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Dissimilatory sulfur cycling in oxygen minimum zones: an emerging metagenomics perspective

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Abstract

Biological diversity in marine OMZs (oxygen minimum zones) is dominated by a complex community of bacteria and archaea whose anaerobic metabolisms mediate key steps in global nitrogen and carbon cycles. Molecular and physiological studies now confirm that OMZs also support diverse micro-organisms capable of utilizing inorganic sulfur compounds for energy metabolism. The present review focuses specifically on recent metagenomic data that have helped to identify the molecular basis for autotrophic sulfur oxidation with nitrate in the OMZ water column, as well as a cryptic role for heterotrophic sulfate reduction. Interpreted alongside marker gene surveys and process rate measurements, these data suggest an active sulfur cycle with potentially substantial roles in organic carbon input and mineralization and critical links to the OMZ nitrogen cycle. Furthermore, these studies have created a framework for comparing the genomic diversity and ecology of pelagic sulfur-metabolizing communities from diverse low-oxygen regions.

Introduction

Oxygen gradients substantially affect ecosystem structure and biogeochemical cycling in the world's oceans [1–3]. Notably, in marine OMZs (oxygen minimum zones), steep declines in dissolved oxygen (from >200 μM to <100 nM) create redox gradients where diverse electron acceptors and donors are used for microbial energy metabolism [4,5]. Bacteria that reduce inorganic nitrogen compounds in place of oxygen are particularly abundant in OMZs, where up to half of the oceanic fixed nitrogen loss occurs through heterotrophic denitrification and anammox (anaerobic ammonium oxidation) [6–8]. Although the microbial and genetic basis of these processes is becoming better understood [9], far less is known about how OMZ nitrogen metabolism is coupled to the cycling of other elements, notably sulfur.

Within the last half-decade, exciting new evidence has confirmed that OMZs support a diverse microbial community that uses inorganic sulfur compounds for energy metabolism. This assemblage appears enriched in autotrophic bacteria that oxidize reduced inorganic sulfur compounds with nitrate [4,10,11], the initial step of so-called 'autotrophic denitrification' [12]. In addition, this community contains sulfate-reducing heterotrophs, which may provide sulfide for autotrophic denitrifiers, as well as remineralized ammonium for anammox [13]. Together, these patterns imply critical links between pelagic OMZ sulfur and nitrogen metabolism. However, our knowledge of the micro-organisms driving this sulfur cycle draws primarily from marker gene surveys, which to date have targeted only a subset of the diverse low-oxygen regions in the global ocean. Recently, the metagenomes (community DNA) of a dominant sulfur-oxidizing lineage (SUP05) [14] and of an OMZ bacterioplankton community in the ETSP (Eastern Tropical South Pacific) [13,15] have helped to describe the functional genetic basis of OMZ sulfur cycling. The present mini-review presents our emerging perspective of the OMZ sulfur-metabolizing community, based largely on these metagenomic data. In so doing, it highlights an imperative for future studies to explore the genomic diversity, biogeography and *in situ* physiological activity of these important functional group(s).

Key words: autotrophic denitrification, Eastern Tropical South Pacific (ETSP), oxygen minimum zone, sulfate reduction, sulfur oxidation, SUP05.

Abbreviations used: anammox, anaerobic ammonium oxidation; ETSP, Eastern Tropical South Pacific; OMZ, oxygen minimum zone; Rm, *Candidatus* 'Ruthia magnifica'; RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; Vo, *Candidatus* 'Vesicomyosocius okutanii'.

