

Modulation and adaptation of gut microbial metabolic functions under probiotic and postbiotic treatment using a novel *in vitro* anaerobic pseudo-colon system

Nehal¹, Prangya Ranjan Rout², Priyankar Dey^{1*}

¹ Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala 147004, Punjab, India

² Department of Biotechnology, Dr. B.R. Ambedkar National Institute of Technology, Jalandhar, India

*Corresponding author

Email: priyankar.dey@thapar.edu; priyankardey28@gmail.com

Mob: +91-9064275660

Supplementary Table 1: Characteristics of the fecal matter donor

Category	Features
Age	20-40
Sex	Male/Female
Type of diet	Vegetarian
Antibiotic usage history	>12 months
Dietary fiber intake	No
Probiotic intake	No
Western diet	Yes
Smoking	No
Drinking	No
Medical history	No
Family history of chronic disease	No
Diarrhea history	No
Physical activity	Yes

Supplementary Table 2: List of common phyla identified under various treatments.

Treatment	Total	Phylum
Control, butyrate, and <i>L. plantarum</i>	4	Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes
<i>L. plantarum</i>	1	Tenericutes

Supplementary Table 3: List of common orders identified under various treatments.

Treatment	Total	Orders
Control, butyrate, and <i>L. plantarum</i>	13	Acidaminococcales, Clostridiales, Bacillales, Alteromonadales, Tissierellales, Enterobacterales, Veillonellales, Selenomonadales, Eggerthellales, Lactobacillales, Bifidobacteriales, Bacteroidales, Micrococcales
Control and butyrate	2	Burkholderiales, Pseudomonadales
Butyrate and <i>L. plantarum</i>	5	Desulfovibrionales, Coriobacteriales, Vibrionales, Streptomycetales, Thiotrichales
Control	2	Rhodocyclales, Rickettsiales
Butyrate	8	Sphingomonadales, Erysipelotrichales, Rhizobiales, Haloplasmatales, Marinilabiliales, Chromatiales, Rhodobacterales, Oceanospirillales
<i>L. plantarum</i>	7	Aeromonadales, Corynebacteriales, Neisseriales, Chitinophagales, Flavobacteriales, Myxococcales, Acholeplasmatales

Supplementary Table 4: List of common genera identified under various treatments.

Treatment	Total	Genera
Control, butyrate, and <i>L. plantarum</i>	41	<i>Dorea</i> , <i>Lactonifactor</i> , <i>Enterococcus</i> , <i>Shigella</i> , <i>Marseillibacter</i> , <i>Clostridium</i> , <i>Escherichia</i> , <i>Dialister</i> , <i>Coprococcus</i> , <i>Oscillibacter</i> , <i>Alistipes</i> , <i>Eubacterium</i> , <i>Bacillus</i> , <i>Citrobacter</i> , <i>Blautia</i> , <i>Bifidobacterium</i> , <i>Anaerococcus</i> , <i>Cronobacter</i> ,

		<i>Salmonella, Oscillospira, Lachnoclostridium, Roseburia, Acidaminococcus, Megasphaera, Serratia, Gemmiger, Ruthenibacterium, Prevotella, Bacteroides, Intestinimonas, Eggerthella, Parabacteroides, Anaerofustis, Pantoea, Ruminococcus, Faecalibacterium, Tyzzerella, Mitsuokella, Klebsiella, Enterobacter, Lactobacillus</i>
Control and butyrate	9	<i>Flintibacter, Mobilitalea, Tissierella, Dakarella, Christensenella, Peptoniphilus, Lactococcus, Lachnospira, Parasutterella</i>
Control and <i>L. plantarum</i>	5	<i>Streptococcus, Yersinia, Butyrivibrio, Averyella, Odoribacter</i>
Butyrate and <i>L. plantarum</i>	6	<i>Raoultella, Erwinia, Collinsella, Streptomyces, Butyricoccus, Alkaliphilus</i>
Control	20	<i>Orientia, Vagococcus, Acetivibrio, Tatumella, Psychrobacter, Leuconostoc, Anaerocolumna, Lonsdalea, Dickeya, Candidatus Azobacteroides, Subdoligranulum, Kluyvera, Listeria, Siccibacter, Macrococcus, Weissella, Pectobacterium, Alicyclobacillus, Desulfuribacillus, Tetrigenococcus</i>
Butyrate	52	<i>Pseudoflavonifractor, Hespellia, Facklamia, Pseudomonas, Romboutsia, Oxalobacter, Cohnella, Ruminiclostridium, Catabacter, Natranaerovirga, Thermophagus, Neofamilia, Asaccharospora, Murimonas, Microbacterium, Bittarella, Arsenophonus, Finegoldia, Kocuria, Haloplasma, Anaerostipes, Colwellia, Dielma, Flavonifractor, Intestinibacter, Desulfovibrio, Hydrogenoanaerobacterium, Xenorhabdus, Halomonas, Erysipelatoclostridium, Morganella, Anaeromassilibacillus, Keratinibaculum, Proteus, Negativicoccus, Clostridioides, Eisenbergiella, Rhodopseudomonas, Sodalis, Gordonibacter, Bilophila, Paeniclostridium, Anaerotaenia, Peptostreptococcus, Clostridiisalibacter, Lagierella, Thioalkalicoccus, Anaerofilum, Dysgonomonas, Anaerotruncus, Psychrobium, Porphyromonas,</i>
<i>L. plantarum</i>	12	<i>Pseudobutyrvibrio, Micrococcus, Angelakisella, Candidatus Phytoplasma, Rahnella, Veillonella, Anaeromyxobacter, Gordonia, Aeromonas, Vibrio, Cellulosilyticum, Peptoclostridium</i>

