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Intact polar lipids as organic biomarkers of viable extraterrestrial life

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The detection of viable life is a key objective for both in situ astrobiological experiments on extraterrestrial bodies, and for planetary protection protocols on Earth following sample return. We evaluated the potential for common bacterial and archaeal intact polar lipids (IPLs) to be used as viable life markers owing to their particularly fast rate (hours to days) of post-mortem degradation. We determined the chemical signatures of IPLs using pyrolysis-gas chromatography-mass spectrometry experiments (with and without derivatisation), to simulate the results of in situ pyrolytic organic analyses. Monoglycerides, formed as pyrolysis products of typical bacterial IPLs, were the strongest signature for the parent compounds, and were identified in both the pyrolysis of a phospholipid mixture and freeze-dried bacterial culture. Our results demonstrate that relatively rapid and elegant pyrolysis techniques can reveal the presence of IPLs, and therefore evidence of viable life in extraterrestrial samples.

Biological lipids serve a variety of cellular functions including their use as structural components of cell membranes. To enable self-assembly, amphiphilic membrane lipids consist of a hydrophilic polar head group and hydrophobic aliphatic side chains, all linked to a connecting backbone – most commonly glycerol. As a result of evolutionary divergence and adaptation, the composition of these membrane lipids varies across biological taxa[1], enabling their use as biomarkers for particular organisms[2]. On the domain scale, the cellular membrane lipids of bacteria and eukarya are generally composed of fatty-acid derived tail groups ester-linked to the glycerol backbone, whereas archaeal lipids exhibit ether-linked branched tail groups derived from isoprenoids[3].

Despite being susceptible to some degradational processes, lipids or their diagenetic derivatives are relatively well-preserved on geological timescales[4]. The endurance of lipid-derived molecules during diagenetic processes has made them a particular focus of astrobiological studies seeking biomarkers diagnostic of past life[4–7]. Intact polar lipids (IPLs) are the pristine, undegraded membrane lipids found in living cells that provide the most detailed taxonomic information[2,8]. Many IPLs are rapidly enzymatically degraded upon death of an organism[9–11]. Incubations of natural sediments exhibited 69% degradation of an ester-bound bacterial IPL after 4 days, compared with 4% of an archaeal ether-bound IPL[12]. Consideration of the degradation products indicates that post-mortem degradation of ester-bound IPLs initiates with the hydrolysis of the ester bond to the side chains[12,13]. For astrobiological studies, we propose the term viable life marker or "VLM" for the diagnostic molecular signatures of living organisms. The rapid post-mortem degradation of IPLs has already led to their consideration as molecular evidence for active life[10,14], constituting a potential VLM.

On Earth, the structure and abundance of IPLs is most commonly investigated by solvent extraction of relevant samples followed by liquid-based chromatography and mass spectrometry[14–16]. However, because of the difficulties associated with transporting solvents and concerns over forward contamination, the solvent extraction and liquid-based chromatography techniques conventionally used for IPL characterisation on Earth are less practical for in situ extra-terrestrial astrobiological experiments[17]. Moreover, the direct analysis of IPLs using gas chromatography-mass spectrometry (GC-MS) is problematic as many IPLs, including phospholipids, are not stable in the gas phase[10], requiring offline derivatisation or hydrolysis in order to be amenable to GC[18].

Instead, in extraterrestrial environments, in situ organic analysis is typically performed through analytical pyrolysis, eliminating the requirement for solvents[17,19]. Pyrolysis is performed by raising the sample material to temperatures above 500 °C followed by transfer of the evolved compounds into a system for characterisation, typically GC-MS[20–22]. These elevated temperatures cause thermochemical decomposition of the organic material in the sample, potentially obscuring diagnostic compounds. However, through experiments using standard organic compounds, combined with an understanding of pyrolysis mechanisms, the characteristic products of biomarkers in analytical pyrolysis can be established, providing a signature for their presence in the pyrolysis of natural samples[23,24]. In situ extraterrestrial pyrolysis methods have previously been simulated in lab experiments using on-line pyrolysis followed by GC-MS (pyrolysis-GC-MS)[25].

