Chemical Geology 386 (2014) 209–217

Contents lists available at ScienceDirect

Chemical Geology

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

Production of branched tetraether lipids in Tibetan hot springs: A possible linkage to nitrite reduction by thermotolerant or thermophilic bacteria?☆

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ARTICLE INFO

Article history:
Received 18 April 2014
Received in revised form 7 August 2014
Accepted 8 August 2014
Available online 17 August 2014

Editor: J. Fein

Keywords:
Branched glycerol dialkyl glycerol tetraethers
Hot springs
Tibet
Nitrite reduction

ABSTRACT

Branched glycerol dialkyl glycerol tetraethers (bGDGTs) are produced by bacteria and originally identified from soils and peat bogs; recently, however, in situ production of bGDGTs in hot springs has been reported. Consequently, we designed this study to evaluate the linkage between the distribution of bGDGTs, their biological sources and inferred metabolic processes based on the distribution and abundance of bGDGTs, pertinent water chemistry, the *nirS* gene (possibly in *Beta*- and *Gamma-proteobacteria*) and available 16S rRNA (tag) gene pyrosequencing data from 37 Tibetan hot springs. The absolute and relative concentrations of intact polar and core bGDGTs suggest that bGDGTs are predominantly produced in situ in these hot springs. Redundancy analyses revealed correlations between the distribution of bGDGTs and concentrations of ammonium, nitrite, and nitrate and the abundance of *nirS* gene, which are better reflected in the core bGDGT fraction than in the respective intact polar bGDGT fraction. Reanalysis of published bacterial 16S rRNA gene sequences showed that residence of members of the bacterial phyla *Proteobacteria* and *Bacteroidetes* correlated positively with a new methylation index ($R_{(III + II)/I}$) of bGDGTs. Some representatives of these taxa examined in this study are capable of *nirS*-encoded nitrite reduction, suggesting that bGDGT-synthesizing bacteria might be affiliated with these two phyla in Tibetan hot springs.

1. Introduction

Branched glycerol dialkyl glycerol tetraethers (bGDGTs; structures presented in Fig. 1) were discovered in samples from peat bogs and identified as a new group of cell membrane lipids (Sinninghe Damsté et al., 2000). The bGDGTs exhibit a mixture of archaeal (transmembrane tetraethers) and bacterial (branched alkyl chains) traits; nevertheless, the bGDGTs have 1,2-di-*O*-alkyl-*sn*-glycerol stereochemistry (Weijers et al. 2006a), which is diagnostic for lipids of bacteria (Wächtershäuser, 2003 and references therein).

The bGDGTs are ubiquitous in soils and peat deposits (Sinninghe Damsté et al., 2000; Schouten et al., 2000; Weijers et al., 2006a, 2006b; Peterse et al., 2009a, 2010, 2012; Liu et al., 2010) and have also been identified in lakes (e.g. Powers et al., 2004; Blaga et al., 2009), coastal marine sediments (Schouten et al., 2000; Hopmans et al., 2004) and hot springs (e.g. Schouten et al., 2007; He et al., 2012; Hedlund et al., 2013; Zhang et al., 2013). In an empirical study of about 130 soils worldwide, Weijers et al. (2007a) found significant linear correlations between bGDGT compositions and annual mean air temperature (MAT) or soil pH, which are expressed by two novel indices, the Methylation of Brached Tetraethers (MBT) index and the Cyclisation of Branched Tetraethers (CBT) index. The MBT and CBT indices have been successfully applied to reconstruct past continental temperatures and soil pHs (Weijers et al., 2007b, 2007c, 2011; Ballantyne et al., 2010; Bendle et al., 2010; Fawcett et al., 2011; Peterse et al., 2011b; Rueda et al., 2009; Schouten et al., 2008; Tyler et al., 2010; Zhou et al.,

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http://dx.doi.org/10.1016/j.chemgeo.2014.08.015

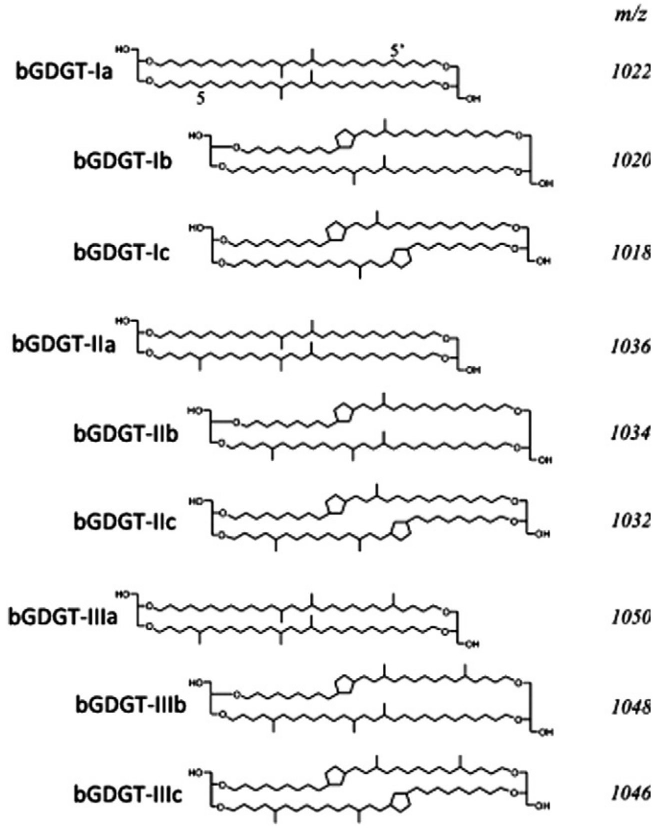

Fig. 1. The structures of bacterial core lipids examined in Tibetan hot springs.

