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, 2011)

"Reprogramming the Expression of the Double-Stranded RNA Mitovirus OnuMV1c from the Mitochondria to the Cytoplasm in the Fungal Pathogen *Ophiostoma Novo-ulmi***"**

Department of Biology

Thursday, August 20, 2015
10:00 A.M.
Hickman Building
Room 110

Supervisory Committee:
Dr. Will Hintz, Department of Biology, University of Victoria (Supervisor)
Dr. Patrick von Aderkas, Department of Biology, UVic (Member)
Dr. John Taylor, Department of Biology, UVic (Outside Member)

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Chair of Oral Examination:
Dr. Sudhakar Ganti, Department of Computer Science, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Dutch elm disease (DED) is a debilitating wilt disease that has decimated elm populations globally. The current epidemic of this disease is caused by the ascomycete fungal pathogen *Ophiostoma novo-ulmi*. A number of strategies have been used to attempt to mitigate the effects of DED but none have met any sustainable success, and the disease continues to have severe ecological and economic impacts. For several years our research group has focused on the development of control strategies at the genetic level. Recently we have been examining the use of naturally occurring fungal viruses (mycoviruses) to induce hypovirulence. Attenuation of fungal pathogenicity using mycoviruses has been well studied in other fungal systems, but has yet to be developed for *O. novo-ulmi*. Though a diversity of mitochondrial viruses have been discovered in European *O. novo-ulmi* populations, the high diversity of vegetative compatibility (vc) types in these populations prevents viral transmission and thus limits the possibility of viral control strategies. The North American populations of *O. novo-ulmi* exhibit much less vc diversity than their European counterparts and are therefore ideal for genetic control strategies.

A candidate virus, OnuMV1c, was found in an isolate of *O. novo-ulmi* (93-1224) at the western Canadian disease front and its genome has been sequenced. OnuMV1c is a mitochondrial virus and has a single-stranded positive RNA genome that encodes an RNA-dependent RNA polymerase (RdRp) involved in its replication as a double-stranded RNA molecule. It exists in *O. novo-ulmi* mitochondria in both its single-stranded and double-stranded form.

We are currently exploring engineering OnuMV1c such that it may carry a RNA interference cassette in addition to its own complement of genes in order to examine the possibility of its use as an enhanced hypovirus. RNA interference (RNAi) is a cytoplasmic process and therefore in order to use OnuMV1c to carry an RNAi cassette we needed to reprogram the viral genome such that it could be expressed in the cytoplasm rather than the mitochondria. The objectives of the master's research were to 1) genetically engineer OnuMV1c to express in the cytoplasm using a cDNA reverse genetics approach, and 2) test the functionality of the re-engineered cDNA OnuMV1c virus (MV1cCyt).

The first objective was accomplished by modifying codons in the RdRp sequence of OnuMV1c such that the sequence could be translated in the cytoplasm. This genetically engineered cytoplasmic version of OnuMV1c, named MV1cCyt, was flanked with exogenous promoter and terminator sequences to drive its transcription. The entire construct was engineered as a cDNA molecule and was cloned into the fungal transformation vector pAN7-1, which was used to transform *O. novo-ulmi* protoplasts. The second objective was achieved through the use of strand-specific RT-PCR, a technique that allowed the detection of both the positive and negative strands of MV1cCyt. Results indicated that while four individual cell lineages contained MV1cCyt cDNA stably integrated into the nuclear genome, only one transformant was able to produce double-stranded MV1cCyt RNA. These results have important implications for the use of OnuMV1c as an engineered hypovirus and represent the first step towards the development of a biological control strategy for Dutch elm disease.