Complete genome sequence of the sulfur-oxidizing chemolithoautotrophic Sulfurovum lithotrophicum 42BKT[^1]

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Abstract

A sulfur-oxidizing chemolithoautotrophic bacterium, *Sulfurovum lithotrophicum* 42BKT[^1]T, isolated from hydrothermal sediments in Okinawa, Japan, has been used industrially for CO\(_2\) bio-mitigation owing to its ability to convert CO\(_2\) into C\(_5\)H\(_8\)NO\(_4\) at a high rate of specific mitigation (0.42 g CO\(_2\)/cell/h). The genome of *S. lithotrophicum* 42BKT[^1]T comprised of a single chromosome of 2217,891 bp with 2217 genes, including 2146 protein-coding genes and 54 RNA genes. Here, we present its complete genome-sequence information, including information about the genes encoding enzymes involved in CO\(_2\) fixation and sulfur oxidation.

Keywords: Complete genome, Sulfur-oxidizing bacterium, Chemolithoautroph, CO\(_2\) bio-mitigation, *Sulfurovum* lithotrophicum

Introduction

**Epsilonproteobacteria** are well-known chemolithoautotrophic bacteria found in deep-sea hydrothermal fields that play significant roles in sulfur, nitrogen, and hydrogen flux [1, 2].

*Sulfurovum lithotrophicum* 42BKT[^1]T is a sulfur-oxidizing member of **Epsilonproteobacteria** that was isolated from deep-sea hydrothermal sediments in Okinawa, Japan [3]. Strain 42BKT[^1]T is a Gram-negative, non-motile, and coccoid-to-short-rod-shaped bacterium that utilizes CO\(_2\) as a carbon source, S or S\(_2\)O\(_3\)^\(^2-\) as electron donors, and O\(_2\) and NO\(_3\)^\(^-\) as electron acceptors [3, 4]. Recent studies have focused on its potential industrial applications for CO\(_2\) bio-mitigation, reporting that this strain could convert CO\(_2\) into C\(_5\)H\(_8\)NO\(_4\) at a high specific mitigation rate of ~0.42 g CO\(_2\)/cell/h [4].

The CO\(_2\)-bio-mitigation ability of *S. lithotrophicum* can be improved and optimized through genetic engineering; however, the present lack of genetic knowledge of *S. lithotrophicum* renders the genetic engineering of this strain difficult. Here, we presented a preliminary description and the general features of *S. lithotrophicum* 42BKT[^1]T, along with its genome-sequence annotations and interactions with other *Sulfurovum* species. This information would be helpful for improving the use of chemolithoautotrophic bacteria, including *Sulfurovum* species, in industrial applications in CO\(_2\) bio-mitigation.

Organism information

**Classification and features**

A representative 16S rRNA gene of *S. lithotrophicum* 42BKT[^1]T was compared with that of other species using NCBI BLAST [5]. Figure 1 shows the phylogenetic tree with *S. lithotrophicum* 42BKT[^1]T, constructed based on the 16S rRNA sequence. This strain shared 99.1% (1393/1406 bp) and 95.1% (1312/1379) sequence identity with the 16S rRNA genes of *Sulfurovum* sp. NBC37[^6] and *Sulfurovum aggregans* Monchim33[^7], respectively.

*S. lithotrophicum* 42BKT[^1]T is a Gram-negative, non-motile, coccoid-to-short-rod-shaped bacterium that is 0.5–1.2 μm in length and 0.4–0.8 μm in width (Fig. 2). The 42BKT[^1]T strain is a mesophilic, facultative anaerobe that requires sea salt to grow and can use NH\(_4\)Cl as a nitrogen source. Normal growth occurs at a
temperature of 10–40 °C, pH of 5.0–9.0, and salinity of 5–60 g/l [3]. The basic details of its genome sequence are shown in Table 1.

