









Metagenome Sequencing to Explore Phylogenomics of Terrestrial Cyanobacteria

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ABSTRACT Cyanobacteria are ubiquitous microorganisms with crucial ecosystem functions, yet most knowledge of their biology relates to aquatic taxa. We have constructed metagenomes for 50 taxonomically well-characterized terrestrial cyanobacterial cultures. These data will support phylogenomic studies of evolutionary relationships and gene content among these unique algae and their aquatic relatives.

Cyanobacteria, or blue-green algae, are found in nearly all aquatic and terrestrial habitats exposed to sunlight, ranging from marine systems, freshwater bodies, and thermal springs to soils and rock surfaces. They represent important ecosystem components, which perform crucial roles in biogeochemical cycling. However, knowledge of their taxonomy, phylogenomics, and physiology has been largely based on aquatic taxa. In the past 20 years, our team has discovered and described terrestrial cyanobacteria collected from extreme environments, including desert soils, ephemerally wet rock walls, and tropical damp cave walls, greatly enriching our understanding of cyanobacterial systematics (1–15). Reference strains of genus and species types are maintained in two algae culture collections at John Carroll University (JCU) and New Mexico State University (NMSU). The cultures are nonaxenic and represent unialgal polycultures containing primarily cyanobacteria and heterotrophic microbial associates. We used shotgun metagenomics of selected strains to support future investigation of the phylogenomic relationships of these unique algae.

Fifty unialgal polycultures of cyanobacterial reference strains from the JCU and NMSU culture collections were selected, representing species taxonomically evaluated within the last 2 decades (Fig. 1). Detailed protocols for biomass growth, tissue harvesting, and DNA extraction can be found at protocols.io (dx.doi.org/10.17504/protocols.io.brg4m3yw). Briefly, biomass of each taxon was grown in liquid Z8 medium (16) and harvested after several weeks to months depending on the growth rate. Harvesting included cleaning, biomass concentration, biomass flash-freezing in liquid nitrogen, and storage at -80°C . We extracted DNA using the Qiagen DNeasy PowerLyzer microbial kit. The extraction procedure included an initial bead-beating step in a Precellys homogenizer for 45 s at 5,000 rpm, repeating four times. Samples were then processed following the manufacturer's protocol for the kit and stored at -20°C until mailing to the Joint Genome Institute (JGI).

Plate-based DNA library preparation for Illumina sequencing was performed using KAPA Biosystems high-throughput library preparation kit p/n KK8235 on a PerkinElmer Sciclone next-generation sequencing (NGS) robotic liquid handling system. Then, 200 ng of sample DNA was sheared to 300 bp using a Covaris LE220 focused

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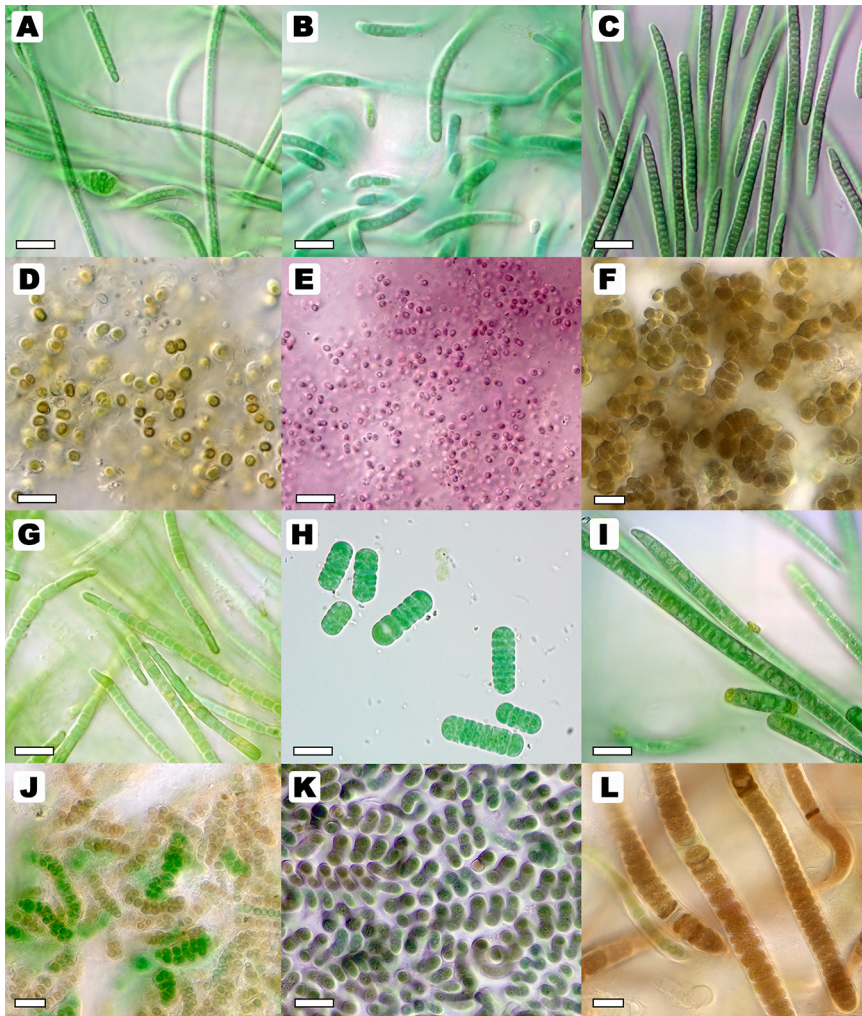