2011; Zink et al., 2010). The bGDGTs recovered from aquatic systems in earlier studies were inferred to be attributed to contamination by erosion of the catchment soils; however, recent studies demonstrated that bGDGTs can be synthesized in situ in aquatic environments such as lakes (Sinninghe Damsté et al., 2009; Tierney and Russell, 2009; Sun et al., 2011; Tierney et al., 2011; Wang et al., 2012), fjords (Peterse et al., 2009b), estuaries (Zhu et al., 2011; Zhang et al., 2012) and hot springs (He et al., 2012; Hedlund et al., 2013; Zhang et al., 2013).

Upon cell death, intact polar lipids are degraded quickly into more recalcitrant core lipids by cleavage of the polar head groups; therefore, intact polar lipids were proposed to originate from living cells (Harvey et al., 1986; White et al., 1977). The head groups of intact polar (IP) bGDGTs have been identified to be sugars, phosphates, or a mixture of both (Liu et al., 2010; Peterse et al., 2011a). It has been recently suggested that the IP-bGDGTs may remain intact over a longer time than originally suspected (Liu et al., 2010; Schouten et al., 2010).

Although bGDGTs are widely distributed in nature, their source bacteria are still elusive. Weijers et al. (2009) combined organic geochemical- and diverse culture-independent techniques to propose *Acidobacteria* as potential biological sources of bGDGTs in peat bogs but failed to identify specific organisms. Examination of a suite of pure cultures of *Acidobacteria* identified only one bGDGT (bGDGT-I; Fig. 1) in the membranes of three aerobic strains (Sinninghe Damsté et al., 2011). However, abundant bGDGTs have been isolated from the anoxic layers of peat bogs (Weijers et al., 2006a; Peterse et al., 2011a), suggesting that other acidobacterial groups may contribute significantly to the detectable pool of bGDGTs. Recently, Zhang et al. (2013) reported a significant positive correlation between bGDGT abundance and the presence of certain thermophilic bacteria in a Great Basin hot spring, suggesting that bGDGTs in this hot spring may be produced in situ by thermophiles. Hedlund et al. (2013) expanded this study to other hot springs of the Great Basin and demonstrated that most bGDGTs are more abundant in hot springs than in surrounding desert soils, again suggesting that bGDGTs can be produced by thermophiles.

Here, we report the spatial distributions of core (C)- and IP-bGDGTs in Tibetan hot springs to evaluate an allochthonous or autochthonous origin of these lipids. We also examined the relationships between bGDGTs and environmental factors and attempted to link abundances of bGDGTs and the proteobacterial *nirS* gene (encoding the cytochrome cd1 nitrite reductase, Zumft, 1997 and Throbäck et al., 2004) in community DNA. Our data and the reanalysis of 16S rRNA gene pyrosequencing data published by Wang et al. (2013) implicate that the source organisms producing more bGDGTs with a high degree of methylation and less bGDGTs with a low degree of methylation in the studied hot springs may be bacteria affiliated with the phyla *Proteobacteria* and *Bacteroidetes*, some representatives of which have the capacity for *nirS*-encoded nitrite reduction as a major physiological trait.

2. Material and methods

2.1. Sampling

Thirty-seven surface sediment samples from hot springs and five surrounding surface soil samples were collected from Gulu (GL), Naqu (NQ), Guozu (GZ), Nima (NM) and Qucai (QC) regions on the Tibetan Plateau in the northwest of China (Fig. 2). The sampling locations, temperature and water chemical parameters were summarized in Table 1. Samples were frozen immediately in liquid nitrogen in the field and further stored at −80 °C in the lab. Temperature and water chemical parameters (i.e. pH, ammonium, nitrite and nitrate) in Table 1 have been reported in Li et al. (2013).

2.2. Lipid extraction and analysis

The total lipids were extracted using a sonication method and separated into apolar (alkane) and polar (C- and IP-bGDGTs) fractions following the procedure of Li et al. (2013). One aliquot of the polar fraction was directly screened by high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS) (1200 Series/6460 Triple Quad, Agilent Technologies, Santa Clara, California, USA) when the other aliquot was acid hydrolyzed and subsequently analyzed on HPLC-APCI-MS. Conditions for HPLC-APCI-MS were set as stated in Li et al. (2013). The abundance of IP-bGDGTs was obtained using a subtraction method. Two samples, GL-S-1 and NM-2S, contained non-detectable C- and IP-bGDGTs but detectable archaeal tetraether lipids (Li et al., 2013).

The MBT and CBT calculations, based on the bGDGT distributions, were according to Weijers et al. (2007a):

$$\mathrm{MBT} = [\mathrm{Ia} + \mathrm{Ib} + \mathrm{Ic}]/[\mathrm{Ia} + \mathrm{Ib} + \mathrm{Ic} + \mathrm{IIa} + \mathrm{IIb} + \mathrm{IIc} + \mathrm{IIIa} + \mathrm{IIIb} + \mathrm{IIIc}] \quad (1)$$

$$\mathrm{CBT} = -\mathrm{LOG}([\mathrm{Ib} + \mathrm{IIb}]/[\mathrm{Ia} + \mathrm{IIa}]) \quad (2)$$

The Roman numerals in the equations referred to the bGDGT structures in Fig. 1.

2.3. Statistical analysis

Redundancy analysis (RDA) was performed using the software CANOCO for Windows version 4.5. The nine bGDGTs as response variables and environmental factors and log-transformed values of proteobacterial *nirS* gene copy numbers as explanatory variables were transferred into CANOCO software. Firstly, the lengths of the bGDGT composition gradients for four axes were measured by the detrended correspondence analysis (DCA) in the software CANOCO. As all of these lengths were shorter than 3 (data not shown), the RDA analysis based on a linear ordination method was suggested to be selected for