Biomarkers allow detection of nutrient limitations and respective supplementation for elimination in *Pichia pastoris* fed-batch cultures

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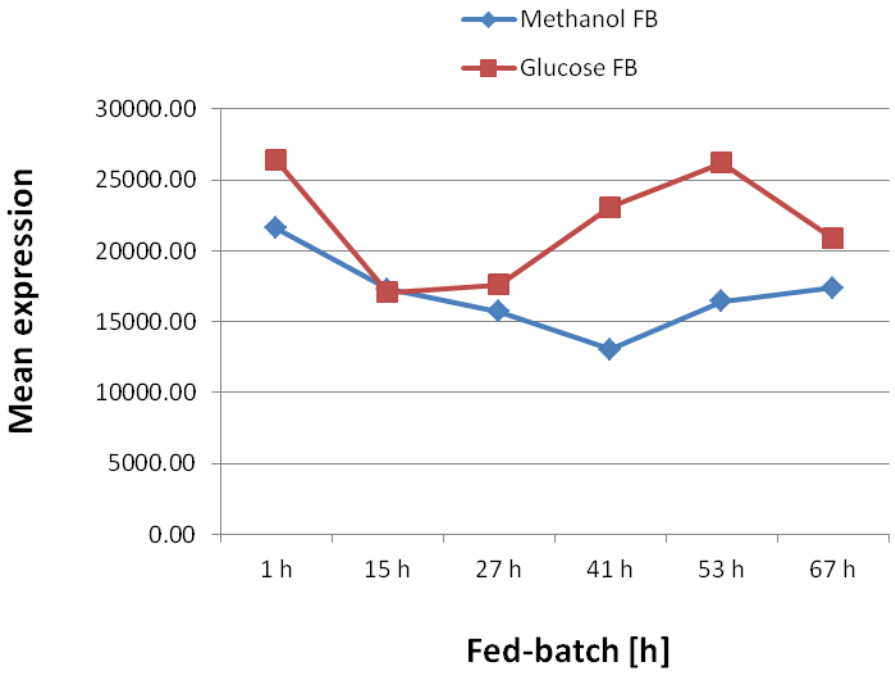
Additional Figures and Tables



Additional Figure S1: Mean expression values (microarray data) of the carboxypeptidase B during the methanol and glucose fed-batch process

Additional Table S2: Description of biological function of marker genes adapted from *Saccharomyces* Genome Database (SGD)

ORF name	Description
PHO84	High-affinity inorganic phosphate (Pi) transporter; also low-affinity manganese transporter; regulated by Pho4p and Spt7p
PHO89	Plasma membrane Na+/Pi cotransporter; active in early growth phase; transcription regulated by inorganic phosphate concentrations and Pho4p
VTC1	Regulatory subunit of the vacuolar transporter chaperone (VTC) complex; VTC complex is involved in membrane trafficking, vacuolar polyphosphate accumulation, microautophagy and non-autophagic vacuolar fusion
PHO80	Cyclin; interacts with cyclin-dependent kinase Pho85p; regulates the response to nutrient levels and environmental conditions, including the response to phosphate limitation and stress-dependent calcium signaling
PHO81	Cyclin-dependent kinase (CDK) inhibitor; regulates Pho80p-Pho85p and Pcl7p-Pho85p cyclin-CDK complexes in response to phosphate levels
GDE1	Glycerophosphocholine (GroPCho) phosphodiesterase; hydrolyzes GroPCho to choline and glycerolphosphate, for use as a phosphate source and as a precursor for phosphocholine synthesis
PHO5	Repressible acid phosphatase; 1 of 3 repressible acid phosphatases that also mediates extracellular nucleotide-derived phosphate hydrolysis; induced by phosphate starvation and coordinately regulated by PHO4 and PHO2
DAL4	Allantoin permease; expression sensitive to nitrogen catabolite repression and induced by allophanate, an intermediate in allantoin degradation
DAL5-2	Allantoate permease; ureidosuccinate permease; also transports dipeptides, though with lower affinity than for allantoate and ureidosuccinate; expression is constitutive but sensitive to nitrogen catabolite repression
DUR3-1	Plasma membrane transporter for both urea and polyamines; expression is highly sensitive to nitrogen catabolite repression and induced by allophanate, the last intermediate of the allantoin degradative pathway
DUR3-2	Plasma membrane transporter for both urea and polyamines, expression is highly sensitive to nitrogen catabolite repression and induced by allophanate, the last

