

1 **Title: Sulfur oxidation genes in diverse deep-sea viruses**

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17 **One sentence summary:** Viruses of ubiquitous sulfur-oxidizing bacteria in the dark oceans
18 possess genes for oxidation of elemental sulfur.

19 **Abstract :**

20 Viruses are the most abundant biological entities in the oceans and a pervasive cause of mortality
21 of microorganisms that drive biogeochemical cycles. Although the ecological and evolutionary
22 impacts of viruses on marine phototrophs are well-recognized, little is known about their impact
23 on ubiquitous marine lithotrophs. Here we report 18 genome sequences of double-stranded DNA
24 viruses that putatively infect widespread sulfur-oxidizing bacteria. Fifteen of these viral genomes
25 contain auxiliary metabolic genes for the alpha and gamma subunits of reverse dissimilatory
26 sulfite reductase (*rdsr*). This enzyme oxidizes elemental sulfur, which is abundant in the
27 hydrothermal plumes studied here. Our findings implicate viruses as a key agent in the sulfur
28 cycle and as a reservoir of genetic diversity for bacterial enzymes that underpin chemosynthesis
29 in the deep oceans.

30

31 **Main text:**

32 Chemolithoautotrophic bacteria are ubiquitous in the dark oceans (1), where they serve as
33 a sink for CO₂ (2) through primary production that equals up to 53% of the particulate organic
34 carbon exported from the photic zone (3). Uncultured sulfur-oxidizing bacteria of the SUP05
35 clade are among the most abundant and widespread marine chemolithoautotrophs, fixing carbon
36 and oxidizing reduced sulfur species and hydrogen in diverse marine environments such as
37 hydrothermal vent plumes (4), hydrothermal vent-associated animals (5, 6), and oxygen
38 minimum zones (7), where they underpin cryptic links between the sulfur and nitrogen cycles
39 (8). Although viruses are abundant in these deep-sea ecosystems (9), little is known about
40 viruses that infect lithotrophic primary producers.

41 We conducted shotgun metagenomic sequencing on samples from five different
42 hydrothermal vent plumes and associated deep ocean waters at the Eastern Lau Spreading Center
43 (Lau Basin) in the Western Pacific Ocean and one plume at Guaymas Basin in the Gulf of
44 California (10, 11) (Table S1). *De novo* assembly of sequence reads and binning by
45 tetranucleotide signatures (12) revealed discrete genomic ‘bins’ (Fig. S1). Five bins (henceforth
46 Lau77, Lau85, Lau87, Lau218 and Lau220) contained 18 viral genome sequences of putative
47 SUP05 viruses. Phylogeny of the viral large terminase gene (*terL*) (Fig. S2) (which reflects
48 phage DNA packaging mechanisms (13)), synteny with well-characterized phage of known
49 taxonomy (Fig. S3), and results of protein sequence similarity searches against public sequence
50 databases (Fig. S4) indicated that the five viruses belonged to three marine viral families of the
51 orders *Caudovirales* (dsDNA viruses, no RNA stage), *Podoviridae*, *Siphoviridae* and *Myoviridae*
52 (Table S2).

53 Fifteen of the 18 viral genomes (from four of the five SUP05 viral genomic bins)
54 contained genes encoding the alpha (*rdsrA*) and gamma (*rdsrC*) subunits of the reverse-acting
55 dissimilatory sulfite reductase (Rdsr) complex for elemental sulfur oxidation (Fig. 1). No other
56 *rdsr* genes or other sulfur oxidation genes were present on the viral genomes. Analysis of
57 bacterial genome bins recovered from Lau and Guaymas metagenomes revealed co-localized
58 *rdsr* genes in the order *rdsrABEFHCMKLJOPN* in the Gammaproteobacteria Lau10 (SUP05),
59 Lau62 (EC-01-9C-26), and Lau60 (unclassified). The deltaproteobacterium Lau20 (Sar324) (14)
60 possessed only *rdsrAB*. Regions flanking the bacterial *rdsr* genes showed no similarity to the
61 viral genome sequences, suggesting that viral *rdsr* genes were derived from selective retention
62 rather than recent homologous recombination with bacterial genomic DNA.

