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# *Salmonella typhimurium* LPS Confers Its Resistance to Antibacterial Agents and Acquired Invasion of Human Epithelial Cells: Complementation of *rfaE* Gene for ADP-L-Glycero-D-Manno-Heptose Biosynthesis of Lipopolysaccharide

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It is becoming increasingly apparent that microorganisms have developed the ability to interact with host cell receptor molecules to induce their own internalization. *Salmonella* is a highly invasive organism that can penetrate the intestinal brush border, seed, and proliferate within the reticuloendothelial system of its mammalian host. The invasive pathogenic bacterium, *Salmonella typhimurium,* is able to induce its own internalization into non-phagocytic cells. It is known that this ability to invade intestinal epithelial cells is an important early step in the pathogenic cycle of *Salmonella.* The initial step in the pathogenesis of samonellosis consists of bacterial attachment and entry into the epithelial cells that line the upper intestinal tract. Following adhesion and invasion, *Salmonella* remains in membrane-bound compartments during transit through epithelial cells to deeper tissues .

The lipopolysaccharide (LPS) of *S. typhimurium* functions as an important virulence determinant in *Salmonella* infections. *Salmonella* LPS is involved in immune evasion, attachment to epithelial cells and bactericidal antibodies. LPS is a molecule consisting of lipid A and oligosaccharide core domain as an outer membrane component in enteric and nonenteric Gram-negative bacteria. Lipid A of *S. typhimurium* consists of five to seven saturated fatty acids attached to a -1,6-linked glucosamine disaccharide. This is attached to the inner core with two 3-deoxy-D-*manno*-octulosonic acid (keto-deoxyoctonate; KDO) units followed by two units of heptose; the outer core region and the O antigen are attached to one of the heptose units. LPS activates complement and some forms are potent toxins, leading to LPS often being called endotoxin. The structural variation of LPS is based on changes in LPS biosynthesis as well as external modification of the LPS molecules by a surface-bound sialyltransferases. The carbohydrate structures in the outercore region typically mimic those of host cell glycosphingolipids, which may favour immune escape. LPS variation plays a multi-faceted role in the establishment of an infection; it allows the entry of the bacteria into human mucosal cells, while it can also switch to an LPS phenotype which results in bacteria resistant to the host immune defence. The molecular basis of this adaptive mechanism is that the amount of sialylation depends on oligosaccharide variation.

Our group at first time cloned the *rfaE* gene, which is involved in ADP-L-*glycero*-D-*manno*-heptose biosynthesis from *S. typhimurium,* using the *rfaE* mutant which produces heptose-deficient LPS having only lipid A and KDO. The cloned *rfaE* gene encoded a polypeptide of 477 amino acid residues with a molecular mass of 53 kDa. The *rfa* (*Waa*) gene cluster of *S. typhimurium* encodes enzymes for core oligosaccharide biosynthesis of LPS.

In this present study, the antibacterial mechanism of enterobacter *S. typhimurium* was studied. The *rfa* (*Waa*) gene cluster of *S. typhimurium* is encoding core oligosaccharide biosynthesis of lipopolysaccharide (LPS). Among *rfa* gene cluster, our group have first cloned the *rfaE* gene, which is involved in ADP-L-*glycero*-D-*manno*-heptose biosynthesis. The *rfaE* mutant synthesizes heptose-deficient LPS; its LPS consists of only lipid A and 3-deoxy-D-*manno*-octulosonic acid (KDO), and mutants, which make incomplete LPS, are rough mutants. *S. typhimurium* deep-rough mutants affected in the heptose region of the inner core show reduced growth rate, sensitivity to high temperature and hypersensitivity to hydrophobic antibiotics such as baicalin. Thus, we have complemented the cloned *rfaE* gene into *S. typhimurium rfaE* mutant strain SL1102 (*rfaE543*), which makes heptose-deficient LPS and has a deep rough phenotype. The complementation gave the smooth phenotype to the SL1102 strain. The sensitivity of SL1102 to bacteriophages was also recovered to wild-type strain, indicating that LPS is used for their receptor for bacteriophage infection. The permeability barrier of SL1102 to hydrophobic antibiotics such as novobiocin and baicalin was restored to that of wild-type, suggesting that antibiotic resistance of the wild type strain is highly correlated with their LPS. Through a agar diffusion assay, the growth-inhibition activity of baicalin was fully observed in mutant SL1102 strain, however, only a half of the inhibitory activity was detected in *rfaE*-complemented SL1102 strain. Furthermore, LPS produced by *rfaE*complemented SL1102 strain was indistinguishable from LPS biosynthesis of smooth strains.

On the other hand, author reports the functional analysis of LPS for bacterial infection into human epithelial cells using complementation with the *S. typhimurium rfaE* gene. Invasion of host cells is essential for the pathogenicity of *Salmonella*. Author group has recently reported the cloning and function of the *rfaE* gene of *S. typhimurium* previously implicated in lipopolysaccharide (LPS)-inner-core biosynthesis (Jin *et al.*, 2001; Kim, 2002). The *rfaE* gene is involved in ADP-L-*glycero*-D-*manno*-heptose biosynthesis. The *rfaE* mutant synthesizes heptose-deficient LPS (Re-LPS); its LPS consists of only lipid A and 3-deoxy-D-*manno*-octulosonic acid (KDO), and mutants, which make incomplete LPS, are rough mutants. *S. typhimurium* deep-rough mutants affected in the heptose region of the inner core show reduced growth rate and sensitivity to high temperature. Complementation of *S. typhimurium rfaE* mutant strain SL1102 (*rfaE543*) with the *rfaE* gene demonstrated conclusively that the gene gave the smooth phenotype to the SL1102 strain. LPS produced by *rfaE*complemented SL1102 strain was indistinguishable from that of smooth strains. Infection experiments *in vitro* demonstrated that the complementation of *S. typhimurium rfaE* gene into the mutant restored invasive potentials of human Chang epithelial cells, human larynx epidermal carcinoma HEp-2 cells and intestinal epithelial Henle-407 cells. These data imply that the LPS phenotype is a critical factor for *Salmonella* invasiveness and invasion of cultured epithelial cells by *S. typhimurium* is accompanied by *Salmonella* lipopolysaccharide.