Public Abstract

First Name:Jason

Middle Name:Lawrence

Last Name:Furrer

Adviser's First Name:Mark

Adviser's Last Name:McIntosh

Co-Adviser's First Name:

Co-Adviser's Last Name:

Graduation Term:FS 2006

Department:Microbiology- Medicine

Degree:PhD

Title:Enterobactin Export in *Escherichia Coli* Via P43 (EntS) and Associated Components

Iron is one of the most environmentally abundant and versatile of the transition metal elements and is required for the growth of nearly all cells. Bacteria have need of iron for a range of metabolic functions including amino acid synthesis, DNA synthesis, and most importantly – some virulence traits. It is well known that the virulence of organisms such as *Escherichia coli*, *Shigella*, and *Salmonella* are all increased by excess iron. It is therefore important to establish the relationships between iron and bacteria. Despite the abundance in the environment, iron is problematic to acquire. Iron forms insoluble complexes in certain environments, which severely limits concentrations. Mammalian cells further sequester iron to prevent bacterial iron acquisition, thus reducing available free iron to almost non-existent in human serum. Faced with this drastic iron shortage in a pathogenic situation, bacteria must find alternative means of iron acquisition for survival. One mechanism developed by bacteria to acquire iron is the use of siderophores. Siderophores, literally meaning “iron carriers,” are small, potent iron-binding molecules. Over 500 different siderophores have been described for bacteria. Despite the extensive body of work done on these significant siderophore systems, in no organism to date has there been a defined export mechanism. The most studied siderophore system is the enterobactin system of *E.coli*, which can successfully compete against all known proteins for iron with unparalleled affinity. The goal of this dissertation work is to illuminate the mechanisms of enterobactin export in *E.coli* and apply results to other organisms. We characterized a membrane protein (*E.coli* P43) that serves as the export pump for enterobactin. To prove this, we created and tested a P43-null mutant for ability to export enterobactin. Further evidence was provided by monitoring cells for enterobactin release with thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Final confirmation of P43 involvement in enterobactin export came from creating a novel inverted membrane vesicle system to monitor direct transport of enterobactin by fluorescence. Secondly, this work addressed the fact that P43, as an inner membrane protein, would likely only allow enterobactin to cross one of the two *E.coli* membranes and we characterized potential interaction partner proteins for P43. These findings are significant because proteins similar to P43 have been reported in other siderophore systems, suggesting bacteria utilize similar methods of export. Understanding siderophores is significant due to their potential therapeutic uses including reduction of metal imbalances in the body (implicated in Alzheimer’s disease, cardiac disorders, and thalasseimas) or for the creation of new treatments for bacterial diseases using antibiotics coupled to siderophores as “Trojan horse” molecules. Finally, there is an additional goal to my graduate education. During my graduate training program and with the help of my committee, I have pursued a Graduate Minor degree in College Teaching. This section attempts to address education questions such as “how do we best teach microbiology to our students” and “how do we prepare our microbiology graduate students to become teacher-scholars” by considering the place of teacher training in a graduate education, experiences with teaching and learning, earning a minor in addition to a scientific doctorate, and additional coursework and preparations incorporated into my training. We feel it is important to leave a trail for future graduate students that may be interested in teaching and for myself to better understand what it means to be a member of the professoriate involved in the academic pursuits of teaching, research, and service.