

**Restoration of ethanol-induced *Bifidobacterium pseudocatenulatum*
depletion ameliorates alcoholic liver disease**

Supplementary Materials

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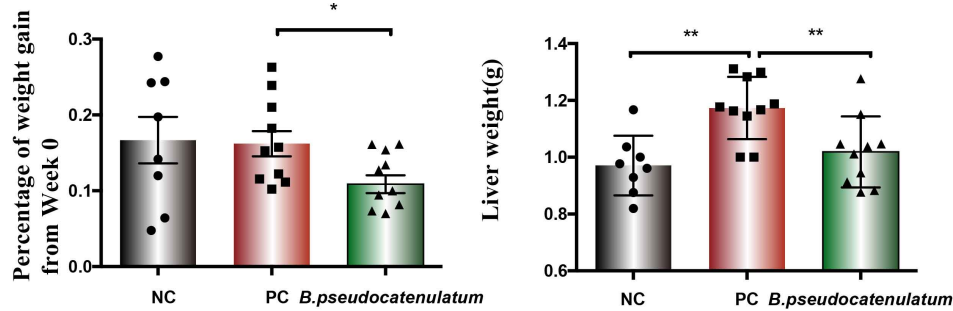
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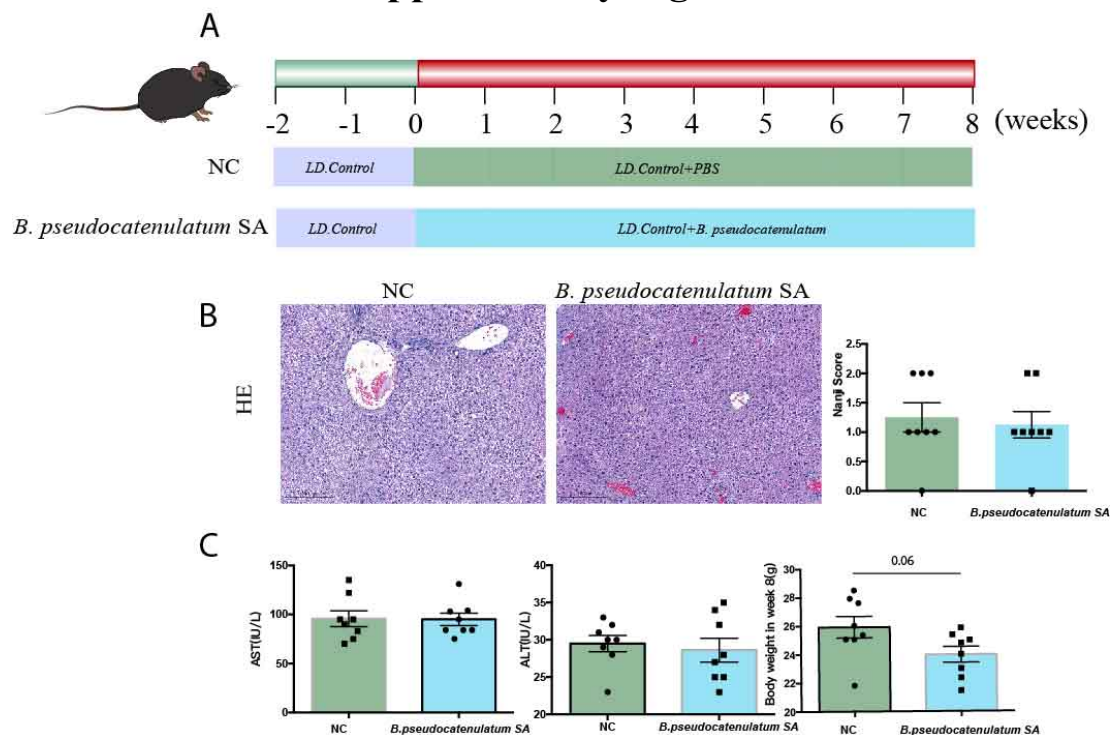
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Supplementary Figure 1



Supplementary Figure1. Effects of *Bifidobacterium pseudocatenulatum* administration on body weight gain and liver weight in mice. Data are presented as the mean $\pm$ SEM (N: Normal Control [NC]=8, Positive Control [PC]=10, *B. pseudocatenulatum*=10). Statistical analysis was performed using one-way ANOVA followed by Bonferroni post hoc correction (for equal variances) or Dunnett's T3 test (for unequal variances). * $P < 0.05$, ** $P < 0.01$

Supplementary Figure 2



Supplementary Figure2. Safety Assessment of *B. pseudocatenulatum*

(A) Schematic of the Lieber-DeCarli control diet feeding model and the experimental design for *Bifidobacterium pseudocatenulatum* intervention.