Supplementary Table 5: List of metabolites identified in Control.

S. No.	Name of compounds	Mol. Formula	Mol. Wt.	RT (min)	Abundance (%)	Probability
1	2,3-Hexanediol	C ₆ H ₁₄ O ₂	118.17	7.924	0.060	26.2
2	1,2-Pentanediol	C ₅ H ₁₂ O ₂	104.15	8.0302	0.070	23.1
3	Methylmalonic acid	C ₄ H ₆ O ₄	118.09	8.1011	0.904	29.4
4	2-Methyl-3-hexanol	C ₇ H ₁₆ O	116.20	8.16	1.446	24.7
5	Phenylacetyl glycine	C ₁₀ H ₁₁ NO ₃	193.20	8.3648	0.071	44.1
6	Glu-Gly	C ₇ H ₁₂ N ₂ O ₅	204.18	21.6791	0.087	9.03
7	2-Butanol	C ₄ H ₁₀ O	74.12	21.6989	0.123	26.5
8	Octanoic acid	C ₈ H ₁₆ O ₂	144.21	21.7553	53.851	74.6
9	Nonanoic acid	C ₉ H ₁₈ O ₂	158.24	24.4351	0.205	44.5
10	Indole	C ₈ H ₇ N	117.15	27.0348	8.677	22.5
11	Capric acid	C ₁₀ H ₂₀ O ₂	172.26	27.1154	28.618	73.9
12	L-Saccharopine	C ₁₁ H ₂₀ N ₂ O ₆	276.29	32.0619	0.193	50
13	Myristic acid	C ₁₄ H ₂₈ O ₂	228.37	36.2736	3.578	42.7
14	Octadecanamide	C ₁₈ H ₃₇ NO	283.5	36.3335	0.230	34.9
15	DL-Phenylalanine	C ₉ H ₁₁ NO ₂	165.19	43.5152	0.044	12.2
16	Indole-3-lactic acid	C ₁₁ H ₁₁ NO ₃	205.21	46.5729	0.033	27.9
17	Fumaric acid	C ₄ H ₄ O ₄	116.07	47.1228	0.022	42.2
18	Trp-His	C ₁₇ H ₁₉ N ₅ O ₃	341.4	47.4081	0.033	50.5
19	Homovanillic acid	C ₉ H ₁₀ O ₄	182.17	47.913	0.026	23.6
20	Thr-Tyr	C ₁₃ H ₁₈ N ₂ O ₅	282.29	48.2163	0.063	17.2
21	Thymidine-5'-phosphate	C ₁₀ H ₁₅ N ₂ O ₈ P	322.21	48.6118	0.027	69.1
22	Nonadecanoic acid	C ₁₉ H ₃₈ O ₂	298.5	48.8573	0.043	29.5
23	Isobutylamine	C ₄ H ₁₁ N	73.14	49.0322	0.050	25.7
24	Arg-Leu-Lys	C ₁₈ H ₃₇ N ₇ O ₄	415.5	49.8728	0.090	61
25	Gln-Arg-Arg	C ₁₇ H ₃₄ N ₁₀ O ₅	458.5	50.0658	0.074	80.4
26	α-D-Glucose 1,6-bisphosphate	C ₆ H ₁₄ O ₁₂ P ₂	340.12	50.3915	0.053	63.5
27	Cys-Ser	C ₆ H ₁₂ N ₂ O ₄ S	208.24	50.6495	0.078	47.4
28	D-Lyxose	C ₅ H ₁₀ O ₅	150.13	50.9484	0.061	70
29	Asn-Asn-Lys	C ₁₄ H ₂₆ N ₆ O ₆	374.39	51.1268	0.109	20.8
30	Urea	CH ₄ N ₂ O	60.056	51.32	0.040	34.4
31	D-Galactose	C ₆ H ₁₂ O ₆	180.16	51.626	0.098	56.5
32	Enterodiol	C ₁₈ H ₂₂ O ₄	302.4	51.7081	0.037	36.8
33	2-Furoic acid	C ₅ H ₄ O ₃	112.08	51.7661	0.049	32.4
34	Lys-Leu	C ₁₂ H ₂₅ N ₃ O ₃	259.35	53.1088	0.057	15.1
35	Met(O)-Val-Arg	C ₁₆ H ₃₂ N ₆ O ₅ S	420.5	53.3107	0.134	14.7
36	N2,N2-Dimethylguanaine	C ₇ H ₉ N ₅ O	179.18	53.686	0.061	16.1
37	Phe-gly	C ₁₁ H ₁₄ N ₂ O ₃	222.24	53.8849	0.049	26.7
38	Ile-Pro-Lys	C ₁₇ H ₃₂ N ₄ O ₄	356.5	54.3381	0.064	16.6
39	Phosphoramidon	C ₂₃ H ₃₄ N ₃ O ₁₀ P	543.5	54.8336	0.048	17.7
40	Trp-glu	C ₁₆ H ₁₉ N ₃ O ₅	333.34	54.998	0.142	74.5
41	Trp-Ile	C ₁₇ H ₂₃ N ₃ O ₃	317.4	55.8552	0.153	49
42	Stearic acid	C ₁₈ H ₃₆ O ₂	284.5	55.9046	0.026	24.9
43	beta-Lactose	C ₁₂ H ₂₂ O ₁₁	342.30	56.9592	0.073	48.8
44	Riboflavin	C ₁₇ H ₂₀ N ₄ O ₆	376.4	57.6106	0.052	37.4

