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Complete genome sequence of the thermophilic sulfate-reducing ocean bacterium *Thermodesulfatator indicus* type strain (CIR29812^T^)

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Keywords: strictly anaerobic, motile, Gram-negative, thermophilic, sulfate-reducing, chemolithoautotrophic, black smoker, *Thermodesulfobacteria, Thermodesulfobacteriaceae*, GEBA

Thermodesulfatator indicus Moussard *et al*. 2004 is a member of the Thermodesulfobacteriaceae, a family in the phylum Thermodesulfobacteria that is currently poorly characterized at the genome level. Members of this phylum are of interest because they represent a distinct, deep-branching, Gram-negative lineage. *T. indicus* is an anaerobic, thermophilic, chemolithoautotrophic sulfate reducer isolated from a deep-sea hydrothermal vent. Here we describe the features of this organism, together with the complete genome sequence, and annotation. The 2,322,224 bp long chromosome with its 2,233 protein-coding and 58 RNA genes is a part of the ***G****enomic* ***E****ncyclopedia of* ***B****acteria and* ***A****rchaea* project.

Introduction

The genus *Thermodesulfatator* currently contains two species, both of which are anaerobic, thermophilic, chemolithoautotrophic sulfate reducers isolated from deep-sea hydrothermal vents [1,2]. Strain CIR29812^T^ (= DSM 15286 = JCM 11887) is the type strain of the species Thermodesulfatator indicus [1]. The strain was isolated from a chimney fragment taken from a black smoker in the Kairai vent field, Central Indian Ridge [1]. The genus name was derived from a combination of the Greek term *thermos*, hot, and the Neo-Latin *desulfatator*, sulfate-reducer, meaning the thermophilic sulfate-reducer [1]; the species epithet was derived from the Latin adjective *indicus*, referring to the Indian Ocean, from where the strain was isolated [1]. The other species in this genus is T. atlanticus, which was isolated from the wall of a chimney at the

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Rainbow vent field on the Mid-Atlantic Ridge [2]. The major difference between the two Thermodesulfatator species is that T. indicus is strictly chemolithoautotrophic, while T. atlanticus is able to utilize organic carbon sources [2]. Here we present a summary classification and a set of features for T. indicus CIR29812^T, together with the description of the genomic sequencing and annotation.

Classification and features

A representative genomic 16S rRNA sequence of *T. indicus* CIR29812^T was compared using NCBI BLAST [3,4] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [5] and the relative frequencies of taxa and keywords (reduced to their stem [6]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Desulfovibrio* (22.5%), *Thermodesulfatator* (22.0%), *Thermodesulfobacterium* (16.9%), *Methylococcus* (10.9%) and *Thermodesulforhabdus* (5.7%) (38 hits in total). Regarding the two hits to sequences from members of the species, the average identity within HSPs was 99.9%, whereas the average coverage by HSPs was 95.8%. Among all other species, the one yielding the highest score was "*Geothermobacterium ferrireducens*" (AF411013), which corresponded to an identity of 90.1% and an HSP coverage of 64.7%. (Note that the Greengenes database uses the INSDC (= EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification. The highest-scoring environmental sequence was AJ874315 ('continuous enrichment hydrothermal black chimney clone 850'), which showed an identity of 96.7% and an HSP coverage of 93.9%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'spring' (6.2%), 'microbi' (4.8%), 'hot' (4.2%), 'nation, park' (2.7%) and 'yellowston' (2.6%) (212 hits in total). These keywords fit reasonably well to the habitat of a thermophilic sulfate-reducer. Environmental samples which yielded hits of a higher score than the highest scoring species were not found.

Figure 1 shows the phylogenetic neighborhood of *T. indicus* in a 16S rRNA based tree. The sequences of the two 16S rRNA gene copies in the genome differ from each other by two nucleotides, and differ by up to four nucleotides from the previously published 16S rRNA sequence (AF393376).

T. indicus cells are Gram-negative rods with a length of 0.8-1.0 µm and a width of 0.4-0.5 µm [1]. An electron micrograph of *T. indicus* is shown in Figure 2. Cells are motile with a single polar flagellum and can be found separately or in groups of two or three cells [1]. The temperature range for growth is 55-80°C with an optimum at 70°C [1]. The salinity range is 10-35 g/L NaCl, with an optimum of 25 g/L NaCl [1]. The pH range is 6.0-6.7 with 6.25 as the optimum [1]. *T. indicus* is strictly anaerobic and strictly chemolithoautotrophic, growing with H_2 as electron donor, sulfate as electron acceptor, and CO_2 as the carbon source [1]. Some organic compounds stimulated growth [1]. Ammonium, nitrate, peptone and tryptone could serve as nitrogen sources [1].

