Supplementary material I: Biomass composition

Growth is modelled as a sink flux of biomass components, in a proportion that matches the biomass composition. This macromolecular composition of the cell was partly measured and partly estimated from literature data. Cells were collected and analyzed from the same fermentation experiments that were used to measure steady-state fluxes. Total RNA was measured by the method of Benthin (1); total carbohydrates by the method of Herbert et al. (2). Total lipid was measured by the method of Idzard et al (3). Dry weight was measured by washing cells with phosphate buffered saline(pH 6.2) and drying them at 55° C on a pre-weighted filter until a steady weight was achieved. Pellet hydrolysates were used to measure amino acid composition (analyzed by Ansynth BV, Roosendaal, The Netherlands). Total DNA was calculated from an average DNA content of 1.8 molecules per cell (based on the genomic distribution of microarray probe signal). Cell number to biomass ratio was measured in a cell counter (Assistant, Sondheim Germany) and amounded to $3.1 \cdot 10^{12}$ cells gDW^{-1}.

Biomass components that are explicitly taken into account in the biomass equation, are protein, RNA, DNA, lipids, cell wall components (peptidoglycan, polysaccharides and (lipo)teichoic acids) and vitamins. Although vitamins do not make a quantitative impact, their inclusion in the biomass equation ensures that deletions that interfere with metabolism of essential vitamins are also lethal in the model [ref]. The amino acid composition of the protein fraction has also been measured, and we found no differences in composition within the dilution rate range of 0.1-0.5 h^{-1}. Ash, *i.e.* inorganic compounds and ions, as well as intracellular metabolites, have not been taken into account in the model.

The biomass equation consists of biomass components and the growth-associated ATP consumption. The latter is discussed in the main text. Based on the data shown in **Table SI-1**, the biomass equation used in the model is:

2.45 protein + 0.279 RNA + 0.062 DNA + vitamins + 0.081 lipids + 0.129 polysaccharides + 0.146 peptidoglycans + 0.014 wall teichoic acids + 0.013 lipoteichoic acids + 27.4 ATP + 27.4 H_2O -> biomass + 27.4 ADP + 27.4 H^+

The stoichiometric coefficients have unit mmol gDW^{-1}.

component	CDM 25 mM Glc (D 0.1 – 0.5 h^{-1})			source
	fraction % (w/w)	MW g mol^{-1}	mol. coeff. mmol gDW^{-1}	
DNA	1.9	307.7	0.062	This study
RNA	9.0	323.2	0.278	This study
Total Protein	29.9			This study
free protein	26.1	107.4	2.45	
crossed-linked to PG	3.8			
lipids	6.3	799.6	0.081	This study and (4,5)
Polysaccharides	9.9	766	0.129	This study and (6)
Peptidoglycans	14.5	992	0.146	This study and (6)
Wall teichoic acids	13.8	10014	0.014	This study and (6)
Lipoteichoic acids	4.1	3153	0.013	(7)
rest	14.4			

Table SI-1. overall macromolecular composition of the cell biomass used in the model.

Cell wall

The main constituent of the cell wall is the peptidoglycan network, consisting of polysaccharide chains of N-acetyl muramic acid and N-acetyl glucosamine, interconnected via a linker pentapeptide. In *B. subtilis* the last amino acid of this pentapeptide is D-alanine, whereas in vancomycin-resistant LAB, among which *L. plantarum*, this last D-ala is replaced by a D-lactate moiety (8). The unit for peptidoglycan is therefore diphospho-N-acetylmuramoyl-(N-acetylglucosamine)-L-alanyl-D-glutamyl-meso-2,6-diaminopimeloyl-D-alanyl-D-lactate.

Teichoic acids, specific components of the cell wall of Gram-positive bacteria, form a diverse group of polymers of phosphorylated sugars or alcohols (8)]. The polymer is attached, via a linkage unit (9), to peptidoglycan (wall teichoic acids) or anchored into the membrane via undecaprenyl diphosphate (lipoteichoic acid). The composition of the linkage unit and the polymer differs for each species (9). *L. plantarum* produces wall teichoic acid consisting of ribitol-phosphate, with additional D-alanine and glucose substitutions. Lipoteichoic acids contain poly-glycerol phosphate, also with D-alanine and glucose substitutions (8,10). Since the exact number of substitutions are not known for *L. plantarum*, we have not included these in the model, except for glucose substitutions in wall teichoic acid, for which we could find data, see below.

