SUPPLEMENTAL MATERIALS AND METHODS

Preparation of Xylulose-5-phosphate

Xylulose-5-phosphate (Xyl5P) was prepared by the ATP-dependent conversion of D-xylulose to Xyl5P catalyzed by xylulose kinase from *E. coli* (EcXK). EcXK was expressed in *E. coli* BL21a and isolated as reported recently (6). The purified enzyme fractions displayed a specific activity of 20 U/mg determined at pH 7.5 under conditions described elsewhere (6). D-Xylulose was prepared at a purity of 90% by applying a protocol described previously (15). Preparation of Xyl5P was carried out in 1.5 mL Eppendorf tubes (working volume was 1 mL). Equimolar amounts of ATP and xylulose (107 mM of each) were applied in 100 mM HEPES/KOH buffer pH 7.5 containing 150 mM NaCl. Xyl5P production was initiated by the addition of EcXK (final concentration 50 U/mL) and reaction mixtures were incubated at ambient temperature for 15 h. EcXK was separated by ultrafiltration (Vivaspin 4 concentrator, cut off of 10 kDa, Sartorius Stedim Biotech, S. A., Aubagne, France). Concentrations of ADP and ATP were determined from the reaction solution by LC-MS/MS which contained 48 ± 5 mM ATP and 56 ± 6 mM ADP (= [Xyl5P]).

SUPPLEMENTAL TABLES

Table S1. Collection of strains used in this study

Strain	Genotype	Ref.
BP000	CEN.PK 113-5D ura3::(GPDp-XKSI-CYCIt, GPDp-*Ct*XR-Wt-CYCIt, GPDp-*Gm*XDH-CYCIt)	(19)
BP10001	CEN.PK 113-5D ura3::(GPDp-XKSI-CYCIt, GPDp-*Ct*XR-Dm-CYCIt, GPDp-*Gm*XDH-CYCIt)	(19)
CEN.PK 113-7D	*MAT*a *URA*3 *HIS*3 *LEU*2 *TRP*1 *MAL*2-8^{c} *SUC*2	(26)

Table S2. Collection of chemicals used for metabolite identification by LC-MS/MS along with their corresponding masses of mother and daughter ions.[a]

Compound	mother ion / daughter ion
Glucose-6-phosphate	259 / 97 (265 / 97)[b]
Fructose-6-phosphate	259 / 97 (265 / 97)
6-Phosphogluconate	275 / 79 (281 / 79)
Ribulose-5-phosphate	229 / 97 (234 / 97)
Ribose-5-phosphate	229 / 97 (234 / 97)
Xylulose-5-phosphate	229 / 97 (234 / 97)
Fructose-1,6-bisphosphate	339 / 97 (345 /97)
Glyceraldehyde-3-phosphate	169 / 97 (172 / 97)
3-Phosphoglycerate	185 / 79 (188 / 79)
Dihydroxyacetone phosphate	169 / 97 (172 / 97)
Glycerol-3-phosphate	171 / 79 (174 / 79)
Phosphoenolpyruvate	167 / 79 (170 / 79)
NADH	664.3 / 79 (685 / 79)
NAD^+	662.3 / 540 (683 / 555)
NADPH	744 / 79 (765 / 79)
$NADP^+$	742 / 620 (763 / 635)
ATP	506 / 159 (515.9 / 159)
ADP	426 / 79(436 / 79)
AMP	346 / 79 (356 / 79)
GTP	522 / 79 (531.9 / 79)
GDP	442 / 79 (452 / 79)
GMP	362 / 79(372 / 79)
Glutamate	146 / 102 (151 / 106)
Glutamine	145 / 127 (150 / 132)
Aspartate	132 / 115 (136 / 119)
Arginine	173 / 131 (179 / 136)
Tyrosine	180 / 163 (189 / 172)
Tryptophane	203 / 116 (214 / 124)
Serine	104 / 74 (107 / 76)
Methionine	148 / 47 (153 / 48)

[a]A mixture containing all compounds listed but Xyl5P was applied (see text). Xyl5P was prepared enzymatically as described in Supplemental Materials and Methods.[b] Values in parentheses indicate masses of mother and daughter ions from ^{13}C-labeled counterparts.

