Ultra-Rapid Pressure Digestion and Label-Free Quantitative Proteomics of *Yersiniae* Infected Mice Tissues

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Overview

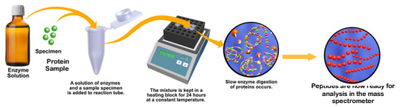
- PCT assisted rapid digestion was demonstrated on 7 standard proteins using a pressure of 20,000 psi for 1, 2, and 5 min at room temperature, 1 h at 37 °C and 50 °C, and overnight at 37 °C. Results showed similar digestion coverage across all conditions.
- A 20,000 psi PCT trypsin digestion on 10 biological replicate lung and spleen tissue samples infected with either sham (control), *Y. pestoides F*, *Y. pestis CO92*, or *Y. pseudotuberculosis* produced reproducible digestions that when analyzed via LC-MS on an LTQ-Orbitrap, identified 107 host proteins that were significantly and specifically changed in abundance due to the specific infection.

Introduction

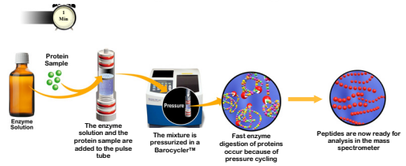
- Traditional proteomic sample preparation methods typically utilize urea and heat for sample protein denaturation followed by sample dilution and 5-12 h trypsin digestion.
- The application of Pressure Cycling Technology (PCT) to denature and enhance trypsin digestion kinetics for proteins has been shown to yield equivalent peptide coverage compared to traditional sample preparation methods.[1]
- Here we demonstrate the application and reproducibility of ultra-rapid PCT digestion on standard protein samples and a full label-free quantitative proteome evaluation of mice lung and spleen tissues after infection by three different *Yersinia*e strains.

Methods

Traditional Protein Digestion System



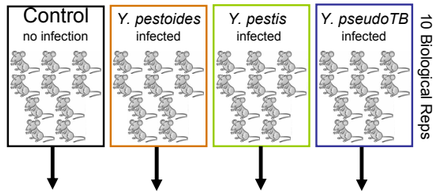
Pressure Cycling Protein Digestion



Albumin
α-Casein
β-Casein
Cytochrome *c*
Lactalbumin
Myoglobin
Ovalbumin

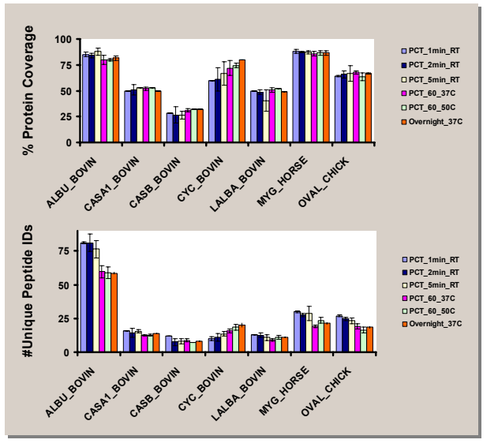
→ µLC-MS/MS

150 µL PCT MicroTubes
3 replicates at 20,000 psi



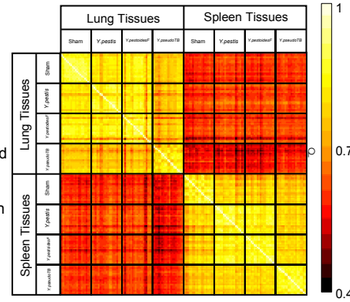
Results

Standard Protein Digestions: Traditional vs. PCT



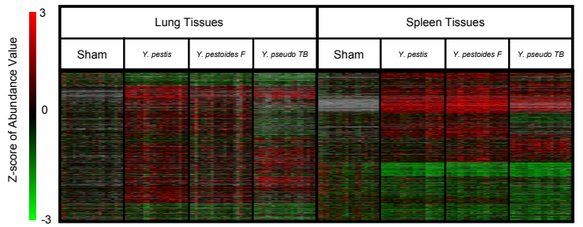
Pearson's Pairwise Correlation of LC-MS runs

Correlated at the protein level (using the most present and abundant peptide across all runs to represent the protein abundance.



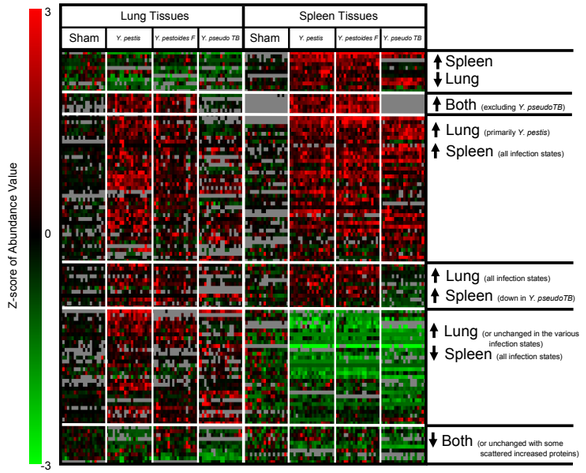
	Lung	Spleen
Avg. Biological Replicate Correlation	0.85	0.86
Avg. Instrument Replicate Correlation	0.89	0.91

Heat Map of Z-score of Abundance Values Relative to the Sham Values



1257 mouse proteins identified that passed peak matching filters of ± 2.5 ppm for the mass and ± 1% for the normalized LC elution time.

Heat Map of 107 Most Significantly Changed Proteins



107 mouse proteins identified in heat map above were deemed significant provided at least 3 peptides per protein were identified, the values passed an ANOVA test with a p-value cutoff of 0.01 (for lung and spleen datasets), and at least one infection group (in lung or spleen) showed an average of at least a 2-fold change in abundance value.

Conclusions

- Ultra-rapid PCT digestion at 20,000 psi for 1, 2, and 5 min at room temperature, 60 min at 37 °C and 50 °C and an overnight trypsin digestion on a mixture of 7 standard proteins (bovine serum albumin, α-casein, β-casein, cytochrome c, lactalbumin, myoglobin, and ovalbumin) showed high reproducibility among triplicate samples with no significant variation between experiments.
- A 1-min ultra-rapid PCT digestion at 20,000 psi was successfully applied to a complex proteome of lungs and spleens of 10 biological replicates of mice infected with three different strains of *Yersinia*e.
- Use of the ultra-rapid PCT digestion allowed label-free quantitation between complex mammalian tissue samples.
- All LC-MS analyses between the "like" biological replicates and technical replicates of the same sample showed a high level of reproducibility.
- Using the ultra-rapid pressure assisted digestion,107 significantly changed proteins related to the host reaction to the infection of the three different strains of *Yersinia*e were identified using a digestion time of only 1 min without the use of chaotropes or heat.

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References

1. D López-Ferrer, K Petritis, K Hixson, T Heibeck, R Moore, M Belov, D Camp II, and RD Smith. Application of Pressurized Solvents for Ultra Fast Trypsin Hydrolysis in Proteomics: Proteomics on the Fly. *J Proteome Res* 2008, 7:3276-3281.

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