Supplemental Material to

Core Fluxome and meta fluxome of lactic acid bacteria under cocoa pulp fermentation simulating conditions

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1. Carbon mass isotopomer fractions of secreted products lactate and acetate in pure cultures of lactic acid bacteria

Table S1: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in pure cultures of *L. fermentum* NCC 575 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	32.0 ± 0.0	94.2 ± 0.0	96.9 ± 0.1
		Sim.	39.3	94.1	96.8
	m+1	Exp.	1.3 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
		Sim.	1.3	3.1	3.1
	m+2	Exp.	2.7 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
		Sim.	1.8	0.1	0.0
	m+3	Exp.	64.0 ± 0.0	2.7 ± 0.0	0.0 ± 0.0
		Sim.	57.6	2.7	0.0
Acetate	*m*+0	Exp.	50.1 ± 0.3	94.4 ± 0.0	49.1 ± 0.0
		Sim.	39.8	95.1	43.3
	m+1	Exp.	3.1 ± 0.1	2.8 ± 0.0	49.7 ± 0.0
		Sim.	2.0	2.1	56.1
	m+2	Exp.	49.5 ± 0.4	2.8 ± 0.0	1.1 ± 0.0
		Sim.	58.2	2.8	0.1

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

Table S2: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in pure cultures of *L. fermentum* NCC 528 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	80.7 ± 0.0	96.0 ± 0.0	96.8 ± 0.1
		Sim.	81.2	95.9	96.8
	m+1	Exp.	2.6 ± 0.1	3.0 ± 0.0	3.1 ± 0.1
		Sim.	2.6	3.1	3.1
	m+2	Exp.	1.0 ± 0.1	0.1 ±0.0	0.0 ± 0.0
		Sim.	0.5	0.1	0.1
	m+3	Exp.	15.7 ± 0.0	0.9 ± 0.0	0.0 ± 0.0
		Sim.	15.7	0.9	0.0
Acetate[b]	*m*+0	Exp.	78.6 ± 0.0	95.2 ± 0.0	79.6 ± 0.0
		Sim.	82.1	96.9	86.6
	m+1	Exp.	3.3 ± 0.0	3.0 ± 0.0	19.2 ± 0.0
		Sim.	2.1	2.1	13.3
	m+2	Exp.	18.1 ± 0.0	1.8 ± 0.0	1.1 ± 0.0
		Sim.	15.8	0.1	0.1

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

Table S3: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in pure cultures of *L. plantarum* NCC 2829 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	69.1 ± 0.3	36.6 ± 0.0	84.5 ± 0.1
		Sim.	69.5	36.0	84.6
	m+1	Exp.	2.5 ± 0.1	1.3 ± 0.0	3.1 ± 0.0
		Sim.	2.3	1.2	3.0
	m+2	Exp.	1.1 ± 0.1	1.1 ± 0.0	12.2 ± 0.0
		Sim.	0.9	1.9	12.3
	m+3	Exp.	27.3 ± 0.1	61.0 ± 0.0	0.2 ± 0.0
		Sim.	27.4	60.8	0.1
Acetate	*m*+0	Exp.	69.1 ± 0.1	86.0 ± 0.2	74.1 ± 0.1
		Sim.	77.6	80.1	78.2
	m+1	Exp.	3.0 ± 0.1	2.7 ± 0.1	22.8 ± 0.1
		Sim.	2.1	2.1	21.2
	m+2	Exp.	27.9 ± 0.2	11.4 ± 0.3	3.1 ± 0.1
		Sim.	20.3	17.8	0.1

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

Table S4: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in pure cultures of *L. plantarum* NCC 1295 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	32.6 ± 0.0	65.3 ± 0.0	66.6 ± 0.0
		Sim.	33.3	65.2	66.4
	m+1	Exp.	1.3 ± 0.0	2.2 ± 0.0	2.4 ± 0.0
		Sim.	1.1	2.1	2.8
	m+2	Exp.	2.6 ± 0.0	0.7 ± 0.0	30.7 ± 0.0
		Sim.	2.0	1.0	30.5
	m+3	Exp.	63.5 ± 0.0	31.7 ± 0.0	0.4 ± 0.0
		Sim.	63.6	31.7	0.3
Acetate[b]	*m*+0	Exp.	49.8 ± 0.0	84.2 ± 0.0	62.5 ± 0.0
		Sim.	37.1	86.0	49.7
	m+1	Exp.	3.8 ± 0.0	3.2 ± 0.0	24.9 ± 0.0
		Sim.	2.0	2.1	38.5
	m+2	Exp.	46.4 ± 0.0	12.6 ± 0.0	12.6 ± 0.0
		Sim.	60.9	11.9	11.7

