Electronic Supplementary Material (ESI) for Food & Function.

In vitro Colon Fermentation Behaviors of Ca^{2+} Cross-Linked Guluronic Acid Block from Sodium Alginate

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Supplementary data

Characterization of materials: Weight-average molecular weight (Mw), polydispersity (Mw/Mn), and radius of gyration (Rg) of guluronic acid block from sodium alginate (Lot No. W201502) presented here were characterized using GPC-MALLS. Guluronic acid block length was characterized using ^{1}H NMR, and the purity of guluronic acid was characterized using ion chromatography.

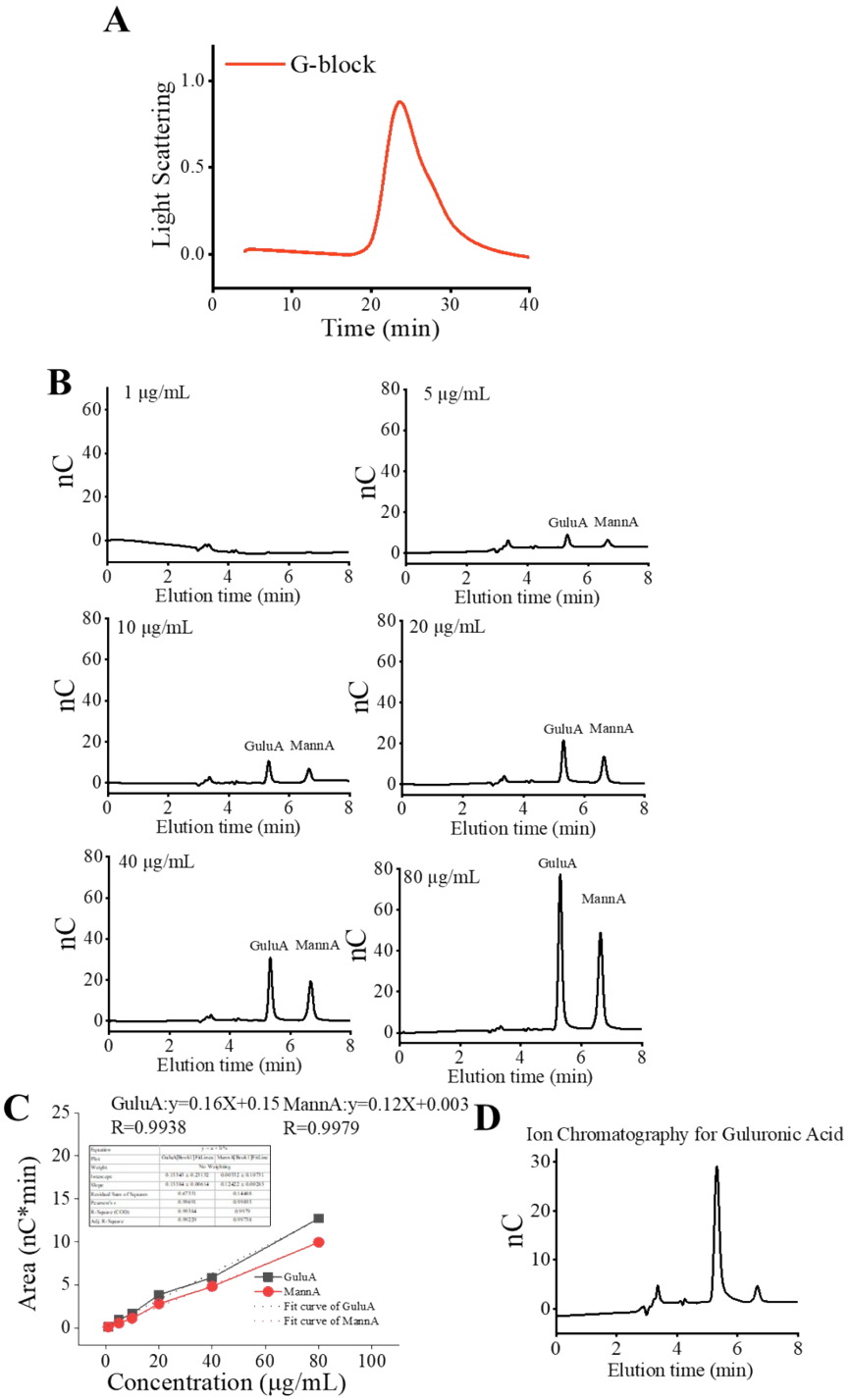

Fig. S1. A, GPC response curve of Guluronic acid block; B, Ion-exchange chromatograms of the guluronic and mannuronic acid standards (1, 5, 10, 20, 40, and 80 μg/mL); C, Fitting curves of standards; D, Ion-exchange chromatograms of guluronic acid monosaccharide from guluronic acid blocks.

