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Rapid direct lipid profiling of bacteria using desorption electrospray ionization mass spectrometry

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Dedicated to Mike Gross on the occasion of his birthday and in appreciation of his powerful contributions to instrumentation and to ion chemistry.

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ABSTRACT

Desorption electrospray ionization (DESI) was employed to measure lipids directly from sixteen bacterial samples without extraction or other sample preparation. Differentiation of different bacterial species and some sub-species was achieved using either the positive or the negative ion mode DESI mass spectra covering the mass/charge range up to *m*/*z* 1000. The data were confirmed by electrospray mass spectrometry (ESI-MS) of lipid extracts from the same bacterial samples. Although the signals were lower, the quality of the direct ionization DESI spectra compared favorably with that of the ESI spectra extracts prepared using chloroform/methanol. The use of unit mass resolution in these experiments allows for overlaps of nominally isobaric and isomeric lipids at particular *m*/*z* values. Tandem mass spectrometry was performed to validate the presence of particular lipids falling into several classes of phospholipids, including phosphatidylethanolamines (PE), phosphatidylglycerols (PG) and lysophospholipids. In addition, lysyl-phosphatidylglycerol (LPG) and lipopeptides were observed in the cases of gram-positive bacteria. DESI-MS lipid profiling was applied to the characterization of four different bacterial species including thirteen *Salmonella* strains. Two bacterial samples *Escherichia coli* K-12 and *Salmonella typhimurium INSP24* were also grown in three different media. Data were compared using principal component analysis (PCA), which indicated that the different species are readily distinguished and that different growth media do not prevent bacterial species differentiation in the cases examined. Several different *Salmonella* strains are also distinguishable from each other based on the PCA results.

1. Introduction

The rapid identification of bacteria is of increasing interest in food, biosafety and medicinal analysis, including the identification of infectious agents and the rapid detection of bio-warfare agents [1,2]. Traditional methods such as nucleic acid hybridization [3], polymerase chain reaction (PCR) [3,4], enzyme-linked immunosorbent assays (ELISA) [5,6], immunofluorescence assays (IFAs) [7] or chemotaxonomic fingerprinting by chromatographic [8] and other methods [9] can differentiate bacteria at the species and sub-species level reliably, but require hours to days to perform [10]. Since the mid-1970s, mass spectrometry has received attention for bacterial detection and differentiation due to its capability when used in conjunction with chromatographic separation to identify particular compounds in complex mixtures. Fatty acids [11], proteins [12] and complex lipids [13] have all been used in distinguishing bacterial samples. More recently, the use of thermal degradation, especially pyrolysis-mass spectrometry, often in the presence of chemical derivatizing agents, has been used to generate, ionize and characterize characteristic compounds such as fatty acid methyl esters [11] or fatty acid monomers released from membrane phospholipids of whole cells [14]. As a standard method in taxonomy, fatty acid profiling based on measurement of the bacterial fatty acid methyl ester (FAME) composition [15,16] has allowed distinction of gram-positive from gram-negative bacteria as well as more specific species identification based on comparison of mass spectra with reference spectra. The next stage in the application of mass spectrometry to bacterial identification involved the use of matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry to record protein profiles of whole bacteria [12,17–23]. MALDI-MS was also applied to obtain abundant, accurate mass ion signals of the phospholipids in whole cell bacteria [24,25]. Electrospray ionization mass spectrometry also has seen some use in proteomics-based approaches to bacterial identification [26,27].

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The lipid composition of the cell membranes varies greatly among bacteria and provides important information that allows bacterial species to be differentiated [28]. As a major component of biological cell membranes, lipids form the lipid bilayers which control cell permeability [29,30]. Lipidomics, an emerging field first defined by Han and Gross, has received much attention and been driven in good measure by advances in mass spectrometry [31]. Lipid profiles can provide valuable information for bacterial identification as already shown in several prior studies [14,32–34], although an ongoing concern which has emerged is the effect of the growth media on the lipid profile. For complementary bacterial characterization utilizing the lipid membrane components information from whole bacteria, pyrolysis-mass spectrometry [35] and MALDI-TOF [24,25] have greatly reduced the amount of sample pretreatment since they require only controlled high temperature or matrix respectively.

Recently, preliminary reports have appeared on the use of ambient ionization methods, desorption electrospray ionization (DESI) [36–38], direct analysis in real time (DART) [39], to provide direct and rapid in situ analysis under ambient conditions on unmodified samples, including analyzing the characteristic lipid components of intact bacteria. In the DESI method, a spray of charged droplets impacts the sample surface, picks up organic molecules, ionizes them and delivers them to the mass spectrometer as scattered microdroplets [40,41]. In previous DESI bacteria studies, a limited number of fatty acids, phospholipids and lipopeptides were detected in vivo from bacterial biofilms growing on agar nutrient [38] or by harvesting bacterial samples suspended in water [37]. In this study, by examining harvested bacterial samples suspended in 70% ethanol after transfer of the sample to a microscope slide, we are able to record high quality DESI mass spectra of different bacterial species and sub-species samples. Many of these can be distinguished based on the distribution of several major classes of lipids, including phosphatidylethanolamines (PE), phosphatidylglycerols (PG), and lysophospholipids. The identifications of these marker compounds were confirmed by tandem mass spectrometry in both the positive and negative ion mode using collision-induced dissociation (CID). In the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, lysyl-phosphatidylglycerol (LPG) and lipopeptides were also observed. Principal component analysis (PCA) was applied to evaluate the reproducibility of the lipid profile data and investigate the differentiation of the bacterial species and strains as well as to investigate the effects of bacteria growth media.

