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Oxygen limitation can trigger the production of branched GDGTs in culture

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Supplementary Information

The Supplementary Information includes:

- Extended Methods
- Tables S-1 and S-2
- Figures S-1 and S-2
- Supplementary Information References

All data and code are available at https://github.com/KopfLab/2021_halamka_et_al and archived with DOI 10.5281/zenodo.4651686.

Extended Methods

Culturing

Edaphobacter aggregans strain Wbg-1 (DSM 19364; Koch *et al.*, 2008) and *Acidobacterium capsulatum* strain 161 (DSM 11244) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). *E. aggregans* was grown on yeast extract (YE) at 20 °C and pH 5.0 in phosphate-buffered *Acidobacterium* subdivision 1 medium with (PSYL5) or without (PYL5) added sucrose (1.8 g/L KH_2PO_4, 0.2 g/L $MgSO_4 \cdot 7\ H_2O$, 1 g/L YE, +/- 30 g/L sucrose) (Campanharo *et al.*, 2016). *A. capsulatum* was grown at 20 °C at pH 4.3 in DSMZ 269 rich medium with glucose. Both strains were grown routinely in aerobic culture tubes (20.8 % O_2). For suboxic growth, the strains were grown in 100 mL bottles continuously stirred and flushed at 100-133 SmL/min with a sterile hydrated blend of N_2 and Air using digital mass flow controllers (Alicat Scientific) at appropriate ratios to obtain 1.04 % and 5.2 % O_2 (v/v) in the headspace (throughout the manuscript we refer to 1.04 % O_2 as 1 %, 5.2 % as 5 %, and 20.8 % as 21 %). Attempts to study *E. aggregans* at O_2 mixing ratios < 1 % were unsuccessful because of cell clumping and insufficient growth yield for lipid analysis. Attempts to grow *E. aggregans* anaerobically by fermentation were likewise unsuccessful, matching previous physiological observation for many Acidobacteria (Eichorst *et al.*, 2018). No attempts were made to grow *E. aggregans* with alternative electron acceptors because the organism does not have the genetic potential for anaerobic respiration. *E. aggregans* was grown aerobically on plates containing PSYL5 with 1.5 g/L agar. Culture experiments were conducted in biological triplicates. For liquid cultures, optical density (OD) was measured at 600 nm (Thermofisher, Genesys 30 Visible Spectrophotometer) and growth rates were calculated by non-linear least squares fitting of OD measurements to a logistic function.

Sample Preparation

Cells from liquid culture were harvested in stationary phase by centrifugation (5000 RPM for 3 minutes). Cells from plate culture were harvested by scraping with a spatula once no more visible growth was observed. Harvested cells were lyophilized overnight and then physically disrupted in 2 mL microcentrifuge tubes by vortexing with methanol and 250 µL of 100 µm muffled glass beads for 10 minutes at 3000 rpm using a Disruptor Genie (Scientific Industries, SI-DD38). This additional cell disruption step increased fatty acid extraction yields for *E. aggregans* by an estimated >1000 % for iso-C15:0 and iso-diabolic acid. Excess MeOH was evaporated and 25 µg 23:0 PC (1,2-ditricosanoyl-sn-glycero-3-phosphocholine), 25 µg 24:0 FA (tetracosanoic acid), and 25 ng C46 GTGT (Huguet *et al.*, 2006) were added to all samples as internal quantification standards. Lipids were extracted for 90 minutes at 65 °C with 500 µL 3N HCl in MeOH (33 % final water content) to cleave tetraether headgroups and transesterify fatty acid esters to fatty acid methyl esters (FAMEs). Samples were cooled for 10-minutes before the addition of 500 µL methyl tert butyl ether. The upper organic phase was extracted 3 times with 500 µL n-hexane and total lipid extracts (TLEs) were evaporated under N_2. TLEs were resuspended in n-hexane for solid phase extraction and separated into 3 lipid classes using 500 mg aminopropyl columns (Sigma Aldrich Discovery DSC-NH2): FAMEs and ketones were eluted using 6 mL of n-hexane followed by 7 mL of 4:1 Hexane:DCM; alcohols including tetraether core lipids were eluted using 7 mL of 9:1 DCM:Acetone; free acids were eluted using 8 mL of 2.5 % formate in DCM. The fatty acid fraction was only used to verify quantitative derivatization to FAMEs during the initial extraction step.

