Supplementary Material

Postbiotics from *Pichia kudriavzevii* promote intestinal health performance through regulation of *Limosilactobacillus reuteri* in weaned piglets

Zhenting Zhang[1,2], Qiujin Guo[1], Jing Wang[1], Hongyan Tan[1], Xuexia Jin[1], Yurong Fan[1], Jiali Liu[1], Shumiao Zhao[1], Jinshui Zheng[3], Nan Peng[1] *

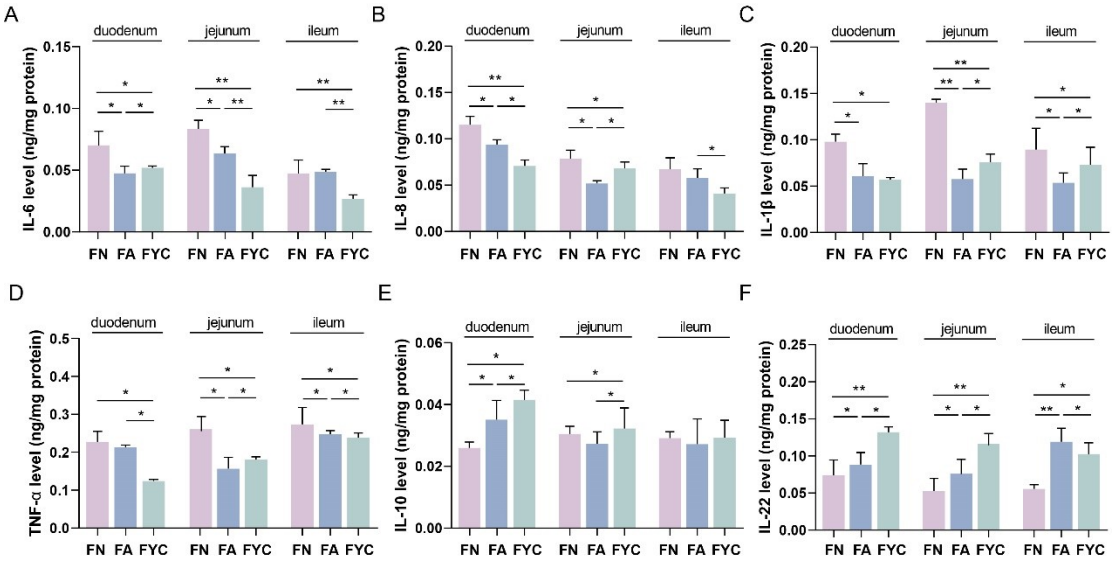
[1] State Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, Hubei, P.R. China

[2] The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, School of Public Health, Guizhou Medical University, Guiyang 550025, Guizhou, P.R. China.

