Department of Molecular and Cellular Biology

Graduate Seminar MCB*6500

Friday, Feb. 3, 2017 in SSC 1511 @ 12 noon

presented by:

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Characterization of the putative 1,2-glucosyltransferase, WapB, in *P. aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen known to cause disease in debilitated hosts, especially in those with cystic fibrosis. The core oligosaccharide (OS) is a main constituent of the lipopolysaccharide (LPS) in *P. aeruginosa* and is divided into inner and outer core. The outer core can be synthesized as one of two glycoforms and both consist of D-glucose (D-Glc), L-rhamnose (L-Rha), and D-galactosamine (GalN). Glycoform 1 is "uncapped", meaning that it contains a terminal glucose (Glc^{IV}) and is not an appropriate acceptor for O-antigen. Glycoform 2 is capped, hence, it lacks Glc^{IV} and is substituted with an O-antigen bonded to L-Rha. The focus of my project is to characterize WapB, a putative 1,2-glucosyltransferase that transfers Glc^{IV} to the uncapped core. A knockout mutant of *wapB* has been shown to form a truncated LPS core. At present, little is known regarding the enzymatic properties or protein structure of WapB. I will test the hypothesis that WapB is a 1,2-glucosyltransferase whose activity is to transfer a Glc residue from the substrate UDP-Glc to the terminal locus of the core OS of certain *P. aeruginosa* serotypes. Since glycosyltransferases tend to be insoluble when expressed and purified, I will design truncated versions of WapB that lack the C-terminal hydrophobic residues and determine the optimal conditions for expression and purification. A Malachite green-based assay will be carried out for determining enzyme-substrate kinetics. I will also initiate crystallization screens to study the 3D protein structure and to understand its enzymatic mechanisms, i.e., with time permitting.