

Supplementary Materials

Functional and metabolomic profiling supports rational selection of probiotic strains for the gut–skin axis

Tamara Aleksandrzyk-Piekarczyk^{1,*}, Aleksandra Tymoszevska², Mariusz Radkiewicz¹, Agnieszka K. Szczepankowska¹, Emilia Samborowska¹, Anna Koryszewska-Bagińska³, Bożena Cukrowska⁴

¹Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland

²Microbiota Lab, Department of Pharmaceutical Microbiology and Bioanalysis, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1B, 02-097 Warsaw, Poland

³Department of Medical Biology, Medical University of Warsaw, Litewska 14/16, 00-575 Warsaw, Poland

⁴Department of Research and Development, Nordic Biotic Ltd., Złota Street 59, 00-120 Warsaw, Poland

*Corresponding author, tamara@ibb.waw.pl

Table S1. Antagonistic activity scores (spot-on-lawn assay). Semi-quantitative inhibition scores (0–1) for each producer–indicator combination are shown for three independent biological replicates (t1–t3; separate cultures prepared on different days), together with the mean ± SD (standard deviation) and coefficient of variation (CV = SD/mean). CV is reported as NA (not applicable) when mean = 0. Score definition: 0, no inhibition; 0.1–0.3, weak; 0.4–0.6, moderate; 0.7–1.0, strong.

Indicator	Producer	Replicates (t1,t2,t3)	Mean ± SD	CV
P. acnes 2334	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	L. fermentum LF160	0.2, 0.3, 0.2	0.23 ± 0.06	0.25
P. acnes 2334	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2334	L. rhamnosus SCN01	1, 0.9, 1	0.97 ± 0.06	0.06
P. acnes 2400	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2400	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2400	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2400	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2400	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2400	L. casei LC130	0.2, 0.3, 0.2	0.23 ± 0.06	0.25
P. acnes 2400	L. fermentum LF160	1, 1, 0.8	0.93 ± 0.12	0.12
P. acnes 2400	L. paracasei LPC100	1, 0.9, 1	0.97 ± 0.06	0.06
P. acnes 2400	L. plantarum SCN02	0.3, 0.2, 0.3	0.27 ± 0.06	0.22
P. acnes 2400	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
P. acnes 2400	L. rhamnosus SCN01	0.8, 0.7, 0.7	0.73 ± 0.06	0.08
P. granulosum 2462	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
P. granulosum 2462	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
P. granulosum 2462	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
P. granulosum 2462	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
P. granulosum 2462	L. acidophilus LA120	0.3, 0.2, 0.3	0.27 ± 0.06	0.22
P. granulosum 2462	L. casei LC130	0.7, 0.9, 0.8	0.80 ± 0.10	0.12
P. granulosum 2462	L. fermentum LF160	0.5, 0.5, 0.5	0.50 ± 0.00	0.00
P. granulosum 2462	L. paracasei LPC100	0.9, 0.7, 0.9	0.83 ± 0.12	0.14
P. granulosum 2462	L. plantarum SCN02	0.8, 0.6, 0.9	0.77 ± 0.15	0.20
P. granulosum 2462	L. reuteri LU150	0.5, 0.8, 0.8	0.70 ± 0.17	0.25
P. granulosum 2462	L. rhamnosus SCN01	1, 1, 1	1.00 ± 0.00	0.00
S. aureus 25923	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	L. casei LC130	0.2, 0.3, 0.2	0.23 ± 0.06	0.25
S. aureus 25923	L. fermentum LF160	0.5, 0.5, 0.5	0.50 ± 0.00	0.00
S. aureus 25923	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. aureus 25923	L. rhamnosus SCN01	1, 1, 0.9	0.97 ± 0.06	0.06
S. aureus 29213	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA

Indicator	Producer	Replicates (t1,t2,t3)	Mean ± SD	CV
S. aureus 29213	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	L. fermentum LF160	0.8, 0.8, 0.7	0.77 ± 0.06	0.08
S. aureus 29213	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. aureus 29213	L. rhamnosus SCN01	1, 1, 0.9	0.97 ± 0.06	0.06
S. aureus 6538	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	B. lactis BIO40	0.2, 0.1, 0.2	0.17 ± 0.06	0.35
S. aureus 6538	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	L. fermentum LF160	0.8, 0.8, 0.7	0.77 ± 0.06	0.08
S. aureus 6538	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	L. plantarum SCN02	0.3, 0.2, 0.3	0.27 ± 0.06	0.22
S. aureus 6538	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. aureus 6538	L. rhamnosus SCN01	0.5, 0.5, 0.4	0.47 ± 0.06	0.12
S. epidermidis 10.2	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. fermentum LF160	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 10.2	L. rhamnosus SCN01	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. fermentum LF160	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 13.12	L. rhamnosus SCN01	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	L. fermentum LF160	0.2, 0.2, 0.1	0.17 ± 0.06	0.35
S. epidermidis 38.2	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 38.2	L. rhamnosus SCN01	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	B. bifidum BF030	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	B. breve SCN03	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	B. lactis BIO40	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	B. longum BL020	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. acidophilus LA120	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. casei LC130	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. fermentum LF160	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. paracasei LPC100	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. plantarum SCN02	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. reuteri LU150	0, 0, 0	0.00 ± 0.00	NA
S. epidermidis 9.20	L. rhamnosus SCN01	0, 0, 0	0.00 ± 0.00	NA