**Chemotaxonomic data**

The major cellular fatty acids that were present in strain 42BKT\(^T\) included C\(_{16}\):1 (53.7%), C\(_{16}\):0 (31.3%), and C\(_{18}\):0 (15.0%) [3]. It did not contain C\(_{14}\):0, C\(_{14}\):1, or C\(_{18}\):1, whereas *S. aggregans* Monchim33\(^T\) contains 7.7, 5.9, and 9.4%, respectively, of these fatty acids [3, 7], and *Sulfurimonas autotrophica* OK\(^T\) contains 8.4% of C\(_{14}\):0 and 9.4% of C\(_{18}\):1 [8]. *S. lithotrophicum* 42BKT\(^T\) can fix CO\(_2\) via the reductive tricarboxylic acid (TCA) cycle, although the gene encoding phosphoenolpyruvate (PEP) carboxylase is not annotated in its genome. Sulfur or S\(_2\)O\(_3\)^2− are oxidized by bacteria of the genus *Sulfurovum*; *S. lithotrophicum* 42BKT\(^T\) can oxidize S\(_2\)O\(_3\)^2− only using a sulfide-quinone reductase, whereas *Sulfurovum* sp. NBC37–1 oxidizes S\(_2\)O\(_3\)^2− using a sulfide-quinone reductase or a sulfide dehydrogenase.

**Genome sequencing information**

**Genome project history**

*S. lithotrophicum* 42BKT\(^T\) was selected for sequencing based on its ability to convert CO\(_2\) into C\(_5\)H\(_8\)NO\(_4\)^−, which can be industrially used for CO\(_2\) bio-mitigation. The draft sequencing and annotation were performed by ChunLab, Inc. (Seoul, Korea). The genome project was deposited in the Genomes OnLine Database [9] under the accession number Gp0118364. The complete genome sequence was also deposited in GenBank [10] under the accession number CP011308. Table 2 contains the details of the project and its association with MIGS version 2.0 compliance [11].

**Growth conditions and genomic DNA preparation**

*S. lithotrophicum* 42BKT\(^T\) was grown in a 125-mL serum bottle (Wheaton Industries, Millville, NJ, USA) with 20 mL of MJ basal medium and filled with a CO\(_2\)/N\(_2\) gas mixture. The bottle was incubated at 29 °C while shaking at 120 rpm (Green Shaker, Vision Scientific Co., Daejeon, Korea) [4]. Genomic DNA was isolated using a QI Amp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions.

**Genome sequencing and assembly**

The genomic library was sequenced using an Illumina MiSeq PE 300 and PacBio 10 K with the Illumina 300-bp paired-end library (Illumina, San Diego, CA, USA) and the PacBio 20 K library (Pacific Biosciences, Menlo Park, CA, USA), respectively. The generated paired-end sequencing
reads (total read length: 2217,891 bp) were assembled using the CLC Genomics Workbench version 7.5.1 (CLC Bio, Aarhus, Denmark) and PacBio SMRT Analysis version 2.3 (Pacific Biosciences), resulting in one contig with an average genome coverage of 852.21 ×.

Genome annotation

The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline [12], which was designed to annotate bacterial genomes. Genome annotation was performed by predicting protein-coding, rRNA, tRNA, ncRNA, and pseudo genes. Phobius [13] was used to predict signal-peptide genes, and TMHMM Server version 2.0 [14, 15] was used to predict transmembrane helix genes [15, 16]. Protein families [17] were investigated using Pfam 29.0 [18], and GeneMarkS+ [19], which uses alignment data for gene prediction, was used as an annotation tool [20].

Genome properties

The genome of *S. lithotrophicum* 42BKTT comprised a single circular chromosome of 2217,891 bp with a GC content of 44.26%. Among the 2217 genes predicted, 2146 (96.80%) were protein-coding DNA sequences, 17 of which were pseudogenes. Among the CDSs, 89.66% were grouped into cluster of orthologous group functional categories. The genome contained a CRISPR array and 54 RNA genes, including 44 tRNAs, 9 rRNAs, and one ncRNA. The properties and statistics of the genome are summarized in Fig. 3 and Tables 3 and 4.

Table 1 Classification and general features of *Sulfurovum lithotrophicum* strain 42BKTT [11]

| MIGS ID | Property | Term | Evidence codea |
|---------|----------|------|----------------|
| Classification | Domain | Bacteria | TAS [29] |
| Phylum | Proteobacteria | TAS [30] |
| Class | Epsilonproteobacteria | TAS [31] |
| Order | Campylobacterales | TAS [32] |
| Family | Helicobacteraceae | TAS [33] |
| Genus | Sulfurovum | TAS [3] |
| Species | Sulfurovum lithotrophicum | TAS [3] |
| Type strain | 42BKTT (CP011308) | TAS [3] |

