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Lipid composition of membranes of *Escherichia coli* by liquid chromatography/tandem mass spectrometry using negative electrospray ionization

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A liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) method using reversed-phase chromatography was developed for the analysis of phospholipids from bacterial extracts of a wild-type strain of *Escherichia coli*. Product ion mass spectra from $[M-H]^-$ precursor ions allowed an identification of individual phospholipid species that includes both fatty acid composition and fatty acyl location on the glycerol backbone using diagnostic product ions. Thus, complete assignment, including *sn-1/sn-2* fatty acyl position, was achieved for this strain of *E. coli*. In addition, the phospholipids were quantified relative to one another using an internal standard method. Copyright © 2007 John Wiley & Sons, Ltd.

Lipids are the main components of membranes and play important roles in bacterial cells such as maintenance of membrane integrity, mediation of signal transduction and the regulation of the cell cycle.^{1–3} Some structures of the different classes of phospholipids found in bacteria are shown in Fig. 1. The polar head group (Y) defines the class of phospholipid (PL), while the two acyl substituents ($R_1CH_2CO_2$ and $R_2CH_2CO_2$) can be any one of a large number of saturated, unsaturated, cyclo or hydroxyl fatty acids. The fatty acyl groups are localized at positions *sn-1* and *sn-2* of the glycerol moiety while the polar head group (Y) is at the *sn-3* position (phosphodiester bond). The molecular structures of PLs have been characterized using a variety of mass spectrometric techniques: Fast-atom bombardment in combination with tandem mass spectrometry (FAB-MS/MS) has been widely used to study the different classes of PLs,^{4–9} then electrospray ionization (ESI)^{10–15} and matrix-assisted laser desorption ionization (MALDI)^{16–21} have been used for the structural analysis of these compounds. More recently, Fourier transform mass spectrometry (FTMS)²² and linear ion trap-orbitrap mass spectrometry²³ have provided very high mass accuracy that permits a detailed and comprehensive characterization of ions. Numerous liquid chromatography/mass spectrometry (LC/MS) methods have also been developed to investigate the composition of PLs from biological material such as bacteria, blood samples, marine sediments and rat liver.^{24–30} Separations by reversed- or normal-phase chromatography in combination with ESI-MS

have shown that normal-phase columns can separate most of the different classes of PLs while reversed-phase (RP) columns separate PLs according to their hydrophobicity, which depends on the length of the fatty acyl chains and the number of potential unsaturated bonds. In addition, acyl chain positions on the glycerol backbone (*sn-1* and *sn-2*) have been determined using FAB-MS/MS^{4,9} and ESI-MS/MS^{12,13,15} in negative ion mode. The product ion mass spectra obtained under high- and low-energy collisions from $[M-H]^-$ precursor ions showed two carboxylate anions corresponding to the two acyl moieties but with the *sn-2* carboxylate ion typically being more abundant than the *sn-1* one. Later studies reported, however, that under ESI low-energy collision-induced dissociation (CID) no preferential losses were observed.³¹ A recent LC/MS analysis using in-source CID (high cone voltage) and the assumption that the *sn-2* carboxylate ion is more abundant than the *sn-1* ion allowed the assignment of the acyl chain position in some species of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). However, the authors suggested that the *sn-1/sn-2* ratio of the abundance of the carboxylate ions depends on the structure of the acyl chains, the nature of the head groups and the cone voltage; hence, calibration curves of cone voltage were needed to differentiate isomeric PLs.²⁷ Cabrera *et al.* improved the identification and the location of the *sn-1* and *sn-2* acyl substituents of phosphatidylglycerols (PGs) using ESI and CID in a quadrupole ion trap mass spectrometer.³² They showed that dissociation

