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ORIGINAL ARTICLE

Characterization of Archaea membrane lipids in radioactive springs using shotgun lipidomics

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Abstract

Lipids from microorganisms, and especially lipids from Archaea, are used as taxonomic markers. Unfortunately, knowledge is very limited due to the uncultivability of most Archaea, which greatly reduces the importance of the diversity of lipids and their ecological role. One possible solution is to use lipidomic analysis. Six radioactive sources were investigated, two of which are surface (Wettinquelle and Radonka) and four deep from the Svornost mine (Agricola, Behounek, C1, and Curie). A total of 15 core lipids and 82 intact polar lipids were identified from the membranes of microorganisms in six radioactive springs. Using shotgun lipidomics, typical Archaea lipids were identified in spring water, namely dialkyl glycerol tetraethers, archaeol, hydroxyarchaeol and dihydroxyarchaeol. Diverse groups of polar heads were formed in archaeal IPLs, whose polar heads are formed mainly by hexose, deoxyhexose, and phosphoglycerol. The analysis was performed using shotgun lipidomics and the structure of all molecular species was confirmed by tandem mass spectrometry. After acid hydrolysis, a mixture of polar compounds was obtained from the polar head. Further analysis by GC–MS confirmed that the carbohydrates were glucose and rhamnose. Analysis by HPLC–MS of diastereoisomers of 2-(polyhydroxyalkyl)-3-(*O*-tolylthiocarbamoyl) thiazolidine-4(*R*)-carboxylates revealed that both L-rhamnose and D-glucose are present in spring samples only in varying amounts. The glycoside composition depends on the type of spring, that is, Wettinquelle and Radonka springs are basically shallow groundwater, while the samples from the Svornost mine are deep groundwater and do not contain glycosides with rhamnose. This method enables quick screening for characteristic Archaea lipids, allowing decisions on whether to pursue further analyses, such as metagenomic analysis, to directly confirm the presence of Archaea.

Keywords Radioactive springs · Archaea · Glycerol dialkyl glycerol tetraethers · Intact polar lipids · L-rhamnose · D-glucose

Introduction

Studies of microorganisms that inhabit springs of extreme composition provide information on the origin, evolution, and biodiversity of life on Earth. The number of prokaryotes in these waters has been reported to range from about 10^3 to 10^5 microbial cells per milliliter (Itävaara et al. 2016). A special type of groundwater is water containing radioactive isotopes, especially ^{226}Ra and ^{222}Rn produced by radioactive decay of radioactive minerals containing uranium and thorium. Radon is a radioactive gaseous element that emits α particles and thus generates free radicals whose interaction with biological molecules causes the peroxidation of cellular lipids (Timkina et al. 2024). So far, radon waters have been mainly monitored in terms of chemical composition (Kamenova-Totzeva et al. 2018; Alomari et al. 2019) or the influence of radon content on the properties of drinking or spa water (Etani et al. 2017; Di Carlo et al. 2019; Piao et al. 2020). These springs occur in a limited number of places around the world with varying radon content. Examples include Paralana spring in the area of Mount Painter in the south of the Australian Flinders Ranges (Anitori et al. 2002), "Franz-Josef-Quelle" springs Bad Gastein, near Salzburg,

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Austria (Weidler et al. 2007), or spring near the city Ramsar in northern Iran (Asgarani et al. 2012). The Ore Mountains (German: Erzgebirge, Czech: Krušné hory) lie along the Czech–German border and are characterized by rich deposits of uranium and other radioactive elements that break down into radon, which causes the radioactivity of groundwater. At the foot of these mountains are the Wettinquelle mineral spring in Bad Brambach, Germany (Wagner et al. 2007), springs in the Jáchymov spa (Joachimstahl), respectively four springs in the Svornost mine (Agricola, Behounek, C1 and Curie) (Krejbichová 1999), or the Radonka spring near Plesná, Czech Republic (Turnová 2019). However, the microbial ecology of waters containing radioactive isotopes remains largely unexplored. The Dourioux and Montagne springs located in the French Massif Central were characterized by specific bacterial populations, which correlates with the radioactivity (less than 4 kBq/L) in these springs (Holub et al. 2024).

