Organic Geochemistry 124 (2018) 63–76

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

An overview of the occurrence of ether- and ester-linked *iso*-diabolic acid membrane lipids in microbial cultures of the Acidobacteria: Implications for brGDGT paleoproxies for temperature and pH

Jaap S. Sinninghe Damsté [a,b,*], W. Irene C. Rijpstra [a], Bärbel U. Foesel [c,1], Katharina J. Huber [c], Jörg Overmann [c,d], Satoshi Nakagawa [e], Joong Jae Kim [f], Peter F. Dunfield [f], Svetlana N. Dedysh [g], Laura Villanueva [a]

[a] NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, The Netherlands
[b] Utrecht University, Faculty of Geosciences, Department of Earth Sciences, P.O. Box 80.021, 3508 TA Utrecht, The Netherlands
[c] Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Inhoffenstraße, B38124 Braunschweig, Germany
[d] German Center for Integrative Biodiversity Research (iDiv) Jena Halle Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany
[e] Kyoto University, Graduate School of Agriculture, Division of Applied Biosciences, Laboratory of Marine Environmental Microbiology, Gokasho, Uji City, Kyoto 611-0011, Japan
[f] University of Calgary, Department of Biological Sciences, 2500 University Dr. NW, Calgary T2N 1N4, Canada
[g] Research Center of Biotechnology of the Russian Academy of Sciences, Winogradsky Institute of Microbiology, Prospect 60-letya Octyabrya 7/2, Moscow 117312, Russia

ARTICLE INFO

Article history:
Received 14 May 2018
Received in revised form 21 June 2018
Accepted 12 July 2018

Keywords:
Acidobacteria
Membrane lipids
Iso-diabolic acid
brGDGTs
Bacteria
Methylation

ABSTRACT

13,16-Dimethyl octacosanedioic acid (*iso*-diabolic acid) is a major membrane-spanning lipid of subdivisions (SDs) 1, 3 and 4 of the Acidobacteria, a highly diverse phylum within the Bacteria. It has been suggested that these lipids are potential building blocks for the orphan bacterial glycerol dialkyl glycerol tetraethers (GDGT) that occur widely in a variety of environmental settings. Here, we expand the knowledge on the occurrence of *iso*-diabolic acid in Acidobacteria by examining the lipid composition of six strains belonging to SDs 6, 8, 10, and 23 of the Acidobacteria, not previously analyzed for these lipids. In addition, we examined 12 new strains belonging to SDs 1, 3 and 4. Acid hydrolysis of total cell material released *iso*-diabolic acid in substantial quantities (25–39% of all fatty acids) from the strains of SDs 1 and 3 (except "*Candidatus* Solibacter usitatus"), and, for the first time, strains of SD 6 (6–25%), but not from SDs 8, 10, and 23. The monoglycerol ether derivative of *iso*-diabolic acid was only dominantly present in SD 4 strains (17–34%), indicating that the occurrence of ether-bound *iso*-diabolic acid is mainly restricted to SD 4 species. Methylated *iso*-diabolic acid derivatives were encountered in SDs 1, 3, 4, and 6, but only SD 4 species produced 5-methyl *iso*-diabolic acid derivatives, whereas the other SDs formed 6-methyl *iso*-diabolic acids. This suggests that the position of methylation of *iso*-diabolic acid may be controlled by the phylogenetic affiliation within the Acidobacteria and thus may not be a direct but an indirect response environmental to environmental conditions as inferred from the bacterial GDGT distributions in soil, peat and rivers.

1. Introduction

Unusual glycerol dialkyl glycerol tetraethers (GDGTs) with *n*-alkyl chains containing 2–4 methyl groups (so-called branched GDGTs; brGDGTs; e.g., structures **1**–**3** in Fig. 1) were identified for the first time in peat by isolation and structure determination by NMR spectroscopy (Sinninghe Damsté et al., 2000). Hints for the existence of such structures were already obtained much earlier when selective ether cleavage was applied to sedimentary organic matter of the Messel Oil Shale (Chappe et al., 1980). However, only the introduction of liquid chromatography coupled to mass spectrometry, enabling the analysis of intact GDGTs in complex environmental samples (Hopmans et al., 2000), made it possible to reveal their structural diversity and distribution. It has now been demonstrated that these brGDGTs are ubiquitous in soil, peat, lake water and sediments, river water and sediments, hot springs and coastal marine sediments (see Schouten et al., 2013 for a

* Corresponding author at: NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, The Netherlands.
E-mail address: jaap.damste@nioz.nl (J.S. Sinninghe Damsté).
[1] Present address: Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Research Unit Comparative Microbiome Analysis, Neuherberg, Germany.

https://doi.org/10.1016/j.orggeochem.2018.07.006
0146-6380/

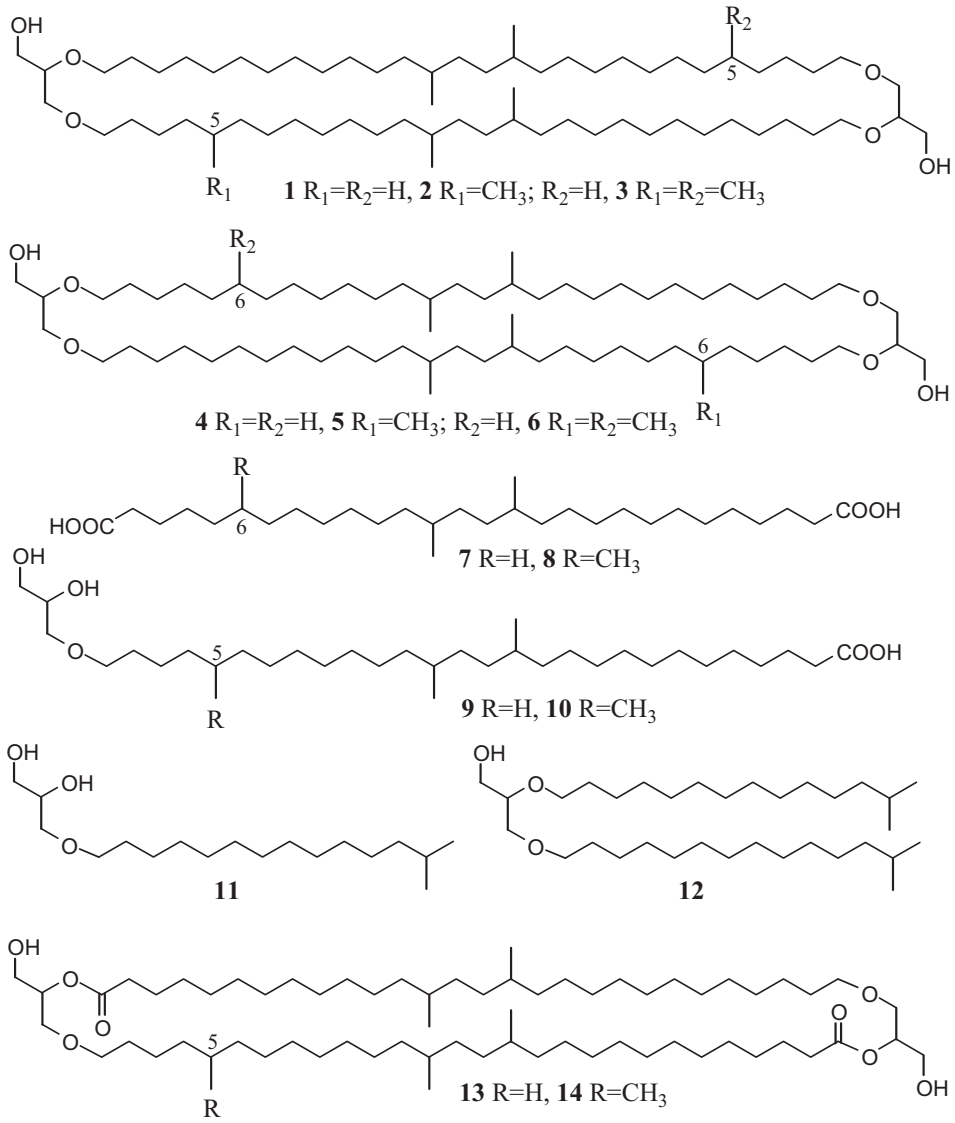

Fig. 1. Structures of lipids mentioned in the text. Note that structures **13** and **14** are hypothetical and have been proposed on results from acid hydrolysis experiments of cell material of SD 4 acidobacterial strains (Sinninghe Damsté et al., 2014).

review), but their microbial sources are still unclear. This is troublesome because of their potential extensive application in geochemistry and paleoclimatology (Schouten et al., 2013).

The assessment of the stereochemistry of the glycerol units in brGDGTs isolated from peat revealed that it is opposite to that of archaeal isoprenoidal GDGTs, suggesting that they are derived from Bacteria (Weijers et al., 2006). The abundance of Acidobacteria in both peat and soil environments, where brGDGTs are also abundant, suggested that these bacteria may be a biological source of the brGDGTs (Weijers et al., 2009). The bacteria producing brGDGTs are supposed to be heterotrophs based on the natural stable carbon isotopic composition of the alkyl building blocks of brGDGTs occurring in peat (Pancost and Sinninghe Damsté, 2003), soil (Weijers et al., 2010; Colcard et al., 2017), lake water and sediments (Weber et al., 2015; Colcard et al., 2017) and "natural labelling experiments" (Oppermann et al., 2010; Weijers et al., 2010). Many of the isolated species of Acidobacteria are, indeed, heterotrophic (see Kielak et al., 2016; Dedysh and Sinninghe Damsté, 2018 for reviews).

Acidobacteria is a diverse phylum of the domain Bacteria, whose members are especially abundant in soils and peat. On the basis of environmental 16S rRNA gene sequences (Barns et al., 2007), Acidobacteria have been divided into 26 subdivisions (SDs). However, at present only seven SDs (i.e., 1, 3, 4, 6, 8, 10, and 23) contain taxonomically characterized representatives (Kielak et al., 2016; Dedysh and Sinninghe Damsté, 2018). Molecular ecological studies based on 16S rRNA genes have indicated that, in wetlands, the most abundant Acidobacteria are affiliated with SDs 1 and 3 (Serkebaeva et al., 2013), whereas in lakes SDs 1, 6, and 7 are more abundant (Zimmermann et al., 2012). In soils, SDs 1, 3, 4 and 6 are more dominant (Janssen, 2006; Jones et al., 2009; Foesel et al., 2014; Naether et al., 2012).

The hypothesis that Acidobacteria may be a source of brGDGTs in the environment was supported by the presence of the uncommon membrane-spanning lipid, 13,16-dimethyl octacosanedioic acid (*iso*-diabolic acid; **7**), as a major lipid in 13 species of SDs 1 and 3 of the Acidobacteria (Sinninghe Damsté et al., 2011). *Iso*-diabolic acid can be considered as a potential building block of the brGDGTs but occurs predominantly ester- and not ether-bound in the SDs 1 and 3 of the Acidobacteria. However, in two of the 13 analyzed strains of SD 1, ether-bound *iso*-diabolic acids, including brGDGT **1,** were also detected after hydrolysis of the

cells, albeit in small amounts. A subsequent study revealed that *iso*-diabolic acid also occurs in SD 4 but that in six out of seven strains ether-bound *iso*-diabolic acid **9** was much more abundant than *iso*-diabolic acid itself (Sinninghe Damsté et al., 2014). This suggested that these acidobacteria produce the hypothetical membrane-spanning diester/diether **13**, a compound that is even more structurally similar to the brGDGTs present in the environment than *iso*-diabolic acid itself. However, brGDGTs were not detected in SD 4 Acidobacterial strains. Hence, a clear microbial source for brGDGTs has still not been identified. The structural diversity of brGDGTs in the environment is complex; they may also contain cyclopentane moieties (Weijers et al., 2006) and occur with additional methyl groups at positions other than the C-5 position (Liu et al., 2012; De Jonge et al., 2013; Ding et al., 2016). Therefore, there is a need for further screening of potential bacterial brGDGT producers since, ultimately, there is a strong need to test brGDGT-derived organic proxies using controlled culture experiments.

