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Rapid discrimination of bacteria using a miniature mass spectrometer†

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Bacteria colonies were analyzed using paper spray ionization coupled with a portable mass spectrometer. The spectra were averaged and processed using multivariate analysis to discriminate between different species of bacteria based on their unique phospholipid profiles. Full scan mass spectra and product ion MS/MS data were compared to those recorded using a benchtop linear ion trap mass spectrometer.

Introduction

In situ microorganism analysis and discrimination is important in a number of scientific and medical fields; however, despite this need, current biochemical techniques are time-consuming.[1,2] So despite the superiority of techniques such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), which successfully differentiate strains of microorganisms,[3,4] alternative methods which rapidly discriminate at the species level are of interest. This is particularly the case for methods capable of *in situ* analysis. Among other methods of analysis are "nose" sensors (*e.g.* a metal–oxide sensor array),[5] laser-induced breakdown spectroscopy,[6] Raman spectroscopy – surfaced enhanced Raman, in particular[7–11] – and Fourier transform infrared spectroscopy.[12,13] In this study, we explore the role of mass spectrometry for *in situ* bacterial discrimination.

Over time, portable mass spectrometers have shown significant improvement in analytical figures of merit (*e.g.* duty cycle, sensitivity, resolution, mass range, dynamic range and robustness).[14–16] Also, with the development of the discontinuous atmospheric pressure interface (DAPI),[17] ambient ionization[18–20] and improved vacuum technology,[14,21,22] portable instruments have decreased in size while simultaneously showing improvements in performance. Ambient ionization in particular has provided a way to quickly analyze chemicals *in situ* without significant sample preparation prior to mass analysis.[18,19,23] It has proven amenable to portable mass spectrometry measurements of pesticides,[24,25] clinical samples,[14] corrosion inhibitors,[26] foodstuffs[25] and other dangerous and illicit compounds.[14,27,28] Previous work with miniature mass spectrometers has largely been limited to demonstrating small molecule analysis (*i.e.* $<m/z$ 500).[15,29] Exceptions include detection of the immunosuppressant tacrolimus (m/z 826, sodium adduct),[14] proteins, peptides, alkaloids, and phospholipids using resonant ejection at low frequency,[30] as well as small protein analysis using a miniature mass spectrometer with a continuous atmosphere pressure inlet.[31] This study seeks to advance routine mass analysis by miniature mass spectrometers into the realm of meso-size biomolecules (*i.e.* m/z 700–1000), specifically phospholipids, and to apply this to microorganism characterization.

Phospholipid analysis using benchtop mass spectrometers has proven to be a powerful tool for rapid disease identification[32,33] and also bacteria analysis[34–37] when combined with multivariate statistical methods such as principal component analysis and linear discriminant analysis. Common mass spectrometry techniques for bacterial discrimination are based on small molecule identification using gas chromatography mass spectrometry (GC/MS) and on protein analysis by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS)[38–41] or liquid chromatography mass spectrometry (LC/MS).[42–44] Previous studies have demonstrated the use of ambient ionization techniques including desorption electrospray ionization (DESI),[45] low temperature plasma (LTP),[46] rapid evaporative ionization mass spectrometry (REIMS),[47] paper spray mass spectrometry (PS-MS),[34,35] and direct analysis in real time (DART)[48] for the discrimination of bacteria.[49] This study demonstrates similar experiments using PS-MS with a portable mass spectrometer. Four Gram positive and four Gram negative bacteria were analyzed using paper spray ionization and a ninth species was analyzed to validate the mass range.

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Experimental

Instrumentation

Mass spectrometric analysis in the negative ion mode was performed using the Mini 12 mass spectrometer.[14] This instrument was employed in the full scan mode to speciate bacteria and in the MS/MS mode for lipid identification. This ion trap-based instrument has all of the scan functions of its benchtop counterpart. Negative ion mode was chosen because the authors were examining phospholipids however positive ion mode analysis of lipids has been previously demonstrated.[50]

Chemicals and materials

Inoculation loops were purchased from Copan Diagnostics, Inc. (Murrieta, CA). Copper clips were purchased from McMaster-Carr (Chicago, IL), and Whatman 1 filter paper was purchased from Whatman International Ltd (Maidstone, England). All chemicals were purchased from Sigma Aldrich (St Louis MO).

Microorganism culturing

All bacterial samples were donated by bioMérieux, Inc. (Hazelwood, MO) and stored at −80 °C. They were cultured on trypticase soy agar supplemented 5% sheep blood (TSAB) purchased from Remel (Lenexa, KS). Aliquots of bacteria were streaked on TSAB plates with sterile inoculation loops and incubated at 37 °C for approximately 24 hours in a VWR forced air incubator (Chicago, IL).