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Sulfur-oxidizers in the OMZ water column

Molecular surveys have uncovered a ubiquitous sulfur-oxidizing community in the pelagic zone of suboxic marine waters. Marker gene sequences [e.g. 16S rRNA gene, reverse dissimilatory sulfite reductase (*dsrAB*)] with high similarity to those of known sulfur-oxidizing autotrophs (thioautotrophs) have been found during episodes of sulfide enrichment in the shelf waters off Namibia [10], the Baltic Sea [16] and a fjord in the North Pacific (Saanich Inlet, British Columbia) [11], as well as under more permanently sulfidic conditions in the Cariaco Basin [17] and Black Sea [18]. Interestingly, thioautotrophic taxa have also been detected at high abundance in zones where free sulfide does not regularly accumulate, notably in the water column of the permanent OMZ in the ETSP upwelling zone [4,15]. Although the taxonomic richness of these pelagic communities remains an open question, gene surveys have revealed diverse phylotypes within a site, with strong representation by thioautotrophic members of the Gamma- and Epsilon-proteobacteria, as well as the Bacteroidetes/Chlorobi group (green sulfur bacteria) [4,10,13,15].

Although the environmental factors controlling the relative abundance of these taxa are not well understood, the anoxic core of the OMZ appears particularly favourable

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for Gammaproteobacteria related to the thioautotrophic symbionts of deep-sea clams. This symbiont-like clade, containing members of the GSO (gammaproteobacterial sulfur-oxidizer) cluster [10,18] and referred to as 'SUP05' after its original detection [14,19], has appeared repeatedly in gene surveys from diverse suboxic regions [4,20,21]. A key study by Walsh et al. [14] reconstructed the metagenome of a SUP05 isolate from sulfidic waters in the Saanich Inlet, where SUP05-like cells can represent up to one-third of all bacteria. The SUP05 metagenome was shown to be similar in identity and content to the genomes of two bacterial symbionts from sulfide-rich hydrothermal vents, Rm (*Candidatus* 'Ruthia magnifica') and Vo (*Candidatus* 'Vesicomyosocius okutanii') [22,23]. Like the symbionts, SUP05 contains genes mediating carbon fixation via the Calvin cycle and oxidation pathways for diverse reduced sulfur species (sulfide, sulfite, elemental sulfur and thiosulfate). The metagenome also encodes membrane-bound nitrate reductase (*narGH*), NO-forming nitrite reductase (*nirK*) and N_2O-forming nitric oxide reductase (*norCB*), implying that SUP05 catalyses key steps in denitrification. Building upon other studies indirectly linking pelagic sulfur-oxidizers to nitrate reduction [10], these data confirmed a genomic basis for the chemolithoautotrophic oxidation of reduced sulfur with inorganic nitrogen compounds in an OMZ water column, so-called 'autotrophic denitrification'.

The SUP05 metagenome and Rm/Vo genomes enable valuable comparisons with OMZ molecular datasets. Recently, sequences matching (by BLAST) diverse thioautotrophic bacteria and archaea were detected at high abundance in metagenome and metatranscriptome (RNA) samples collected from the same site in the ETSP OMZ in 2008 [15] and 2010 [13]. In the 2008 dataset, 'symbiont-like' sequences with top matches to genes in either the SUP05 or Rm/Vo genomes steadily increased in number from oxic to anoxic depths, accounting for over 6% of total protein-coding DNA sequences at the OMZ core (200 m) (Figure 1). Notably, symbiont-like genes encoding NarGH and proteins of the reverse *dsr* pathway, most of which were most similar to SUP05 homologues, accounted for substantial fractions (up to half) of all *nar* and *dsr* genes and transcripts at this depth. In contrast, denitrification genes mediating nitrite and nitric oxide reduction (*nirK* and *norCB*), although abundant and highly transcribed, were not strongly represented by SUP05-like sequences (Figure 1) (*nirK* and *norCB* homologues are absent from the Rm/Vo genomes). Similarly, sequences with top matches to SUP05 or Rm/Vo genes encoding RubisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), the key enzyme of carbon fixation in these bacteria, were not detected in the ETSP OMZ core (Figure 1). However, the most abundant RubisCO sequence at this depth was most similar to RubisCO from an environmental clone (marine bacterium 560, Monterey Bay), which, upon further examination, is closely related to SUP05 and Rm/Vo RubisCO. Together, these data provide strong evidence for nitrate-based sulfur oxidation by a SUP05-like population in the ETSP OMZ, but also suggest a need to quantify the contribution of these cells to carbon fixation and subsequent steps of denitrification. Indeed, consistent with these molecular results, bottle experiments using seawater from the ETSP OMZ recently confirmed that sulfide exposure causes a substantial increase in microbial nitrate reduction to nitrite, but has a much smaller effect on N_2O and N_2 production [13]. However, these effects were variable across depths and sites, reflecting heterogeneity in the activity or genetic composition of the underlying microbial community.

