Non-contiguous finished genome sequence and description of *Sulfurimonas hongkongensis* sp. nov., a strictly anaerobic denitrifying, hydrogen- and sulfur-oxidizing chemolithoautotroph isolated from marine sediment

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Here, we report a type strain AST-10 representing a novel species *Sulfurimonas hongkongensis* within *Epsilonproteobacteria*, which is involved in marine sedimentary sulfur oxidation and denitrification. Strain AST-10^T (= DSM 22096^T = JCM 18418^T) was isolated from the coastal sediment at the Kai Tak Approach Channel connected to Victoria Harbour in Hong Kong. It grew chemolithoautotrophically using thiosulfate, sulfide or hydrogen as the sole electron donor and nitrate as the electron acceptor under anoxic conditions. It was rod-shaped and grew at 15-35°C (optimum at 30°C), pH 6.5-8.5 (optimum at 7.0-7.5), and 10-60 g L^{-1} NaCl (optimum at 30 g L^{-1}). Genome sequencing and annotation of strain AST-10^T showed a 2,302,023 bp genome size, with 34.9% GC content, 2,290 protein-coding genes, and 42 RNA genes, including 3 rRNA genes.

Introduction

The genus *Sulfurimonas* was formally proposed in 2003, and included only one species, *Sulfurimonas autotrophica* OK10^T, at that time [1]. Since then, several novel species have been identified, such as *Sulfurimonas paralvinellae* GO25^T [2], *Sulfurimonas denitrificans* DSM 1251^T (reclassified, previously known as *Thiomicrospira denitrificans*) [2], and *Sulfurimonas gotlandica* GD1^T [3]. Here, we report another novel species, *Sulfurimonas hongkongensis* AST-10^T, isolated from coastal sediment, and describe its features, together with the genome sequencing and annotation.

Currently, all known *Sulfurimonas* members were isolated from marine sediments except for strain GD1 from deep seawater [4]. The most widely shared feature of *Sulfurimonas* members is chemolithoautotrophy; strains can grow by oxidizing hydrogen gas, elemental sulfur, hydrogen sulfide, or thiosulfate [1-7]. In our previous studies, anoxic sulfur-oxidizing bacteria were demonstrated to dominate the nitrate induced marine sediment remediation process [8-10]. Phylogenetic analysis based on 16S rRNA genes showed that *Epsilonproteobacteria* closely related to *S. denitrificans* constituted the major bacterial population during such remediation of the sediment at Kai Tak Approach Channel, Hong Kong, China. Strain AST-10^T was isolated from the sediment and named *Sulfurimonas hongkongensis* sp. nov., based on its unique physiological and phylogenetic characteristics.

Classification and features

Sediment was collected 10-50 cm below the seawater/sediment interface at the Kai Tak Approach Channel connected to Victoria Harbor in Hong Kong, China. Sewage and industrial effluent had been discharged there for decades until the installation of a new sewage collection system in the late 1990s. The long lasting sulfate-reducing conditions resulted in a high sulfide concentration in the sediment, where an AVS (Acid-Volatile Sulfide) of 198 μmol g^{-1} had been measured [8]. The pore water after centrifugation at 4,000 rpm for 15 min had a pH of 7.89 and a salinity of 2.9%.

Enrichments were prepared by adding 20 g of wet sediment (32.0% dry matter) to serum bottles containing 70 mL of sterilized seawater, purged with N_2 and incubated for at least 24 h at room temperature. Potassium nitrate (1 g L^{-1}) and sodium phosphate, monobasic (0.1 mmol L^{-1}), were then added from sterilized stock solutions. The bottles were incubated at 28°C in a water bath for 72 h. The enrichments were plated onto agar plates of DSM113-S medium, a salinity modified version of DM113 medium that is recommended by DSMZ for nitrate-reducing and sulfide-oxidizing bacteria. One liter of DSM113-S contained: KH_2PO_4 (2.0 g), KNO_3 (4.0 g), NH_4Cl (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.8 g), $Na_2S_2O_3 \cdot 5H_2O$ (5.0 g), $NaHCO_3$ (1.0 g), $FeSO_4 \cdot 7H_2O$ (2.0 mg), NaCl (25.0 g) and 2 ml of trace element solution SL-4. Solid media contained 1.5% bacterial agar from Difco. All media were sterilized by autoclaving and cooled under N_2 atmosphere. Colonies formed on plates were picked and further purified by re-streaking single colonies on agar plates for more than 20 rounds (4-10 d $round^{-1}$). A colony isolated and purified from the above process was defined as strain AST-10^T.

