**Analyzing fungi in the stool of non-Western humans**

To determine whether fungi were present at different levels in non-Western societies, we analyzed ribosomal small subunit amplicon sequencing data generated by Yatsunenko et al. (1) from 474 total subjects within Malawi, Venezuela, and the United States. PCR primers were non-specific and sequencing was deep enough to detect non-bacterial 18S rRNA genes, which we analyzed. Fungal reads were detected at very low levels in all countries and age groups (0.00001-0.00018%; Tables S6 and S7), and the fraction of reads mapping to fungi was similar between children and adults. Reads matching *Aspergillus* and *Penicillium* were the most abundant overall. The percent of fungal reads was lower in the United States (0.000013%) than in Malawi (0.00016%; p < 0.0001 by Tukey's multiple comparison test following ANOVA) or Venezuela (0.000059%; p = 0.17), and despite slightly higher sequencing depth for United States samples, the percent of US subjects containing any fungal reads (7.3%) was also lower than in Malawi (39.8%; p < 0.0001 by binomial logistic regression) or Venezuela (22.7%; p < 0.0001). The higher incidence of fungi in Malawi and Venezuela in this large amplicon dataset is consistent with the shotgun sequencing findings originally reported by the authors (Yatsunenko et al. Fig S7), and suggests that fungi may be more abundant in the GI tracts of non-Western populations. However, due to the overall low abundance of fungi, deeper sequencing is needed to confirm this finding.

**Table S6. Comparison of the number of fungi detected in Yatsunenko et al. ribosomal SSU amplicon data from stool collected in different countries.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Malawi** | | **United States** | | **Venezuela** | |
| **Adults**a | **Children** | **Adults** | **Children** | **Adults** | **Children** |
| Total Reads | 6.3 x 107 | 1.0 x 108 | 2.8 x 108 | 3.7 x 108 | 5.9 x 107 | 1.3 x 108 |
| Total Subjects | 31 | 62 | 136 | 180 | 34 | 63 |
| Fungal Reads | 0.00014% | 0.00018% | 0.00002% | 0.00001% | 0.00002% | 0.00008% |
| Subjects with Fungi | 45% | 37% | 10% | 5% | 21% | 24% |

aAdults ≥ 18, children < 18 years old.

**Methods**

The only known large dataset of fecal microbiomes from non-Western cultures was that of Yatsunenko et al (1). The ribosomal small subunit V4 amplicon sequences (NCBI ERX115521), containing 1,010,810,512 total Illumina HiSeq reads from 506 subjects' stool samples (474 with metadata) were analyzed for fungi. The primers used in this study were likely 515F and 806R: as examined using PrimerProspector (2) and the Silva database (3), 515F perfectly matches most fungal sequences, but 806R is biased against fungi, particularly the Ascomycota.

Using Bowtie2 (4), reads were mapped against all fungal genomes in NCBI (downloaded July 19, 2016) that was cleaned of bacterial contamination as described previously (5). Positive fungal hits were refined and assigned a taxonomic name by using BLASTN (6) to run reads against a custom non-redundant GenBank and NCBI nr database. Statistical calculations were performed in GraphPad Prism (ANOVA and Tukey's multiple comparison test) or R using the lattice package (binomial logistic regression).

**References**

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