University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

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BSc Hons (University of Victoria, 2012)

"Carrageenan Desulfation and Depolymerization by the Marine Isolate *Pseudoalteromonas* sp. PS47"

Department of Biochemistry and Microbiology

Thursday, December 6, 2018
9:00 A.M.
Clearihue Building
Room B017

Supervisory Committee:
Dr. Alisdair Boraston, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Francis Nano, Department of Biochemistry and Microbiology, UVic (Member)
Dr. John Burke, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Thomas Fyles, Department of Chemistry, UVic (Outside Member)

External Examiner:
Dr. Nicole Koropatkin, Department of Microbiology & Immunology, University of Michigan

Chair of Oral Examination:
Dr. Kieka Mynhardt, Department of Mathematics and Statistics, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Carrageenans are sulfated polysaccharides found in the cell walls of red algae with 20–30% of the dry weight coming from sulfate esters. The understanding of how heterotrophic bacteria desulfate and depolymerize carrageenan has become a rather arduous endeavor as there are 15 different classes of carrageenan distinguished by the degree of sulfation and the presence or absence of a unique galactose derivative, the 3,6-anhydro-D-galactose. The depolymerization of carrageenan requires the removal of the sulfate substituents, a role fulfilled by sulfatases, which hydrolyze sulfate esters playing a key role in the regulation of sulfation states that determine the function of sulfated biomolecules. Through structural, mechanistic, and sequence-based studies a highly conserved sulfate-binding motif has been identified among sulfatases; however, the molecular determinants for substrate specificity remain largely speculative. Additionally, the largest sulfatase family S1, requires a unique catalytic residue resulting from a post-translationally modified cysteine in order to be functional thus making them difficult to study in vitro. Using a strain of *Pseudoalteromonas* sp. PS47 isolated in the Boraston Lab I show that the depolymerization of carrageenan is dependent on the degree of sulfation and that recognition of the leaving group is the driving force behind S1 specificity. With little information on the recognition of sulfated biomolecules, the X-ray crystal structures of the three sulfatases from PS47; PsS1_19A, PsS1_19B, and PsS1_NC in complex with their biological substrates provides a deeper understanding of how carbohydrate specific sulfatases recognize their cognate substrate and how this recognition of the leaving group can be extended to other S1 sulfatase families. Furthermore, I show that an *exo*-acting glycoside hydrolase (PsGH42) requires desulfation of carrageenan oligosaccharides before it can hydrolyze the β-glycosidic linkage, a new specificity of family 42. This research demonstrates how carrageenan depolymerization is entirely dependent on the functionality and specificity of the sulfatases found within the carrageenan utilization locus.