Here, we expand the knowledge on the occurrence of *iso*-diabolic acid in Acidobacteria by examining the lipid composition of six strains belonging to SDs 6, 8, 10, and 23 of the Acidobacteria, not previously studied for the presence of these lipids, completing the screening of all SDs for which cultured representatives are available. In addition, we examined 12 novel strains belonging to SDs 1, 3 and 4. We also detected *iso*-diabolic acid or its glycerol ether derivative methylated at the C-5 or C-6 positions and identified potential genes involved in ether bond formation. Lastly, we discuss the implications of our findings for the use of the brGDGTs as paleoenvironmental proxies for temperature and pH.

2. Materials and methods

2.1. Cultures

The acidobacterial strains used in this study are listed in Table 1. *Vicinamibacter silvestris* Ac_5_C6^T and *Luteitalea pratensis* HEG_-6_39^T (both SD 6) were grown at DSMZ using the conditions previously described (Huber et al., 2016; Vieira et al., 2017). *Arenimicrobium luteum* Ac_12_G8^T, *Stenotrophobacter roseus* Ac_15_C4^T, *Brevitalea deliciosa* Ac_16_C4^T, *Stenotrophobacter namibiensis* Ac_17_F2^T, and *Tellurimicrobium multivorans* Ac_18_E7^T, all belonging to SD 4, were also grown at DSMZ using conditions as previously described (Pascual et al., 2015; Wüst et al., 2016). Biomass was harvested by centrifugation (9000*g*, 30 min; Avanti-J26 XPI, Beckman Coulter), frozen (−20 °C, overnight), and lyophilized (0.05 mbar, −30 °C).

Acidicapsa sp. CE1 and CE14, *Granulicella aggregans* TPB6028^T, *Granulicella* sp. AF10, and *Paludibaculum fermentans* P105^T were grown at the Winogradsky Institute of Microbiology using conditions as previously described (Pankratov and Dedysh, 2010; Pankratov, 2012; Kulichevskaya et al., 2014; Belova et al., 2018). '*Thermotomaculum hydrothermale*' AC55 (SD 10) was grown at Kyoto University using conditions as previously described (Izumi et al., 2012). *Telmatobacter* spp. strains 15–8A and 15–28 were isolated from the sediment of a warm, acidic geothermal pool (pH 5.0, 29.7 °C) in Reporoa, New Zealand, using a mineral salts medium as previously described (Sharp et al., 2014; site LOR16, see Supplementary Table S1).

Cell material of "*Ca.* Koribacter versatilis" Ellin345 (SD 1), "*Ca.* Solibacter usitatus" Ellin6076 (SD 3), *Holophaga foetida* TMBS4^T, *Geothrix fermentans* H-5 ^{T} (both SD 8), and *Thermoanaerobaculum aquaticum* MP01^T (SD 23) was available from previous studies (Losey et al., 2013; Sinninghe Damsté et al., 2017), where details on the conditions of their cultivation have been described.

2.2. Lipid analysis

Lyophilized cells were hydrolyzed with 1.4 N HCl in methanol by refluxing for 3 h, using our previously described procedure (Sinninghe Damsté et al., 2011). Fatty acids in the extracts were converted into methyl esters using diazomethane, and an aliquot was subsequently silylated with N,O-*bis*(trimethylsilyl)-trifluoroacetamide (BSTFA) in pyridine at 60 °C for 20 min. This fraction was subsequently analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS) using conditions previously described (Sinninghe Damsté et al., 2011). Another aliquot of the methylated extract was separated over an activated Al_2O_3 column using dichloromethane (DCM) and DCM/methanol (1:1, v/v) into an apolar and polar fraction, respectively. The apolar fraction was used to determine the double bond positions of the mono-unsaturated fatty acid methyl esters (FAMEs) using the mass spectra of their dimethyl disulfide derivatives as described by Nichols et al. (1986). The polar fraction was dissolved in hexane/*iso*-propanol (99:1, v/v), filtered through a 0.45 μm polytetrafluorethylene filter, and analyzed by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry

Table 1
Acidobacterial strains studied for their lipid composition.

Acidobacterium	SD	origin	Reference
Ca. Koribacter versatilis Ellin345 (=DSM 22529)	1	Pasture soil	Joseph et al. (2003)
Telmatobacter sp. 15-8A	1	Geothermal spring sediment	Dunfield, unpublished
Telmatobacter sp. 15-28	1	Geothermal spring sediment	Dunfield, unpublished
Acidicapsa sp. CE1	1	*Spaghnum* peat	Pankratov (2012)
Acidicapsa sp. CE14	1	lichen *Cladonia* sp.	Pankratov (2012)
Granulicella aggregans TPB6028^T (=DSM 25274^T)	1	*Spaghnum* peat	Pankratov and Dedysh (2010)
Granulicella sp. AF10	1	forested tundra soil	Dedysh, unpublished
Paludibaculum fermentans P105^T (=DSM 26340^T)	3	Littoral wetland	Kulichevskaya et al. (2014)
Ca. Solibacter usitatus Ellin6076	3	Pasture soil	Joseph et al. (2003)
Arenimicrobium luteum Ac_12_G8 ^{T} (= DSM 26556^T)	4	Savannah soil	Wüst et al (2016)
Stenotrophobacter roseus Ac_15_C4^T (= DSM 29891^T)	4	Fallow soil	Pascual et al. (2015)
Brevitalea deliciosa Ac_16_C4 ^{T} (= DSM 29892^T)	4	Savannah soil	Wüst et al (2016)
Stenotrophobacter namibiensis Ac_17_F2^T (= DSM 29893^T)	4	Woodland soil	Pascual et al. (2015)
Tellurimicrobium multivorans Ac_18_E7^T (= DSM 26557^T)	4	Fallow soil	Pascual et al. (2015)
Vicinamibacter silvestris Ac_5_C6 ^{T} (= DSM 29464^T)	6	Savannah soil	Huber et al. (2016)
Luteitalea pratensis HEG_-6_39 (= DSM 100886^T)	6	Grassland soil	Vieira et al. (2017)
Holophaga foetida TMBS4^T (= DSM 6591^T)	8	Anoxic mud	Liesack et al. (1994)
Geothrix fermentans H-5^T (= DSM 14018^T)	8	Aquifer	Coates et al. (1999)
'*Thermotomaculum hydrothermale*' AC55 (= DSM 24660)	10	Hydrothermal vent	Izumi et al. (2012)
Thermoanaerobaculum aquaticum MP01^T (= DSM 24856^T)	23	Hot spring	Losey et al. (2013)

(HPLC–APCI-MS) for branched GDGTs using a method described elsewhere (Hopmans et al., 2016).

2.3. Characterization of the branching position of methylated iso-diabolic acid

Cells of *Vicinamimibacter silvestris* Ac_5_C6^T were base hydrolyzed with 1 N KOH (96% methanol). The obtained extract, containing the *iso*-diabolic acids, was treated with $LiAlH_4$ in dioxane to convert acid groups into alcohol moieties. The formed diols were converted to alkyl iodides by treating them with HI following a procedure described elsewhere (Schouten et al., 1998) and the formed iodides were reduced to hydrocarbons with PtO_2 in hexane supplying H_2 (Kaneko et al., 2011). The formed hydrocarbons were analyzed with GC and GC–MS. Kovats retention indices were determined by co-injection with a mixture of *n*-alkanes.

2.4. Identification of ether-lipid biosynthetic genes and phylogenetic analyses

Specific biosynthetic genes were identified in acidobacterial (meta)genomes with PSI-BLAST (Position-Specific iterated BLAST) searches at the protein level (www.ncbi.com) using two iteration steps using in most cases the annotated proteins of *Myxobacterium xanthus* DK 1622 (NC_008095.1) as query sequences.

Nearly complete 16S rRNA gene sequences of the acidobacterial strains investigated were obtained from the ARB SILVA database (https://www.arb-silva.de/) and aligned with ClustalW (Thompson et al., 1994). A phylogenetic tree was generated with MEGA 6 (Tamura et al., 2013) using the Neighbor-joining method (Saitou and Nei, 1987); bootstrapping values were based on 1000 replicates and are shown next to the branches (Felsenstein, 1985). Evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969). The analysis involved 44 nucleotide sequences, and a total of 1587 positions in the final dataset. Putative and annotated partial homologs of the ElbD protein were aligned by Muscle (Edgar, 2004) in the MEGA 6 software (Tamura et al., 2013) and edited manually. Phylogenetic reconstruction was performed by maximum likelihood in PhyML v3.0 (Guindon et al., 2010) using the best model according to AIC indicated by the MEGA 6 software (Tamura et al., 2013).

3. Results

A total of 20 strains of bacteria belonging to Acidobacteria SDs 1, 3, 4, 6, 8, 10, and 23 were (re)analyzed for their lipid composition using direct acid hydrolysis of cell material, a method that allows not only the quantification of the more common fatty acids, but also of the specific membrane-spanning lipids that occur in Acidobacteria, i.e. *iso*-diabolic acid and its derivatives (see Sinninghe Damsté et al., 2011). Phylogenetic relationships of most of these strains to other cultured and uncultured Acidobacteria, based on 16S rRNA and functional genes, have recently been presented elsewhere (e.g., Kielak et al., 2016; Sinninghe Damsté et al., 2017).

3.1. Fatty acids and ether lipids released by acid hydrolysis

Three examples of typical gas chromatograms of total lipid fractions obtained after acid hydrolysis of cells (i.e., for *Arenimicrobium luteum* Ac_16_C4, SD 4, *Vicinamibacter silvestris* Ac_5_C6^T, SD 6, and '*Thermotomaculum hydrothermale*' AC55, SD 10) are shown in Fig. 2. All investigated acidobacterial strains contained *iso*-$C_{15:0}$ as a dominant fatty acid (6–55% of all lipids, on average 31%; Table 2), with its unsaturated counterpart, *iso*-$C_{15:1\Delta 9c}$, also present in relatively high abundance (7–17%; Table 2) in all studied SD 4 strains and in *Ca.* S. usitatus (SD 3). The structurally related *iso*-$C_{17:0}$ only occurs in relatively high fractional abundances in the SD 10 and 23 strains (24–33%; Table 2), but its unsaturated counterpart, *iso*-$C_{17:1\ \Delta 9c}$, is sometimes a dominant fatty acid as well. Other predominant fatty acids are monounsaturated C_{16} and C_{18} fatty acids (Table 2).