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

2. Carbon mass isotopomer fractions of secreted products lactate and acetate in mixed cultures of lactic acid bacteria

Table S5: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in mixed cultures of *L. fermentum* NCC 575 grown cocoa in pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	36.6 ± 0.0	91.4 ± 0.0	94.6 ± 0.0
		Sim.	36.6	91.3	94.5
	m+1	Exp.	1.3 ± 0.0	2.8 ± 0.0	3.0 ± 0.0
		Sim.	1.2	3.0	3.1
	m+2	Exp.	2.3 ± 0.0	0.1 ± 0.0	2.4 ± 0.0
		Sim.	1.8	0.2	2.3
	m+3	Exp.	59.9 ± 0.0	5.6 ± 0.0	0.0 ± 0.0
		Sim.	60.4	5.5	0.0
Acetate	*m*+0	Exp.	44.7 ± 0.0	93.7 ± 0.1	46.8 ± 0.0
		Sim.	38.4	92.6	39.5
	m+1	Exp.	4.0 ± 0.0	3.0 ± 0.0	51.8 ± 0.0
		Sim.	2.0	2.1	59.8
	m+2	Exp.	51.3 ± 0.0	3.3 ± 0.0	1.4 ± 0.0
		Sim.	59.6	5.3	0.1

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

Table S6: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in mixed cultures of *L. fermentum* NCC 528 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	47.0 ± 0.0	58.6 ± 0.0	72.5 ± 0.1
		Sim.	41.9	57.4	71.9
	m+1	Exp.	1.5 ± 0.0	2.0 ± 0.0	2.5 ± 0.1
		Sim.	1.4	1.9	2.5
	m+2	Exp.	1.9 ± 0.0	0.8 ± 0.0	24.8 ± 0.0
		Sim.	1.7	1.2	24.8
	m+3	Exp.	49.5 ± 0.0	38.6 ± 0.0	0.2 ± 0.0
		Sim.	55.0	39.5	0.2
Acetate[b]	*m*+0	Exp.	65.3 ± 0.1	89.7 ± 0.0	70.9 ± 0.0
		Sim.	36.9	89.7	37.5
	m+1	Exp.	3.5 ± 0.1	3.1 ± 0.0	23.8 ± 0.0
		Sim.	2.0	2.1	61.8
	m+2	Exp.	31.3 ± 0.0	7.3 ± 0.0	5.3 ± 0.0
		Sim.	61.1	8.2	0.7

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

Table S7: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in mixed cultures of *L. plantarum* NCC 2829 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	39.4 ± 0.0	90.9 ± 0.0	96.6 ± 0.0
		Sim.	39.3	91.0	96.5
	m+1	Exp.	1.4 ± 0.0	2.8 ± 0.0	3.0 ± 0.0
		Sim.	1.3	3.0	3.1
	m+2	Exp.	2.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.0
		Sim.	1.8	0.2	0.4
	m+3	Exp.	57.1 ± 0.0	6.2 ± 0.0	0.0 ± 0.0
		Sim.	57.6	5.9	0.0
Acetate	*m*+0	Exp.	50.2 ± 0.0	91.2 ± 0.0	51.0 ± 0.0
		Sim.	39.8	92.2	40.4
	m+1	Exp.	3.9 ± 0.0	3.0 ± 0.0	47.6 ± 0.0
		Sim.	2.0	2.1	58.9
	m+2	Exp.	45.9 ± 0.0	5.8 ± 0.0	1.3 ± 0.0
		Sim.	58.1	5.7	0.6

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

Table S8: Experimental and predicted mass isotopomer distributions of lactate and acetate [% of total pool] in mixed cultures of *L. plantarum* NCC 1295 grown in cocoa pulp simulation medium

Analyte	Mass isotopomer fraction	Source[a]	Labeled substrate		
			[$^{13}C_6$]glucose	[$^{13}C_6$]fructose	[1,2-$^{13}C_2$]glucose
Lactate	*m*+0	Exp.	38.6 ± 0.1	90.9 ± 0.0	95.9 ± 0.0
		Sim.	38.6	90.9	95.8
	m+1	Exp.	1.3 ±0.0	2.8 ± 0.0	3.0 ± 0.0
		Sim.	1.3	3.0	3.1
	m+2	Exp.	2.2 ± 0.0	0.2 ± 0.0	1.1 ± 0.0
		Sim.	1.8	0.2	1.1
	m+3	Exp.	57.9 ± 0.0	6.2 ± 0.0	0.0 ± 0.0
		Sim.	58.3	6.0	0.0
Acetate	*m*+0	Exp.	57.9 ± 0.0	91.4 ± 0.0	51.2 ± 0.0
		Sim.	39.8	92.0	40.3
	m+1	Exp.	3.9 ± 0.0	2.9 ± 0.0	47.5 ± 0.0
		Sim.	2.0	2.1	59.0
	m+2	Exp.	46.7 ± 0.0	5.7 ± 0.01	1.3 ±0.0
		Sim.	58.2	5.9	0.5

[a]Experimental data (Exp.) were obtained from GC/MS measurements of $TBDMS_2$-lactate and acetic acid (pentyl ester) after correction factor analysis. Predicted data (Sim.) correspond to model simulations at the optimized flux distribution.

