

Table S1. Metabolites identified in *E. coli* strains.

	Metabolites	Assignments	¹H chemical shifts (ppm)	¹³C chemical shifts (ppm)
1	Leucine	α CH; β CH ₂ ; γ CH; δ CH ₃ ; δ' CH ₃	3.74 (m); 1.70(m); 0.96 (d); 0.87 (d)	56.2; 42.6; 24.4; 22.12
2	Ethanol	α CH ₂ ; β CH ₃	3.64 (m); 1.17 (t)	60.3; 19.6
3	Lactic acid	α CH; β CH ₃	4.10 (q); 1.31 (d)	71.4; 22.9
4	Alanine	α CH; β CH ₃	3.77 (q); 1.47 (d)	53.1; 18.9
5	Isoleucine	α CH; β CH; γ CH ₂ ; γ' CH ₃ ; δ CH ₃	3.71 (d); 2.01 (m); 1.63 (m); 1.03 (d); 1.00 (t)	61.88; 39.0; 24.6; 18.1; 14.8
6	Acetic acid	CH ₃	1.91 (s)	26.1
7	Glutamic acid	α CH; β CH ₂ ; γ CH ₂	3.74 (m); 2.09 (m); 2.34 (m)	57.6; 29.8; 36.4
8	Methionine	α CH; β CH ₂ ; γ CH ₂ ; SCH ₃	3.85 (dd); 2.18 (m); 2.63 (t); 2.10 (m)	56.8; 32.7; 31.6; 16.6
9	Pyruvic acid	CH ₃	2.36 (s)	29.2
10	α -ketoglutaric acid	α CH ₂ ; β CH ₂	3.01 (t); 2.44 (t)	38.8; 33.4
11	Aspartic acid	α CH; β CH ₂	3.91 (dd); 2.73 (dd)	55.1; 39.4
12	Tyrosine	C ₁ H; C ₂ H ₂ ; C ₄ H; C ₅ H	3.93 (dd); 3.17 (dd); 7.19 (d); 6.85 (m)	59.0; 38.3; 133.5; 115.9
13	Phosphorylcholine	α CH ₂ ; β CH ₂ ; NCHN	4.15 (d); 3.57 (d); 3.20(s)	60.6; 68.9; 56.5
14	Betaine	CH ₃ ; CH ₂	3.25 (s); 3.89 (s)	55.9; 68.6
15	β -D-glucose	C ₁ H ₂ ; C ₂ H; C ₃ H; C ₄ H; C ₅ H; C ₆ H	3.89 (m); 3.47 (dd); 3.46 (dd); 3.40 (m); 3.23 (dd); 4.69 (d)	63.4; 79.6; 79.5; 72.5; 77.0; 97.5
16	Threonine	α CH; β CH; γ CH ₃	3.54 (d); 4.23 (m); 1.30 (d)	63.4; 68.3; 20.3
17	Valine	α CH; β CH; γ CH ₃ ; γ' CH ₃	3.60 (d); 2.26 (m); 0.98 (d); 1.03 (d)	63.3; 31.9; 19.4; 20.8
18	Lysine	α CH; β CH ₂ ; γ CH ₂ ; δ CH ₂ ; ϵ CH ₂	3.75 (t); 1.88 (m); 1.46 (m); 1.72 (m); 3.02 (t)	57.5; 32.7; 24.0; 29.1; 42.1
19	Arginine	α CH; β CH ₂ ; γ CH ₂ ; δ CH ₂	3.76 (t); 1.91 (dd); 1.68 (m); 3.24 (t)	57.3; 30.5; 26.5; 43.3
20	Phenylalanine	C ₁ H; C ₂ H ₂ ; C ₄ H; C ₅ H; C ₆ H	3.98 (dd); 3.19 (dd); 7.37 (m); 7.42 (m); 7.32 (m)	58.9; 39.2; 130.4; 131.8; 132.1
21	Histidine	α CH; β CH ₂ ; NCHC; NCHN	4.00 (dd); 3.26 (dd); 7.14 (d); 8.03 (d)	57.3; 30.16; 120.0; 138.4
22	Ribose-5-phosphate	C ₁ H ₂ ; C ₂ H; C ₃ H; C ₄ H; C ₅ H	3.88 (m); 4.07 (m); 4.23 (t); 4.05 (m); 5.40 (m)	67.5; 84.4; 77.2; 78.0; 98.9
23	Uridine	C ₁ H; C ₂ H; C ₃ H; C ₄ H; C ₅ H ₂ ; ¹ C ₁ H; ¹ C ₂ H	5.90 (d); 4.35 (dd); 4.23 (dd); 4.12 (dd); 3.86 (dd); 7.86 (d); 5.89 (d)	92.1; 76.5; 72.1; 87.1; 63.6; 144.6; 105.1

