**Supplementary materials**

1. **Supplementary Figure Captions**

**Supplementary Figure S1**. Bacterial sequence curation and analysis. Sequenced 16S rRNA gene V4 amplicons generated from DNA samples on a MiSeq. MiSeq-generated Fastq files were quality-filtered and clustered into 97% similarity operational taxonomic units (OTUs) using the mothur software package http://www.mothur.org]. The resulting dataset had 26292 OTUs. An average of 19887 quality-filtered reads were generated per sample. Sequencing quality was determined using FastQC 0.11.5, and visualized.

**Supplementary Figure S2**. Fungal sequence curation and analysis. Sequenced ITS2 amplicons generated from DNA samples on a MiSeq. MiSeq-generated Fastq files were quality-filtered and clustered into 97% similarity operational taxonomic units (OTUs) using the mothur software package http://www.mothur.org]. The resulting dataset had 4158 OTUs. An average of 10331 quality-filtered reads were generated per sample. Sequencing quality was determined using FastQC 0.11.5, and visualized.

**Supplementary Figure S3**. Plots of the Constrained Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance matrix between young, middle and older age groups for bacteria (A) and fungi (B). CAP1 and CAP2 represent the canonical principal coordinates axes 1 and 2.

**Supplementary Figure S4**. Taxonomic composition of gut microbiota. (A) Bacterial composition of gut microbiota represented by the abundance (percentage) of genera per each age group per each sample in each comparison group. (B) Fungal composition of gut microbiota represented by the abundance (percentage) of genera per each age group per each sample in each comparison group.

**Supplementary Figure S5.** Stacked bar plot of the 15 most abundant species among human samples. Sorted according to the species with the highest mean abundances among samples (*i.e.,* *Faecalibacterium prausnitzii*) in decreasing order.

1. **Supplementary Tables**

**Supplementary Table S1**. Demographic data of the study population including age, ethnicity, gender, and diet type.

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics | Young (20-29 yrs.)  subjects (n=18) | Middle age (30-49 yrs.)  subjects (n=13) | Older subjects (≥50 yrs.)  (n=20) |
| Age, years Mean( SD, range) | 23.17 (3.40, 9) | 40.54 (7.07, 19) | 66.30 (6.90, 24) |
| Ethnicity Emirati (%) | 100% | 100% | 100% |
| Gender (M %, F %) | (11.11, 88.89) | (38.46, 61.54) | (25%, 75%) |
| Diet (High Fiber Content %) | 55.56% | 69.23% | 60% |

**Supplementary Table S2.** Pairwise PERMANOVA comparisons for age-BMI, age-diet, age-probiotics and age-gender combinations.

|  |  |  |  |
| --- | --- | --- | --- |
| **Factor** | **Pairwise comparison** | **PREMANOVA p-value for Bacteria\*** | **PREMANOVA p-value for Fungi\*** |
| **BMI** | Young.Normal vs Older.Normal | 0.057 | 0.012 |
| Young.Overweight vs Older.Overweight | 0.005 | 0.008 |
| Young.Normal vs Young.Overweight | 0.572 | 0.916 |
| Older.Normal vs Older.Overweight | 0.628 | 0.077 |
| **Fiber Diet** | Young.Low vs Older.Low | 0.004 | 0.001 |
| Young.High vs Older.High | 0.037 | 0.012 |
| Young.Low vs Young.High | 0.076 | 0.158 |
| Older.Low vs Older.High | 0.361 | 0.807 |
| **Probiotics** | Young.Yes vs Older.Yes | 0.001 | 0.008 |
| Young.No vs Older.No | 0.009 | 0.001 |
| Young.Yes vs Young.No | 0.917 | 0.030 |
| Older.Yes vs Older.No | 0.166 | 0.462 |
| **Gender** | Young.Female vs Older.Female | 0.001 | 0.001 |
| Young.Male vs Older.Male | 1.000 | 0.178 |
| Young.Female vs Young.Male | 0.900 | 0.284 |
| Older.Male vs Older.Female | 0.983 | 0.160 |

Table S4. Gene abundance of UniRef mapped genes from shotgun metagenomics sequencing data analysis across 10 fecal microbiomes of young and older human subjects.

Enclosed as a separate file.

