University of Victoria | Graduate Studies

Notice of the Final Oral Examination for the Degree of Master of Science

of

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BSc (University of Victoria, 2013)

"Investigation of a Putative Type I Secretion System and Potential Substrates in *Treponema pallidum*, the Causative Agent of Syphilis"

Department of Biochemistry and Microbiology

Monday, July 4, 2016
1:00 P.M.
Clearihue Building
Room A207

Supervisory Committee:
Dr. Caroline Cameron, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Caren Helbing, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Steve Perlman, Department of Biology, UVic (Outside Member)

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Dr. Robert Chow, Department of Biology, UVic

Chair of Oral Examination:
Dr. Michelle Bonner, Department of Political Science, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Recent bioinformatic analyses identified an operon encoding a potential Type I Secretion System (T1SS) in *Treponema pallidum* that we hypothesize functions to export key treponemal virulence factors that may contribute to the unique invasiveness and pathogenesis of this spirochete. The membrane fusion protein component (MFP) of T1SSs in other organisms has been shown to play a role in substrate recognition. Hence, the objective of this project is to use the putative MFP, Tp0965, of the potential *T. pallidum* T1SS to investigate protein-protein interactions with the *T. pallidum* virulence factor pallilysin (Tp0751) and assess the possibility of the latter being a T1SS substrate. Moreover, protein-protein interactions between Tp0965 and a *T. phagedenis* lysate are investigated with the goal of identifying putative T1SS substrates in this spirochete that could result in the discovery of novel *T. pallidum* virulence factors via sequence similarity.

Plate-based binding studies and pull-down assays showed a low level of interaction between recombinant Tp0965 and the previously characterized host-component-binding protease, pallilysin, suggesting that the export of this virulence factor could occur via the putative T1SS.

Additionally, bioinformatic analyses of the related but cultivable model spirochete *Treponema phagedenis* predicted the presence of a potential T1SS homologous to the putative T1SS in *T. pallidum*. Thus, a more global and unbiased pull-down assay using "bait" Tp0965 and a "prey" *T. phagedenis* lysate was carried out, followed by mass spectrometric analysis to identify putative novel T1SS substrates with potential homologs in *T. pallidum*. We successfully identified a *T. phagedenis* protein, TphBig, that showed evidence of an interaction with Tp0965. TphBig seems to possess characteristics of a T1SS substrate suggesting it may be secreted via this system in *T. phagedenis.* Upon extensive bioinformatic analysis, it was found that TphBig showed weak sequence similarity as well as some structural similarity to the *T. pallidum* protein, Tp0854.

Tp0854 is predicted to contain a sialidase and a phosphatase domain with an RTX motif, which is characteristic of some T1SS substrates. Thus, it was hypothesized that if Tp0854 had characteristics of a T1SS, it may interact with Tp0965. Therefore, the phosphatase domain containing the RTX motif was produced recombinantly and plate-based binding studies indeed suggested an interaction with Tp0965, confirming the *in silico*-predicted interaction.

Future experiments to characterize the potential T1SS in *T. pallidum* will comprise the functional and structural characterization of the novel putative T1SS substrate Tp0854, including the identification of Tp0965-interacting sites, as well as the functional characterization of the sialidase and phosphatase domains. Moreover, a potential experiment that could be carried out as a more definite test for T1SS substrate secretion, is the heterologous expression of *T. pallidum* pallilysin and/or Tp0854 in an *E. coli* strain harbouring an endogenous T1SS and test for secretion. Similarly, the reconstitution of the *T. pallidum* putative T1SS in liposomes could be used to further investigate the secretion of pallilysin and/or Tp0854 via this system.

Additionally, the optimized unbiased pull-down technique could be further applied to detect more protein-protein interactions within *T. pallidum* and potentially lead to the identification of more virulence factors that may be secreted via the T1SS.

These studies constitute the first investigation of a putative T1SS and its substrates within *T. pallidum*. Thus, insight gained will lead to a better understanding of the mechanisms facilitating *T. pallidum* host invasion and may reveal new potential vaccine targets to prevent bacterial dissemination and chronic infection.