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### Monte-Carlo modeling of the central carbon metabolism of *Lactococcus lactis*: insights into metabolic regulation

Murabito, E.; Verma, M.; Bekker, M.; Bellomo, D.; Westerhoff, H.V.; Teusink, B.; Steuer, R.

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**SUPPLEMENTAL TEXT S1**

for

**Monte-Carlo Modeling of the Central Carbon Metabolism of *Lactococcus Lactis*:  
Insights into Metabolic Regulation**

by Murabito et al. (2014)

**Determination of extracellular fluxes:** Fluxes for lactate, acetate, formate, pyruvate and ethanol were calculated using their fermentation broth concentration, dilution rate (0.5 h<sup>-1</sup>) and steady state bacterial cell dry weight. Fluxes are shown in Table S1.

Table S1: Extracellular fluxes measured in the current study.

<b>Reaction ID</b>	<b>Chemical equation</b>	<b>Flux (mM/min)</b>	<b>Source</b>
LDH	PYR + NADH → LAC + NAD	4.68E+02	Measured in this study
ADH	ACALD + NADH → ETOH + NAD	5.96E+01	Measured in this study
ACK	ACPH + ADP → ACETATE + ATP	4.67E+01	Measured in this study
ACLACD	ACLAC → Acetoin	1.42E+02	Measured in this study
BDH	Acetoin + NADH → Butanediol + NAD	1.42E+02	Measured in this study
PTS	GLCo + PEP → G6P + PYR	4.29E+02	Measured in this study

**Intracellular metabolites:** Steady state intracellular metabolites concentrations were gathered from previously published articles in various journals. The steady state data and its sources are given in Table S2.

Table S2: Steady state concentrations of intracellular metabolites.

<b>Metabolite</b>	<b>Conc. (mM)</b>	<b>Source</b>
PYR	9.00	Ana et al., (2000)
PHI	5.00	Current study
NAD	8.40	Garrigues et al., (1997)
ADP	6.10	Current study
G6P	2.50	Garrigues et al., (1997)
GAP	2.40	Ana et al., (2000)
COA	0.08	
PEP	2.10	Garrigues et al., (1997)
DHAP	6.12	Ana et al., (2000)
Acetoin	0.1	
G3P	0.6	Garrigues et al., (1997)
F6P	5.0	Ana et al., (2000)
ACALD	0.0004	
ACPH	0.00	
G2P	0.5	Garrigues et al., (1997)
G13P2	0.6	Garrigues et al., (1997)
ACLAC	0.1	
FBP	45.0	Ana et al., (2000)
NADH	0.7	Garrigues et al., (1997)
ATP	3.9	Current study
ACCOA	0.92	Lall et al., (2011)
LAC	75	Ana et al., (2002)
FMT	0.1	Ana et al., (2002)
ETOH	0.1	Ana et al., (2002)
Butanediol	0.1	Current study
ACETATE	0.1	Ana et al., (2002)
GLCo	29	Current study
GLC	0.1	Model calculated
BIOM	1.0	Assumed
PHIo	7.0	

**Metabolic regulations:** Regulation of various glycolytic enzymes by intra/extra cellular metabolites was adopted from previously published reports in literature. Details of regulations are shown in Table S3 with parameters and its source.

Table S3: List of metabolic regulations in the glycolysis of *L. lactis*:

Reaction	Regulator	Regulation	Source
re21: GLCo + PEP = G6P + PYR	FBP	Inhibitor	Neves et al., (2005); Voit et al., (2006)
re7: GAP + NAD + PHI = G13P2 + NADH	NADH	Inhibitor	Neves et al., (2005); Voit et al., (2006)
re11: PRP + ADP = PYR + ATP	PHI (Pi) FBP	Inhibitor Activator	Neves et al., (2005) Neves et al., (2005); Voit et al., (2006)
re13: PYR + COA = ACCOA + FMT	DHAP GAP	Inhibitor Inhibitor	Neves et al., (2005) Neves et al., (2005); Voit et al., (2006)
re15: ACALD + NADH = ETOH + NAD	ATP	Inhibitor	Neves et al., (2005)
re12: PYR + NADH = LAC + NAD	PHI FBP NADH/NAD ratio	Inhibitor Activator Inhibitor	Neves et al., (2005) Neves et al., (2005) Garrigues et al., (1997)

**Table S4 – List of equilibrium constants.** The equilibrium constants are calculated using group contribution method.

Reaction ID	Chemical equation	Keq
GLT	GLCo → GLC	1.00E+00
HXK	GLC + ATP → G6P + ADP	5.81E+02
PGI	G6P → F6P	3.71E+00
PFK	F6P + ATP → FBP + ADP	5.81E+02
ALD	FBP → DHAP + GAP	1.97E+00
TPI	DHAP → GAP	1.00E+00
GAPDH	GAP + PHI + NAD → G13P2 + NADH	1.67E+00
PGK	G13P2 + ADP → G3P + ATP	1.53E+02
PGM	G3P → G2P	9.98E-01
ENO	G2P → PEP	4.59E+00
PYK	PEP + ADP → PYR + ATP	4.83E+06
LDH	PYR + NADH → LAC + NAD	3.19E+04
PDH	PYR + CoA → ACCoA + FMT	5.47E+03
ACALDH	ACCoA + NADH → ACALD + NAD + CoA	3.49E-04
ADH	ACALD + NADH → ETOH + NAD	1.51E+04
PTA	ACCoA + PHI → ACPH + CoA	1.52E-03
ACK	ACPH + ADP → ACETATE + ATP	4.79E+03
ATPase	ATP → ADP + PHI	9.99E+04
AS	PYR → ACLAC	4.11E+07
ACLACD	ACLAC → Acetoin	3.19E+01
BDH	Acetoin + NADH → Butanediol + NAD	4.98E+02
Redox Balance	NAD → NADH	1.00E+05
PTS	GLCo + PEP → G6P + PYR	4.83E+06

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