Electronic Supplementary Material (ESI) for Molecular BioSystems.

Construction and Simulation of *Bradyrhizobium diazoefficiens* USDA110 Metabolic Network: Comparison between the Free-living and Symbiotic States

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Table S1. Basic properties of *i*YY1101

Features	*i*YY1101
Compartment	2
Genes	1101
Metabolites	661
Cytoplasmic metabolites	660
Extracellular metabolites	106
Reaction	1031
Metabolic reactions	821
Cytoplasmic reactions	810
Extracellular reactions	11
Irreversible	520
Reversible	301
Spontaneous	22
Transport reactions	106
Exchange reactions	99
Sink reactions	3
Objective function	5
Associated with genes	715
Not associated with genes	316

Table S2. Essential gene predicted in the FL state

Essential gene							
bll0006	bll1476	bll5072	bll6605	blr0515	blr1315	blr3302	blr5099
bll0187	bll2399	bll5331	bll6606	blr0581	blr1381	blr3307	blr6489
bll0188	bll2481	bll5379	bll6607	blr0650	blr1383	blr3312	blr6490
bll0477	bll3807	bll5521	bll7272	blr0653	blr1393	blr3797	blr6784
bll0522	bll4423	bll5919	bll7408	blr0683	blr1479	blr3884	blr7371
bll0549	bll4585	bll6497	bll7514	blr0685	blr1481	blr4088	blr7377
bll0566	bll4608	bll6501	bll7752	blr0716	blr1482	blr4119	blr7382
bll0822	bll4758	bll6510	bll7953	blr0738	blr1483	blr4362	blr7454
bll1396	bll4800	bll6512	bll7969	blr0745	blr1484	blr4810	blr7524
bll1418	bll4805	bll6595	bll8126	blr0746	blr1485	blr4842	blr7525
bll1419	bll4847	bll6600	blr0216	blr1120	blr1499	blr4843	blr8104
bll1421	bll4849	bll6601	blr0512	blr1140	blr2398	blr4844	blr8106
bll1422	bll4852	bll6602	blr0513	blr1228	blr2489	blr5037	blr8134
bll1475	bll4856	bll6604	blr0514	blr1256	blr2631		

Table S3. Essential gene predicted in the SNF state

Essential gene							
bll0566	bll3461	blr1744	blr1761	blr1774	blr2764	blr3731	bsr1757
bll2399	bll7752	blr1745	blr1769	blr2037	blr2766	blr4486	bsr1775
bll2481	blr0468	blr1746	blr1770	blr2038	blr2768	blr5037	bsr2765
bll2759	blr0469	blr1747	blr1771	blr2398	blr2769	bsr0470	bsr2770
bll2760	blr1743	blr1759	blr1773	blr2763	blr3730		

Table S4. Essential genes associated with symbiotic nitrogen fixation detected by simulated single-gene-deletion

Synonym code	Gene name	Description	EC number	Pathway
blr2037	*nif*A	Nif-specific regulatory protein	-	Nitrogen metabolism
blr1759	*nif*B	FeMo cofactor biosynthesis protein	-	Nitrogen metabolism
blr1743	*nif*D	Nitrogenase molybdenum-iron protein subunit alpha	1.18.6.1	Nitrogen metabolism
blr1745	*nif*E	Nitrogenase molybdenum-cofactor synthesis protein	-	Nitrogen metabolism
blr1769	*nif*H	nitrogenase reductase	1.18.6.1	Nitrogen metabolism
blr1744	*nif*K	Nitrogenase molybdenum-iron protein subunit beta	1.18.6.1	Nitrogen metabolism
blr1746	*nif*N	Nitrogenase molybdenum-cofactor biosynthesis protein NifN	-	Nitrogen metabolism
blr1770	*nif*Q	Molybdenum processing protein	-	Nitrogen metabolism
blr4486	*nif*R	Nitrogen regulation protein	-	Nitrogen metabolism
blr1771	*nif*W	Nitrogenase stabilizing/protective protein	-	Nitrogen metabolism
blr1747	*nif*X	Iron-molibdenum cofactor processing protein	-	Nitrogen metabolism
blr1761	*nif*Z	Iron-sulfur cofactor synthesis protein	-	Nitrogen metabolism
blr2038	*fix*A	Electron transfer flavoprotein subunit beta	-	Nitrogen metabolism
blr1773	*fix*B	Electron transfer flavoprotein subunit alpha	-	Nitrogen metabolism
blr1774	*fix*C	Electron transfer flavoprotein-quinone oxidoreductase	1.5.5.-	Nitrogen metabolism
blr2768	*fix*H	-	-	Nitrogen metabolism
blr2769	*fix*I	E1-E2 type cation ATPase	3.6.3.4	Nitrogen metabolism
bll2759	*fix*J	Response regulator FixJ	-	Nitrogen metabolism
bll2760	*fix*L	Two-component oxygen-sensor histidine kinase	2.7.13.3	Nitrogen metabolism
blr2763	*fix*N	Cbb3-type cytochrome c oxidase subunit I	1.9.3.1	Nitrogen metabolism
blr2764	*fix*O	Cbb3-type cytochrome c oxidase subunit II	1.9.3.1	Nitrogen metabolism
blr2766	*fix*P	Cbb3 oxidase subunit III	-	Nitrogen metabolism
bsr2765	*fix*Q	Cbb3 oxidase subunit IV	-	Nitrogen metabolism
bsr2770	*fix*S	-	-	Nitrogen metabolism
bsr1757	*fix*U	Nitrogen fixation protein	-	Nitrogen metabolism
bsr1775	*fix*X	Ferredoxin	-	Nitrogen metabolism
bll3461	*pca*J	3-oxoadipate CoA-transferase subunit B	2.8.3.8	Propanoate metabolism
blr5037	*hem*B	Delta-aminolevulinic acid dehydratase	4.2.1.24	Porphyrin and chlorophyll metabolism
blr2398	*hem*C	Porphobilinogen deaminase	2.5.1.61	Porphyrin and chlorophyll metabolism
bll2399	*hem*E	Uroporphyrinogen decarboxylase	4.1.1.37	Porphyrin and chlorophyll metabolism
bll2481	*hem*F	Coproporphyrinogen III oxidase	1.3.3.3	Porphyrin and chlorophyll metabolism
bll7752	*hem*H	Ferrochelatase	4.99.1.1	Porphyrin and chlorophyll metabolism
bll0566	-	Uroporphyrinogen III synthase	4.2.1.75	Porphyrin and chlorophyll metabolism
blr0468	*ccm*B	Heme exporter protein B	-	ABC transporters
blr0469	*ccm*C	Heme exporter protein C	-	ABC transporters
bsr0470	*ccm*D	Heme exporter protein D	-	ABC transporters
blr3730	*dct*B	C4-dicarboxylate transport sensor histidine kinase DctB	2.7.13.3	Two-component system
blr3731	*dct*D	C4-dicarboxylate transport sensor histidine kinase DctD	2.7.13.3	Two-component system

