Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary files for:

A metabolic trade-off between phosphate and glucose utilization in *Escherichia coli*

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Materials and Methods

Bacterial strains and growth media

The bacterial strains used in this study are described in Table S3. Strains BW6041 and BW6043 were obtained by streaking a day 19 sample on Luria-agar plates and randomly picking colonies. The insertion Tn10 conferring tetracycline resistance in *sucC* and *kanR* genes was accomplished by using the method described previously in Yu *et al*. (2000). Briefly, using primer sets with flanking sucC and kanR target sequences, linear Tn10 DNA were synthesized by PCR from BW3345 and recombine into DY330 after electroporation. For the construction of *sucC*:Tn*10*, the primer set used was sucCtetF1(5'-GCACCG GTGGGTTATGCCTGTACTACTCCGACTCGACATCTTGGTTACCG-3') and sucCtetR1 (5'-GCCGCTGTCAGCCAGTTTCTTCGCGCCGAGCAAGAGGGTCATTATATTTCG-3') and for construction of kanR::Tn*10,* the primer set used was kanTetF1 (5'-GGA TTATCA ATACCATATTTTTGAAAAAGCCGCAAGAGGGTCATTATATTTCG-3') and kanTetR1 (5'-GAGGCCGCGATTAAATTCCAACATGGATGCTGACTCGACATCTTGGTTACCG-3'). The mutated genes were then transferred into appropriate recipients by P1 phage transductions.

All phosphate limited (Pi-limited) bacterial cultures used T-salts minimal medium supplemented with D-glucose (0.2% w/v) and 35 μM KH_2PO_4 for chemostats and 1 mM KH_2PO_4 for batch cultures. For glucose limitation, Minimal Medium A (MMA) was supplemented with 0.02% w/v D-glucose for chemostats and 0.2% w/v for batch cultures.

For long-term chemostats, *E. coli* strain BW2952 was grown for 8 h in T-salts medium supplemented with 1 mM KH_2PO_4 and 0.2% w/v D-glucose and inoculated (1 mL) into 80-mL chemostats containing T-salts medium, 35 μM KH_2PO_4 and 0.2% D-glucose (Wang et al. 2010). The chemostat cultures were maintained at a dilution date of 0.1 h for 32 days at 37 °C. Samples for NMR were collected directly every 2-3 days and filtered through a Millex-GP syringe filter unit with 0.22 μm pore size.

Analysis of extracellular metabolites using Nuclear Magnetic Resonance (NMR) spectroscopy

The 1.5 ml chemostat aliquots were freeze-dried and resuspended in 550 μl D_2O containing 1 mM sodium 3-trimethylsilyl-D_4-propionate (TSP) and 5 mM sodium azide. The D_2O provided a frequency lock for the spectrometer and reduced the solvent signal, and the TSP served as an internal chemical shift reference ($\delta = 0$). The spectra were acquired according to

a protocol modified from Beckonert *et al*. (2007) on a Bruker Avance DRX600 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany), with a magnetic field strength of 14.1 T (^{1}H resonance frequency 600 MHz) using a 5-mm inverse probe. A one-dimensional NOESY pulse sequence was used for water suppression and data were acquired into 64K data points over a spectral width of 12 kHz, with eight dummy scans and 128 scans per sample. Apodisation (0.5 Hz exponential function), Fourier transform, phase correction, and chemical shift referencing were carried out in iNMR 3 (Mestrelab). The data were then exported to Matlab for further analysis. Metabolite assignments were based on previous studies and an online database (Behrends et al. 2009, Ulrich et al. 2008).

Identification of a clone with an increased fermentative activity and genome re-sequencing

Three independent clones were isolated from the day-19 glycerol stock sample after streaking on L-agar plates. The purified clones were then inoculated separately into Pi-limited chemostats. Samples were taken for excreted fermentation products by withdrawing 5 mL cultures, filtered through a Millex-GP syringe filter unit with 0.22 μm pore size and stored at -20^{o}C until analysis. The supernatant samples were then analysed by HPLC (Agilent) in Rezex ROA-organic acid column (Phenomenex). The mobile phase used for separation of fermentation products was 0.005 N H_2SO_4 at flow rate of 0.5 mL/min at ambient temperature. The separated compounds were detected using the UV detector at 210 nm. Lactate and succinate were identified on the basis of the retention time of known standards.