The potential for pyrolysis-GC-MS to distinguish biotic from abiotic organic assemblages for application in extraterrestrial settings has been well established[26], but the characteristic products of IPLs in pyrolysis have not been specifically identified and targeted in such experiments. In this study,

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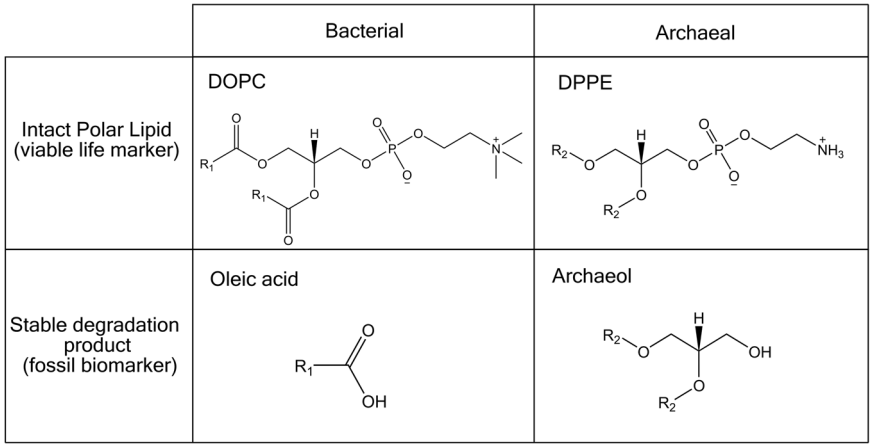

Fig. 1 | Standards used in this study to represent typical bacterial and archaeal intact polar lipids and their post-mortem degradation products. DOPC = 1,2-Dioleoyl-sn-glycero-3-phosphocholine, DPPE = 1,2-di-O-phytanyl-sn-glycero-3-phosphoethanolamine, R_1 = 9-octadecene, R_2 = phytane

we aimed to identify the thermochemical decomposition products of IPLs using pyrolysis-GC-MS to simulate the in situ pyrolysis process, with the motivation that these signature products could constitute a chemical marker to distinguish viable life in extraterrestrial samples. This could have potential applications for *in-situ* life detection experiments and analysis of returned samples.

Techniques for distinguishing viable life, such as detecting VLMs, are of importance for planetary protection protocols aiming to mitigate the risk of the introduction of harmful organisms to Earth from returned terrestrial samples[27]. The issue is especially pressing due the plans for Mars sample return in the near future[28]. Analyses including cultivation, DNA sequencing, microscopic imaging and liquid-based chromatography have been proposed but have various individual limitations, especially regarding sample-size requirements and contamination risk[29].

We selected lipid standards to exemplify typical microbial IPLs and the products of their post-mortem degradation. We identified products of pyrolysis that were unique to the IPLs, enabling their potential use as a signature of viable life. Then, by pyrolysing natural samples, we were able to determine whether these signatures could be discerned in complex organic mixtures. Further details of the methodology are outlined in the Methods section."

Results

Selected lipid standards

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-di-O-phytanyl-sn-glycero-3-phosphoethanolamine (DPPE) were selected as standards to represent a typical bacterial and archaeal IPL respectively. 9-Octadecenoic (oleic) acid standards and 1,2-di-O-phytanyl-sn-glycerol (archaeol) were selected to represent the geologically stable products of their post-mortem degradation[5,12,30] (Fig. 1).

Pyrolysis of bacterial lipid standards

Table 1 displays the products resulting from pyrolysis-GC-MS or pyrolysis-derivatisation-GC-MS of DOPC and oleic acid standards. Compounds eluting after oleic acid were observed exclusively in the pyrolysis of DOPC (Supplementary Fig. 1). In particular, 9-octadecenoic acid, 2,3-dihydroxypropyl ester (glyceryl monooleate), 2 trimethylsilyl (TMS) derivative and 9-octadecenoic acid, 3-hydroxypropenyl ester (dehydrated glyceryl monooleate) formed during the pyrolysis of DOPC, with and without the derivatisation step respectively.