FIG 1 Light micrographs of representative cyanobacterial species maintained in unialgal cultures and studied in this project. Images represent cell morphologies in the stationary phase of the cyanobacterial life cycle. Wet mounts of cyanobacterial biomass were observed using a Zeiss AxioImager.A2 microscope equipped with Nomarski differential interference contrast optics and a Zeiss AxioCam 305 color camera at $\times 400$ or $\times 1,000$ magnification. (A) *Nodosilinea* sp. strain WJT8-NPBG4; (B) *Myxocorys chilensis* ATA2-1-KO14; (C) *Trichocoleus desertorum* ATA4-8-CV12; (D) *Cyanosarcina radialis* HA8281-LM2; (E) *Aphanocapsa lilacina* HA4352-LM1; (F) *Pleurocapsa minor* HA4340-MV1; (G) *Kastovskya adunca* ATA6-11-RM4; (H) *Hormoscilla* sp. strain CMT-3BRIN-NPC48; (I) *Microcoleus vaginatus* WJT46-NPBG5; (J) *Mojavia pulchra* JT2-VF2; (K) *Spirirestis rafaensis* WJT71-NPBG6; (L) *Brasilonema octagenarum* HA4186-MV1. Bars, 10 μm (A–E, G–I, and K) and 20 μm (F, J, and L).

ultrasonicator. The sheared DNA fragments were size selected by double solid-phase reversible immobilization (SPRI), and then the selected fragments were end repaired, A tailed, and ligated with Illumina-compatible sequencing adaptors from IDT containing a unique molecular index barcode for each sample library. The prepared libraries were quantified with a KAPA Biosystems quantitative PCR (qPCR) kit on a Roche LightCycler 480 real-time PCR instrument. Genomic libraries were sequenced with a NovaSeq instrument (Illumina, San Diego, CA) using NovaSeq XP V1 reagent kits and an S4 flow cell following a 2×150 -bp indexed run recipe. Demultiplexed reads were processed with BBDuk v38.87 (17) to remove contaminants, trim adapter sequence and “G” homopolymers ≥ 5 in size at the ends, quality trim reads, and remove reads with ≥ 4 “N” bases, with an average quality score of < 3 , or with a length of < 51 bp. After processing, 2,021,674,638 reads remained (per sample range, 24,625,170 to 59,843,005; mean \pm standard deviation, $40,433,493 \pm 8,547,361$).

Cleaned reads were assembled with metaSPAdes v3.13.0 (18) (--meta option) into contigs with parameters for a minimum contig length of 2 kb (-m 2000 option) and

TABLE 1 Metadata, accession numbers, and metagenome statistics for the 50 cyanobacterial cultures investigated in this study^a

