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# Diagnostic amide products of amino lipids detected in the microaerophilic bacteria *Lutibacter* during routine fatty acid analysis using gas chromatography

Nicole J. Bale [a,*], Michel Koenen [a], Subhash Yadav [a], Ellen C. Hopmans [a], Laura Villanueva [a], Jaap S. Sinninghe Damsté [a,b], Stefan Schouten [a,b]

[a] NIOZ Royal Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, Utrecht University, Texel, the Netherlands
[b] Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Utrecht, the Netherlands

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ABSTRACT

Analysis of fatty acids in the form of fatty acid methyl esters (FAMEs) using gas chromatography (GC) is routine within microbiology but still some compounds remain unidentified. During characterization of the FAMEs of two strains of the microaerophilic bacterium *Lutibacter* sp., recently isolated from the Black Sea, a series of compounds, eluting after the regular FAMEs, were detected. We identified these compounds using GC–mass spectrometry (GC–MS) and an authentic standard, to be amino acids glycine-linked via an amide bond to β-hydroxy fatty acids (i.e. glycine β-hydroxy fatty acid amides). Analysis of the intact polar lipids of the *Lutibacter* species by ultra-high performance liquid chromatography–high resolution mass spectrometry (UHPLC–HRMS) showed that the glycine β-hydroxy fatty acid amides are derived from glycine lipids (also known as cytolipins), which are amino acid lipids. Amino acid lipids represent an under-studied, but potentially significant, group of microbial membrane lipids and our results provide a rapid way to detect the presence of glycine lipids during routine fatty acid analysis by GC. Furthermore, glycine β-hydroxy fatty acid amides represent easily detectable biomarker lipids for glycine lipid-producing microorganisms in natural environments.



## 1. Introduction

Analysis of fatty acid methyl esters (FAMEs) is a routine investigation carried out in fields such as environmental microbiology and biogeochemistry to characterize microbial strains and then to trace fatty acids as biomarker lipids in the environment. These fatty acids are typically released by base hydrolysis from intact polar lipids (IPLs), the majority of which are diacyl glycerol lipids, including phospholipids such as phosphatidylethanolamine (PE) and phosphatidylcholine (PCs) (Fig. 1A). The FAMEs are mostly identified by comparison of retention times with standard FAME mixtures (Eder, 1995), which often results in incomplete identification of all compounds released by hydrolysis. Further characterization of FAMEs by GC–mass spectrometry (GC–MS) can result in additional identifications, provided that reference mass spectra are available either in the literature or in mass spectral libraries.

Here, we report the detection of a series of compounds as part of FAME analysis of two strains of the microaerophilic bacterium *Lutibacter* sp. (Bacteriodetes), recently isolated from 2000 m depth in the Black Sea. We identified these compounds using GC–MS and by comparison with an authentic standard. Furthermore, we analyzed the distribution of IPLs present in these strains using ultra high performance liquid chromatography–high resolution mass spectrometry (UHPLC–HRMS) to investigate the source polar lipids of these of compounds.

## 2. Methods

### 2.1. Isolation and cultivation of Lutibacter strains B1 and B2

*Lutibacter* sp. strain B1 and B2 was cultivated from Black Sea water samples (2000 m depth; 42° 53.78′ N, 30° 40.72′ E) collected in March 2017. Both strains were purified by repeated streaking on agar medium containing pyruvate (2.0 g $l^{-1}$); tryptone (2.0 g $l^{-1}$); yeast extract (1.0 g $l^{-1}$); $CaCl_2{\cdot}2H_2O$ (1.0 g $l^{-1}$), NaCl (20.0 g $l^{-1}$), $MgCl_2{\cdot}6H_2O$ (3.6 g $l^{-1}$), $MgSO_4{\cdot}7H_2O$ (4.3 g $l^{-1}$) and KCl (0.5 g $l^{-1}$) at pH 7.0. Routine laboratory cultivation of both strains was performed under microaerophilic conditions at 25 °C in the liquid medium mentioned above.

* Corresponding author.
*E-mail address:* nicole.bale@nioz.nl (N.J. Bale).

