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Cryptic metabolisms in anoxic subseafloor sediment

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Abstract

Microbial gene expression in anoxic subseafloor sediment was recently explored in the Baltic Sea and the Peru Margin. Our analysis of these data reveals diverse transcripts encoding proteins associated with neutralization of reactive oxygen species, including catalase, which may provide an in situ source of oxygen. We also detect transcripts associated with oxidation of iron and sulfur, and with reduction of arsenate, selenate and nitrate. Given limited input of electron acceptors from outside the system, these results suggest that the microbial communities use an unexpectedly diverse variety of electron acceptors. Products of water radiolysis and their interactions with sediment continuously provide diverse electron acceptors and hydrogen. Cryptic microbial utilization of these oxidized substrates and H2 may be an important mechanism for multi-million-year survival under the extreme energy limitation in subseafloor sediment.

Introduction

Marine sediment is estimated to host a third of Earth’s Bacteria and Archaea (Kallmeyer et al., 2012; Bar-On et al., 2018). Although cells are abundant, mean per-cell rates of metabolic activity are generally very low in subseafloor sediment (>1 meter below seafloor (mbsf)) (D’Hondt et al., 2002; Jørgensen, 2011; Lomstein et al., 2012). Despite their low metabolic rates, cells inhabiting subseafloor sediment are generally alive (Schippers et al., 2005; Ramírez et al., 2018) and capable of growth (Morono et al., 2011).

In anoxic subseafloor sediment along continental margins, sulfate reduction, fermentation, and methanogenesis, fueled primarily by organic matter oxidation, are the dominant net metabolic activities (D’Hondt et al., 2002). Along continental margins, where rates of anaerobic activity are relatively high, detectable dissolved sulfate and net sulfate reduction are typically limited to sediment within and above the sulfate–methane transition zone (SMTZ) (D’Hondt et al., 2002; Orsi et al., 2013). Below this zone, dissolved sulfate is usually below detection and net respiration is primarily hydrogenotrophic methanogenesis (D’Hondt et al., 2002).

The energetic processes that enable long-term microbial survival in deeply buried marine sediment are not fully understood. Long-term survival in this habitat may be influenced by cryptic energy-yielding activities that are not represented by net activities traditionally recognized from dissolved chemical profiles. For example, water radiolysis is a ubiquitous process in marine sediment (Sauvage et al., 2021). Water radiolysis, and the interaction of its products with sediment, generates diverse biologically accessible chemicals (Sauvage et al., 2021), leading us to hypothesize that radiolytic products may be used by sedimentary microbes for energy. To explore this possibility, we analysed the only publicly available metatranscriptomes from deep (5–159 mbsf range) anoxic sediment, sampled from the Peru Margin (Orsi et al., 2013) and the Baltic Sea (Zinke et al., 2017; Zinke et al., 2019). Using a custom set of hidden Markov models (HMMs (Johnson et al., 2010)), we targeted genes associated with reactive oxygen...
species neutralization and energy-yielding reactions in deeply buried, energy-starved, anoxic sedimentary environments.

**Results**

**Geochemical profiles**

Geochemical profiles show that all sites in this study are anoxic (Fig. 1). Detailed geochemical descriptions are found elsewhere (Shipboard Scientific Party, 2003; Expedition 347 Science Party, 2015a; Expedition 347 Science Party, 2015b). Briefly, at Baltic Sea Integrated Ocean Drilling Program Sites M0059 and M0063 (Fig. 1A and B), sulfate disappearance in the shallowest sediment (Expedition 347 Science Party, 2015a; Expedition 347 Science Party, 2015b) suggests that dissolved inorganic carbon (DIC) is the predominant net electron acceptor throughout most of the sediment column. At Peru Margin Ocean Drilling Program Site 1229 (Fig. 1C), sulfate penetrates deeper, and, at greater depths, diffuses upward from brine in deeper sediment (Shipboard Scientific Party, 2003). Sulfate is below detection and methane concentrations are high between 30 and 90 mbsf at Site 1229, indicating net methanogenesis (net DIC reduction) between those depths.