Table S5. Essential gene pairs predicted in the FL state

Essential gene pairs			
bll0007-bll4274	bll4389-blr3778	blr0428-blr8101	blr3755-blr2585
bll0416-blr0488	bll4607-blr1632	blr0428-blr4385	blr3755-blr2586
bll0466-blr5690	bll5033-blr5751	blr0429-blr0206	blr3792-bll6435
bll0501-blr4687	bll5464-bll1630	blr0429-blr8101	blr3796-bll6631
bll1200-blr4838	bll5610-blr3972	blr0429-blr4385	blr3989-bll0553
bll1200-blr3552	bll5785-blr1119	blr0495-bll0415	blr4582-blr1098
bll1631-bll5465	bll5968-bll5145	blr0654-blr0652	blr5101-bll5021
bll2049-blr4809	bll7052-bll0466	blr0655-blr1323	blr5747-blr5690
bll2654-bll4746	bll7596-blr5702	blr0739-bll4859	blr5747-bll7052
bll4070-blr5702	bll7967-bll4570	blr1326-blr3294	blr6225-bll1397
bll4070-bll7596	bll8013-blr0744	blr1488-blr7029	blr6298-bll6010
bll4124-blr7471	blr0428-blr0206	blr2582-blr3755	

Table S6. Essential gene pairs predicted in the SNF state

Essential gene pairs			
bll0439-blr0512	bll0442-blr0512	bll0453-bll0441	blr0512-bll1188
bll0439-blr0513	bll0442-blr0513	bll0453-bll0442	blr0513-bsl1187
bll0439-blr0514	bll0442-blr0514	bll0453-bll0443	blr0513-bll1188
bll0439-blr0515	bll0442-blr0515	bll0455-bsl1187	blr0514-bsl1187
bll0440-blr0512	bll0443-blr0512	bll0455-bll1188	blr0514-bll1188
bll0440-blr0513	bll0443-blr0513	bll0455-bll0439	blr0515-bsl1187
bll0440-blr0514	bll0443-blr0514	bll0455-bll0440	blr0515-bll1188
bll0440-blr0515	bll0443-blr0515	bll0455-bll0441	blr1488-blr7029
bll0441-blr0512	bll0453-bsl1187	bll0455-bll0442	blr2036-bll2623
bll0441-blr0513	bll0453-bll1188	bll0455-bll0443	blr2767-blr5778
bll0441-blr0514	bll0453-bll0439	bll0466-bll1200	blr5747-bll1200
bll0441-blr0515	bll0453-bll0440	blr0512-bsl1187	