Supplementary Table 6: List of metabolites identified in Butyrate treatment.

S. No.	Name of compounds	Mol. Formula	Mol. Wt.	RT (min)	Abundance (%)	Probability
1	N-acetylglycinamide	C ₉ H ₉ NO ₃	179.17	7.6555	0.077	28.5
2	1-Octanol	C ₈ H ₁₈ O	130.23	7.7225	0.032	12.6
3	2,3-Hexanediol	C ₆ H ₁₄ O ₂	118.17	7.7389	0.182	36.8
4	Thr-Leu	C ₁₀ H ₂₀ N ₂ O ₄	232.28	7.8204	0.198	27.1
5	Propionamide	C ₃ H ₇ NO	73.09	7.8518	0.084	45.1
6	N-acetylputrescine	C ₆ H ₁₄ N ₂ O	130.19	8.0494	0.129	64
7	Methylmalonic acid	C ₄ H ₆ O ₄	118.09	8.2637	0.564	70.2
8	Phenylacetyl glycine	C ₁₀ H ₁₁ NO ₃	193.20	8.3053	0.042	44.5
9	2-Methyl-3-hexanol	C ₇ H ₁₆ O	116.20	8.3401	0.339	24.3
10	4-Methylbenzoic acid	C ₈ H ₈ O ₂	136.15	8.4808	0.021	14.4
11	Hydroxyacetone	C ₃ H ₆ O ₂	74.08	10.0918	0.074	29.3
12	Valeric acid	C ₅ H ₁₀ O ₂	102.13	14.2926	10.493	23.5
13	Caproic acid	C ₆ H ₁₂ O ₂	116.16	15.4717	0.139	55.1
14	Propan-1-one	C ₃ H ₅ O	57.07	18.1136	0.423	42.7
15	Octanoic acid	C ₈ H ₁₆ O ₂	144.21	21.607	48.360	74
16	Nonanoic acid	C ₉ H ₁₈ O ₂	158.24	24.3185	0.319	43.2
17	Lys-Met	C ₁₁ H ₂₃ N ₃ O ₃ S	277.39	26.8983	0.032	29.1
18	Indole	C ₈ H ₇ N	117.15	26.9175	11.486	25.7
19	Capric acid	C ₁₀ H ₂₀ O ₂	172.26	26.9832	25.021	49.6
20	Isoleucyl-Arginine	C ₁₂ H ₂₅ N ₅ O ₃	287.36	31.8084	0.051	76.7
21	L-Saccharopine	C ₁₁ H ₂₀ N ₂ O ₆	276.29	31.9405	0.454	35.8
22	3-Phenylpropylamine	C ₉ H ₁₃ N	135.21	36.1169	0.033	26.3
23	Octadecanamide	C ₁₈ H ₃₇ NO	283.5	36.2266	0.254	67
24	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.42	40.1035	0.107	39
25	Methyl propionate	C ₄ H ₈ O ₂	88.11	46.1293	0.054	30.7
26	Homogentisic acid	C ₈ H ₈ O ₄	168.15	48.6986	0.078	33.6
27	alpha-D-Ribofuranose	C ₅ H ₁₀ O ₅	150.13	49.0306	0.061	15.7
28	Urea	CH ₄ N ₂ O	60.056	50.4521	0.213	20.8
29	Ethanol	C ₂ H ₆ O	46.07	51.2717	0.057	21.3
30	Methyl acetate	C ₃ H ₆ O ₂	74.08	52.718	0.133	17.8
31	Acetophenone	C ₈ H ₈ O	120.15	52.8678	0.082	5.37
32	p-Cresol	C ₇ H ₈ O	108.14	53.1454	0.052	11.3
33	Pyrazine	C ₄ H ₄ N ₂	80.09	53.3875	0.025	8.04
34	2-Nitrobutane	C ₄ H ₉ NO ₂	103.12	53.5019	0.092	29.7
35	Homovanillic acid	C ₉ H ₁₀ O ₄	182.17	54.0746	0.061	7.07
36	Trp-Ile	C ₁₇ H ₂₃ N ₃ O ₃	317.4	55.0232	0.030	49
37	D-Galactose	C ₆ H ₁₂ O ₆	180.16	55.5676	0.040	13.9
38	Stearic acid	C ₁₈ H ₃₆ O ₂	284.5	57.4794	0.058	23.7
39	Ethyl heptanoate	C ₉ H ₁₈ O ₂	158.24	57.5556	0.051	7.58

