

Supplementary materials

Table S1 The main composition of the drink and diet

Group	Drink	Diet
YC	Drink water freely	Standard diet: water 9.2%, crude protein 22.1%, crude fat 5.3%, crude fibre 3.0%, crude ash 6.0%, calcium 1.25%, total phosphorus 0.75%.
OC	Drink water freely	
RJ	Drink RJ solution: water 63.26%, water-soluble protein 73.67 mg/g, reducing sugars 80.46 mg/g.	

YC: Young control group; OC: Old control group, RJ: Royal jelly plus old group.

Table S2 The sequences of primers used in this study

Gene name	Primer sequences
<i>P16</i>	Forward 5'- GATGGACGTTTCAGGTGGCATA -3' Reverse 5'- GCTTAGCAATGAAACTGCGAAGT -3'
<i>P53</i>	Forward 5'- TCAC,kAGCGTCTGTTGACATTT -3' Reverse 5'- ACCAAGCTCATTACCCTGACA -3'
<i>Klotho</i>	Forward 5'- GGACATTTCCCTGTGACTTTGC -3' Reverse 5'- AGAGAGAGTAGTGTCCACTTGAACGT -3'
<i>Sirt7</i>	Forward 5'- CCTGCATCCCTAACAGAGAG -3' Reverse 5'- AGCTGGACCCTAAACACAGG -3'
<i>TNF-α</i>	Forward 5'-CCCTCACACTCACAAACCACC-3' Reverse 5'-CTTTGAGATCCATGCCGTTG-3'
<i>IL-6</i>	Forward 5'-ACAACCACGGCCTTCCCTAC-3' Reverse 5'- GCACAACCTTTTTCTCATTTCAC-3'
<i>IL-10</i>	Forward 5'- AATAAGCTCCAAGACCAAGGTGT-3' Reverse 5'- CATCATGTATGCTTCTATGCAGTTG-3'
<i>ZO-1</i>	Forward 5'- GGGAAAACCCGAAACTGATG-3' Reverse 5'- GCTGTACTGTGAGGGCAACG-3'
<i>OCCUDIN</i>	Forward 5'- ATAATGGGAGTGAACCCGACG-3' Reverse 5'- CGATCCATCTTTCTTCGGGTTT-3'

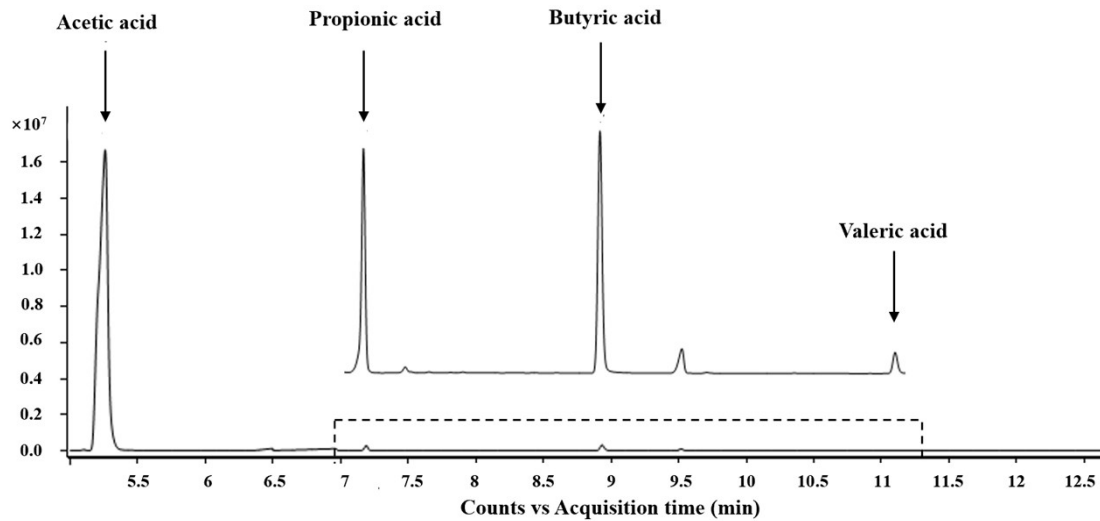


Fig S1 Total ion chromatogram of short-chain fatty acid standard

Table S3 Retention time, mass-to-charge ratio, standard curve, correlation coefficient R^2 , and linear range of short-chain fatty acid standards

