

Post-transcriptional Modification of RNA: Effect on Biology and Virulence of *Salmonella*

Amin A. Fadl

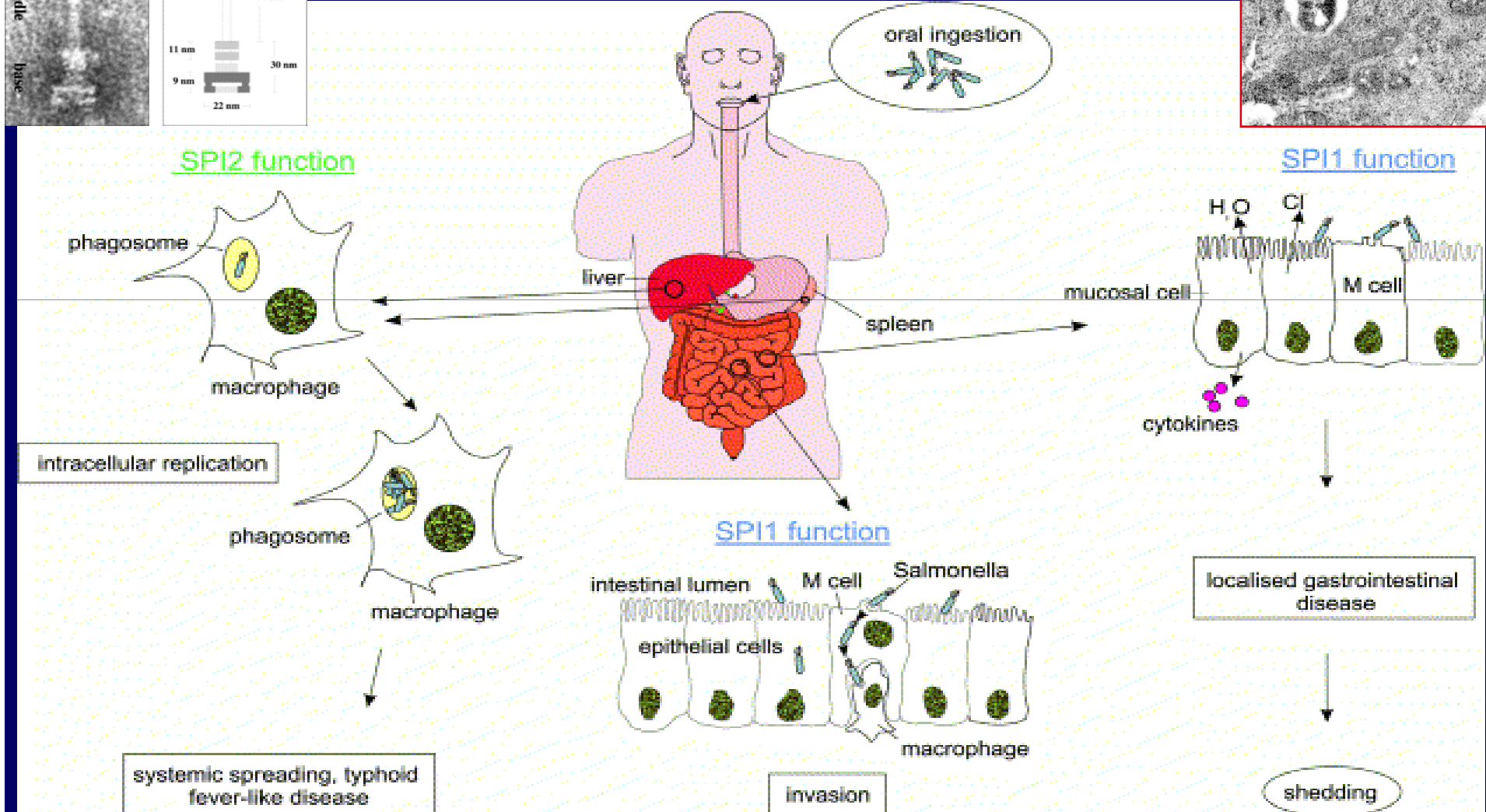
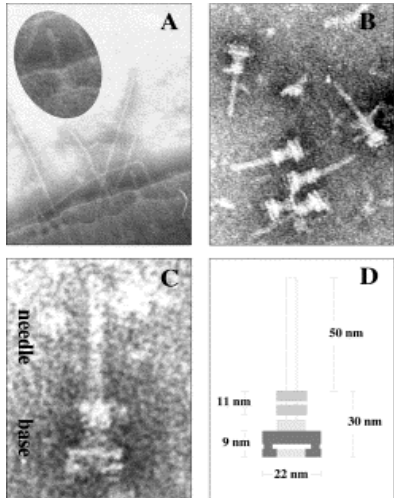
University of Wisconsin-Madison



THE UNIVERSITY
of
WISCONSIN
MADISON

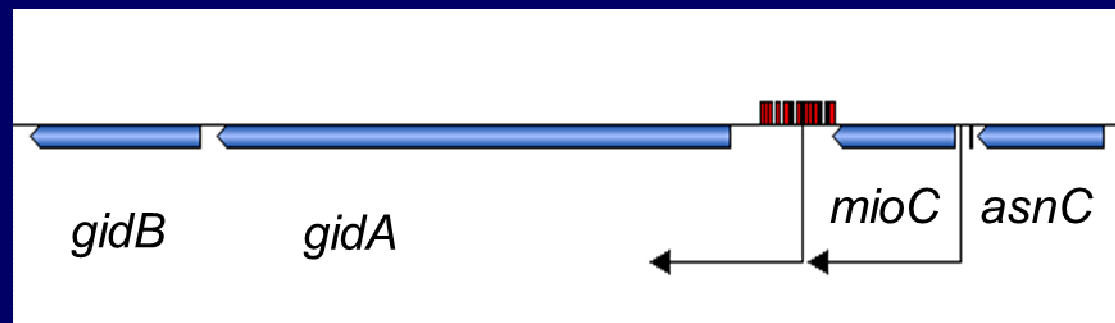


Salmonella Pathogenesis



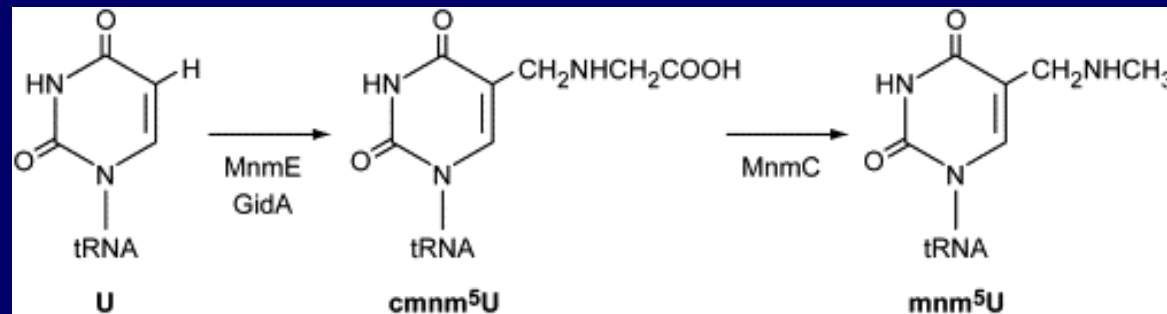
tRNA Modification Enzymes

- ❖ tRNAs are key molecules of translational machinery that ensure decoding of successive codons in mRNA inside the ribosome.
- ❖ Post-transcriptional tRNA modification is found in all organisms and is required for tRNA functions, control gene expression
- ❖ Glucose-inhibited division gene (**GidA**, MnmG), methylaminomethyl (**MnmE**), Ribosomal Small Subunit Methyltransferase (RsmG, **GidB**)



GidA (MnmG)

- ❖ Thought to be involved in cell division and chromosome replication (filamentous) in *Escherichia coli*
- ❖ Recent studies suggested a role in gene regulation and tRNA modification
- ❖ GidA complexes with MnmE to catalyze tRNA modification (addition of cmm group onto the C5 carbon of uridine at position 34 (U34) of tRNAs)