Archaea are considered the most primitive forms of life on Earth and play a key role in global ecosystem functions. They can be found in any terrestrial or aquatic environment habitat, although many known species are extremophiles and isolated from anaerobic, hypersaline, acidic, and hydrothermally and geothermally heated environments once thought to not support life. Despite the morphological similarity, Archaea are biochemically different from bacteria. A distinct and characteristic feature of Archaea is the composition and organization of their plasma membrane. Archaeal membrane lipids consist of branched isoprenoids that are ether-linked to the glycerol-1-phosphate backbone, while lipids in bacteria Eukarya are typically based on straight-chain fatty acids that are ester attached to the enantiomeric glycerol-3-phosphate backbone (Rezanka et al. 2023). Glycerol dialkyl glycerol tetraethers (GDGT) and archaeol (AR) are the most common lipids in the core membrane of archaeal cells (Gambacorta et al. 1995). Crenarchaeol was used as a diagnostic biomarker for ammonia-oxidizing Archaea (Damsté et al. 2002). GDGTs are made up of a series of compounds with different numbers of cyclopentane rings on a biphytane skeleton (GDGT-0 to GDGT-8). Unique GDGTs with four cyclopentane rings and one cyclohexane ring (crenarchaeol and crenarchaeol regioisomer) are considered diagnostic biomarkers of ammonia-oxidizing Archaea (De La Torre et al. 2008).

Some Archaea, e.g., *Pyrococcus* or *Thermococcus* species, such as *Thermococcus gammatolerans*, *Thermococcus marinus*, *Thermococcus kodakarensis*, or *Pyrococcus furiosus* are known to be resistant to ionizing radiation (Confalonieri and Sommer 2011). Lipid analysis was performed in *T. kodakarensis* and core lipids and mainly archaeol-type IPLs, 2Hex-AR (dihexose-AR), phosphatidylglycerol-AR (PG-AR) or tetraethers such as phosphatidyl glycerol-GDGT, 2Hex-GDGT, and many others were identified (Meador et al. 2014). In the article by Tourte et al. (2020), they have been described in the core lipids of *Thermococcus* and *Pyrococcus*, for example, PG-AR, GDGT-0, GDGT-3, and 2Hex-GDGT-PG.

Intact polar lipids (IPLs) consisting of core lipid skeletons and various polar head groups are more labile lipid components that are considered biomarkers for living microorganisms (Sturt et al. 2004). Intact polar lipids of Archaea include a wide variety of structures in which both polar head groups (e.g., glyco-, phospho-, and glycophospho-based head groups) and several combinations of diether and tetraether core lipids result in a diverse array of IPLs (Fig. 1S). Therefore, IPLs are widely used as a tool to estimate microbial biomass in soil, water column, and subsurface water (springs) (Lipp et al. 2008; Schubotz et al. 2009; Rethemeyer et al. 2010). For example, Rossel et al. 2007, 2011) analyzed the composition of archaeal IPLs in various cold seep samples and found that Archaea produced diglycosidic GDGTs, intact polar archaeol with glycosidic and phospho-headgroups or only phospho-headgroups (Rossel et al. 2007, 2011).

Several sugars have been found in the main glycosidic group of Archaea, including hexoses-glucose, galactose, mannose, and gulose, and modified carbohydrates such as (*N*)-acetylglucosamine and sulfated sugars (see Koga and Morii 2005 and references therein). To fully determine their structure, it is necessary to determine the absolute configuration of monosaccharides. One of the possible methods is the reaction of enantiomers to form diastereoisomers, e.g., with chiral 2-butanol. Acetylated-2-butyl derivatives were then obtained by derivatization and analyzed by capillary gas chromatography (Gerwig et al. 1978). In the last decade, liquid chromatography (HPLC) methods have been developed for the analysis of derivatized saccharides that can be used for the separation of diastereomeric derivatives (Tanaka et al. 2007; Wang et al. 2012; Liu et al. 2022).

The reaction of aldoses with L-cysteine methyl ester and *O*-tolyl isothiocyanate yields methyl 2-(polyhydroxyalkyl)−3-(*O*-tolylthiocarbamoyl)thiazolidine-4(*R*)-carboxylates. The RP-HPLC of the resulting derivatives makes it possible to distinguish the D- and L-enantiomers of monosaccharides. Using different ion ratios in tandem MS, individual enantiomers can be identified for compounds that have a similar retention time (tR).

