Instructions for use

Title

Draft genome sequence of Desulfoplanes formicivorans Pf12B(T), a sulfate-reducing bacterium of the family Desulfomicrobiaceae

Author(s)

Watanabe, Miho; Kojima, Hisaya; Fukui, Manabu

Citation

Standards in genomic sciences, 12, 34

https://doi.org/10.1186/s40793-017-0246-2

Issue Date

2017-06-05

Doc URL

http://hdl.handle.net/2115/66940

Rights

Creative Commons Attribution 4.0 International License

Rights(URL)

http://creativecommons.org/licenses/by/4.0/

Type

article

File Information

s40793-017-0246-2.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP
Draft genome sequence of *Desulfoplanes formicivorans* Pf12B\(^T\), a sulfate-reducing bacterium of the family *Desulfomicrobiaceae*

Miho Watanabe\(^1\),\(^2\)*, Hisaya Kojima\(^1\) and Manabu Fukui\(^1\)

**Abstract**

*Desulfoplanes formicivorans* strain Pf12B\(^T\) is the type strain of the type species in the genus *Desulfoplanes*, which is the one of the genera in the family *Desulfomicrobiaceae* within the order *Desulfovibrionales*. This deltaproteobacterium was isolated from a blackish meromictic lake sediment. *D. formicivorans* strain Pf12B\(^T\) is a Gram-negative, motile and sulfate-reducing bacterium. Cells of strain Pf12B\(^T\) are characterized by possession of vibroid morphology and red fluorescent pigment. Here we describe the features, draft genome sequence and annotation of this organism, the sole species of the genus *Desulfoplanes*. The genome comprised 3,000,979 bp, 2,657 protein-coding genes and 58 RNA genes.

**Keywords:** Bacteria, Gram-negative, Anaerobe, Sulfate-reducer, *Desulfomicrobiaceae*

**Introduction**

Strain Pf12B\(^T\) (= NBRC 110391\(^T\) = DSM 28890\(^T\)) is the type strain of *Desulfoplanes formicivorans*, which is the type species of the genus *Desulfoplanes* in the family *Desulfomicrobiaceae*. The family *Desulfomicrobiaceae* was proposed by Kuever et al. (2006) and contained only one genus, *Desulfomicrobium*. The genus *Desulfoplanes* was later added to this family because of the phylogenetic position [1]. All members of the family *Desulfomicrobiaceae* including *D. formicivorans* are sulfate reducers and incomplete oxidizers, which are unable to completely oxidize organic matters to CO\(_2\). All known strains of the genus *Desulfomicrobiaceae* have rod- or ellipsoidal-shaped morphology and they all lack desulfoviridin, which is a red fluorescent pigment [2–4]. In contrast, *D. formicivorans* strain Pf12B\(^T\) was characterized by vibroid morphology and possession of red fluorescent pigment.

In this study we summarize the features of *D. formicivorans* strain Pf12B\(^T\) and provide an overview of the draft genome sequence and annotation of this strain.

**Organism Information**

**Classification and features**

*D. formicivorans* strain Pf12B\(^T\) was isolated from the anaerobic sediments of a meromictic lake [1, 5]. Cells of this strain are Gram-negative, motile, non-spore-forming and vibroids (Fig 1, Table 1). Under UV illumination, cell lysate of the strain exhibited red fluorescence suggesting the presence of desulfoviridin. Temperature range for growth is 13–50 °C, with an optimum temperature at 42–45 °C. NaCl concentration for growth is 0.5–8% (w/v) and optimal concentration is 1–4% (w/v). This bacterium is strictly anaerobic and is capable of respiration and fermentation. Sulfate, thiosulfate and sulfite are used as electron acceptors for growth. Nitrate is not used for respiration. Pyruvate, malate and fumarate are used for fermentative growth.

Phylogenetic relationship of *D. formicivorans* strain Pf12B\(^T\) and all members of the family *Desulfomicrobiaceae* are shown in the 16S rRNA gene phylogenetic tree (Fig. 2). *D. formicivorans* strain Pf12B\(^T\) is assigned to the family *Desulfomicrobiaceae*. 

---

* Correspondence: m.watanabe@pop.lowtem.hokudai.ac.jp
1 The Institute of Low Temperature Science, Hokkaido University, Nishi 8, Kita 19, Kita-ku Sapporo, Hokkaido 060-0819, Japan
2 Postdoctoral Research Fellow of the Japan Society for the Promotion of Science, Chiyoda-ku, Tokyo 102-8471, Japan

© The Author(s). 2017 Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Desulfomicrobiaceae but forms a well-separated branch among other cultivated relatives of the same family.