24	ADP	C ₁ H; C ₂ H; C ₃ H; C ₄ H; C ₅ H ₂ ; N ¹ CHN; N ² CHN	5.94 (m) ; 4.58 (d); 4.22 (d) ; 4.11 (d) ; 4.12 (m) ; 8.54 (s) ; 8.30 (s)	87.1 ; 73.7; 70.1 ; 65.4 ; 83.3 ; 140.4 ; 148.4
25	Pyroglutamic acid	αCH; βCH ₂ ; γCH ₂	4.16 (dd) ; 2.25 (m); 2.38 (m)	61.0 ; 28.0; 32.3
26	ATP	C ₁ H; C ₂ H; C ₃ H; C ₄ H; C ₅ H ₂ ; N ¹ CHN; N ² CHN	6.15 (d) ; 4.82 (d); 4.64 (t); 4.42 (m) ; 4.28 (m) ; 8.55 (s); 8.24 (s)	89.3 ; 77.0; 73.0; 86.6 ; 67.7 ; 142.5; 155.4
27	Cyclic AMP	C ₁ H; C ₂ H; C ₃ H; C ₄ H; C ₅ H ₂ ; N ¹ CHN; N ² CHN	6.13 (d); 4.34 (d) ; 4.73 (d); 4.75 (d); 4.47 (m); 8.17 (s) ; 8.19 (s)	94.3; 72.2 ; 75.0; 79.9; 69.9; 155.4 ; 142.4
28	Adenosine	C ₁ H; C ₂ H; C ₃ H; C ₄ H; C ₅ H ₂ ; N ¹ CHN; N ² CHN	6.03 (d); 4.74 (s); 4.43 (dd) ; 4.29 (dd) ; 3.91 (dd) ; 8.30 (s); 8.18 (s)	91.1; 76.7; 73.5 ; 88.6 ; 64.4 ; 143.3; 155.3
29	α-D-glucose	C ₁ H ₂ ; C ₂ H; C ₃ H; C ₄ H; C ₅ H; C ₆ H	3.82 (m) ; 3.81 (m) ; 3.52 (m) ; 3.70 (m) ; 3.72 (m) ; 5.22 (d)	63.3 ; 74.3 ; 74.9 ; 75.6 ; 63.5 ; 94.9
30	Glucose-1-phosphate	C ₁ H; C ₂ H; C ₃ H; C ₄ H; C ₅ H; C ₆ H ₂	5.44 (dd) ; 3.47 (d) ; 3.77 (m) ; 3.38 (t) ; 3.91 (m) ; 3.80 (m)	96.5 ; 75.1 ; 75.9 ; 72.5 ; 74.8 ; 63.5

Note: **Bold** chemical shifts indicate identified peaks in the spectra. Multiplicity: s: singlet; d: doublet; dd: doublet of doublets; t: triplet; q: quartet; m: multiplet.

Table S2. Summary of fit of orthogonal projection to latent structures-discriminant analysis (OPLS-DA) models.