Supplementary Table 7: List of metabolites identified in *L. plantarum* treatment.

S. No.	Name of compounds	Mol. Formula	Mol. Wt.	RT (min)	Abundance (%)	Probability
1	Betaine	C ₅ H ₁₁ NO ₂	117.15	4.1793	0.024	28.8
2	DL-Ethionine	C ₆ H ₁₃ NO ₂ S	163.24	4.2122	0.022	40.1
3	Alanine	C ₃ H ₇ NO ₂	89.09	4.2458	0.036	73.6
4	Methyl pyrimidine-5-carboxylate	C ₆ H ₆ N ₂ O ₂	138.12	4.2656	0.022	11.9
5	Homovanillic acid	C ₉ H ₁₀ O ₄	182.17	5.2206	0.109	28.2
6	Lysine	C ₆ H ₁₄ N ₂ O ₂	146.19	5.4636	0.025	72.3
7	Uridine	C ₉ H ₁₂ N ₂ O ₆	244.20	5.7022	0.043	27.3
8	2-Hydroxypyridine	C ₅ H ₅ NO	95.1	6.1698	0.118	51.1
9	Methylguanidine	C ₂ H ₇ N ₃	73.1	7.833	0.037	27.3
10	Phenylacetyl glycine	C ₁₀ H ₁₁ NO ₃	193.2	8.1402	0.049	47.7
11	Methanesulfonic acid	CH ₄ O ₃ S	96.11	8.5056	0.048	28
12	Indole-3-carboxylic acid	C ₉ H ₇ NO ₂	161.16	18.1573	0.420	21.5
13	Octanoic acid	C ₈ H ₁₆ O ₂	144.21	21.6176	53.794	72.5
14	Indole	C ₈ H ₇ N	117.15	23.5046	1.584	35.6
15	Nonanoic acid	C ₉ H ₁₈ O ₂	158.24	24.3247	0.340	48.1
16	Capric acid	C ₁₀ H ₂₀ O ₂	172.26	26.9902	28.224	69.9
17	Gln-Glu	C ₁₀ H ₁₇ N ₃ O ₆	275.26	31.8192	6.991	63.8
18	Methyl 2-methylprop-2-enoate	C ₉ H ₁₄ O ₄	186.2	31.8298	0.046	8.26
19	Nonane	C ₉ H ₂₀	128.25	31.8862	0.437	25.5
20	2-Isopropylmalic acid	C ₇ H ₁₂ O ₅	176.17	31.9143	0.119	14.7
21	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.42	40.1122	0.417	20.4
22	3-Methyl-2-pentenedioic acid	C ₆ H ₈ O ₄	144.12	42.9318	0.224	18.3
23	9-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282.5	43.3253	0.255	10.6
24	Myristic acid	C ₁₄ H ₂₈ O ₂	228.37	46.165	0.061	42.5
25	Cinnamic acid	C ₉ H ₈ O ₂	148.16	47.0509	0.033	5.2
26	Pyridine	C ₅ H ₅ N	79.1	47.7396	0.096	13.4
27	Met(O)-Val-Arg	C ₁₆ H ₃₂ N ₆ O ₅ S	420.5	48.1956	0.041	11.1
28	Lys-Cys-Lys	C ₁₅ H ₃₁ N ₅ O ₄ S	377.5	48.4125	0.056	29.7
29	Propylamine	C ₃ H ₉ N	59.11	49.0277	0.041	8.41
30	D-Galactose	C ₆ H ₁₂ O ₆	180.16	49.4444	0.079	45.5
31	Methyl propionate	C ₄ H ₈ O ₂	88.11	49.7088	0.042	62.5
32	Tyr-Ala	C ₁₂ H ₁₆ N ₂ O ₄	252.27	50.2452	0.070	20
33	Methyl phenylacetate	C ₉ H ₁₀ O ₂	150.17	50.5284	0.146	11.2
34	Propan-1-one	C ₃ H ₅ O	57.07	50.5679	0.210	11.6
35	Leucine	C ₆ H ₁₃ NO ₂	131.17	50.7106	0.053	14.4
36	Arginine	C ₆ H ₁₄ N ₄ O ₂	174.2	50.8919	0.031	8.33
37	Octadecane	C ₁₈ H ₃₈	254.5	51.1097	0.223	5.83
38	Hypoxanthine	C ₅ H ₄ N ₄ O	136.11	51.6056	0.086	23.7
39	12-Hydroxystearic acid	C ₁₈ H ₃₆ O ₃	300.5	51.7046	0.070	17.9
40	Nonadecanoic acid	C ₁₉ H ₃₈ O ₂	298.5	51.9828	0.134	12.5
41	Deoxycholic acid	C ₂₄ H ₄₀ O ₄	392.6	52	0.060	26.9
42	Propionic acid	C ₃ H ₆ O ₂	74.08	52.1469	0.041	8.08
43	1-Heptene	C ₇ H ₁₄	98.19	52.7441	0.242	15.6
44	p-Cresol	C ₇ H ₈ O	108.14	53.3247	0.073	31.8