Name	Retention time (min)	mass-to-charge ratio (m/z)	Calibration curve	Correlation coefficient R^2	Linear range ($\mu\text{g/mL}$)
Acetic acid ($\mu\text{g/mL}$)	5.27	117	$y = 3.7000x + 838004.87$	1.0000	100~10000
Propionic acid ($\mu\text{g/mL}$)	7.19	131	$y = 1.3721x + 17843$	0.9976	2~1000
Butyric acid ($\mu\text{g/mL}$)	8.94	145	$y = 1.6716x + 15802$	0.9991	2~1000
Valeric acid ($\mu\text{g/mL}$)	11.07	159	$y = 1.6041x + 4472.6$	0.9956	0.5~100

Table S4 α diversity analysis of feces among the three groups

Group	ACE	Chao1	Shannon	Simpson
YC	616.44±176.76 ^a	636.53±232.99 ^a	4.47±0.41 ^b	0.85±0.05 ^b
OC	600.80±116.72 ^a	586±112.08 ^a	5.85±0.30 ^a	0.96±0.01 ^a
RJ	559.03±90.60 ^a	552.80±86.57 ^a	5.64±0.38 ^a	0.94±0.38 ^a

Notice: The values under the same parameter with different letters (i.e., a–b) are significantly different ($p < 0.05$).

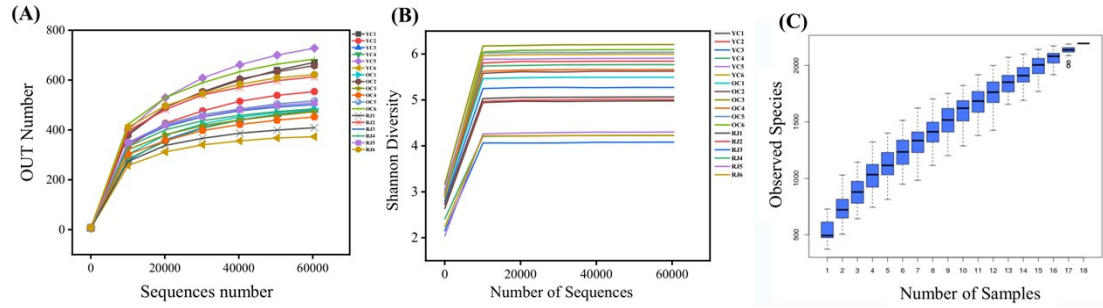


Fig. S2 Rarefaction analysis (A), Shannon index (B) and species accumulation (C) of fecal samples (n = 6).

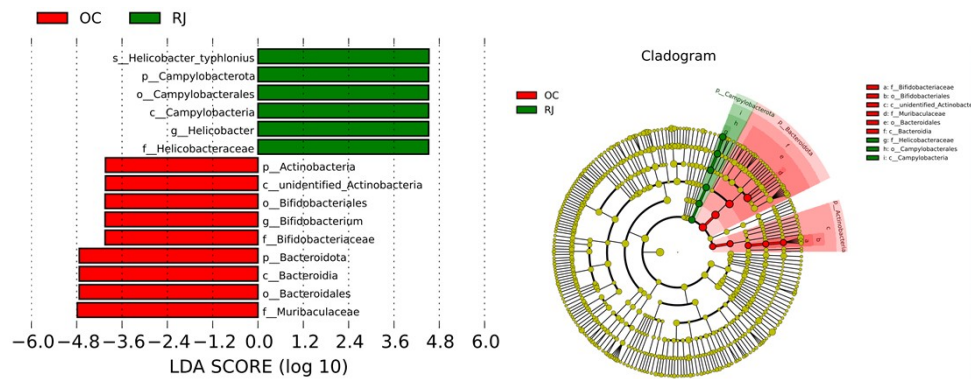


Fig S3. LEfSe analyses of gut microbiota data between OC and RJ groups. Differentially expressed taxa with the LDA scores > 4 and p values < 0.05. (A) The taxonomic histogram shows the LDA scores calculated for characteristics at the OUT level. (B) The taxonomic cladogram shows the relative abundance of OTUs. Circles represent phylogenetic levels from phylum (innermost circle) to species (outermost circle) and the diameter of each circle is proportional to the taxon's abundance.

