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Dynamic Quantitative Characterization of *Cyanothece* sp. ATCC 51142

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Overview

- The cell cycling aspects of *Cyanothece* represent a unique model system for investigating overall characterization and application of quantitative proteomic methodologies.
- Relative abundance quantitative studies clearly revealed distinct cyclic protein abundance patterns, and initial dynamic quantitative studies have shown great potential for utilization in protein turnover characterization.

Introduction

- The strain *Cyanothece* sp. ATCC 51142 was isolated from benthic waters off the Gulf Coast and is a unicellular strain approximately ~4 µm in diameter along the major axis.
- This organism fixes nitrogen at very high rates and has developed a type of temporal regulation in which N_2 fixation and photosynthesis occur at different times throughout a diurnal cycle that includes very high levels of CO_2 fixation and storage.
- These aspects of carbon and nitrogen storage make *Cyanothece* almost unique among well-studied cyanobacteria and make it a key candidate for broad proteomic characterization.
- Of particular interest is to utilize an approach to provide dynamic quantitative information based upon protein turnover measurements.

Methods

- *Cyanothece* cells were grown under 12 h dark/12 h light conditions at 30°C with ambient air bubbling. Timepoint samples were collected every 2 h for 48 h.
- Membrane and cytosolic proteins of each timepoint were isolated and tryptically digested, after which timepoint samples were subjected to capillary liquid chromatography (LC) combined with tandem MS (MS/MS) analysis to initially identify peptides and create an accurate mass and time (AMT) tag database.[1]
- Fragmentation spectra were identified and filtered and the peptide identifications formed the AMT tag database. [2]
- Quantitative information was initially derived from the LC-MS/MS analysis using a spectral count approach to determine protein cycling events.
- Dynamic quantitation experiments were performed using a time-course pulse metabolic labeling strategy that utilizes incorporation of stable isotope labeled amino acids to identify new protein synthesis. For these initial experiments *Cyanothece* cell cultures were grown in constant light and + nitrate to measure initial incorporation of the heavy label.
- The *Cyanothece* genome has just recently been sequenced at the Washington University Genome Sequencing Center (St. Louis, MO), and the finished assembly independently confirmed using an optical restriction map generated by OpGen, Inc. (Madison, WI).

Results

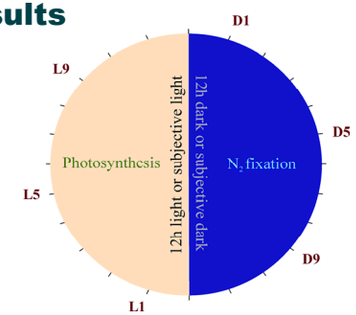

Figure 1. Initial study design was based upon a 48 h double diurnal cycle (12 h dark/ 12 h light), with a total of 24 time points taken (one every 2 h) for protein isolation, digestion, and LC-MS/MS analysis.

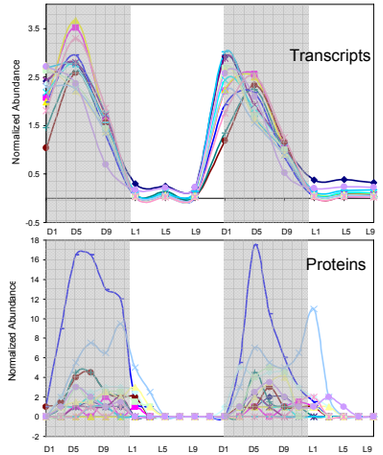

Figure 2. Using a spectral count approach, the cycling characteristics of nitrogenases (based on their measured abundances) were clearly observed, which provided a high correlation with previously measured transcriptomic analyses, including a noticeable lag period between observed induction of mRNA and protein abundance.

Cyanothece Proteome Characterization

Benefits of utilizing proteomics studies to guide genomic annotation

- 27,592 unique peptides detected from 2768 proteins, ~50% coverage of predicted proteome.
- 53 additional non-predicted proteins were identified with multiple unique peptide identifications based upon searching LC-MS/MS data against normally removed lower confidence annotated ORFs.
- 457 out of 1930 hypothetical proteins (24%) were reannotated as "unknown" due to detection of multiple peptide sequences.

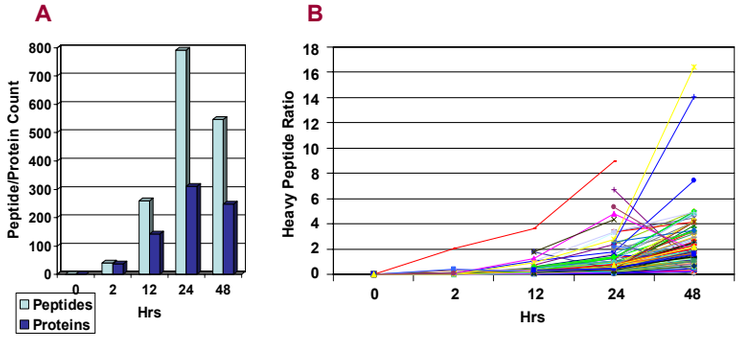

Figure 3. Initial demonstration of a metabolic-based pulse stable isotopic label incorporation using a heavy labeled Leu residue introduced into the culture media at time point 0 followed by steady state constant light (non-cycling) growth conditions. A) Total number of heavy/light labeled peptides/protein pairs identified across the timeframe. B) Incorporation ratio (heavy/light) for the 200 top proteins across the same timeframe.

Conclusions

- Initial LC-MS/MS based relative protein abundance measurements were able to clearly track key cycling proteins across the diurnal cycle.
- There was significant benefit in using proteomics data with genomic sequencing to guide annotation efforts.
- Initial amino acid based metabolic labeling studies have shown potential for obtaining protein synthesis turn-over information that will provide dynamic quantitative proteome information for correlation with diurnal cycle time course studies.

Acknowledgements

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References

1. Jacobs, J.M., et al. "Ultra-sensitive, high throughput, and quantitative proteomic measurements." *International Journal of Mass Spectrometry* **240**, 195-212 (2005)
2. Qian, W.J., et al. "Probability-based evaluation of peptide and protein identifications from tandem mass spectrometry and SEQUEST analysis: The human proteome" *J. Proteome Res.* **4**, 53-62 (2005).

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