45	l-Isoleucine	C ₆ H ₁₃ NO ₂	131.17	55.235	0.053	21.8
46	Phenazine	C ₁₂ H ₈ N ₂	180.2	55.3391	0.048	48.1
47	sn-Glycero-3-phosphoethanolamine	C ₅ H ₁₄ NO ₆ P	215.14	55.6819	0.255	31.4
48	Methyl acetate	C ₃ H ₆ O ₂	74.08	56.2061	0.062	29.2
49	Trp-Cys	C ₁₄ H ₁₇ N ₃ O ₃ S	307.37	56.6033	0.041	36.2
50	5-Methyltetrahydrofolic acid	C ₂₀ H ₂₅ N ₇ O ₆	459.5	57.2967	0.038	52.3
51	Acetic acid	C ₂ H ₄ O ₂	60.05	57.3383	4.234	18

Supplementary Table 8: List of common metabolites identified under various treatments.

Treatment	Total	Metabolites
Control, butyrate, and <i>L. plantarum</i>	7	Indole, Homovanillic acid, Octanoic acid, Capric acid, Phenylacetyl glycine, D-Galactose, Nonanoic acid
Control and butyrate	8	Trp-Ile, Methylmalonic acid, Stearic acid, 2,3-Hexanediol, 2-Methyl-3-hexanol, Urea, L-Saccharopine, Octadecanamide
Control and <i>L. plantarum</i>	3	Myristic acid, Met(O)-Val-Arg, Nonadecanoic acid
Butyrate and <i>L. plantarum</i>	5	Palmitic acid, Methyl propionate, Methyl acetate, Propan-1-one, p-Cresol
Control	26	Thr-Tyr, Lys-Leu, Riboflavin, Fumaric acid, Phosphoramidon, Indole-3-lactic acid, beta-Lactose, Phe-gly, Gln-Arg-Arg, 2-Furoic acid, Arg-Leu-Lys, Thymidine-5'-phosphate, Cys-Ser, Ile-Pro-Lys, DL-Phenylalanine, Enterodiol, 1,2-Pentanediol, D-Lyxose, Trp-glu, 2-Butanol, Trp-His, Glu-Gly, Isobutylamine, Asn-Asn-Lys, N ₂ ,N ₂ -Dimethylguanidine, alpha-D-Glucose 1,6-bisphosphate
Butyrate	19	4-Methylbenzoic acid, Ethanol, Acetophenone, Homogentisic acid, Valeric acid, Caproic acid, Hydroxyacetone, Propionamide, Isoleucyl-Arginine, 2-Nitrobutane, N-acetylputrescine, 1-Octanol, alpha-D-Ribofuranose, Thr-Leu, Ethyl heptanoate, Lys-Met, N-acetylglycinamide, Pyrazine, 3-Phenylpropylamine
<i>L. plantarum</i>	36	3-Methyl-2-pentenedioic acid, Nonane, Phenazine, DL-Ethionine, Lysine, Indole-3-carboxylic acid, Betaine, Trp-Cys, 1-Heptene, l-Isoleucine, Arginine, Octadecane, 2-Isopropylmalic acid, Leucine, Methylguanidine, Deoxycholic acid, Gln-Glu, 9-Octadecenoic acid, 12-Hydroxystearic acid, Propylamine, Cinnamic acid, Alanine, Uridine, Lys-Cys-Lys, 2-Hydroxypyridine, Pyridine, sn-Glycero-3-phosphoethanolamine, Hypoxanthine, Tyr-Ala, Methyl phenylacetate, Methyl pyrimidine-5-carboxylate, Methanesulfonic acid, 5-Methyltetrahydrofolic acid, Methyl 2-methylprop-2-enoate, Propionic acid, Acetic acid

Supplementary Table 9: Metabolite class enrichment in Control.