With the advancement of analytical techniques-particularly increasingly powerful, albeit more costly, mass spectrometers and their integration with chiral chromatography-we can anticipate new insights, such as an enhanced understanding of carbohydrate chirality (L versus D) in intact polar lipids. Additionally, the analysis of lipids and Archaea taxonomy using next-generation sequencing methods (e.g., Illumina, Pacific Biosciences, or NanoPore) will enable the identification of new species and genera. Unfortunately,

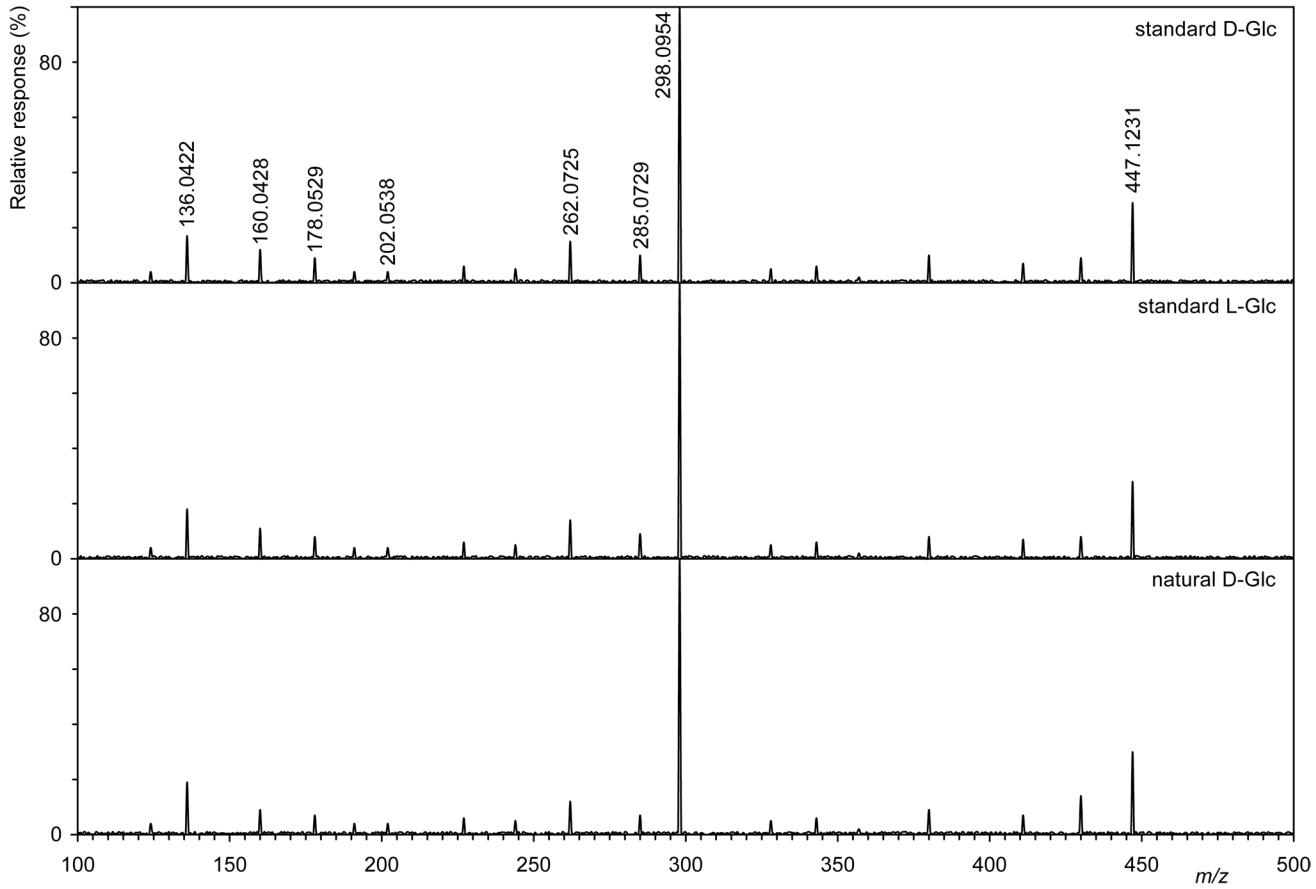

Fig. 1 Tandem MS of ions from $[M+H]^+$ of standard and natural derivatives of glucose

Archaea remain challenging to cultivate compared to most bacteria and eukaryotes, which limits the amount of biomass that can be obtained.

The samples used in this study were collected exclusively in the natural environment and mainly obtained from radioactive sources, where archaeal IPLs are very diverse. Archaeal IPLs were tentatively identified on mass spectra. This study provides extensive information for the rapid and direct characterization of archaeal membrane IPLs. Furthermore, D-glucose and L-rhamnose were identified as the two monosaccharides in different proportions depending on the sampling site. This method can serve as a valuable tool for researchers studying microbial lipids in natural environments.