The ETSP meta-omic datasets also highlight the potential for genomic variation in SUP05-like populations. The relative abundance of different SUP05-like genes varied by over three orders of magnitude in the ETSP DNA datasets discussed above (following corrections for gene length variation). This variation is likely to be due in part to differential representation of genes in sequence databases, which would bias the identification of SUP05-like genes during BLAST searches (e.g. RubisCO sequences from OMZ thioautotrophs will not match symbiont-like genes if more closely related homologues are present in the BLAST database). Alternatively, the patchy recovery of genes could indicate that the ETSP SUP05-like population harbours a unique set of genes. SUP05-like sequences from this site shared ~70% amino acid identity with the Saanich Inlet metagenome (Table 1). Not surprisingly, sequences matching SUP05 genes annotated as 'hypothetical' were less conserved than genes with known protein products and were recovered less frequently, potentially reflective of elevated rates of gene loss or sequence change in this genome fraction (although other explanations are possible). Interestingly, however, hypothetical genes were significantly more highly expressed than known genes (Table 1), consistent with other studies indicating the potentially high functional importance of uncharacterized, or peripheral, components of the genome [24–26]. Comparative metagenomic studies should help to clarify to what extent these genes contribute to niche-specific variation in SUP05-like cells from diverse low-oxygen waters.

A role for pelagic microbial sulfate reduction

The connection between the ETSP OMZ sulfur-oxidizer community and its reduced sulfur source is unclear. Sulfide, which stimulates nitrate reduction in this community [13], has not been readily detected in OMZ waters, raising the question of how pelagic oxidizers obtain their sulfide. In most sulfidic marine habitats (e.g. anoxic sediments, closed anoxic basins such as the Black Sea), sulfide originates from microbial sulfate reduction. However, sulfide production by this process would presumably be rare in the OMZ water column where alternative, more energetically favourable, electron acceptors (e.g. nitrite, nitrate or even low amounts of oxygen) are available and would be used preferentially over sulfate for microbial respiration [27]. Indeed, in instances when sulfide has been detected in OMZs, it either has diffused from underlying anoxic sediments or is associated with layers where nitrate and nitrite are lacking [10,28,29].

Figure 1 | **Relative abundance of metagenome (DNA, top) and metatranscriptome (RNA, bottom) sequences matching key protein-coding genes at four depths in the ETSP OMZ**

'Symbiont-like' sequences match (as top BLASTX hit) genes in either the SUP05 metagenome, or the symbiont genomes of Rm or Vo (see the text). Sequences matching genes of 'other taxa' are shown for comparison. The 'all genes' panel excludes genes matching other taxa. Abundance is shown as a proportion of total protein-coding sequence reads in each dataset. Depths: oxycline = 50 m; sub-oxycline = 85 m; upper OMZ = 110 m; OMZ core = 200 m. Data taken from [15].