In addition to these common bacterial fatty acids, the less common, later-eluting (Fig. 2) membrane-spanning lipid, 13,16-dimethyloctacosanedioic acid (or *iso*-diabolic acid **7**) was detected in varying amounts (1–47% of total lipids; Table 2). This work represents the first report of its occurrence in SD6 strains, where the fractional abundance of *iso*-diabolic acid amounts to ca. 30% (Table 2). *Iso*-diabolic acid was not detected in the strains of SD 8, 10, and 23 (Table 2). In the five studied strains of SD 4 the fractional abundance of *iso*-diabolic acid was low (1–2%; Table 2). Strikingly, however, in these strains acid hydrolysis released substantial amounts of 1-monoalkyl glycerol ethers (MGE) (e.g., Fig. 2a). The ether lipids were MGE derivatives of the abundant saturated fatty acids, with a dominance of *iso*-$C_{15:0}$ MGE (**11**) (15–20%; Table 2) and the MGE derivative **9** of *iso*-diabolic acid (17–34%; Table 2). The full structural identification of MGE **9** has previously been described (Sinninghe Damsté et al., 2014).

3.2. Methylated iso-diabolic acid derivatives

In addition to *iso*-diabolic acid **7** and its MGE derivative **9**, two related components containing an additional methyl group, i.e. **8** and **10**, were also detected in some of the strains (Table 2). This was apparent from their mass spectra, which revealed a shift of several fragment ions in the high *m*/*z* region by 14 Da. The position of the additional methyl group in the *iso*-diabolic acid MGE derivative **10** occurring in SD 4 Acidobacteria has previously been determined to be at the C-5 position (Sinninghe Damsté et al., 2014). The five newly analyzed strains of SD 4 all contained this methyl derivative, sometimes in relatively high fractional abundance (e.g., 13% in *A. luteum* Ac_12_G8^T; Table 2).

Both SD 6 species contained a methylated *iso*-diabolic acid; in one case even in relatively high fractional abundance (14% for *V. silvestris*; Table 2). To identify the position of the additional methyl group in the methylated *iso*-diabolic acid of *V. silvestris*, it was converted to the corresponding hydrocarbon (see Methods). The formed component was identified as 6,13,16-trimethyloctacosane as indicated by comparison of its mass spectral analysis and relative retention time data (Table 3) with earlier reported data (Sinninghe Damsté et al., 2000). This revealed that the position of methylation of the methylated *iso*-diabolic acid in SD 6 strains (i.e., C-6) is different from the one in the *iso*-diabolic acid MGE derivative **10** occurring in SD 4 Acidobacteria (i.e., C-5; Sinninghe Damsté et al., 2014).

In the examination of the SD 1 and 3 strains we came across two species that also produced methylated *iso*-diabolic acid (i.e. '*Ca.* Koribacter versatilis' and *Paludibaculum fermentans*), although these components were only encountered in a second batch culture (Table 2), suggesting that their formation may depend on the growth phase of the culture. By comparison of their mass spectra and relative retention time data (Table 3) with 6-methyl *iso*-diabolic acid **8** of *V. silvestris*, it was concluded that they are also methylated at C-6. Comparison of these data with that of the tentatively identified 5-methyl *iso*-diabolic acid in *Chloracidobacterium thermophilum* (Sinninghe Damsté et al., 2014; Table 3) revealed that this identification is most likely incorrect since it has a Kovats index that is substantially higher than would be expected for 5-methyl *iso*-diabolic acid. It is likely that this

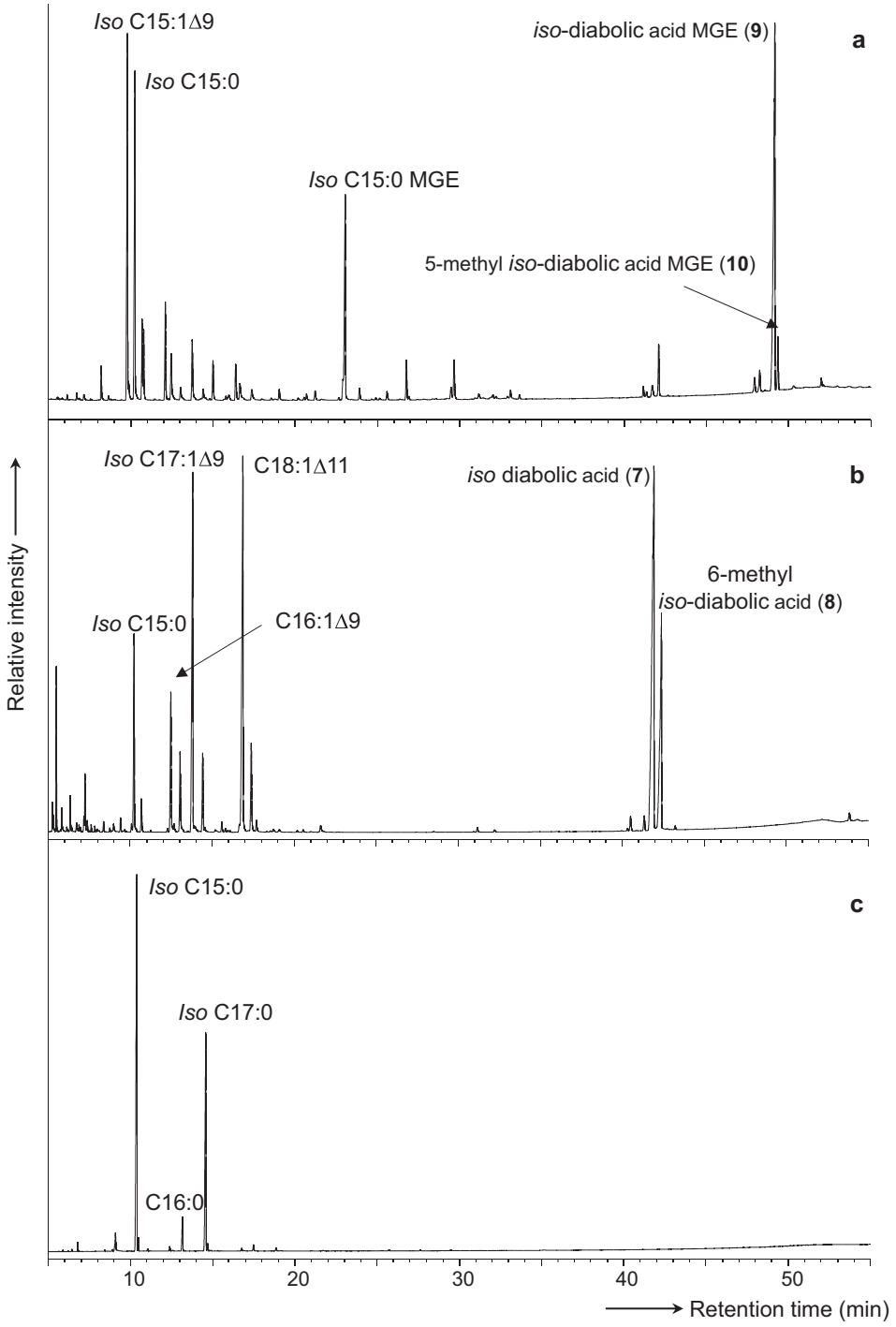

Fig. 2. Gas chromatograms of lipids released after acid hydrolysis of whole cell material of (a) *Tellurimicrobium multivorans* Ac_18_E7^T (SD4), (b) *Vicinamibacter silvestris* Ac_5_C6^T, (SD6) and (c) '*Thermotomaculum hydrothermale*' AC55 (SD10). Carboxylic groups were derivatized to the corresponding methyl esters and alcohol moieties were derivatized to trimethyl silyl ethers prior to gas chromatographic analysis. Numbers refer to structures shown in Fig. 1.

component is a homologue of *iso*-diabolic acid, i.e. 13,16-dimethyl nonacosanoic acid (Table 2).

3.3. Branched GDGTs

The acid-hydrolyzed biomass of some of the acidobacterial cultures (see Table 2) was also analyzed for the presence of GDGTs by HPLC–APCI-MS using selected ion monitoring (SIM). However, we were unable to identify any branched GDGTs **1**–**6** in the species investigated.

3.4. Bioinformatic search for ether lipid biosynthetic genes

Recently, Lorenzen et al. (2014) described the first gene cluster in the bacterial domain that is involved in the biosynthesis of fatty acid-derived ether lipids in myxobacteria. It is composed of four genes (*elbB* – *elbE*; where elb stands for ether lipid biosynthesis), where the gene encoding the multifunctional enzyme ElbD plays a key role. Prompted by this discovery, we searched acidobacterial genomes and metagenomes (for a description see Sinninghe Damsté et al., 2017) for the genes of this cluster. In three (meta)

Table 2
Relative abundance of fatty acids and ether lipids after acid hydrolysis of cell material and general characteristics of the membrane lipids in strains of the different subdivisions of the studied *Acidobacteria*.

Component[a]	% of total lipids[b] in strain[c]																				
	SD1								SD3		SD4					SD6		SD8		SD 10	SD 23
	1[d]	1[d]	2	3	4	5	6	7	8[d]	9	10[e]	11[e]	12[e]	13[e]	14[e]	15[e]	16	17	18	19	20[e]
Fatty acids																					
C11:0																			3.2	2.4	
Iso C13											0.3		1.5	1.5	0.7			15.1	0.3	0.7	
Iso C14	0.6																		2.4		1.7
C14:1 Δ5	0.3																				
C14:1 Δ9	0.4	0.5					0.2	0.8													
C14:0	1.3	0.6			0.7		0.6	1.9	0.3							0.2		1.1	2.3	1.5	0.4
Iso C15:1 Δ7c			0.4	0.4														8.8	12.9		
Iso C15:1 Δ7t																		0.7			
Iso C15:1 Δ9c	0.7	1.1	0.3	0.3						10.8	10.3	17.0	6.8	6.8	6.5						
Iso C15:1 Δ9t											0.4	1.0			0.5						
Iso C15	31.9	35.3	41.3	42.1	38.3	53.0	28.1	18.7	25.1	47.2	20.2	15.3	23.6	25.6	30.6	6.1	25.1	29.3	50.1	55.5	28.3
Anteiso C15					0.3			0.3	0.1									4.2	4.1	1.2	4.1
C15:1Δ7	3.2																				
C15:1Δ9							0.5	0.5	0.2												
C15:0	2.7								0.3												
Iso C16:1Δ7																			0.9		
Iso C16:1Δ9					0.6														0.6		
Iso C16			0.2	0.2				0.3			0.5		0.8			0.1		0.9	5.5	0.4	12.7
C16:1Δ7	9.4								7.0									1.6	1.9		
C16:1Δ9c	12.2	18.9					22.5	27.0	17.5	4.3	2.8	2.6	5.7	7.2	0.3	5.1	12.6	1.7	1.6		
C16:1Δ9t							0.6	0.4	0.4								0.5				
C16:1(Δ10)+Δ11	0.4								0.2							0.4					
C16:0	4.4	1.7	0.7	0.7		1.1	5.3	7.5	5.3	1.6	0.5	0.8	0.9	3.3		2.8	2.6	10.9	9.8	3.9	5.3
Iso C17:1Δ9c	1.0	1.2	19.1	18.6	12.3	13.0	0.9	0.5	1.4	23.9	1.0	3.7	5.8	4.9		15.1	8.8	3.6[f]	3.3[f]		
Iso C17:1Δ9t					0.3	1.1															
10-methyl-C16:0																		2.9			
Iso C17:0	0.4	0.2	4.5	4.6	10.3	8.6	2.9	1.6	2.5	4.2		0.5	1.8	2.4		2.8	3.8	1.5	0.7	32.6	23.7
Anteiso C17					0.8	1.5	0.3	0.4	0.6							0.1				0.9	11.0
C17:1Δ9	1.9									0.9			0.9	0.4			0.5				
C17:0							0.5	0.2	0.3	0.6											
Iso C18:0																					9.1
C18:1Δ9	2.4	4.2			0.7				1.0		1.9	1.1	0.9	1.3			13.7	1.0			
C18:1Δ11									0.3							19.5					
C18:1Δ13									0.4												
C18:0	1.5	1.5			0.5		0.6	0.7	0.7	1.1	0.5	0.8		0.9		3.4	3.0	4.4	0.4	0.8	1.4
Iso C19:1Δ11			0.6	0.6							0.3[g]										
Iso C19																					1.6
Anteiso C19:0																					0.7
C20:0											1.1					0.4					
Iso C21:0											1.9	1.0	2.0	2.5	2.3						
Iso-diabolic acid (**7**)	25.3	31.6	32.8	32.6	35.2	21.6	37.3	38.9	34.1		1.7	1.2	1.4	1.3	1.4	30.4	28.5				
6-methyl *iso*-diabolic acid (**8**)		3.0						0.3	2.2							13.7	0.9				
Ethers																					
2-*iso* C_{15}-MGE										0.7								5.7			
1-*iso* C_{15}-MGE (**11**)										2.4	20.3	15.1	15.1	20.0	18.7			1.8			
2-C16:1-MGE																		0.6			
2-C16:0-MGE																		0.3			
Iso C16-MGE													0.8								
1-C16-MGE											1.5	0.6	2.0					1.0			
2-*iso* C17:1-MGE																		0.4			
1-*iso* C17-MGE												2.9	1.0					0.5			
1,2-*iso* C15-DGE (**12**)										2.3								1.5			
1,2-ante*iso* C15-																		0.3			