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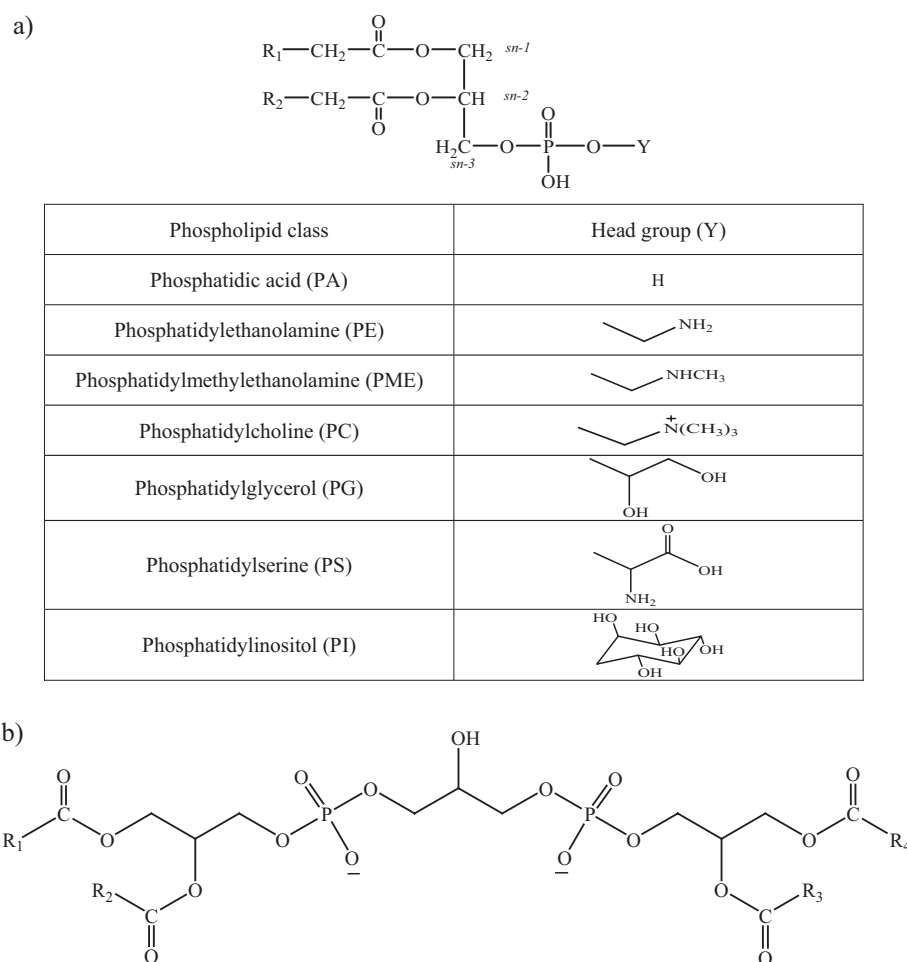


Figure 1. Structures of the main lipids classes: (a) phospholipids and (b) cardiolipins.

of the deprotonated PGs gives three diagnostic product ions, namely $[M-H-RCHCO]^-$, $[M-H-RCHCO-H_2O]^-$ and $[M-H-74-RCH_2COOH]^-$, and that the product ions from the *sn*-2 position are more abundant than those from the *sn*-1 position; this allows the unmistakable assignment of the acyl positions, since the comparison of the relative abundances of the carboxylate ions is not always sufficient.

As a leading model organism, lipids from the membranes of *Escherichia coli* have been widely analyzed.^{9,10,12,13,20,21,33} FAB, ESI, and MALDI-MS were used for PLs while gas chromatography electron ionization (GC/EI)-MS experiments were carried out for fatty acid mixtures.^{21,34} It has been shown that the PLs present in the bacterial membrane of *E. coli* are mainly PEs and PGs although cardiolipins are also present in relatively minor amounts.^{9,11,21}

Despite the extensive characterization of the PLs of *E. coli*, the positions of all the acyl chains have not been reported. In this work, we present a RP-HPLC/ESI-MS/MS method to analyze bacterial PLs using an ion trap mass spectrometer that allows (i) the identification of the PLs, (ii) the identification of the fatty acyl substituents of each PL, (iii) the assignment of the positions of acyl chains, and (iv) the relative quantification of the PLs by means of an internal standard. The determination of *sn*-1/*sn*-2 substituents was achieved by adapting the previously described method,³²

which gives fragmentation patterns that can be used to characterize PGs and PEs.

EXPERIMENTAL

Reagents and chemicals

Standards of PGs (PG C12:0/C12:0, PG C16:0/C16:0) and PEs (PE C12:0/C12:0, PE C16:0/C16:0, PE C16:0/C18:2 and PE C18:0/C18:0) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Stock solutions of these standards ($1 \mu\text{g } \mu\text{L}^{-1}$) were prepared in $\text{CH}_3\text{CN}/\text{CHCl}_3$ (1:1, v/v), and then diluted in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (80:20, v/v) to a final concentration of $10 \text{ ng } \mu\text{L}^{-1}$.

HPLC-grade acetonitrile, methanol, chloroform and ammonium acetate were supplied by VWR (Fontenay-sous-Bois, France).

Deionized water (18 M Ω) was obtained from a Milli-Q apparatus (Waters, Saint-Quentin-Yvelines, France).

Bacterial organisms and culture conditions

LM 3118, a wild-type strain of *E. coli* (K12),³⁵ was grown in nutrient broth (Luria broth) at 37°C on a rotary shaker (15–20 mL culture) overnight. Luria broth³⁶ was from the recipe of Miller (5 g yeast extract, 10 g peptone tryptone, 10 g NaCl). The pH was maintained at 6.95 automatically by

titration with 5% H₂SO₄ or 5% NaOH. Peptone tryptone and yeast extract were from Difco (Detroit, MI, USA), NaCl from Sigma. The medium was made in distilled water and autoclaved under standard conditions. Then, cells were harvested by centrifugation at 10 000 g for 10 min at 3°C. The pellet was recovered and dispersed in 1 ml of deionized water.

Sample preparation

Lipids were extracted from pelleted cells according to the method of Bligh and Dyer.³⁷ 2 ml of CHCl₃/CH₃OH (2:1, v/v) were added to 1 ml of bacterial suspension, samples were vortex-mixed, centrifuged at 1000 g for 10 min and the chloroform-rich (bottom) phase containing the PLs was then removed with a Pasteur pipette. The extraction step was repeated twice and the collected organic phase was evaporated and stored at -20°C.