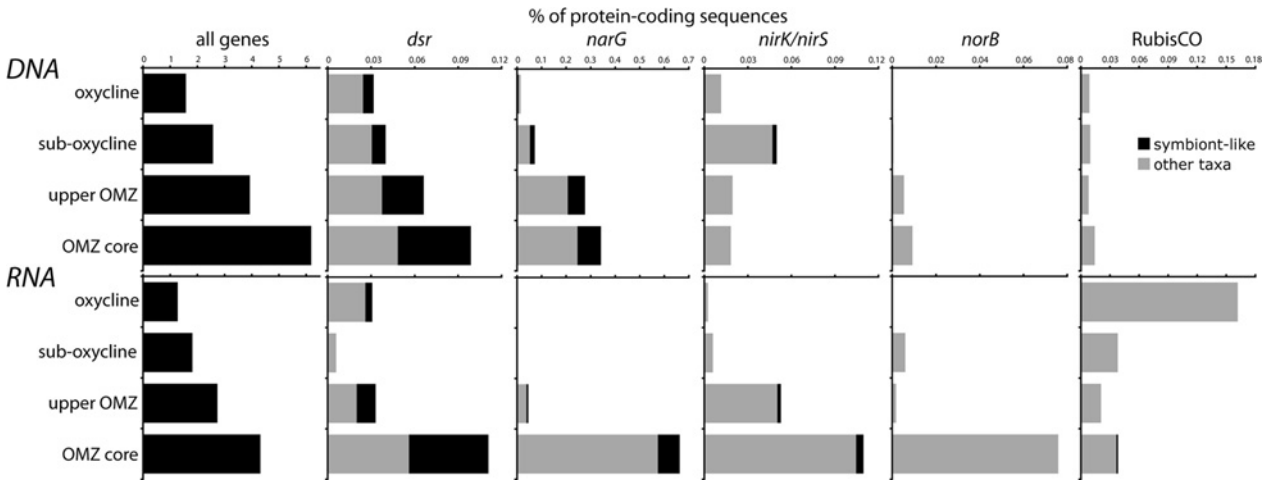

Table 1 | **Sequences matching SUP05 genes encoding known and hypothetical proteins in ETSP OMZ metagenomes (DNA) and metatranscriptomes (RNA)**

Known proteins are SUP05 genes annotated in NCBI with a known protein product ($n = 1002$) [14]. Hypothetical proteins are SUP05 genes annotated in NCBI as 'hypothetical' ($n = 454$) [14]. Data for 2008 and 2010 are from [15] and [13] respectively; numbers in parentheses are total counts of 454 pyrosequencing reads with significant BLASTX matches to protein-coding genes in the NCBI-nr database.

	2010 DNA 150 m ($n = 957\,153$)		2008 DNA 200 m ($n = 274\,463$)		2008 RNA 200 m ($n = 39\,218$)	
Parameter	**Known**	**Hypothetical**	**Known**	**Hypothetical**	**Known**	**Hypothetical**
SUP05 genes*	885	284	834	223	344	63
Recovery (%)†	88	63	83	49	34	14
Abundance‡	0.0078	0.0058	0.0041	0.0036	0.0078	0.0123
Amino acid identity§	72.5	62.5	73.9	64.2	69.7	78.5
Expression‖	na	na	na	na	2.3	4.8

*Number of SUP05 genes detected in 2008/2010 datasets (as best matches in BLASTX against NCBI-nr).

†Percentage of total known or hypothetical SUP05 genes.

‡Mean abundance, where abundance equals read counts normalized to dataset size (protein-coding read counts) and gene length (in kb).

§Mean amino acid identity in BLAST-aligned regions (gaps not included).

‖Mean expression ratio (abundance in RNA/abundance in DNA).

Despite these predictions, bottle experiments recently demonstrated a strong role for sulfate reduction to sulfide in the water column of the ETSP OMZ off northern Chile [13]. Sulfate reduction rates in incubations containing radiolabelled sulfate matched or exceeded rates of denitrification and anammox, suggesting that the respiration of organic matter with sulfate could contribute substantially to total carbon mineralization in the OMZ (up to 33%), as well as provide an important supply of ammonium for anammox. Metagenomic datasets from this study revealed sequences matching the dissimilatory sulfur-metabolism genes (e.g. *aprA*, encoding adenosine 5′-phosphosulfate reductase) of sulfate-reducing Deltaproteobacteria (e.g. *Desulfatibacillum* and *Desulfobacterium* spp.), as well as diverse genes from known sulfur-oxidizing bacteria. Together with coupled measurements of high biological sulfide-oxidation rates, these experiments suggested a cryptic sulfur cycle in which sulfate reduction provides sulfide that is immediately reoxidized by a diverse oxidizer community. This tight coupling would be analogous to what has been observed in some benthic habitats where high rates of sediment sulfate reduction are balanced by efficient sulfide oxidation in thioautotrophic bacterial mats, thereby preventing sulfide accumulation [30,31].