Supplementary figures

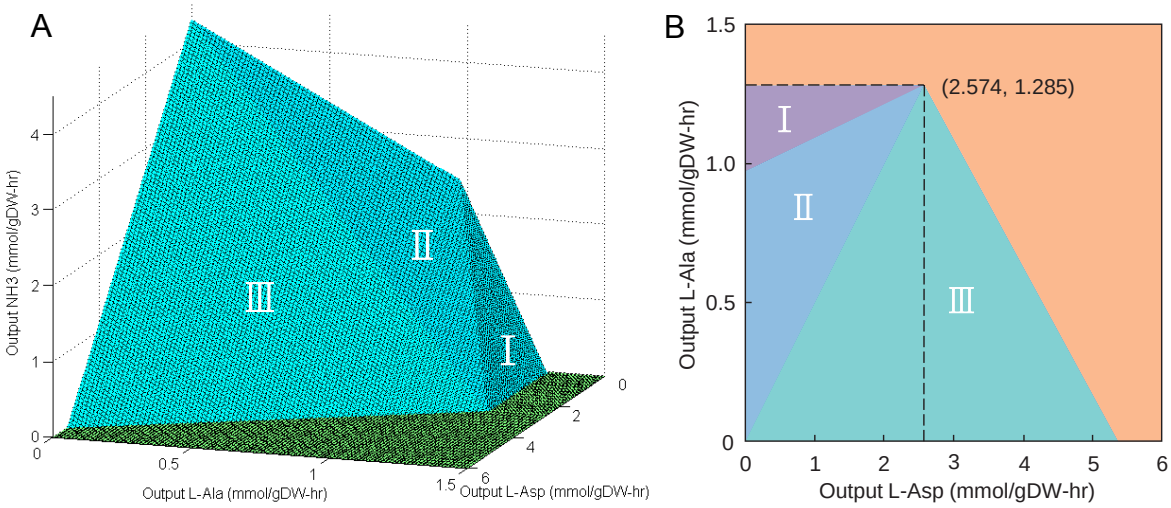

Fig. S1. Phenotype phase plane for the three kinds of nitrogen output, NH_3, L-alanine and L-aspartate, and the trade-offs between them. The phase planes are shown in 3D plot (A) and 2D plot (B), respectively. Regions denoted by I, II and III characterize different quantitative output modes of NH_3 influenced by the output fluxes of L-alanine and L-aspartate. The SNF metabolic system has maximum nitrogen output rate at the intersecting line of plane II and III (A), *i.e.*, when the output ratio of L-aspartate and L-alanine was 2.574: 1.285 = 2: 1, the SNF system has maximum nitrogen output.

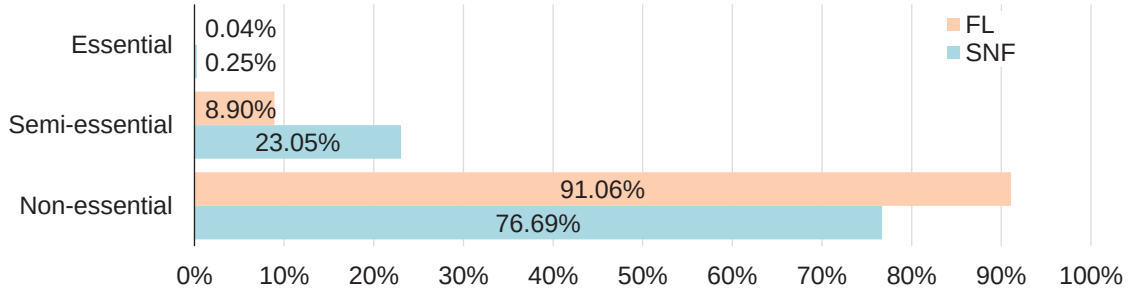

Fig. S2. The proportions of gene pairs with different lethality in different metabolic models. FL, free living; SNF, symbiotic nitrogen fixation.