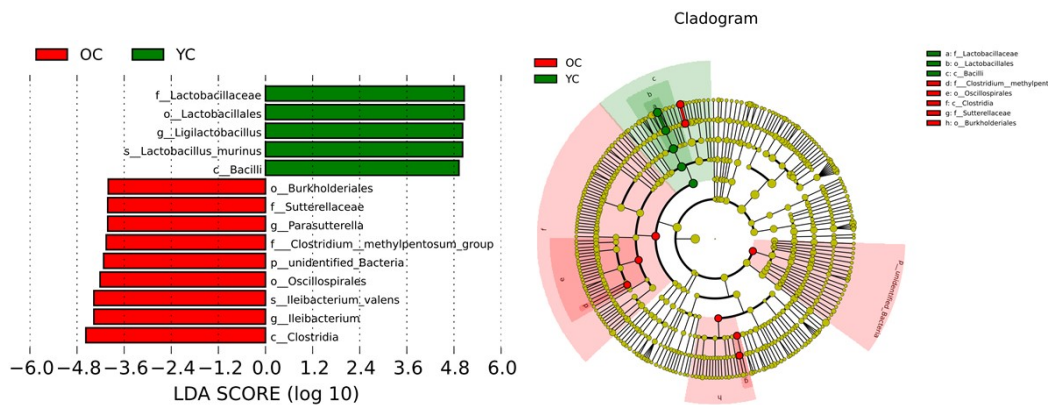
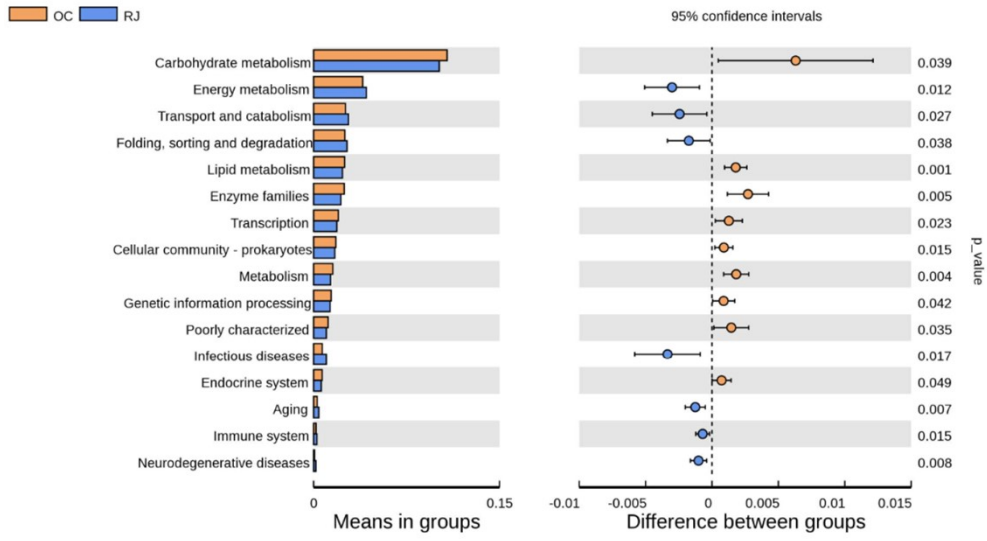


Fig S4. LEfSe analyses of gut microbiota data between OC and YC groups. Differentially expressed taxa with the LDA scores > 4 and p values < 0.05. (A) The taxonomic histogram shows the LDA scores calculated for characteristics at the OUT level. (B) The taxonomic cladogram shows the relative abundance of OTUs. Circles represent phylogenetic levels from phylum (innermost circle) to species (outermost circle) and the diameter of each circle is proportional to the taxon's abundance.