Table S3. Summary of metabolite pools obtained for CEN.PK 113-7D, BP000 and BP10001. [a]

Metabolite	BP10001 on xylose	BP000 on xylose	CEN.PK 113-7D on xylose	CEN.PK 113-7D on glucose
Central carbon metabolism				
Xyl5P	0.5 ± 0.1	0.3 ± 0.1	0.03 ± 0.05	0.7 ± 0.1
Ru5P	0.39 ± 0.03	0.3 ± 0.1	n.d. [b]	0.3 ± 0.1
Rib5P	0.21 ± 0.03	0.18 ± 0.03	0.051 ± 0.005	0.39 ± 0.05
S7P[c]	31 ± 5	23 ± 6	0.08 ± 0.03	26 ± 5
6PG	0.014 ± 0.004	0.013 ± 0.001	0.007 ± 0.003	1.5 ± 0.2
Glc6P	0.31 ± 0.02	0.24 ± 0.02	0.039 ± 0.005	13 ± 1
Fru6P	0.07 ± 0.01	0.07 ± 0.02	0.031 ± 0.004	1.1 ± 0.1
Fru(1,6)P_2	0.30 ± 0.03	0.29 ± 0.03	n.d. [b]	35 ± 6
Ga3P	0.17 ± 0.01	0.15 ± 0.02	n.d. [b]	0.54 ± 0.02
DHAP	0.4 ± 0.1	0.4 ± 0.1	n.d. [b]	5.5 ± 0.5
G3P	0.26 ± 0.05	0.23 ± 0.03	0.08 ± 0.01	1.4 ± 0.1
2PG + 3PG	0.50 ± 0.05	0.50 ± 0.03	0.3 ± 0.1	6.1 ± 0.3
2PG[d]	0.04	0.04	0.026	0.52
3PG[d]	0.46	0.46	0.274	5.58
PEP	0.28 ± 0.01	0.28 ± 0.02	0.09 ± 0.01	0.7 ± 0.1
Redox metabolism				
NADH	0.7 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.7 ± 0.2
NAD^+	1.8 ± 0.2	1.2 ± 0.1	0.8 ± 0.1	2.4 ± 0.6
NADPH	0.07 ± 0.02	0.05 ± 0.02	0.014 ± 0.005	0.09 ± 0.01
$NADP^+$	0.08 ± 0.01	0.069 ± 0.001	0.014 ± 0.002	0.25 ± 0.10
Energy metabolism				
ATP	9.5 ± 1.0	7.3 ± 0.3	0.20 ± 0.01	13 ± 1
ADP	4.3 ± 0.4	4.0 ± 0.3	0.56 ± 0.1	4.7 ± 0.7
AMP	2.2 ± 0.1	2.3 ± 0.1	3.9 ± 0.3	1.1 ± 0.2
AXP pool	16 ± 1.5	14 ± 1	4.8 ± 0.4	19 ± 2
Energy charge	0.73	0.68	0.11	0.82
GTP	1.7 ± 0.1	1.7 ± 0.1	n.d. [b]	3.0 ± 0.2
GDP	0.8 ± 0.1	0.9 ± 0.1	0.20 ± 0.04	0.9 ± 0.1
GMP	0.58 ± 0.04	0.8 ± 0.1	1.5 ± 0.1	0.12 ± 0.03
GXP pool	3.0 ± 0.2	3.4 ± 0.3	1.7 ± 0.1	4.0 ± 0.3
Amino acids				
Glu	96 ± 4	78 ± 8	127 ± 8	75 ± 2
Gln	41 ± 3	26 ± 2	8 ± 1	35 ± 3
Asp	4.9 ± 0.3	3.0 ± 0.2	4.6 ± 0.3	10.3 ± 0.4
Arg	71 ± 5	43 ± 7	53 ± 3	104 ± 4
Tyr	8.4 ± 0.5	5.9 ± 0.3	1.2 ± 0.2	1.2 ± 0.2
Trp	1.20 ± 0.04	1.2 ± 0.1	0.5 ± 0.1	0.20 ± 0.05
Ser	2.3 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	4.4 ± 0.4
Met	0.11 ± 0.02	0.21 ± 0.02	0.56 ± 0.01	0.16 ± 0.02