In the experiment without derivatisation, the dehydrated form of glyceryl monooleate was identified by the low intensity peak at m/z = 338 in the mass spectra (Supplementary Fig. 2A). This peak was attributed to the molecular ion of the compound, being 18 Da less than the complete monoglyceride, reflecting a loss of H_2O during pyrolysis. This process of dehydration during analytical pyrolysis has been previously established for alcohols generally[31]. The elimination reaction will have produced multiple olefinic positional isomers which are unlikely to be chromatographically separated[32]. In the experiment with derivatisation, glyceryl monooleate, 2TMS derivative could be confidently identified due to the high intensity peak for the characteristic ion at m/z = 397[33] (Supplementary Fig. 2B). In experiments where the 1-monoglyceride (α isomer) was observed, the 2-monoglyceride (β isomer) was absent or observed at very low abundance. Therefore, all monoglycerides described in these results are in the structural arrangement of the 1-monoglyceride regioisomer.

Table 1 | Products arising from the pyrolysis of bacterial IPL standards DOPC and oleic acid.

Pyrolysis product	Pyrolysis-GC-MS		Pyrolysis-derivatisation-GC-MS	
	DOPC (IPL)	Oleic acid	DOPC	Oleic acid
Cyclooctene	✓	✓		
8-Heptadecene	✓	✓		
Oleic acid, methyl ester	✓	✓	✓	
Oleic acid	✓	✓	✓(1TMS)	✓(1TMS)
Dehydrated glyceryl monooleate	✓✓			
Glyceryl monooleate			✓✓(2TMS)	
N,N-dimethyloleamide	✓✓			
Phosphoric acid			✓✓(3TMS)	

Tick marks indicate identification of the compound in the total ion chromatogram (TIC). Pyrolysis products with double ticks were derived from DOPC but not oleic acid, giving them the potential to be diagnostic of the presence of IPLs. *TMS* Trimethylsilyl derivative, the adjacent number refers to the number of TMS groups present.

Pyrolysis of archaeal lipid standards

Table 2 outlines the products arising from the pyrolysis-GC-MS and pyrolysis-derivatisation-GC-MS of archaeal lipid standards archaeol and DPPE. Without a derivatisation step, no products were exclusive to DPPE. With the derivatisation step, the formation of glycerol, 3TMS derivative and phosphoric acid, 3TMS derivative was exclusive to the pyrolysis of DPPE (Table 2, Supplementary Fig. 3).

Pyrolysis of natural samples

To explore the response of IPLs in a natural sample we selected soy lecithin, a natural mixture of phospholipids extracted from soybeans[34]. Pyrolysis of the lecithin sample resulted in the formation of fatty acids and their corresponding (dehydrated) monoglycerides (Table 3). In the experiment without derivatisation, the response for dehydrated monoglycerides was relatively low (Supplementary Fig. 4). In contrast, in the experiment with

Table 2 | Products arising from the pyrolysis of archaeal IPL standards DPPE and archaeol.

Pyrolysis product	Pyrolysis-GC-MS		Pyrolysis-derivatisation-GC-MS	
	DPPE (IPL)	Archaeol	DPPE	Archaeol
Phytene-2	✓	✓	✓	✓
Phytol	✓	✓	✓	✓
Phytanol	✓	✓	✓(1TMS)	✓(1TMS)
Glycerol			✓✓(3TMS)	
Phosphoric acid			✓✓(3TMS)	

Tick marks indicate observation of the compound in the TIC. Pyrolysis products with double ticks were derived from DPPE but not archaeol, giving them the potential to be diagnostic of the presence of IPLs. *TMS* Trimethylsilyl derivative.

Table 3 | Products arising from the pyrolysis of the lecithin sample.

Pyrolysis product	Pyrolysis-GC-MS	Pyrolysis-derivatisation-GC-MS
Palmitic acid	✓	✓(1TMS)
Linoleic acid	✓	✓(1TMS)
Oleic acid	✓	✓(1TMS)
Stearic acid	✓	✓(1TMS)
Dehydrated glyceryl monopalmitate	✓	
Dehydrated glyceryl monolinoleate	✓	
Dehydrated glyceryl monooleate	✓	
Glyceryl monopalmitate		✓(2TMS)
Glyceryl monolinoleate		✓(2TMS)
Glyceryl monooleate		✓(2TMS)
Glyceryl monostearate		✓(2TMS)
Phosphoric acid		✓(3TMS)

Tick marks indicate observation of the compound in the TIC. *TMS* Trimethylsilyl derivative.

derivatisation, all of the derivatised monoglycerides expected (based on the identification of their respective free fatty acids) were detected with significant response (Supplementary Fig. 4). As with the pyrolysis of DOPC, (dehydrated) monoglycerides were significantly easier to discern in the experiment with derivatisation due to the greater intensity of characteristic ions in their mass spectra.

