Minireview of the genome of *Ciceribacter thiooxidans*

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ABSTRACT

For the following minireview different aspects of Ciceribacter thiooxidans' genome were analysed. These include the number of proteins and RNA's encoded in the genome, their type and structural features, Chargaff's second parity rule, GC-count, CDS's distribution on + and - strand of the DNA molecules, Shine-Dalgarno sequences, stop-codon usage, GC skew and cumulative GC skew of the DNA molecules.

INTRODUCTION

Ciceribacter thiooxidans is a nitrate-reducing thiosulfate oxidizing bacterium. It lives in sulfide-rich anoxic sediment in rivers. Ciceribacter thiooxidans play an important role in nitrogen and sulfur cycling in diverse ecosystems. It belongs to the Rhizobiaceae family most of which strains are nodule-forming symbiotic microorganisms with nitrogen-fixing abilities. Cells are Gram-negative, facultatively chemolithotrophic, facultatively anaerobic, non-spore-forming and rod-shaped (1.0–2.0×0.6–0.8 μm) with a flagellum. Colonies are circular, convex, entire, glistening and semi-translucent, optimal pH is 7.5, nitrate is reduced to the nitrogen gas [1].

MATERIAL AND METHODS

GCF_014126615.1_ASM1412661v1_assembly_stats.txt - file containing different characteristics of the bacteria genome including but not limited to: number of DNA molecules in the cell, GC content, number of scaffolds, length of all DNA molecules present in the cell. It can be found in the supplementary materials (Fig. S1)

GCF_014126615.1_ASM1412661v1_feature_table.txt - file containing information about all the genes and what they encode. There is additional information about their transcripts, such as name and length of the proteins or the type of RNA's. It can be found in the supplementary materials (Fig. S2). For convenience information from this file is also presented in the Google Sheets file which can be found in the supplementary materials (Fig. S3).

The script written in Python 3.9 was used to calculate the number of each nucleotide, GC skew, 20 nucleotides long sequences before translation initiation starting point and most common 6-mers in them, number of occurrences for each stop-codon. This script can be found in the supplementary materials (Fig. S4)

GCF_014126615.1_ASM1412661v1_genomic.fna - fasta file containing entire DNA sequence of the bacteria. It can be found in the supplementary materials (Fig. S5)

To calculate p-value based on the value of chi-squared test script on the website was used. The link to the website can be found in the supplementary materials (Fig. S6)

GCF_014126615.1_ASM1412661v1_cds_from_genomic.fna.gz - file containing information about all CDS in the genome of *Ciceribacter thiooxidans.* It can be found in the supplementary materials (Fig. S7)

To assess whether Chargaff's second parity rule applies to the genome of the *Ciceribacter thiooxidans* Pearson's chi-squared test with significance level $\alpha = 0.05$ was used.

To assess whether protein coding genes are distributed randomly between + and - strands of the genome of the *Ciceribacter thiooxidans* Pearson's chi-squared test with significance level $\alpha = 0.05$ was used.

RESULTS

Basic genome information

The genome contains 2 DNA molecules: 1 chromosome and 1 plasmid. The length of the chromosome is 3661327 nucleotides. The length of the plasmid is 1382059 nucleotides. Both molecules have approximately the same GC-count of 63% and 62% respectively. Genome coverage is 129,0x (Fig. S1).

