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Supplementary information

Peat-based gnotobiotic plant growth systems for Arabidopsis microbiome research

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Supplementary Methods

DNA extraction

Three rosettes from individual FlowPots were aseptically harvested, pooled in a collection tube containing three 3 mm zirconium beads, frozen in liquid nitrogen, and stored at -80°C until further processing. Immediately prior to DNA extraction, frozen plant tissues were homogenized using a Tissuelyser (Qiagen, 30 Hz for 2 x 1 min). Samples were then taken up into the lysis solution of a PowerSoil kit (MoBio) collection tube and heated to 70°C for 5 min. Remaining DNA extraction steps were performed according to the manufacturer's instructions. DNA was quantified and checked for quality using a ND-1000 UV Nanodrop spectrophotometer (Thermo Scientific).

Amplification of 16S rRNA gene fragments

16S rRNA gene fragments were amplified from total DNA using primers 799F and 1192R modified with 5' Fluidigm CS-adapter sequences: CS1-799F [5'-ACACTGACGACATGGTTCTACA-AACMGGATTAGATACCCKG-3'] and CS2-1192R [5'-TACGGTAGCAGAGACTTGGTCT-ACGTCATCCCCACCTTCC-3']. Each sample was amplified in triplicate in a 25 ul reaction volume containing AccuPrime HiFi Polymerase (0.15 µl, Thermo Fisher), DMSO (1.00 µl), forward and reverse primers (200 nM each), 10X AccuPrime Buffer II (2.50 µl, Thermo Fisher), template DNA (~20 ng), and DNA-free water. PCR was performed using the following parameters: hot start 94°C (1 min); 30 cycles of [94°C (20 s), 53°C (30 s), 68°C (45 s)], and a final extension at 68°C (2 min). Amplicon DNA was gel purified, pooled by sample, then checked for quality using a spectrophotometer. Illumina adapters and barcodes were added during a second PCR according to the Access Array workflow (Fluidigm). CS-16S fusion primers were used during secondary PCR to increase fidelity and improve library quality. The concentration of barcoded amplicon products was normalized using SequalPrep DNA Normalization Plates (Invitrogen) and subsequently pooled. DNA concentration was determined by Qubit dsDNA and Kapa qPCR after pooling and paired-end Illumina

sequencing was performed at the Michigan State University Research Technology Support Facility.

Bioinformatic analysis of 16S rRNA gene fragment amplicons

The output files from the Illumina sequencing software were quality-checked using FastQC, and further processed using the preprocessing tools from the Ribosomal Database Project pipeline[1]. Briefly, sequences were demultiplexed, trimmed to a consistent length with adapters removed, and filtered to remove all sequences with a q PHRED quality score below. Singleton reads were discarded and all paired-end reads from were assembled using the RDP-modified version of PANDAseq[2]. Arabidopsis reads were identified and removed using BBDuk (http://jgi.doe.gov/data-and-tools/bb-tools/). Sequences were then rarified using USEARCH, and clustered at a 97% identity cutoff using UPARSE, with singletons removed[3]. Chimeric sequences were identified and removed using UCHIME [4] and counts of each OTU were determined by mapping reads back to '.uc' files. Representative OTU sequences were extracted and aligned with the RDP Infernal 1.1 alignment model and taxonomy was assigned using the RDP classifier classification from the RDP database[1].

References

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