Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-248-AC4, 2019

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Cryptic role of tetrathionate in the sulfur cycle: A study from Arabian Sea oxygen minimum zone sediments" *by* Subhrangshu Mandal et al.

Subhrangshu Mandal et al.

wriman@jcbose.ac.in

Received and published: 20 September 2019

Anonymous Referee #3

Referee's Comment: The manuscript describes the analysis of inorganic sulfur compound cycling microbial populations in two sediment cores from the Indian Ocean. Particular focus is on populations driving the metabolism of thiosulfate and tetrathionate (thiosulfate reducing/tetrathionate forming, tetrathionate reducing, and tetrathionate oxidising groups). The study used a range of geochemical measurements, slurry incubations, microbiology (isolation of sulfur cycling microorganisms and assessment of their capabilities to transform thiosulfate and tetrathionate) as well as molecular bio-

Printer-friendly version

Discussion paper

logical approaches (metagenomics and metatranscriptomics). The biogeochemistry of sulfur compounds in sediments is a complex web of chemical and biological transformations, there is a need to better understand the role of individual species of inorganic sulfur compounds as well as the metabolic pathways and microbial groups involved in their transformations. As such, this is a topic of high interest, especially if, as the title implies, some of the transformations may be of a cryptic (not easily identified) nature.

Authors' Response: We thank the Reviewer for appreciating the underlying science of this study.

Referee's Comment: My overarching impression of the manuscript is that it is not easy to follow the story and that it would benefit from revising the structure. It is lacking a clear approach to the analysis and presentation of the data. Even starting in the introduction, I would suggest that, given the focus on the various enzymes being instrumental in the transformations of thiosulfate and tetrathionate, the introduction should provide a brief overview of the most important enzymes involved (and their encoding genes) and perhaps contain a schematic conceptual overview illustrating the most important points.

Authors' Response: We agree with your concerns, and so in the revised version of the manuscript have overhauled the entire text by providing the warranted information (regarding the enzymes and genes which appear to be instrumental in the transformations of thiosulfate and tetrathionate) in the Introduction, making the rationales for the analyses clearer, and discussing the results in their proper context under separate Results / Discussions sections. A schematic conceptual overview illustrating the most important points was already there in the form of Figure 4 in the previous manuscript and the same has now been updated with respect to the altered data components of the paper.

Referee's Comment: It would be beneficial and aid readability, if a clear overview of the basic findings was shown perhaps as depth profiles showing key chemical parameters

Printer-friendly version

Discussion paper

of the cores under investigation.

Authors' Response: We agree, and have now incorporated new figures showing depth profiles of all the important geochemical and microbiological parameters and comprehensively discussed their implications under a new Discussion section 4.1 titled "Trends of geomicrobial parameters down the sediment-depths corroborated sulfur cycle functions centered on tetrathionate".

Referee's Comment: With a view of the diversity and metagenomics analysis, I have two key criticisms: (i) revolving around the specific use of metagenomics read data for taxonomic assignment and (ii) extrapolating from that assignment to physiological properties of entire genera of bacteria. In that context, I have to say that I think it is a pity the authors did not carry out a diversity analysis of the sediment samples based on pooled 16S rRNA amplicon sequencing in parallel to the metagenomics/metatranscriptomics, because the ribosomal RNA gene survey would provide a much better and more robust diversity analysis than the assignment of taxonomy based on random metagenomic reads. Although a taxonomic assignment of a random metagenomic read is possible, it is fraught with major uncertainty, unless a closely related organism's genome is available in a database. As that is not the case for the vast majority of microorganisms found in nature at present, the taxonomic assignment of metagenomic reads is a bound to provide unreliable/unresolved taxonomies and lead to poor estimates of the abundance of specific types of bacteria. This affects data shown in Tables S8-13 as well as Fig 1 and 2.