Materials and methods

Standards

D-Galactose (D-Gal), L-galactose (L-Gal), D-glucose (D-Glc), L-glucose (L-Glc), D-mannose (D-Man), L-mannose (L-Man), L-rhamnose (L-Rha), methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl-α-L-rhamnoside, methyl-β-L-rhamnoside, L-cysteine methyl ester hydrochloride, and *p*-tolyl isothiocyanate (4-methylphenyl isothiocyanate) were purchased from Sigma-Aldrich (now Merck, Prague, Czech Republic). Other chemicals used were also purchased from Merck.

Collections of water and extraction of lipids

For the analysis of total lipids in each spring, see Table 1S, a 1.5-L sterile bottle was filled with water. The bottles were immediately transported to the laboratory and the cells were filtered on 0.22-µm membrane filters (VWR, USA). Briefly, after the addition of an internal standard (β-L-gulopyranosyl-caldarchaetidyl-glycerol from *Thermoplasma acidophilum*, $[C_{95}H_{189}O_{16}P+H]^+$ at *m/z* 1618.3786 (Matreya, now Cayman Europe, Estonia)) all filters were extracted according to Bligh and Dyer (1959), a mixture of methanol (MeOH)/dichloromethane (DCM)/phosphate buffer (pH 7.4; 2:1:0.8, v/v/v) in an ultrasonic bath (2 × 15 min), followed by two-time extractions according to Markham and Jaworski (2007) with isopropanol/hexane/water (55:20:25 v/v/v). The supernatants were combined, and DCM and water were added.

The DCM phase was collected and dried under the flow of N_2.

Shotgun analysis

The linear ion trap quadrupole-LTQ-Orbitrap (LTQ-Orbitrap) Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was equipped with a heated electrospray interface (HESI) in positive and negative ionization modes. The range of mass spectrometer (MS) scan was performed on the Fourier transform (FT) cell and recorded within a window between 100 and 2000 Da. The mass resolution was set at 105,000, and the ion spray voltage was + 3.5 kV in positive ionization mode. The ionization mode used the following parameters: N_2 was used as a nebulizer gas and set at 18 arbitrary units (sheath gas) and 7 arbitrary units (auxiliary gas); ion source temperature, 250 °C; capillary temperature, 230 °C; capillary voltage, 50 V; and tube lens voltage, 170 V. Helium was used as a collision gas for collision-induced dissociation (CID) experiments. The CID normalization energy of 35% was used to fragment the parent ions. The MS/MS product ions were detected in a high-resolution FT mode.

The MS spectrometer was calibrated using a Pierce LTQ Orbitrap positive ion calibration solution (Thermo Fisher Scientific, San Jose, CA, USA). For mass spectral acquisition, the internal lock mass was used: *m/z* 413.2662 $[M + Na]^+$, i.e., diisooctyl phthalate in ESI^+. The mass accuracy was better than 1.0 ppm. Core lipid structures and also intact polar lipids were confirmed with the help of the LIPID MAPS Lipidomics Gateway spectral database (Conroy et al. 2023) and previously published descriptions of positive mass spectra of core lipids and IPLs (Liu et al. 2012; Knappy et al. 2015; Horai et al. 2019).

Identification of carbohydrates from IPLs

Methanolysis

The mixture of 2 mL of anhydrous methanol and 280 µL of acetyl chloride was mixed. After 5 min, the reagent was added dropwise to total lipids (~0.5 µg) and heated for 16 h (overnight) at 80 °C in tubes sealed with Teflon-lined septa in screw caps. Methanolic HCl was evaporated under a stream of nitrogen at 40 °C. The evaporation step was repeated twice more each time after adding 250 µL of MeOH.

Silylation

A mixture containing 100 µL hexamethyldisilazane and 50 µL trimethylchlorosilane was added to the methylglycoside mixture in 1 mL of pyridine. The mixture was shaken vigorously for about 1 min and then allowed to stand for 10 min. The reagents were removed in a stream of nitrogen at room temperature, 100 µL of hexane was added to the mixture, and the trimethylsilyl-methyl glycosides were identified by GC–MS. The standards of TMS methylglycosides were prepared from commercially obtained methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-rhamnopyranoside, and methyl β-D-rhamnopyranoside.