2. Experimental

2.1. Sample preparation

All bacterial samples used were supplied by the Food and Drug Administration (FDA). The sixteen bacterial samples analyzed are listed in Table S-1 in the supplementary data. Three media were used to grow the bacteria, LB (Luria-Bertani) agar, TSA (Trypticase/tryptic Soy Agar) and BHI (Brain, Heart Infusion) agar. Media are listed in order of increasing nutritional value. Unless specifically mentioned, all reported spectra were taken from bacteria grown on LB agar. The bacteria were harvested and samples were suspended in 70% ethanol. Cell concentrations were about 10^6 cfu/ml and then 3 μL of the suspended cells were evenly deposited onto glass slides for DESI analysis. No other sample preparation was performed.

To perform comparative ESI-MS analysis and to validate the DESI method, the method of Bligh and Dyer [42] was used for lipid extraction. Chloroform/methanol solution (1/2, v/v; 187.5 μL) was added to the bacterial suspension (10^6 cfu/ml, 50 μL) and vortexed for 10–15 min. Then chloroform (62.5 μL) was added to extract the organic material and shaken for 1 min followed by the addition of 1 M NaCl solution (62.5 μL). The sample was mixed for another minute before centrifugation for 3 min. The lower phase was collected and vacuum dried, then redissolved in chloroform/methanol solution (2/1, v/v; 50 μL) prior to analysis. All these reagents used were obtained from Mallinckrodt Chemicals (St. Louis, MO, USA).

The standard lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (16:0, 18:1 PG) used in the experiments to verify the bacterial compound structure was purchased from Avanti Polar Lipids (Alabaster, AL, USA).

2.2. Mass spectrometry

A desorption electrospray ionization (DESI) source (prototype Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) fitted to a linear ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA) was used in this study. The DESI source delivered nebulized solvent onto the glass surface at an incident angle of 55° from a distance of about 3 mm using a spray voltage of ±4.5 kV and a solvent flow rate of 3 μL/min. The inlet capillary temperature was set at 200 °C. The N_2 gas pressure was maintained at 120 psi. Microscope glass slides were used as surfaces; no charge build-up was observed. Different spray solvents, including methanol:water (1:1, v:v), acetonitrile:methanol:water (1:2:1, v:v:v), methanol:chloroform:ammonium acetate (9:1:0.1%, v/v/w) and methanol:water:formic acid (1:1:0.1%, v:v:v), were examined. All the data shown here were recorded using methanol:water (1:1, v:v) as spray solvent.

To confirm the structural composition of the compounds as assigned in the spectra, collision-induced dissociation (CID) experiments were performed using an isolation window of 1.5–2 mass/charge units and 25–35% (manufacturer's unit) collision energy. To validate the DESI data, comparative ESI-MS data was recorded on a bacterial lipid extract at a spray voltage of 4.5 kV; heated capillary temperature of 250 °C; sheath gas (N_2) flow rate of 20 units. The sample flow rate was set to 3 μL/min. All the ESI mass spectra shown are an average of approximately 50 consecutive scans.

2.3. Statistical analysis

In-house software was used to perform PCA which can reduce a complex data set to smaller set of dimensions to reveal patterns in the data, and express the data in such a way as to highlight similarities and differences. The analysis was performed directly using the raw data in the case of the bacteria DESI mass spectra (all mass spectra covered the range between m/z 100 and 1000). Typically, the first two principal components encompassed more than 95% of the total variance.

3. Results and discussion

3.1. Direct DESI-MS of bacteria

Samples of whole bacteria were suspended in 70% ethanol at concentrations of about 10^6 cfu/ml and transported in this form. To record mass spectra, 3 μL of the suspension (~3000 bacteria) was deposited onto a glass microscope slide for DESI analysis. Both positive and negative ion mode DESI mass spectra were recorded for each bacterial sample. Among several spray solvents examined, methanol:water (1:1, v:v) led to good signal of the bacterial samples in both ion modes. For gram-negative bacteria, fatty acids (FA), PG and lyso-phosphatidylglycerols (lyso-PG) appeared as the main peaks only in negative ion mode spectra. PE and lyso-phosphatidylethanolamines (lyso-PE) are observed as major ions in positive ion mode spectra. Only a few lyso-PE are present in the