Pyrolysis of the freeze-dried bacteria *Shewanella frigidimarina* resulted in the formation of a wide range of products, similar to those identified in previous studies[35]. Due to the variety of complex compounds formed, often coeluting from the column, extracted ion chromatograms (EICs) were produced to investigate the presence of the signatures of bacterial IPLs, using target ions established in the soy lecithin experiments. Hexadecanoic (palmitic) acid was identified as the most abundant fatty acid. Hence, EICs were produced at target ions for the corresponding (dehydrated) monoglyceride: glyceryl monopalmitate (m/z = 239 and m/z = 371 for the underivatised and derivatised compound, respectively).

In the pyrolysis-GC-MS of the bacterial sample without derivatisation, the EIC at m/z = 239 exhibited a peak at a retention time closely matching (within 0.03 min) the elution of dehydrated glyceryl monopalmitate in the lecithin experiment (Fig. 2A, B, Supplementary Table 3). Though the mass spectrum associated with this peak would be inconclusive in isolation, the matching retention time increases confidence in the identification of the compound as dehydrated glyceryl monopalmitate. Owing to the low signal to noise ratio in this EIC, selected ion monitoring (SIM) was employed at three characteristic ions for the compound resulting in a greater signal clarity for the compound at the expected retention time (Fig. 2A, B).

In the results from pyrolysis-derivatisation-GC-MS of the bacterial sample, the EIC at m/z = 371 exhibited a peak at a retention time closely matching that of glyceryl monopalmitate, 2TMS derivative in the lecithin experiment (Fig. 2C, D, Supplementary Table 3). In contrast to the experiment without derivatisation, the mass spectrum associated with this peak by itself would likely be sufficient to be confidently attributed to the compound due to the detection of many other characteristic fragment ions. A retention time that matched the compound observed in previous experiments further increased confidence in its identification as the monoglyceride.

Discussion

By identifying products of DOPC pyrolysis that were not observed in the pyrolysis of oleic acid (i.e. its post-mortem degradation product), the distinguishing signatures of bacterial IPLs in pyrolysis can be constrained (Table 1). The observation of (dehydrated) glyceryl monooleate is the most pertinent of these species as its structure retains an intact ester bond from the parent IPL molecule. IPL ester bonds have been shown to be rapidly hydrolysed upon death of an organism[9,12], thus their presence in the monoglyceride is indicative of viable life in the pyrolysed sample. While the parent phospholipid would not be stable in the gas phase, the monoglyceride is a relatively volatile product of pyrolysis that is amenable to GC and retains sufficient structural information to act as a VLM. Using the detection of monoglycerides as a VLM can be generalised for any ester-linked IPL with a glycerol backbone, although the composition of the monoglyceride would vary with the composition of the tail groups of the parent IPL. A conceptual summary of these interpretation steps is displayed in Fig. 3.

Both the dehydrated and derivatised forms of the monoglycerides observed in the pyrolysis experiments exhibit the intact ester bond inherited from the parent IPL, validating their use as a VLM. Therefore, for convenience, we will henceforth use the term 'monoglyceride' to refer to both the dehydrated and derivatised forms of these compounds. Derivatisation likely improves sensitivity and certainty for the detection of the monoglycerides arising from the pyrolysis of bacterial IPLs. Because, compared to its underivatised counterpart, the mass spectrum of the derivatised monoglyceride is more clearly distinguished (Supplementary Fig. 2). The formation of more distinct mass spectra during pyrolysis-GC-MS has already been established as a general advantage of incorporating a silylation derivatisation step into pyrolytic organic analysis[21,36,37], and the technique will be employed by future in situ pyrolysis experiments on Mars[38].

