ZYMO RESEARCH

Microbiome Sequencing Service Report:
Shotgun Metagenomic Sequencing

Table of Contents

1. Workflow Checklist

Sample Received	✓
Sample Quality Evaluated	✓
Sample Prepared for Sequencing	✓
Next-Gen Sequencing	✓
Sequence Quality Check	✓
Bioinformatics Processing	✓
Data/Results	✓

Ver 8.3

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2. Methods

The samples were processed and analyzed with the ZymoBIOMICS® Shotgun Metagenomic Sequencing Service (Zymo Research, Irvine, CA).

DNA Extraction: If DNA extraction was performed, one of three different DNA extraction kits was used depending on the sample type and sample volume and were used according to the manufacturer's instructions, unless otherwise stated. The kit used in this project is marked below.

☐ ZymoBIOMICS® DNA Miniprep Kit (Zymo Research, Irvine, CA)

☐ ZymoBIOMICS® DNA Microprep Kit (Zymo Research, Irvine, CA)

☒ ZymoBIOMICS®-96 MagBead DNA Kit (Zymo Research, Irvine, CA)

☐ N/A (DNA Extraction Not Performed)

Additional Notes: N/A

Library Preparation: Genomic DNA samples were profiled with shotgun metagenomic sequencing. Sequencing libraries were prepared with the option marked below.

☐ KAPA™ HyperPlus Library Preparation Kit (Kapa Biosystems, Wilmington, MA) with up to 100 ng DNA input following the manufacturer's protocol using internal single-index 8 bp barcodes with TruSeq® adapters (Illumina, San Diego, CA)

☒ Illumina® DNA Library Prep Kit (Illumina, San Diego, CA) with up to 500 ng DNA input following the manufacturer's protocol using unique dual-index 10 bp barcodes with Nextera® adapters (Illumina, San Diego, CA)

All libraries were pooled in equal abundance. The final pool was quantified using qPCR and TapeStation® (Agilent Technologies, Santa Clara, CA).

Sequencing: The final library was sequenced on the platform marked below.

☐ NextSeq® (Illumina, San Diego, CA)

☒ NovaSeq® (Illumina, San Diego, CA)

Control Samples: The ZymoBIOMICS® Microbial Community Standard (Zymo Research, Irvine, CA) was used as a positive control for each DNA extraction, if performed. The ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research, Irvine, CA) was used as a positive control for each library preparation. Negative controls (i.e. blank extraction control, blank library preparation control) were included to assess the level of bioburden carried by the wet-lab process.

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2. Methods

Bioinformatics Analysis: Raw sequence reads were trimmed to remove low quality fractions and adapters with Trimmomatic-0.33 (Bolger et al., 2014): quality trimming by sliding window with 6 bp window size and a quality cutoff of 20, and reads with size lower than 70 bp were removed. Antimicrobial resistance and virulence factor gene identification was performed with the DIAMOND sequence aligner (Buchfink et al., 2015). Microbial composition was profiled with Centrifuge (Kim et al., 2016) using bacterial, viral, fungal, mouse, and human genome datasets. Strain-level abundance information was extracted from the Centrifuge outputs and further analyzed: (1) to perform alpha- and beta-diversity analyses; (2) to create microbial composition barplots with QIIME (Caporaso et al., 2012); (3) to create taxa abundance heatmaps with hierarchical clustering (based on Bray-Curtis dissimilarity); and (4) for biomarker discovery with LEfSe (Segata et al., 2011) with default settings ($p > 0.05$ and LDA effect size > 2).

3. References

Bolger, A.M., Lohse, M., and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114-2120.

Buchfink, B., Xie, C., Huson, D.H. (2015) Fast and sensitive protein alignment using DIAMOND. *Nature Methods* **12**:59-60.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335-336.

Kim, D., Song, L., Breitwieser, F.P., Salzberg, S.L. (2016) Centrifuge: rapid and sensitive classification of metagenomic sequences. *Genome Res* **12**:1721-1729.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C. (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* **12**: R60.

