Supporting Information

A High-Quality Genome-Scale Model for *Rhodococcus opacus* Metabolism

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Supplemental Table 1. Wildtype phenol ^{13}C-MFA vs. genome-scale model predicted fluxes.

Pathway	Reaction	MFA	FBA	pFBA	E-Flux2	SPOT
Substrate Uptake	Phenol.ext -> AcCoA + SucCoA	100	100.00	100.00	100 ± 0	100 ± 0
EMP Pathway	G6P <-> F6P	-0.48	-1.82	-1.82	-1.07 ± 0.42	0 ± 0
	F6P + ATP -> FBP	-2	-13.96	-13.96	-2.16 ± 2.45	-2.31 ± 3.27
	FBP <-> DHAP + GAP	-2	-13.96	-13.96	-2.16 ± 2.45	-2.31 ± 3.27
	DHAP <-> GAP	-2	-19.63	-19.63	-5.5 ± 3.68	-4.28 ± 2.27
	GAP <-> G1,3P	-10.79	-37.86	-37.86	-16.26 ± 7.85	-9.23 ± 13.06
	G1,3P <-> 3PG + ATP + NADH	-10.79	-37.86	-37.86	-16.26 ± 7.85	-9.23 ± 13.06
	3PG <-> 2PG	-16.25	-46.34	-46.34	-22.4 ± 10.13	10.16 ± 19.55
	2PG <-> PEP	-16.25	-46.34	-46.34	-22.4 ± 10.13	26.33 ± 30.74
	PEP <-> Pyr + ATP	30	22.57	0.00	2.99 ± 4.23	127.45 ± 94.34
Pentose Phosphate Pathway	6PG <-> Ru5P + CO2 + NADPH	0.46	0.00	0.00	0 ± 0	0 ± 0
	Ru5P <-> X5P	-1.19	-7.10	-7.10	1.89 ± 1.21	-2.31 ± 3.27
	Ru5P <-> R5P	1.65	7.09	7.09	16.34 ± 4.29	4.28 ± 2.27
	X5P <-> TKC2 + GAP	-1.19	-4.67	-4.67	-8.84 ± 2.38	-4.62 ± 6.53
	TKC2 + E4P <-> F6P	-1.19	-4.67	-4.67	-8.84 ± 2.38	-4.62 ± 6.53
	TKC2 + R5P <-> S7P	0	-2.42	-2.42	10.73 ± 2.6	2.31 ± 3.27
	TAC3 + GAP <-> F6P	0	-2.42	-2.42	10.73 ± 2.6	2.31 ± 3.27
	S7P <-> TAC3 + E4P	0	-2.42	-2.42	10.73 ± 2.6	2.31 ± 3.27
ED Pathway	G6P <-> 6PG + NADPH	-0.58	0.00	0.00	0 ± 0	-8.11 ± 11.46
	6PG <-> KDPG	-1.04	0.00	0.00	0 ± 0	0 ± 0
	KDPG <-> Pyr + GAP	-1.04	0.00	0.00	0 ± 0	0 ± 0
TCA Cycle	Pyr <-> AcCoA + CO2 + NADH	59.23	0.00	0.00	24.8 ± 21.7	26.91 ± 10.44
	OAC + AcCoA <-> Cit	114.44	84.30	84.30	93.76 ± 42.93	89.92 ± 56
	Cit <-> ICit	114.44	84.30	84.30	93.76 ± 42.93	89.92 ± 56
	ICit <-> AKG + CO2 + NADPH	113.47	77.54	77.54	91.04 ± 43.98	-59.32 ± 16.85
	AKG <-> SucCoA + CO2 + NADH	108.61	66.98	66.98	60.21 ± 15.29	0 ± 0
	SucCoA <-> Suc + ATP	207.48	163.39	163.39	171.1 ± 29.83	33.04 ± 25.28
	Suc <-> Fum + FADH2	208.58	173.75	173.75	187.32 ± 43.94	182.28 ± 57.93
	Fum <-> Mal	209.67	182.96	182.96	192.76 ± 42.81	182.28 ± 57.93
	Mal <-> OAC + NADH	171.02	182.96	160.39	155.43 ± 54.84	174.59 ± 66.41
Glyoxylate Shunt	ICit -> Glyox + Suc	0.97	6.77	6.77	2.72 ± 3.84	149.24 ± 65.78
	Glyox + AcCoA -> Mal	0.97	0.00	0.00	1.61 ± 2.27	0 ± 0
Anaplerotic Pathways	Pyr + CO2 + ATP -> OAC	0	0.00	0.00	-6.57 ± 4.97	31.01 ± 42.87
	Mal -> Pyr + CO2 + NADH	39.62	0.00	22.57	38.94 ± 12.57	7.69 ± 10.88
	PEP + CO2 -> OAC	48.9	-75.38	-52.81	-29.2 ± 9.19	-114.97 ± 54.26

	OAC + ATP -> PEP + CO2	97.77	75.38	52.81	29.2 ± 9.19	114.97 ± 54.26
Acetate Formation	AcCoA <-> Ac + ATP	-1.23	-2.14	0.00	0.21 ± 0.3	-94.94 ± 39.52
Energy Reactions	NADH <-> NADPH	14.43	0.00	0.00	0 ± 0	0 ± 0
	ATP -> ATP.ext	680.09	0.00	0.00	0 ± 0	0 ± 0
Transport	CO2 -> CO2.ext	376.46	227.03	227.03	306.07 ± 94.06	277.47 ± 51.81
	O2.ext -> O2	433.7	368.99	368.99	428.93 ± 91.15	356.67 ± 76.94
	NH3.ext -> NH3	25.75	0.87	0.87	0.52 ± 0.2	0 ± 0
	SO4.ext -> SO4	0.42	0.87	0.87	0.52 ± 0.2	0 ± 0
Biomass Reaction	0.515*Ala + 0.174*Arg + 0.153*Asn + 0.439*Asp + 0.03*Cys + 0.314*Glu + 0.314*Gln + 0.399*Gly + 0.059*His + 0.155*Ile + 0.302*Leu + 0.181*Lys + 0.059*Met + 0.138*Phe + 0.147*Pro + 0.176*Ser + 0.201*Thr + 0.067*Tyr + 0.28*Val + 0.048*Trp + 9.035*AcCoA + 0.245*R5P + 0.325*MEETHF + 0.225*G6P + 0.071*F6P + 1.02*GAP + 0.43*3PG + 0.083*Pyr + 0.051*PEP + 0.087*AKG + 0.223*OAC + 33.25*ATP + 19.3*NADPH -> Biomass	4.69	11.30	11.30	6.67 ± 2.63	0 ± 0

Supplemental Table 2. PVHG6 phenol ^{13}C-MFA vs. genome-scale model predicted fluxes.