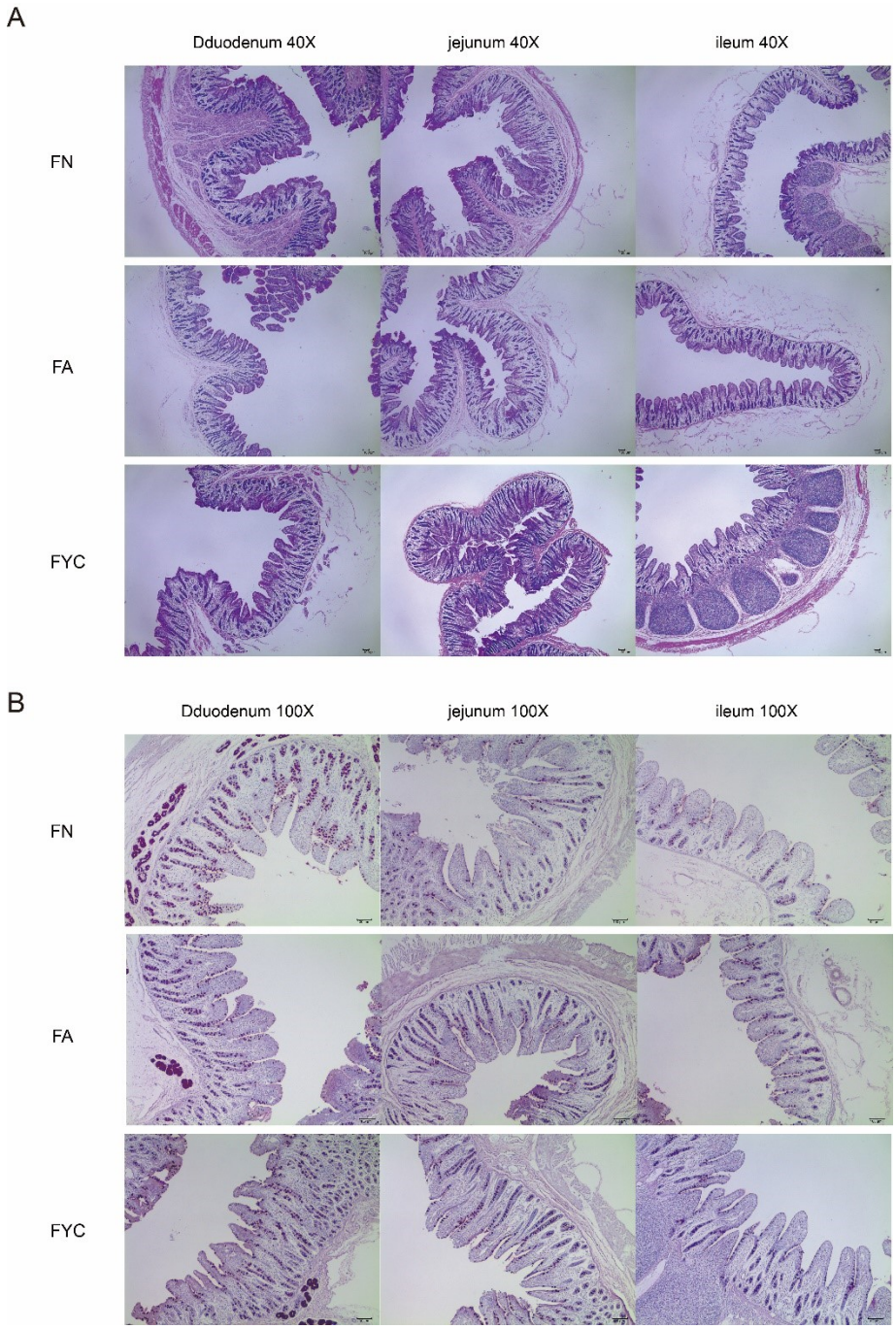
[3] State Key Laboratory of Agricultural Microbiology, College of Informatics, Huazhong Agricultural University, Wuhan, 430070, Hubei, P.R. China

*Correspondence should be addressed to Nan Peng: Tel: +86 27 8728 1267, Fax: +86 27 8728 0670, Email: nanp@mail.hzau.edu.cn

