The role of *wzz* genes in *Salmonella typhimurium* virulence

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Abstract

Salmonella is a genus of Gram-negative bacteria responsible for food-borne enteritis and systemic fever. Salmonellosis continues to be a serious health burden worldwide. Lipopolysaccharide (LPS) is the dominant lipid component of the outer membrane of *Salmonella*. LPS is composed of lipid A, an oligosaccharide core, and a polymer of O units known as O antigen. Wzz proteins control the length of O antigen by an unknown mechanism. While O antigen is essential for *S. typhimurium* virulence, the significance of length regulation by Wzz is not known.

This study investigates the pathogenic relevance of the *wzz* genes in *S. typhimurium*. In addition to the previously recognised *wzz* gene (wzz_{ST}), a second gene with this function (wzz_{fepE}) was identified in the *S. typhimurium* genome. Whereas wzz_{ST} specifies the production of long LPS chains with a modal length of 16-35 O antigen repeat units, wzz_{fepE} conferred very long chains containing >100 O antigen repeat units. Strains carrying mutations in one or both *wzz* genes were constructed to investigate the role of *wzz* genes in virulence. It was found that *wzz*-controlled regulation of O antigen length was essential for resistance to the bactericidal activity of serum complement, while studies in the mouse model of infection found that *wzz* genes are essential for full virulence.

The *wzz* double mutant was complemented with heterologous *wzz* genes from a variety of bacterial sources. Despite variable sequence similarity of the encoded Wzz proteins each was functional in the *S. typhimurium* host, generating a panel of isogenic O antigen length variants. This panel of variants was used to define a minimum length requirement for complement resistance and identified relationships between O antigen length and complement consumption.

Finally, the regulation of O antigen chain length was investigated. It was found that growth either in iron limiting conditions or in serum caused an increased production of LPS with very long O antigen chains (conferred by wzz_{fepE}), resulting in increased complement resistance. The constitutive activation mutation of the *phoPQ* regulator (*phoP*^{c}) results in an altered O antigen distribution phenotype consistent with down-regulation of *wzz* genes. However PhoPQ had no effect on production of Wzz_{ST} as determined by immunoblotting with an anti-Wzz_{ST} antiserum.

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