New Signaling Pathways and Metabolites for Host: Pathogen Communication During Gastrointestinal Infections

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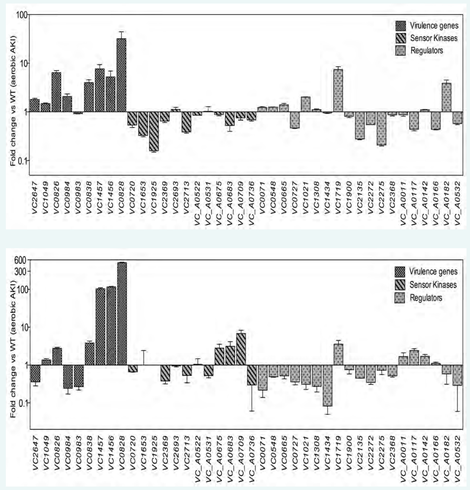
Summary:

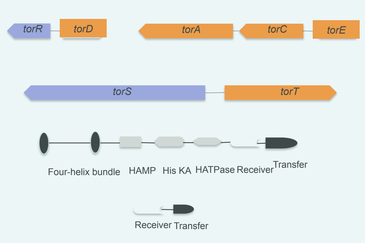
We report discovery of several bacteria two-component signaling pathways (TCS) and their cognate signals (host metabolites), which regulate toxin production in *Vibrio cholerae* and other enteric bacteria by mutually exclusive and novel non-canonical mechanisms. We also report two novel high-throughput and systems-biology portable assays to measure host and bacterial RNA expression profiles and metabolites of infected mice, and generate a "molecular signature" of diarrheal diseases. We first utilized high-throughput screening to identify bacterial TCS pathways regulating cholera toxin production, followed by genomics, digital gene-expression technology, RNA-Seq, metabolomics, proteomics and animal models to decipher their detailed mechanism. One TCS pathway acts a phosphorylation-mediated switch between bacterial virulence gene expression and host metabolism. It activates bacterial toxin production during hypoxia via a non-phosphorylated response regulator, and represses host metabolic process detrimental to pathogenesis in it's phosphorylated form. A second TCS pathway senses host potassium levels, and activates toxin production via tyrosine phosphorylation of its response regulator. Thus, this pathway can switch between aspartate and tyrosine phosphorylation of its cognate response regulator to modulate bacterial virulence and pathogenesis. Using sequencing and 2D LC/ESI/MS/MS proteomics, **Significance:** These data integrates bacterial signaling and host responses *in vivo* to identify a new landscape of host-pathogen interactions, metabolites and mechanisms for infectious diseases. We also report two novel RNA and metabolic profiling approaches that can be ported into systems-biology platforms, which will have wide utility in microbiology research.

Screening technologies

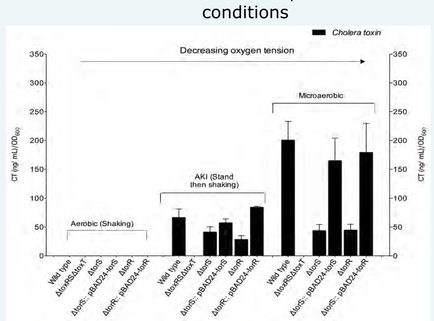
NanoString screen for TCSs expressed under virulent conditions



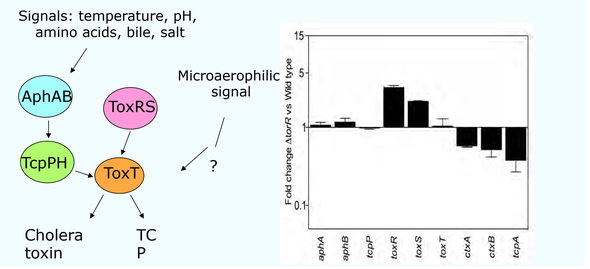
V. cholerae Tor TCSc (TTCSs)



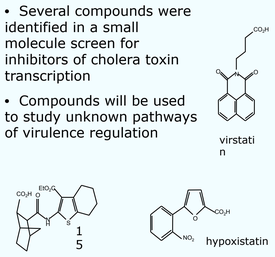
TTCSs is essential for virulence expression under hypoxic conditions

