https://doi.org/10.5217/ir.2021.00124 • Intest Res 2022;20(2):260-268

See "One-year clinical efficacy and safety of indigo naturalis for active ulcerative colitis: a real-world prospective study" on pages 260-268.

Supplementary Material

DNA extraction

Mucosal bacterial DNA was extracted from the mucosal samples using NucleoSpin® Tissue XS (Macherey-Nagel, Düren, Germany) and 0.1-mm Zirconia/Silica beads in a TissueLyser (Qiagen Inc., Chatsworth, CA, USA) vibrating at 25 times per second for 1 minute.

16S ribosomal RNA gene amplification and sequencing

16S rRNA gene sequencing was conducted as described previously with minor modifications.[1,2] Briefly, the extracted bacterial DNA was used as the template to amplify the V4 region of each 16S rRNA gene using the primer pair 515F/806R, which included the Illumina flowcell adapter sequences. The reverse primer also contained a 12-base barcode sequence. Paired-end sequencing of the polymerase chain reaction amplicons was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) using custom primers.

Processing of sequence reads and statistical analysis

Raw Illumina FASTQ files were demultiplexed, quality filtered, and analyzed using QIIME v1.9.1 software.[3] The open-reference strategy was used to pick operational taxonomic units (OTUs), using UCLUST (Haas et al.[4]) at a minimum sequence identity of 97% against the Greengenes 13_8 reference (DeSantis et al.[5]) to cluster the preprocessed sequences into OTUs, which are defined by the intrinsic phenotypic similarities that constitute candidate taxa. Reads without hits in the reference database were randomly subsampled and clustered *de novo*. Chimeric sequences were removed using ChimeraSlayer (Segata et al.[6]). Alpha diversity, which described the microbial diversity within samples, was measured by Observed Species, Phylogenetic Diversity Whole Tree, and Chao 1. The measured alpha diversities were compared between before and after indigo naturalis treatment using a non-parametric 2 samples *t*-test and the default number of Monte Carlo permutations (999). Beta diversity, which described the diversity in a microbial community between different samples, was evaluated using Principal Coordinate Analysis that was based on the weighted UniFrac distances. The significance of the distance between 2 groups was calculated using permutational multivariate analysis of variance. To determine potential biomarker OTUs, which differ in the abundance and occurrence between sample groups, we performed linear discriminant analysis (LDA) effect size (LEfSe) (Afgan et al.[7]) via the Galaxy framework (http://huttenhower.sph.harvard.edu/galaxy) (Giardine et al.[8]). We considered differences in abundance as statistically significant when the logarithmic LDA score was >2.0.

REFERENCES

1. Hirano A, Umeno J, Okamoto Y, et al. Comparison of the microbial community structure between inflamed and non-inflamed sites in patients with ulcerative colitis. J Gastroenterol Hepatol 2018;33:1590-1597.
2. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621-1624.
3. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335-336.
4. Haas BJ, Gevers D, Earl AM, et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 2011;21:494-504.

5. DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimerachecked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 2006;72:5069-5072.
6. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.
7. Afgan E, Baker D, van den Beek M, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res 2016;44:W3-W10.
8. Giardine B, Riemer C, Hardison RC, et al. Galaxy: a platform for interactive large-scale genome analysis. Genome Res 2005;15:1451-1455.