DGE *Iso*-diabolic acid-MGE (**9**)											29.3	33.5	21.0	17.3	25.6						
5-methyl *iso*-diabolic acid-MGE (**10**)											5.5	2.9	8.0	4.6	13.2						
Mono-unsaturation (%)[h]	31	25	19	18	13	13	24	28	27	39	19	29	22	22	9	37	34	18	21	0	0
Membrane-spanning (%)[h]	26	35	34	33	36	22	38	40	37	0	35	36	29	22	38	47	31	0	0	0	0
Ether moieties (%)[h]	0	0	0	0	0	0	0	0	0	4	34	31	28	25	33	0	0	9	0	0	0
Methylation iso-diabolic acid (%)	0	8	0	0	0	0	0	0	6	na	15	8	26	19	32	30	3	na	na	na	na
Iso C_{15}-based (%)	59	73	77	77	76	76	67	59	63	63	88	87	76	74	97	53	57	46	63	57	30

[a] Bold numbers refer to structures in Fig. 1; MGE = monoalkyl glycerol ether; DGE = dialkylglycerol ether.
[b] Normalized on the sum of the FID-integrated GC peak areas of all the lipids listed. Values for major components (i.e., >5%) are underlined.
[c] Strains: (1) *Ca.* Koribacter versatilis Ellin345; (2) *Telmatobacter* sp. 15-8A; (3) *Telmatobacter* sp. 15–28; (4) *Acidicapsa* sp. CE1; (5) *Acidicapsa* sp. CE14; (6) *Granulicella aggregans* TPB6028^T; (7) *Granulicella* sp. AF10; (8) *Paludibaculum fermentans* P105^T; (9) *Ca.* Solibacter usitatus Ellin6076, (10) *Brevitalea deliciosa* Ac_16_C4^T; (11) *Tellurimicrobium multivorans* Ac_18_E7^T; (12) *Stenotrophobacter roseus* Ac_15_C4^T; (13) *Stenotrophobacter namibiensis* Ac_17_F2^T; (14) *Arenimicrobium luteum* Ac_12_G8^T; (15) *Vicinamibacter silvestris* Ac_5_C6^T; (16) *Luteitalea pratensis* HEG_-6_39^T; (17) *Holophaga foetida* TMBS4^T; (18) *Geothrix fermentans* H-5^T; (19) '*Thermotomaculum hydrothermale*' AC55; (20) *Thermoanaerobaculum aquaticum* MP01^T.
[d] Different cultures of the same species.
[e] Strains that were tested for the presence of brGDGTs.
[f] Also contains some *iso* C17:1 Δ7.
[g] Also contains some *iso* C19:1 Δ9.
[h] Calculated on a molar basis.

Table 3
Kovats retention indices[a] of *iso*-diabolic acids and the corresponding hydrocarbons for different strains[b] of SDs 1, 3, 4 and 6 of *Acidobacteria.*

Carbon skeleton	Hydrocarbon				Diacid			
	SD1 (1)	SD3 (2)	SD4[c] (3)	SD6 (4)	SD1 (5)	SD3 (2)	SD4 (6)	SD6 (4)
13,16-Dimethyloctacosane	2856, 2863	2856, 2864	2855, 2862	2856, 2862	3489	3484, 3490	3487	3483, 3489
6,13,16-Trimethyloctacosane	–	–	–	2900[d], 2905	3525	3517, 3525	–	3521, 3525
5,13,16-Trimethyloctacosane	–	–	2909	–	–	–	–	–
13,16-Dimethylnonasacosane	–	–	–	–	–	–	3588	–

[a] Measured on a 50 m CP-Sil5 CB capillary column (0.32 mm i.d., d_f = 0.12 μm). In most cases two values are given for two closely eluting components likely representing two different diastereomers. Two GC-separable diastereomers were also reported for synthetic 13,16-dimethyloctacosane (Chappe et al., 1980). The retention index of the most abundant isomer is underlined.
[b] Strains: (1) *Acidobacteriaceaea* A2_4c; (2) *Paludibaculum fermentans* P105^T; (3) *Brevitalea aridisoli* Ac_11_E3^T; (4) *Vicinamibacter silvestris* Ac_5_C6^T; (5) *Ca.* Koribacter versatilis Ellin345; (6) *Chloroacidobacterium thermophilum* B^T. Strains (1), (3), and (6) have been characterized previously (Sinninghe Damsté et al., 2011, 2014).
[c] The hydrocarbons measured had previously been obtained by subjecting a fraction containing MGEs **9** and **10** $LiAlH_4$ reduction followed by HI degradation and hydrogenation (Sinninghe Damsté et al., 2014).
[d] Because of co-elution with the *n*-C_{29} alkane used for the measurement of the Kovats retention indices, this value is less accurate.

genomes of SD 4 the *elbB – elbE* gene cluster was identified and has a close resemblance to the organization of this cluster in deltaproteobacteria (Fig. 3a). The *elbD* gene of the acidobacteria was 43–48% similar (at the protein level) to that of *Myxococcus xanthus* DK 1622 and phylogenetically it is associated with the cluster formed by the deltaproteobacteria (Fig. 3b). The *elbD* gene of Acidobacterium OLB17, an acidobacterium present in the sludge of a wastewater treatment plant, was divided into two adjacent genes (Fig. 3a). The *elbB – elbE* gene cluster was not identified in the three available genomes of *Chloracidobacterium thermophilum*, which has also been classified as an SD 4 acidobacterium (Bryant et al., 2007; Tank and Bryant, 2015).

4. Discussion

4.1. Occurrence of iso-diabolic acid

In this study we determined the occurrence of the membrane spanning lipid *iso*-diabolic acid in four previously uninvestigated SDs of the Acidobacteria for which cultured relatives are currently available (i.e., SDs 6, 8, 10 and 23). Unfortunately, the number of isolated strains in these SDs is rather limited. Nevertheless, a clear-cut division between the occurrence of *iso*-diabolic acid was observed; the two species of SD 6 did contain *iso*-diabolic acid in considerable amounts (30–45% including the methylated derivatives), whereas the SD 8, 10, and 23 strains did not contain *iso*-diabolic acid. The data of newly studied strains from SDs 1, 3, and 4 (Table 2) confirmed the previous conclusion (Sinninghe Damsté et al., 2011, 2014) that they are all capable of producing *iso*-diabolic acid. An exception is the SD 3 acidobacterium "*Ca.* Solibacter usitatus": it is the only species of all 40 strains from SDs 1, 3, 4, and 6 examined so far where *iso*-diabolic acid was not detected. Remarkably, it is also characterized by a high abundance (i.e., almost 50%; Table 2) of *iso*-C_{15} fatty acid, the presumed building block of *iso*-diabolic acid (Sinninghe Damsté et al., 2011). This is one of the highest abundances of this fatty acid detected so far in acidobacteria. Perhaps this species is lacking the as yet unknown gene(s) required for the coupling of two *iso*-C_{15} fatty acid