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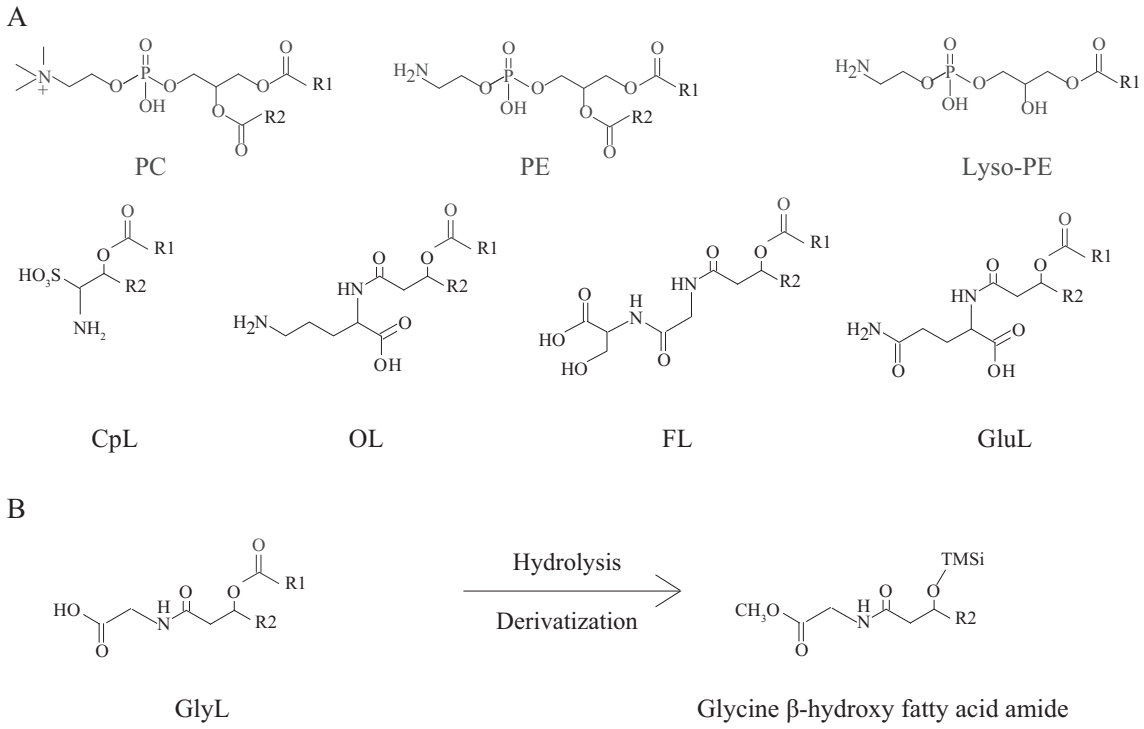


**Fig. 1.** Structures of intact polar lipids (IPLs) detected. (A) PC, PE, lyso-PEs, capnine lipid (CpL), ornithine lipid (OL), flavolipin (FL) and glutamine lipid (GluL). (B) Putative scheme showing the hydrolysis of a glycine lipid (GlyL, also known as cytolipin) to glycine β-hydroxy fatty acid amide. R1 and R2 represent alkyl moieties.

### 2.2. Lipid extraction and analysis

The extracts for fatty acid (FA) analysis were obtained as described previously (Bale et al., 2019). Briefly, freeze-dried *Lutibacter* biomass was hydrolyzed by refluxing for 1 h with 1 M KOH in methanol before neutralization with a 2 M HCl/methanol (1:1, v/v) solution. The FAs in the resulting extract were derivatized firstly by methylation (diazomethane in diethyl ether, removed under a stream of $N_2$) before being derivatized with pyridine (10 μl) and N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) (10 μl). The derivatized extracts were brought to a final volume with ethyl acetate, to a concentration of 1 mg $ml^{-1}$. FA methyl ester (FAME) quantification and identification were as per Bale et al. (2019) and FAMEs were identified based on literature data and library mass spectra. Double bond positions were determined, where possible, using dimethyldisulfide (DMDS) derivatization of the FAMEs. To this end, extracts were derivatized in hexane (100 μl) with DMDS (Merck ≥ 99%; 100 μl) and $I_2$ in ether (60 mg $ml^{-1}$; 20 μl) and heated overnight at 40 °C. Hexane (400 μl) was then added with $Na_2S_2O_3$ (5% aqueous solution; 200 μl) to deactivate the iodine. The hexane layer was removed, and the aqueous phase washed with hexane (×2). The hexane layers were combined and analyzed by GC–MS as described above. The double bond positions were identified through comparisons with literature data and library mass spectra.

In order to confirm the identity of a range of unknown compounds, we used a commercially available glycine β-hydroxy fatty acid amide standard, commendamide (Sanbio, Uden, The Netherlands), which was hydrolyzed, derivatized and analyzed in the same manner as the *Lutibacter* lipid extracts. Double bond positions in the unknown compounds were tentatively identified using the same method as described above, making comparisons with the MS fragmentation pattern of established unsaturated FAMEs.

Intact polar lipids (IPLs) were also extracted from freeze-dried biomass *Lutibacter* using a modified Bligh-Dyer procedure, as described in Bale et al. (2019). Before analysis the extract was re-dissolved in a mixture of methanol:dichloromethane (9:1, v/v) which contained as an internal standard a platelet activating factor (PAF) standard (1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, 5 ng on column). Aliquots were filtered through 0.45 μm regenerated cellulose syringe filters (4 mm diameter; Grace Alltech, Deerfield, IL, USA).

Analysis of extracts was carried out using an UHPLC–HRMS according to the reversed phase method of Wörmer et al. (2013) using a system and settings as described in Bale et al. (2019). IPLs were quantified in terms of their MS peak area response, which does not necessarily reflect the actual relative abundance of the different IPLs due to variable response behavior.

## 3. Results

Two *Lutibacter* sp. strains (B1 and B2), isolated from water collected at 2000 m depth of the Black Sea (Y. Subhash, unpublished results), were cultivated at 25 °C for 3 days. Analysis of the FAMEs released by base hydrolysis of the biomass extract by GC–MS revealed that in both strains the major fatty acids (>5%) were *iso* $C_{15:0}$, *iso* $C_{15:1\omega11}$ and β-hydroxy *iso* $C_{15:0}$, while the minor fatty acids (1–5%) were *iso* $C_{13:0}$, *anteiso* $C_{15:1\omega11}$, *anteiso* $C_{15:0}$, $C_{15:0}$, *iso* $C_{17:0}$, α-hydroxy *iso* $C_{15:0}$ and β-hydroxy *iso* $C_{17:0}$ (Table 1). Besides these fatty acids, a series of nine compounds (A–I, Fig. 2) eluted between 24 and 28 min and accounted for ca. 20% of all GC-amenable compounds. The compounds had molecular ions of either *m/z* 401, 415, 427 or 429 with fragment ions representing losses from the molecular ion of 15, 59, 90 or 130 Da, respectively (e.g., Fig. 3). The losses of 15 Da ($CH_3$) and 59 Da ($COOCH_3$) are typical for the carboxylic acids groups of FAMEs (Capella et al., 1968). Applying the Nitrogen Rule (Pellegrin, 1983), that a loss with an even nominal mass contains an uneven number of N atoms, we assume that the uncommon loss of 90 Da is a fragment that