Gas chromatography-mass spectrometry

TMS methyl glycosides were analyzed using a high-performance 7890A gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a fused silica capillary column (SPB®−5 Capillary GC Column, L × I.D. 30 m × 0.25 mm × 0.25 µm, bonded; poly(5% diphenyl/95% dimethyl siloxane phase)) coupled to an Agilent 7000 MS/MS (Agilent) with a chemical ionization source (CI). The temperature program was as follows: 100 °C for 1 min, then increased at 5 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. Positive chemical ionization mass spectra (PCI; full scan mode) were recorded in normal mode, CI reagent ammonia at 1.3 mL/min (Rezanka et al. 2019). All spectra were scanned in the range of *m/z* 50–600.

Hydrolysis and derivatization of saccharide

A total of 100 µL of solution containing ~0.5 µg/mL of total lipids was prepared in methanol. Total lipids were hydrolyzed with 350 µL HCl (3.5 M) in a screw cap vial, the hydrolyzate was lyophilized, and the lyophilized samples were derivatized.

One hundred microliters of a solution containing ~0.5 µg/mL monosaccharide was dissolved in 120 µL of anhydrous pyridine and L-cysteine methyl ester (50 µg/mL) was added to standard monosaccharide solutions or sample to react in a screw cap vial for 1 h at 60 °C. The reaction mixture was incubated with 80 µL of phenyl isothiocyanate at 60 °C for 1 h. Anhydrous pyridine was added to a final volume of 200 µL and then the mixture was left at room temperature overnight before LC–MS analysis.

Identification of D- and L-enantiomers of saccharides

LC equipment consisted of a 1090 Win system, a PV5 ternary pump, and an automatic injector (HP 1090 series, Agilent, Santa Clara, CA, USA) and Luna Omega 1.6 µm, C18, 100 Å, LC column 150 × 2.1 mm. The Glc derivative with tR 11.27 min was used as an internal standard, with a flow rate of 0.50 mL/min, an injection volume of 7 µL, and a column temperature of 27 °C. Methyl 2-(polyhydroxyalkyl)−3-(*O*-tolylthiocarbamoyl)-thiazolidine-4(*R*)-carboxylates (four monosaccharides, that is, Gal, Glc, Man, and Rha) were

separated using a solvent gradient program with the solvent mixture from 45% A (water with 0.05% formic acid) and 55% B (acetonitrile/methanol/isopropanol (50:25:25, v/v/v) with 0.05% formic acid) to 100% B within 20 min was used and hold for 5 min. The composition was returned to the initial conditions for 20 min.

Results and discussion

The water from six springs (see Materials and Methods, Table 1S) was filtered and all the biomass from the filters was extracted. However, due to the varying relative hydrophobicities of the lipid molecules, it was not possible to extract all lipid classes from the biomass (de Jesus and Filho 2020). The extraction method according to Bligh and Dyer (1959) is not suitable for very polar lipids, such as mono- to oligo-glycosides of lipids, as has already been discussed in several papers (Markham et al. 2006; Markham and Jaworski 2007; Shiva et al. 2018). Therefore, after extraction according to Bligh and Dyer (1959) another extraction of polar lipids was performed using a mixture of the lower phase of isopropanol/hexane/water (55:20:25 v/v/v) (Markham et al. 2006; Markham and Jaworski 2007; Ebert et al. 2018). Total lipids were analyzed by shotgun lipidomics using high-resolution mass spectrometry (HR-MS) with positive ESI on an Orbitrap instrument (see "Materials and Methods").

The representation of individual lipids, whether core lipids or IPLs, is shown in Table 2S. It can be clearly seen from this table that the weight amount of the molecular species of lipids differs for individual sources. The pair of springs, Wettinquelle and Radonka, which spontaneously emerge to the surface, contain up to an order of magnitude more IPLs than the four springs from the Svornost mine (Jáchymov). This phenomenon can be explained by the fact that the waters of the Wettinquelle and Radonka springs are essentially shallow groundwater enriched with radon, whereas the waters of the Svornost mine springs are deep underground waters that have been isolated from the surface zone and atmospheric oxygen for a longer time. The origin of dissolved inorganic substances is similar for both types, resulting from the incongruent dissolution of minerals in granites (Paces and Smejkal 2004).