LC/MS/MS analyses

HPLC/MS/MS analyses were performed using an Applied Biosystems (Courtaboeuf, France) chromatographic system consisting of a 140B pump coupled to an Esquire-LC ion trap mass spectrometer (Bruker Daltonics, Wissembourg, France). Data acquisitions and processing were carried out using an Esquire Control 6.16 and Data Analysis 3.0 data system. LC separations were obtained on a C18 X-Terra column (50.0 mm × 2.1 mm i.d., 3.5 μm particle size; Waters) at room temperature using the following linear gradient; 50% (hold 5 min) to 100% CH₃CN in 20 mm ammonium acetate buffer (pH 6.7) in 30 min. The flow rate was 0.200 ml min⁻¹ and injections were carried out with a Rheodyne model 7125 (Interchim, Montluçon, France) valve equipped with a 20 μL loop. The optimized ESI parameters for negative ion detection were capillary voltage set at +4 kV, end plate at +3.5 kV. The declustering potentials through the capillary exit (V_{CE}) and skimmer (V_{SK1}) voltages were, respectively, -134 V and -52 V. Nitrogen was used as the drying (9 L min⁻¹, 300°C) and nebulizing (30 psi) gas.

The ESI and MS/MS parameters were optimized before LC injections by infusing commercial standards as well as biological samples, which were flow injected at 3 μL min⁻¹ by means of a syringe pump (Cole-Palmer, Vernon Hills, IL, USA).

LC/MS and LC/MS/MS experiments were recorded in the same run using the standard scan mode at normal resolution; the scan speed was 13000 *m/z* units s⁻¹ and the mass resolution was 0.6 *m/z* units at half peak height (FWHM) over a range of *m/z* 50–1500.

Helium was the buffer gas and the pressure in the ion trap was 1.2 × 10⁻⁵ mbar. The ion accumulation in the trap was monitored so that charge space phenomenon was reduced. Spectra were averages of five scans. CID experiments were acquired using helium as collision gas, with an isolation width of 4 *m/z* units and a resonant excitation voltage of 1.0 V.

The relative quantifications of PLs were performed using PE C12:0/C12:0 as the internal standard (IS); the cells pellets were spiked with 50 μL of the stock solution (1 μg μL⁻¹) of PE C12:0/C12:0 before the extraction step. For each sample, *intra-day* precision was determined by triplicate LC/MS

injections on the same day while *inter-day* precision was obtained from replicate runs on different days.

RESULTS AND DISCUSSION

Optimization of LC/MS/MS conditions: analyses of standards and application to bacterial extracts

Negative ESI-MS and -MS/MS parameters were first optimized by direct infusion of standard solutions of PEs and PGs as well as infusion of cell extracts. Then, an LC/MS/MS method using a reversed-phase column (C18) and a linear gradient from 50% to 100% CH₃CN in 20 mm ammonium acetate buffer (pH 6.7) was developed using a mixture of the commercial standards of PLs (data not shown). Using this method, bacterial extracts from the wild-type strain of *E. coli* were analyzed. Three *intra-day* LC/MS/MS injections and two *inter-day* series of injections were carried out, including PE C12:0/C12:0 as IS for relative quantification.

Figure 2(a) shows the extracted ion chromatograms of [M-H]⁻ ions from LC/MS analysis. Although LC separation was achieved for the mixture of standards (data not shown), some co-elution occurred for the biological samples because of the high number of PLs present in *E. coli*. If the isobaric forms are ignored, up to 20 species (10 PGs and 10 PEs) could be detected, while if the isobaric forms are taken into account, up to 30 phospholipids could be detected (Table 1). For example, the PG C31:1 (*m/z* 705) can be either PG C14:0/cyC17:0 or PG C15:0/C16:1.

Concerning the order of elution of PLs, in our chromatographic conditions it is apparent that:

1. For identical acyl groups, the PG class elutes first; e.g., PG C14:0/C16:1 has a retention time (*t_R*) of 16.0 min while PE C14:0/C16:1 elutes at 19.1 min. A similar observation can be made for PG C16:0/C16:1 (*t_R* = 19.3 min) and PE C16:0/C16:1 (*t_R* = 22.6 min).
2. In the same class of PLs, one unsaturation leads to an increase in polarity and consequently a decrease in retention time; for instance, PE C16:0/C18:1 (*t_R* = 25.9 min) elutes after PE C18:1/C16:1 (*t_R* = 23.1 min) whatever the substituent position.
3. Isobaric forms co-eluted and could not be distinguished.