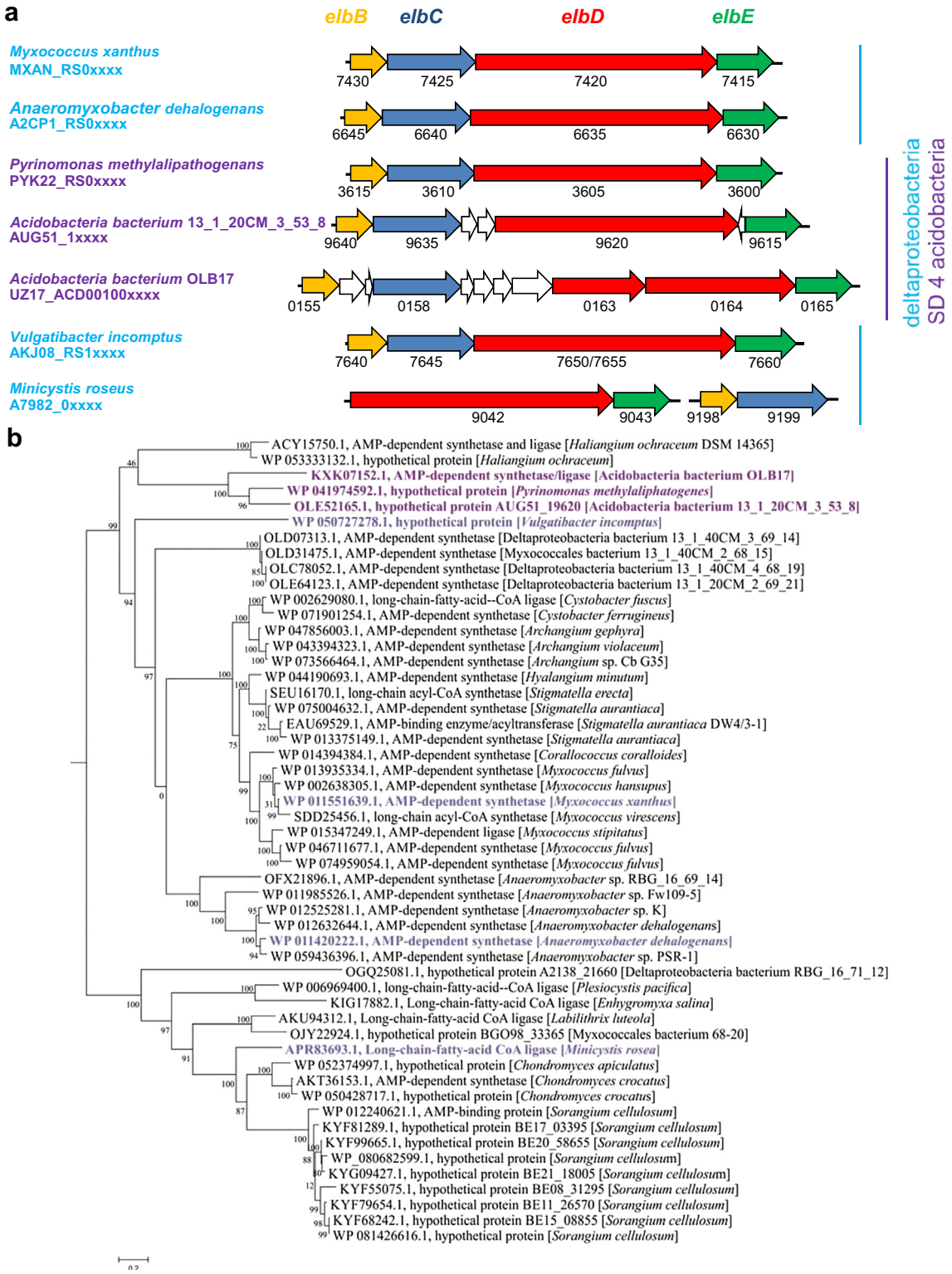

Fig. 3. Identification of the ether lipid biosynthesis gene cluster (*elbB – elbD*) in genomes and metagenomes of SD 4 Acidobacteria. (a) Constitution of the gene cluster in comparison with four species of the deltaproteobacteria including *M. xanthus* DK 1622, where this gene cluster was first identified (Lorenzen et al., 2014). This shows a highly similar arrangement of this gene cluster in SD 4 Acidobacteria and the deltaproteobacteria. The genes were identified by BLAST protein searches using the protein sequences of *elbB – elbD* of *M. xanthus* as queries; the various genes are indicated by different colors. Stippled lines indicate a distance between the genes. The numbers refer, in combination with the code below the species name, to the locus tags in the annotated genomes from the NCBI database. These numbers typically increase by 5 for every next gene. (b) Phylogenetic tree of the ElbD proteins encoded by the SD 4 acidobacterial genomes of a cultured strain and environmental genomes in comparison with those of a number of selected deltaproteobacteria revealing their close relatedness. The tree was constructed using the maximum likelihood method with a LG model plus gamma distribution and invariant sites (LG + G + I). The analysis included 2531 positions in the final dataset. The scale bar represents the number of amino acid substitutions per site. Branch support was calculated with the approximate likelihood ratio test (aLRT) and values ≥50% are indicated on the branches.

moieties resulting in the formation of *iso*-diabolic acid, or perhaps the culture conditions used did not lead to expression of these unknown gene(s).

The lipid profiles of the five additional strains of SD 4 predominantly reveal *iso*-diabolic acid in an ether-bound form (Table 2), confirming earlier findings of SD 4 strains (cf. Sinninghe Damsté et al., 2014). The single exception remains the SD 4 acidobacterium *C. thermophilum*, which does produce *iso*-diabolic acid but not in an ether-bound form (Sinninghe Damsté et al., 2014). In the 16S rRNA gene tree *C. thermophilum* is also clearly separated from the other SD 4 Acidobacteria (Fig. 4). This distinct taxonomic position of *C. thermophilum* is consistent with its physiological capabilities. It is the only known phototrophic member of the Acidobacteria (Bryant et al., 2007), while all other known species are organotrophs (Kielak et al., 2016). It is also the only SD 4 acidobacterium that produces hopanoids (Sinninghe Damsté et al., 2017; Fig. 4), signifying its unique (chemo)taxonomic position. Interestingly, we identified the *elbB – elbE* gene cluster, which is presumed to be responsible for ether bond formation in bacteria (Lorenzen et al., 2014), in all available whole genomes and (almost) complete metagenomes of SD 4 Acidobacteria, but not in *C. thermophilum* or any other acidobacterium belonging to other SDs. This fits well with the chemotaxonomic observations: only SD 4 Acidobacteria (excepting *C. thermophilum*) produce *iso*-diabolic acid bound through an ether linkage to glycerol at position C-1 in substantial amounts.

Although myxobacteria produce an array of ether lipids (Ring et al., 2006), Lorenzen et al. (2014) showed that the *elbB – elbE* gene cluster controlled the production of 1-O-13-methyl tetradecyl glycerol (**11**), even though the exact biochemical mechanism remains unclear. This MGE may also be an important intermediate product in the biosynthesis of ether lipids in SD 4 Acidobacteria. The phylogeny of these genes (e.g., Fig. 3b) suggests that the ancestor of the SD 4 Acidobacteria obtained the capacity to produce these ether lipids through lateral gene transfer of this *elbB – elbE* gene cluster from the deltaproteobacteria. Indeed, many of the species of the order *Myxococcales*, which show the closest relationship with the SD 4 Acidobacteria in terms of ElbD phylogeny (Fig. 3b), have been isolated from soil. Soil is also a niche for SD 4 Acidobacteria, which would enable the exchange of genes. The SD 4 Acidobacteria forming the branch composed of *C. thermophilum* (Fig. 4) are an exception: the lateral gene transfer event may have

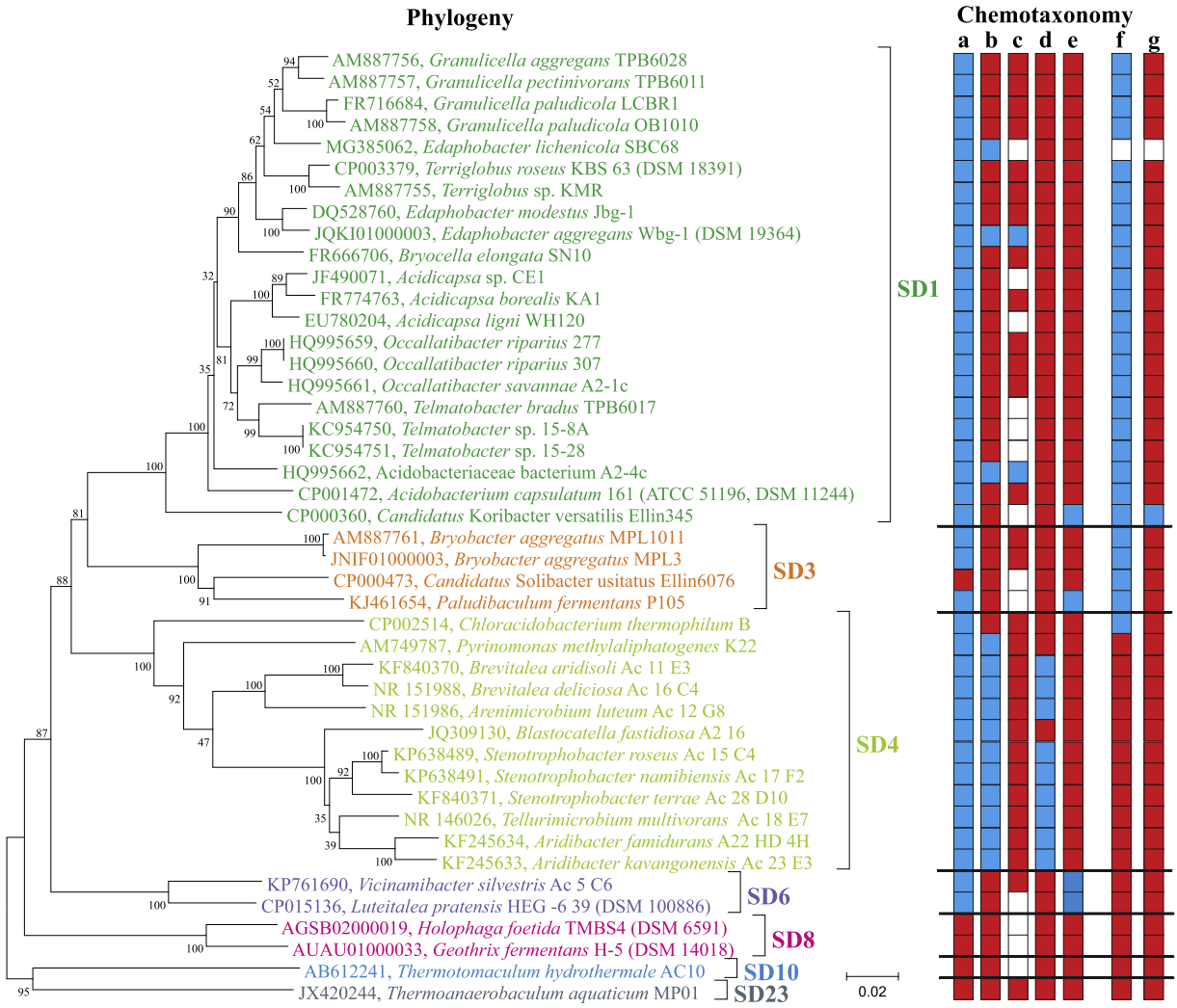

Fig. 4. Chemotaxonomic traits of Acidobacteria indicated in a phylogenetic tree of the nearly complete 16S rRNA gene sequences of the Acidobacteria that have been analyzed in this and previous studies (Sinninghe Damsté et al., 2011, 2014). The various SDs are indicated in different colors. Chemotaxonomic traits indicated are the occurrence of (a) *iso*-diabolic acid acid, (b) *iso*-diabolic acid MGE, (c) brGDGTs, (d) 5-methyl *iso*-diabolic acid MGE, (e) 6-methyl *iso*-diabolic acid and, for reference (data of Sinninghe Damsté et al., 2017), (f) hopanoids, and (g) methylated hopanoids. Red color indicates absence, blue color indicates confirmed presence, white color means not determined. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Scale bar indicates 2% sequence divergence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

happened after the evolution of this group or it may have lost this capacity.

4.2. General chemotaxonomy

When we compare all available lipid profiles of Acidobacteria from this and previous studies (Sinninghe Damsté et al., 2011, 2014) (see Supplementary Tables; 46 strains in total), a consistent pattern emerges. The general chemotaxonomic signature, i.e. ester- or ether-bound *iso*-diabolic acid present in SD 1, 3, 4 and 6 but not in SD 8, 10, and 23 Acidobacteria, generally fits with the 16S rRNA gene molecular phylogeny of the Acidobacteria since SDs 1, 3, 4 and 6 are more closely related to each other than to SDs 8, 10, and 23 (Fig. 4). This also becomes evident when the contribution of the membrane-spanning lipids is calculated; in SDs 1, 3, 4 and 6 these typically amount to 25–40%, whereas in SDs 8, 10, and 23 membrane-spanning lipids do not occur (Fig. 5c). The degree of ether linkages in the membrane (Fig. 5d) is generally low; only the SD 4 Acidobacteria have on average 30% with ether linkages (excepting *C. thermophilum*; see Section 4.1).

However, there are some strains that produce small amounts of ether lipids. Two SD 1 acidobacterial species, *Edaphobacter aggregans* Wbg-1 ^{T} and *Acidobacteriaceae* bacterium strain A2-4c, produce small amounts of ether-bound *iso*-diabolic acid and *iso*-C_{15} MGE (Sinninghe Damsté et al., 2011). The SD 3 acidobacterium "*Ca.* Solibacter usitatus" produces small amounts of the *iso*-C_{15} MGE (predominantly at position 1 but also at position 2; Table 2) and an 1,2-dialkyl glycerol ether (DGE) containing two the *iso*-C_{15} units. These ether lipids together with some others (Table 2) also occur in the SD 8 acidobacterium *Holophaga foetida* TMBS4^{T} in small relative amounts. This suggests that membrane ether lipids in these bacteria can perhaps also be produced by biochemical pathways other than that catalyzed by the enzymes encoded by the *elbB* – *elbE* gene cluster, which was exclusively detected in SD 4 acidobacteria.