Lipidomics of Archaea

Briefly, a total of 96 typical Archaea lipids were identified (Schouten et al.2013). Of these 96 lipids, there were a total of 81 IPLs, which prove the presence of living Archaea cells (Wu et al. 2019). Their identification is mainly based on high-resolution tandem mass spectrometry positive electrospray ionization, which are based on $[M+H]^+$ type ions, which are formed during positive ionization. The tandem mass spectrum is mainly characterized by the presence of the adduct ($[M+H]^+$), which is the base peak. Furthermore, two significant and typical GDGT-0 ions are present, i.e., ions $[M+H\text{-}18]^+$ and $[M+H\text{-}74]^+$. The first of them arises from the loss of one water molecule, the second then from one of the glycerols from the ion at *m/z* 1302.3230. Other ions that show the structure of GDHT-0 are ions resulting from the loss of one biphytane unit. Similarly to IPL, glycoside ions were characteristic, whether it is a molecular species at *m/z* 1626.4283, which is a glycoside with a disaccharide bound to GDGT-0 or a disaccharide containing one deoxyhexose and one hexose again bound to GDGT-0. It is clear from the tandem mass spectra that the polar head is preferentially split off, in this case, the loss of saccharides. This corresponds to the presence of ions, which are formed by the gradual loss of hexose/deoxyhexose, water, and glycerol. The values m/z and ion structures are shown in Tables 3S and 4S. Similarly, other molecular species found in water from radioactive springs were identified using tandem MS.

Table 3S describes the identification of individual molecular species, i.e., the ions obtained by the tandem MS 2Hex-GTGD-0. In Table 4S, the ions resulting from the tandem MS of DHexHex-GTGD-0 (DHex) are further described. A more detailed description of the cleavage is given below. Based on the neutral loss of the ion ~ 162.053 Da, it can be assumed that it is a glycoside containing two hexoses and the aglycon GDGT-0 and in the second case there is a gradual neutral loss of 162.053 and 146.058 Da, which confirms the structure glycoside with one hexose and one deoxyhexose (de Souza et al. 2009; Pitcher Angela et al. 2011). Similarly, other molecular species were identified by tandem MS and are listed in Table 2S.

Structure of carbohydrates

The glycosyl composition was determined by combined gas chromatography–mass spectrometry (GC–MS) of the trimethylsilyl (TMS) methyl glycosides produced from total lipids by acid methanolysis and silylation (Merkle and Poppe 1994). Four major peaks were identified whose retention times corresponded to the standards of TMS α-methylglucoside, TMS β-methylglucoside, TMS α-methylrhamnoside, and TMS β-methylrhamnoside. In the area of molecular ions of positive chemical ionization mass spectrometry (PCI-MS), there were abundant ions of type $[M+H]^+$ and the ammonium adduct ion $[M+NH_4]^+$. Other ions were formed by the gradual loss of neutral molecules, predominantly methanol and trimethylsilanol; see below. Ions at *m/z* 207 and at *m/z* 217, whose structure is similar to that of electron impact MS, were also found (Bleton et al. 1996). The base peak of both methyl glucosides identified by PCI-MS was an ion of type $[M+H\text{-}CH_3OH\text{-}TMSiOH]^+$ at *m/z* 361. Further abundant ions were $[M+NH_4]^+$ at *m/z* 500, $[M+NH_4\text{-}CH_3OH]^+$ at *m/z* 468, $[M+NH_4\text{-}CH_3OH\text{-}TMSiOH]^+$ at *m/z* 378, $[M+NH_4\text{-}CH_3OH\text{-}2\times TMSiOH]^+$ at *m/z* 288,

$[M+NH_4\text{-}CH_3OH\text{-}3\times TMSiOH]^+$ at *m/z* 198, etc. (Bleton et al. 1996). The following values were found for both rhamnosides: $[M+NH_4]^+$ at *m/z* 412, $[M+NH_4\text{-}CH_3OH]^+$ at *m/z* 380, $[M+NH_4\text{-}CH_3OH\text{-}TMSiOH]^+$ at *m/z* 290, $[M+NH_4\text{-}CH_3OH\text{-}2TMSiOH]^+$ at *m/z* 200, $[M+NH_4\text{-}CH_3OH\text{-}3TMSiOH]^+$ at *m/z* 110, and the ion $[M+H]^+$ at *m/z* 395 was also found. GC–MS/PCI allowed us to confirm that the only two saccharides present in total lipids were glucose and rhamnose. To further elucidate and confirm the absolute structure of glucose and rhamnose, the derivatization and the separation of diastereoisomers of methyl 2-(polyhydroxyalkyl)-3-(*O*-tolylthiocarbamoyl)-thiazolidine-4(*R*)-carboxylates were performed using RP-HPLC/tandem MS.