Authors' Response: We agree that rRNA gene sequence analyses provide more robust taxonomic diversity analysis than the assignment of taxonomy based on identity of shotgun metagenomic reads; but on the flip side we have to bear in mind that such data only remain qualitative and give estimates of alpha diversity. We have already published the comprehensive, 16S rRNA gene sequence based, taxonomic diversity analyses for almost 100 sediment-samples from six SSK42 cores, including SSK42/5 and SSK42/6 [Fernandes et al., 2018, Enhanced carbon-sulfur cycling in the sedi-

Printer-friendly version

Discussion paper

ments of Arabian Sea oxygen minimum zone center. Sci. Rep. 8: 8665]. But such PCR amplified sequence data are not theoretically suitable for interpreting any quantitative population ecology trends or community metabolic functions such as the ones we have revealed in this paper from shotgun metagenomic sequence datasets (by directly annotating the same via BLAST search against the non-redundant protein sequence database, or assembling and annotating genes within the contigs by HMMER search against the EggNOG database).

We also agree with your concerns that taxonomic assignment from shotgun metagenomic reads, though possible, is fraught with major uncertainty, unless a closely related organism's genome is available in a database. With regard to this issue we can assure you that the parameters we have used to classify reads using the Best Hit Classification algorithm [BlastX search with minimum 45 nucleotides (15 amino acids) alignment and $\geq$60% identity, and maximum e-value allowed 1e–5] are stringent enough to assign taxonomic affiliation to homologs of metabolically diverse genes, irrespective of their intrinsic levels of conservation, in a reliable manner up to the genus level. This stringency level of search parameters is considered optimum across the literature because it neither exaggerates diversity not fails to resolve taxonomies for most categories of genes.

Anyway, there is no doubt that these are virtually never-dying debates, so to lay all apprehensions to rest we have carried out whole genome shotgun sequencing and annotation for the three tetrathionate-forming isolates Halomonas sp. MCC 3301, Methylophaga sp. MTCC 12599 and Pseudomonas bauzanensis MTCC 12600; the two tetrathionate-oxidizing isolates Halothiobacillus sp. SB14A, and Pusillimonas ginsengisoli MTCC12558; and the tetrathionate-reducing isolate Enterobacter sp. RVSM5a. Subsequently we have mapped the metagenomic sequence data from the 25 distinct sample-sites of SSK42/5 and SSK42/6 separately onto each of above mentioned de novo sequenced genomes – remarkably, significant percentages of the metagenomic read-sets were found in this way to match sequences from the individual genomes.

Printer-friendly version

Discussion paper

The data, which clearly give a picture of the relative abundances of the strains in each of the 25 distinct sediment-samples have been presented in the form of a new heat map figure.

Referee's Comment: My second criticism is the assumption in the paper that entire genera of bacteria always share specific physiological capacities with respect to the sulfur transformations of interest. While this is true for some genera (and a parameter used in systematics), for many genera this is not necessarily the case. Therefore, suggesting that genus A or B are tetrathionate producing bacteria or -oxidising bacteria, will likely overestimate the abundance of that specific metabolic type based on that assumption.

Authors' Response: So for as direct taxonomic annotations of raw metagenomic reads, followed by functional/metabolic classification of taxa, are concerned please note that we have all along clarified (already in the initial manuscript) that for one category of genera each and every member strain in the literature is known to possess tetrathionate-forming/oxidizing/reducing property, so the presence of such genera is more definitely indicative of the concerned processes in situ, whereas for another category of genera only some (and not all) member strains are known to possess tetrathionate-forming/oxidizing/reducing property, so their presence indicates further additional possibilities of such processes in situ. Furthermore, it may be noted that to keep this discrimination explicit, trends of relative abundance for the first category were depicted in Figs. 1 and 2 (these data are clearly free from diversity/abundance over-estimation), while those for the second category were all presented separately in Supplementary Tables S8-S13 (these data are likely to involve unknown proportions of diversity/abundance over-estimation, and so have been kept in isolation from the definitive estimates given in Figs. 1 and 2).

We have now edited the text in such a way as to make these issues more clearly comprehendible.

Referee's Comment: On the other hand, the metagenomics data can reveal the abundance of specific types of genes, as has been done here, and potentially identify the types of bacteria potentially contributing to the cycling of specific compounds. Too much of the discussion of inorganic sulfur metabolism in these sediments is based on the broad assumption of taxonomy, and too little is made of the specific genes found and listed in various supplementary tables. There are still limitations of our understanding of the genetics of sulfur transformations and it would be useful to perhaps illustrate whether the enzymes/genes driving specific transformations of sulfur compounds in some of the taxa mentioned (eg Salmonella) have actually been identified. Very little is done with the metatranscriptome data, it only gets a few mentions, but there is no clear overview of what has been found in which layer, how many reads were analysed and generally which bacteria were transcriptionally active with respect to sulfur cycling.