Genome sequencing information
Genome project history
D. formicivorans strain Pf12B^T was selected for genome sequencing on the basis of its 16S rRNA gene-based phylogenetic position in the family Desulfomicrobiaceae (Fig. 2). A summary of the genome sequencing project information and its association with MIGS version 2.0 compliance [6] are shown in Table 2. The genome consists of 26 contigs, which has been deposited at DDBJ/EMBL/GenBank under accession number BDFE00000000.

Growth conditions and genomic DNA preparation
D. formicivorans strain Pf12B^T (DSM 28890) was grown on bicarbonate-buffered sulfide-reduced medium [7] containing 28 mM sulfate, 10 mM formate and 0.5 g l^-1 yeast extract at 45 °C. Genomic DNA was extracted from collected cells using Wizard® genomic DNA purification kit (Promega).

Genome sequencing and assembly
The genome of strain Pf12B^T was sequenced using paired-end Illumina sequencing at Hokkaido System Science Co., Ltd. (Japan). From a library with 350 bp inserts, the 10,511,386 reads were generated. After trimming of the reads, a total of 9,393,309 high-quality filtered paired end reads with a hash length of 95 bp were obtained. Reads were assembled de novo using Velvet version 1.2.08 into 26 high quality scaffolds. Gap closing analysis in these scaffolds was performed using Platanus version 1.2.1.

| Table 1 Classification and general features of Desulfoplanes formicivorans strain Pf12B^T according to MIGS recommendations |
|-----------------|-----------------|-----------------|-----------------|
| MIGS ID | Property | Term | Evidence code |
| --- | --- | --- | --- |
| Classification | Domain Bacteria | TAS [6] |
| | Phylum Proteobacteria | TAS [18] |
| | Class Deltaproteobacteria | TAS [19, 20] |
| | Order Desulfovibrionales | TAS [20, 21] |
| | Family Desulfomicrobiaceae | TAS [4, 20] |
| | Genus Desulfoplanes | TAS [1] |
| | Species Desulfoplanes formicivorans | TAS [1] |
| | Type strain: Pf12B^T (DSM 28890) | |
| Gram stain | negative | TAS [1] |
| Cell shape | vibroid | TAS [1] |
| Motility | motile | TAS [1] |
| Sporulation | nonsporulating | TAS [1] |
| Temperature range | 13–50 °C | TAS [1] |
| Optimum temperature | 42–45 °C | TAS [1] |
| pH range; Optimum | 6.1–8.6; 7.0–7.5 | TAS [1] |
| Carbon source | organic acids | TAS [1] |
| MIGS-6 Habitat | Brackish meromictic lake sediment | TAS [1] |
| MIGS-6.3 Salinity | 10–40 g NaCl /l | TAS [1] |
| MIGS-22 Oxygen requirement | obligate anaerobic | TAS [1] |
| MIGS-15 Biotic relationship | free-living | TAS [1] |
| MIGS-14 Pathogenicity | non-pathogen | NAS |
| MIGS-4 Geographic location | Kushiro, Hokkaido, Japan | TAS [1, 5] |
| MIGS-5 Sample collection | May 2012 | TAS [5] |
| MIGS-4.1 Latitude | 42°58'20.6"N | TAS [5] |
| MIGS-4.2 Longitude | 144°24'6.6"E | TAS [5] |
| MIGS-4.4 Altitude | NA | |

Evidence codes: - 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). NA; not available.

Genome annotation
Draft genome sequences were automatically annotated using the MiGAP [8]. In the pipeline, RNAmmer [9] and tRNAscan-SE [10] were used to identify rRNA and tRNA genes, respectively. MetaGene Annotator [11] was used to predict ORFs likely to encode proteins (CDSs), and functional annotation was performed based on...
reference databases, including RefSeq, TrEMBL, and COGs. Manual annotation was performed using IMC-GE software (In Silico Biology; Yokohama, Japan). Putative CDSs were confirmed again by a sequence similarity search using the BLASTP tool. Putative CDSs possessing BLASTP matches with more than 70% coverage and 35% identity and E-values less than $1 \times e^{-5}$ were considered potentially functional genes. When these cut-off values were not satisfied, the CDSs were annotated as hypothetical proteins. Transcription start sites of predicted proteins were corrected based on multiple sequence alignments. If the distance between CDSs was larger than 500 bp, further ORF extraction for coding genes was performed.

The protein-coding genes in the genome were also subjected to analysis on WebMGA [12] for the COGs and Protein family (Pfam) annotations. Transmembrane helices and signal peptide prediction were analyzed using Phobius [13]. CRISPR loci were distinguished using the CRISPR Recognition Tool [14].

**Genome properties**

The total genome of strain *D. formicivorans* strain Pf12B<sup>T</sup> was 3,000,979 bp in size with a GC content of 49.81% (Table 3). It was predicted to contain 2,715 genes including 2,657 protein-coding genes and 58 RNA genes (for tRNA and rRNA). Approximately 83% of the predicted genes were assigned to COG functional categories. The distribution of genes into COGs functional categories is presented in Table 4.