Metabolite	Class	MSR (control. no strain. medium only)	<i>B. bifidum</i> BF030	<i>B. breve</i> SCN03	<i>B. lactis</i> BI040	<i>B. longum</i> BL020	<i>L. acidophilus</i> LA120	<i>L. casei</i> LC130	<i>L. fermentum</i> LF160	<i>L. paracasei</i> LPC100	<i>L. plantarum</i> SCN02	<i>L. reuteri</i> LU150	<i>L. rhamnosus</i> SCN01
FA 18:1	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
FA 18:2	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
FA 20:1	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
FA 20:2	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
FA 20:3	Fatty Acids	< LOD	< LOD	2.1	1.43	< LOD	< LOD	< LOD	2.76	< LOD	< LOD	1.95	< LOD
FA 20:4n-6 (AA)	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
FA 20:5n-3 (EPA)	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
FA 22:6n-3 (DHA)	Fatty Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
GABA	Biogenic Amines	59.7	59.6	60.2	51.2	62	60.5	61.3	55.6	59	151	56.6	71
GCA	Bile Acids	0.004	0.003	0.001	< LOD	0.002	0.005	0.003	0.006	0.004	0.002	0.002	0.003
GCDCA	Bile Acids	< LOD	0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
GDCA	Bile Acids	< LOD	0	0	0	< LOD	0	0	< LOD	< LOD	< LOD	< LOD	< LOD
GLCA	Bile Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
GLCAS	Bile Acids	< LOD	0.001	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
GUDCA	Bile Acids	< LOD	< LOD	< LOD	< LOD	0.001	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Gln	Aminoacids	7.09	131	606	238	959	152	6.03	382	9.41	31.8	34.1	6.78
Glu	Aminoacids	2500	2458	2819	2802	3274	2293	2632	1557	1996	1521	2170	2487
Gly	Aminoacids	5129	6459	7003	7448	6522	5689	8189	6186	5532	5583	6127	6982
HArg	Aminoacids Related	0.254	0.223	0.213	0.274	0.221	0.192	0.32	0.523	0.263	0.62	0.671	0.313
HipAcid	Carboxylic Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
His	Aminoacids	159	197	238	226	227	168	216	142	126	130	2.5	181
Histamine	Biogenic Amines	0.69	0.608	0.494	0.561	0.543	0.976	0.251	0.52	0.72	0.504	177	0.764
Hypoxanthine	Nucleobases Related	7.9	45.1	13.5	32.3	10.4	26.6	32.1	< LOD	14.3	< LOD	5.06	35.8
Ile	Aminoacids	763	917	987	1059	866	912	1218	768	770	680	792	1083
Ind-SO4	Indoles Derivatives	0.014	0.017	0.012	0.018	0.016	0.019	< LOD	0.013	0.012	0.025	0.012	0.018
Indole	Indoles Derivatives	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Kynurenine	Aminoacids Related	< LOD	0.195	< LOD	< LOD	< LOD	< LOD	0.325	< LOD	0.163	< LOD	< LOD	0.035
Lac	Carboxylic Acids	3234	48513	21620	20162	40423	17610	118597	70895	88540	143233	64565	88144
Leu	Aminoacids	1949	1831	1909	2120	1793	1939	2196	1479	1684	1451	1435	1973
Lys	Aminoacids	468	792	608	545	771	545	445	453	284	361	262	524
Met	Aminoacids	163	339	271	374	266	1.65	356	174	3.07	188	148	188
Nitro-Tyr	Aminoacids Related	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
OH-GlutAcid	Carboxylic Acids	85.4	25.4	26.1	37.5	103	17	326	28.2	168	57.2	82.3	89.4
Orn	Aminoacids Related	27.5	72.5	39.1	23.2	55.3	50.5	11.9	714	21	41.9	662	27.1
PAG	Aminoacids Related	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
PEA	Biogenic Amines	1.09	0.432	0.484	0.489	0.517	1.37	0.468	0.472	1.25	0.43	0.481	1.23
Phe	Aminoacids	745	842	861	927	675	801	856	532	744	391	446	936
PheAlaBetaine	Aminoacids Related	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Pro	Aminoacids	457	528	600	958	696	556	1322	406	802	601	810	888