Identification of PLs present in the LM 3118 strain of *E. coli*

Despite the lack of chromatographic resolution, it was possible to differentiate and identify each PL using their MS/MS spectra. Thus, 13 PGs and 17 PEs were identified taking into account the isobaric forms (Table 1). In negative ion mode, abundant [M-H]⁻ ions were observed for these two classes of PLs: the [M-H]⁻ ions are even mass for PEs and odd for PGs. The product ion mass spectra of the two classes of PLs showed some common characteristic ions (Table 1 and Scheme 1):

1. Two abundant carboxylate anions allowed the identification of both acyl moieties. Their fragmentation mechanisms have been previously described.^{15,27} For instance,

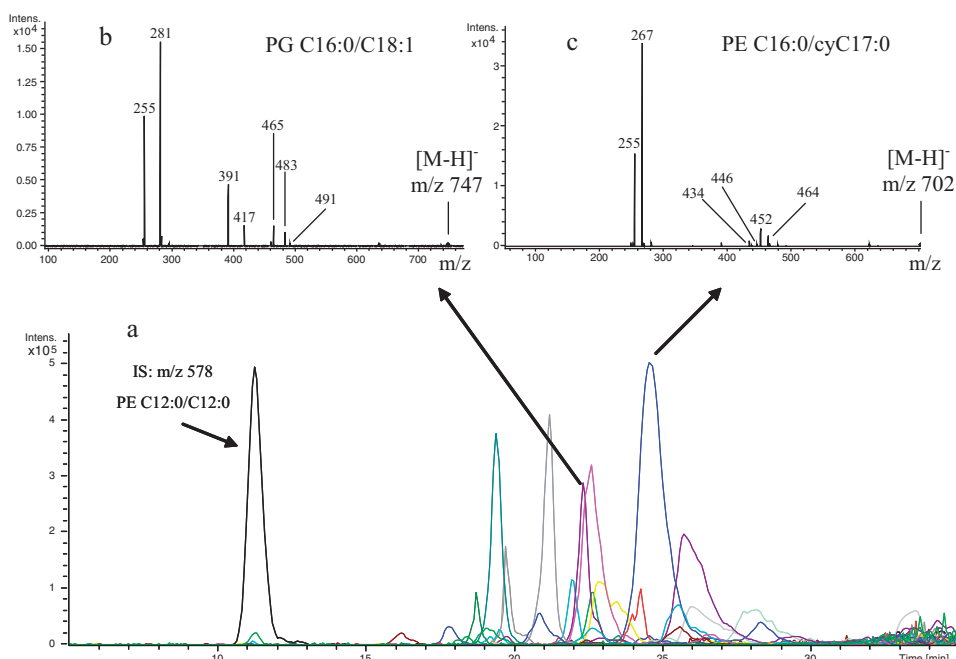


Figure 2. (a) LC/MS analysis of the wild-type strain (LM 3118): extracted ion chromatograms of $[M-H]^-$ ions. (b) Product ion mass spectrum of the precursor ion at m/z 747 corresponding to $[M-H]^-$ of PG C16:0/C18:1 eluted at 22.2 min. (c) Product ion mass spectrum of the precursor ion at m/z 702 corresponding to $[M-H]^-$ of PE C16:0/cyC17:0 eluted at 24.5 min.

the MS/MS spectra of the precursor ions at m/z 747 and 702 are reported in Figs. 2(b) and 2(c), respectively. These two ions correspond to $[M-H]^-$ ions of PLs eluted at 22.2 and 24.5 min. The CID spectrum of m/z 747 shows abundant product ions at m/z 255 and 281 corresponding, respectively, to the carboxylate anions of C16:0 and C18:1 fatty acids (FAs). In the same way, the precursor ion at m/z 702 generates carboxylate ions of C16:0 and cyC17:0 (m/z 255 and 267). (Note that CX:Y describes the composition of the fatty acyl group where X indicates the number of carbon atoms and Y designates the number of double bonds, cyC17:0 referring to a cyclopropyl group.) The structural elucidation of fatty acyl moieties has been performed by GC/MS³⁸ and is in agreement with reported data.^{12,13,20,21,38} The relative abundance of m/z 255 (carboxylate anion from C16:0) is nearly 60% of that of m/z 281 (carboxylate anion from C18:1) which is the base peak in the CID spectrum of m/z 747, assuming that C16:0 and C18:1 are, respectively, the *sn*-1 and *sn*-2 FAs.¹² Similarly, m/z 255 has a relative abundance of 50% of m/z 267 (base peak in the CID spectrum of m/z 702) assuming that C16:0 and cyC17:0 are the *sn*-1 and *sn*-2 FAs. Therefore, m/z 747 should be identified as PG C16:0/C18:1 and m/z 702 as PE C16:0/cyC17:0. However, these assumptions needed to be confirmed and this was achieved by additional structural information (explained in the next section). By analyzing all the acquired CID spectra, we derived a general formula linking the masses of the carboxylate moieties and the molecular masses of the PGs or PEs, i.e. $M_{PG} = M_{RCOO^-_{sn-1}} + M_{RCOO^-_{sn-2}} + 212$ and $M_{PE} = M_{RCOO^-_{sn-1}} + M_{RCOO^-_{sn-2}} + 181$.

