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Lipid compositions in *Escherichia coli* and *Bacillus subtilis* during growth as determined by MALDI-TOF and TOF/TOF mass spectrometry

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ABSTRACT

Lipids in *Escherichia coli* and *Bacillus subtilis* were analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and TOF/TOF tandem mass spectrometry. Lipids were extracted from bacterial cells using an equal volume mixture of dichloromethane, ethanol, and water, which formed a biphasic system with the lipids in the organic layer. The resulting mass spectra of the extracts from both bacteria showed a series of peaks corresponding to sodiated phospholipids – primarily phosphatidylethanolamines (PEs) and phosphatidylglycerols (PGs). The relative amounts of the phospholipids and the fatty acid compositions inferred from the spectra were in good agreement with previously reported values from GC/MS and thin-layer chromatography studies. *E. coli* and *B. subtilis* were easily differentiated by dissimilarities in the composition and relative amounts of the phospholipids present as well as by the presence of lysyl-phosphatidylglycerol and diglycosyl diglycerides solely in the *B. subtilis* mass spectra. Changes in lipid content in the bacteria during their growth phases were also monitored. In *E. coli*, the spectra indicated an increase in the amount of the unique C_{cy-17} fatty acid (in which the fatty acid chain contains a cyclopropane ring) formed during exponential growth. During stationary growth, the spectra indicated an increase in the amount of saturated fatty acids. In *B. subtilis*, the phospholipid composition remained relatively unchanged during exponential growth, but the amount of PG slightly decreased while the amount of PE slightly increased during stationary growth. No significant changes were observed for the lysyl-phosphatidylglycerols or glycolipids during the exponential or stationary growth phases.

1. Introduction

Interest in the characterization of bacteria and other microorganisms arises from a number of different concerns – taxonomic identification, differentiation of pathogenic from non-pathogenic species, monitoring metabolic processes, or analyzing the structure of the cell membrane [1–3]. While many of the studies that address these issues use some type of chromatography or physical–chemical methods, direct analysis by mass spectrometry has proven to be a valuable tool in many cases. Its speed and sensitivity allow for rapid analysis with minimal sample preparation and, as a result, it has been quite successful in providing useful information on a wide variety of microorganisms [4–7].

Over the past decade, a significant portion of mass spectrometry studies on bacteria have focused on the peptides and proteins within the cells. In whole-cell matrix-assisted laser desorption/ionization (MALDI) analysis, for example, bacteria are harvested and analyzed without any processing and the resulting mass spectra yield characteristic peptide/protein fingerprint patterns that are specific to a given bacterium – allowing differentiation of bacteria to the genus, species, and even strain level [6–11]. Biomarkers in bacterial RNA and DNA and recombinant proteins have been identified by this method as well [12,13].

There has also been widespread interest in the development of methods for analyzing lipid profiles from bacteria. A variety of methods have been developed to address questions ranging from taxonomic identification to sorting out the important roles lipids play in the structures of cell membranes or in the various metabolic and communication processes within the cell [14–16]. Mass spectrometry based methods for the analysis of bacterial lipids have primarily focused on answering questions related to taxonomy. The earliest methods developed could be broadly categorized as being either pyrolysis/MS or GC/MS [17–19]. Pyrolysis–mass spectrometry has largely fallen out of favor. However the GC/MS approach is still commonly used for bacterial taxonomy. Such studies typically involve the separation and analysis of the fatty acid esters (usually

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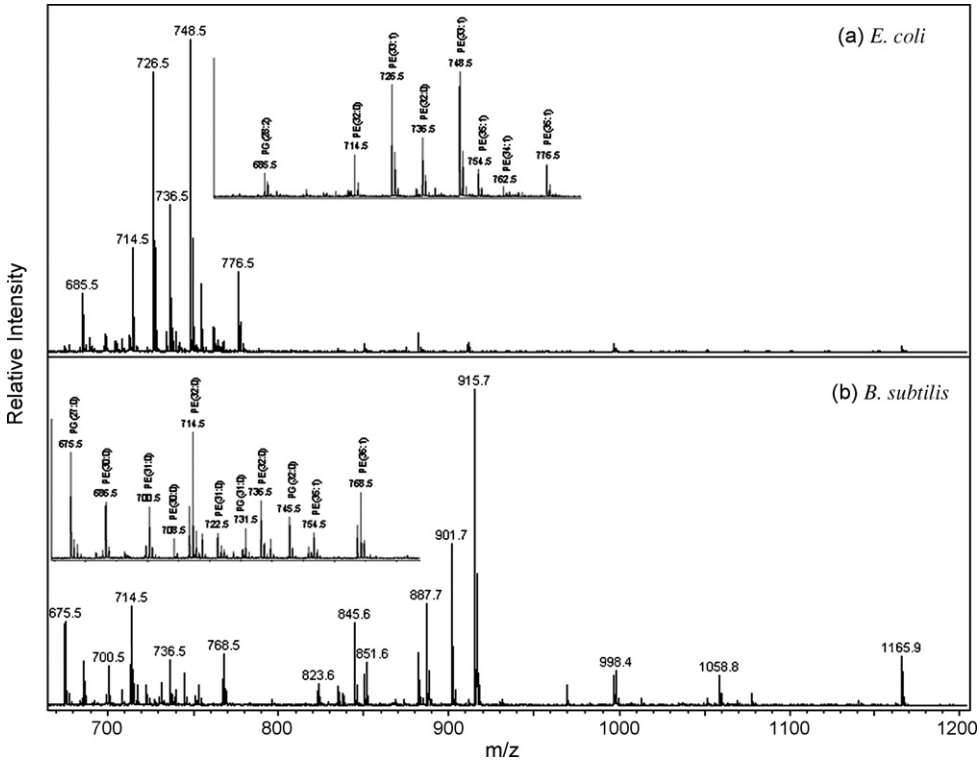

Fig. 1. Mass spectra of the lipid extracts from *Escherichia coli* and *Bacillus subtilis* (cells collected during stationary phase). Insets show the expanded phospholipids region of the spectra.

methyl esters) produced by reactive hydrolysis of the lipids. A comparison of the fatty acid profile from an unknown organism with a library of profiles often provides an acceptable bacterial identification [4]. However, with both techniques information regarding the specific fatty acids associated with the lipid moieties is lost.