(B) Assessment of hepatic histopathology: Hematoxylin and Eosin (H&E) staining.

(C) Hepatic levels of triglycerides, and serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

(N: Normal Control [NC]=8, *B. pseudocatenulatum* Safety Assessment [*B. pseudocatenulatum* SA]=8)

Statistical analysis was performed using independent samples t-test. * $P < 0.05$ and ** $P < 0.01$ compared to the NC group.

Supplementary Table 1**Metabolite levels varied between the PC and *B. pseudocatenulatum* groups**

Metabolite	VIP	P value	FC	Average RT	Quant mass	Peak areas (LI09)	Peak areas (PC)
Digalacturonic Acid	2.742	0.000	1.493	18.27	111	1078.66	722.53
Cerotinic Acid	2.707	0.000	0.539	27.23	117	4850.61	8998.03
Behenic acid	2.669	0.000	0.512	28.95	117	33039.38	64559.16
11-Eicosenoic acid	2.556	0.000	0.422	25.1	367	9384.10	22259.86
Lignoceric Acid	2.572	0.000	0.552	32.29	117	27587.49	49966.57
Daidzein	2.407	0.001	0.498	29.94	383	2033.56	4080.65
3,4-Dihydroxy-phenylacetic Acid	2.349	0.002	0.699	16.38	233	344.04	492.21
18-Methylnon- adecane-1,2-diol	2.227	0.003	0.529	27.27	355	5933.47	11222.44
6-Deoxyglucose	2.086	0.003	1.385	15.31	85	35927.43	25944.44
Z Phthalate	2.194	0.003	0.600	30.75	411	1778.24	2964.04
2-Aminoethyl methacrylate	2.089	0.003	1.498	26.23	174	102534.08	68448.64
Alkane	2.047	0.004	1.392	14.84	85	115231.14	82780.47
Inositol-4-Mono- phosphate	1.998	0.005	1.475	26.89	519	11024.99	7475.63
Pipecolic Acid	2.040	0.006	1.612	10.76	156	1722.47	1068.73
Nonadecanoic acid	2.043	0.006	0.777	23.84	117	5814.94	7482.04
Aspartate	1.962	0.007	1.255	11.47	373	4060.60	3236.10
Galacturonic Acid	2.013	0.007	0.034	23.63	243	1040.59	30717.44
Pantothenic acid	1.987	0.009	0.621	18.6	291	3031.11	4884.49
Montanic Acid	1.941	0.011	0.668	30.61	117	12493.03	18703.48
Chenodeoxy- cholic Acid	1.919	0.014	1.371	24.36	85	2201.42	1605.24
Palmitelaidic acid	1.783	0.015	0.486	18.78	117	3514.14	7228.23
5-Hydroxy-pentanoic	1.756	0.016	1.373	10.28	85	9931.79	7233.01

Metabolite	VIP	<i>P</i> value	FC	Average RT	Quant mass	Peak areas (LI09)	Peak areas (PC)
acid							
Kynurenine	1.752	0.017	1.296	15.16	111	2136.04	1647.89
Uridine	1.718	0.019	0.055	26.26	85	4290.04	77735.01
Octadecylglycerol	1.808	0.020	0.423	29.78	205	7888.87	18629.01
Xanthosine	1.682	0.025	2.051	32.93	325	7936.44	3868.93
Deoxycholic Acid	1.683	0.026	0.529	39.73	255	180302.67	340887.83
N-Acetylputrescine	1.678	0.027	1.248	10.14	111	7378.85	5912.19
Hydroxycarbamate	1.639	0.029	1.204	10.68	221	2006.96	1667.51
5-Aminovaleric acid	1.605	0.032	1.760	14.01	174	44121.99	25066.08
Urea	1.563	0.038	14.547	9.13	147	191422.18	13158.68
1-Monoolein	1.469	0.040	1.291	31.03	113	6795.88	5263.98
Dodecanoic acid	1.671	0.041	0.810	14.18	257	4352.21	5371.29
Cysteine-Glycine	1.496	0.041	14.840	13.8	257	22970.20	1547.88
Urocanic Acid	1.615	0.043	0.676	18.53	267	1721.90	2547.62
2-imidazolidinone	1.486	0.048	1.938	10.33	215	525.69	271.24
N-Acetyl-5-Hydroxytryptamine	1.399	0.048	34.731	13.66	103	18095.22	521.01