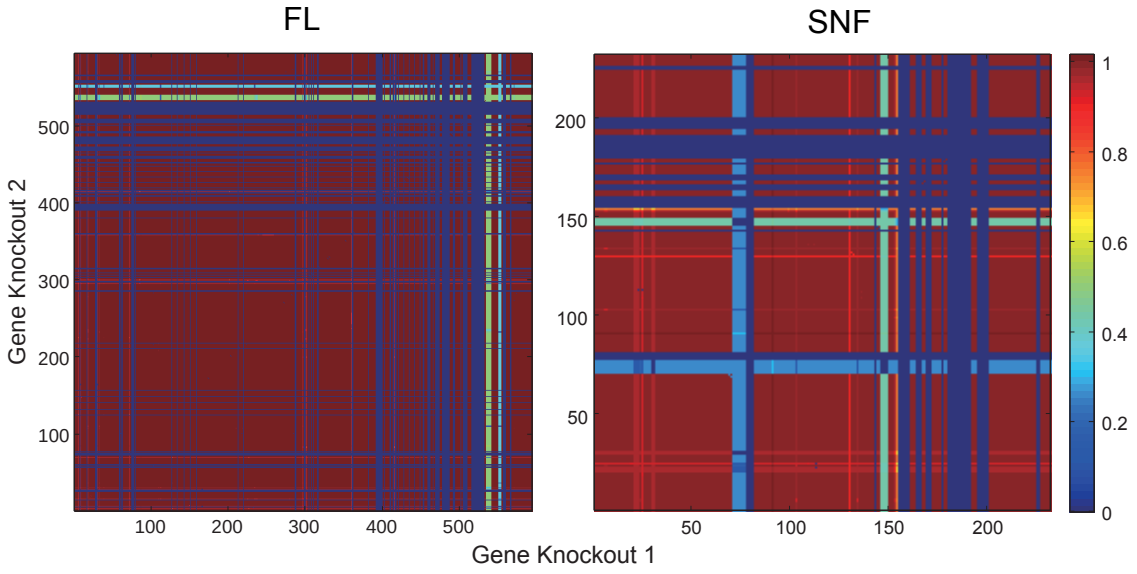

Fig. S3. Simulated double-gene-knockout to the FL and SNF models. Only genes proved to be related with fluxes by FVA were considered. Colors represent the ratio of optimal value of objective function of a double-gene-deletion strain to that of the wild-type strain.

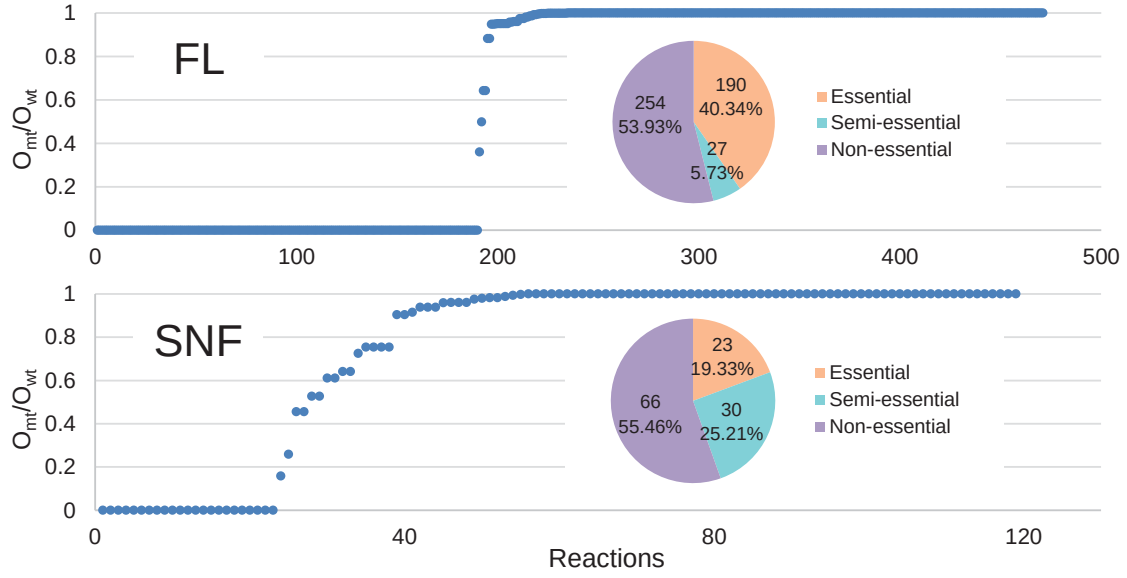

Fig. S4. Simulated single-reaction-knockout to the FL and SNF models. The horizontal axis represents reactions in the metabolic models; only reactions proved to be related with fluxes by FVA were considered; the vertical axis represents the ratio of optimal value of objective function of a single-reaction-deletion strain to that of the wild-type strain. O_{mt}/O_{wt}, the ratio of the optimal objective function value for a single reaction-deletion strain to that for the wild-type strain. Pie charts show the proportions of three kinds of reactions: essential, semi-essential and non- essential.

Supplementary Methods: Method of determining the biomass objective function for *B. diazoefficiens* USDA110

All of the free-living and normally-growing microorganisms need to maintain the biomass synthesis, so the biomass reaction was used as the objective function (OF) of the FL model for the free-living rhizobium. Determining biomass reaction means determining all known biomass constituents and their fractional contributions to the overall cellular biomass and energy consumption during biomass synthesis which includes growth-associated ATP maintenance (GAM) and non-growth-associated ATP maintenance (NGAM). In this study, all of them were determined by literature mining step by step.

1 Biomass composition of *B. diazoefficiens*

Through mining and integrating information in literatures (1-6)，Biomass composition of *B. diazoefficiens* USDA 110 was determined (Table 1).

Table 1 Biomass composition of *B. diazoefficiens*

Component	Cellular content% (w/w)	References
Protein	50.1	(4,5)
DNA	3.0	(1, 2)
RNA	8.5	(1, 2)
Phospholipids	0.9	(3, 4)
Poly-beta-Hydroxybutyrate (PHB)	15.9	(3)
Peptidoglycan	2.5	(6)
Lipopolysaccharides (LPS)	3.4	(6)
Capsular polysaccharide & Extracellular polysaccharides (CPS & EPS)	15.7	(5)
Total	100	-

We suppose the total DNA content of a single cell is approximately equal to the DNA content of biomass.