---

**Table 2** Project information

| MIGS ID | Property Term | Term |
|---------|---------------|------|
| MIGS 31 | Finishing quality | High-quality draft |
| MIGS 28 | Libraries used | TruSeq Nano DNA Library prep kit |
| MIGS 29 | Sequencing platforms | Illumina Hiseq paired-end |
| MIGS 30 | Fold coverage | 370 x |
| MIGS 30 | Assemblers | Velvet version 1.2.08 |
| MIGS 32 | Gene calling method | Microbial Genome Annotation Pipeline (MiGAP) |
| Locus Tag | BDFE01000001-BDFE01000026 |
| Genbank ID | BDFE00000000 |
| GenBank Date of Release | June 30, 2016 |
| BIOPROJECT | PRJDB4875 |
| MIGS 13 | Source Material Identifier | DSM 28890 |
| Project relevance | Ecology and evolution |

**Table 3** Genome statistics

| Attribute | Value | % of Total |
|-----------|-------|------------|
| Genome size (bp) | 3,000,979 | 100.00 |
| DNA coding (bp) | 2,596,072 | 86.51 |
| DNA G + C (bp) | 1,494,788 | 49.81 |
| DNA scaffolds | 26 | - |
| Total genes | 2,715 | 100.00 |
| Protein coding genes | 2,657 | 97.86 |
| RNA genes | 58 | 2.14 |
| Pseudo genes | NA | NA |
| Genes in internal clusters | NA | NA |
| Genes with function prediction | 1888 | 69.54 |
| Genes assigned to COGs | 2255 | 84.87 |
| Genes with Pfam domains | 2110 | 79.41 |
| Genes with signal peptides | 356 | 13.40 |
| Genes with transmembrane helices | 570 | 21.45 |
| CRISPR repeats | 2 | 0.07 |

NA, not available
Insights from the genome sequence

The draft genome provides interesting phylogenetic and metabolic information, including phylogeny of dsr genes, which are essential for dissimilatory sulfate reduction. The dsrAB genes are frequently used as marker genes to evaluate phylogenetic relationship of sulfate-reducing bacteria, as well as to reveal their diversity and distribution in environments. Phylogenetic analysis based on DsrAB amino acid sequence was performed to disclose the phylogenetic position of *D. formicivorans* strain Pf12B^T^ among sulfate reducers belonging to the families *Desulfovibrionales* and *Desulfobacterales* (Fig. 3). In the resulting phylogenetic tree, *D. formicivorans* strain Pf12B^T^ was clearly separated from all members of the family *Desulfomicrobiaceae*. This result partially conflicts with the 16S rRNA gene phylogeny, and this contradiction may represent a new case of lateral gene transfer event which frequently has been found among dissimilatory sulfate-reducing and sulfur-oxidizing bacteria [15].

Conclusions

Draft genome sequence of *D. formicivorans* strain Pf12B^T^ described here is the first published genome sequence of a member of the genus *Desulfoplanes*, which is a newly proposed taxon in the family *Desulfomicrobiaceae*. The genome of the strain Pf12B^T^ consists of 2,657 protein-coding genes and 58 RNA genes. DsrAB phylogenetic tree shows the strain Pf12B^T^ is located in the independent position, which is distant from a cluster of *Desulfobacteraceae* species.

Table 4 Number of genes associated with general COG functional categories

| Code | Value | %age | Description |
|------|-------|------|-------------|
| J    | 156   | 5.75 | Translation, ribosomal structure and biogenesis |
| A    | 0     | 0.00 | RNA processing and modification |
| K    | 102   | 3.76 | Transcription |
| L    | 110   | 4.05 | Replication, recombination and repair |
| B    | 1     | 0.04 | Chromatin structure and dynamics |
| D    | 29    | 1.07 | Cell cycle control, Cell division, chromosome partitioning |
| V    | 25    | 0.92 | Defense mechanisms |
| T    | 210   | 7.74 | Signal transduction mechanisms |
| M    | 169   | 6.23 | Cell wall/membrane biogenesis |
| N    | 105   | 3.87 | Cell motility |
| U    | 93    | 3.43 | Intracellular trafficking and secretion |
| O    | 110   | 4.05 | Posttranslational modification, protein turnover, chaperones |
| C    | 222   | 8.18 | Energy production and conversion |
| G    | 116   | 4.27 | Carbohydrate transport and metabolism |
| E    | 234   | 8.62 | Amino acid transport and metabolism |
| F    | 65    | 2.39 | Nucleotide transport and metabolism |
| H    | 101   | 3.72 | Coenzyme transport and metabolism |
| I    | 51    | 1.88 | Lipid transport and metabolism |
| P    | 122   | 4.50 | Inorganic ion transport and metabolism |
| Q    | 37    | 1.36 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 258   | 9.51 | General function prediction only |
| S    | 168   | 6.19 | Function unknown |
| -    | 459   | 16.91 | Not in COGs |