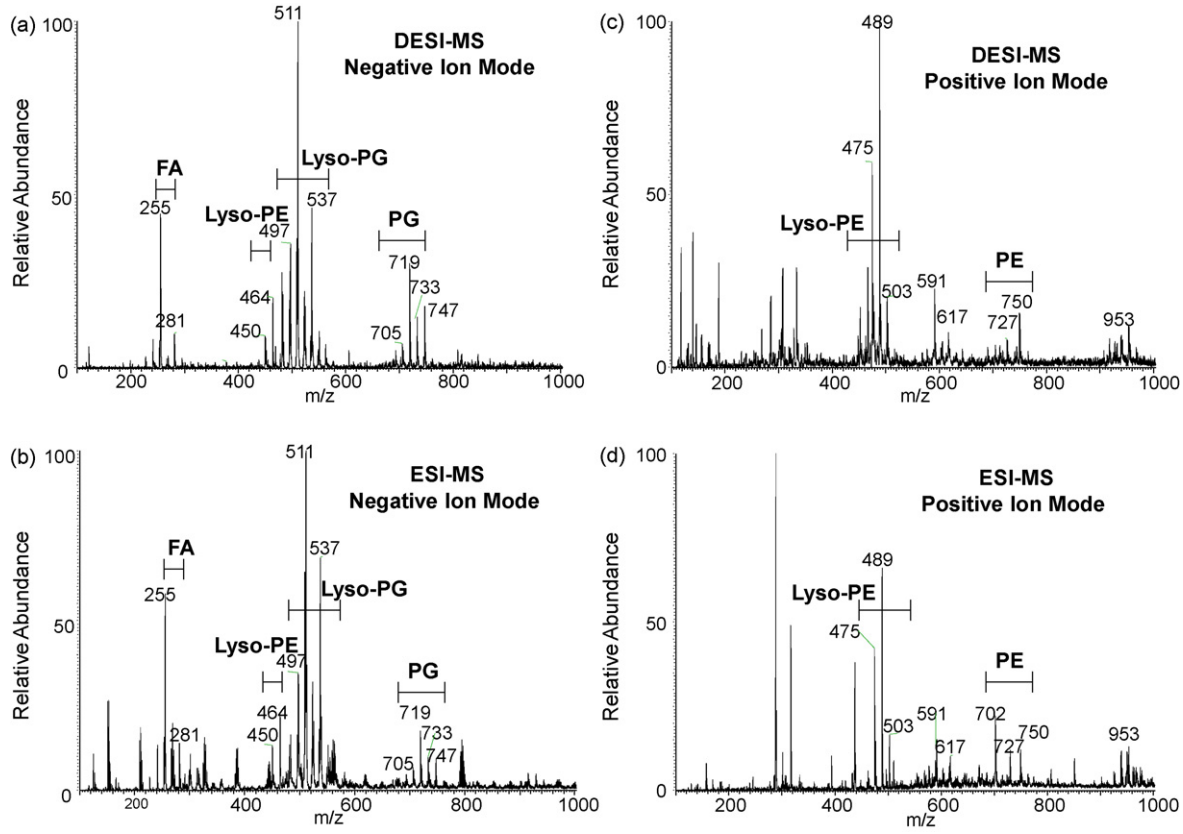

Fig. 1. Comparisons of direct DESI-MS analysis of *E. coli* K12 suspension sample and ESI-MS analysis of lipid extracts from *E. coli* K12. (a) Negative ion mode DESI mass spectrum, (b) negative ion mode ESI mass spectrum, (c) positive ion mode DESI mass spectrum, and (d) positive ion mode ESI mass spectrum.

negative ion mode. In positive ion mode spectra, PE compounds are present in the sodiated form consistent with the high salt concentrations of the samples. Fig. 1a and c show typical data for the case of *Escherichia coli* K12.

To validate the DESI-MS method for direct detection of a characteristic distribution of polar lipids in the bacterial samples, the corresponding ESI-MS of a lipid extract of the bacteria was recorded. Fig. 1 compares the mass spectra of bacterial cells (directly deposited as a suspension on glass slides) by DESI-MS and the corresponding ESI mass spectra of the extract. Negative ion mode spectra recorded using both methods (Fig. 1a and b) show a high degree of similarity in terms of signal due to particular lipids and in terms of overall spectral quality. There is a difference of approximately 100 in terms of total signal in favor of ESI of the extract over DESI of the bacterial suspension sample but the S/N properties are very similar. Note that 2 ± 1 μL bacterial samples (ca. 1000–3000 cfu) were needed to record the DESI spectra with an average of 3–5 consecutive scans in 1 s and signal intensity was in the range of 10^4 counts, and 1 μL bacterial extracts (ca. 1000 cfu) were used to record ESI spectra using an average of 50 consecutive scans in 20 s and signal intensity was in the range of 10^6 counts. The differences in sample work and time of analysis are noteworthy. The two positive ion mode spectra (Fig. 1c and d) also show a high degree of similarity, with PE and lyso-PE observed as major compounds. All the chemical assignments made in the figures are confirmed by tandem mass spectrometry as discussed later. It is noteworthy that the directly recorded DESI spectra are of similar quality (signal/noise ratio and resolution) to the ESI data recorded on the bacterial extracts.

DESI mass spectra of the same bacterial species grown on different LB culture plates over variable lengths of time and analyzed on different time intervals after harvest yielded highly similar spectra (Fig. S-1 in the supplementary data). In the case of the gram-negative bacteria lipid extracts, however, after being stored at room temperature for 1 day, the relative abundance of the phospholipids in the *m*/*z* 700 region (PE and PG) had diminished in the ESI mass spectra (data not shown). These results strongly suggest that before DESI-MS analysis of bacterial suspension sample, the lipid bilayers were protected in the cell membrane over a long period in order to be able to display the observed highly reproducible profiles in DESI-MS analysis. Gram-negative bacteria have thinner cell walls comprising of only 20% peptidoglycan with a lipopolysaccharide layer above; the phospholipid bilayer of the cell lies below. Although the solvent 70% ethanol may dissolve away parts of the outer lipopolysaccharide, the cells are not lysed to any great degree. In contrast, gram-positive bacteria have thicker cell walls, 90% of which is comprised of peptidoglycan [43], thus the lipid bilayer is well covered and protected. So gram-positive bacteria are even less damaged and may still be viable after ethanol suspension.

