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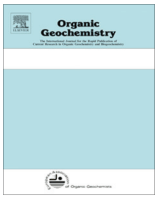

An overview of the occurrence of ether- and ester-linked *iso*-diabolic acid membrane lipids in microbial cultures of the Acidobacteria: Implications for brGDGT paleoproxies for temperature and pH

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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

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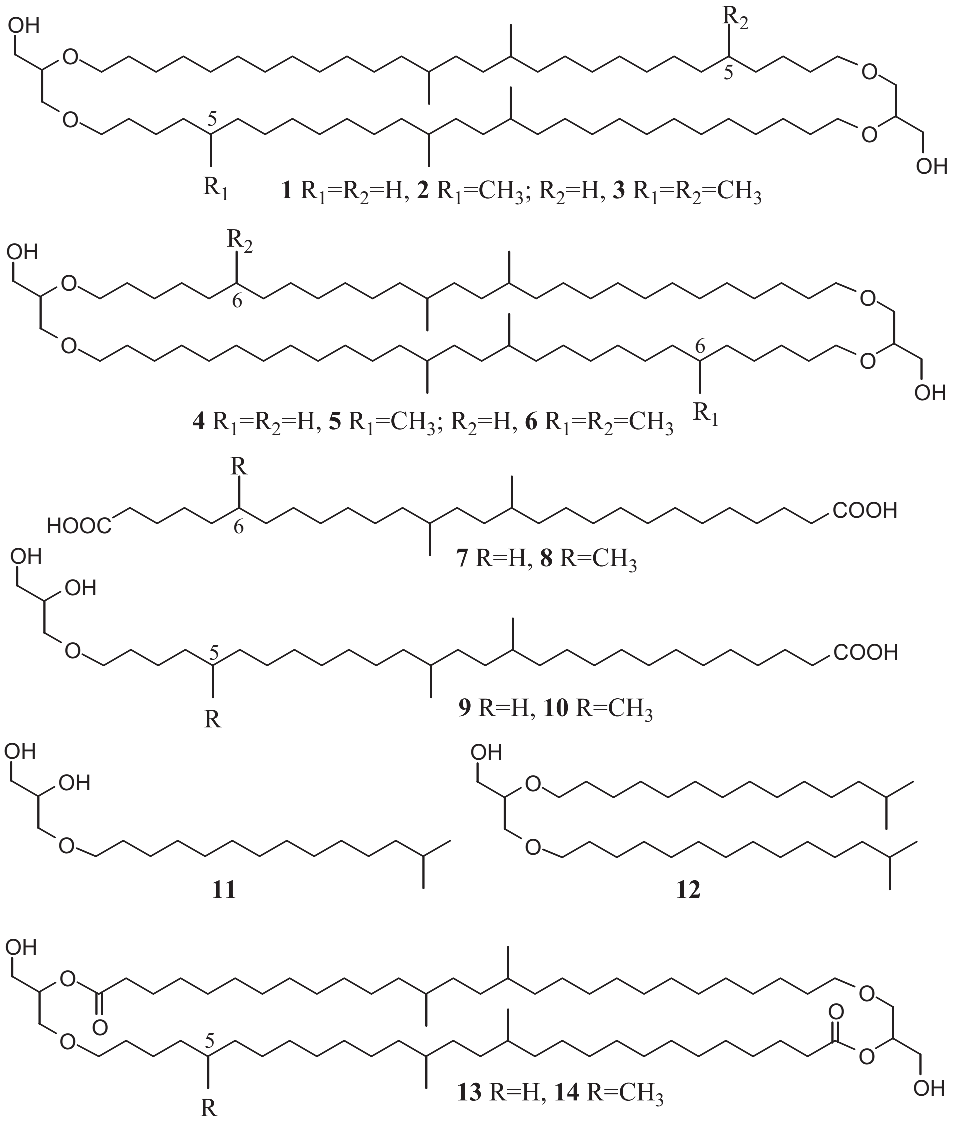

Fig. 1. Structures of lipids mentioned in the text. Note that structures **13** and **14** are hypothetical and have been proposed on results from acid hydrolysis experiments of cell material of SD 4 acidobacterial strains (Sinninghe Damsté et al., 2014).

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