Metabolite	Class	MSR (control. no strain. medium only)	<i>B. bifidum</i> BF030	<i>B. breve</i> SCN03	<i>B. lactis</i> BI040	<i>B. longum</i> BL020	<i>L. acidophilus</i> LA120	<i>L. casei</i> LC130	<i>L. fermentum</i> LF160	<i>L. paracasei</i> LPC100	<i>L. plantarum</i> SCN02	<i>L. reuteri</i> LU150	<i>L. rhamnosus</i> SCN01
ProBetaine	Aminoacids Related	0.549	0.735	0.646	0.77	0.743	0.859	0.717	0.732	0.811	0.811	0.659	0.802
Putrescine	Biogenic Amines	2.09	3.84	2.45	2.55	3.49	3.01	2.85	2.76	2.13	3.17	2.04	3.22
SDMA	Aminoacids Related	2.15	4.01	6.22	3.57	2.9	3.41	8.55	5.59	7.5	10.7	2.79	8.06
Sarcosine	Aminoacids Related	6.66	6.44	6.26	6	6.63	7.47	6.46	6.3	7.41	6.58	6.3	8.46
Ser	Aminoacids	945	961	1112	1256	1145	1157	1252	833	855	522	908	1154
Serotonin	Biogenic Amines	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Spermidine	Biogenic Amines	4.22	9.98	6.03	5.1	8.2	3.28	1.77	7.68	< LOD	0.792	0.682	< LOD
Spermine	Biogenic Amines	0.62	2	0.738	0.53	1.22	0.617	0.504	1.39	< LOD	1.12	0.412	< LOD
Suc	Carboxylic Acids	501	696	683	642	1310	772	932	14466	792	2091	5533	921
TCA	Bile Acids	0.006	0	0.001	0.002	0.002	0.006	0.003	0.006	0.005	0.004	0.002	0.003
TCDCa	Bile Acids	< LOD	< LOD	0.005	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
TDCA	Bile Acids	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
TLCA	Bile Acids	< LOD	0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
TMAO	Amine Oxides	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
TMCA	Bile Acids	0.004	< LOD	0.003	< LOD	< LOD	< LOD	< LOD	0.003	< LOD	0.005	0.003	< LOD
Taurine	Aminoacids Related	26.1	25.5	27	27.9	24.8	26.4	26.3	24.8	22.7	23.6	24.3	27.1
Thr	Aminoacids	850	1034	1079	1250	1063	923	1201	753	817	671	878	968
Trigonelline	Alkaloids	0.846	1.07	0.882	1.02	1.01	1.08	0.963	0.969	1.06	1	0.785	1.26
Trp	Aminoacids	154	183	153	173	118	120	168	138	90.9	90.9	114	147
TrpBetaine	Aminoacids Related	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Tyr	Aminoacids	250	322	301	418	272	263	303	148	215	71	139	290
Val	Aminoacids	1092	1505	1411	1948	1407	1295	2076	1194	1362	1269	1518	1762
Xanthine	Nucleobases Related	13.8	20	< LOD	15.9	< LOD	26.1	< LOD	< LOD	< LOD	< LOD	< LOD	5.85
alpha-AAA	Aminoacids Related	1.98	2.24	2.54	2.43	3.5	2.74	3.61	2.67	1.74	3.8	3.07	1.68
beta-Ala	Biogenic Amines	5.27	5.42	4.79	4.96	5.04	5.63	5.09	4.22	4.5	2.71	4.61	5.81
c4-OH-Pro	Aminoacids Related	32.7	28.8	28.4	30.2	31.4	31.8	27.7	27.1	31.5	28.7	28.8	38.4
p-Cresol-SO4	Cresols	0.494	0.372	0.405	0.409	0.344	0.44	0.281	0.404	0.347	0.256	0.323	0.391
t4-OH-Pro	Aminoacids Related	76.7	73	96.6	109	99.1	86.6	153	77.7	110	69.1	81	140

22
23
24
25
26

Table S3. TEER time course and Dunnett-adjusted multiple comparisons versus matched control after exposure of Caco-2 monolayers to live bacterial strains.
(A) TEER values were exported from CellZscope, normalized for each insert to its post-stabilization baseline (t = 0; 100%), and reported as % of baseline; the first 6 h of recording were omitted due to instrument stabilization. (B) Statistical inference was performed in GraphPad Prism (v10.6.1) using a mixed-effects model (REML) with Geisser - Greenhouse correction, followed by Dunnett's multiple comparisons test versus the matched control at each time point (Dunnett p_{adj} values reported).
Table S3A_summary (mean ± SD and n)

