Draft genome sequence of *Arthrospira platensis* C1 (PCC9438)

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Arthrospira platensis is a cyanobacterium that is extensively cultivated outdoors on a large commercial scale for consumption as a food for humans and animals. It can be grown in monoculture under highly alkaline conditions, making it attractive for industrial production. Here we describe the complete genome sequence of *A. platensis* C1 strain and its annotation. The *A. platensis* C1 genome contains 6,089,210 bp including 6,108 protein-coding genes and 45 RNA genes, and no plasmids. The genome information has been used for further comparative analysis, particularly of metabolic pathways, photosynthetic efficiency and barriers to gene transfer.

Introduction

Arthrospira platensis is a cyanobacterium that contains large amounts of proteins, vitamins, lipids and pigments [1]. It is widely used as a human food and an animal feed. In addition, its extracts can enhance the immune system and promote health [1,2]. As the natural habitat is soda lakes, *Arthrospira* spp are cultivated under highly alkaline conditions in open ponds on a large commercial scale. This condition can minimize and sometimes prevent the culture from contamination [3]. Unlike many plant food products whose nutritional value rapidly deteriorates at high temperatures, the nutritional value of *Arthrospira* products is maintained even when the cells are processed at high temperatures [4]. In contrast to many cyanobacteria, there is no report of toxicity of *Arthrospira* for humans, animals or environments [4].

The genome sequences of *Arthrospira* spp. have been the subject of immense interest due to the beneficial properties of these organisms in the biotechnology and environmental fields [5,6]. *A. platensis* C1 is the fifth complete genome report for a member of the genus *Arthrospira*. *A. platensis* C1 has long been used as a laboratory strain for physiological and molecular studies due to its non-gliding property, which enables single colonies formation. This property facilitates studies at the molecular level and strain improvement, particularly, the development of a transformation system. Currently, a successful transformation system for *Arthrospira* has not yet been established.

Thus the genome sequences may help to identify barriers responsible for the instability of the transformants. Here, we present a summary classification and a set of features of *A. platensis* C1 together with the complete genomic sequence and its annotation.

Classification and features

Historically, the classification of the *Arthrospira* and *Spirulina* genera [Figure 1] was a subject of controversy. For the commercial strain, *Arthrospira* or *Spirulina* was used interchangeably. Both *Arthrospira* and *Spirulina* are similar in morphological characters; cylindrical, multicellular, filamentous cyanobacteria with an open, left-handed helical shape [Table 1]. They both belong to the Phylum *Cyanobacteria*, Order *Oscillatoriales* and Family *Oscillatoriaceae* [13]. However, they can be differentiated by the presence of cell septa: *Arthrospira* possess septa, whereas *Spirulina* do not [14].

Table 1. Classification and general features of *A. platensis* C1 according to the MIGS recommendations [8]

MIGS ID	Property	Term	Evidence Code
		Domain *Bacteria*	TAS [9]
		Phylum *Cyanobacteria*	TAS [10,11]
		Class *Cyanobacteria*	TAS [11,12]
	Current Classification	Order *Oscillatoriales*	TAS [13]
		Family *Oscillatoriaceae*	TAS [13]
		Genus *Arthrospira*	TAS [11]
		Species *Arthrospira platensis C1*	TAS [11]
	Gram stain	Negative	TAS [14]
	Cell shape	Spiral	TAS [14]
	Motility	None	
	Sporulation	None	
	Temperature range	20 – 40	TAS [14]
	Optimum temperature	30-35	TAS [14]
MIGS-22	Relationship to Oxygen	Aerobic	TAS [3]
	Carbon source	Phototroph, Mixotroph	TAS [3]
	Energy source	Phototroph	TAS [3]
MIGS-6	Habitat (EnvO)	Fresh water	TAS [3]
MIGS-6.1	Temperature	20 - 40	TAS [3]
MIGS-6.2	pH	8.0 -10.0	TAS [3]
MIGS-6.3	Salinity	0.06	TAS [3]
MIGS-10	Extrachromosomal elements	None	TAS [6]
MIGS-11	Estimated Size	6.08 Mb	IDA
MIGS-14	Known Pathogenicity	None	NAS
MIGS-15	Biotic Relationship	Free living	NAS
MIGS-4	Geographic Location	Not reported	NAS
MIGS-4.1	Latitude	Not reported	NAS
MIGS-4.2	Longitude	Not reported	NAS
MIGS-4.3	Depth	Not reported	NAS
MIGS-4.4	Altitude	Not reported	NAS

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 [15]. 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.