Table S1 Molecular and chemical parameters of guluronic acid block

Sample	Mw×10^3 Da	Mw/Mn	Rg (nm)	G-length	GuluA%
G-block	2.281	2.718	39.2	9.5	91.23%

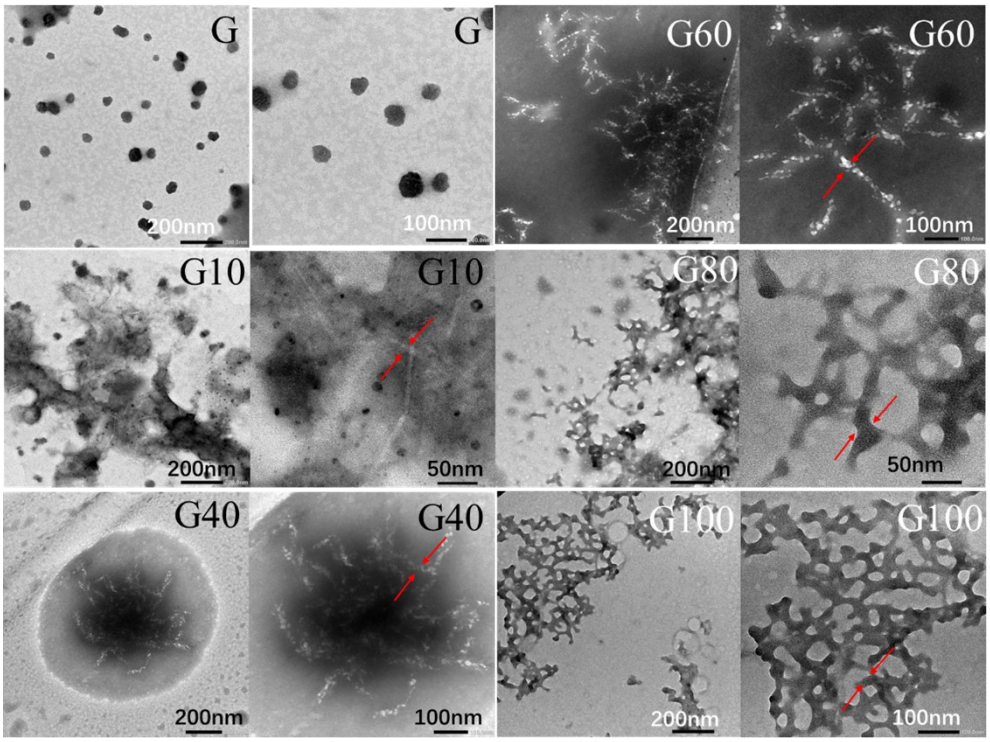

Fig. S2. Representative TEM photographs of GB and the cross-linked GB after *in vitro* mimic gastrointestinal digestion. G, G10, G40, G60, G80, and G100 represented the G cross-linked at calcium-ion concentrations of 0, 10, 40, 60, 80, and 100 mmol/L, respectively.

Table S2 The composition of *in vitro* culture medium

Class	Compound	Content (/L)
Nitrogen source	Yeast extracts	4.0 g
Nitrogen source	Peptone	2.0 g
Nitrogen source	L-cysteine	0.46 g
Salts & Minerals	$MgSO_4$	0.01g
Salts & Minerals	NaCl	0.1g
Salts & Minerals	$CaCl_2$	0.01g
Salts & Minerals	$NaHCO_3$	0.50 g
Vitamins	Vitamin k_1	0.002 g
Buffer compounds	K_2HPO_4	0.04 g
Buffer compounds	KH_2PO_4	0.04 g
Others	Resazurin	0.001 g
Others	Hemin	0.02 g
Others	Bile acids	0.5 g
Others	Tween 80	2.0 mL