Pathway	Reaction	MFA	FBA	pFBA	E-Flux2	SPOT
Substrate Uptake	Phenol.ext -> AcCoA + SucCoA	100	100.00	100.00	100 ± 0	100 ± 0
EMP Pathway	G6P <-> F6P	-0.48	-1.82	-1.82	-1.63 ± 0.09	0 ± 0
	F6P + ATP -> FBP	-2	-13.96	-13.96	-8.39 ± 1.76	0 ± 0
	FBP <-> DHAP + GAP	-2	-13.96	-13.96	-8.39 ± 1.76	0 ± 0
	DHAP <-> GAP	-2	-19.63	-19.63	-13.48 ± 1.48	-13.34 ± 4.15
	GAP <-> G1,3P	-10.79	-37.86	-37.86	-29.86 ± 0.58	0 ± 0
	G1,3P <-> 3PG + ATP + NADH	-10.79	-37.86	-37.86	-29.86 ± 0.58	0 ± 0
	3PG <-> 2PG	-16.25	-46.34	-46.34	-40.91 ± 8.54	79.5 ± 14.47
	2PG <-> PEP	-16.25	-46.34	-46.34	-40.91 ± 8.54	167.68 ± 44.33
	PEP <-> Pyr + ATP	30	22.57	0.00	0 ± 0	504.17 ± 9.15
Pentose Phosphate Pathway	6PG <-> Ru5P + CO2 + NADPH	0.46	0.00	0.00	0 ± 0	0 ± 0
	Ru5P <-> X5P	-1.19	-7.10	-7.10	-2.23 ± 2.1	0 ± 0
	Ru5P <-> R5P	1.65	7.09	7.09	14.65 ± 5.25	13.34 ± 4.15
	X5P <-> TKC2 + GAP	-1.19	-4.67	-4.67	-8.34 ± 2.68	0 ± 0
	TKC2 + E4P <-> F6P	-1.19	-4.67	-4.67	-8.34 ± 2.68	0 ± 0
	TKC2 + R5P <-> S7P	0	-2.42	-2.42	6.11 ± 4.79	0 ± 0
	TAC3 + GAP <-> F6P	0	-2.42	-2.42	6.11 ± 4.79	0 ± 0
	S7P <-> TAC3 + E4P	0	-2.42	-2.42	6.11 ± 4.79	0 ± 0
ED Pathway	G6P <-> 6PG + NADPH	-0.58	0.00	0.00	0 ± 0	0 ± 0
	6PG <-> KDPG	-1.04	0.00	0.00	0 ± 0	0 ± 0
	KDPG <-> Pyr + GAP	-1.04	0.00	0.00	0 ± 0	0 ± 0
TCA Cycle	Pyr <-> AcCoA + CO2 + NADH	59.23	0.00	0.00	0 ± 0	0 ± 0
	OAC + AcCoA <-> Cit	114.44	84.30	84.30	84.38 ± 2.26	508.69 ± 90.5
	Cit <-> ICit	114.44	84.30	84.30	84.38 ± 2.26	508.69 ± 90.5
	ICit <-> AKG + CO2 + NADPH	113.47	77.54	77.54	83.23 ± 3.84	-127.61 ± 102.08
	AKG <-> SucCoA + CO2 + NADH	108.61	66.98	66.98	73.72 ± 20.9	0 ± 0
	SucCoA <-> Suc + ATP	207.48	163.39	163.39	170.52 ± 4.53	-83.44 ± 149.06
	Suc <-> Fum + FADH2	208.58	173.75	173.75	174.9 ± 2.78	552.86 ± 43.41
	Fum <-> Mal	209.67	182.96	182.96	183.18 ± 2.33	552.86 ± 43.41
	Mal <-> OAC + NADH	171.02	182.96	160.39	152 ± 9.31	541.85 ± 30.82
Glyoxylate Shunt	ICit -> Glyox + Suc	0.97	6.77	6.77	1.15 ± 1.63	636.3 ± 192.47
	Glyox + AcCoA -> Mal	0.97	0.00	0.00	0.71 ± 1	0 ± 0
Anaplerotic Pathways	Pyr + CO2 + ATP -> OAC	0	0.00	0.00	0 ± 0	303.33 ± 24.38
	Mal -> Pyr + CO2 + NADH	39.62	0.00	22.57	31.89 ± 7.99	11 ± 15.56
	PEP + CO2 -> OAC	48.9	-75.38	-52.81	-46.71 ± 8.22	-336.49 ± 37.03

	OAC + ATP -> PEP + CO2	97.77	75.38	52.81	46.71 ± 8.22	336.49 ± 37.03
Acetate Formation	AcCoA <-> Ac + ATP	-1.23	-2.14	0.00	0 ± 0	-32.13 ± 23.72
Energy Reactions	NADH <-> NADPH	14.43	0.00	0.00	0 ± 0	0 ± 0
	ATP -> ATP.ext	680.09	0.00	0.00	0 ± 0	0 ± 0
Transport	CO2 -> CO2.ext	376.46	227.03	227.03	264.47 ± 19.25	594.05 ± 8.41
	O2.ext -> O2	433.7	368.99	368.99	402.25 ± 25.03	697.38 ± 3.71
	NH3.ext -> NH3	25.75	0.87	0.87	0.78 ± 0.04	0 ± 0
	SO4.ext -> SO4	0.42	0.87	0.87	0.78 ± 0.04	0 ± 0
Biomass Reaction	0.515*Ala + 0.174*Arg + 0.153*Asn + 0.439*Asp + 0.03*Cys + 0.314*Glu + 0.314*Gln + 0.399*Gly + 0.059*His + 0.155*Ile + 0.302*Leu + 0.181*Lys + 0.059*Met + 0.138*Phe + 0.147*Pro + 0.176*Ser + 0.201*Thr + 0.067*Tyr + 0.28*Val + 0.048*Trp + 9.035*AcCoA + 0.245*R5P + 0.325*MEETHF + 0.225*G6P + 0.071*F6P + 1.02*GAP + 0.43*3PG + 0.083*Pyr + 0.051*PEP + 0.087*AKG + 0.223*OAC + 33.25*ATP + 19.3*NADPH -> Biomass	4.69	11.30	11.30	10.15 ± 0.56	0 ± 0

Supplemental Table 3. Wildtype glucose ^{13}C-MFA vs. genome-scale model predicted fluxes.