All studied acidobacteria contain *iso*-C_{15} fatty acid as an important building block for the membrane lipids. Even in acidobacteria that do not produce *iso*-diabolic acid (SDs 8, 10, and 23), *iso*-C_{15} fatty acid-derived lipids still represent 30–56% of the lipids (Fig. 5b). The degree of unsaturation of the membrane lipids varies considerably (Fig. 5a). However, this is likely related to the adjustment to physiological conditions rather than to the genetic make-up since all the strains that do not contain unsaturated lipids are thermophilic (SD 4: *C. thermophilum* and *Pyrinomonas methylaliphatogenes*; SD 10: *'Thermotomaculum hydrothermale'*; SD 23: *Thermoanaerobaculum aquaticum*). It is well known that bacteria adjust the degree of unsaturation of lipids according to growth temperature with increasing fractional abundances of unsaturated lipids at low temperatures (e.g., Chintalapati et al., 2004).

4.3. Acidobacteria as a potential source for brGDGTs.

BrGDGTs occur ubiquitously in soil, peat bogs, lakes, and coastal marine sediments (see Schouten et al., 2013 for a review), suggesting that their source must be a common and abundant group of bacteria in the environment. Acidobacteria have been proposed as a potential candidate group for the production of brGDGTs because both occur abundantly in peat bogs (Weijers et al., 2009). This prompted our earlier work on cultures of Acidobacteria (Sinninghe Damsté et al., 2011). The identification of the presumed "building block" of brGDGTs (*iso*-diabolic acid) in all investigated cultures of SDs 1 and 3 Acidobacteria was an important step in this respect. However, *iso*-diabolic acid occurred predominantly in an ester-bound form and not in an ether-bound form. Trace amounts of brGDGT **1** were detected in two SD 1 strains but the amounts were so low that other Acidobacteria were considered as the predominant producers of the brGDGTs. A subsequent study of seven different strains of SD 4 Acidobacteria revealed that they also do not produce brGDGTs (Sinninghe Damsté et al., 2014). However, six of the seven investigated strains produced lipids in which *iso*-diabolic acid occurs ether-bound to a glycerol moiety (i.e., MGE **5**) in high relative amounts. Hence, the hypothetical diester/diether lipid **13**, composed of two esterified MGE **9** units, is thought to be an important building block of the membrane of most members of SD 4. It has the closest structural resemblance to brGDGTs **1**; only the two ester bonds at the *sn2* position have to be changed into ether bonds. The results of the six other strains of SD 4 Acidobacteria reported here confirm these findings since they also contain relatively high amounts of MGE **9/10**. However, the examination of the other SDs (i.e., SDs 6, 8, 10, and 23) did not lead to new clues towards the biological origin of the brGDGTs. SD 6, an environmentally significant SD (e.g., Jones et al., 2009; Zimmermann et al., 2012), does produce *iso*-diabolic acid but only in an ester-bound form like most members of SDs 1 and 3, and the other SDs investigated in this study do not produce *iso*-diabolic acid at all. Furthermore, we were able to identify a gene cluster that potentially codes the enzymes involved in the production of the ether bond at the *sn1*-position in all but one SD 4 species for which genome data are available (Fig. 3). However, this gene cluster is not present in any of the other genomes of isolated Acidobacteria or in environmental genomes of Acidobacteria, suggesting that the occurrence of ether-bound *iso*-diabolic acid is limited to most members of SD 4. Although new isolates of as yet uncultivated SDs of Acidobacteria may still reveal the production of brGDGTs, the quest for the bacterial producers of brGDGTs in the environment may need to be extended to other bacterial phyla. Unfortunately, this quest is hampered by the limited knowledge of the enzymes that are involved in the biosynthesis of *iso*-diabolic acid, specifically the reaction step in which two C_{15} *iso*-fatty acids are condensed. This knowledge would enable the search of (meta)genomic data for the potential to biosynthesize *iso*-diabolic acid.

In the environment brGDGTs are not only found as the parent component **1** but also with additional methylation of the alkyl chain(s). Initially, this additional methyl group was identified to be positioned at C-5 based on the isolation of **2** from a Dutch peat and subsequent structural determination by NMR spectroscopy (Sinninghe Damsté et al., 2000). Subsequently, other brGDGT isomers with additional methyl groups at different positions have been identified (e.g., Liu et al., 2012; Weber et al., 2015; Ding et al., 2016) of which the 6-methyl brGDGTs **4**–**6** (De Jonge et al., 2013, 2014a) are the most abundant in addition the 5-methyl brGDGTs **1**–**3**. However, SD 1 and 3 Acidobacteria did not contain *iso*-diabolic acid with an additional methyl substituent (Sinninghe Damsté et al., 2011). The detection of 5-methyl *iso*-diabolic acid-MGE **9** in five out of seven species of SD 4 Acidobacteria (Sinninghe Damsté et al., 2014) bridged this gap. This is confirmed by our analysis of six additional species of SD 4 Acidobacteria; all species contain 5-methyl *iso* diabolic acid-MGE **10**, sometimes in relatively high abundance (Table 2). The SD 6 acidobacterium *Vicinamibacter silvestris* also contains substantial amounts of a methylated *iso*-diabolic acid but in this case the methyl group is not positioned at C-5 but at C-6. Smaller amounts of 6-methyl *iso*-diabolic acid **8** were also encountered in two species of SDs 1 and 3 and the other SD 6 acidobacterium examined, *Luteitalea pratensis* (Table 2). *V. silvestris* was isolated from an alkaline (pH 7.5–8.0) savannah soil, has a wide pH tolerance (4.7–9.0), and grows optimally at pH 7.0 (Huber et al., 2016). *L. pratensis* displays a narrower pH tolerance (5.3–8.3) (Vieira et al., 2017) and grows optimally at a pH of ca. 6.1–7.5. These strains are phylogenetically (16S rRNA gene) closely related to 11 other isolated strains from a slightly alkaline soil (George et al., 2011). Hence, in contrast to the other Acidobacteria, SD 6 strains seem better

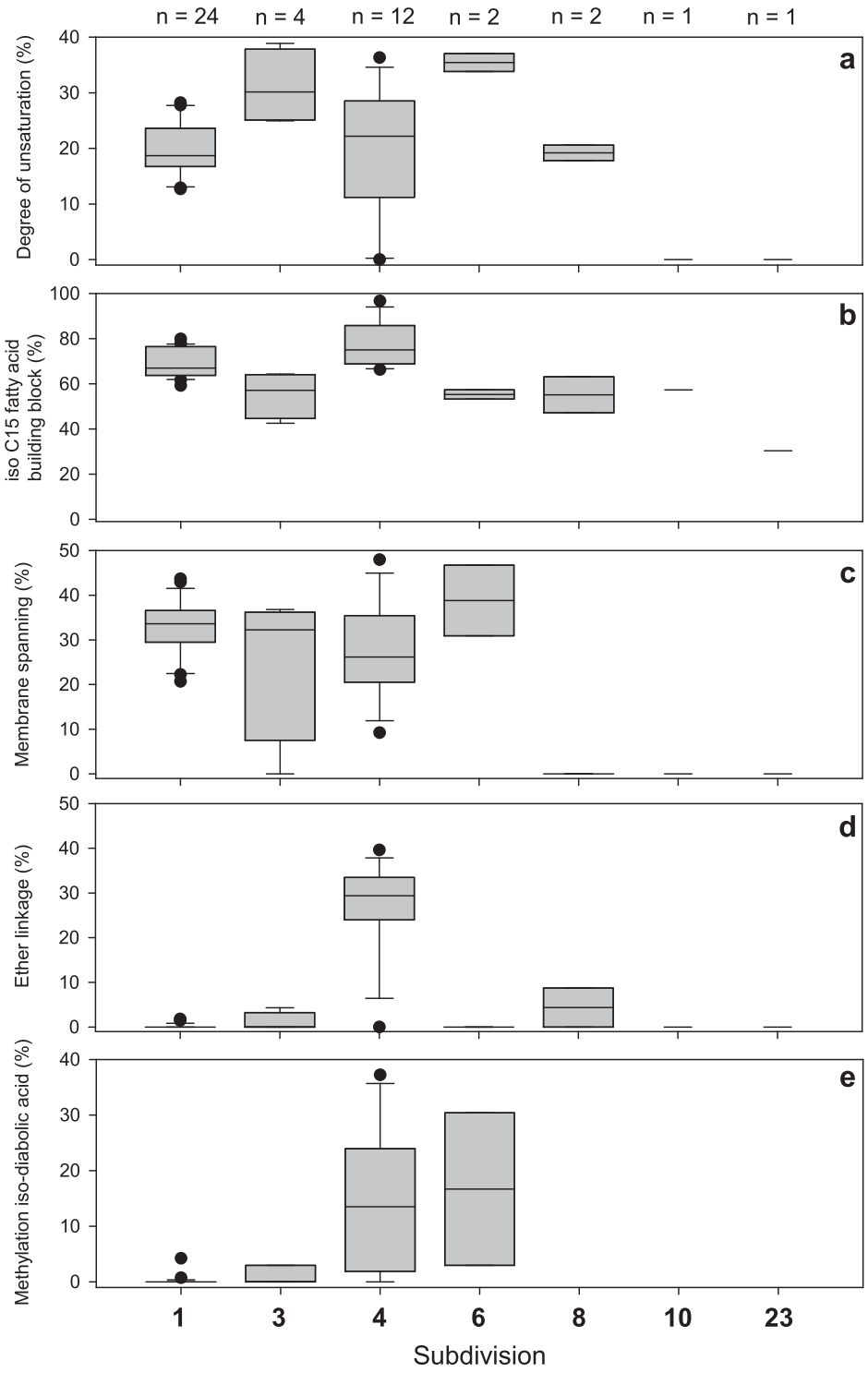

Fig. 5. Box plots for various characteristics of the membrane lipid composition of SDs 1, 3, 4, 6, 8, 10 and 23 based on this and previous studies (Sinninghe Damsté et al., 2011, 2014). (a) The degree of unsaturation (i.e., one double bond). (b) The contribution of the C_{15} *iso* fatty acid as a presumed building block to the membrane, assuming that also esterified and ether-linked *iso*-diabolic acid and its methylated counterparts, are derived from the *iso*-C_{15} fatty acid. (c) The contribution of membrane-spanning lipids (i.e., *iso*-diabolic acid and its methylated counterparts) to the membrane. (d) Contribution of ether instead of ester linkages to the membrane. (e) The degree of methylation (in addition to the two mid-chain methyl groups) of esterified and ether-linked *iso*-diabolic acid in the membrane. Since SD 8, 10, and 23 Acidobacteria do not contain *iso*-diabolic acid values cannot be given for these bacteria. The data are derived from 46 different strains summarized in the Supplementary Information. In cases where two separate batch cultures of one strain were studied, the average values were used to compile this plot.

adjusted to more alkaline conditions than most other Acidobacteria of SDs 1 and 3, which commonly do not grow at a pH > 7.0 (see Kielak et al., 2016 for an overview). In soils, river water, and lakes, 6-methyl brGDGTs are found in higher relative abundances at higher pH conditions (De Jonge et al., 2014a,b; Yang et al., 2015; Russell et al., 2018). Hence, the detection of 6-methyl *iso*-diabolic acid-MGE in SD 6 Acidobacteria isolated from slightly alkaline soils is in line with what we observe for brGDGTs in the environment. This, together with the fact that the additional methyl groups of the methylated *iso*-diabolic acid are at exactly

the same position as those of the environmental brGDGTs (i.e., at C-5 and C-6), would still be in line with the hypothesis that brGDGTs derive from Acidobacteria.

