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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, *S. aureus* and *S.* Typhimurium in three food modeling media. In addition, heat map 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 four hours 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.

Keywords

Foodborne bacteria detection; Volatile Organic Compound profiling; Secondary Electrospray Ionization-Mass Spectrometry; Biomarkers; *Escherichia coli* O157:H7; *Staphylococcus aureus*; *Salmonella* Typhimurium

1. Introduction

During the past three decades, *E. 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

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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 hrs 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) strains detections, which involving both serological tests and polymerase chain reaction (PCR) procedures, could take up to 28 pieces of equipment and materials and may also take 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 *Escherichia 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 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 *S. aureus*, *E.coli* from brain heart infusion agar (Whittaker et al., 2003) or the use of NMR study of *Brassica rapa* metabolic response to *B. subtilis*, *S. aureus*, *Escherichia 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 *A. 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.*, *Staphylococcus aureus*, *Escherichia 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 that 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 *Salmonella* Typhimurium detection from food modeling media (Senecal et al., 2002), *Salmonella* 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 directly 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. *P. 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 was 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 one minute via displacement by CO2 (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 one minute 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 to the peak of greatest intensity. SAS version 9.2 and JMP version 9 (SAS Institute Inc., Cary, NC, USA) was used to conduct principal component analysis (PCA), generate PCA scores, loading plots and biplots, as well as to determine the statistical significance of observed PCA scores difference. Peaks between 40 and 150 *m/z* (mass-to-charge ratio) and greater than a 1 % relative intensity threshold (after blank subtraction) were used as variables for PCA, while all experimental replicates were used as observations. The heatmap was generated using Matlab (MathWorks Inc.). Common logarithm values of peak relative intensities from the average spectra of each bacterial strain were used for the generation of this graphical representation of VOC spectra profile data.

3. Results and discussion

3.1. Headspace VOC profiling for the detection of foodborne pathogenic bacteria

Three foodborne pathogenic bacteria, *E. coli* O157:H7 (EC O157:H7), *S. aureus* ATCC 25923 (SA), and *S. enterica* serovar Typhimurium ST5383 (ST), were grown aerobically at 37°C for 16 h in meat extract medium (MEM), vegetable extract medium (VEM), and apple extract medium (AEM). The full scan spectra (40–150 m/z) of bacterial culture headspace VOCs from these three bacteria in the three different food modeling media are shown in Fig. 1 a–c. We observed that the different bacterial species produce a unique global VOC profile consisting of dominant peaks, which are labeled in each spectrum, as well as bacterium-specific peaks. Some of the peaks we detected have been reported by others using similar mass spectrometry technologies. For example, Bunge and colleagues detected peaks m/z = 91, 117, 118 and 119 in the headspace of *E. coli* bacteria using PTR-MS (Bunge et al.,

2008), and Rudzinski, Allardyce, and their colleagues (Rudzinski et al., 2004; Allardyce et al., 2006) reported compounds detected as peak 59, 61 and 103 produced by *S. aureus* via SIFT-MS.

We used the multivariable analysis method, principal component analysis (PCA), to establish statistical evidence for distinction of the spectral data. The principal component analysis score of the absolute intensities for all peaks (40 – 150 *m/z*) of mass spectra from the headspace volatiles of the three bacteria from MEM, VEM and AEM are plotted in Fig. 1d–f. A clear separation between the species can be seen in each medium ($P < 0.0001$ in MEM, and $P < 0.05$ in VEM and AEM).

To further compare the VOC profiles from different pathogenic bacteria headspace, presence/absence analyses of peaks from Figures 1a–c were conducted. Peaks are considered the same when the associated fragmentation spectra are identical. EC O157:H7 has 6 core peaks (m/z of 65, 91, 92, 117, 118, and 119) that are found in the headspace of the culture in all three media, and with similar relative intensities. There are 23 core peaks for SA, and 11 core peaks for ST. These core peaks constitute a conserved fundamental fingerprint for these organisms under all of the conditions tested. A comparison of data from these conserved fingerprints (data not shown) indicates that most of the peaks produced in the culture headspace are unique to the specific bacterium. These unique peaks could be used as distinguish signals for detection and identification of these bacteria.