3. Fermentation profiles of mixed cultures of lactic acid bacteria

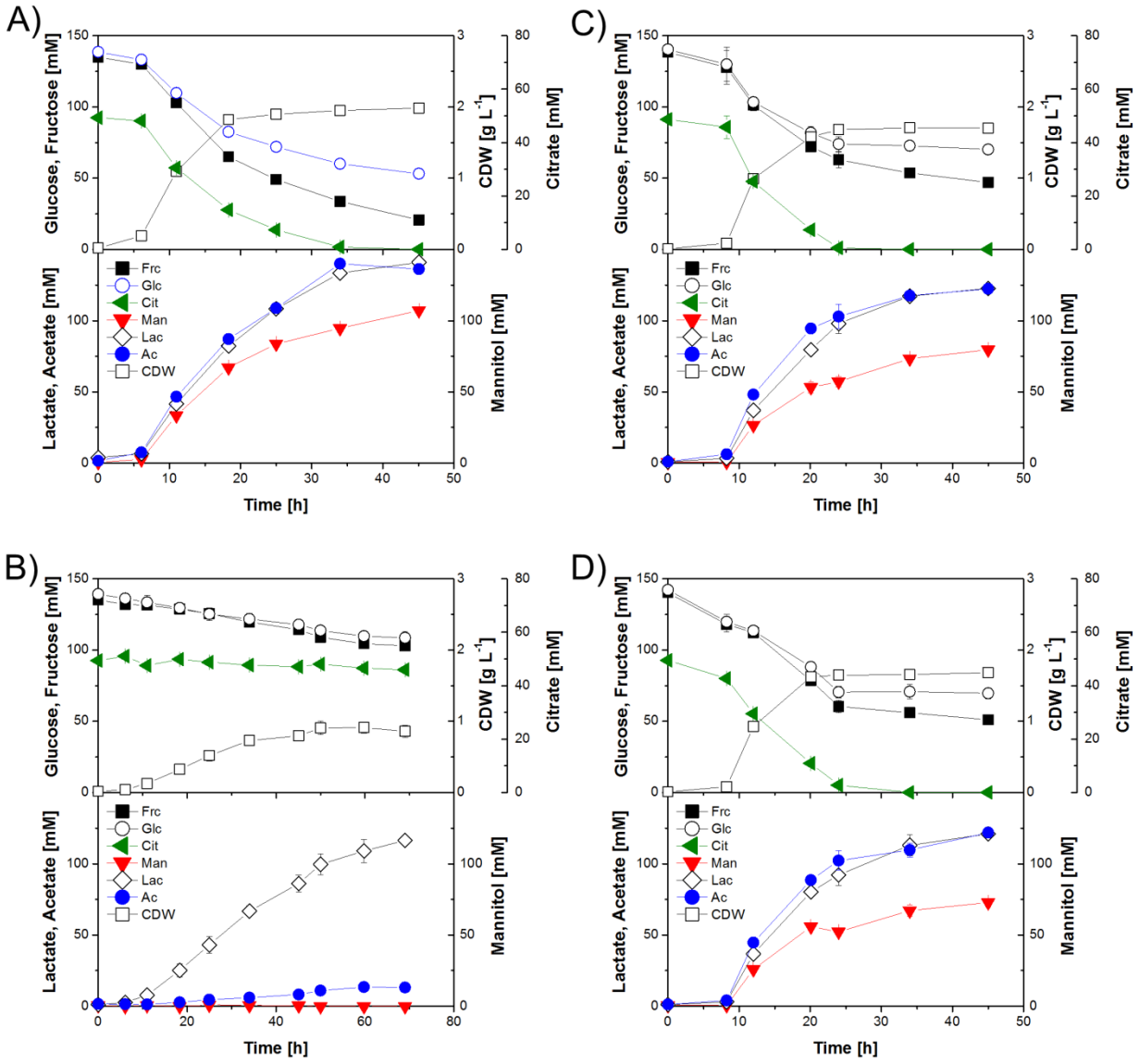

Figure S1: Fermentation profiles of mixed cultures of *L. fermentum* NCC 575 (A), *L. fermentum* NCC 528 (B), *L. plantarum* NCC 2829 (C), and *L. plantarum* NCC 1295 (D) in cocoa pulp simulation medium (refer to table 1 for more detailed information). The data represent mean values from two replicates and their corresponding standard deviations, respectively. Glc: glucose, Frc: fructose, Man: mannitol, Cit: citrate, Lac: lactate, Ac: acetate, CDW: cell dry weight.