Fig. 3 Phylogenetic tree based on DsrAB amino acid sequence of *D. formicivorans* strain Pf12B^T^ and members of the orders *Desulfovibrionales* and *Desulfobacterales*. The tree was constructed by the Maximum-Likelihood method with MEGA version 5.1 [16] based on ClustalX version 2.1 [17] aligned protein sequences. Bootstrap values (percentages of 1000 replications) of ≥ 50% are shown at nodes.
Abbreviations
CRISPR: Clustered regularly interpaced short palindromic repeat; Dsr: Dissimilatory sulfite reductase; MiGAP: Microbial Genome Annotation Pipeline

Acknowledgements
This study was supported by a grant-in-aid for Research Fellow of Japan Society for the Promotion Science to MW and JSPS KAKENHI Grant Number 22370005 to MF.

Authors’ contributions
MF and HK designed and supervised the study. MW characterized the strain and carried out all the bioinformatics analysis. MW and HK drafted the manuscript. All authors discussed the data and read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 6 July 2016 Accepted: 26 May 2017
Published online: 05 June 2017

References
1. Watanabe M, Kojima H, Fukui M. Desulfoplanes formicivorans gen. nov., sp. nov., a novel sulfate-reducing bacterium isolated from a brackish meromictic lake, and emended description of the family Desulfomicrobiaceae. Int. J Syst Evol Microbiol. 2015;65:1902–7.
2. Jones HE, Skyring GW. Effects of enzymic assay conditions on sulfite reduction catalysed by desulfovivridin from Desulfovibrio gigas. Biochim Biophys Acta. 1975;377:52–60.
3. Kuever J, Galushko A. The Family Desulfomicrobiaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The Prokaryotes. 4th ed. Deltaproteobacteria and Epsilonproteobacteria. Berlin Heidelberg: Springer; 2014. p. 97–102.
4. Kuever J, Rainey FA, Widdel F, Family II. Desulfomicrobiaceae fam. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Bergey’s Manual of Systematic Bacteriology, volume 2: part C. 2nd ed. New York: Springer; 2005. p. 944.
5. Kubo K, Kojima H, Fukui M. Vertical distribution of major sulfate-reducing bacteria in a shallow eutrophic meromictic lake. Syst Appl Microbiol. 2014;37:510–9.
6. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A. 1990;87:4576–9.
7. Widdel F, Bak F. Gram-negative mesophilic sulfate-reducing bacteria. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH, editors. The Prokaryotes. 2nd ed. New York: Springer; 1992;2:352–78.
8. Sugawara H, Ohyama A, Mori H, Kurokawa K. Microbial Genome Annotation Pipeline (MiGAP) for diverse users. Software Demonstrations S001-1-2L. In: 20th Int. Conf. Genome Inform. (GIW2009) Poster Software Demonstrations, Yokohama, Japan.
9. Lagesen K, Hallin P, Redlund EA, Stærfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 2007;35:3100–8.
10. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 1997;25:955–64.
11. Noguchi H, Taniguchi T, Itoh T. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res. 2008;15:387–96.
12. Wu S, Zhu Z, Fu L, Niu B, Li W. WebMGA: a customizable web server for fast metagenomic sequence analysis. BMC Genomics. 2011;12:444.
13. Kall L, Krogh A, Sonnhammer ELL. Advantages of combined transmembrane topology and signal peptide prediction: the Phobius web server. Nucleic Acids Res. 2007;35:W429–32.
14. Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyprides NC, Hugenholtz P. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinformatics. 2007;8:209.
15. Müller AL, Kjeldsen KU, Ratter T, Pester M, Loy A. Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. ISME J. 2015;9(5):1152–65.
16. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGAS: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9.
17. Larkin MA, Blacksheds G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23:2947–8.
18. Garrity GM, Holt JG. Taxonomic Outline of the Archaea and Bacteria. In: Boone DR, Castenholz RW, editors. Bergey’s Manual of Systematic Bacteriology, vol. 1. 2nd ed. New York: Springer; 2001. p. 155–66.
19. Kuever J, Rainey FA, Widdel F, Class II. Deltaproteobacteria class. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Bergey’s Manual of Systematic Bacteriology, volume 2: part C. 2nd ed. New York: Springer; 2005. p. 948–9.
20. Editor L. Validation List No. 107. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol. 2006;56(1):1–6.
21. Kuever J, Rainey FA, Widdel F, Order II. Desulfovibionales ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Bergey’s Manual of Systematic Bacteriology, volume 2: part C. 2nd ed. New York: Springer; 2005. p. 925–6.