Other pyrolysis products exclusive to the IPL were also observed including N,N-dimethyloleamide and phosphoric acid, 3TMS derivative. However, these products can be formed by secondary reactions[39] or do not exhibit sufficient intact bonding to serve as a conclusive signature for the presence of IPLs, precluding their use as a VLM. Table 1 also includes the products resulting from the pyrolysis-GC-MS of both DOPC and oleic acid (e.g. cyclooctene). As these compounds arise from the pyrolysis of both the IPL and the product of its enzymatic hydrolysis, they lack the potential to be used to discern the presence of an IPL.

Pyrolysis of naturally derived lecithin demonstrated that monoglycerides can be detected as pyrolysis products from a sample containing a mixture of different IPLs with varying tail groups. In the chromatograms of all experiments, a greater response for a particular fatty acid was associated with a greater response for the corresponding monoglyceride (Supplementary Fig. 4), providing guidance for detecting monoglycerides in natural samples: by identifying the most abundant fatty acid in preliminary pyrolysis experiments, the corresponding monoglyceride will be expected to be at greatest abundance. Hence, its structure and properties can be deduced and then targeted with increased specificity.

Results from the pyrolysis of a bacterial culture indicated the potential for monoglycerides to be detected during the pyrolysis of cellular samples.

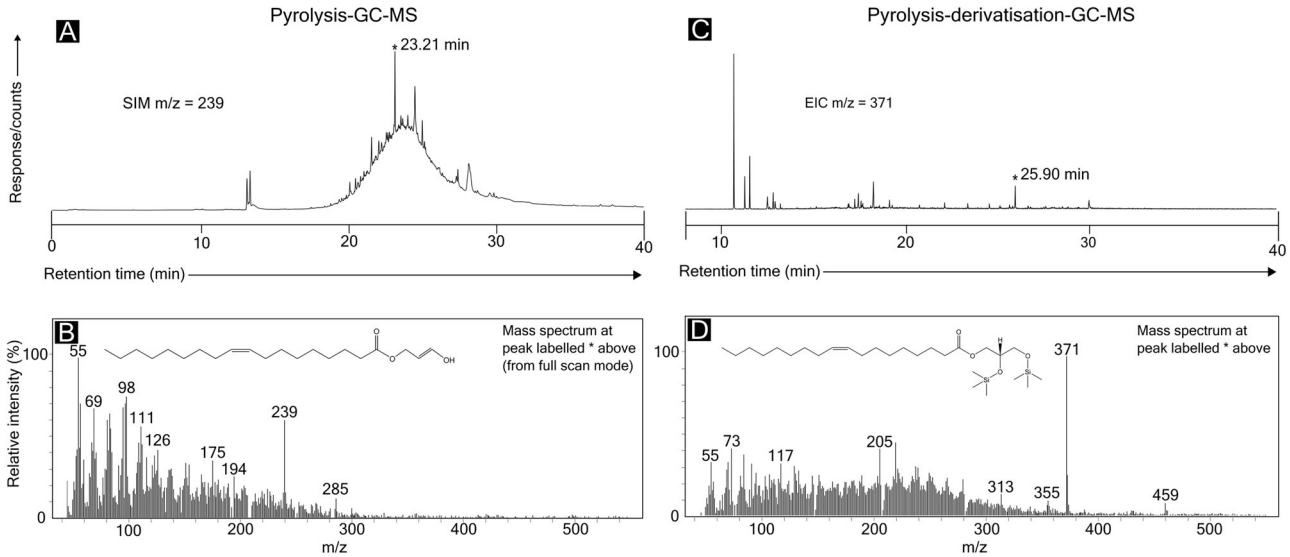

Fig. 2 | Chromatograms and mass spectra acquired during the pyrolysis of a Shewanella frigidimarina bacterial culture indicating the presence of monoglycerides. A Chromatogram acquired from the pyrolysis-GC-MS of the bacteria in selected ion (SIM) mode. m/z = 239 was selected due to it being the most intense peak in the spectra of the dehydrated monoglyceride. The chromatogram displays a peak with a retention time matching the elution of the dehydrated monoglyceride in the soy lecithin experiment. **B** Spectrum acquired in an identical experiment using full scan mode, at the retention time of the peak labelled with * in **A**. **C** Extracted ion chromatogram (EIC) acquired from the pyrolysis-derivatisation-GC-MS of the bacteria, showing a peak with a retention time matching the elution of the derivatised monoglyceride in previous experiments. **D** Spectrum acquired at the peak labelled with * in **C**.