DNA content of a single cell = 9.1×10^{-15} g (1).

Cell volume = $\pi \times (0.4)^2 \times 2$=1.0048 $(\mu m)^3$ (7).

We suppose the cell density equals to the density of water and the moisture content of cell is 70% (8)，thus the proportion of DNA in biomass is：

$9.1\times10^{-15}/[1.0048\times10^{-12}\times(1-0.70)]$ = 3.02%.

2 Amino acid component proportions of *B. diazoefficiens* biomass

Amino acid composition (mol%) of proteins in *B. diazoefficiens* has been measured (9); the proportions of amino acids in biomass is (using L-Ala as an example):

Content (mmol/g DW) = 12.2%×50.1%×1000/107.602 = 0.568 mmol/g DW

The proportions of amino acids in *B. diazoefficiens* biomass were shown in Table 2.

Table 2 Amino acid component proportions of *B. diazoefficiens* biomass

Component	Composition (mol%) [a)]	MM(g/mol)	Modified MM(g/mol) [b)]	Weight (g (if total 1 mol Amino acid))	Content (mmol/g DW)
L-Ala	12.2	89	71	8.653	0.568
L-Arg	4.3	175	157	6.744	0.200
L-Asn	4.5	132	114	5.125	0.210
L-Asp	4.5	132	114	5.125	0.210
L-Cys	0.5	121	103	0.514	0.023
L-Gln	5.2	146	128	6.649	0.242
L-Glu	5.2	146	128	6.649	0.242
Gly	9.3	75	57	5.296	0.433
L-His	1.6	155	137	2.190	0.074
L-Ile	4.5	131	113	5.080	0.210
L-Leu	7.4	131	113	8.354	0.345
L-Lys	9.0	147	129	11.598	0.419
L-Met	2.7	149	131	3.533	0.126
L-Phe	3.7	165	147	5.434	0.172
L-Pro	3.8	115	97	3.682	0.177
L-Ser	6.3	105	87	5.476	0.293
L-Thr	5.4	119	101	5.449	0.251
L-Trp	0.5	204	186	0.929	0.023
L-Tyr	2.7	181	163	4.397	0.126
L-Val	6.8	117	99	6.725	0.317
Total	100.1	-	-	107.602	4.661

MM: Molecular Mass

[a)] In relevant literatures, composition of cysteine is 'trace'; tryptophan data is absent; aspartate and asparagine are represented by 'aspartic acid'; glutamatea and glutamine are represented by 'glutamic acid'. Assume both of the composition of cysteine and tryptophan are 0.5%; composition of aspartate and asparagine are equivalent; so as to glutamatea and glutamine.

[b)] During protein synthesis process, addition of one amino acid is accompanied with one H_2O loss, so the modified molar mass = molar mass of amino acid - molar mass of water.

3 ODNs component proportions of *B. diazoefficiens* biomass

The GC-content of *B. diazoefficiens* USDA110 is 64.1% (10). We suppose biomass has equal proportions of G and C as well as A and T, and the proportions of deoxynucleotides (ODNs) in *B. diazoefficiens* biomass were shown in Table 3.

Table 3 ODNs component proportions of *B. diazoefficiens* biomass

Component	Composition (mol%)	MM(g/mol)	Modified MM(g/mol)[a)]	Weight (g (if total 1 mol DNA))	Content (mmol/g DW)

dAMP	18	331	313	56.34	0.0175
dCMP	32	307	289	92.48	0.0311
dGMP	32	347	329	105.28	0.0311
dTMP	18	322	304	54.72	0.0175
Total	100	-	-	308.82	-

[a)] During DNA synthesis process, addition of one deoxyribonucleotide is accompanied with one H_2O loss, so the modified molar mass = molar mass of deoxyribonucleotides - molar mass of water.

4 Nucleotide component proportions of *B. diazoefficiens* biomass

We suppose the total RNA of *B. diazoefficiens* USDA110 only contains rRNA, tRNA and mRNA, and their proportions in the total RNA were 80%, 15% and 5%, respectively (11-13). The proportions of nucleotides of rRNAs, tRNAs and mRNAs were obtained from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/Bradyrhizobium_diazoefficiens_USDA_110_uid57599/). The proportions of nucleotides in *B. diazoefficiens* biomass were shown in Table 4.

Table 4 Nucleotide component proportions of *B. diazoefficiens* biomass

Component	Composition (mol%)	MM(g/mol)	Modified MM(g/mol)[a)]	Weight (g (if total 1 mol RNA))	Content (mmol/g DW)
AMP	24.0	347	329	78.96	0.0631
CMP	24.7	323	305	75.335	0.0649
GMP	31.1	363	345	107.295	0.0817
UMP	20.2	324	306	61.812	0.0531
Total	100	-	-	323.402	-

[a)] During RNA synthesis process, addition of one nucleotide is accompanied with one H_2O loss, so the modified molar mass = molar mass of nucleotides - molar mass of water.

