Biomass composition of *L. reuteri*

Protein:DNA:RNA:Lipid:LTA:CPS:PG ratio of biomass is not known for reuteri. Lipoteichoic acid (LTA) from *L. lactis* (Oliveira, Nielsen, & Förster, 2005) is used because *L. plantarum* also produces Wall teichoic acid (WTA), which *L. reuteri* and *L. lactis* do not. Peptidoglycan in *L. plantarum* (Teusink et al., 2006) was calculated from *Bacillus cereus*, so here peptidoglycan from *L. lactis* is used. Protein ratio was experimentally measured for *L. reuteri* in this study. Otherwise numbers from *L. plantarum* were used for the macromolecular composition.

Component		**Fraction (% w/w)**	**Norm Fraction (% w/w)**	**Molar mass ($g\ mol^{-1}$)**	**Coefficent ($mmol\ gDW^{-1}$)**	**Source**
Protein	PROT_LRE_c	35,4%	43,0%	129,9	3,311	*L. reuteri* (This study)
DNA	DNA_LRE_c	1,9%	2,3%	309,5	0,075	*L. plantarum* (Teusink et al., 2006)
RNA	RNA_LRE_c	9,0%	10,9%	321,6	0,340	*L. plantarum* (Teusink et al., 2006)
Lipids	LIP_LRE_c	6,3%	7,7%	795,1	0,096	*L. plantarum* (Teusink et al., 2006)
Lipoteichoic acid	LTAtotal_LRE_c	8,0%	9,7%	5290,4	0,018	*L. lactis* (Oliveira et al., 2005)
Polysaccharides	CPS_LRE_c	9,9%	12,0%	648	0,186	*L. plantarum* (Teusink et al., 2006)
Peptidoglycan	PGlac2_c	11,8%	14,3%	992	0,145	*L. lactis* (Oliveira et al., 2005)
	Total	82,3%	100%			

0.186 CPS_LRE_c + 0.075 DNA_LRE_c + 0.096 LIP_LRE_c + 0.018 LTAtotal_LRE_c + 0.145 PGlac2_c + 3.311 PROT_LRE_c + 0.340 RNA_LRE_c + 10.2 atp_c + 1e-05 btn_c + 0.0002 coa_c + 10.2 h2o_c + 0.0002 nad_c + 1e-06 pydx5p_c + 1e-05 thf_c + 1e-05 thmpp_c + 0.0002 udcpdp_c + 0.00001 adeadocbl_c --> 10.2 adp_c + 10.2 h_c + 10.2 pi_c

Flux through ATPM is fixed at: 0.36 mmol h^{-1} gDW^{-1} (*L. plantarum* (Teusink et al., 2006))

Protein

Amino acid composition analyzed following cell pellet hydrolysis (Ansynth).

Amino Acid	**Fraction $g/g_{total\ a.a.}$**	**Fraction g/gDW**	**Fraction g/gDW (only protein)**	**MW (g/mol)**	**Fraction mole/gDW**	**Molar ratio**
Ala	10,4%	5,2%	0,2%	89,1	2,25E-05	0,7%*
Arg	4,7%	2,4%	2,4%	174,2	1,35E-04	4,1%
Asp + Asn	13,4%	6,7%	6,7%	132,1	5,08E-04	
Asp						7,7%
Asn						7,7%
Cys	0,8%	0,4%	0,4%	121,2	3,15E-05	1,0%
Glu + Gln	13,8%	6,9%	4,8%	146,6	3,27E-04	
Glu						2,7%*