Fig. 2 shows DESI mass spectra of four *Salmonella* strains in both the negative and positive ion modes. These spectra show very marked differences to those of *E. coli* K12 displayed in Fig. 1. Different species of bacteria examined directly by DESI using methanol/water as the spray solvent show very different lipid distributions [36,37]. As illustrated in Fig. 2, the differences among the four *Salmonella* strains spectra are also obvious, indicating that the spectra of microorganisms recorded by DESI-MS contain distinctive information, even among different strains of the same species. The uniqueness and high reproducibility (Fig. S-1, supplementary data) of different bacteria spectra allow pattern recognition based on mass spectrometric profiling of microorganisms [36].

Negative ion DESI mass spectra of gram-positive bacteria bacteria (*B. subtilis*, *S. aureus*) are displayed in Fig. 3. Compared to the negative ion DESI mass spectra of gram-negative bacteria (*E.*

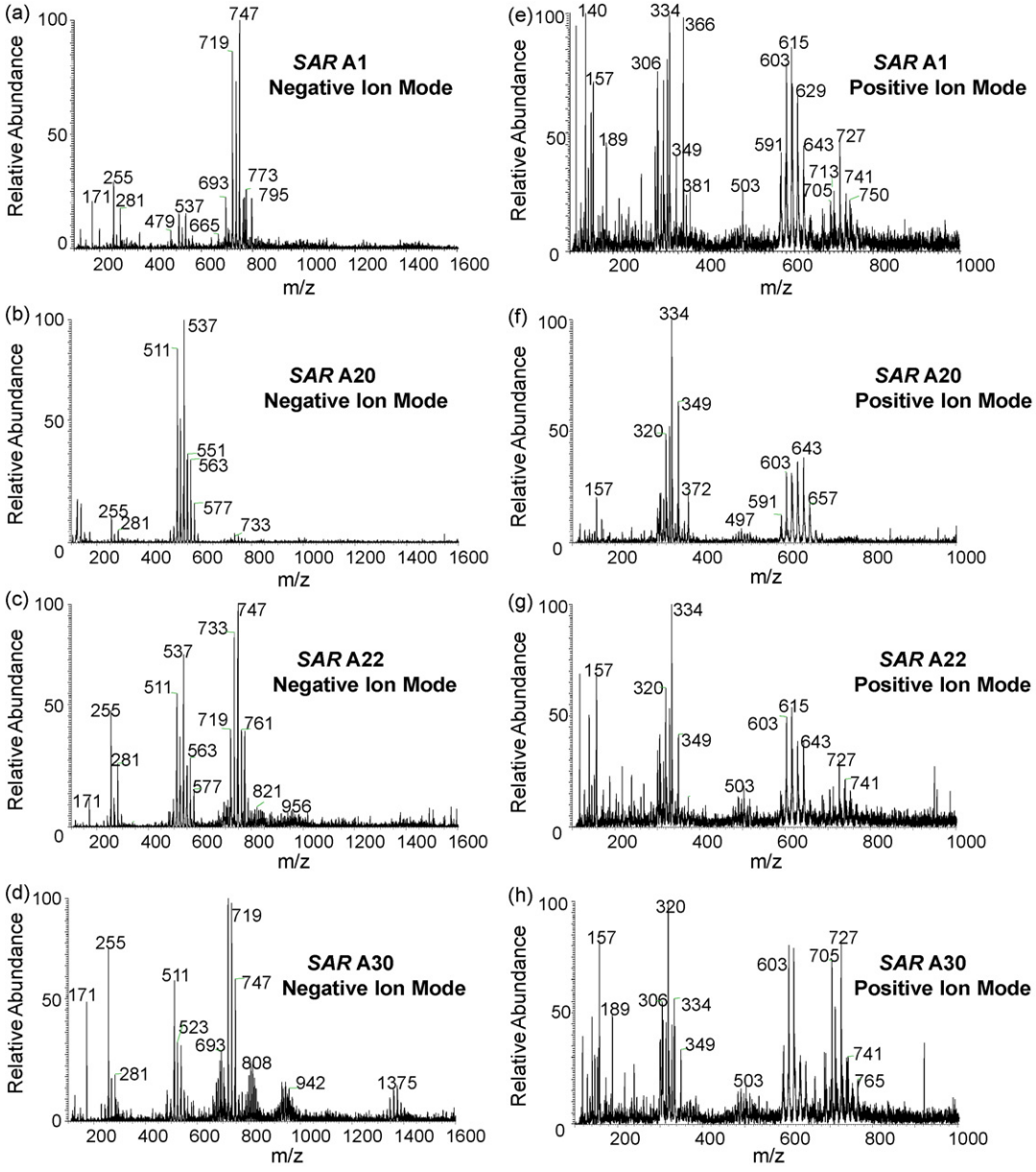

Fig. 2. DESI mass spectra of different *Salmonella* strains at both negative and positive ion modes. (a) *SAR* A1 at negative ion mode, (b) *SAR* A20 at negative ion mode, (c) *SAR* A22 at negative ion mode, (d) *SAR* A30 at negative ion mode, (e) *SAR* A1 at positive ion mode, (f) *SAR* A20 at positive ion mode, (g) *SAR* A22 at positive ion mode and (h) *SAR* A30 at positive ion mode.

