Additional File 1

Fermentation stage-dependent adaptations of *Bacillus licheniformis* during enzyme production

Sandra Wiegand, Birgit Voigt, Dirk Albrecht, Johannes Bongaerts, Stefan Evers,

Michael Hecker, Rolf Daniel and Heiko Liesegang

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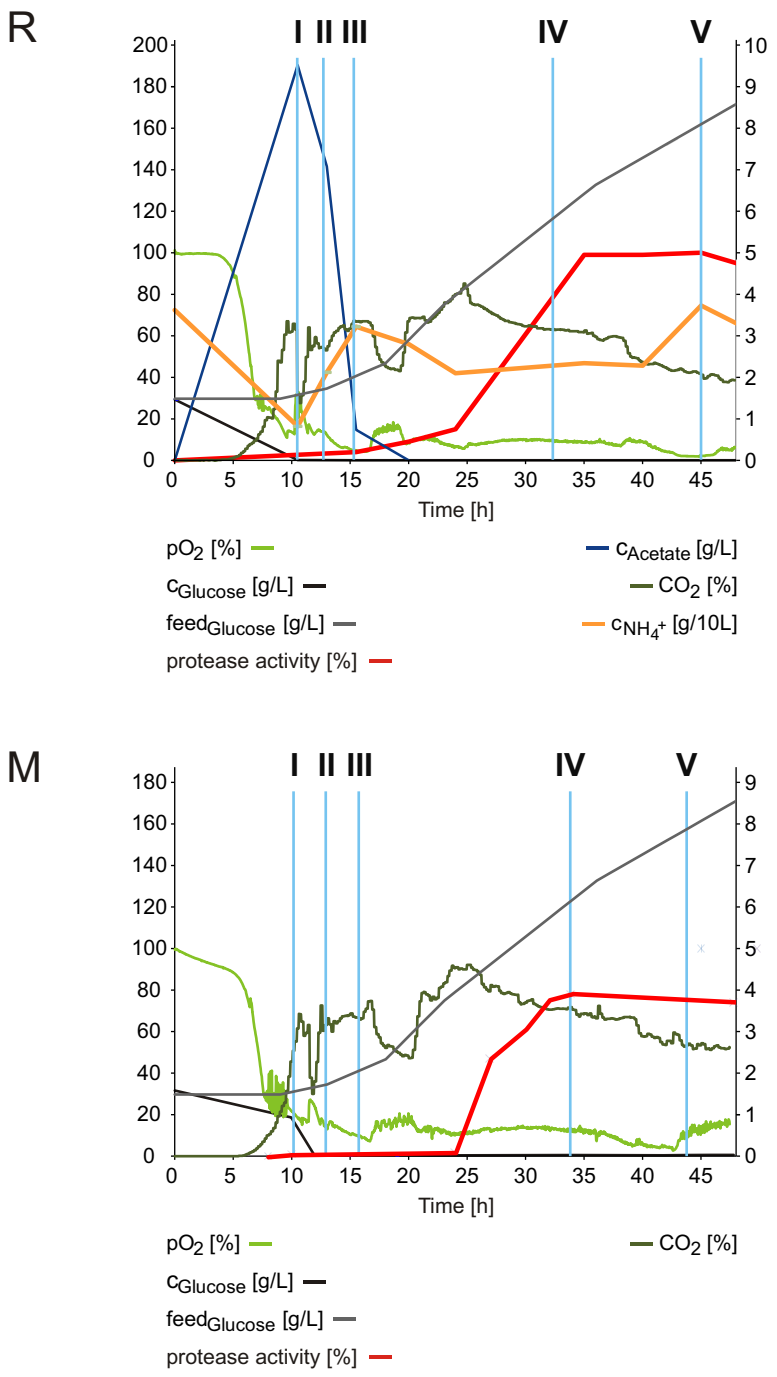


Figure S1 Protease production and process parameters. Process parameters are shown for fermentations R and M (please refer to Figure 1 for replicate L). Oxygen partial pressure pO_2 [%], glucose concentration $c_{Glucose}$ [g/L], supplied glucose $feed_{Glucose}$ [g/L] and normalized protease activity [%] are displayed on the left y-axis, whereas acetate concentration $c_{Acetate}$ [g/L] (only for fermentation R), carbon dioxide content CO_2 [%], and ammonium concentration c_{NH4+} [g/10L] (only for fermentation R) are scaled on the right y-axis. Process time t [h] is given on the x-axis. The sampling points I to V are indicated by light blue lines.