More recent studies have used electrospray ionization (ESI) to generate both positive and negative ions of intact phospholipids and, with the use of collision induced dissociation, information about the types of phospholipids present as well as their fatty acid composition has been obtained [20,21]. A few studies have also been done using MALDI-TOF, with the emphasis on rapidly obtaining spectra of phospholipids from unprocessed, whole bacterial cells [19,22–24]. However, in these studies identification of a given phospholipid was dependent on previous GC/MS data to identify the phosphatidyl head-groups and fatty acids.

One concern with the analysis of lipids is that their composition and relative amounts in a given bacterium are dependent on environmental conditions, such as temperature, pH, nutrients in the medium, or growth phase [25–30]. While this may cause difficulties in using lipid profiles for taxonomy and for differentiating very similar bacteria species, it does provide the opportunity to follow growth changes and metabolic processes and could still be relevant in differentiating bacteria in significantly different groups such as a Gram-positive from a Gram-negative bacterium. In this paper, we report on the analysis of lipids extracted (in a simple process) from *Escherichia coli* and *Bacillus subtilis* at various times during their exponential and stationary growth phases using MALDI-TOF mass spectrometry and identification of the types of lipids using MALDI-TOF/TOF tandem mass spectrometry.

2. Experimental

E. coli (Invitrogen Top10) and *B. subtilis* (BD99) were grown at 37 °C while shaking in 50 mL of Luria-Bertani broth (*B. subtilis* was supplemented with 0.05% glucose). Bacteria typically progress through four growth phases – lag phase, log phase (or exponential phase), stationary phase, and death phase. Growth curves were established utilizing a Klett-Summerson photoelectric colorimeter in which the turbidity of the sample (which is proportional to the bacterial population) is measured as the absorbance at 660 nm. Aliquots of cells were collected during early log, mid-log, and late log phases, which correspond to the initial entry, mid-point, and exit from the exponential growth phase, respectively. Additionally, overnight cultures of each isolate were collected (24 h growth), corresponding to an extended stationary/death phase. Cells were harvested by centrifugation at 4 °C and washed twice with ice-cold 75% ethanol.

The extraction of the lipids in the bacterial cells was based on the method described by Folch et al. [31] and Bligh and Dyer [32]. However, instead of using chloroform and methanol, the cells were mixed with 30 mL of a 1:1:1 (v:v:v) mixture of dichloromethane, ethanol, and water. The mixture was shaken for 1–2 min and allowed to stand overnight. The result was a biphasic system, with the bottom, organic layer containing the lipids. This layer was then transferred by pipette to another container and the solvent evaporated under nitrogen until approximately 1 mL remained. One microliter of the lipid extract was then mixed with 1 μL of 1 M 2,5-dihydroxy benzoic acid (DHB) (in 90% methanol with 0.1% formic acid) and spotted onto a MALDI target.

Mass spectra were obtained on an Ultraflex II MALDI-TOF/TOF mass spectrometer equipped with a smartbeam™ solid state laser (Bruker Daltonik, Bremen, Germany) operated in positive-ion, reflectron mode. The laser power was adjusted to a point slightly above the ionization threshold of the sample and fired at a rate of 10 Hz with ~1000–1500 laser shots accumulated per scan. Five to eight replicate samples were obtained for each bacteria species. In each case, the cells were grown and harvested and the lipids extracted under similar conditions to minimize changes in lipid composition due to outside factors. Three to five mass spectra were obtained and averaged for each individual sample using 1000–1500

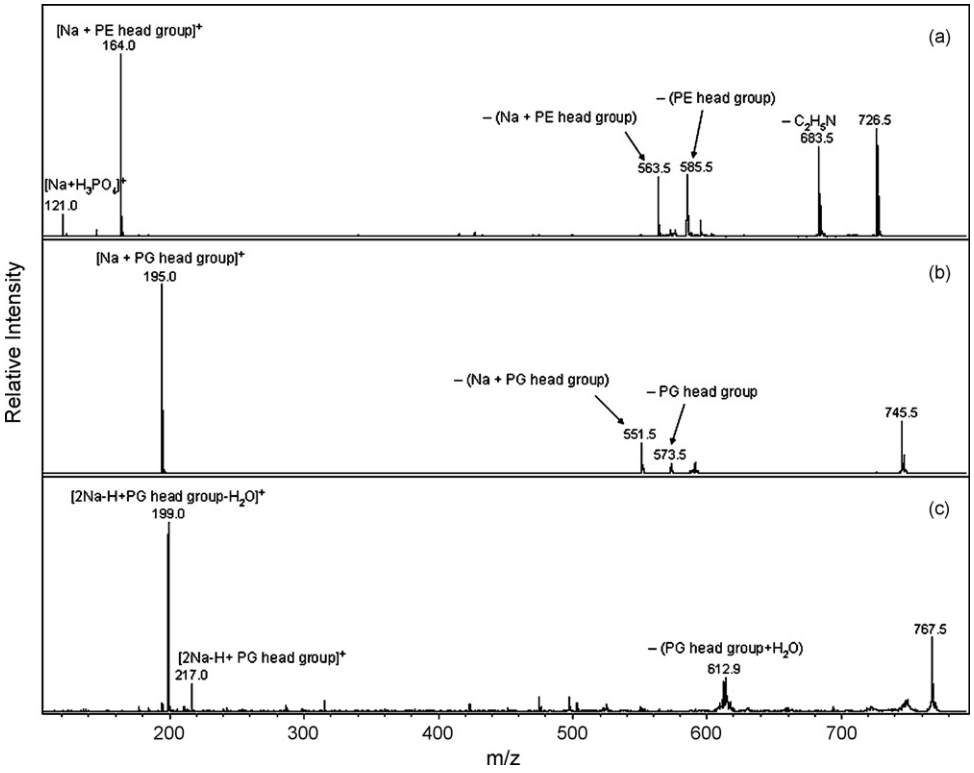

Fig. 2. MS/MS spectra of selected phospholipids using "LIFT" TOF/TOF. Phospholipid head-groups can be easily identified from the characteristic fragment ions formed by cleavage of the phosphate–glycerol bond. Phospholipids with multiple sodium ions can also be easily detected.