Supplementary Table 2

Metabolite levels varied between the PC and NC group

Metabolite	VIP	<i>P</i> value	FC	Average RT	Quant mass	Peak areas (NC)	Peak areas (PC)
Galactinol	2.610	0.000	0.374	29.53	204	7769.12	2907.60
Isochlorogenic Acid	1.686	0.003	1.339	24.44	415	694.48	929.68
Inositol-4-Monophosphate	1.631	0.006	1.901	26.89	519	3932.63	7475.63
18-Methyl-nonadecane-1,2-diol	1.880	0.008	0.651	27.27	355	17235.34	11222.44
Hexose-6-Phosphate	1.578	0.024	0.504	17.18	269	3422.56	1725.46

Metabolite	VIP	<i>P</i> value	FC	Average RT	Quant mass	Peak areas (NC)	Peak areas (PC)
Trans-4-Hydroxy-L-Proline	1.555	0.026	0.700	11.72	158	1517.59	1062.96
Glycerol-1-Phosphate	1.566	0.040	0.777	13.69	211	3400.35	2642.46
Uracil	2.005	0.001	2.307	10.42	241	26784.32	61798.54
Glycerol-Alpha-Phosphate	2.071	0.001	0.520	17.99	299	1473.97	766.07
Beta-Glycerophosphoric acid	1.519	0.045	0.769	15.26	243	6759.74	5197.76
Urocanic Acid	1.970	0.001	1.911	18.53	267	1333.37	2547.62
Daidzein	2.213	0.001	0.566	29.94	383	7215.15	4080.65
Fructose	2.422	0.000	3.331	13.45	103	359.96	1198.97
D-Arabinose	1.797	0.004	1.899	14.75	103	82129.85	155976.21
Glucose	1.656	0.010	2.099	18.09	159	4533.11	9516.32
Galacturonic Acid	1.649	0.011	31.812	23.63	243	965.59	30717.44
1-Kestose	1.747	0.011	0.531	30.03	361	1097.04	582.74
Shikimic Acid	1.721	0.013	0.673	12.12	204	1580.50	1063.46
N-Acetylgalactosamine	1.616	0.013	2.670	20.17	205	5716.19	15262.07
Trehalose-6-Phosphate	1.639	0.021	0.662	34.07	243	1433.54	949.33
Allose	1.448	0.025	1.577	21.19	319	4094.10	6456.24
cis-4-Hydroxycyclohexylacetic acid	1.327	0.043	1.721	13.82	170	1330.82	2290.65
Sedoheptulose	1.294	0.049	1.442	21.13	319	3943.05	5684.57
Indoxyl Sulfate	1.796	0.005	0.571	15.76	277	2351.36	1341.94
Hypoxanthine	1.963	0.001	2.009	16.06	265	25328.75	50888.19
Adenine	2.056	0.003	0.305	16.81	264	8824.45	2692.16
Stigmasterol	1.521	0.050	0.062	39.39	129	114725.94	7077.85
Pseudo Uridine	1.904	0.002	2.630	24.39	217	2367.23	6225.89
Cerotinic Acid	2.585	0.000	0.477	27.23	117	18846.00	8998.03
Lignoceric Acid	2.254	0.000	0.599	32.29	117	83389.80	49966.57

