Complete genome sequence of *Desulfomicrobium baculatum* type strain (X²)

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*Desulfomicrobium baculatum* is the type species of the genus *Desulfomicrobium*, which is the type genus of the family *Desulfomicrobiaceae*. It is of phylogenetic interest because of the isolated location of the family *Desulfomicrobiaceae* within the order *Desulfovibrionales*. *D. baculatum* strain X² is a Gram-negative, motile, sulfate-reducing bacterium isolated from water-saturated manganese carbonate ore. It is strictly anaerobic and does not require NaCl for growth, although NaCl concentrations up to 6% (w/v) are tolerated. The metabolism is respiratory or fermentative. In the presence of sulfate, pyruvate and lactate are incompletely oxidized to acetate and CO₂. Here we describe the features of this organism, together with the complete genome sequence and annotation. This is the first completed genome sequence of a member of the deltaproteobacterial family *Desulfomicrobiaceae*, and this 3,942,657 bp long single replicon genome with its 3494 protein-coding and 72 RNA genes is part of the Genomic Encyclopedia of Bacteria and Archaea project.

Introduction

Strain X² (DSM 4028 = CCUG 34229 = VKM B-1378) is the type strain of the species *Desulfomicrobium baculatum*, which is the type species of the genus *Desulfomicrobium*. Strain X² was first described as *Desulfovibrio baculatus* by Rozano-va and Nazina [1,2], and later transferred to the novel genus *Desulfomicrobium* (currently containing seven species) [3] (Figure 1) because several phenotypic traits were not consistent with the definition of the genus *Desulfovibrio*. In
Fermented. Vitamins are not required for growth [3]. *D. baculatum* strain 9974 (DSM 1743) is also able to use ethanol as a substrate [10] and sulfur as an electron acceptor [6]. The use of ethanol as an electron donor for sulfate respiration depends on supplementing the medium with the trace elements tungstate or molybdate [10]. Sulfate uptake in symport with sodium ions has been shown in strain 9974, unlike in other fresh water sulfate reducers which use protons [11]. Distinctive features of *D. baculatum* strain XT are: (i) NaCl is not required for growth [3], (ii) fermentation of fumarate and malate to succinate and acetate is preferred against utilization of these substrates as electron donors for sulfate reduction [9], (iii) sulfur is not used as an electron acceptor and (IV) molecular nitrogen can be assimilated [3].

A desulfoviridin-type dissimilatory sulfite reductase, which is a hallmark feature of the genus Desulfovibrio, is absent in strain XT, however a sulfite reductase of the desulforubidin-type was reported for strain 9974 [12]. Cells of *D. baculatum* strain XT contain c- and b-type cytochromes [3]. The tetraheme cytochrome c₅₅ of strain 9974, which is thought to play a role in sulfur reduction and the coupling of electron transfer to hydrogenases, has been analyzed in some detail using advanced biophysical methods [13-15]. Strain 9974 also contains several distinct [NiFeSe] hydrogenases that are located in different cellular compartments [16]. The crystal structure of the periplasmic [NiFeSe] hydrogenase of this strain has been determined [17] and it is proposed that the selenium ion in the active center plays a role in the oxygen-tolerant hydrogen production of this enzyme, which distinguishes it from most [NiFe] hydrogenases [18]. An active selenocysteine system for usage of the 21st amino acid has been studied in detail for *D. baculatum* strain 9974 [19-21]. Pyridoxal-5'-phosphate, the prosthetic group of selenocysteine synthases, is bound to a distinct lysine residue (Lys295) within the active site of the enzyme of this strain [20].

Figure 1 shows the phylogenetic neighborhood of *D. baculatum* strain XT in a 16S rRNA based tree. Analysis of the two 16S rRNA gene sequences in the genome of strain XT indicated that the two genes are almost identical (1 bp difference), and that both genes differed by one nucleotide from the previously published 16S rRNA sequence generated from DSM 4028 (AJ277894).

### Classification and features

Cells of *D. baculatum* strain XT are short rods with rounded ends of 0.6 x 1-2 μm (Figure 2). Cells stain Gram-negative, are motile by a single polar flagellum, and do not form endospores. The metabolism is strictly anaerobic and can be respiratory or fermentative [3,9]. Temperature range for growth is 2-41°C (optimum 28-37°C) and NaCl concentrations of 0-6% (w/v) are tolerated (optimum 1% w/v). Sulfate, sulfite and thiosulfate are used as electron acceptors and are reduced to H₂S. Nitrate is not reduced. Simple organic compounds are incompletely oxidized to acetate [3]. Malate, fumarate and pyruvate can be fermented with succinate and acetate as end products. Carbohydrates are not fermented. Vitamins are not required for growth [3].
Figure 1. Phylogenetic tree of *D. baculatum* strain X*T* and all type strains of species within members of the family *Desulfomicrobiaceae*, inferred from 1,457 aligned characters [22, 23] of the 16S rRNA gene sequence under the maximum likelihood criterion [24]. The tree was rooted with all members from the *Desulfonatronaceae*, another family in the order *Desulfovibrionales*. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1,000 bootstrap replicates if larger than 60%. Strains with a genome-sequencing project registered in GOLD [25] are printed in blue; published genomes in bold.