laser shots rastered over the entire spot on the MALDI target. To test the variability in the spectra, the relative intensity of a given peak was calculated as a percentage of the sum of the 10–15 highest intensity peaks in the spectrum. For sample replicates, the average relative intensity of a given peak varied less than 5%.

MS/MS spectra were obtained using the "LIFT" technique described elsewhere (LIFT-TOF/TOF) [33]. In brief, fragment ions are generated by unimolecular dissociation of the precursor ions produced during the MALDI process, similar to a post-source decay experiment; no collision gas was used. Ions leave the source with 8 kV of kinetic energy and a given precursor ion with its jointly migrating fragments are isolated using a timed ion gate. The ions are then "lifted" in potential by 19 kV in the LIFT cell so that the fragment ions have kinetic energies within 30% of the parent. This kinetic energy difference is small enough that all of the ions can be separated in the reflector and, therefore, the unimolecular fragments can be detected with their corresponding parent ion in a single spectrum. This effectively eliminates the need to paste several segments together, as is done in traditional post-source decay experiments.

3. Results and discussion

Typical mass spectra of the lipid extracts from cells collected overnight (during the stationary phase) of *E. coli* and *B. subtilis* are shown in Fig. 1. The two species can be easily distinguished by visual inspection of the spectra. Higher *m*/*z* ions are prominent in the spectrum for *B. subtilis* but are absent from the corresponding *E. coli* spectrum. Differences are also apparent in the types of phospholipids present. Insets in Fig. 1 show expanded regions of the spectra from *m*/*z* 680 to 780, where phospholipids are expected. Several more peaks are observed in this region for *B. subtilis* and most of them have *m*/*z* values that differ from those observed for *E. coli*.

In order to identify the peaks in the spectra, LIFT-TOF/TOF mass spectrometry was employed [33]. Fig. 2 shows examples of the MS/MS spectra of selected phospholipid ions. The predominant fragmentation of phospholipids involves the cleavage of the phosphate–glycerol bond, resulting in the elimination of a characteristic phosphatidyl head-group, thus facilitating identification [34]. For example, Fig. 2a is the MS/MS spectrum of *m*/*z* 726.5 and shows a significant fragment ion at *m*/*z* 164, which is consistent with the sodiated polar head-group of phosphatidylethanolamine (PE): $[Na + (C_2H_5N)H_3PO_4]^+$. The other fragment ions in the spectrum also indicate the presence of PE, with *m*/*z* 683.5 corresponding to the loss of ethanolamine (C_2H_5N) and *m*/*z* 585.5 and 563.5 corresponding to the loss of the ethanolamine phosphate head-group $[(C_2H_5N)H_3PO_4]$ and the sodiated head-group $[Na + (C_2H_5N)H_3PO_4]$, respectively. Similar MS/MS spectra were observed for all of the significant even mass ions observed in the phospholipid region (from *m*/*z* 670 to 780) of the spectrum. This observation is in line with previous studies that have indicated that PE is a major component in bacterial lipids (up to 80% in *E. coli*) [35]. Fig. 2b is the MS/MS spectrum of *m*/*z* 745.5. It shows a predominant fragment ion at *m*/*z* 195.0, which is consistent with the sodiated polar head-group of phosphatidylglycerol (PG): $[Na + (HOCH_2CHOHCH_2)H_3PO_4]^+$. PGs are reportedly the second most abundant phospholipids in bacteria [35]. The fragment ion peaks at *m*/*z* 573.5 and 551.5 correspond to the loss of the glycerol phosphate head-group $[(HOCH_2CHOHCH_2)H_3PO_4]$ and the sodiated head-group $[Na + (HOCH_2CHOHCH_2)H_3PO_4]$, respectively. The LIFT-TOF/TOF data can also be helpful for identifying ions containing more than one alkali metal ion. For example, Fig. 2c shows the MS/MS spectrum from *m*/*z* 767.5, which is 22 Da higher than that of *m*/*z* 745.5 and suggests the addition of a second Na ion. The predominant fragment in this spectrum is *m*/*z* 199.0 with fragment ions observed at *m*/*z* 217.0 and 612.9 as well. The *m*/*z* 217.0 ion is consistent with a di-sodiated glycerol phos-

Table 1
Assignments of peaks in the mass spectra of *E. coli* and *B. subtilis*.

m/z	*E. coli*	*B. subtilis*
675.5		Na + PG (27:0)
685.5	Na + PG (28:2)	Na + PG (28:2)
686.5	Na + PE (30:0)	Na + PE (30:0)
700.5		Na + PE (31:0)
708.5		2Na–H + PE (30:0)
712.5	Na + PE (32:1)	
713.5		Na + PG (30:2)
714.5	Na + PE (32:0)	Na + PE (32:0)
717.5		Na + PG (30:0)
722.5		2Na–H + PE (31:0)
726.5	Na + PE (33:1)	
731.5		Na + PG (31:0)
734.5	2Na–H + PE (32:1)	
736.5	2Na–H + PE (32:0)	2Na–H + PE (32:0)
739.5		2Na–H + PG (30:0)
740.5	Na + PE (34:1)	
745.5		Na + PG (32:0)
748.5	2Na–H + PE (33:1)	
753.5		2Na–H + PG (31:0)
754.5	Na + PE (35:1)	
755.5		Na + PG (33:2)
762.5	2Na–H + PE (34:1)	
767.5		2Na–H + PG (32:0)
768.5		Na + PE (36:1)
776.5	2Na–H + PE (35:1)	
823.6		H + LPG (30:0)
851.6		H + LPG (32:0)
887.7		Na + DGDG (30:0)
901.7		Na + DGDG (31:0)
915.7		Na + DGDG (32:0)

