University of Victoria | Graduate Studies

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

of

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MSc (Mumbai University, 2010)

"Temperature Sensitive *Mycobacterium Tuberculosis* as a Potential Vaccine Candidate"

Department of Biochemistry and Microbiology

Tuesday, May 12, 2015
10:00 A.M.
Engineering and Computer Science Building
Room 128

Supervisory Committee:
Dr. Francis Nano, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Caroline Cameron, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Perry Howard, Department of Biology, UVic (Outside Member)

External Examiner:
Dr. Réal Roy, Department of Biology, UVic

Chair of Oral Examination:
Dr. Sarah Macoun, Department of Education, Psychology and Leadership Studies, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Mycobacterium tuberculosis remains one of the most common worldwide causes of illness and death due to an infectious disease. The emergence of multiple and extreme-drug resistant strains has increased the need to find an effective vaccine for tuberculosis. The goal of our research group is to engineer a temperature-sensitive (TS) *M. tuberculosis* strain that can be used as a tool in vaccine development. The first approach to create TS *M. tuberculosis* involves the integration of the essential gene *ligA* encoding a TS NAD+ dependent DNA ligase, which was taken from the psychrophilic organism *Pseudoalteromonas haloplanktis*. The integration and functioning of *ligA* was demonstrated in the fast-growing organism *Mycobacterium smegmatis*. This strain had a TS phenotype with growth limited to below 37°C. The strain was found to have a stable TS phenotype and did not mutate to a temperature-resistant form at a detectable level. Following experiments with the fast growing *M. smegmatis*, the integration of the *ligA* gene was attempted in slow-growing *M. tuberculosis*. Merodiploids of *M. tuberculosis* containing both the psychrophilic and the WT *ligA* gene in its chromosome were obtained.

The second approach used for the development of TS *M. tuberculosis* was the directed evolution of native *M. tuberculosis* essential genes. An advantage of this approach is that the gene encoding the essential protein will resemble the native *M. tuberculosis* gene and thus will closely match the native transcriptional and translational rates. A system to screen and select for TS essential genes engineered by directed evolution was designed, where the essential gene on the chromosome of *E. coli* was knocked out and this gene was supplied on a conditionally replicating plasmid. As a first step in developing this directed evolution approach, a family of conditionally replicating plasmids were created and tested in an essential gene knock-out strain of *E. coli*.