5 Lipid component proportions of *B. diazoefficiens* biomass

Generally, composition of the lipids in biomass is relatively complex. As to *B. diazoefficiens*, the most abundant lipids is poly-beta-hydroxybutyrate (PHB), which may account for 15.9% of the dry weight of cell (3) and 81.7% of the total lipids (4). Other lipids include phospholipids, aliphatic hydrocarbons, free fatty acids and fatty alcohols. The main energy-storage substance in *B. diazoefficiens* is PHB, while glycerolipids that is the main energy-storage lipids in most organisms is rare in *B. diazoefficiens* (about 0.07% (w/w)) and can be ignored (4). Among other major lipids, phospholipids is the only one in biomass component; further, pathways related to aliphatic hydrocarbons and fatty alcohols metabolism were not included in our model，hence, it could be assumed that PHB and phospholipids were the exclusive components of lipids in *B. diazoefficiens*.

Compositions of phospholipids in *B. diazoefficiens* have been detected (14). Biosynthetic pathways related to long-chain, unsaturated and odd-chain fatty acids were not included in our model, therefore we suppose that the phospholipid molecules are only composed of hexadecanoic acids (C16:0). The proportions of phospholipids in *B. diazoefficiens* biomass were shown in Table 5.

Table 5 Phospholipid component proportions of *B. diazoefficiens* biomass

Component [a)]	Composition (w/w)	MM(g/mol)	Content (mmol/g DW)
PE	54.6	692	0.00710
PC	24.5	523	0.00422
PG	13.7	627	0.00197
CL	7.2	1354	0.00048
Total	100	-	-

[a)] In relevant literature, mono-methyl-phosphatidylethanolamine (MMPE) is one of the components of phospholipid in *B. diazoefficiens* USDA110, but its proportion is small (3.4%) and the corresponding metabolic pathway is unknown, so it was treated as PE. PE: phosphatidylethanolamine, PC: phosphatidylcholine, PG: phosphatidylglycerol, CL: cardiolipin.

Precursors of PHB is (R)-3-Hydroxybutanoate whose molar mass is 104. During PHB synthesis process, addition of one (R)-3-Hydroxybutanoate is accompanied with one H_2O loss, so proportion of PHB in B. *diazoefficiens* biomass is:

15.9%×1000/ (104-18) = 1.8488 mmol/g DW。

6 Peptidoglycan component proportions of B. *diazoefficiens* biomass

Precursors of peptidoglycan is (N-acetylmuramoyl-(N-acetylglucosamine)-L-alanyl-D-glutamyl-meso-2,6-diaminopimeloyl-D-alanyl) in Gram-negative bacteria, whose molar mass is 981. During peptidoglycan synthesis process, addition of two monomers is accompanied with three H_2O loss, so proportion of peptidoglycan in B. *diazoefficiens* biomass is:

2.5%×1000/(981-27) = 0.0262 mmol/g DW.

7 Lipopolysaccharide component proportions of *B. diazoefficiens* biomass

Lipopolysaccharides (LPS) in *B. diazoefficiens* USDA110 are composed of (lipid A) and polysaccharide side chains. Compositions of LPS in *B. diazoefficiens* have been detected (15). The proportions of compositions of LPS in *B. diazoefficiens* biomass were shown in Table 6.

Table 6 LPS component proportions of *B. diazoefficiens* biomass

Component [a)]	Composition (w/w)	MM(g/mol)	Modified MM(g/mol) [b)]	Content (mmol/g DW)
Fucose	0.209	164	146	0.0434
Xylose	0.056	150	132	0.0126
Arabinose	0.053	150	132	0.0121
Mannose	0.198	180	162	0.0373
Glucose	0.065	180	162	0.0123
Glucosamine	0.026	179	161	0.0049
Galacturonate	0.070	194	176	0.0122
KDO [c)]	0.019	238	220	0.0027
Lipid A	0.305	1797	1797	0.0058
Total	1.000	-	-	-

[a] In relevant literatures, the metabolic pathways of fucosamine and quinovosamine are unknown and proportion of quinovosamine is small, so quinovosamine is not considered and fucosamine is treated as fucose.

[b] During LPS synthesis process, addition of one monosaccharide is accompanied with one H_2O loss, so the modified molar mass = molar mass of monosaccharide - molar mass of water.

[c] KDO: 2-Keto-3-Deoxy-D-manno-Octonate.

8 CPS and EPS component proportions of *B. diazoefficiens* biomass

Capsular polysaccharide (CPS) and extracellular polysaccharides (EPS) can be secreted by *B. diazoefficiens* USDA110 when it is cultured on a medium containing carbohydrates. Their compositions and the proportion of each component are almost the same (15, 16), so they can be combined as one component. Biosynthetic pathways of one of the key components of CPS and EPS, 4-O-methyl-galactose, was not included in our model, however, it can be treated as galactose. Compositions of CPS and EPS in *B. diazoefficiens* have been detected (15-17). The proportions of CPS and EPS in *B. diazoefficiens* biomass were shown in Table 7.