PE, phosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysyl-phosphatidylglycerol; DGDG, diglycosyl diacylglycerol. Numbers in parenthesis indicate the number of carbon atoms and double bonds, respectively, in the fatty acid side chains.

phate head-group $[2Na–H + (HOCH_2CHOCHCH_2)H_3PO_4]^+$ while *m/z* 199.0 can be assigned to the loss of H_2O from that species. The fragment ion at *m/z* 612.9 is consistent with the loss of (PG head-group – H_2O). A summary of the phospholipid ions identified in the mass spectra for *E. coli* and *B. subtilis* using the LIFT-TOF/TOF MS/MS data is given in Table 1. In each case, the phospholipids are present as sodium or di-sodium adducts; no protonated species were observed.

Unfortunately, the LIFT-TOF/TOF spectra did not yield sufficient ion signals to allow unambiguous assignments of the individual fatty acid side chains present in a given phospholipid. Thus, only the total fatty acid side chain composition of the phospholipids is given in Table 1. Lack of fragments corresponding to fatty acids has been previously observed in positive-ion MALDI MS/MS analysis of phospholipids but there has been some success reported using negative-ion collision induced dissociation [36,37]. However, the signal from negative ions was not sufficient to perform LIFT-TOF/TOF experiments in this study.

It has been reported that C_{16} and C_{17} carbon chains comprise approximately 70–80% of the total fatty acid content in both *E. coli* and *B. subtilis* [25–29,38]. One difference is that the C_{17} fatty acid side chain in *E. coli* contains a cyclopropane ring (i.e., methylene-hexadecanoic acid, C_{cy-17}) [25–27,39] while that in *B. subtilis* is primarily the branched chain: anteisoheptadecanoic acid ($C_{a-17:0}$) [38]. This difference is observed in the mass spectra of the two bacteria shown in Fig. 1 as the spectrum for *E. coli* shows a prominent peak corresponding to PE (33:1), *m/z* 726.5, that is absent in the *B. subtilis* spectrum. The most likely combination of fatty acid side chains for this phospholipid is $C_{16:0} + C_{cy-17}$ as $C_{17:0}$ is not known to be present in *E. coli* and $C_{16:1}$, $C_{14:0}$ and C_{cy-19} are present in small amounts [25–27,39].

Another difference between the two bacteria species is the presence of several peaks between *m/z* 800 and 950 in the mass spectrum for *B. subtilis* that are absent in the *E. coli* spectrum. MS/MS analysis indicated these peaks can be grouped into two main lipid classes, both of which are known to be prevalent in Gram-positive bacteria, such as *B. subtilis*, but rare in Gram-negative bacteria, such as *E. coli*. LIFT-TOF/TOF spectra for the first group, consisting of *m/z* 823.6 and 851.6, are shown in Fig. 3. In both cases, the spectra show a prominent fragment ion corresponding to neutral loss of 300 Da along with the complementary protonated fragment ion at *m/z* 301.1. Gram-positive bacteria are known to contain amino acid esters of phosphatidylglycerol, such as lysine, alanine, or ornithine [28,40] and the neutral loss of 300 Da is consistent with loss of a lysyl + glycerol phosphate head-group, with the fragment ion at

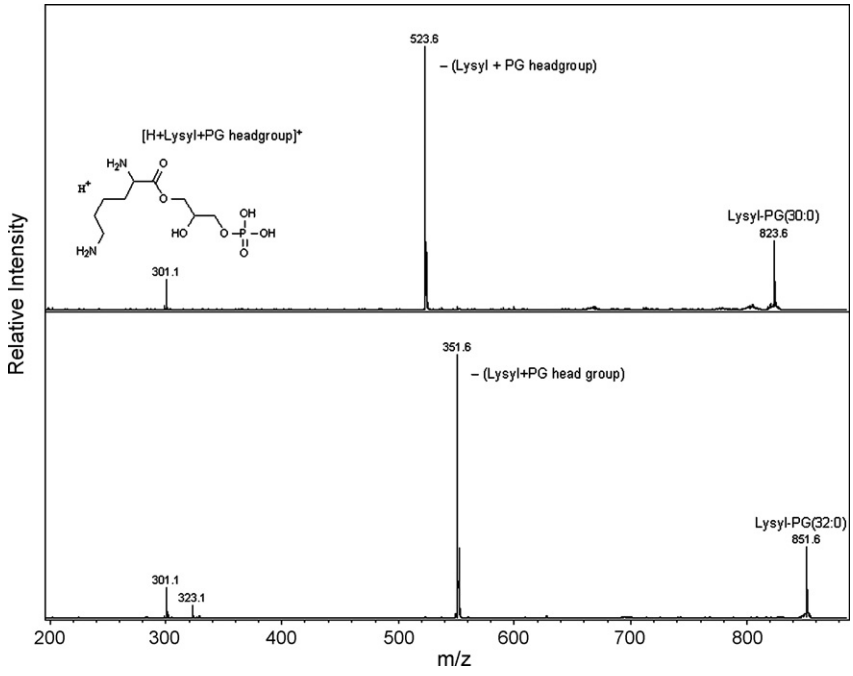

Fig. 3. MS/MS (LIFT-TOF/TOF) spectra of *m/z* 823.6 and 851.6, showing the characteristic loss of a lysyl-glycerolphosphate moiety.