**Table 1**
Fatty acids, hydroxy fatty acids and glycine β-hydroxy fatty acids amides identified in *Lutibacter* sp. strains B1 and B2 after base hydrolysis.

| Lipid | | Retention time (min) | Relative abundance (%) | |
|---|---|---|---|---|
| | | | B1 | B2 |
| Fatty acids | *iso* C13:0 | 6.2 | 4.1 | 3.8 |
| | *anteiso* C13:0 | 6.3 | 0.3 | 0.4 |
| | *iso* C14:1 ω10 | 7.3 | 0.0 | 0.1 |
| | *iso* C14:0 | 7.7 | 0.7 | 0.7 |
| | *n*-C14:0 | 8.3 | 0.2 | 0.2 |
| | *iso* C15:1 ω11 | 9.1 | 18.6 | 17.9 |
| | *anteiso* C15:1 ω11 | 9.1 | 1.1 | 1.4 |
| | *iso* C15:0 | 9.6 | 28.7 | 27.3 |
| | *anteiso* C15:0 | 9.6 | 3.3 | 3.2 |
| | *n*-C15:0 | 10.2 | 1.6 | 1.5 |
| | *iso* C16:1 ω11 | 10.9 | 0.1 | 0.1 |
| | *iso* C16:0 | 11.4 | 0.5 | 0.5 |
| | *iso* C17:1 ω6 | 13.0 | 0.2 | 0.2 |
| | *iso* C17:1 ω8 | 13.0 | 0.1 | 0.1 |
| | *iso* C17:0 | 13.5 | 1.1 | 1.0 |
| | *anteiso* C17:0 | 13.6 | 0.1 | 0.1 |
| Hydroxy fatty acids | *iso* β-OH C14:0 | 9.9 | 0.3 | 0.3 |
| | β-OH *n*-C14:0 | 11.8 | 0.1 | 0.1 |
| | *iso* β-OH C15:0 | 13.9 | 11.8 | 10.8 |
| | *iso* α-OH C15:0 | 14.0 | 3.5 | 4.3 |
| | *anteiso* β-OH C15:0 | 14.1 | 0.3 | 0.3 |
| | *anteiso* α-OH C15:0 | 14.1 | 0.2 | 0.2 |
| | β-OH *n*-C15:0 | 14.6 | 0.2 | 0.2 |
| | α-OH *n*-C15:0 | 14.7 | 0.1 | 0.1 |
| | *iso* β-OH C16:0 | 16.0 | 0.8 | 0.8 |
| | β-OH *n*-C16:0 | 16.7 | 0.4 | 0.4 |
| | *iso* β-OH C17:1 | 17.3 | 0.1 | 0.1 |
| | *anteiso* β-OH C17:1 | 17.5 | 0.1 | 0.1 |
| | *iso* β-OH C17:0 | 18.0 | 2.5 | 2.5 |
| | *anteiso* β-OH C17:0 | 18.2 | 0.3 | 0.3 |
| Glycine β-hydroxy fatty acids amides | Glycine *iso* β-OH C15:0 amide (A) | 24.1 | 1.7 | 1.9 |
| | Glycine *anteiso* β-OH C15:0 amide (B) | 24.2 | 0.1 | 0.1 |
| | Glycine β-OH *n*-C15:0 amide (C) | 24.8 | 0.4 | 0.4 |
| | Glycine *iso* β-OH C16:0 amide (D) | 26.0 | 2.0 | 2.3 |
| | Glycine β-OH *n*-C16:0 amide (E) | 26.7 | 1.3 | 1.5 |
| | Glycine *iso* β-OH C17:1 ω6 amide (F) | 27.2 | 0.1 | 0.1 |
| | Glycine *anteiso* β-OH C17:1 ω6 amide (G) | 27.4 | 0.3 | 0.3 |
| | Glycine *iso* β-OH C17:0 amide (H) | 28.0 | 11.7 | 13.0 |
| | Glycine *anteiso* β-OH C17:0 amide (I) | 28.0 | 1.1 | 1.2 |

