Fate of different *Listeria monocytogenes* strains on different whole apple varieties during long-term simulated commercial storage

**SUMMARY**

This project will determine the fate of *Listeria monocytogenes* (Lm) on apples during long-term simulated commercial storage. Some of the key questions to be answered include: 1) Do different foodborne outbreak strains of Lm differ in their ability to survive on apples? 2) Does Lm survival differ when apples are contaminated from water versus direct contact with equipment surfaces (crates, brushes)? 3) Does storing apples in air versus a controlled atmosphere (low oxygen and low carbon dioxide) affect Lm survival? 4) Does the apple variety (Gala, Granny Smith, Honeycrisp) and region in which the apples are grown (Washington, Michigan, Pennsylvania) affect how Lm attaches and survives on apples; and 5) Does apple waxing affect Lm survival?

**OBJECTIVES**

1. The microbiological safety of whole and sliced apples has been questioned recently due to multiple recalls for *Listeria monocytogenes* (Lm) and two outbreaks of listeriosis from caramel apples. Whole Gala and Honeycrisp apples were recalled in December 2017 due to Lm contamination, suggesting extended survival of this pathogen. As outlined in Figure 1, this 2-year project will:
   - Assess the survival of eight L. monocytogenes strains grown planktonically or as a biofilm on three unwaxed apple varieties (Gala, Granny Smith, Honeycrisp) from two different harvest seasons and three different growing regions (Washington, Michigan, Pennsylvania) during air (21% O₂) or controlled atmosphere (1.5% O₂, 0–3% CO₂) storage; and
   - Determine the survival of L. monocytogenes on apples after waxing.

**METHODS**

The *Listeria monocytogenes* strains used in this study were selected to represent diverse lineages, genotypes, and sources, and include strains from apple-associated outbreaks, major outbreaks via other vehicles, and from apples (Table 1). The strains were first tagged genetically with unique genetic barcodes that were cloned into a shuttle barcoding vector pTZ200.mix, which was then inserted into a specific chromosomal locus of each strain. Chromosomal strain tagging with unique fluorescent tags has been pursued with red-shifted GFP (green fluorescent protein), derived from pKVi1.

**RESULTS TO DATE**

We succeeded in stable chromosomal tagging of six different strains of *L. monocytogenes*. Sequence analysis of PCR products from the tag integration region confirmed that the barcode was unique for each strain (Figure 2). An initial red-shifted GFP fusion construct has been created and chromosomally inserted in the serotype 4b strain 2011L-2858, implicated in the 2011 cantaloupe-associated outbreak, resulting in strain 2011L-2858-GFP. Testing is ongoing to confirm and optimize the fluorescence of strain 2011L-2858-GFP.

**BENEFITS TO INDUSTRY**

Positive impacts of the anticipated outcomes include: (i) identification of specific knowledge gaps pertaining to environmental conditions and processes (e.g., waxing) that impact Lm’s capacity to adhere and persist on apples; (ii) elucidation of strain-specific differences in Lm adherence and subsequent fate on apples; (iii) clarification of the impact of apple variety, production region, and growing season on Lm contamination of apples; and (iv) clarification of the role that more resistant, surface-grown Lm cells, as could occur in the field and especially in the packinghouse, may play on contamination of apples during processing. These findings will inform the industry of the presently unknown risks associated with different components of apple production and packing, and aid in the design and validation of Lm-targeting interventions to better ensure apple safety.

![Graphical outline of the project.](image)

![Sequence content of the barcodes in the six *L. monocytogenes* strains. The PCR product harboring the unique 29–30 nucleotide barcode was sequenced, and the unique sequence content is indicated in the colored region. Flanking sequences are identical among all strains, and correspond to the backbone of the vector pTZ200.mix. The row of Ns in the pTZ200.mix corresponds to the variable region. The Lm strains are as indicated in Table 1, with the number after the hyphen indicating the colony that was sequenced.](image)

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**LENGTH OF FUNDING**

January 1, 2019 – December 31, 2020

**Table 1. Listeria monocytogenes strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype (lineage)</th>
<th>Genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>2011L-2858</td>
<td>4b (I)</td>
<td>ST1</td>
<td>Caramel Apple outbreak, 2011</td>
</tr>
<tr>
<td>2010L-1723</td>
<td>4b (I)</td>
<td>ST7</td>
<td>Caramel Apple outbreak, 2010</td>
</tr>
<tr>
<td>2011L-6680</td>
<td>V2a (I)</td>
<td>ST78</td>
<td>Celery outbreak, 2011</td>
</tr>
<tr>
<td>2011L-2858</td>
<td>V2b (I)</td>
<td>ST5</td>
<td>Cantaloupe outbreak, 2011</td>
</tr>
<tr>
<td>F2365</td>
<td>4b</td>
<td>ST1</td>
<td>California cheese outbreak, 1985</td>
</tr>
<tr>
<td>H7858</td>
<td>4b</td>
<td>ST16</td>
<td>Hot dog outbreak, 1958-59</td>
</tr>
</tbody>
</table>

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