Figure 2. Scanning electron micrograph of *D. baculatum* X*T* (Manfred Rohde, Helmholtz Centre for Infection Biology, Braunschweig)
The cellular fatty acid patterns of *D. baculatum* strain X^T^ and the accompanying strains 5174, 9974 and H.L21 [26] were found to be dominated by anteiso- (ai) and iso-methyl branched unsaturated and saturated fatty acids. The most abundant fatty acid is iso-17:1 cis7 (24.2–28.6%), followed by 18:1 cis11 (6.4–12.2%), iso-15:0 (8.2–11.6%), ai-17:0 (4.5–8.3%), ai-15:0 (5.2–7.7%), 18:0 (3.9–7.1%) and 16:0 (3.6–5.7%). Less abundant fatty acids are iso-15:1 (3.1–4.0%), 16:1 cis7 (2.2–5.0%), ai-17:1 (2.4–4.1%), 18:1 cis9 (2.6–4.3%), iso-16:1 (0.5–2.2%), and 17:0 (0.2–0.3%). Branched chain, hydroxylated fatty acids are also present, 3-OH iso-15:0 (1.4–2.4%), 3-OH ai-15:0 (0.7–1.2%), and 3-OH iso-17:0 (1.2–2.2%), which may be derived from a lipopolysaccharide. The polar lipid composition of *D. baculatum* strain X^T^ has not been investigated. The respiratory quinone composition of *D. baculatum* strain X^T^ has also not been investigated, but the presence of MK-6 has been reported in *D. macestii* and *D. norvegicum* [7, 27].

### Table 1. Classification and general features of *D. baculatum* X^T^ in accordance to the MIGS recommendations [28]

| MIGS ID | Property                  | Term                                                                 | Evidence code |
|---------|---------------------------|----------------------------------------------------------------------|---------------|
|         | Current classification    |                                                                       |               |
|         | Domain                    | Bacteria                                                             |               |
|         | Phylum                    | Proteobacteria                                                       |               |
|         | Class                     | Deltaproteobacteria                                                  |               |
|         | Order                     | Desulfovibrionales                                                  |               |
|         | Family                    | Desulfomicrobiaceae                                                 |               |
|         | Genus                     | Desulfomicrobium                                                     |               |
|         | Species                   | Desulfomicrobium baculatum                                           | TAS [29]      |
|         | Type strain               | X                                                                    |               |
|         | Gram stain                | negative                                                             | TAS [1]       |
|         | Cell shape                | rod-shaped                                                            | TAS [1]       |
|         | Motility                  | motile, single polar flagellum                                       | TAS [1]       |
|         | Sporulation               | non-sporulating                                                      | TAS [1]       |
|         | Temperature range          | mesophilic                                                           | TAS [1]       |
|         | Optimum temperature        | 28–37°C                                                              | TAS [1]       |
|         | Salinity                  | 10 g NaCl/l                                                          | TAS [1]       |
|         | MIGS-22                   | Oxygen requirement                                                   |               |
|         |                            | strictly anerobic                                                    | TAS [1]       |
|         | MIGS-6                    | Habitat                                                              |               |
|         |                            | freshwater to brackish anoxic sediments                              | TAS [1]       |
|         | MIGS-15                   | Biotic relationship                                                  |               |
|         |                            | free-living                                                          | NAS           |
|         | MIGS-14                   | Pathogenicity                                                        |               |
|         |                            | none                                                                 | NAS           |
|         | MIGS-4                    | Biosafety level                                                      |               |
|         |                            | 1                                                                    | TAS [30]      |
|         | MIGS-4.1                  | Isolation                                                            |               |
|         |                            | water-saturated manganese carbonate ore                              | TAS [1]       |
|         | MIGS-4.2                  | Geographic location                                                 |               |
|         |                            | not reported                                                         |               |
|         | MIGS-4.3                  | Sample collection time                                               |               |
|         |                            | 1975 or earlier                                                      | IDA           |
|         | MIGS-4.4                  | Latitude – Longitude                                                 |               |
|         |                            | not reported                                                         |               |
|         | MIGS-4.5                  | Depth                                                                |               |
|         |                            | not reported                                                         |               |