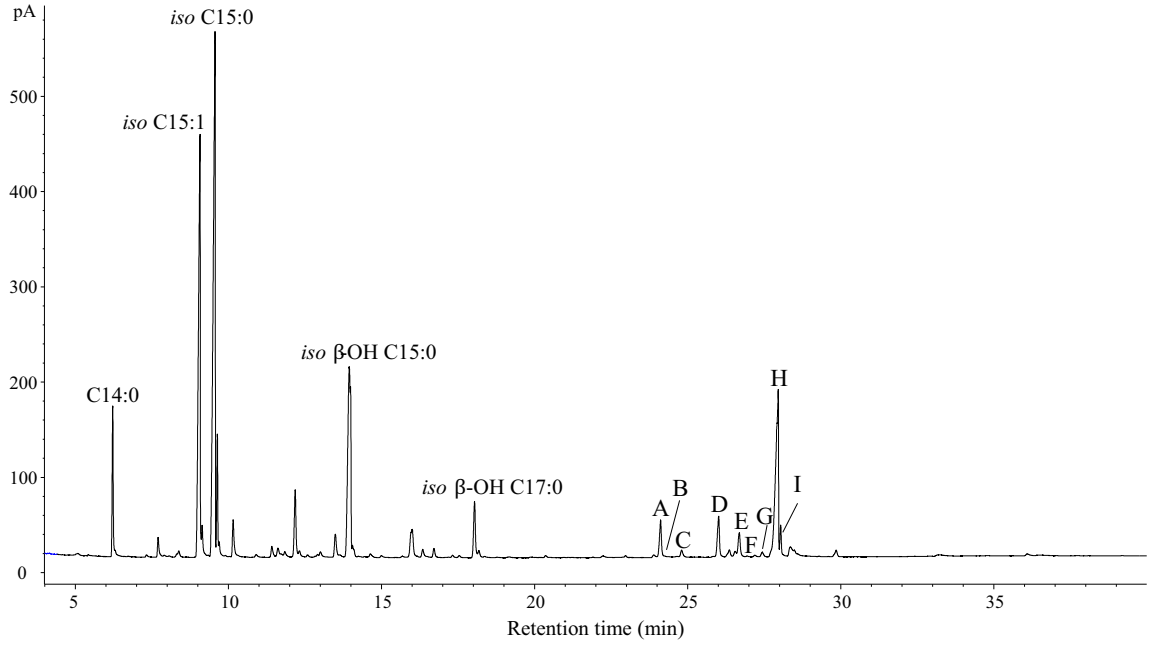


**Fig. 2.** GC–MS chromatogram of *Lutibacter* sp. silylated fatty acid methyl esters (FAMEs).

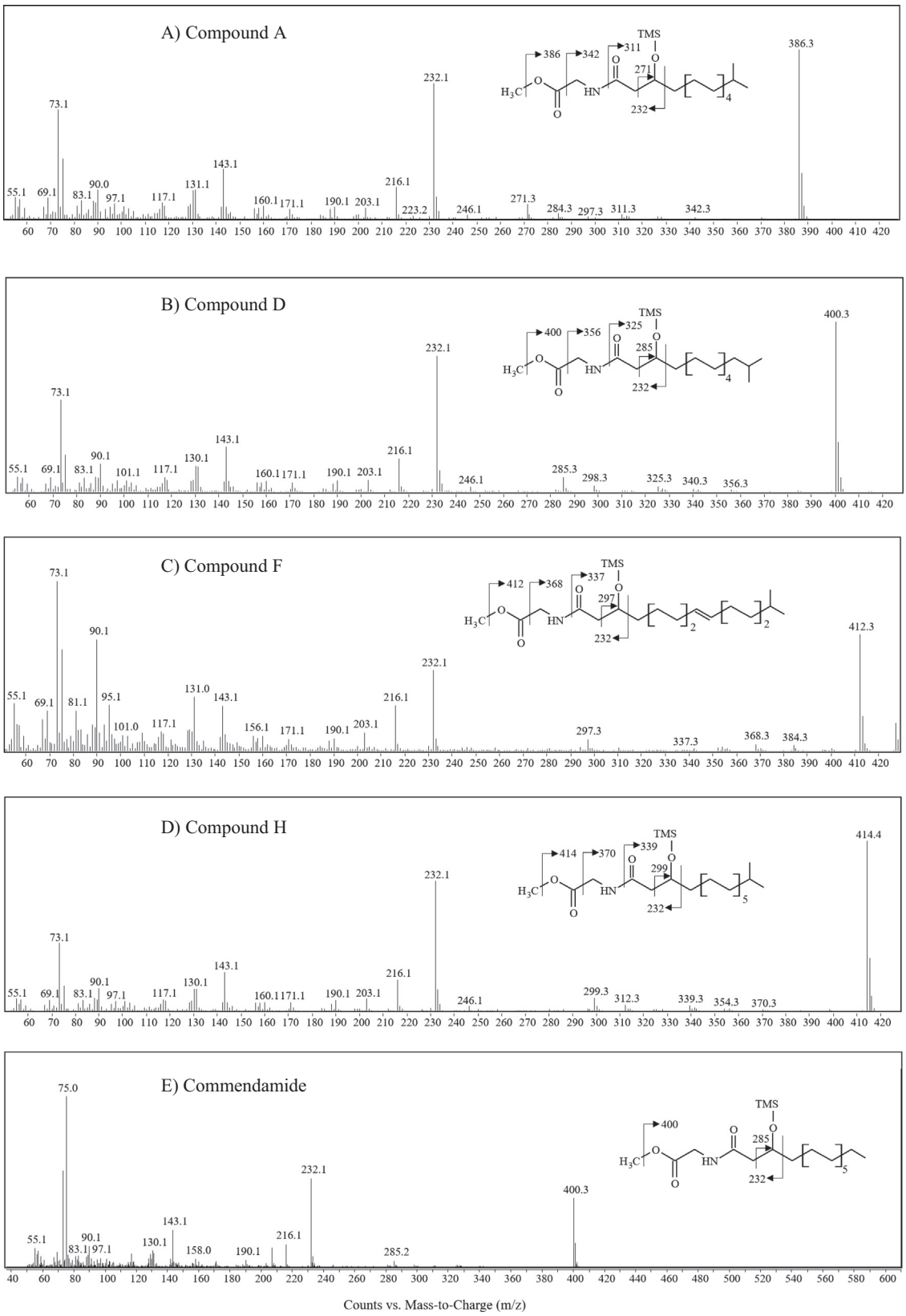


**Fig. 3.** GC–MS spectra of (A–D), GC–MS spectra of novel compounds A, D, F, H and E, a commercially available glycine β-hydroxy fatty acid amide standard, commendamide.

