

Title: Factors Affecting the Isolation, Culturability, and Resuscitation of Dormant Cells of *Mycobacterium paratuberculosis*

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Objective:

To focus on determining the roles of novel bacterial Resuscitation Promoting Factors (RPF) in resuscitating growth of dormant or Viable-But-Not-Culturable (VNBC) cells of *Mycobacterium paratuberculosis*, and in promoting recovery and growth capacity of stressed and vegetative cells.

What is to be done?

The research has 6 components:

1. Formation of VNBC cells of *M. paratuberculosis*
2. Resuscitation of the VNBC cells
3. Detection of viable cells using the polymerase chain reaction (PCR)
4. Morphological properties of the *M. paratuberculosis* VNBC cells
5. Green fluorescent protein (GFP) in tracking cell viability
6. The transcription profile of *M. paratuberculosis* rpf-genes

Why is it to be done?

Mycobacterium paratuberculosis (sub. *avium*) causes Johne's disease in cattle and transmission of MAP from infected cattle to humans has been implicated as a factor in Crohn's disease. The beef industry needs to reduce or eliminate Johne's disease and prevent possible transmission to humans through the consumption of beef. There is a need for basic and applied research to meet this objective. This is a basic project that addresses the challenging issue of what makes MAP difficult to identify either in infected cattle or on food products. Thus it will address both animal health and food safety issues of the effects of MAP.

How is it to be done?

The first component of this study will provide the basis for the remaining research. This work will use the related *Mycobacterium* species - *M. avium* and will test temperature shock and nutrient limitations, in particular. Component 2 will take RPF, that has been found in other bacteria, e.g. *Micrococcus luteus*, and test its ability to resuscitate VNBC cells of *M. paratuberculosis*. For the next component, real time PCR is to be used to examine expression of dormant-phase associated genes and to track cell viability in VNBC cultures before and during resuscitation. Differences in cell morphology and structure will be examined in component 4 of the study using various dyes and staining methods together with electron microscopy. In component 5, GFP expressing cells of *M. paratuberculosis* and *M. avium* will be subjected to shock techniques by which VNBC cells are formed and resuscitated so that GFP non-viable non-culturable cells may be used to give insights as to factors affecting viability in natural environments. For the final section of the research, promoter regions for RPF like genes in *M.*

paratuberculosis will be cloned into vectors carrying promoter-less genes that will be transformed into *M. avium*. The promoter-less genes will be selected so that they could become candidates for vaccines in further development of this technology.