	Metabolite Set	Total	Hits	Expect	P value	Holm P	FDR
	Amino acids, peptides, and analogues	3220	13	0.601	1.41E-14	1.26E-11	1.26E-11
	Fatty acids and conjugates	941	6	0.175	2.32E-8	2.07E-5	1.03E-5
	Dicarboxylic acids and derivatives	75	2	0.014	9.32E-5	0.0827	0.0276
	Alloxazines and isoalloxazines	8	1	0.00149	0.00149	1.0	0.304
	Purine nucleosides	11	1	0.00205	0.00205	1.0	0.304
	Dibenzylbutanediol lignans	11	1	0.00205	0.00205	1.0	0.304
	Carbohydrates and carbohydrate conjugates	1600	3	0.297	0.00329	1.0	0.368
	Pyrimidine deoxyribonucleotides	19	1	0.00354	0.00354	1.0	0.368
	Furoic acid and derivatives	20	1	0.00373	0.00372	1.0	0.368
	Carboximidic acids	27	1	0.00504	0.00502	1.0	0.447
	Ureas	37	1	0.0069	0.00688	1.0	0.556
	Indolyl carboxylic acids and derivatives	55	1	0.0103	0.0102	1.0	0.757
	Carbonyl compounds	975	2	0.182	0.0143	1.0	0.982
	Indoles	133	1	0.0248	0.0245	1.0	1.0
	Methoxyphenols	137	1	0.0255	0.0252	1.0	1.0
	Alcohols and polyols	309	1	0.0576	0.056	1.0	1.0
	Amines	350	1	0.0653	0.0632	1.0	1.0

Supplementary Table 10: Metabolite class enrichment in Butyrate treatment.

	Metabolite Set	Total	Hits	Expect	P value	Holm P	FDR
	Fatty acids and conjugates	941	7	0.166	3.26E-10	2.9E-7	2.9E-7
	Carbonyl compounds	975	5	0.172	8.28E-7	7.36E-4	3.68E-4
	Amino acids, peptides, and analogues	3220	7	0.569	1.37E-6	0.00122	4.07E-4
	Carboximidic acids	27	2	0.00477	1.06E-5	0.00942	0.00236
	Carboxylic acid derivatives	236	2	0.0417	8.2E-4	0.726	0.146
	Phenylacetic acids	8	1	0.00141	0.00141	1.0	0.21
	Cresols	23	1	0.00406	0.00406	1.0	0.516
	Ureas	37	1	0.00654	0.00652	1.0	0.725
	Dicarboxylic acids and derivatives	75	1	0.0133	0.0132	1.0	1.0
	Pyrazines	117	1	0.0207	0.0205	1.0	1.0
	Indoles	133	1	0.0235	0.0232	1.0	1.0
	Methoxyphenols	137	1	0.0242	0.0239	1.0	1.0
	Carbohydrates and carbohydrate conjugates	1600	2	0.282	0.0324	1.0	1.0
	Alcohols and polyols	309	1	0.0546	0.0532	1.0	1.0
	Fatty alcohols	535	1	0.0945	0.0903	1.0	1.0
	Benzoic acids and derivatives	624	1	0.11	0.105	1.0	1.0
	Fatty acid esters	1650	1	0.291	0.254	1.0	1.0

Supplementary Table 11: Metabolite class enrichment in *L. plantarum* treatment.

	Metabolite Set	Total	Hits	Expect	P value	Holm P	FDR
	Fatty acids and conjugates	941	10	0.217	1.87E-14	1.66E-11	1.66E-11
	Amino acids, peptides, and analogues	3220	11	0.743	1.57E-10	1.39E-7	6.98E-8
	Carboxylic acid derivatives	236	3	0.0544	2.4E-5	0.0213	0.00711
	Carboxylic acids	39	2	0.009	3.84E-5	0.034	0.00854
	Alkanes	105	2	0.0242	2.8E-4	0.248	0.0498
	Pyrimidine ribonucleosides	5	1	0.00115	0.00115	1.0	0.171
	Cinnamic acids	8	1	0.00185	0.00184	1.0	0.231
	Pyridines	9	1	0.00208	0.00207	1.0	0.231
	Cresols	23	1	0.00531	0.00529	1.0	0.523
	Guanidines	31	1	0.00715	0.00713	1.0	0.634
	Indolecarboxylic acids and derivatives	45	1	0.0104	0.0103	1.0	0.754
	Unsaturated aliphatic hydrocarbons	47	1	0.0108	0.0108	1.0	0.754
	Organosulfonic acids and derivatives	48	1	0.0111	0.011	1.0	0.754
	Pterins and derivatives	77	1	0.0178	0.0176	1.0	1.0
	Hydropyridines	95	1	0.0219	0.0217	1.0	1.0
	Indoles	133	1	0.0307	0.0302	1.0	1.0
	Methoxyphenols	137	1	0.0316	0.0311	1.0	1.0
	Purines and purine derivatives	196	1	0.0452	0.0442	1.0	1.0
	Benzodiazines	200	1	0.0461	0.0451	1.0	1.0
	Bile acids, alcohols and derivatives	273	1	0.063	0.0611	1.0	1.0