The 16S phylogenetic tree shown in Figure 1 indicated that strain AST-10^T is a member of the genus *Sulfurimonas*, (Table 1). An online BLAST query in NCBI using the 16S rRNA gene sequence from strain AST-1^T showed a relatively low identity to all currently identified *Sulfurimonas* species, including *S. denitrificans* DSM 1251^T (97% identity), *S. gotlandica* GD1^T (95% identity), *S. autotrophica* OK10^T (95% identity), and *S. paralvinellae* GO25^T (94% identity). Using the commonly accepted criterion of a 97% 16S rDNA sequence similarity cut-off for defining species [19,20], strain AST-10^T could accordingly be identified as a novel species within the genus *Sulfurimonas*.

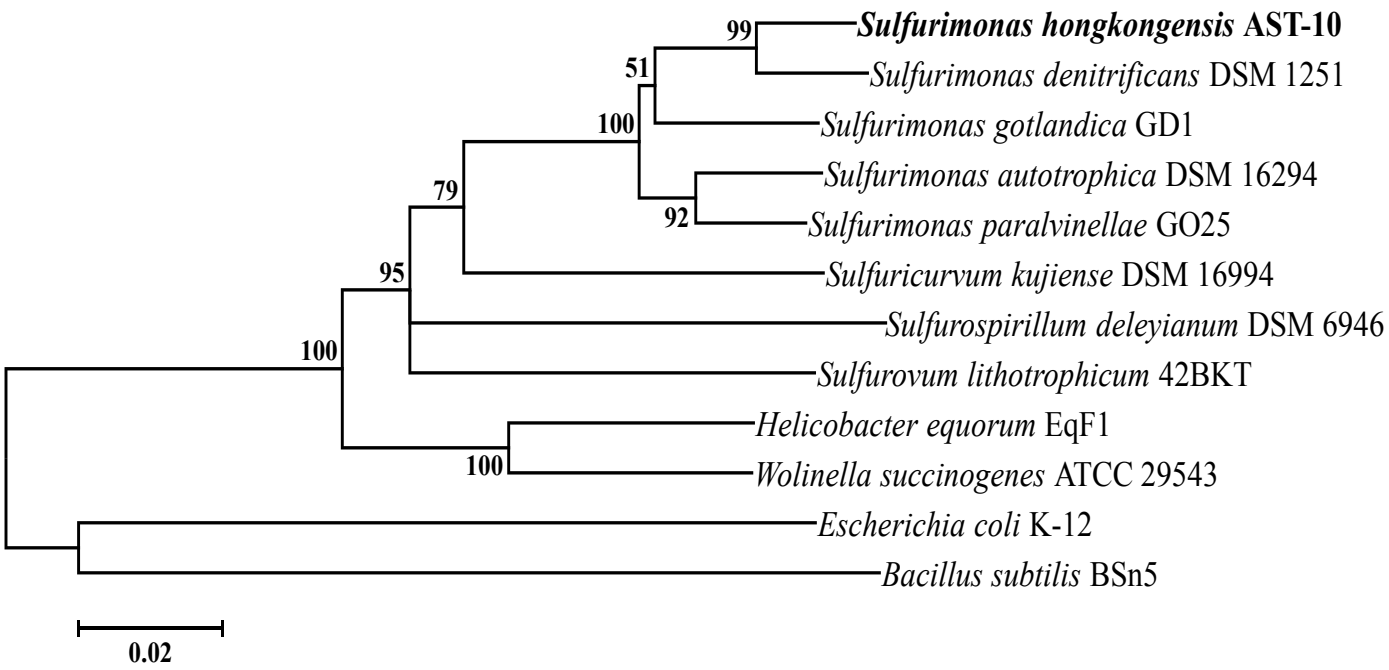

Figure 1. Phylogenetic tree highlighting the position of *Sulfurimonas hongkongensis* relative to the other species within the *Helicobacteriaceae*. The neighbor-joining tree was constructed using MEGA 5.05 and tested with 1,000 bootstrap replicates. Bootstrap values over 50% are shown and the scale bar 0.02 represents 2% nucleotide substitution. All reference sequences can be exactly searched and retrieved from NCBI GenBank based on the full name of each strain.