coli K12 as Fig. 1a and *SAR* A1 as Fig. 2a), much lower phospholipid signals are observed in the gram-positive bacteria, which are consistent with the structural characteristics of gram-positive bacteria cell membrane. It is easy to differentiate gram-positive and gram-negative species based on the DESI mass spectra since abundant FA, lyso-PE, lyso-PG, PG and PE peaks are shown in gram-negative bacteria; however, lipopeptide, LPG and very limited PG, PE, lyso-PG are shown in gram-positive bacteria (Table S-1, supplementary data).

3.2. Lipid identification

Cyclic lipopeptide biomarkers such as kurstakins, iturins, surfactins, polymyxins, bacitracin have received much attention [44–46]. LPG is a known class of membrane lipids in several gram-positive bacteria but rarely reported for gram-negative bacteria [47]. Both the lipopeptide and LPG are particularly valuable for studying gram-positive bacteria. In the case of *B. subtilis* DESI mass spectrum (Fig. 3a), the C15 lipopeptide surfactin is clearly evident from the peak at m/z 1035 due to the intact deprotonated molecule. This is also easily observable in the presence of the signal due to other lipids. In the m/z 600–900 region (shown expanded 10 times), PG and LPG peaks are present, consistent with the literature [48]. Peaks at m/z 693, 707 and 721 correspond to $[PG\ (30:0)\text{-}H]^-$, $[PG\ (31:0)\text{-}H]^-$, $[PG\ (32:0)\text{-}H]^-$ respectively. LPGs at m/z 847($[LPG\ (32:1)\text{-}H]^-$), m/z 877 ($[LPG\ (34:0)\text{-}H]^-$) and m/z 891 ($[LPG35:0\text{-}H]^-$) have also been reported [48]. In Fig. 3b, DESI mass spectra of *S. aureus* display intense PG peaks at m/z 693, 721, 749 ($[PG\ (34:0)\text{-}H]^-$) as well as weak surfactin lipopeptides in a group around m/z 1035. *S. aureus* also displays an LPG signal at m/z 837 consistent with reports in the literature [49]. Both these types of ions are detected with fair intensity in the DESI mass spectra.

To confirm the structures of lipid components from bacteria, DESI tandem mass spectra were recorded. Characteristic fragmen-

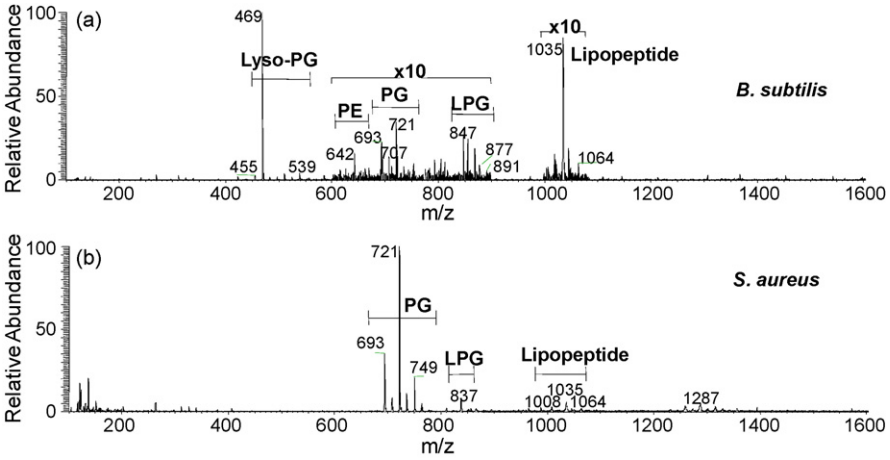

Fig. 3. Negative ion mode DESI mass spectra of gram-positive bacteria with major compound categories shown. (a) *B. subtilis* DESI spectrum with strong lyso-PG peak at *m*/*z* 469, regions *m*/*z* 600–900 and 1000–1100 expanded by 10 times showing PE, PG, LPG and a relatively strong C15 surfactin lipopeptide signal at *m*/*z* 1035. (b) *S. aureus* DESI spectrum with strong PG peaks in the *m*/*z* 700 range and some LPG and lipopeptides.

tations of several lipids, including PG, PE and lysophospholipids, were observed in these MS/MS spectra. Fig. 4 shows the negative ion mode product ion tandem mass spectrum (MS/MS) of the standard compound 16:0, 18:1 PG (Fig. 4a), compared with the corresponding spectrum of the compounds which give rise to the ions at *m*/*z* 747 in *SAR* A1 (Fig. 4b) and in *E. coli* K12 (Fig. 4c). The three spectra are almost identical, indicating that the compounds responsible for *m*/*z* 747 in both bacteria are 16:0, 18:1 PG. Dissociation of $[M\text{-}H]^-$ is dominated by signals due to the fatty acid units. Loss of a neutral carboxylic acid at the *sn*-2 position gives a fragment at *m*/*z* 465. The products of this fragmentation pathway are slightly more abundant than those due to the loss of a ketene molecule at the *sn*-2 position (to give *m*/*z* 483). The similar loss of a neutral carboxylic acid at *sn*-1 position is much less abundant and shown at ion *m*/*z* 491. The relative abundance of the *sn*-1 or *sn*-2 position carboxylic acid loss can be used to determine the structures of other unknown PG compounds in bacteria. The ions at *m*/*z* 391 and 417 are the further loss of a dehydrated glycerol (74 Da) from $[M\text{-}R_2COOH]^-$ and