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Mėmelio fermentuotas tyrimai

Zymo Research Corporation, JAV, 2022-07-14

Kodas	Produkto pavadinimas
MF-1	Fermentuotas avižų produktas su spirulina 1 L
MF-2	Fermentuotas avižų produktas su cinamonu 1 L
MF-3	Fermentuotas avižų produktas su ciberžole 1 L
MF-4	Fermentuotas avižų produktas su kmynais 1 L
MF-5	Fermentuotas pankolių produktas 0,5 L
X	**Kito gamintojo** fermentuotas avižų produktas 1 L

Taxonomy Summary. Current Level: Class

View Figure (.pdf) View Legend (.pdf)

View Table (.tsv)
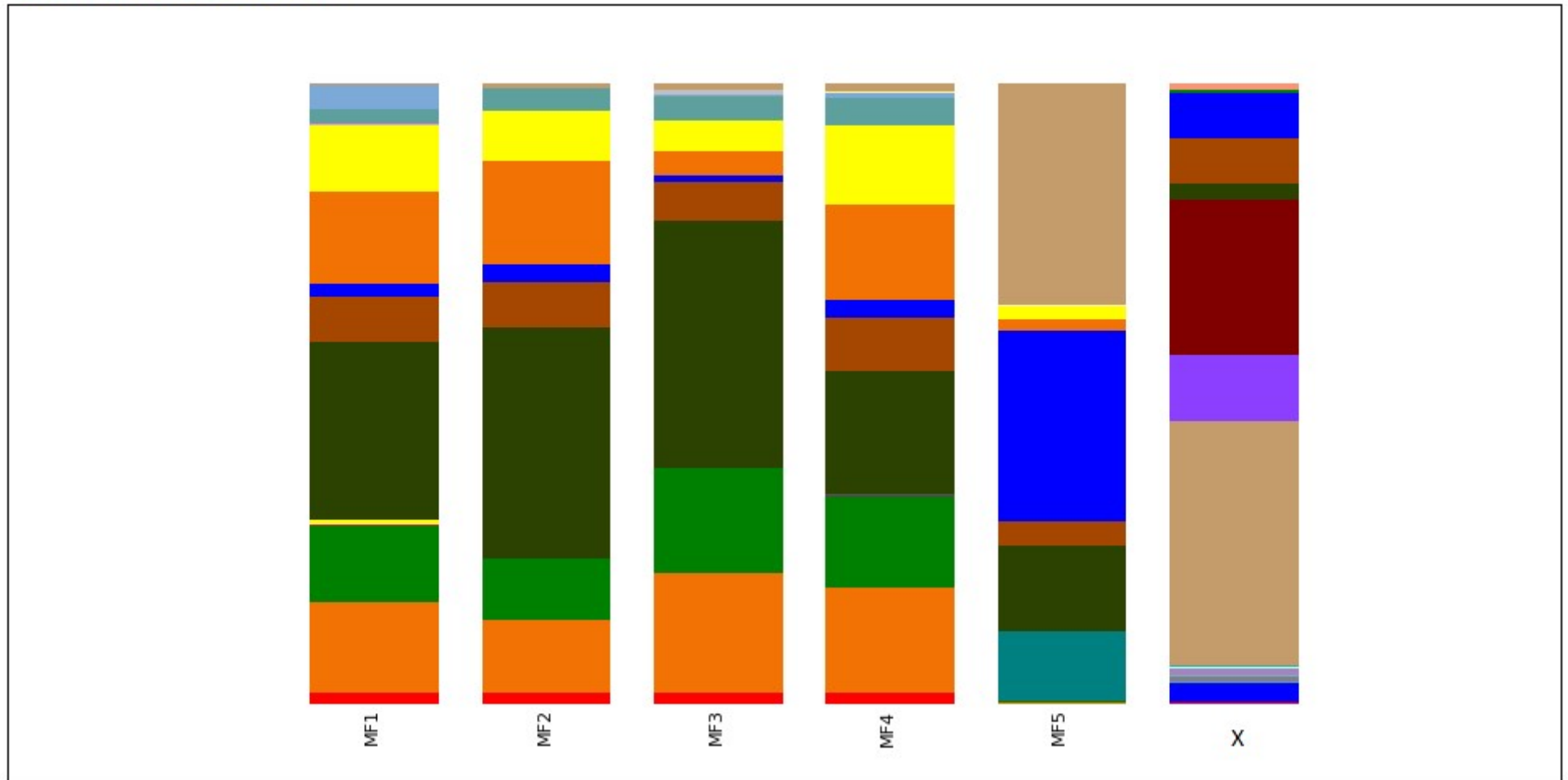
Legend	Taxonomy	Total %	MF1 %	MF2 %	MF3 %	MF4 %	MF5 %	X %
	k__Bacteria;p__Actinobacteria;o__Bifidobacteriales	21.3%	28.7%	23.3%	37.9%	33.6%	0.5%	3.4%
	k__Bacteria;p__Actinobacteria;o__Propionibacteriales	0.1%	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%
	k__Bacteria;p__Cyanobacteria;o__Oscillatoriales	0.1%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%
	k__Bacteria;p__Firmicutes;o__Bacillales	0.5%	0.0%	0.0%	0.0%	0.1%	0.0%	2.7%
	k__Bacteria;p__Firmicutes;o__Clostridiales	6.6%	0.0%	0.0%	0.0%	0.0%	0.0%	39.5%
	k__Bacteria;p__Firmicutes;o__Lactobacillales	63.8%	66.2%	75.7%	60.1%	63.9%	63.5%	53.3%
	k__Bacteria;p__Proteobacteria;o__Rhodospirillales	1.0%	3.9%	0.0%	0.0%	0.9%	0.1%	1.1%
	k__Eukaryota;p__Ascomycota;o__Saccharomycetales	6.7%	0.3%	0.9%	1.9%	1.4%	35.8%	0.0%
	k__Viruses;p__unknown;o__unknown	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxonomy Summary. Current Level: Family