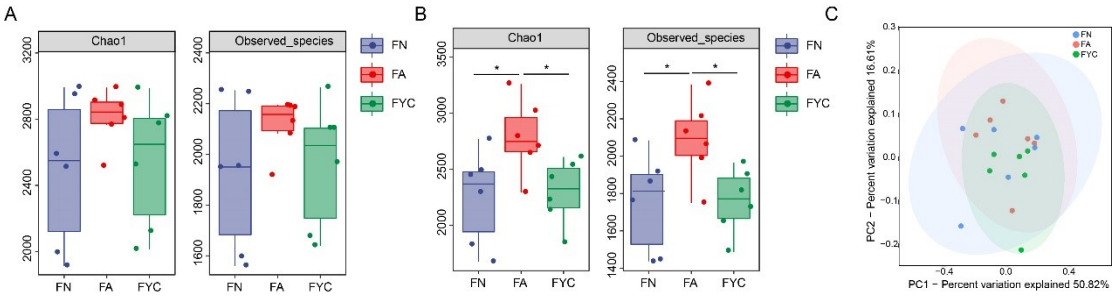
Figures



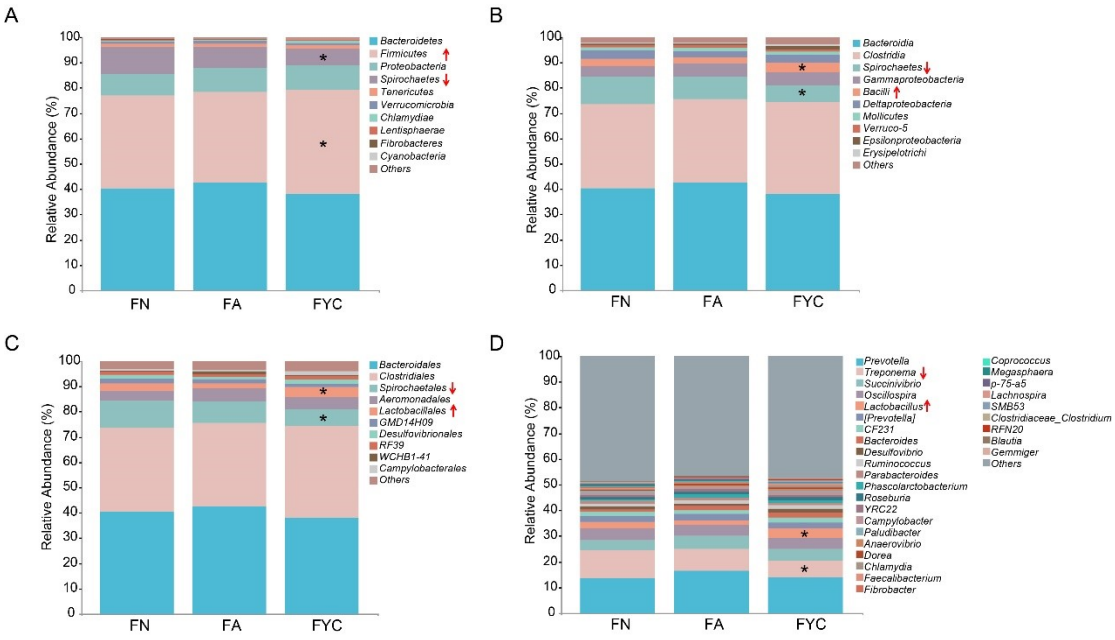
Supplementary Figure 1. Supplementation of *P. kudriavzevii* postbiotics reduced intestinal inflammation in early-weaned piglets. The concentration of intestinal pro-inflammatory factors (**A-D**) and anti-inflammatory factors (**E** and **F**) in the duodenum, jejunum, and ileum of the piglets from the groups of early-weaned piglets fed on a normal diet (FN), a normal diet with antibiotics (FA), or a normal diet with 0.5% yeast culture (FYC). Data are shown as mean ± SD. Statistical analyses were performed using one-way ANOVA. Significance, ** $p < 0.01$, * $p < 0.05$.