Metabolite	VIP	<i>P</i> value	FC	Average RT	Quant mass	Peak areas (NC)	Peak areas (PC)
Montanic Acid	1.912	0.006	0.672	30.61	117	27812.62	18703.48
4-Hydroxy-butyric Acid	1.686	0.008	1.254	9.01	281	323.28	405.45
Palmitic Acid	1.430	0.022	1.166	19.2	313	990786.04	1155070.76
2-Hydroxy-caproic acid	1.438	0.027	2.965	9.05	159	270.72	802.73
Arachidonic acid	1.324	0.047	0.622	24.32	91	14825.45	9222.88
Capric acid	1.027	0.049	0.701	11.85	229	1231.87	863.38
Delta-Tocopherol	2.396	0.000	0.527	33.38	439	1810.46	953.93
Noradrenaline	1.662	0.043	0.722	16.33	266	553.08	399.26
Piceatannol	2.327	0.000	0.623	34.86	145	1390.40	865.55
Cysteine-Glycine	1.646	0.009	1.450	13.8	257	1067.15	1547.88
Isoleucine	1.619	0.011	2.349	9.84	158	23814.21	55942.13
Valine	1.591	0.012	2.073	8.8	144	39918.63	82750.61
L-Phenylalanine	1.507	0.020	2.523	14.02	218	8074.10	20369.44
Ornithine	1.468	0.032	0.611	16.24	174	2408.75	1471.28
Proline	1.258	0.033	1.355	9.89	142	53293.34	72233.26
Saccharopine	1.258	0.036	1.712	26.62	221	1359.36	2327.69
5-Aminova-leric acid	1.510	0.039	0.548	14.01	174	45726.17	25066.08
L-Glutamic acid	1.266	0.041	2.102	13.93	246	10600.70	22282.61
Xanthosine	1.521	0.018	16.381	32.93	325	236.18	3868.93
1-Methylinosine	1.298	0.039	1.343	41.89	131	3017.15	4050.99
Udp-Glucuronic Acid	1.361	0.037	2.529	16.26	109	4201.83	10624.99
Pantothenic acid	1.857	0.002	2.019	18.6	291	2419.49	4884.49

Supplementary Table 3

Metabolite levels varied between the NC and *B. pseudocatenulatum* group

Metabolite	VIP	<i>P</i> value	FC	Average RT	Quant mass	Peak areas (LI09)	Peak areas (NC)
Cerotinic Acid	2.387	0.000	0.257	27.23	117	4850.61	18846.00
Lignoceric Acid	2.384	0.000	0.331	32.29	117	27587.49	83389.80
Inositol-4- Monophosphate	2.368	0.000	2.803	26.89	519	11024.99	3932.63
Galactinol	2.337	0.000	0.319	29.53	204	2478.61	7769.12
Daidzein	2.296	0.000	0.282	29.94	383	2033.56	7215.15
Xanthosine	2.188	0.000	33.603	32.93	325	7936.44	236.18
18-Methyl-nonad- ecane-1,2-diol	2.181	0.000	0.344	27.27	355	5933.47	17235.34
1-Monoolein	2.073	0.000	1.631	31.03	113	6795.88	4165.60
Arachidonic acid	2.036	0.000	0.343	24.32	91	5087.11	14825.45
Adenine	1.983	0.000	0.265	16.81	264	2342.86	8824.45
Alkane	2.023	0.000	1.560	14.84	85	115231.14	73853.49
Octadecylglycerol	1.985	0.000	0.275	29.78	205	7888.87	28663.09
6-Deoxyglucose	2.008	0.000	1.555	15.31	85	35927.43	23097.38
4-Aminobutyric Acid	1.939	0.001	0.428	11.79	117	471.45	1100.97
Montanic Acid	1.991	0.001	0.449	30.61	117	12493.03	27812.62
Piceatannol	1.932	0.001	0.540	34.86	145	750.72	1390.40
Sulfuric Acid	1.889	0.001	0.558	10.95	227	769.69	1379.56
Isocitric Acid	1.816	0.002	1.438	12.8	111	2638.95	1835.43
Aspartate	1.789	0.003	1.389	11.47	373	4060.60	2924.18
Putrescine	1.809	0.003	0.459	16.61	174	2732.65	5953.84
Nonadecanoic acid	1.772	0.003	0.674	23.84	117	5814.94	8632.20
N-Acetylornithine	1.786	0.003	0.557	30.07	174	1015.56	1822.40
Udp-Glucuronic Acid	1.728	0.004	2.713	16.26	109	11398.40	4201.83
11-Eicosenoic acid	1.735	0.005	0.316	25.1	367	9384.10	29695.02
5-Hydroxypentanoic acid	1.721	0.005	1.451	10.28	85	9931.79	6842.66
L-Phenylalanine	1.654	0.007	1.975	14.02	218	15946.13	8074.10
N-Acetylputrescine	1.663	0.008	1.311	10.14	111	7378.85	5627.29
Hexose-6-Phosphate	1.578	0.009	0.489	17.18	269	1672.23	3422.56

