**Abstract**

The EnvZ/OmpR two-component system controls expression of the SsrA/B two-component system located on *Salmonella* Pathogenicity Island 2. SPI-2 encodes a type III secretion system which functions as a nanomachine to inject bacterial effector proteins into eukaryotic cells. During the intracellular phase of infection, *Salmonella* switch from secreting type three secretion system structural components to secreting effectors into the macrophage cytoplasm, enabling *Salmonella* to replicate in the phagocytic vacuole. Major questions remain regarding how bacteria survive the acidified vacuole and how acidification affects bacterial secretion. To address these questions, we employed a DNA-based FRET biosensor (“I-switch”) to measure bacterial cytoplasmic pH and immunofluorescence to monitor effector secretion during infection. Surprisingly, we observed a rapid drop in bacterial cytoplasmic pH upon phagocytosis that was not predicted by current models. Cytoplasmic acidification was completely dependent on the OmpR response regulator, but did not require known OmpR-regulated genes. Secretion followed acidification and blocking acidification of the macrophage vacuole resulted in a neutralized bacterial cytoplasm, which in turn blocked secretion. Based upon these findings, we developed a novel model for *Salmonella* infection involving an acid-dependent secretion process. Thus, when *Salmonella* is in an acidified intracellular compartment, its cytoplasm also acidifies. This acidification requires OmpR and drives activation and assembly of the SPI-2 type three secretion system as well as effector secretion. Supported by VA 1I01BX000372, NIH R01GM079 and MBI, NUS.

**References**


Wang et al., The inner membrane histidine kinase EnvZ senses osmolality via helix-coil transitions in the cytoplasm. *EMBO J.* 31(11): 2648-2659. PMC22543870.