	Amines	350	1	0.0807	0.0776	1.0	1.0
	Carbonyl compounds	975	1	0.225	0.202	1.0	1.0
	Carbohydrates and carbohydrate conjugates	1600	1	0.368	0.309	1.0	1.0
	Glycerophosphoethanolamines	3080	1	0.711	0.512	1.0	1.0

Supplementary Table 12: List of common metabolic classes identified under various treatments.

Treatment	Total	Metabolite class
Control, butyrate, and <i>L. plantarum</i>	6	Carbonyl compounds, Indoles, Methoxyphenols, Fatty acids and conjugates, Amino acids, peptides, and analogues, Carbohydrates and carbohydrate conjugates
Control and butyrate	4	Alcohols and polyols, Ureas, Carboximidic acids, Dicarboxylic acids and derivatives
Control and <i>L. plantarum</i>	1	Amines
Butyrate and <i>L. plantarum</i>	2	Cresols, Carboxylic acid derivatives
Control	6	Pyrimidine deoxyribonucleotides, Alloxazines and isoalloxazines, Dibenzylbutanediol lignans, Purine nucleosides, Indolyl carboxylic acids and derivatives, Furoic acid and derivatives
Butyrate	5	Fatty alcohols, Phenylacetic acids, Benzoic acids and derivatives, Fatty acid esters, Pyrazines
<i>L. plantarum</i>	15	Unsaturated aliphatic hydrocarbons, Hydropyridines, Pyrimidine ribonucleosides, Carboxylic acids, Organosulfonic acids and derivatives, Cinnamic acids, Alkanes, Indolecarboxylic acids and derivatives, Benzodiazines, Pyridines, Guanidines, Bile acids, alcohols and derivatives, Purines and purine derivatives, Glycerophosphoethanolamines, Pterins and derivatives

Supplementary Table 13: List of metabolic pathways enriched in Control.

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact
Lipoic acid metabolism	1/28	0.57354	0.24144	1.0	0.96487	0.02703
Phenylalanine, tyrosine and tryptophan biosynthesis	1/23	0.57432	0.24085	1.0	0.96487	0.04167
Tryptophan metabolism	1/10	0.57432	0.24085	1.0	0.96487	0.0
Galactose metabolism	1/39	0.65855	0.18141	1.0	0.96487	0.02439
Riboflavin metabolism	1/17	0.74243	0.12935	1.0	0.96487	0.11111
D-Amino acid metabolism	1/25	0.77839	0.1088	1.0	0.96487	0.05882
Purine metabolism	1/76	0.80318	0.095184	1.0	0.96487	0.0
Phenylalanine metabolism	2/35	0.83768	0.076924	1.0	0.96487	0.02857
Pyruvate metabolism	1/27	0.85046	0.070348	1.0	0.96487	0.02381
Citrate cycle (TCA cycle)	1/20	0.85046	0.070348	1.0	0.96487	0.0
Arginine biosynthesis	1/16	0.85046	0.070348	1.0	0.96487	0.0
Alanine, aspartate and glutamate metabolism	1/22	0.85046	0.070348	1.0	0.96487	0.0
Butanoate metabolism	1/18	0.85046	0.070348	1.0	0.96487	0.0
Other carbon fixation pathways	1/24	0.85046	0.070348	1.0	0.96487	0.0
Pyrimidine metabolism	1/51	0.96487	0.015533	1.0	0.96487	0.04225
One carbon pool by folate	1/20	0.96487	0.015533	1.0	0.96487	0.0

Supplementary Table 14: List of metabolic pathways enriched in Butyrate treatment.

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact
Lipoic acid metabolism	1/28	0.57356	0.24142	1.0	0.78637	0.02703
Phenylalanine, tyrosine and	1/23	0.57409	0.24102	1.0	0.78637	0.04167

tryptophan biosynthesis						
Tryptophan metabolism	1/10	0.57409	0.24102	1.0	0.78637	0.0
Arginine and proline metabolism	1/29	0.63738	0.1956	1.0	0.78637	0.0
Fatty acid degradation	1/41	0.65107	0.18637	1.0	0.78637	0.02083
Fatty acid biosynthesis	1/46	0.65107	0.18637	1.0	0.78637	0.01923
Biosynthesis of unsaturated fatty acids	1/2	0.65107	0.18637	1.0	0.78637	0.0
Purine metabolism	2/76	0.65971	0.18065	1.0	0.78637	0.01124
Propanoate metabolism	1/26	0.68808	0.16236	1.0	0.78637	0.04167
Pentose phosphate pathway	1/26	0.71486	0.14578	1.0	0.78637	0.02941
Glycolysis or Gluconeogenesis	1/29	0.72588	0.13913	1.0	0.78637	0.02703
Pyruvate metabolism	1/27	0.72588	0.13913	1.0	0.78637	0.02381
Galactose metabolism	1/39	0.80319	0.095181	1.0	0.80319	0.02439

Supplementary Table 15: List of metabolic pathways enriched in *L. plantarum* treatment.