2. $[M-H-RCHCO]^-$ and $[M-H-RCHCO-H_2O]^-$ ions result from ketene elimination followed by a neutral loss of water from either the *sn*-1 or *sn*-2 position (Scheme 1(a)). A preferential loss of the acyl group in the *sn*-2 position has been previously observed: this was described for PGs in an ion trap mass spectrometer³² and for PEs under low-energy CID conditions in a triple quadrupole mass spectrometer.¹² By analyzing an authentic commercial standard of PE C16:0/C18:2 (precursor ion at m/z 714), we verified that the MS/MS experiment performed using our ion trap mass spectrometer did indeed give product ion mass spectra showing a more abundant $[M-H-RCHCO_{sn-2}]^-$ ion (m/z 452) than $[M-H-RCHCO_{sn-1}]^-$ (m/z 476); this confirms the preferential elimination of the ketene from the *sn*-2 position (Fig. 3). This elimination of $RCHCO_{sn-2}$ can be explained by steric hindrance being more important for the *sn*-2 fatty acyl substituent. With regard to the PLs of *E. coli* (Figs. 2(b) and 2(c)), m/z 747 (PG C16:0/C18:1) yields product ions at m/z 491, 483 and 465. Likewise, m/z 702 (PE C16:0/cyC17:0) shows product ions at m/z 464, 452 and 434. In both case these ions correspond, respectively, to $[M-H-RCHCO_{sn-1}]^-$, $[M-H-RCHCO_{sn-2}]^-$ and $[M-H-RCHCO_{sn-2}-H_2O]^-$. One additional product ion was detected at m/z 446 for the PE which is consistent with $[M-H-RCHCO_{sn-1}-H_2O]^-$. As the m/z 483 ion is more abundant than m/z 491 and m/z 452 is more abundant than 464, we can confirm that C18:1 and cyC17:0 constitute the *sn*-2 FAs of the PG and PE, respectively. Such ions were observed in the product ion spectra for all the selected deprotonated molecules of PGs and PEs and

Table 1. Identification of phospholipids (PLs) present* in the studied strains of *E. coli*; product ions observed in negative ESI-MS/MS. The values written in bold correspond to more abundant species

t_r (min)	$[M-H]^-$	PLs	RCH_2 $CO_2^- sn-1$	RCH_2 $CO_2^- sn-2$	$[M-H-74-R_1]$ CH_2CO_2H $sn-1]^-$	$[M-H-74-R_2]$ CH_2CO_2H $sn-2]^-$	$[M-H-R_1]$ $CHCO_2 sn-1]^- /$ $[M-H-R_1]$ $CHCO_2 sn-1-H_2O]^-$	$[M-H-R_2]$ $CHCO_2 sn-2]^- /$ $[M-H-R_2]$ $CHCO_2 sn-2-H_2O]^-$
11.3	578	PE C12:0/C12:0	199	199			396/378	396/378
16.0	691	PG C14:0/C16:1	227	253	389	363	481/-	455/437
17.7	705	PG C14:0/cyC17:0	227	267	403	363	—	455/437
		PG C15:0/C16:1	241	253	—	377	—	469/451
18.4	634	PE C14:0/C14:0	227	227	—	—	—	—
		PE C16:0/C12:0	199	255	—	—	—	452/-
18.5	693	PG C16:0/C14:0	255	227	363	391	—	483/465
19.1	660	PE C14:0/C16:1	227	253	—	—	450/-	424/-
19.3	719	PG C14:0/C18:1	227	281	—	363	-/491	-/437
		PG C15:0/cyC17:0	241	267	403	377	—	469/-
		PG C16:0/C16:1	255	253	389	391	481/463	483/465
19.9	745	PG C18:1/C16:1	281	253	389	417	483/463	509/491
20.9	674	PE C14:0/cyC17:0	227	267	—	—	464/-	424/-
		PE C15:0/C16:1	241	253	—	—	450/-	438/420
21.0	733	PG C16:0/cyC17:0	255	267	403	391	495/477	483/465
21.8	721	PG C16:0/C16:0	255	255	391	391	483/465	483/465
22.2	747	PG C16:0/C18:1	255	281	417	391	-/491	483/465
22.6	688	PE C15:0/cyC17:0	241	267	—	—	-/446	438/420
		PE C16:0/C16:1	255	253	—	—	450/432	452/434
22.7	773	PG C18:1/C18:1	281	281	417	417	509/491	509/491
23.1	714	PE C18:1/C16:1	281	253	—	—	450/432	478/460
		PE cyC17:0/cyC17:0	267	267	—	—	464/446	464/446
24.1	761	PG C16:0/cyC19:0	255	295	431	391	-/505	483/465
24.5	702	PE C16:0/cyC17:0	255	267	—	—	464/446	452/434
25.3	690	PE C16:0/C16:0	255	255	—	—	452/434	452/434
25.9	716	PE C16:0/C18:1	255	281	—	—	478/460	452/-
26.8	742	PE cyC19:0/cyC17:0	295	267	—	—	464/-	492/-
		PE C18:1/C18:1	281	281	—	—	478/460	478/460
27.7	730	PE C16:0/cyC19:0	255	295	—	—	-/474	452/434
		PE C18:0/cyC17:0	283	267	—	—	—	480/-
		PE C17:0/C18:1	269	281	—	—	478/-	466/-

*The phospholipid that eluted at 11.3 min is not present in *E. coli* but present on the EIC (extracted in chromatogram) as it corresponds to the internal standard PE C12:0/C12:0.

