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Rapid and non–destructive detection of *Pectobacterium carotovorum* causing soft rot in stored potatoes through volatile biomarkers sensing

Rajeev Sinha [a], Lav R. Khot [a, *], Brenda K. Schroeder [b], Yongsheng Si [a]

[a] Center for Precision and Automated Agricultural Systems, Biological Systems Engineering, Washington State University, Pullman, WA, USA
[b] Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID, USA

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ABSTRACT

Early disease detection plays a significant role in the pre– and post–production management of specialty crops, that often are stored for several months prior to consumption. Potato is one of the most important specialty crops of the United States. However, soft rot in potatoes due to pathogenic infections during bulk storage, accounts for substantial losses to the industry. This study was aimed at assessing the applicability of an emerging technology, portable field asymmetric ion mobility spectrometry (FAIMS), towards early detection of soft rot in potatoes during bulk storage. The FAIMS senses mobility of ions pertinent to volatile organic compounds (VOCs) released from inoculated tubers. In this study, potato tubers, inoculated with *Pectobacterium carotovorum* causing soft rot, were analyzed using FAIMS over a 30–day period in storage. Sterile water inoculated tubers were considered as healthy controls. Results suggest that FAIMS can detect soft rot as early as two days after inoculation (DAI) by effectively capturing VOCs associated with rot progression. The activity of pathogen and associated VOCs release was maximum during the second week after inoculation. A principal component analysis showed a clear distinction between the healthy and *P. carotovorum* inoculated tubers. Classification models, quadratic discriminant analysis and Naïve Bayes with leave–one–out cross validation confirmed the validity of FAIMS response with accuracies between 83 and 100% for both healthy and *P. carotovorum* inoculated tubers.

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1. Introduction

Currently, there are approximately 7.4 billion people worldwide, of which around 0.8 billion are undernourished. There has been a decrease in the prevalence of undernourishment from 18.6% in 1990–92 to a projected estimate of 10.8% in 2014–16 (Godfray et al., 2010; FAO Statistical Pocketbook, 2015). Such reduction rate has been attributed to the technological advancements for production agriculture and post–production processes. Postharvest crop loss is one of the most important factors which contributes to global food insecurity. Twenty–four percent of the total crop loss worldwide, and 9% in developed nations occurs during produce handling and storage (Lipinski et al., 2013). Plant diseases are often responsible for major postharvest crop losses (Sciumbato, 1993; Beuve et al., 1999; Thinlay et al., 2000; Flood, 2010). Therefore, effective disease detection and proper postharvest management of crops are key to increasing global food security.

Potato is one of the major staple food crops of the world and the United States (U.S.) is the fifth largest potato producer in the world, with a productivity of about 47.2 metric ton ha^{-1}, reported in 2014 (FAOSTATS, 2015). Around 7.5% of the potatoes produced in the U.S. are lost due to associated storage issues during potato bulk storage. Bacterial soft rot of tubers, caused by members of the soft rot Enterobacteriaceae (SRE), are significant contributors to the storage losses (Olsen et al., 2006). *P. carotovorum* is one major group of bacteria within the SRE that causes soft rot in potatoes. During bulk storage, soft rot is commonly a result of a favorable micro–climatic conditions within the potato pile producing localized “hot spots”. The pathogen can spread quickly to healthy tubers located below such hot spots in the pile, which is facilitated by intense respiration activity and the heat released from rotting tubers (Olsen et al., 2006; Inglis et al., 2011).

During normal growth, most plants produce intrinsic volatile organic compounds (VOCs) released through leaves, flowers and fruits (Tholl et al., 2006). In recent years, the sensing of VOCs for monitoring and early detection of diseases in plants and produce

* Corresponding author.
E-mail address: lav.khot@wsu.edu (L.R. Khot).

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has emerged as a promising tool (Cardoza et al., 2002; Toome et al., 2010; Laothawornkitkul et al., 2010; Jansen et al., 2011; Spadafora et al., 2016). Headspace sampling followed by Gas Chromatography–Mass Spectrometry (GC–MS), GC–Flame Ionization Detector (GC–FID), and electronic noses for disease detection in plants and agricultural produce are techniques that have been evaluated and reported by many researchers (Doughty et al., 1996; Arshak et al., 2004; Deng et al., 2004; Ebel et al., 2006; Blasioli et al., 2010; Copolovici and Niinemets, 2010; Konduru et al., 2015).

Analysis of volatiles emitted by *P. carotovorum* inoculated potato tubers can be used for rapid detection of diseases in storage facilities. Varns and Glynn (1979) investigated the feasibility of early disease detection in potato tubers inoculated with *P. carotovorum,* previously known as *Erwinia carotovora*. The GC–MS analysis identified acetone, ethanol and 2–butanone as volatile biomarkers that were produced in large concentrations. Waterer and Pritchard (1984) investigated *Corynebacterium sepedonicum*, now *Clavibacter sepedonicus* (causing ring rot of potatoes) and *E. carotovora* (causing soft rot of potatoes) and reported associated volatile biomarkers to be ethanol, methanol and acetaldehyde. Similarly, de Lacy Costello et al. (1999) studied volatiles associated with *E. carotovora*, *Bacillus polymyxa* and *Arthrobacter* sp., using GC–MS and reported 22 unique volatiles indicative of soft rot caused by *E. carotovora*. Some key volatile biomarkers identified were acetone, 2–propenal, 2–butanone, 1–butanol and 2–pentanone. Kushalappa et al. (2002) investigated volatile release pattern from potatoes (variety: Russet Burbank) inoculated with different subsp. of *E. carotovora* at 3, 4 and 5 days after inoculation (DAI) using the GC–FID technique. They reported varying volatiles' fingerprints based on the retention time (RT) to classify different pathogen groups. Other studies also used different gas analysis techniques to detect potato tuber soft rot under storage conditions (Ouellette et al., 1990; Lyew et al., 1999, 2001; British Potato Council, 2000; Lui et al., 2005). Most of these studies used a headspace sampling method followed by laboratory based GC–MS/GC–FID analysis to characterize the volatiles. Although accurate, the above techniques are not intended for real–time detection, require skilled labor and are often time consuming, costly and do not offer system portability.