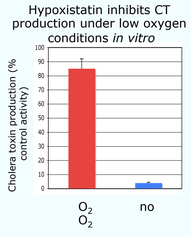


Non-classical regulation of *V. cholerae* virulence by the TTCSs



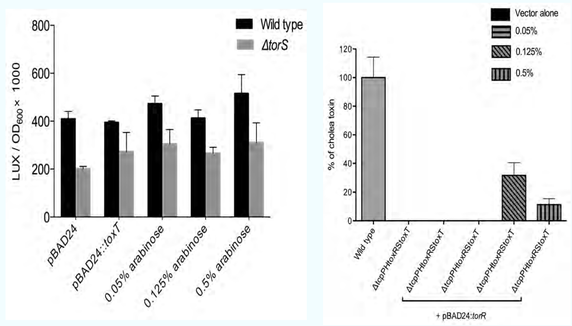
- Several compounds were identified in a small molecule screen for inhibitors of cholera toxin transcription
- Compounds will be used to study unknown pathways of virulence regulation



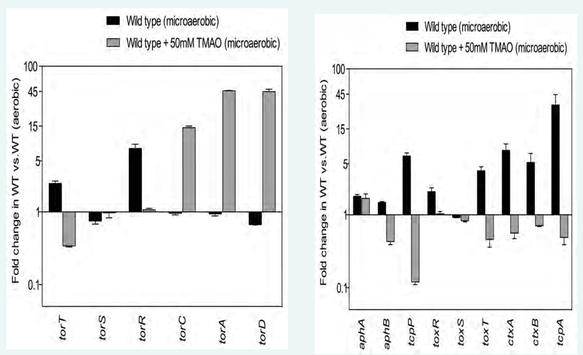


Overexpression of the **classical virulence pathway** in the **sensor and regulator** knockout strains is **insufficient** to restore virulence expression

Overexpression of the **regulator** in the **classical virulence pathway knockout strain*** is **sufficient** to restore virulence expression

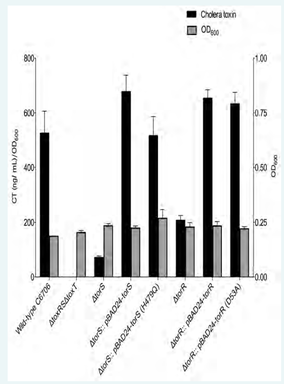


Dual signals: oxygen and/or TMAO regulate virulence and TTCSs expression

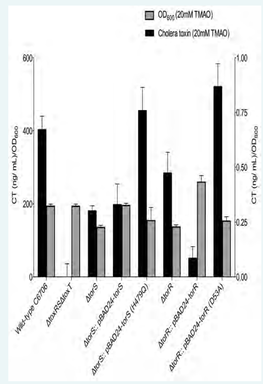


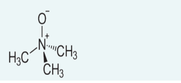
New pathway for cholera disease

Phosphorylation of the TTCSs is **dispensable** for **regulation of the classical virulence pathway**

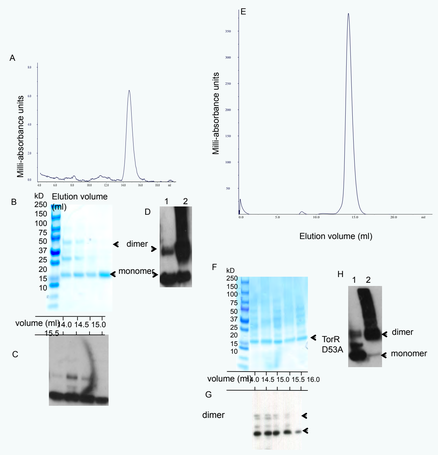


Phosphorylation of the TTCSs **is required TMAO metabolism** and **represses expression** of the **classical virulence pathway**