In vitro Virulence Potentials

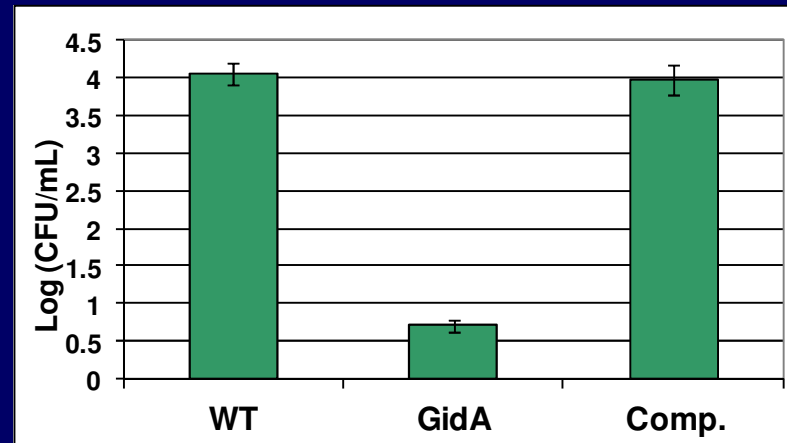
WT

GidA

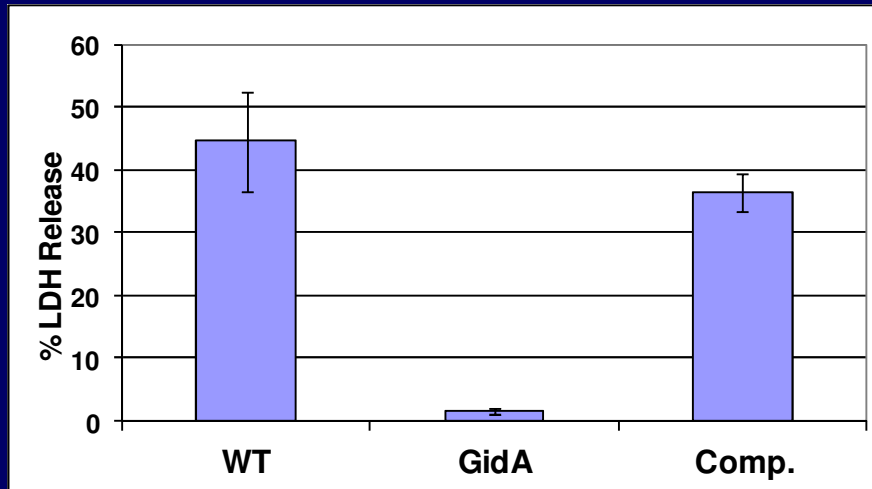
GidA-compl



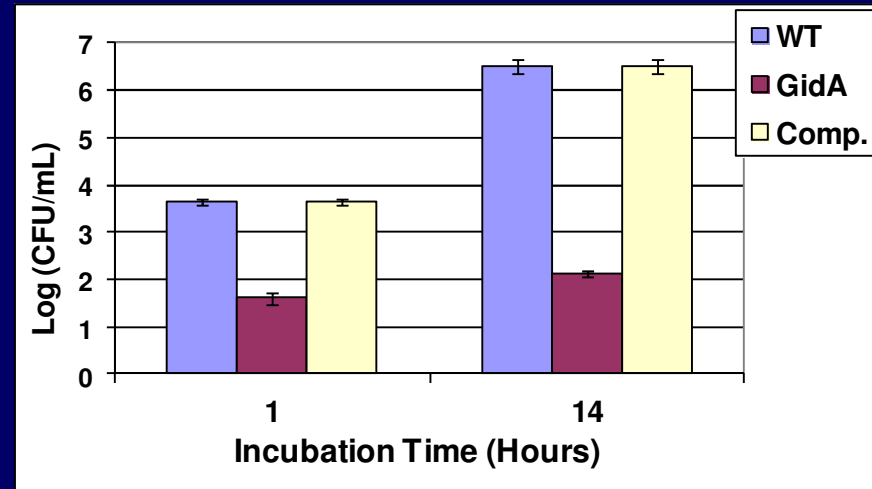
Filamentous morphology



Defective in invasion of intestinal cells



Inhibit cytotoxicity in murine macrophages



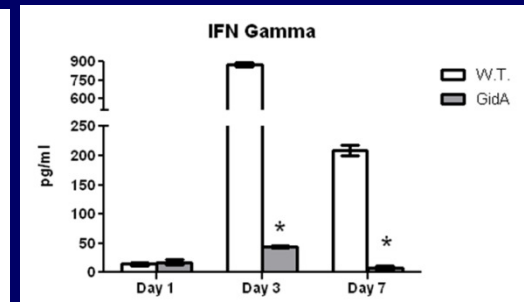
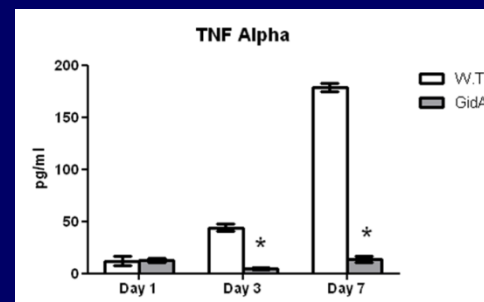
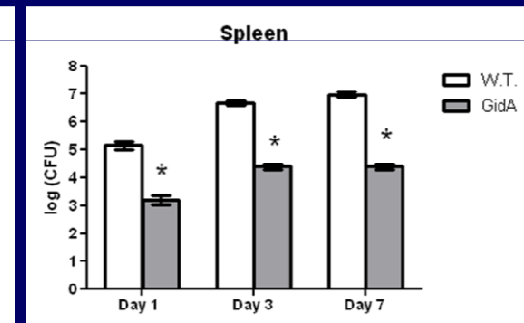
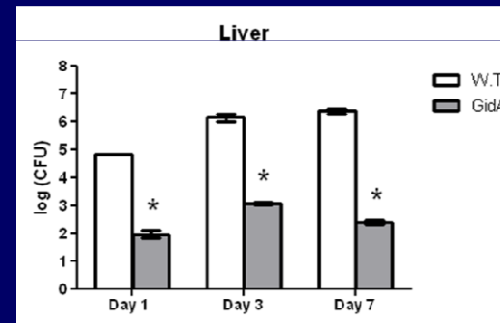
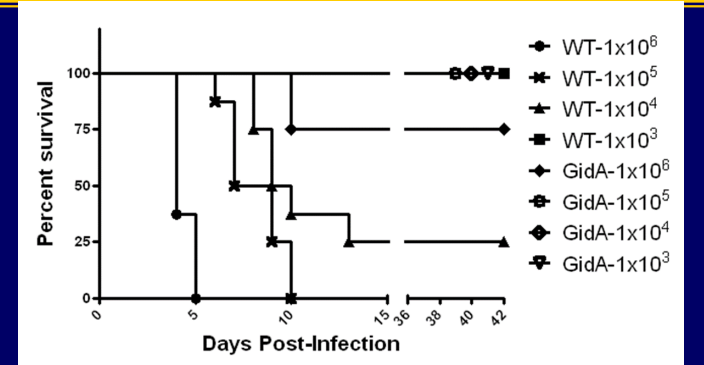
Decreased replication in macrophages

In vivo Virulence of GidA mutant

❖ Increased LD₅₀

❖ Decreased systemic replication

❖ Marked reduction in induction of cytokines



Transcriptome Profiling: 154 genes down-regulated and 124 up-regulated. Confirmed by real-time RT-PCR

Gene Name	Gene #	Microarray FC	RT-PCR FC	Semi-quantitative Western blots
<i>spaP</i>	STM2890	-9.61	-10.85	
<i>prgJ</i>	STM2872	-4.36	-9.85	
<i>fepE</i>	STM0589	4.53	4.54	
<i>hscC</i>	STM0659	3.78	2.63	
<i>yhjC</i>	STM3607	4.22	2.46	
<i>ssaN</i>	STM1415	2.96	4.59	
<i>yebK</i>	STM1887	2.86	2.36	
<i>invF</i>	STM2899	-11.79	-9.83	
<i>invE</i>	STM2897	-12.20	-7.57	
<i>motA</i>	STM1923	-5.05	-2.09	
<i>spaQ</i>	STM2889	-9.49	-3.50	
<i>invA</i>	STM2896	-10.21	-2.16	
<i>prgH</i>	STM2874	-6.86	-3.82	
<i>fliD</i>	STM1960	-4.79	-4.26	
<i>fliC</i>	STM1959	-5.64	-14.89	
<i>cheW</i>	STM1920	-8.71	-4.86	
<i>mukB</i>	STM0994	2.10	3.46	
<i>mreB</i>	STM3374	-2.54	-2.27	
<i>parA</i>	PSLT052	7.07	18.64	
<i>parB</i>	PSLT053	5.45	5.08	

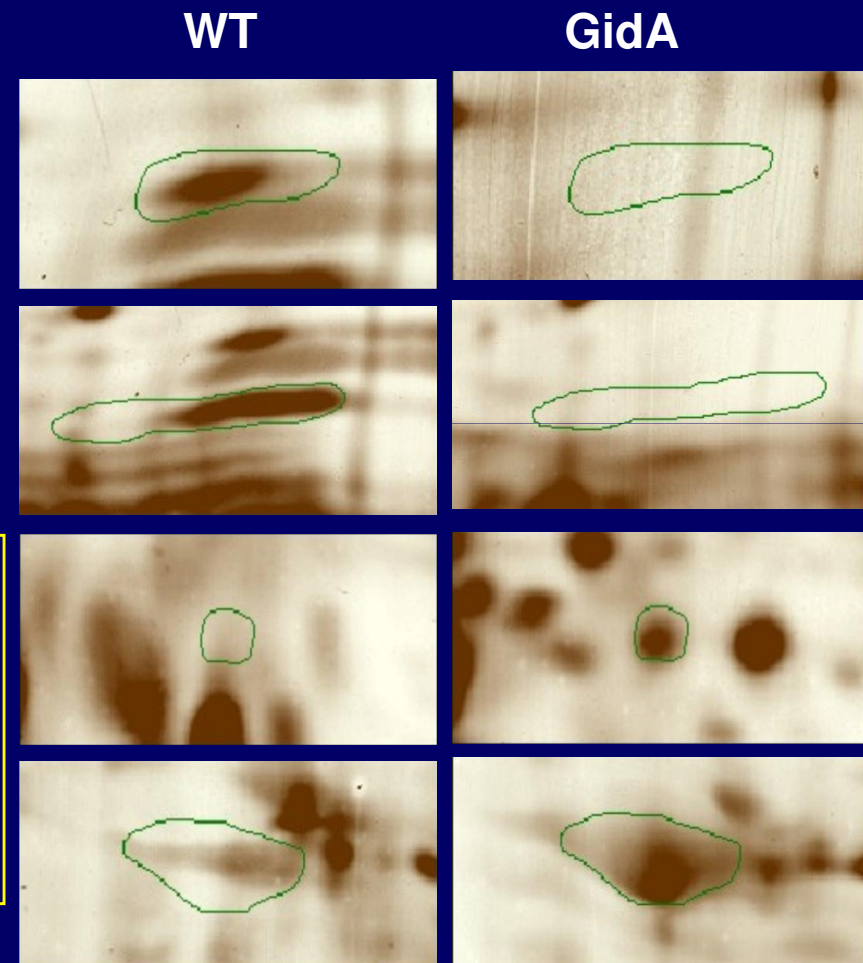
	WT	GidA
MotB	100 ± 3.2	50 ± 1.2
PrgH	100 ± 3.2	56 ± 3.1
FliC	100 ± 5.7	9 ± 1.5

- Assay proteins involved in invasion and motility
- Quantitate using densitometry