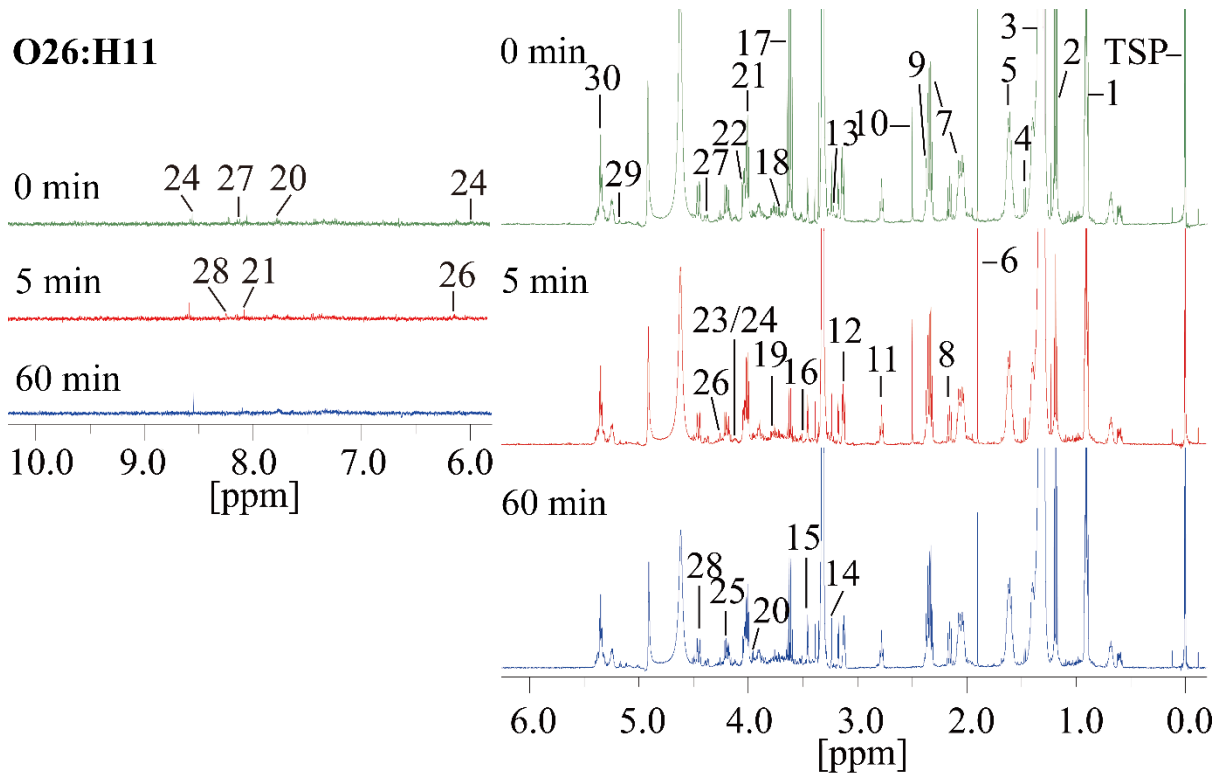
Compared group	Serotype	Principal component 1		Principal component 2	
		R ² Y	Q ²	R ² Y	Q ²
5 min vs. 0 min	O26	1.00	0.94	1.00	0.97
	O45	1.00	0.92	1.00	0.96
	O103	0.97	0.87	1.00	0.93
	O111	0.99	0.86	1.00	0.92
	O121	0.91	0.69	1.00	0.83
	O145	0.98	0.89	1.00	0.94
60 min vs. 0 min	O26	1.00	0.96	1.00	0.98
	O45	1.00	0.95	1.00	0.97
	O103	0.99	0.97	1.00	0.99
	O111	1.00	0.97	1.00	0.99
	O121	0.99	0.93	1.00	0.97
	O145	1.00	0.95	1.00	0.98

Table S3. Metabolic pathway analysis for the screened metabolites in each *E. coli* strain.

