Correlations between biomarkers of varying bioavailability and putative hydrocarbonoclastic bacteria in an Early-Eocene marlstone sedimentary record

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Abstract

We examined the possibility that, during short-term refrigerated storage, microbial communities continue to biodegrade individual lipid biomarkers in an intact core section with Early Eocene consolidated marlstone sediments from the hydrothermal system overlying the Chicxulub impact crater (Yucatán, Mexico). Amplicon sequencing of environmental 16S rRNA obtained from the core samples revealed an increase in the relative abundance of predominant amplicon sequence variants (ASVs) assigned to the bacterial genera *Halomonas* and *Marinobacter* compared to immediately frozen marlstone rock samples from the same core. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

(PICRUSt2) predicted that these members of the rock-associated microbiomes have the genomic potential to anaerobically degrade hydrocarbons *via* dissimilatory nitrate reduction to ammonia and denitrification. These taxa showed strong Pearson Correlation Coefficients PCC (Pearson's *r*-values) with the most bioavailable non-sulfurized compounds *e.g.,* polycyclic aromatic hydrocarbons (PAHs) and isorenieratane, vs. moderate correlations with compounds including hopanes, *n*-alkanes and steranes that have undergone early abiotic diagenetic sulfurization. These results suggest that non-sulfurized lipid biomarkers (notably PAHs and isorenieratane) may be subject to continued biodegradation in sediments during short-term refrigerated storage.

Keywords: microbial degradation, biomarkers, PAHs, nitrate reduction, abiotic sulfurization

1. Introduction

Dormant as well as active microbial communities are present in subseafloor sediments up to several km deep (Inagaki et al., 2015). For molecular biological analysis of this deep biosphere, sediments from freshly split cores are immediately subsampled and flash frozen to stop microbial growth, which would otherwise render the samples unsuitable for downstream analysis. In contrast, in the field of organic geochemistry, sediment intervals are often subsampled from intact or split cores after months to years of refrigerated storage. However, it remains unknown if under such conditions, a subset of sedimentary microbial communities may continue to biodegrade paleo-environmentally diagnostic biomarkers. Here, we report preliminary results on the increase in the relative abundance of bacteria that harbor the genomic potential to continue to degrade individual biomarkers in refrigerated Early Eocene marlstone sediments from a hydrothermal system overlying the Chicxulub Impact Crater (Yucatán, Mexico).

2. Materials and Methods

The core section used for this study was recovered from the Chicxulub Impact Crater (Yucatán, Mexico; Hole M0077A) in May of 2016 by the International Ocean Discovery Program (IODP) and International Continental Scientific Drilling Program (ICDP) Expedition 364 (Gulick et al., 2017). Core catcher samples were recovered at low resolution (every 9 meters) throughout the Cenozoic interval of this core and were immediately frozen (Cockell et al., 2021). The taxonomic and relative abundance data of microbial communities from these properly stored samples were available for comparison (Cockell et al., 2021). The remaining intact core sections were capped and transported refrigerated (4 °C) in the dark to the core facility of MARUM (Bremen, Germany), where four months later, the core sections were split in half, subsampled, and described in detail by the IODP-ICDP 364 Science Party. For our study, 43 subsamples were obtained aseptically from a 12-m-long split core section between ~506.23 and 518.3 meters below the seafloor (mbsf) spanning the Early Eocene (~48.3-48.8 Ma) (Gulick et al., 2017) for paired analysis of lipid biomarkers and the rock-associated microbiome. Total lipids were extracted and fractionated into saturated, aromatic, and polar fractions after Schaefer et al. (2020). Polar fractions were further treated with Raney nickel to release the C-S bound compounds (biomarkers) that were sequestered during early sulfurization (Melendez et al., 2013). The saturated, aromatic, and desulfurized polar fractions in each sample were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and metastable reaction monitoring (MRM) to identify and quantify lipid biomarkers (Schaefer et al., 2020). The methods used for DNA extraction and microbial community profiling through Illumina MiSeq sequencing of the environmental 16S rRNA genes have been described in detail by Cockell et al. (2021). We furthermore predicted gene functions based on the recovered 16S rRNA gene sequences using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Douglas et al., 2019). Sparse Partial Least

Square (sPLS) regression using the R package *mixOmics* (version 6.13.22) was performed to reveal the correlation, which can be seen as a robust approximation of the Pearson Correlation Coefficent (PCC) (Rohart et al., 2017), between the microbial communities and predicted gene functions vs. biomarkers. In addition, we analyzed the correlation between the downcore distribution of the microbial communities and predicted gene functions with the unspecific bulk total organic carbon (TOC) content (see Schaefer et al. (2020) for comparison).