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact
Lipoic acid metabolism	1/28	0.57354	0.24144	1.0	0.98507	0.02703
Taurine and hypotaurine metabolism	1/10	0.57529	0.24011	1.0	0.98507	0.14286
Methane metabolism	1/26	0.57529	0.24011	1.0	0.98507	0.08
Glyoxylate and dicarboxylate metabolism	1/37	0.57529	0.24011	1.0	0.98507	0.02941
Glycolysis or Gluconeogenesis	1/29	0.57529	0.24011	1.0	0.98507	0.02703
Phenylalanine, tyrosine and tryptophan biosynthesis	1/23	0.57847	0.23772	1.0	0.98507	0.04167
Tryptophan metabolism	1/10	0.57847	0.23772	1.0	0.98507	0.0
Fatty acid degradation	1/41	0.59278	0.22711	1.0	0.98507	0.02083

Fatty acid biosynthesis	1/46	0.59278	0.22711	1.0	0.98507	0.01923
Biosynthesis of unsaturated fatty acids	1/2	0.59278	0.22711	1.0	0.98507	0.0
Pyruvate metabolism	2/27	0.60763	0.21636	1.0	0.98507	0.04762
Sulfur metabolism	2/20	0.65633	0.18288	1.0	0.98507	0.04545
Purine metabolism	1/76	0.67058	0.17355	1.0	0.98507	0.02247
Other carbon fixation pathways	2/24	0.67734	0.1692	1.0	0.98507	0.08333
Galactose metabolism	1/39	0.68086	0.16694	1.0	0.98507	0.02439
Valine, leucine and isoleucine biosynthesis	3/22	0.70389	0.1525	1.0	0.98507	0.1
Valine, leucine and isoleucine degradation	2/22	0.73896	0.13138	1.0	0.98507	0.15384
Pyrimidine metabolism	1/51	0.78139	0.10713	1.0	0.98507	0.02817
Propanoate metabolism	1/26	0.79641	0.098862	1.0	0.98507	0.04167
Alanine, aspartate and glutamate metabolism	1/22	0.83331	0.079193	1.0	0.98507	0.04
Selenocompound metabolism	1/15	0.83331	0.079193	1.0	0.98507	0.0
One carbon pool by folate	2/20	0.88557	0.052777	1.0	0.98507	0.06061
Arginine and proline metabolism	1/29	0.892	0.049636	1.0	0.98507	0.07407
Arginine biosynthesis	1/16	0.892	0.049636	1.0	0.98507	0.0
D-Amino acid metabolism	3/25	0.89264	0.049323	1.0	0.98507	0.17646
Glycine, serine and threonine metabolism	1/33	0.94417	0.024948	1.0	0.98507	0.0
Lysine degradation	1/22	0.98507	0.0065347	1.0	0.98507	0.10526
Lysine biosynthesis	1/13	0.98507	0.0065347	1.0	0.98507	0.0

Supplementary Table 16: List of common metabolic pathways identified under various treatments.

Treatment	Total	Metabolic pathways
Control, butyrate, and <i>L. plantarum</i>	6	Phenylalanine, tyrosine and tryptophan biosynthesis, Lipoic acid metabolism, Pyruvate metabolism, Purine metabolism, Galactose metabolism, Tryptophan metabolism
Control and <i>L. plantarum</i>	6	Alanine, aspartate and glutamate metabolism, Pyrimidine metabolism, One carbon pool by folate, D-Amino acid metabolism, Arginine biosynthesis, Other carbon fixation pathways
Butyrate and <i>L. plantarum</i>	6	Biosynthesis of unsaturated fatty acids, Fatty acid degradation, Glycolysis or Gluconeogenesis, Propanoate metabolism, Fatty acid biosynthesis, Arginine and proline metabolism
Control	4	Citrate cycle (TCA cycle), Phenylalanine metabolism, Butanoate metabolism, Riboflavin metabolism
Butyrate	1	Pentose phosphate pathway
<i>L. plantarum</i>	10	Glyoxylate and dicarboxylate metabolism, Selenocompound metabolism, Taurine and hypotaurine metabolism, Methane metabolism, Valine, leucine and isoleucine biosynthesis, Sulfur metabolism, Lysine biosynthesis, Lysine degradation, Valine, leucine and isoleucine degradation, Glycine, serine and threonine metabolism

Supplementary Figure 1. Comparison of gut microbial functions between the butyrate and *L. plantarum* treated groups.