Pathway	Reaction	^{13}C-MFA	FBA	pFBA	E-Flux2	SPOT
Substrate Uptake	Gluc.ext + ATP -> G6P	100	100.00	100.00	100 ± 0	100 ± 0
EMP Pathway	G6P <-> F6P	-1.61	93.77	93.77	73.47 ± 4.8	64.54 ± 6.86
	F6P + ATP -> FBP	0	78.19	78.19	41.94 ± 3.45	48.75 ± 7.31
	FBP <-> DHAP + GAP	0	78.19	78.19	41.94 ± 3.45	48.75 ± 7.31
	DHAP <-> GAP	0	58.87	58.86	14.64 ± 2.11	29.17 ± 3.13
	GAP <-> G1,3P	86.56	132.67	132.67	99.33 ± 3.39	148.75 ± 7.31
	G1,3P <-> 3PG + ATP + NADH	86.56	132.67	132.67	99.33 ± 3.39	148.75 ± 7.31
	3PG <-> 2PG	78.52	120.74	120.74	62.14 ± 7.65	148.75 ± 7.31
	2PG <-> PEP	78.52	120.74	120.74	62.14 ± 7.65	150.21 ± 6.61
	PEP <-> Pyr + ATP	19.02	100.00	115.70	61.53 ± 5.12	151.49 ± 6.5
Pentose Phosphate Pathway	6PG <-> Ru5P + CO2 + NADPH	6.62	0.00	0.00	3.13 ± 3.27	0 ± 0
	Ru5P <-> X5P	2.12	-7.75	-7.75	5.19 ± 3.33	31.58 ± 1.93
	Ru5P <-> R5P	4.5	7.74	7.74	1.68 ± 0.82	20.46 ± 1.36
	X5P <-> TKC2 + GAP	2.12	-4.64	-4.64	1.88 ± 1.67	15.79 ± 0.96
	TKC2 + E4P <-> F6P	0.15	-4.64	-4.64	1.88 ± 1.67	15.79 ± 0.96
	TKC2 + R5P <-> S7P	1.96	-3.12	-3.12	-12.24 ± 1.29	15.79 ± 0.96
	TAC3 + GAP <-> F6P	1.96	-3.12	-3.12	-12.24 ± 1.29	15.79 ± 0.96
	S7P <-> TAC3 + E4P	1.97	-3.12	-3.12	-12.24 ± 1.29	15.79 ± 0.96
ED Pathway	G6P <-> 6PG + NADPH	100	0.00	0.00	0.71 ± 5.02	-38.03 ± 5.75
	6PG <-> KDPG	93.38	0.00	0.00	15.87 ± 7.23	35.46 ± 6.86
	KDPG <-> Pyr + GAP	93.38	0.00	0.00	15.87 ± 7.23	35.46 ± 6.86
TCA Cycle	Pyr <-> AcCoA + CO2 + NADH	135.41	0.00	0.00	17.7 ± 5.56	0 ± 0
	OAC + AcCoA <-> Cit	69.04	48.60	48.60	43.86 ± 4.95	28.41 ± 5.95
	Cit <-> ICit	69.04	48.60	48.60	43.86 ± 4.95	28.41 ± 5.95
	ICit <-> AKG + CO2 + NADPH	69.04	48.60	48.60	43.86 ± 4.95	26.1 ± 7.2
	AKG <-> SucCoA + CO2 + NADH	61.6	110.11	110.11	76.59 ± 4.11	0 ± 0
	SucCoA <-> Suc + ATP	59.88	36.07	36.07	29.85 ± 4.97	26.1 ± 7.2
	Suc <-> Fum + FADH2	61.6	110.11	110.11	32.83 ± 4.97	28.41 ± 5.95
	Fum <-> Mal	63.27	120.00	120.00	42.09 ± 4.97	28.95 ± 5.37
	Mal <-> OAC + NADH	25.97	120.00	120.00	30.67 ± 1.46	28.95 ± 5.37
Glyoxylate Shunt	ICit -> Glyox + Suc	0	0.00	0.00	0 ± 0	2.32 ± 3.27
	Glyox + AcCoA -> Mal	0	0.00	0.00	0 ± 0	0 ± 0
Anaplerotic Pathways	Pyr + CO2 + ATP -> OAC	0	6.25	21.96	38.35 ± 3.29	1.28 ± 1.8
	Mal -> Pyr + CO2 + NADH	37.31	0.00	0.00	11.42 ± 4.03	0 ± 0
	PEP + CO2 -> OAC	55.51	15.70	0.00	-4.09 ± 5.79	-1.28 ± 1.8

	OAC + ATP -> PEP + CO2	0	0.00	0.00	4.09 ± 5.79	1.28 ± 1.8
Acetate Formation	AcCoA <-> Ac + ATP	-1.89	-0.32	0.00	2.28 ± 0.16	-12.53 ± 1.13
Energy Reactions	NADH <-> NADPH	21.31	0.00	0.00	0 ± 0	0 ± 0
	ATP -> ATP.ext	527.47	0.00	0.00	0 ± 0	0 ± 0
Transport	CO2 -> CO2.ext	264.28	139.50	139.50	168.9 ± 0.6	234.81 ± 11.01
	O2.ext -> O2	198.14	195.96	195.96	208.5 ± 2.08	196.99 ± 11.87
	NH3.ext -> NH3	39.38	0.59	0.59	0.56 ± 0	0 ± 0
	SO4.ext -> SO4	0.64	0.59	0.59	0.56 ± 0	0 ± 0
Biomass Reaction	0.515*Ala + 0.174*Arg + 0.153*Asn + 0.439*Asp + 0.03*Cys + 0.314*Glu + 0.314*Gln + 0.399*Gly + 0.059*His + 0.155*Ile + 0.302*Leu + 0.181*Lys + 0.059*Met + 0.138*Phe + 0.147*Pro + 0.176*Ser + 0.201*Thr + 0.067*Tyr + 0.28*Val + 0.048*Trp + 9.035*AcCoA + 0.245*R5P + 0.325*MEETHF + 0.225*G6P + 0.071*F6P + 1.02*GAP + 0.43*3PG + 0.083*Pyr + 0.051*PEP + 0.087*AKG + 0.223*OAC + 33.25*ATP + 19.3*NADPH -> Biomass	7.18	13.52	13.52	12.66 ± 0.02	0 ± 0

Supplemental Table 4. Biomass composition of *Bacillus subtilis* [1] and *R. opacus* with glucose and *R. opacus* with phenol. The numbers in the table represent the mass percent of biomass made of that macromolecule class.

Species (condition)	Protein	Lipid	Carbohydrate
B. subtilis (G)	65.61	17.52	0.092
R. opacus (G)	38.84	14.08	17.21
R. opacus (P)	24.04	40.26	14.64

Supplemental Table 5. *R. opacus PD630* biomass reaction reactants for phenol and glucose conditions. The reactant coefficients in the biomass reaction for the *B. subtilis* [1] genome-scale model, and the custom biomass reactions for *R. opacus* on phenol and glucose.

Metabolite	Category	*B. subtilis*	*R. opacus* (G)	*R. opacus* (P)
10-Formyltetrahydrofolate	carbon carrier	0.00022	0.00022	0.00022
L-Alanine	protein	0.49872	0.18277	0.29529
S-Adenosyl-L-methionine	carbon carrier	0.00022	0.00022	0.00022
L-Arginine	protein	0.28717	0.10524	0.17003
L-Asparagine	protein	0.23403	0.08577	0.13857
L-Aspartate	protein	0.23403	0.08577	0.13857
ATP	energy molecule	52.54715	52.54715	52.54715
Calcium	salt	0.00505	0.00505	0.00505
Chloride	salt	0.00505	0.00505	0.00505
CoenzymeA	carbon carrier	0.00056	0.00056	0.00056
Co2+	salt	0.00010	0.00010	0.00010
CTP	energy molecule	0.12962	0.12962	0.12962
Cu2+	salt	0.00069	0.00069	0.00069
L-Cysteine	protein	0.08891	0.03258	0.05264
DATP	nucleic acid	0.02540	0.02540	0.02540
DCTP	nucleic acid	0.02623	0.02623	0.02623
DGTP	nucleic acid	0.02623	0.02623	0.02623
DTTP	nucleic acid	0.02540	0.02540	0.02540
FAD	energy molecule	0.00022	0.00022	0.00022
Fe2+	salt	0.00652	0.00652	0.00652