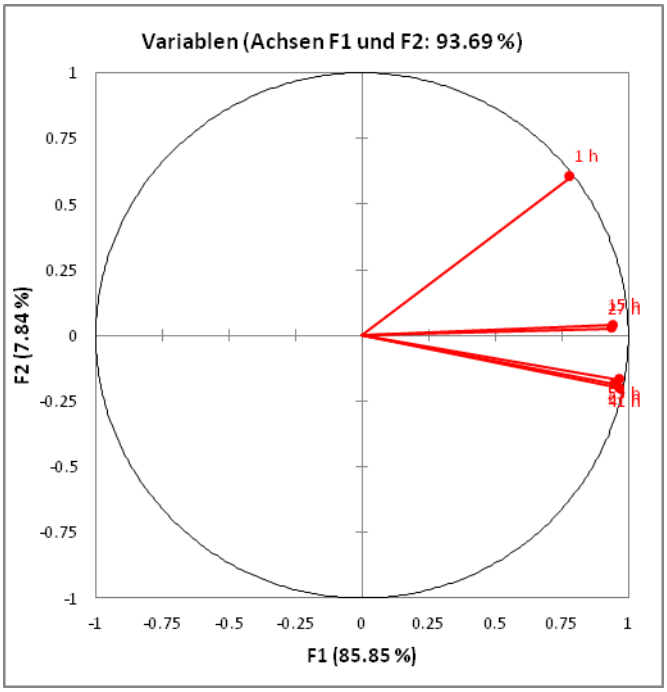
	intermediate of the allantoin degradative pathway
TPO2	Polyamine transporter of the major facilitator superfamily; specific for spermine; localizes to the plasma membrane
PUT1	Proline oxidase; nuclear-encoded mitochondrial protein involved in utilization of proline as sole nitrogen source; PUT1 transcription is induced by Put3p in the presence of proline and the absence of a preferred nitrogen source
CAR1-1	Arginase, catabolizes arginine to ornithine and urea; expression responds to both induction by arginine and nitrogen catabolite repression
GLT1	NAD(+)-dependent glutamate synthase (GOGAT); synthesizes glutamate from glutamine and alpha-ketoglutarate; with Gln1p, forms the secondary pathway for glutamate biosynthesis from ammonia; expression regulated by nitrogen source; assembles into filaments as cells approach stationary phase and under cytosolic acidification and starvation conditions
DAL1	Allantoinase; converts allantoin to allantoate in the first step of allantoin degradation; expression sensitive to nitrogen catabolite repression
SEO1-1	Putative permease, member of the allantoate transporter subfamily of the major facilitator superfamily
GAT1	Transcriptional activator of nitrogen catabolite repression genes; contains a GATA-1-type zinc finger DNA-binding motif; activity and localization regulated by nitrogen limitation and Ure2p
YBR139W	Putative serine type carboxypeptidase; role in phytochelatin synthesis; green fluorescent protein (GFP)-fusion protein localizes to the vacuole; expression induced by nitrogen limitation in a GLN3, GAT1-independent manner
JLP1-1	Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase; involved in sulfonate catabolism for use as a sulfur source; contains sequence that resembles a J domain (typified by the E. coli DnaJ protein); induced by sulphur starvation
MET32	Zinc-finger DNA-binding transcription factor; involved in transcriptional regulation of the methionine biosynthetic genes; targets strong transcriptional activator Met4p to promoters of sulfur metabolic genes; feedforward loop exists in the regulation of genes controlled by Met4p and Met32p
MET4	Leucine-zipper transcriptional activator; responsible for regulation of sulfur amino acid pathway; requires different combinations of auxiliary factors Cbf1p,