Ambient ionization and mass spectrometry

Eight species of bacteria were analyzed in this study; *B. subtilis* was used to establish the mass range of the Mini 12. The phospholipid profiles of three Gram positive species, *S. aureus*, *S. epidermidis*, and *S. agalactiae*, and four Gram negative bacteria, *P. aeruginosa*, *E. coli*, *A. baumannii*, and *A. lwoffii* were compared *via* PCA analysis. Each sample (a sub-colony of cultured bacteria) was placed on a triangular piece of Whatman 1 filter paper and 5 μL of DMF was added to lyse the membrane. Upon drying, 20–30 μL of ethanol was spotted as the spray solvent. 3.5 kV was applied to the paper and mass analysis began. Using approximately 5–10 seconds of spraying, the full scan mass spectra were collected for ions in the range *m*/*z* 100–840 (each spectrum was the average of 10 scans). The bacterial analysis was replicated 6 times across multiple days to determine day-to-day variability. (Note: 6 replicates were arbitrarily chosen because 2 replicates were run for 3 days to test robustness of the procedure) MS/MS product ion spectra were recorded on major ions in the full scan spectrum. The authors used SNV, baseline correction and normalization of the raw data in order to better prepare it for PCA in Matlab within the *m*/*z* 400–800 *via* total ion current; the normalized spectra were then imported into Origin for PCA analysis.

Results and discussion

Paper spray ionization was used with a miniature mass spectrometer (Mini 12) in the negative ion mode to analyze all eight bacterial samples. The average mass spectrum for each species was normalized by total ion count. There is a clear visual distinction between most of the species of bacteria based on their lipid signals (~*m*/*z* 700 and greater). Even though members of the same genus (*e.g. A. lwoffii vs. A. baumannii* and *S. epidermidis vs. S. aureus*) have similar profiles, they can still be differentiated by comparing relative peak abundances for the different lipids.

Fig. 1 compares data from the Mini 12 and data from a conventional benchtop linear ion trap (LTQ) for *P. aeruginosa*. Resolution suffers in the move to the portable instrument, but overall lipid profiles are similar. Fatty acid dimer signals (*m*/*z* 500–700), on the other hand, are dissimilar there are no fatty acid peaks above baseline in the Mini 12 spectrum. This is interpreted as being the result of higher energy input into the ions generated in the miniature instrument, a known phenomenon.[51] Fig. S1 and S2† show a comparable trend for *S. aureus* and *B. subtilis*. Lipid peaks are observed at similar relative intensities between the benchtop and portable instruments.

Six full scan mass spectra for each of the bacteria species were normalized with respect to the entire data set (42 spectra) and subjected to PCA analysis. Fig. 2 shows, in 2D, a separation between many of the species on both instruments, though groupings are tighter for the LTQ data. *E. coli*, *S. aureus*, *S. epidermidis*, and *A. baumannii* are particularly well distinguished, whereas *A. lwoffii* and *S. agalactiae* show moderate separation on the Mini. With the LTQ, *A. baumannii* and were poorly resolved from *S. agalactiae*.

Tandem mass spectrometry plays a key role in compound identification as well as noise reduction and quantitation; as such, it is imperative that this capability be retained in the move to miniaturize mass spectrometers. For example, the

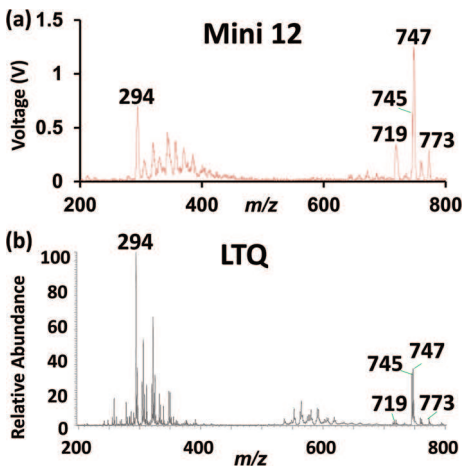

Fig. 1 Mass spectra of *P. aeruginosa* obtained in the negative ion mode using paper spray and a (a) miniature mass spectrometer, (b) benchtop linear ion trap mass spectrometer.