| Gram stain | Negative | TAS [3] |
| Cell shape | Coccolid to short rods | TAS [3] |
| Motility | None-motile | TAS [3] |
| Sporulation | Not reported | NAS |
| Temperature range | 10–40 °C | TAS [3] |
| Optimum temperature | 28–30 °C | TAS [3] |
| pH range; Optimum | 6.5–7.0 | TAS [3] |
| Carbon source | Deep-sea hydrothermal vent | TAS [3] |
| MIGS-6 | Habitat | Deep-sea hydrothermal vent | TAS [3] |
| MIGS-6.3 | Salinity | 0.5–6% NaCl (w/v) | TAS [3] |
| MIGS-22 | Oxygen requirement | Facultatively anaerobic | TAS [3] |
| MIGS-15 | Biotic relationship | Symbiont | TAS [3] |
| MIGS-14 | Pathogenicity | Not reported | NAS |
| MIGS-4 | Geographic location | Okinawa, Japan | TAS [3] |
| MIGS-5 | Sample collection | April 2002 | TAS [3] |
| MIGS-4.1 | Latitude | 27° 47′ 38″ N | TAS [3] |
| MIGS-4.2 | Longitude | 126° 53′ 87″ E | TAS [3] |
| MIGS-4.4 | Altitude | −1033 m | TAS [3] |

aEvidence 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). These evidence codes are from the Gene Ontology project [34].

Insights from the genome sequence

*S. lithotrophicum* 42BKTT is a sulfur-oxidizing bacterium that can fix CO₂ through the reductive TCA cycle. Here, we focused on investigating its abilities for CO₂ fixation and sulfur oxidation (sox), based on its genome sequence.

So far, six pathways have been associated with CO₂ fixation: the Calvin-Benson-Bassham or reductive pentose pathway, the reductive TCA cycle or reverse citric acid cycle, the reductive acetyl CoA or Wood-Ljungdahl pathway, the 3-hydroxypropionate pathway.

Table 2 Project information

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS-31 | Finishing quality | Completely finished |
| MIGS-28 | Libraries used | Illumina 300-bp paired-end library, PacBio 20 K library |
| MIGS-29 | Sequencing platforms | Miseq PE 300, PacBio 10 K |
| MIGS-31.2 | Fold coverage | 852.21 x |
| MIGS-30 | Assemblers | CLC Genomics Workbench v.7.5.1, SMRT Analysis v.2.3 |
| MIGS-32 | Gene-calling method | Prodigal 2.6.2 |
| Locus Tag | YH65 |
| Genbank ID | CP011308.1 |
| Genbank Date of Release | 08/20/2015 |
| GOLD ID | Gp0118364 |
| BIOPROJECT | PRJNA279430 |
| MIGS-13 | Source-material identifier | 42BKTT / ATCC BAA-797T |
| Project relevance | CO₂ fixation |
or malyl CoA pathway, the 3-hydroxypropionate/4-hydroxy-butyrate cycle, and the dicarboxylate/4-hydroxybutyrate cycle [21, 22]. Similar to the majority of Epsilonproteobacteria, S. lithotrophicum 42BKTT can also grow chemoautotrophically through its adenosine triphosphate citrate lyase, 2-oxoglutarate:ferredoxin oxidoreductase, and pyruvate:ferredoxin oxidoreductase via the reductive TCA cycle [23–25]. We annotated these three key enzymes, as well as other relevant enzymes such as malate dehydrogenase, fumarate hydratase, fumarate reductase, isocitrate dehydrogenase, aconitate hydratase, PEP synthase, and PEP carboxylase, in the genome sequence of 42BKT T. Notably, Sulfurovum sp. NBC37–1 and Candidatus Sulfurovum sediminum AR could also assimilate CO₂ via the reductive TCA cycle [6, 26].

S. lithotrophicum 42BKTT is known to oxidize or S₂S O₃⁻ via a sox system using SoxB, SoxXA, SoxYZ, and Sox(CD)₂ periplasmic proteins [27]. These enzymes catalyze the oxidation of S or S₂O₃⁻ using horse cytochrome c as the final electron acceptor [28]. Here, we confirmed the presence of SoxA, SoxB, SoxZ, SoxY, and SoxX genes in the 42BKTT genome.

