


SHORT GENOME REPORT

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Complete genome sequence of the sulfur-oxidizing chemolithoautotrophic *Sulfurovum lithotrophicum* 42BKT^T

Wooyoung Jeon^{1,2}, Lia Priscilla^{1,3}, Gyuyeon Park^{1,2}, Heeseok Lee^{1,2}, Narae Lee⁴, Dongyup Lee⁴, Hyuksung Kwon⁵, Iksung Ahn⁵, Changha Lee⁵, Hongweon Lee^{1,2} and Jungoh Ahn^{1,2*} 

Abstract

A sulfur-oxidizing chemolithoautotrophic bacterium, *Sulfurovum lithotrophicum* 42BKT^T, isolated from hydrothermal sediments in Okinawa, Japan, has been used industrially for CO₂ bio-mitigation owing to its ability to convert CO₂ into C₅H₈NO₄⁻ at a high rate of specific mitigation (0.42 g CO₂/cell/h). The genome of *S. lithotrophicum* 42BKT^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 CO₂ fixation and sulfur oxidation.

Keywords: Complete genome, Sulfur-oxidizing bacterium, Chemolithoautotroph, CO₂ 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^T is a sulfur-oxidizing member of *Epsilonproteobacteria* that was isolated from deep-sea hydrothermal sediments in Okinawa, Japan [3]. Strain 42BKT^T is a Gram-negative, non-motile, and coccoid-to-short-rod-shaped bacterium that utilizes CO₂ as a carbon source, S or S₂O₃²⁻ as electron donors, and O₂ and NO₃⁻ as electron acceptors [3, 4]. Recent studies have focused on its potential industrial applications for CO₂ bio-mitigation, reporting that this strain could convert CO₂ into C₅H₈NO₄⁻ at a high specific mitigation rate of ~0.42 g CO₂/cell/h [4].

The CO₂-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^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 CO₂ bio-mitigation.

Organism information

Classification and features

A representative 16S rRNA gene of *S. lithotrophicum* 42BKT^T was compared with that of other species using NCBI BLAST [5]. Figure 1 shows the phylogenetic tree with *S. lithotrophicum* 42BKT^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. NBC37-1 [6] and *Sulfurovum aggregans* Monchim33^T, respectively.

S. lithotrophicum 42BKT^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^T strain is a mesophilic, facultative anaerobe that requires sea salt to grow and can use NH₄Cl as a nitrogen source. Normal growth occurs at a

* Correspondence: ahnjo@kribb.re.kr

¹Biotechnology Process Engineering Center, KRIBB, 40 Yeongudanji-ro, Cheongju 363-883, South Korea

²Bioprocess Department, University of Science and Technology, 217 Gajeong-ro Yuseong-gu, Daejeon, South Korea

Full list of author information is available at the end of the article



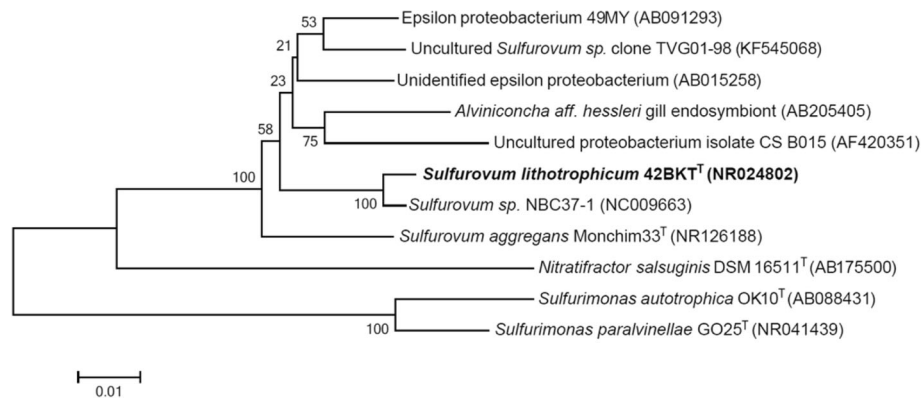


Fig. 1 Phylogenetic tree showing the relative position of *Sulfurovum lithotrophicum* 42BKT^T, based 16S rRNA gene sequence. All sites were informative and free of gaps. Evolutionary history was inferred using the neighbor-joining method [35]. The tree was built using the maximum composite-likelihood method [36]. The percentage of replicate trees with the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the corresponding branches [37]. Evolutionary analyses were conducted in MEGA6 [38]. Corresponding GenBank accession numbers are shown in brackets next to the strain name