Evidence codes – IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [31]. If the evidence code is IDA, then the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.
The genome was sequenced using a combination of Sanger and 454 sequencing platforms. All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website. 454 Pyrosequencing reads were assembled using the Newbler assembler version 1.1.02.15 (Roche). Large Newbler contigs were broken into 4,375 overlapping fragments of 1,000 bp and entered into assembly as pseudo-reads. The sequences were assigned quality scores based on Newbler consensus q-scores with modifications to account for overlap redundancy and to adjust inflated q-scores. A hybrid 454/Sanger assembly was made using the parallel phrap assembler (High Performance Software, LLC). Possible mis-assemblies were corrected with Dupfinisher or transposon bombing of bridging clones [32]. Gaps between contigs were closed by editing in Consed, custom primer walk or PCR amplification. 731 Sanger finishing reads were produced to close gaps, to resolve repetitive regions, and to raise the quality of the finished sequence. The error rate of the completed genome sequence is less than 1 in 100,000. Together all sequence types provided 37.2 x coverage of the genome.

### Genome annotation

Genes were identified using Prodigal [33] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using JGI’s GenePRIMP pipeline [34]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, Uni-
Desulfovicrobium baculatum type strain (XT)

Prot, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation were performed within the Integrated Microbial Genomes (IMG-ER) platform [35].

**Genome properties**
The genome is 3,942,657 bp long and comprises one circular chromosome with a 58.7% GC content (Table 3 and Figure 3). Of the 3,565 genes predicted, 3,494 were protein coding genes, and 71 RNAs; 58 pseudogenes were also identified. 74.9% of the genes were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The properties and the statistics of the genome are summarized in Table 3. The distribution of genes into COGs functional categories is presented in Table 4.

### Table 3. Genome Statistics

| Attribute                        | Value     | % of Total |
|----------------------------------|-----------|------------|
| Genome size (bp)                 | 3,942,657 |            |
| DNA Coding region (bp)           | 3,572,336 | 90.61%     |
| DNA G+C content (bp)             | 2,312,250 | 58.65%     |
| Number of replicons              | 1         |            |
| Extrachromosomal elements        | 0         |            |
| Total genes                      | 3565      |            |
| RNA genes                        | 71        | 2.02%      |
| rRNA operons                     | 2         |            |
| Protein-coding genes             | 3494      | 97.98%     |
| Pseudo genes                     | 58        | 1.63%      |
| Genes with function prediction   | 2675      | 75.01%     |
| Genes in paralog clusters        | 357       | 12.82%     |
| Genes assigned to COGs           | 2689      | 75.41%     |
| Genes assigned Pfam domains      | 2688      | 75.38%     |
| Genes with signal peptides       | 723       | 20.27%     |
| Genes with transmembrane helices | 897       | 25.15%     |
| CRISPR repeats                   | 0         |            |

### Table 4. Number of genes associated with the 21 general COG functional categories

| Code | Value | %     | Description                                      |
|------|-------|-------|--------------------------------------------------|
| J    | 166   | 4.8   | Translation, ribosomal structure and biogenesis  |
| A    | 0     | 0.0   | RNA processing and modification                  |
| K    | 154   | 4.4   | Transcription                                    |
| L    | 116   | 3.3   | Replication, recombination and repair            |
| B    | 3     | 0.1   | Chromatin structure and dynamics                 |
| D    | 35    | 1.0   | Cell cycle control, mitosis and meiosis          |
| Y    | 0     | 0.0   | Nuclear structure                                |
| V    | 38    | 1.1   | Defense mechanisms                               |
| T    | 325   | 9.3   | Signal transduction mechanisms                   |
| M    | 221   | 6.3   | Cell wall/membrane biogenesis                    |
| N    | 108   | 3.1   | Cell motility                                    |
| Z    | 0     | 0.0   | Cytoskeleton                                     |
### Table 4. Number of genes associated with the 21 general COG functional categories (cont.)

| Code | Value | %   | Description                                                   |
|------|-------|-----|--------------------------------------------------------------|
| W    | 0     | 0.0 | Extracellular structures                                     |
| U    | 82    | 2.3 | Intracellular trafficking and secretion                      |
| O    | 122   | 3.5 | Posttranslational modification, protein turnover, chaperones |
| C    | 241   | 6.9 | Energy production and conversion                             |
| G    | 126   | 3.6 | Carbohydrate transport and metabolism                        |
| E    | 266   | 7.6 | Amino acid transport and metabolism                          |
| F    | 68    | 1.9 | Nucleotide transport and metabolism                          |
| H    | 135   | 3.9 | Coenzyme transport and metabolism                            |
| I    | 52    | 1.5 | Lipid transport and metabolism                               |
| P    | 137   | 3.9 | Inorganic ion transport and metabolism                       |
| Q    | 34    | 1.0 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 319   | 9.1 | General function prediction only                             |
| S    | 221   | 6.3 | Function unknown                                             |
| -    | 805   | 23.0| Not in COGs                                                  |

**Figure 3. Graphical circular map of the genome.** From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.
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