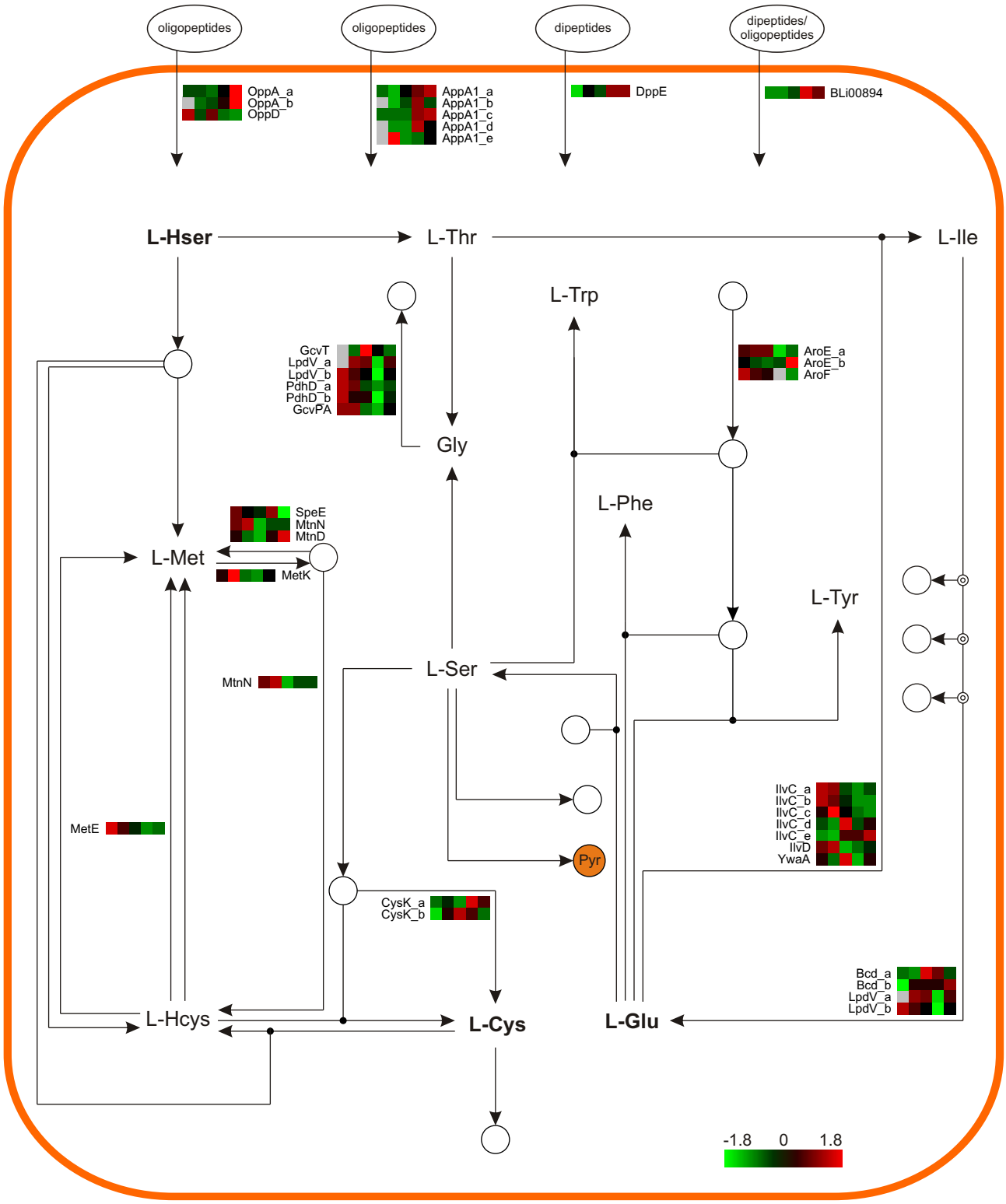


Figure S2 Proteome of the amino acid metabolism – Part I. Heat map representation of Z-score transformed protein spot volumes of proteins involved in amino acid transport and metabolism. In cases where a specific protein is assigned to more than one spot, the particular spots are indicated by an underscore, followed by an ordering letter. Statistically not significant values are indicated by light grey boxes. Pyr: Pyruvate. For the corresponding transcriptome data please refer to Figure 5.

