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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-
TABLE 1 Summary of sample information and metatranscriptome annotation statistics

| Microcosm condition | Replicate no. | Read count | Total bases (Mbp) | N50 (bp) | Contig count | Total gene count | GC content (%) | No. of CDS 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 | 3300031502 |
| 4 | 195,630,988 | 16.2 | 4,635 | 22,239 | 29,758 | 56.122 | 28,287 | 95.06 | 63.51 | 19.32 | 3300031503 |
| 5 | 199,215,548 | 18.3 | 4,979 | 25,842 | 33,517 | 54.725 | 31,752 | 94.73 | 64.26 | 18.54 | 3300031504 |
| 6 | 208,163,376 | 17.7 | 8,064 | 29,944 | 37,213 | 59.167 | 35,178 | 94.53 | 64.7 | 21.33 | 3300031507 |
| 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 | 97.84 | 66.7 | 23.29 | 3300031493 |
| 6 | 185,064,556 | 37.7 | 15,320 | 61,975 | 78,324 | 53.737 | 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 | 24.25 | 3300031483 |
| 2 | 187,827,806 | 5.2 | 1,425 | 6,656 | 9,257 | 48.703 | 9,083 | 98.12 | 67.91 | 24.25 | 3300031476 |
| 3 | 227,366,660 | 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 | 28.1 | 3300031492 |
| 6 | 199,479,246 | 7.8 | 1,184 | 7,120 | 11,581 | 48.025 | 11,370 | 98.18 | 68.79 | 22.29 | 3300031497 |
| 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 | 98.84 | 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 |

*CDS, coding DNA sequence.*

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

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