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Metatranscriptomic Sequencing of a Cyanobacterial Soil-Surface Consortium with and without a Diverse Underlying Soil Microbiome

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ABSTRACT Soil surface consortia are easily observed and sampled, allowing examination of their interactions with soil microbiomes. Here, we present metatranscriptomic sequences from Dark Green 1 (DG1), a cyanobacterium-based soil surface consortium, in the presence and absence of an underlying soil microbiome and/or urea.

Microbial inoculants can establish unpredictably in soils, due to factors including competition with established microorganisms (1); however, inoculants that form visible surface films provide unique opportunities to track survival. In 2013, cyanobacterium-based soil surface consortia from Pennsylvania were enriched to develop surface film-forming inoculants (2). One consortium, Dark Green 1 (DG1), was enriched in culture over 2 years without added nitrogen or carbon, and abundant members include *Cylindrospermum* spp. and six nonphotosynthetic taxa (3).

We introduced DG1 to soils containing low- or high-diversity microbiomes, with or without urea added. Soil was collected from Penn State's Agronomy Research Farm (4), sieved to 2 mm, and twice autoclaved (45 min, 24-h interval). To one portion, nonautoclaved soil was reintroduced at 5% (vol/vol) to establish a high-diversity microbiome. Inoculated and uninoculated soil was dispensed into 12 petri dishes each (10 by 15 mm; 25 g dry soil/dish). An even fructose/maltose/glucose/galactose/ribose mixture was added to microcosms at 2 g carbon/kg dry soil. Six microcosms from each soil type received urea at 150 (start of incubation) and 50 mg nitrogen/kg dry soil (pre-DG1 addition), generating four treatments. The microcosms were dark incubated for 43 weeks at 21°C.

DG1 was grown in modified BG-11 medium under continuous fluorescent lighting (average 1,865 lux) and moderate agitation at 21°C (4). The cultures were pelleted at 5,500 rpm in 50-ml Falcon tubes, the medium was removed, and sterile deionized (DI) water was added (3:1 [vol/vol]) to resuspend the mixture. We pipetted 3-ml suspension across the surface of each soil sample and incubated the microcosms under constant fluorescent light for 5 weeks at 21°C.

RNA was extracted from the excised biofilms using the RNeasy PowerSoil total RNA kit (Qiagen), assessed on an Agilent BioAnalyzer at the Penn State Genomics Core (RNA integrity no. [RIN], >7), and shipped to the Joint Genome Institute (JGI). Metatranscriptome library preparation was performed on a Sciclone NGS robot (PerkinElmer) using Illumina's Ribo-Zero rRNA removal kits (equimolar bacteria/yeast/plant root) and the TruSeq stranded total RNA high-throughput (HT) kit, with 100 ng/sample RNA and 10 PCR cycles for library amplification. Libraries were quantified with KAPA library quan-

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TABLE 1 Summary of sample information and metatranscriptome annotation statistics

Microcosm condition	Replicate no.	Read count	Total bases (Mbp)	N_{50} (bp)	Contig count	Total gene count	GC content (%)	No. of CDS[a] genes	CDS genes (%)	Genes with predicted protein product (%)	Genes assigned to enzymes (%)	IMG taxon no.
High diversity plus urea	1	219,723,368	96.8	47,412	169,357	215,733	60.503	212,099	98.32	65.23	21.51	3300031481
	2	231,138,230	48.2	19,281	79,209	98,703	55.355	95,648	96.9	64.74	21.1	3300031495
	3	225,572,282	21.2	5,785	29,055	39,260	57.064	37,559	95.67	63.59	19.47	3300031499
	4	195,630,988	16.2	4,635	22,239	29,758	56.122	28,287	95.06	63.51	19.32	3300031502
	5	199,215,548	18.3	4,979	25,842	33,517	54.725	31,752	94.73	64.26	18.54	3300031503
	6	208,163,376	17.7	8,064	29,994	37,213	59.167	35,178	94.53	64.7	21.33	3300031504
High diversity	1	186,588,010	23.3	13,149	43,867	51,454	58.69	49,065	95.36	60.35	19.61	3300031484
	2	197,781,214	22.8	8,201	35,746	45,006	55.917	43,032	95.61	62.99	19.18	3300031487
	3	247,118,632	13.3	4,935	21,236	25,866	53.513	23,991	92.75	62.46	18.6	3300031491
	4	232,297,572	23.4	7,461	35,593	45,664	55.476	43,666	95.62	64.19	19.32	3300031490
	5	177,035,364	58.6	25,717	97,967	124,267	60.664	121,385	97.68	67.6	23.29	3300031493
	6	185,064,556	37.7	15,320	61,975	78,183	58.324	75,871	97.04	66.4	21.89	3300031476
Low diversity plus urea	1	290,517,454	16.5	3,481	19,787	29,350	53.737	28,862	98.34	70.16	24.1	3300031488
	2	187,827,806	5.2	1,425	6,656	9,257	48.703	9,083	98.12	67.91	24.25	3300031483
	3	227,368,660	5.7	1,368	6,887	9,570	49.214	9,399	98.21	70.24	23.12	3300031475
	4	207,822,514	10.9	1,766	10,912	17,267	51.587	17,007	98.49	70.42	24.02	3300031494
	5	225,974,356	25.2	3,578	24,957	41,645	58.976	41,127	98.76	70.57	25.81	3300031492
	6	199,479,246	7.8	1,184	7,120	11,581	48.025	11,370	98.18	68.79	22.29	3300031479
Low diversity	1	213,606,582	12.6	1,048	10,670	18,251	50.188	18,028	98.78	69.56	23.57	3300031498
	2	222,910,458	13.7	831	11,117	19,560	50.393	19,244	98.38	68.84	22.89	3300031489
	3	243,278,232	19.8	2,155	19,353	30,794	54.187	30,424	98.8	68.14	23.87	3300031477
	4	205,533,538	10	957	8,594	14,312	47.57	14,080	98.38	68.04	21.68	3300031482
	5	196,564,512	11.1	1,221	10,284	16,357	48.782	16,095	98.4	68.89	22.5	3300031497
	6	228,387,134	12.1	1,841	12,242	19,161	51.531	18,891	98.59	69.25	24.08	3300031480

[a] CDS, coding DNA sequence.

tification kits on a Roche LightCycler 480. Sequencing was performed on an Illumina NovaSeq using XP v1 reagent kits following a 2 × 150-nucleotide (nt) indexed run recipe.

Default parameters were used for all software unless otherwise noted. BBDUK (v38.26) removed (i) contaminants, (ii) adapter sequences and right read segments where quality was equal to 0, (iii) reads with *N* bases, a mean quality score of <10, or minimum length of ≤51 bp or 33% of full length, and (iv) rRNA (5). The filtered reads were assembled using MEGAHIT v1.1.2 (–k list, 23, 43, 63, 83, 103, 123) (6). The filtered reads were mapped to contigs using BBMap (v38.25, ambiguous=random) to estimate coverage (5). Genes were identified and annotated in IMG/M v4 (7, 8). Taxonomic assignments for transcripts were determined by selecting the options "compare genomes" and "phylogenetic distribution" at a percent identity of ⩾60% and normalized by estimated gene copies. Table 1 presents the annotation statistics for the metatranscriptomes.

Initial analysis suggests fewer cyanobacterium transcripts when high-diversity microbiomes are present, particularly with urea. Of interest will be the frequency of transcripts indicating interspecific interactions.

Data availability. Metatranscriptome sequences are available through the JGI Genomes OnLine Database (GOLD) under project identifier Gs0132857.

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