APPLICATION OF MASS SPECTROMETRY IN DETECTION OF FOODBORNE PATHOGENS

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Abstract – Conventional culture-based methods of foodborne pathogen detection can be very time-consuming. The current research focused on investigating the ability of MALDI-TOF MS and GC-MS platforms to rapidly detect three pathogens (*Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella enterica*) directly from selective enrichment broths containing spiked minced meat samples, obviating the need for culturing on solid media. Minced beef samples were spiked with the pathogen of interest (10 cfu/ mL of final enrichment broth), enriched in selective broth and sampled at different incubation times. The enrichment broths were analysed using MALDI-TOF MS and GC-MS. Meat samples spiked with as low as 10 colony-forming units of *L. monocytogenes* and *S. enterica* per mL of selective broth culture were detected by MALDI-TOF MS within 30 h and 18 h of incubation, respectively. Analysis of the samples by GC-MS enabled discrimination of the spiked meat samples from the control samples after 24 h, 18 h and 12 h of incubation for *L. monocytogenes*, *S. enterica* and *E. coli* O157:H7, respectively. In conclusion, both MALDI-TOF MS and GC-MS can be used to detect foodborne pathogens directly from selective enrichment broth saving the overall time and cost associated with pathogen detection.

Key Words –GC-MS, MALDI-TOF MS, spiked meat

List of Abbreviations:

MALDI-TOF MS: (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry)
GC-MS (Gas Chromatography-Mass Spectrometry)

I. INTRODUCTION

The fresh meat industry has often faced expensive product recalls following foodborne outbreaks of pathogens like *Salmonella* species, *Escherichia coli* and *Listeria monocytogenes* [1]. Considering the short shelf-life and perishability of red meat products, there is a continuous need for specific, sensitive, rapid, and cost-effective detection techniques for such pathogens from complex food matrixes. Currently, a wide range of methods are available for pathogen detection which include biochemical, immunological and molecular platforms. However, routine testing in food laboratories is still largely dependent on culture-based techniques that rely on long enrichment periods using different selective and confirmatory culture media [2]. The recent emergence of mass spectrometry based technologies such as MALDI-TOF MS for the detection and characterisation of clinically important bacteria has transformed microbial diagnostics. This technology was originally developed for detecting bacteria of clinical significance from solid culture media. Previously, we have introduced an improvement to this technique by adopting it for the detection of the foodborne pathogen, *Listeria monocytogenes*, directly from selective enrichment broth (obviating the need for culturing on solid media) containing complex food matrices [3]. In the current study, direct detection of three red meat pathogens, namely *L.*

monocytogenes, *Salmonella enterica* and *E. coli* O157:H7, from selective enrichment broth was investigated. The current study also evaluated the application of a more recent whole-cell metabolomics approach using GC-MS as a novel method of providing timely, accurate and reliable identification of the three meat pathogens. We have previously demonstrated that a simple metabolomics-based approach can facilitate the rapid detection of *L. monocytogenes* grown in laboratory media and a complex food matrix [4]. The current project investigated the metabolome of spiked red meat samples in selective enrichment media and the data obtained were mined using chemometrics to identify potential biomarkers.

II. MATERIALS AND METHODS

a) Bacterial strains and culture media

For experiments involving the direct detection of the pathogens from enrichment broth, a standard *L. monocytogenes* strain (ACM 98; obtained from the Australian Collection of Microorganisms) and reference strains of *Salmonella enterica* subsp. enterica serovar Typhimuirum (ATCC 13311) and *E. coli* O157:H7 (ATCC 43895) were used. For all spiking experiments, a 24 h culture of each pathogen on Brain Heart Infusion agar (BHIA) was adjusted to a 0.5 McFarland Standard. The standardized culture was used to spike a 1/10 dilution of a minced beef sample (not containing the test pathogen) in selective enrichment broth to achieve the required spiking load (10 cfu/mL of final enrichment broth). The spiked foods were enriched in Oxoid Novel Enrichment (ONE) broth for *L. monocytogenes* (OBL, Oxoid, UK), Muller-Kauffmann Tetrathionate-Novobiocin broth (MKTTn, Micromedia, Australia) for *S. enterica* and Tryptic Soy Broth modified with novobiocin (TSBn) for *E. coli* O157:H7. Unspiked meat samples were used as controls in all experiments.