Table 1. Classification and general features features of *Sulfurimonas hongkongensis* AST-10 based on the MIGS recommendations [11]

MIGS ID	Property	Term	Evidence code[a]
	Current classification	Domain *Bacteria*	TAS [12]
		Phylum *Proteobacteria*	TAS [13]
		Class *Epsilonproteobacteria*	TAS [14,15]
		Order *Campylobacterales*	TAS [14,16]
		Family *Helicobacteraceae*	TAS [14,17]
		Genus *Sulfurimonas*	TAS [1-3]
		Species *Sulfurimonas hongkongensis*	IDA
		Type strain AST-10	IDA
	Gram stain	Gram-negative	TAS [1]
	Cell shape	Rod-shaped, 0.2-0.4 μm x 0.5-1.2 μm	IDA
	Motility	Not reported	
	Sporulation	No	NAS
	Temperature range	15-35°C	IDA
	Optimum temperature	30°C	IDA
	Carbon source	HCO_3^-, CO_2	IDA
	Energy source	H_2, HS^- or $S_2O_3^{2-}$	IDA
	Terminal electron receptor	NO_3^-	IDA
MIGS-6	Habitat	Coastal sediment	IDA
MIGS-6.3	Salinity	10-60 g L^{-1} NaCl, optimum at 30 g L^{-1}	IDA
MIGS-22	Oxygen	Strict anaerobe	IDA
MIGS-15	Biotic relationship	Free living	IDA
MIGS-14	Pathogenicity	Not reported as a pathogen	NAS
MIGS-4	Geographic location	Kai Tak Approach Channel, Hong Kong	IDA
MIGS-5	Sample collection time	July, 2006	IDA
MIGS-4.1	Latitude – Longitude	22.33°N – 114.19°E	TAS
MIGS-4.2			
MIGS-4.3	Depth	10-50 cm depth of coastal sediment	IDA
MIGS-4.4	Altitude	below sea surface	IDA

[a]Evidence codes - IDA: Inferred from Direct Assay; 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 [18].

Cell morphology was examined by Scanning Electron Microscopy (SEM). As shown in Figure 2, the cells of AST-10^T were rod-shaped, 0.2-0.4 μm in diameter, and 0.5-1.2 μm in length. On solid medium, AST-10^T grew and formed small, white, transparent, round shaped colonies with smooth boundaries.

