SUPPORTING INFORMATION

Lactobacillus kefiranofaciens JKSP109 and *Saccharomyces cerevisiae* JKSP39 isolated from Tibetan kefir grain co-alleviated AOM/DSS induced inflammation and colorectal carcinogenesis

Xuejun Zeng[1,2], Hang Jia[1,2], Yiheng Shi[1,2], Ke Chen[1,2], Zhouli Wang[1,2], ZhenpengGao[1,2], Yahong Yuan[1,2*], Tianli Yue[1,2,3*]

[1]College of Food Science and Engineering, Northwest A & F University, Yangling, 712100, China

[2]Laboratory of Quality & Safety Risk Assessment for Agri-products (Yangling), Ministry of Agriculture, Yangling, 712100, China

[3]College of Food Science and Technology, Northwest University, Xi'an, 710069, China

* Corresponding author.

Tianli Yue

E-mail: yuetl@nwsuaf.edu.cn

Tel/ Fax: 029-87092261

Institute: College of Food Science and Engineering, Northwest A&F University

Address: 22. Xi-nong Road, Yangling, Shaanxi, 712100, China.

Yahong Yuan

E-mail: Yyh324@126.com

Tel/ Fax: 029-87092261

Institute: College of Food Science and Engineering, Northwest A&F University

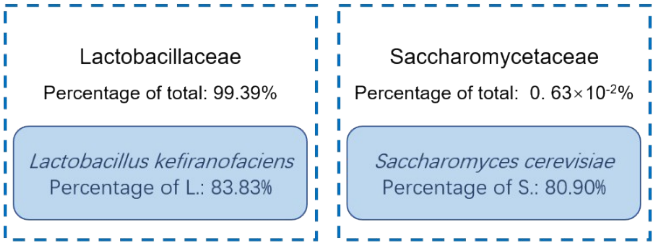
Address: 22. Xi-nong Road, Yangling, Shaanxi, 712100, China.

1 The microbial species in Tibetan kefir grain

The appearance of Tibetan kefir grain that used in this study was shown in Fig S1A. The microbial composition of Tibetan kefir grain was analyzed by metagenome. The most abundant bacteria and yeast were listed in Fig. S1B, and the top 20 species in Tibetan kefir grain were showed in Fig. S1B.

A

B



C

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%

Fig. S1 The composition of Tibetan kefir grain. (A) A photograph of Tibetan kefir grain that used in this study. (B) The relative abundance of Lactic acid bacteria and yeast at family level. And the percentages of the most abundant species within the Lactobacillaceae and Saccharomycetaceae

families are shown in a small box with a blue background. (C) The top 20 species in Tibetan kefir grain.

2 Occult blood in feces detection and viscera index

Orthotolidine method was used to detect the occult blood. Briefly, a bamboo stick was used to pick up a little amount of feces and put it on the sterilized filter paper, then droplets with 2-3 drops of o-tolidine (0.15 L/L, 150 mL o-tolidine add glacial acetic acid to 1 L). After that, 2-3 drops of 3% H_2O_2 were added to the feces. The scores were judged by the color of the reaction: 0 for does not turn blue for 2 minutes, 2 for immediately appears blue-green, 4 for immediately appears dark blue-green. Results showed that the occult blood of Mice in CRC group were serious, but LK with SC treatment can reduce the occult blood in stool. (Fig. S2A) The weight of heart and kidney was steady, there was no significant difference among five groups. AOM/DSS can induce the volume and weight of spleen changed (Fig. S2B). In CRC group, the liver and lung weight were significantly increased (liver: $p < 0.01$; lung: $p < 0.001$), and the thymus weight was significantly decreased ($p < 0.01$). Treatment with LK made the weight loss of thymus is less than CRC group ($p < 0.05$) but had little effect on liver and lung weight. Treatment with SC significantly decreased the ratio of lung ($p < 0.05$) compared to CRC but had little effect on liver and thymus weight. Treatment with the mix of LK and SC significantly decreased the liver and lung index compared to CRC group (liver: $p < 0.05$; lung: $p < 0.05$), but had little effect on thymus weight.