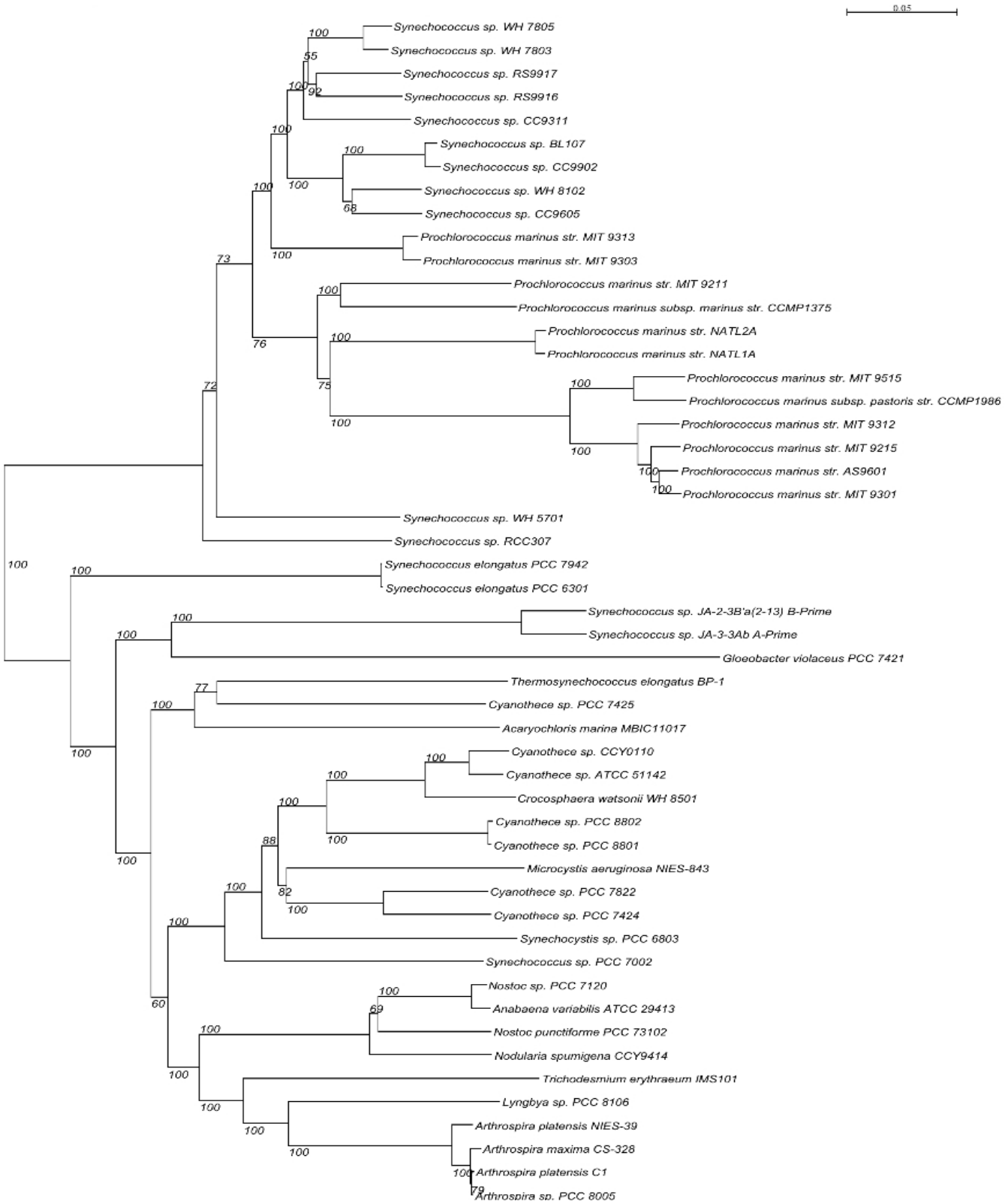

Figure 1 The phylogenetic tree of 51 cyanobacterial concatenated ribosomal proteins. The main topology is in agreement with earlier inferences of the phylogeny of this taxon with the 16s rRNA based on the GTR+G+I substitution model [7]. The tree is built using the Neighbor-Joining method and 1,000 re-samplings to calculate bootstrap values. *A. platensis* C1 was clustered together with other strains in the order *Oscillatoriales* and was clearly separated from related species in the order *Nostocales*. The conserved, concatenated ribosomal protein phylogenetic tree indicated the monophyly of this *Arthrospira* genus.

Chemotaxonomy

Arthrospira platensis C1 or *Arthrospira* sp. PCC 9438, as designated based on its morphology by Pasteur Institute, Paris, France, was originally classified as *Spirulina platensis* C1 [1,3]. This reclassification was in agreement with the presence of γ-linolenic acid (GLA) in the fatty acid profile, a chemotaxonomic marker of *Arthrospira*, while GLA is absence in *Spirulina* [16-18].

The phylogenetic tree of cyanobacteria was reconstructed with evolutionary information embedded in conserved, concatenated ribosomal proteins. *A. platensis* C1 was positioned into the genus *Arthrospira* with 100% of the bootstrapping value. The closest strain is *Arthrospira sp.* PCC 8005 with 97.43% sequence identity, whereas the other strains of this genus share 94.93-96.58% sequence identity. The nearest related order is *Nostocales* with approximately 70% sequence identity.

Genome sequencing and annotation

Genome project history

Arthrospira genome sequencing projects have been carried out in 5 research centers: Genoscope, France; the DOE Joint Genome Institute, USA; the National Institute of Technology and Evaluation (NITE), Japan; the University of Applied Sciences, Switzerland, and King Mongkut's University of Technology