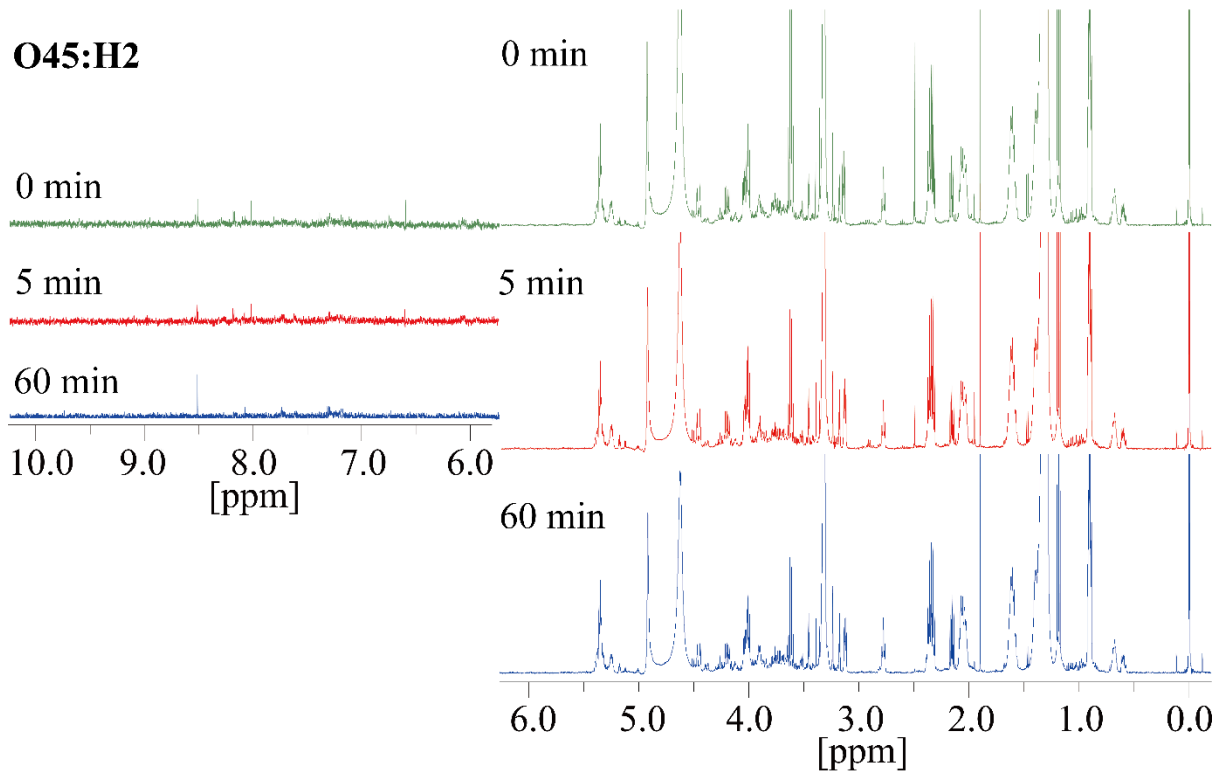
Strain	Pathway name	Matching	P value	FDR
O26:H11 (ATCC BAA-2196)	Aminoacyl-tRNA biosynthesis	5/45	0.000	0.003
	Arginine biosynthesis	2/16	0.011	0.321
	Valine, leucine and isoleucine biosynthesis	2/22	0.020	0.321
	Alanine, aspartate and glutamate metabolism	2/22	0.020	0.321
	Valine, leucine and isoleucine degradation	2/23	0.022	0.321
	Pyruvate metabolism	2/26	0.027	0.321
	Carbapenem biosynthesis	1/3	0.030	0.321
	Glycolysis / Gluconeogenesis	2/29	0.033	0.321
	Purine metabolism	3/73	0.034	0.321
O45:H2 (ATCC BAA-2193)	Aminoacyl-tRNA biosynthesis	6/45	0.000	0.000
	Valine, leucine and isoleucine biosynthesis	3/22	0.002	0.061
	Valine, leucine and isoleucine degradation	3/23	0.002	0.061
	Glycolysis / Gluconeogenesis	3/29	0.004	0.091
	Alanine, aspartate and glutamate metabolism	2/22	0.027	0.357
	Carbapenem biosynthesis	1/3	0.036	0.357
	Novobiocin biosynthesis	1/3	0.036	0.357
	D-Alanine metabolism	1/3	0.036	0.357
	Pyruvate metabolism	2/26	0.037	0.357
O103:H11 (ATCC BAA-2215)	Aminoacyl-tRNA biosynthesis	7/45	0.000	0.000
	Arginine biosynthesis	3/16	0.000	0.013
	Alanine, aspartate and glutamate metabolism	3/22	0.001	0.023
	Valine, leucine and isoleucine biosynthesis	2/22	0.016	0.265
	Valine, leucine and isoleucine degradation	2/23	0.018	0.265
	Pyruvate metabolism	2/26	0.023	0.265
	Carbapenem biosynthesis	1/3	0.028	0.265
	D-Alanine metabolism	1/3	0.028	0.265
	Arginine and proline metabolism	2/29	0.028	0.265
	Glyoxylate and dicarboxylate metabolism	2/37	0.044	0.376
O111 (ATCC BAA-2440)	Aminoacyl-tRNA biosynthesis	6/45	0.000	0.000