Time_h	L. acidophilus LA120 mean	L. acidophilus LA120 SD	L. acidophilus LA120 n	L. fermentum LF160 mean	L. fermentum LF160 SD	L. fermentum LF160 n	L. paracasei LPC100 mean	L. paracasei LPC100 SD	L. paracasei LPC100 n	L. plantarum SCN02 mean	L. plantarum SCN02 SD	L. plantarum SCN02 n	L. rhamnosus SCN01 mean	L. rhamnosus SCN01 SD	L. rhamnosus SCN01 n	Control Lactobacillus mean	Control Lactobacillus SD	Control Lactobacillus n	B. bifidum BF030 mean	B. bifidum BF030 SD	B. bifidum BF030 n	B. breve SCN03 mean	B. breve SCN03 SD	B. breve SCN03 n	Control Bifidobacterium	Control Bifidobacterium SD	Control Bifidobacterium n
0.0	100.0	0.0	2.0	100.0	0.0	3.0	100.0	0.0	2.0	100.0	0.0	3.0	100.0	0.0	3.0	100.0	0.0	3.0	100.0	0.0	2.0	100.0	0.0	2.0	100.0	0.0	2.0
3.0	93.32	0.28	2.0	95.9	4.8	3.0	92.92	5.77	2.0	92.5	2.05	3.0	103.15	2.77	3.0	93.48	3.24	3.0	98.96	0.43	2.0	98.14	4.31	2.0	95.23	0.69	2.0
6.0	93.68	1.04	2.0	95.29	5.06	3.0	91.85	7.68	2.0	91.58	2.86	3.0	115.18	4.77	3.0	92.66	5.48	3.0	100.28	4.6	2.0	100.34	5.61	2.0	95.36	1.8	2.0
9.0	96.05	0.28	2.0	97.76	5.14	3.0	94.06	7.85	2.0	93.36	2.09	3.0	128.36	8.88	3.0	93.08	5.98	3.0	103.55	5.97	2.0	103.93	4.08	2.0	97.56	4.6	2.0
12.0	98.0	2.0	2.0	101.4	6.6	3.0	98.15	6.88	2.0	96.39	3.25	3.0	138.56	11.66	3.0	94.13	5.57	3.0	108.66	8.21	2.0	109.46	1.17	2.0	103.81	4.81	2.0
15.0	101.82	2.47	2.0	106.73	7.61	3.0	102.57	4.31	2.0	99.98	4.54	3.0	147.77	14.88	3.0	97.4	6.2	3.0	116.3	9.33	2.0	116.9	5.38	2.0	111.69	7.17	2.0
18.0	104.23	4.68	2.0	112.1	10.54	3.0	107.39	0.6	2.0	105.11	7.34	3.0	157.79	20.55	3.0	99.57	8.73	3.0	123.23	10.01	2.0	124.48	10.71	2.0	120.04	7.33	2.0
21.0	106.75	4.94	2.0	116.0	11.92	3.0	111.65	0.94	2.0	109.84	9.57	3.0	163.42	23.36	3.0	101.34	11.29	3.0	127.2	13.49	2.0	129.24	16.72	2.0	127.89	10.72	2.0
24.0	108.2	5.57	2.0	119.89	13.35	3.0	115.89	3.34	2.0	112.68	12.47	3.0	172.36	28.57	3.0	103.36	12.17	3.0	135.07	13.5	2.0	135.51	19.64	2.0	137.13	10.72	2.0
27.0	110.74	6.12	2.0	124.17	14.89	3.0	119.77	7.41	2.0	117.58	13.42	3.0	182.4	34.94	3.0	103.87	13.54	3.0	139.62	17.75	2.0	141.2	21.96	2.0	144.97	14.38	2.0
30.0	113.2	5.75	2.0	127.9	17.37	3.0	124.82	10.66	2.0	121.85	16.75	3.0	189.9	40.15	3.0	106.5	14.09	3.0	148.46	16.8	2.0	149.11	27.39	2.0	151.68	20.1	2.0
33.0	114.16	7.1	2.0	131.22	18.86	3.0	128.63	14.17	2.0	125.87	19.0	3.0	197.01	45.89	3.0	107.25	13.98	3.0	155.18	18.82	2.0	154.05	35.45	2.0	159.48	20.61	2.0
35.0	116.52	6.63	2.0	132.92	19.2	3.0	131.86	15.12	2.0	129.29	21.58	3.0	199.99	47.81	3.0	108.07	13.56	3.0	156.97	25.3	2.0	157.76	39.49	2.0	162.95	22.84	2.0