Table 7 CPS and EPS component proportions of *B. diazoefficiens* biomass

Component	Composition (mol%)	MM(g/mol)	Modified MM(g/mol) [a]	Weight (g (if total 1 mol CPS or EPS))	Content (mmol/g DW)
Mannose	19.73	180	153	30.19	0.185
Galactose	18.94	180	153	28.98	0.177
Glucose	41.93	180	153	64.16	0.393
Galacturonate	19.39	194	167	32.38	0.182
Total	100.00	-	-	155.71	-
Correct total [b]				167.64	

[a] During CPS and EPS synthesis process, addition of one monosaccharide is accompanied with one and a half H_2O loss, so the modified molar mass = molar mass of monosaccharide – 1.5 × molar mass of water.

[b] In CPS and EPS, 28.4% of all the glycosyl is acetylated and the acetyl donor is acetyl-CoA, so the added molar mass by acetylation is 42 and the average molar mass of CPS or EPS monomer is 155.71 × (1-28.4%) + (155.71+42) × 28.4% = 167.64. In the biomass, acetyl component of CPS and EPS is 15.7%×28.4% × 1000/167.64 = 0.266 mmol/g DW.

9 Total component proportions of *B. diazoefficiens* biomass

In summary, the same components were combined，and the total component proportions of *B. diazoefficiens* biomass were shown in Table 8.

Table 8 Total component proportions of *B. diazoefficiens* biomass

Component	Content (mmol/g DW)	Component	Content (mmol/g DW)
L-Ala	0.568	dTMP	0.0175
L-Arg	0.2	AMP	0.0631
L-Asn	0.21	CMP	0.0649
L-Asp	0.21	GMP	0.0817

L-Cys	0.023	UMP	0.0531
L-Gln	0.242	PE	0.0071
L-Glu	0.242	PC	0.00422
Gly	0.433	PG	0.00197
L-His	0.074	CL	0.00048
L-Ile	0.21	Arabinose	0.0121
L-Leu	0.345	Fucose	0.0434
L-Lys	0.419	Galactose	0.177
L-Met	0.126	Galacturonate	0.1942
L-Phe	0.172	Glucosamine	0.0049
L-Pro	0.177	Glucose	0.4053
L-Ser	0.293	Mannose	0.2223
L-Thr	0.251	Xylose	0.0126
L-Trp	0.023	KDO	0.0027
L-Tyr	0.126	Lipid A	0.0058
L-Val	0.317	PHB	1.8488
dAMP	0.0175	Peptidoglycan	0.0262
dCMP	0.0311	Acetyl-CoA	0.266
dGMP	0.0311		

10 GAM and NGAM of *B. diazoefficiens*

GAM of the biomass reaction was mainly composed of the energy consumption related to the biosynthesis of biomacromolecules such as proteins, nucleic acids and polysaccharides. Biosynthesis of biomacromolecules related to the GAM in our model includes proteins, DNA, RNA, polysaccharide side chains of LPS, CPS and EPS. Energy consumption related to the biosynthesis of proteins, DNA and RNA in *B. diazoefficiens* have been detected (6). During the synthesis process of CPS, EPS and polysaccharide side chains of LPS, addition of one monosaccharide is accompanied with three high energy bonds consumption. Composition and proportion of *B. diazoefficiens* GAM were shown in Table 9.

Table 9 Composition and proportion of *B. diazoefficiens* GAM

Component	wt%	Total mmol	mmol ATP/mmol	Total
Protein	0.501	4.6610	4.324	20.154
DNA	0.030	0.0972	3.365	0.327
RNA	0.085	0.2628	2.406	0.632
LPS	0.034	0.1375	3	0.413
CPS & EPS	0.157	0.9370	3	2.811
Total	-	-	-	24.337

Generally, NGAM requirements are hard to be accurately estimated and often represented in metabolic models by the reaction ATP + H_2O=>ADP + Pi + H^+ whose lower bound was set as the NGAM. A proper reference value of NGAM could not be found in the metabolic models of closely

related species, so the metabolic model of *E.coli*, *i*JO1366, was consulted (18), whose NGAM is 3.0 mmol*gDW^{-1}*h^{-1}.