[a] Values are shown in µmol/gCDW and represent averages of six biological samples obtained from two individual fermentations. [b] n.d., not detectable; [c] Values relate to a MS signal corrected by corresponding internal standard signal per gCDW. [d] Concentrations were calculated assuming the interconversion of 2PG and 3PG catalyzed by GPM to be at equilibrium (9).

Table S4. Summary of thermodynamic analysis.

			CEN.PK 113-7D on glucose	CEN.PK 113-7D on xylose	BP10001 on xylose	BP000 on xylose
Enzyme	K_{eq}	Ref.	$\Delta\Delta G$ [a]	$\Delta\Delta G$ [a]	$\Delta\Delta G$ [a]	$\Delta\Delta G$ [a]
PGI	0.31	(25)	-3.3 ± 0.4	2.4 ± 0.7	-0.8 ± 0.5	-0.3 ± 1
PFK	800	(10)	-11 ± 1	n.d. [b]	-15 ± 1	-15 ± 1
FBA	0.099 mM [c]	(27)	-2.6 ± 1	n.d. [b]	-0.2 ± 1	-0.4 ± 1
GA3PDH-PGK	1.83 mM^{-1} [c]	(5)	-0.1 ± 2	n.d. [b]	-0.2 ± 1.7	-0.2 ± 1.6
GPM	0.19	(9)	-0.02 ± 0.3	-0.02 ± 1.8	-0.02 ± 0.5	-0.02 ± 0.3
ENO	4.5	(31)	-4.6 ± 0.5	-2.4 ± 1.1	-0.6 ± 0.3	-0.6 ± 0.3
PYK [d]	6500	(2)	-16 ± 3	-17 ± 4	-15 ± 2	-15 ± 2
TPI	22	(27)	-1.9 ± 0.3	n.d. [b]	-5.6 ± 0.7	-5 ± 1
G3PDH	4300	(2)	-21 ± 2	n.d. [b]	-20 ± 2	-21 ± 2
G6PDH-lactonase	1×10^5	(3)	-37 ± 1	-33 ± 3	-37 ± 2	-37 ± 1
GND	77 mM [c]	(28)	-9 ± 2	n.d. [b]	-1 ± 2	-2 ± 2
RKI	1.2	(3)	0.3 ± 1.2	n.d. [b]	-2.0 ± 0.6	-1.6 ± 1.3
RPE	1.8	(3)	0.8 ± 1.2	n.d. [b]	-0.9 ± 0.7	-1.5 ± 2
TKL-TAL	22.9	(3)	-4.7 ± 1.6	n.d. [b]	-18 ± 2	-16 ± 4
ADK1	0.8	(1)	-0.5 ± 1.4	2.6 ± 2.5	0.9 ± 0.9	0.7 ± 0.6

[a] $\Delta\Delta G$ values refer to the difference of ΔG_{Keq} - ΔG_{MAR}, where ΔG, K_{eq} and MAR denote Gibbs free energy, equilibrium constant and mass action ratio, respectively. Values are shown in kJ/mol. Reactant concentrations were taken from Supplemental Table S3. [b] n.d., not determined; [c] A value of 2.38 mL per gCDW was used to calculate molar concentrations (7). [d] Pyruvate concentrations of 0.6 mM and 2 mM were used as lower and upper bounds for calculation of ΔG_{MAR} (24, 29).