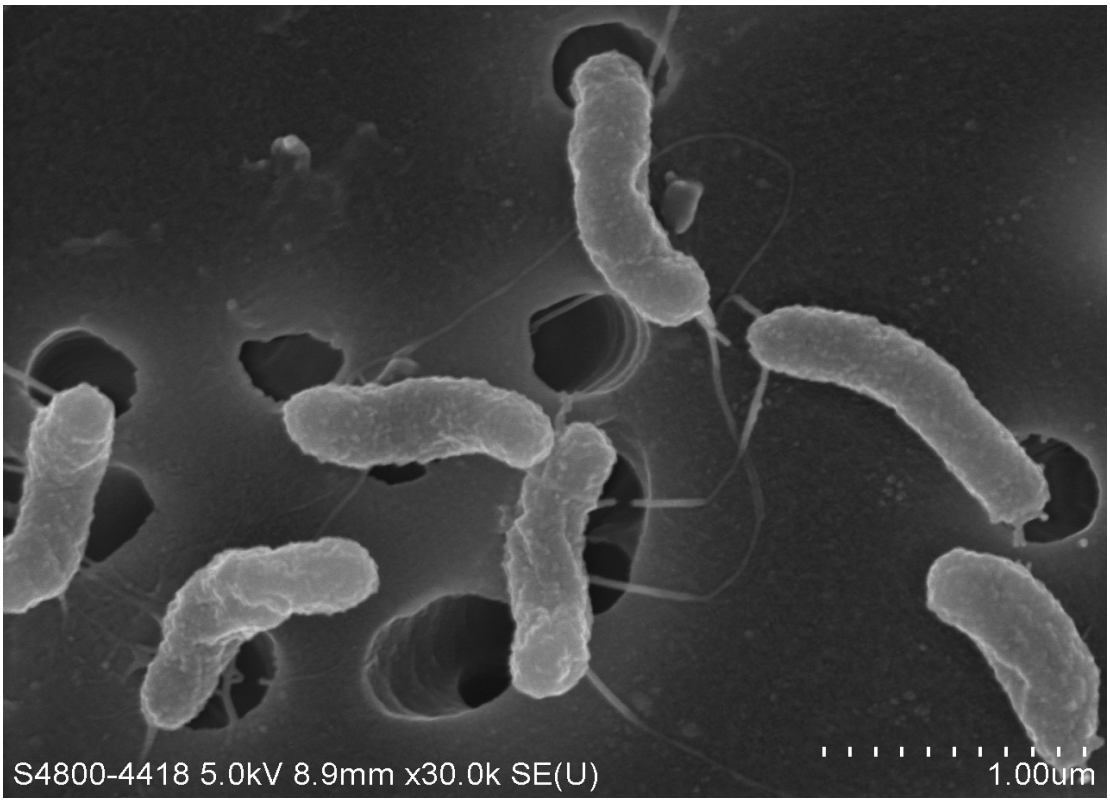

Figure 2. Scanning electron micrograph of *Sulfurimonas hongkongensis* AST-10^T. The scale bar represents 1.0 μm.

Physiology

Effects of temperature, pH, and salinity on the growth of strain AST-10^T were investigated, showing that it grew at 15-35°C (optimum at 30°C), pH 6.5-8.5 (optimum at 7.0-7.5), and 10-60 g L^{-1} NaCl (optimum at 30 g L^{-1}). The generation time of strain AST-10^T under optimal conditions was tested as 6.1 h. It was significantly shorter than other species, such as *S. paralvinellae* GO25^T and *S. denitrificans* DSM 1251^T. The cell yield of strain AST-10^T was 5.2 g dry weight per mole of $S_2O_3^{2-}$. This value is similar to that of its *Epsilonproteobacterial* relative *S. denitrificans* DSM 1251^T (5.72 g), but only about one-half of the *Betaproteobacterial* *Thiobacillus denitrificans* (11.6 g). Such difference in growth efficiency might be attributed to the different pathways used for carbon fixation and metabolism.

To determine whether electron acceptors other than NO_3^- would sustain the growth of strain AST-10^T, SO_4^{2-}, NO_2^-, Fe^{3+}, and O_2 were separately tested with $S_2O_3^{2-}$ as the sole electron donor. No growth was observed using any of these electron acceptors. $S_2O_3^{2-}$, HS^-, and H_2 can support the growth of strain AST-10^T as electron donors, however, acetate, lactate, malate, formate, pyruvate, glucose, glycerol, and yeast extract cannot. Hence, strain AST-10^T was a chemolithoautotroph, using NO_3^- as an electron acceptor and $S_2O_3^{2-}$, HS^-, or H_2 as an electron donor. The time course of $S_2O_3^{2-}$ oxidation and NO_3^- reduction during strain AST-10^T growth was monitored. N_2 was the dominant denitrification product, no accumulation of N_2O and NO_2^- was detected, when it was cultivated using DSM113-S at 30°C and pH 7.5. Significant production of insoluble S^0 occurred when it was cultured with an excess amount of $S_2O_3^{2-}$ (molar ratio of $S_2O_3^{2-}/NO_3^- > 2$). SO_4^{2-} became the dominant oxidation product under excess NO_3^- conditions (molar ratio of $S_2O_3^{2-}/ NO_3^- < 0.25$). This was quite similar to the well-characterized strain *Thiomicrospira* CVO [21]. But for *S. denitrificans* DSM 1251^T, no accumulation of insoluble S^0 was observed even under a high molar ratio of $S_2O_3^{2-}/NO_3^-$ [5].

Chemotaxonomy

Cellular fatty acid composition was analyzed using the cells grown in DSM113-S medium at 30°C in the late-exponential phase. The major cellular fatty acids of strain AST-10^T were $C_{14:0}$ (4.8%), $C_{16:0}$ (32.8%), 2-OH $C_{16:0}$ (9.5%), $C_{16:1}$ (14.6%), $C_{18:0}$ (16.9%), and $C_{18:1}$ (19.2%). This composition was

generally similar to those of *S. paralvinellae* GO25^T and *S. autotrophica* OK10^T. However, 2-OH $C_{16:0}$ was a unique fatty acid, differentiating AST-10^T from other species within the genus of *Sulfurimonas*.

Genome sequencing and annotation

Genome project history

The strain was selected for genome sequencing on the basis of its 16S rRNA gene-based phylogenetic position within the genus *Sulfurimonas* (Table 1). It is the first sequenced genome of *Sulfurimonas hongkongensis* sp. nov. A summary of the genome sequencing project information is shown in Table 2. The genome consists of 28 contigs, which has been deposited at DDBJ/EMBL/GenBank under accession number AUPZ00000000. The version described in the present study is the first version.