Metabolite	VIP	<i>P</i> value	FC	Average RT	Quant mass	Peak areas (LI09)	Peak areas (NC)
Hydroxycarbamate	1.587	0.011	1.308	10.68	221	2006.96	1533.97
Valine	1.569	0.012	1.767	8.8	144	70545.38	39918.63
L-Lysine	1.553	0.012	0.572	17.56	174	7042.27	12308.59
Trehalose-6-Phosphate	1.578	0.012	0.522	34.07	243	747.62	1433.54
Isoleucine	1.544	0.014	1.974	9.84	158	46998.35	23814.21
Kynurenine	1.575	0.014	1.354	15.16	111	2136.04	1578.01
2-Aminoethyl methacrylate	1.535	0.016	1.434	26.23	174	102534.08	71508.54
Indoxyl Sulfate	1.502	0.016	0.561	15.76	277	1319.91	2351.36
Glycerol-Alpha-Phosphate	1.543	0.018	0.558	17.99	299	823.17	1473.97
Isochlorogenic Acid	1.452	0.022	1.245	24.44	415	864.61	694.48
3-Epicholic Acid	1.433	0.026	0.355	39.5	253	98289.36	276684.06
4-Hydroxybutyric Acid	1.416	0.027	1.237	9.01	281	399.97	323.28
Z Phthalate	1.363	0.034	0.674	30.75	411	1778.24	2637.70
Tripropylene glycol monomethyl ether	1.378	0.040	1.173	18.33	117	2423.02	2065.22
Trans-4-Hydroxy-L-Proline	1.256	0.043	0.696	11.72	158	1056.71	1517.59
Hydroxytyrosol	1.320	0.043	1.596	15.6	267	1698.28	1063.81
Dodecanoic acid	1.285	0.045	0.809	14.18	257	4352.21	5381.25
Phytosphingosine	1.265	0.046	0.582	29.26	204	45767.60	78684.98
Adipic Acid	1.309	0.047	1.210	10.02	111	7323.67	6052.13
1-Kestose	1.281	0.049	0.601	30.03	361	658.90	1097.04

Differential metabolites are ranked by ascending *P* values. RT: retention time, VIP: variable weight value, FC: fold change, and abundance is indicated by the detected peak's area.

Supplementary Table 4

PCR primer sets for RT-PCR analysis of the indicated genes

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
IL-1 β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
IL-4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT

IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
IL-10	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG
TNF- α	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
CCL2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
TLR2	CACCACTGCCCCGTAGATGAAG	AGGGTACAGTCGTCGAACTCT
TLR4	GCAGGATCATGGCATGTCAAC	ATCTGGGTGAGGTTACAGCCT
TLR-5	ATGTGGACACGGAAGAGACAA	ACCATCGAAACCCAAAGACTC
TLR-7	ATGGCATGGCTTACACCACC	GAGGCCAATTTTGTCTCCACA
TLR-9	ACAACCTCTGACTTCGTCCACC	TCTGGGCTCAATGGTCATGTG
TLR-12	GAGGGAGTACAGCCCTCAGA	AGGGAACCCCTAGCAGAGAG
Myd88	AGGACAAACGCCGGAACTTTT	GCCGATAGTCTGTCTGTTCTAGT
ZO-1	GCCGCTAAGAGCACAGCAA	GCCCTCCTTTTAACACATCAGA
Occludin	TGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
GAPDH	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
ACTIN	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA
CD36	ATGGGCTGTGATCGGAACTG	TTTGCCACGTCATCTGGGTTT
FASN	GGCTCTATGGATTACCCAAGC	CCAGTGTTTCGTTCTCGGA
ACC	GATGAACCATCTCCGTTGGC	GACCCAATTATGAATCGGGAGTG
FASN	GGCTCTATGGATTACCCAAGC	CCAGTGTTTCGTTCTCGGA
CPT1A	AGATCAATCGGACCCTAGACAC	CAGCGAGTAGCGCATAGTCA
SREBP1C	CTTTGGCCTCGCTTTTCGG	TGGGTCCAATTAGAGCCATCTC
PPAR- α	AACATCGAGTGTGCAATATGTGG	CCGAATAGTTCGCCGAAAGAA
PPAR- γ	GGAAGACCACTCGCATTCCTT	GTAATCAGCAACCATTGGGTCA
