Food Poisoning and Typhoid Fever – Proteomic Exploration of Virulence in *Salmonella typhimurium* and *typhi*

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Overview

***Salmonella enterica*, serovar Typhimurium (*S. typhimurium*) causes food poisoning and the *typhi* serovar *(S. typhi)* is the causative agent of typhoid fever. To understand the systems involved in virulence of these highly similar organisms, comprehensive and quantitative proteome studies were conducted (using the accurate mass and time tag approach) with samples cultured under several conditions.**

For *S. typhimurium*, a comparison of the data from each set of samples revealed specific increases in 54 proteins in MgM culture (mimics host intracellular growth conditions). In particular, both virulence-related propanediol utilization proteins and Mg2+ transport proteins were strongly upregulated in MgM.

The two serovars Typhimurium and Typhi are highly similar in terms of their genetic makeup, and the high-throughput proteomic studies showed 656 proteins with similar abundance when these organisms are grown in similar culture conditions. However, *S. typhi* data showed significant expression increases for some proteins, mostly occurring in groups involved in the type III secretion system.

The findings suggest that *Salmonella enterica*, serovar Typhimurium and Typhi exhibit unique protein compositions when culture in a medium mimicking intracellular growth.

Introduction

- ***Salmonella typhimurium***
  - Major cause of Salmonellosis, usually presents as food poisoning
  - ~40,000 reported US cases/year (potentially ~30 times higher)
  - Life threatening in the immune impaired, infants, and elderly
  - Increased resistance to antibiotics
  - Can infect humans and other animals
- ***Salmonella typhi***
  - Causes Typhoid Fever
  - Fevers over 100 °F in adults
  - Potentially fatal if not treated with antibiotics
  - Specific to human host

Figure 1. The effects of infection by the bacteria studied, comparing virulence of the serovars.

Methods

Sample Preparation

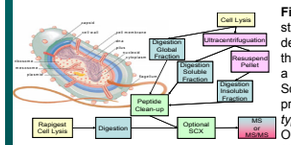


Figure 2. Cell lines were grown in standard LB media and Mg-depleted media (MgM), which is thought to mimic the environment of a macrophage in a host cell. Global, Soluble, Insoluble, and Rapigest preps were used for the *typhimurium* and *typhi* samples. Only Rapigest was used for the strain comparisons.

Instrument Analysis

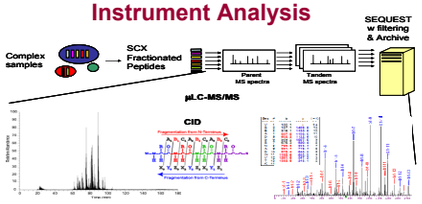


Figure 3. Peptide identification by LC-MS/MS. SEQUEST identifications were required to pass the filters used by Washburn et al (1) and in addition, have a minimum discriminant score (2) of 0.9. Proteins with 3 or more distinct peptide sequences are represented, and the qualitative protein abundance is represented by a spectrum count for all peptides seen.

Data Analysis

Normalization and Clustering

Peptide counts for each protein were processed by one of 2 methods prior to clustering using a Kmeans clustering algorithm:

1. Missing values were filled with the value "1", and all values divided by the sum of the peptide observations in all conditions.
2. Missing values were left blank, and a Z-score was applied to the observations across conditions (see Figure 4).

Immunoblot Verification

Western Blot Validation for pdu proteins in *typhimurium*

- Blots generated at OHSU using standard protocol procedures.
- pduB::HA, pduC::HA, and pduO::HA strains each cultured in LB log, LB stationary, and MgM, recording optical density (O. D.; 600 nm)
- Cells harvested by cold centrifugation, washed 2X with an equal volume of ice-cold DPBS, resuspended in 100 µL of Laemmli 1X sample buffer, and boiled for 5 min.
- Protein samples were normalized to culture O. D. and loaded onto 12% Tris-Cl SDS-PAGE gels (Invitrogen).
- Electrophoresis to PVDF membranes (Millipore), blocked in Tris-buffered saline (TBS) plus 5% powdered non-fat dry milk for 1 h, probed with an anti-HA monoclonal antibody (Covance, 1:1000, in block solution) for 1 h, washed 3-5 min with TBS, probed with a sheep-anti-mouse 2° antibody (Amersham, in block solution) for 30 min, and finally washed 3-5 min with TBS. The immune complexes were detected via chemiluminescence by using Perkin Elmer's "Western Lightning" reagents, and then exposed to XAR Biofilm (Kodak).

Results

S. typhimurium

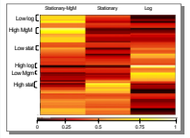


Figure 4. Protein abundances of 3 growth phases. Individual proteins are shown in rows. Normalized scale is 0 (low) to 1 (high), represented by the black to white color gradient.

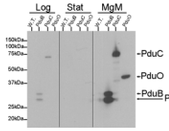


Figure 5. Immunoblot analysis of PduB/C/O protein levels from *S. typhimurium* log phase LB, stationary phase LB, and MgM cultures. No Pdu proteins were detected during stationary phase in rich medium, while PduB (and PduB' a naturally occurring version missing the N-terminal), and PduC were only present at low levels during log phase growth. Exposure to MgM media strongly induced the synthesis of all four proteins.