Lipid Analysis

Tetraethers were analysed in the Organic Geochemistry Laboratory at the University of Colorado Boulder on a Thermo Scientific Ultimate 3000 HPLC coupled to a Q Exactive Focus Orbitrap-Quadrupole MS with APCI source using previously published methods (Hopmans *et al.*, 2016) with the following modification: the initial eluent gradient was 14 % 90:1 Hexane:IPA instead of 18 % 90:1 Hexane:IPA in order to achieve better separation between isomers. The compounds were confirmed together with retention time and MS2 spectra generated by data dependent acquisition mode (ddMS2). FAMEs were analysed on a DB-5 capillary column (Agilent Technologies, 30 m length, 0.25 mm I.D., 0.25 µm film thickness) with a Thermo Trace 1310 Gas Chromatograph (2 min at 80 °C, ramped to 140 °C at 20 °C/min, ramped to 325 °C at 5 °C/min, held for 10 min at 325 °C) equipped with PTV injector and coupled to a TSQ 8000 Evo triple quadrupole MS on full scan mode. Cellular tetraether abundances were calculated relative to FAMEs using the C24 and C46 internal standards.

Hydrogenation

To determine if any of the tetraether structures identified in this study contained unsaturations, a hydrogenation experiment was conducted on a portion of the alcohol fraction of *E. aggregans*. The sample was suspended in 1.5 mL of ethyl acetate in a 2 mL glass vial, with an excess of platinum catalyst added to the vial. The sample was then connected to a pressured H_2 gas manifold for 90 minutes. The resulting sample was run on the HPLC-MS using the same method described below for all other samples and compared to a non-hydrogenated fraction of the same sample.

Supplementary Tables

Table S-1 Lipid data.

Table S-1 is available for download (Excel) from the online version of the article at http://www.geochemicalperspectivesletters.org/article2132.

Table S-2 Growth rate data.

Organism	% O_2	C source	Replicate	Growth rate [d^{-1}]	*K* [OD600]
e.agg	21	1 g/L YE	rep1	0.54	0.60
e.agg	21	1 g/L YE	rep2	0.59	0.54
e.agg	21	1 g/L YE	rep3	0.58	0.57
e.agg	21	1 g/L YE + 30 g/L sucrose	rep1	0.87	1.56
e.agg	21	1 g/L YE + 30 g/L sucrose	rep2	0.89	1.54
e.agg	21	1 g/L YE + 30 g/L sucrose	rep3	0.92	1.51
e.agg	5	1 g/L YE + 30 g/L sucrose	rep1	0.88	1.20
e.agg	5	1 g/L YE + 30 g/L sucrose	rep2	0.91	1.23
e.agg	5	1 g/L YE + 30 g/L sucrose	rep3	0.88	1.16
e.agg	1	1 g/L YE	rep1	0.53	0.37
e.agg	1	1 g/L YE	rep2	0.52	0.29
e.agg	1	1 g/L YE	rep3	0.64	0.30
e.agg	1	1 g/L YE + 30 g/L sucrose	rep1	0.38	0.44
e.agg	1	1 g/L YE + 30 g/L sucrose	rep2	0.55	0.36
e.agg	1	1 g/L YE + 30 g/L sucrose	rep3	0.58	0.35
a.cap	1	DSMZ 269	rep1	1.10	0.20
a.cap	1	DSMZ 269	rep2	1.31	0.19
a.cap	1	DSMZ 269	rep3	0.94	0.25