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	High-quality draft
MIGS-28	Libraries used	Paired-end 500 bp shotgun library
MIGS-29	Sequencing platforms	Illumina HiSeq 2000
MIGS-31.2	Fold coverage	3,011 ×
MIGS-30	Assemblers	CLC Genomics Workbench 6.0.2
MIGS-32	Gene calling method	GeneMarkS+
	Genbank ID	AUPZ00000000
	Genbank date of release	August 13, 2013
	Project relevance	Ecology and Evolution

Growth conditions and DNA isolation

As described above, the strain was grown in DSM113-S medium under anoxic condition with optimal growth at 30°C, pH7.0-7.5, and NaCl 30 g L^{-1}. The genomic DNA used for shotgun sequencing was prepared by DSMZ.

Genome sequencing and assembly

The genome shotgun sequencing project was finished by BGI (Beijing Genomics Institute). Briefly, DNA was first mechanically fragmented with an enrichment size of ~500 bp. Then the DNA fragmentation was gel purified and quality checked. The recycled DNA was used for shotgun library construction, which was finally sequenced on an Illumina HiSeq 2000 platform using the paired-end 150 bp sequencing strategy.

A total of 6,932,096,700 bp of raw sequence was obtained, which was assembled with CLC Genomics Workbench 6.0.2 using a word size of 40 bp. The draft genome was finally assembled into 28 contigs with a 2,302,023 bp genome size and more than 3,000 fold genome coverage (Table 3).

Genome annotation

The draft genome was annotated by NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Protein-coding genes with function prediction were calculated based on the PGAP result. The COGs (Clusters of Orthologous Groups) functional annotation was conducted by PRSBLAST search against COGs database with an E-value cutoff 1e-10 [22,23]. Pfam domains were annotated using HMMER 3.0 program on Pfam database with an E-value cutoff 1e-10 [24,25]. SignalP 4.1 Server was employed to analyze proteins with signal peptide [26]. TMHMM Server 2.0 was used to predict transmembrane helices in proteins [27].

Genome properties

The draft genome of *Sulfurimonas hongkongensis* AST-10^T was assembled into 28 contigs with a total size of 2,302,023 bp and a GC content of 34.9%. 2,332 genes were annotated, 2,290 of which were protein-coding genes. The remaining 42 genes were RNA genes including 3 rRNA genes. A total of 1,146 of the protein-coding genes were assigned putative functions. The remaining 1,144 protein-

coding genes were annotated as hypothetical proteins. The AST-10^T genome properties and statistics are summarized in Tables 2-4 and Figure 3.

Table 3. Nucleotide content and gene count levels of the genome

Attribute	Value	% of total[a]
Genome size (bp)	2,302,023	100%
DNA coding region (bp)	2,127,855	92.4%
DNA G+C content (bp)	803,203	34.9%
Number of contigs	28	
Contig N50 (bp)	235,215	
Total genes[b]	2332	100%
RNA genes	42	1.8%
rRNA genes	3	0.1%
tRNA genes	39	1.7%
Protein-coding genes	2290	98.2%
Pseudo genes	0	0.0%
Frameshifted genes	0	
Protein-coding genes with function prediction	1146	50.0%
Protein-coding genes assigned to COGs	1700	74.2%
Protein-coding genes assigned Pfam domains	1516	66.2%
Protein-coding genes with signal peptides	155	6.8%
Protein-coding genes with transmembrane helices	565	24.7%

[a]The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome, [b]Also includes 54 pseudogenes and 5 other genes.

Table 4. Number of genes associated with the 25 general COG functional categories

Code	Value	%age[a]	Description
J	130	5.7	Translation
A	0	0.0	RNA processing and modification
K	64	2.8	Transcription
L	89	3.9	Replication, recombination and repair
B	0	0.0	Chromatin structure and dynamics
D	16	0.7	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	27	1.2	Defense mechanisms
T	163	7.1	Signal transduction mechanisms
M	138	6.0	Cell wall/membrane biogenesis
N	68	3.0	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	58	2.5	Intracellular trafficking and secretion
O	69	3.0	Posttranslational modification, protein turnover, chaperones
C	128	5.6	Energy production and conversion
G	52	2.3	Carbohydrate transport and metabolism
E	134	5.9	Amino acid transport and metabolism
F	55	2.4	Nucleotide transport and metabolism
H	97	4.2	Coenzyme transport and metabolism
I	42	1.8	Lipid transport and metabolism
P	101	4.4	Inorganic ion transport and metabolism
Q	17	0.7	Secondary metabolites biosynthesis, transport and catabolism
R	158	6.9	General function prediction only
S	94	4.1	Function unknown
-	590	25.8	Not in COGs

[a] The total is based on the total number of protein coding genes in the annotated genome.