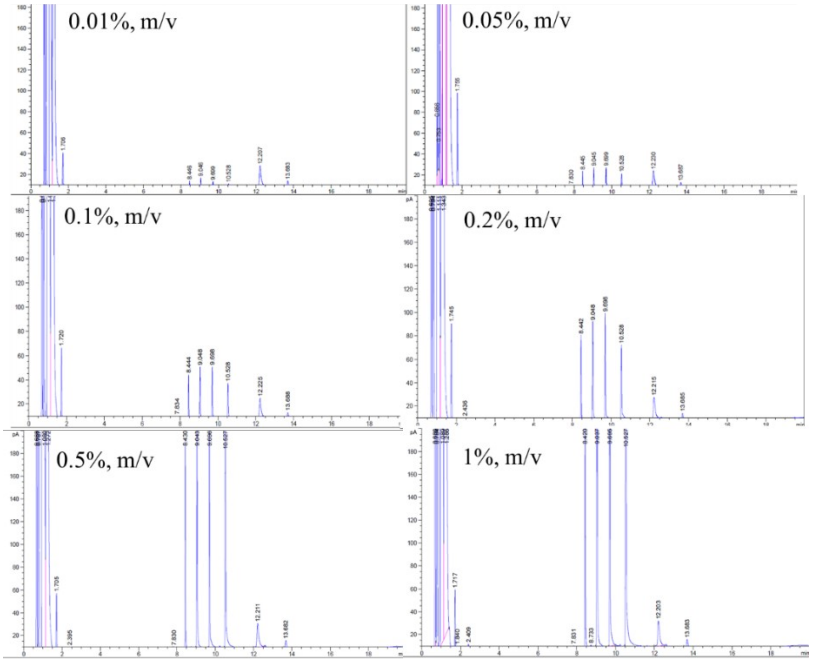

Fig. S3 Gas chromatograms of the acetic, propionic, butyric, and valeric acid standard.

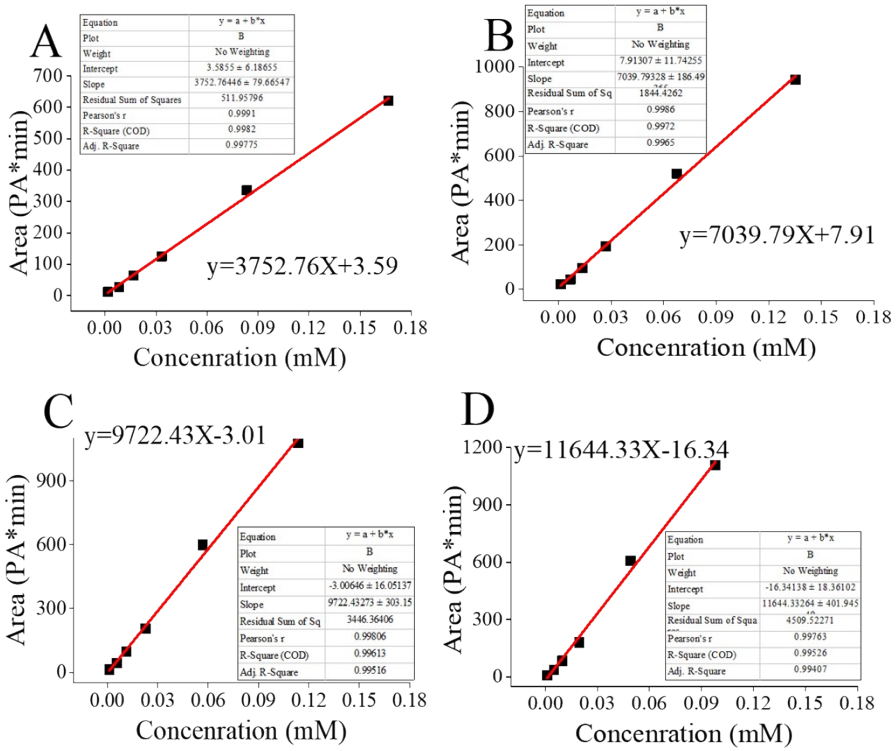

Fig. S4. Fitting curve of SCFA standards. A, acetic, B, propionic, C, butyric, and D, valeric acid.

