

Genomic sequence analysis of *Dissulfurirhabdus thermomarina* SH388 and proposed reassignment to Dissulfurirhabdaceae fam. nov.

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Short report

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Abstract

Here, we report the draft genome sequence of *Dissulfurirhabdus thermomarina* SH388. Improved phylogenetic and taxonomic analysis of this organism using genome-level analyses supports assignment of this organism to a novel family within the Desulfobacterota phylum. Additionally, comparative genomic and phylogenetic analyses contextualize the convergent evolution of sulfur disproportionation and potential extracellular electron transfer in this organism relative to other members of the Desulfobacterota.

Background:

The disproportionation of intermediate valence sulfur species (converting substrates such as sulfite, thiosulfate, and elemental sulfur into products of sulfate and sulfide) is a poorly understood microbial metabolism that has been implicated in the production and preservation of extremely depleted sulfur isotope signatures in the rock record (e.g. Canfield and Thamdrup 1994; Canfield and Teske, 1996). Despite its potential significance for understanding paleoredox proxies (e.g. Johnston et. al., 2005), this metabolism is poorly understood (e.g. Finster 2008) and the genetic capacity for sulfur disproportionation is largely indistinguishable from that for sulfate reduction based on sequence data alone (Anantharaman et. al., 2018). Mechanisms for elemental sulfur disproportionation are not well understood, yet this process is of significant interest particularly due to its ability to utilize an insoluble extracellular substrate. Most of the diversity of organisms shown to be capable of disproportionating elemental sulfur are members of the Desulfobulbaceae family of Desulfobacterota (formerly Deltaproteobacteria) (Finster 2008). However, a small number of distantly related organisms are also capable of elemental sulfur disproportionation, though it remains unknown whether these lineages use the same or different pathways for sulfur disproportionation, and whether these pathways have evolved convergently or if their distribution is the result of a single evolutionary innovation followed by horizontal gene transfer. In order to resolve these possibilities, we report here the draft genome sequence of *Dissulfurirhabdus thermomarina* SH388. This organism was first isolated from a shallow marine hydrothermal vent and was shown to be capable of disproportionating sulfur compounds (including elemental sulfur and sulfite) (Slobodkina et. al., 2016) but is of uncertain phylogenetic affinity within the Desulfobacterota and is not closely related to elemental sulfur disproportionators within the Desulfobulbaceae. Understanding the genetics and evolutionary history of sulfur metabolism in *D. thermomarina*, in contrast to members of the Desulfobulbaceae, may therefore provide insight into the diversity, mechanism, and evolution of sulfur disproportionation metabolisms more broadly.

Methods:

Genome sequencing and analysis followed methods described previously (Bertran et al. 2020a, Bertran et al. 2020b) and summarized here. Purified genomic DNA of strain DSM100025 was ordered from the DSMZ and submitted to MicrobesNG for sequencing. Cultures were grown anaerobically at 50 °C on Medium 1210a with strain-specific modifications before genomic DNA extraction with a JetFlex genomic

DNA purification kit from Genomed. DNA libraries were prepared with a Nextera XT library prep kit on a Hamilton Microlab Star automated liquid handling system. Libraries were sequenced on an Illumina HiSeq using a 250 bp paired end protocol. Reads were adapter trimmed using Trimmomatic 0.30 (Bolget et al. 2014) and de novo assembly was performed using SPAdes version 3.7 (Bankevich et al. 2012). Annotation was performed using RAST v2.0 (Aziz et. al., 2008). Genome completeness was estimated with CheckM v1.0.12 (Parks et. al., 2015), and likelihood of presence or absence of metabolic pathways was estimated with MetaPOAP v1.0 (Ward et. al., 2018). The taxonomic assignment of the genome was verified with GTDB-Tk v0.3.2 (Parks et. al., 2018). Hydrogenase proteins were classified with HydDB (Søndergaard et. al., 2016).