Two clones, one with increased fermentative activity and one with wild-type level, were chosen for whole genome re-sequencing as previously described (Maharjan et al. 2012).

Estimation of competitive fitness

Fitness comparisons were made against a tetracycline-resistant derivative of BW6041 vs BW6043. Three to four chemostat competition experiments were conducted as previously described (Wang et al. 2010, Maharjan et al. 2010). The reported relative fitness was based on the equations given in Dykhuizen and Hartl (1983), measured in terms of Malthusian parameters, with the reported selection coefficient (*S*) determined from the slope of the linear regression of ln[*p(t)/q(t*)], where *p(t)* and *q(t*) represent the relative frequencies of the strains from at a given time point. At least five different time points within 24 hours of competitions were used for estimating the selection coefficient.

Figure S1. Formate, lactate, and succinate are more closely correlated to each other than to acetate in the exometabolome of evolving cultures. (Ellipses represent 95% confidence intervals.)

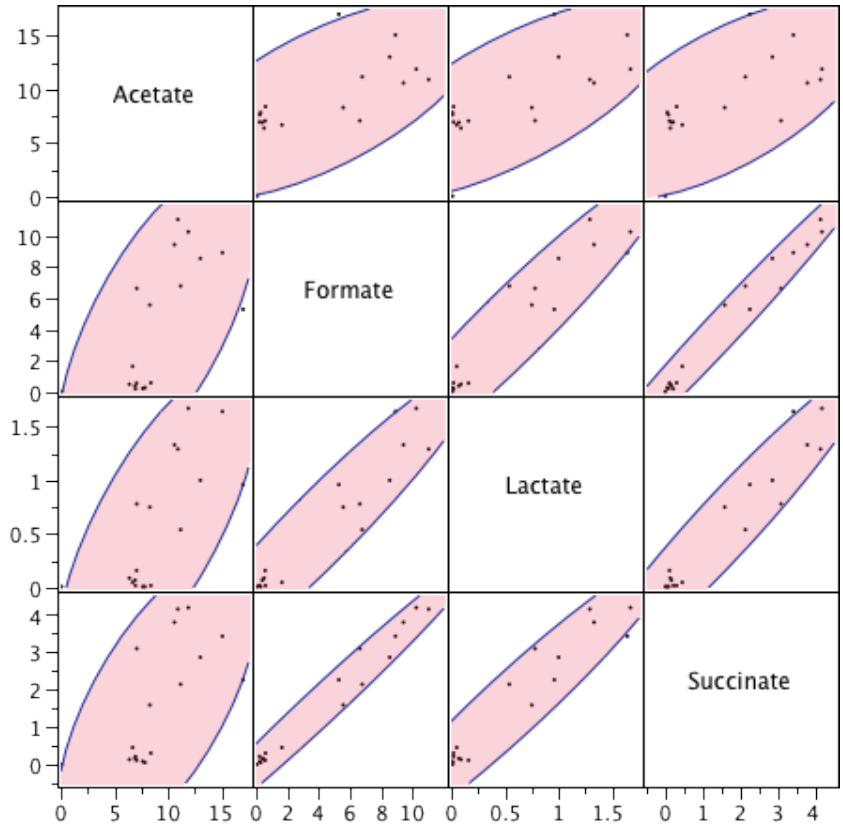

Figure S2. Glucose uptake and fermentation product production (both normalised by carbon atoms in the molecules) over time for chemostat 1.