Recently, a new emerging technology known as field asymmetric ion mobility spectrometry (FAIMS) is being used in volatile based analysis. FAIMS characterizes volatiles by fingerprinting the ion mobility of constituent ions of volatiles instead of chemically analyzing the VOCs composition. Researchers have studied FAIMS applications in the medical, food and dairy industry (Arasaradnama et al., 2014; Zhao et al., 2015). Alexander et al. (2014) used differential mobility spectrometry (DMS), which is synonymous to FAIMS, to detect huanglongbing (HLB) disease by analyzing the VOCs released from the infected citrus trees. Rutolo et al. (2014) evaluated the feasibility of detection of potato (variety: Maris Piper) soft rot caused by *P. carotovorum* using this technology. However, no study quantified the temporal progression of soft rot in potato tubers under storage conditions. More studies that evaluate portable FAIMS for detecting potato rot in different cultivars, and during varied bulk storage types and environments are needed. Moreover, studies are needed to understand the ability of FAIMS to characterize and quantify the release of volatile biomarkers specific to soft rot on a temporal basis.

In this study, the pattern of release of VOCs by *P. carotovorum* inoculated potato tubers was studied using FAIMS technology. The hypothesis is that the portable FAIMS can detect volatile biomarkers of soft rot before symptoms are visible on the tubers, leading towards early detection and effective disease management. The specific objectives of the study were to assess the applicability of FAIMS towards: 1) detection of potato soft rot caused by *P. carotovorum*, 2) assessing how early the disease symptoms can be detected during storage, and 3) monitoring of the temporal progression of the disease and characterization of associated volatile biomarkers.

2. Materials and methods

2.1. Sample preparation

Russet Burbank is one of the most common potato varieties grown in the Pacific Northwest states of the U.S., with yield ranging from 28 to 67 metric ton ha^{-1} (Potato Association of America (2015)). This variety is considered to be an industry standard and the harvested tubers are often bulk stored for up to 12 months. Therefore, potato tubers (variety: Russet Burbank) from the 2015 growing season and stored in a commercial bulk storage facility (AgriNorthwest Inc., Prescott, WA, U.S.) were used in this study. Tubers were inoculated with *P. carotovorum*. *P. carotovorum* subsp. *carotovorum* strain Ec101 was grown overnight in 5 mL nutrient broth at 28 °C with agitation at 200 rpm. A 0.5 mL aliquot of the culture was then added to 250 mL nutrient broth and incubated overnight at 28 °C with agitation at 200 rpm. Cells were harvested by centrifugation (48,800 × G for 10 min), washed with sterile distilled water, and re–suspended in sterile distilled water to an optical density (OD_{600}) of 0.3 (approximately 1×10^8 CFU mL^{-1}). The cells were then harvested by centrifugation and re–suspended in sterile distilled water at 1/10th the volume (approximately 1×10^9 CFU mL^{-1}) (Vidaver, 1976; Dung et al., 2014). A high concentration of inoculum was used to ensure consistent rot of the tubers. The inoculum was placed in a 15 mL sterile tube and a 22–gauge needle was completely immersed in the inoculum and then used to puncture the surface of sterilized tubers (to a depth of 2.5 cm) five times on the same surface about 1 cm apart. Before inoculation, the tubers were washed with tap water and the injection sites were sterilized by cleaning the surface using a cotton swab with 95% ethanol. A similar protocol was followed to inoculate a set of tubers with sterile water, which were used as healthy controls in the experiment.

2.2. FAIMS set up

2.2.1. The existing set up

Portable FAIMS is an emerging technology for gas analysis. The constituent gases are differentiated based on the different mobility of the constituent ions in a variable electric field. The volatiles to be analyzed are fed into the ionization chamber using carrier gas, which was lab air in this study. These are then ionized using a radioactive source (Ni^{63}) by a charge transfer process and both positive and negative ions are generated. The ion clouds mark their way to the electrode channel where application of a radio frequency (RF) waveform causes the ions to move in a zig–zag trajectory under the varying electrical field. Based on the ion mobility, many ions collide with the electrode plate losing their charge and some ions collide with the detector plate generating an ion current. In addition to the RF waveform, a compensation voltage (CV) (DC Voltage) was also added to modify the RF waveform to shift the trajectories of the ions, so that they reach the detector plate and do not lose their charge through collision with the electrode plates. The value of the CV was increased from −6 V to 6 V in 512 steps, thus generating a spectrum of ion currents corresponding to each CV value. At the same time, the applied RF waveform, known as dispersion field (DF) was varied from 0 to 100% and a 3D spectrum of ion current, CV and DF was generated for both the positive and negative ions in the analyte. Therefore, using FAIMS for detection of desired analytes in a sample comprises three different steps. The