contains N and we postulated that it represents $NH_3CH_2COOCH_3$, which corresponds to a methylated glycine. Furthermore, the loss of 130 Da likely indicates the presence of a $CH_2CONHCH_2COOCH_3$ moiety. Based on this, we deduced that the compounds A–I contain a glycine polar moiety attached, via an amide linkage, to a β-hydroxy fatty acid (Fig. 1B), collectively designated glycine β-hydroxy fatty acid amides. This is supported by the diagnostic fragment ion at *m*/*z* 232, which likely corresponds to the glycine

β-hydroxy fatty acid amide moiety released by fragmentation next to the silylated β-hydroxy group (Fig. 3). The $[M]^+$ of these compounds indicates that the aliphatic parts of the molecules are either a β-OH $C_{15:0}$ (*m*/*z* 401), β-OH $C_{16:0}$ (*m*/*z* 415), β-OH $C_{17:1}$ (*m*/*z* 427) or β-OH $C_{17:0}$ (*m*/*z* 429) (Fig. 3). The double bond in β-OH $C_{17:1}$ was tentatively identified as ω6, as described in the method section. Supporting our interpretation is the similarity of the proposed structures for D, E and H with those reported for a series of compounds in the marine bacterium *Cytophaga* sp. SANK71996 (Morishita et al., 1997). In order to further confirm the identification of these compounds as glycine β-hydroxy fatty acid amides, we acquired a commercially available glycine β-hydroxy fatty acid amide standard, commendamide (Cohen et al., 2015), which was derivatized and analyzed by GC–MS (Fig. 3E). The strong similarity of its mass spectrum with those of the compounds A–I (cf. Fig. 3A–D) provides robust support for the assigned structures.

The intact polar lipids (IPLs) of the two *Lutibacter* sp. strains were analyzed by UHPLC-HRMS (Fig. 4). The major polar lipids were phosphatidylethanolamines (PEs), capnine lipids (CpL), flavolipins (FL), ornithine lipids (OL), and glycine lipids (GlyL), also known as cytolipins. Additionally, glutamine lipids (GluL), lyso-PEs (in which one of the FA chains is not present) and phosphatidylcholines (PCs) were present in trace levels (Table 2; see Fig. 1 for structures). Identification of (lyso) PEs, FLs, GlyLs, OLs and PCs were based on comparison of their diagnostic fragmentations in $MS^2$ with those described in the literature (Sturt et al., 2004; Moore et al., 2016). The CpLs and GluLs were identified based on their accurate masses and $MS^2$ characteristic fragment ions (Table 2). FLs, OLs, GlyLs and GluLs are all glycerol-free amino acid lipids consisting of an amino acid moiety, linked via an amide bond to a β-hydroxy fatty acid, esterified to another fatty acid (cf. Fig. 1).

## 4. Discussion

We surmise that the release of glycine β-hydroxy fatty acid amides by base hydrolysis of *Lutibacter* sp. is likely due to the hydrolysis of the GlyLs present in these species (see Fig. 1B for theoretical scheme). Indeed, several of the glycine β-hydroxy fatty acid amides reported here were reported previously to be generated from GlyLs extracted from a *Cytophaga* sp. (Morishita et al., 1997). Due to their substantially larger amino acid moieties, the remaining three amino lipids, FLs, OLs and GluLs, would not produce amide products detectable by gas chromatography.