Species	Available at culture collections	Strain ID	Alternative strain ID	BioProject no.	SRA run no.	JGI IMG ^b	No. of reads	No. of contigs	N ₅₀ (bp)	GC content (%)	Habitat	Location
<i>Aetokthonos hydrillicola</i>	NMSU, JCU, CCALA	B3-Florida	CCALA 1050	PRJNA617093	SRR12347192	3300036376	56,817,878	54,864	184,060	63.45	Aquatic epiphyte	South Carolina, USA
<i>Aphanocapsa lilacina</i>	NMSU, JCU	HA4352-LM1		PRJNA677053	SRR13206895	3300039303	58,991,043	38,358	112,017	63.64	Cave rock wall	Hawaii, USA
<i>Aphanocapsa</i> sp.	NMSU, JCU	G5E-SYN-MK-11-07L		PRJNA621664	SRR11678123	3300035009	26,644,370	1,052	87,507	48.55	Desert wet wall	Utah, USA
<i>Aphanothece saxicola</i>	NMSU, JCU	G5E-SYN-MK-01-06B		PRJNA621658	SRR11676827	3300035003	50,225,611	8,874	272,324	66.57	Desert wet wall	Utah, USA
<i>Aphanothece</i> sp.	NMSU, JCU	CMT-3BRIN-NPC111		PRJNA617094	SRR12347633	3300036407	41,058,001	13,407	164,105	59.69	Desert soil	California, USA
<i>Brasilonema angustatum</i>	NMSU, JCU	HA4187-MV1		PRJNA617095	SRR12347719	3300036389	51,374,669	22,657	78,161	54.18	Tropical soil	Hawaii, USA
<i>Brasilonema octogenerum</i>	NMSU, JCU	HA4186-MV1		PRJNA621669	SRR11678155	3300035014	41,935,410	2,727	183,890	63.68	On wood in tropics	Hawaii, USA
<i>Calothrix</i> sp.	NMSU, JCU	F12-JR17		PRJNA617096	SRR12347730	3300036390	26,844,447	1,488	168,980	56.22	Desert soil	California, USA
<i>Chroococcus</i> sp.	NMSU, JCU	CMT-3BRIN-NPC107		PRJNA653492	SRR12688413	3300037833	47,424,890	59,802	35,531	63.26	Desert soil	California, USA
<i>Cyanomargarita calcaria</i>	NMSU, JCU	G5E-NOS-MK-12-04C		PRJNA621661	SRR11676926	3300035006	30,710,984	5,791	264,901	58.92	Desert wet wall	Utah, USA
<i>Cyanosarcina radialis</i>	NMSU, JCU	HA8281-LM2		PRJNA653493	SRR12688412	3300037834	39,248,498	48,900	35,006	63.37	Cave rock wall	Hawaii, USA
<i>Desmonostoc geniculatum</i>	NMSU, JCU	HA4340-LM1		PRJNA621655	SRR11676647	3300035000	51,944,514	29,336	130,707	61.24	Cave rock wall	Hawaii, USA
<i>Desmonostoc vinosum</i>	NMSU, JCU	HA7617-LM4		PRJNA650882	SRR12951526	3300038554	36,690,552	6,487	166,368	60.96	Cave rock wall	Hawaii, USA
<i>Drouetiella hepatica</i>	NMSU, JCU	UHER 2000/2452		PRJNA617097	SRR12347860	3300036391	42,783,418	20,061	28,986	65.05	Rock surface	Kosice, Slovakia
<i>Gloeocapsa</i> sp.	NMSU, JCU	UFS-A4-WI-NPMV-4B04		PRJNA617098	SRR12347921	3300036443	29,499,512	17,169	32,766	60.53	Desert soil	Utah, USA
<i>Goleter apudmare</i>	NMSU, JCU, CCALA	HA4340-LM2	CCALA 1075	PRJNA621659	SRR11676930	3300035004	36,243,261	714	190,367	59.38	Cave rock wall	Hawaii, USA
<i>Hassallia</i> sp.	NMSU, JCU	WJT32-NPBG1		PRJNA617099	SRR12347922	3300036392	33,287,445	4,616	21,632	58.06	Desert soil	California, USA
<i>Hormoscilla</i> sp.	NMSU, JCU	CMT-3BRIN-NPC48		PRJNA650881	SRR12951525	3300038553	42,048,713	5,825	143,678	59.26	Desert soil	California, USA
<i>Iphinoe</i> sp.	NMSU, JCU	HA4291-MV1		PRJNA617100	SRR12349226	3300036393	31,352,639	32,008	107,621	62.85	Tropical soil	Hawaii, USA
<i>Kaiparowitsia implicata</i>	NMSU, JCU	G5E-PSE-MK54-09C		PRJNA650883	SRR13242888	3300038555	43,375,246	4,576	456,261	63.35	Desert wet wall	Utah, USA
<i>Kastovskya adunca</i>	NMSU, JCU, CCALA	ATA6-11-RM4	CCALA 1025	PRJNA621676	SRR11678224	3300034631	42,257,751	47,939	14,667	63.07	Desert soil	Atacama, Chile
<i>Komarekiella atlantica</i>	NMSU, JCU	HA4396-MV6		PRJNA650825	SRR12951531	3300038556	39,748,068	24,886	121,785	64.40	Tropical vernal pool	Hawaii, USA