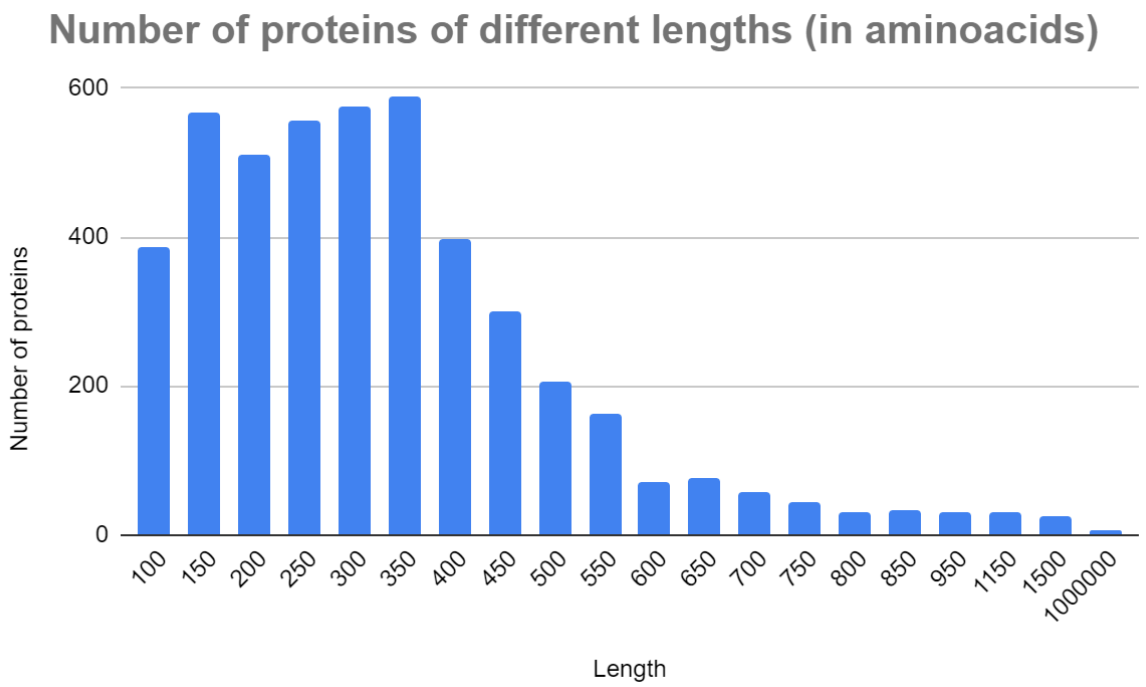
Table 1. Length and GC-count in DNA of *Ciceribacter thiooxidans*

	Chromosome	Plasmid
Length	3661327	1382059
GC-count	63%	62%

Proteome information

There are a total of 4661 proteins encoded in the genome of *Ciceribacter thiooxidans*. The following histogram made in Google Sheets depicts the number of proteins of different lengths (Histogram 1).

Histogram 1. Number of proteins of each length in the proteome of *Ciceribacter thiooxidans.* X-axis - the length of the proteins (from the number below the left box to the number below the current box), Y-axis - the number of such proteins.



Out of those 4661 proteins 3452 are encoded in the chromosome and 1209 are encoded in the plasmid. There are a total of 2233 proteins encoded on the + strand of the DNA and 2428 encoded on the - strand of the DNA molecule. There are 56 ribosomal, 595 transporter and 491 hypothetical proteins. Transporters make up 12,77% of all proteins and hypothetical proteins make up 10,53% (Fig. S3). More detailed information about the number of different proteins in the chromosome and plasmid can be found in Table 2.

Table 2. Information about the proteins encoded in different DNA molecules of *Ciceribacter thiooxidans*

	Chromosome	Plasmid
Number of proteins on + strand	1638	595
Number of proteins on - strand	1814	614
Number of transporter proteins	343	252
Number of ribosomal proteins	56	0
Number of hypothetical proteins	375	116
Percentage of transporter	9.93%	20.84%

proteins		
Percentage of hypothetical proteins	10.86%	9.59%

RNA's

There are a total of 68 genes which encode RNA's. This is expectedly much fewer than the number of protein-encoding genes. All RNA's are encoded in the chromosome DNA. Information about the types of RNA's is presented in Table 3.