11 The determined biomass reaction

In sum, the determined biomass reaction for *B. diazoefficiens* USDA110 is:

0.266 Acetyl-CoA[c] + 0.568 L-Ala[c] + 0.0631 AMP[c] + 0.0121 Arabinose[c] + 0.2 L-Arg[c] + 0.21 L-Asn[c] + 0.21 L-Asp[c] + 24.337 ATP[c] + 0.00048 CL[c] + 0.0649 CMP[c] + 0.023 L-Cys[c] + 0.0175 dAMP[c] + 0.0311 dCMP[c] + 0.0311 dGMP[c] + 0.0175 dTMP[c] + 0.0434 Fucose[c] + 0.177 Galactose[c] + 0.1942 Galacturonate[c] + 0.242 L-Gln[c] + 0.0049 Glucosamine[c] + 0.4053 Glucose[c] + 0.242 L-Glu[c] + 0.433 Gly[c] + 0.0817 GMP[c] + 15.885 H2O[c] + 0.074 L-His[c] + 0.21 L-Ile[c] + 0.0027 KDO[c] + 0.345 L-Leu[c] + 0.0058 Lipid A[c] + 0.419 L-Lys[c] + 0.2223 Mannose[c] + 0.126 L-Met[c] + 0.00422 PC[c] + 0.0071 PE[c] + 0.0262 Peptidoglycan[c] + 0.00197 PG[c] + 1.8488 PHB[c] + 0.172 L-Phe[c] + 0.177 L-Pro[c] + 0.293 L-Ser[c] + 0.251 L-Thr[c] + 0.023 L-Trp[c] + 0.126 L-Tyr[c] + 0.0531 UMP[c] + 0.317 L-Val[c] + 0.0126 Xylose[c] → 24.337 ADP[c] + 0.266 CoA[c] + 24.337 H[c] + 24.337 Pi[c]

References

1. T. Bisseling, R. C. van Den Bos and A. van Kammen, *Microbiology*, 1977, **101**, 79-84.
2. F. J. Bergersen, *Microbiology*, 1958, **19**, 812-823.
3. S. A. Kim and L. Copeland, *Appl. Environ. Microb.*, 1996, **62**, 4186-4190.
4. T. Gerson and J. J. Patel, *Appl. Microbiol.*, 1975, **30**, 193-198.
5. A. Fabra, J. Angelini, A. Donolo, M. Permigiani and S. Castro, *Anton. Leeuw. Int. J. G.*, 1998, **73**, 223-228.
6. I. Thiele and B. Ø. Palsson, *Nat. Protoc.*, 2010, **5**, 93-121.
7. W. X. Chen and E. T. Wang, *Chinese Rhizobia*, Science Press, 2011.
8. M. A. Islam, E. A. Edwards and R. Mahadevan, *PLoS Comput. Biol.*, 2010, **6**, el000887.
9. T. T. Lillich and G. H. Elka, *J. Appl. Bact.*, 1973. **36**, 315-319.
10. T. Kaneko, Y. Nakamura, S. Sato, K. Minamisawa, T. Uchiumi, S. Sasamoto, A. Watanabe, K. Idesawa, M. Iriguchi, K. Kawashima, M. Kohara, M. S. Matsumoto, S. Shimpo, H. Tsuruoka, T. Wada, M. Yamada and S. Tabata, *DNA Res.*, 2002, **9**, 189-197.
11. T. Kampers, P. Friedhoff, J. Biernat, E. M. Mandelkow and E. Mandelkow, *FEBS Lett.*, 1996, **399**, 344-349.
12. M. C. Hansen, A. K. Nielsen, S. Molin, K. Hammer and M. Kilstrup, *J. Bacteriol.*, 2001, **183**, 4747-4751.
13. A. J. Westermann, S. A. Gorski and J. Vogel, *Nat. Rev. Microbiol.*, 2012, **10**, 618-630.
14. P. S. Miclea, M. Péter, G. Végh, G. Cinege, E. Kiss, G. Váró, I. Horváth and I. Dusha, *Mol. Plant. Microbe. In.*, 2010, **23**, 638-650.
15. V. Puvanesarajah, F. M. Schell, D. Gerhold and G. Stacey, *J. Bacteriol.*, 1987, **169**, 137-141.
16. J. M. Andrew and D. B. Wolfgang, *Plant Physiol.*, 1980, **66**, 158-163.
17. K. Minamisawa, *Plant Cell Physiol.*, 1989, **30**, 877-884.
18. J. D. Orth, T. M. Conrad, J. Na, J. A. Lerman, H. Nam, A. M. Feist and B. Ø. Palsson, *Mol. Syst. Biol.*, 2011, **7**, 535-543.

Supplementary files

Supplementary file S1. Important information during the reconstruction of metabolic network of *B. diazoefficiens* USDA110, including reaction sources, genome annotation, details of reactions and metabolites and the gaps unable to be filled. (XLS)

Supplementary file S2. Detailed results of the phenotypic microarray experiment and corresponding in silico predictions. (XLS)

Supplementary file S3. The reconstructed genome-scale metabolic network model of *B. diazoefficiens* USDA110, iYY1101. (XML)

Supplementary file S4. The constraint-based metabolic network model of *B. diazoefficiens* USDA110 specific for the FL state. (XML)

Supplementary file S5. The constraint-based metabolic network model of *B. diazoefficiens* USDA110 specific for the SNF state. (XML)

Supplementary file S6. Detailed results of simulation including essentiality analysis for genes and reactions and metabolic control analysis. (XLS)