	Valine, leucine and isoleucine biosynthesis	3/22	0.001	0.019
	Valine, leucine and isoleucine degradation	3/23	0.001	0.019
	Glycolysis / Gluconeogenesis	2/29	0.023	0.358
	Carbapenem biosynthesis	1/3	0.025	0.358
	Novobiocin biosynthesis	1/3	0.025	0.358
O121:H19 (ATCC BAA-2219)	Aminoacyl-tRNA biosynthesis	6/45	0.000	0.000
	Arginine biosynthesis	3/16	0.000	0.009
	Alanine, aspartate and glutamate metabolism	2/22	0.013	0.307
	Pantothenate and CoA biosynthesis	2/24	0.016	0.307
	Arginine and proline metabolism	2/29	0.023	0.307
	Carbapenem biosynthesis	1/3	0.025	0.307
	Novobiocin biosynthesis	1/3	0.025	0.307
O145 (ATCC BAA-2192)	Aminoacyl-tRNA biosynthesis	6/45	0.000	0.000
	Valine, leucine and isoleucine biosynthesis	3/22	0.001	0.048
	Valine, leucine and isoleucine degradation	3/23	0.002	0.048
	Arginine biosynthesis	2/16	0.013	0.271
	Alanine, aspartate and glutamate metabolism	2/22	0.023	0.357
	Pantothenate and CoA biosynthesis	2/24	0.028	0.357
	Pyruvate metabolism	2/26	0.032	0.357
	Carbapenem biosynthesis	1/3	0.033	0.357
	Glycolysis / Gluconeogenesis	2/29	0.039	0.366
	Purine metabolism	3/73	0.043	0.366