**Energy-yielding reactions**

We detected protein-encoding transcripts (PETs) linked to diverse energy-yielding metabolisms, derived largely from Chloroflexi and Firmicutes in the Baltic Sea subseafoor sediment and Actinobacteria and Proteobacteria in the Peru Margin subseafoor sediment (Fig. 2). Among these are PETs related to the reduction of sulfite, nitrate (as reported by Orsi et al., 2013), arsenate and selenate. In the Peru Margin sediment, iron oxidation is predicted at depth from the presence of the cyc2 gene fragments (McAllister et al., 2020). Sulfur oxidation is predicted from the presence of sdo [sulfur dioxygenase (Liu et al., 2014)] and sqr [sulfide-quinone oxidoreductase (Griesbeck et al., 2002)] gene transcripts in the Baltic Sea sediment (12–42 mbsf) and in all but one sample (5 mbsf) from the Peru Margin. Our detection of sdo transcripts is consistent with recent radiotracer-based recognition of a cryptic sulfur cycle in ‘sulfate-depleted’ marine sediment (Jørgensen et al., 2019).

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**Fig 1.** Sulfate (cyan), methane (red) and cell counts (black) profiles for sites (A) M0059E, (B) M0063E and (C) 1229D. Data from Expedition 347 Science Party (2015a), Expedition 347 Science Party (2015b) and Shipboard Scientific Party (2003).

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Reactive oxygen species neutralization

We detected PETs for neutralization of ROS in all surveyed metatranscriptomes (Fig. 2). PETs were identified for diverse ROS neutralization genes, including peroxiredoxin, hydroperoxide reductase and superoxide dismutase (Fig. 2). PETs homologous to the dye-decolorizing peroxidase (Dyp), which performs hydrogen peroxide-dependent oxidation of recalcitrant carbon polymers such as lignin (Chen et al., 2015), and heme peroxidase (Hansel et al., 2012), implicated in indirect extracellular manganese oxidation by Roseobacter spp. (Andeer et al., 2015), were also detected. Predicted taxonomic affiliations of transcripts indicate lineage-specific expression of ROS-related PETs (Fig. 2). Firmicutes, Chloroflexi and Proteobacteria drive expression patterns. Cyanobacteria, Chlorobi, Bacteroides, Aquificae and various Archaeal PETs are present but rare. Baltic Sea ROS-related PETs belong to Firmicutes, Chloroflexi, and, to a smaller degree, Proteobacteria. In the Peru Margin, catalase transcripts are derived mostly from Proteobacteria.

Discussion

Catabolic activities

PETs related to the oxidation of iron and sulfur were detected in our survey (Fig. 2). In principle, sulfur can be oxidized with manganese or iron oxides as the electron acceptor (Jørgensen et al., 2019). In contrast, energy-yielding microbial oxidation of iron typically requires highly oxidized electron acceptors, such as oxygen, hydrogen peroxide, superoxide and nitrate (Jørgensen et al., 2020). However, all of these samples are from much greater sediment depths than oxygen and nitrate appear to penetrate from the overlying ocean (D’Hondt et al., 2004). And while oxidized manganese can also serve as an electron acceptor for oxidizing iron, there is no evidence, as far as we know, that Mn(IV) and Fe(II) can be metabolically coupled by microorganisms. We also found nar transcripts exclusively in the 91-mbsf Peru Margin sample, the approximate depth where downward-diffusing methane is intercepted by sulfate diffusing up from brine below (D’Hondt et al., 2004), corroborating a previous report (Orsi et al., 2013). Overall, our survey of catabolic metabolisms suggests the presence of a cryptic source of oxidizing power in deep anoxic sediment.

ROS-related activities

The presence of ROS-related PETs in deep sediment also indicates widespread neutralization and use of ROS in this system. The detection of transcripts encoding dye-decolorizing peroxidase suggests the presence and microbial use of H₂O₂ to oxidize substrates and degrade recalcitrant organic polymers (Chen et al., 2015). Moreover, heme peroxidases, thought to be secreted from
cells, react with $\text{H}_2\text{O}_2$ and produce superoxide, which can then oxidize Mn(II) to Mn(IV). Interestingly, fungal peroxidases, including manganese and heme peroxidases, are also thought to result in extracellular manganese oxidation (Hansel et al., 2012). Our observations, despite the known limitations of transcriptional surveys (see Notes on transcriptomic interpretation in the SI), support that $\text{H}_2\text{O}_2$ and perhaps other, more transient, ROS are present, at least ephemeronously, in this ecosystem.