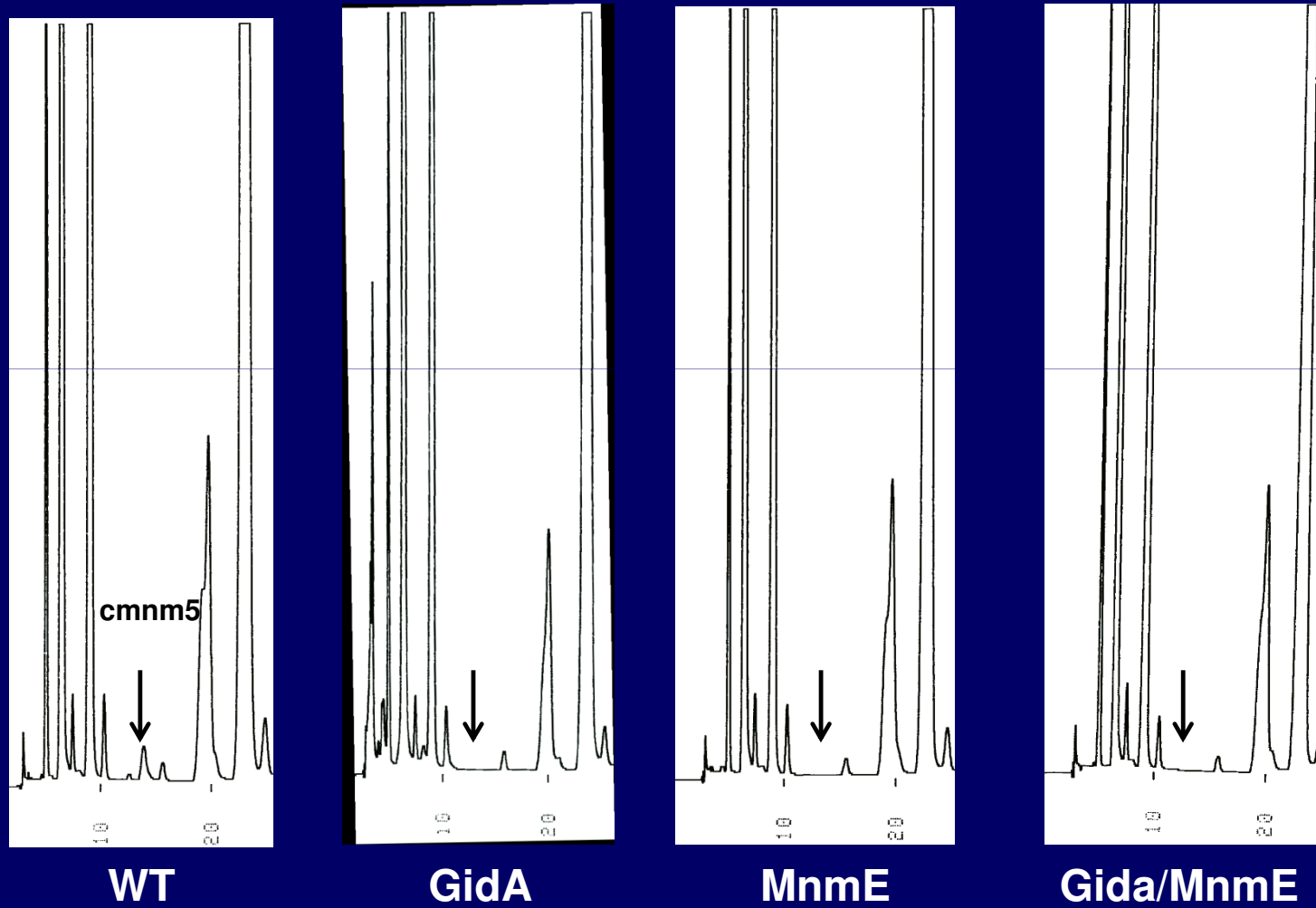
Proteomic Analysis

- ❖ 170 proteins altered
- ❖ 117 proteins down-regulated
- ❖ 53 proteins up-regulated

Identified by MALDI-MS including **MalE** (maltose-binding protein), **YghA** (an oxidoreductase help *Salmonella* survive inside macrophages), **Tpx** (a thiol peroxidase, help *Salmonella* survive within macrophages), tpx (H₂O₂ survival)

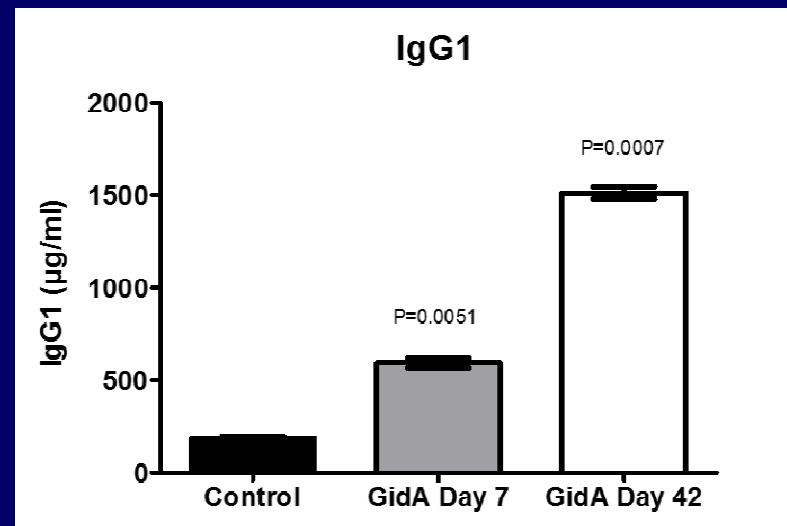
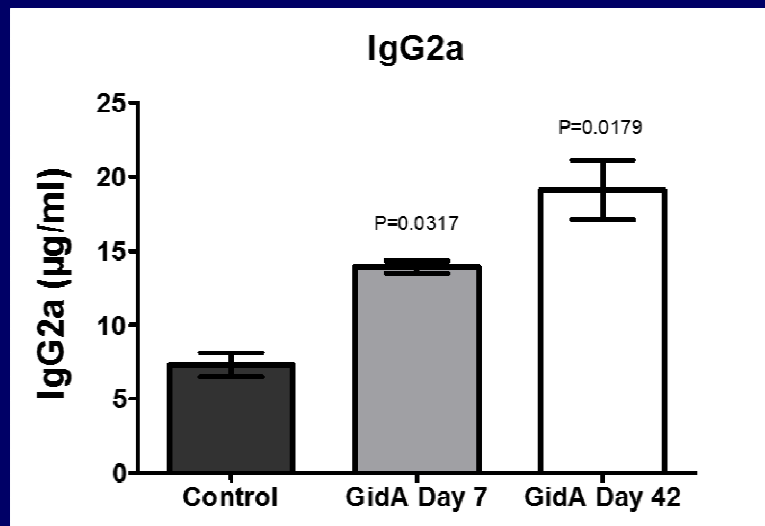


HPLC analysis of enzymatic digests of tRNA isolated from WT and various mutants. Arrow indicates peak corresponding to 5-methylaminomethyl (cmnm5) in the WT and missing in mutants.



Immunization study

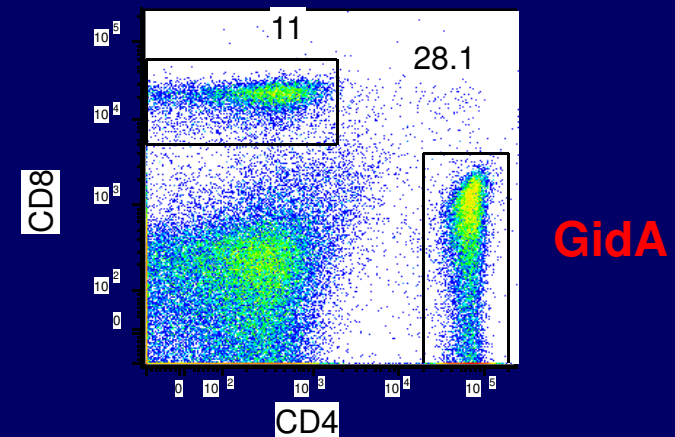
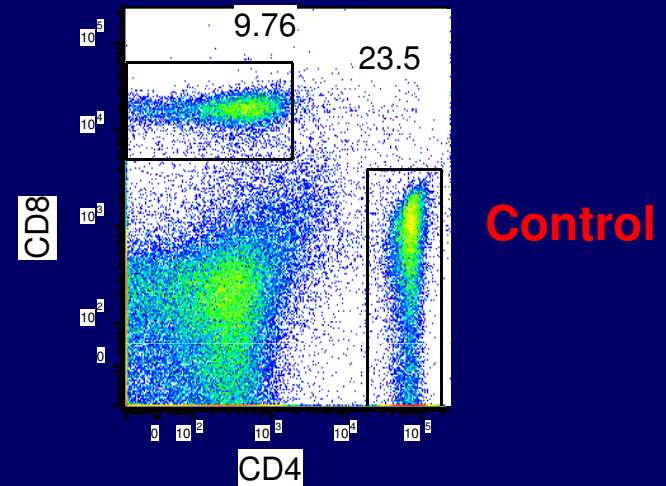
- ❖ Mice immunized with *gidA* mutant was protected against *Salmonella* WT lethal dose
- ❖ Th1/Th2 immune response



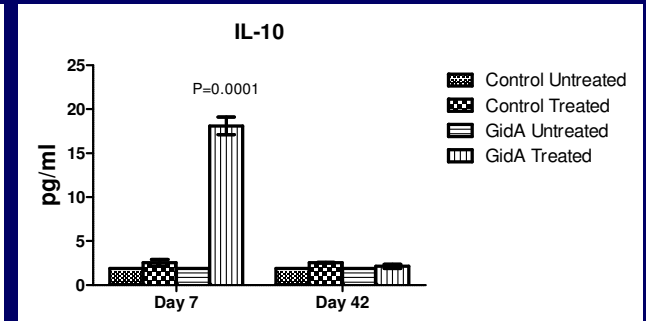
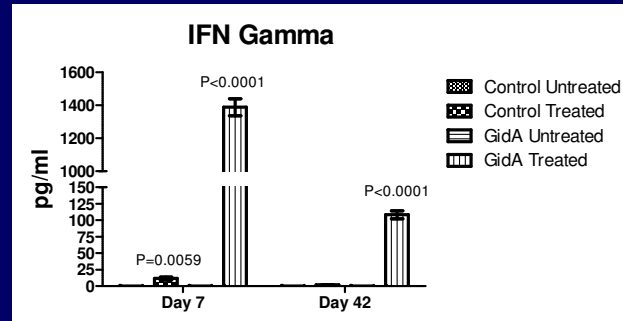
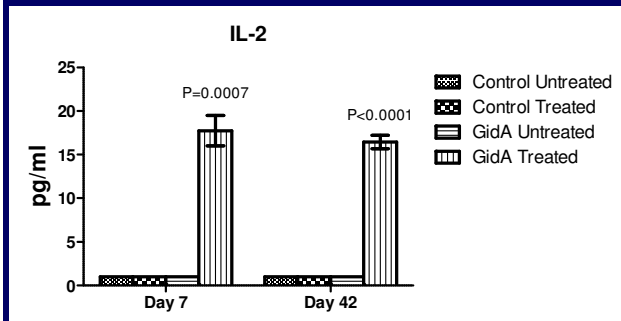
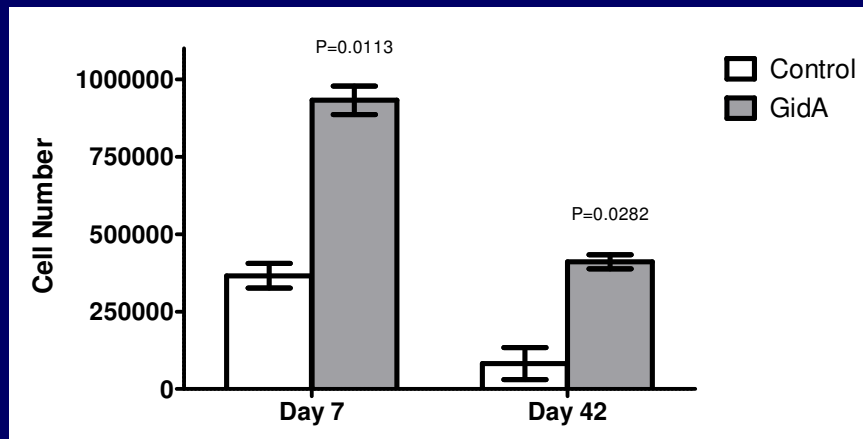
T cell Populations