b) Detection of pathogens using MALDI-TOF MS

To investigate the ability of MALDI-TOF MS to identify bacteria from selective enrichment broth, pilot experiments were performed with different selective broths (without spiked meat) specific for the target bacteria. For detection of *L. monocytogenes* from spiked meat samples, a primary enrichment of 24 h in OBL was followed by a secondary enrichment of 6 h (in OBL). For *S. enterica*, a single enrichment was performed in MKTTn broth for 18 h. One mL aliquots (in duplicate) of the selective enrichment broth were sampled and processed as per the protocol previously reported [3]. In the case of *E.* coli O157:H7, since the enrichment broths tested yielded no identification, a primary enrichment was performed in TSBn for 6 h which was followed by incubation on Rainbow Agar (RA) for a further 18 h (37°C). Individual colonies showing characteristic morphologies of *E. coli* O157:H7 were analyzed using MALDI-TOF MS. Mass spectral peak lists for the test samples were obtained in the mass range of 2 to 20 KDa using the Launchpad software (version 2.9, Shimadzu) and exported to the SARAMIS database (version 4.10) for identification. In the current study, since well-characterized isolates were used, identifications exceeding a 75% confidence value were considered as reliable identifications for all experiments.

c) Detection of pathogens using GC-MS

In the case of *L. monocytogenes*, the enrichment broth containing spiked meat samples was sampled for GC-MS analysis after 18 and 24 h of incubation (37°C). After 24 h of incubation, 1 mL of the enrichment broth was transferred to 9 mL of sterile OBL. This secondary enrichment broth was sampled for GC-MS analysis after a further 6 h of incubation. For experiments with *S. enterica* and *E. coli* O157:H7, broths were sampled after 12 and 18 h of incubation (42°C for MKTTN and 41°C for TSBN). Five mL aliquots were taken at each sampling point and the samples were processed as per the protocol of Karpe *et al.* [5]. An Agilent 7890A GC/MSD chromatograph coupled with a 30 m HP-5MS Ultra Inert capillary column, 250 µm ID, 0.25 µm film thickness was used for the analysis of all the samples. Samples (n=5) were analysed using the MassHunter software. The metabolites were identified using GC-MS reference libraries (Golm, Fiehn, NIST 11). The data were initially normalized using the internal standard and then analysed using the chemometric data analysis software SIMCA 13 (Umetrics, Sweden). Initially, Principal Component Analysis (PCA) was undertaken, which is an unsupervised

approach to find statistically significant differences between datasets. PCA was followed by partial least square discriminate analysis (PLS-DA). In addition, volcano plots were produced via the free-to-use online software MetaboAnalyst version 2.0 and used to assist in identifying the potential biomarkers based on metabolite fold changes (ratio of metabolites across the control and spiked samples).

III. RESULTS AND DISCUSSION

Direct identification of pathogens from selective enrichment broths would save valuable time and expense by eliminating the need to further test pathogen-negative meat samples.

a) Detection of pathogens using MALDI-TOF MS

The 30 h detection scheme (24 h primary enrichment in OBL followed by 6 h secondary enrichment in OBL at 37°C) developed previously in our laboratory [3] was able to successfully detect *L. monocytogenes* from spiked minced beef samples. In the case of *S. enterica*, prior to the meat spiking experiments, different selective broths including One Broth *Salmonella* (OBS), Rappaport-Vassiliadis Soya Peptone broth (RVS) and MKTTn broth were assessed to verify the detection of *S. enterica* using MALDI-TOF MS. Identification was successful only from MKTTn broth, albeit at very low confidence scores (75 % to 84 %) highlighting the need for a customised database incorporating spectra obtained from broth cultures. For the detection of *Escherichia coli* O157: H7, three selective enrichment broths, namely EC broth, EC broth with 4-methylumbelliferyl-β-D-glucuronide (MUG) and TSBn broth were evaluated. None of the broths tested gave consistent identifications. Thus, an alternate scheme of detection was proposed which included a 6 h enrichment of the spiked meat sample in TSBn (41°C) followed by plating on the chromogenic RA culture media for a further 18 h incubation period. The typical grey colonies obtained for *E. coli* O157:H7 were then analysed using the direct spotting method. Positive identifications were obtained for the pathogen using this 24 h detection scheme. In the case of a naturally contaminated meat samples, further confirmation of the presence of the Shiga toxin gene would be required using molecular methods.