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 10^T, another chemolithoautotrophic bacteria, contains 8.4% of C_{14:0} and 9.4% of C_{18:1} [8]. *S. lithotrophicum* 42BKT^T can fix CO₂ via the reductive tricarboxylic acid (TCA) cycle, although the gene encoding phosphoenolpyruvate (PEP) carboxylase is not annotated in its genome. Sulfur or S₂O₃²⁻ are oxidized by bacteria of the genus

Sulfurovum; *S. lithotrophicum* 42BKT^T can oxidize S²⁻ only using a sulfide-quinone reductase, whereas *Sulfurovum* sp. NBC37-1 oxidizes S²⁻ 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 CO₂ into C₅H₈NO₄, which can be industrially used for CO₂ 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 CO₂/N₂ 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 QIAmp 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

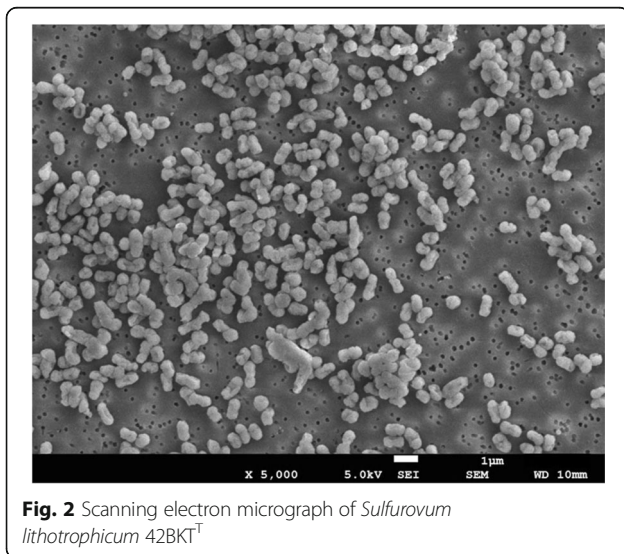


Fig. 2 Scanning electron micrograph of *Sulfurovum lithotrophicum* 42BKT^T

Table 1 Classification and general features of *Sulfurovum lithotrophicum* strain 42BKT^T [11]

MIGS ID	Property	Term	Evidence code ^a
	Classification	Domain <i>Bacteria</i>	TAS [29]
		Phylum <i>Proteobacteria</i>	TAS [30]
		Class <i>Epsilonproteobacteria</i>	TAS [31]
		Order <i>Campylobacteriales</i>	TAS [32]
		Family <i>Helicobacteraceae</i>	TAS [33]
		Genus <i>Sulfurovum</i>	TAS [3]
		Species <i>Sulfurovum lithotrophicum</i>	TAS [3]
		Type strain: 42BKT ^T (CP011308)	TAS [3]
	Gram stain	Negative	TAS [3]
	Cell shape	Coccioid 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	Sodium bicarbonate	TAS [4]
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]

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

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×
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	42BKT ^T / ATCC BAA-797 ^T
	Project relevance	CO ₂ fixation

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] 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* 42BKT^T 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, 5.