- ❖ CD4⁺ levels of immunized mice on day 42 (28.1%) compared to levels in control (23.5%)
- ❖ No difference in CD8⁺ levels
- ❖ No difference in CD44⁺ and CD62L⁺ in both CD4⁺ and CD8⁺

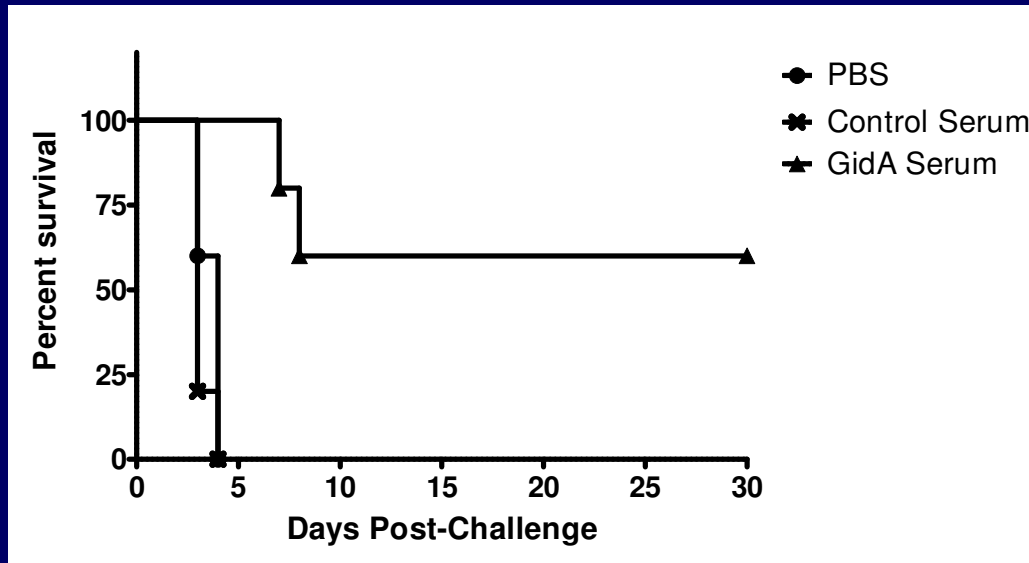
Day 42



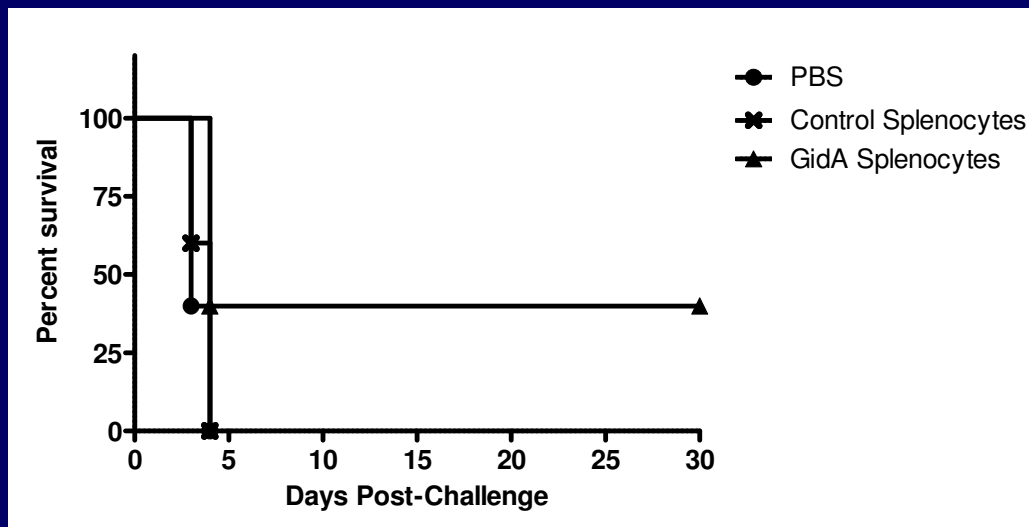
Lymphocytes Proliferation & Cytokines



Passive Immunization



Serum



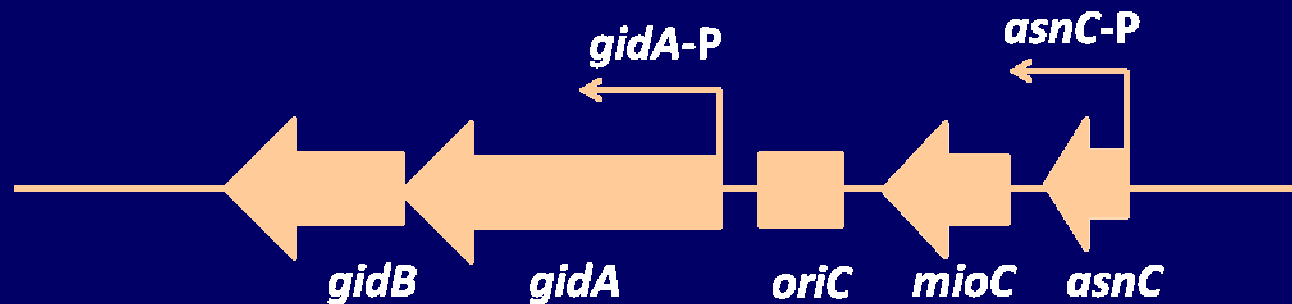
Splenocytes

Summary 1

- ❖ Deletion of *gidA* severely affected the morphology and the virulence of *Salmonella*
- ❖ Increase in Th1 and Th2 with marked level of Th2 in the sera of immunized mice
- ❖ Lymphocytes from immunized mice showed a strong response to *Salmonella* antigen
- ❖ Passive immunization with lymphocytes and sera provided protection against lethal dose challenge

Regulation of *gidAB* Operon

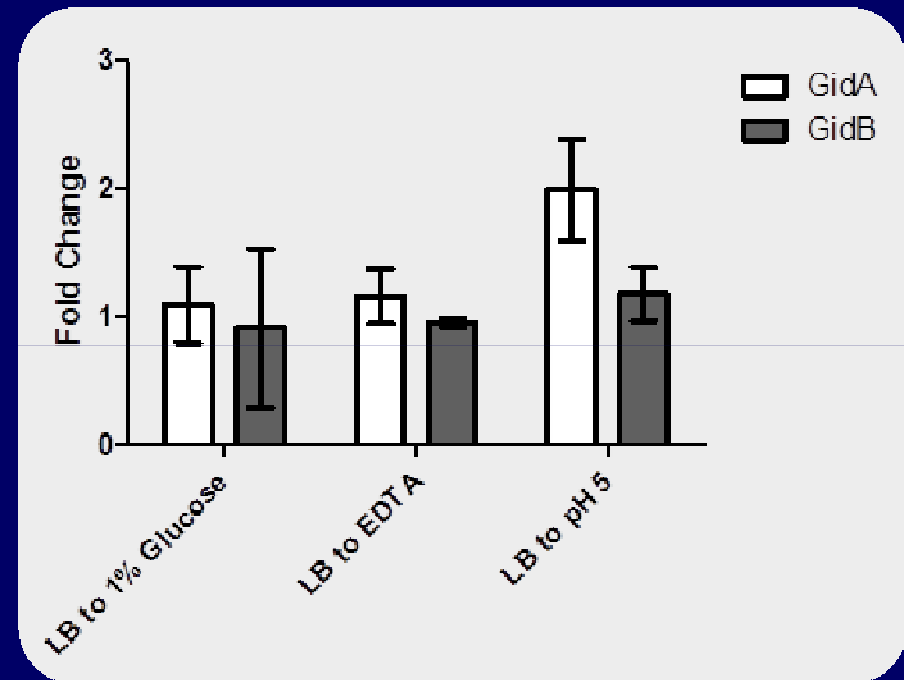
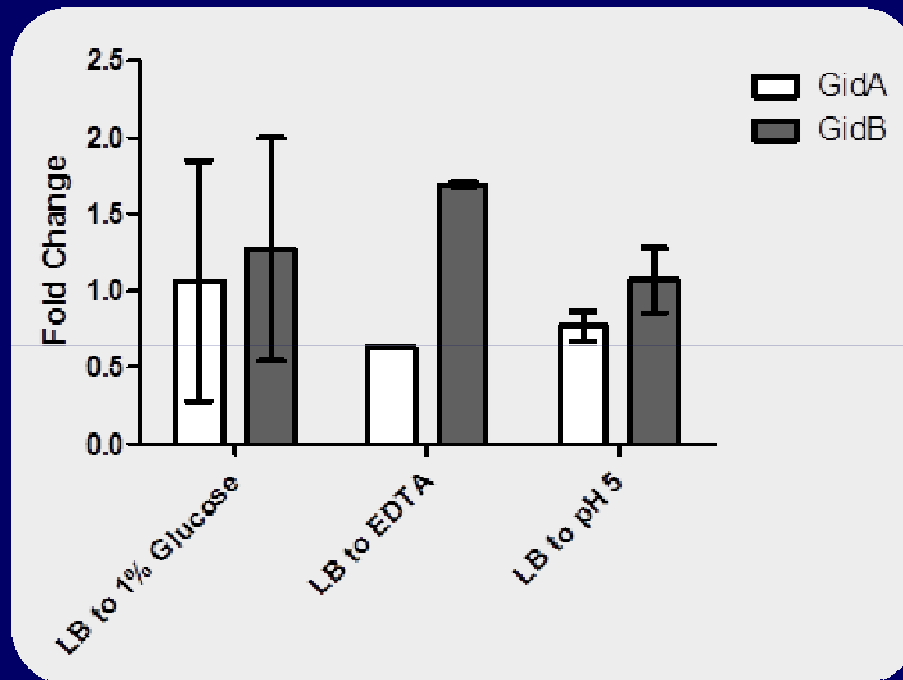
- ❖ Increased filamentous morphology of *gidA* mutant in growth media supplemented with glucose
- ❖ *gidA* thought to be modulated by the AsnC
- ❖ Bioinformatic analysis indicated two promoters