Thonburi (KMUTT), Thailand, using various *Arthrospira* strains (*A. platensis* PCC8005, *A. maxima* CS-328, *A. platensis* NIES- 39, *A. platensis* Paraca and *A. platensis* C1 (this study), respectively). In this study, the genome of *A. platensis* C1 has been sequenced, and the results provide data that can be used for the further study of its biological functions.

The genome project is deposited in the GenBank Database (NCBI ID 67617 and accession number AFXD00000000). DNA sequencing and finishing were performed in collaboration between the Genome Institute, BIOTEC-NSTDA, Thailand and Kazusa DNA Research Institute, Japan. The genome assembly and annotation steps were performed in collaboration between KMUTT, the Genome Institute, BIOTEC-NSTDA, Chiang Mai University, Thailand and Kazusa DNA Research Institute. The summary of the project information is shown in Table 2.

Table 2 Genome sequencing project information

MIGS-ID	Property	Term
MIGS-31	Finishing quality	Draft
MIGS-28	Libraries used	Genomic libraries: Sanger (one each of 2 and 5 kb library), standard 454 pyrosequence library and BAC library
MIGS-29	Sequencing platforms	454 and Sanger Technology
MIGS-31.2	Sequence coverage	28×
MIGS-30	Assemblers	Newbler version 2.3, Phrap
MIGS-31.3	Contigs	63
MIGS-32	Gene calling method	Glimmer 3.0
	GOLD ID	Gi09635
	NCBI project ID	67617
	Database: IMG-GEBA	2507262036
	Source material identifier	PCC9438
	Project relevance	Biotechnological

Growth conditions and DNA isolation

A. platensis strain C1 was obtained from Prof. Avigad Vonshak (Algal Biotechnology, Ben-Gurion University of the Negev, Israel). The cells were routinely grown at 35 °C with constant shaking at 150 rpm in Zarrouk's medium [19], under aeration of 1% (v/v) CO_2 and illumination of 100 µE m^{-2} s^{-1} from fluorescent lamps. For stock cultures, cells were transferred into new Zarrouk's medium [19] every two weeks.

