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, the 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. This information can direct existing and future management practices to optimize the quality and food safety of pears simultaneously. We will use every opportunity to communicate our research and findings to stakeholders throughout the project through presentations, open-access peer-reviewed literature, and publications that target pear growers and packers. Effective communication of our research findings will guide postharvest handling practices that will minimize the likelihood of outbreaks and preserve the long-term quality of fresh pears during storage.

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 three, six, and nine 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
Figure 1 presents a project overview. Conventional market quality pears (Green Anjou) will be harvested in Washington State, processed (drenched, packed) and prepared for storage, either in bulk bins (typically for short-term storage) (e.g., **Figure 2**), or wrapped in tissue and placed in 40-lb boxes (for long-term storage) (e.g., **Figure 3**). 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), both using the manufacturers' protocols. Co-inoculation experiments will be conducted over shelf life as follows: *Listeria monocytogenes* (L; control): L+yeast (treatment 1), L+mold (treatment 2), and L+bacterium (treatment 3).

Results to Date
The internal project team (Strawn, Hamilton, den Bakker, and Critzer), and the new CPS initiative advisory group (which has been named the "Engagement Team") met to outline project goals, objectives, timelines, and discuss deliverables and potential pitfalls as well as opportunities for engagement beyond the outlined project. Our industry partners sent our first shipment of pears from the 6-month storage time-point to the University of Georgia, where laboratory analyses are ongoing. Briefly, Den Bakker and team are performing DNA extraction and sequencing on the 6-month pear samples. Strawn and Hamilton traveled to Washington on a scoping trip to visit and engage with the Pacific Northwest Pear industry. Briefly, the trip included observation of pear storage, conversations with industry, and learning about pear storage practices.

Benefits to the Industry
This project will provide a broader understanding of how the quality of fresh pears may impact 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). Populations of *Listeria* spp. are expected to decline during storage, but its survival is expected to be impacted by the bacterial, yeast, or mold co-inoculant. Findings from this proposed research will help provide recommendations for industry storage practices that optimize both quality and safety and guide future research efforts toward enhancing postharvest management practices that achieve this goal.

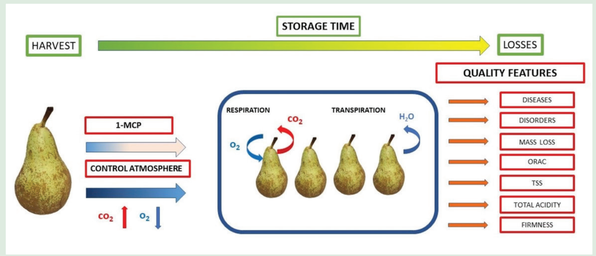

Figure 1. Project overview: Long-term storage of pears

Figure 2. Harvested Green Anjou in bins awaiting transport to storage

Figure 3. Green Anjou wrapped in paper