3. Results and discussion

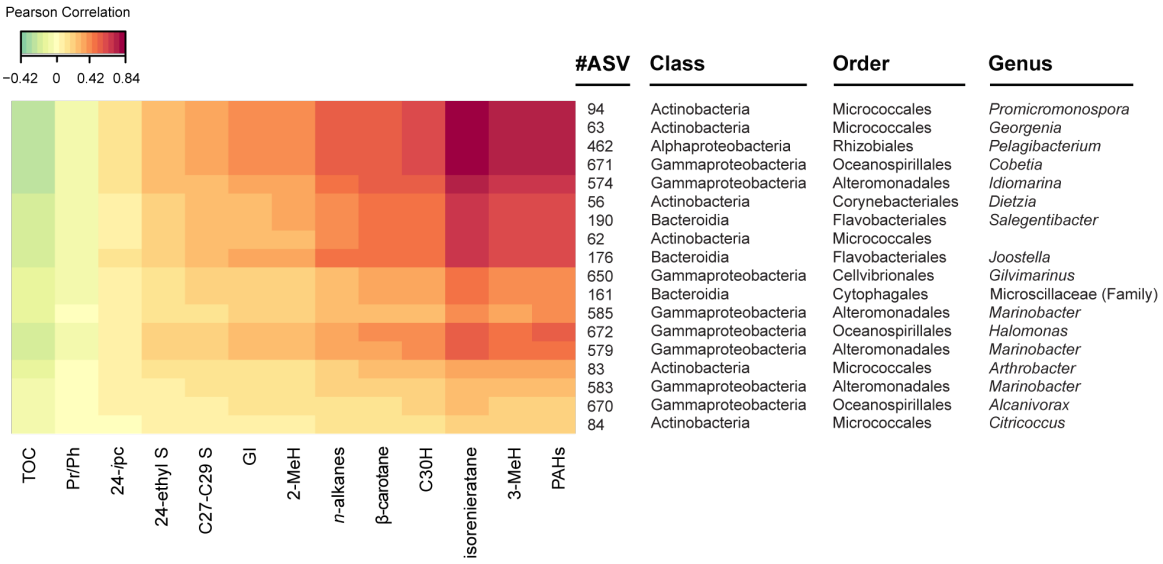

Figure 1 *Clustered Image Map (CIM; prepared in mixOmics) showing Pearson's r values between the downcore variability of selected biomarkers (horizontal axis) and individual amplicon sequence variants (ASVs) (vertical axis) which were recovered from the 43 analyzed Late Eocene marlstone samples. The ASVs were arbitrarily numbered and were assigned to class, order, and genus (or family) level. Only ASVs that revealed biologically relevant correlations (Pearson's r>0.2 or r< -0.2) with downcore quantitative changes in individual biomarker compositions are shown. See the color scale bar above the figure as a reference for the Pearson correlations between ASVs and biomarkers in the CIM, which varied between r= -0.42 (most negative correlation/green) to r=0.84 (highest positive correlation/dark red).*

Abbreviations: 3-MeH (3-methylhopane); $C_{30}H$ (C_{30} αβ hopane); 2-MeH (2-methylhopane); GI (Gammacerane index); C_{27}-C_{29} S (C_{27}-C_{29} Steranes); 24-ethyl S (24-ethyl dimethylsterane); 24-ipc S (24-ipc sterane).

The lack of a correlation between bacterial taxa and TOC content implies that most of the organic carbon in the analyzed early Eocene sedimentary rocks is recalcitrant and no longer bioavailable (Figure 1). Instead, a subset of the deep biosphere microbiome described in more detail below showed a strong positive correlation with specific biomarkers, notably isorenieratane and PAHs (r = ~ 0.8, Figure 1). The 3-methylhopane, C_{30} hopanes and $C_{13\text{-}35}$ *n*-alkanes may also be influenced by these microbial communities (r > ~ 0.5), whereas regular C_{27}-C_{29} steranes, 24-ethyl dimethyl steranes and 24-isopropylcholestanes (24-*i*pc steranes) may be less influenced by microbial attack (Pearson's r = ~ 0.2 - 0.5). This agrees with the observation from the desulfurized polar fractions that more steranes were sequestered by sulfur during early diagenesis, compared to hopanes and *n*-alkanes (Figure S1). Based on these results, we predict that the susceptibility of these compounds to microbial degradation in the Early Eocene marlstone is highest for compounds with the lowest degree of early sulfurization, *i.e.*, PAHs followed by isorenieratane, hopanes, *n*-alkanes and steranes.