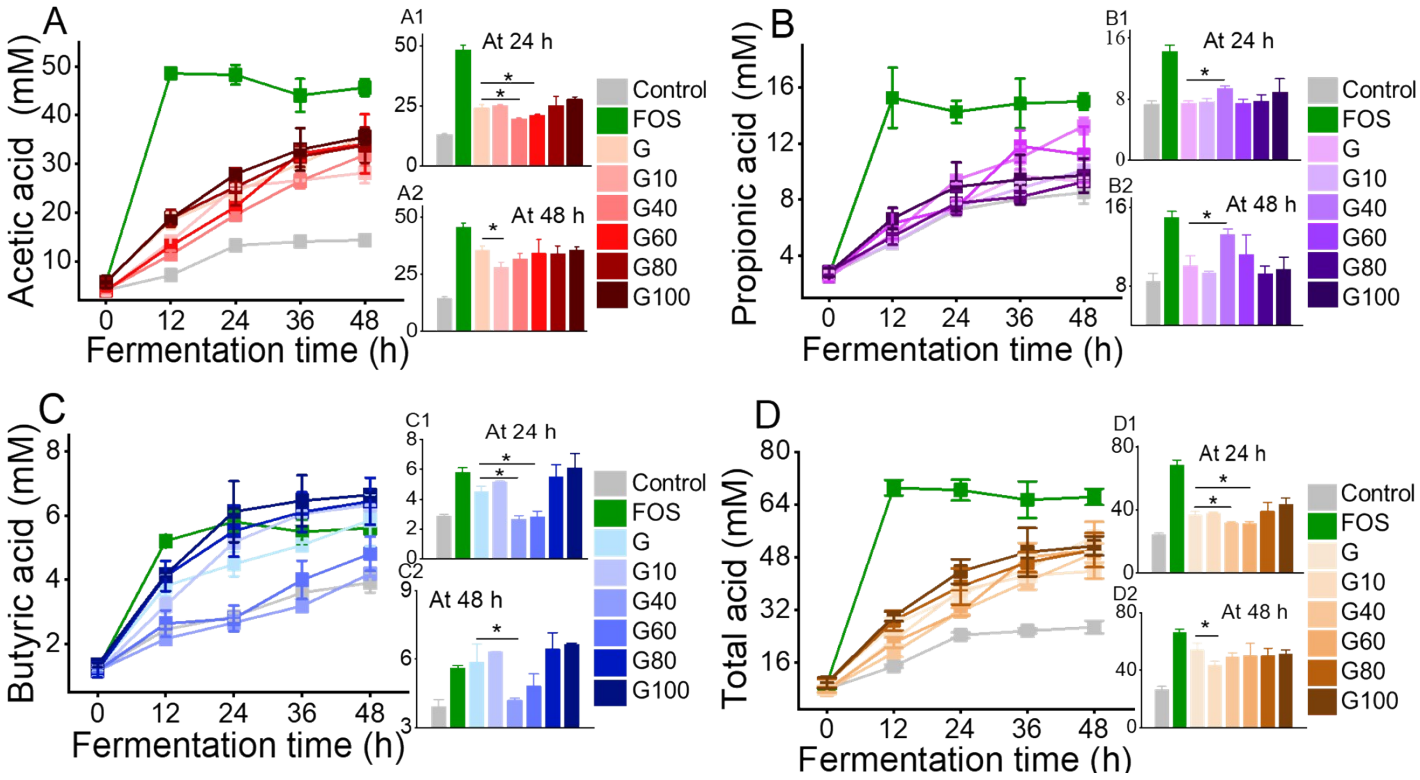

Fig. S5. Tendency of SCFA concentration in GB and cross-linked GB during *in vitro* fermentation.

Table S3 Variations of Bound and Free calcium concentration in GB and cross-linked GB during *in vitro* fermentation

State	Control	FOS	G	G10	G40	G60	G80	G100
Bound								
0	1.99±0.33	3.09±0.33	2.24±0.56	12.84±0.56	17.83±0.95	27.31±2.23	13.27±0.56	13. 91±1.16
12	0.14±0.51	0.31±0.51	0.92±0.22	12.83±1.32	15.47±1.02	27.20±0.23	12.43±1.32	12.81±1.25
24	0.32±0.38	0.11±0.38	0.24±0.08	5.37±0.88	8.08±0.67	26.16±5.15	5.26±0.67	5.55±0.48
36	0.45±1.08	0.35±1.08	0.15±0.05	2.31±0.45	6.68±0.98	5.62±0.48	4.17±0.88	4.46±0.88
48	0.32±0.25	0.42±0.25	0.15±0.04	3.34±0.14	2.31±0.77	5.30±0.45	2.54±0.77	2.20±0.14
Free								
0	8.55±0.33	9.14±0.33	8.38±0.56	12.77±1.23	15.45±3.76	20.98±6.23	12.45±1.76	11.45±2.11
12	8.80±0.87	8.78±0.51	8.88±0.87	12.08±0.96	19.73±3.21	22.13±8.23	12.73±2.21	12.35±1.74
24	8.63±2.26	8.50±0.38	8.99±2.26	14.54±1.52	20.27±0.52	22.65±0.59	12.27±0.52	13.81±0.52
36	8.59±1.08	8.56±1.08	8.67±0.45	15.51±1.33	25.33±0.54	27.30±0.62	18.33±0.54	17.33±0.54
48	8.91±0.25	8.84±0.25	9.70±0.26	18.55±1.78	28.33±0.24	36.53±1.09	19.33±0.24	19.33±0.24

