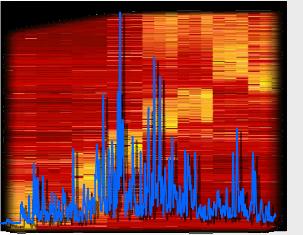


Detection of Cellular Pathways Utilizing Quantitative Protein Profiling in *Yersinia pestis*, *Shewanella oneidensis*, and *Rhodobacter sphaeroides*

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Overview

Proteomic profiling has progressed beyond providing qualitative inventories of proteins to the quantitative measures of changes in protein abundance. Important to microbial systems is the changes of metabolic pathways as a result of stress and environment. Quantitative approaches have been applied for protein profiling in microbial systems thus enabling a more comprehensive analysis of changes in protein abundance. We have applied these approaches to determination of culture dependent expression of cellular pathways of three microbial systems, the type III secretion system in *Yersinia pestis*, the electron transport chain in *Shewanella oneidensis*, and the photosynthetic reaction center in *Rhodobacter sphaeroides*.



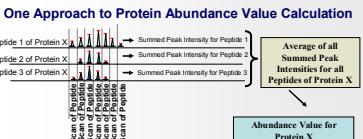
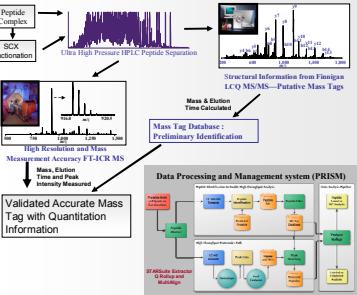
Methods

- Peptides generated from proteolytic digest of a protein
- Identifications enabled by the availability of sequenced genome
- High accuracy mass measurements from FTICR MS measurements
- Provide unique biomarkers for nearly all proteins

Mass tag = Biomarker



Accurate Mass and Time (AMT) Tag Data Processing Pipeline



Introduction

Microorganisms employ various metabolic pathways to adapt to cellular stress and environmental conditions, and quantitative measures of these pathways can indicate virulence responses, adaptation pathways and critical metabolic functions. Utilizing the AMT tag approach, both isotopic labeling and label-free approaches enabled the quantification of large numbers of proteins directly from crude cell lysates.

Shewanella oneidensis

- Gram negative, Respiratory generalist and facultative anaerobe
- General interest because it can oxidize organic matter using metals such as Fe(II) or Mn(II/IV) as the electron acceptors.
- Potential applications in the bioremediation of metals
- Can also reduce soluble U(VI) to the insoluble U(IV) form.
- Ability to reduce U prevents further U mobility in groundwater and subsequent contamination of down-gradient water resources.
- Can function to enzymatically reduce and precipitate a diverse range of heavy metals and radionuclides.
- A thorough understanding of how MR-1 responds to changes in electron acceptor type and concentration and the enzymatic pathways involved in these reactions is critical for effectively using metal-reducing bacteria for remediation.

Rhodobacter sphaeroides

- Gram negative purple nonsulfur eubacterium
- Member of the α-3 subdivision of the Proteobacteria.
- Rhodobacter sphaeroides* is a well-studied and free-living microbe that is capable of growth under a variety of conditions, e.g., autotrophic, phototrophic, and anaerobic heterotrophic, by utilizing a variety of electron acceptors such as dimethyl sulfoxide (DMSO), trimethyl-amine-N-oxide (TMAO) and nitrate
- R. sphaeroides* is known for its diverse abilities, e.g., metal reduction, nitrogen fixation, and the sequestering of carbon dioxide.
- Capable of producing copious amounts of H₂ and therefore a potential source of renewable energy
- Many of these abilities are linked to *R. sphaeroides*' photosynthetic apparatus that has served as a model for anoxygenic photosynthesis.