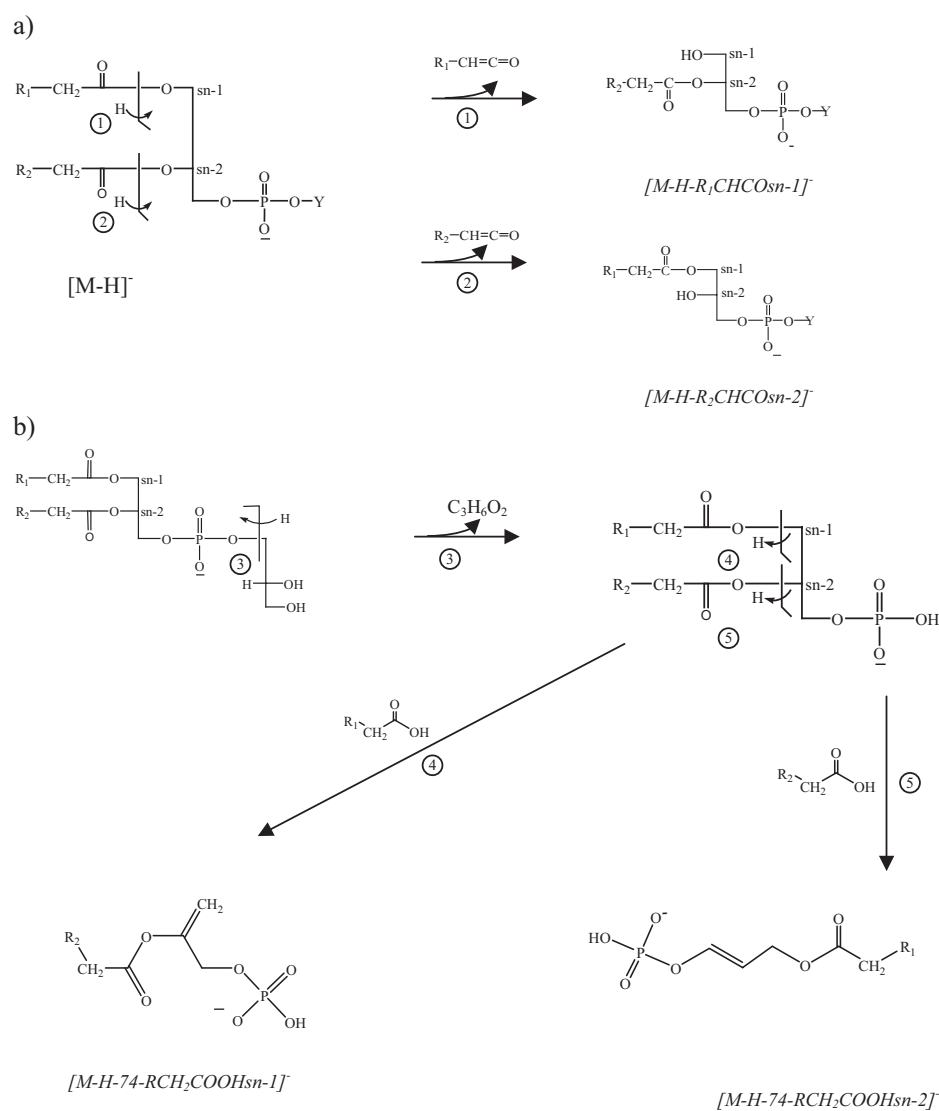
differences in their relative abundances allowed both acyl substituent groups to be positioned on the glycerol backbone. The ensemble of data in Table 1 shows a high complexity in PL composition because some precursor ions are a mixture of isobaric forms; e.g., the $[M-H]^-$ ion at m/z 742 is a mixture of PEcyC17:0/cyC19:0 and PE C18:1/C18:1. Nevertheless, it was possible to distinguish major from minor forms by comparing the relative abundances of the product ions (carboxylate and $[M-H-RCHCO]^-$) generated from each form.

In the case of PGs, some additional product ions are detected which imply the successive neutral losses of 74 Da and FA residues (Scheme 1(b)). The neutral loss of 74 Da has been assigned to $C_3H_6O_2$, presumably derived from the glycerol moiety.^{4,32} Thus, the two product ions, $[M-H-74-RCH_2COOH_{sn-1}]^-$ and $[M-H-74-RCH_2COOH_{sn-2}]^-$, can be used to characterize the PG class. Once more, the most abundant ion involves the $sn-2$ fatty acid loss. For example, the product ions at m/z 391 and 417 from the m/z 747 precursor ion correspond, respectively, to $[M-H-74-RCH_2COOH_{sn-2}]^-$ and $[M-H-74-RCH_2COOH_{sn-1}]^-$. The relative abundance of m/z 417, which is less than that of m/z 391, gives

additional evidence that C16:0 and C18:1 are at the $sn-1$ and $sn-2$ positions, respectively.

