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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-
tification kits on a Roche LightCycler 480. Sequencing was performed on an Illumina NovaSeq using XP v1 reagent kits following a 2/5 H11003 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 H11021 10, or minimum length of H11349 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 H32897 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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REFERENCES