Legend	Taxonomy	Total %	MF1 %	MF2 %	MF3 %	MF4 %	MF5 %	X %
(red)	k__Bacteria;p__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	21.3%	28.7%	23.3%	37.9%	33.6%	0.5%	3.4%
(blue)	k__Bacteria;p__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Propionibacterium	0.1%	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%
(orange)	k__Bacteria;p__Cyanobacteria;o__Oscillatoriales;f__Microcoleaceae;g__Arthrospira	0.1%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%
(dark green)	k__Bacteria;p__Cyanobacteria;o__Oscillatoriales;f__Microcoleaceae;g__Limnospira	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
(purple)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Anoxybacillus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%
(yellow)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus	0.2%	0.0%	0.0%	0.0%	0.1%	0.0%	1.0%
(cyan)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Geobacillus	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	1.3%
(pink)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Parageobacillus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%
(teal)	k__Bacteria;p__Firmicutes;o__Bacillales;f__unknown;g__unknown	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
(brown)	k__Bacteria;p__Firmicutes;o__Clostridiales;f__Clostridiaceae;g__Clostridium	6.6%	0.0%	0.0%	0.0%	0.0%	0.0%	39.5%
(gray)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus	1.8%	0.0%	0.0%	0.0%	0.0%	0.0%	10.7%
(green)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	59.5%	63.6%	72.2%	56.0%	59.4%	63.5%	42.6%
(salmon)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	2.5%	2.6%	3.6%	4.1%	4.6%	0.0%	0.0%
(light blue)	k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter	1.0%	3.9%	0.0%	0.0%	0.8%	0.0%	1.1%
(light orange)	k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Gluconobacter	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%
(light green)	k__Eukaryota;p__Ascomycota;o__Saccharomycetales;f__Pichiaceae;g__Pichia	6.7%	0.3%	0.9%	1.9%	1.4%	35.8%	0.0%
(light purple)	k__Viruses;p__unknown;o__unknown;f__unknown;g__unknown	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxonomy Summary. Current Level: Genus