Insights from the genome sequence

S. lithotrophicum 42BKT^T 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

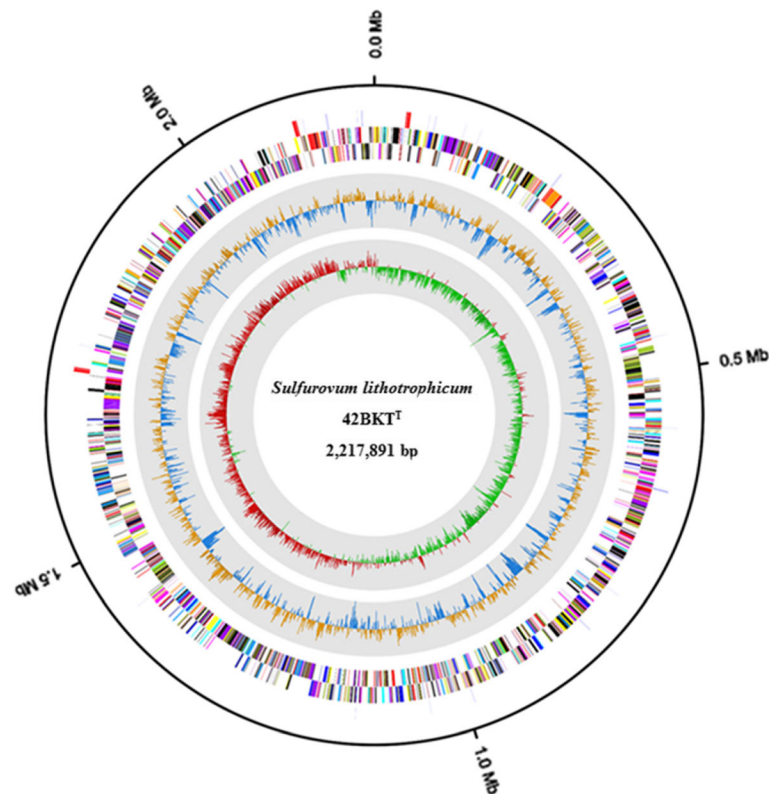


Fig. 3 Genome map of *Sulfurovum lithotrophicum* 42BKT^T. From the outer to the inner circle: RNA regions (rRNA, red; tRNA, lavender), CDS on the reverse strand (colored based on COG categories), CDS on the forward strand (colored based on COG categories), G + C skew (blue/goldenrod), and GC ratio (green/red)

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	

or malyl CoA pathway, the 3-hydroxypropionate/4-hydroxybutyrate cycle, and the dicarboxylate/4-hydroxybutyrate cycle [21, 22]. Similar to the majority of *Epsilonproteobacteria*, *S. lithotrophicum* 42BKT^T 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 42BKT^T is known to oxidize or S₂S O₃²⁻ via a sox system using SoxB, SoxXA, SoxYZ, and Sox(CD)₂ periplasmic proteins [27]. These enzymes catalyze the oxidation of S or S₂O₃²⁻ 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 42BKT^T genome.

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

Code	Value	% age ^a	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

^aPercentage of the total number of protein-coding genes in the genome

Table 5 Species in the genus *Sulfurovum*

Species (isolation source)	Genome size (Mb)	Accession no.	CDS	GC (%)	Reference
<i>Sulfurovum lithotrophicum</i> 42BKT ^T (Deep-sea hydrothermal sediment)	2.21	CP011308	2092	44.3	This report
<i>Sulfurovum</i> sp. NBC37-1 (Deep-sea hydrothermal vent)	2.56	AP009179	2466	43.8	[6]
<i>Candidatus Sulfurovum sediminum</i> AR (Marine sediment)	2.12	AJLE01000000	2114	39.2	[26]

Conclusions

To the best of our knowledge, this is the first report describing the genome sequence of *S. lithotrophicum* 42BKT^T, 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* 42BKT^T assimilates CO₂ via the reductive TCA cycle and oxidizes S or S₂O₃²⁻ 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 CO₂ 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. LP and HL performed the phylogenetic analysis. 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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Author details

¹Biotechnology Process Engineering Center, KRIBB, 40 Yeongudanji-ro, Cheongju 363-883, South Korea. ²Bioprocess Department, University of Science and Technology, 217 Gajeong-ro Yuseong-gu, Daejeon, South Korea. ³Chemical Engineering Study Program, Faculty of Industrial Technology, Institut Teknologi Bandung, Jl.Ganesa No. 10, Bandung 40132, Indonesia. ⁴Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore. ⁵Department of Chemical and Biomolecular Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, South Korea.

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