3.2. VOC biomarker (core peak) discovery for EC O157:H7 and non-O157 strains

Non-O157:H7 *E. coli* strains have also been associated with foodborne outbreaks. We used SESI-MS to profile VOCs in the headspace of nine outbreak, Shiga toxin-producing EC strains (STEC; *E. coli* O6, O26, O45, O84, O103, O111, O121, O145, and O157: NM), as well as the non-pathogenic K12 strain to determine whether or not these ten strains can also be differentiated from SA and ST, based on their VOC profile. Heat map analysis (Fig. 2) of SESI-MS relative peak intensity shows that the six major peaks associated with EC O157:H7 (m/z of 65, 91, 92, 117, 118 and 119) are present in all eleven EC strains, suggesting that there is a core VOC fingerprint for *E. coli* that identifies it as EC, and differentiates it from SA and ST. SESI-MS VOC headspace analysis for VEM and AEM also showed that all six core fingerprint peaks are present for all eleven EC strains (data not shown). These peaks therefore can be considered core VOC fingerprint peaks for these *Escherichia coli* serovars.

We also conducted a time series study to determine how early *E. coli* strain core fingerprint peaks can be detected when grown in MEM using SESI-MS. The six core EC VOC biomarkers were monitored every 2 h, up to 8 h, for all eleven EC strains. The EC O157:H7 headspace VOC profile time-series data is shown here and is representative of all the ECs included in this study. At hour two, four (m/z 91, 117, 118 and 119) of the six EC O157:H7 core VOC fingerprint peaks are detected by SESI-MS. Other EC serovars also produced measurable amounts of a subset of the six core VOCs (data not shown). At hour four, all six core VOC fingerprint peaks were easily measurable for all serovars, e.g., EC O157:H7 (Fig. 3).

3.3. EC serovars/strains differentiation based on VOC profiling

While the eleven EC strains tested in this study have six core peaks in common, there are some peaks that can be used to differentiate between these bacteria at the serovar level. For example, the heatmap (Fig. 2) shows that EC O145 uniquely produced peak 59, and EC O157:H7 produced peak 70, which is not present in other EC strains when grown on MEM. Fig. 6 shows the PCA score plot and loading plot constructed based on the spectral data from all eleven EC strains grown on MEM. Three out of eleven strains, EC O157:H7, EC O145, and EC K12, are differentiable based on the VOC profiles generated in MEM ($p < 0.0001$ for both PC 1 and PC 2 for EC O157:H7 and EC O145, and $p<0.05$ for PC 2 in EC K12). According to the Fig. 4 loading plot as well as the heatmap (Fig. 2), we can see that peaks such as 41,42, 43, 53, 55 and 59 provide information to separate EC O145 from other strains, while peaks 70, 120 and 147 drive EC O157:H7 clustering. EC O157:H7 and EC O145 are also distinguishable from each other after growth on VEM and AEM (data not shown). While in MEM, the EC K12 VOC fingerprint is distinguishable from all STECs. However, EC K12 is not distinguishable from the STECs in VEM and AEM. In a previous study, Posse and colleagues analyzed both O157 and non-O157 STEC strains for serotyping and metabolic profiling, to try to correlate carbohydrate fermentation and STEC serotype (Possé et al., 2007). Similar to our work, they found a correlation between serotyping and metabolic profiling. However, Posse and colleagues, like this study, found that some serotypes have a similar metabolic profile under the tested growth conditions and thus could not be separated metabolically.

4. Conclusion

Using the SESI-MS technique, we demonstrate clear distinction between EC O157:H7, SA, and ST based on their VOC profiles on each of three different food modeling media. We detected six core peaks (m/z of 65, 91, 92, 117, 118 and 119) for *E. coli* which could be recognized as headspace VOC biomarkers for the species. All six peaks were observed after four hours growth on MEM for all *E. coli* serovars and strains tested. In addition, we were also able to distinguish EC O157:H7 from other non-O157 EC serovars/strains (EC K12, O6, O26, O45, O84, O103, O111, O121, O145 and O157: NM), in all three food modeling media. This study suggests that SESI-MS has the potential to be used as an analytical method for foodborne pathogenic bacteria detection and differentiation, being able to differentiate EC O157:H7 from its close relatives. While limiting factors, such as labor and time involved for isolation of individual bacteria and a pre-enrichment step still exist at this stage, this technology could be used to directly sample and identify singular and multiple pathogen contaminants.