Supplementary Figures

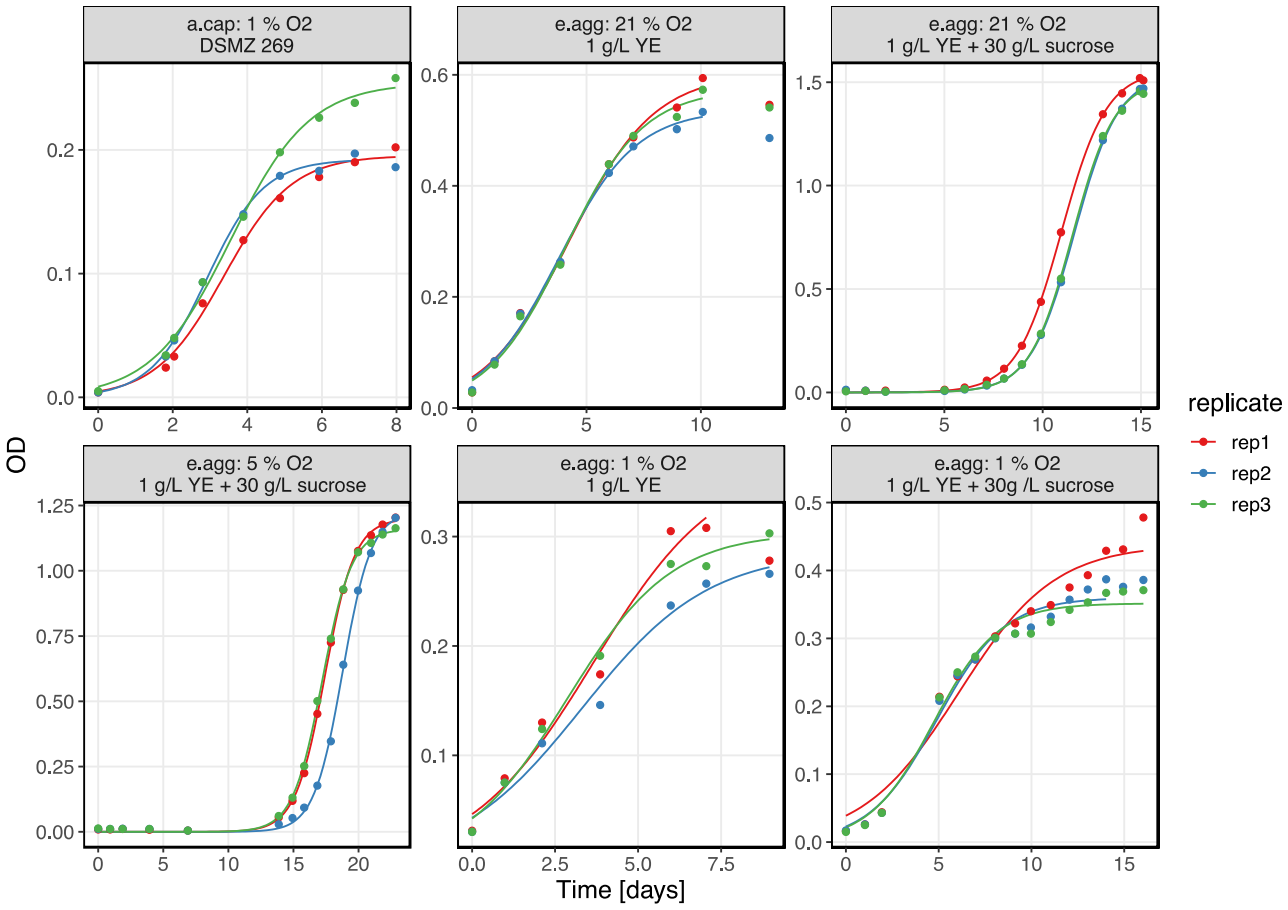

Figure S-1 Growth curves from *E. aggregans* and *A. capsulatum* liquid culture experiments. Panels are labelled with organism name, oxygen content, and carbon source. Colours represent biological replicates. Data points (circles) are optical density measurements at 600 nm *vs*. time (in days). Lines are fitted growth curves estimated by fitting optical density measurements to the following logistic equation, where t is time, OD is the optical density, and fit parameters μ and K represent the growth rate and carrying capacity (max OD), respectively:

$$OD_t = \frac{K}{1 + (K/OD_{t0} - 1) \cdot e^{-\mu t}}$$

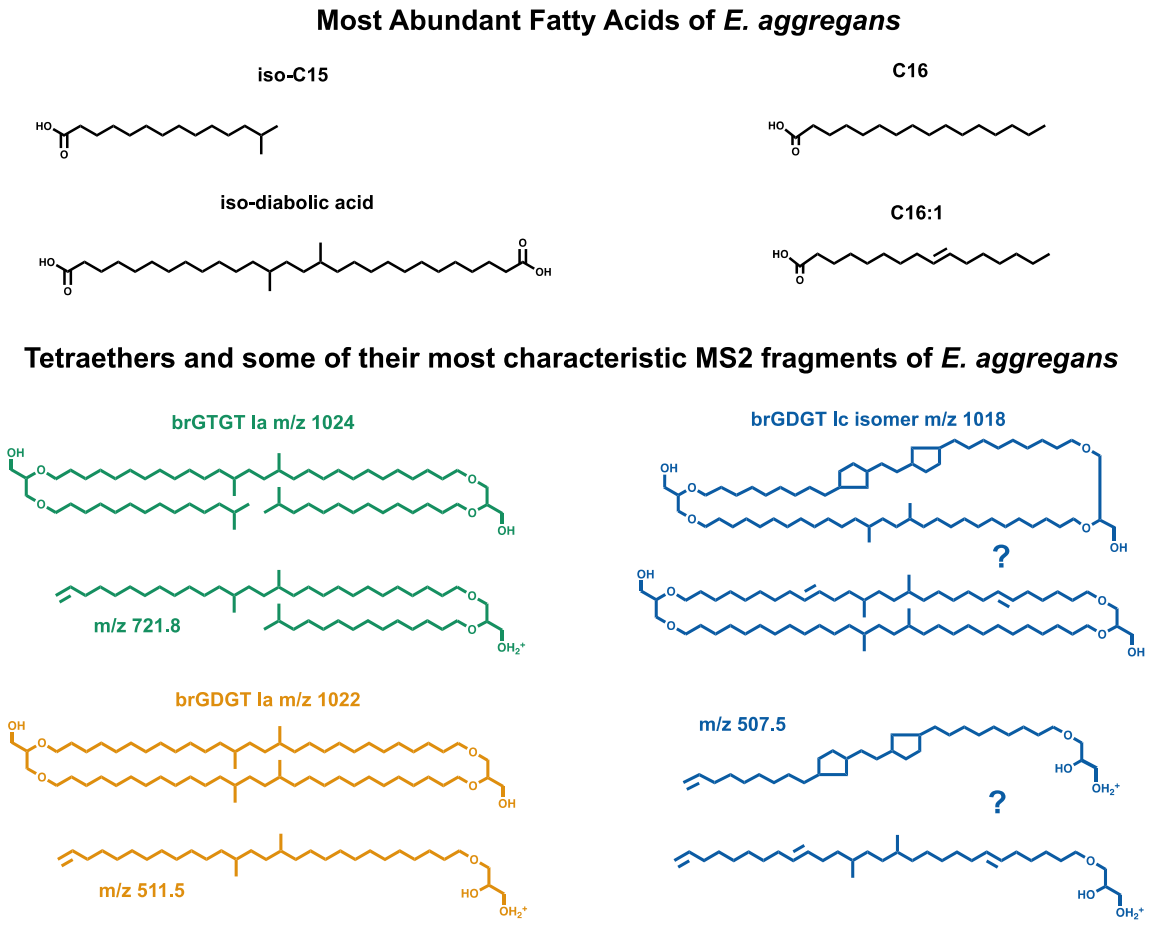

Figure S-2 Structures of the most abundant fatty acids and tetraethers (with characteristic fragments) of *E. aggregans*.

Supplementary Information References

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