	Met28p, Met31p and Met32p
SUL1	High affinity sulfate permease of the SulP anion transporter family; sulfate uptake is mediated by specific sulfate transporters Sul1p and Sul2p, which control the concentration of endogenous activated sulfate intermediates
MUP1-1	High affinity methionine permease; integral membrane protein with 13 putative membrane-spanning regions; also involved in cysteine uptake
MUP3	Low affinity methionine permease; similar to Mup1p
YCT1	High-affinity cysteine-specific transporter; green fluorescent protein (GFP)-fusion protein localizes to the endoplasmic reticulum

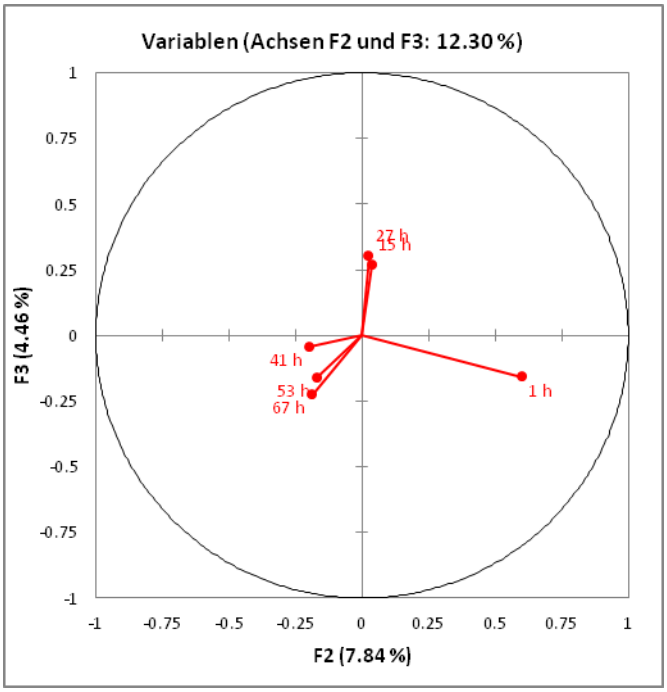
Additional Table S3: qPCR primer sequences

Primer name	Primer sequence
ACT1_Fwd	CCTGAGGCTTTGTTCCACCCATCT
ACT1_Rev	GGAACATAGTAGTACCACCGGACATAACGA
JLP1-1_Fwd	AGCATGGGACAACAGAAG
JLP1-1_Rev	TCAGCAAGAGGGGTAATTCG
MET32_Fwd	TTCTCCGACATCCTCCAT
MET32_Rev	AAGCCCTTCTGACATCTG
SUL1_Fwd	GATTTTACCTCCGCCAACTG
SUL1_Rev	CTGTCTCTCCTCTTCTTGTGT
MUP1-1 Fwd	GTCATCAAGTCACCAATCGT
MUP1-1 Rev	AGGCAGCAACACAGTGAA
PHO84-1_Fwd	AACCTCCCCAATACCACAAC
PHO84-1_Rev	GGCTCCCACTTTACCAGAA
VTC1_Fwd	AATTTCCGCTGGTCTGT
VTC1_Rev	GGCCCAAAACGGTCAT
PHO81_Fwd	AGCTCTCGTCAAACTATTCAG
PHO81_Rev	CAAACACCACACCACTCA
PHO5_Fwd	TGCTTACCGAGAGATTATTGTG
PHO5_Rev	AGGGCCAGAGGAACAGTCA
DUR3-1 _Fwd	CAGCATCCTCCTTCCTTATAC
DUR3-1_Rev	AAGCAGCAACACTAAACCAA
TPO2_Fwd	CGGTCTTGCCTGTGGTATT
TPO2_Rev	ACCGAAGCTCCCCATATC

