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## Involvement of TolC protein in the export of siderophore enterobactin in *Escherichia coli*

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To acquire the necessary iron against harsh competition in the environment, iron starved bacteria synthesize, excrete and retrieve iron scavenging molecules termed siderophores, one of which is enterobactin. TolC protein may play a vital role in the secretion of enterobactin. Enterobactin molecules destined for secretion must cross both the inner (cytoplasmic) and outer membranes and the intervening periplasmic space, believed to be a distance of at least 130Å across. TolC resembles a trans-periplasmic tunnel embedded in the outer membrane of the cell. It is open to the external environment but is closed at its periplasmic entrance. In order for the cell to export enterobactin, TolC is recruited by substrate specific membrane complexes (translocases) in the periplasmic space and inner membrane. When TolC is recruited, the entrance is opened to allow substrate passage through a continuous machinery spanning the entire cell envelope, from the cytosol to the external environment. PCR primers specific for TolC were designed to amplify the TolC gene. The quality of the PCR product was confirmed using agarose gel electrophoresis. The TolC gene was cloned into a pBAD directional TOPO vector containing an N-terminal His-tag and a gene for kanamycin resistance. The recombinant vector was then transformed into One Shot TOP10 competent *Escherichia coli* cells. Transformants were selected for by plating on LB medium supplemented with kanamycin. Transformed colonies were analyzed using PCR and restriction digestion. Positive transformants were selected and expression was induced with arabinose. SDS-PAGE assay with His-tag In-gel stain revealed TolC expression. Furthermore, analysis of TolC-null mutations using high performance liquid chromatography (HPLC) reveals that the TolC mutant secretes little, if any, enterobactin. However, some levels of breakdown products 2,3-dihydroxybenzoylserine (DHBS) monomer, dimer, and trimer are observed. These data establish that TolC may be a critical component of the *E. coli* enterobactin secretion machinery and may represent a type of siderophore export mechanism previously undescribed. TolC family proteins are ubiquitous among gram-negative bacteria, and the conserved apertures present a possible chemotherapeutic target in multidrug-resistant pathogens.