

SUPPLEMENTAL METHODS

DNA Isolation

Each sample collection swab was placed in 300 μ l of Yeast Cell Lysis Solution (Epicentre MasterPure Yeast DNA Purification kit) and 0.5 μ l of ReadyLyse Lysozyme solution (Epicentre) was added before incubation for 1 hour at 37°C with shaking. Samples were then processed with bead beating for 10 minutes at maximum speed on a vortex mixer with 0.5 mm glass beads (MoBio), followed by a 30 minute incubation at 65°C with shaking. Subsequent steps were performed as previously described¹.

16S rRNA Library Preparation

Amplification of 16S rRNA genes followed previously published protocols².

16S rRNA Sequence Processing and Analyses

FASTQ files were generated from raw BCL files using ‘configureBclToFastq.pl’ (Illumina Inc.) and paired-ends were assembled using the PANDAseq Assembler³. QIIME 1.6.0⁴ was used for the initial stages of sequence analysis: potential sequencing artifacts outside of the 248-255 base pair length window were removed, sequences were clustered into OTUs (operational taxonomic units, a proxy for ‘species’) using the UCLUST method⁵ at 97% sequence similarity, sequences were taxonomically classified using the RDP classifier⁶ at a confidence threshold of 0.8, unclassified sequences and sequences derived from plastids were removed, samples with less than 2500 sequences were removed from the data set, each 16S amplicon pool was subsampled at an even depth of 2500 sequences for downstream processing, alpha diversity metrics (Faith’s Phylogenetic Diversity index⁷ and observed species-level OTUs) and a beta diversity metric (Bray-Curtis index) were calculated for each sample.

References

1. Gardner SE, Hillis SL, Heilmann K, et al. 2013. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. *Diabetes* 62:923-930.
2. Caporaso JG, Lauber CL, Walters WA, et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 6:1621-1624.
3. Masella AP, Bartram AK, Truszkowski JM, et al. 2012. PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics* 13:31.
4. Caporaso JG, Kuczynski J, Stombaugh J, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7:335-336.
5. Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460-2461.
6. Wang Q, Garrity GM, Tiedje JM, et al. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261-5267.
7. Faith DP. 1992. Conservation Evaluation and Phylogenetic Diversity. *Biol Conserv* 61:1-10.