Table 3. RNA genes in the genome of *Ciceribacter thiooxidans*

RNA genes	Number of this genes
All RNA	68
tRNA	55
rRNA	9
tmRNA	1
RNase_P_RNA	1
SRP_RNA	1
6s/SsrS RNA	1

It should be noted that some tRNA genes have multiple copies- there are 5 tRNA's-Met with anticodon CAT, 3 tRNA's-Ala with anticodon CGC, 3 tRNA's-Ile with anticodon GAT, 2 tRNA's-Asp with anticodon GTC, 2 tRNA's-Arg with anticodon TCT, 3 tRNA's-Ala with anticodon TGC and 2 tRNA's-Glu with anticodon TTC. This should be accounted for by the researcher who decides to use *Ciceribacter thiooxidans* as the system for the eukaryotic proteins synthesis (Fig. S3).

Chargaff's second rule

Chargaff's second rule states that complementary nucleotides are met with almost equal frequencies in single stranded DNA [2]. Chargaff's second rule was tested on the chromosome and plasmid of the *Ciceribacter thiooxidans* using Pearson's chi-squared test with significance level $\alpha = 0.05$ [3]. Number of each nucleotide was calculated using a script written in Python (Fig. S4) and FASTA-file containing genome of *Ciceribacter thiooxidans* (Fig. S5). P-value was calculated using software on the website listed in the supplementary materials (Fig. S6). The results are presented in Table 4

Table 4. Number of different nucleotides, Chi-squared test value and corresponding p-value for chromosome and plasmid of *Ciceribacter thiooxidans.* AT or GC distribution is the distribution of these nucleotides in the DNA strand, expected ratio is 1:1.

	Chromosome	Plasmid
Number of adenines	675729	263164
Number of thymines	675896	261529
Number of cytosines	1149049	430111
Number of guanines	1160653	427255
Chi-squared test value for AT distribution	0.0206	5.0948
Chi-squared test value for GC distribution	58.3000	9.5137
p-value for AT distribution	0.885874	0.023998
p-value for GC distribution	10^{-10}	0.002039

P-value was above chosen significance level only for AT distribution in the chromosome, which means there is no statistical difference between expected and observed number of adenines and thymines in that molecule. The GC and AT distribution in the plasmid are below the significance level, but are still pretty close to it. The most notable difference is in the GC distribution in the chromosome, where p-value is 10^{-10}.

CDS's distribution

The hypothesis that there should be the same number of protein-coding genes on + and - strands of the DNA was tested using Pearson's chi-squared test with the significance level $\alpha = 0.05$ [3]. The results are presented in Table 5.

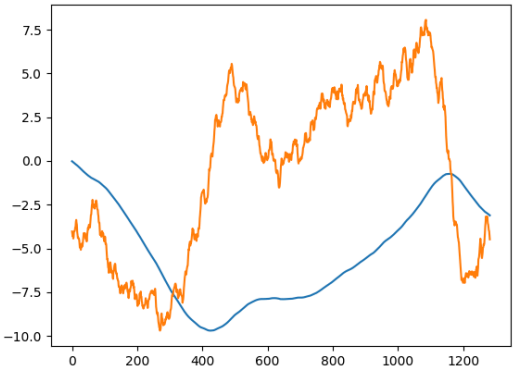
Table 5. Number of proteins encoded on + and - strand of chromosome and plasmid of *Ciceribacter thiooxidans*, corresponding chi-squared test value and p-value.

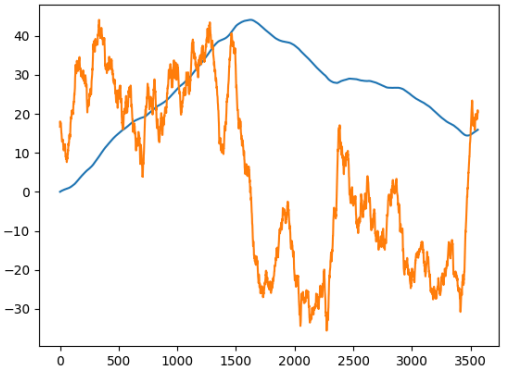
	Chromosome	Plasmid
Number of CDS's on + strand	1638	595
Number of CDS's on - strand	1814	614
Chi-squared test value	8.9733	0.2986
p-value	0.00274	0.584761

The statistical difference is only observed in the chromosome, where p-value is 0.00274, meaning CDS's are not distributed randomly between its + and - strand.

