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$^{-1}$) and steady state bacterial cell dry weight. Fluxes are shown in Table S1.

**Table S1: Extracellular fluxes measured in the current study.**

| Reaction ID | Chemical equation             | Flux (mM/min) | Source                  |
|-------------|--------------------------------|---------------|-------------------------|
| 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.

| Metabolite | Conc. (mM) | Source          |
|------------|------------|-----------------|
| 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 it 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 | 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   |
| PKF         | 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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