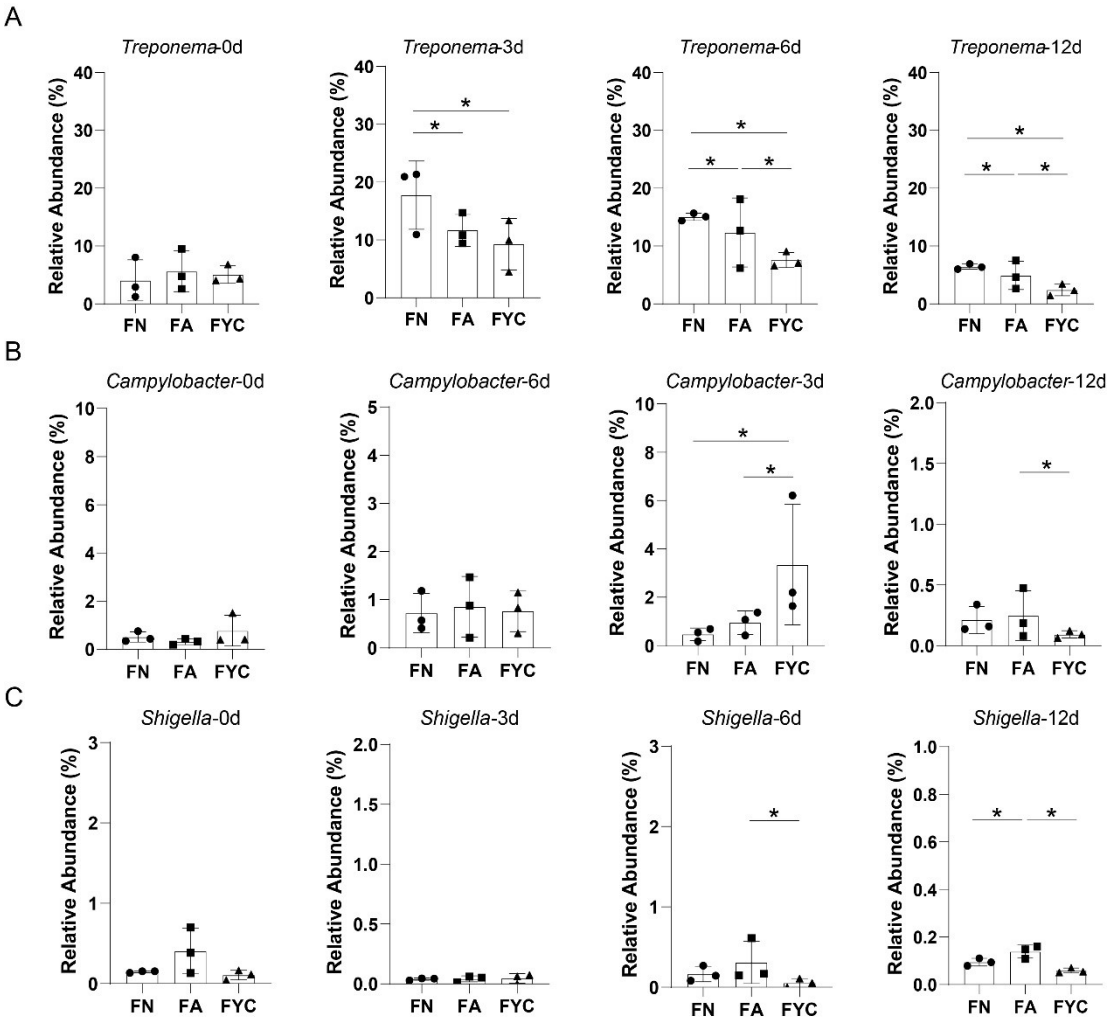
Supplementary Figure 2. The effects of yeast postbiotics supplementation on duodenum, jejunum, and ileum histological morphology. (**A**) HE staining; (**B**) PAS staining. Normal diet (FN), a normal diet with antibiotics (FA), or a normal diet with 0.5% yeast culture (FYC). The images of the intestinal morphology at 40 × (HE) and 100 × (PAS) magnification (scale bars, 100 μm) are shown.



