Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2017-289-RC1, 2017
© Author(s) 2017. This work is distributed under
the Creative Commons Attribution 4.0 License.

Biogeosciences
Discussions
Open Access
EGU

Interactive comment on "Benthic Archaea as potential sources of tetraether membrane lipids in sediments across an oxygen minimum zone" *by* Marc A. Besseling et al.

Anonymous Referee #1

Received and published: 30 September 2017

The manuscript by Besseling et al. describes the distribution of archaeal intact polar tetraether lipids and their covariation with archaeal 16S rRNA genes in the Arabian Sea. Given the ever-expanding diversity of uncultivated archaea discovered by next-gen sequencing, "next gen" lipidomics techniques such as UHPLC-high-resolution mass spectrometry techniques need to be further developed and applied to allow lipid source assignments that in turn will improve the use of intact polar lipid biomarkers and improve our understanding of the mechanisms of, and problems with, proxies such as TEX86. The high-throughput sequencing methods used by the authors are state of the art. However, the methodology for intact polar lipid analysis employed by the authors is sub-par. While I agree that the use of high-resolution mass spectrometry represents

Printer-friendly version

Discussion paper

a significant improvement over previous analyses on the same samples, the chromatographic method used by the authors does not satisfy this potential. In fact, I have reason to believe that the results of this study are severely flawed by the choice of chromatographic method. Thus, I cannot recommend publication of this work in Biogeosciences in the current form.

I urge the authors to re-analyze their data using more appropriate reversed phase HPLC-MS methods. Previous studies have shown that diol column HPLC-MS and other normal phase methods lead to severe underestimation of the abundances of glycosidic GDGTs (Wörmer et al., 2013; Zhu et al., 2013). This would explain the unusually high abundances of HPH-GDGT reported in the present study and the lack of other ubiquitous compounds such as hydroxylated and unsaturated GDGT (Liu et al., 2012; Zhu et al., 2014b) and BDGT/PDGT (Becker et al., 2016; Meador et al., 2015; Zhu et al., 2014a). Alternatively, re-analysis of the samples along with authentic standards would help to correct for different ionization efficiencies based on headgroup types. If the authors did perform such a correction (and if they did not do this) they need to acknowledge this in the methods and discussion sections.

Judging from the supplementary spreadsheet, the authors may have considered a wider range of archaeal lipids, not exclusively traditional intact polar tetraethers, than reported in the manuscript. If so, where are these data? However, it is questionable if this comprehensive analysis is possible with the author's choice of chromatography. A more comprehensive analysis of the archaeal lipidome (e.g., by analyzing intact polar archaeols including core lipid structural modifications) in these samples would enhance source assignments and comparison to previous studies (Meador et al., 2015; Yoshinaga et al., 2015), and provide new biomarkers or source assignment for groups such as MCG and Woesearchaeota. This analysis would then satisfy the author's conclusion that the known diversity of archaeal IPLs was significantly expanded.

Further, because the authors used a column different from the one described in their method reference, the suitability of the chromatography for IPL analysis cannot be as-

sessed. The authors should provide annotated TIC or EIC traces for representative samples in supplementary figures. Importantly, the type of chromatography used here does not provide chromatographic separation of IPLs by core lipid type (e.g., GDGT-0, GDGT-1), but the way the authors present their data implies the contrary. If the different core lipid types of each IPL co-eluted in their analyses, the relative abundances reported would be questionable. At the very least the authors would need to do an isotope peak correction and then report in detail how this was done (correction factors for each IPL-core lipid combination). It would be much better to use a method than can actually chromatographically separate IPL-GDGTs by core lipid structure (Zhu et al., 2013). Additionally, the method used for this study likely cannot distinguish between the core lipid crenarchaeol and it's regioisomer. However, crenarchaeol more abundant than the regioisomer in MH GDGT and HPH GDGT but lower than its regioisomer in DH GDGT in thaumarchaeal cultures (Elling et al., 2017). This limitation needs to be addressed either by using a different chromatographic method or at least by discussing this issue in the manuscript.