**Supplementary Table S4**. The list of statistically significant genes and reactions that show differential abundance between the young and older individuals.

|  |  |  |
| --- | --- | --- |
| Gene\_Family | p-value | p-adj\* |
| 6.3.2.8 | 1.66E-09 | 7.93E-07 |
| 6.3.2.9 | 4.19E-08 | 1.00E-05 |
| 5.1.1.3 | 9.00E-08 | 1.43E-05 |
| 6.3.2.10 | 1.98E-07 | 2.37E-05 |
| 6.3.2.9|unclassified | 9.93E-05 | 0.0095 |
| 6.3.2.8|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | 0.0003 | 0.0205 |
| 5.1.1.3|unclassified | 0.0005 | 0.0272 |
| 1.3.1.98|unclassified | 0.0005 | 0.0272 |
| 6.3.2.10|unclassified | 0.0004 | 0.0272 |
| 6.3.2.4|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | 0.0007 | 0.0333 |
| 6.3.2.8|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | 0.0009 | 0.0386 |
| 2.5.1.7|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | 0.0011 | 0.0395 |
| 6.3.2.8|unclassified | 0.001 | 0.0395 |
| 6.3.2.8|g\_\_Blautia.s\_\_Ruminococcus\_obeum | 0.0012 | 0.0403 |
| 6.3.2.10|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | 0.0013 | 0.0403 |
| 2.5.1.7|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | 0.0017 | 0.0456 |
| 6.3.2.8|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | 0.0017 | 0.0456 |
| 6.3.2.9|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | 0.0015 | 0.0456 |
| 6.3.2.4|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | 0.0019 | 0.0487 |
| 5.1.1.3|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | 0.0025 | 0.0593 |
| 6.3.2.10|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | 0.0029 | 0.0666 |
| 6.3.2.4|unclassified | 0.004 | 0.087 |
| 6.3.2.9|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | 0.0044 | 0.0923 |
| 2.5.1.7|unclassified | 0.0049 | 0.0934 |
| 6.3.2.8|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_longum | 0.0047 | 0.0934 |
| 6.3.2.4|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_longum | 0.0056 | 0.1021 |
| 6.3.2.4|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | 0.0062 | 0.109 |
| 6.3.2.10|g\_\_Dorea.s\_\_Dorea\_longicatena | 0.0066 | 0.1123 |
| 2.5.1.7|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | 0.0078 | 0.1285 |
| 6.3.2.10|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_longum | 0.0082 | 0.1311 |
| 6.3.2.10|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | 0.009 | 0.1395 |
| 6.3.2.4|g\_\_Blautia.s\_\_Ruminococcus\_obeum | 0.0097 | 0.1433 |
| 6.3.2.10|g\_\_Bacteroides.s\_\_Bacteroides\_vulgatus | 0.0099 | 0.1433 |
| 2.5.1.7|g\_\_Blautia.s\_\_Ruminococcus\_obeum | 0.0108 | 0.1512 |
| 2.5.1.7|g\_\_Dorea.s\_\_Dorea\_longicatena | 0.0117 | 0.1578 |
| 2.5.1.7|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_longum | 0.0122 | 0.1578 |
| 6.3.2.9|g\_\_Bacteroides.s\_\_Bacteroides\_uniformis | 0.012 | 0.1578 |
| 6.3.2.8|g\_\_Parabacteroides.s\_\_Parabacteroides\_merdae | 0.0129 | 0.1621 |
| 6.3.2.9|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_longum | 0.0133 | 0.1628 |
| 5.1.1.3|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | 0.0142 | 0.1693 |
| 5.1.1.3|g\_\_Bacteroides.s\_\_Bacteroides\_uniformis | 0.0148 | 0.1726 |
| 6.3.2.8|g\_\_Bacteroides.s\_\_Bacteroides\_vulgatus | 0.0154 | 0.1755 |
| 6.3.2.9|g\_\_Parabacteroides.s\_\_Parabacteroides\_merdae | 0.016 | 0.1783 |
| 6.3.2.4|g\_\_Dorea.s\_\_Dorea\_longicatena | 0.0178 | 0.1936 |
| 5.1.1.3|g\_\_Blautia.s\_\_Ruminococcus\_obeum | 0.0199 | 0.1937 |