Chemotaxonomy

The major respiratory quinone found in *T. indicus* is menaquinone with seven isoprene subunits (MK-7) [1]. The major phospholipids are phosphatidylinositol and phosphatidyl-ethanolamine. Phosphatidylglycerol and three unidentified phospholipids are present in lesser amounts [1]. The major fatty acids are $C_{18:0}$ and $C_{18:1}$, and hydroxylated fatty acids are also present [1]. *T. indicus* was found to be sensitive to tetracycline, ampicillin, chloramphenicol, and rifampicin, and resistant to penicillin, kanamycin, and streptomycin [1].

Genome sequencing and annotation

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position [23], and is part of the ***G**enomic **E**ncyclopedia of **B**acteria and **A**rchaea* project [24]. The genome project is deposited in the Genomes On Line Database [13] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

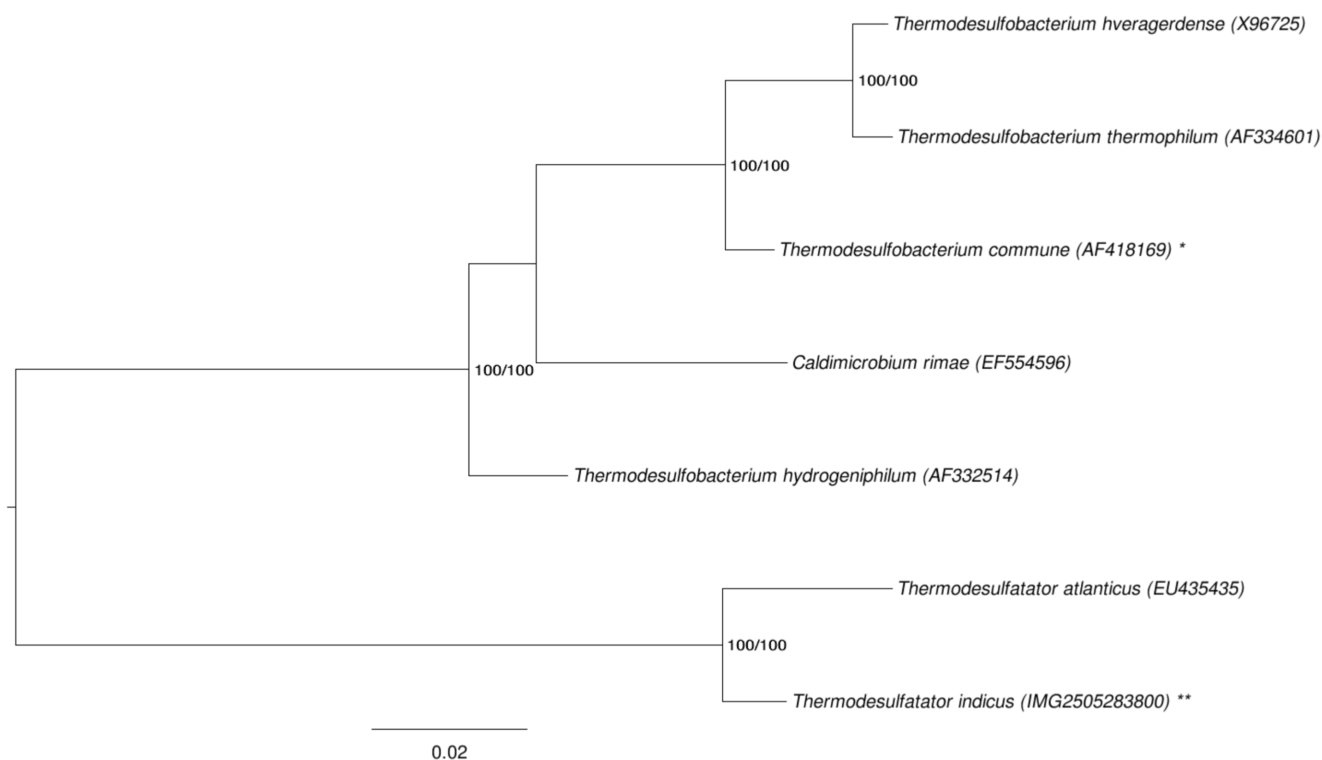

Figure 1. Phylogenetic tree highlighting the position of *T. indicus* relative to the type strains of the other species within the phylum *Thermodesulfobacteria*. The tree was inferred from 1,475 aligned characters [7,8] of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [9]. Rooting was done initially using the midpoint method [10] and then checked for its agreement with the current classification (Table 1). The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 1,000 ML bootstrap replicates [11] (left) and from 1,000 maximum-parsimony bootstrap replicates [12] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [13] are labeled with one asterisk, those also listed as 'Complete and Published' with two asterisks.