Fe3+	salt	0.00758	0.00758	0.00758
L-Glutamine	protein	0.25549	0.09363	0.15127
L-Glutamate	protein	0.25549	0.09363	0.15127
Glycine	protein	0.59478	0.21797	0.35217
Glycerol teichoic acid n45 unlinked unsubstituted	lipid	0.00097	0.00223	0.00078
Glycerol teichoic acid n45 unlinked D ala substituted	lipid	0.00097	0.00223	0.00078
Glycerol teichoic acid n45 unlinked glucose substituted	lipid	0.00097	0.00223	0.00078
GTP	energy molecule	0.20883	0.20883	0.20883
H2O	water	47.18485	47.18485	47.18485
L-Histidine	protein	0.09198	0.03371	0.05446
L-Isoleucine	protein	0.28206	0.10337	0.16701
Potassium	salt	0.18950	0.18950	0.18950
L-Leucine	protein	0.43740	0.16030	0.25898
Lipoteichoic acid synthesis n24 linked glucose substituted	lipid	0.00005	0.00011	0.00004
Lipoteichoic acid synthesis n24 linked N acetylglucosamine substituted	lipid	0.00005	0.00011	0.00004
Lipoteichoic acid synthesis n24 unliked D alanine substituted	lipid	0.00005	0.00011	0.00004
Lipoteichoic acid synthesis n24 linked unsubstituted	lipid	0.00005	0.00011	0.00004
L-Lysine	protein	0.33316	0.12209	0.19726
L-Methionine	protein	0.14921	0.05468	0.08834
Magnesium	salt	0.00842	0.00842	0.00842
510-Methylenetetrahydrofolate	carbon carrier	0.00022	0.00022	0.00022

Mn2+	salt	0.00067	0.00067	0.00067
Menaquinol8	energy molecule	0.00010	0.00010	0.00010
NAD	energy molecule	0.00178	0.00178	0.00178
NADP+	energy molecule	0.00043	0.00043	0.00043
Peptidoglycan subunit of *Bacillus subtilis*	carbohydrate	0.00971	0.14773	0.17367
L-Phenylalanine	protein	0.17987	0.06592	0.10650
L-Proline	protein	0.21461	0.07865	0.12707
Pyridoxal-5-phosphate	energy molecule	0.00022	0.00022	0.00022
Riboflavin	energy molecule	0.00022	0.00022	0.00022
L-Serine	protein	0.20950	0.07678	0.12405
Sulfate	salt	0.00421	0.00421	0.00421
5678-Tetrahydrofolate	carbon carrier	0.00022	0.00022	0.00022
Thiaminediphosphate	energy molecule	0.00022	0.00022	0.00022
L-Threonine	protein	0.24629	0.09026	0.14583
L-Tryptophan	protein	0.05519	0.02022	0.03268
L-Tyrosine	protein	0.13388	0.04906	0.07927
UTP	energy molecule	0.13990	0.13990	0.13990
L-Valine	protein	0.41083	0.15056	0.24325
Zinc	salt	0.00033	0.00033	0.00033

Supplemental Table 6. A mapping of reactions from ^{13}C-MFA to genome-scale model reactions.

Pathway	Reaction	Genome-Scale Model Reaction Ids
Substrate Uptake	Gluc.ext + ATP -> G6P	reverse_EX_glc__D_e
	Phenol.ext -> AcCoA + SucCoA	PHEMOX
EMP Pathway	G6P <-> F6P	PGI
	F6P + ATP -> FBP	PFK or reverse_FBP
	FBP <-> DHAP + GAP	FBA
	DHAP <-> GAP	TPI
	GAP <-> G1,3P	GAPD or reverse_GAPDi_nadp
	G1,3P <-> 3PG + ATP + NADH	reverse_PGK
	3PG <-> 2PG	reverse_PGM
	2PG <-> PEP	ENO
	PEP <-> Pyr + ATP	ACMANApts or reverse_PPS or PYK or FRUptspp or MALTptspp or DHAPT or SUCpts or GLCptspp or MNLpts or FRUpts2pp or SBTpts or SUCptspp or GLCpts or ACGApts or FRUpts2 or GALpts or MALTpts or GLCADpts or MANptspp or FRUpts or ARBTpts or TREptspp or GALTpts or TREpts or ASCBpts or GAMpts or MANpts or ACGAptspp or FUCpts
Pentose Phosphate Pathway	6PG <-> Ru5P + CO2 + NADPH	GND
	Ru5P <-> X5P	RPE
	Ru5P <-> R5P	reverse_RPI
	X5P <-> TKC2 + GAP	TKT2
	TKC2 + E4P <-> F6P	TKT2
	TKC2 + R5P <-> S7P	TKT1
	TAC3 + GAP <-> F6P	TALA
	S7P <-> TAC3 + E4P	TALA
ED Pathway	G6P <-> 6PG + NADPH	G6PDH2r and PGL
	6PG <-> KDPG	EDD
	KDPG <-> Pyr + GAP	EDA
TCA Cycle	Pyr <-> AcCoA + CO2 + NADH	PDH
	OAC + AcCoA <-> Cit	CS or reverse_CITL
	Cit <-> ICit	ACONT or (ACONTa and ACONTb)
	ICit <-> AKG + CO2 + NADPH	ICDHx or ICDHyr
	AKG <-> SucCoA + CO2 + NADH	AKGDH or reverse_KGD2
	SucCoA <-> Suc + ATP	SUCBZT2 or OCOAT1 or SUCBZT1 or reverse_SUCOAS or BZSCT or OCOAT4 or 3OADPCOAT

	Suc <-> Fum + FADH2	reverse_FRD3 or reverse_FRD2 or reverse_ASPO5 or reverse_FRD or SUCD1 or reverse_FRDx
	Fum <-> Mal	FUM
	Mal <-> OAC + NADH	MDH or MDH2 or MDH3 or MDH3_1 or DMALRED
Glyoxylate Shunt	ICit -> Glyox + Suc	ICL
	Glyox + AcCoA -> Mal	MALS
Anaplerotic Pathways	Pyr + CO2 + ATP -> OAC	PC or reverse_OAADC
	Mal -> Pyr + CO2 + NADH	ME1 or ME2
	PEP + CO2 -> OAC	PPC or reverse_PEPCK_re
	OAC + ATP -> PEP + CO2	PEPCK_re
Acetate Formation	AcCoA <-> Ac + ATP	BUTCT or A4HBCT or ACOXT or reverse_ACS or reverse_ACACCT
Biomass Formation	AKG + NADPH + NH3 -> Glu	
	Glu + ATP + NH3 -> Gln	
	Glu + ATP + 2*NADPH -> Pro	
	Glu + CO2 + Gln + Asp + AcCoA + 5*ATP + NADPH -> Arg + AKG + Fum + Ac	
	OAC + Glu -> Asp + AKG	
	Asp + 2*ATP + NH3 -> Asn	
	Pyr + Glu -> Ala + AKG	
	3PG + Glu -> Ser + AKG + NADH	
	Ser <-> Gly + MEETHF	
	Gly <-> CO2 + MEETHF + NADH + NH3	
	Thr <-> Gly + AcCoA + NADH	
	Ser + AcCoA + 3*ATP + 4*NADPH + SO4 -> Cys + Ac	
	Asp + Pyr + Glu + SucCoA + ATP + 2*NADPH -> LLDAP + AKG + Suc	
	LLDAP -> Lys + CO2	
	Asp + 2*ATP + 2*NADPH -> Thr	
	Asp + METHF + Cys + SucCoA + ATP + 2*NADPH -> Met + Pyr + Suc + NH3	
	Pyr + Pyr + Glu + NADPH -> Val + CO2 + AKG	
	AcCoA + Pyr + Pyr + Glu + NADPH -> Leu + CO2 + CO2 + AKG + NADH	
	Thr + Pyr + Glu + NADPH -> Ile + CO2 + AKG + NH3	

