University of Victoria | Graduate Studies

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

of

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"Characterization of Psychrophilic Alleles of Essential Genes as Means of Generating Temperature-Sensitive Strains of Mesophilic Organisms"

Department of Biochemistry and Microbiology

April 7, 2016
2:00 P.M.
David Turpin Building
Room A144

Supervisory Committee:
Dr. Francis Nano, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Christopher Upton, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Martin Boulanger, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Kim Juniper, School of Earth and Ocean Sciences, UVic (Outside Member)

External Examiner:
Dr. Karl Klose, Department of Biology, University of Texas at San Antonio

Chair of Oral Examination:
Dr. Astri Wright, Department of Anthropology, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Essential genes are involved in control of the basic metabolism of their host. These genes encode elements involved is such crucial processes as DNA replication, transcription, translation or biosynthesis of important molecules. What makes essential genes unique is the fact that they cannot be lost from the genome. If any of them becomes inactivated it would result in inevitable death of an organism. Because of their role they can be efficiently used to control the survival of genetically modified organisms. Specific regulatory mechanisms can be applied to modulate the activity of essential genes, which prevents an organism from growing at determined conditions. Such mechanisms are called “kill switches” and have been developed in recent years as a response to significant development in the field of molecular biology.

Proteins encoded by psychrophilic organisms are characterized by decreased resistance to thermal denaturation. This is believed to be a result of adaptation to low-temperature environment, where mutations that destabilize the protein structure are not selected against. For these reasons they often cannot perform their functions at moderate temperatures, which are typical for mesophilic organisms. At the same time psychrophilic proteins do not display any inhibition at permissive conditions.

Use of psychrophilic alleles of essential genes has been proposed as a method of rendering modified organisms incapable of surviving at elevated temperatures. This allows generation of attenuated strains of pathogenic bacteria or generally safe versions of laboratory organisms. A temperature-sensitive organism can be created by substituting a single essential gene in mesophilic organism with its psychrophilic homologue. This can be facilitated by using the host’s native recombination system or through the use of plasmid based allele shuffling mechanisms.

The objective of this work was to analyze a number of psychrophilic alleles of various essential genes for their ability to cause temperature-sensitive phenotype in mesophilic bacterium *Francisella novicida*. The special attention has been placed on investigating psychrophilic alleles of bacterial DNA ligase. Furthermore a selected psychrophilic strain has been characterized as a potential source of multiple temperature-sensitive alleles of essential genes. Finally the secondary focus was to develop a simple and robust mechanism allowing efficient exchange of alleles of essential genes in the mesophilic host.