Yersinia pestis

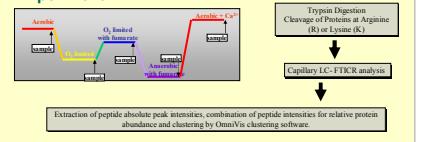
- Causative agent of plague
- Gram negative, highly communicable, enteric bacterium
- Known bioterrorist and biowarfare threat agent.
- The Type III secretion virulence mechanism of *Y. pestis* is made up of a syringe-like secretion structure (injectionome)
- Injectionome is made up of a basal protein complex that provides a port through the inner membrane and periplasmic space, an outer membrane channel protein (YscC), and the "needle" structure necessary to pierce the host cell membrane.

Results

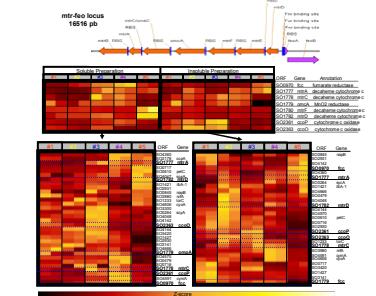
Shewanella oneidensis

The purpose of this study was to determine the effect of different electron acceptors on the expressed electron transport chain pathway in the organism and specifically the cytochrome proteins therein.

Experiment

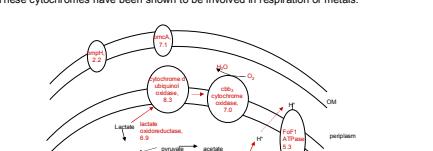


Cytochrome response to Oxygen Levels in *Shewanella* Culture Media



Fumarate reductase: Increases in abundance under anaerobic and suboxic conditions. This induction pattern is confirmed by both microarray and 2-D gel experiments.
MtrD/MtrC (SO1780/SO1782): These MtrC-like & MtrA-like decamer cytochromes increase in abundance in aggregated cells. These results suggest that the function of these uncharacterized proteins may require close cell contact.

MtrA/MtrC SO1775/SO1779: These proteins are induced under anaerobic conditions. These cytochromes have been shown to be involved in respiration of metals.

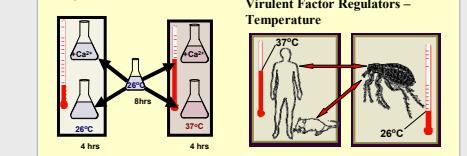


TCA cycle and electron transport chain of the *S. oneidensis* MR-1. Relationship of the cytochromes in *Shewanella* as part of the electron transport chain to the TCA cycle are shown. Changes observed in expression of proteins areas shown. Red indicates an increase in expression under suboxic vs. aerobic culturing conditions while blue indicates reduced expression.

Yersinia pestis

The purpose of this experiment was to utilize *in vitro* culture conditions to induce the expression of the Type III Secretion System (TTSS) and identify other proteins which co-expressed with these virulence factors.

Experiment



An abundance heat map with Z-score used to compare 963 proteins from *Yersinia pestis* versus culture condition and cell fractionation.

Proteins represented are those that were observed with at least two unique peptide identifications in a single LC-MS analysis.

The relative z-score of each triplicate analysis from each culture condition (26°C w/o Ca²⁺, 26°C w/ Ca²⁺, 37°C w/o Ca²⁺, 37°C w/ Ca²⁺) and cell fraction preparation (all inclusive global preparation, soluble preparation, insoluble preparation) is also shown side by side. The darkest colored data represent proteins that are not observed by any peptide identification in the particular analysis. A lighter color represents higher relative z-score and thus a higher relative abundance for each observed protein.

Relative z-score cluster plot of 146 proteins observed to cluster with similar relative abundance changes as nine known virulence associated proteins.