| 5.1.1.3|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_adolescentis | 0.0207 | 0.1937 |
| 5.1.1.3|g\_\_Alistipes.s\_\_Alistipes\_indistinctus | 0.0195 | 0.1937 |
| 6.3.2.4|g\_\_Parabacteroides.s\_\_Parabacteroides\_merdae | 0.0227 | 0.1937 |
| 6.3.2.4|g\_\_Coprococcus.s\_\_Coprococcus\_comes | 0.0215 | 0.1937 |
| 6.3.2.4|g\_\_Bacteroides.s\_\_Bacteroides\_caccae | 0.0209 | 0.1937 |
| 6.3.2.8|g\_\_Eubacterium.s\_\_Eubacterium\_siraeum | 0.0219 | 0.1937 |
| 6.3.2.8|g\_\_Dorea.s\_\_Dorea\_longicatena | 0.0225 | 0.1937 |
| 6.3.2.8|g\_\_Bacteroides.s\_\_Bacteroides\_uniformis | 0.022 | 0.1937 |
| 6.3.2.8|g\_\_Bacteroides.s\_\_Bacteroides\_ovatus | 0.0216 | 0.1937 |
| 6.3.2.9|g\_\_Eubacterium.s\_\_Eubacterium\_rectale | 0.0224 | 0.1937 |
| 6.3.2.9|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_adolescentis | 0.0184 | 0.1937 |
| 2.5.1.7|g\_\_Bacteroides.s\_\_Bacteroides\_vulgatus | 0.0234 | 0.196 |
| 5.1.1.3|g\_\_Escherichia.s\_\_Escherichia\_coli | 0.0247 | 0.1963 |
| 6.3.2.4|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_adolescentis | 0.0245 | 0.1963 |
| 6.3.2.4|g\_\_Bacteroides.s\_\_Bacteroides\_vulgatus | 0.026 | 0.1963 |
| 6.3.2.4|g\_\_Bacteroides.s\_\_Bacteroides\_dorei | 0.0262 | 0.1963 |
| 6.3.2.8|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_adolescentis | 0.0267 | 0.1963 |
| 6.3.2.9|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | 0.0266 | 0.1963 |
| 6.3.2.10|g\_\_Dorea.s\_\_Dorea\_formicigenerans | 0.026 | 0.1963 |
| 6.3.2.10|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_adolescentis | 0.0257 | 0.1963 |
| 5.1.1.3|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_longum | 0.0271 | 0.1965 |
| 2.5.1.7|g\_\_Bacteroides.s\_\_Bacteroides\_uniformis | 0.0287 | 0.2044 |
| 6.3.2.10|g\_\_Blautia.s\_\_Ruminococcus\_obeum | 0.0306 | 0.2151 |
| 2.5.1.7|g\_\_Alistipes.s\_\_Alistipes\_shahii | 0.0318 | 0.2158 |
| 6.3.2.9|g\_\_Bacteroides.s\_\_Bacteroides\_ovatus | 0.0315 | 0.2158 |
| 6.3.2.10|g\_\_Bacteroides.s\_\_Bacteroides\_uniformis | 0.032 | 0.2158 |
| 2.5.1.7|g\_\_Bifidobacterium.s\_\_Bifidobacterium\_adolescentis | 0.0334 | 0.2219 |
| 2.5.1.7|g\_\_Eubacterium.s\_\_Eubacterium\_siraeum | 0.0356 | 0.2289 |
| 2.5.1.7|g\_\_Akkermansia.s\_\_Akkermansia\_muciniphila | 0.0356 | 0.2289 |
| 5.1.1.3|g\_\_Eubacterium.s\_\_Eubacterium\_rectale | 0.0366 | 0.2289 |
| 6.3.2.8|g\_\_Bacteroides.s\_\_Bacteroides\_dorei | 0.036 | 0.2289 |
| 6.3.2.9|g\_\_Alistipes.s\_\_Alistipes\_shahii | 0.0369 | 0.2289 |
| 2.5.1.7|g\_\_Eubacterium.s\_\_Eubacterium\_rectale | 0.0392 | 0.2345 |
| 6.3.2.10|g\_\_Alistipes.s\_\_Alistipes\_shahii | 0.0386 | 0.2345 |
| 6.3.2.10|g\_\_Akkermansia.s\_\_Akkermansia\_muciniphila | 0.0392 | 0.2345 |
| 6.3.2.4|g\_\_Alistipes.s\_\_Alistipes\_indistinctus | 0.0404 | 0.2356 |
| 6.3.2.10|g\_\_Coprococcus.s\_\_Coprococcus\_comes | 0.0401 | 0.2356 |
| 6.3.2.8|g\_\_Coprococcus.s\_\_Coprococcus\_comes | 0.0432 | 0.2488 |
| 6.3.2.4|g\_\_Escherichia.s\_\_Escherichia\_coli | 0.0444 | 0.2525 |
| 6.3.2.8|g\_\_Akkermansia.s\_\_Akkermansia\_muciniphila | 0.0456 | 0.2566 |
| 6.3.2.9|g\_\_Akkermansia.s\_\_Akkermansia\_muciniphila | 0.0466 | 0.2588 |
| 6.3.2.9|g\_\_Dorea.s\_\_Dorea\_formicigenerans | 0.0473 | 0.26 |
| 6.3.2.9|g\_\_Coprococcus.s\_\_Coprococcus\_comes | 0.0482 | 0.2619 |
| 5.1.1.3|g\_\_Bacteroides.s\_\_Bacteroides\_vulgatus | 0.0496 | 0.2662 |