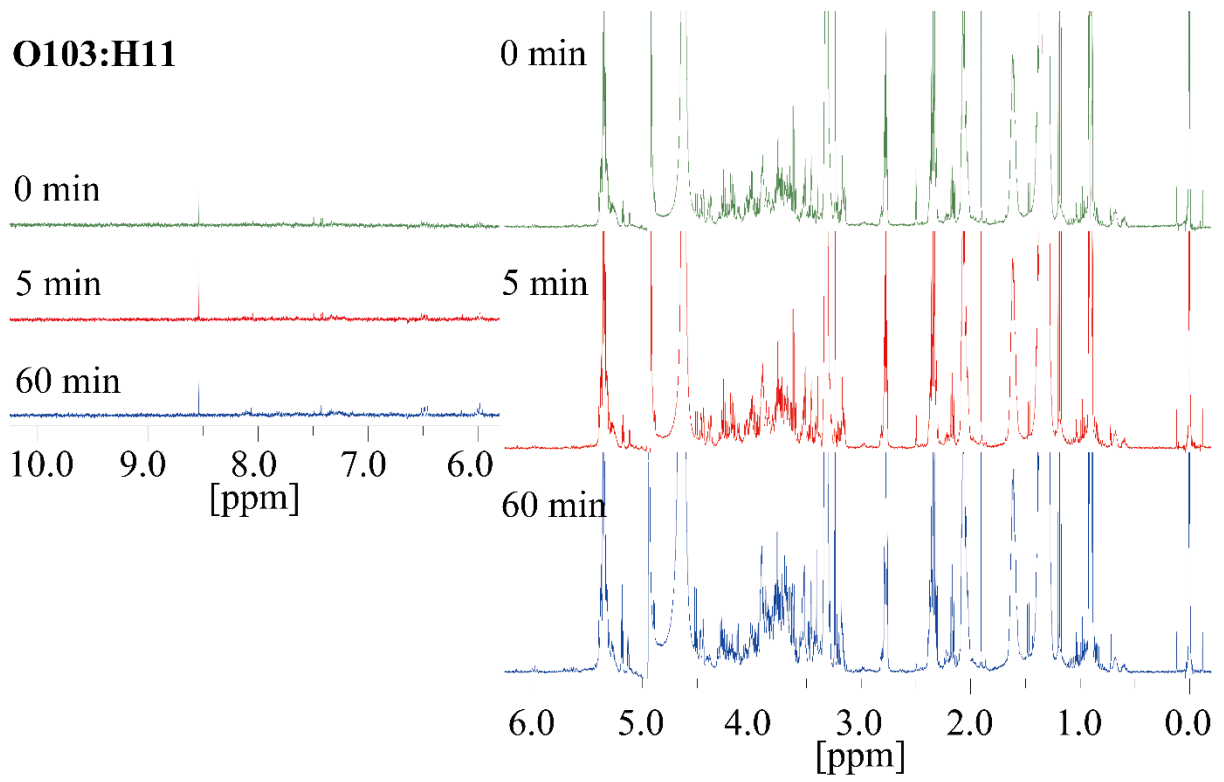
O26:H11



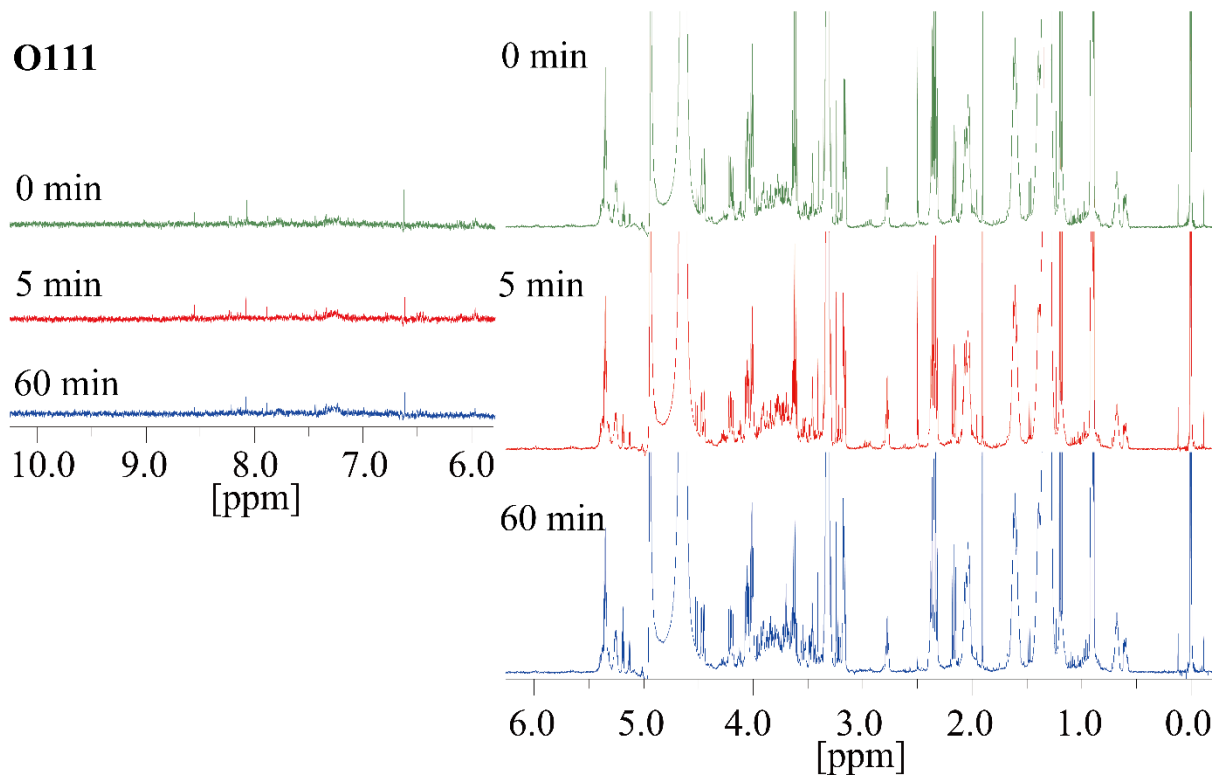
O45:H2



O103:H11



O111



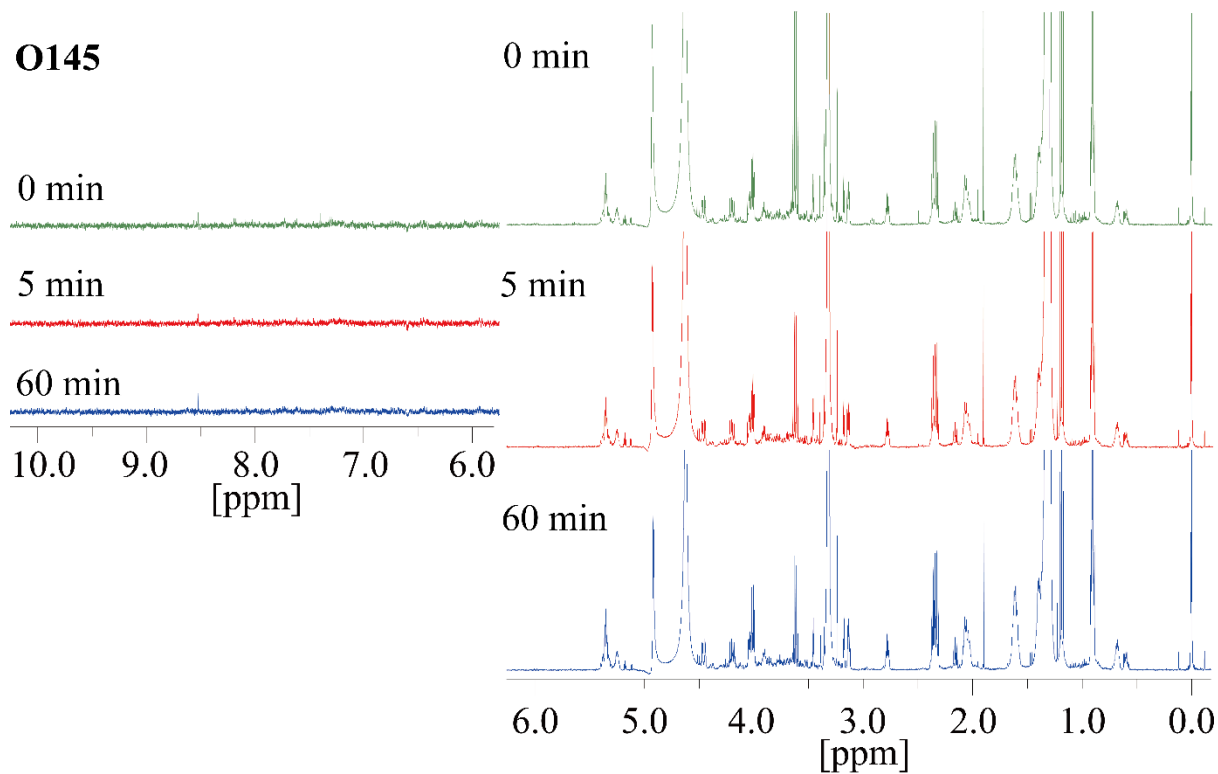
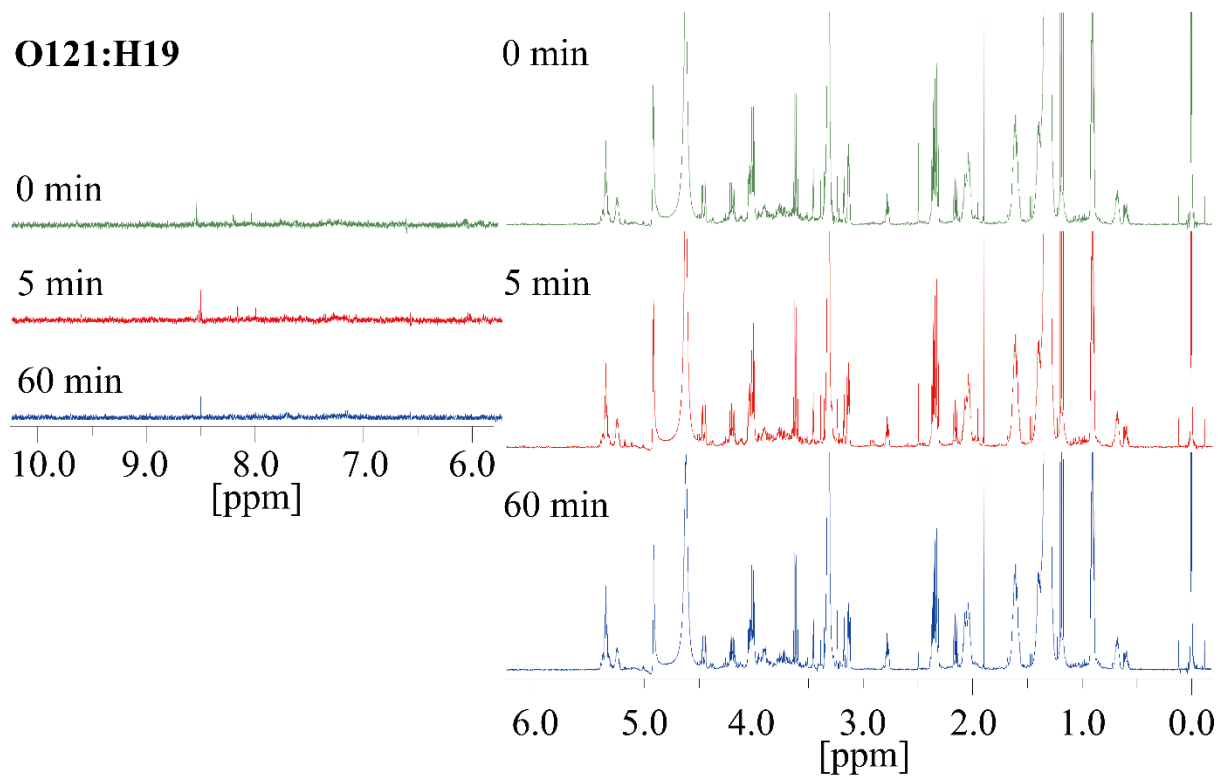


Fig. S1. Typical ^1H nuclear magnetic resonance (NMR) spectra of *E. coli* O26:H11 (ATCC BAA-2196), O45:H2 (ATCC BAA-2193), O103:H11 (ATCC BAA-2215), O111 (ATCC BAA-2440), O121:H19 (ATCC BAA-2219) and O145 (ATCC BAA-2192) in wheat flour treated at 60 °C for 0, 5 and 60 min.