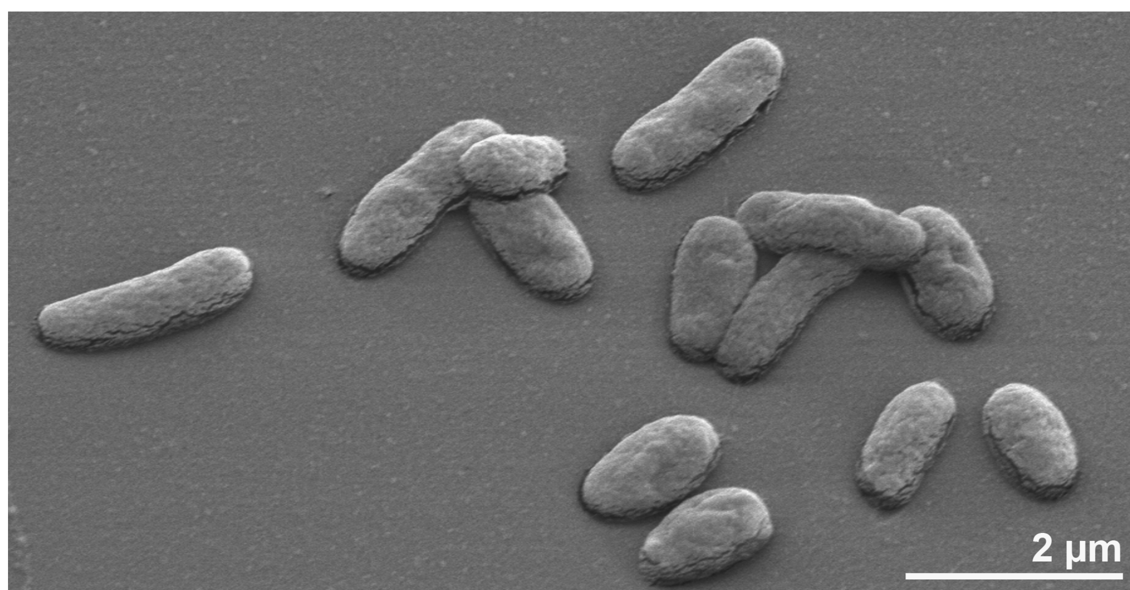

Figure 2. Scanning electron micrograph of T. indicus CIR29812^T

Table 1. Classification and general features of *T. indicus* CIR29812ᵀ according to the MIGS recommendations [14].

MIGS ID	Property	Term	Evidence code
	Current classification	Domain *Bacteria*	TAS [15]
		Phylum *Thermodesulfobacteria*	TAS [16]
		Class *Thermodesulfobacteria*	TAS [17,18]
		Order *Thermodesulfobacteriales*	TAS [17,19]
		Family *Thermodesulfobacteriaceae*	TAS [17,20]
		Genus *Thermodesulfatator*	TAS [1]
		Species *Thermodesulfatator indicus*	TAS [1]
		Type-strain CIR29812	TAS [1]
	Gram stain	negative	TAS [1]
	Cell shape	small rods	TAS [1]
	Motility	motile *via* single polar flagellum	TAS [1]
	Sporulation	non-sporulating	TAS [1]
	Temperature range	thermophile, 55-80°C	TAS [1]
	Optimum temperature	70°C	TAS [1]
	Salinity	10-35 g NaCl per liter, optimum at 25 g	TAS [1]
MIGS-22	Oxygen requirement	strictly anaerobic	TAS [1]
	Carbon source	CO_2	TAS [1]
	Energy metabolism	chemolithoautotrophic	TAS [1]
MIGS-6	Habitat	deep-sea hydrothermal vent field	TAS [1]
MIGS-15	Biotic relationship	free living	TAS [1]
MIGS-14	Pathogenicity	none	NAS
	Biosafety level	1	TAS [21]
MIGS-23.1	Isolation	chimney fragment from black smoker	TAS [1]
MIGS-4	Geographic location	Kairai vent field, Central Indian Ridge	TAS [1]
MIGS-5	Sample collection time	April 2001	TAS [1]
MIGS-4.1	Latitude	-25.317	TAS [1]
MIGS-4.2	Longitude	70.033	TAS [1]
MIGS-4.3	Depth	2,420 m	TAS [1]
MIGS-4.4	Altitude	-2,420 m	TAS [1]

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. If the evidence code is IDA, then the property was directly observed for a living isolate by one of the authors or an expert mentioned in the acknowledgements [22].