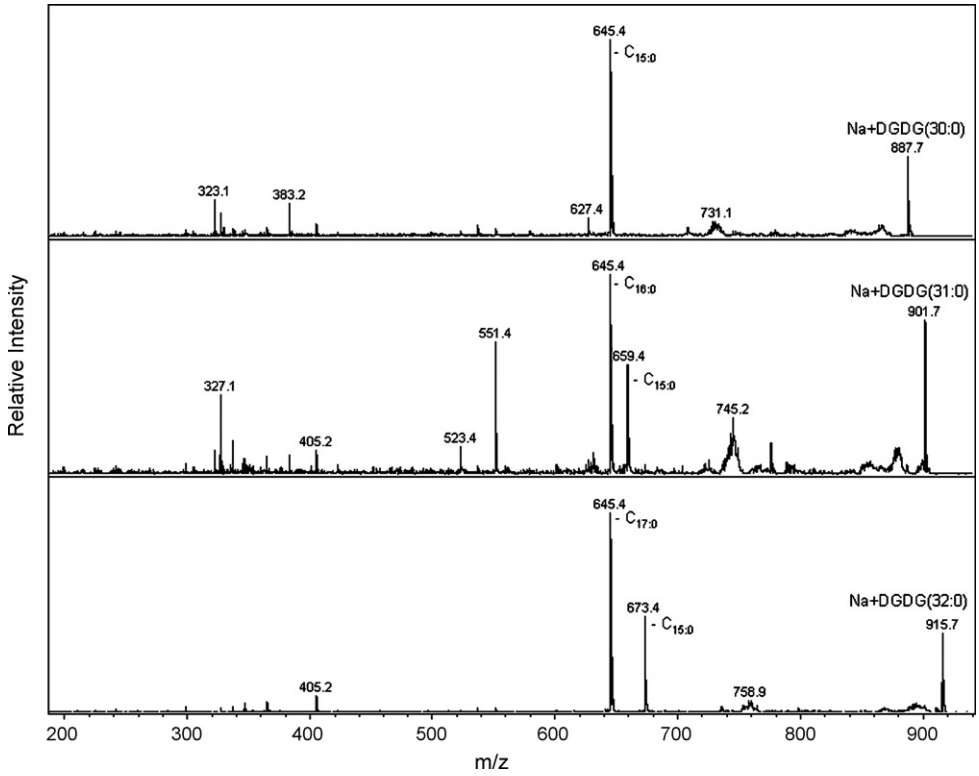

Fig. 4. MS/MS (LIFT-TOF/TOF) spectra of *m/z* 887.7, 901.7, and 915.7, identified as diglycosyl diglycerides. Fragment ions due to the loss of $C_{15:0}$, $C_{16:0}$, and $C_{17:0}$ are observed.

m/z 301.1 corresponding to the protonated LPG head-group. The two precursor ions can then be identified as the protonated lysyl-phosphatidylglycerol moieties: H + LPG (30:0) and H + LPG (32:0). As with the phospholipids, the MS/MS spectra did not contain fragments unique to a given fatty acid and, thus, only the total fatty acid composition can be determined.

LIFT-TOF/TOF spectra for the second group, consisting of *m/z* 887.7, 901.7, and 915.7, are shown in Fig. 4. The MS/MS spectrum

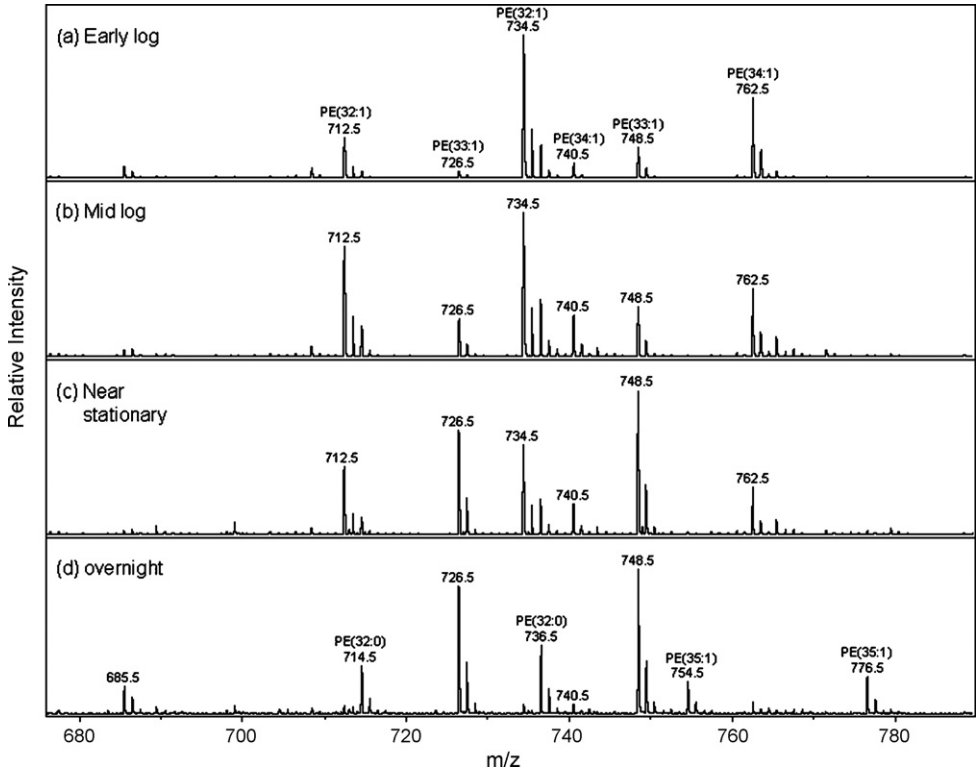

Fig. 5. Mass spectra of the phospholipids extracted from *E. coli* at various points in the growth process. An increase in the relative amount of $C_{cy\text{-}17}$ (*m/z* 726.5) is observed in later stages of growth as well as a shift from PE (32:1) to PE (32:0).