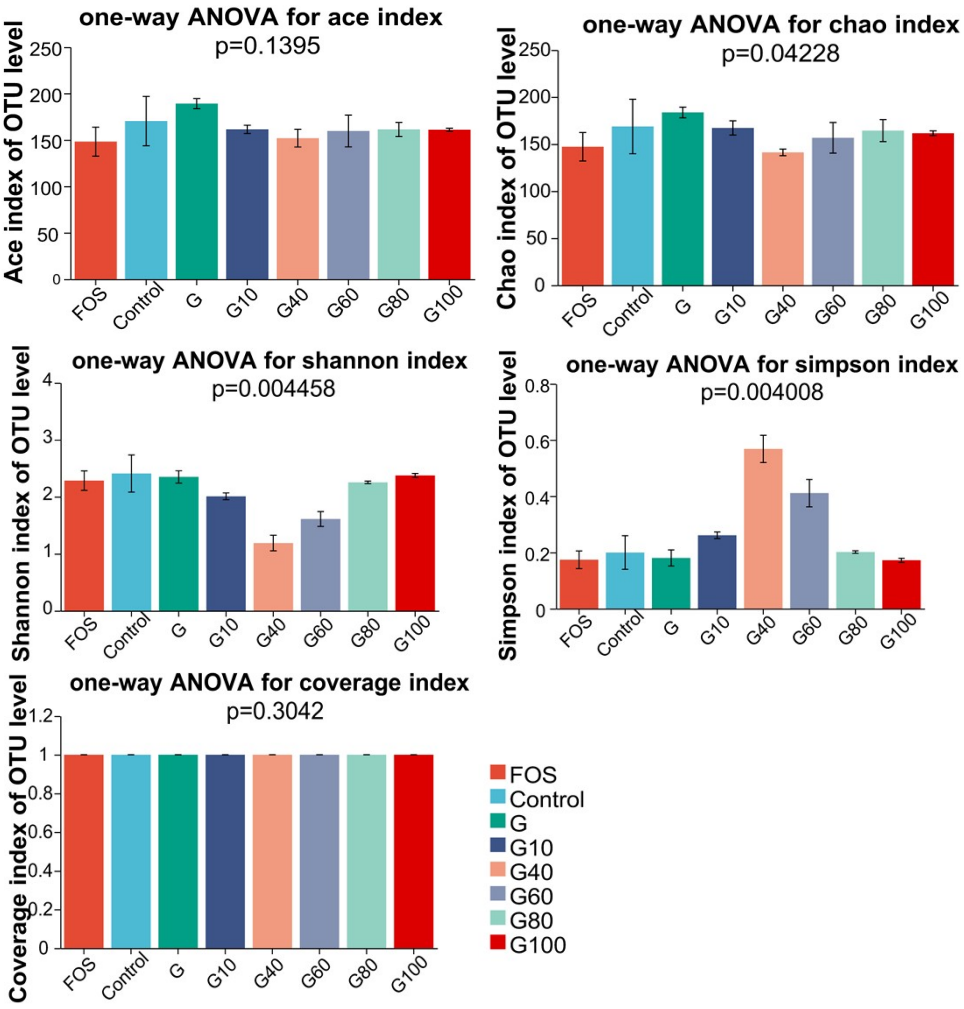

Fig. S6. Alpha diversity index of all groups after 36h' fermentation.