The results obtained for this strain of *E. coli* show that the 13 PGs and 17 PEs are principally constituted of C12:0, C14:0, C16:0, C16:1, C18:0 and C18:1 FAs, and, to a lesser extent, of C15:0, cyC17:0 and cyC19:0 FAs. These three FAs contain an odd number of carbons and are not usually found in animal cells but are characteristic of the enterobacteriaceae and of *E. coli* in particular.^{11,12,21} Isobaric forms were present for PG C31:1 (m/z 705), PG C32:1 (m/z 719), PE C28:0 (m/z 634), PE C31:1 (m/z 674), PE C32:1 (m/z 688), PE C34:2 (m/z 714), PE C35:1 (m/z 730) and PE C36:2 (m/z 742). These data are in accordance with previous reports.^{11,13,21}

It is noteworthy that LC separation helps solve the problem of selective ionization occurring for direct injections of different classes of PLs: it has been previously demonstrated that, for a mixture of PGs and PEs directly infused and studied in negative ESI-MS, deprotonated molecules of PGs give stronger signals than PEs,^{11,12} even if the PEs represent the major components in the sample. Under our LC conditions, 17 chromatographic peaks from PE species were readily detectable and the most abundant peak of the chromatographic profile corresponded to PE C16:0/cyC17:0 (m/z 702) (Fig. 2(a)).



Relative quantification

The addition of PE C12:0/C12:0 as internal standard (IS) ($t_R = 11.3$ min) allows the relative quantification of PLs within the LM 3118 strain. Figure 4 displays the two series of triplicate injections. As LC separation overcame the problem of the different ionization properties of the phospholipid classes, the ratios between the relative abundances of deprotonated molecules from a given PL to that of the IS could be determined (RA_{PL}/RA_{IS}). In Fig. 4, these ratios are plotted as a percentage of the highest ratio obtained, namely $RA_{PE\ C16:0/cyC17:0(m/z\ 702)}/RA_{IS}$. For relative quantification, the isobaric forms were not discriminated. The intra-day precisions of the analytical method are shown: the relative standard deviation varied from 1 to 14% (%RSD) and depended on chromatographic peak intensity and ion accumulation in the ion trap. The inter-day precision varied from 2 to 33%. However, the two series show similar profiles. The worst values of %RSD were obtained for the lowest and the highest chromatographic peaks and this may be due to either a signal that was too weak (a low signal to noise ratio)

or a charge space phenomenon caused by the accumulation of too many ions in the ion trap (in the case of the highest chromatographic peak). The samples were therefore diluted to find a compromise between avoiding the loss of signal from the lowest chromatographic peak and limiting the charge space phenomenon for the highest chromatographic peak. This compromise maximizes reproducibility. From the quantification, we can note that:

- nine PLs are present in large amounts and they correspond to PE C32:1 (m/z 688), PE C33:1 (m/z 702), PE C34:2 (m/z 714), PE C34:1 (m/z 716), PE C35:1 (m/z 730), PE C36:2 (m/z 742), PG C32:1 (m/z 719), PG C33:1 (m/z 733) and PG C34:1 (m/z 747),
- PE C16:0/cyC17:0 (m/z 702) is the highly predominant PL,
- minor species consist of the six following PLs: PE C28:0 (m/z 634), PE C30:1 (m/z 660), PG C30:1 (m/z 691), PG C30:0 (m/z 693) and PG C31:1 (m/z 705).

These results show that, as expected for *E. coli*, PEs constitute the major class of phospholipids, in both number