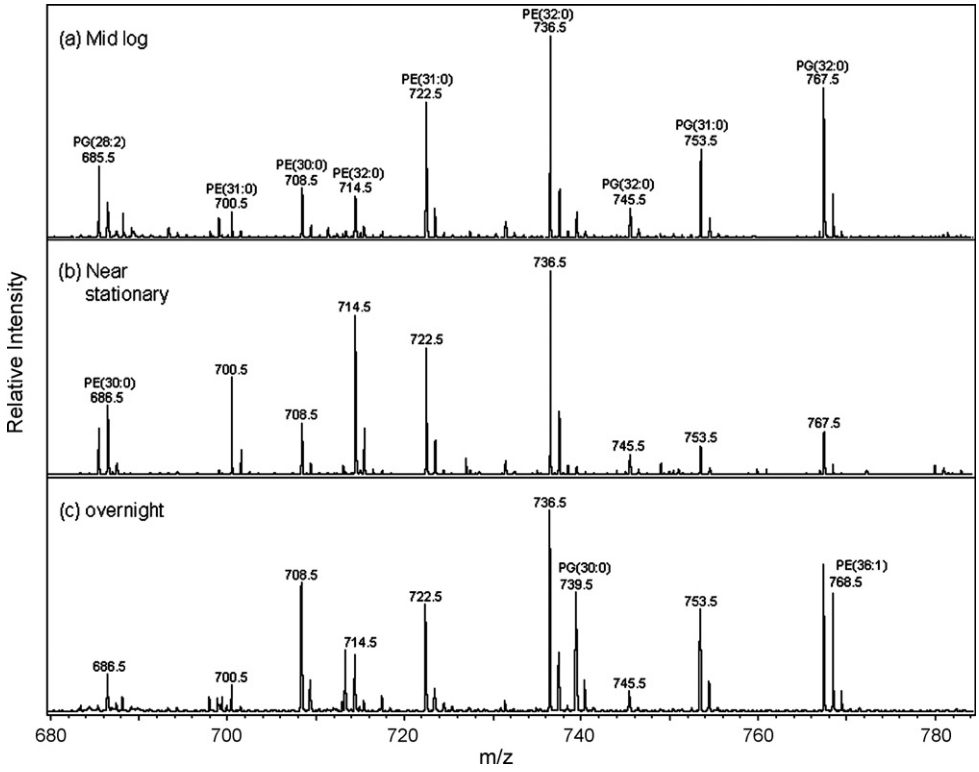

Fig. 6. Mass spectra of the phospholipids extracted from *B. subtilis* at various points in the growth process. An increase in the amount of PE relative to PG is observed during the later, stationary growth phase.

for *m*/*z* 887.7 shows a prominent fragment ion at *m*/*z* 645.4, which is consistent with the loss of a $C_{15:0}$ fatty acid. The MS/MS spectrum for *m*/*z* 901.7 shows two prominent fragments, at *m*/*z* 645.4 and 659.4, which are consistent with the loss of $C_{16:0}$ and $C_{15:0}$ fatty acids. The MS/MS spectrum for *m*/*z* 915.7 also shows two fragments, at *m*/*z* 645.4 and 673.4, which are consistent with the loss of $C_{17:0}$ and $C_{15:0}$ fatty acids. These are the only lipid species where the loss of a given fatty acid side chain is observed in the MS/MS spectra. One type of lipid that is widespread among Gram-positive bacteria are glycolipids and, in particular, glycosyl diglycerides [15]. Assuming *m*/*z* 887.7 consists of two $C_{15:0}$ fatty acids, the three precursor ions can thus be assigned as the sodiated diglycosyl diglycerides: DGDG (30:0), DGDG (31:0), and DGDG (32:0), respectively [41–43]. Absent the MS/MS data, the composition of the 30:0, 31:0 and 32:0 species might have been presumed to be from ($C_{16:0}$ + $C_{14:0}$), ($C_{16:0}$ + $C_{15:0}$) and ($C_{16:0}$ + $C_{16:0}$) respectively since $C_{16:0}$ is the most prominent fatty acid observed in *B. subtilis* [25–29,38].

Lipid composition in the two bacterial species was also monitored as a function of growth phase. Cells were collected during early log, mid-log, and late log during the exponential growth phase, at the transition from exponential to stationary growth phase, and overnight (during the stationary phase). Mass spectra of the lipids extracted from *E. coli* at selected stages of growth are shown in Fig. 5. During the exponential growth phase (Fig. 5a–c), increases in the relative intensities of *m*/*z* 726.5 and 748.5 of 25% are observed. This difference is five times higher than the average variation in intensity observed for replicate samples. These two peaks have been identified as Na + PE (33:1) and 2Na–H + PE (33:1), which most likely contain the $C_{cy\text{-}17:0}$ fatty acid. This result is consistent with previous studies that have also reported an increase in the proportion of cyclopropane fatty acids during growth of *E. coli*, especially near the transition to the stationary phase [25,27].

Comparison of Fig. 5a–c to d shows two other trends worth noting. Peaks at *m*/*z* 712.5 (Na + PE (32:1)) and 734.5 (2Na–H + PE (32:1)) disappear, apparently being replaced by peaks at *m*/*z* 714.5 and 736.5. This would seem to suggest growth-phase dependent saturation of a specific fatty acid from (32:1) to (32:0). A previous gas chromatography study that analyzed fatty acid profiles during growth of *E. coli* AB301 reported that the amount of $C_{16:0}$ remained fairly constant during exponential growth while the amount of $C_{16:1}$ decreased, but changes during the stationary growth phase were not measured [27]. The second trend apparent in the comparison between Fig. 5a–c and d is the sudden appearance of peaks at *m*/*z* 754.5 and 776.5, assigned to Na + PE (35:1) and 2Na–H + PE (35:1), respectively.