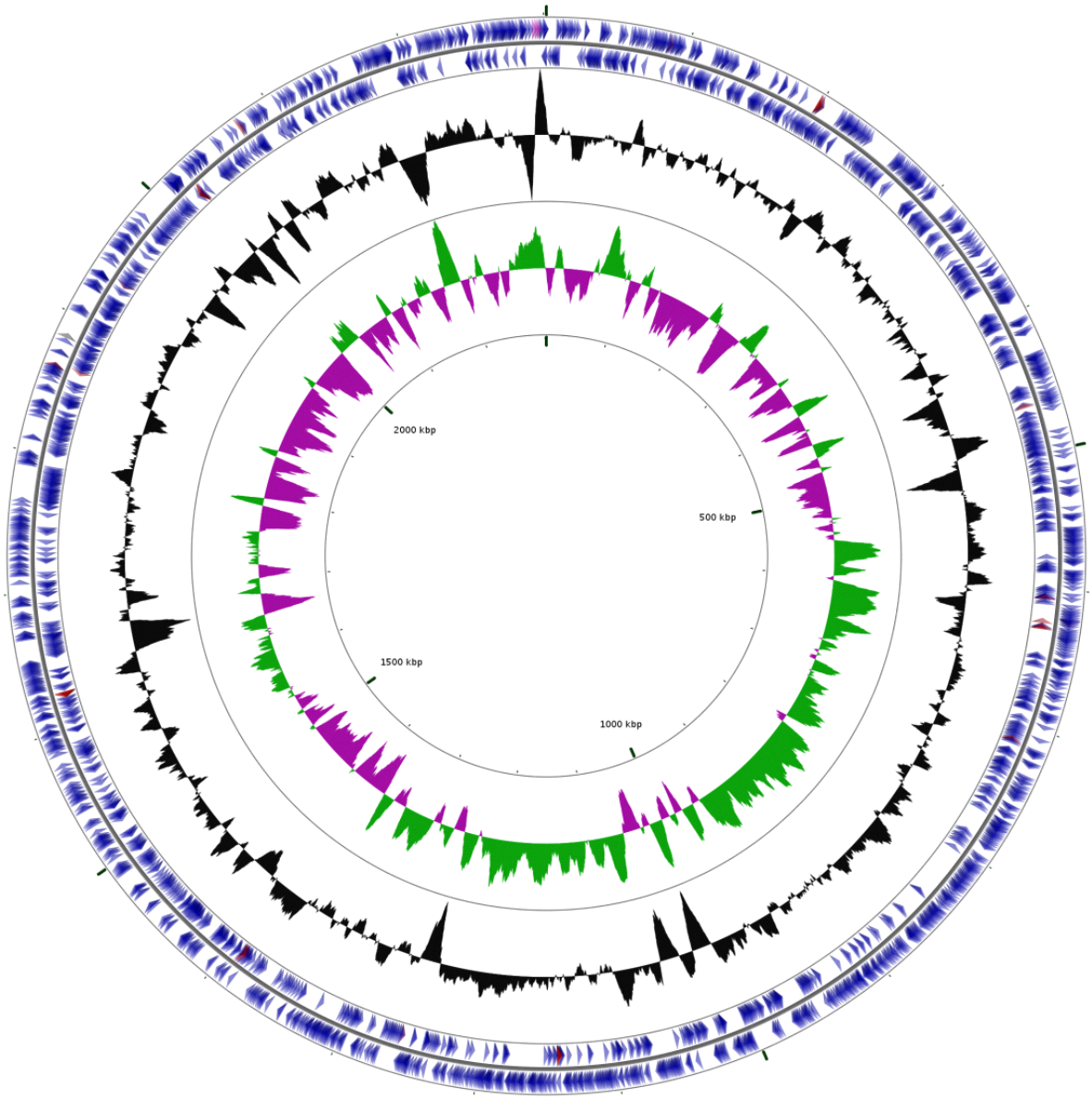

Figure 3. Graphical circular map of the *Sulfurimonas hongkongensis* AST-10 genome. Seen from the outside to the inside: genes on forward strand, genes on reverse strand, GC content, GC skew. The graphical map was plotted on the CGview Server.