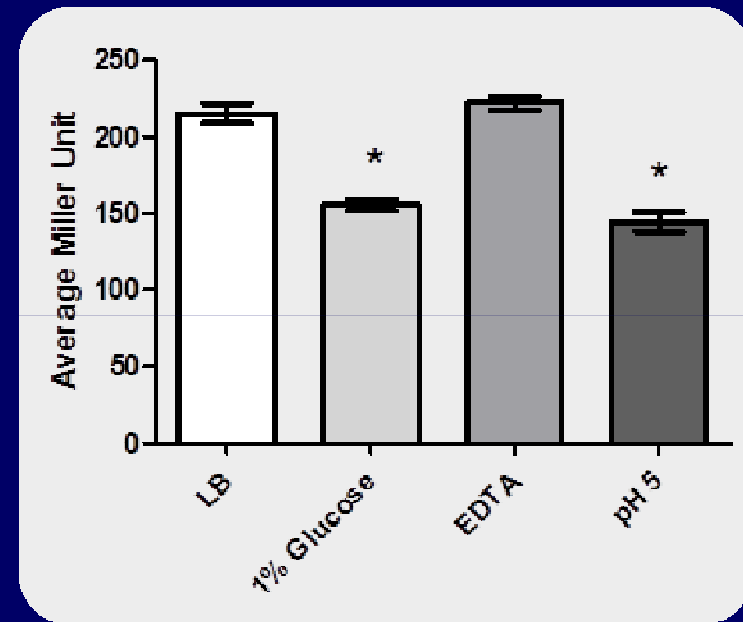
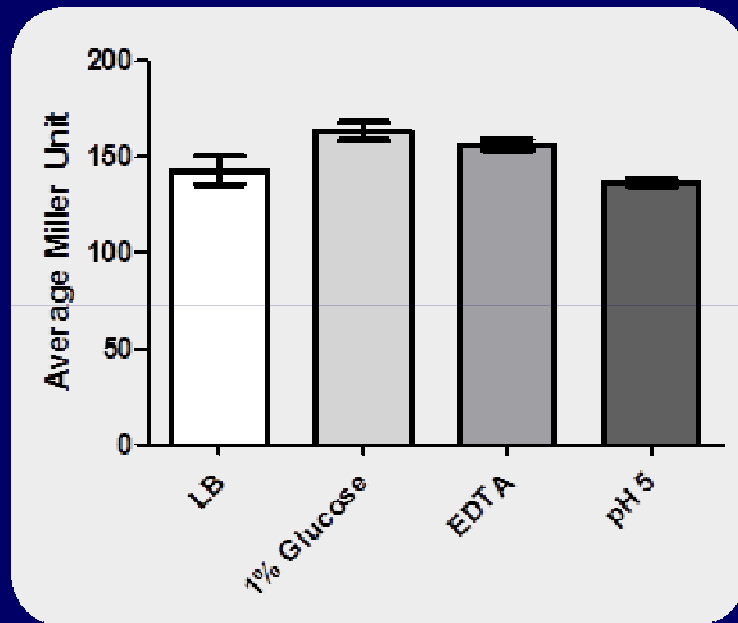
Transcriptional analysis of *gidAB*

- ❖ Real-time RT-PCR to detect *gidA* and *gidB* expression under different environmental conditions
- ❖ *gidA* and *asnC* promoter activity under different conditions using lacZ fusion assay
- ❖ Effect of *asnC* deletion on *GidA* expression

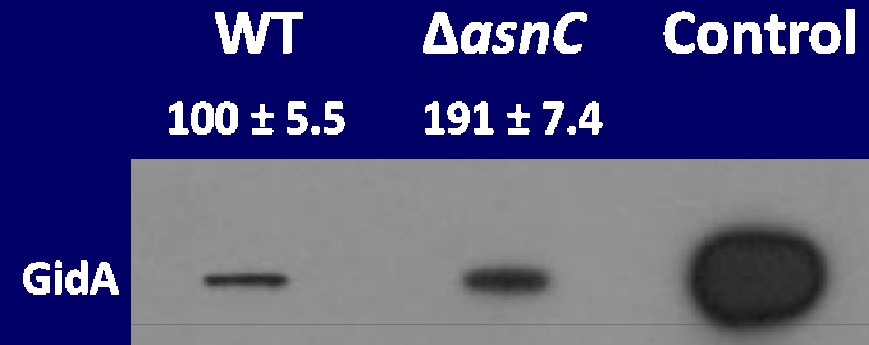
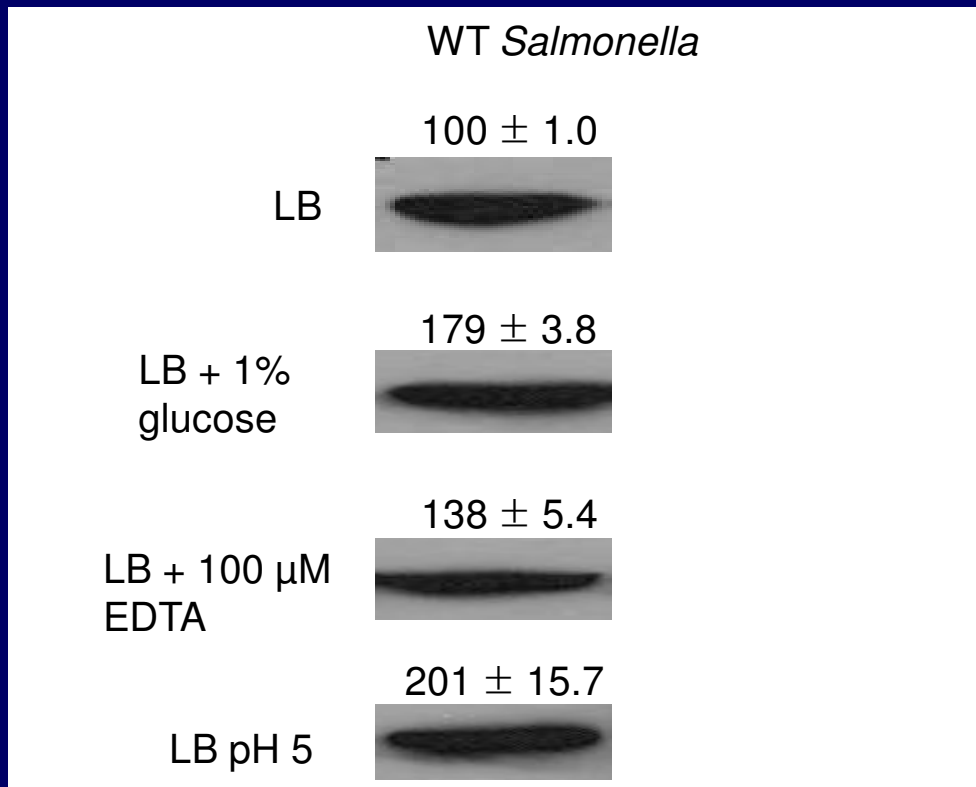
No significant difference in *gidA* and *gidB* expression at transcriptional level using real-time RT-PCR



The *lacZ* assay indicated no significant *gidA* promoter activity (left), while *asnC* promoter (right) showed a significant decrease in activity in media supplemented with 1% glucose and under acidic pH 5.

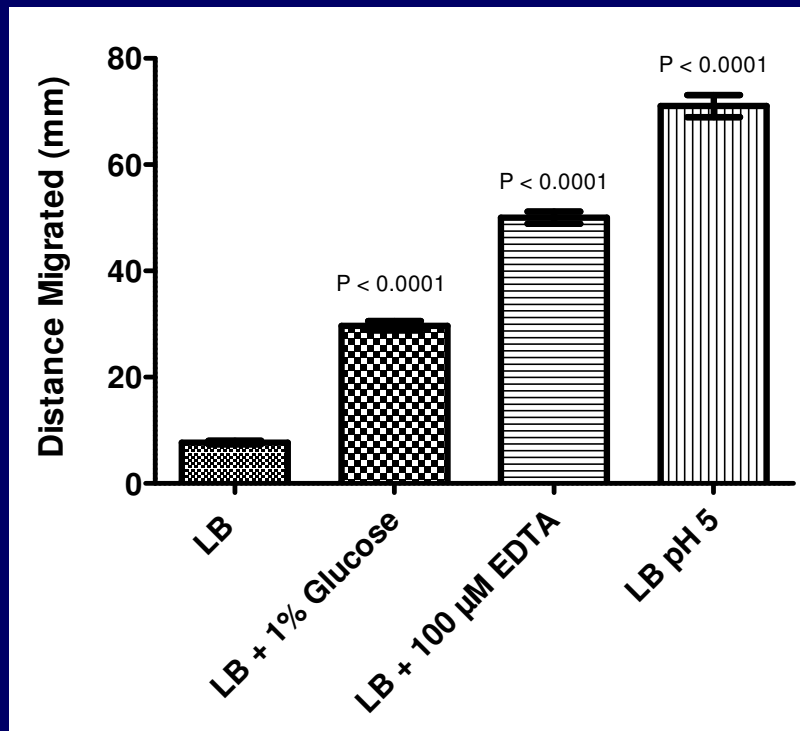
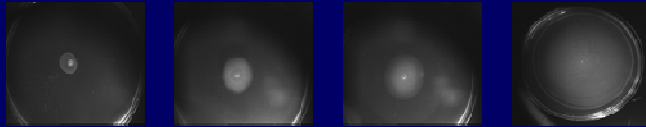


