LIGHTNING TALK:

Brian Lowrance

Structural and functional characterization of the *Clostridioides difficile* glycosyl hydrolase CcsZ

Brian Lowrance, Joel T. Weadge

Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada

Microbial life often exists in the form of diverse communities residing within a protective exopolysaccharide matrix known as a biofilm. Biofilms support these communities by sequestering water and nutrients, providing resilience against external stressors including mechanical force, UV radiation and desiccation, and also contributing to immune evasion, antibiotic resistance and accelerated cell growth. Within the medical industry, the impact of microbial biofilm formation in disease pathogenesis and antimicrobial resistance are becoming increasingly evident, making research into biofilm biosynthesis a rapidly growing point of interest. Various organisms employ different cellular materials and biosynthetic machinery to produce these exopolysaccharide matrices. Cellulose biofilms are one form that are increasingly discovered in nature and the biosynthetic mechanisms have been observed and characterized in the Gram-negative model organisms *Escherichia coli* and *Salmonella*, where they were labelled the Bacterial cellulose synthesis complex (Bcs). Gram-positive organisms, including the medically relevant *Clostridioides difficile* and related *Clostridium botulinum*, *Clostridium tetani* and *Clostridium perfringins,* are also known to produce biofilms composed of cellulose, yet the underlying mechanisms of biosynthesis remain elusive. *C. difficile,* among others, has been identified to possess a putative cellulose synthesis operon, encoding for the Clostridial cellulose synthase complex (Ccs), featuring the GH5 family glucanase CcsZ that we propose functions in the essential regulation of polymer length and release from the cell. Herein, we characterize the structure and function of CcsZ and explore its role in biofilm biosynthesis. Accordingly, mass spectroscopy and *in vitro* experimentation have verified that CcsZ exhibits endoglucanase activity while functioning optimally at a pH of 4.5 and displays a preference for cellulose over other β1,4-linked glucans. The crystal structure was solved and elucidates the core catalytic residues responsible for hydrolysis as well as the $(\alpha/\beta)_8$ TIM barrel, which differs from its Gram-negative, GH8 counterpart (BcsZ) that exhibits a $(\beta/\beta)_6$ barrel. To compliment these results, we are conducting kinetic analyses with the native protein, in addition to active site mutants, to provide insight into the mechanism of action. Taken together, this will provide the basis for our understanding of Gram-positive cellulose biosynthesis and act as a foundation to expand our knowledge that may lead to new measures to counteract infections caused *C. difficile*, other Clostridia and Gram-positive bacteria as a whole.