27

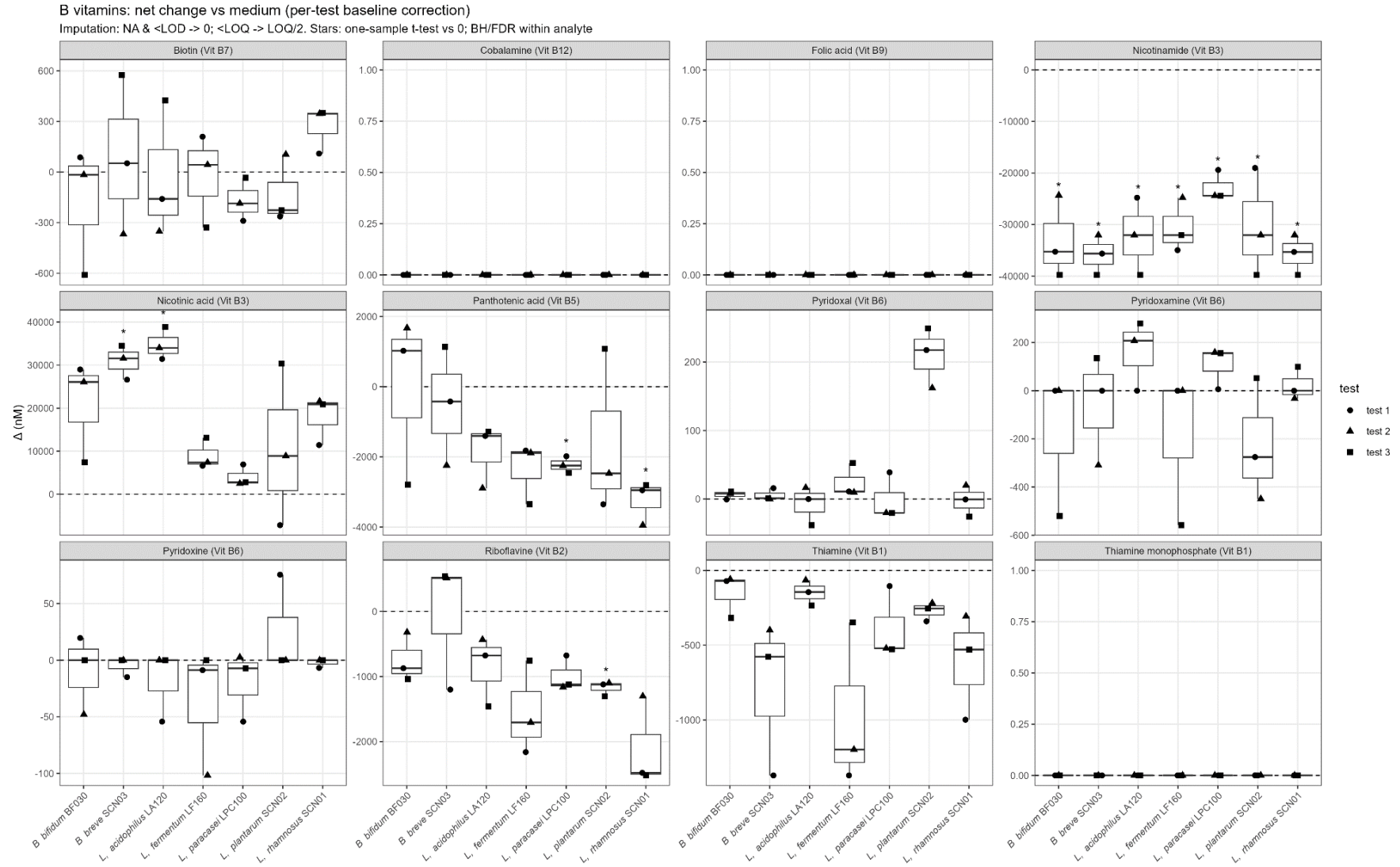
Table S3B_stats (mean difference vs matched control and Dunnett-adjusted p)

Time_h	L. acidophilus LA120 mean_diff	L. acidophilus LA120 p_adj	L. fermentum LF160 mean_diff	L. fermentum LF160 p_adj	L. paracasei LPC100 mean_diff	L. paracasei LPC100 p_adj	L. plantarum SCN02 mean_diff	L. plantarum SCN02 p_adj	L. rhamnosus SCN01 mean_diff	L. rhamnosus SCN01 p_adj	B. bifidum BF030 mean_diff	B. bifidum BF030 p_adj	B. breve SCN03 mean_diff	B. breve SCN03 p_adj
0.0	0.0		0.0		0.0		0.0		0.0		0.0		0.0	
3.0	0.16	>0.9999	-2.42	0.9132	0.56	>0.9999	0.98	0.985	-9.67	0.056	-3.74	0.0528	-2.91	0.6672
6.0	-1.02	0.9957	-2.63	0.952	0.81	>0.9999	1.08	0.997	-22.52	0.0198	-4.92	0.4844	-4.99	0.5604
9.0	-2.97	0.8537	-4.67	0.7729	-0.98	0.9998	-0.28	>0.9999	-35.28	0.0214	-5.98	0.5433	-6.36	0.4126
12.0	-3.88	0.7379	-7.28	0.5443	-4.02	0.9184	-2.26	0.9506	-44.43	0.0308	-4.86	0.7405	-5.66	0.4521
15.0	-4.41	0.7347	-9.32	0.4592	-5.17	0.7384	-2.57	0.9593	-50.37	0.0459	-4.61	0.8223	-5.2	0.6845
18.0	-4.66	0.8919	-12.53	0.4848	-7.82	0.556	-5.54	0.865	-58.22	0.07	-3.2	0.913	-4.45	0.8576
21.0	-5.41	0.9081	-14.66	0.501	-10.31	0.5451	-8.5	0.7893	-62.08	0.0775	0.69	0.9976	-1.36	0.9932
24.0	-4.84	0.9513	-16.53	0.4835	-12.53	0.4853	-9.32	0.8243	-69.0	0.1015	2.06	0.9796	1.62	0.9924
27.0	-6.87	0.8934	-20.31	0.4139	-15.9	0.4636	-13.71	0.6539	-78.54	0.1221	5.35	0.9265	3.77	0.9708
30.0	-6.7	0.9055	-21.4	0.4541	-18.32	0.4853	-15.35	0.6713	-83.4	0.1472	3.22	0.9783	2.58	0.9916
33.0	-6.91	0.9102	-23.97	0.4126	-21.38	0.5033	-18.62	0.5935	-89.76	0.1696	4.29	0.9667	5.43	0.9751
35.0	-8.45	0.8236	-24.84	0.3914	-23.79	0.4676	-21.22	0.5614	-91.91	0.1769	5.98	0.9571	5.18	0.9815