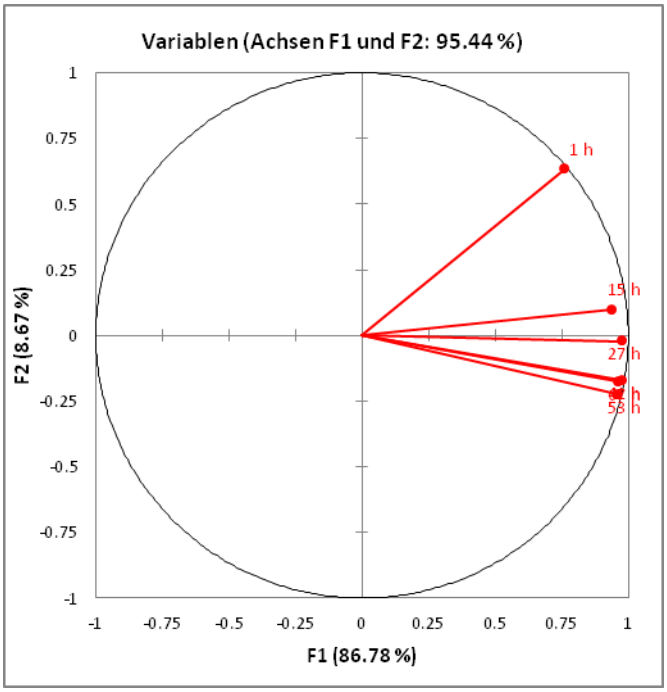
PUT1_Fwd	ATGGCAGACGATGTTACAC
PUT1_Rev	CCGTTCTCCTCCAATCTTCT
DAL1_Fwd	GGAATTGCATCGGTCGGTTT
DAL1_Rev	GGCAGTGTTCAGCGAAGT



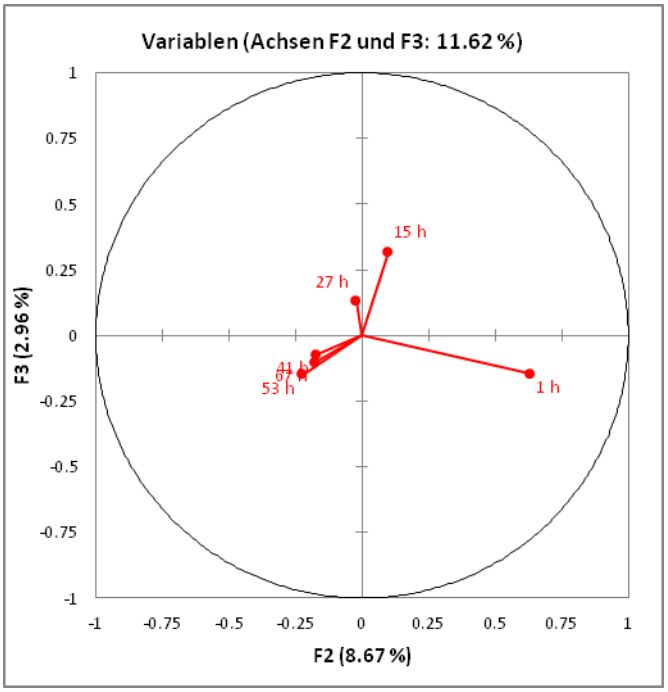
Additional Figure S4: Principal component analysis (variable factor map; F2 vs. F1) of the methanol fed-batch (1 h, 15 h, 27 h, 41 h, 53 h and 67 h after starting the methanol fed-batch) referred to the glycerol fed-batch (log2 fold change).



Additional Figure S5: Principal component analysis (variable factor map; F3 vs. F2) of the methanol fed-batch (1 h, 15 h, 27 h, 41 h, 53 h and 67 h after starting the methanol fed-batch) referred to the glycerol fed-batch (log2 fold change).



Additional Figure S6: Principal component analysis (variable factor map; F2 vs. F1) of the glucose fed-batch (1 h, 15 h, 27 h, 41 h, 53 h and 67 h after starting the glucose fed-batch) referred to the glycerol fed-batch (log2 fold change).



Additional Figure S7: Principal component analysis (variable factor map; F3 vs. F2) of the glucose fed-batch (1 h, 15 h, 27 h, 41 h, 53 h and 67 h after starting the glucose fed-batch) referred to the glycerol fed-batch (log2 fold change).