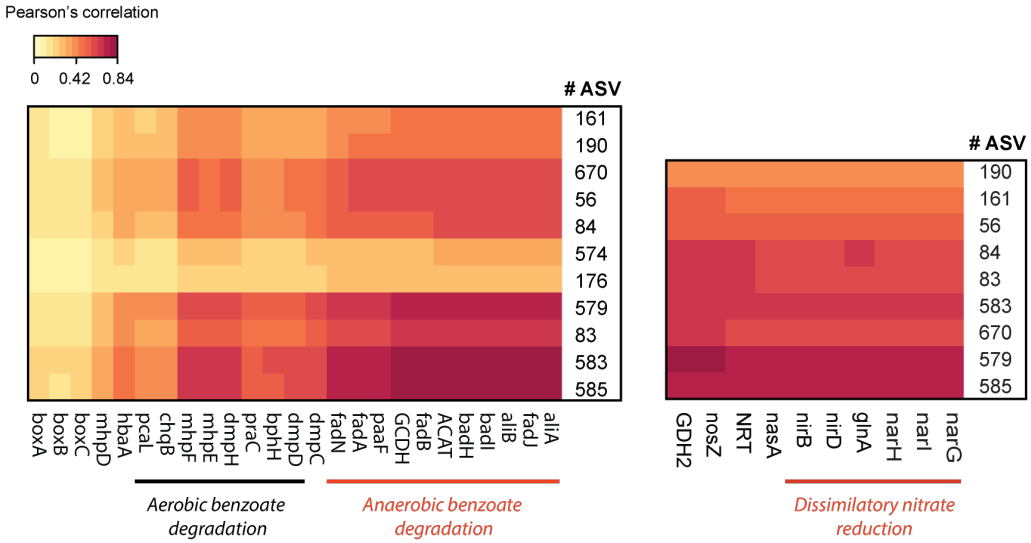

Figure 2 *CIM showing the Pearson's r-values between individual ASVs from Figure 1 and predicted functional genes related to benzoate degradation and dissimilatory nitrate reduction (horizontal axis) in the analyzed 43 Early Eocene marlstone samples. See the color scale bar above the figure as a reference for the Pearson's r values between ASVs and predicted genes in the CIM, which varied between r= 0 (no correlation/light yellow) to r=0.84 (highest positive correlation/dark red).*

The 18 ASVs representing bacterial taxa that may be involved in the biodegradation of these biomarkers, notably PAHs, (Figure 1), include presumably facultative anaerobic members of the genera *Marinobacter* and *Halomonas* (Gammaproteobacteria). Benzoyl-CoA is a compound that is formed as a central intermediate in the anaerobic degradation of aromatic substrates (Harwood et al., 1992). The majority of the ASVs that were identified as possible biomarker degraders, notably *Marinobacter* and *Halomonas* (Figure 1), also revealed the strongest Pearson correlations with the predicted genes *ali*A, *ali*B and *hba*A as well as *bad*H and *bad*I (Figure 2). However, other ASVs (e.g.,) in Figure 1 did not exhibit significant correlations with predicted genes in Figure 2. The bacterial biomarker degraders most likely represent facultative anaerobes since the genes *ali*A, *ali*B and *hba*A are involved in the activation of benzoate to benzoyl-CoA in bacteria growing anaerobically with benzoate as a carbon and energy source (Egland et al., 1995). The genes *bad*H and *bad*I participate in β-oxidation in the upper pathway of benzoyl-CoA degradation. Moreover, a subset of these ASVs showed strong Pearson's *r*-values with the predicted genes *nir*B and *nir*D, implying that the degradation of benzoate and/or aromatic compounds (*e.g.*, PAHs) in the refrigerated early Eocene consolidated marlstone sediments is coupled with dissimilatory nitrate reduction to ammonia (Figure 2). Combined, our results suggest that the short-term refrigerated storage of consolidated deep subsurface sedimentary rocks resulted in an increased relative abundance of putative hydrocarbonoclastic denitrifying bacteria (*e.g.*, a ~5-folder increase in *Halomonas*

related ASVs compared to Cockell et al., 2021), which would have the potential to continue to biodegrade the more labile non-sulfurized paleo-environmentally diagnostic biomarkers.

