

SUPPLEMENTARY INFORMATION

Disproportionate carbon dioxide efflux in bacterial metabolic paths of assimilated organic substrates leads to variable carbon use efficiency

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Content: **Table S1-Table S7**

Table S1 | Extracellular substrate depletion (in mmol C) in *Pseudomonas putida* mt-2 cultures grown on 1:1 carbon equivalent mixture of (left) glucose:ferulate or (right) glucose:*p*-coumarate. Values represent mean and standard deviation obtained from three biological replicates. Grey boxes are for values that were not found.

Time (h)	Glucose:ferulate				Glucose: <i>p</i> -coumarate			
	Ferulate (mmol C)		Glucose (mmol C)		<i>p</i> -Coumarate (mmol C)		Glucose (mmol C)	
	mean	SD	mean	SD	mean	SD	mean	SD
0.00	49.67	0.00	49.89	0.08	49.90	0.01	49.65	0.02
2.50	49.36	3.23	40.23	0.05	47.69	0.92	47.00	2.65
4.00	48.47	0.98	10.84	0.04	45.51	1.90	23.42	9.00
4.50	44.77	0.51	0.63	0.01	36.10	6.46	5.46	4.86
5.25	39.15	0.82			0.62	1.07	0.02	0.02
5.75	32.75	1.78					0.05	0.08
6.50	16.77	1.01						
7.25	7.60	0.66						
8.00	0.90	0.18						
8.46	0.90	0.18						

Table S2 | Intracellular metabolite labeling after assimilation of [U-¹³C₆]-glucose and unlabeled *p*-coumarate in *Pseudomonas putida* mt-2 cells. Values represent mean and standard deviation obtained from three biological replicates. Grey boxes are for values that were not found. Metabolite abbreviations are as follows: glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-bisphosphate (FBP), dihydroxyacetone-phosphate (DHAP), ribose-5-phosphate (R5P), xylulose-5-phosphate (Xu5P), sedoheptulose-7-phosphate (S7P), phosphoenolpyruvate (PEP), 3-phosphoglycerate (3-PG), and α-ketoglutarate (α-KG).

Pathway		Mean					Standard Deviation				
EDEMP Pathway	# C	Gluconate	G6P	F6P	FBP	DHAP	Gluconate	G6P	F6P	FBP	DHAP
	0	0.003	0.035	0.023	0.082	0.076	0.003	0.049	0.030	0.039	0.065
	1	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.001
	2	0.000	0.003	0.000	0.000	0.010	0.000	0.003	0.000	0.000	0.008
	3	0.000	0.038	0.035	0.023	0.910	0.000	0.007	0.003	0.032	0.057
	4	0.001	0.021	0.021	0.005		0.000	0.005	0.000	0.007	
	5	0.013	0.033	0.028	0.031		0.002	0.002	0.011	0.028	
6	0.982	0.870	0.892	0.860		0.000	0.042	0.016	0.028		
PP Pathway	# C	R5P	Xu5P	S7P		R5P	Xu5P	S7P			
	0	0.041	0.043	0.009		0.046	0.049	0.012			
	1	0.001	0.001	0.000		0.001	0.001	0.000			
	2	0.027	0.029	0.001		0.006	0.004	0.000			
	3	0.016	0.016	0.002		0.000	0.001	0.000			
	4	0.035	0.027	0.031		0.011	0.009	0.009			
	5	0.880	0.884	0.032		0.030	0.039	0.006			
	6			0.051				0.005			
7			0.873				0.009				
Downstream ED Pathway	# C	PEP	3-PG	Pyruvate		PEP	3-PG	Pyruvate			
	0	0.134	0.110	0.394		0.057	0.043	0.003			
	1	0.061	0.043	0.066		0.006	0.007	0.001			
	2	0.073	0.037	0.069		0.002	0.005	0.005			
3	0.732	0.810	0.471		0.065	0.032	0.010				