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	Four genomic libraries: one 454 pyrosequence standard library, two 454 PE libraries (7 and 11 kb insert sizes), one Illumina library
MIGS-29	Sequencing platforms	Illumina GAii, 454 GS FLX Titanium
MIGS-31.2	Sequencing coverage	183.8 × Illumina; 126.8 × pyrosequence
MIGS-30	Assemblers	Newbler version 2.3-PreRelease-6-30-2009-gcc-3.4.6, Velvet version 1.0.13, phrap
MIGS-32	Gene calling method	Prodigal
	INSDC ID	CP002683
	GenBank Date of Release	November 21, 2011
	GOLD ID	Gc01827
	NCBI project ID	40057
	Database: IMG-GEBA	2505119042
MIGS-13	Source material identifier	DSM15286
	Project relevance	Tree of Life, GEBA, Bioenergy

Growth conditions and DNA isolation

T. indicus strain CIR29812^T, DSM 15286, was grown anaerobically in DSMZ medium 383 (*Desulfobacterium* medium) [25] at 70°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram-positive DNA purification kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer with modification st/LALM for cell lysis as described in Wu *et al*. 2009 [24]. DNA is available through the DNA Bank Network [26].

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [27]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly consisting of 49 contigs in one scaffold was converted into a phrap [28] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (427.0 Mb) was assembled with Velvet [29] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 298.3 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [28] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution (C. Han, unpublished), Dupfinisher [30], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 95 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI (A. Lapidus, unpublished). The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 310.6 × coverage of the genome. The final assembly contained 759,221 pyrosequence and 11,861,111 Illumina reads.

Genome annotation

Genes were identified using Prodigal [31] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [32]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. These data sources were combined to assert a product description for each predicted protein. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE [33], RNAMMer [34], Rfam [35], TMHMM [36], and SignalP [37].

Genome properties

The genome consists of a 2,322,224 bp long circular chromosome with a 42.4% G+C content (Table 3 and Figure 3). Of the 2,291 genes predicted, 2,233 were protein-coding genes, and 58 RNAs; 38 pseudogenes were also identified. The majority of the protein-coding genes (73.2%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

Table 3. Genome Statistics

Attribute	Value	% of Total[a]
Genome size (bp)	2,322,224	100.00%
DNA coding region (bp)	2,101,503	90.50%
DNA G+C content (bp)	985,214	42.43%
Number of replicons	1	
Extrachromosomal elements	0	
Total genes	2,291	
RNA genes	58	
rRNA operons	2	
tRNA genes	49	
Protein-coding genes	2,233	100.00%
Pseudo genes	38	1.70%
Genes with function prediction (proteins)	1,678	75.15%
Genes in paralog clusters	959	42.95%
Genes assigned to COGs	1,845	82.62%
Genes assigned Pfam domains	917	41.07%
Genes with signal peptides	351	15.72%
Genes with transmembrane helices	499	22.35%
CRISPR repeats	3	

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.