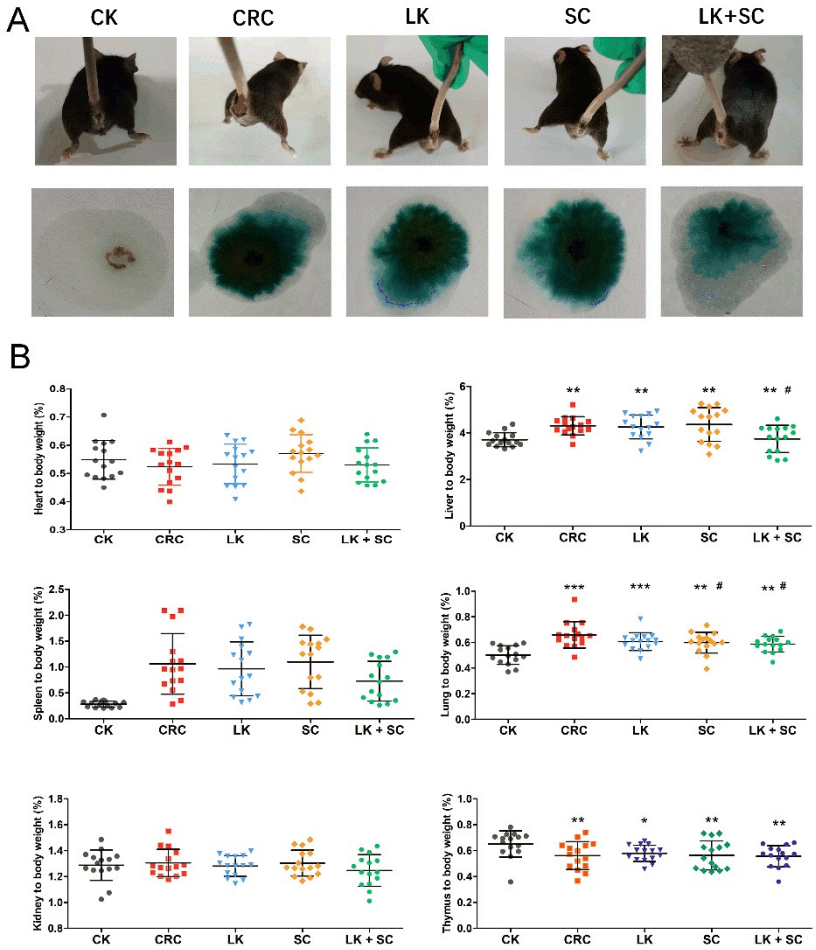


Fig. S2 Fecal occult blood and viscera index. (A) The fecal occult blood in different groups. (B) The viscera index (heart, liver, spleen, lung, kidney and thymus) of mice in five groups. Symbol "*" for versus CK group, and symbol "#" for versus CRC group. The data are presented with mean ± standard deviation (SD), $p < 0.05$, * or #; $p < 0.01$, ** or ##; $p < 0.001$, *** or ###.

2 Histopathology score

Table S1 Histopathology score evaluation

Score	Inflammation	Mucosal damage	Crypt damage	Range of lesions(%)
0	None	None	None	0
1	Mild	Mucous layer	1/3	1~25
2	Moderate	Submucosa	2/3	26~50
3	Severe	Muscularis and serosa	100%	51~75
4	-	-	100% with epithelium loss	76~100

3 qRT-PCR primers

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-IL-6	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'
Mus-Occludin	Forward-5'- TTGAAAGTCCACCTCCTTACAGA-3'
	Reverse-5'- CCGGATAAAAAGAGTACGCTGG-3'
Mus-Claudin-1	Forward-5'-GGGGACAACATCGTGACCG-3'
	Reverse-5'-AGGAGTCGAAGACTTTGCACT-3'
Mus-Ki67	Forward-5'-ATCATTGACCGCTCCTTTAGGT-3'
	Reverse-5'-GCTCGCCTTGATGGTTCCT-3'
Mus-NF-κB	Forward-5'-ATGGCAGACGATGATCCCTAC-3'
	Reverse-5'-TGTTGACAGTGGTATTTCTGGTG-3'

4 The comparation of tested index in three treatment groups

Table S3 The detail data for tested indexes

	CK	CRC	LK	SC	LK+SC
Body weight (endpoint)	27.91	25.03	25.69	26.20	26.91
DAI (endpoint)	-	6.33	5.33	4.93	4.53
Colon length (cm)	6.79	5.64	6.44	6.75	6.65
Tumor multiplicity	-	3.47	2.80	2.40	2.07
tumor incidence	-	100%	100%	100%	93.30%
> 4 mm tumor (%)	-	38.20%	23.20%	16.07%	11.07%
F/B	0.40	1.10	0.99	0.63	0.89
A/B	1.47	5.34	2.26	4.62	2.94
Claudin-1 (RNA)	0.95	0.54	0.94	0.95	1.06
Occludin (RNA)	0.95	0.20	0.91	1.15	1.08
ZO-1 (RNA)	0.95	0.60	1.13	0.94	1.14
Claudin-1 (Protein) (% of control)	1.30	0.59	1.21	1.08	1.25
Occludin (Protein) (% of control)	1.31	0.47	0.74	0.69	0.67
ZO-1 (Protein) (% of control)	1.58	0.33	0.58	0.66	1.03
TNF-α (RNA)	0.92	3.72	1.56	1.67	1.76
IL-6 (RNA)	0.88	2.05	1.46	1.37	1.08
IL-17a (RNA)	0.95	3.76	3.21	2.93	2.06
TNF-α(Pro) (pg/ml)	261.56	529.51	246.54	382.09	333.91
IL-6 (Pro) (pg/ml)	45.97	84.31	70.79	60.55	50.23
IL-17a (Pro) (pg/ml)	19.68	27.08	26.55	23.24	17.79
Ki67 (RNA)	1.02	2.13	1.24	1.51	0.99
NF-κB (RNA)	0.95	2.51	1.44	2.77	1.72

Ki67 (Protein) (% of control)	0.90	2.12	1.00	0.89	0.72
NF-κB (Protein) (% of control)	0.64	1.78	0.91	0.99	0.78
Acetic acid (mg/g)	26.24	26.21	27.54	30.90	31.24
Propionic acid (mg/g)	4.96	4.81	5.36	5.47	5.63
Isobutyric acid (mg/g)	0.34	0.27	0.37	0.29	0.35
Butyric acid (mg/g)	1.33	1.21	1.43	1.91	1.82
Isovaleric acid (mg/g)	0.29	0.28	0.69	0.54	0.73
Valeric (mg/g)	0.39	0.35	0.55	0.43	0.50
Sum of SCFAs (mg/g)	33.55	33.13	35.94	39.53	40.26