**Supplementary Table S5.** List of genes enriching pathway PWY-6386 (UDP-N-acetylmuramoyl-pentapeptide biosynthesis II).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene ID | Gene name | Reaction id | Reaction EC | Enzymatic activity | p-adj\* | Species |  |  |  |  |  |  |
| G-11845 | murZ | UDPNACETYLGLUCOSAMENOLPYRTRANS-RXN | 2.5.1.7 | UDP-N-acetylglucosamine 1-carboxyvinyltransferase II | 0.0395431 | 2.5.1.7|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | | | | |  |  |
|  |  |  |  |  | 0.0403042 | 2.5.1.7|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | | | |  |  |  |
| G-11846 | murI | GLUTRACE-RXN | 5.1.1.3 | glutamate racemase | 0.0000143 | 5.1.1.3 |  |  |  |  |  |  |
|  |  |  |  |  | 0.0272091 | 5.1.1.3|unclassified |  |  |  |  |  |  |
| G-11847 | ddl | DALADALALIG-RXN | 6.3.2.4 | D-alanine:D-alanine ligase | 0.0487391 | 6.3.2.4|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | | | |  |  |  |
|  |  |  |  |  | 0.0333478 | 6.3.2.4|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | | | | |  |  |
| G-9474 | murB | UDPNACETYLMURAMATEDEHYDROG-RXN | 1.3.1.98 | UDP-N-acetylenolpyruvoylglucosamine reductase | 0.02721 | 1.3.1.98|unclassified |  |  |  |  |  |  |
| G-11843 | murC | UDP-NACMUR-ALA-LIG-RXN | 6.3.2.8 | UDP-N-acetylmuramoyl--L-alanine ligase | 0.0000008 | 6.3.2.8 |  |  |  |  |  |  |
|  |  |  |  |  | 0.0403042 | 6.3.2.8|g\_\_Blautia.s\_\_Ruminococcus\_obeum | | | |  |  |  |
|  |  |  |  |  | 0.0456316 | 6.3.2.8|g\_\_Collinsella.s\_\_Collinsella\_aerofaciens | | | |  |  |  |
|  |  |  |  |  | 0.0205323 | 6.3.2.8|g\_\_Eubacterium.s\_\_Eubacterium\_hallii | | | |  |  |  |
|  |  |  |  |  | 0.0385965 | 6.3.2.8|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | | | | |  |  |
|  |  |  |  |  | 0.0395431 | 6.3.2.8|unclassified |  |  |  |  |  |  |
| G-9476 | murD | UDP-NACMURALA-GLU-LIG-RXN | 6.3.2.9 | UDP-N-acetylmuramoylalanine--D-glutamate ligase | 0.00001 | 6.3.2.9 |  |  |  |  |  |  |
|  |  |  |  |  | 0.0456316 | 6.3.2.9|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | | | | |  |  |
|  |  |  |  |  | 0.0094958 | 6.3.2.9|unclassified |  |  |  |  |  |  |
| G-9475 | murF | 6.3.2.10-RXN | 6.3.2.10 | UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase II | 0.0000237 | 6.3.2.10 |  |  |  |  |  |  |
|  |  |  |  |  | 0.0403042 | 6.3.2.10|g\_\_Faecalibacterium.s\_\_Faecalibacterium\_prausnitzii | | | | |  |  |
|  |  |  |  |  | 0.0272091 | 6.3.2.10|unclassified |  |  |  |  |  |  |
| G-11844\* | murA | UDPNACETYLGLUCOSAMENOLPYRTRANS-RXN | 2.5.1.7 | UDP-N-acetylglucosamine 1-carboxyvinyltransferase I | Did not pass filter |  |  |  |  |  |  |  |
| G-9473\* | murE | 6.3.2.10-RXN | 6.3.2.10 | UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase I | Did not pass filter |  |  |  |  |  |  |  |
| \*p-adj: adjusted p-value from the Wilcoxon's signed rank test for assessing the statistical significance of differentially abunadant genes between young and older age groups. | | | | | | | | | | | | |