4.4. Implications for the use of brGDGTs in paleoenvironmental assessment

The distribution of brGDGTs in sediments and soils is used to reconstruct past pH and temperature based on a set of empirical relationships (e.g., Weijers et al., 2007; Peterse et al., 2010; De Jonge et al., 2014a; Sinninghe Damsté, 2016; Naafs et al., 2017; Dearing Compton-Flood et al., 2018; Russell et al., 2018), which are thought to reflect the ability of bacteria in soil, peat, lake water and marine sediments to adjust their brGDGT-based membrane composition in response to temperature and pH. Three main responses have been noted:

(1) an increase in the relative abundance of the 6-methyl brGDGTs at increasing pH;
(2) an increase in the number of cyclopentane moieties with increasing pH; and
(3) an increase in the degree of methylation at position C-5 of the brGDGTs with decreasing temperature.

With respect to (1), as discussed in the previous section, the biosynthesis of 6-methyl *iso*-diabolic acid by SD 6 Acidobacteria that are more common in alkaline soils, does provide biological support for this empirical observation and for the use of compositional changes in brGDGT distributions to reconstruct past pH. With respect to (2), our data do not provide support for this empirical relationship simply because the formation of cyclopentane moieties by an internal cyclization reaction as proposed for the formation of cyclized brGDGTs (Weijers et al., 2006) has not been observed for *iso*-diabolic acid or its derivatives in any of the cultures studied. With respect to (3), the two thermophilic species (i.e., *P. methylaliphatogenes* and *C. thermophilum*) within the group of studied SD 4 species produce no additionally methylated *iso*-diabolic acid or its derivatives. In contrast, nine out of the ten mesophilic SD 4 *Acidobacteria* produce these components (Table 2; Sinninghe Damsté et al., 2014), with *Stenotrophobacter terrae*, *S. roseus*, and *Arenimicrobium luteum* containing them in the highest relative abundance (i.e., 26–32%). Among these members of SD 4, *S. terrae* has the lowest optimal growth temperature range (Foesel et al., 2013; Losey et al., 2013; Crowe et al., 2014; Huber et al., 2014; Wüst et al., 2016; Pascual et al., 2015; Tank and Bryant, 2015). These results indeed suggest that the degree of methylation may be an adaption to temperature. It should be noted, however, that most of the non-thermophilic members of SD 4 were isolated from Namibian savannah soils and still have optimal growth temperatures at >30 °C, which is still fairly high and probably explains why the degree of methylation is still <25% in most cases. Furthermore, it should be tested in culture if the degree of additional methylation of *iso*-diabolic acid is indeed a physiological response. Such experiments have been performed with the thermophile, *P. methylaliphatogenes* (Sinninghe Damsté et al., 2014), in the 50–69 °C range but, although some changes in membrane lipid composition were observed, additional methylation of *iso*-diabolic acid was not observed. For the studied species of SDs 1 and 3 the degree of methylation is much lower (up to 8%), highly variable between different batches of cultures (Table 2), and 6-methyl *iso*-diabolic acid occur in only two of 26 examined species (Table 2; Sinninghe Damsté et al., 2011). This is surprising since the optimal growth temperature range (see Kielak et al., 2016 for an overview) is typically lower than that of the studied SD 4 species. Although temperature experiments need to be performed to test if additional methylation of *iso*-diabolic acid may also be a physiological response, these data suggest that there is also a genetic control (i.e., not all Acidobacteria may be able to synthesize methylated *iso*-diabolic acid). Quite a number of the SD 1 and 3 acidobacterial strains have been isolated from peat bogs (Kielak et al., 2016). Furthermore, environmental studies also show SD 1 Acidobacteria to occur abundantly in these environments (Weijers et al., 2009; Serkebaeva et al., 2013). This may perhaps explain why in peat systems the brGDGT distribution is often dominated by the tetramethylated core structure (Sinninghe Damsté et al., 2000; Weijers et al., 2006, 2009) and why the degree of methylation of brGDGTs shows a different response with temperature (Naafs et al., 2017). Altogether, this may indicate that the differences we observe in brGDGT distributions in the environment may not only reflect physiological responses but may also be related to compositional changes in the (acido)bacterial species. This may explain why different environments have different temperature calibrations for brGDGTs.

5. Conclusions

Detailed investigation of 46 acidobacterial strains from seven SDs has revealed that only two of them produce small amounts of brGDGTs. However, the strains of SDs 1, 3, 4, and 6 do produce the presumed building block of brGDGTs, *iso*-diabolic acid, as an important constituent of their membranes. In members of SD 4 *iso*-diabolic acid occurs predominantly in an ether-bound form and is connected at the C-1 position to the glycerol moiety. A specific gene cluster present in all but one available (meta)genomes of the SD 4 species is probably responsible for the formation of this ether bond and is probably inherited through lateral gene transfer from deltaproteobacteria. This gene cluster is absent in known genomes of cultured species of other SDs and in Acidobacteria genomes recovered from metagenomes, including those belonging to SDs for which no cultured relatives are available. This suggests that the quest for the biological sources of brGDGTs should be extended into other bacterial phyla. Methylation of *iso*-diabolic acid occurs at exactly the same positions (i.e., C-5 and C-6) as observed for brGDGTs in the environment. Most SD 4 species produce the glycerol ether derivative of 5-methyl *iso*-diabolic acid, while members of SD 6, and to a much lesser extent SDs 1 and 3 produce 6-methyl *iso*-diabolic acid. These observations support the empirical use of the degree of methylation of 5-methyl brGDGTs as a temperature proxy and the use of the relative abundance of the 6-methyl brGDGTs as a pH proxy.

Acknowledgements

We thank David Naafs and an anonymous referee for their reviews. This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement number 694569 – MICROLIPIDS). JSSD also receives funding from the Netherlands Earth System Science Center (NESSC) and Soehngen Institute for Anaerobic Microbiology (SIAM) through gravitation grants from the Dutch Ministry for Education, Culture and Science (grant numbers 024.002.001 and 024.002.002). SND was supported by the Russian Science Foundation (project no. 16-14-10210).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.orggeochem.2018.07.006.

Associate Editor—**Philip Meyers**

References

Barns, S.M., Cain, E.C., Sommerville, L., Kuske, C.R., 2007. Acidobacteria phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. Applied and Environmental Microbiology 73, 3113–3116.

Belova, S.E., Suzina, N.E., Rijpstra, W.I.C., Sinninghe Damsté, J.S., Dedysh, S.N., 2018. *Edaphobacter lichenicola* sp. nov., a member of the family Acidobacteriaceae from lichen-dominated forested tundra. International Journal of Systematic and Evolutionary Microbiology 68, 1265–1270.

Bryant, D.A., Costas, A.M.G., Maresca, J.A., Chew, A.G.M., Klatt, C.G., Bateson, M.M., Tallon, L.J., Hostetler, J., Nelson, W.C., Heidelberg, J.F., Ward, D.M., 2007. *Candidatus* Chloracidobacterium thermophilum: An aerobic phototrophic acidobacterium. Science 317, 523–526.

Chappe, B., Michaelis, W., Albrecht, P., 1980. Molecular fossils of Archaebacteria as selective degradation products of kerogen. Physics and Chemistry of the Earth 12, 265–274.

Chintalapati, S., Kiran, M.D., Shivaji, S., 2004. Role of membrane lipid fatty acids in cold adaptation. Cellular and Molecular Biology 50, 631–642.

Coates, J.D., Ellis, D.J., Gaw, C.V., Lovley, D.R., 1999. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer. International Journal of Systematic Bacteriology 49, 1615–1622.

Colcord, D.E., Pearson, A., Brassell, S.C., 2017. Carbon isotopic composition of intact branched GDGT core lipids in Greenland lake sediments and soils. Organic Geochemistry 110, 25–32.

Crowe, M.A., Power, J.F., Morgan, X.C., Dunfield, P.F., Lagutin, K., Rijpstra, W.I.C., Vyssotski, M., Sinninghe Damsté, J.S., Houghton, K.M., Ryan, J.L.J., Stott, M.B., 2014. *Pyrinomonas alimethylogenes* gen. nov. sp. nov., a novel group 4 thermophilic Acidobacteria from geothermal soils. International Journal of Systematic and Evolutionary Microbiology 64, 220–227.

Dearing Crampton-Flood, E., Peterse, F., Munsterman, D., Sinninghe Damsté, J.S., 2018. Extracting Pliocene continental air temperature evolution in North Western Europe from tetraether lipids archived in North Sea Basin sediments. Earth and Planetary Science Letters 490, 193–205.

Dedysh, S.N., Sinninghe Damsté, J.S., 2018. Acidobacteria. In: eLS. John Wiley & Sons Ltd, Chichester. http://doi.org/10.1002/9780470015902.a0027685.

De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E., Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched glycerol dialkyl glycerol tetraethers in peat using HPLC-MS^2, GC-MS and GC-SMB-MS. Organic Geochemistry 54, 78–82.

De Jonge, C., Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2014a. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: Implications for palaeoclimate reconstruction. Geochimica et Cosmochimica Acta 141, 97–112.

De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Sinninghe Damsté, J.S., 2014b. In-situ produced branched glycerol dialkyl glycerol tetraethers in suspended particulate matter from the Yenisei River, Eastern Siberia. Geochimica et Cosmochimica Acta 125, 476–491.

Ding, S., Schwab, V.F., Ueberschaar, N., Roth, V.N., Lange, M., Xu, Y., Gleixner, G., Pohnert, G., 2016. Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl glycerol tetraethers in lake sediments. Organic Geochemistry 102, 52–58.

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5, 113.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Foesel, B.U., Rohde, M., Overmann, J., 2013. *Blastocatella fastidiosa* gen. nov., sp. nov., isolated from semiarid savanna soil – The first described species of Acidobacteria subdivision 4. Systematic and Applied Microbiology 36, 82–89.

Foesel, B.U., Nägele, V., Naether, A., Wüst, P.K., Weinert, J., Bonkowski, M., Lohaus, G., Polle, A., Alt, F., Oelmann, Y., Fischer, M., Friedrich, M.W., Overmann, J., 2014. Determinants of *Acidobacteria* activity in German grassland and forest soils. Environmental Microbiology 16, 658–675.

George, I.F., Hartmann, M., Liles, M.R., Agathos, S.N., 2011. Recovery of as-yet-uncultured soil Acidobacteria on dilute solid media. Applied and Environmental Microbiology 77, 8184–8188.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59, 307–321.

Hopmans, E.C., Schouten, S., Pancost, R.D., van der Meer, M.T., Sinninghe Damsté, J. S., 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. Rapid Communications in Mass Spectrometry 14, 585–589.

Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved chromatography on GDGT-based palaeoproxies. Organic Geochemistry 93, 1–6.

Huber, K.J., Wüst, P.K., Rohde, M., Overmann, J., Foesel, B.U., 2014. *Aridibacter famidurans* and *Aridibacter kavangonensis*, two novel species of Acidobacteria subdivision 4 isolated from semiarid savanna soil. International Journal of Systematic and Evolutionary Microbiology 64, 1866–1875.

Huber, K.J., Geppert, A.M., Wanner, G., Fösel, B.U., Wüst, P.K., Overmann, J., 2016. The first representative of the globally widespread subdivision 6 Acidobacteria, *Vicinamibacter silvestris* gen. nov., sp. nov., isolated from subtropical savannah soil. International Journal of Systematic and Evolutionary Microbiology 66, 2971–2979.