28 **Figure S1. Net metabolite changes in cell-free spent MRS media relative to run-matched uninoculated controls (targeted LC-MS/MS; n = 3 independent**
 29 **biological runs).** Panels show (A) B vitamins, (B) organic acids (“SCFA” panel; including lactate, selected branched-chain fatty acids (BCFAs) and C6 acids), (C)
 30 polyamines/biogenic amines and related metabolites, and (D) amino acids and related compounds. Values are plotted as baseline-corrected net changes (Δ) in native
 31 concentration units (nM for vitamins/selected analytes; μ M for organic acids and amino acids). Boxplots summarize distributions across runs (median, IQR), with
 32 individual run values overlaid (tests 1–3); dashed line denotes $\Delta = 0$. Asterisks indicate BH-adjusted significance for a two-sided one-sample test versus 0 within each
 33 analyte (* $p_{adj} < 0.05$; ** $p_{adj} < 0.01$; *** $p_{adj} < 0.001$). See Methods for data processing and baseline correction.

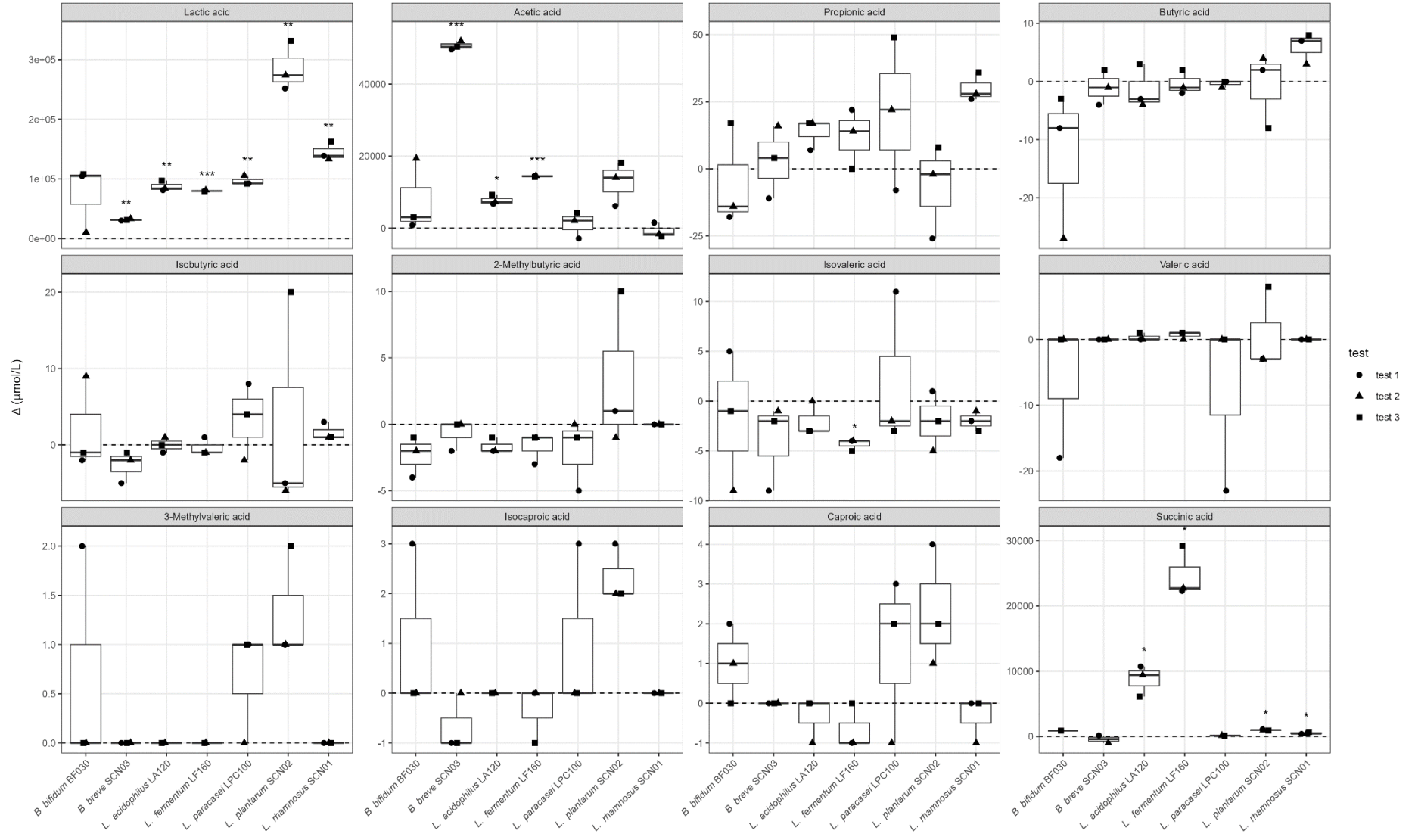
34
 35
 36

(A)