### Table 3 Genome statistics

| Attribute                     | Value   | % of total |
|-------------------------------|---------|------------|
| Genome size (bp)              | 2217,891| 100.00     |
| DNA coding (bp)               | 2,028,222| 91.44      |
| DNA G + C (bp)                | 981,638 | 44.26      |
| DNA scaffolds                 | 1       |            |
| Total genes                   | 2217    | 100.00     |
| Protein-coding genes          | 2146    | 96.80      |
| RNA genes                     | 54      | 2.44       |
| Pseudo genes                  | 17      | 0.77       |
| Genes in internal clusters    | NA      | NA         |
| Genes with function prediction| 1559    | 70.32      |
| Genes assigned to COGs        | 1979    | 89.26      |
| Genes with Pfam domains       | 1770    | 79.84      |
| Genes with signal peptides    | 412     | 18.58      |
| Genes with transmembrane helices| 513    | 23.14      |
| CRISPR repeats                | 1       |            |

**Fig. 3** Genome map of *Sulfurovum lithotrophicum* 42BKTT. From the outer to the inner circle: RNA regions (rRNA, red; tRNA, lavender), CDS on the reverse strand (colored based on COG categories), CDS on the forward strand (colored based on COG categories), G + C skew (blue/goldenrod), and GC ratio (green/red).
Conclusions
To the best of our knowledge, this is the first report describing the genome sequence of *S. lithotrophicum* 42BKTT\(^1\), which comprised a circular chromosome of 2217,891 bp (44.26% GC content) with 2217 genes, among which 2146 were CDSs, 17 were pseudogenes, and 54 were RNA genes. *S. lithotrophicum* 42BKTT\(^1\) assimilates CO\(_2\) via the reductive TCA cycle and oxidizes S or S\(_2\)O\(_3\)\(^2\) via the sox system. The details of the genome sequence of this strain could provide potential strategies to enhance the industrial application of such bacteria for CO\(_2\) bio-mitigation.

Abbreviations
CDS: Coding DNA sequence; COG: Cluster of orthologous group; PEP: Phosphoenolpyruvate; TCA: Tricarboxylic acid

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Authors’ contributions
WJ and GP performed the microbial cultivation and genomic DNA isolation. WJ, LP, and NL performed sequencing and data analysis. WJ, LP, and JA drafted the manuscript. DL, HK, IA, CL, HL, and JA edited the manuscript. All the authors have read and approved the final manuscript.

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

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

| Code | Value | % age* | Description |
|------|-------|--------|-------------|
| J    | 138   | 6.43   | Translation, ribosomal structure, and biogenesis |
| A    | 0     | 0.00   | RNA processing and modification |
| K    | 47    | 2.19   | Transcription |
| L    | 94    | 4.38   | Replication, recombination, and repair |
| B    | 1     | 0.05   | Chromatin structure and dynamics |
| D    | 14    | 0.65   | Cell cycle control, cell division, chromosome partitioning |
| V    | 18    | 0.84   | Defense mechanisms |
| T    | 88    | 4.10   | Signal-transduction mechanisms |
| M    | 144   | 6.71   | Cell wall/membrane/envelope biogenesis |
| N    | 6     | 0.28   | Cell motility |
| U    | 39    | 1.82   | Intracellular trafficking and secretion |
| O    | 95    | 4.43   | Post-translational modification, protein turnover, chaperones |
| C    | 138   | 6.43   | Energy production and conversion |
| G    | 53    | 2.47   | Carbohydrate transport and metabolism |
| E    | 119   | 5.55   | Amino acid transport and metabolism |
| F    | 60    | 2.80   | Nucleotide transport and metabolism |
| H    | 85    | 3.96   | Coenzyme transport and metabolism |
| I    | 43    | 2.00   | Lipid transport and metabolism |
| P    | 106   | 4.94   | Inorganic ion transport and metabolism |
| Q    | 22    | 1.03   | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 143   | 6.66   | General function prediction only |
| S    | 526   | 24.51  | Function unknown |
| -    | 238   | 11.09  | Not in COGs |

*Percentage of the total number of protein-coding genes in the genome

Table 5 Species in the genus *Sulfurovum*

| Species (isolation source) | Genomic size (Mbs) | Accession no. | CDS | GC (%) | Reference |
|---------------------------|--------------------|---------------|------|--------|-----------|
| *Sulfurovum* lithotrophicum 42BKTT\(^1\) (Deep-sea hydrothermal sediment) | 2.21 | CP011308 | 2092 | 44.3 | This report |
| *Sulfurovum* sp. NBC37–1 (Deep-sea hydrothermal vent) | 2.56 | AP009179 | 2466 | 43.8 | [6] |
| *Candidatus* Sulfurovum sediminum AR (Marine sediment) | 2.12 | AJLE01000000 | 2114 | 39.2 | [26] |
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