	PEP + PEP + E4P + Glu + ATP + NADPH -> Phe + CO2 + AKG PEP + PEP + E4P + Glu + ATP + NADPH -> Tyr + CO2 + AKG + NADH Ser + R5P + PEP + E4P + PEP + Gln + 3*ATP + NADPH -> Trp + CO2 + GAP + Pyr + Glu R5P + FTHF + Gln + Asp + 5*ATP -> His + AKG + Fum + 2*NADH	
1-Carbon metabolism	MEETHF + NADH -> METHF MEETHF -> FTHF + NADPH	
Oxidative Phosphorylation	NADH + 0.5*O2 -> 2*ATP FADH2 + 0.5*O2 -> ATP	
Energy Reactions	NADH <-> NADPH ATP -> ATP.ext	reverse_NADTRHD ATPM
Transport	CO2 -> CO2.ext O2.ext -> O2 NH3.ext -> NH3 SO4.ext -> SO4	EX_co2_e reverse_EX_o2_e reverse_EX_so4_e SO4t2
Biomass Reaction	0.515*Ala + 0.174*Arg + 0.153*Asn + 0.439*Asp + 0.03*Cys + 0.314*Glu + 0.314*Gln + 0.399*Gly + 0.059*His + 0.155*Ile + 0.302*Leu + 0.181*Lys + 0.059*Met + 0.138*Phe + 0.147*Pro + 0.176*Ser + 0.201*Thr + 0.067*Tyr + 0.28*Val + 0.048*Trp + 9.035*AcCoA + 0.245*R5P + 0.325*MEETHF + 0.225*G6P + 0.071*F6P + 1.02*GAP + 0.43*3PG + 0.083*Pyr + 0.051*PEP + 0.087*AKG + 0.223*OAC + 33.25*ATP + 19.3*NADPH -> Biomass	Growth or Growth_Phenol or Growth_Glucose

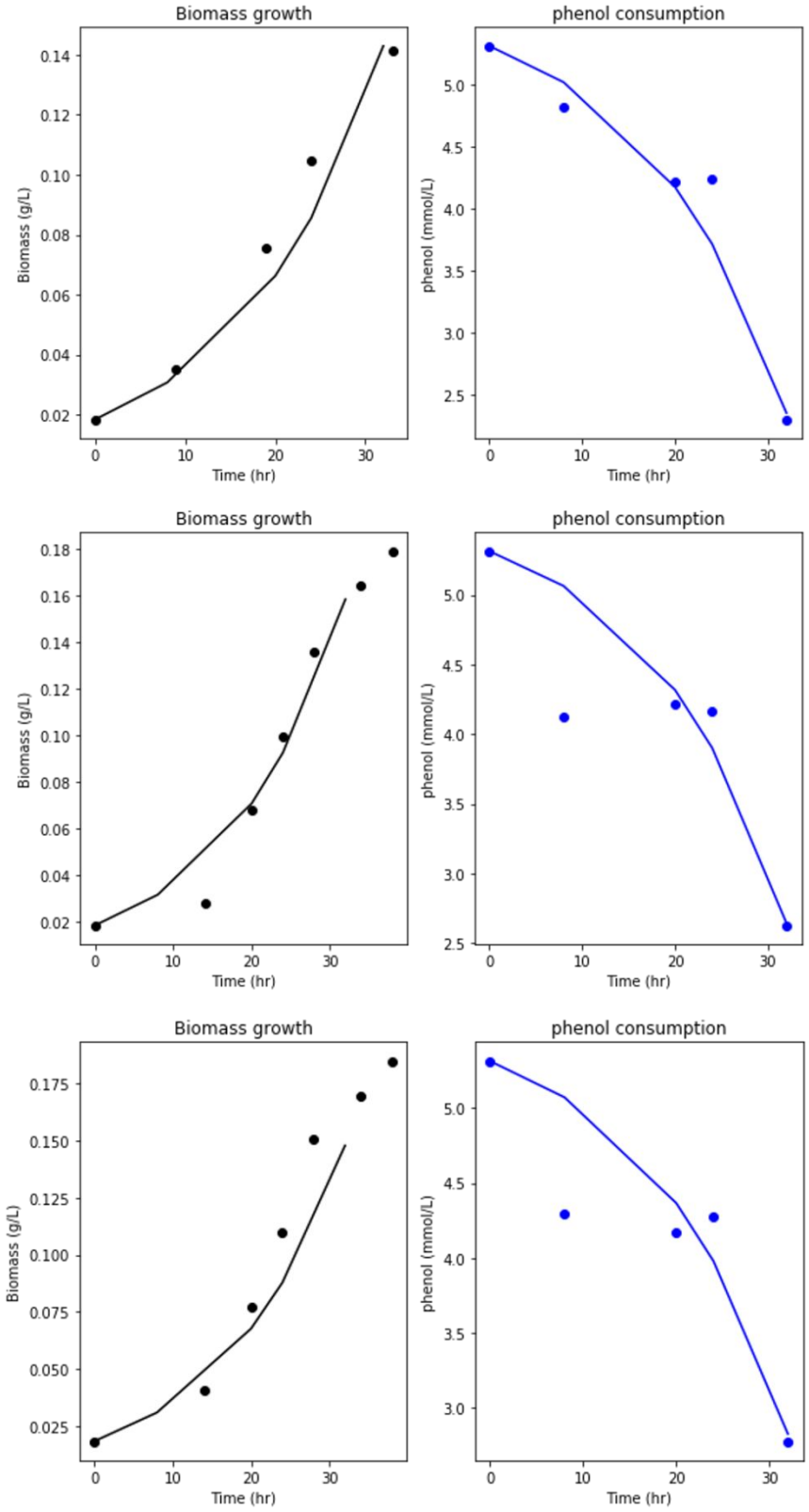
Supplemental Table 7. Description of the notebooks used to generate and test iGR1773.

