AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2021– 2023

TITLE: Immunomodulatory and Vaccine Potential of *Mycobacterium avium* subsp*. paratuberculosis* Novel Antigens

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COOPERATORS:

SUMMARY/ABSTRACT: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is an etiological agent of a chronic and highly contagious enteritis in ruminants known as Johne's. MAP infection causes the biggest losses in the dairy industry. The key for efficient management and prevention of MAP infection is to have effective vaccine. The only available MAP vaccine in the US cannot protect animals from being infected, and it merely limits the progression of the disease.

The foundation of the vaccine development lays in understand the molecular mechanisms of MAP pathogenesis and, more specifically, finding the right bacterial antigens that can initiate the strong protective immunity in animals. In attempts to identify MAP antigens produced within the biologically relevant environments, we investigated bacterial secreted proteome within bovine intestinal and mammary gland cells as well as in the milk. Ten potential targets were selected through bioinformatic analysis as potential antigens. Some selected MAP genes have high similarity to *Mycobacterium tuberculosis* proteins that has been demonstrated to provoke immunostimulation *in vitro* and *in vivo*.

OBJECTIVES:

1. To evaluate MAP candidate antigens for immunogenisity and for stimulation of protective innate immunity.
2. To establish the cellular mechanism(s) induced by effective antigens.

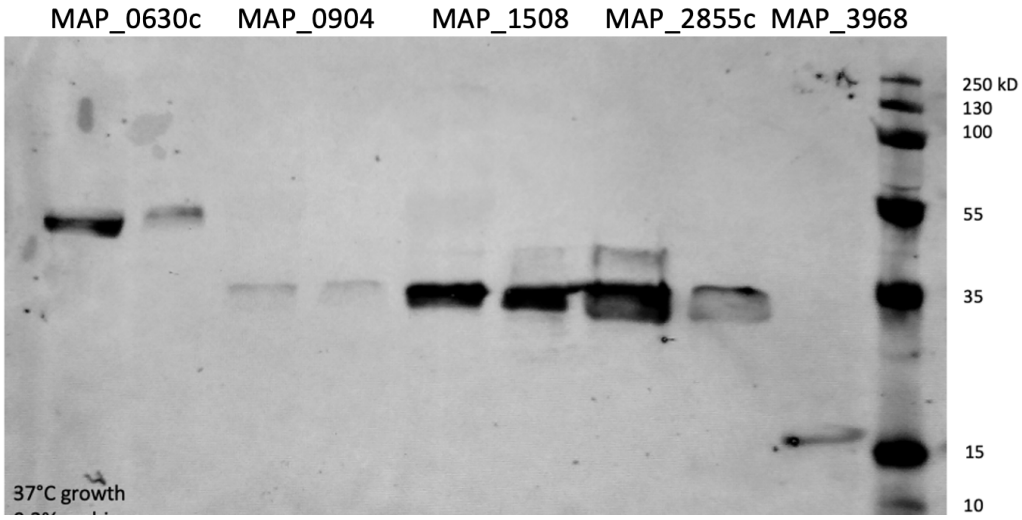
PROCEDURES:

To create recombinant proteins. Ten MAP genes selected are MAP_0630c, MAP_0904, MAP_0966c, MAP_1508, MAP_2855c, MAP_3666c, MAP_3968, MAP_1609c/fbpB, MAP_3145c/tetA, wag31).

To test if antigens can activate macrophage protective innate immunity. One of the main functions of macrophage is to immediately clear invading organism before they establish infection; however, MAP is a successful pathogen that learned how to avoid killing by these immune cells. Macrophage monolayers will be incubated with antigens and then infected with MAP. Bacterial intracellular growth will be monitored to establish if immune responses activated by antigens can limit bacterial growth and attenuate MAP survival within the host cells.

To evaluate antigens for immunogenicity. The Raw264.7 macrophages that are widely used in MAP research will be incubated with antigens and secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12, MCP1) will be measured using ELISA kits.

SIGNIFICANT ACCOMPLISHMENTS TO DATE: We have constructed all 10 genes into 2 overexpression vector pET14b (His-tag at N-terminus) and pET6HN_C (HN-tag at C-terminus). Many mycobacterial proteins have signal proteins either on the N- or C-terminus that is cleaved when protein is secreted (released) out of the bacterial cell. Because mycobacterial signal peptides differ from other Gram-positive pathogens, the identification of the location of the signal peptides is not possible through existing bioinformatic tools. Therefore, to make sure we have the tags remained (without losing it during the protein cleavage process) on the protein for the purification purpose and increase our chances for mycobacterial protein production in the *E. coli* system, we created 2 construct versions of each gene (20 clones in total). So far, we have successfully purified 5 MAP recombinant proteins shown in the figure 1 as the western blot image.



We are currently troubleshooting to resolve the expression problems for other five genes by changing expression conditions in *E. coli*.

To test if antigens can activate protective innate immunity in macrophages, currently, we are measuring the intracellular MAP growth dynamics. RAW264.7 cells were pretreated with purified 5 recombinant proteins and then infected with MAP over 7 days. MAP infection without pretreatment served as a control. Results are pending, as it takes two months for the MAP pathogen to grow on agar growth media. By recording the viable intracellular bacterial colony forming units from each condition on the 7H10 agar plates, we can draw a conclusion if selected antigens can stimulate macrophage innate killing mechanisms against MAP.

Currently, we are initiating experiments to determine the production of pro-inflammatory cytokines in pretreated cells with five antigens. These experiments will help us to assess antigenic and immunomodulatory properties of selected five recombinant proteins.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES: At the end of the project, we plan to publish a peer-reviewed paper. If produced recombinant proteins will stimulate innate immunity that leads to MAP attenuation, we will have strong preliminary data that will help to apply to future USDA funding.