**Potential source of oxidants**

We report the presence of PETs associated with neutralization of ROS, as well as PETs associated with reduction of arsenate, selenate and nitrate in deep subseafloor sediment, where DIC and sulfate are typically assumed to be the primary terminal electron acceptors. PETs associated with oxidation of sulfur and iron are also expressed, indicating that these communities are primed to utilize these energy-generating pathways. We have considered whether these results could reflect signatures left over from the oxygenated world (i.e. constitutive functional gene expression rather than a true physiological response to the environment). Constitutive expression of functional genes from early in the sediment history might conceivably occur if microbes persist from that time without reproduction (D’Hondt et al., 2014; Kirkpatrick et al., 2019). However, recent studies have identified active cell division by dominant lineages of anaerobic subseafloor microbes (Atribacteria and Chloroflexi) in subseafloor sediment as old as 8 million years (Vuillemin et al., 2020a; Vuillemin et al., 2020b). In actively reproducing cells, the transcriptomic profiles change as net growth increases (Vuillemin et al., 2020a, Vuillemin et al., 2020b) and constitutive expression of functional genes should be a minor component of the transcriptome compared to the transcriptome that is activated as a part of the true physiological response to the environment. In accordance with this line of reasoning, residual transcription of oxygen utilization genes (e.g. terminal oxidases) is not observed in our analysis, indicating that transcripts not useful in the anoxic sediment are either not expressed or expressed at levels below detection limit.

Our observations collectively suggest the presence of an in situ source of biologically available oxidizing power in deep anoxic sediment. Although redox-active antimicrobials (RAAs) might explain PETs for ROS (Imlay, 2008), the independent detection of PETs related to diverse catabolic redox activities suggests that the ROS transcriptional patterns may not be exclusively driven by RAAs. Unexpected redox processes can be directly and indirectly supported by water radiolysis, which is ubiquitous in marine sediment and other wet geological environments (Lin et al., 2006; Blair et al., 2007). Water radiolysis appears to be the predominant source of electron donors ($\text{H}_2$) and diverse electron acceptors in marine sediment older than a few million years (Sauvage et al., 2021). Reaction of oxidized radiolytic products with sedimentary minerals, as well as various catalases and peroxidases, may generate redox species that are otherwise generally absent from sediment beneath the SMTZ [e.g. hydrogen peroxide, sulfite/sulfate, iron(III)].

**Concluding remarks**

Our results provide evidence for potential cryptic redox cycling in deeply buried marine sediment. Using a small, highly curated database of marker genes of biogeochemical significance, we predict the occurrence of unexpectedly diverse microbial activities, including various forms of catabolic metabolism and oxidative defence, that go undetected by standard geochemical measurements in this habitat. This study greatly expands the potential metabolic diversity associated with anoxic subseafloor sediment and highlights reactions that may provide long-term (multi-million-year scale) support for microbial communities buried in deep anoxic sediment.

**Materials and methods**

**Sample collection**

Information regarding collection, handling and processing of samples for the original transcriptomic studies can be found in the following publications: Peru Margin - Orsi et al., 2013; Baltic Sea - Zinke et al., 2017; Zinke et al., 2019 (see the Extended Methods section of the SI for additional details).

**Data acquisition and processing**

Metatranscriptomic data from Peru Margin and Baltic Sea marine sediment were downloaded from the Sequence Read Archive (SRA) using the SRA Toolkit v.2.8.2 (SRA Toolkit Development Team, NCBI). Site metadata, sequence trimming, assembly and read map details are provided in the Extended Methods section of the SI.

**Annotation**

To identify transcripts relevant to cryptic metabolisms and ROS neutralization, we developed two novel bioinformatics tools: LithoGenie and RosGenie. These software packages and the HMM libraries employed by each tool are public: https://github.com/Arkadiy-Garber/LithoGenie and https://github.com/Arkadiy-Garber/RosGenie. Software details,
including marker gene selection, HMM calibrations and contaminant screening are provided in the Annotation section of the SI.

Acknowledgements

We thank Andreas P. Teske for insightful commentary and suggestions on an early draft of this manuscript. We also thank two anonymous reviewers for their thoughtful comments. This project was funded in part by the US National Science Foundation, through the Center for Dark Energy Biosphere Investigations (C-DEBI; grant NSF-OCE-0939564). This is C-DEBI publication 569. This is JISAO contribution no. 2020-1070; PMEL contribution no. 5104.

Funding Information

G.A.R. was funded by an NSF C-DEBI post-doctoral fellowship. S.D. and G.A.R. were funded by the U.S. National Science Foundation through grants NSF-OCE-1130735 and NSF-OCE-0939564. S.M.M. was funded by the Joint Institute for the Study of the Atmosphere and Ocean (JISAO) under the NOAA Cooperative Agreement NA15OAR4320063.

Author Contributions


References


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Appendix S1.** Supporting information.