Authors' Response: We absolutely agree that there are major limitations in global understanding of the genetics of sulfur transformations; therefore, we have now reviewed in the new Introduction all the enzymes/genes that have been identified thus far as drivers of specific transformations of sulfur compounds in the taxa mentioned/considered in the text (including Salmonella). In this context it is noteworthy that corroborating your assumption, the genome of the current tetrathionate-reducing isolate belonging to the genus Enterobacter was found not to encompass the typical tetrathionate reductase (ttr) genes. For the records, we have now also added a detailed analysis of the six new genomes in relation to the homologs of sulfur-transformation genes present.

As for the metatranscriptome analyses, we have now added new data identifying which tetrathionate-metabolizing genes in general, and those matching homologs from the genomes of the new isolates in particular, were there in the metatranscriptome. In the revised manuscript we have also included dedicated sections for in depth metatranscriptomic methodology where the all the read statistics have been given. Notably, however, metatranscriptome was analyzed for only 275 cmbsf of SSK42/6, so the data

Printer-friendly version

Discussion paper

obtained thereof are not applicable to the other layers of the two sediment packages.

Referee's Comment: Regarding the transformations measured in slurry experiments, there needs to be a more complete reporting of the activities measured (or not) in all sediment samples. This should be shown comprehensively, not as currently done in Tables S14-S21, which suggest that only a few subsamples had certain activities. I am also not convinced that a 30-day incubation period of the slurries, some incubating sediments from a completely anoxic system under aerobic conditions (!), is providing the sort of activity data that would be supportive of suggesting that these key biological reactions are linked up in a cryptic cycle. The mentioning of alternative sources of oxygen from cryptic sources such as perchlorate is pointing to an unpublished study by the same authors. If this is crucial for the understanding of the functioning of this system and the microbiological activities required for the cryptic cycling of these compounds, these aspects should be incorporated here or the other study needs to be published. Alternatively, it is possible that the activities are due to facultative anaerobic bacteria in these sediments that have reverted back to an aerobic lifestyle given suitable incubation conditions and a 30-day period to wake up.

Authors' Response: We have now reported the slurry culture data as graphs plotted against sediment-depths. Notably, Whatever may be the actual tetrathionate formation/oxidation rate of the SSK42 sediment-samples in vitro or in situ, results of the slurry culture experiments illustrated that tetrathionate-forming and oxidizing bacteria of SSK42/5 and SSK42/6 were alive in situ. The issue of potential in situ active state of the tetrathionate-metabolizing bacteria was addressed mainly by the metatranscriptomic data both assembled and generally annotated as well as mapped against tetrathionate metabolizing genes of the isolates.

As for the data illustrating the feasibility of aerobic metabolism in these sediment horizons, please note that the same constitute a completely separate paper of our group, under consideration elsewhere, and those data are too voluminous to be incorporated here. Anyway, we have added in this revised manuscript that genes for

Printer-friendly version

Discussion paper

aerobic respiration by aa3-/cbb3-type cytochrome-c oxidases (coxABCD / ccoNOPQ) and cytochrome-bd ubiquinol oxidase (cydABX / appX) were identified in the assembled metatranscriptome from 275 cmbsf of SSK42/6 in general, and the portions of the metatranscriptomic dataset in particular which matched with sequences from the tetrathionate-oxidizing isolates, thereby suggesting that potential activity of this aerobic metabolic process is possible in this environment (the relevant gene and transcript Tables have also been incorporated in the revised manuscript as Supplementary Materials).

Specific comments Referee's Comment: Introduction: The introduction would benefit from a description of relevant metabolic pathways and enzymes targeted by the analysis of this paper Line 73: define mbsl Line 75 what was the diameter of these cores?

Authors' Response: We agree, and have now incorporated the warranted information in the Introduction. The diameter of all SSK42 cores was 12 cm; this information has been incorporated alongside the full-forms of the oceanographic units.