b) *Detection of pathogens using GC-MS*

Approximately 100-120 metabolites were identified across all samples for *L. monocytogenes, S. enterica* and *E. coli* O157:H7. Initially, PCA was performed for the data obtained using GC-MS. While the PCA models showed some discrimination between the spiked and non-spiked meat samples, a PLS-DA analysis was performed for each incubation time to strengthen the predictive capability of the PCA models. Statistically significant discrimination between the control (meat enriched in selective broth) and spiked samples (artificially contaminated meat enriched in selective broth) was obtained at 24 h of incubation for *L. monocytogenes,* 12 h for *E. coli* O157:H7 and 18 h for *S. enterica.* Here, the authors would like to highlight that the GC-MS based metabolomics approach provided a more rapid differentiation between the spiked and control samples in comparison with the MALDI-TOF MS based schemes. The PLS-DA scatter plot differentiating between the control samples and samples spiked with *L. monocytogenes* is depicted in Fig. 1.

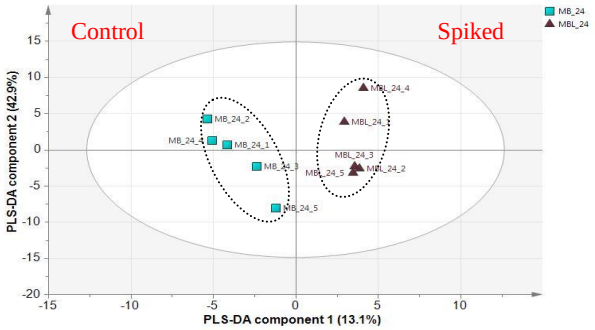

Fig. 1 PLS-DA scatter plot (R^2X=91.5%, R^2Y=100%, Q^2=91.7%) of *L. monocytogenes* inoculated in minced meat sample (10 cfu/mL spiking load) analysed using GC-MS. MB24 denotes samples obtained from non-spiked meat control after 24 h of incubation and MBL24 denotes the samples obtained from spiked meat samples post 24 h of incubation. The PLS-DA eclipse (solid line) represents the 95% confidence interval. The dotted line eclipse highlights groupings within the PLS-DA plot.

A volcano plot was generated using MetaboAnalyst for the discrimination between

the control and spiked samples based on their metabolite fold change. Potential biomarker metabolites for *L. monocytogenes*, *S. enterica* and *E. coli* O157:H7 were identified from the volcano plots. Some of the potential biomarker metabolites identified in the meat sample spiked with *L. monocytogenes* are outlined in Table 1.

Table 1 Potential biomarker metabolites identified (p-value <0.05) in meat sample spiked with *L. monocytogenes* post 24 h of incubation in OBL

Metabolite features identified by GC-MS	Fold Change	*p*-value
2,6-Dihydroxybenzoic acid 3TMS	77.4640	5.60E-06
Guanosine (5TMS)	15.5800	1.13E-05
Benzeneacetic acid, 3,4-tris[(trimethylsilyl)oxy]-, TMS	15.1690	5.82E-03
Adenine-TMS	6.8596	6.16E-04
D-Fructose (5 TMS)	5.2500	1.33E-01
D-Glucose (5TMS)	0.1568	1.99E-04
D-Arabinose (4 TMS)	0.2140	2.13E-02
Cadaverin (4 TMS)	0.3711	2.40E-02
Inositol (1 TMS)	0.3284	7.79E-02

Overall in the GC-MS analysis, many common compounds such as amino acids and cadaverine (generally produced due to putrefaction of animal tissue) were found, which is in accordance with Cavellos-Cavellos *et al.* [6]. Similar to their findings, a single biomarker metabolite exclusive to a pathogen (qualitative analysis) would be difficult to find. However, by performing a targeted metabolomics study, we can use a combination of compounds to develop rapid diagnostic tests for these pathogens.

IV. CONCLUSION

In summary, MALDI-TOF MS and a whole-cell metabolomics approach using GC-MS are potentially rapid methods for detection of meat pathogens directly from selective enrichment broth cultures. However, the proposed methodologies will need to be further validated by testing naturally contaminated meat samples. Since MALDI-TOF MS is an already established technology that has been readily adopted by clinical laboratories, its adoption by food laboratories should be relatively straightforward. Considering that this technology can be a universal system for detecting numerous microbes, developing a database which is specific for meat pathogens and spoilage microbes (that are cultured on different culture media of relevance to food laboratories) would be beneficial to the meat industry.

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