Table S5. Summary of results obtained from metabolic control analysis performed for enzymes constituting the xylose pathway and from analysis of coenzyme usage of XR in BP000 and BP10001[a]

	BP10001	BP000	Ref.
XR[b]			
K_{NADH} [μM]	41	38	(18)
$K_{i,NADH}$ [μM]	30	19	(18)
K_{NADPH} [μM]	128	3	(18)
$K_{i,NADPH}$ [μM]	64	1	(18)
K_{xylose}^{NADH} [mM]	106	142	(18)
K_{xylose}^{NADPH} [mM]	722	96	(18)
V_{max}^{NADH} [s^{-1}]	12	11	(18)
V_{max}^{NADPH} [s^{-1}]	30	13	(18)
v_{NADH} [s^{-1}]	5.7 ± 0.1	2.7 ± 0.1	
v_{NADPH} [s^{-1}]	0.19 ± 0.03	3.4 ± 0.1	
$v_{NADH} + v_{NADPH}$ [s^{-1}]	5.9	6.1	
v_{NADH}/v_{NADPH}	30 ± 5 (1.2)[c] *≥ 1.3*[d]	0.8 ± 0.2 (0.03)[c] *≥ 0.96*[d]	
ε_{NADH}[e]	0.11	-0.06	
ε_{NADPH}[e]	-0.01	0.11	
ε_{xylose}[e]	0.45	0.44	
XDH			
K_{NAD+} [μM]	140	140	(16)
$K_{i,NAD+}$ [μM]	780	780	(16)
$K_{xylitol}$ [mM]	12	12	(16)
v [%][f]			
xylitol 50 mM	60 ± 2	53 ± 2	
xylitol 150 mM	74 ± 1	67 ± 2	
$\varepsilon_{xylitol}$ / ε_{NAD+}[e]			
xylitol 50 mM	0.29 / 0.26	0.32 / 0.34	
xylitol 150 mM	0.12 / 0.20	0.14 / 0.27	
XK[g]			
K_{ATP} [mM]	1.55	1.55	(21)
v [%][f]	72 ± 2	66 ± 1	

Table S5 (continued). Summary of results obtained from metabolic control analysis performed for enzymes constituting the xylose pathway and from analysis of coenzyme usage of XR in BP000 and BP10001[a]

[a]Reactant concentrations (µmol/gCDW) from Supplemental Table S3 were transformed into mmol/L by assuming a cell volume of 2.38 mL/gCDW (7). [b]A rate equation accounting for the simultaneous utilization of NADH and NADPH was applied to XR catalyzed reduction of xylose (20). An intracellular concentration for xylose of 133 mM (= mM xylose in the medium (8)) was assumed. [c]Values in parentheses were obtained by using 508 µM NADPH (17). [d]Values depict lower bounds of v_{NADH}/v_{NADPH} obtained from FB analysis using a genome-scale network model (14). Note that the genome-scale network was always compatible with a 100% usage of NADH by XR while a 100% usage of NADPH was not. [e]Elasticity coefficients ε were obtained from the relationship $[S]\delta v/(v\delta[S])$, in which [S] and v corresponded to the concentration of a reactant and the respective steady-state rate constant, respectively (23). Differences in v (δv) were calculated by presuming an increase of reactant concentration by 1% ($\delta[S] = 0.01[S]$). [f]Values of steady-state rates represent the degree of rate saturation relative to the maximal turnover number (=100%). [g]Estimates for v were obtained from the Michaelis Menten rate equation.

Table S6. Summary of metabolic control analysis of reactions catalyzed by PFK, PYK, TKL and TAL[a]