Notebook	Task of Notebook
Notebook A: Uptake Reactions	Add metabolites and reactions to model for phenol and other aromatic monomer uptake
Notebook B: Biomass Reactions	Define and add biomass reactions for *R. opacus* growth on glucose and phenol
Notebook C: Annotation and Curation	Add annotations for reactions, metabolites, and genes based on BiGG universal model.
Notebook D: Experimental Consumption and Growth Rates	Calculate consumption and growth rates from time course fermentations with phenol and glucose
Notebook E: FBA and pFBA simulations	Find flux solutions using FBA and pFBA
Notebook F: Glucose E-Flux2 simulation	Find glucose E-Flux2 flux solutions
Notebook G: Glucose SPOT simulation	Find glucose SPOT flux solutions
Notebook H: Phenol E-Flux2 simulation	Find phenol E-Flux2 flux solutions
Notebook I: Phenol SPOT simulation	Find phenol SPOT flux solutions
Notebook J: ATP Maintenance Calculation	Calculate ATP maintenance for glucose and phenol

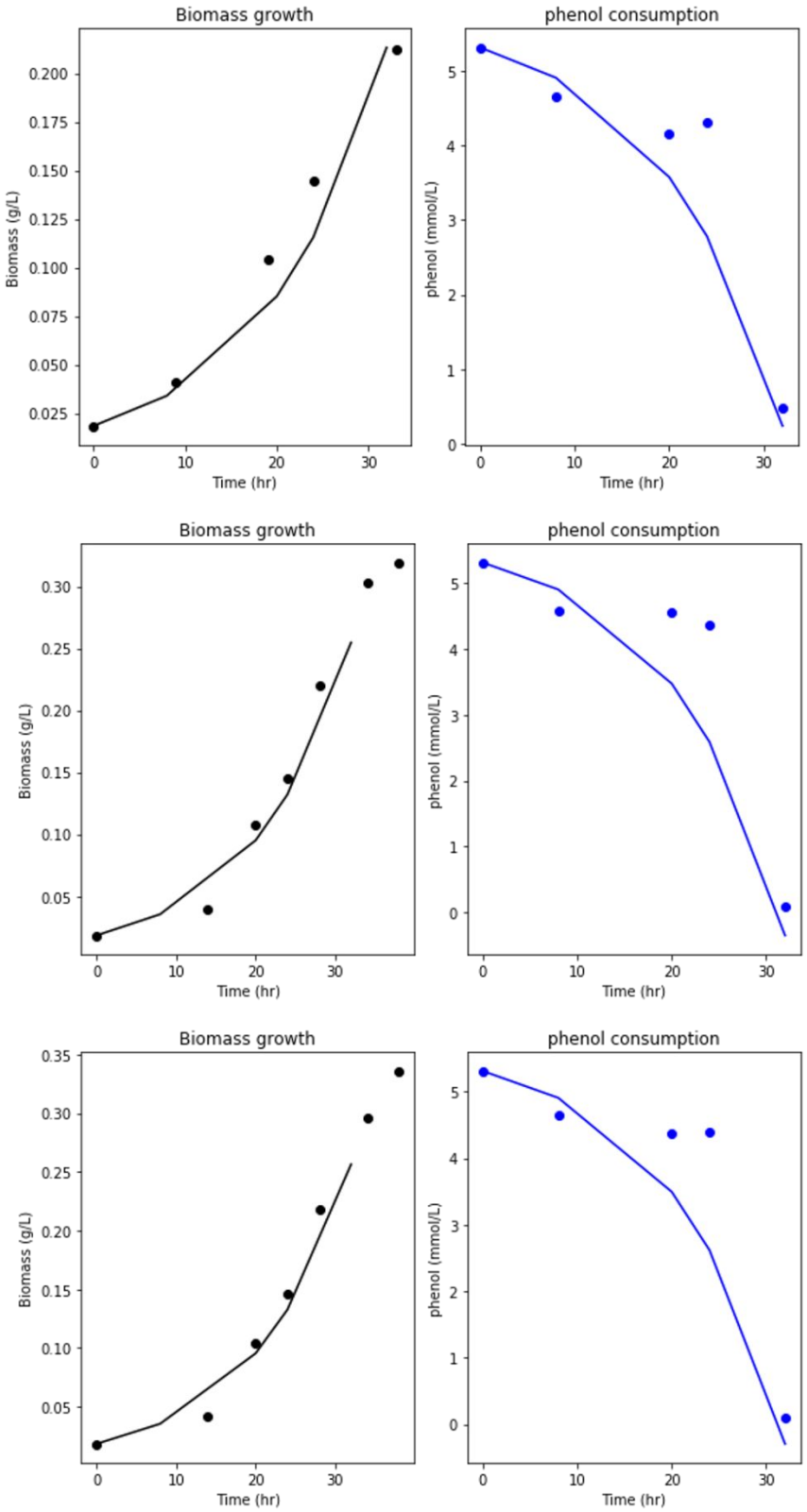
Supplemental Table 8. A Mapping of abbreviations to metabolite names.

Abbreviation	Metabolite
3PG	3-phosphoglycerate
6PG	6-phosphogluconate
AcCoA	acetyl-CoA
αKG	α-ketoglutarate
Asx	aspartate and asparagine
Cit	citrate
DHAP	dihydroxyacetone phosphate
E4P	erythrose-4-phosphate
Fum	fumarate
F6P	fructose-6-phosphate
FBP	fructose-1,6-bisphosphate
G1P	glucose-1-phosphate
G1,3P	glycerate-1,3-phosphate
G2P	glycerate-2-phosphate
G6P	glucose-6-phosphate
GAP	glyceraldehyde-3-phosphate
Glx	glutamate and glutamine
ICit	isocitrate
KDPG	2-dehydro-3-deoxy-phosphogluconate
Mal	malate
OAC	oxaloacetate
PEP	phosphoenolpyruvate
Pyr	pyruvate
R5P	ribose-5-phosphate
Ru5P	ribulose-5-phosphate
Suc	succinate
SucCoA	succinyl-CoA
TA-C3	3-carbon fragment transferred by transaldolase