For the shotgun genome library preparation, a total DNA sample was sheared by nebulization with nitrogen at 45 psi for 1 min and purified using a QIAGEN Purification kit (QIAGEN, Valencia, CA). DNA fragments with sizes greater than 300 bp were selected by Ampure Bead purification (Beckman Coulter, Brea, CA) and blunt-ended with T4 DNA polymerase and T4 polynucleotide kinase (454 Life Sciences, Roche, Branford, CT). After DNA purification, 454-library adaptors were added to both ends of the fragments, which were immobilized using Library Immobilization Beads for further DNA sequencing.

For paired-end library preparation, a total DNA sample was sheared by nebulization with nitrogen at 10 psi for 2 min to obtain 2.2 kb fragments with hairpin adaptors added to both ends of the DNA fragments. The DNA fragments with hairpin adaptors were immobilized using Library Immobilization Beads and ligated to paired-end adaptors. DNA libraries were linked to the capture beads and amplified by emulsion PCR (Roche Applied Science protocol).

Genome sequencing and assembly

The genome of *A. platensis* C1 was sequenced using a hybrid method between the 454 Life Sciences technology on the Genome Sequencer (GS) FLX System and BigDye Terminator v3.1 Cycle sequencing. Pyrosequencing reads were assembled using the Newbler *de novo* sequence assembly software version 2.0.0 (Roche). The Phred/Phrap/Consed software package [20] was used for sequence assembly and quality assessment in the finishing process. The remaining gaps between contigs were closed by custom primer walk or PCR amplification and then editing in Consed. The final assembly contains 739,684 reads from pyrosequencing and 45,959 reads from Sanger sequencing, resulting in 28× coverage of the genome. Employing *A. maxima* CS-328 contigs [21] as a reference, the *A. platensis* C1 circular genome of 6.08 Mb total size with 1 scaffold and 63 gaps has been constructed.

Table 3 Genome statistics

Attributed	Value	% of Total
Genome size (bp)	6,089,210	100.00%
DNA coding region (bp)	4,951,337	81.31%
G+C content (bp)	2,651,568	44.68%
DNA scaffolds	1	100.00%
Total genes	6,153	100.00%
Protein coding genes	6,108	100.00%
RNA genes	45	0.73%
Genes with protein function prediction	3,757	61.06%
Genes with enzymes	952	15.47%
Genes with Transporter Classification	345	5.61%
Genes assigns to KEGG pathways	1,012	16.45%
Genes assigned to KEGG Orthology (KO)	1,837	29.86%
Genes assigned to COGs	3,459	56.22%
Genes assigned to Pfam	3,529	57.35%
Genes assigned to TIGRfam	1,180	19.18%
Genes assigned to InterPro	4,244	68.97%
Genes assigned in paralog clusters	1,048	17.03%
Genes assigned with signal peptides	570	9.26%
Genes assigned with transmembrane proteins	1,094	17.78%

Genome annotation

In agreement with the result from the Integrated Microbial Genomes Expert Review (IMG-ER) platform [22], all the genes in the *A. platensis* C1 genome were identified using the GLIMMER 3.0 program in our Microbial Inhouse Annotation Pipeline. Initial criteria for automated functional assignment required a minimum of 50% residue identity with over 80% length match for BLASTP alignments to the NCBI nonredundant database, InterPro, SwissProt, SignalP, COG, and KEGG databases. The tRNAscan-SE tool [23] was used to find tRNA genes, whereas ribosomal RNAs were found by using the tool RNAmmer [24]. Additional gene prediction analysis and functional annotation were performed within IMG-ER platform [22] and a round of manual curation, including confirmation with proteomic data [25,26].