Identification of D- and L-enantiomers of glucose and rhamnose

Multiple ion monitoring (MRM) of the derivatized hydrolysate showed two peaks at 11.32 min (D-Glc) and 14.37 min (L-Rha), that is, SIM at *m/z* 285.073 (structure of ion, see Fig. 2S or 3S). The sugar groups of the total extracts were tentatively determined as a mixture of L-rhamnose and D-glucose by comparing the tR with the tR of the monosaccharide standards. The result was further confirmed by tandem MS of ions from $[M+H]^+$, see Fig. 1.

The fragmentation pathway of the hexose derivative is shown in Fig. 2S, so we only briefly present here. The hexose derivatives produced characteristic fragment ions at *m/z* 298.0955 $[447.1254\text{-}C_8H_7NS]^+$, 285.0726 $[447.1254\text{-}C_6H_{10}O_5]^+$, 262.0744 $[298.0955\text{-}2H_2O]^+$, 178.0532 $[298.0955\text{-}C_4H_8O_4]^+$, 160.0427 $[178.0532\text{-}H_2O]^+$, and 136.0427 $[298.0955\text{-}C_6H_{10}O_5]^+$ from the protonated ion *m/z* 447.1254 ($[M+H]^+$). A similar pathway for the deoxyhexose derivative was previously described (Liu et al. 2022). The scheme of the tandem MS is shown in Fig. 3S. In addition, characteristic fragment ions from the $[M+Na]^+$ adduct ions (a derivative of hexose) were also identified. The characteristic fragment ions produced by the hexoses at *m/z* 469.1074 ($[M+Na]^+$) were at *m/z* 320.0774, 200.0346, and 143.0311. The fragmentation pathways of these characteristic fragment ions from the $[M+Na]^+$ adduct ion were similar