Tricarboxylic acid Cycle	# C	aKG	Citrate	Aspartate	Succ	Malate	α-KG	Citrate	Aspartate	Succ	Malate
		0	0.236	0.347	0.182	0.819	0.931	0.054	0.057	0.021	0.003
1	0.118	0.093	0.146	0.035	0.012	0.009	0.023	0.007	0.001	0.017	
2	0.295	0.145	0.197	0.089	0.000	0.022	0.030	0.004	0.008	0.000	
3	0.159	0.173	0.325	0.024	0.041	0.019	0.004	0.010	0.002	0.021	
4	0.135	0.126	0.150	0.031	0.016	0.042	0.020	0.035	0.004	0.001	
5	0.057	0.095				0.024	0.015				
6		0.020					0.027				

Table S3 | Intracellular metabolite labeling after assimilation of [U-¹³C₆]-glucose and unlabeled ferulate in *Pseudomonas putida* mt-2 cells. Values represent mean and standard deviation obtained from three biological replicates. Grey boxes are for values that were not found. Metabolite abbreviations are as provided in table S1.2.

Pathway		Mean					Standard Deviation				
EDEMP Pathway	#	Gluconate	G6P	F6P	FBP	DHAP	Gluconate	G6P	F6P	FBP	DHAP
	C										
	0	0.002	0.004	0.002	0.008	0.005	0.001	0.006	0.002	0.011	0.007
	1	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.003
	3	0.000	0.031	0.030	0.000	0.992	0.000	0.001	0.003	0.000	0.004
	4	0.000	0.025	0.021	0.000		0.000	0.004	0.004	0.000	
5	0.011	0.030	0.029	0.000		0.001	0.015	0.006	0.000		
6	0.986	0.909	0.917	0.992		0.000	0.005	0.016	0.011		
PP Pathway	#	R5P	Xu5P	S7P			R5P	Xu5P	S7P		
	C										
	0	0.000	0.000	0.000			0.000	0.000	0.000		
	1	0.002	0.000	0.000			0.003	0.000	0.000		
	2	0.021	0.000	0.002			0.005	0.000	0.001		
	3	0.000	0.000	0.003			0.000	0.000	0.000		
	4	0.036	0.016	0.022			0.003	0.010	0.002		
	5	0.941	0.983	0.026			0.001	0.010	0.002		
6			0.042					0.005			
7			0.905					0.006			
Downstream ED Pathway	#	PEP	3-PG	Pyruvate			PEP	3-PG	Pyruvate		
	C										
	0	0.118	0.118	0.370			0.020	0.020	0.025		
	1	0.083	0.083	0.072			0.010	0.010	0.010		
2	0.093	0.093	0.075			0.007	0.007	0.012			
3	0.706	0.706	0.483			0.036	0.036	0.003			
Tricarboxylic acid Cycle	#	aKG	Citrate	Aspartate	Succ	Malate	α-KG	Citrate	Aspartate	Succ	Malate
	C										
0	0.172	0.231	0.204	0.781	0.939	0.014	0.051	0.010	0.022	0.026	

1	0.133	0.104	0.187	0.040	0.018	0.005	0.008	0.003	0.001	0.001
2	0.303	0.171	0.231	0.085	0.000	0.002	0.015	0.024	0.007	0.000
3	0.184	0.218	0.255	0.032	0.019	0.006	0.017	0.030	0.005	0.018
4	0.147	0.134		0.061	0.024	0.006	0.005		0.010	0.009
5	0.061	0.096				0.006	0.024			
6		0.046					0.012			

Table S4 | Protein abundance for enzymes in central carbon metabolism and substrate uptake pathways. Values represent log₂ fold changes in protein levels for *Pseudomonas putida* mt-2 cells grown on glucose (G), glucose:*p*-coumarate (G:C), and glucose:ferulate (G:F) relative to internal standard; and log₂ fold changes in protein levels of glucose:*p*-coumarate cells (GC/G) or glucose:ferulate cells (GF/G) relative to glucose-grown cells. Data were obtained from four biological replicates. Not found, NF; Not applicable, NA.