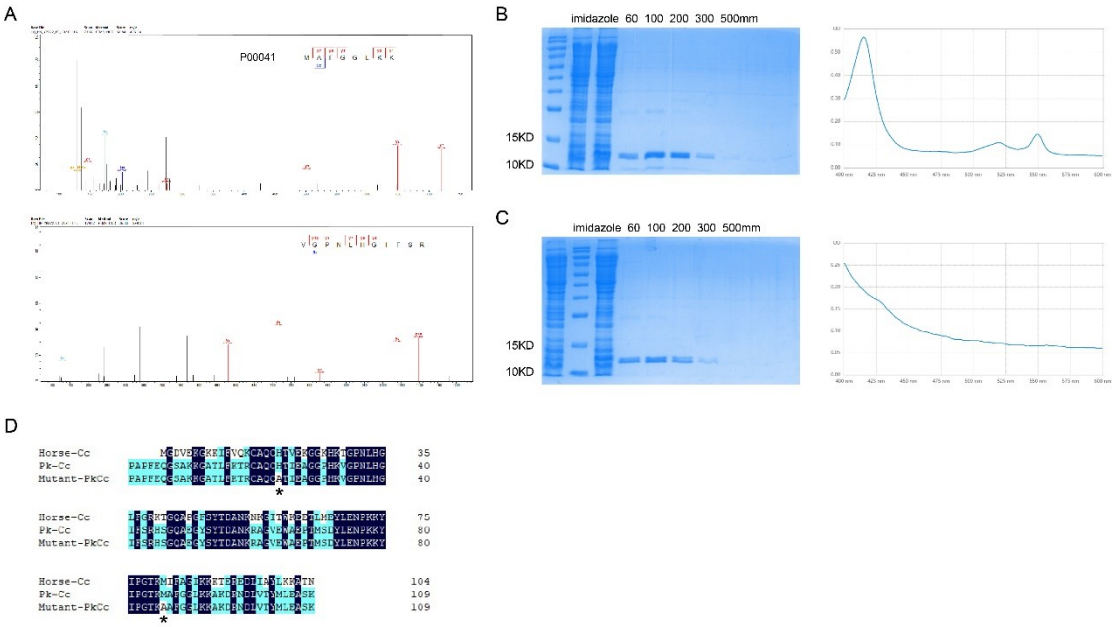
Supplementary Figure 3. The alpha and beta diversities of the intestinal bacterial communities. (**A**) Bacterial alpha diversity based on Chao 1 and observed species index (from 0 to 3 days) or (**B**) from 6 to 12 days. (**C**) Scatterplot from PCoA in bacterial communities based on the weighted UniFrac distance (from 0 to 3 days). One-way ANOVA with adjustment for multiple comparisons was conducted. Significance, $*p < 0.05$.



Supplementary Figure 4. Supplement of yeast postbiotics affected the intestinal bacterial taxonomic compositions. The shifts in the relative abundance of bacterial phylum (**A**), class (**B**), order (**C**), and genus (**D**). One-way ANOVA with adjustment for multiple comparisons was conducted. FN, FA, and FYC: groups of early-weaned piglets fed on a normal diet (FN), a normal diet with antibiotics (FA), and a normal diet with 0.5% yeast culture (FYC). Statistical analyses were performed using one-way ANOVA. Significance, * $p < 0.05$.



Supplementary Figure 5. The relative abundances of pathogenic bacteria, including *Treponema* (A), *Campylobacter* (B), and *shigella* (C), in different groups at four time points. FN, FA, and FYC: groups of early-weaned piglets fed on a normal diet (FN), a normal diet with antibiotics (FA), and a normal diet with 0.5% yeast culture (FYC). Statistical analyses were performed using one-way ANOVA. Significance, * $p < 0.05$.



Supplementary Figure 6. Identification of the proteins from *P. kudriavzevii* postbiotics. (**A**) Characteristics of the peptides of cytochrome c. Red represents the peptides of cytochrome c. (**B**) SDS-PAGE analysis of heterogeneously expressed wild-type and detection of the characteristic absorption peak of cytochrome c. M, protein markers. (**C**) SDS-PAGE analysis of heterogeneously expressed mutant and detection of the characteristic absorption peak of cytochrome c. M, protein markers. (**D**) Sequence alignment of different cytochrome c. Horse-Cc was from the heart of a horse. Pk-Cc was expressed from *E. coli*. Mutant-Cc was expressed in *E. coli* with haem iron ligands changed**, *** represent mutant sites. Statistical analyses were performed using one-way ANOVA. Significance, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Tables

Table S1 CDM-medium composition (final pH 7).

