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Bacterial communities associated with a polydomous arboreal ant: Inter-nest variation and interaction with the phyllosphere of a tropical tree

Appendix S1 - Library Preparation and DNA Sequencing

The preparation of libraries for bacterial amplicon sequencing was carried out using the specific oligonucleotides 341F and 806R targeting the V3/V4 region of the 16S rRNA gene in a two-step PCR protocol (WANG & QIAN 2009, CAPORASO & al. 2012). The primers used in the first PCR, in addition to containing a specific target region for V3/V4, also encompass a region corresponding to a partial Illumina adapter based on the TruSeq structure (Illumina, USA). The presence of this adapter allows for a second PCR that adds indexing sequences following established procedures (CAPORASO & al. 2011). Indexing is performed with unique dual indices for each sample in the second PCR. Two microliters of extracted DNA from each sample were used as a template in the first PCR reaction. PCR reactions were carried out using Platinum Taq (Invitrogen, USA) under the following conditions: 95 °C for 5 min, 25 cycles of 95 °C for 45s, 55 °C for 30s, and 72°C for 45s, with a final extension of 72 °C for 2 min for PCR 1. For PCR 2, the conditions were 95 °C for 5 min, 10 cycles of 95 °C for 45s, 66 °C for 30s, and 72 °C for 45s, with a final extension of 72 °C for 2 min. All PCR reactions were performed in triplicate. The final PCR products were purified using Neobeads® (Sera-Mag™ magnetic beads), and an equivalent volume of each sample was added to the sequencing pool. In each round of PCR, a Negative Reaction Control (NRC) was included. For each Receiving Order (RO), a Negative Extraction Control (NEC) was also included. The final DNA concentration of the library pool was estimated using Picogreen dsDNA (Invitrogen, USA), and then diluted for quantification by qPCR using the Collibri™ Library Quantification Kit (Invitrogen, USA), which had been optimized for Illumina libraries. The sequencing pool was adjusted to a final concentration of 11 pM (for V2 kits) or 17.5 pM (for V3 kits) and sequenced on the MiSeq system (Illumina, USA), using the Illumina sequencing primers provided with the manufacturer's kit. The paired-end 500-cycle runs were performed using V2x500 or V3x600 sequencing kits (Illumina, USA) with >100,000 reads coverage per sample.

Although negative controls were not sequenced, all samples were processed in a facility with clean room using sterile techniques and reagents. The lack of contamination in previous projects conducted in the same facility provides confidence in the results. For future studies, it is strongly recommended that negative controls be sequenced to provide an additional layer of confidence in the results.

References

WANG, Y. & QIAN, P.Y. 2009: Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. – PLoS ONE 4.

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CAPORASO, J.G., LAUBER, C.L., WALTERS, W.A., & al. 2011: Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. – Proceedings of the National Academy of Sciences 108:4516–4522.