Given these fundamental limitations, major parts of the methods, results, and discussion sections need to be revised. However, HPLC-MS (re-)analysis with a different method could be achieved within a day or two, given that the current study only includes 8 samples. I hope that the authors will agree that this approach has the potential to greatly expand the significance of their work with only moderate additional effort.

Other comments: Line 27: DeLong et al. report only on Antarctic samples. You may want to choose a reference that discusses a more diverse set of environments (e.g. DeLong and Pace, 2013; Schleper et al., 2005) or a collection of references e.g., (DeLong, 1992; Fuhrman et al., 1992; Teske and Sørensen, 2008).

Line 31: Lloyd et al. only provide data on two archaeal groups. You may rather cite a paper that actually discusses archaeal diversity, such as Teske and Sørensen (2008) or Teske (2013) some of the more recent literature, e.g., Hug et al. (2016) or Spang et al. (2017).

Printer-friendly version

Discussion paper

Line 40-42: What are the metabolisms of the other archaeal groups?

Line 59-61: What about the Lincoln et al. (Lincoln et al., 2014) paper?

Line 86: Why was this standard added? Was it used for any correction?

Line 90: Specify the modifications.

Line 159-161: Two DH isomers were also reported earlier by Elling et al. (2014; 2017).

Line 166-171: Did you detect crenarchaeol regioisomer? If not, why not? Co-elution with crenarchaeol? This should be pointed out here or elsewhere.

Line 172-173: The way the % values are used in this section is very confusing (. . .37.6% of 36.6%) etc. It could help to provide a further table in addition to Table 2 that shows the samples arranged by depth or headgroup type instead of sorting by core GDGT type.

Line 196: What does "p=1.00" represent?

Line 254-260: How were the transcripts analyzed? I did not find this information in the methods section. How long were the samples stored before analysis?

Line 270-274: This sentence is a bit long and complex. Revise?

Line 281: "Differences"?

Line 297: Rather "overlying"?

Line 319-321: Could you point out whether there is any evidence for the occurrence of Woesearchaeota in the water column and whether you would expect this group and the other archaeal groups to be present in oxic vs. anoxic environments (or both).

Line 353-357: I disagree with the statement that the diversity of detected (detectable) IPLs was greatly enhanced by this study. There are two IPL types described in addition to those reported by (Lengger et al., 2012). However, the diversity of IPL-GDGTs reported by previous studies is much higher, e.g. Yoshinaga et al. (2015), with re-

spect to both the headgroup types as well as the structural modifications in the core lipid such as hydroxylation, unsaturation, methylation, monoalkylation, trialkylation, or substitution of glycerol with butanetriol or pentanetriol. Many of these would likely be detectable in the presented samples using different methods.

Line 359-360: GDGT-0 and crenarchaeol are also dominant core lipids of MH-GDGT in Thaumarchaeota (Elling et al., 2014; Elling et al., 2015; Elling et al., 2017; Schouten et al., 2008). MH-GDGT also appears to be the dominant membrane lipid in many Thaumarchaeota. Also, how does your interpretation fit to the results of Lincoln et al. (2014) who suggested production of crenarchaeol by other archaeal groups?

Line 360: Qin et al. did not study IPLs. A more appropriate reference would be Elling et al. (2017).

Line 360-363: I am quite confident that the predominance of HPH-GDGTs in the sediment is an artifact of the chromatographic method that results in severe underestimation of MH-GDGT (and potentially DH-GDGT) relative abundances. However, the notion that higher HPH-content relates to higher activity was also supported by culture studies (Elling et al., 2014).

Line 363: Neither Harvey et al. or Schouten et al. discuss HPH IPLs and do not provide any experimental evidence for degradation rates of HPH versus MH or DH or any other GDGT IPL types. Rephrase.

Line 369-371: Do you think these fossil IPLs extracellular or intracellular (Braun et al., 2016)? Is some of the DNA also fossil? Would the DNA be preserved differently (degradation rates) than the IPLs and how would this affect the interpretation of your results?

Line 372-373: How did the 0/crenarchaeol ratio change for the anoxic sites? If they change in a similar way, does that point to different sources of 0 versus crenarchaeol or to accumulation of fossil IPLs?