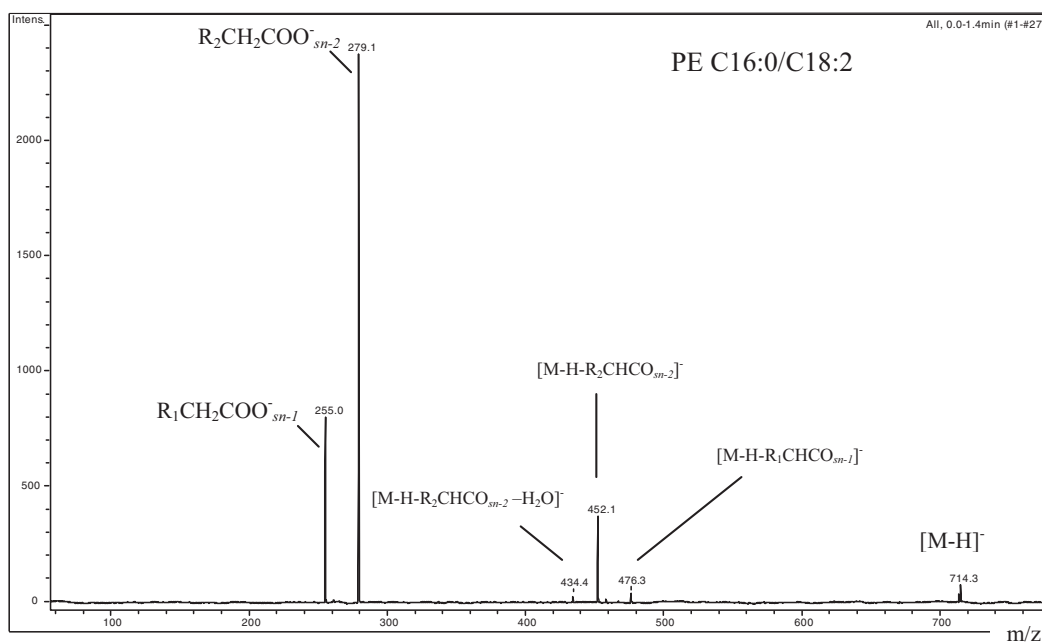


Figure 3. Product ion mass spectrum of the $[M-H]^-$ ion of standard PE C16:0/C18:2.

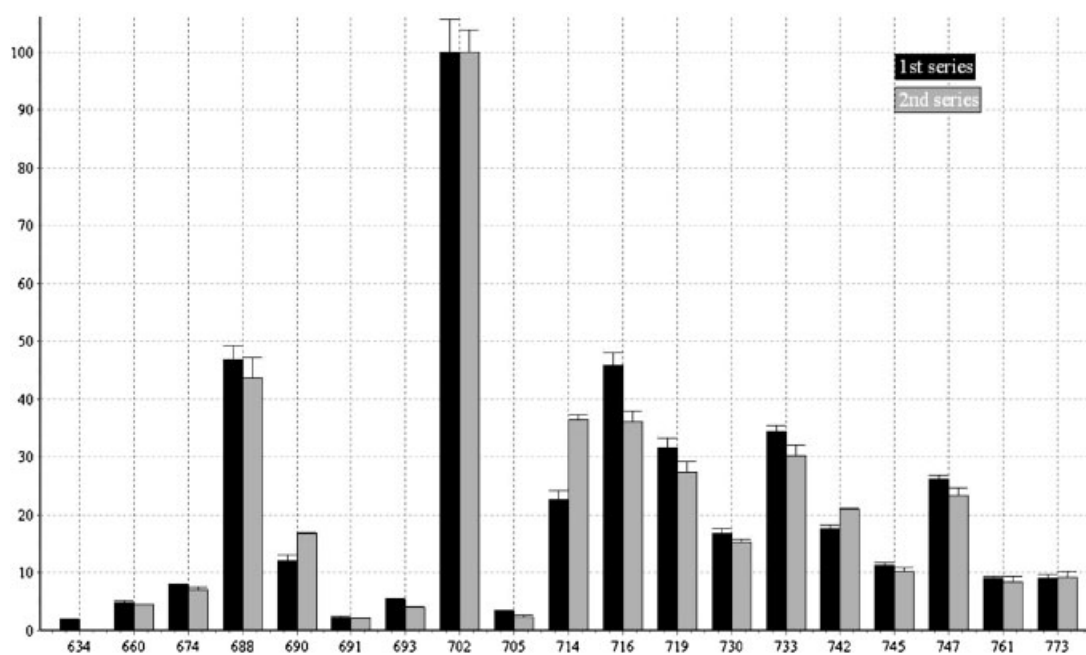


Figure 4. Relative composition of phospholipids in the LM 3118 strain of *E. coli*. Values are means \pm RSD.

and quantity, present in the LM 3118 strain grown under the culture conditions reported above. In this strain, the main FAs are C14:0, C16:1, C16:0, cyC17:0 and C18:1.

CONCLUSIONS

This study showed that complex lipid mixtures extracted from bacterial cell extracts can be analyzed by LC/ESI-MS/MS in an ion trap mass spectrometer in the negative ionization mode. Separation of PLs was achieved using a C_{18}

reversed-phase column. Individual PL species were identified and their fatty acyl substituents were positioned on the glycerol backbone. These PLs comprised 17 PEs and 13 PGs including isobaric forms. In negative ion mode, PG and PE undergo common fragmentation processes: the principal product ions are carboxylate ions, then ions formed by neutral loss of RCHCO followed by H_2O elimination from the $[M-H]^-$ ion. The preferential elimination of the ketene from the *sn*-2 position allows the substitution positions to be determined for both acyl groups. In the case of PGs,

additional product ions corresponding to $[M-H-74-RCH_2COOH]^-$ were also observed which confirm their identifications.

A lipid profile was obtained by relative quantification using PE C12:0/C12:0 as internal standard. This showed that (i) PEs constitute the major components of the *E. coli* membranes, (ii) PE C16:0/cyC17:0 is the most common species, and (iii) C14:0, C16:1, C16:0, cyC17:0 and C18:1 constitute almost all of the fatty acyl substituents.

Further analysis is needed for the cardiolipins since these are minor constituents of *E. coli* membranes that could not be detected under our chromatographic conditions owing to insufficient elution from our reversed-phase column.

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