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Short communication

Detection of *Escherichia coli* via VOC profiling using secondary electrospray ionization-mass spectrometry (SESI-MS)

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ABSTRACT

Escherichia coli O157:H7 (EC O157:H7), as well as its recently emerging non-O157 relatives, are a notorious group of pathogenic bacteria associated with foodborne outbreaks. In this study, we demonstrated that secondary electrospray ionization mass spectrometry (SESI-MS) could be a rapid and accurate detection technology for foodborne pathogens. With SESI-MS volatile organic compound (VOC) profiling, we were able to detect and separate a group of eleven *E. coli* strains from two major foodborne bacteria, *Staphylococcus aureus* and *Salmonella* Typhimurium in three food modeling media. In addition, heatmap analysis of relative peak intensity show that there are six core peaks (*m*/*z* of 65, 91, 92, 117, 118 and 119) present and at a similar intensity in all eleven *E. coli* strains at the experimental conditions we tested. These peaks can be considered conserved VOC biomarkers for *E. coli* species (robustly produced after just 4 h of growth). Bacterial strain-level differentiation was also attempted via VOC profiling, and we found that EC O157:H7 and EC O145 were differentiable from all other EC strains under the conditions investigated.

1. Introduction

During the past three decades, *Escherichia coli* (EC) O157:H7 has evolved from a clinical research topic to a global public health concern (Mead and Griffin, 1998). The bacterium can cause severe, acute hemorrhagic diarrhea and abdominal cramps, and can result in complications such as hemolytic uremic syndrome (HUS). While EC O157:H7 is the most notorious member of the group of pathogenic *E. coli* strains that cause foodborne illness, the frequency of its lesser known relatives is increasing in foodborne outbreaks. Recent examples include the 2012 multistate *E. coli* O26 outbreak linked to raw clover sprouts from Jimmy John's Gourmet Sandwich (CDC, 2012), the 2011 European sprout *E. coli* O104 outbreak (CDC, 2011), the 2010 multistate outbreak of *E. coli* O145 linked to shredded romaine lettuce (CDC, 2010a), and the 2009 multistate outbreak of *E. coli* O157:H7 infections associated with beef (CDC, 2010b). Given the impact of both O157 and non-O157 strains on foodborne illness, the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) announced in September 2011 that it was taking action to prohibit sales of ground beef, or its precursors, that are contaminated with *E. coli* serogroups O26, O103, O45, O111, O121 and O145, in addition to previously regulated O157:H7 (USDA, 2011). Therefore, technologies to accurately detect and monitor these pathogenic *E. coli* strains are required.

Conventional methods used for detecting foodborne bacteria, such as selective medium screening and biochemical tests, remain the gold standard. A major drawback, however, is that these methods are often laborious and slow. For example, the FDA bacteriological analytical manual guideline for *E. coli* O157:H7 bacteria screening from food requires fourteen media and reagents, and takes 24 h for a positive result and three days for full confirmation (Feng et al., 2011). Similarly, the USDA microbiology laboratory guidebook (USDA, 2012) suggested methods for Shiga toxin-producing *E. coli* (STEC) strain detection, which involves both serological tests and polymerase chain reaction (PCR) procedures, could take up to 28 pieces of equipment and materials and one to three days to provide positive identification. Other methods, such as enzyme-linked immunosorbent assay (ELISA) detection for *E. coli* in water samples (e.g., Pappert et al., 2010), PCR detection for *Salmonella* spp., *Listeria monocytogenes*, and *E. coli* O157:H7 in meat samples (e.g., Kawasaki et al., 2005), and loop-mediated isothermal amplification detection for *E. coli* strains from various food samples (e.g., Wang et al., 2012), have been used for the detection of foodborne bacteria. While useful, these methods are often coupled to

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culture enrichment procedures to ensure detection of metabolically-active cells. Emerging techniques show promise in the detection of foodborne bacteria. For example, the infrared spectroscopy of fatty acid methyl esters profiling of *Staphylococcus aureus*, *E. coli* from brain heart infusion agar (Whittaker et al., 2003) or the use of NMR study of *Brassica rapa* metabolic response to *Bacillus subtilis*, *S. aureus*, *E. coli*, *Salmonella* Typhimurium and *Shigella flexneri* contamination via ^{1}H NMR and 2D NMR spectroscopy (Jahangir et al., 2008).

Mass spectrometry (MS) also shows promise as a pathogen detection and monitoring tool that might ultimately be fast, accurate, and high-throughput (for a review, see Sauer and Kliem, 2010). There are several MS technologies that have been put forward as tools to identify bacteria on and in food. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) proteomics study of *E. coli* and *Aeromonas hydrophila* isolated from lettuce (Holland et al., 2000), *Serratia marcescens*, *Stenotrophomonas maltophilia*, and *Pseudomonas fragi* isolated from seafood (Bohme et al., 2010), can successfully distinguish between different genus and species; gas chromatography mass-spectrometry (GC–MS) has been used for identifying EC O157:H7 and *Salmonella* spp. in ground beef and chicken via their metabolite profiles (Cevallos-Cevallos et al., 2011); and fatty acid analysis via two dimensional GC–MS has been used for *Bacillus* spp., *S. aureus*, *E. coli* and *Salmonella enterica* after growth on a trypticase soy agar (Gardner et al., 2011). An ideal technology, however, can respond in real-time, and can directly sample contaminated food products.