Other major components of the *Lactobacillus* cell wall are polysaccharides and a protein S-layer. The latter may constitute 15-20% of the total protein content (8). The composition of the polysaccharides differ between species and even strains. The sugar composition of *L. plantarum*'s cell wall has been measured in great detail (6), and this allows for a detailed reconstruction of the cell wall components.

component	part of:	μmol/g cell wall	μmol/g teichoic acid
muramic acid	peptidoglycan	345	
Mur6P	linker of wall teichoic acid	31	
mannosamine	linker of wall teichoic acid	17	74
glycerol	linker of wall teichoic acid	34	84
ribitol	unit wall teichoic acid	480	2100
Pi	several	950	2090
glucose	CPS and wall teichoic acid	829	2720
galactose	CPS	292	
rhamnose	CPS	641	

Table SI-2: composition of *L. plantarum* cell wall components according to (6).

Conclusions drawn from this data set:

1. Based on the muramic acid and wall teichoic acid data, there are approximately 10 times more peptidoglycan units than there are teichoic acid molecules. With MWs of 992 and 10014 for PG and WTA, respectively, the masses of PG and WTA are similar.
2. The average chain length of wall teichoic acid is estimated as the moles of ribitol divided by moles of linker molecule. Based on total cell wall numbers (3rd column), the length is 18, based on the teichoic acid numbers (4th column) the length would be 28. An average chain length of 25 ribitol molecules was therefore taken as a reasonable estimate. Hence,

wall teichoic acid was modeled as glycerol phosphate-N-acetylmannosaminyl-glucosamine $(ribitol)_{25}$.

3. all ribitol molecules are substituted with glucose. This is based on the amount of glucose found in the teichoic acid preparation (4^{th} column).
4. The polysaccharide composition in L. plantarum is not known, but the rhamnose:galactose ratio in **Table S1-2** is close to 2:1. The glucose content was estimated by subtracting the glucose amount in the cell wall by the amount of ribitol (based on conclusion 2). The resulting 349 μmol/g cell wall is close to the amount of galactose, making an average composition of CPS in L. plantarum glucose:rhamnose:galactose:phosphate = 1:2:1:1. The same ratios were found for the exopolysaccharide composition of *Lactococcus lactis* (11).

We have measured the total carbohydrate content of *L. plantarum* at four different growth rates, resulting in a constant carbohydrate content of 13.4 ± 1% (w/DW) when averaged. Since the assay measures unsubstituted hexose only (2), we took the carbohydrate content as representative of total glucose, galactose and rhamnose present in the cell wall. From this assumption we could estimate that 1 g DW contains 0.42 g cell wall (0.46 g when also the lipoteichoic acids are included), a high but not unrealistic number for a rod-shaped, gram-positive, lactic acid bacterium[1]. From the total cell wall content and conclusions 1-4 we could calculate the amount of individual cell wall components as detailed in **Table SI-1**. These components together form 0.38 g/gDW of the 0.42 g/gDW of total cell wall, leaving 0.04 g/gDW for the protein S-layer. This amount would comprise 15% of the total protein content, in line with estimates from (8).

Protein composition

Protein content was measured both by measuring the individual amino acids in a total hydrolysate of the cell pellet, and was 29.9 ± 0.4 % (w/DW) when averaged over 4 dilution rates: no trend was observed. Total protein determinations using the BCA method confirmed the relatively low protein content of 30%. To further validate the measurements, we measured the total protein content of *L. lactis* with the same BCA method and found it to be 45%, in line with reported values (12). We therefore must conclude that *L. plantarum* has a relatively low protein content (and a high cell wall content). This observation was independent of the concentration of glucose in the feed (**Table SI-3**). Since both total protein level and the individual amino acid levels were highly comparable, we concluded that the biomass composition under these conditions are very similar.

The amino acid measurements included amino acids in peptidoglycans (alanine and glutamate). In the model, we have corrected for the peptidoglycan contribution to the total amino acid content of the pellet (see **Tabel S1-1**). Production of charged tRNAs costs 2 ATP per attached amino acid. Assembly of AA-tRNAs into polypeptides includes 2 GTP per amino acid for binding the ribosome and for translocation of the amino acid; furthermore there are additional

[1] The numbers from *L. plantarum* can be compared to data from L. lactis (12, 13), taking into account the difference in cell surface to cell volume ratio caused by the difference in cell morphology, *L. lactis* being sphere-shaped and *L. plantarum* being rod-shaped with rounded ends. The cell surface to volume ratio of *L. plantarum* is 3.7 μm^{-1} (14). The cell surface to volume ratio for a sphere is $3/r$ where r is the radius. When the volumes of both organisms are assumed to be similar, the reported value of 6 μm^3 for *L. plantarum* (14) can be used to estimate the radius of *L. lactis*, using $V = 4/3\pi r^3$. With $V = 6\ \mu m^3$, $r = 1.13\ \mu m$, the cell surface to cell volume ratio then becomes 2.7 μm^{-1}. Hence, the surface of lactobacilli would be 1.4 times larger than that of a lactococci. This number fits very well with the relative lipid contents, being 0.043 g gDW^{-1} for *L. lactis* (12), and 0.063 g gDW^{-1} for *L. plantarum* (**Table S1-1**), a ratio of 1.5. When taking peptidoglycan, polysaccharides and lipoteichoic acids together (from (13)), they constitute 31.8% (w/DW) of L. lactis biomass. Our estimate for L. plantarum of 46.1% is 1.45 times higher, which also fits the differences in cell morphology.