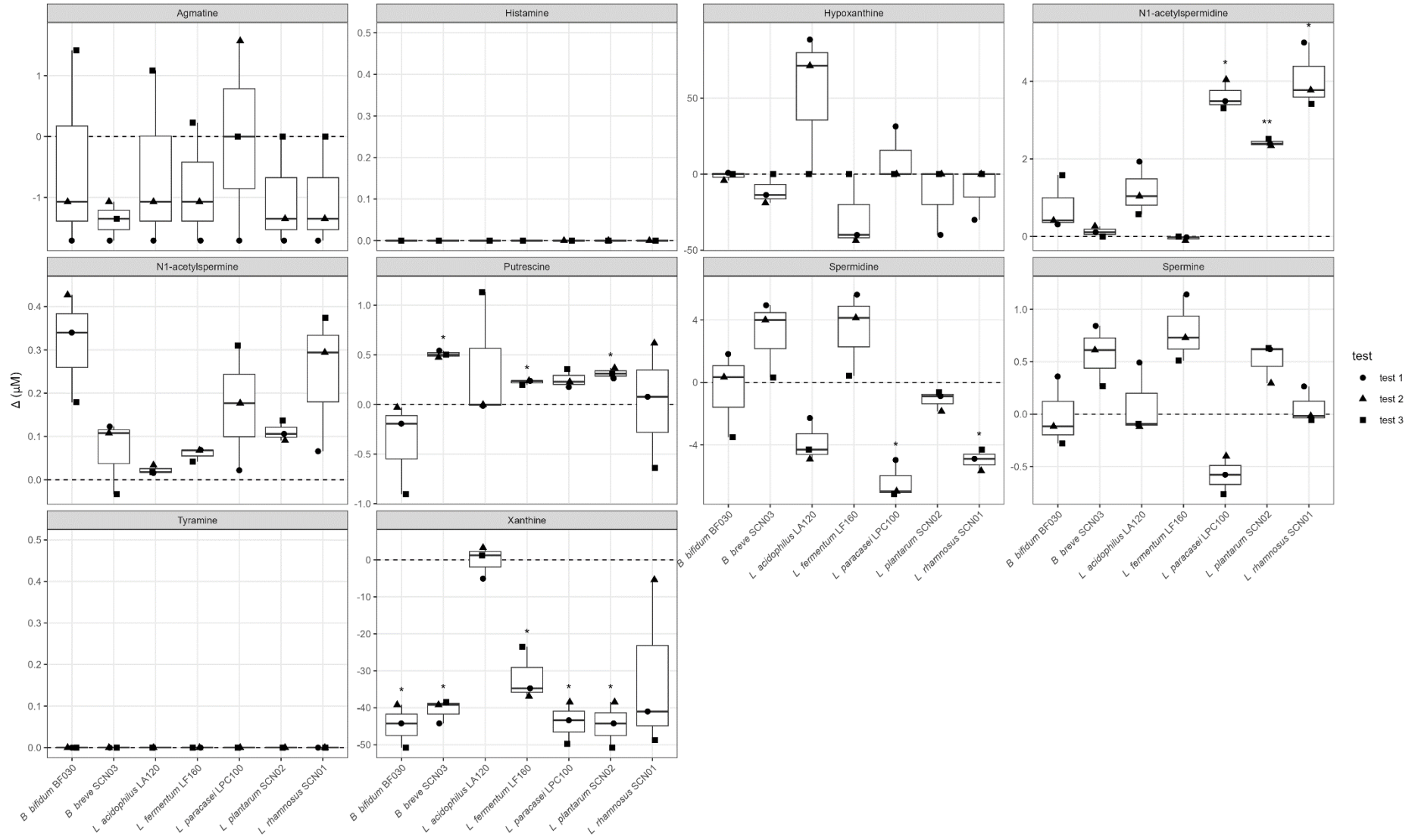
SCFAs: net change vs medium (per-test baseline correction)