GidA Expression in *Salmonella* Grown Under Different Conditions

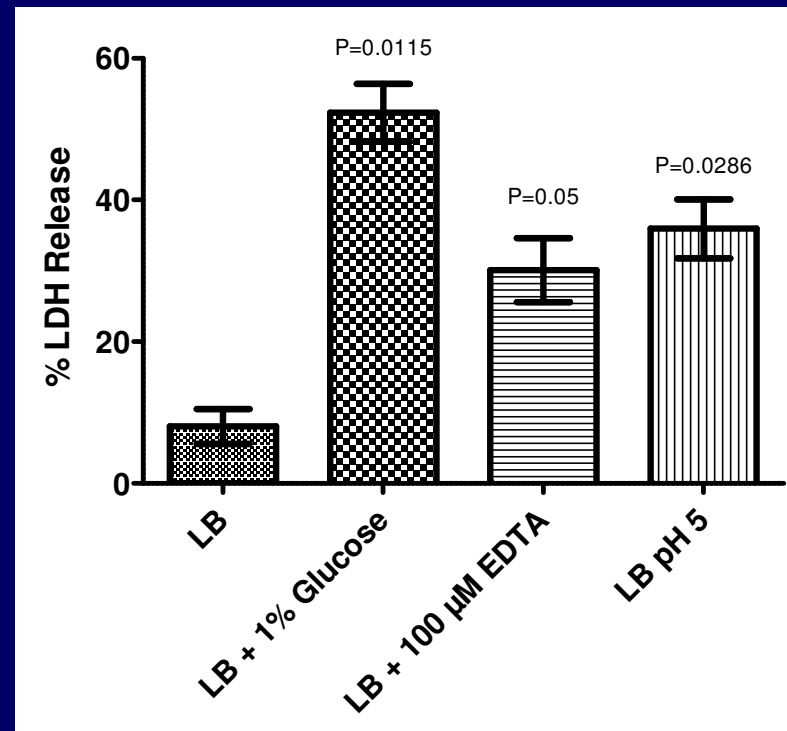


Deletion of *Salmonella asnC* increased GidA Expression

Virulence Assays under Growth Conditions



Motility



Cytotoxicity

Summary 2

- ❖ **Transcriptional analysis, using real-time RT-PCR, indicated no significant difference in *gidA* expression under various conditions.**
- ❖ **No *gidA* promoter activity, *asnC* promoter showed decreased activity under glucose and acidic pH.**
- ❖ **Significance increase in *GidA* protein expression under different conditions and when *asnC* deleted.**
- ❖ **Suggested that *GidA* expression is modulated by environmental condition and by the *AsnC* mostly at post-transcriptional level**

Acknowledgements



ITALIA

Nick

Katie

Megan

?

Dan

Jackie

Dareen

Acknowledgements (Collaborators)

- ❖ **Ralph Albrecht, Ph.D., Animal Sciences**
- ❖ **Mark Cook, Ph.D., Animal Sciences**
- ❖ **Philip Bochsler, DVM, Ph.D., WVDL**
- ❖ **Ogi Okwumabua, Ph.D., WVDL**
- ❖ **Richard Gourse, Ph.D., Bacteriology**
- ❖ **Charles Lauhon, Ph.D., School of Pharmacy**
- ❖ **Ashok Chopra, Ph.D., Microbiology & Immunology, UTMB**

Thank you. Question...comment?



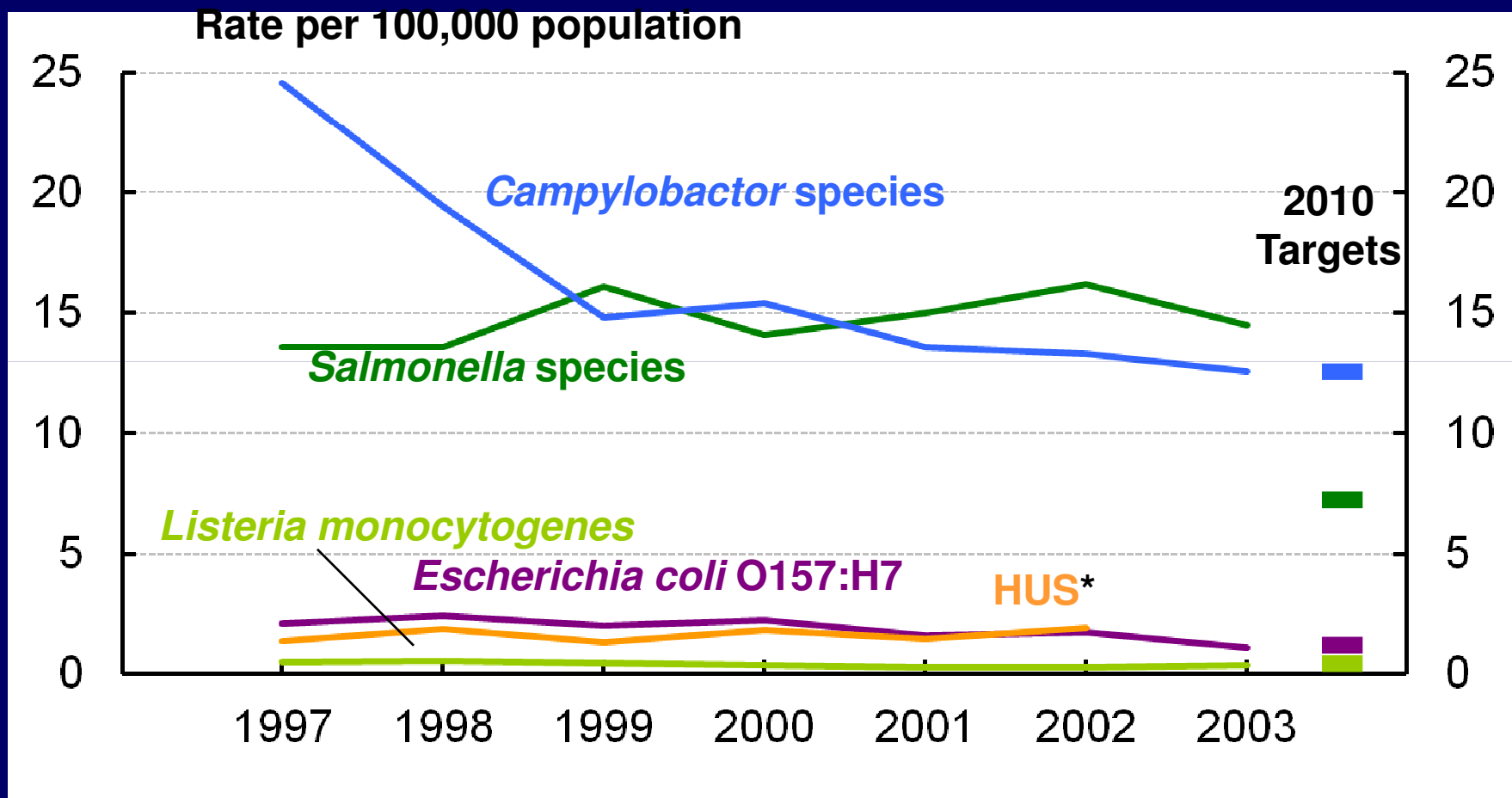
Post-transcriptional Modification of RNA: Effect on Biology and Virulence of *Salmonella*

Amin A. Fadl

University of Wisconsin-Madison



Background & Significance



Source: Foodborne Disease Active Surveillance Network (FoodNet)

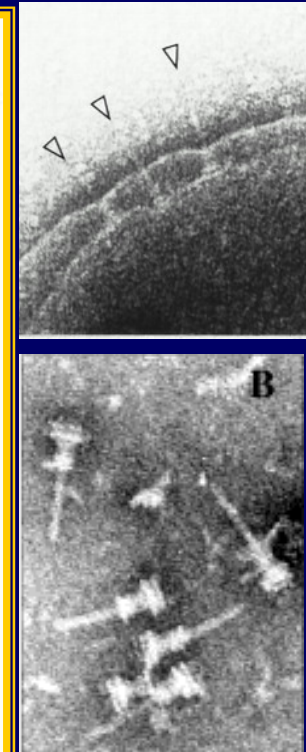
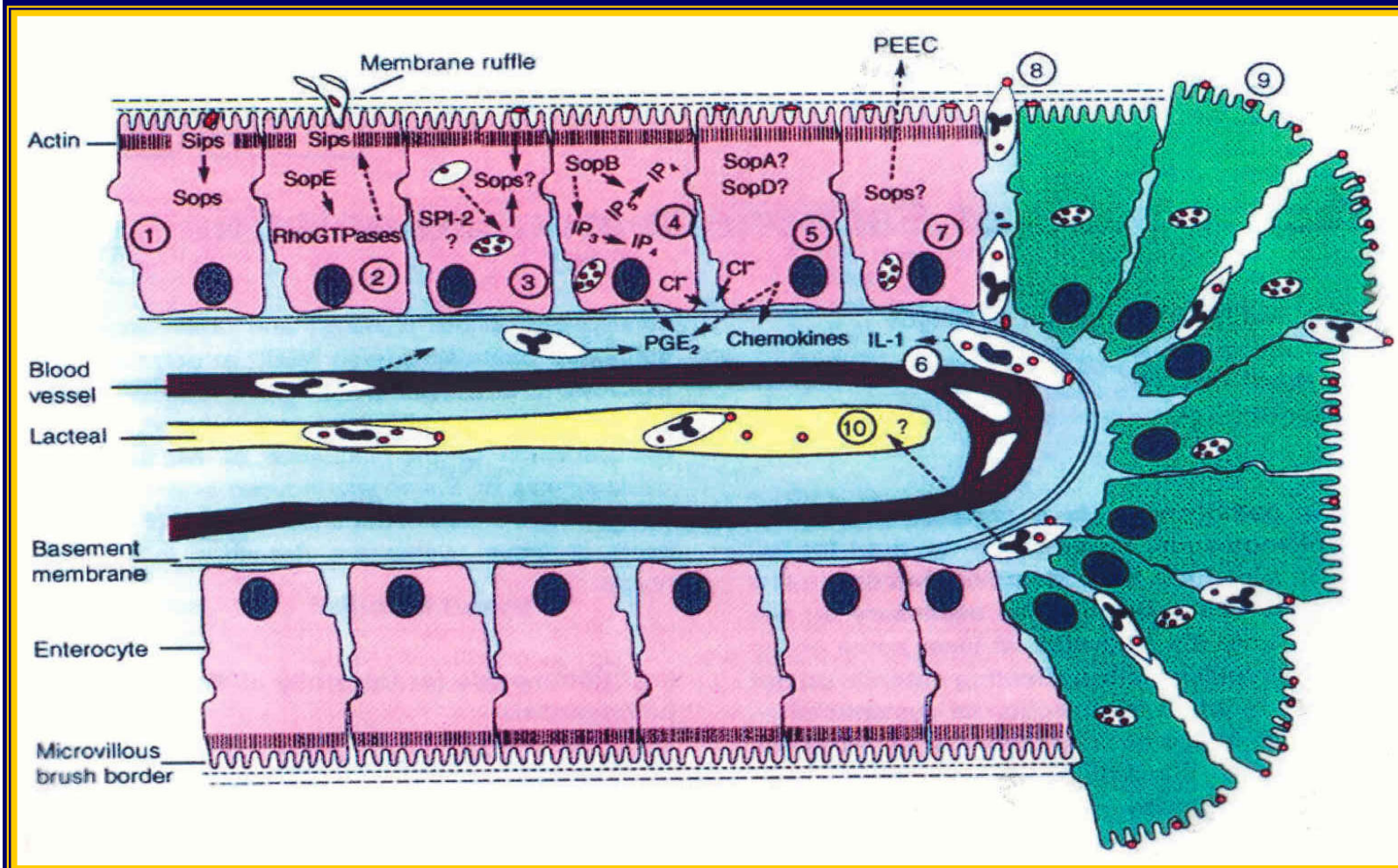
Background & Significance

	Cases	%
Overall foodborne illness	76,000,000	
Bacterial foodborne illness	4,200,000	5.5
Foodborne salmonellosis	1,400,000	1.7
salmonellosis from SE	194,408	0.25
Egg association: 40 to 80%	77,000- 155,000	< 0.20

Out of 1.4 million cases of salmonellosis, 95% (1.3 million) associated with food; 20% (234,000) from SE (about 75% associated with eggs).