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Highlights

- First report of SESI-MS based method for foodborne bacteria detection
- *E. coli* can generate different VOC profiles than *S. aureus* and *S.* Typhimurium
- A group of eleven *E. coli* strains were separated from two major foodborne bacteria
- Six VOC biomarkers were discovered for *E. coli* species
- *E. coli* O157:H7 and *E. coli* O145can be detected among other *E. coli* serotypes via SESI-MS

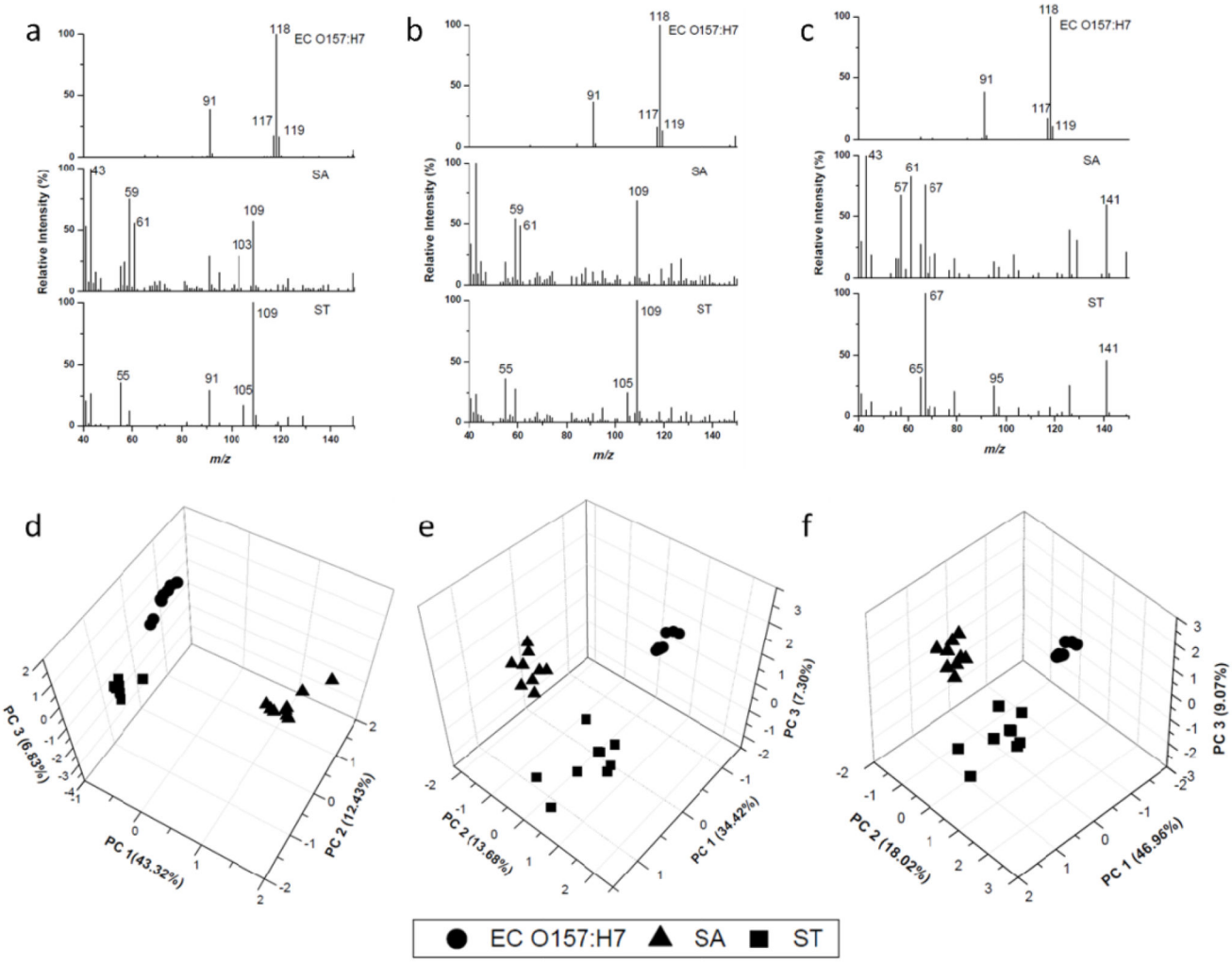

Fig. 1.