Gln						7,1%
Gly	5,2%	2,6%	2,6%	75,1	3,50E-04	10,6%
His	2,1%	1,1%	1,1%	155,2	6,93E-05	2,1%
Ile	4,9%	2,5%	2,5%	131,1	1,89E-04	5,7%
Leu	7,3%	3,7%	3,7%	131,1	2,81E-04	8,5%
Lys	9,4%	4,7%	4,7%	146,2	3,23E-04	9,8%
Met	2,7%	1,3%	1,3%	149,2	8,93E-05	2,7%
Phe	3,5%	1,8%	1,8%	165,2	1,08E-04	3,3%
Pro	3,0%	1,5%	1,5%	115,1	1,33E-04	4,0%
Ser	3,8%	1,9%	1,9%	105,1	1,80E-04	5,4%
Thr	5,1%	2,5%	2,5%	119,1	2,13E-04	6,4%
Trp	0,9%	0,5%	0,5%	204,2	2,31E-05	0,7%
Tyr	3,4%	1,7%	1,7%	181,2	9,30E-05	2,8%
Val	5,4%	2,7%	2,7%	117,2	2,33E-04	7,0%
Total	**100%**	**50,2%**	**43,0%**	**129,9**	**3,31E-03**	**100%**

*Glutamate and alanine in lipoteichoic acid and peptidoglycan are subtracted from measured values.

Alanine in lipoteichoic acid:

mmol LTA / gDW	0,018
mol alanine / mol LTA	15,3
mol alanine in LTA / gDW	0,0003
Alanine molar mass (g/mol)	89,094
g alanine in LTA / gDW	**0,0245**

Alanine in peptidoglycan:

mmol PGlac2 / gDW	0,145
mol alanine / mol LTA	2
mol alanine in LTA / gDW	0,0003
Alanine molar mass (g/mol)	89,094
g alanine in LTA / gDW	**0,0258**

Glutamate in Peptidoglycan:

mmol PGlac2 / gDW	0,145
mol glutamate / mol LTA	1
mol glutamate in LTA / gDW	0,0002
Glutamate molar mass (g/mol)	147,13
g glutamate in LTA / gDW	**0,0213**

Protein reaction:

0.306 atp_c + 2 gtp_c + 2.306 h2o_c + 0.0062 alatrna_c + 0.0412 argtrna_c + 0.0775 asntrna_c + 0.0775 asptrna_c + 0.0096 cystrna_c + 0.0721 glntrna_c + 0.0189 glutrna_c + 0.1068 glytrna_c + 0.0211 histrna_c + 0.0578 iletrna_c + 0.0857 leutrna_c + 0.0986 lystrna_c + 0.0272 mettrna_c + 0.0329 phetrna_c + 0.0404 protrna_c + 0.0548 sertrna_c + 0.0650 thrtrna_c + 0.0070 trptrna_c +

0.0284 tyrtrna_c + 0.0711 valtrna_c --> PROT_LRE_c + 0.306 adp_c + 2.0 gdp_c + 2.306 h_c + 2.306 pi_c + 0.0062 trnaala_c + 0.0412 trnaarg_c + 0.0775 trnaasn_c + 0.0775 trnaasp_c + 0.0096 trnacys_c + 0.0910 trnaglu_c + 0.1068 trnagly_c + 0.0211 trnahis_c + 0.0578 trnaile_c + 0.0857 trnaleu_c + 0.0986 trnalys_c + 0.0272 trnamet_c + 0.0329 trnaphe_c + 0.0404 trnapro_c + 0.0548 trnaser_c + 0.0650 trnathr_c + 0.0070 trnatrp_c + 0.0284 trnatyr_c + 0.0711 trnaval_c

DNA

Calculated from JCM1112 genome

Nucleotide	Ratio	MW (g mol^{-1})	MW * Ratio (g mol^{-1})
dAMP	0,30560617	331,2	101,2
dTMP	0,30560617	304,2	93,0
dCMP	0,19439383	289,2	56,2
dGMP	0,19439383	304,2	59,1
		Average MW of DNA	**309,5**

DNA reaction:
1.37 atp_c + 0.310284595735 datp_c + 0.180906354753 dctp_c + 0.207484087701 dgtp_c + 0.301324961811 dttp_c + 1.37 h2o_c --> DNA_LRE_c + 1.37 adp_c + 1.37 h_c + 1.37 pi_c + ppi_c

RNA

Calculated from JCM1112 genome, assuming equal transcription

Nucleotide	Ratio	MW (g mol^{-1})	MW * Ratio (g mol^{-1})
AMP	0,31434679	329,2	103,5
UMP	0,29106444	306,2	89,1
CMP	0,18074957	305,2	55,2
GMP	0,21383921	345,2	73,8
		Average MW of RNA	**321,6**