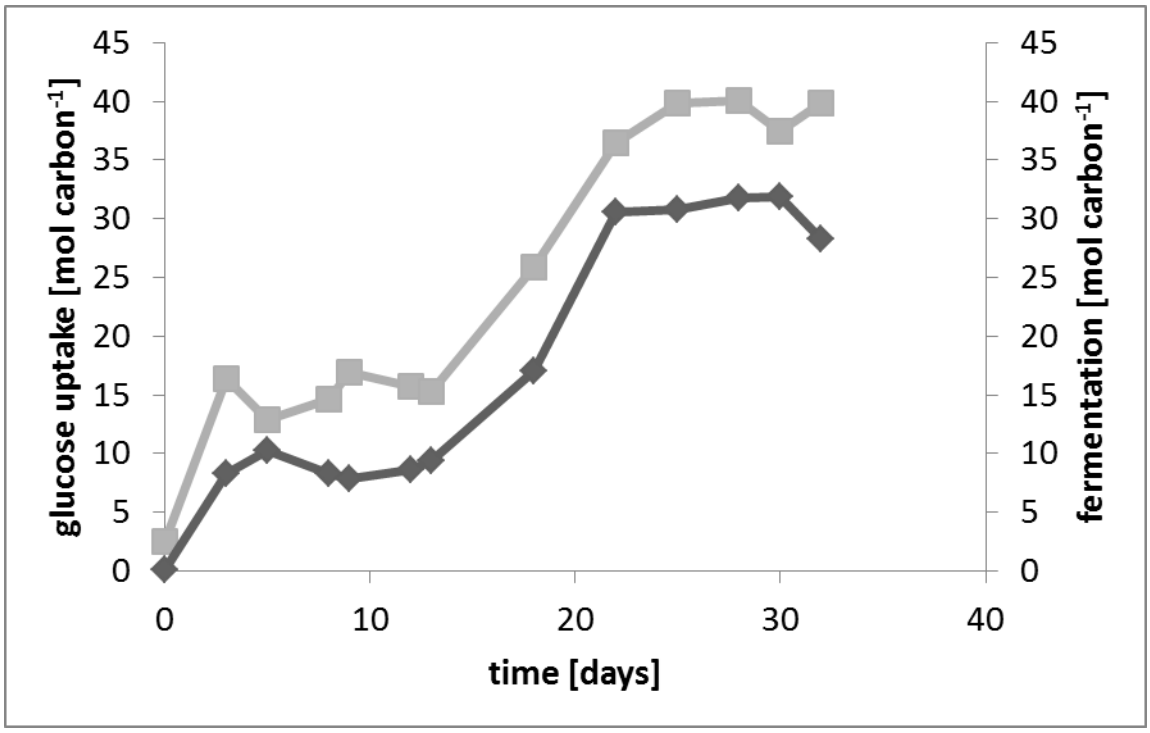

Table S1. Optical densities (OD_{600}) of the chemostats.

Time (day)	1	2
1	0.318	0.298
2	0.229	0.219
3	0.157	0.157
4	0.163	0.163
5	0.147	0.147
7	0.145	0.145
8	0.141	0.141
9	0.147	0.139
10	0.149	0.149
12	0.138	0.138
13	0.103	0.143
14	0.125	0.125
16	0.129	0.129
17	#N/A	0.131
18	#N/A	0.111
19	0.121	#N/A
22	0.099	#N/A
23	0.124	0.124
24	0.131	0.131
25	#N/A	0.146
26	0.141	0.131
27	#N/A	0.139
30	0.126	#N/A
32	#N/A	0.129

Table S2. Concentrations of glucose and fermentation products.

