Oxygen limitation can trigger the production of branched GDGTs in culture

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Abstract

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are ubiquitous and well preserved sedimentary biomarkers. These compounds serve as important palaeoenvironmental indicators due to strong empirical correlations between brGDGT distributions and temperature and pH in modern environments. However, the mechanistic link between temperature, pH, and brGDGT production has been impossible to ascertain thus far due to the absence of a clear biological source for brGDGTs. Here, we report that oxygen limitation triggers brGDGT production in at least one cultured species of Acidobacteria and confirm for the first time the biosynthesis of three structural varieties of brGDGTs, including an uncharacterised isomer of brGDGT Ic. This discovery helps explain why brGDGT producers have been so difficult to identify and provides a pathway towards uncovering the genetic basis and biological function of brGDGTs, which will lead to a more comprehensive understanding of their palaeoenvironmental significance. If the oxygen effects observed here apply more broadly, the empirical calibrations for brGDGT-based temperature and pH reconstructions may currently be missing the effects of oxygen as a relevant and possibly dominant control in the environmental distributions of brGDGTs.

Introduction

Methodological advances in sample preparation and analysis over the past decade have highlighted the vast global distribution of brGDGTs across terrestrial, aquatic, hydrothermal, and sedimentary systems (Lincoln et al., 2013; De Jonge et al., 2014; Weber et al., 2018; Wang et al., 2019). The relative abundances of structurally unique brGDGTs have been explored in many of these settings in efforts to calibrate brGDGTs as a palaeoenvironmental proxy by establishing empirical correlations with temperature and pH, such as the Methylation index of Branched Tetraethers (MBT) and the Cyclization index of Branched Tetraethers (CBI), respectively (Weijers et al., 2007; Peterse et al., 2012; Naafs et al., 2017). Prior work has demonstrated that many Acidobacteria, a diverse and widespread phylum of soil bacteria, synthesise the potential brGDGT precursor iso-diabolic acid (13,16-dimethyl octacosanedioic acid) in large quantities (Sinninghe Damsté et al., 2018), and that some members of subdivision 1 (SD 1) Acidobacteria, including Edaphobacter aggregans, produce trace amounts of at least one brGDGT (Sinninghe Damsté et al., 2011). However, the lack of cultured organisms that consistently produce branched tetraethers raises the question of how these compounds are so structurally diverse and abundant in nature yet so elusive in the laboratory.

Here we investigated the effects of molecular oxygen availability (O₂) on brGDGT production to test the hypothesis that brGDGT production may require a specific environmental constraint. Many Acidobacteria including E. aggregans harbour high affinity terminal oxidases in their genomes (Eichorst et al., 2018). These types of oxidases often have half-saturation constants at low nM concentrations of O₂, likely enabling survival and growth in micro-aerobic habitats (Pitcher and Watmough, 2004). Such low O₂ availability is common in many soil, peat, and sedimentary environments and prior work on brGDGT distribution and production across oxygen gradients suggests that some environmental source organisms may preferentially grow at oxic/anoxic transitions (Liu et al., 2014; Weber et al., 2018; Martinez-Sosa and Tierney, 2019). To study the effects of O₂ limitation, we examined the tetraether and fatty acid membrane composition of E. aggregans grown in a simplified yeast extract medium under O₂ conditions ranging from fully aerated (21 % O₂) to severely O₂ limited (1 % O₂). An excess additional carbon source (sucrose) was either added or omitted to control for the potential effects of growth rate.

Results and Discussion

The effects of O₂ limitation. E. aggregans produced similar fatty acids under all growth conditions with the sum of just four fatty acids (iso-diabolic acid, iso-C15:0, C16:0, and C16:1) constituting over 90 % of the fatty acid fraction (Fig. 1, Table S-1), consistent with previous observations in a different growth medium (91 %; Sinninghe Damsté et al., 2011). Although the relative abundance of individual fatty acids differed significantly between culture conditions, the hypothesised brGDGT precursor