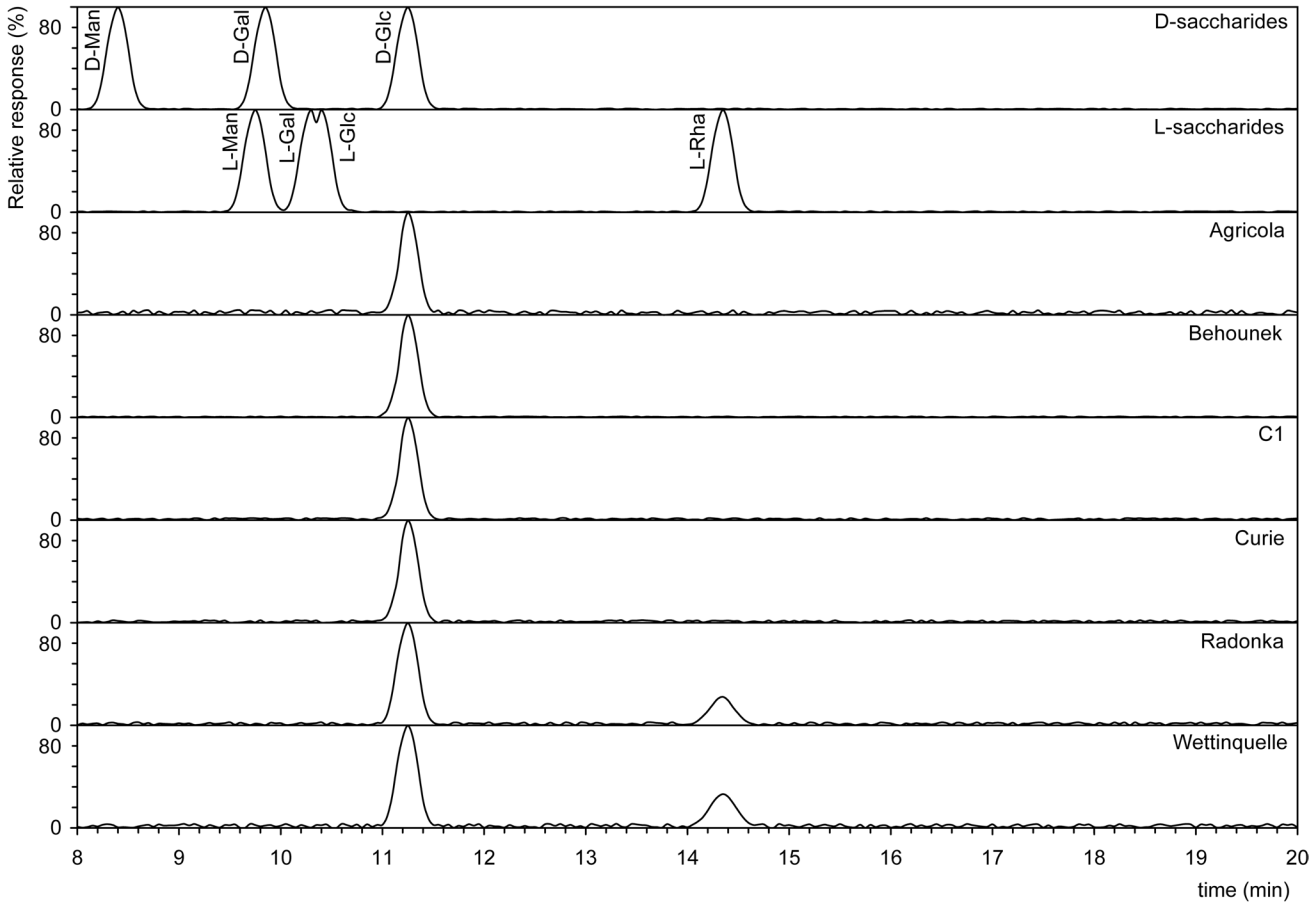

Fig. 2 LC-tandem MS (ion at *m/z* 285.0726) of L- and D-monosaccharides and hydrolysate from six springs as 2-(polyhydroxyalkyl)−3-(*O*-tolylthiocarbamoyl)-thiazolidine-4(*R*)-carboxylates

to those of the protonated ion $[M+H]^+$, see some structures in Fig.2S. Separation of individual derivatives of standards, i.e., methyl 2-(polyhydroxyalkyl)-3-(*O*-tolylthiocarbamoyl)-thiazolidine-4(*R*)-carboxylates, except for the separation of two hexose derivatives, i.e., L-Gal and L-Glc, was not a problem (Liu et al. 2022). On the basis of these results, it was determined that the D enantiomer of glucose and the L enantiomer of rhamnose are present in natural total lipids in different proportions. From two springs that rose to the surface, i.e., Wettinquelle spring in Bad Brambach and Radonka spring near the town of Plesná, a D-Glc versus L-Rha ratio of 1.0:0.3 was found, while in four springs from the Svornost mine (Jáchymov) rhamnose was not found at all, see Fig. 2.

Conclusion

Lipidomic analysis of microorganism membranes obtained from six springs in the Ore Mountains along the Czech–German border identified more than 90 molecular species of lipids that are characteristic of Archaea. Intact polar lipids (IPLs), of which more than 80 have been identified, are considered biomarkers for living microorganisms (Sturt et al. 2004), predominantly Archaea (Rossel et al. 2007, 2011). Furthermore, after hydrolysis of the polar head, two monosaccharides, i.e. glucose and rhamnose, were identified. Deoxyhexose (L-Rha) was identified as a component of the polar head, to the best of our knowledge, for the first time. In the analysis of diastereoisomers, D-glucose and L-rhamnose were found to be present in the samples. Wettinquelle spring was previously analyzed using ribosomal RNA genes, but only bacteria were identified (Wagner et al. 2007). Furthermore, in the springs of the Svornost mine only bacteria were obtained by cultivation (Smrhova et al. 2022), while Weidler et al. (2007) identified in the "Franz-Josef-Quelle" spring (Bad Gastein), more than twice as many Archaea clones as bacteria. Therefore, it can be assumed that it will be possible to identify Archaea in the six springs on both sides of the Ore Mountains using metagenomics.

The method employed-lipidomic analysis-offers a very fast, highly accurate, and much more cost-effective way to identify Archaea. Up to 30 analyses can be conducted within an hour, even considering potential sample preparation steps like cell filtration and lipid extraction. This means that several hundred samples can be analyzed in a single day. The results could even be semi-quantified with the use of an appropriate internal standard. An internal standard, such as any IPLs (see Table 2S), can be prepared by culturing certain Archaea, which will be the focus of our future efforts.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12223-024-01235-3.

Author contribution Michal Řezanka, Pavel Řezanka, and Tomáš Řezanka have designed and conducted the experiments, analyzed the data, written the manuscript, and provided valuable contributions in project administration, conceptualization, review, and editing of the manuscript, and supervision of this whole project. Lucie Kyselová contributed to manuscript writing, preparation of figures, and review. All authors read and approved the final manuscript.

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Data Availability Data will be made available on request.

Declarations

Competing interests The authors declare no competing interests.

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