63 Phylogenetic analyses indicated that all viral *rdsrA* genes recovered affiliated with
64 SUP05 Gammaproteobacteria (74-96% amino acid identity, Fig. S5) and were distinct from
65 *rdsrA* genes of other bacteria (Fig. 2). We identified two distinct groups of *rdsrA* sequences that
66 each included both viral and bacterial sequences. All viral *rdsrA* genes fall into Group one except
67 for Lau85, which contained two copies of *rdsrA* with one representative in each group. Bacterial
68 representatives of Group one included the SUP05 GB-1 and GB-2 from Guaymas as well as
69 *Bathymodiolus* mussel symbionts (6). Group two was populated by SUP05 from oxygen
70 minimum zones (7) and symbionts of deep-sea clams (5). The tight phylogenetic clustering of
71 *rdsrA* gene sequences of three distinct phage families with SUP05 bacteria in two separate
72 lineages suggests that the phage *rdsrA* genes originated from SUP05 and were transferred to
73 viruses. These observations are analogous to those of core photosynthesis genes in
74 cyanobacterial phages and other microbe-derived auxiliary metabolic genes (15, 16) (e.g. *psbA*,
75 *psbD*, *mazG*) that are similar but not identical to known hosts, forming sub-clusters distinct from
76 host proteins (17, 18).

77 The amino acid sequences deduced from the viral *rdsrA* and *rdsrC* genes indicated the
78 capacity to serve as functional sulfur-oxidizing enzymes. Phage RdsrA contained all conserved
79 sulfite reductase residues and secondary structure elements for α -helix and β -sheets (Fig. S6).
80 Similarly, a multiple alignment of RdsrC amino acid sequences indicated highly conserved
81 residues across two distinct groups (Fig. S7). We also identified other genes in the viral genomes
82 with high sequence identity to SUP05, including iron-sulfur cluster proteins, cytochromes, and
83 sulfur relay proteins (Table S3). The existence of these additional SUP05-like genes on viral
84 genomes supports their specificity to SUP05 bacteria and suggests a role for viral genes in
85 supplementing host metabolism.

86 Elemental sulfur is a key and stable intermediate of bacterial oxidation of reduced sulfur
87 compounds and constitutes a ‘bottleneck’ in the sulfur cycle (19). Sulfur-oxidizing bacteria with
88 an incomplete Sox pathway, such as SUP05, form intracellular or extracellular globules of
89 elemental sulfur that serve as a store of electron donor for energy metabolism (Fig. 3) (4, 19, 20,
90 21). The presence of *rdsrA* and *rdsrC* on viral genomes may offer selective advantages to the
91 viruses by supplementing host pathways for oxidation of this sulfur during infection. First,
92 enhanced expression of *rdsrA* could replenish proteins involved in a rate limiting reaction in the
93 host, as previously demonstrated with cyanobacterial phage D1 proteins involved in
94 photosynthesis (22). Second, phage *rdsrC* could maintain or increase high transcription levels to
95 ensure efficient delivery of sulfur-substrate to the RdsrAB complex during infection. Thus,
96 phage auxiliary metabolic genes that can supplement or sustain sulfur oxidation metabolism in
97 their hosts may ensure continued viral infection and replication.

98 A coupled bioenergetic-thermodynamic reaction path model (23) indicated that aerobic
99 elemental sulfur oxidation potentially accounted for 80-93% of the total lithotrophic energy
100 available in the ABE and Mariner hydrothermal plumes at Lau Basin at two temperatures that
101 were representative of the plumes studied here (Fig. 4A, Fig. S8). The model predicted a minor
102 role for 19 other lithotrophic microbial metabolisms considered, including oxidation of other
103 reduced sulfur species such as hydrogen sulfide, sulfite and thiosulfate. These results were
104 consistent with a previous model of hydrothermal plumes that found elemental sulfur to be the
105 most abundant source of chemosynthetic energy (24), and with our previous work (4, 25, 26),
106 which was based on the same approach. Our model predicted greater relative energy yields for
107 more energetically favored metabolisms such as sulfur oxidation because we assumed that
108 metabolic reactions occurred in sequence, from most to least energetically favored, and enabled

109 reactions to explicitly modify the product and reactant pools. Although there is some uncertainty
110 regarding the speciation of sulfur present in the plume, elemental sulfur is a central intermediate
111 in the oxidation of other reduced forms of sulfur used by SUP05 (Fig. 3). X-ray fluorescence
112 maps showed that sulfur was abundant in the plumes (Fig. 4B, Fig. S8), while microprobe X-ray
113 diffraction showed elemental sulfur was widely present in particle aggregates with other
114 crystalline phases, such as pyrite (Fig. 4C, Fig. S8). Although we did not conclusively identify
115 intracellular elemental sulfur, our results showed that elemental sulfur presented an abundant
116 source of energy for SUP05 bacteria in hydrothermal plumes and deep ocean waters of Guaymas
117 and Lau basins.