energy costs related to mRNA synthesis and turnover, and for proofreading (15). Hence, protein synthesis was modelled as:

$\Sigma\, x_i$ AA-tRNA$_i$ + 0.306 ATP + 2 GTP + 2.306 H_2O -> protein + $\Sigma\, x_i$ tRNA$_i$ + 0.306 ADP + 2 GDP + 2.306 P_i + 2.306 H^+

where x_i is the molar fraction of amino acid i in the free amino acid fraction (column 4, **Table SI-3**).

amino acid	amount CDM 25 mM Glc % (g/DW)	amount CDM 100 mM Glc % (g/DW)	molar fraction in free protein
Alanine	4.22 ± 0.08	4.19 ± 0.20	0.125[a]
Arginine	1.50 ± 0.11	1.35 ± 0.06	0.039
Asx [b]	3.33 ± 0.06	3.17 ± 0.10	
Aspartic acid			0.06
Asparagine			0.06
Cystine	0.13 ± 0.01	0.11 ± 0.01	0.011
Glx [b]	5.17 ± 0.23	5.19 ± 0.25	
Glutamic acid			0.023[a]
Glutamine			0.083
Glycine	1.16 ± 0.06	1.13 ± 0.05	0.084
Histidine	0.56 ± 0.01	0.57 ± 0.02	0.017
Isoleucine	1.18 ± 0.06	1.10 ± 0.04	0.043
Leucine	2.13 ± 0.08	2.04 ± 0.08	0.078
Lysine	2.06 ± 0.10	1.92 ± 0.07	0.066
Methionine	0.70 ± 0.02	0.67 ± 0.02	0.022
Phenylalanine	1.22 ± 0.04	1.07 ± 0.04	0.034
Proline	0.94 ± 0.03	1.00 ± 0.08	0.04
Serine	1.19 ± 0.03	1.20 ± 0.06	0.056
Threonine	1.58 ± 0.03	1.45 ± 0.05	0.064
Tryptophane	0.26 ± 0.03	0.29 ± 0.00	0.006
Tyrosine	1.11 ± 0.03	0.94 ± 0.02	0.028
Valine	1.44 ± 0.08	1.51 ± 0.03	0.06
Total protein	29.89 ± 0.43	29.19 ± 0.57	

Table SI-3. Amino acid composition of the protein fraction of the biomass. In columns 2 and 3, amino acid amounts in cell pellet hydrolysates ± SD are given for 4 chemostats averaged over dilution rates of 0.1-0.5 h^{-1}, at two glucose concentrations. In the fourth column, the molar fraction of each amino acid in the free protein fraction is shown, as it is incorporated into the protein synthesis reaction.

[a]Glutamate and alanine levels were adjusted for peptidoglycan content.

[b] Asx indicates the sum of asparagines and aspartic acid, Glx the sum of glutamine and glutamic acid: only the sum of these compounds could be measured. Equimolar amounts of aspartic acid and asparagines in Asx and glutamic acid and glutamine in Glx were assumed.

Lipid composition

Cellular lipids of *lactobacilli* deviate from most other gram positives (16) and are mainly composed of three types of phospholipids, the acyl chain of which are composed of 6 different fatty acids (**Table SI-4**).

compound	% (mol/mol total lipid)[a]
Phospholipids	
phosphatidylglycerol	75[b]
1-lysyl phosphatidylglycerol	23
Cardiolipin	2
Fatty acids	
Tetradecenoic acid (14:0)	3
Hexadecanoic acid (16:0)	26
Hexadecanoic acid (16:1)	12
Octodecanoic acid (18:0)	2
Octodecanoic acid (18:1)	32
Cyclopropanoyl octadecanoic acid	25

Table SI-4. lipid composition of L. plantarum based on (5,16).
[a] original measurements of phospholipids were in % of total ^{32}P radioactivity and converted on the basis of P content
[b] 7% of phospholipids were not identified, but most likely are based on phoshatidylglycerol and hence this phospholipid fraction was increased to supplement to 100%.

Based on these data, an average fatty acyl chain was defined, and with this acyl chain, an average phospholipid was defined having a chemical formula of $C_{42.87}H_{81.08}N_{0.46}O_{10.37}P_{1.02}$ with a molecular weight of 799.6 g mol^{-1}. The latter number was used to convert mass-% of lipids (**Table SI-1**) into a molar coefficient for the biomass equation.