* Cost \$23 billion (*Salmonella* \$2.65 billion)

Pathogenic mechanisms associated with *Salmonella* infections

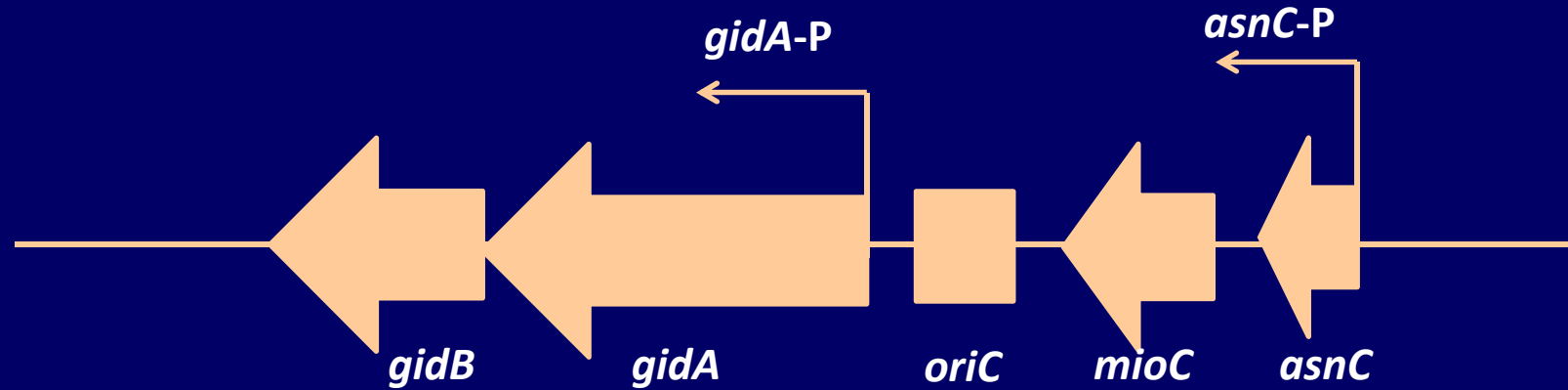


(Wallis and Galyov, 2000)

Altered genes/proteins expression of factors associated in cell division in the *gidA* mutant compared to the WT

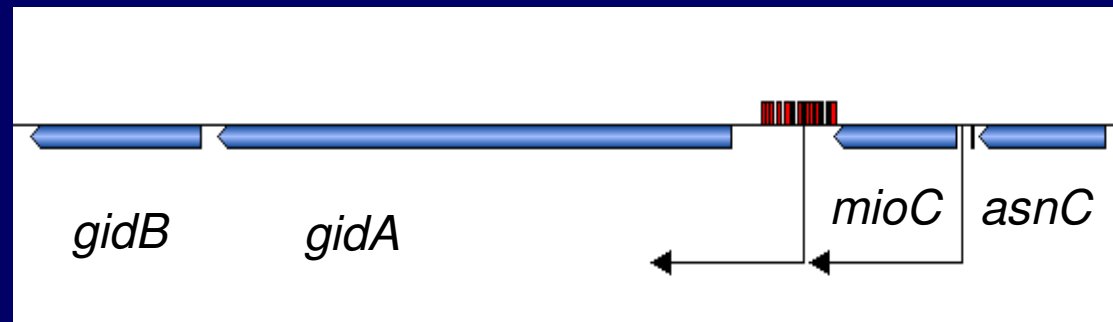
Gene Name	Gene #	Microarray FC	RT-PCR FC	WT	GidA
<i>mreB</i>	STM3374	-2.54	-2.27	100 ± 5.7	63 ± 2.1
<i>recX</i>	STM2828	-2.34	-5.28	100 ± 0.6	66 ± 1.5
<i>mukB</i>	STM0994	2.10	3.46	100 ± 8.6	115 ± 1.4
<i>parB</i>	PSLT053	5.45	5.08	100 ± 1.2	135 ± 1.7
<i>parA</i>	PSLT052	7.07	18.64	100 ± 1.4	461 ± 2.1
<i>xerC</i>	STM3949	2.08	9.56		
<i>yhbC</i>	STM3288	-2.35	-2.43		
-	STM1015	3.91	7.62		
-	STM2626	3.79	3.00		

gidAB Operon



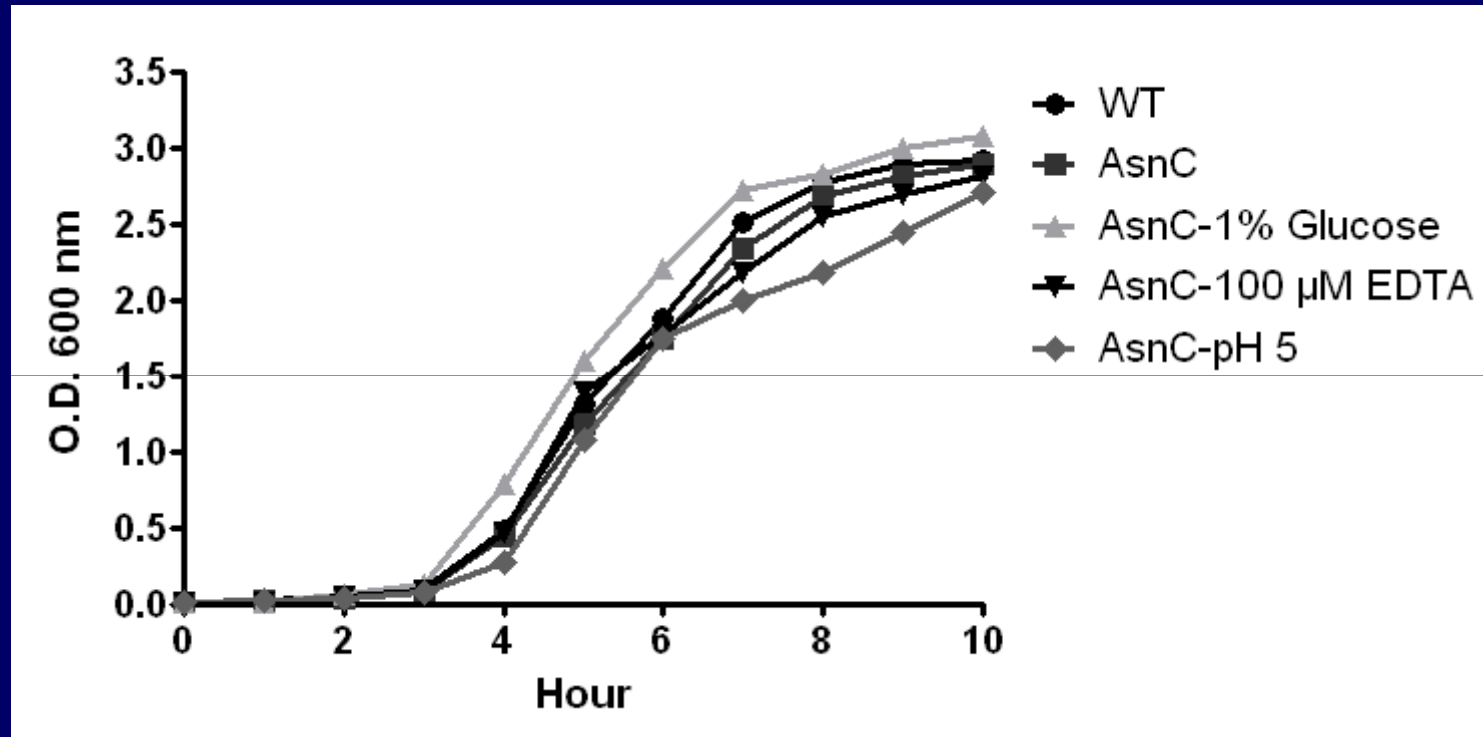
- Contains *gidA* and *gidB* genes

Regulation of *gidAB* operon



- *gidA* thought to be modulated by the AsnC

AsnC growth curve

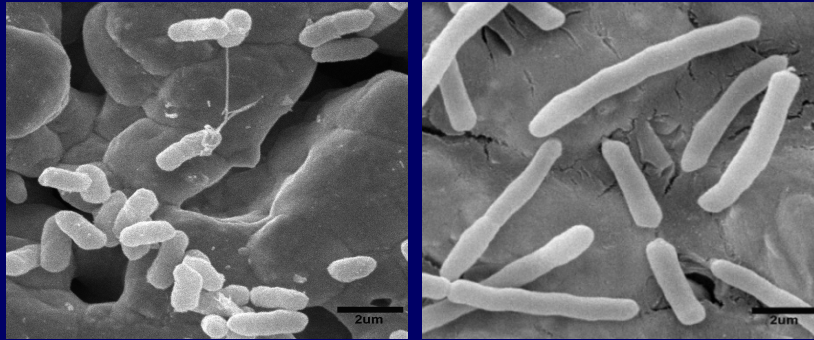


Mechanism of Filamentous Morphology

WT

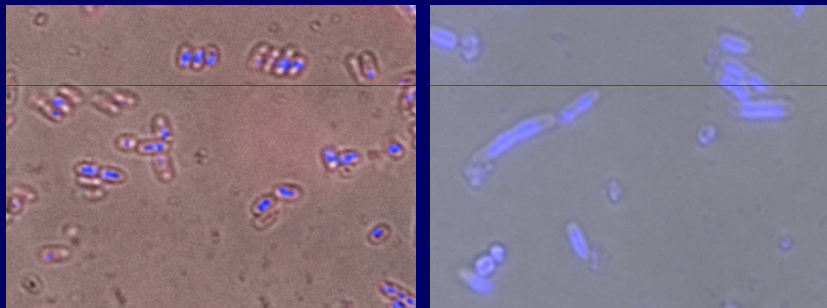
GidA

A



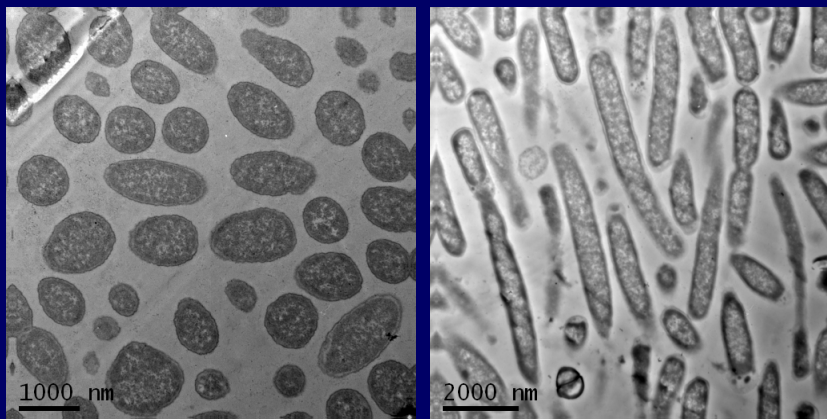
The majority of the *gidA* mutant cells appear to be one filament, with few signs of constriction.