Printer-friendly version

Discussion paper

Line 380: Typo. “Acid hydrolysis”

Line 384-386: You should discuss here or elsewhere that some cultures exist from the Thermoplasmatales cluster, although most clades remain uncultivated. E.g. there are many (acidophilic, thermoacidophilic) Thermoplasmatales cultures for which lipids have been analysed and all of them produce GDGTs. Further, the recently cultivated Methanomassiliicoccales (closely related to the uncultivated TMEG group) have been shown to produce IPL-GDGTs such as MH-GDGT-0 (Becker et al., 2016). It is thus very likely that the rest of the uncultivated Thermoplasmatales-like archaea can produce GDGTs.

Line 388: The way these papers are referenced is highly misleading. Four of the five references do not relate to Woesearchaeota and the inference of Villanueva et al. (2017) that Woesearchaeota do not produce GDGTs is circumstantial at best without knowledge of the actual GDGT biosynthetic pathway. Rephrase.

Line 392-394: Becker et al. (2016) showed that BDGTs are detectable in a globally distributed set of marine sediments. It is therefore likely that these compounds would be present in your samples. I suspect that you would detect these compounds using different chromatographic conditions. Recently, Thermoplasma-related methanogens (Methanomassiliicoccales) have been identified as a source of BDGTs in the environment (Becker et al., 2016). Meador et al. (2015) also identified further phosphatidic and MH, DH-GDGTs in MCG-rich samples. Could the MCG be sources of these compounds in your samples?

Line 416: As stated earlier, DH-GDGT-0 isomers have been detected in Thaumarchaeota (Elling et al., 2014; Elling et al., 2015).

Line 422: Typo. “sediments”

Line 423: Also Elling et al. (2014).

Line 430: Please provide experimental evidence for the “recalcitrant nature of glyco-

Printer-friendly version

Discussion paper

sidic bonds".

Line 433-434: Again, very misleading references, as in Line 388. These works do not discuss the GDGT lipid biosynthetic pathway. Delete the sentence or rephrase.

Line 435: I disagree: Could you please specify how the source assignment of IPL-GDGTs has been improved?

Figure 1: It would be very helpful to have another set of two plots (surface, subsurface) next to A and B that shows summed IPLs by headgroup type or a synthesis of the data in Table 2.

References

Becker K. W., Elling F. J., Yoshinaga M. Y., Söllinger A., Urich T. and Hinrichs K.-U. (2016) Unusual Butane- and Pentanetriol-Based Tetraether Lipids in Methanomassiliicoccus luminyensis, a Representative of the Seventh Order of Methanogens ed. V. Müller. Appl. Environ. Microbiol. 82, 4505–4516. Braun S., Morono Y., Becker K. W., Hinrichs K.-U., Kjeldsen K. U., Jørgensen B. B. and Lomstein B. A. (2016) Cellular content of biomolecules in sub-seafloor microbial communities. Geochim. Cosmochim. Acta 188, 330–351. DeLong E. F. (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci. U. S. A. 89, 5685–5689. DeLong E. F. and Pace N. R. (2013) Environmental diversity of Bacteria and Archaea. Syst. Biol. 50, 470–478. Elling F. J., Könneke M., Lipp J. S., Becker K. W., Gagen E. J. and Hinrichs K.-U. (2014) Effects of growth phase on the membrane lipid composition of the thaumarchaeon Nitrosopumilus maritimus and their implications for archaeal lipid distributions in the marine environment. Geochim. Cosmochim. Acta 141, 579–597. Elling F. J., Könneke M., Mußmann M., Greve A. and Hinrichs K.-U. (2015) Influence of temperature, pH, and salinity on membrane lipid composition and TEX86 of marine planktonic thaumarchaeal isolates. Geochim. Cosmochim. Acta 171, 238–255. Elling F. J., Könneke M., Nicol G. W., Stieglmeier M., Bayer B., Spieck E., de la Torre J. R., Becker K. W., Thomm M., Prosser J. I., Herndl G. J., Schleper C. and Hinrichs K.-U. (2017) Chemotaxonomic