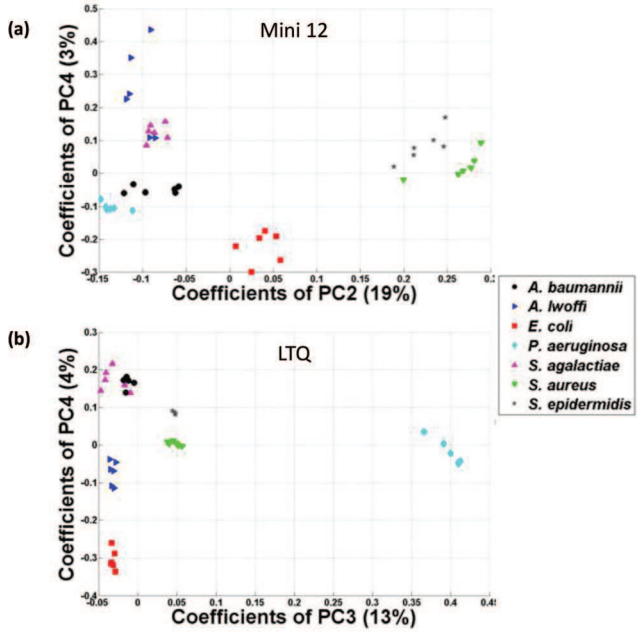

Fig. 2 Negative mode PCA score plot of six replicates each of 7 bacteria species. Species are indicated by colour and symbol: *A. lwoffii* (blue right pointing triangle), *A. baumannii* (black circle), *P. aeruginosa* (cyan diamond), *E. coli* (red square), *S. aureus* (green downward triangle), *S. epidermidis* (gray asterisk), and *S. agalactiae* (purple upward pointing triangle).

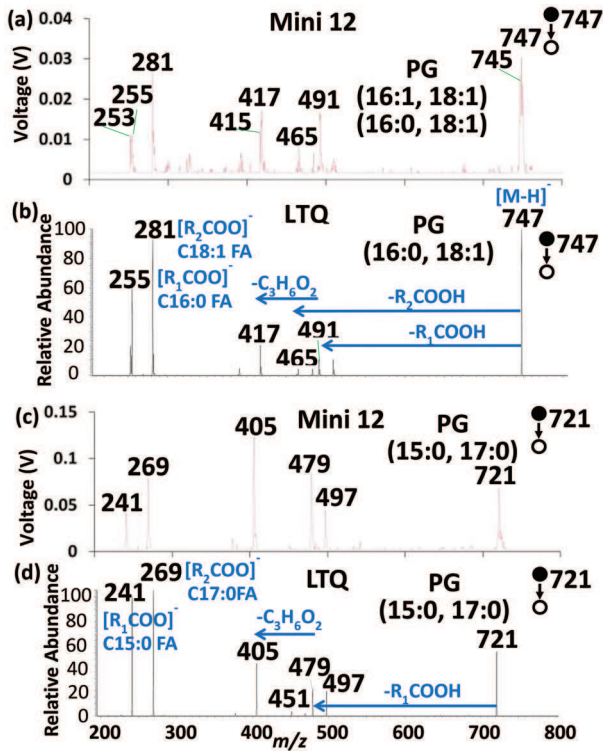

Fig. 3 Negative ion mode MS/MS of *m*/*z* 747 in *P. aeruginosa* and *m*/*z* 721 in *S. aureus* using paper spray and a (a) (c) miniature mass spectrometer, (b) (d) benchtop linear ion trap mass spectrometer.

MS/MS product ion spectra of *m*/*z* 747 in *P. aeruginosa* and *m*/*z* 721 in *S. aureus*, two typical phosphoglycolipids (PGs) observed from microorganisms, are shown in Fig. 3. As before, the primary peaks observed are similar in both mass and intensity on both the miniature instrument and the benchtop LTQ (note that similar intensity requires tuning of collision energy, activation time, and pressure). Fig. S3† shows tandem mass spectra of a lipopeptide (surfactin) produced by *B. subtilis* and the most of the high mass fragments of surfactin are also nominally the same.

Conclusions

This work demonstrates that small mass spectrometers allow the analysis of meso-size molecules by reproducibly analyzing lipids from bacteria. Although there were day-to-day variations, significant differences in the lipid profiles of the several species of bacteria were measured. Aside temporal from variation, sampling with inoculation loops may have been another source of error because the amount of bacteria was hard to precisely control. The authors estimate that the bacteria concentration on the paper was approximately 10^9 CFUs mL^{-1}. *In situ* analysis of bacteria without culturing will need to be pursued in the future. Although for many applications this will prove very difficult due to low levels of bacteria, it has potential to be applied to direct analysis of microorganisms at high concentrations for environmental protection, food safety, and clinical studies.

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