Conclusion

Description of *Sulfurimonas hongkongensis* sp. nov.

Sulfurimonas hongkongensis (hong.kong.en'sis. N.L. fem. adj. *hongkongensis* pertaining to Hong Kong, the city where the type strain was isolated).

Strain AST-10^T is rod-shaped with size of 0.2-0.4 µm x 0.5-1.2 µm. It is an obligate anaerobe and occurs singly. The temperature range for growth is 15-35^{o}C, optimum at 30^{o}C. The pH range for growth is 6.5-8.5, optimum at 7.0-7.5. The salinity range for growth is 10-60 g L^{-1}, and optimum at 30 g L^{-1}. Strictly chemolithoautotrophic growth occurs with H_2, HS^- or $S_2O_3^{2-}$ as an electron donor and with nitrate as an electron acceptor. Nitrate is reduced to N_2, and reduced sulfur compounds are oxidized into S^0 or SO_4^{2-} (depending on molar ratio of $S_2O_3^{2-}/NO_3^-$). The major cellular fatty acids are $C_{14:0}$, $C_{16:0}$, 2-OH $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$, with $C_{16:0\ 2\text{-}OH}$ as a unique fatty acid different from other species in the genus *Sulfurimonas*.

The type strain AST-10^T = DSM 2096^T = JCM 18418^T, was isolated from coastal sediment at the Kai Tak Approach Channel connected to Victoria Harbour in Hong Kong, China. The GC content of the genome is 34.9%. The genome sequence has been deposited at DDBJ/EMBL/GenBank under accession number AUPZ00000000.

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References

1. Inagaki F, Takai K, Kobayashi H, Nealson KH, Horikoshi K. *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing epsilonproteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. *Int J Syst Evol Microbiol* 2003; **53**:1801-1805. PubMed http://dx.doi.org/10.1099/ijs.0.02682-0
2. Takai K, Suzuki M, Nakagawa S, Miyazaki M, Suzuki Y, Inagaki F, Horikoshi K. *Sulfurimonas paralvinellae* sp. nov., a novel mesophilic, hydrogen- and sulfur-oxidizing chemolithoautotroph within the *Epsilonproteobacteria* isolated from a deep-sea hydrothermal vent polychaete nest, reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans* comb. nov. and emended description of the genus *Sulfurimonas*. *Int J Syst Evol Microbiol* 2006; **56**:1725-1733. PubMed http://dx.doi.org/10.1099/ijs.0.64255-0
3. Labrenz M, Grote J, Mammitzsch K, Boschker HT, Laue M, Jost G, Glaubitz S, Jurgens K. *Sulfurimonas gotlandica* sp. nov., a chemoautotrophic and psychrotolerant epsilonproteobacterium isolated from a pelagic Baltic Sea redoxcline, and an emended description of the genus *Sulfurimonas*. *Int J Syst Evol Microbiol* 2013; **63**:4141-4148. PubMed http://dx.doi.org/10.1099/ijs.0.048827-0
4. Grote J, Schott T, Bruckner CG, Glockner FO, Jost G, Teeling H, Labrenz M, Jurgens K. Genome and physiology of a model *Epsilonproteobacterium* responsible for sulfide detoxification in marine oxygen depletion zones. *Proc Natl Acad Sci USA* 2012; **109**:506-510. PubMed http://dx.doi.org/10.1073/pnas.1111262109
5. Sievert SM, Scott KM, Klotz MG, Chain PS, Hauser LJ, Hemp J, Hugler M, Land M, Lapidus A, Larimer FW, *et al*. Genome of the epsilonproteobacterial chemolithoautotroph *Sulfurimonas denitrificans*. *Appl Environ Microbiol* 2008; **74**:1145-1156. PubMed http://dx.doi.org/10.1128/AEM.01844-07
6. Sikorski J, Munk C, Lapidus A, Ngatchou Djao OD, Lucas S, Glavina Del Rio T, Nolan M, Tice H, Han C, Cheng JF, *et al*. Complete genome sequence of *Sulfurimonas autotrophica* type strain (OK10). *Stand Genomic Sci* 2010; **3**:194-202. PubMed
7. Bruckner CG, Mammitzsch K, Jost G, Wendt J, Labrenz M, Jurgens K. Chemolithoautotrophic denitrification of *Epsilonproteobacteria* in marine pelagic redox gradients. *Environ Microbiol* 2013; **15**:1505-1513. PubMed http://dx.doi.org/10.1111/j.1462-2920.2012.02880.x
8. Shao M, Zhang T, Fang HH. Autotrophic denitrification and its effect on metal speciation during marine sediment remediation. *Water Res* 2009; **43**:2961-2968. PubMed http://dx.doi.org/10.1016/j.watres.2009.04.016
9. Zhang M, Zhang T, Shao MF, Fang HH. Autotrophic denitrification in nitrate-induced marine sediment remediation and *Sulfurimonas denitrificans*-like bacteria. *Chemosphere* 2009; **76**:677-682. PubMed http://dx.doi.org/10.1016/j.chemosphere.2009.03.066
10. Shao MF, Zhang T, Fang HH, Li X. The effect of nitrate concentration on sulfide-driven autotrophic denitrification in marine sediment. *Chemosphere* 2011; **83**:1-6. PubMed http://dx.doi.org/10.1016/j.chemosphere.2011.01.042
11. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, *et al*. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; **26**:541-547. PubMed http://dx.doi.org/10.1038/nbt1360
12. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains *Archaea*, *Bacteria*, and *Eucarya*. *Proc Natl Acad Sci USA* 1990; **87**:4576-4579. PubMed http://dx.doi.org/10.1073/pnas.87.12.4576
13. Garrity GM, Bell JA, Lilburn T. Phylum XIV. *Proteobacteria* phyl. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 2, Part B, Springer, New York, 2005, p. 1.
14. Validation List No. 107. List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* 2006; **56**:1-6. PubMed http://dx.doi.org/10.1099/ijs.0.64188-0
15. Garrity GM, Bell JA, Lilburn T. Class V. *Epsilonproteobacteria* class. nov. In: Garrity GM,