RNA reaction:
0.710810743235 atp_c + 0.187747387186 ctp_c + 0.21245220899 gtp_c + 0.288989660589 utp_c --> RNA_LRE_c + 0.4 adp_c + 0.4 h_c + 0.4 pi_c + ppi_c

Lipids (Liu, Hou, Zhang, Zeng, & Qiao, 2014)

Fatty acid	Ratio	MW (g mol^{-1})	Formula	MW * Ratio (g mol^{-1})
C14:0	0,0687	228,38	$C_{14}H_{28}O_2$	15,68971
C16:0	0,2566	256,43	$C_{16}H_{32}O_2$	65,79994
C16:1	0,0258	254,41	$C_{16}H_{30}O_2$	6,563778
C18:0	0,075	284,48	$C_{18}H_{36}O_2$	21,336
C18:1	0,1591	282,47	$C_{18}H_{34}O_2$	44,94098
C18:2	0,3519	280,45	$C_{18}H_{32}O_2$	98,69036
C18:3	0,0418	278,44	$C_{18}H_{30}O_2$	11,63879
cyc19:0	0,0211	296,49	$C_{19}H_{36}O_2$	6,255939
			Average MW of fatty acids	**270,9155**

Fatty acids:

agly3p_LRE_c + 0.0211 cpocdacp_c + 0.0258 hdeACP_c + 0.075 ocdacp_c + 0.0418 ocdctrACP_c + 0.3519 ocdcyaACP_c + 0.1591 octeACP_c + 0.2566 palmACP_c + 0.0687 tdeacp_c --> ACP_c + pa_LRE_c

Phospholipids ratio from *L. plantarum* used here, as no *L. reuteri* data was available

Phospholipid	**Ratio**	**MW with average fatty acid (g mol^{-1})**	**MW * Ratio (g mol^{-1})**
Phosphatidylglycerol	0,75	751,95	563,96
1-lysyl phosphatidylglycerol	0,23	882,14	202,89
Cardiolipin	0,02	1411,81	28,24
Average MW of phospholipids			**795,1**

Lipid reaction:
0.02 clpn_LRE_c + 0.23 lyspg_LRE_c + 0.75 pg_LRE_c --> LIP_LRE_c

Lipoteichoic acid (Bron, Baarlen, & Kleerebezem, 2011; Kleerebezem et al., 2010; Walter et al., 2007)

20 glycerol phosphate (Gro-P) residues

Gro-P residues were substituted with d-alanyl esters (74-79%, average 76,5%)

Gro-P residues were substituted with glycosyl residues (6%)

Component	**Average molar ratio**	**Molar mass (g mol^{-1})**
Diglucosyl diacylglycerol	1	906,3
Glycerol phosphate	20	154,0
D-alanine	15,3	71,1
D-glucose	1,2	180,2
Average molar mass of Lipoteichoic acid		**5290,4**

LTA reaction:
0.175 LTA_LRE_c + 0.765 LTAala_LRE_c + 0.06 LTAglc_LRE_c --> LTAtotal_LRE_c

L. reuteri does not produce Wall teichoic acid

Polysaccharides (Ksonzeková et al., 2016)

Glucan homopolysaccharides (merely glucose units)

D-glucose, 4 units, 162 g mol^{-1} per unit → 648 g mol^{-1}

Polysaccharide reaction:
4.0 h2o_c + 4.0 udpg_c <=> CPS_LRE_c + 5.0 h_c + 3.0 udp_c + ump_c