Pathway	Protein	Log ₂ _G	Log ₂ _GF	Log ₂ _GC	Log ₂ (GF/G)		Log ₂ (GC/G)	
					mean	<i>p</i> -value	mean	<i>p</i> -value
Initial Glucose Catabolism	GTSC	-0.680	NF	NF	NA	NA	NA	NA
	GAD	-0.723	NF	0.024	NA	NA	0.747	NA
	GNUK	-0.355	0.047	-0.495	0.401	0.363	-0.141	0.394
	KGUD	-0.903	NF	-0.784	NA	NA	0.119	0.398
	KGUK	-0.565	-0.194	-0.239	0.371	0.375	0.326	0.382
Pentose Phosphate Pathway	PGL	-1.219	-0.206	-0.537	1.012	0.204	0.682	0.318
	ZWFA	-0.706	-0.220	-0.075	0.486	0.336	0.631	0.309
	RPE	1.048	NF	0.300	NA	NA	-0.748	0.389
	RPIA	-0.110	0.198	0.267	0.309	0.388	0.378	0.372
	TKTA	0.230	0.294	0.317	0.064	0.394	0.087	0.389
	TAL	NF	NF	0.180	NA	NA	NA	NA
Reverse-EMP Pathway	PGI	-0.107	NF	-0.362	NA	NA	-0.255	NA
	FBP	0.197	0.104	0.287	-0.093	0.396	0.090	NA
	FBA	0.020	-0.164	-0.224	-0.184	0.368	-0.244	0.321
	TPIA	0.319	0.453	0.421	0.135	0.394	0.103	0.396
ED Pathway	EDD	-0.925	0.329	-0.115	1.253	0.027	0.809	0.319
	EDA	-0.069	0.703	0.638	0.772	0.359	0.707	0.036
Downstream ED Pathway	GAPA	-0.745	0.459	-0.103	1.204	0.010	0.642	0.247
	GAPB	-0.153	-0.118	-0.188	0.035	0.398	-0.035	0.398
	PGK	0.319	0.375	0.222	0.056	0.398	-0.097	0.396
	PGM	1.402	1.923	1.015	0.521	0.387	-0.387	0.368
	ENO	-0.155	-0.242	-0.343	-0.087	0.396	-0.188	0.383
	PPSA	0.237	0.179	0.142	-0.058	0.396	-0.094	0.391
	PYKA	-0.256	-0.019	0.146	0.237	0.383	0.402	0.349
Anaplerotic	MAEB	-0.173	-0.083	-0.047	0.090	0.392	0.126	0.387

Reactions	OADA	0.394	0.782	0.143	0.388	NA	-0.250	NA
	PYCB	-0.237	0.235	0.349	0.472	0.343	0.586	0.325
Glyoxylate Shunt	GLCB	0.612	0.403	0.567	-0.208	0.389	-0.044	0.398
	ACEA	2.130	1.288	1.521	-0.843	0.108	-0.610	0.182
TCA Cycle	ACEE	-0.458	-0.019	-0.264	0.439	0.234	0.194	0.366
	GLTA	-1.318	-1.435	-0.912	-0.116	0.399	0.407	0.395
	ACNB	0.409	0.077	0.289	-0.332	0.370	-0.120	0.393
	IDH	-0.230	-0.267	-0.091	-0.037	0.398	0.139	0.392
	SUCA	0.307	0.063	0.023	-0.244	0.359	-0.285	0.323
	SUCB	0.305	0.156	0.306	-0.150	0.393	0.001	0.399
	LPDG	0.364	0.308	0.347	-0.056	0.396	-0.017	0.399
	SUCC	0.311	0.009	0.247	-0.302	0.322	-0.064	0.395
	SUCD	0.582	0.518	0.668	-0.064	0.398	0.086	0.397
	SDHA	0.747	0.112	0.252	-0.635	0.247	-0.495	0.295
	SDHB	1.117	0.386	0.871	-0.731	0.343	-0.246	0.388
	SDHD	0.452	0.354	0.084	-0.098	0.398	-0.369	0.384
	FUMA	-0.322	-0.496	-0.436	-0.174	0.391	-0.114	0.391
	MQO	-0.441	-0.257	-0.459	0.183	0.392	-0.019	0.399
MDH	-0.561	-0.381	-0.163	0.180	0.388	0.397	0.318	
Initial Aromatic Catabolism	FCS	NF	0.209	-0.009	NA	NA	NA	NA
	ECH	NF	0.218	0.207	NA	NA	NA	NA
	VDH	NF	-0.182	0.092	NA	NA	NA	NA
	VANB	NF	-1.213	NF	NA	NA	NA	NA
	VANA	NF	-0.862	2.310	NA	NA	NA	NA
	POBA	NF	NF	-0.406	NA	NA	NA	NA
Ortho-cleavage pathway	PCAG	NF	0.155	-0.609	NA	NA	NA	NA
	PCAH	NF	-0.996	-1.473	NA	NA	NA	NA
	PCAB	NF	-0.139	-0.193	NA	NA	NA	NA
	PCAC	NF	-0.801	-1.085	NA	NA	NA	NA
	PCAD	NF	-0.529	-0.496	NA	NA	NA	NA
	PCAF	NF	-0.103	-0.492	NA	NA	NA	NA
Para-cleavage pathway	PRAD	-0.923	-0.788	-0.554	-0.234	NA	0.369	0.050
	PRAG	-0.004	0.297	0.515	-0.218	0.008	0.519	0.031