Mass spectra for cells collected from *B. subtilis* at various stages of growth are shown in Fig. 6. No significant (and consistent) changes were observed during the exponential growth phase, which is in agreement with previous studies [28,29], but the mass spectra do indicate an increase in the amount PE, at the expense of PG, during the stationary phase. For example, the peak at *m*/*z* 685.5 (Na + PG (28:2)) decreases while that of *m*/*z* 686.5 (Na + PE (30:0)) increases and the peak at *m*/*z* 767.5 (2Na + PG (32:0)) decreases while that of *m*/*z* 768.5 (Na + PE (36:1)) increases. This trend was also reported during the growth phases of *B. stearothermophilus*, which is similar to *B. subtilis* except it lacks amino acid esters of PG and has a higher cardiolipin concentration [30]. During exponential growth, the phospholipids content remained relatively unchanged. However, during the transition to stationary growth and afterwards, the amount of PE increased while that of PG decreased (although there was a corresponding increase in the amount of cardiolipin, which was attributed to the conversion of PG).

4. Conclusion

MALDI-TOF and MALDI-TOF/TOF analyses of various lipids extracted from *E. coli* and *B. subtilis* cells collected at various times in the growth phase are reported. Phospholipids containing phos-

phatidylethanolamine and phosphatidylglycerol were observed for both bacteria, with noticeable differences in the relative amounts of each phospholipid and their fatty acid compositions. The $C_{cy\text{-}17}$ fatty acid (containing a cyclopropane ring) was easily identified in *E. coli* while lysyl-phosphatidylglycerols and diglycosyl diglycerides were observed in the *B. subtilis* extracts. During the growth of *E. coli*, the mass spectra indicated an increase in the amount of $C_{cy\text{-}17}$ during the exponential growth phase and an increase in the amount of saturated fatty acids during the stationary growth phase. In *B. subtilis*, no significant changes were observed in the spectra during the exponential growth phase but the relative amount of PE increased while that of PG decreased during the stationary growth phase.

These rapid and direct analyses from simple lipid extracts from two species of bacteria demonstrate the detection of two types of biology-related and experimentally useful differences in lipid profiles. The detection of differences in the profiles from two very dissimilar organisms is not unexpected, but it was obtained using a very simple procedure and the lipid profiles were entirely consistent with previously reported profiles obtained using much more laborious methods. The ability to follow the subtle, but well established, changes in the lipid profiles associated with the growth phase of the bacterium demonstrates the potential of this or similar approaches for studying changes in the bacterial lipidome. Although much more work will be needed to prove this, the detection of these relatively small changes in the lipid profiles during growth is also consistent with differentiation of more closely related organisms than the two used in this study.