Peptidoglycan

Assumed the same as *L. plantarum*

Peptidoglycan reaction:
uaagmdalac_c --> PGlac2_c + udcpdp_c

Glycogen

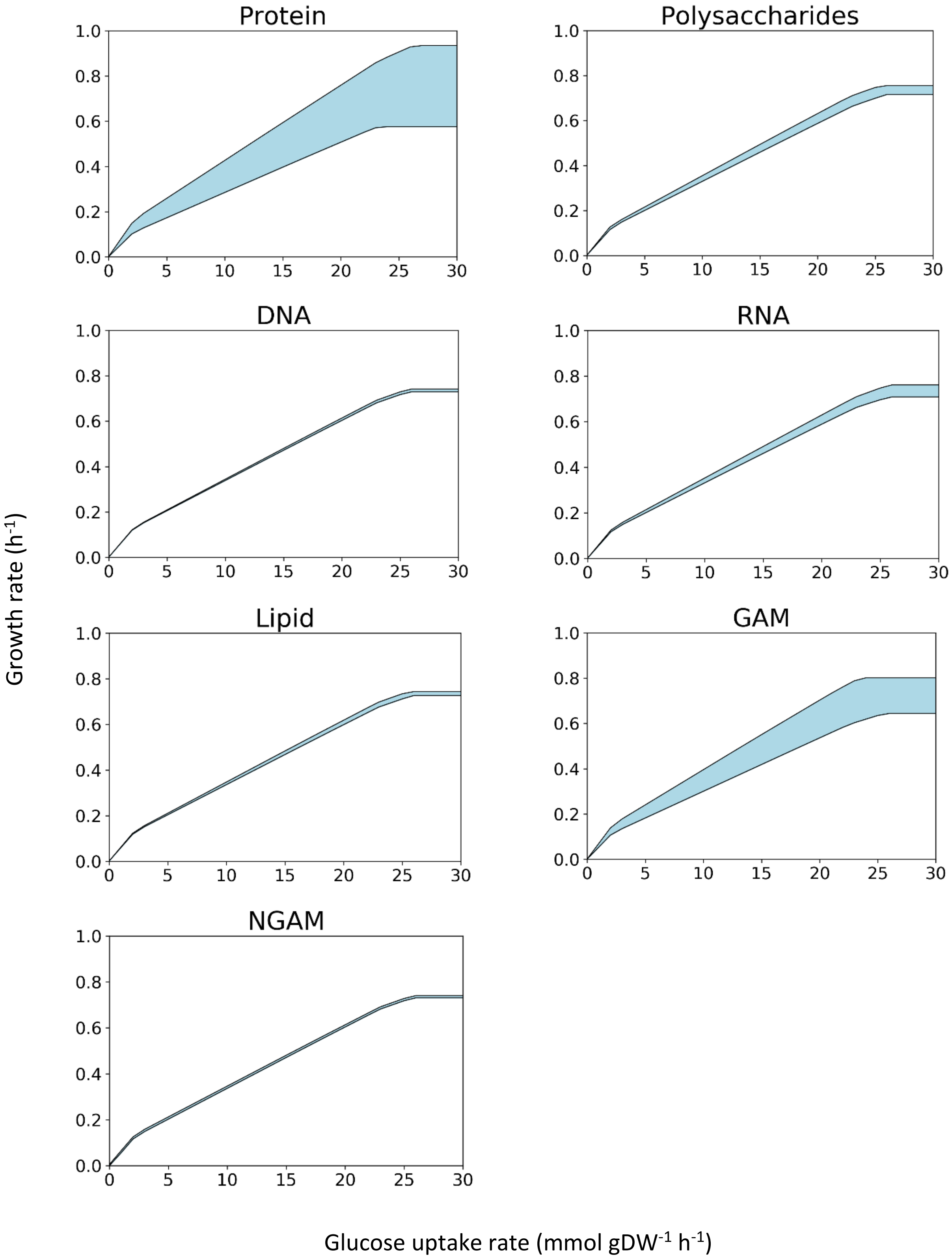
Glycogen in *B. subtilis* biomass was negligible (Dauner & Sauer, 2001; Dauner, Storni, & Sauer, 2001). Assumed the same here.

ATP requirements

No data for *L. reuteri* available. Non-growth associated maintenance (NGAM) assumed the same as *L. plantarum.* Growth associated maintenance (GAM) was calculated by constraining model with experimental uptake- and secretion rates of metabolites, and growth rate of *L. reuteri*.

Sensitivity analysis

The effect of 50% variation in biomass and energy component coefficients on predicted growth rate was investigated. Growth rate was most sensitive to change in the protein and GAM components of the biomass.



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