Phylogenetic analyses followed methods described previously (Ward et. al., 2019a, Ward et. al., 2019b) and summarized here. Additional *Desulfobulbales* genomes were downloaded from the NCBI Genbank and WGS databases. Protein sequences used in analyses (see below) were identified locally with the `tblastn` function of BLAST+ (Camacho et. al., 2009), aligned with MUSCLE (Edgar 2004), and manually curated in Jalview (Waterhouse 2009). Positive BLAST hits were considered to be full length (e.g. > 90% the shortest reference sequence from an isolate genome) with e-values greater than $1e^{-20}$. Phylogenetic trees were calculated using RAxML (Stamatakis 2014) on the Cipres science gateway (Miller et. al., 2010). Transfer bootstrap support values were calculated by BOOSTER (Lemoine et. al., 2018), and trees were visualized with the Interactive Tree of Life viewer (Letunic and Bork 2016). Taxonomic assignment was confirmed with GTDB-Tk (Parks et. al., 2018). Histories of vertical versus horizontal inheritance of metabolic genes were inferred by comparison of organismal and metabolic protein phylogenies (Doolittle 1986, Ward et. al., 2018a, Ward et. al., 2019c).

Results:

The *Dissulfurirhabdus thermomarina* genome was sequenced at ~ 85x coverage as 508907 reads. The assembled genome was recovered as 413 contigs (292 > 500 nt, 194 > 2500 nt) for a total of 2536728 nt with 70.82% GC and an N50 of 14884. It encodes 2791 coding sequences and 53 RNAs. The genome was estimated by CheckM to be 96.75% complete with 1.94% redundancy of which 33.33% is due to heterogeneity.

Discussion:

When first isolated, *D. thermomarina* was placed in the Deltaproteobacteria but was not assigned at lower taxonomic ranks. Following genome-wide taxonomic analysis with GTDB-Tk, *D. thermomarina* is robustly placed in the *Dissulfuribacteriales* order (phylum *Desulfobacterota*, class *Dissulfuribacteria*), but does not cluster with any characterized families within this order. *D. thermomarina* is sufficiently divergent from its closest characterized relative, *Dissulfuribacter thermophilus*, to suggest that these organisms represent separate families within the *Dissulfuribacteriales*. We therefore propose assignment of *D. thermomarina* as the type species of a novel family, *Dissulfurirhabdaceae*, within the *Dissulfuribacteriales* order of *Desulfobacterota*.

Sulfur disproportionators are expected to encode the same marker genes as sulfate reducers (e.g. Anantharaman et. al., 2018); consistent with this expectation, *D. thermomarina* encodes sulfate adenylyltransferase, adenylylsulfate reductase, dissimilatory sulfite reductase, and the complex DsrMKJOP, which is associated with sulfite reduction. Known sulfur disproportionators in the Desulfobulbaceae family of Desulfobacterota encode AprB proteins with a truncated tail; this conserved truncation has been proposed as a molecular marker of the capacity for sulfur disproportionation (Bertran 2019). *D. thermomarina* encodes an AprB protein with a similar truncation despite encoding an AprB protein that is only distantly related to those of the Desulfobulbaceae (Fig. 2). This AprB truncation appears to have independently evolved in *D. thermomarina*, reinforcing interpretations of the association between this marker and the capacity for disproportionation; moreover, the apparently independent acquisition of the AprB truncation and the capacity for disproportionation in *D. thermomarina* and members of the Desulfobulbaceae suggests that this might be a more widespread trait that has evolved convergently multiple times in ancestrally sulfate reducing lineages.

D. thermomarina is capable of autotrophic growth (Slobodkina et. al., 2016) and encodes CO dehydrogenase/acetyl-CoA synthase, suggesting it makes use of the reductive acetyl-CoA (Wood-Ljungdahl) pathway like many sulfate reducing bacteria (Berg 2011). The genome encodes a hydrogenase annotated by HydDB as a Group 1c NiFe hydrogenase associated with anaerobic respiratory uptake of H₂. *D. thermomarina* does not encode canonical proteins for aerobic respiration or denitrification, consistent with its inability to use O₂ or nitrate as electron acceptors in culture (Slobodkina et. al., 2016). However, this organism encodes a bd O₂ reductase; while these enzymes can in some cases be coupled to aerobic respiration (e.g. in *Nitrospira*, Palomo et al. 2018), they are often found in obligate anaerobes (e.g. Ward et. al., 2015) in which they are likely used for O₂ detoxification and oxidative stress tolerance (Forte et. al., 2017).