Unanswered questions

Metagenomic data, coupled with marker gene surveys and process rate measurements, confirm that sulfur-metabolizing microbial populations are abundant and important for elemental cycling in OMZs. However, several critical questions remain unanswered. The phylogenetic diversity of these micro-organisms within an OMZ is uncertain. Our metagenomic analyses of the ETSP revealed temporally and spatially variable abundances of functional genes matching diverse sulfur-oxidizing and -reducing taxa, in addition to SUP05-like bacteria [13,15]. However, the fragmentary nature of meta-omic DNA and RNA sequences (<350 bp) prohibits accurate phylogenetic identification of the organisms involved. Indeed, some proteins involved in dissimilatory sulfur metabolism (e.g. Dsr and Apr) contain homologues in both oxidative and reductive pathways, and discriminating between these homologues will probably require comparative analyses of full-length gene sequences [32].

The ubiquity, microhabitat distribution and phylogenetic affiliation of OMZ sulfate-reducing bacteria also remain ambiguous. In the ETSP OMZ, metagenomics identified sequences matching known sulfate-reducing taxa [13]. However, reducer-like sequences were a minor fraction of total reads (~2%) relative to sequences matching known sulfur-oxidizers (up to 16%). The disconnect between these values may reflect biases imposed during collection, as bacterioplankton samples were pre-filtered (cells >1.6 μm excluded) before sequencing, thereby excluding particle- or aggregate-associated communities. Targeted analyses of distinct microbial size fractions may reveal sulfate reducers preferentially associated with aggregates, perhaps due to the higher organic matter content or lower oxygen concentrations in these microhabitats. Metatranscriptomic characterizations of these bacteria, directly coupled with sulfate reduction measurements, would help to resolve the taxonomic identity and genetic basis for sulfate reduction in OMZ waters.

Finally, geographic variation in genome content remains uncharacterized for OMZ sulfur-metabolizing bacteria. Our knowledge of the genomes of pelagic OMZ sulfur-oxidizers is based almost exclusively on the SUP05 and ETSP OMZ metagenomes. SUP05-like sequences in these datasets share homology with the Rm and Vo bacteria, both of which live in symbiosis with clams at hydrothermal vents in the Pacific [22,23]. However, this family of clams (Vesicomyidae) occurs worldwide in diverse reducing habitats. The associated symbiont lineages have evolved in parallel with their hosts [33,34], but form distinct evolutionary clades related to geography and the extent of genomic recombination and symbiont integration into the host [35,36]. Complex genomic rearrangements and biogeographic structuring are also likely in the free-living relatives of these bacteria (e.g. SUP05-like clades). Metagenomics coupled with expanded environmental sampling will enable comparisons of gene content and phylogeographic subdivisions across diverse sulfur-oxidizer lineages, potentially revealing niche-specific adaptations to diverse OMZ settings. Such data should guide more intensive physiological characterizations of key functional genes (e.g. via heterologous expression systems or proteomics), as well as attempts to culture OMZ sulfur-metabolizing bacteria.

OMZ expansions and overall declines in oceanic dissolved oxygen are predicted in response to future climate change and human activity [37–39], thereby making it imperative to fully understand the biological response to shifting oxygen conditions. The studies highlighted in the present paper reveal complex communities of dissimilatory sulfur-oxidizing and sulfate-reducing micro-organisms in the OMZ water column. The genomic diversity, *in situ* physiology, and spatial and temporal distributions of these communities remain virtually undescribed for most OMZs. Integrated molecular and physiological studies will be critical for determining the extent to which OMZ sulfur transformations are linked to nitrogen and carbon cycling (e.g. via denitrification, carbon fixation and carbon and ammonium remineralization), and consequently the overall structure and function of low-oxygen waters.

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