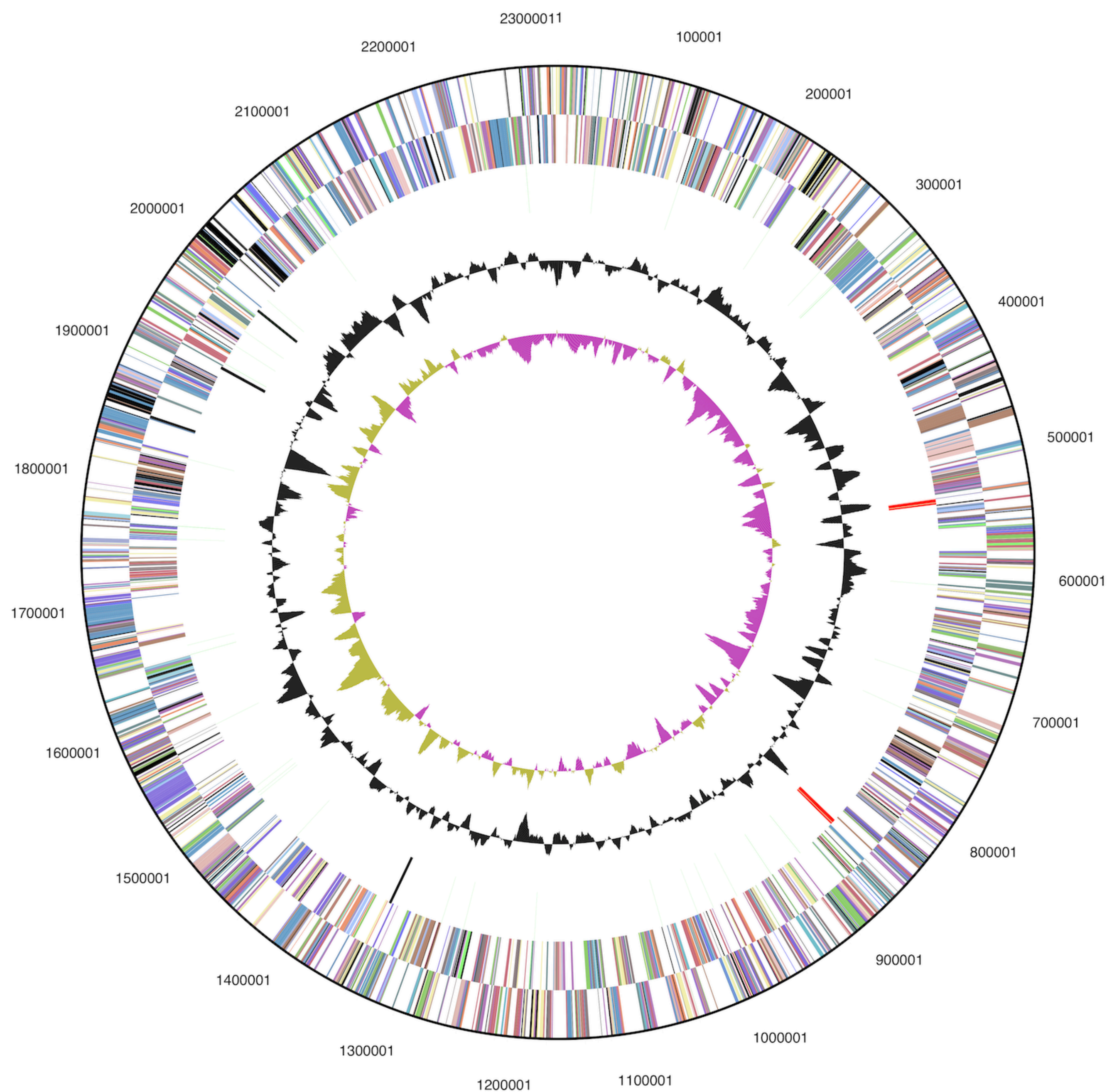

Figure 3. Graphical map of the chromosome. From outside to the center: Genes on forward strand (colored by COG categories), Genes on reverse strand (colored by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

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

Code	value	%age[a]	Description
J	155	6.9	Translation, ribosomal structure and biogenesis
A	2	0.1	RNA processing and modification
K	72	3.2	Transcription
L	144	6.4	Replication, recombination and repair
B	2	0.1	Chromatin structure and dynamics
D	35	1.6	Cell cycle control, cell division, chromosome partitioning
Y	0	0.0	Nuclear structure
V	17	0.8	Defense mechanisms
T	114	5.1	Signal transduction mechanisms
M	129	5.8	Cell wall/membrane biogenesis
N	84	3.8	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	83	3.7	Intracellular trafficking and secretion, and vesicular transport
O	88	3.9	Posttranslational modification, protein turnover, chaperones
C	151	6.8	Energy production and conversion
G	67	3.0	Carbohydrate transport and metabolism
E	166	7.4	Amino acid transport and metabolism
F	58	2.6	Nucleotide transport and metabolism
H	123	5.5	Coenzyme transport and metabolism
I	39	1.7	Lipid transport and metabolism
P	82	3.7	Inorganic ion transport and metabolism
Q	19	0.9	Secondary metabolites biosynthesis, transport and catabolism
R	225	10.1	General function prediction only
S	152	6.8	Function unknown
-	388	17.4	Not in COGs

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

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