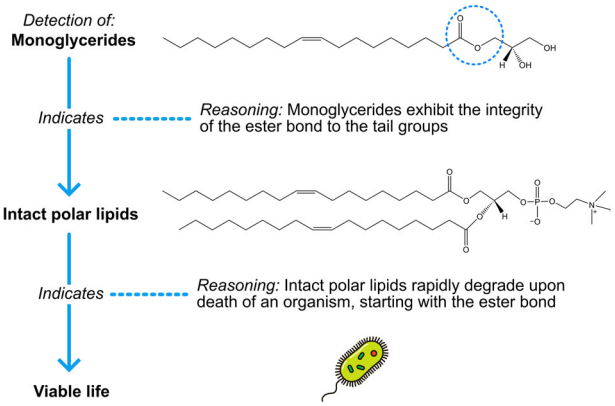

Fig. 3 | The generalised reasoning for the detection of a monoglyceride in the pyrolysis of a sample as evidence for the presence of viable life.

Currently, astrobiological space missions equipped with pyrolysis-GC-MS instrumentation are believed to lack the capability to discriminate between the signatures of viable and deceased life[21]. The results of these pyrolysis experiments demonstrate the potential for typical bacterial IPLs to be detected by these in situ analyses and, thereby, recognise viable extraterrestrial life. Although no single product of thermochemical decomposition could be used to conclusively support the presence of an entire IPL structure, the ester bond preserved in a monoglyceride has been specifically demonstrated to be labile upon death of the host organism. Further to this, bacterial life is not viable without this bond being intact to enable the formation of a cohesive cell membrane. Therefore, the observation of monoglycerides as a pyrolysis product is sufficient to function as a VLM, especially if observed alongside the other signature pyrolysis products of bacterial IPLs outlined here (free fatty acids, phosphoric acid, etc.).

Archaeal type ether-linked IPLs have less potential to be distinguished from their post-mortem degradation products. The most significant difference between the pyrolysis products of a typical archaeal IPL and the post-mortem degradation product (archaeol) was the identification of phosphoric acid and glycerol TMS derivatives following pyrolysis-derivatisation of the IPL. The structure of these pyrolysis products can only support the possibility of the presence of an IPL and their observation is insufficient to independently establish the presence of living biomass. However, the characteristic pyrolysis products from archaeal type ether-linked IPLs could still act as independent biosignatures, although not necessarily of viable life, as abiotic synthesis of systematically branched chain compounds such as phytol has not been observed[13], and their detection should prompt follow-up investigations by other techniques.

One additional use case of the technique is assessing returned extraterrestrial samples for the presence of active or viable life, which is a priority for planetary protection protocols[40,41]. For instance, the committee on space research (COSPAR) has developed a sample safety assessment framework (SSAF) to analytically exclude the possibility of the presence of life in samples returned from Mars prior to release and distribution[29]. The SSAF incorporates a test sequence of increasingly involved analyses with the aim of establishing sufficient confidence in the absence of life in a returned sample, while minimising damage and the risk of contamination.

Once a Martian sample is returned to Earth, many of the limitations associated with in situ organic analyses will no longer apply[42,43]. The detection of IPLs could be performed using wet chemistry techniques such as solvent extraction and liquid chromatography. However, the use of the pyrolytic signatures of IPLs as a VLM may constitute a valuable screening step in any sample safety test sequence. Pyrolysis-GC-MS holds significant advantages over liquid-based techniques, even when considering analyses of returned samples. Pyrolysis is a relatively simple procedure, which requires small samples, minimal sample preparation and releases organic material from the sample without the need for multi-stage wet chemical procedures[25]. Screening by pyrolysis-GC-MS can, therefore, provide valuable data with minimal exposure of the sample to the terrestrial environment, thereby reducing the likelihood of hazardous Martian organisms being released from the sample into the terrestrial biosphere.