Headspace VOC spectra comparisons (a–c) and principal component analysis (PCA) score plots (d–f) of *E. coli* O157:H7 (EC O157:H7.), *S. aureus* (SA), and *S.* Typhimurium (ST) in three different food modeling media: meat extract medium (MEM, left), vegetable extract medium (VEM, middle) and apple extract medium (AEM, right) (37°C, 16 h). Nine replicates were used for each bacterium in each medium.

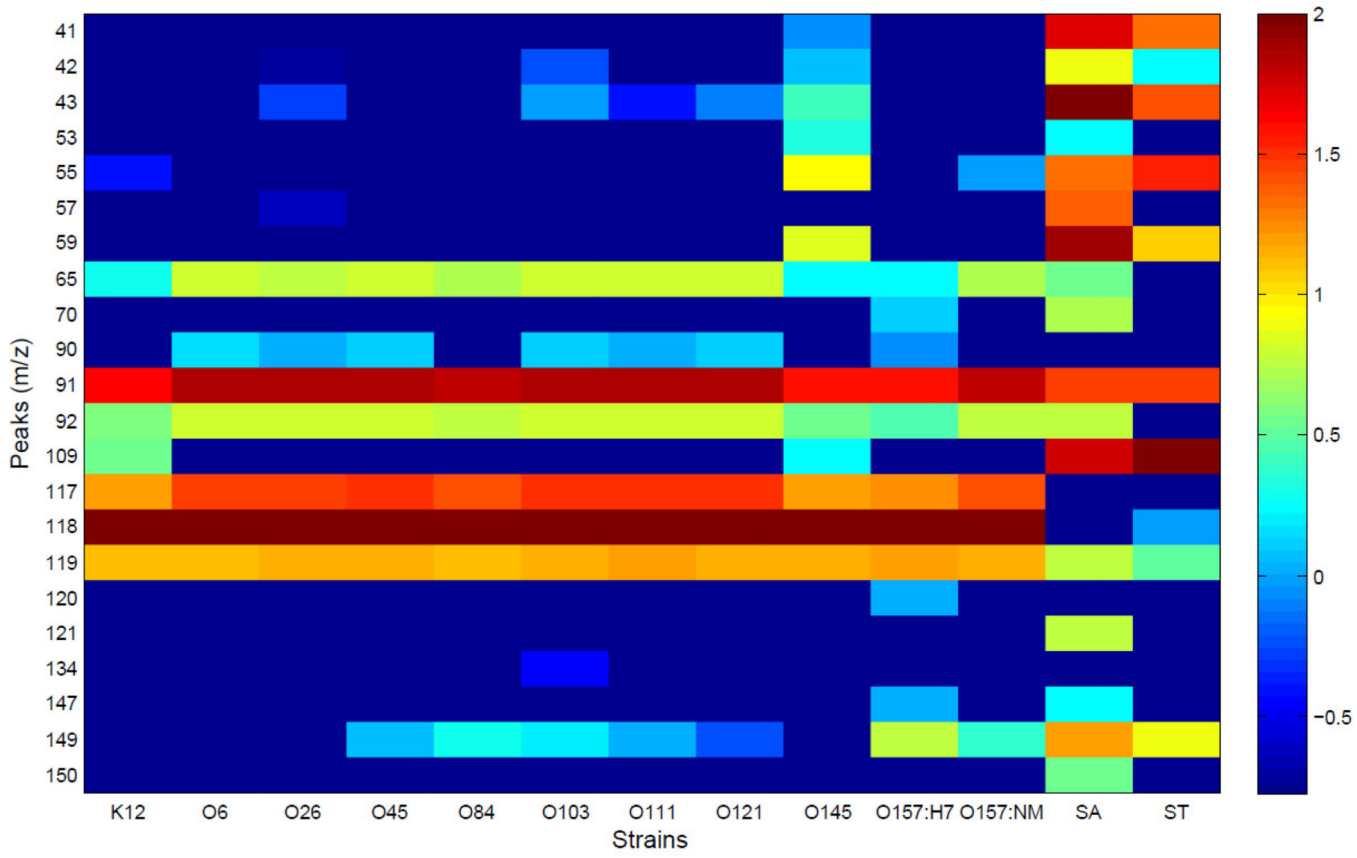

Fig. 2.

