Electronic Supplementary Material (ESI) for Food & Function.

SUPPORTING INFORMATION

DNA extraction of TKGs

Microbial DNA was extracted using a FastDNA® SPIN Kit (MP Biomedicals, USA). Briefly, samples (3 g) were placed into 10.0 ml tubes that containing lysing matrix E, a mixture of silica particles and ceramic designed to high-efficiently lyse all organisms covering bacteria, yeast, fungi, eubacterial spores and endospores. Homogenization of sample was carried out in the presence of MT buffer and sodium phosphate buffer in a FastPrep® Instrument with pyrolysis matrix E within 40 seconds. After the lysis process, samples are centrifuged to remove cell debris and lysing matrix. DNA in supernatant is purified with a silica-based GENECLEAN® procedure using SPIN filters. And the eluted DNA was stored at -20 °C before use.

Scanning electron microscopy (SEM) of TKGs

Tibetan kefir grainwas added to 500 μL of sterile saline solution and then ground. A sample of each (2 μL) was added to a silicon wafer and dried. Each sample was placed in a 30 g/L glutaraldehyde solution (solvent: 0.1 M phosphate buffer, pH 7.0) and incubated for 12 h at 4°C. The grain samples were then washed three times with phosphate buffer saline solution for 10 min. Samples were then dehydrated in an ethanol gradient (30%, 50%, 70%, 80%, 90%, and 100% ethanol solutions) for 10 min each. Samples were then maintained in 100% ethanol until dried and coated with gold. Scanning electron microscopy (FEI, nova nano SEM450) was used to observe the preparations.

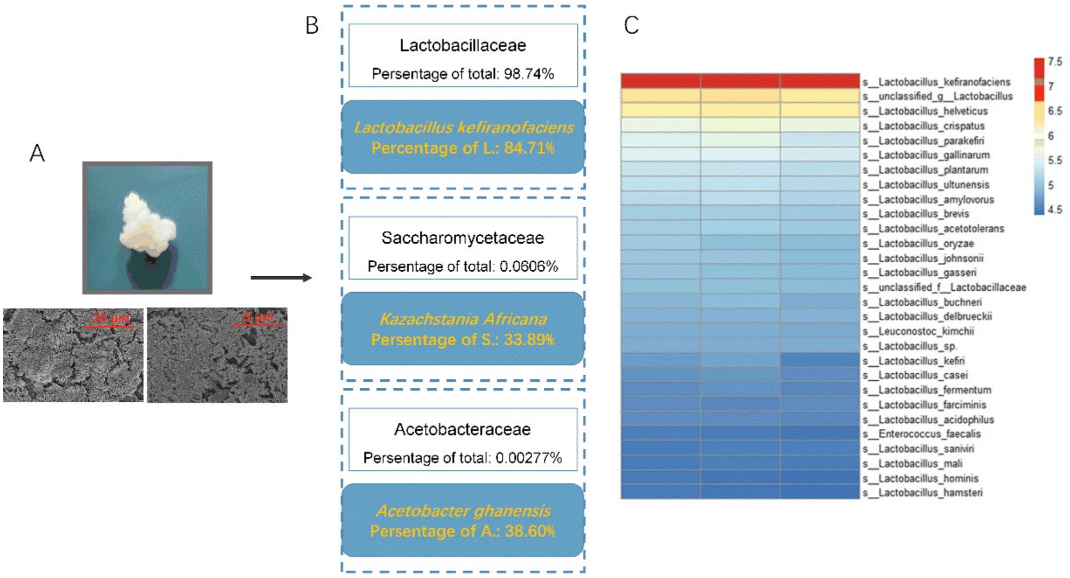

Fig. S1 Metagenomic analysis of Tibetan kefir grain. (A) Morphological characteristics of Tibetan kefir grain. (B) The relative abundance of Lactic acid bacteria, yeast and acetic acid bacteria at family level. And the percentages of the most abundant species within the Lactobacillaceae, Saccharomycetaceae, and Acetobacteraceae families are shown in a small box with a blue background. (C) The top 30 species in Tibetan kefir grain.