The detection of dormant microorganisms, such as bacterial endospores, is crucial for planetary protection protocols since these viable forms of life are typically resilient to harsh extraterrestrial environmental conditions and could survive sterilisation procedures[44]. As phospholipids are considered essential for the formation of a functional spore inner membrane[45], we propose that IPL signatures are likely to be detectable in the pyrolysis of viable endospore material. Further experimental investigation

should verify this hypothesis. Additionally, future experimentation of the technique on a range of microbial species, especially those considered to be viable in space conditions, will be important to confirm the general application of IPL signatures as a marker of viable life for planetary protection protocols.

We have demonstrated that pyrolysis-GC-MS can serve as an elegant and rapid method for producing evidence to support the presence or absence of viable life in a returned Martian sample. Pyrolysis-GC-MS could be incorporated into future sample safety sequences, to provide data that informs decisions on further sample processing.

The novel method to distinguish viable and deceased biology in pyrolysis outlined in this study comes with significant considerations and limitations. Firstly, this method relies upon the assumption that IPLs can be used as a VLM due to their rapid degradation upon death of an organism. However, this rate of degradation is dependent on the specific chemical structure of the IPL as well as the environmental conditions it endures following death of the host organism[8,9,12]. Therefore, the lability of the parent IPL in its particular geochemical setting must be considered if its characteristic products of pyrolysis are to be used as a VLM. Similarly, the rapid post-mortem degradation of IPLs is biologically mediated[12], raising the possibility of increased preservation potential if enzymatic activity is inhibited. Though these factors may compound the post-mortem stability of IPLs, this is unlikely to entirely preclude their use as VLMs because even the slowest recorded IPL degradation rates are significant when compared with the geological preservation times typically observed for fossil life.

Secondly, pyrolysis of extraterrestrial samples will differ from the experiments in this study due to the presence of inorganic materials. For example, the Martian surface exhibits a variety of minerals, such as iron oxides and perchlorates, that have previously been shown to obfuscate the signatures of diagnostic organic compounds in pyrolysis experiments[24,46]. Further experiments, closely simulating specific extraterrestrial environments, may be important for future applications of the technique. Additionally, the influence of abiotic organic matter, such as that sourced from meteoritic infall[47] or hydrothermal processes[48], on this IPL detection technique warrants further investigation. Fortunately, our recognition of characteristic fragments and their mass spectrometry responses enables the use of selected ion monitoring to tackle sensitivity limitations.

Finally, the structural diversity of IPLs poses a further challenge for their detection using in situ pyrolysis. Variations in the structures of IPLs will change the products formed during pyrolysis of the compound. We have established the pyrolytic signatures of typical bacterial and archaeal IPLs. Yet this is by no means exhaustive of all IPL structures. Variations in the head group, tail group or backbone of an IPL would result in different pyrolytic signatures. Future investigation on a wider variety of IPLs would allow for these signatures to be determined. However, the possibility of any extraterrestrial organisms varying beyond the molecular biology known on Earth makes accounting for all possible IPL signatures impossible. While the compartmentalisation of a cellular membrane is considered an essential component for life[49], extraterrestrial organisms could well have evolved to use different membrane chemistries. Nevertheless, the suggestion that phospholipids may have evolved independently in archaeal and bacterial lineages[50], promotes the possibility of convergent Darwinian evolution tending towards such biochemistry given suitable conditions. Hence, we argue that using known terrestrial biomarkers of viable life as an "educated guess" represents a present-day and reasonable approach to extraterrestrial life detection until more data become available.

As a result of these limitations, the lack of the detection of the VLMs outlined in this study should not be used as definitive evidence against the presence of any viable life in a sample, only as an investigative step to lower the likelihood of "life as we know it" being present. On the other hand, we argue that a clear detection of monoglyceride IPL signatures in pyrolysis experiments constitutes strong evidence towards the presence of viable life.

