The Francisella Pathogenicity Island (FPI) is a segment of the Francisella genome that contains the coding genes involved in the pathogenicity of F. tularensis. In previous studies, when the FPI transcriptional regulator mglA was knocked out, immune response levels were lower than infection with the wild strain. This suggests that without the FPI, F. tularensis does not stimulate wild-type levels of cytokine secretion. The FPI protein PdpD was knocked out and found to be non-essential to cellular growth or reproduction. However, when this protein is knocked out, the difference in secretion of the cytokine TNF-α between the PdpD knockout strain and the wild-type is greater than in the other knockout strains. This project will continue to focus on how Francisella tularensis impacts the immune response including the possible role of PdpD function.

Materials and Methods

- Macrophage cells (J774A.1) were infected with Francisella tularensis novocida (donated by Dr. Francis Nano, University of Victoria, Victoria, British Columbia, Canada).
- RNA from the macrophage cells was extracted at 2, 5, 12, and 24 hours post-infection using TRIzol reagent (Invitrogen, Carlsbad CA) as per manufacturer instructions.
- The RNA was reverse transcribed into cDNA using reverse-transcription-polymerase-chain-reaction (rt-PCR) later used in qt-PCR.
- The cDNA from the rt-PCR was used as the template for quantitative-polymerase-chain-reaction (qt-PCR) in order to quantify the expression levels of IL1-β. The primers used targeted the IL1-β gene and the β-actin gene.
- The results from the qt-PCR was then analyzed using the 2-ΔΔCT method. The 2-ΔΔCT method represents data as a fold change in expression that normalizes (scales) to an internal reference and compares to a control.

Results

The dissociation curve is a graph showing the point at which a double-stranded DNA molecule dissociates into single-stranded DNA. A dissociation curve is unique to each DNA molecule. Ideally, the peak of the curves for each line should be in a tight grouping, as in Figure (1). Also, the dissociation curves for the negative controls should be a tight band of relatively flat lines across the graph. However, the results were less than ideal; Figure (2) and Figure (3) (the negative controls) have clear dissociation curve peaks deviating from the flat band. In figures (4) and (5), there are multiple peaks for one sample set; these results suggest contamination. Due to contamination, all results are invalid.

Discussion

The results were invalid due to contamination. Because the qt-PCR results shown clearly exhibit contamination in all samples and controls, contamination maybe due to outside DNA contaminating all of the samples. In the most recent qt-PCR, however, the β-actin negative controls came back without any contamination, suggesting that the IL-1β primers are contaminated. Both contamination scenarios are likely, to resolve the contamination issues buying new primers and taking more care in the procedure will likely suffice.

Conclusions

Data is inconclusive due to contamination.

Works Cited


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