MF1   MF2   MF3   MF4   MF5   X

Legend	Taxonomy	Total %	MF1 %	MF2 %	MF3 %	MF4 %	MF5 %	X %
■ (red)	k__Bacteria;p__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium animalis	1.2%	1.8%	1.7%	1.8%	1.8%	0.1%	0.3%
■ (blue)	k__Bacteria;p__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium pseudolongum	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	3.1%
■ (orange)	k__Bacteria;p__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium thermacidophilum	10.5%	14.7%	11.8%	19.3%	16.8%	0.2%	0.0%
■ (dark green)	k__Bacteria;p__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium;s__Bifidobacterium thermophilum	9.0%	12.2%	9.8%	16.9%	15.0%	0.3%	0.0%
■ (purple)	k__Bacteria;p__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Propionibacterium;s__Propionibacterium freudenreichii	0.1%	0.1%	0.0%	0.0%	0.1%	0.1%	0.0%
■ (yellow)	k__Bacteria;p__Cyanobacteria;o__Oscillatoriales;f__Microcoleaceae;g__Arthrospira;s__Arthrospira platensis	0.1%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (cyan)	k__Bacteria;p__Cyanobacteria;o__Oscillatoriales;f__Microcoleaceae;g__Limnospira;s__Limnospira indica	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (pink)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Anoxybacillus;s__Anoxybacillus flavithermus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (teal)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Anoxybacillus;s__Anoxybacillus geothermalis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%
■ (brown)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus circulans	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (gray)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus coagulans	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.9%
■ (green)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus licheniformis	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%
■ (salmon)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus paranthracis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (light blue)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus smithii	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
■ (tan)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Bacillus;s__Bacillus subtilis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (light green)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Geobacillus;s__Geobacillus lituanicus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
■ (light purple)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Geobacillus;s__Geobacillus stearothermophilus	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%
■ (pale yellow)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Geobacillus;s__Geobacillus zalihae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
■ (light gray)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Parageobacillus;s__Parageobacillus caldoxylosilyticus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (magenta)	k__Bacteria;p__Firmicutes;o__Bacillales;f__Bacillaceae;g__Parageobacillus;s__Parageobacillus thermoglucosidasius	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%
■ (sky blue)	k__Bacteria;p__Firmicutes;o__Bacillales;f__unknown;g__unknown;s__[Flavobacterium] thermophilum	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
■ (tan/brown)	k__Bacteria;p__Firmicutes;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__Clostridium butyricum	6.6%	0.0%	0.0%	0.0%	0.0%	0.0%	39.5%
■ (olive)	k__Bacteria;p__Firmicutes;o__Clostridiales;f__Clostridiaceae;g__Clostridium;s__Clostridium perfringens	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (purple)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Enterococcaceae;g__Enterococcus;s__Enterococcus faecium	1.8%	0.0%	0.0%	0.0%	0.0%	0.0%	10.7%
■ (mauve)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus acidophilus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
■ (teal)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus amylovorus	1.9%	0.0%	0.0%	0.0%	0.0%	11.2%	0.0%
■ (dark red)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus crispatus	4.1%	0.0%	0.0%	0.0%	0.0%	0.0%	24.8%
■ (dark green)	k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus delbrueckii	33.7%	38.6%	37.2%	40.0%	19.8%	12.7%	2.7%

Taxonomy							
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus crispatus	4.1%	0.0%	0.0%	0.0%	0.0%	0.0%	24.8%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus delbrueckii	23.7%	28.6%	37.3%	40.0%	19.8%	13.7%	2.7%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus fermentum	6.8%	7.4%	7.2%	6.1%	8.6%	4.0%	7.4%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus kisonensis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus mucosae	7.8%	2.0%	2.9%	1.2%	2.8%	30.8%	7.2%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus paracasei	8.8%	14.8%	16.9%	3.9%	15.4%	1.7%	0.1%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus rapi	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus reuteri	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus rhamnosus	6.4%	10.8%	8.0%	4.9%	12.7%	2.1%	0.1%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Lactobacillus timonensis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus sp.	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus;s__Streptococcus thermophilus	2.4%	2.5%	3.6%	4.1%	4.6%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter aceti	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter fabarum	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter oryzifermentans	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter pasteurianus	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.9%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter persici	0.8%	3.9%	0.0%	0.0%	0.8%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter pomorum	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter senegalensis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Acetobacter;s__Acetobacter tropicalis	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Gluconobacter;s__Gluconobacter cerinus	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%
k__Eukaryota;p__Ascomycota;o__Saccharomycetales;f__Pichiaceae;g__Pichia;s__Pichia kluyveri	0.2%	0.0%	0.0%	0.8%	0.0%	0.1%	0.0%
k__Eukaryota;p__Ascomycota;o__Saccharomycetales;f__Pichiaceae;g__Pichia;s__Pichia kudriavzevii	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Eukaryota;p__Ascomycota;o__Saccharomycetales;f__Pichiaceae;g__Pichia;s__Pichia manshurica	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%
k__Eukaryota;p__Ascomycota;o__Saccharomycetales;f__Pichiaceae;g__Pichia;s__Pichia membranifaciens	6.5%	0.2%	0.8%	1.1%	1.3%	35.7%	0.0%
k__Viruses;p__unknown;o__unknown;f__unknown;g__unknown;s__Streptococcus satellite phage Javan607	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
k__Viruses;p__unknown;o__unknown;f__unknown;g__unknown;s__Streptococcus satellite phage Javan613	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%