Replicate	Day	glucose	acetate	ethanol	formate	lactate	propionate	succinate
		mM	mM	mM	mM	mM	mM	mM
1	0	10.68	0.03	0.01	0.06	0.01	0.00	0.00
1	3	8.38	3.83	0.01	0.12	0.01	0.01	0.11
1	5	8.96	4.62	0.01	0.33	0.01	0.01	0.16
1	8	8.67	3.81	0.01	0.22	0.04	0.01	0.09
1	9	8.28	3.52	0.02	0.28	0.05	0.01	0.07
1	12	8.48	3.89	0.01	0.32	0.09	0.01	0.06
1	13	8.56	3.69	0.02	0.91	0.03	0.01	0.25
1	18	6.79	4.57	0.03	3.07	0.41	0.01	0.87
1	22	5.02	6.57	0.02	5.40	0.92	0.01	2.29
1	25	4.47	6.55	0.01	5.64	0.92	0.01	2.30
1	28	4.42	8.30	0.02	4.91	0.90	0.01	1.88
1	30	4.86	8.03	0.02	5.56	0.80	0.02	1.94
1	32	4.46	9.36	0.02	2.92	0.53	0.02	1.24
2	0	11.46	0.04	0.01	0.00	0.01	0.00	0.00
2	2	7.66						
2	6	8.55	4.21	0.02	0.13	0.01	0.00	0.04
2	8	8.88						
2	10	8.85	4.32	0.01	0.16	0.00	0.01	0.03
2	13	8.8						
2	19	5.76	3.90	0.01	3.65	0.43	0.01	1.70
2	23	5.03	5.09	0.03	4.98	0.55	0.01	1.88
2	26	4.97	6.01	0.01	6.08	0.71	0.01	2.27
2	29	6.46	6.15	0.01	3.73	0.30	0.01	1.17
2	30	6.26	6.32	0.02	3.85	0.30	0.01	1.20

Table S3. List of strains used in this study

Strain	Isolate/Relevant genotype	References
MC4100	F^- *araD139* Δ(*argF-lac*)*U169 rspL150 deoCl relA1 thiA ptsF25 flb5301 rbsR*	
BW2952	MC4100 *malG*::λ*plac*Mu55 ϕ(*malG*::*lacZ*)	Ferenci et al. (2009)
BW3788	BW2952 *sucC*::Tn10	This study
BW5318	BW2952 *rpoS3767*	Maharjan et al. (2013)
BW3789	BW5318 *sucC:Tn10*	This study
BW6041	A chemostat evolved isolate - isolated from a 19th day glycerol stock sample	This study
BW6042	BW6041 kanR::Tn10	This study
BW6043	A chemostat evolved isolate - isolated from a 19th day glycerol stock sample	This study
BW6044	BW6043 kanR::Tn10	This study
DY330	W3110 Δ*lacU169 gal490* λ*cl857* Δ(*cro-bioA*)	Yu et al. (2000)

Table S4. List of mutations identified by whole genome re-sequencing in two evolved isolates

Isolate	Region	Gene[a]	Product	Genome position[b]	Nucleotide		Codon		Amino acid	
					Reference	change to	Reference	change to	Reference	change to
BW6041	Noncoding	(7) isrC	Small RNA with unkonwn function	1961815	G	T	NA	NA	NA	NA
	Coding	rpoS	RNA polymerase, sigma S (sigma 38) factor	2751004	T	C	GAT	GAC	I	V
	Coding	rpoS	RNA polymerase, sigma S (sigma 38) factor	2751045	A	C	CAG	CCG	L	R
BW6043	Noncoding	(142) sucC	Intergene between sucB and sucC	664852	A	T	NA	NA	NA	NA
	Noncoding	(141) sucC	Intergene between sucB and sucC	664853	A	C	NA	NA	NA	NA
	Noncoding	(7b) isrC	small RNA with unknown function	1961815	G	T	NA	NA	NA	NA
	Coding	rpoS	RNA polymerase, sigma S (sigma 38) factor	2751004	T	C	GAT	GAC	I	V
	Coding	rpoS	RNA polymerase, sigma S (sigma 38) factor	2751045	A	C	CAG	CCG	L	R
	Noncoding	(164)BWG_3698	Aminoglycoside phosphotransferase	4139083	C	T	NA	NA	NA	NA

[a] Numbers in parentheses indicate the position of mutation relative to genes.

[b] Position of mutations in the genome is based on the genome sequence of the ancestral strain BW2952. Bold entries are shared mutations between these two strains.

NA stands for non-applicable

References for supplementary material

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