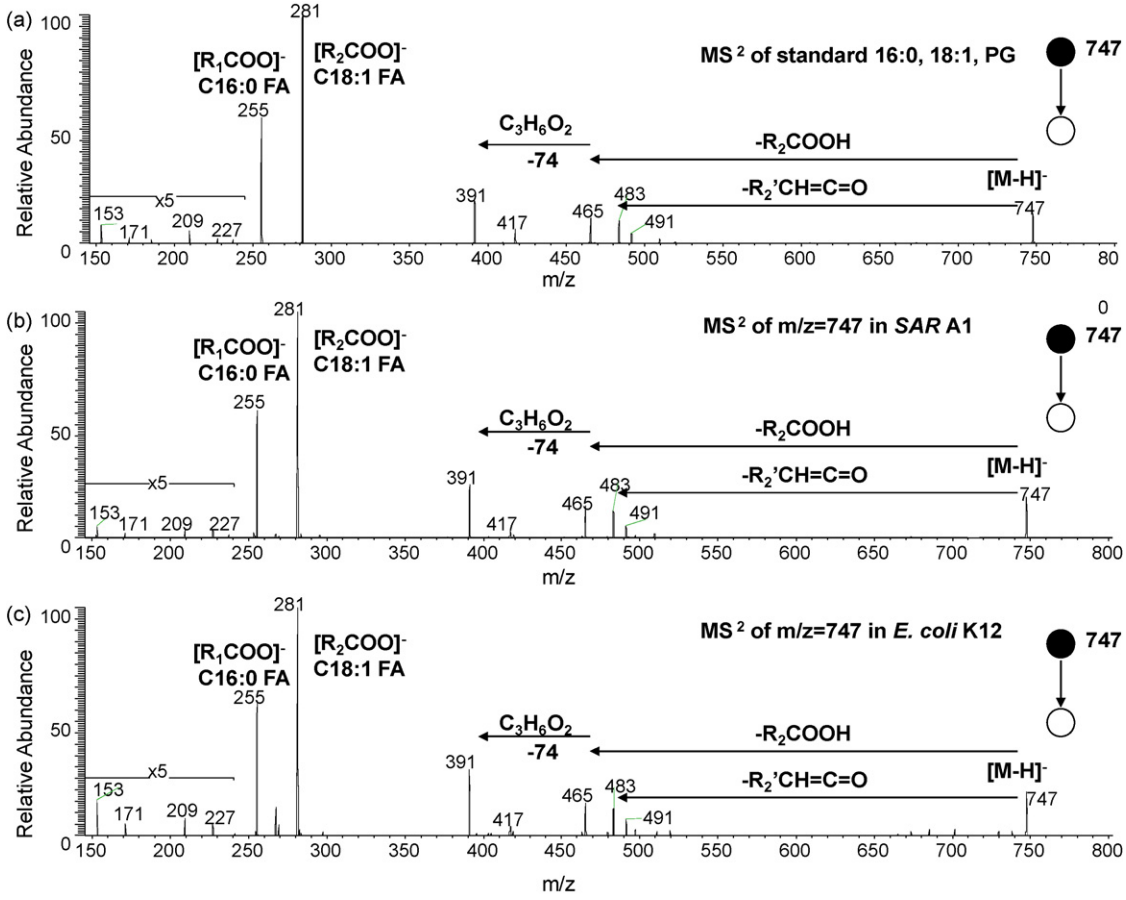

Fig. 4. Negative ion mode MS/MS of (a) 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (16:0, 18:1 PG), (b) *m*/*z* 747 in *SAR* A1(Fig. 2a) and (c) *m*/*z* 747 in *E. coli* K12 (Fig. 1a) with *m*/*z* 150–240 region expanded by 5 times showing characteristic PG fragmentation ions.