118 The abundance and diversity of viruses infecting SUP05 bacteria in hydrothermal plumes
119 suggests that chemolithoautotrophs in the deep sea face viral predation pressures similar to
120 photosynthetic microbes in the surface waters (27). The remarkable synteny and conservation of
121 the four viruses studied here (95-99% genome nucleotide identity) across hydrothermal vent
122 environments and ocean basins suggests that these viruses are ubiquitous in marine environments
123 dominated by SUP05 bacteria. Analyses of the Pacific Ocean Virome dataset (28) (Fig. S9),
124 which notably contains viral communities from oxygen minimum zones dominated by SUP05
125 (7), revealed the presence of *rdsrA* and *rdsrC* genes (Table S5), consistent with the prevalence of
126 phage-encoded sulfur oxidation beyond hydrothermal plumes and in the wider pelagic oceans.

127 To date, deep-sea SUP05 has evaded growth in laboratory cultures, thus direct host-phage
128 manipulations and validation of the underlying mechanisms of phage-influenced sulfur oxidation
129 remain a challenge. Yet, this study demonstrates the sequence-based elucidation of microbial
130 community dynamics through the discovery of phages that infect a widespread deep-sea
131 bacterium. The existence of *rdsr* genes in viral genomes reveals a mechanism for horizontal

132 transfer of genes associated with sulfur cycling (29) and implicates viruses in the evolutionary
133 dynamics of a central step in the planetary cycling of sulfur.

134

135 **Supplementary Materials**

136

137 www.sciencemag.org

138 Materials and Methods

139 Supplementary Text

140 Figs. S1 to S10

141 Tables S1 to S6

142 References (30-80)

143 Database S1

144

145 **References and Notes**

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194

195 **FIGURE CAPTIONS**

196 **Fig. 1**

197 Gene content of 15 phage genomes from three viral families retrieved from Lau and Guaymas
198 basins. Colored nested circles represent syntenous viral genomes/contiguous genomic fragments
199 from locations indicated in the legend. Grey boxes on outermost circle indicate predicted genes
200 of the four viruses. *rdsrA* and *rdsrC* genes are highlighted in yellow.

201

202 **Fig. 2**

203 **A.** Phylogenetic tree of *rdsrA* genes inferred by Maximum Likelihood. **B.** Detailed view of the
204 SUP05 *rdsrA* clade. Group one and Group two sub-clades are shown on the right. Sequences are
205 colored by geographical origin; Blue – Guaymas Basin; Red – Kilo Moana (Lau Basin); Green –
206 Tahi Moana (Lau Basin); Purple – ABE (Lau Basin); Brown – Tui Malila (Lau Basin); Orange –
207 Mariner (Lau Basin).

208

209 **Fig. 3.**

210 Schematic of the sulfur oxidation pathway in SUP05 bacteria. Grey box indicates the reaction
211 impacted by SUP05 viruses. Key genes are shown in italics: *sqr* (sulfide quinone reductase), *sox*

212 (sulfur oxidation), *rdsr* (reverse dissimilatory sulfite reductase), *apr* (adenosine 5'-
213 phosphosulfate reductase), and *sat* (sulfate adenylyltransferase).

214 **Fig. 4**

215 **A.** Modeled free energies of catabolic reactions as a percentage of total available free energy in
216 the ABE hydrothermal plume at 2.5 and 5°C. Total available free energy in the plume is
217 normalized per kilogram plume fluid (p.f.) and per kilogram vent fluid (v.f.). **B.** Distribution of
218 iron (displayed in red) and sulfur (displayed in green) in particles collected at 0.5 m above the
219 ABE vent. Locations where elemental sulfur was detected by micro-probe X-ray diffraction
220 measurements are indicated as spots 0, 1, 2, and 19. **C.** Radially integrated diffractograms with
221 elemental sulfur peaks annotated (*) at 22.0, 25.7, and 34.1 degrees 2-theta.