Comparison of the average SESI-MS peak intensity (six replicates) of *E. coli* strains K12, O6, O26, O45, O84, O103, O111, O121, O145, O157:H7, and O157: NM, and SA and ST after growth on MEM (37°C, 16 h). Colors represents the common logarithm value of each peak's relative intensity.

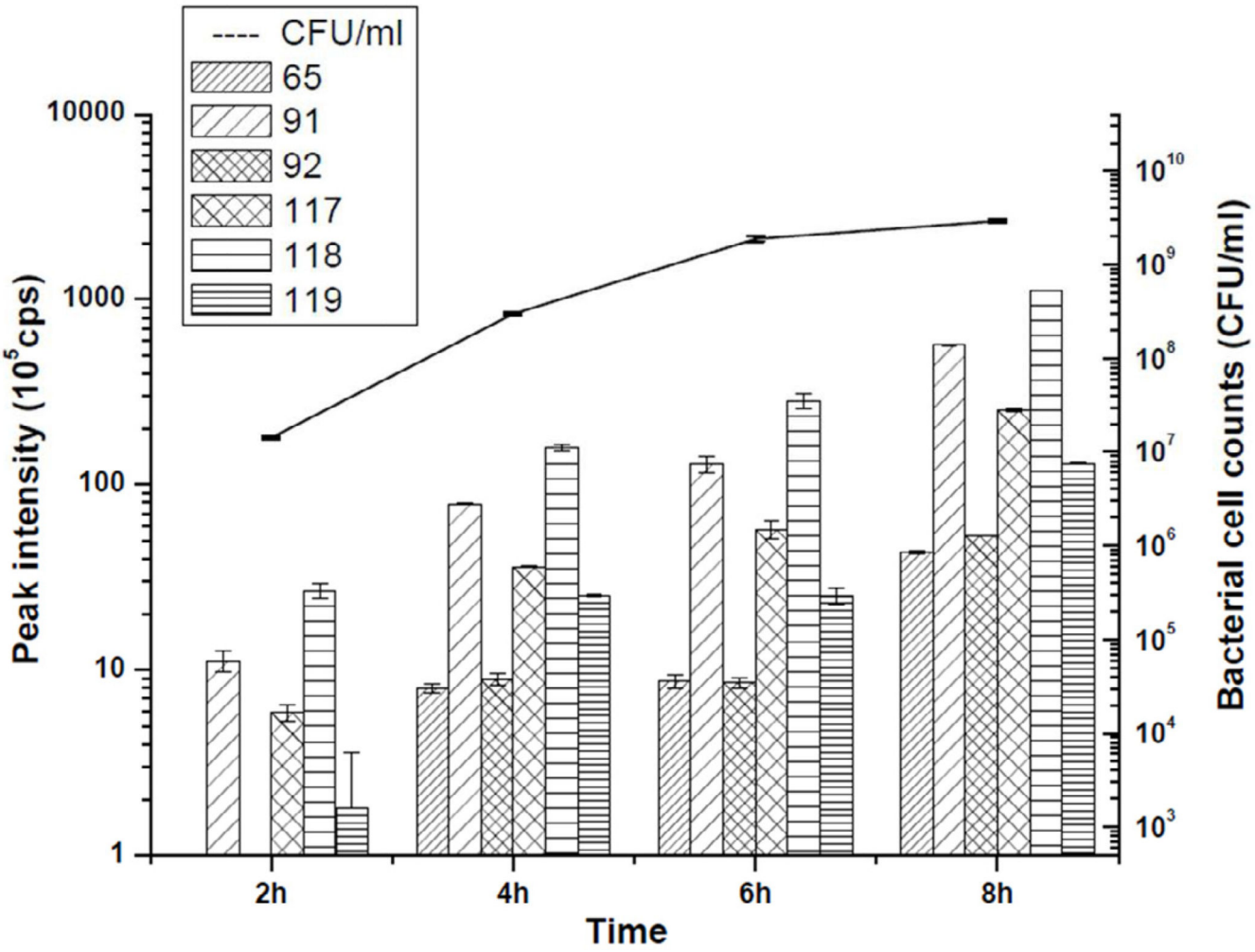

Fig. 3.