Stars: one-sample t-test vs 0; BH/FDR within each metabolite



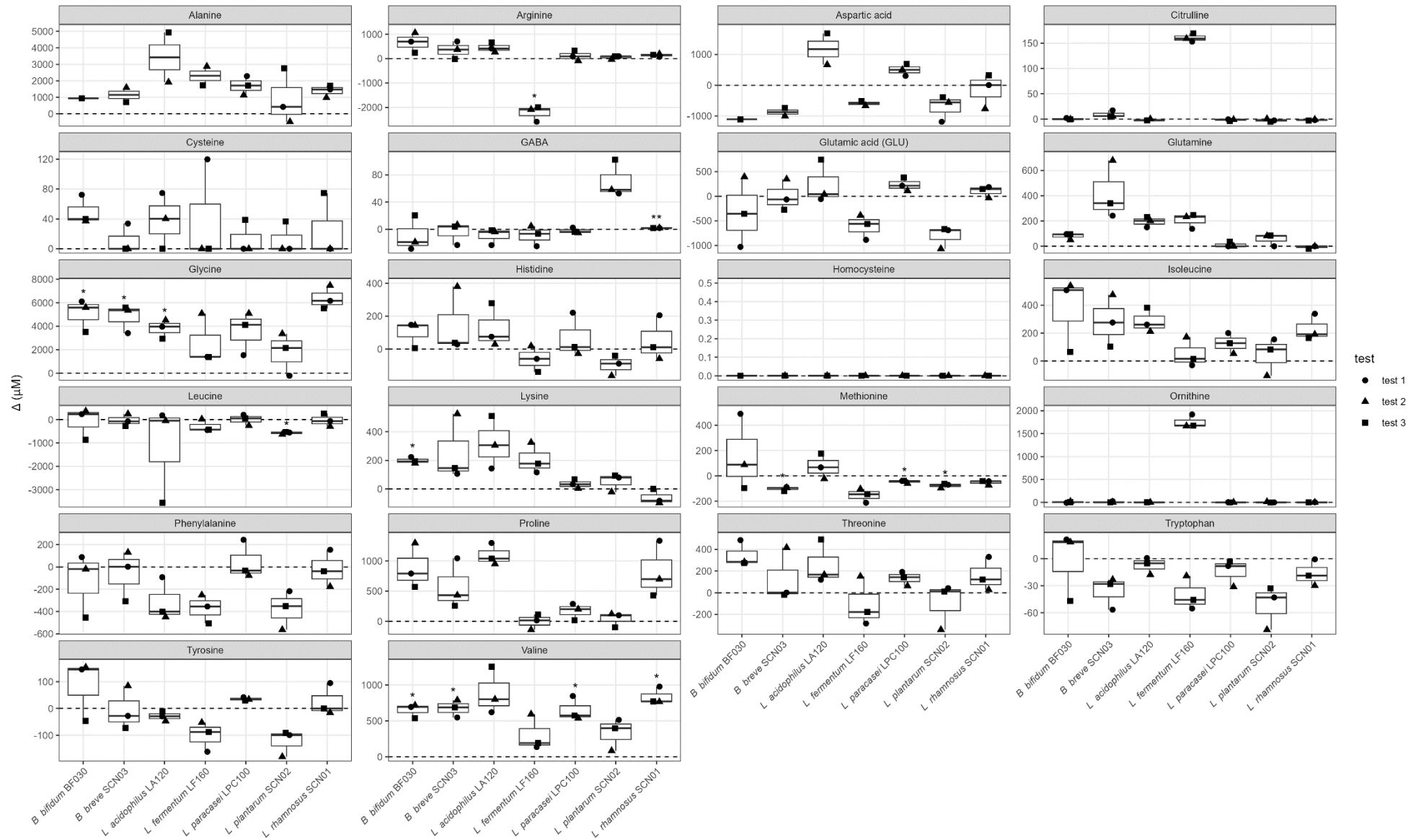
Polyamines, Biogenic Amines, and Other Nitrogen-Containing Metabolites

All raw tests used for per-test correction (sample TESTx - MSR TESTx). Then, per strain, the 3 raw tests are relabeled as test 1–3. BLANK=missing; NA/<LOD=0; <LOQ=LOQ/2 when possible (else 0). Stars: t-test vs 0; BH/FDR within metabolite.



Amino acids & amino acid-related

All raw tests used for per-test correction (sample TEST_x - MSR TEST_x). Then, per strain, the 3 raw tests are relabeled as test 1-3. BLANK=missing; NA/<LOD=0; <LOQ=LOQ/2 when possible (else 0). Stars: t-test vs 0; BH/FDR within metabolite.



41 **Figure S2. TEER-based assessment of Caco-2 epithelial resistance following exposure to *Bifidobacterium* strains.** (A) Time course of TEER over 0–35 h, expressed
 42 as percentage of the post-stabilization baseline at $t = 0$ (baseline = 100%). Data are shown as mean \pm SD across independent biological experiments; points indicate
 43 measurements plotted every 3 h and lines connect mean values (shaded bands indicate \pm SD). (B) Time course shown as in (A) but restricted to the early phase (0–21
 44 h) to highlight transient differences versus control. (C) Endpoint TEER at 35 h. Dots represent independent biological experiments; diamonds indicate the mean;
 45 horizontal segments denote mean \pm SD. (D) Endpoint TEER at 9 h, displayed as in (C), to capture early effects. For panels (C–D), n denotes the number of independent
 46 biological experiments per condition. The dashed line marks the baseline (100%) (horizontal in panels A–B; vertical in panels C–D).

