Rapid detection of preexisting internal *Leuconostoc* spp. spoilage population in fresh-cut carrots during storage

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Abstract
During an extended period of abnormally short quality retention in mixed component packaged salads, due primarily to rapid decay, a root-cause investigation was undertaken. From an initial investigative assessment, this study focused on identification of the primary underlying microbiological cause and validation of a rapid detection screening of raw material. Analysis of several lots of raw, unconsumed product, approx. 6 cm vertically peeled carrot plugs, revealed the accumulation of an aseptic slime in the shipping bag void space and around the extremely softened plug surface. This premature diagnostic signal of lactic acid bacteria (*LAB*) spoilage, specifically *Leuconostoc*, developed in cold storage (2.5°C) after two weeks. Efforts were undertaken to determine whether the *Leuconostoc* was internalized in raw material or primarily environmental contamination with a proliferating reservoir of *LAB* in the primary processing and packaging environment. Polymerase chain reaction (PCR) primers specific for the amplification of a sequenced region of the *Leuconostoc mesenteroides* ss ribosomal RNA gene confirmed the taxonomic identity. Total initial *LAB* and *Leuconostoc* bacterial populations isolated from symptomatic carrots ranged from log$_{10}$ 7.5–8.8 and log$_{10}$ 3.5–4.0 CFU/g carrot tissue weight respectively, and increased log$_{10}$ 2.5–3.5 CFU/g respectively in population density on asymptomatic and symptomatic raw carrot material during two week refrigerated storage.

Introduction
Although lactic acid bacteria (*LAB*) are usually utilized extensively in dairy technology and for commercial fermentation of vegetables, such as carrots and cabbage, indigenous and naturally present *LAB* can also cause detrimental decay and spoilage on the raw product material if population growth becomes unmanageable (Gardner et al., 2001). This prolific population growth can result from processing failures involving neglected sanitation protocols and abusive postharvest storage conditions, which ultimately lead to the proliferation of spoilage *LAB* in the surrounding environment. Such rampant microbial growth can also initiate product spoilage before the “sell-by” or “use-by” date in grocery stores, amounting to considerable economic loss and marred commercial reputation amongst consumers. This troublesome form of decay (and resultant product deterioration) is undeniably one of the major causes of decay of the fresh-cut produce, drastically shortens the microbologically shelf-life and aesthetic quality of fresh-cut produce during storage and after packaging, especially when abusive storage conditions persist and weaken the physiology of the fresh-cut produce.

Rapid and accurate detection of LAB contamination sites during the manufacture of fresh-cut produce are necessary to quickly prevent or target reservoir accumulation and suppression of spoilage LAB. Environmental LAB spoilage microbes of fresh cut produce are typically potentially derived from residual puddles of water or unclean liquid runoff as sources of contamination. From samples taken, isolated populations of *LAB* will most likely indicate a source. For further identification into the genus *Leuconostoc* and species *Leuconostoc mesenteroides*, several other traditional biochemical assays are used, but have slowly been replaced by faster and more accurate genotypic tests, such as those included in this study. With the intention of rapidly diagnosing the microbial cause of this real-world postharvest processing concern, a rapid genetic detection method was developed for future utilization in commercial and industrial screening purposes of *LAB* populations as potential problems in postharvest storage of minimally processed carrot.

Materials and Methods
Four groups of raw product samples from CA fresh-cut facility were tested, each three months apart.

Symptomatic (final product) samples

\[\text{Lot A: 1.6, 2.3, 1.3, 3.04}\]
\[\text{Lot B: 2.25, 2, 2.47, 3}\]
\[\text{Lot C: 2.47, 1.9, 1.9, 2.81}\]
\[\text{Lot D: 1.6, 1.3, 2, 3.35}\]

Asymptomatic (raw product carrot material) samples

\[\text{Lot E: 3.47, 3, 2.3, 3.11}\]

Group 3

\[\text{Lot F: 1.5, 0.5, 0, 10}^{-5}\]
\[\text{Lot G: 7.9, 4.3, 2, 10}^{-5}\]
\[\text{Lot H: 3, 2, 10}^{-5}\]

Isolation and investigation of LAB from all groups of samples

Stimulating low levels of *LAB* and *Leuconostoc* populations in asymptomatic raw carrot tissue material in order to identify and verify the major microbial culprit behind this spoilage, 4 independent samplings were performed and further aimed to improve the efficiency for analysis and rapid screening of symptomatic carrot plug lots. Testing for rapid *LAB* identification used, including PCR, was to amplify the presence of a region of the 16S rRNA gene of *Leuconostoc*. Previous tests for identifying spoilage organisms like *LAB* have included cumbersome biochemical tests requiring days to weeks for adequate and diagnostic results. After sampling symptomatic raw carrot plugs, asymptomatic plugs, and carrot plugs of different peeled treatments, *Leuconostoc*, the notorious spoilage microbe of raw carrots, was discovered for all treatments. Common microbiological techniques were used to quantify and identify overall *LAB* and *Leuconostoc* populations, and PCR confirmed the presence of *Leuconostoc*, offering a rapid screening of symptomatic or asymptomatic carrot tissue for early warning of potential spoilage in storage.

Conclusions
This study was prompted by the observation of diagnostic spoilage signs and symptoms on raw, fresh-cut carrots in storage, indicative of proliferative populations of LAB, specifically *Leuconostoc*. In order to identify and verify the major microbial culprit behind this spoilage, 4 independent samplings were performed and further aimed to improve the efficiency for analysis and rapid screening of symptomatic carrot plug lots. Testing for rapid *LAB* identification used, including PCR, was to amplify the presence of a region of the 16S rRNA gene of *Leuconostoc*. Previous tests for identifying spoilage organisms like *LAB* have included cumbersome biochemical tests requiring days to weeks for adequate and diagnostic results. After sampling symptomatic raw carrot plugs, asymptomatic plugs, and carrot plugs of different peeled treatments, *Leuconostoc*, the notorious spoilage microbe of raw carrots, was discovered for all treatments. Common microbiological techniques were used to quantify and identify overall *LAB* and *Leuconostoc* populations, and PCR confirmed the presence of *Leuconostoc*, offering a rapid screening of symptomatic or asymptomatic carrot tissue for early warning of potential spoilage in storage.