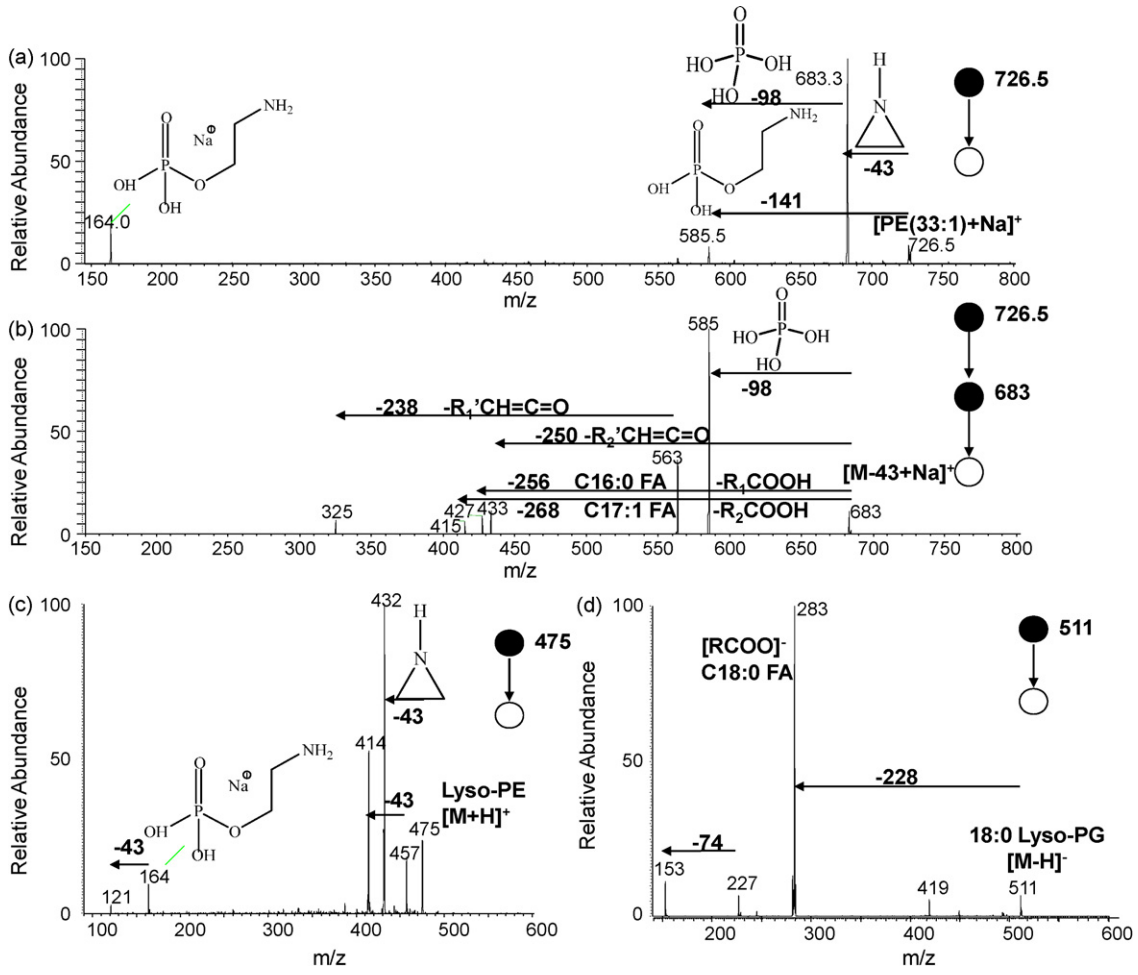

Fig. 5. (a) Positive ion mode MS/MS of precursor ion *m*/*z* 726.5 [PE(33:1) + Na]$^+$ present in *SAR* A1 (as *m*/*z* 727 in Fig. 2e), (b) positive ion mode MS/MS/MS of precursor ion *m*/*z* 683 resulting from MS/MS of precursor ion *m*/*z* 726.5[16:0, 17:1 PE + Na]$^+$ present in *SAR* A1, (c) positive ion mode MS/MS of lyso-PE compound *m*/*z* 475 present in *E. coli* K12(Fig. 1c) and (d) negative ion mode MS/MS of compound *m*/*z* 511(18:0 lyso-PG) present in *SAR* A1(Fig. 2a).

[M-R_2′CH = C = O]. The PG characteristic fragment ions are observed at *m*/*z* 153([$C_3H_5OOPO_3H$]$^-$), 171([$C_3H_7O_2OPO_3H$]$^-$), 209([M-H-R_1COOH-R_2COOH]$^-$) and 227([M-H-R_1CH = C = O-R_2COOH]$^-$ + [M-H-R_2CH = C = O-R_1COOH]$^-$) as described in the literature [50].

Fig. 5a presents the product ion MS/MS positive ion spectrum for the sodium adduct of one of the PE compounds detected in *SAR* A1 (Fig. 2e). The most abundant ions correspond to the loss of neutral ethyleneamine (43 Da) and ethanolamine phosphate (141 Da), respectively. The ion observed at *m*/*z* 164 is the phosphoethanolamine sodium adduct. The neutral loss of 141 Da is characteristic of ethanolamine phospholipids and has been used for the determination of PE molecular species in mixtures [51,52]. In comparison with PG negative ion mode MS/MS, less informative fragmentation is observed in PE positive ion mode MS/MS to indicate the existence of fatty acid groups. To confirm the fatty acid groups of PE in the positive ion mode, MS/MS/MS (Fig. 5b) was carried out using the product ion *m*/*z* 683 resulting from MS/MS of [PE (33:1) + Na] $^+$ (Fig. 5a) as the precursor for the next step. From Fig. 5b, the two fatty acid groups and their ketenes loss fragments are clearly displayed and there is also a neutral loss of phosphoric acid (98 Da). The MS/MS/MS experiments provided adequate structure information of PE. Compound at *m*/*z* 726.5 in *SAR* A1 (shown as *m*/*z* 727 in Fig. 2e) is identified on the basis of ms/ms data as [16:0, 17:1 PE + Na]$^+$.

