Department of Agriculture, Trade and Consumer Protection

Division of Agricultural Development

Agricultural Development & Diversification Program (ADD)

Grant Project Final Report

Contract Number: 17081

Grant Project Title: Rapid Diagnostic Method for Detection of Mycobacterium paratuberculosis

Amount of Funding Awarded: $24,000

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Paradigm
Diagnostics, Inc.

Memo

To: Mike Bandli

From: Alan Olstein

Re: Final project summary.

This project summary will summarize and appraise the objective results as well as provide a forecast of the potential research targets for any future projects contemplated.

Initially the project focused on using a high sensitivity approach to earlier detection of M. paratuberculosis cells in veterinary samples. Both DNA and cellular receptor targets in the MAP cell population suffer from the same potential drawbacks, i.e. the ability to distinguish viable from non-viable cells and the presence of assay interferents in the sample necessitates further cellular purification. This is a task readily undertaken with a faster growing cell culture, but with such slow growing cell species sub-culturing as a preliminary step to detection significantly draws out the time-to results. An excerpt of one of the early progress reports points up this fact:

Among the problems encountered were the inability of the assay to distinguish between Mycobacterium smegmatis (a non-pathogenic mycobacterium) and the pathogen, Mycobacterium paratuberculosis. Early work attempted to reproduce results reported by Cyprinski (Uof Wisconsin, Madison) which suggested that there were receptors for mammalian growth hormone on the surface of the pathogenic species that were not present in the non-pathogen (smegmatis). Using porcine growth hormone modified with a fluorescent marker, we noted and photo documented that the attachment sites did exist and that it appeared that the pathogen clumped together and the non-pathogens did not. However, during conversations with experts at the University of Wisconsin, we were told that the non-pathogens did clump in some situations and that was not a reliable method of separating them.

Based upon papers published by Olsen et al[1] and Chauhan and Mande[2] we propose to develop a chemiluminescent detection method for the enzyme, alkyl hydroperoxide reductase which appears to have unique characteristics associated with the virulence of the *M. paratuberculosis* pathogen. Stratman et al[3] have disclosed a phage binding protein the structure of which exhibits high specific binding to the cell wall of *M. paratuberculosis.* We have succeeded in preparing a chemiluminescent derivative of this specific peptide, which has been used in binding studies with *M. paratuberculosis* and *M. smegmatis.* The results of these tests were inconclusive but sufficiently encouraging to repeat, in light of the subsequent discovery that the instrument used was far out of calibration and probably contributed to the equivocal results. <u>Next steps include repeating these experiments.</u>

We never got to the repeating the experiments stage of this work. The growth rate of these organisms makes maintaining continuity on these projects difficult especially when staffed incompletely. We continue to believe that a rational development approach to screening for the presence of this pathogen involves a detailed understanding of the organisms' physiology and biochemistry. As can be gleaned from the literature there are suggestions of potential enzyme marker which may be specific to the pathogen. In particular, enzyme indicators which could be incorporated into the growth medium might permit more facile analysis and identification of positive cultures. We have had considerable success with this approach to the development of a simple indicator

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[1] Olsen, I. et al. *Infect. and Immun.* **68(2)** 801-808 (2000).
[2] Chauhan, R. and Mande, S. *Biochem. J.* **354** 209-215 (2001)
[3] Stratman, J. et al. *J. Clin. Microbial.* **40(11)** 4244-4250 (2002).

broth for high through-put screening of environmental samples for *Listeria spp*. We have also applied this approach to the development of a screening medium for detection of *Salmonella spp*. as well. This is the sort of approach that is needed to succeed in the development of a practical MAP screening tool, but it can only be accomplished once some standard organisms cultures are available, and the optimum growth, cultural requirements for this pathogen are better understood.

Thank you for the opportunity to work on this challenging project. We hope the information developed during the course of this project will be of use to researchers attempting to develop practical products of use to the dairy industry and the State of Wisconsin.