E. coli core VOC fingerprint peak intensity as a function of time (bar chart, left axis, standard error indicated) during EC O157:H7 growth on MEM (37°C, 16h). The plate count data for each time-point are also shown (right axis, standard error indicated).

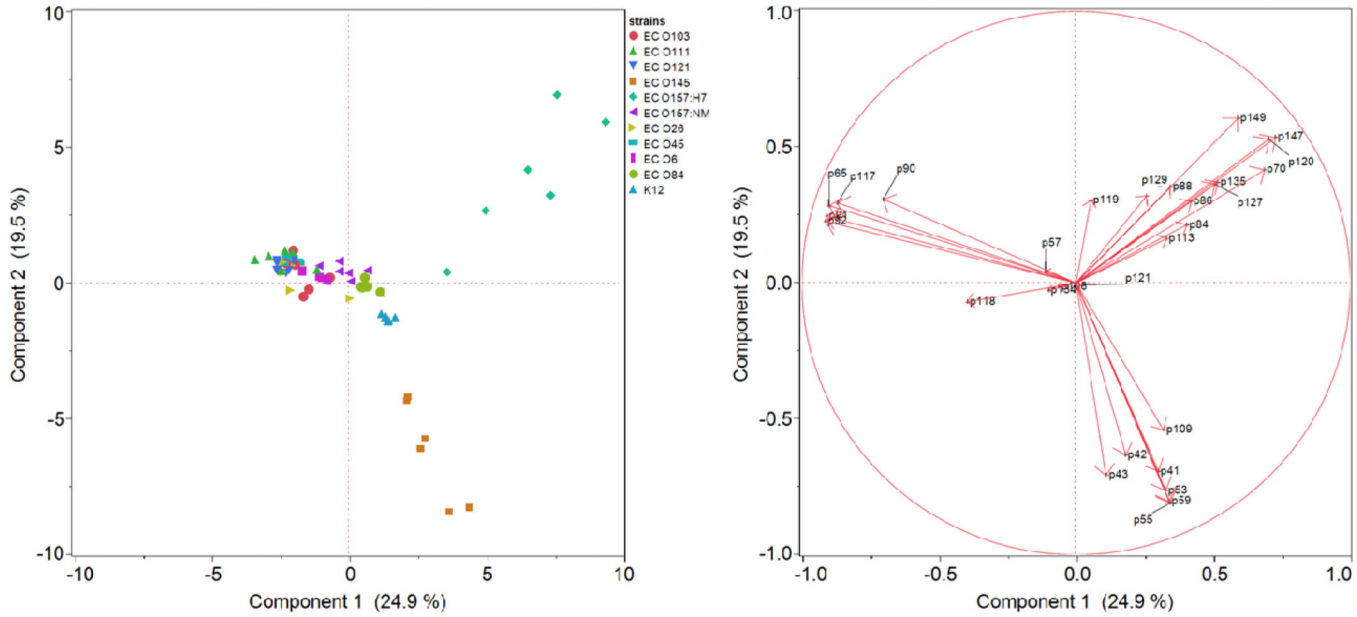

Fig. 4.

PCA score plot (left) and loading plot (right) of SESI-MS data from *E. coli* K12, O6, O26, O45, O84, O103, O111, O121, O145, O157:H7, and O157: NM after growth on MEM (37°C, 16 h).

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*
	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**, Yale University
Staphylococcus aureus	ATCC 25923	ATCC
Salmonella enterica	ST5383, Typhimurium	Yale University

* NYSDH: New York State Department of Health

** CGSC: Coli Genetic Stock Center