Fig. 5c and d shows the MS/MS product ion spectrum of lyso-PE and lyso-PG detected in bacteria *E. coli* K12 positive ion mode (Fig. 1c) and *SAR* A1 negative ion mode (Fig. 2a), respectively. Fig. 5c presents the MS/MS of precursor ion at *m*/*z* 475 in *E. coli* K12. The 43 Da ethyleneamine loss and the *m*/*z* 164 peak of phosphoethanolamine sodium adduct are present. These are indicative of the characteristic fragmentations of ethanolamine phospholipids. Fig. 5d is the MS/MS product ion spectrum of *m*/*z* 511 (18:0 lyso-PG) in *SAR* A1. As mentioned before, *m*/*z* 153 and 227 are characteristic fragment ions of PG type compounds. Lysophospholipids have only one fatty acid group, which may be in *sn*-1 or *sn*-2 position.

3.3. PCA

Statistical tools were used to compare the DESI mass spectra recorded for different species and to use the underlying patterns for discrimination between them. PCA is a widely used, easily implemented method of comparing populations with multiple variables. Fig. 6 shows the score plots of first principal component (PC1) against the second (PC2) for seven different bacteria whose DESI mass spectra were measured. *B. subtilis* and *S. aureus* are both gram-positive bacteria, while *E. coli* K12 and *Salmonella* (*SAR* A1, *SAR* A20, *SAR* A22 and *SAR* A30) are gram-negative. Each mass spectrum was exported as a set of raw intensities and normalized before being used to construct the data matrix input to PCA. Fig. 6a shows the PCA score plot of the negative ion mode mass spectra. The gram-positive and gram-negative bacteria were clearly distinguished using PCA. Fig. 6b presents the positive ion mode mass spectra PCA score plot,

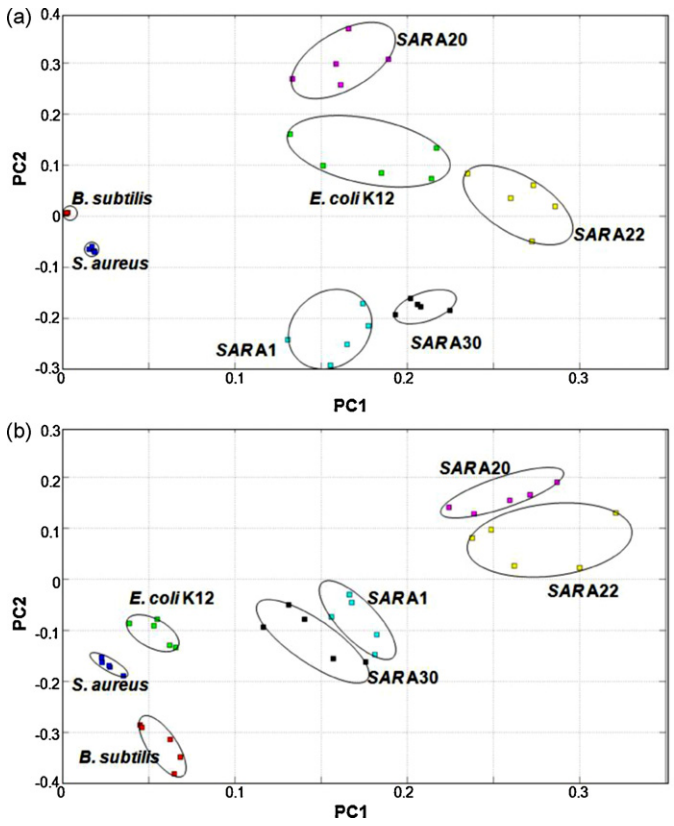

Fig. 6. Score plots of PCA results of (a) negative ion mode and (b) positive ion mode DESI mass spectra of *B. subtilis*, *S. aureus*, *E. coli* K12, and four *Salmonella* strains (*SAR* A1, *SAR* A20, *SAR* A22 and *SAR* A30).

where the four *Salmonella* strains are well separated from all other species on the plot. It should be noted that, among the 16 different bacteria which are listed as Table S-1 (supplementary data), some *Salmonella* stains were not well separated in the PCA plot.

To investigate the effect of changes in the growth medium, *E. coli* K12 and *SAR* A1 grown on three different media (LB agar, TSA and BHI agar) were harvested and analyzed. PCA results for both their negative and positive ion mode data (Fig. S-2, supplementary data) indicate that the three media give indistinguishable DESI mass spectra in both the positive and negative ion modes. Species and the extent tested strain distinctions can still be made.

4. Conclusions

For each bacterial sample analyzed by DESI, both positive and negative ion mode mass spectra were recorded and lipids representative of several major classes were characterized by tandem mass spectrometry. PCA was applied to analyze the MS data, and it showed that the different species (*B. subtilis*, *S. aureus*, *E. coli* and *Salmonella*) and several *Salmonella* strains are well separated, although not all the *Salmonella* strains can be clearly differentiated. The gram-positive and gram-negative bacteria tested here were easily distinguished using DESI-MS in the negative ion mode. PCA results for bacteria harvested from different media indicate that there were no obvious media effects in our study to cause interference in species distinctions.

The DESI-based direct sampling and ionization method provides a simple and robust approach to differentiate major bacterial species, which can be potentially utilized in a variety of applications such as the screening of food for contamination. This study here identified some of the main lipid components using the DESI mass spectra of bacteria. Future experiments will use reactive DESI with different reagents for the selective sampling of lipids in bacteria, or alternatively sampling of other compounds to provide complementary chemical information in bacteria profiling.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.06.014.

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