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TABLE 1 (Continued)

Species	Available at culture collections	Strain ID	Alternative strain ID	BioProject no.	SRA run no.	JGI IMG ^b	No. of reads	No. of contigs	<i>N</i> ₅₀ (bp)	GC content (%)	Habitat	Location
<i>Lyngbya</i> sp.	NMSU, JCU	HA4199-MV5		PRJNA621654	SRR11676618	3300034630	37,379,750	5,629	214,210	61.23	Tropical soil	Hawaii, USA
<i>Microcoleus vaginatus</i>	NMSU, JCU	WJT46-NPBG5		PRJNA677056	SRR13207170	3300039305	40,531,812	28,602	165,791	60.22	Desert soil	California, USA
<i>Mojavia pulchra</i>	NMSU, JCU, CCALA	JT2-VF2	CCALA 691	PRJNA650826	SRR12951532	3300038557	54,924,618	10,021	208,763	62.75	Desert soil	California, USA
<i>Myxococcus californica</i>	NMSU, JCU, UTEX	WJT36-NPBG1	UTEX B 3157	PRJNA621674	SRR11678221	3300035019	32,998,795	9,403	81,746	64.17	Desert soil	California, USA
<i>Myxococcus chilensis</i>	NMSU, JCU, UTEX	ATA2-1-KO14	UTEX B 3158	PRJNA621667	SRR11678144	3300035012	46,028,284	19,551	69,559	60.50	Desert soil	Coquimbo, Chile
<i>Nodosilinea</i> sp.	NMSU, JCU	WJT8-NPBG4		PRJNA621656	SRR11676645	3300035001	36,650,447	2,944	173,054	65.12	Desert soil	California, USA
<i>Nostoc desertorum</i>	NMSU, JCU, CCALA	CM1-VF14	CCALA 693	PRJNA617101	SRR12349250	3300036444	55,466,988	7,058	211,685	62.42	Desert soil	California, USA
<i>Nostoc indistinguedum</i>	NMSU, JCU, CCALA	CM1-VF10	CCALA 692	PRJNA621666	SRR11678133	3300035011	34,734,084	13,226	98,409	54.74	Desert soil	California, USA
<i>Oscillatoria princeps</i>	NMSU	RMCB-10		PRJNA621670	SRR11678154	3300035015	35,756,581	29,084	28,454	61.51	Surface water	Lower Austria, Austria
<i>Oscillatoria tanganyikae</i>	NMSU, JCU	F16-MK23		PRJNA621665	SRR11678132	3300035010	41,220,548	7,027	306,031	59.66	Desert soil	California, USA
<i>Pegethrix bostrychoides</i>	NMSU, JCU	G5E-TBD4-15B		PRJNA617102	SRR12349533	3300036394	34,506,500	2,385	397,454	61.39%	Desert wet wall	Utah, USA
<i>Pelatocladus maniholoensis</i>	NMSU, JCU	HA4357-MV3		PRJNA653494	SRR12687981	3300037800	30,851,680	553	52,911	40.09%	Cave rock wall	Hawaii, USA
<i>Plectolyngbya</i> sp.	NMSU, JCU	WJT66-NPBG17		PRJNA617103	SRR12349534	3300036395	24,625,170	9,370	10,169	53.14	Desert soil	California, USA
<i>Pleurocapsa minor</i>	NMSU, JCU	G5E-CHR-MK-17-07R		PRJNA677054	SRR13207074	3300039304	44,764,195	25,094	40,205	64.01	Desert wet wall	Utah, USA
<i>Pleurocapsa minor</i>	NMSU, JCU	HA4230-MV1		PRJNA617104	SRR12349680	3300036396	59,843,005	708	316,355	63.57	Tropical rock wall	Hawaii, USA
<i>Scytolyngbya</i> sp.	NMSU, JCU	HA4215-MV1		PRJNA617105	SRR12349737	3300036104	33,289,893	8,107	243,918	60.55	Freshwater stream	Hawaii, USA
<i>Scytonema hyalinum</i>	NMSU, JCU	WJT4-NPBG1		PRJNA617106	SRR12349874	3300036520	40,826,558	35,954	57,285	64.29	Desert soil	California, USA
<i>Scytonematopsis contorta</i>	NMSU, JCU, UTEX	HA4267-MV1	UTEX 2964	PRJNA653495	SRR12688486	3300037859	41,139,075	2,560	124,805	50.66	Tropical vernal pool	Hawaii, USA
<i>Spirirestis rafaensis</i>	NMSU, JCU	WJT71-NPBG6		PRJNA683097	SRR13242887	3300042538	30,810,560	12,769	49,002	61.71	Desert soil	California, USA
<i>Stenomitos rutilans</i>	NMSU, JCU	HA7619-LM2		PRJNA617107	SRR12350322	3300036446	39,342,385	29,421	125,980	59.27	Cave rock wall	Hawaii, USA
<i>Symplocastrum torsivum</i>	NMSU, JCU, CCALA, UTEX	CPER-KK1	CCALA 1031 UTEX B 3163	PRJNA617108	SRR12350505	3300036521	45,589,224	7,498	188,807	61.87	Grassland soil	Colorado, USA
<i>Tildenella nuda</i>	NMSU, JCU	ZEHNDER 1965/U140		PRJNA621657	SRR11676687	3300035002	43,732,034	26,865	163,389	64.07	Temperate wet wall	Zurich, Switzerland