	glucose-growing	xylose-metabolizing		
	CEN.PK 113-7D	BP10001	BP000	Ref.
PFK[b]				(10)
v [%]	63 ± 3	2.7 ± 0.6	3.2 ± 1.6	
K_{ATP} [mM]	0.28 ± 0.04	0.26 ± 0.02	0.24 ± 0.01	
K_{Fru6P} [mM]	0.24 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	
L	0.003 ± 0.002	4.2 ± 0.6	4.0 ± 1.4	
ε_{Fru6P}^{c}	0.36 ± 0.04	1.8 ± 0.1	1.8 ± 0.1	
ε_{ATP}^{c}	-0.11 ± 0.04	-0.41 ± 0.01	-0.31 ± 0.02	
ε_{ADP}^{c}	-0.01 ± 0.01	-0.04 ± 0.01	-0.064 ± 0.002	
ε_{AMP}^{c}	0.13 ± 0.04	0.46 ± 0.01	0.39 ± 0.03	
PYK[d]				
K_{PEP} = 0.09 mM; K_{ADP} = 0.18 mM				(32)
v [%]	70 ± 3	52 ± 1	51 ± 2	(13)
ε_{PEP}^{c}	0.24 ± 0.06	0.43 ± 0.02	0.43 ± 0.0.2	
ε_{ADP}^{c}	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.01	
TKL				
K_{Xyl5P} = 0.07 mM; K_{Rib5P} = 0.15 mM				(30)
v [%]	42 ± 4	28 ± 4	21 ± 5	(4)
ε_{Xyl5P}^{c}	0.19 ± 0.02	0.25 ± 0.04	0.4 ± 0.1	
ε_{Rib5P}^{c}	0.48 ± 0.03	0.63 ± 0.03	0.66 ± 0.04	
K_{Ga3P} = 4.9 mM; K_{Fru6P} = 1.8 mM				(22)
v [%]	0.9 ± 0.1	0.02 ± 0.01	0.02 ± 0.01	(4)
ε_{Ga3P}^{c}	0.96 ± 0.01	0.99 ± 0.01	0.99 ± 0.01	
ε_{Fru6P}^{c}	0.79 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	
TAL[e, f]				
K_{Fru6P} = 0.32 mM				(11)
v [%]	59 ± 2	8 ± 1	8 ± 2	
K_{Ga3P} = 0.22 mM				(12)
v [%]	71 ± 1	44 ± 1	41 ± 1	

Table S6 (continued). Summary of metabolic control analysis for reactions catalyzed by PFK, PYK, TKL and TAL[a]

[a]Reactant concentrations (µmol/gCDW) from Supplemental Table S3 were transformed into mmol/L by assuming a cell volume of 2.38 mL/gCDW (7). Values of steady-state rates represent the degree of rate saturation relative to the maximal turnover number (=100%). [b]Kinetic parameters were calculated from the allosteric kinetic model reported for PFK (10). [c]Elasticity coefficients ε were obtained from the relationship $[S]\delta v / (v\delta[S])$. [S] and v corresponded to the concentration of a reactant and the respective steady-state rate constant, respectively (23). Differences in v (δv) were calculated by presuming an increase of reactant concentration by 1% ($\delta[S] = 0.01[S]$). [d]Values representing high affinity Michaelis constants for PEP and ADP were applied as $Fru(1,6)P_2$ pools were present at saturating concentrations (K_a = 0.014 mM (32)) in both glucose-growing and xylose-metabolizing cells. [e]Estimates for v were obtained from the Michaelis Menten rate equation. [f]Data predicted that the preferred net reaction of TAL-TKL in xylose-metabolizing cells was 2Xyl5P + Rib5P → 2Fru6P + Ga3P

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Pathways for utilization of L-arabinose and D-xylose. Solid lines indicate oxidoreductive pathways typical of yeast and fungi while dashed lines indicate pathways typical of bacteria. The following abbreviations were used: *XylA* – xylose isomerase, *XYL1* - aldose reductase, *XYL2* – xylitol dehydrogenase, *XYL3* – xylulose kinase, *lad1* – L-arabitol 4-dehydrogenase, *lxr1* – L-xylulose reductase, *araA* – arabinose isomerase, *araB* – L-ribulokinase, *araD* – L-ribulose-5-phosphate 4-epimerase

Figure S2. Elution profiles of pentose 5-P isomers (A) and respective ^{13}C-compounds (B). Black and grey solid lines indicate elution profiles obtained for metabolite extracts of CEN.PK 113-7D grown on glucose and BP10001 metabolizing xylose, respectively. Elution profiles of metabolite extracts of CEN.PK 113-7D and BP000 cultivated on xylose are shown as grey and black dashed lines, respectively. Dotted lines indicate the retention times.