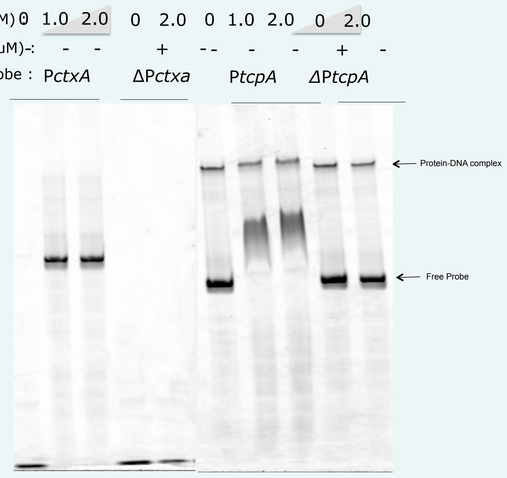




Phosphorylation-independent dimerization of the response regulator

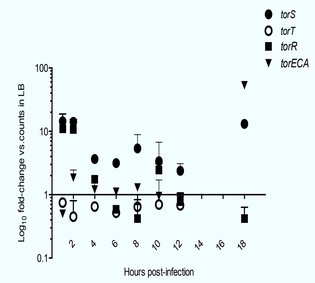


EMSA confirms **response regulator binding sites** in **promoter regions** of **cholera toxin and pilus genes**



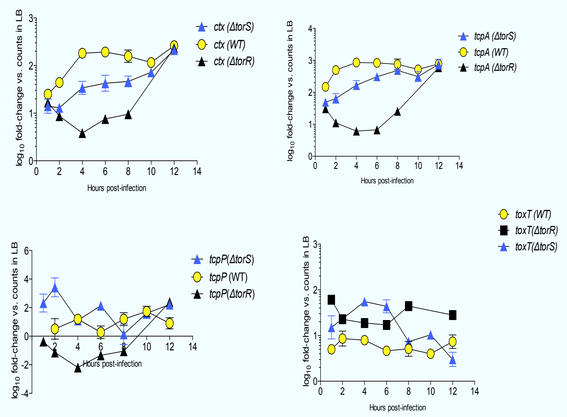
In vivo NanoString and Metabolomics

In the first two hours*: Sensor and regulator transcripts are up regulated in bacteria** *recovered from small intestines of* ***orogastrically infected mice

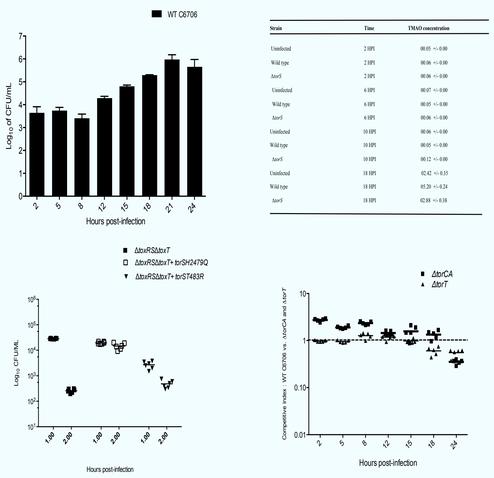


***At 18 HPI and later*: Sensor and regulator transcripts are NOT EXPRESSED. While the TMAO metabolic genes are UPREGULATED.**

Expression of classical virulence pathways are **not downregulated in** sensor or regulator knockout strains **during the course of infection**



Non-classical regulation



Strain	Time	TMAO concentration	
Uninfected	2 HPI	00.05	+/- 0.00
Wild type	2 HPI	00.06	+/- 0.00
ΔtorS	2 HPI	00.06	+/- 0.00
Uninfected	6 HPI	00.07	+/- 0.00
Wild type	6 HPI	00.05	+/- 0.00
ΔtorS	6 HPI	00.06	+/- 0.00
Uninfected	10 HPI	00.06	+/- 0.00
Wild type	10 HPI	00.05	+/- 0.00
ΔtorS	10 HPI	00.12	+/- 0.00
Uninfected	18 HPI	02.42	+/- 0.35
Wild type	18 HPI	05.26	+/- 0.24
ΔtorS	18 HPI	02.88	+/- 0.38