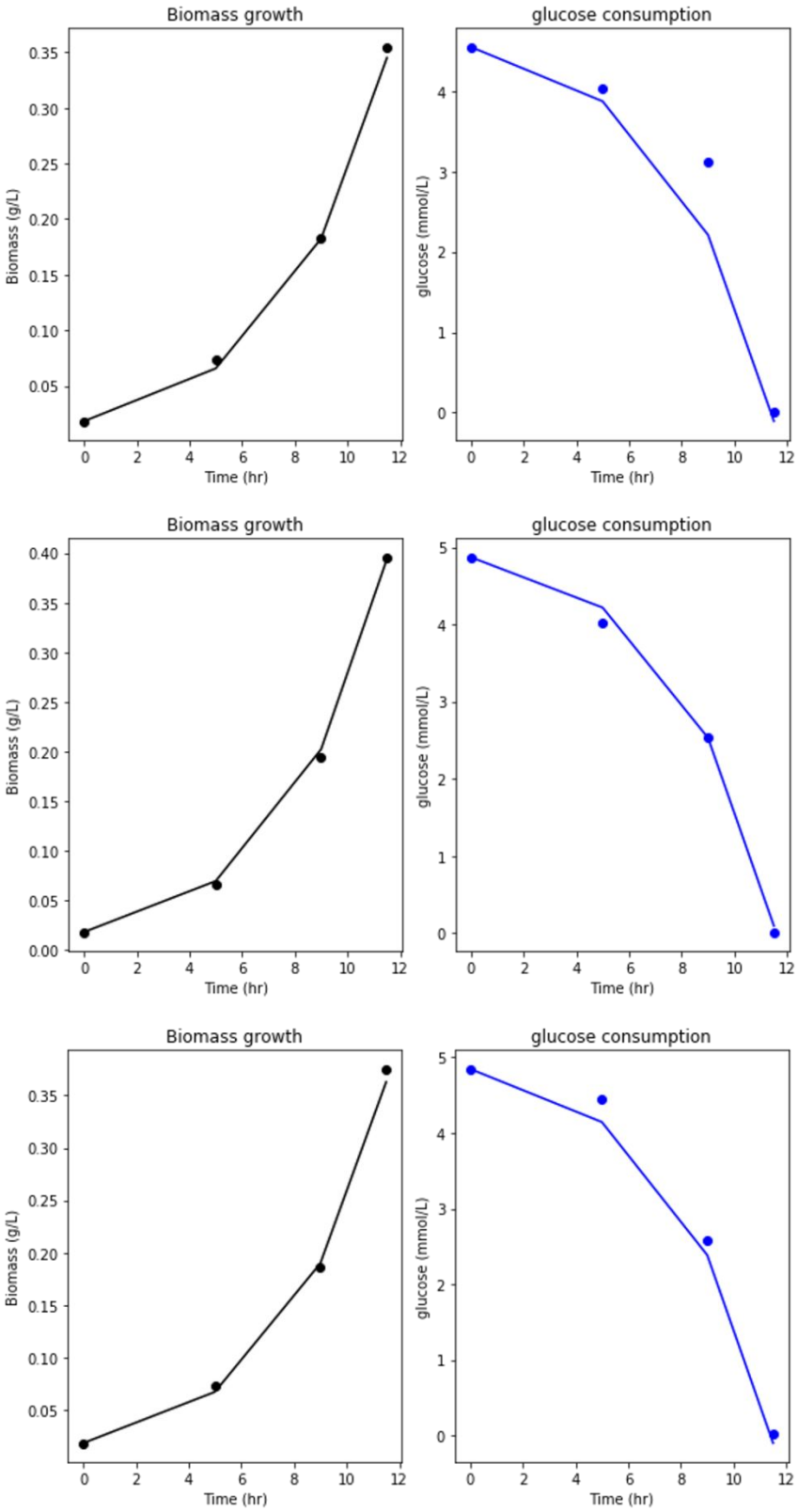
TK-C2	2-carbon fragment transferred by transketolase
X5P	xylulose-5-phosphate



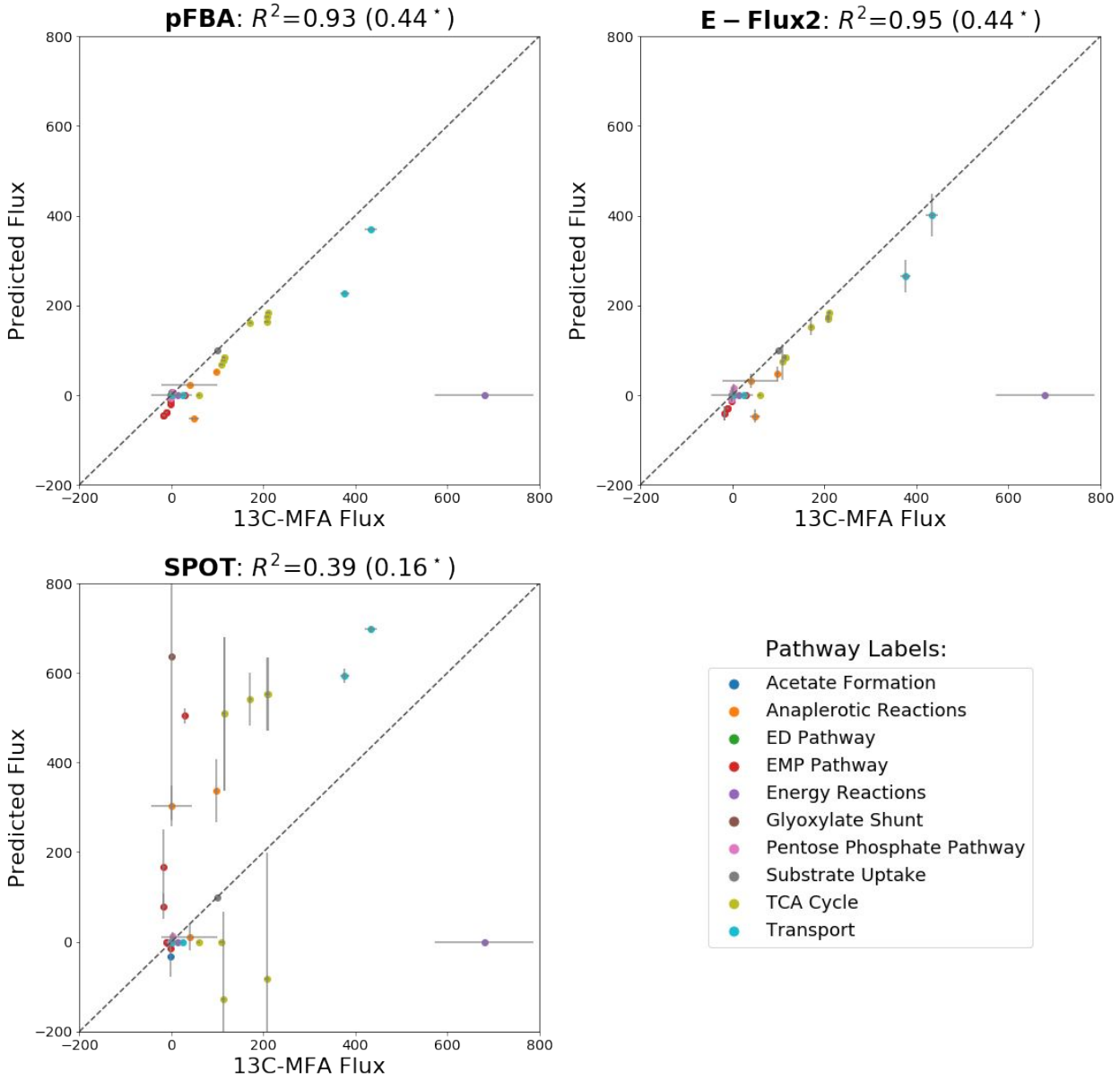
Supplemental Figure 1. WT phenol growth and consumption data. The points on the graphs represent measured data. The lines are the model fitting.