Brenner DJ, Krieg NR, Staley JT (eds), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 2, Part C, Springer, New York, 2005, p. 1145.

16. Garrity GM, Bell JA, Lilburn T. Order I. *Campylobacterales* ord. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 2, Part C, Springer, New York, 2005, p. 1145.
17. Garrity GM, Bell JA, Lilburn T. Family II. *Helicobacteraceae* fam. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 2, Part C, Springer, New York, 2005, p. 1168.
18. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al*. Gene ontology: tool for the unification of biology. *Nat Genet* 2000; **25**:25-29. PubMed http://dx.doi.org/10.1038/75556
19. Stackebrandt E, Goebel BM. A Place for DNA-DNA Reassociation and 16s Ribosomal-RNA Sequence-Analysis in the Present Species Definition in Bacteriology. *Int J Syst Bacteriol* 1994; **44**:846-849. http://dx.doi.org/10.1099/00207713-44-4-846
20. RossellóoMora R. Amann R. The species concept for prokaryotes. *FEMS Microbiol Rev* 2001; **25**:39-67. PubMed http://dx.doi.org/10.1016/S0168-6445(00)00040-1
21. Gevertz D, Telang AJ, Voordouw G, Jenneman GE. Isolation and characterization of strains CVO and FWKO B, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. *Appl Environ Microbiol* 2000; **66**:2491-2501. PubMed http://dx.doi.org/10.1128/AEM.66.6.2491-2501.2000
22. Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* 2000; **28**:33-36. PubMed http://dx.doi.org/10.1093/nar/28.1.33
23. 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. PubMed http://dx.doi.org/10.1186/1471-2164-12-444
24. Finn RD, Clements J, Eddy SR. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Res* 2011; **39**:W29-W37. PubMed http://dx.doi.org/10.1093/nar/gkr367
25. Punta M, Coggill PC, Eberhardt RY, Mistry J, Tate J, Boursnell C, Pang N, Forslund K, Ceric G, Clements J, *et al*. The Pfam protein families database. *Nucleic Acids Res* 2012; **40**:D290-D301. PubMed http://dx.doi.org/10.1093/nar/gkr1065
26. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* 2011; **8**:785-786. PubMed http://dx.doi.org/10.1038/nmeth.1701
27. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* 2001; **305**:567-580. PubMed http://dx.doi.org/10.1006/jmbi.2000.4315