S. typhimurium LT2 MgM proteins of interest:

- multiple Mg2+ transport protein
- multiple propanediol utilization proteins
- PhoR, a sensory kinase in the two-component regulatory system

S. typhi

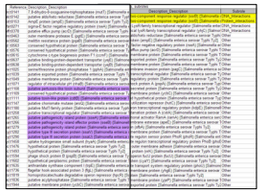
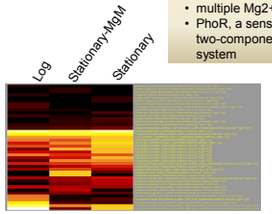


Figure 6. Highly expressed in MgM included putative pathogenicity island proteins as well as ssrB an virulence protein (virK)



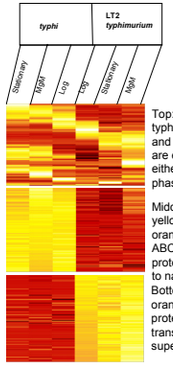
S. typhi TY2 Condition Clusters

MgM proteins of interest are:

- multiple Mg2+ transport protein
- PhoR, a sensory kinase in the two-component regulatory system

Figure 7. 42 Proteins were expressed by *typhi* that were not linked to any *typhimurium* proteins by homology. Graphic shows protein values from peptide observations counts, with no normalization procedure, on a scale from 0 to 87 – black through yellow to white.

S. typhimurium and *typhi:* Serovar Comparison



Top: Protein abundances across growth phases for typhi and typhimurium. Proteins (2455 total records) are listed in rows and phases (columns) are ordered by correlation. Proteins are expressed most similarly within the growth phases of either serovar and least similar to the opposite serovar. Log phase is most similar between the two serovars.

Middle: A cluster of highly expressed proteins in typhi (left, yellow) that are minimally expressed in typhimurium (right, orange). This protein cluster contained 113 proteins, including ABC transporters, putative cyroplasmic and periplasmic proteins, and outer membrane porins (OmpC, OmpS, OmpN, to name a few).

Bottom: A cluster of minimally expressed proteins in typhi (left, orange) that are highly expressed in typhi (right, yellow),This protein cluster contained 123 proteins including putative transcriptional regulators and cytoplasmic proteins, ABC superfamily proteins, and flagellar biosynthesis proteins.

Figure 9. Protein cluster showing similar expression in the Stationary and MgM phases of both serovars. A total of 49 proteins (in rows) are shown.

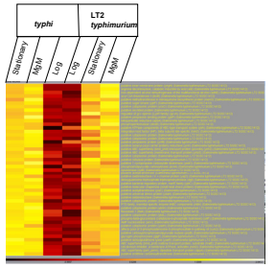


Figure 10. Common proteins between typhi and typhimurium. Homology was determined using a BLAST analysis of the protein file typhi against that for typhimurium. Protein matches had a maximum e value of 0.4 and a minimum percent identity of 60%.

Proteins unchanged between strains

- Protein chain elongation factor Tu
- Chaperone HSP70 in biosynthesis/cell division (dnaK)
- Chaperone HSP60
- Enolase (eno)
- Malate dehydrogenase (mdh)

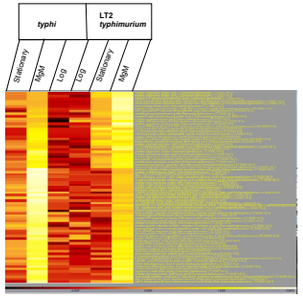


Figure 11. Protein cluster showing similar expression in the MgM phases of both serovars. A total of 75 proteins are shown.

Strain Comparison

Differences in *S. typhimurium* strains (LT2 and 14028)

- **LT2** is a laboratory strain
- **14028** is a more virulent strain
- **Some major differences**
  - **Propanediol utilization proteins**
  - 5 times as common in 14028 strain versus LT2 for MgM medium
  - A regulatory mutant supports the propanediol utilitization as a virulence factor
  - **Fels-1 prophage**
  - Absent in **14028**

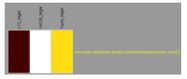


Figure 12. Proteins showing similar expression between the more virulent typhimurium strain and typhi (nmpC). Unknown function.

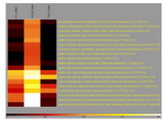


Figure 13. Proteins more highly expressed in the 14028 strain included propaendiol utilization proteins.

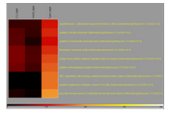
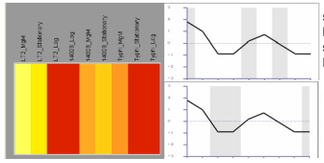


Figure 14. Proteins more highly expressed in typhi included phage shock and ABC superfamily proteins.

A Focus on ssrB



ssrB is increased in MgM (upper panel, shaded) and Stationary Phases in all 3 strains.

ssrB is decreased in Log Phase (lower panel, shaded) in all 3 strains.

Figure 15. Expression patterns for ssrB, part of a 2-component regulation system in pathogenicity island III.

Conclusions

- **Upregulation of pdu proteins in *typhimurium* in host-mimicking culture condition**
- **Sensory kinase proteins and Mg^{2+} transport proteins observed in both *typhimurium* and *typhi* under growth conditions that mimic a host intracellular environment**
- **High expression of pathogenicity island proteins, virK, and the regulator ssrB in *typhi* in host-mimicking culture condition**
- **75 proteins of similar expression patterns between *typhi* and *typhimurium* that are highly expressed in the host-mimicing culture condition**
- **Some strain-specific expression patterns observed in the MgM condition**
- **SsrB is more highly expressed in stationary and MgM phases of cell growth for both the virulent strain of *typimurium* and *typhi***

Acknowledgements

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2. Strittmatter, E. et al. 2004. Application of Peptide LC Retention Time Information in a Discriminant Function for Peptide Identification by Tandem Mass Spectrometry. *J Prot Res* 3, 760-769.

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