It is known that volatile organic compounds (VOCs) generate characteristic odors for certain bacteria, and these VOCs can be used for species identification (e.g., Schöller et al., 1997; Bunge et al., 2008). VOCs produced by bacteria have been targeted for detection by GC–MS, such as *E. coli* O157:H7 and *S.* Typhimurium detection from food modeling media (Senecal et al., 2002), *S.* Typhimurium VOC detection from a pork product (Yun et al., 2010), proton transfer reaction-mass spectrometry (PTR-MS) for *Pseudomonas* spp., *Enterococcus* spp. on beef and pork (Mayr et al., 2003), as well as selected ion flow tube-mass spectrometry (SIFT-MS) for psychrotrophic bacteria and H_2S-producing bacteria (Noseda et al., 2008). Secondary electrospray ionization mass spectrometry (SESI-MS), with its direct gas sampling ability, exhibits some of the best features of a desired laboratory analytical tool, such as real-time analysis and the potential for high-throughput sample analysis, and only requires minimal instrument modification to the standard, commercially-available mass spectrometer instrument. SESI-MS has a sensitive detection limit at parts per trillion (ppt) and sometimes lower (Martínez-Lozano et al., 2009), and it has been applied to the detection of explosive gaseous samples (Martínez-Lozano et al., 2009), human breath vapor (Martínez-Lozano and de la Mora, 2007, 2008), as well as the identification of clinically-relevant pathogens (e.g., *Pseudomonas aeruginosa* and *S. aureus*; Zhu et al., 2010).

The aim of this study is to demonstrate that SESI-MS is a feasible analytical tool to rapidly detect and distinguish EC O157:H7 and non-O157 *E. coli* from *S. aureus* and *S.* Typhimurium. With SESI-MS VOC profiling, we were able to detect and separate a group of 11 *E. coli* serotypes (including EC O157:H7) from *S. aureus* and *S.* Typhimurium in three food modeling media (meat extract medium, vegetable extract medium, and apple extract medium). We report six conserved VOC biomarker peaks for *E. coli* species (peaks 65, 91, 92, 117, 118 and 119), which are present by 4 h growth for all *E. coli* serotypes/strains we tested. Finally, EC O157:H7 and EC O145 were always differentiable from all other EC strains in the conditions investigated in this study. This current study examines monocultures in an effort to identify the common and unique volatile biomarkers for the bacteria investigated using SESI-MS. We envision a future where the technology can be deployed to directly sample a food matrix, i.e., without isolation of individual bacteria, with the output being both bacteria constituent identity as well as a quantity determination.

2. Materials and methods

2.1. Bacterial strains, medium, and growth condition

The strains used in this study are listed in Table 1. Biochemical tests (BD Enterotube II Prepared Media Tubes, Franklin Lakes, NJ) and antigen-specificity stereotyping (Oxoid Dryspot Seroscreen, Oxoid, Cambridge, UK; Remel Wellcolex *E. coli* O157:H7 Kit, Remel, Lenexa, KS; Adsorbed monovalent O single antisera, Statens Serum Institut, Copenhagen S Denmark) were used to confirm the genus and serotype of strains. Unless otherwise indicated in the text, strains were cultured aerobically for 16 h at 37 °C in 50 mL of food modeling medium (final OD >1 for all samples). Three food modeling media were used: meat extract medium (MEM) (Sigma, St. Louis, MO), vegetable extract medium (VEM) (Sigma, St. Louis, MO), and apple extract medium (AEM) (Spectrum, New Brunswick, NJ). For the time course study, plate counts (Tryptic soy agar, BD Diagnostics; 37 °C; 24 h) for each time point were generated.

2.2. Secondary electrospray ionization-mass spectrometry (SESI-MS)

The VOC mass spectra were collected using SESI-MS, as previously reported (Zhu et al., 2010). Briefly, bacterial culture headspace VOCs were introduced into a customized SESI-MS reaction chamber for 1 min via displacement by CO_2 (99.99%; 2 L/min) at ambient temperature. Formic acid (0.1% (v/v)) was used as the electrospray solution, delivered at a flow rate of 5 nL/s through a non-conductive silica capillary (40 μm ID) with a sharpened needle tip. The operation voltage was ~3.5 kV. Spectra were collected within 1 min as an accumulation of 20 scans in single-quadrupole positive-ion mode. The system was flushed with CO_2 between samples until the spectrum returned to background levels.

2.3. Data processing and analysis

Analyst 1.4.2 software (Applied Biosystems) was used for spectra collection and processing. Mass spectra shown in each figure are the average spectra of all replicates for each bacterial strain. Spectra have been blank-subtracted (the blank spectrum is the spectrum generated by medium without bacteria presence) and normalized

Table 1
Bacterial strains used in this study.

Genus and species	Strain ID, serotype	Source/origin
Escherichia coli	07-4434-1, O6	Wadsworth center, NYSDH[a]
	11-26983-1, O103	Wadsworth center, NYSDH
	11-25799-3, O111	Wadsworth center, NYSDH
	11-20403-1, O121	Wadsworth center, NYSDH
	11-18318, O157: NM	Wadsworth center, NYSDH
	11-25813-1, O26	Wadsworth center, NYSDH
	08-2175-14, O84	Wadsworth center, NYSDH
	11-26550-11, O45	Wadsworth center, NYSDH
	IDR1000013809, O145	Wadsworth center, NYSDH
	ATCC 43895, O157:H7	ATCC
	K12	CGSC[b], Yale University
Staphylococcus aureus	ATCC 25923	ATCC
Salmonella enterica	ST5383, Typhimurium	Yale University

[a] NYSDH: New York State Department of Health.
[b] CGSC: Coli Genetic Stock Center.