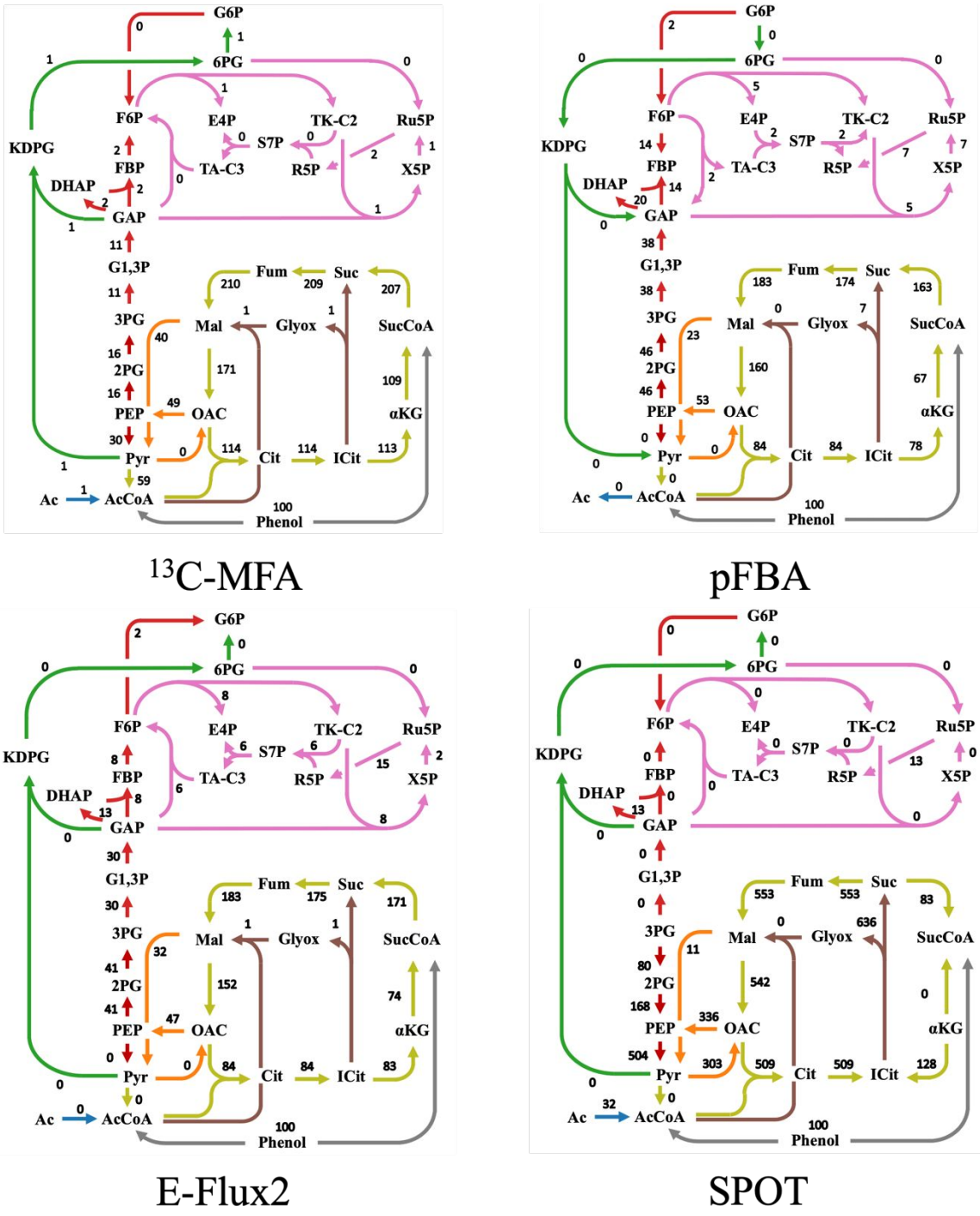
Supplemental Figure 2. PVHG6 phenol growth and consumption data. The points on the graphs represent measured data. The lines are the model fitting.



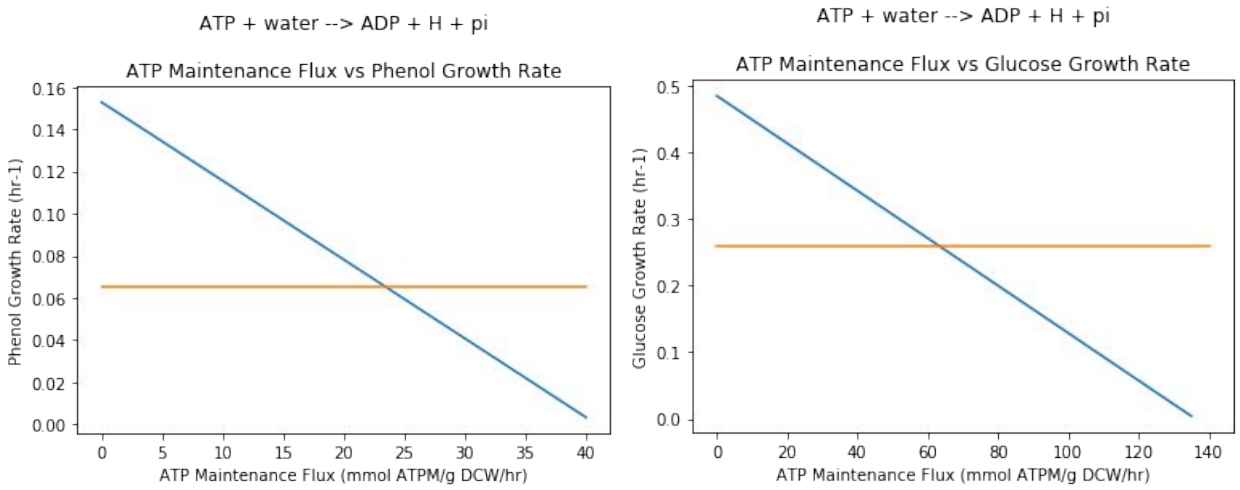
Supplemental Figure 3. WT glucose growth and consumption data. The points on the graphs represent measured data. The lines are the model fitting.



Supplemental Figure 4. Phenol metabolism flux predictions based on PVHG6 strain transcripts. The y-axis represents the predicted flux by each of the COBRA methods (FBA, pFBA, E-Flux2, and SPOT) and the x-axis represents the flux measured via ^{13}C-MFA. The fluxes are normalized to the carbon source uptake (units are mmol reaction / 100 mmol substrate uptake). The first R^2 value does not include ATP maintenance reaction and the R^2 value in parentheses includes the ATP maintenance reaction. The x-axis error bars are 90% confidence intervals, and if applicable, the y-axis error bars are standard deviations of flux predictions made from three biological replicates of transcriptomics data.



Supplemental Figure 5. Phenol flux maps based on PVHG6 transcripts. *R. opacus* PD630's flux map predictions when phenol is the sole carbon source. The flux values are relative flux distributions based on 100 mmol of phenol consumed by the cell to generate 100 mmol of influx toward both acetyl-CoA and succinyl-CoA.



Supplemental Figure 6. FBA prediction of ATP maintenance vs. growth rate. These plots represent how the genome-scale model and flux balance analysis were used to calculate ATP maintenance flux. The blue lines represent the predicted growth rate for a given ATP maintenance flux. The orange lines represent the experimental growth rate of wildtype *R. opacus*. The intersection of these two lines gives the FBA prediction for ATP maintenance flux.

References

1. Oh, Y.-K.; Palsson, B. O.; Park, S. M.; Schilling, C. H.; Mahadevan, R., Genome-scale Reconstruction of Metabolic Network in Bacillus subtilis Based on High-throughput Phenotyping and Gene Essentiality Data*. *Journal of Biological Chemistry* **2007,** *282* (39), 28791-28799.