GC-skew

GC skew and cumulative GC skew of both chromosome and plasmid of *Ciceribacter thiooxidans* were analysed using code written in Python (Fig. S4) and FASTA-file containing genome of *Ciceribacter thiooxidans* (Fig. S5). The results are presented on the following plots.





Plot 1 (left). GC skew (orange) and cumulative GC skew (blue) of plasmid of *Ciceribacter thiooxidans.* X-axis - position in the genome (in thousands nucleotides). Y-axis - value of cumulative GC skew (blue). GC skew value (orange) was multiplied for it to be visible on the plot, values on Y axis do not represent values of the GC skew. **Plot 2 (right).** GC skew (orange) and cumulative GC skew (blue) of the chromosome of *Ciceribacter thiooxidans.* X-axis - position in the genome (in thousands nucleotides). Y-axis - value of cumulative GC skew (blue). GC skew value (orange) was multiplied for it to be visible on the plot, values on Y-axis do not represent values of the GC skew.

It is known that the maximum value of the cumulative skew corresponds to the terminus, and the minimum value corresponds to the origin of replication [4]. As can be seen from our plots, in the plasmid origin of replication is at around 425000th nucleotide, terminus at around 1158000th nucleotide; in the chromosome origin is at around 1625000th nucleotide, terminus at around 3471000th nucleotide.

6-mers upstream of the translation initiation point

Using script written in Python (Fig. S4) twenty nucleotide long sequences before the translation initiation point were analysed in both chromosome and plasmid DNA of *Ciceribacter thiooxidans.* Ten of the most frequent 6-mers for each DNA were calculated using protocol written in Python (Fig. S4) and FASTA-file containing genome of *Ciceribacter thiooxidans* (Fig. S5). The results are presented in Table 6.

Table 6. 10 most frequent 6-mers in the twenty nucleotide long sequence before the initiation point in the chromosome and plasmid of *Ciceribacter thiooxidans.*

	Chromosome	Plasmid
6-mer and number of occurrences	219 GGAGGA 219 AGGAGA 198 AAGGAG 196 GAGGAG 168 GAAGGA 167 GGAGAA 162 AGGAGG 152 AAAGGA 149 GGGAGG 141 GAAAGG	133 GGAGGA 111 GAGGAG 101 AGGAGA 97 GGGAGG 74 GGAGAA 70 AGGAGG 70 AAGGAG 64 AGGGAG 61 GAGGAA 60 CGGAGG

These 6-mers are Shine-Dalgarno sequences [5]. They bind to the 16S rRNA and allow the ribosome to start protein synthesis. As can be seen from Table 5, 19 out of 20 most frequent Shine-Dalgarno sequences consist only of A and G.

Stop codon usage

Number of occurrences for each stop codon was calculated using protocol written in Python (Fig. S5) for both plasmid and chromosome of *Ciceribacter thiooxidans.* The sequences of CDS's used for this can be found in supplementary materials (Fig. S7). The results are presented in Table 7.