Genome properties

The genome of *A. platensis* C1, with a total of 6.08 Mbp (6,089,210 base pairs), contains 44.68% G+C (Table 3) and, in agreement with the findings of Fujisawa *et al*. (2010) [6], no plasmid DNA. Our results confirm the presence of a single genome in *A. platensis* C1. The *A. platensis* C1 circular genome of 6.08 Mbp was compared with the *A. maxima* CS-328 and *A. platensis* NIES-39 genomes [6]. A total of 6,153 open reading frames (orfs) were predicted. Of these, 3,757 were annotated as coding for known protein functions and 45 for RNA genes (6 for rRNA and 39 for tRNA). The distribution of genes into COGs is presented in Table 4.

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

Code	Value	% age	Description
J	161	4.22	Translation, ribosomal structure and biogenesis
A	0	-	RNA processing and modification
K	164	4.29	Transcription
L	459	12.02	Replication, recombination and repair
B	2	0.05	Chromatin structure and dynamics
D	49	1.28	Cell cycle control, cell division, chromosome partitioning
Y	0	-	Nuclear structure
V	98	2.57	Defense mechanisms
T	337	8.82	Signal transduction mechanisms
M	234	6.13	Cell wall/membrane/envelope biogenesis
N	54	1.41	Cell motility
Z	0	-	Cytoskeleton
W	0	-	Extracellular structures
U	71	1.86	Intracellular trafficking, secretion, and vesicular transport
O	180	4.71	Posttranslational modification, protein turnover, chaperones
C	181	4.74	Energy production and conversion
G	149	3.9	Carbohydrate transport and metabolism
E	201	5.26	Amino acid transport and metabolism
F	66	1.73	Nucleotide transport and metabolism
H	157	4.11	Coenzyme transport and metabolism
I	69	1.81	Lipid transport and metabolism
P	148	3.88	Inorganic ion transport and metabolism
Q	71	1.86	Secondary metabolite biosynthesis, transport and catabolism
R	525	13.75	General function prediction only
S	443	11.6	Function unknown
-	2694	43.78	Not in COG

Table 5. Genome statistics comparison among Arthrospira spp.

Genome Name	*A. platensis C1*	*A. platensis NIES-39*	*A. maxima* CS-328	*A. platensis Paraca*	*A. platensis*
Genome size (bp)	6,089,210	6,788,435	6,003,314	4,997,563	6,145,553
Total genes	6,153	6,676	5,730	5,401	5,718
Protein-coding genes	6,108	6,630	5,690	5,370	5,675
Protein with function prediction	3,757	2,542	3,315	3,023	3,023
RNA genes	45	46	40	31	43
Enzymes	952	905	889	816	882
% Enzymes	15.47%	13.56%	15.51%	15.11%	15.42%
Transporter Classification	345	NA	NA	NA	NA
% Transporter Classification	5.61%	NA	NA	NA	NA
KEGG pathways	1,012	993	931	904	954
% KEGG pathways	16.45%	14.87%	16.25%	16.74%	16.68%
KEGG Orthology (KO)	1,837	1,702	1,623	1,492	1,658
% KEGG Orthology (KO)	29.86%	25.49%	28.32%	27.62%	29.00%
COGs	3,459	3,570	3,306	3,050	3,234
%COGs	56.22%	53.48%	57.70%	56.47%	56.56%
Pfam	3,529	3,598	3,564	3,431	3,526
%Pfam	57.35%	53.89%	62.20%	63.53%	61.66%
TIGRfam	1,180	1,213	1,160	1,185	1,203
%TIGRfam	19.18%	18.17%	20.24%	21.94%	21.04
InterPro	4,244	3,969	3,938	4,207	4,294
%InterPro	68.97%	59.45%	68.73%	77.89%	75.10%
Signal peptides	570	1,319	545	559	1,150
%signal peptides	9.26%	19.76%	9.51%	10.35%	20.11%
Transmembrane proteins	1,094	1,123	1,053	1,094	1,057
%Transmembrane proteins	17.78%	16.82%	18.38%	20.26%	18.49%
COG clusters	1,569	1,566	1,491	1,489	1,472
KOG clusters	729	723	722	737	717
Pfam clusters	1,740	1,732	1,702	1,729	1,730
TIGRfam clusters	932	936	902	924	931

NA: Information not available.