In summary, we have demonstrated the capability of pyrolysis-based organic analyses to detect the signatures of common IPLs. Due to the rapid post-mortem degradation of IPLs, we now have a tool for recognising the chemical signatures of viable life using readily available, highly sensitive analytical techniques. The detection of IPLs may have important applications for in situ life detection experiments that regularly employ pyrolysis as a method for organic analysis. IPL recognition also has utility for planetary protection and sample return missions. Although wet chemistry procedures can be used to identify the presence of IPLs on Earth, we have demonstrated how pyrolysis can reveal similarly useful information. As a result, this technique could also offer a simple and rapid screening method to aid the examination of returned Martian samples for the presence of potentially hazardous viable life.

Methods

Sample preparation

Lipid standards of 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-di-O-phytanyl-sn-glycero-3-phosphoethanolamine (DPPE) and 1,2-di-O-phytanyl-sn-glycerol (archaeol) were obtained from Avanti Polar Lipids. 9-Octadecenoic (oleic) acid was obtained from Merck Sigma-Aldrich. To prepare standard solutions, 2 mg of each lipid standard was dissolved in 2 ml dichloromethane (1 mg/ml) and stored at −20 °C. To prepare the soy lecithin samples, the capsule shell of a commercial supplement (Holland and Barrett, UK) was pierced and then 2 mg of the interior soy lecithin material was dissolved in 2 ml dichloromethane (1 mg/ml). A viable, freeze-dried bacterial culture of the strain *Shewanella frigidimarina* (a gamma-proteobacteria of the pseudomonadota phylum) was purchased from DSMZ GmbH.

Pyrolysis-GC-MS

Approximately 20 µl of each standard solution, or 0.5 mg of freeze-dried bacterial culture, was deposited individually onto a quartz wool plug in a quartz pyrolysis tube and then left at room temperature for any solvent to evaporate. In the pyroprobe, the sample was heated to 650 °C at a rate of 20 °C ms^{-1} and then held at this temperature for 15 s in a flow of helium. Following pyrolysis, the interface was raised from 150 °C (100 °C for oleic acid) to 280 °C at a rate of 60 °C min^{-1}. The GC injector operated with a 10:1 split at 270 °C and with a helium constant column flow rate of 1.1 ml min^{-1}. Separation was performed on a DB-5MS ultra inert column (J&W; 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness). After being held for 2 min at 40 °C, the GC oven temperature was ramped to 310 °C at a rate of 10 °C min^{-1} and then held at this temperature for 9 min. Mass spectra were acquired in electron impact (EI) mode (70 eV) over a scan range from 50 to 550 mass/charge (m/z). To increase sensitivity during the experiments pyrolysing freeze-dried bacteria, selected ion monitoring (SIM) was employed. The monitored ions were m/z = 98.1, 116.1 and 239.3, each with a dwell time of 100 ms. Compounds were identified using the NIST mass spectral database and relevant literature.

Pyrolysis-derivatisation-GC-MS

An additional derivatisation step was performed using hexamethyldisilazane (HMDS) to produce trimethylsilyl (TMS) derivatives. The standard pyrolysis-GC-MS protocol was followed as outlined above but with the additional injection of 5 µl HMDS to the pyrolysis tube immediately prior to pyrolysis and the use of an 8 min solvent delay on the mass spectrometer. For the freeze-dried bacteria, 8 µl HMDS was added to ensure sufficient derivatisation of the sample.

Controls

For the pyrolysis-derivatisation-GC-MS experiments, to establish the pyrolysis products deriving from HMDS, control samples were analysed by pyrolyzing quartz wool loaded with 5 µl HMDS. Products detected in the analysis of the HMDS control eluted early in the experiment (< 15 min), and were dominated by organic compounds containing silane and amine moieties resulting from thermal degradation or polymerisation of the HMDS. Contaminants such as phthalates were identified and attributed to procedural exposure to plastic laboratory equipment. Siloxanes from column-bleed were also recognised as contaminants. Occasional minor lipid

components were excluded if their presence could only by explained by impurities in the standard. For clarity, all of these compounds were disregarded from the results presented herein and are not annotated in the figures.

All experiments were replicated at least once, and up to five times for the most important data, to ensure that any significant signals detected were not the result of contamination or methodological error.

Data availability

Data available on request from the authors.

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Author contributions

S.H. performed experiments and wrote the main manuscript text. M.A.S. provided conceptual and experimental guidance, assisted with data interpretation and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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