Figure S3. Heat maps representing changes of intracellular metabolite levels of xylose-metabolizing cells (BP000 and BP10001) and xylose-resting (CEN.PK 113-7D) cells relative to glucose-growing cells. Fold-change of metabolite pools is displayed in shades of red (decrease) and blue (increase). PPP, PP pathway; UG, upper glycolysis; LG, lower glycolysis; GM, glycerol metabolism; EM, energy metabolites; RM, redox metabolites; AA, amino acids; xPG, sum of 2PG and 3PG

SUPPLEMENTAL FIGURES

Figure S1

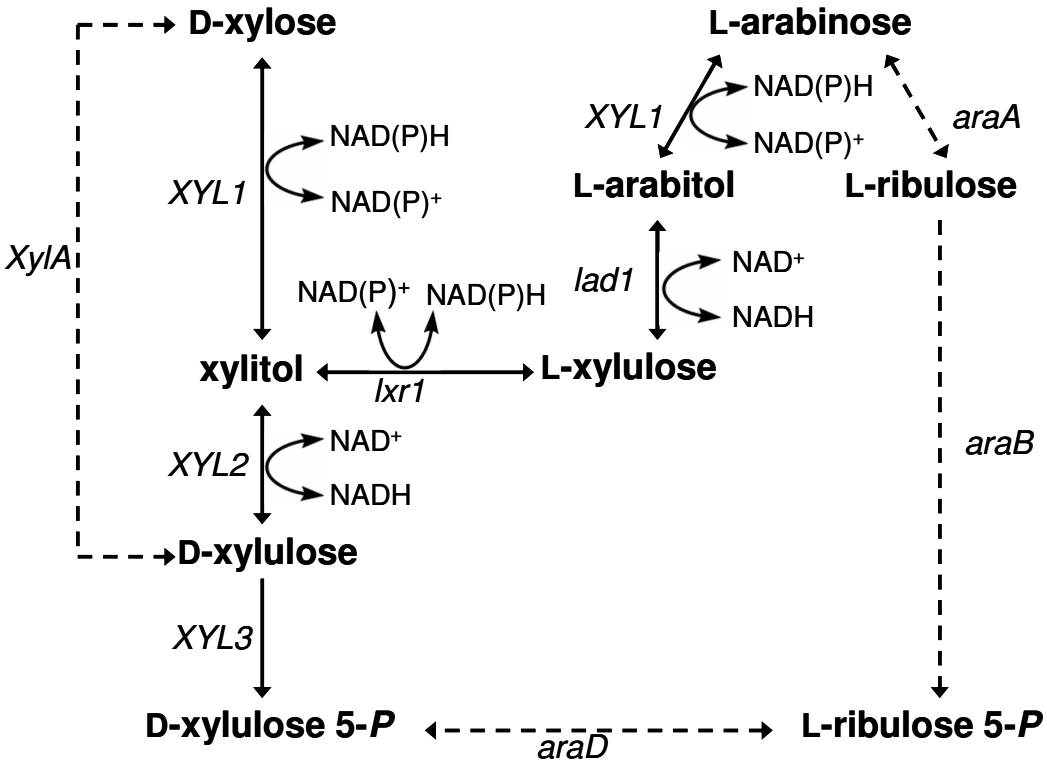
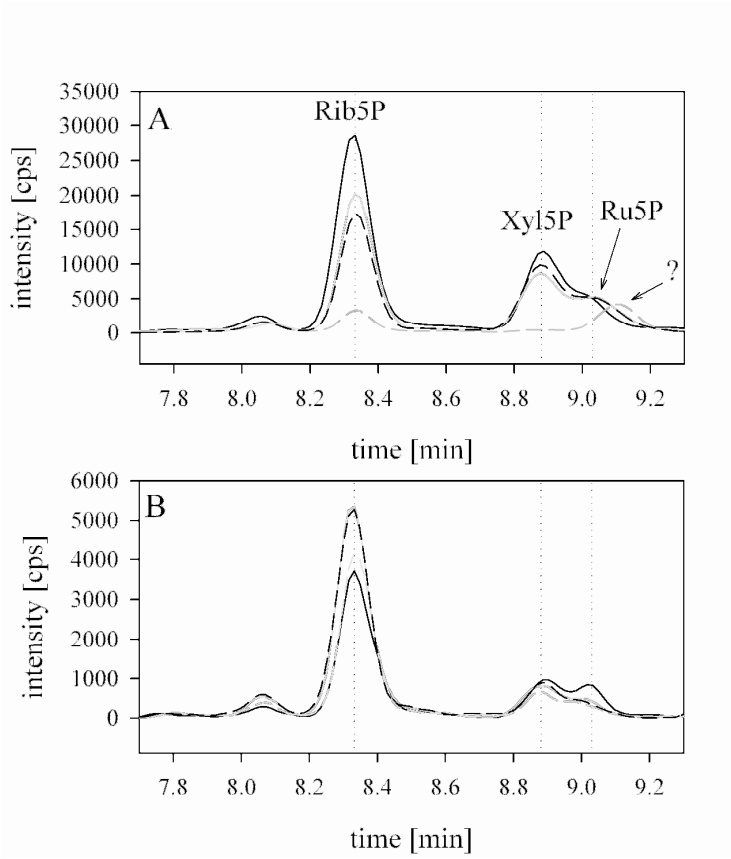