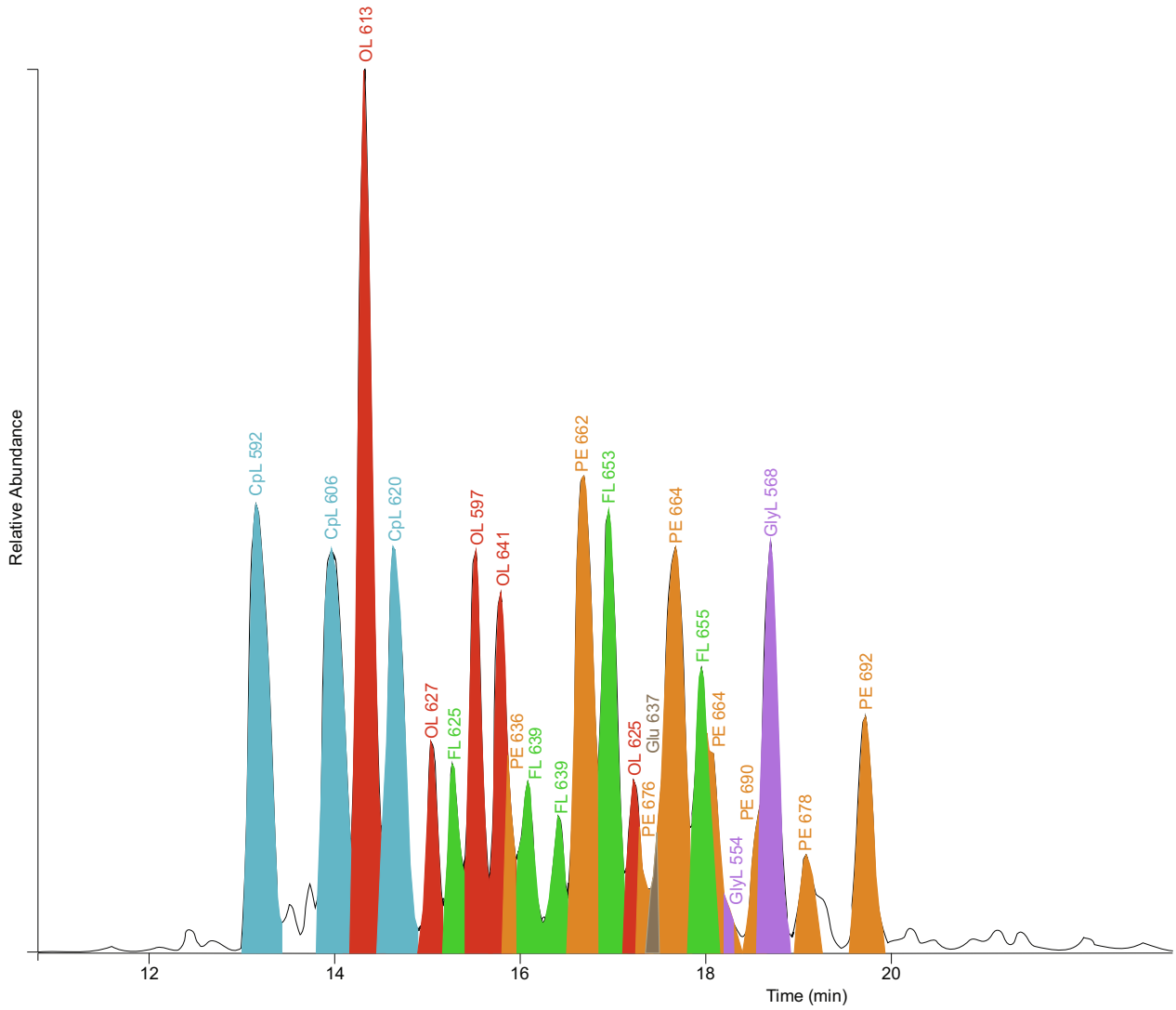


**Fig. 4.** Partial base peak chromatogram of the UHPLC–HRMS analysis of intact polar lipids in the *Lutibacter* sp. strain B1. See text for explanation of peak label abbreviations. Trace level compounds are not indicated.

**Table 2**
Masses and relative abundance of intact polar lipids (IPLs) in *Lutibacter* sp. strains B1 and B2 as determined by UHPLC–HRMS.