Izumi, H., Nunoura, T., Miyazaki, M., Mino, S., Toki, T., Takai, K., Sako, Y., Sawabe, T., Nakagawa, S., 2012. *Thermotomaculum hydrothermale* gen. nov., sp. nov., a novel heterotrophic thermophile within the phylum Acidobacteria from a deep-sea hydrothermal vent chimney in the Southern Okinawa Trough. Extremophiles 16, 245–253.

Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology 72, 1719–1728.

Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME Journal 3, 442–453.

Joseph, S.J., Hugenholtz, P., Sangwan, P., Osborne, C.A., Janssen, P.H., 2003. Laboratory cultivation of widespread and previously uncultured soil bacteria. Applied and Environmental Microbiology 69, 7210–7215.

Jukes, T.H., Cantor, C.R., 1969. Evolution of protein molecules. In: Munro, H.N. (Ed.), Mammalian Protein Metabolism. Academic Press, New York, pp. 21–132.

Kaneko, M., Kitajima, F., Naraoka, H., 2011. Stable hydrogen isotope measurement of archaeal ether-bound hydrocarbons. Organic Geochemistry 422, 166–172.

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., van Veen, J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria: Moving beyond genes and genomes. Frontiers in Microbiology 7, 744.

Kulichevskaya, I.S., Suzina, N.E., Rijpstra, W.I.C., Sinninghe Damsté, J.S., Dedysh, S.N., 2014. *Paludibaculum fermentans* gen. nov., sp. nov., a facultative anaerobe capable of dissimilatory iron reduction from subdivision 3 of the Acidobacteria. International Journal of Systematic and Evolutionary Microbiology 64, 2857–2864.

Liesack, W., Bak, F., Kreft, J.-U., Stackebrandt, E., 1994. *Holophaga foetida* gen. nov., sp. nov., a new, homoacetogenic bacterium degrading methoxylated aromatic compounds. Archives of Microbiology 162, 85–90.

Liu, X.L., Summons, R.E., Hinrichs, K.U., 2012. Extending the known range of glycerol ether lipids in the environment: Structural assignments based on tandem mass spectral fragmentation patterns. Rapid Communications in Mass Spectrometry 26, 2295–2302.

Lorenzen, W., Ahrendt, T., Bozhüyük, K.A., Bode, H.B., 2014. A multifunctional enzyme is involved in bacterial ether lipid biosynthesis. Nature Chemical Biology 10, 425–427.

Losey, N.A., Stevenson, B.S., Busse, H.-J., Sinninghe Damsté, J.S., Rijpstra, W.I.C., Rudd, S., Lawson, P.A., 2013. *Thermoanaerobaculum aquaticum* gen. nov., sp. nov., the first cultivated member of Acidobacteria subdivision 23, isolated from a hot spring. International Journal of Systematic and Evolutionary Microbiology 63, 4149–4157.

Naafs, B.D.A., Inglis, G.N., Zheng, Y., Amesbury, M.J., Biester, H., Bindler, R., Blewett, J., Burrows, M.A., del Castillo Torres, D., Chambers, F.M., Cohen, A.D., Evershed, R.P., Feakins, S.J., Gałkal, M., Gallego-Sala, A., Gandois, L., Gray, D.M., Hatcher, P. G., Honorio Coronado, E.N., Hughes, P.D.M., Huguet, A., Könönen, M., Laggoun-Défarge, F., Lähteenoja, O., Lamentowicz, M., Marchant, R., McClymont, E., Pontevedra-Pombal, X., Ponton, C., Pourmand, A., Rizzuti, A.M., Rochefort, L., Schellekens, J., De Vleeschouwer, F., Pancost, R.D., 2017. Introducing global peat-specific temperature and pH calibrations based on brGDGT bacterial lipids. Geochimica et Cosmochimica Acta 208, 285–301.

Naether, A., Foesel, B., Nägele, V., Wüst, P., Weinert, J., Bonkowski, M., Alt, F., Oelmann, Y., Polle, A., Lohaus, G., Gockel, S., Hemp, A., Kalko, E., Linsenmair, K.E., Pfeiffer, S., Renner, S., Schöning, I., Weisser, W., Wells, K., Fischer, M., Overmann, J., Friedrich, M., 2012. Environmental factors affect acidobacterial communities below the subgroup level in grassland and forest soils. Applied and Environmental Microbiology 78, 7398–7406.

Nichols, P.D., Guckert, J.B., White, D.C., 1986. Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC–MS of their dimethyl disulphide adducts. Journal of Microbiological Methods 5, 49–55.

Oppermann, B.I., Michaelis, W., Blumenberg, M., Frerichs, J., Schulz, H.M., Schippers, A., Beaubien, S.E., Kruger, M., 2010. Soil microbial community changes as a result of long-term exposure to a natural CO_2 vent. Geochimica et Cosmochimica Acta 74, 2697–2716.

Pancost, R.D., Sinninghe Damsté, J.S., 2003. Carbon isotopic compositions of prokaryotic lipids as tracers of carbon cycling in diverse settings. Chemical Geology 195, 29–58.

Pankratov, T.A., 2012. Acidobacteria in microbial communities of the bog and tundra lichens. Microbiology 81, 51–58.

Pankratov, T.A., Dedysh, S.N., 2010. *Granulicella paudicola* gen. nov., sp. nov., *Granulicella pectinivorans* sp. nov., *Granulicella aggregans* sp. nov. and *Granulicella rosea* sp. nov., acidophilic, polymers-degrading acidobacteria from *Sphagnum* peat bogs. International Journal of Systematic and Evolutionary Microbiology 60, 2951–2959.

Pascual, J., Wüst, P.K., Geppert, A., Foesel, B.U., Huber, K.J., Overmann, J., 2015. Novel isolates double the number of chemotrophic species and allow the first description of higher taxa in Acidobacteria subdivision 4. Systematic and Applied Microbiology 38, 534–544.

Peterse, F., Nicol, G.W., Schouten, S., Sinninghe Damsté, J.S., 2010. Influence of soil pH on the abundance and distribution of core and intact polar lipid-derived branched GDGTs in soil. Organic Geochemistry 41, 1171–1175.

Ring, M.W., Schwär, G., Thiel, V., Dickschat, J.S., Kroppenstedt, R.M., Schulz, S., Bode, H.B., 2006. Novel iso-branched ether lipids as specific markers of developmental sporulation in the myxobacterium *Myxococcus xanthus*. Journal of Biological Chemistry 281, 36691–36700.

Russell, J.M., Hopmans, E.C., Loomis, S.E., Liang, J., Sinninghe Damsté, J.S., 2018. Distributions of 5- and 6-methyl branched glycerol dialkyl glycerol tetraethers (brGDGTs) in East African lake sediment: Effects of temperature, pH, and new lacustrine paleotemperature calibrations. Organic Geochemistry 117, 56–69.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.

Schouten, S., Hoefs, M.J., Koopmans, M.P., Bosch, H.J., Sinninghe Damsté, J.S., 1998. Structural characterization, occurrence and fate of archaeal ether-bound acyclic and cyclic biphytanes and corresponding diols in sediments. Organic Geochemistry 29, 1305–1319.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. Organic Geochemistry 54, 19–61.

Serkebaeva, Y.M., Kim, Y., Liesack, W., Dedysh, S.N., 2013. Pyrosequencing-based assessment of the *Bacteria* diversity in surface and subsurface peat layers of a northern wetland, with focus on poorly studied phyla and candidate divisions. PLoS One 8, e63994.

Sharp, C.E., Smirnova, A.V., Graham, J.M., Stott, M.B., Khadka, R., Moore, T.R., Grasby, S.E., Strack, M., Dunfield, P.F., 2014. Distribution and diversity of verrucomicrobial methanotrophs in geothermal and acidic environments. Environmental Microbiology 16, 1867–1878.

Sinninghe Damsté, J.S., 2016. Spatial heterogeneity of sources of branched tetraethers in shelf systems: The geochemistry of tetraethers in the Berau River delta (Kalimantan, Indonesia). Geochimica et Cosmochimica Acta 186, 13–31.

Sinninghe Damsté, J.S., Hopmans, E.C., Pancost, R.D., Schouten, S., Geenevasen, J.A.J., 2000. Newly discovered non-isoprenoid glycerol dialkyl glycerol tetraether lipids in sediments. Chemical Communications, 1683–1684.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U., Overmann, J., Dedysh, S.N., 2011. 13,16-Dimethyl octacosanedioic acid (*iso*-diabolic acid), a common membrane-spanning lipid of Acidobacteria subdivisions 1 and 3. Applied and Environmental Microbiology 77, 4147–4154.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Foesel, B., Wüst, P., Overmann, J., Tank, M., Bryant, D., Dunfield, P., Houghton, K., Stott, M., 2014. Ether- and ester-bound *iso*-diabolic acid and other lipids in members of Acidobacteria subdivision 4. Applied and Environmental Microbiology 80, 5207–5218.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Dedysh, S.N., Foesel, B.U., Villanueva, L., 2017. Pheno- and genotyping of hopanoid production in Acidobacteria. Frontiers in Microbiology 8, 968.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30, 2725–2729.

Tank, M., Bryant, D.A., 2015. *Chloracidobacterium thermophilum* gen. nov., sp. nov.: An anoxygenic microaerophilic chlorophotoheterotrophic acidobacterium. International Journal of Systematic and Evolutionary Microbiology 65, 1426–1430.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.

Vieira, S., Luckner, M., Wanner, G., Overmann, J., 2017. *Luteitalea pratensis* gen. nov., sp. nov. a new member of subdivision 6 Acidobacteria isolated from temperate grassland soil. International Journal of Systematic and Evolutionary Microbiology 67, 1408–1414.

Weber, Y., De Jonge, C., Rijpstra, W.I.C., Hopmans, E.C., Stadnitskaia, A., Schubert, C.J., Lehmann, M.F., Sinninghe Damsté, J.S., Niemann, H., 2015. Identification and carbon isotope composition of a novel branched GDGT isomer in lake sediments: Evidence for lacustrine branched GDGT production. Geochimica et Cosmochimica Acta 154, 118–129.

Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology 8, 648–657.

Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. Geochimica et Cosmochimica Acta 71, 703–713.

Weijers, J.W.H., Panoto, E., van Bleijswijk, J., Schouten, S., Rijpstra, W.I.C., Balk, M., Stams, A.J.M., Sinninghe Damsté, J.S., 2009. Constraints on the biological source(s) of the orphan branched tetraether membrane lipids. Geomicrobiology Journal 26, 402–414.

Weijers, J.W.H., Wiesenberg, G.L.B., Bol, R., Hopmans, E.C., Pancost, R.D., 2010. Carbon isotopic composition of branched tetraether membrane lipids in soils suggest a rapid turnover and a heterotrophic life style of their source organism(s). Biogeosciences 7, 2959–2973.

Wüst, P.K., Foesel, B.U., Geppert, A., Huber, K.J., Luckner, M., Wanner, G., Overmann, J., 2016. *Brevitalea aridisoli*, *B. deliciosa* and *Arenimicrobium luteum*, three novel species of Acidobacteria subdivision 4 (class Blastocatellia) isolated from savanna soil and description of the novel family Pyrinomonadaceae. International Journal of Systematic and Evolutionary Microbiology 66, 3355–3366.

Yang, H., Lü, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. The 6-methyl branched tetraethers significantly affect the performance of the methylation index (MBT′) in soils from an altitudinal transect at Mount Shennongjia. Organic Geochemistry 82, 42–53.

Zimmermann, J., Portillo, M.C., Serrano, L., Ludwig, W., Gonzalez, J.M., 2012. Acidobacteria in freshwater ponds at Donana national park, Spain. Microbial Ecology 63, 844–855.