B



The *gidA* mutant displaying a filamentous morphology with a defect in chromosome segregation.

C

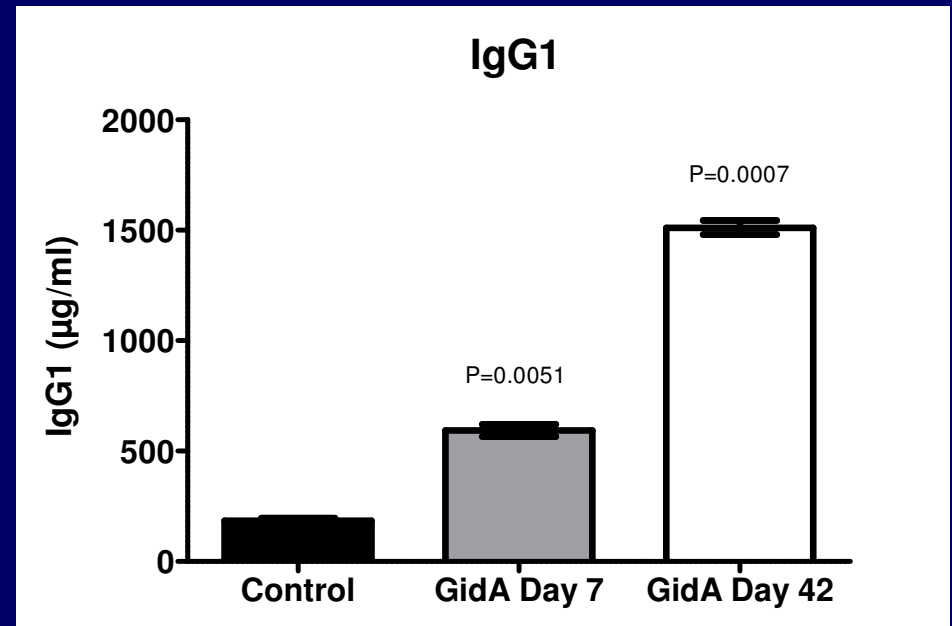
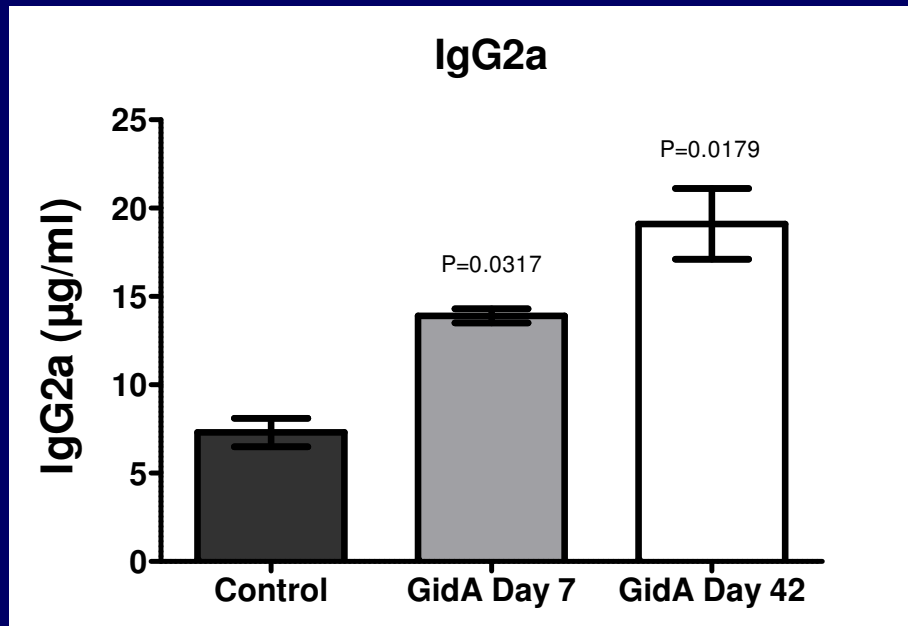


defect in chromosome segregation causing a filamentous morphology in the *gidA* mutant compared to the normal rod-shaped WT.

Summary

- ❖ The *gidA* mutant displayed the following phenotypes:
 - filamentous morphology
 - impaired motility, invasion of T84 intestinal epithelial cells, cytotoxicity, limited replication in macrophages
- ❖ Transcriptome and proteome analyses showed significant alterations in genes/proteins encoding for factors involved in *Salmonella* pathogenesis, indicating regulatory role
- ❖ The *gidA* mutant was attenuated in mice and animals immunized with *gidA* mutant protected from lethal dose of WT *Salmonella*

Th1/Th2 immune response



Deletion of *Salmonella asnC* increased *GidA* Expression



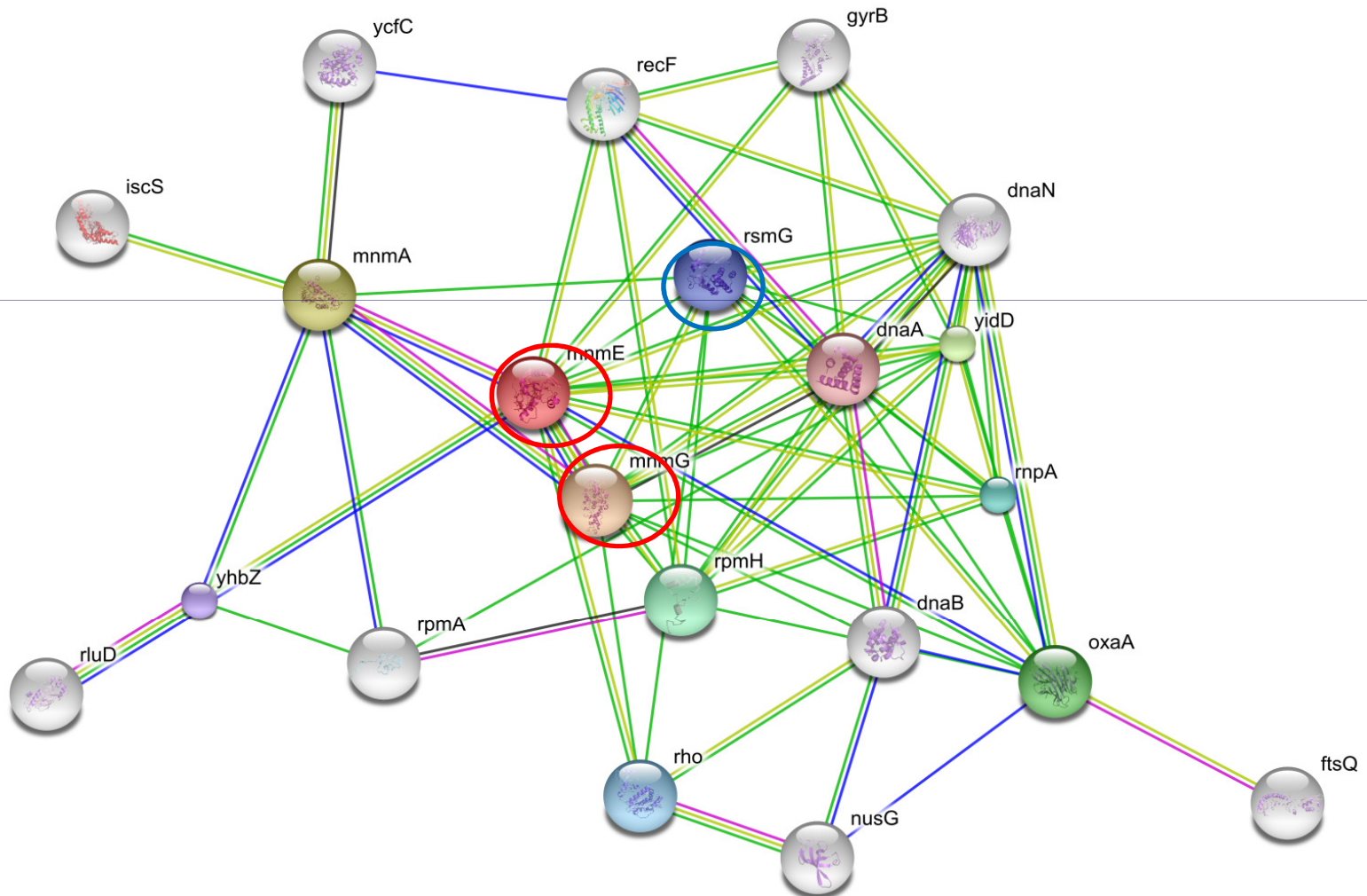
WT-14028 versus *asnC* mutant for *GidA* expression levels

Background & Significance

- ❖ **Model organism to study bacterial genetics and virulence.**
- ❖ **Major cause of food-borne diseases (poultry, meat, dairy products), use as an indicator of how safe a country's food supplies are**
- ❖ **Multiple antibiotic-resistance strains: use in animal feed**
- ❖ **FDA report: half of livestock and poultry feed meals and 16% of complete feeds contaminated with *Salmonella***

Predicted regulatory & functional association for GidA

(STRING 8.3 software, Jensen et al, 2009)



Effects on Biology & Virulence

- ❖ Morphological changes in *Aeromonas* and *E. coli* (filamentous), *Proteus mirabilis* & *Myxococcus* (colonial).
- ❖ Identified to modulate potent virulence factors: *Aeromonas* cytotoxic enterotoxin (Act), quorum sensing (RhlR expression) in *Pseudomonas*, inhibition of SpeB protease expression in *S. pyogenes*, *Shigella flexneri* altered transcription regulator VirF