

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 State, Michigan, Pennsylvania) affect how *Lm* attaches and survives on apples; and 5) Does apple waxing affect *Lm* survival? Answers to these and other questions will assist the apple industry in minimizing the *Lm* risks associated with current apple growing and packing practices.

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:
2. 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
3. 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 the 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 pKV111.

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 1/2b 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.

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

Strain	Serotype (lineage)	Genotype	Source
2014L-6695	4b (I)	ST382	Caramel Apple outbreak, 2014
2014L-6680	4b (I)	ST1	Caramel Apple outbreak, 2014
2010L-1723	1/2a (II)	ST378	Celery outbreak, 2010
2011L-2858	1/2b (I)	ST5	Cantaloupe outbreak, 2011
F2365	4b	ST1	California cheese outbreak, 1985
H7858	4b	ST6	Hot dog outbreak, 1998-99

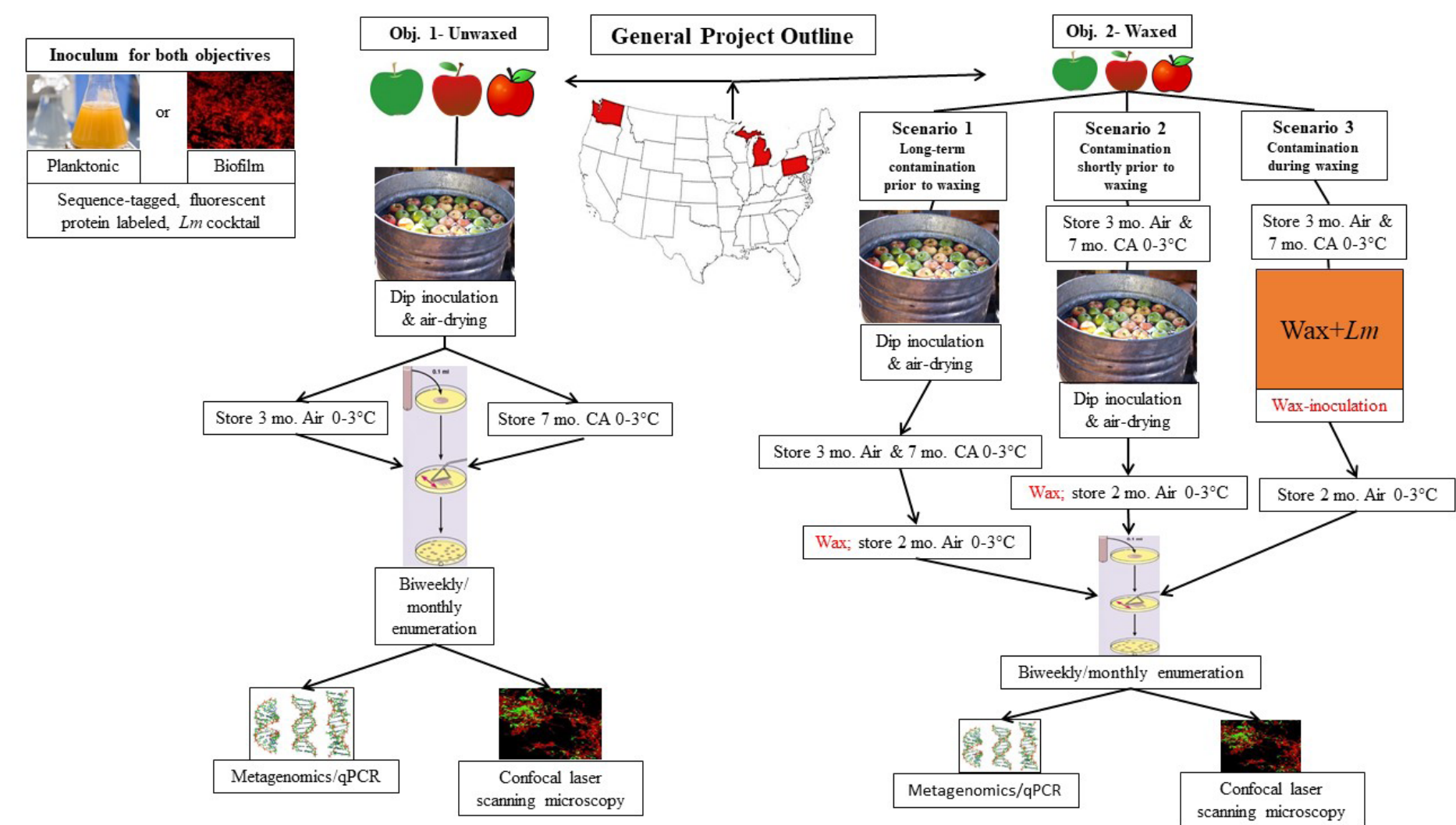


Figure 1. Graphical outline of the project.

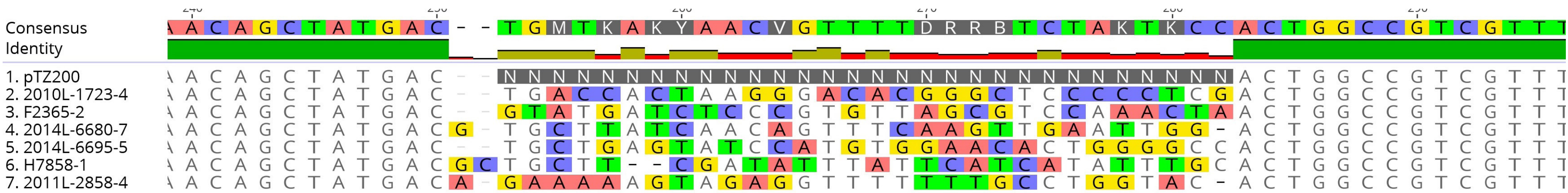


Figure 2. Sequence content of the barcodes in the six *L. monocytogenes* strains. The PCR product harboring the unique 29–30 nucleotide barcode was sequenced, and the unique sequence content is indicated in the colored region. Flanking sequences are identical among all strains, and correspond to the 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.



CONTACT Elliot T Ryser
Michigan State University
E: ryser@msu.edu

Sophia Kathariou
North Carolina State University
E: skathar@ncsu.edu

AUTHORS Elliot T. Ryser, Sophia Kathariou (Co-PI)
Randy Beaudry (Co-PI)
Cameron Parsons, Duncan Matthews
Rania Raftopoulou

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