	Component	Concentration, g/L
Carbon Source	Glucose	15
Amino Acids	L-Arginine	0.72
	L-Asparagine	0.5
	L-Histidine	0.17
	L-Isoleucine	0.24
	L-Leucine	1
	L-Methionine	0.125
	L-Valine	0.7
Vitamins	Niacin	0.01
	Biotin	0.004
	Pantothenate	0.01
	Lipoic acid	0.01
	Folic acid	0.004
	p-Aminobenzoic acid	0.01
	Vitamin B1	0.01
	Vitamin B2	0.01
	Vitamin B6	0.01
	Vitamin B12	0.01
Inorganic Salts	$FeSO_4 \cdot 7H_2O$	0.005
	K_2HPO_4	6.48
	KH_2PO_4	3.12
	$MgCl_2$	0.3864
	NaCl	3
	$ZnSO_4$	0.005
	K_2SO_4	0.023
	Boric acid	0.00075
	$CaCl_2$	0.03
	$CoCl_2 \cdot 6H_2O$	0.00019
	$CuSO_4$	0.00012
	KI	0.00011
	$MnSO_4 \cdot H_2O$	0.00034
	$(NH_4)6Mo_7O_{24} \cdot 4H_2O$	0.00019
Other	L-Glutathione reduced	0.015
	Ammonium citrate dibasic	1.69
	Citric acid$\cdot H_2O$	0.003

81 **Table S2 The component of normal diet, antibiotics and yeast culture supplement.**

Component	FN	FA	FYC
Corn	221.7	220.0	221.7
Whey Powder	150.0	150.0	150.0
Flour	90.0	90.0	90.0
Extruded soybean	100.0	100.0	100.0
Fermented soybean meal Fish	50.0	50.0	45.0
meal	40.0	40.0	40.0
Glucose	50.0	50.0	50.0
Soybean protein concentrate	80.0	80.0	80.0
Soybean oil	30.0	30.0	30.0
Calcium hydrophosphate	6.0	6.0	6.0
L-Lysine Sulphate (70%)	9.0	9.0	9.0
Zinc Oxide	2.1	2.1	2.1
Sodium chloride	3.0	3.0	3.0
Organic acid	3.0	3.0	3.0
L-Threonine (99%)	2.0	2.0	2.0
DL-Methionine (99%)	1.5	1.5	1.5
L-tryptophan (99%)	0.5	0.5	0.5
Choline chloride	0.5	0.5	0.5
Mildew preventive	0.5	0.5	0.5
Antioxidant	0.2	0.2	0.2
1% Premix (S811)	10.0	10.0	10.0
Second head	150.0	150.0	150.0
Enramycin (8%)	-	0.1	-
Quinocetone (50%)	-	0.1	-
Oxytetracycline calcium (20%)	-	1.5	-
Yeast culture	-	-	5.0

82

Table S3 Information of primer pairs designed for qPCR.

Primers	Sequence (5'-3')
P13-epsH1-F	ATTACTCATCAGTCCCATACGCC
P13-epsH1-R	GAATGGCAAAGTGAGGACCAATC
P13-epsJ1-F	GGTTTAGGGTGCTAAATCGTGC
P13-epsJ1-R	AGCTTGTGGTTTTAAGGGCA
P13-epsL-F	CGAAACATTGGCCGCATAGTAG
P13-epsL-R	GTGAAGGTCTTGGGTTAGGAGG
P13-epsD-F	GTGAAGGTCTTGGGTTAGGAGG
P13-epsD-R	TTCGATGAGGATGAGCCATAGC
P13-secA2-F	CACTCATAGACTGGCTGCAATTG
P13-secA2-R	GGGGGCGATTGTCTTAAATCAAG
P13-gtf1-1-F	CCTGAACCCACTGCTTCCAT
P13-gtf1-1-R	TATTTACGGCGAGGGTGGTG
P13-luxS-F	GATCACACAAAGGTTAAGGCACC
P13-luxS-R	GAACAATCAATCACGCCATCCAA