Regulation and analysis of the type III secretion system

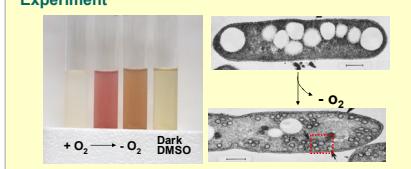
The TTSS is made up of the basal protein complex (injectionome) that provides a port through the inner membrane and periplasmic space, an outer membrane channel protein (YscC), and the "needle" structure necessary to pierce the host cell membrane and participates as a channel to direct Yop proteins into the host cell cytoplasm, which then act on the host cell to promote pathogenesis.

(A) The *Yersinia pestis* bacteria in the extracellular environment of the eukaryotic host (37 °C, ~2 mM [Ca²⁺]). The presence of divalent cation maintains the YopN protein plug conformation in the type III secretion apparatus, keeping virulent yop expression repressed. (B) At the site of eukaryotic cell contact (simulated by the removal of Ca²⁺ from the growth media *in vitro* at 37 °C) YopN is released. LcmC is subsequently released through the secretory pathway and it signals up-regulation of the virulent yop proteins which are then translocated into the eukaryotic cell causing evasion from phagocytosis as well as cytotoxicity of the host cell. (C) Relative z-score cluster plot of 10 model virulent proteins versus the culture conditions under each cell fractionation scheme.

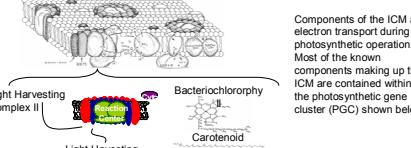
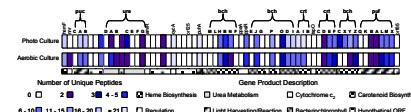
Rhodobacter sphaeroides

The purpose of this experiment was the characterization of the proteins involved in the photosynthesis functions of the organism and those that co-localized with the photosynthetic gene cluster under the appropriate conditions.

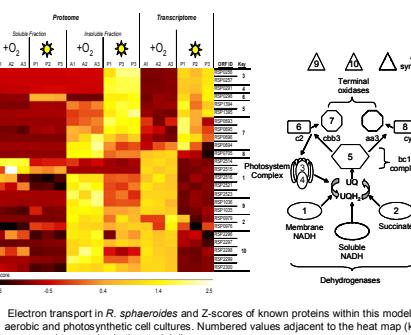
Experiment



Transition of *R. sphaeroides* from aerobic respiration to photosynthesis. All components necessary for photosynthesis are contained within the ICM, which can be separated as a chromophore sub-cellular fraction.



Components of the ICM and electron transport during photosynthetic operation. Most of the known components making up the ICM are contained within the photosynthetic gene cluster (PGC) shown below.



Conclusions

Shewanella oneidensis

- Quantitative measurements of cytochrome abundance indicate that most cytochrome proteins increased in abundance under anaerobic conditions
- Many of these proteins are associated with the electron transport chain, and indicate an increase in the activity in anaerobic respiratory pathways.
- The concomitant decrease of expression of the TCA cycle illustrates the need for increased energy capacity under anaerobic conditions.
- The need for the increased potential is due to the difference in redox potential between oxygen and fumarate.

Rhodobacter sphaeroides

- Proteins associated with the photosynthetic gene cluster were shown to increase in abundance under photosynthetic conditions.
- The proteins were also shown to co-locate into the chromatophore sub-cellular fraction.
- Results indicate the presence of these known photosynthetic proteins as well as a number of protein having function currently not associated with photosynthesis.
- The understanding and manipulation of such proteins could lead to the increased efficiency of coupling photosynthesis to H₂ production for bioenergy.

Yersinia pestis

- Quantification by absolute peak intensity revealed a cluster of proteins associated with virulence under the expected conditions.
- The proteins associated with the type III secretion system were found to have increased abundance at higher temperatures and lower Ca²⁺ concentrations.
- 146 proteins having unknown function also had similar abundance profiles as the type III secretion system
- These other proteins serve as potential biomarkers for virulence as well as targets for future study.

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