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References

[1] F. Priest, B. Austin, Modern Bacterial Taxonomy, 2nd edition, Springer, New York, 1993.
[2] J. Irudayaraj, H. Yang, S. Sakhamuri, Differentiation and detection of microorganisms using Fourier transform infrared photoacoustic spectroscopy, J. Mol. Struct. 606 (2002) 181–188.
[3] G. Gottschalk, Bacterial Metabolism, 2nd edition, Springer, New York, 1986.
[4] C.L. Wilkins, J.O. Lay Jr., J.D. Winefordner (Eds.), Identification of Microorgansisms by Mass Spectrometry, Series: Chemical Analysis: A Series of Monographs on Analytical Chemistry and Its Applications, John Wiley & Sons, New York, 2006.
[5] B.L.M. Baar, Characterisation of bacteria by matrix-assisted laser desorption/ionization and electrospray mass spectrometry, FEMS Microbiol. Rev. 24 (2006) 193–219.
[6] C. Fenselau, D.A. Plamen, Characterization of intact microorgansisms by MALDI mass spectrometry, Mass Spectrom. Rev. 20 (2001) 157–171.
[7] J.O. Lay Jr., MALDI-TOF mass spectrometry of bacteria, Mass Spectrom. Rev. 20 (2001) 172–194.
[8] T.C. Cain, D.M. Lubman, W.J. Weber Jr., A. Vertes, Differentiation of bacteria using protein profiles from matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 8 (1994) 1026–1030.
[9] R.D. Holland, J.G. Wilkes, F. Rafii, J.B. Sutherland, C.C. Persons, K.J. Voorhees, J.O. Lay Jr., in: J.R. Chapman (Ed.), Mass Spectrometry of Proteins and Peptides, Humana Press, Totowa, NJ, 2000, pp. 461–487.
[10] R.J. Arnold, J.P. Reilly, Fingerprint matching of *E. coli* strains with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of whole cells using a modified correlation approach, Rapid Commun. Mass Spectrom. 12 (1998) 630–636.
[11] J.J. Jones, M.J. Stump, R.C. Fleming, J.O. Lay, C.L. Wilkins, Investigation of MALDI-TOF and FT-MS techniques for analysis of *Escherichia coli* whole cells, Anal. Chem. 75 (2003) 1340–1347.
[12] L.F. Marvin, M.A. Roberts, L.B. Fay, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry, Clin. Chim. Acta 337 (2003) 11–21.
[13] M.L. Easterling, C.M. Colangelo, R.A. Scott, I.J. Amster, Monitoring protein expression in whole bacterial cells with MALDI time-of-flight mass spectrometry, Anal. Chem. 70 (1998) 2704–2709.
[14] M. Kates, Bacterial lipids, Adv. Lipid Res. 2 (1964) 17–90.
[15] M.P. Lechevalier, C.W. Moss, Lipids in bacterial taxonomy – a taxonomist's view, Crit. Rev. Microbiol. 5 (1977) 109–210.
[16] J.E. Cronan Jr., Molecular biology of bacterial membrane lipids, Annu. Rev. Biochem. 47 (1978) 13–189.
[17] F. Basile, K.J. Voorhees, T.L. Hadfield, Microorganism Gram-type differentiation based on pyrolysis–mass spectrometry of bacterial fatty acid methyl ester extracts, Appl. Environ. Microbiol. 61 (1995) 1534–1539.
[18] W.W. Christie, Gas chromatography–mass spectrometry methods for structural analysis of fatty acids, Lipids 33 (1998) 343–353.
[19] Y. Ishida, K. Kitagawa, A. Nakayama, H. Ohtani, Complementary analysis of lipids in whole bacteria cells by thermally assisted hydrolysis and methylation-GC and MALDI-MS combined with on-probe sample pretreatment, J. Anal. Appl. Pyrol. 77 (2006) 116–120.
[20] P.B.W. Smith, A.P. Snyder, C.S. Harden, Characterization of bacterial phospholipids by electrospray ionization tandem mass spectrometry, Anal. Chem. 67 (1995) 1824–1830.
[21] G.E. Black, A.P. Snyder, K.S. Heroux, Chemotaxonomic differentiation between *Bacillus cereus* group and *Bacillus subtilis* by phospholipids extracts analyzed with electrospray ionization tandem mass spectrometry, J. Microb. Methods 28 (1997) 187–199.
[22] Y. Ishida, A.J. Madonna, J.C. Rees, M.A. Meetani, K.J. Voorhees, Rapid analysis of intact phospholipids from whole bacterial cells by matrix-assisted laser desorption/ionization mass spectrometry combined with on-probe sample pretreatment, Rapid Commun. Mass Spectrom. 16 (2002) 1877–1882.
[23] Y. Ishida, K. Kitagawa, A. Nakayama, H. Ohtani, On-probe sample pretreatment for direct analysis of lipids in Gram-positive bacterial cells by matrix-assisted laser desorption ionization mass spectrometry, Appl. Environ. Microbiol. 71 (2005) 7539–7541.
[24] J.J. Jones, M.J. Stump, R.C. Fleming, J.O. Lay, C.L. Wilkins, Strategies and data analysis techniques for lipid and phospholipid chemistry elucidation by intact cell MALDI-FTMS, J. Am. Soc. Mass Spectrom. 15 (2004) 1665–1674.
[25] A.G. Marr, J.L. Ingraham, Effect of temperature on the composition of fatty acids in *Escherichia coli*, J. Bacteriol. 84 (1962) 1260–1267.
[26] A.J. DeSiervo, Alterations in the phospholipids composition of *Escherichia coli B* during growth at different temperatures, J. Bacteriol. 100 (1969) 1342–1349.
[27] J.E. Cronan Jr., Phospholipid alterations during growth of *Escherichia coli*, J. Bacteriol. 95 (1968) 2054–2061.
[28] J.A.F. Op den Kamp, I. Redai, L.L.M. van Deenen, Phospholipid composition of *Bacillus subtilis*, J. Bacteriol. 99 (1969) 298–303.
[29] H.H. Daron, Fatty acid composition of lipid extracts of a thermophilic *Bacillus* species, J. Bacteriol. 101 (1970) 145–151.
[30] G.L. Card, Metabolism of phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin of *Bacillus stearothermophilus*, J. Bacteriol 114 (1973) 1125–1137.
[31] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[32] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[33] D. Suckau, A. Resemann, M. Schuerenberg, P. Hufnagel, J. Franzen, A. Holle, A novel MALDI LIFT-TOF/TOF mass spectrometer for proteomics, Anal. Bioanal. Chem. 376 (2003) 952–965.
[34] M. Pulfer, R.C. Murphy, Electrospray mass spectrometry of phospholipids, Mass Spectrom. Rev. 22 (2003) 332–364.
[35] W. Dowhan, Molecular basis for membrane phospholipids diversity: why are there so many lipids? Annu. Rev. Biochem. 66 (1997) 199–232.
[36] Y.-P. Ho, C. Fenselau, Applications of 1.06-μm IR laser desorption on a Fourier transform mass spectrometer, Anal. Chem. 70 (1998) 4890–4895.
[37] J.A. Marto, F.M. White, S. Seldomridge, A.G. Marshall, Structural characterization of phospholipids by matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry, Anal. Chem. 67 (1995) 3894–3979.
[38] T. Kaneda, Fatty acids of the genus *Bacillus*: an example of branched-chain preference, Bacteriol. Rev. 41 (1977) 391–418.
[39] D.W. Grogan, J.E. Cronan Jr., Cyclopropane ring formation in membrane lipids of bacteria, Microbiol. Mol. Biol. Rev. 61 (1997) 429–441.
[40] C. Sohlenkamp, K.A. Galindo-Lagunas, Z. Guan, P. Vinuesa, S. Robinson, J. Thomas-Oates, C.R.H. Raetz, O. Geiger, The lipid lysyl-phosphatidylglycerol is present in membranes of *Rhozobium tropici* CIAT899 and confers increased resistance to polymyxin B under acidic growth conditions, Mol. Plant-Microbe Interact. 20 (2007) 1421–1430.
[41] I. Ishizuka, T. Yamakawa, in: H. Wiegandt (Ed.), New Comprehensive Biochemistry, vol. 10, Elsevier, Amsterdam, 1985, p. 101.
[42] P.S. Sastry, Glycosyl glycerides, Adv. Lipid Res. 12 (1974) 251.
[43] W. Wang, Z. Liu, L. Ma, C. Hao, S. Liu, V.G. Voinov, N.I. Kalinovskaya, Electrospray ionization multiple-stage tandem mass spectrometric analysis of diglycosyldiacylglycerol glycolipids from the bacteria *Bacillus pumilus*, Rapid Commun. Mass Spectrom. 13 (1999) 1189–1196.