The Final Oral Examination
for the Degree of

DOCTOR OF PHILOSOPHY
(Department of Biology)

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2005  PUC University of Goiás, Brazil  Hons. B.Sc.

“Towards control of Dutch elm disease: dsRNAs and the regulation of gene expression in Ophiostoma novo-ulmi”

Thursday, June 20, 2013,
10:00 am
Harry Hickman Bldg, Room 120

Supervisory Committee:
Dr. Will Hintz, Department of Biology, UVic (Supervisor)
Dr. Barbara J. Hawkins, Department of Biology, Uvic (Member)
Dr. Patrick von Aderkas, Department of Biology, Uvic (Member)
Dr. Delano James, Department of Biology, Uvic (Member)
Dr. Caroline Cameron, Department of Biochemistry and Microbiology, UVic, (Outside Member)

External Examiner:
Dr. Richard Winder, Canadian Forest Service, Pacific Forestry Centre

Chair of Oral Examination:
Dr. Subhasis Nandi, Department of Electrical and Computer Engineering, UVic
Abstract

Ophiostoma novo-ulmi is a plant pathogenic fungus that has had a severe impact on forest health around the world with significant economic implications. This fungus affects elm trees (Ulmus spp.) and it is the causative agent of Dutch elm disease (DED). This PhD research has been focusing on battling DED by developing strategies that attack the fungus at the genetic level.

The first phase of this PhD project involved the development of RNA interference (RNAi) as a method for gene regulation. Through the development of a highly efficient RNAi cassette we evaluated the use of RNAi to suppress the expression of the endopolygalacturonase (epg1) locus. This epg1-RNAi cassette significantly reduced the amount of polygalacturonase activity in the fungus and resulted in almost complete degradation of epg1 mRNA as assayed by reverse-transcribed PCR.

When RNAi was first demonstrated to effectively down-regulate gene expression in O. novo-ulmi we recognized the importance of developing a native promoter in order to selectively down-regulate specific gene loci that would allow us to manipulate genes responsible for the parasitic fitness of the fungus and develop conditional-lethal mutants. We addressed this need in the second phase of this research by re-developing a carbon-catabolite regulated promoter (alcA) to drive the expression of an epg1-RNAi cassette. The full length gene sequence for the alcA gene including its promoter was captured by genome walking. The expression of an epg1-RNAi driven by the alcA promoter resulted in the down-regulation of epg expression under glucose starvation, however, normal levels were expressed when transformants were cultured in high glucose media. This improvement in the RNAi cassette offered an attractive genetic tool in that the host could be cultured in the presence of glucose to express normal levels for the selected gene and then, under glucose-depleted conditions for switching off the expression of the selected gene. We can thereby now functionally identify genes related to the health and fitness of fungus and target them for interference.

In the third phase of this research we explored here the use of dsRNA viruses as agents to vector the RNAi cassette. The
development of a biological control for DED requires, at minimum, an agent that is antagonistic to the fungus but is transmissible to extant populations and is highly specific to minimize off-target effects. We intend to explore the engineering of dsRNA viruses for the purposes of understanding and controlling fungal pathogenesis. In previous studies an isolate of \textit{O. novo-ulmi} strain 93-1224 was found in the city of Winnipeg that was infected by two dsRNA mitoviruses. We sequence characterized these two mitoviruses and named them OnuMV1c and OnuMV7.

In the fourth phase of this research we paired OnuMV1c virus with three other species/isolates of \textit{O. ulmi} and two other of \textit{O. himal-ulmi} that lacked dsRNA. We were able to determine that this virus may have a cytoplasmic route and, therefore, may not be dependent on mitochondrial fusion for transmission. We also developed mitochondrial markers of specific optional introns whose presence or absence varied between the isolates analyzed helping us to clearly differentiate species and evaluate whether recombination between mitochondrial types had occurred. We anticipate that the characterization of these mitoviruses will allow us to investigate the potential to re-engineer these naturally occurring viruses such that, in addition to the usual complement of genes for their own replication, they may be engineered to also carry an RNAi cassette to down-regulate genes involved in parasitic fitness.

This research has provided a comprehensive investigation of gene expression and gene manipulation while also providing powerful genetic tools that could be used to understand gene regulation in \textit{O. novo-ulmi}. It has also added to the body of knowledge regarding mitoviruses and their transmission and contributed to their potential use as a biological control for this devastating fungal disease. By enhancing our understanding of \textit{Ophiostoma} gene regulation and transmissible hypovirulence this work advanced efforts to develop a new approach to target DED as well as providing a potential model for the control of other fungal diseases.
Awards, Scholarships, Fellowships
2010  Amelia Leith Memorial Fellowship
2008  “Best Talk” 20th Biology Graduate Symposium, UVIC
2008  Amelia Leith Memorial Fellowship
2001-2004 Undergraduate research grant from the National Council for Scientific and Technological Development (CNPq, Brazil)

Presentations

Publications