Twin-arginine Translocation in *Yersinia*

The substrates and their role in virulence

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Abstract

Pathogenic *Yersinia* cause a manifold of diseases in humans ranging from mild gastroenteritis (*Y. pseudotuberculosis* and *Y. enterocolitica*) to pneumonic and bubonic plague (*Y. pestis*), while all three have a common virulence strategy that relies on a well-studied type III secretion system and its effector proteins to colonize the host and evade immune responses. However, the role of other protein secretion and/or translocation systems in virulence of *Yersinia* species is not well known. In this thesis, we sought to investigate the contribution of twin-arginine translocation (Tat) pathway and its secreted substrates to the physiology and virulence of *Y. pseudotuberculosis.* Tat pathway uniquely exports folded proteins including virulence factors across the cytoplasmic membranes of bacteria. The proteins exported by Tat pathway contain a highly conserved twin-arginine motif in the N-terminal signal peptide. We found that the loss of Tat pathway causes a drastic change of the transcriptome of *Y. pseudotuberculosis* in stationary phase at environmental temperature with differential regulation of genes involved in virulence, carbon metabolism and stress responses. Phenotypic analysis revealed novel phenotypes of the Tat-deficient strain with defects in iron acquisition, acid resistance, copper oxidation and envelope integrity, which we were partly able to associate with the related Tat substrates. Moreover, increased glucose consumption and accumulation of intracellular fumarate were observed in response to inactivation of Tat pathway implicating a generic effect in cellular physiology. We evaluated the direct role of 22 *in silico* predicted Tat substrate mutants in the mouse infection model and found only one strain, Δ*sufI*, exhibited a similar degree of attenuation as Tat-deficient strain. Comparative *in vivo* characterization studies demonstrated a minor defect for Δ*sufI* in colonization of intestinal tissues compared to the Tat-deficient strain during early infection, whereas both SufI and TatC were required for dissemination from mesenteric lymph nodes and further systemic spread during late infection. This verifies that SufI has a major role in attenuation seen for the Tat deficient strain both during late infection and initial colonization. It is possible that other Tat substrates such as those involved in iron acquisition and copper resistance also has a role in establishing infection. Further phenotypic analysis indicated that SufI function is required for cell division and stress-survival. Transcriptomic analysis revealed that the highest number of differentially regulated genes in response to loss of Tat and SufI were involved in metabolism and transport. Taken together, this thesis presents a thorough analysis of the involvement of Tat pathway in the overall physiology and virulence strategies of *Y. pseudotuberculosis*. Finally, we propose that strong effects in virulence render TatC and SufI as potential targets for development of novel antimicrobial compounds.

Keywords
Yersinia pseudotuberculosis, Tat, virulence, Tat substrates, SufI, stress response, metabolism, infection, transcriptome analysis

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