ENTEROBACTIN EXPORT IN *ESCHERICHIA COLI* VIA P43 (ENTS) AND ASSOCIATED COMPONENTS

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ABSTRACT

Ferric iron, critical for the metabolic functions of many microorganisms, is generally insoluble at neutral pH or quickly complexed by host iron storage proteins. To acquire necessary ferric iron against harsh competition in the environment, iron-starved *Escherichia coli* synthesize, excrete and retrieve an iron-scavenging siderophore molecule termed enterobactin. Despite extensive characterization of the enterobactin system, the export machinery allowing enterobactin secretion to the extracellular environment has only recently been identified. *E.coli* membrane protein P43 *(entS)* in the enterobactin gene cluster encodes a Major Facilitator Superfamily (MFS) exporter. A P43 null mutant was unable to efficiently secrete enterobactin to the supernate, but did secrete elevated levels of enterobactin breakdown products as analyzed by TLC, HPLC, and cross-feeding assays. To further evaluate P43 function in enterobactin transport, inverted membrane vesicles were created using French press and incorporated with an iron-binding fluorescent dye, calcein-AM (CA). Differences in siderophore transport were observed between wild-type and the P43-mutant by monitoring CA fluorescence restoration following iron quenching and the addition of enterobactin. Using specific energy poisons in conjunction with this vesicle system, it was determined that proton motive force energy is utilized for this transport. Additional results demonstrate that siderophore transport from the periplasm to the external environment may be due to contributions from several other identified *E.coli* components, such as the multi-drug export system comprised of the outer membrane protein TolC and the translocase AcrAB. These data all demonstrate P43 provides a critical activity for the *E.coli* enterobactin secretion machinery and establish a mechanism for cellular release of siderophore.