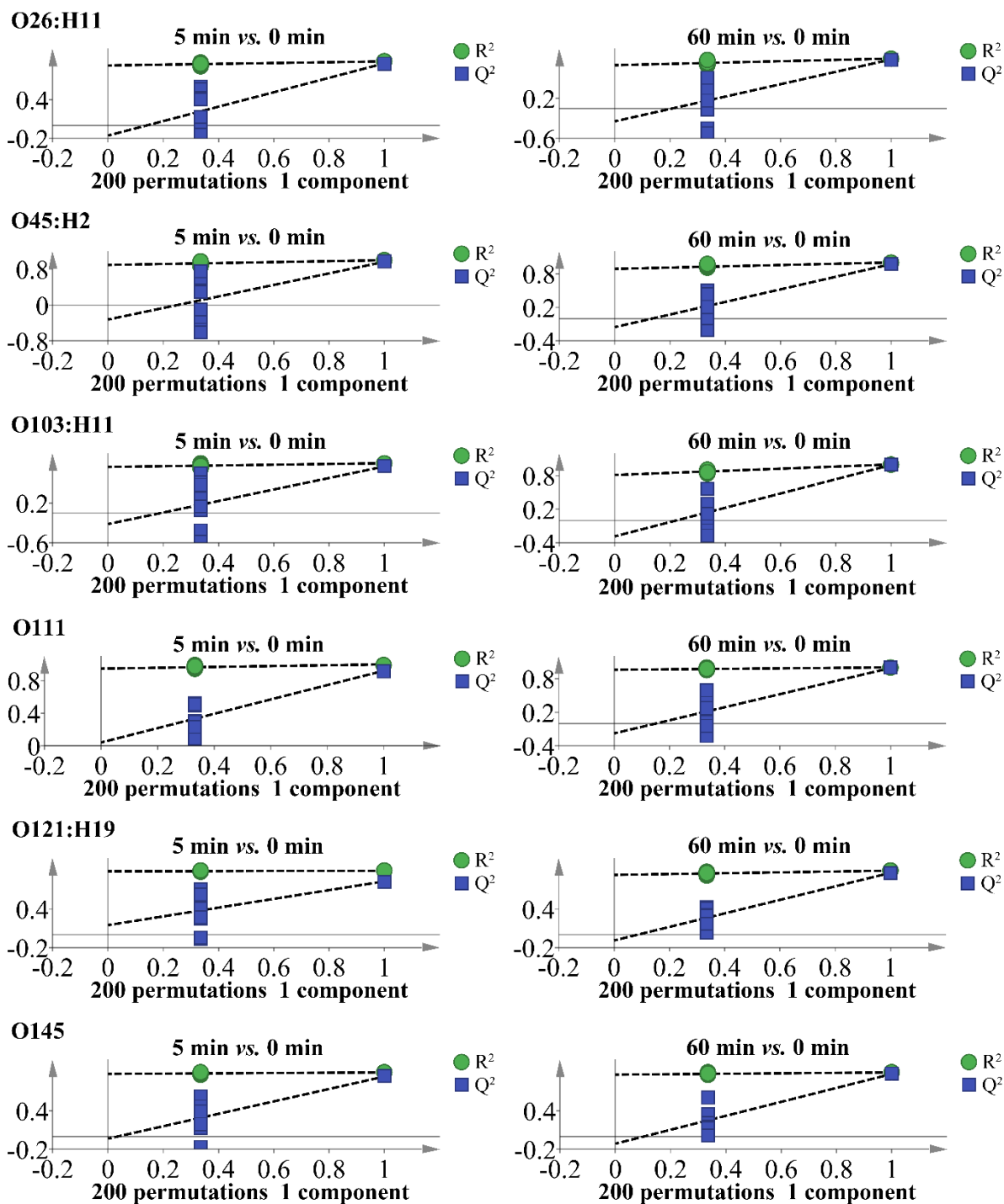


Fig. S2. Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) permutation test plots.