GC-MS-based comparative metabolomics analysis of pathogenic *Salmonella enterica* serovar Typhimurium grown under host environment conditions

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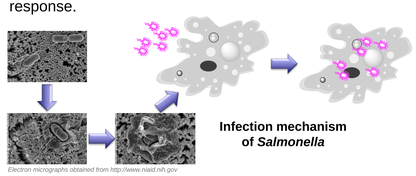
Overview

- *Salmonella* infection is one of the most common enteric diseases in the world and annually costs the US $14.6 billion in medical expenses and lost productivity[1].
- In order to understand metabolic changes during infection, we performed a GC-MS based metabolomics study with a pathogenic *Salmonella* strain under environmental conditions similar to those experienced in the host.
- Two software programs each possessing different deconvoluting algorithms were comparatively employed for data analysis of a pooled sample of *Salmonella* cell lysates.
- Statistical analysis showed levels of metabolites changed accordingly in response to different environmental conditions, and a significant number of metabolites were either up-regulated or down-regulated .

Introduction

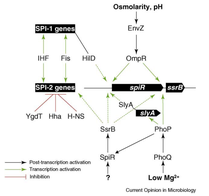
- The pathogenic bacterium *Salmonella* uses complex biological mechanisms to invade and proliferate within host cells while simultaneously avoiding the host immune response.



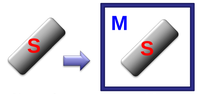
Electron micrographs obtained from http://www.niaid.nih.gov

Infection mechanism of *Salmonella*

- Many regulatory processes are required for these adaptive mechanisms inside the host, and identifying metabolites that are regulated during infection improves a systems level understanding of infection.



Regulatory factors of *Salmonella*[2]

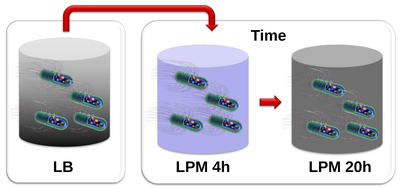


Normal condition — **Inside macrophage**

Expression of virulence factors is activated

Methods

Cell culture design



Strain: *Salmonella enterica* serovar Typhimurium (ATCC 14028; Wild Type)
LB : Luria-Bertani Complex medium
LPM : Low phosphate, low magnesium defined medium (low pH, 5.8)[3]

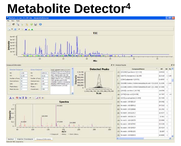
Sample analysis

Quenching / Lysis	Cell washing, Flash frozen, Bead-beat
Extraction	Chloroform/Methanol (2:1, v/v) method
Derivatization	Methoxyamination/Trimethylsilyation
GC-MS analysis	Agilent GC 7890A with MSD 5975C
Data-processing	Deconvolution / Fiehn library* match

***Fiehn Metabolomics library** contains over a thousand EI mass spectra and retention indices for ~700 metabolites.

Data-processing programs

Metabolite Detector[4]

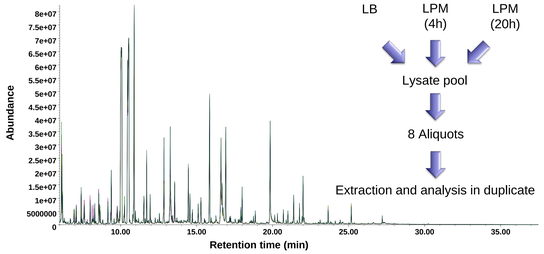


AnalyzerPro

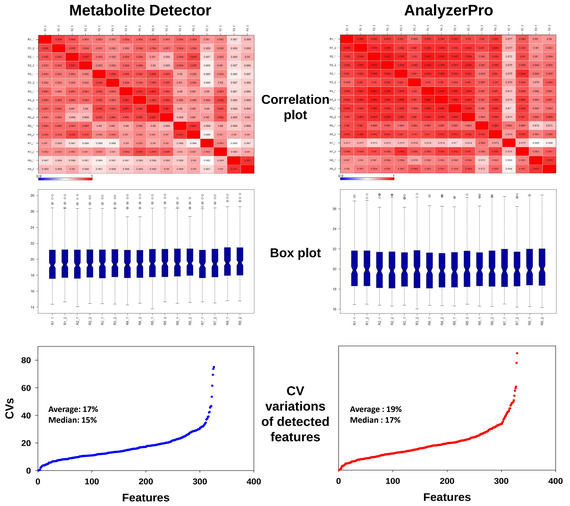
Univ. of Braunschweig, GER (Dr. Karsten Hiller, *et al.)*	**Developer**	SpectralWorks Inc., UK
Consistency in quantification	**Pros**	Less errors in peak detection
Possible missing peaks	**Cons**	Additional library construction
netCDF and TOF data formats	**Format**	Compatible with most formats
Freely available	**Accessibility**	Commercial software

Results

Performance test

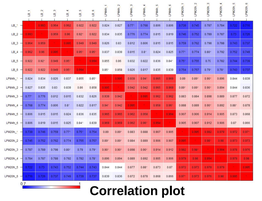


Overlay of GC-MS chromatograms (n = 16) that demonstrate good reproducibility, which enhances peak identification and quantification.

