University of Victoria | Graduate Studies

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

of

MELISSA CID

MSc (Université Louis Pasteur, 2008)

"Host Glycan Degradation by *Streptococcus pneumoniae*"

Department of Biochemistry and Microbiology

Thursday, July 16, 2015
1:00 P.M.
David Strong Building
Room C124

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. Chris Nelson, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Réal Roy, Department of Biology, UVic (Outside Member)

External Examiner:
Dr. Margo M. Moore, Department of Biology, Simon Fraser University

Chair of Oral Examination:
Dr. Patricia MacKenzie, School of Social Work, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Streptococcus pneumoniae is a commensal inhabitant of the human nasopharynx that can sometimes become pathogenic and cause diseases such as pneumonia, otitis media and meningitis. Carbohydrate metabolism is a critical component of *S. pneumoniae* virulence. Among the myriad of carbohydrate-specific pathways involved in the hostpneumococcus interaction, the N-glycan foraging pathway stands out because of its direct implication in numerous aspects of virulence such as fitness, adhesion/invasion and impairment of the host immune response. Much of the literature has been focussed on the importance of step-wise depolymerisation of N-glycans by the enzymes NanA, BgaA and StrH. However, the importance of the liberation of N-glycans from host glycoconjuguates and their intake by the bacterium has yet to be examined. We have identified a Carbohydrate Processing Locus (CPL) that is highly conserved throughout a large number of Firmicutes and whose individual components appear widespread in bacteria that we hypothesize is active on host N-glycans. This locus encodes for two putative α-mannosidases GH92 and GH38, a characterised α-mannosidase GH125, a putative β-hexosaminidase GH20C, a putative α-fucosidase GH29 and a ROK protein. The genomic context of CPL orthologues suggests that an endo-β-N-acetylglucosaminidase (EndoD) and an ABC transporter ($ABC_{N\text{-}glycan}$) are functionally associated with this locus. Based on our bioinformatic analyses and known functions of these proteins we hypothesize that the CPL encodes a concerted pathway responsible for the liberation, transport, and processing of N-glycans. The objective of this research is to characterize the putative components of this pathway and assess their implication in virulence. Specific focus on $ABC_{N\text{-}glycan}$ demonstrated its specificity for a range of N-glycans liberated by EndoD, shedding light on a novel import system for branched N-glycans. Furthermore, we provided evidence that GH92 is an α-1,2-mannosidase that likely removes the terminal mannose residues found on high-mannose N-glycans. EndoD and GH92 are shown to participate in virulence in mice; however, their role in virulence has yet to be determined. This work will significantly advance the construction and validation of a model of Nglycan processing by *S. pneumoniae*. As the components of this model pathway are conserved amongst a wide variety of bacteria, this work is of fundamental relevance to understanding how microbes from various environments degrade and metabolize Nglycans.