(PBI CEO and President)**

**Presented a Talk at**  
**BioPharm America 2010**  
**September 16, 2010 Boston, MA**

**Pressure Cycling Technology (PCT):**  
**A Novel, Enabling Platform**  
**Revolutionizing**  
**Biomarker Discovery**

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*PCT MicroTubes and MicroCaps  
Are Now Available in Convenient,  
Easy-to-Use 96 Well Racks  
and Other Formats*



- 96 MicroTubes or MicroCaps in Bulk
- 96 MicroTubes or MicroCaps in Packets of 8 Each (Original)
- 96 MicroTubes in a Rack (No Caps)
- 96 MicroCaps of 50, 100, or 150 uL in a Rack (No Tubes)
- 96 MicroTubes with 50 uL MicroCaps in a Rack (Pre-capped)
- 96 MicroTubes with 100 uL MicroCaps in a Rack (Pre-capped)
- 96 MicroTubes with 150 uL MicroCaps in a Rack (Pre-capped)

For More Information or to  
Request a PBI Specialist to Contact You  
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