| | Adduct type | Accurate mass ($m/z$) | AEC | Δ (mmu) | CFI ($m/z$) | AEC | Δ (mmu) | R1[a] | R2[a] | Relative abundance (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | B1 | B2 |
| Ornithines (OLs)( | $[M+H]^+$ | 597.5205 | $C_{35}H_{69}N_2O_5^+$ | 0.43 | 115.0868 | $C_5H_{11}ON_2^+$ | 0.21 | $C_{14}H_{29}$ | $C_{12}H_{25}$ | 4.2 | 5.4 |
| | | 599.4990 | $C_{34}H_{67}N_2O_6^+$ | −0.40 | 115.0868 | $C_5H_{11}ON_2^+$ | 0.21 | $C_{13}H_{27}O$ | $C_{12}H_{25}$ | 0.6 | 0.9 |
| | | 611.5360 | $C_{36}H_{71}N_2O_5^+$ | 0.28 | 115.0868 | $C_5H_{11}ON_2^+$ | 0.21 | $C_{14}H_{29}$ | $C_{13}H_{27}$ | 0.8 | 0.8 |
| | | 613.5152 | $C_{35}H_{69}N_2O_6^+$ | 0.14 | 115.0869 | $C_5H_{11}ON_2^+$ | 0.31 | $C_{14}H_{29}O$ | $C_{12}H_{25}$ | 9.6 | 14.7 |
| | | 625.5517 | $C_{37}H_{73}N_2O_5^+$ | 0.26 | 115.0869 | $C_5H_{11}ON_2^+$ | 0.31 | $C_{14}H_{29}$ | $C_{14}H_{29}$ | 1.9 | 2.8 |
| | | 627.5309 | $C_{36}H_{71}N_2O_6^+$ | 0.28 | 115.0868 | $C_5H_{11}ON_2^+$ | 0.21 | $C_{14}H_{29}O$ | $C_{13}H_{27}$ | 2.7 | 2.8 |
| | | 641.5466 | $C_{37}H_{73}N_2O_6^+$ | 0.26 | 115.0869 | $C_5H_{11}ON_2^+$ | 0.31 | $C_{14}H_{29}O$ | $C_{14}H_{29}$ | 3.9 | 4.1 |
| | | | | | | | | | Total | 24 | 31 |
| Flavolipins (FLs) | $[M+H]^+$ | 625.4791 | $C_{35}H_{65}N_2O_7^+$ | 0.45 | 106.0502 | $C_3H_8O_3N^+$ | 0.36 | $C_{14}H_{27}$ | $C_{12}H_{25}$ | 1.9 | 2.3 |
| | | 627.4948 | $C_{35}H_{67}N_2O_7^+$ | 0.48 | 106.0502 | $C_3H_8O_3N^+$ | 0.36 | $C_{14}H_{29}$ | $C_{12}H_{25}$ | 0.7 | 0.7 |
| | | 639.4945 | $C_{36}H_{67}N_2O_7^+$ | 0.17 | 106.0503 | $C_3H_8O_3N^+$ | 0.38 | $C_{14}H_{27}$ | $C_{13}H_{27}$ | 2.8 | 1.7 |
| | | 653.5106 | $C_{37}H_{69}N_2O_7^+$ | 0.63 | 106.0502 | $C_3H_8O_3N^+$ | 0.35 | $C_{14}H_{27}$ | $C_{14}H_{29}$ | 5.8 | 4.9 |
| | | 655.5260 | $C_{37}H_{71}N_2O_7^+$ | 0.42 | 106.0503 | $C_3H_8O_3N^+$ | 0.38 | $C_{14}H_{29}$ | $C_{14}H_{29}$ | 3.4 | 3.1 |
| | | 671.5203 | $C_{37}H_{71}N_2O_8^+$ | −0.23 | 106.0502 | $C_3H_8O_3N^+$ | 0.34 | $C_{14}H_{29}O$ | $C_{14}H_{29}$ | 0.1 | 0.2 |
| | | | | | | | | | Total | 15 | 13 |
| Capnine CpLs) | $[M+H]^+$ | 592.4603 | $C_{32}H_{66}NO_6S^+$ | −0.21 | 124.0063 | $C_2H_6O_3NS^+$ | 0.01 | $C_{14}H_{29}O$ | $C_{14}H_{29}$ | 6.9 | 8.4 |
| | | 606.4760 | $C_{33}H_{68}NO_6S^+$ | −0.24 | 124.0064 | $C_2H_6O_3NS^+$ | 0.11 | $C_{15}H_{31}O$ | $C_{14}H_{29}$ | 6.7 | 7.5 |
| | | 620.4919 | $C_{34}H_{70}NO_6S^+$ | 0.10 | 124.0063 | $C_2H_6O_3NS^+$ | 0.01 | $C_{16}H_{33}O$ | $C_{14}H_{29}$ | 5.3 | 4.6 |
| | | | | | | | | | Total | 19 | 21 |
| Glycine lipids (GlyLs) | $[M+H]^+$ | 538.4470 | $C_{32}H_{60}NO_5^+$ | 0.36 | 76.0400 | $C_2H_6O_2N^+$ | 0.69 | $C_{14}H_{27}$ | $C_{12}H_{25}$ | 1.4 | 1.0 |
| | | 540.4623 | $C_{32}H_{62}NO_5^+$ | 0.03 | 76.0400 | $C_2H_6O_2N^+$ | 0.69 | $C_{14}H_{29}$ | $C_{12}H_{25}$ | 0.8 | 0.5 |
| | | 552.4622 | $C_{33}H_{62}NO_5^+$ | −0.03 | 76.0400 | $C_2H_6O_2N^+$ | 0.71 | $C_{14}H_{27}$ | $C_{13}H_{27}$ | 1.5 | 0.8 |
| | | 554.4781 | $C_{33}H_{64}NO_5^+$ | 0.19 | 76.0400 | $C_2H_6O_2N^+$ | 0.69 | $C_{14}H_{29}$ | $C_{13}H_{27}$ | 1.2 | 0.6 |
| | | 568.4941 | $C_{34}H_{64}NO_5^+$ | 0.19 | 76.0400 | $C_2H_6O_2N^+$ | 0.73 | $C_{14}H_{29}$ | $C_{14}H_{29}$ | 5.4 | 5.0 |
| | | 582.5096 | $C_{35}H_{68}NO_5^+$ | 0.38 | 76.0400 | $C_2H_6O_2N^+$ | 0.69 | $C_{15}H_{31}$ | $C_{14}H_{29}$ | 0.5 | 0.5 |
| | | 596.5251 | $C_{36}H_{70}NO_5^+$ | 0.24 | 76.0400 | $C_2H_6O_2N^+$ | 0.66 | $C_{16}H_{33}$ | $C_{14}H_{29}$ | 0.1 | 0.4 |
| | | | | | | | | | Total | 11 | 9 |
| Glutamine (GluL) | $[M+H]^+$ | 623.4996 | $C_{36}H_{67}N_2O_6^+$ | 0.20 | 147.0764 | $C_5H_{11}O_3N_2^+$ | −0.02 | $C_{14}H_{27}$ | $C_{13}H_{27}$ | 0.5 | 0.3 |
| | | 637.5154 | $C_{37}H_{69}N_2O_6^+$ | 0.37 | 147.0764 | $C_5H_{11}O_3N_2^+$ | −0.02 | $C_{14}H_{27}$ | $C_{14}H_{29}$ | 1.8 | 1.5 |
| | | 639.5308 | $C_{37}H_{71}N_2O_6^+$ | 0.12 | 147.0764 | $C_5H_{11}O_3N_2^+$ | −0.02 | $C_{14}H_{29}$ | $C_{14}H_{29}$ | 0.6 | 0.7 |
| | | | | | | | | | Total | 3 | 2 |
| Lyso PE | $[M+H]^+$ | 440.2770 | $C_{20}H_{43}NO_7P^+$ | −0.19 | | | | $C_{14}H_{29}$ | – | 0.6 | 0.5 |
| PE | $[M+H]^+$ | 634.4428 | $C_{33}H_{65}NO_8P^+$ | −1.42 | | | | $C_{14}H_{27}$ | $C_{12}H_{25}$ | 0.3 | 0.4 |
| | | 636.4607 | $C_{33}H_{67}NO_8P^+$ | 0.81 | | | | $C_{14}H_{29}$ | $C_{12}H_{25}$ | 1.5 | 0.4 |
| | | 648.4599 | $C_{34}H_{67}NO_8P^+$ | 0.02 | | | | $C_{14}H_{29}$ | $C_{13}H_{25}$ | 0.1 | 0.6 |
| | | 650.4761 | $C_{34}H_{69}NO_8P^+$ | 0.61 | | | | $C_{14}H_{29}$ | $C_{13}H_{27}$ | 0.4 | 0.4 |
| | | 662.4756 | $C_{35}H_{69}NO_8P^+$ | 0.06 | | | | $C_{14}H_{27}$ | $C_{14}H_{29}$ | 8.0 | 6.6 |
| | | 664.4914 | $C_{35}H_{71}NO_8P^+$ | 0.21 | | | | $C_{14}H_{29}$ | $C_{14}H_{29}$ | 8.4 | 4.9 |
| | | 676.4912 | $C_{36}H_{71}NO_8P^+$ | −0.03 | | | | $C_{14}H_{29}$ | $C_{15}H_{29}$ | 1.6 | 1.0 |
| | | 678.5073 | $C_{36}H_{73}NO_8P^+$ | 0.43 | | | | $C_{14}H_{29}$ | $C_{15}H_{31}$ | 2.0 | 1.9 |
| | | 690.5070 | $C_{37}H_{73}NO_8P^+$ | 0.13 | | | | $C_{14}H_{27}$ | $C_{16}H_{33}$ | 2.9 | 1.6 |
| | | 692.5228 | $C_{37}H_{75}NO_8P^+$ | 0.32 | | | | $C_{14}H_{29}$ | $C_{16}H_{33}$ | 2.8 | 5.0 |
| | | | | | | | | | Total | 28 | 23 |
| PC | $[M]^+$ | 718.5380 | $C_{39}H_{77}NO_8P^+$ | −0.11 | 184.0734 | $C_5H_{15}O_4NP^+$++ | 0.08 | $C_{29}H_{58}$ (R1 + R2) | | 0.1 | 0.1 |
| | | 730.5378 | $C_{40}H_{77}NO_8P^+$ | −0.29 | 184.0734 | $C_5H_{15}O_4NP^+$++ | 0.08 | $C_{30}H_{58}$ (R1 + R2) | | 0.1 | 0.1 |
| | | | | | | | | | Total | 0.2 | 0.2 |