Figure S2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Ru5P
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8.2
8.4
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4000
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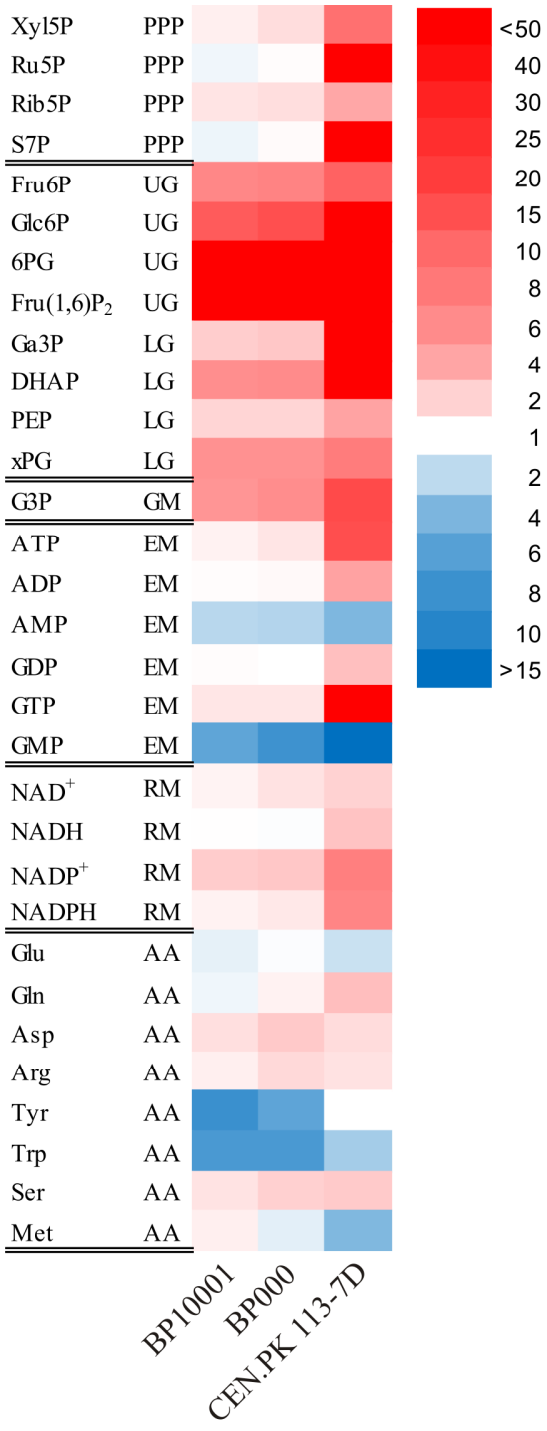
9.0
9.2
time [min]

Figure S3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Ru5P PPP
Rib5P PPP
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Ga3P LG
DHAP LG
PEP LG
xPG LG
G3P GM
ATP EM
ADP EM
AMP EM
GDP EM
GTP EM
GMP EM
NAD+ RM
NADH RM
NADP+ RM
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Asp AA
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