Field evaluation of microfluidic paper-based analytical devices for microbial source tracking

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Summary

In this work to date, we developed a procedure to determine contamination of lettuce (both mature and artificial plants) exposed to airborne fecal matter when placed around animal operation facilities and a manure lagoon for a week. The collected samples were used for DNA extraction and quantitative polymerase chain reaction (qPCR) or quantitative loop-mediated isothermal amplification (qLAMP) to detect *Bacteroidetes* as biomarkers for each animal source. In general, the results confirm the airborne contamination of the lettuce samples. Results on cattle fecal samples show that the samples collected by plastic leaves present a much better correlation between cultivation rows (i.e., at different distances from animal operation facilities) and the threshold cycles in the qPCR data; plants closer to the animal operation facility were more contaminated.

Objectives

1. Establish background levels of fecal and pathogenic contamination in the field to determine the limits of detection that are needed for a field-based assay.
   - Determine the contamination of lettuce leaves exposed to airborne fecal matter from different animal sources, including cattle, sheep, swine, and chicken.
   - Understand the effect of distance from animal operation facilities on fecal contamination,
   - Determine the efficacy of different sampling methods suitable for on-farm microbial detection.
2. Design and test a portable microfluidic paper-based analytical device (μPAD) that can detect contamination and provide results within an hour in the field.

Methods

Work to date has focused on Objective 1. Mature lettuce plants, grown in a greenhouse, and artificial plants (made from plastic sheets) were placed around animal feeding operation facilities or a manure lagoon in Indiana. Ten pots (per spot) were placed at three different distances from each animal operation or lagoon, with three replicates in each row. Lettuce and plastic plants were collected after one week, and the leaves/sheets were either swabbed or surface-washed to collect potential contaminants. Total DNA was extracted from all the samples using a fecal DNA extraction kit. A general *Bacteroides-Prevotella* 16S rRNA gene PCR assay was used to verify the presence of fecal bacterial DNA in each extract prior to screening qPCR/ qLAMP primer sets.

Results to Date

Figure 1 shows that when using swabs on mature leaves, the results indicate high variability across replicates and no correlation between distance from the feeding operation and amount of contaminant (as indicated by the number of cyles needed for amplification). On the other hand, **Figure 2** shows low variability across replicates and a clear distinction between the signals from different rows. The artificial plants have flat surfaces that are easier to swab. Although the swabbing technique could pick up target DNA on mature lettuce leaves, artificial plants better indicate the fecal contaminant risk of the same area. **Figure 3** shows that LAMP possesses a faster amplification rate than qPCR. The short duration of the assay potentially enables contamination detection within an hour in the field.

Benefits to the Industry

This project develops and tests a procedure to determine contamination of fresh produce by aerosolized fecal matter from various animal sources. Such a procedure is useful for assessing risk of planting crops around animal feeding operations. This study also compares efficacy of two different sampling methods suitable for on-farm microbial detection. The sampling method will affect the functionality and user-friendliness of the future biosensors for fresh produce contamination detection.

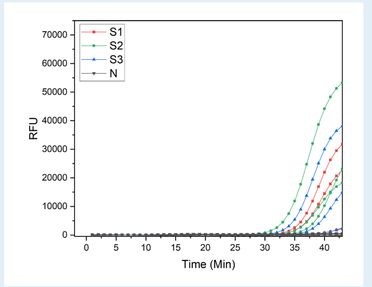

Figure 1.qPCR amplification curve for *Bacteroidetes* primers. "S" represents swab samples collected from lettuce leaf surface during field experiment. The number represents the sample row, from row 1 (closest to the animal facility) to row 3 (furthest from the animal facility). "N" represents no template controls.

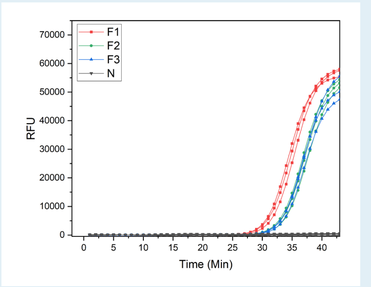

Figure 2. qPCR amplification curve for *Bacteroidetes* primers. "F" represents swab samples collected from artificial plant surfaces during field experiment. The number represents the sample row, from row 1 (closest to the animal facility) to row 3 (furthest from the animal facility). "N" represents no template controls.

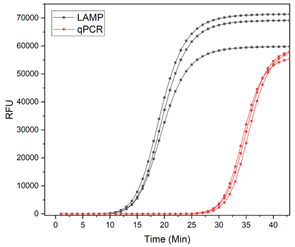

Figure 3. qLAMP and qPCR amplification curves for *Bacteroidetes* primers with the same sample. Samples were collected from the field *experiment* near a cattle farm. The figure shows that LAMP amplification reaches the positive threshold faster than the qPCR amplification.