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iso-diabetic acid was always the dominant membrane core lipid ranging in relative abundance from 40 ± 3 % to 84 ± 2 % with a systematic decrease both at lower O2 and without sucrose. In liquid culture, the organism produced no detectable tetraethers at 21 % and 5 % O2 however, at 1 % O2 E. aggregans produced significant quantities (up to 2.9 ±0.7 %) of up to three different tetraethers (brGDGT Ia, brGTGT Ia (Glycerol Triacyl glycerol Tetraether), and a structural isomer of brGDGT Ic (Fig. 2). Retention time offsets between the newly described tetraethers (brGDGT Ic isomer and brGTGT Ia) and previously described structurally similar tetraethers (brGDGT Ic and C46 G TG) are shown in Figure 2b. These data confirm and quantify for the first time the previously suspected production of brGDGT Ia in E. aggregans (Sinninghe Damsté et al., 2011), and reveal the capability of this species to produce other forms of tetraethers including a potentially cyclised structure. However, the brGDGT Ic isomer we observe is asymmetric with two unsaturation equivalents on the same alkyl chain. BrGDGT Ic commonly found in environmental samples contains only one unsaturation (a pentacyclic ring) on each alkyl chain instead. While the unsaturation equivalents could stem from cyclisation pointing to an isomer of brGDGT Ic with a bicyclic alkyl chain, other structural variations such as double bonds and cyclohexyl rings could produce the observed mass spectrum (see Fig. 2 for two potential structures). While hydrogenation of the tetraethers did not reduce the brGDGT Ic isomer to brGDGT Ia, suggesting cyclisation instead of double bonds, tetraethers with cyclopentyl rings usually elute later under normal phase chromatography (Liu et al., 2016), not earlier as observed here. Future analyses by NMR or ether cleavage and GC-MS with a purified concentrated fraction of this trace membrane component will help determine the exact structure of this new brGDGT Ic isomer. If E. aggregans indeed produces a cyclised brGDGT it must contain a cyclisation pathway different from the archaea, as the previously described archaeal grsAB tetraether cyclisation genes (Zeng et al., 2019) do not have homologues in the E. aggregans genome. The third tetraether found in E. aggregans, brGTGT Ia, is likewise rarely identified in environmental samples but other tri-alkyl tetraethers have been previously observed. Although the exact biosynthetic pathway for branched tetraethers remains unresolved, we note the correlation between the occurrence of brGTGT Ia and elevated quantities of iso-C15.

Environmental implications. The clumping phenotype of E. aggregans highlights the potential importance of micro-scale spatial O2 gradients in brGDGT production and is supported by E. aggregans growth on solid medium. Unlike in liquid cultures, we observed measurable quantities of both brGDGT Ia and the brGDGT Ic isomer in fully oxygenated (21 % O2) plate growth experiments (Table S-1). Aerobic plate growth and colony formation often produce micro-aerophilic environments within colonies, thus a significant portion of the plate culture likely experienced severe O2 limitation thereby triggering brGDGT production. This mode of growth on a solid substrate, rather than in liquid culture, is much more representative of the lifestyle of soil microorganisms in their natural environment (Kolter and
While the mechanistic links between physiology and brGDGT-based production at low O$_2$ is either not a universal trait among Acidobacteria, or that biosynthetic activation thresholds differ between organisms. Future genetic work with *E. aggregans* and additional culturing work with other Acidobacteria will help establish how widespread the genes for brGDGT production are in this phylum and under what conditions brGDGT biosynthesis occurs.

**Palaeoclimate proxies.** Although *E. aggregans* does not make a sufficiently large number of different tetraethers to test the mechanistic links between physiology and brGDGT-based climate proxies such as MBT and CBT, the results presented here show clearly that O$_2$ limitation can be a trigger for brGDGT production. Because modern environmental calibrations rely on the relative distribution of brGDGTs in environmental samples, they are susceptible to abundance changes in any one structure. Although calibration data have demonstrated strong relationships between brGDGT distributions and environmental conditions like temperature and pH, dissolved O$_2$ measurements are limited in existing calibration data (Raberg *et al.*, 2021). It is therefore unknown whether this variable is implicitly captured or mostly unaccounted for in modern calibrations. Recent environmental observations indicate that the abundance of other environmental factors such as salinity is also produced significant quantities of brGDGTs not detected in this study (*e.g.*, *E. aggregans* and *Bradyrhizobium* sp. [Yao *et al.*, 2020]). Holocene palaeoclimate records from the Arctic show a decoupling between known temperature trends and brGDGT-inferred temperatures (*e.g.*, Kusch *et al.*, 2019) suggesting an alternative overriding environmental control that is
unaccounted for. Our results suggest that for at least one source organism dissolved oxygen is the primary gradient to which brGDGT biosynthesis responds.

Conclusions

Our results indicate that O2 availability controls biosynthesis of branched tetraethers by *E. aggregans*, with low O2 required for production. This is the first confirmed organism to consistently produce significant quantities of multiple brGDGTs, thus opening the door to rigorous laboratory examination to elucidate the biosynthetic pathways and biological function of these enigmatic lipids. The identification of the enzymes involved in the synthesis of brGDGTs in *E. aggregans* will aid in the identification of other bacterial species that produce brGDGTs and help uncover the effect that O2 limitation may have on brGDGT biosynthesis and palaeoclimate proxies.

Author Contributions

TAH, ADY and SHK designed the research. TAH, JMM, ADY, JD and ND performed the research. TAH and SHK analysed the data. TAH, JMM and SHK wrote the paper.

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Additional Information

Supplementary Information accompanies this letter at https://www.geochemicalperspectivesletters.org/article2132.

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