Table S5 | Substrate-specific contribution of glucose- and *p*-coumarate- derived carbons towards CO₂ in decarboxylation reactions in *Pseudomonas putida* mt-2 cells calculated from flux contribution analysis. Values represent mean and standard deviation obtained from three biological replicates. Metabolite abbreviations are as provided in table S1.2.

Reaction	From Glucose		From <i>p</i> -Coumarate	
	mean	SD	mean	SD
6-PG → Ru5P + CO ₂	0.988	0.085	0.012	0.001
OAA → PEP + CO ₂	0.580	0.029	0.420	0.021
OAA → Pyruvate + CO ₂	0.580	0.029	0.420	0.021
Pyruvate → Acetyl-CoA + CO ₂	0.472	0.024	0.528	0.026
Isocitrate → α-KG + CO ₂	0.447	0.062	0.553	0.028
α-KG → Succinyl-CoA + CO ₂	0.454	0.023	0.546	0.027

Table S6 | Contribution (in mol CO₂-C/ mol C total C uptake) of glucose and *p*-coumarate towards CO₂ produced in decarboxylation reactions calculated from metabolic flux analysis and flux contribution analysis. Values represent mean and standard deviation obtained from three biological replicates. Metabolite abbreviations are as provided in table S1.2.

Reaction	mol CO ₂ -C/ mol total C uptake			
	From Glucose		From <i>p</i> -Coumarate	
	mean	SD	mean	SD
6-PG → Ru5P + CO ₂	0.255	0.075	0.006	3.5E-08
OAA → PEP + CO ₂	0.034	0.013	0.045	0.013
OAA → Pyruvate + CO ₂	0.001	5.5E-08	0.001	3.6E-06
Pyruvate → Acetyl-CoA + CO ₂	0.082	0.014	0.169	0.023
Isocitrate → α-KG + CO ₂	0.214	0.025	0.485	0.054
α-KG → Succinyl-CoA + CO ₂	0.209	0.025	0.461	0.053
Aromatic <i>Ortho</i> -cleavage	0	0	0.224	0.033
Aromatic <i>Para</i> -cleavage	0	0	0.503	0.054

Table S7 | Contribution (mmol C/g_{CDW}/h) of glucose and *p*-coumarate towards metabolites that serve as biomass precursors calculated from metabolic flux analysis and flux contribution analysis. Values represent mean and standard deviation obtained from three biological replicates. Metabolite abbreviations are as provided in table S1.2. CDW, cell dry weight.

Metabolites	mmol-biomass-C/g _{CDW} /h			
	From Glucose		From <i>p</i> -Coumarate	
	mean	SD	mean	SD
3-phosphoglycerate	0.890	0.134	0.300	0.015
α-ketoglutarate	0.367	0.092	2.20	0.110
Dihydroxyacetone-3-phosphate	0.075	0.015	0.003	0.0001
Erythrose-4-phosphate	0.290	0.058	0.003	0.0002
Glucose-6-phosphate	0.095	0.028	0.009	0.0005
Oxaloacetate	0.804	0.161	3.87	0.193
Phosphoenol-pyruvate	0.581	0.087	0.238	0.012
Pyruvate	0.937	0.140	3.14	0.157
Ribose-5-phosphate	0.880	0.220	0.053	0.003