Detection of *Salmonella enterica*, *Escherichia coli* O157 and *Listeria monocytogenes* through bead based Magpix® fluidics

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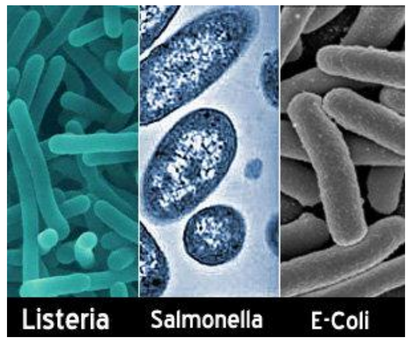
Introduction

The aim of this research is to develop a sensitive diagnostic system that can detect the presence of up to fifty pathogens in a single food sample. The pathogens that are used in this research are *Salmonella enterica, Listeria monocytogenes, and Escherichia Coli O157*. Currently, we are trying to find a correlation between the concentration of the pathogen to the MFI (Median Fluorescence Intensity) values given by the Magpix® machine.



Background

- Genes that have been amplified to use for the purpose of this research are:
  - *invA* (281 base pairs) for *Salmonella enterica*
  - *hlyA* (271 base pairs) for *Listeria monocytogenes*
  - *invA* (363 base pairs) for *Escherichia Coli O157*

Goals

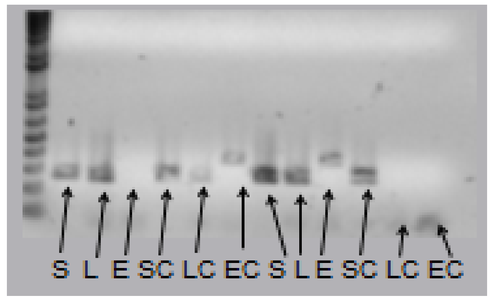
- Multiplex-detect more than one pathogen in a single food sample
- Test the limit of detection (LOD)

Methods

- DNA extraction occurred through the use of Isolation of Genomic DNA from Gram-Positive and Gram-Negative Bacteria protocol with Wizard Genomic DNA purification Kit.
- qPCR was used for DNA amplification and the reaction mixture for amplification included: Absolute Blue mix, bio-tinylated forward and reverse primer, water, and DNA.
- The protocol followed was:
  - 95˚C for 15 minutes
  - 95˚C for 30 sec causes DNA denaturation, at 57.5˚C for 30 seconds causes annealing, and at 72˚C for 30 seconds causes extension. This process of DNA denaturation, annealing, and extension occurs for 40 cycles
  - Lastly, at 72˚C for 7 minutes and then the PCR is programmed to hold at 4˚C.
- Performed gravitation filtration to purify DNA
- Performed gel electrophoresis (picture below) to check the presence of DNA
- Used Nanodrop® to find the concentration of DNA
- By the given concentration, made dilutions in the 96 wells plate and then added beads (with probes attached) to the dilutions

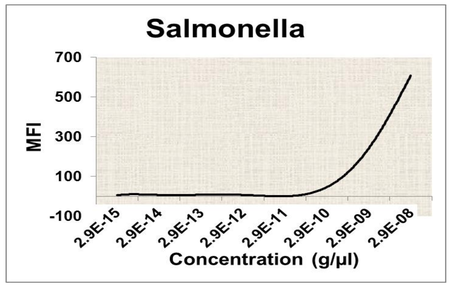
- Performed hybridization by putting the samples in PCR at 95˚C for 5 minutes and then hold for 15 minutes
- Ran the samples in Magpix® and got MFI for corresponding dilutions

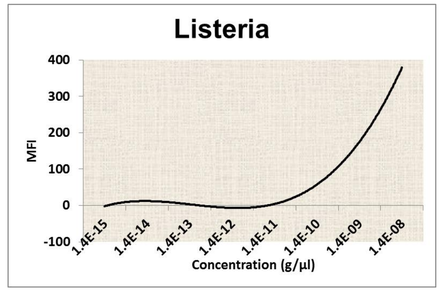
Results and Graphs

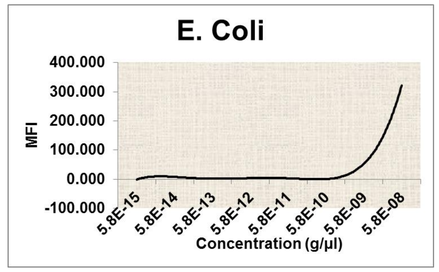


- KEY: Salmonella (S), Salmonella Control (SC), Listeria (L), Listeria Control (LC), E. Coli (E), and E. Coli Control (EC)

- On the gel, each dark band indicates DNA of certain base pairs. On the very left of the gel, there is a DNA ladder, which is separated by 100 base pairs. On the right of the ladder, are the results that we have gotten







Samples were put into the Magpix® and the machines took 50 µl samples from each well and gave the corresponding results. The results and the corresponding graphs indicated that Magpix® can multiplex and detect the presence of pathogens clearly attograms.

Continuous Work

- Work with real food samples starting with apple juice
- Try to extract DNA from media and apple juice and then proceed with the current protocol
- Find the correlation between the CFU (colony forming units) to the MFI values

References

Taniuchi, Mami Verweij, Jaco, & Sethabutr, Orntipa (2011, December). *Multiplex PCR method to detect Cyclospora, Cystoisospora, and Microsporidia in stool samples*. retrieved July 31 2013, from National Center for Biotechnology Information Web Site: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3217099/

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