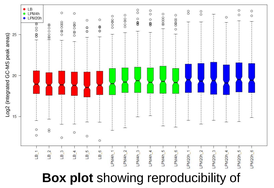


Data processing with two software programs: The generated data sets from each software showed similar results; however, significant differences were found (e.g., missing peaks, incorrect identifications, and etc).

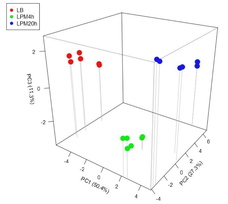
Statistical analysis



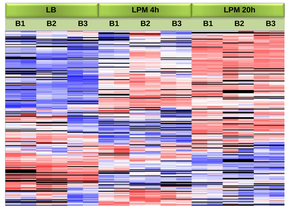
Correlation plot



Box plot showing reproducibility of measured metabolite abundances



Principal Component Analysis showing segregation of sample types based on metabolite profiles



Heat-map analysis showing differential metabolite abundances

Detected features: 244 (average). Each set of detected metabolites from the different conditions were compared statistically. The pattern of detected intracellular metabolites displays metabolic changes by condition.

Metabolite identification

LB → LPM 4h → LPM 20h

Up-regulated		Down-regulated	
metabolites	p value	metabolites	p value
3-hydroxypropanoic acid	1.1E-02	2'-deoxycytidine 5'-MP	2.7E-03
aspartic acid	4.7E-04	3-aminopropionitrile	1.3E-07
D-malic acid	9.0E-04	adenine	1.2E-04
D-mannitol	8.4E-08	cytosine	4.1E-02
glyceric acid	1.9E-10	fructose	9.8E-03
glycine	3.8E-06	glucose-6-phosphate	3.2E-04
L-alanine	7.7E-05	glycerol-1-phosphate	6.0E-03
L-cysteine	1.0E-03	guanosine-5'-MP	9.9E-04
L-glutamic acid	1.0E-03	lactamide	8.5E-03
L-homoserine	4.1E-02	malonic acid	2.2E-05
L-leucine	1.1E-02	nicotinic acid	3.1E-04
L-lysine	2.2E-06	orotic acid	3.9E-04
L-methionine	3.5E-03	pyruvic acid	3.9E-04
L-ornithine	1.4E-02	spermidine	1.4E-08
L-proline	9.8E-03	tyramine	6.5E-05
L-threonine	4.3E-07		
L-tyrosine	3.2E-04		
L-valine	3.2E-07		
N-acetyl-L-glutamic acid	8.8E-03		
pantothenic acid	9.5E-08		
putrescine	6.7E-07		
tartronic acid	4.9E-06		
thymine	3.5E-03		
tyrosine	5.8E-04		
uracil	7.0E-04		

Up-regulated		Down-regulated	
metabolites	p value	metabolites	p value
2'-deoxyadenosine	2.4E-05	3-aminopropionitrile	7.6E-03
2'-deoxycytidine 5'-MP	6.6E-07	3-hydroxypropanoic acid	3.2E-03
aspartic acid	1.8E-02	4-hydroxybenzoic acid	2.1E-03
cytosine	2.2E-06	8-aminocaprylic acid	1.6E-03
DL-isoleucine	2.2E-02	DL-glyceraldehyde	6.3E-03
glyceric acid	4.7E-03	D-malic acid	6.8E-03
glycine	4.3E-05	glycerol 1-phosphate	6.2E-07
guanosine-5'-MP	2.0E-05	hypoxanthine	4.4E-04
L-alanine	2.8E-02	L-threonine	2.1E-04
L-cysteine	8.9E-06	L-valine	2.5E-03
L-glutamic acid	1.1E-05	malonamide	1.7E-03
L-leucine	1.7E-03	malonic acid	2.5E-02
L-lysine	4.6E-08	purine riboside	8.8E-03
L-methionine	4.3E-02	pyruvic acid	2.2E-02
L-ornithine	1.0E-02	ribose	1.9E-02
L-proline	3.5E-02	uracil	6.9E-04
methylmalonic acid	1.8E-02		
N-acetyl-L-glutamic acid	1.9E-03		
nicotinic acid	1.2E-03		
N-methylglutamic acid	9.2E-04		
orotic acid	1.9E-03		
phosphoenolpyruvic acid	2.1E-04		
tyramine	3.3E-04		
tyrosine	8.2E-05		
xanthine	3.9E-05		

Regulated features (p <0.05, *t*-test) among 123 identified metabolites

Conclusions

- Intracellular metabolites of *Salmonella* grown under different conditions were globally measured by GC-MS using the Fiehn metabolomics library.
- Two deconvoluting software programs were comparatively evaluated and the qualities of processed data of both met our requirements for this study.
- Some amino acids and other metabolites were found to be increased or decreased after the transfer of cells from LB to LPM. Some of these eventually returned to their “normal” levels while others did not.
- These results are being incorporated with proteomics and lipidomics data for a systems level understanding of *Salmonella enterica* serovar Typhimurium.

Acknowledgements

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