Printer-friendly version

Discussion paper

characterisation of the thaumarchaeal lipidome. Environ. Microbiol. 19, 2681–2700. Fuhrman J. A., McCallum K. and Davis A. A. (1992) Novel major archaebacterial group from marine plankton. Nature 356, 148–149. Hug L. A., Baker B. J., Anantharaman K., Brown C. T., Probst A. J., Castelle C. J., Butterfield C. N., Hernsdorf A. W., Amano Y., Kotaro I., Suzuki Y., Dudek N., Relman D. A., Finstad K. M., Amundson R., Thomas B. C. and Banfield J. F. (2016) A new view of the tree and life's diversity. , Manuscript submitted for publication. Lengger S. K., Hopmans E. C., Reichart G.-J., Nierop K. G. J., Sinninghe Damsté J. S. and Schouten S. (2012) Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone. Part II: Selective preservation and degradation in sediments and consequences for the TEX86. Geochim. Cosmochim. Acta 98, 244–258. Lincoln S. A., Wai B., Eppley J. M., Church M. J., Summons R. E. and DeLong E. F. (2014) Planktonic Euryarchaeota are a significant source of archaeal tetraether lipids in the ocean. Proc. Natl. Acad. Sci. U. S. A. 111, 9858–9863. Liu X.-L., Lipp J. S., Simpson J. H., Lin Y.-S., Summons R. E. and Hinrichs K.-U. (2012) Mono- and dihydroxyl glycerol dibiphytanyl glycerol tetraethers in marine sediments: Identification of both core and intact polar lipid forms. Geochim. Cosmochim. Acta 89, 102–115. Meador T. B., Bowles M., Lazar C. S., Zhu C., Teske A. and Hinrichs K.-U. (2015) The archaeal lipidome in estuarine sediment dominated by members of the Miscellaneous Crenarchaeotal Group. Environ. Microbiol. 17, 2441–2458. Schleper C., Jurgens G. and Jonuscheit M. (2005) Genomic studies of uncultivated archaea. Nat. Rev. Microbiol. 3, 479–488. Schouten S., Hopmans E. C., Baas M., Boumann H., Standfest S., Könneke M., Stahl D. A. and Sinninghe Damsté J. S. (2008) Intact Membrane Lipids of "Candidatus Nitrosopumilus maritimus," a Cultivated Representative of the Cosmopolitan Mesophilic Group I Crenarchaeota. Appl. Environ. Microbiol. 74, 2433–2440. Spang A., Caceres E. F. and Ettema T. J. G. (2017) Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. Science 357. Teske A. (2013) Marine Deep Sediment Microbial Communities. In The Prokaryotes – Prokaryotic Communities and Ecophysiology (eds. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson). Springer, Berlin, Heidel-

Printer-friendly version

Discussion paper

berg. pp. 123–138. Teske A. and Sørensen K. B. (2008) Uncultured archaea in deep marine subsurface sediments: have we caught them all? ISME J. 2, 3–18. Wörmer L., Lipp J. S., Schröder J. M. and Hinrichs K.-U. (2013) Application of two new LC-ESI-MS methods for improved detection of intact polar lipids (IPLs) in environmental samples. Org. Geochem. 59, 10–21. Yoshinaga M. Y., Lazar C. S., Elvert M., Lin Y.-S., Zhu C., Heuer V. B., Teske A. and Hinrichs K.-U. (2015) Possible roles of uncultured archaea in carbon cycling in methane-seep sediments. Geochim. Cosmochim. Acta 164, 35–52. Zhu C., Lipp J. S., Wörmer L., Becker K. W., Schröder J. and Hinrichs K.-U. (2013) Comprehensive glycerol ether lipid fingerprints through a novel reversed phase liquid chromatography-mass spectrometry protocol. Org. Geochem. 65, 53–62. Zhu C., Meador T. B., Dummann W. and Hinrichs K.-U. (2014a) Identification of unusual butanetriol dialkyl glycerol tetraether and pentanetriol dialkyl glycerol tetraether lipids in marine sediments. Rapid Commun. Mass Spectrom. 28, 332–338. Zhu C., Yoshinaga M. Y., Peters C. A., Liu X.-L., Elvert M. and Hinrichs K.-U. (2014b) Identification and significance of unsaturated archaeal tetraether lipids in marine sediments. Rapid Commun. Mass Spectrom. 28, 1144–1152.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-289, 2017.