4. Implications for lipid biomarker analysis

In this study we provided preliminary insights into specific members of subseafloor microbial communities with the genomic potential to continue to decompose lipid biomarkers in recently cored consolidated marine sedimentary rocks during short-term refrigerated storage. For studies that will target relatively labile lipid biomarkers for paleoenvironmental and paleoclimate reconstructions, it may be necessary to subsample sediment cores shortly after recovery and to immediately store the samples frozen similar to the now standardized protocol for obtaining and storing samples from the deep biosphere for DNA and/or RNA work. However, to provide more direct evidence for the active degradation of lipid biomarkers in refrigerated deep subsurface sedimentary records, future studies could involve time-series experiments to monitor the decline in lipid biomarker content over time and to perform correlation analyses between lipid biomarkers and the composition and relative abundance of extremely short-lived functional gene transcripts (metatranscriptomics) and proteins/enzymes (proteomics). This would show that genes involved in the breakdown of biomarkers are not only actively transcribed, but also translated into functional proteins/enzymes capable of carrying out these processes.

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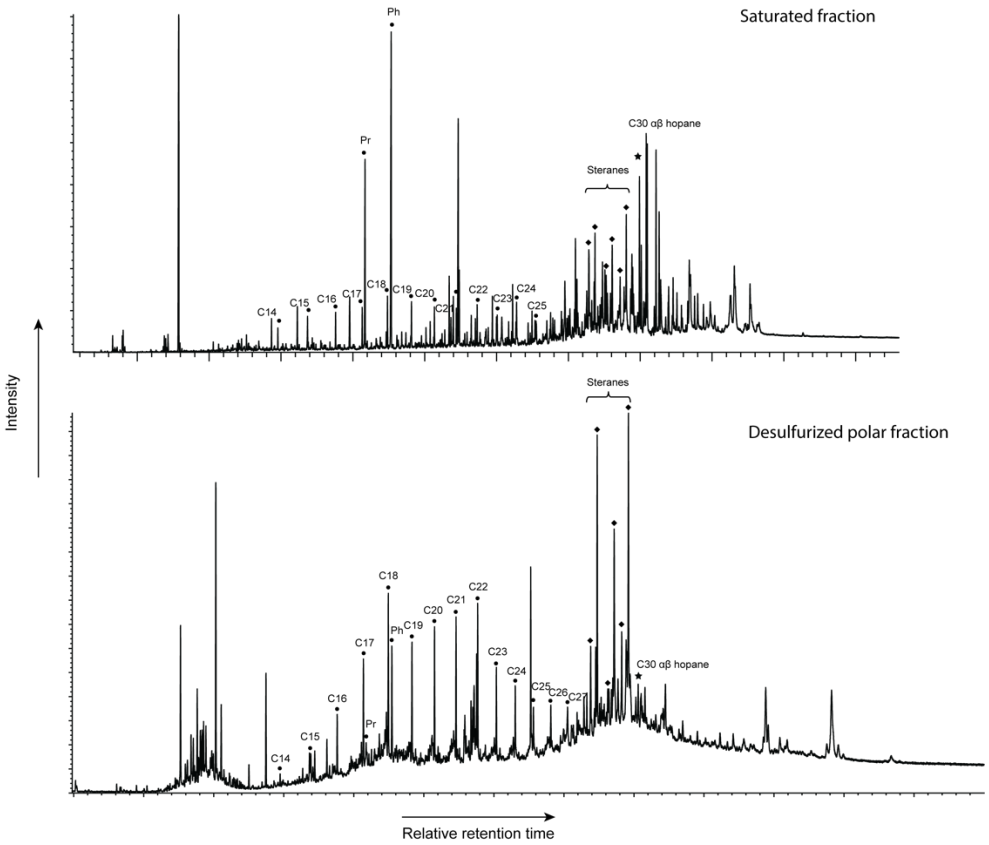
Supplementary materials

Figure S1 *Gas chromatograms of saturated* (top) *and desulfurized polar* (bottom) *fractions of representative sample* (*506.78 mbsf*)*.* Top*: n-C_{17}/pristane: 0.02; n-C_{18}/phytane: 0.01; C_{27}-C_{29} steranes/C_{30} αβ hopanes: 0.17; Bottom: n-C_{17}/pristane: 0.47; n-C_{18}/phytane: 0.09; C_{27}-C_{29} steranes/C_{30} αβ hopanes: 22.99. Isorenieratane was not detected in the desulfurized polar fraction.*


Saturated fraction
Ph
Pr
C30 αβ hopane
Steranes
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
C25
Intensity
Steranes
Desulfurized polar fraction
C18
C22
C21
C20
Ph
C19
C17
C23
C24
C30 αβ hopane
C25
C26
C27
C16
Pr
C15
C14
Relative retention time