[a] R1 and R2 specifies the alkyl moieties of the various IPLs shown in Fig. 1. AEC = Assigned elemental composition. CFI = characteristic fragment ion. mmu = milli mass unit, Δ mmu = (measured mass – calculated mass) × 1000. PE and lyso PE were identified not by a characteristic fragment ion but by a characteristic loss of 141.0185 Da from the $[M+H]^+$ ion.

Glycerol-free amino acid lipids are an under-reported lipid type, despite the genetic evidence that certain amino acid lipids are produced by a wide range of bacteria (Geiger et al., 2010; Vences-Guzmán et al., 2012; Smith et al., 2019). For example, around 25% of sequenced bacterial genomes are predicted to be involved in the synthesis of OLs (Vences-Guzmán et al., 2012). GlyLs have been described in several gram-negative bacteria, including *Cytophaga johnsonae* (Kawazoe et al., 1991), a marine *Cytophaga* sp. (Morishita et al., 1997), the marine *Cyclobacterium marinus* WH (Batrakov et al., 1999), the soil bacterium *Pedobacter heparinus* (Moore et al., 2016), *Aequorivita* sp. isolated from shallow Antarctic sea sediment (Chinanese et al., 2018), and in the anaerobic *Bacteroides thetaiotaomicron* (Lynch et al., 2019).

The high proportion of amino acid lipids (ca. 55–60% of the total peak area) in the two strains of *Lutibacter* sp. isolated from 2000 m depth in the Black Sea raises the possibility that these lipids are a membrane adaption to the extreme pressure and specific redox chemistry of their environment (Geiger et al., 2010). Indeed, studies of OLs and lysine lipids have found that modifications in both their fatty acid composition and amino acid head groups can be related to stress conditions including phosphorus limitation, temperature or pH (Moore et al., 2013, 2015).

Future studies examining the proportions of glycerol free amino lipids such as GlyLs in different species will improve our understanding of which microorganisms produce which specific lipids and under which environmental conditions. Such knowledge is essential in order to better constrain the use of lipid biomarkers both in the present-day environment and as microbial markers in geological studies. The identification of GlyLs and other amino acid lipids typically relies on thin-layer chromatography (TLC) or HPLC–MS methods, which are not available in all microbiology laboratories. In contrast, FAME analysis is a more common technique employed for strain description and in the analysis of environmental samples. Our results show that GlyLs yield GC-amenable compounds which can be readily identified by retention time and confirmed by mass spectrometry. This may result in an increased detection and appreciation of the occurrence of GlyLs in microbial strains and in the environment.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2020.104027.

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