Nucleic acids

The DNA content of the cell was both calculated and directly measured, and these estimates fitted reasonably well. A DNA chromosome of 3.3 Mbp with GC content of 44.5% and an average cell copy number of 1.8 (as judged from microarray hybridisation signals, data not shown), atking $3.1 \cdot 10^{12}$ cells gDW^{-1} (measured by cell count, see Experimental procedures in the main text), results in 0.019 g DNA gDW^{-1}. This is reasonably close to the measured value of 0.013 g DNA gDW^{-1} considering the losses during DNA extraction. We took 0.019 g DNA gDW^{-1} as the more realistic number (Table SI-1). Assembly of precursor dXTP's into dXMP polymers costs 2 ATP: $dXTP + DNA_n \rightarrow dXMP + DNA_{n+1} + PP_i$. Additional ATP costs (1.37 mole ATP per mole nuleotide) were taken into account for a.o. proofreading and unwinding of the DNA helix, as detailed in (13,15).

Total RNA content was measured as detailed in [ref]. Nucleotide composition of the RNA was taken from (15), assuming that the highly conserved ribosomal RNA fraction dominated the total RNA pool. For RNA biosynthesis, the reaction is very similar to that of DNA:

$XTP + RNA_n$ -> $XMP + RNA_{n+1} + PP_i$. Additional ATP costs (0.4 mole ATP per mole nuleotide) were included for discarding segments and modifications, as detailed in (13,15).

DNA assembly was modelled as:
0.22 dGTP + 0.22 dCTP + 0.28 dATP + 0.28 dTTP + 1.37 ATP + 1.37 H_2O -> DNA + 1 PP_i + 1.37 ADP + 1.37 P_i + 1.37 H^+
RNA assembly was modelled as:
0.32 GTP + 0.20 CTP + 0.66 ATP + 0.22 UTP + 0.4 H_2O -> RNA + 1 PP_i + 0.4 P_i + 0.4 ADP + 0.4 H^+

Vitamins and cofactors

Lactic acid bacteria are auxotrophic for many vitamins, and so is *L. plantarum* [ref lacplantcyc]. To model the vitamin requirements, vitamins or vitamin-derived cofactors were included into the biomass equation, with a low molar coefficient (10^{-5} mmol gDW^{-1}) to make sure it was not limiting in the simulations (no quantitative data on intracellular vitamin levels were available). Vitamins and cofactors included in the biomass equation were: molybdenum cofactor, tetrahydrofolate, biotin, pyridoxal 5-phosphate, thiamin pyrophosphate, undecaprenol (lipid II), NAD, and co-enzyme A.

References

1. Benthin, S., Nielsen, J., and Villadsen, J. (1991) *Biotech. Tech.* 5, 39-42
2. Herbert, D., Phipps, P. J., and Strange, R. E. (1971) *Methods in Microbiology* 5B, 265-275
3. Izard, J., and Limberger, R. J. (2003) *J Microbiol Methods* 55(2), 411-418
4. Veerkamp, J. H. (1971) *J Bacteriol* 108(2), 861-867
5. Exterkate, F. A., Otten, B. J., Wassenberg, H. W., and Veerkamp, J. H. (1971) *J Bacteriol* 106(3), 824-829
6. Kojima, N., Araki, Y., and Ito, E. (1985) *Eur J Biochem* 148(1), 29-34
7. Wicken, A. J., Evans, J. D., Campbell, L. K., and Knox, K. W. (1982) *Infect Immun* 38(1), 1-7
8. Delcour, J., Ferain, T., Deghorain, M., Palumbo, E., and Hols, P. (1999) *Antonie Van Leeuwenhoek* 76(1-4), 159-184
9. Araki, Y., and Ito, E. (1989) *Crit Rev Microbiol* 17(2), 121-135
10. Foster, S. J., and Popham, D. L. (2002) Structure and synthesis of cell wall, spore cortex, teichoic acids, S-layers, and capsules. In: A.L., S., Hoch, J. A., and Losick, R. (eds). *Bacillus Subtilis and Its Closest Relatives: From Genes to Cells*, ASM Press, Washington DC
11. Boels, I. C., Van Kranenburg, R., Kanning, M. W., Chong, B. F., De Vos, W. M., and Kleerebezem, M. (2003) *Appl Environ Microbiol* 69(8), 5029-5031
12. Novak, L., and Loubiere, P. (2000) *J Bacteriol* 182(4), 1136-1143
13. Oliveira, A. P., Nielsen, J., and Forster, J. (2005) *BMC Microbiol* 5, 39
14. Dumont, F., Marechal, P. A., and Gervais, P. (2004) *Appl Environ Microbiol* 70(1), 268-272
15. Neidhardt, F. C., and al., e. (1987) *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, American Society for Microbiology
16. O'Leary, W. M., and Wilkinson, S. G. (1988) Gram-positive bacteria. In: Ratledge, C., and Wilkinson, S. G. (eds). *Microbial Lipids*, Academic Press, London