Table 7. Number of occurrences of different stop-codons in chromosome and plasmid of *Ciceribacter thiooxidans*

	Chromosome	Plasmid
Number of occurrences of each stop-codon	TGA 2515 TAA 538 TAG 478	TGA 904 TAA 149 TAG 214

This is an unexpected result, since in most bacteria TAA stop-codon is the most frequent because it is recognised by both RF1 and RF2 [6]. On the other hand, TGA stop-codon is only recognised by RF2. However, during the translation of mRNA which encodes RF2 ribosomal slippage takes place, so this mRNA also encodes another protein - penicillin-binding protein 1A (Fig. S3). This protein is responsible for penicillin resistance [7]. *Ciceribacter thiooxidans* was discovered in the sediment of Pearl River Delta in China [1] which is heavily contaminated by different antibiotics [8]. Therefore when *Ciceribacter thiooxidans* synthesises penicillin-binding protein 1A to protect itself from the high concentration of antibiotics nearby, it also synthesises RF2. It was shown that high concentration of RF2 in the bacteria cell correlates with higher usage of TGA stop-codon [6]. High RF2 concentration caused by water contamination results in much higher TGA stop-codon frequency.

Anticodon usage

Anticodons of tRNA's present in the Ciceribacter thiooxidans genome were analysed (Fig. S3). The results are presented in Table 8.

Table 8. Anticodons of tRNA's for each amino acid. Anticodons of tRNA's present in *Ciceribacter thiooxidans* genome are written in black, anticodons which are not present in any tRNA are highlighted in red. If tRNA with specific anticodon is present in the genome in more than 1 copy, the number of copies is written in the parenthesis after the given anticodon.

Amino acid	Anticodon of tRNA for this amino acid
Arg	CCT, TCT (x2), CCG, ACG, TCG, GCG
Leu	CAA, GAG, TAG, TAA, CAG, AAG
Ile	GAT (x3), CAT, TAT, AAT
Ala	TGC (x3), CGC (x3), GGC, AGC
Met	CAT (x4)
Val	GAC, TAC, AAC, CAC
Lys	CTT, TTT
Ser	TGA, GCT, GGA, CGA, AGA, ACT
Pro	TGG, GGG, CGG, AGG
Glu	TTC (x2), CTC
Asn	GTT, ATT
Cys	GCA, ACA
Trp	CCA
Gly	TCC, CCC, GCC, ACC
Tyr	GTA, ATA
Thr	TGT, GGT, CGT, AGT
Asp	GTC (x2), ATC
Gln	TTG, CTG
His	GTG, ATG
Phe	GAA, AAA

The first observation is that only 41 (CAT anticodon is counted once) out of possible 61 anticodons are present, but obviously all codons encoding amino acids can be translated in the cell. This is achieved because one nucleotide in the anticodon can have more than 1 complementary nucleotide, dictated by Wattson-Crick base pairing rules. This effect is called wobble base pairing, wobble base pairs are presented in Table 9.

Table 9. Wobble base pairing rules. A - adenine, G - guaning, C - cytosine, U - uridine, I - hypoxanthine, k^2C - lysidine

tRNA 5' anticodon base	mRNA 3' codon base (Revised) [9]
A	U, C, G, or (A)
C	G
G	C or U
U	A, G, U, or C
I	A, C, or U
k^2C	A

Another interesting observation is that one tRNA-Ile has CAT anticodon, but tRNA-Ile are only expected to have anticodons GAT, TAT or AAT [10]. CAT anticodon would normally bind to the ATG codon, which encodes methionine. However, the first cytosine in this anticodon is modified by the tRNA lysidine(34) synthetase TilS (Fig. S3), which converts cytosine to lysidine [12]. Lysidine forms base pairs with adenine (Table 9), so the resulting anticodon acts as TAT and can bind to common Ile codons.

SUPPLEMENTARY DATA

S1 GCF_014126615.1_ASM1412661v1_assembly_stats.txt

S2 GCF_014126615.1_ASM1412661v1_feature_table.txt.gz

S3 Genome features

S4 Python code

S5 GCF_014126615.1_ASM1412661v1_genomic.fna.gz

S6 https://www.socscistatistics.com/pvalues/chidistribution.aspx

S7 GCF_014126615.1_ASM1412661v1_cds_from_genomic.fna.gz

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest.

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