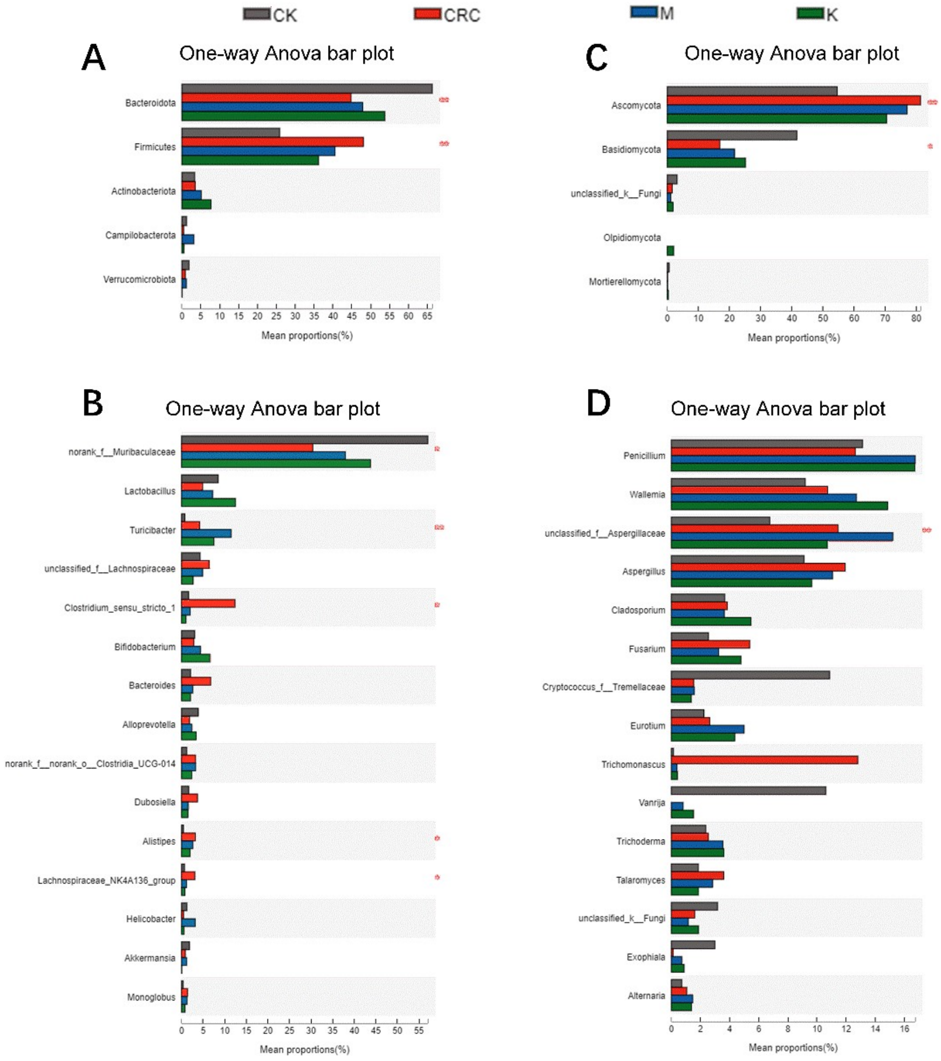

Fig. S2 Relative abundance of gut microbiota at the phylum (top 5) and genus level (top 15). (A) The difference of relative abundance of gut bacteria microbiota at phylum level among four groups. (B) The difference of relative abundance of gut fungal microbiota at phylum level among four groups. (C) The difference of relative abundance of gut bacteria microbiota at genus level among four groups. (D) The difference of relative abundance of gut fungal microbiota at genus level among four groups. The data are presented as mean ± standard deviation (SD), $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

Table S1. The 20 most abundant species within the Tibetan kefir grain communities.

Species	Relative abundance
Lactobacillus kefiranofaciens	83.32 ± 1.29%
unclassified *Lactobacillus*	4.84 ± 0.55%
Lactobacillus helveticus	3.91 ± 0.14%
Lactobacillus crispatus	1.05 ± 0.15%
Lactobacillus parakefiri	0.69 ± 0.17%
Lactobacillus gallinarum	0.62 ±0.02%
Lactobacillus plantarum	0.43 ± 0.05%
Lactobacillus ultunensis	0.40 ± 0.02%
Lactobacillus amylovorus	0.35 ± 0.02%
Lactobacillus acetotolerans	0.27 ± 0.02%
Lactobacillus brevis	0.27 ± 0.01%
Lactobacillus oryzae	0.21 ± 0.03%
Lactobacillus johnsonii	0.21 ± 0.02%
Lactobacillus gasseri	0.20 ± 0.01%
unclassified Lactobacillaceae	0.20 ± 0.01%
Lactobacillus buchneri	0.17 ± 0.01%
Lactobacillus delbrueckii	0.16 ± 0.01%
Lactobacillus sp.	0.15 ± 0.01%
Leuconostoc kimchi	0.14 ± 0.01%
Lactobacillus kefiri	0.11 ± 0.02%

Table S2 List of qRT-PCR primers and amplicon size

Target	Primer sequence
Mus-β-actin	Forward-5’-GCTCTGGCTCCTAGCACCAT-3’
	Reverse-5’-GCCACCGATCCACACAGAGT-3’
Mus-TNF-α	Forward-5’-CCCTCACACTCAGATCATCTTCT-3’
	Reverse-5’-GCTACGACGTGGGCTACAG-3’
Mus-IL6	Forward-5’-GTTGCCTTCTTGGGACTGATGCT-3’
	Reverse-5’-GCCTCCGACTTGTGAAGTGGTATAG-3’
Mus-IL-17A	Forward-5’-TTTAACTCCCTTGGCGCAAAA-3’
	Reverse-5’-CTTTCCCTCCGCATTGACAC-3’
Mus-ZO-1	Forward-5’-GCCGCTAAGAGCACAGCAA-3’
	Reverse-5’-GCCGCTAAGAGCACAGCAA-3’
Claudin-1	Forward-5’-GGGGACAACATCGTGACCG-3’
	Reverse-5’-AGGAGTCGAAGACTTTGCACT-3’
Mus-β-catenin	Forward-5’-GCTCATCCCACTAATGTCCAG-3’
	Reverse-5’-CTGGTCCTCATCGTTTAGCAG-3’
Mus-Ki67	Forward-5’-ATCATTGACCGCTCCTTTAGGT-3’
	Reverse-5’-GCTCGCCTTGATGGTTCCT-3’
Mus-NF-κB	Forward-5’-ATGGCAGACGATGATCCCTAC-3’
	Reverse-5’-TGTTGACAGTGGTATTTCTGGTG-3’