D. thermomarina is capable of disproportionating not only soluble sulfur species such as sulfite but also insoluble elemental sulfur (Slobodkina et. al., 2016). The mechanism of elemental sulfur disproportionation in *D. thermomarina* and other strains capable of this metabolism is not known yet, but likely involves novel extracellular electron transfer pathways. Extracellular multiheme cytochrome proteins are commonly involved in respiratory electron transfer to insoluble mineral substrates (e.g. McGlynn et. al., 2015, Shi et. al., 2016) and could therefore be expected to be involved in disproportionation of elemental sulfur. Analysis of the *D. thermomarina* genome with CXXCH_finder (McGlynn et. al., 2015) recovered 81 proteins with heme binding domains, including one hypothetical protein with 26 heme binding motifs—on par with proteins from bacteria such as *Geobacter* and *Shewanella*, well known for their capacity for extracellular electron transfer and ability to respire extracellular mineral substrates (McGlynn et. al., 2015). This hypothetical protein shows low similarity to other proteins accessible on the NCBI database (< 50% to any sequences), but of all proteins from well-characterized organisms it is most similar to a hypothetical protein from *Thermosulfidibacter takii*, a thermophilic bacterium capable of elemental sulfur reduction. The putative extracellular electron transfer protein from *D. thermomarina* also has notable similarity (~ 30%) to extracellular iron oxide respiratory

system periplasmic decaheme cytochrome c protein components from *Shewanella oneidensis* MR-1 including the protein DsmE associated with respiration of extracellular substrates (e.g. Bewley et. al., 2012). We therefore propose that *D. thermomarina* utilizes extracellular multiheme cytochrome proteins related to those in dissimilatory iron reducing bacteria in order to transfer electrons to insoluble substrates such as elemental sulfur. In contrast, members of the Desulfobulbales that are characterized as being capable of elemental sulfur disproportionation (e.g. *Desulfobulbus propionicus*, *Desulfocapsa thiozymogenes*) encode proteins with no more than 11 or 12 CxxCH motifs, suggesting that different lineages may have evolved multiple different mechanisms to enable the metabolism of insoluble sulfur compounds.

Conclusions:

In addition to better constraining the phylogenetic placement and taxonomy of this organism, the draft genome of *D. thermomarina* provides evidence for the evolutionary history of metabolic pathways it employs. This includes the apparent convergent evolution for the capacity for sulfur disproportionation in different lineages of Desulfobacterota (e.g. *Dissulfurirhabdus* and members of Desulfobulbaceae). These organisms appear to have evolved sulfur disproportionation via similar genetic mechanisms independently from an ancestral state of sulfate reduction. The presence of putative multiheme cytochrome proteins encoded by *D. thermomarina* also suggests that the capacity for elemental sulfur disproportionation may in some cases utilize mechanisms homologous to those used in the respiration of extracellular electron acceptors such as metal oxides as has been well studied in organisms such as *Geobacter* and *Shewanella*, demonstrating the versatility of microorganisms to adapt proteins encoded in their genomes and acquired via horizontal gene transfer in order to develop novel metabolic traits.

Declarations

Ethics approval and consent to participate:

Not applicable

Consent for publication:

Not applicable

Availability of data and materials:

The datasets generated during and analysed during the current study are available in the NCBI repository under project ID PRJNA579145. Raw sequencing data is available in the SRA database under Accession ID SRR11035951 while the assembled genome is available in the WGS database Accession ID of JAAGRR010000000.

Competing interests:

The authors declare that they have no competing interests

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Authors contributions:

LMW, EB, and DTJ conceived of the study. LMW and EB processed samples and analyzed data. LMW wrote the manuscript with assistance from EB and DTJ. All authors read and approved the final manuscript.