Referee's Comment: Line 82: for ease of reading I suggest to always refer to both cores with the full abbreviation, not SSK42/5 and 6 but SSK42/5 and SSK42/6

Authors' Response: We agree, and have now done as warranted.

Referee's Comment: Line 82: I find the description of the N2 shower lacking in detail and find it hard to understand how it would keep the exposed core adequately protected from oxygen.

Authors' Response: We have now overhauled the portion dealing with sampling details in such a way that all confusions emanating from the previous text are now resolved. This said, it is noteworthy that answers to these questions were already there in our previous publication Fernandes et al., 2018, which also dealt with these SSK42 cores (albeit form other perspectives) and was cited amply throughout the manuscript, including the sampling-related section. We had therefore thought that further repetition of the

Printer-friendly version

Discussion paper

details would be unnecessary and also potent causes of unintended self-plagiarism. However, now we understand that as an independent paper this manuscript should carry its own sampling details and have therefore brought back many of those details taking sufficient care of literary repetitiveness. For the records please note the following.

- In order to protect the ASOMZ sediment-samples from aerial oxidation, the entire cores were not split open into two D-shaped halves directly; instead only one ∼15-cm-long C-shaped part of PVC core-liner was removed at a time, as shown in a new Supplementary Figure added to the revised manuscript. We have now restructured the old sentences and written additional new lines to explain this in an unambiguous way. - The 15 cm length exposed at a time for sampling was constantly and closely showered with high-purity N2 emitted from multiple nozzles fitted to multiple nitrogen-generators. This contrivance was sufficient to prevent atomospheric oxidation of the 15 cm exposed surface of the core. - Immediately after the C-shaped longitudinal part of the PVC core-liner was cut open, top one cm of the exposed surface was scrapped off along the core-circumference using sterile scalpels to eliminate potential contaminations from the core-liners' inner-surfaces and/or sea-waters through which the cores had passed. - Subsequently, to sample a particular sediment-depth of the core for microbiological studies, an approximately 5-mm-thick sediment-slice (spanning equally on either side of the core-height marking) was scooped out with a sterile scalpel and put into a sterile polypropylene bottle. The head-space of every sample-containing bottle was flushed with high-pure N2, following which it was sealed with Parafilm - At the same time, for on board extraction of pore-waters, sediment-samples from a particular depth were taken out by inserting sterile 50 ml cut off syringes deep inside the core cross-section, multiple times along the circumference on the exposed 'C half'; the samples were immediately collected in sterile 50 ml centrifuge tubes; and all these were carried out under focused streams of high-pure N2.

Referee's Comment: Line 88: scraped

Authors' Response: We apologise, the typo is now fixed.

Referee's Comment: Line 145 define cmbsf, how thick was that sediment layer?

Authors' Response: cmbsf is now defined; approximately 5-mm-thick sediment-slices were sampled.

Referee's Comment: Line 147 and 190: pooling up, no 'up'

Authors' Response: We agree, now fixed.

Referee's Comment: Line 213: kmer lenghts of . . .

Authors' Response: Thank you, "lengths of" now added.

Referee's Comment: Line 227: text string search?

Authors' Response: Yes.

Referee's Comment: Line 236/7: not every read would represent one of these functional groups. Please reword/revise or explain more clearly what you did. There are no 'genera' of this that and the other, they are genera that contain species some of which have certain physiological characteristics, but not necessarily all of them

Authors' Response: We agree that your concerns hold well in a large number of cases, and exactly for that reason, we had clearly distinguished (already in the initial manuscript) that for one category of genera each and every member strain in the literature is known to possess tetrathionate-forming/oxidizing/reducing property, so the presence of such genera is more definitely indicative of the concerned processes in situ, whereas for another category of genera only some (and not all) member strains are known to possess tetrathionate-forming/oxidizing/reducing property, so their presence indicates further additional possibilities of such processes in situ. Furthermore, it may be noted that to keep this discrimination explicit, trends of relative abundance for the first category were depicted in Figs. 1 and 2 (these data are clearly free from diversity/abundance over-estimation), while those for the second category were all pre-

sented separately in Supplementary Tables S8-S13 (these data are likely to involve unknown proportions of diversity/abundance over-estimation, and so have been kept in isolation from the definitive estimates given in Figs. 1 and 2).