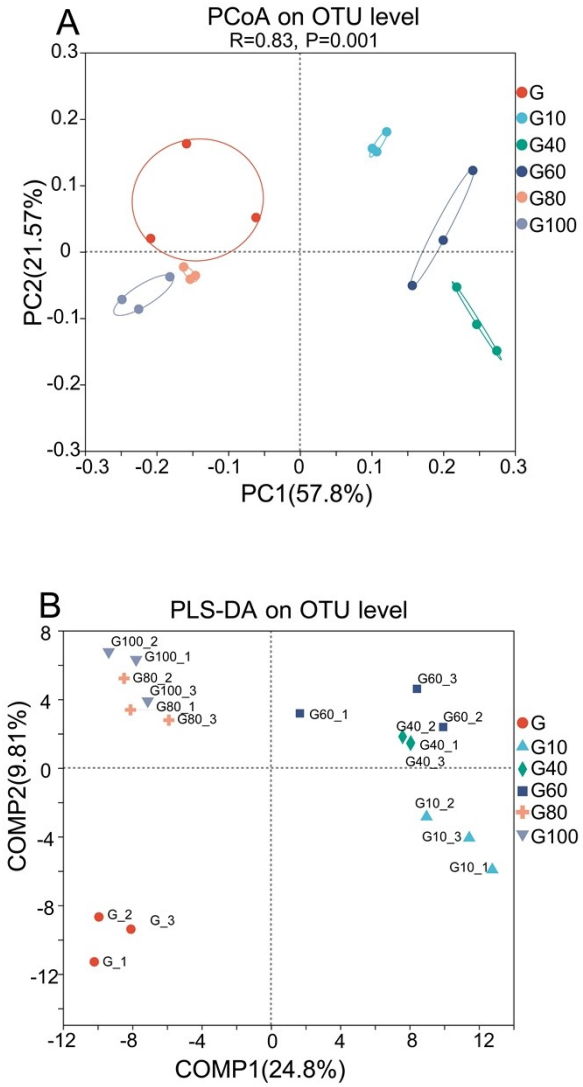

Fig. S7. Beta diversity index of the six experimental groups after 36 h' fermentation.