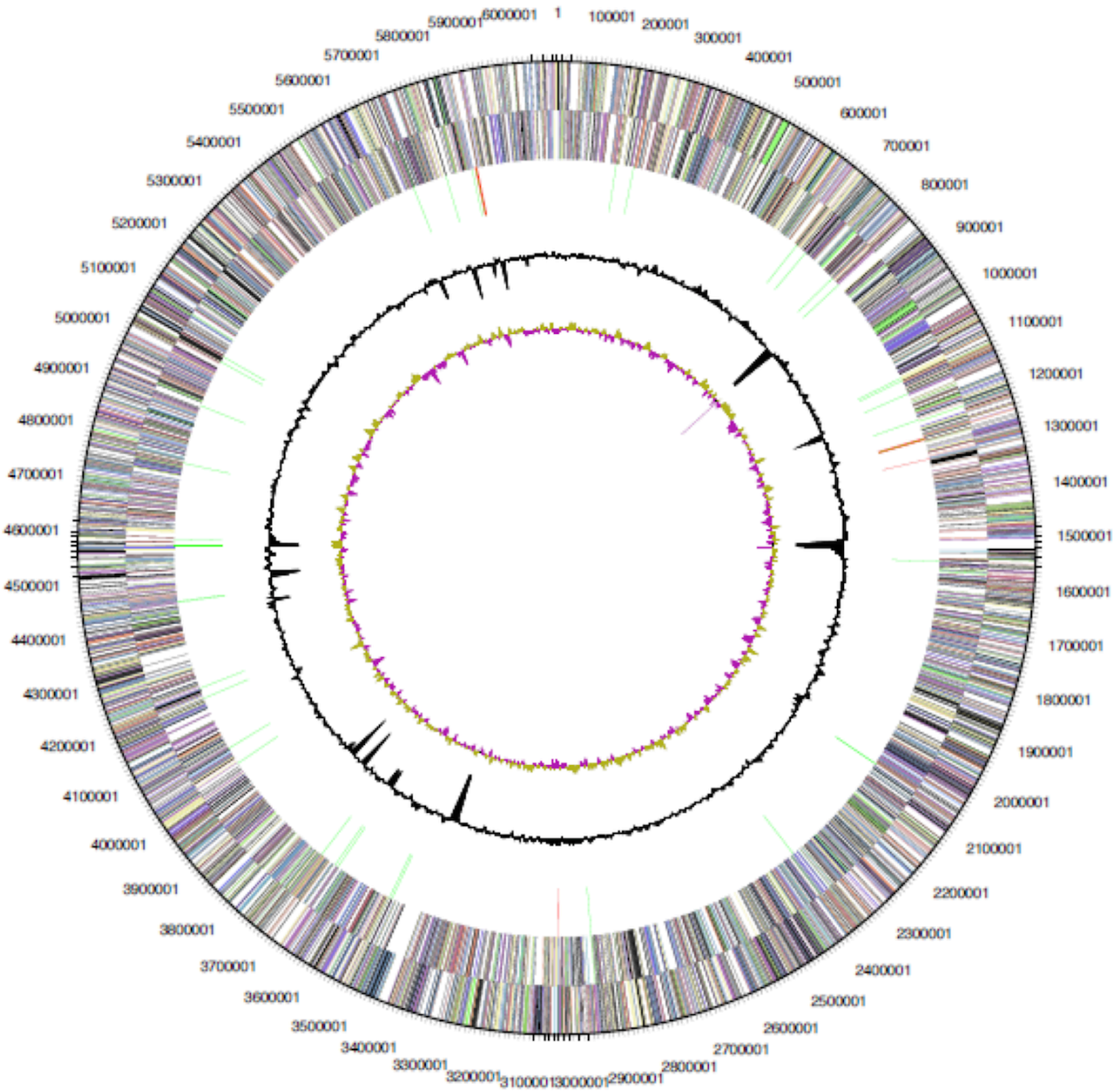

Figure 2 Graphical circular map of the chromosome of *Arthrospira platensis* C1. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