(Continued on next page)

TABLE 1 (Continued)

Species	Available at culture collections	Strain ID	Alternative strain ID	BioProject no.	SRA run no.	JGI IMG ^b	No. of reads	No. of contigs	<i>N</i> ₅₀ (bp)	GC content (%)	Habitat	Location
<i>Tildenella torsiva</i>	NMSU, JCU	UHER 1998/13D		PRJNA653496	SRR12689282	3300037860	51,170,286	22,112	16,291	50.54	Temperate wet wall	Kosice, Slovakia
<i>Timaviella obliquedivisa</i>	NMSU, JCU	G5E-PSE-MK23-08B		PRJNA621673	SRR11678220	3300035018	43,160,626	11,520	86,031	58.41	Desert wet wall	Utah, USA
<i>Tolypothrix brevis</i>	NMSU, JCU	G5E-NOS-MK-07-07A		PRJNA621662	SRR11678125	3300035007	36,087,578	9,013	171,611	59.37	Desert wet wall	Utah, USA
<i>Tolypothrix carrinoides</i>	NMSU, JCU	HA7290-LM1		PRJNA621663	SRR11678124	3300035008	36,281,317	43,124	40,599	59.34	Cave rock wall	Hawaii, USA
<i>Trichocoleus desertorum</i>	NMSU, JCU	ATA4-8-CV12		PRJNA621668	SRR11678143	3300035013	37,070,905	5,401	91,542	60.17	Desert soil	Atacama, Chile
" <i>Trichormus</i> " sp.	NMSU, JCU	ATA11-4-KO1		PRJNA621660	SRR11676933	3300035005	32,384,820	832	238,030	52.69	Desert soil	Tarapaca, Chile

^a Cyanobacterial cultures are available upon request from the research culture collections of Jeffrey R. Johansen (johansen@jcu.edu) at John Carroll University (JCU) and Nicole Pietrasiak (npietras@nmsu.edu) at New Mexico State University (NMSU). The selected strains are also available publicly at the University of Texas Culture Collection of Algae (UTEX) and the Culture Collection of Autotrophic Organisms in Tréboň, Czech Republic (CCALA). Accordingly, alternative UTEX and CCALA strain identifiers are given in the table.

^b JGI IMG/M, Joint Genome Institute Integrated Microbial Genomes and Microbiomes database.

default kmer options for paired 150-bp reads. Across all assemblies, 745,331 metagenomic contigs were retained after quality control using AAFTF vecscreen v0.2.3 (19) (range of assembly GC content, 40.09% to 66.57%; GC content mean \pm standard deviation, 59.94% \pm 4.96%; range of assembly N_{50} , 10,169 to 456,261 bp; N_{50} mean \pm standard deviation, 133,466 \pm 100,540 bp).

Data availability. All reads and assemblies were deposited under the NCBI accession numbers listed in Table 1. We also provided accession identifiers linking to JGI projects associated with each sample, including available IMG (20) data (Table 1).

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