Level 2



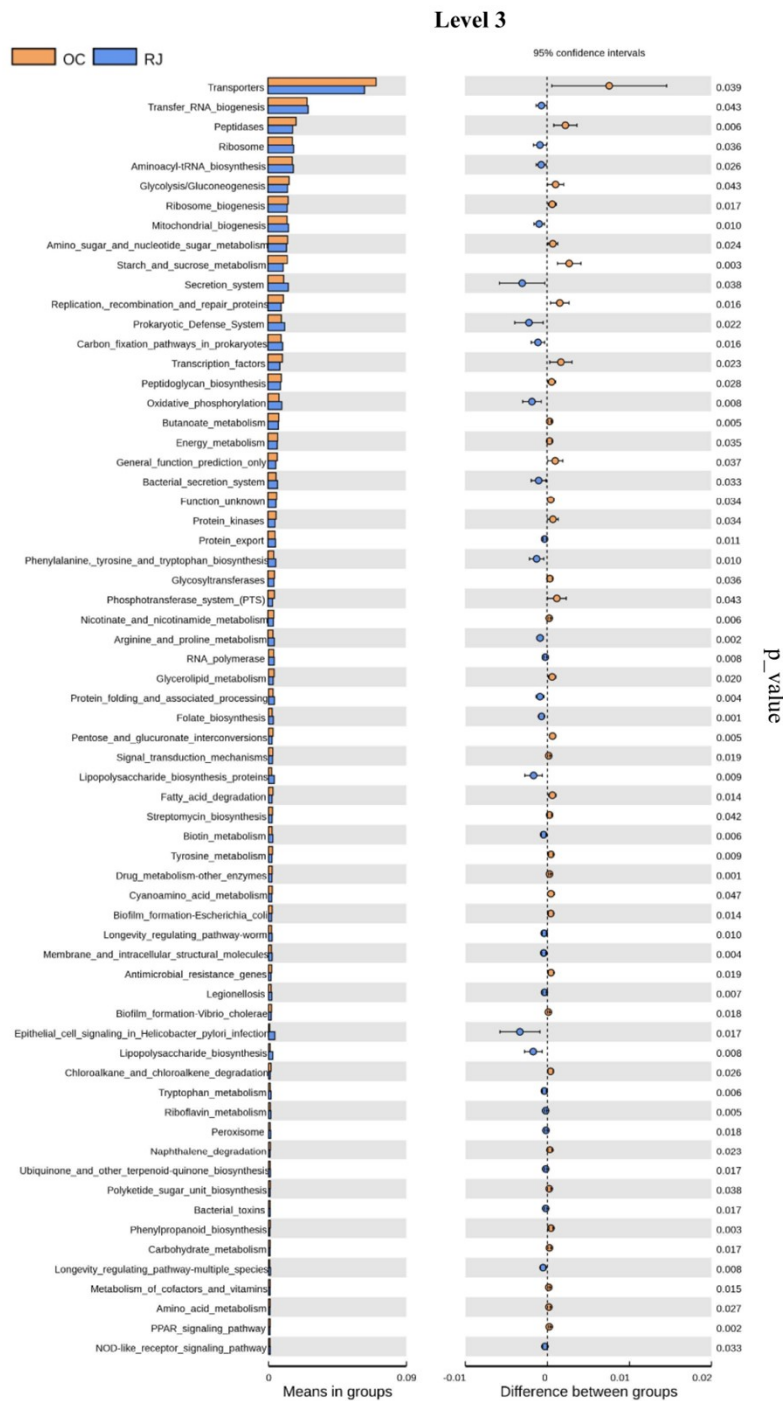


Fig S5. Tax4Fun functional profile of fecal microbiota communities based on KEGG pathway analysis. (A) Functional differences were observed between the OC and RJ group at level 2. (B) Functional abundance of OC vs RJ at level 3 with Welch's t test.