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Figures

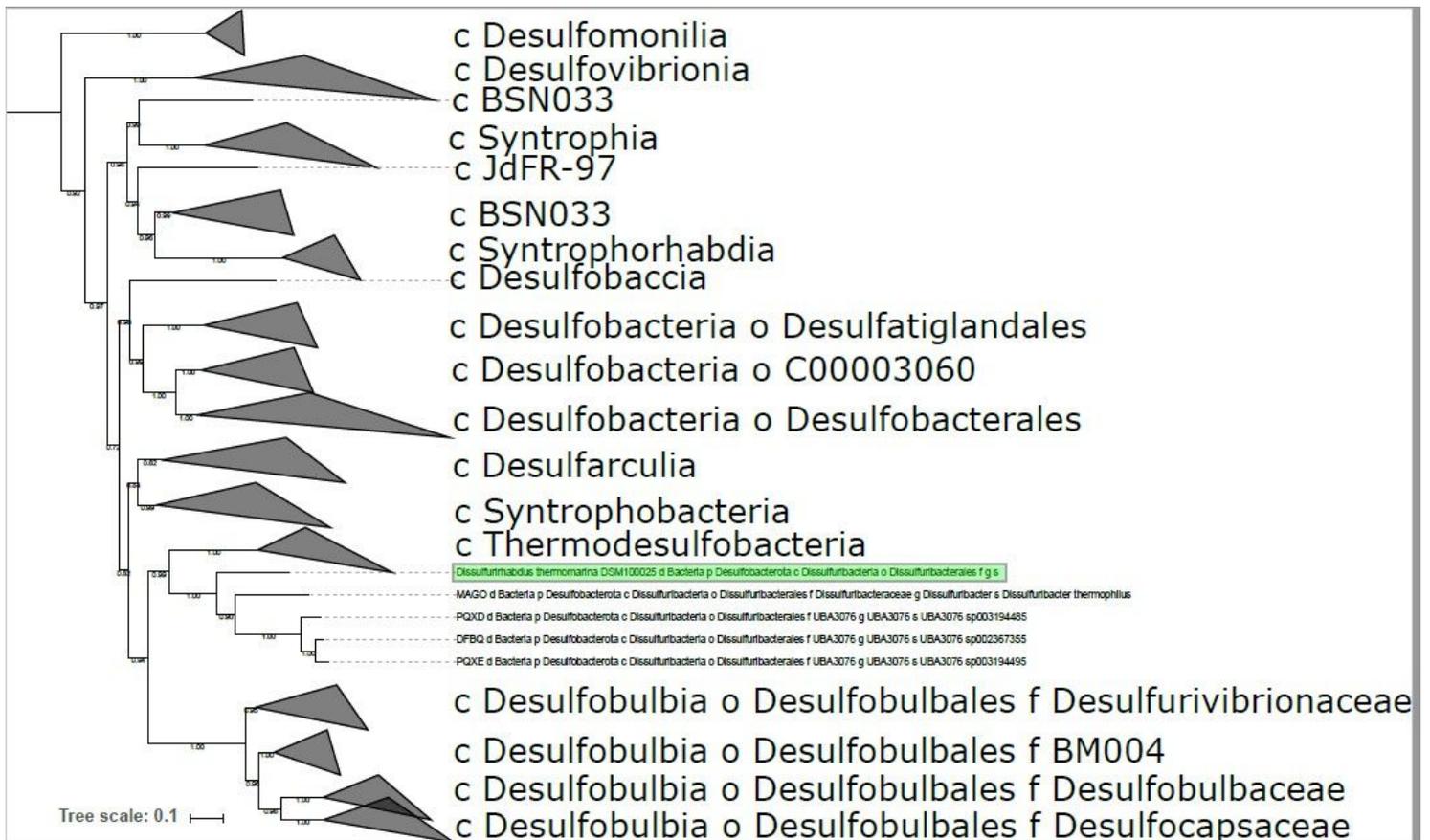


Figure 1

1. Phylogeny of the Desulfobacterota, showing the placement of *Dissulfurhabdus thermomarina* relative to other lineages, built with concatenated ribosomal proteins following methods from Hug et. al., 2016. Strains are labeled with NCBI WGS ID and/or taxonomic assignments made with GTDB-Tk (Parks et. al., 2018).