The genome atlas of *A. platensis* C1 is shown in Figure 2. The atlas was created based on one FASTA file containing the nucleotide sequences in one piece. Together with the annotation file, positions of genes were used for the map.

In the genome atlas, the gene annotation, base content, AT and GC skew, percent AT and some structural properties of the DNA are shown. The structural properties are Position Preference, Stacking Energy and Intrinsic Curvature, all of which are related to the flexibility and strength of the DNA molecule [27].

Genomic comparison

The genome sequences from all *Arthrospira* strains (*A. maxima* CS-328, *A. platensis* NITE-39, *A. platensis* PCC8005, *A. platensis* Paraca, and *A. platensis* C1) provided the data for a comparative genome analysis of these strains. Based on the genome statistics comparison (Table 5), all of the

Arthrospira spp. genomes revealed highly conserved sequences. Interestingly, the number of signal peptides in *A. platensis* C1 has been reported to be the lowest among the *Arthrospira* spp. Further study of these primary sequences may reveal the importance of signal peptides in protein targeting in this cyanobacterial species compared with other laboratory and commercial strains.

Insights from the genome sequence

Analysis of the genome of *Arthrospira* spp. compared to other cyanobacteria confirmed that the *Arthrospira* are non-nitrogen fixing, filamentous cyanobacteria. They are nontoxic, which might be due to the absence of polyketide and non-ribosomal peptide-producing genes.

Genes involved in gliding motility in *A. platensis* NIES-39 (a vigorously motile strain utilizing type IV pili as the major mechanism for gliding) [6] have been compared with those in *A. platensis* C1 (a nonmotile strain). Interestingly, all the genes involved in type IV pili are present in the *A. platensis* C1 genome, however, the lack of gliding ability in *A. platensis* C1 is due to an unknown mechanism. Further studies are needed to elucidate this mechanism of cell motility.

Cellular defense mechanism

Like other genomes of *Arthrospira* spp., the genome of *A. platensis* C1 contains highly interspersed repetitive sequences that account for 9% of its genome. Genome comparisons among cyanobacteria revealed unusual genes involved in defense mechanisms, including restriction and modification enzymes, group II introns, insertion elements and CRISPR. These genes are considered to be major barriers for stable transformation. Therefore, these genes have been targeted for the development of a stable gene transformation system for *Arthrospira* spp. Because of the nonmotile property of *A. platensis* C1, single colonies can be selected and used for further strain improvement and genetic manipulation experiments.

By combining a stable transformation system with the advantage of colony-forming ability, we should be able to harness *A. platensis* C1 for many biotechnological applications using gene manipulation and systems biology.

Transporter Characteristic of *A. platensis* C1

Because of the ability of cyanobacteria to adapt to extremely different habitats, the relationship between their membrane transporter proteins and their habitats has been a focus of interest. Membrane transporters are proteins that allow cell membranes to deliver essential nutrients, eject waste products, and help the cell sense environmental conditions around it [28].

Interestingly, all *Arthrospira* species contain genes for Na+/H+ antiporters. The NapA-type Na+/H+ antiporter homolog, which is reported to be involved in salt tolerance at alkaline pH in some cyanobacteria, is found in all *Arthrospira* genomes. *Arthrospira* species live in high-alkalinity environments, and there are many alkali transporters in their genomes. These results revealed the relationship between the transporters and the lifestyles and niche adaptations of cyanobacteria.

Acknowledgements

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