A metagenomic approach to food safety risk mitigation in pears

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Summary

While existing research has evaluated potential conditions that could support the growth of foodborne pathogens on fruit surfaces, this previous work has primarily been focused on whole, intact fresh apples, leaving the pear industry vulnerable to opportunities for contamination and a lack of science-backed recommendations to prevent contamination or control microbial growth under industry-relevant conditions. Research that does exist to support the long-term storage of fresh pears emphasizes quality considerations only and does not take into account how this could impact food safety risks over time. Thus, we have designed a series of experiments to better understand 1) the pear surface microbiome before storage, 2) how the storage environment impacts the microbiome of marketable and unmarketable pears, and 3) how key players in the microbiome can impact food safety risks throughout pear storage. Results from these studies will yield data for the fresh pear industry on metagenomic profiles of marketable and unmarketable pears.

Objectives

1. Identify culturable microbiological community members (yeast, mold, and lactic acid bacteria) on conventional, whole, intact pears prior to storage.
2. Describe yeast, mold, and lactic acid bacteria composition on marketable and unmarketable conventional, whole, intact pears under two different storage practices at 3, 6, and 9 months in long-term controlled atmosphere cold storage to develop a metagenomic profile and track community composition.
3. Co-inoculate representative yeast, mold, and bacterial community members with *Listeria monocytogenes* under industry-relevant conditions to characterize synergistic and antagonistic effects.

Methods

Conventional market quality pears (Green Anjou), harvested in Washington State, will be processed (drenched, packed) and prepared for storage, either in bulk bins (typically for short-term storage) (**Picture 1**), or wrapped in tissue and placed in 40-lb boxes (for long-term storage). Total microbial communities and cultural populations will be pooled and extracted using the Qiagen DNeasy PowerSoil Pro Kit and targeted regions amplified using PCR. The Illumina platform will be used to sequence PCR amplicons obtained from the V3 and V4 variable regions of the 16S rRNA gene and the ribosomal Internal Transcribed Spacer region (ITS1-5.8S- ITS2: ITS), using the manufacturers' protocols. Co-inoculation experiments will be conducted during shelf life as follows: *L. monocytogenes* (L; control): L+yeast (treatment 1), L+mold (treatment 2), and L+bacterium (treatment 3) (e.g., **Picture 2**).

Results to Date

Preliminary analyses of the bacterial (16S) data show the composition of bacterial communities found on pears is influenced by (i) individual wrapping of the pears and (ii) time (**Figure 1**). Bacterial communities were similar for 3- and 6-month samples, while distinct differences were evident by 9 months. Marketable bulk pears were different than marketable wrapped pears.

Intact and mechanically damaged pears did not support the growth of *L. monocytogenes*. A ca. 1 log reduction was observed throughout 5 months of storage. Pears mechanically damaged and co-inoculated with *Penicillium expansum* (blue mold) showed the greatest log reduction of *L. monocytogenes* (~9 log) (**Figure 2**). Effect of wrapping paper was greatest on pears co-inoculated with *Bacillus thuringiensis* or *L. monocytogenes* only (~3 log reduction).

Benefits to the Industry

This project will provide a broader understanding of how the quality of fresh pears may impact their food safety (through the development of culturable metagenomic profiles at key timepoints during storage) and show how the organisms selected for by the storage environment impact food safety (through the most prevalent isolate characterization and co-inoculation studies with food safety–relevant microorganisms).

No conditions have supported the growth of *L. monocytogenes* through 5 months of storage, even with wounding and decay from *P. expansum*. Wrapped pears also have showed the greatest inhibition of *P. expansum*. Findings from this research will help provide recommendations for industry storage practices that optimize both quality and safety during storage and guide future research efforts toward enhancing postharvest management practices that achieve this goal.

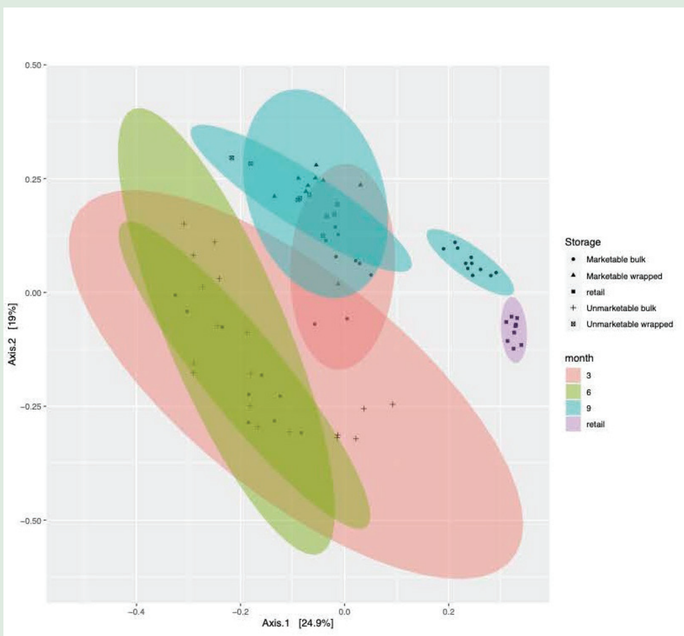

Figure 1. Principal component analysis showing composition of bacterial communities found on pears is influenced by (i) storage of pears (bulk, wrapped; shapes) and (ii) by time (months; colors).

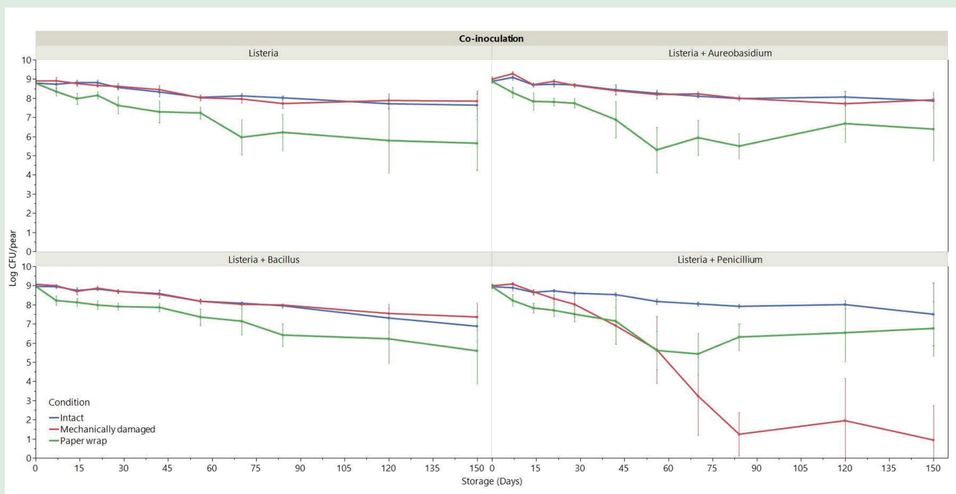

Figure 2: Survival of L. monocytogenes with treatment variables (Listeria, Listeria + Aureobasidium, Listeria + Penicillium, Listeria + Bacillus) for intact, mechanically damaged, and paper wrapped pears.

Picture 1: Bulk pears

Picture 2: Pears inoculated with Listeria + Penicillium individually wrapped in paper.