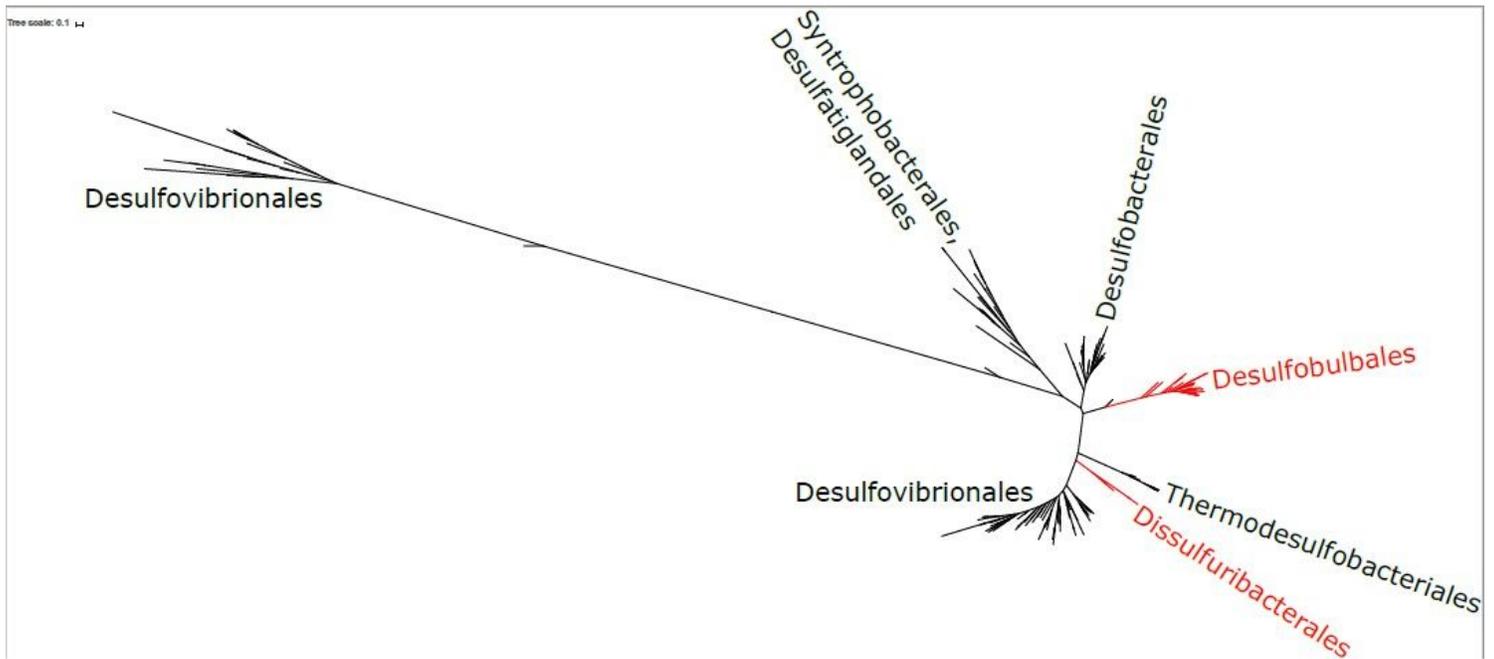


Figure 2

2. Phylogeny of AprB proteins from members of the Desulfobacterota, with sequences from the Dissulfuribacterales (including *Dissulfurirhabdus thermomarina*) and members of the Desulfobulbales highlighted in red. These groups include sulfur disproportionators which encode AprB proteins with a truncated tail, but they are not closely related and are separated by many lineages of non-disproportionating bacteria that encode full-length AprB proteins; it therefore appears that these traits have convergently evolved in the two lineages.