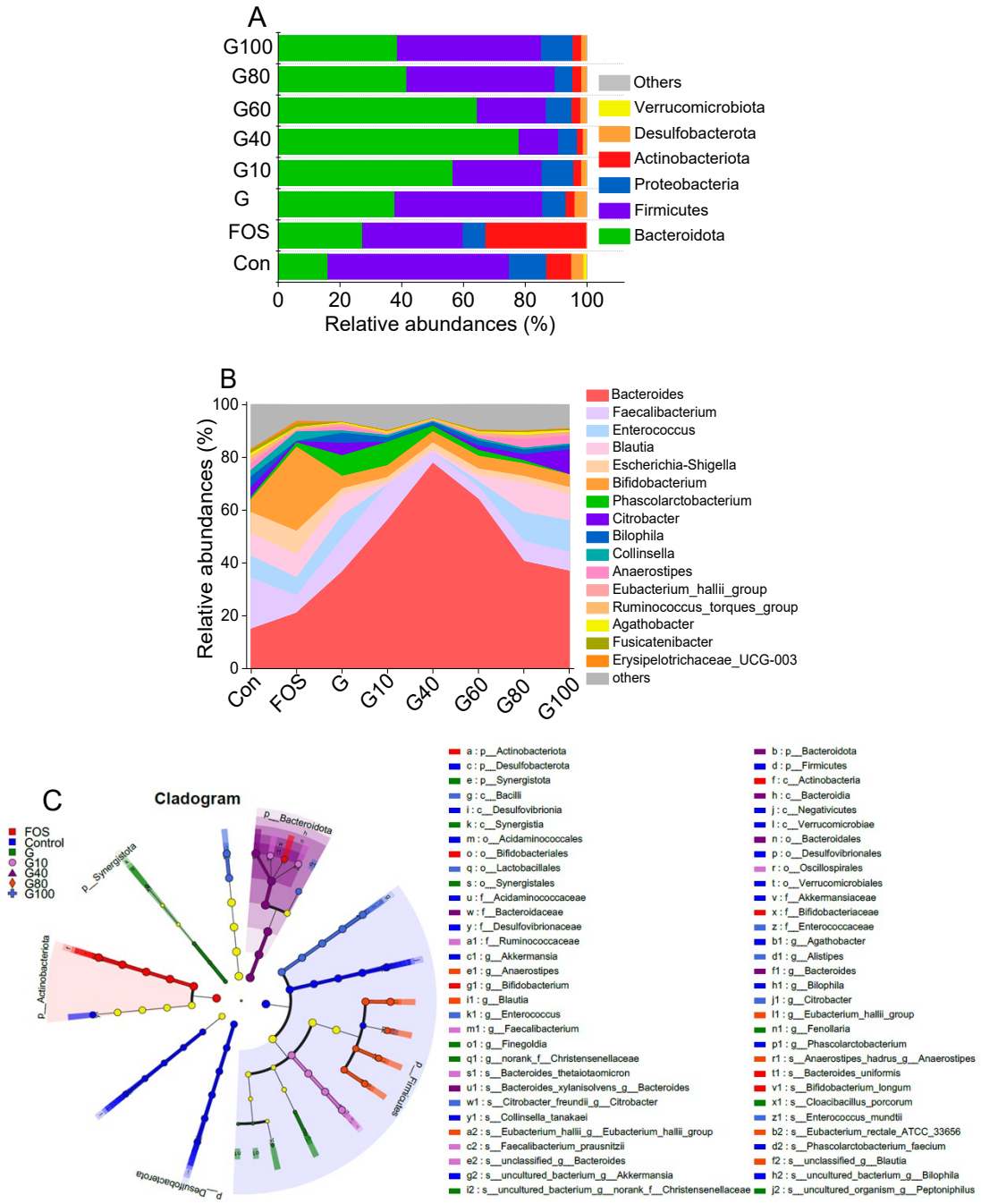

Fig. S8 Microbial community at the phylum level after 36h' fermentation.

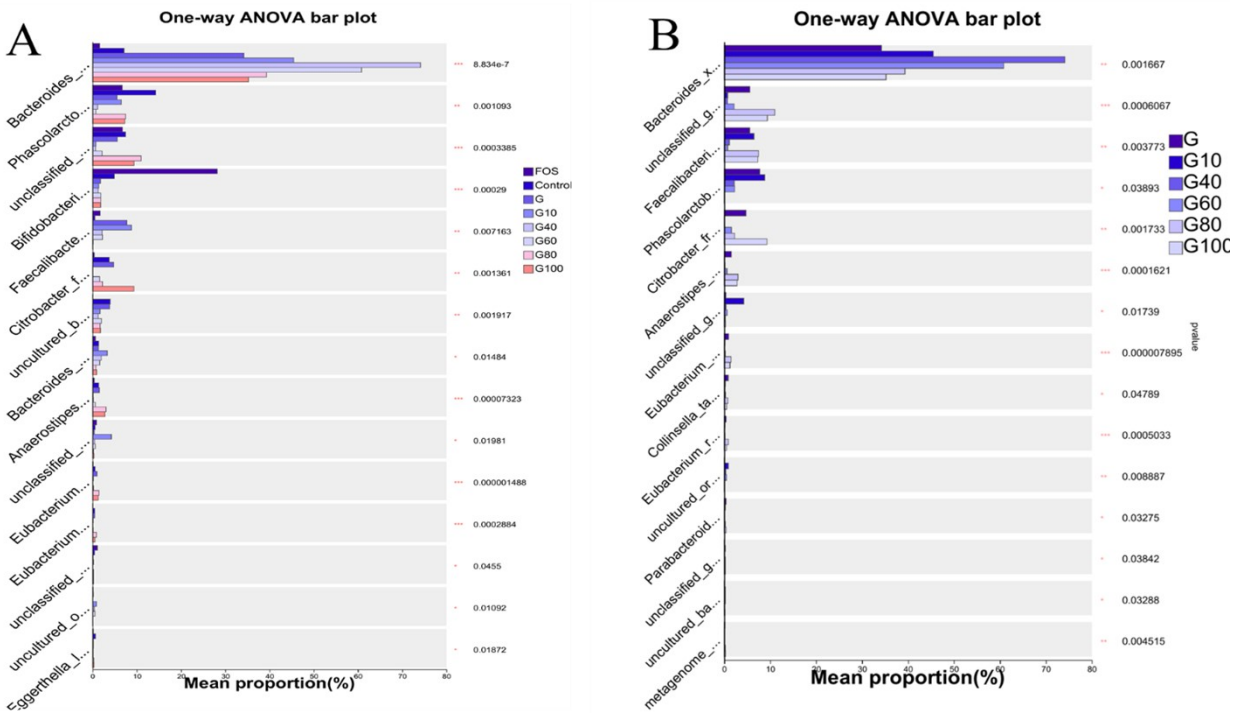

Fig. S9. Significance differences between (A) all groups, (B) experimental groups using one-way ANOVA test.