We have now edited the text in such a way as to make these issues more clearly comprehendible.

Referee's Comment: Line 246 following: No context for why one would assess the aerobic metabolism of these compounds with samples from 275cm below the sediment surface where there is no oxygen.

Authors' Response: Please note that since this study was aimed at revealing potential roles of tetrathionate in the sulfur cycle of marine sediments, plausible abilities to oxidize thiosulfate to tertahionate, and oxidize/reduce tetrathionate, were tested for all the communities present at all individual sediment-depths (from surface to core-bottom) of the sediment horizons explored and not just 275 cmbsf alone. Pure culture isolations and metatranscriptome analysis, however, were done only from 275 cmbsf, which is within the sulphate-methane transition zone where microbiological activity, for any marine sediment horizon, is generally very high and multi-faceted.

Referee's Comment: Line 309: actually described below

Authors' Response: We apologise, the oversight is now fixed.

Referee's Comment: Line 327: reword, genes do not have these activities, they encode enzymes that transform the compounds

Authors' Response: We agree, now fixed.

Referee's Comment: Line 350: I do not think that all of marine Pseudomonas and Halomonas do that

Authors' Response: We agree that not all but majority of the marine strains of Pseudomonas and Halomonas have the metabolic capacity of conversion of thiosulfate into

Printer-friendly version

Discussion paper

tetrathionate. Anyway, we have now overcome this small but important question mark by carrying out whole genome shotgun sequencing and annotation for the current three tetrathionate-forming isolates Halomonas sp. MCC 3301, Methylophaga sp. MTCC 12599 and Pseudomonas bauzanensis MTCC 12600, subsequent to which we have mapped the metagenomic sequence data from the 25 distinct sample-sites of SSK42/5 and SSK42/6 separately onto each of above mentioned de novo sequenced genomes – remarkably, significant percentages of the metagenomic read-sets were found in this way to match sequences from the individual genomes. The data, which clearly give a picture of the relative abundances of the strains in each of the 25 distinct sediment-samples have been presented in the form of a new heat map figure.

Referee's Comment: Table S1- Please clarify what is meant with 1st or 2nd sample fraction

Authors' Response: To sample a particular sediment-depth of a core for microbiological studies, an approximately 5-mm-thick sediment-slice (spanning equally on either side of the core-height marking) was scooped out with a sterile scalpel and put into a sterile polypropylene bottle.

For every sediment-depth, two such sample-replicates or slices - designated for duplicate metagenome (plus other metaomics) analyses - were collected (these were designated as sample replicates 1 and 2); a third slice was taken for all culture-dependent studies (notably, in the revised manuscript, the word "sample fraction" is now replaced by "sample replicate").

Referee's Comment: Table S4- Not a single DoxA encoding gene was identified in any of the samples. Should it perhaps not be listed in Table S4 accordingly? Please state what the significance of the yellow highlighting is in this and the other excel based supplementary tables

Authors' Response: Thanks for the suggestions. We have now removed the mention of doxA and soxD from Tables S3 and S4.

Printer-friendly version

Discussion paper

As the supplementary tables are large and each excel sheet contains large number of genes we have tried to assist visual cognition by alternately highlighting genes with similar metabolic function.

Referee's Comment: Table S6 Define what is meant with prevalence and how it is quantified Unclear what is meant with correlation of metabolic type with sediment depth, when depth is not quantified here

Authors' Response: To reduce the complexity of some of the sentences, the term "prevalence" has been used to denote relative abundance (detailed description for the estimation of relative abundance from metagenomic data, and its synonymy with prevalence, is mentioned in the Methods).

Please note that both the ∼3-m-long cores were explored biogeochemically at 15 to 30 cm intervals, which here is regarded as a sediment depth. In this way, we considered sediment-depth and tetrathionate metabolizing bacterial groups as two quantitative parameters and tested their interdependence by determining Pearson correlation coefficient (CC) and/or Spearman rank correlation coefficient (RCC).

Referee's Comment: Tables S8 to S11 should have totals for the abundance of all types per depth.

Authors' Response: We agree and have now given these totals.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-248, 2019.

Printer-friendly version

Discussion paper