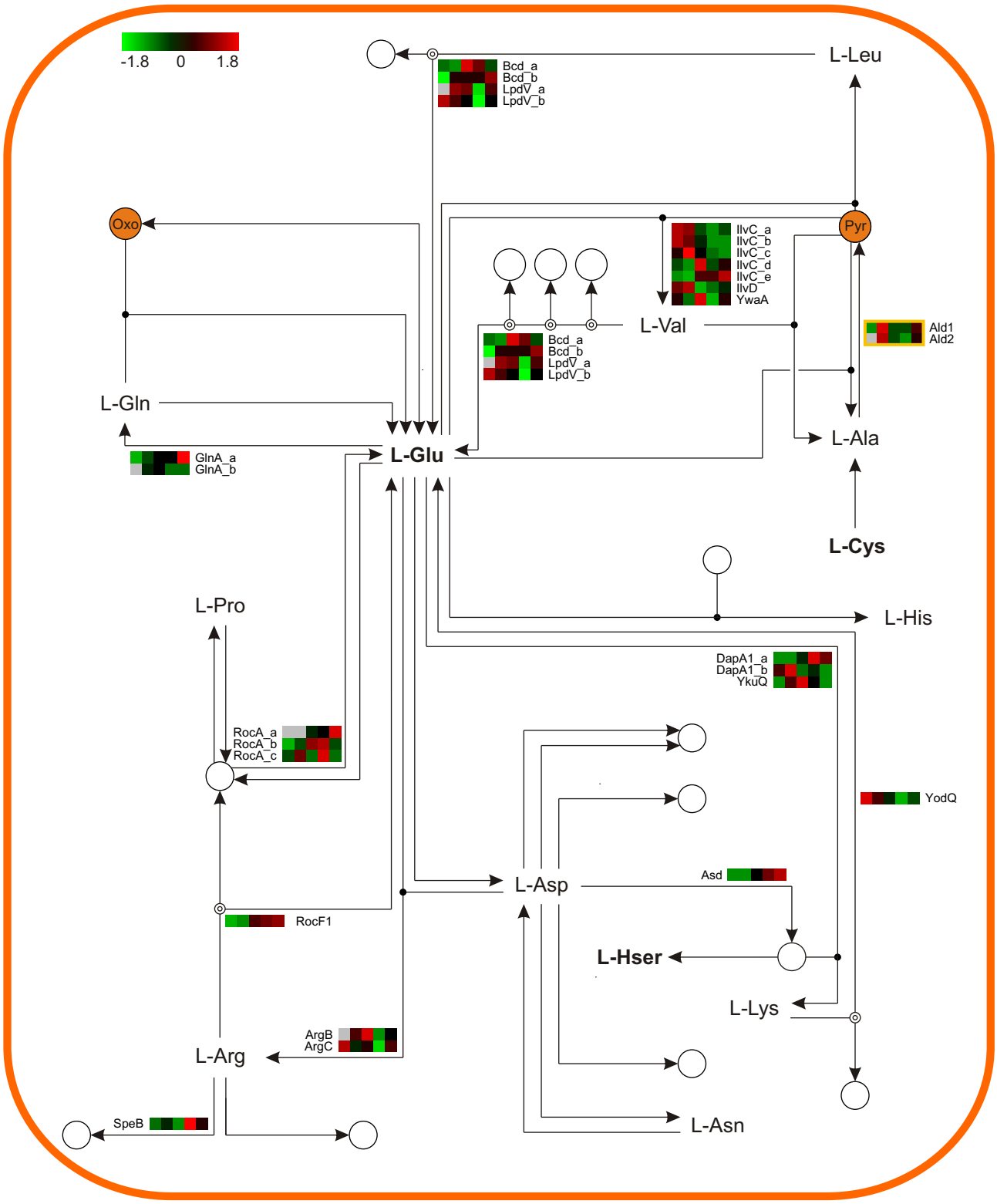
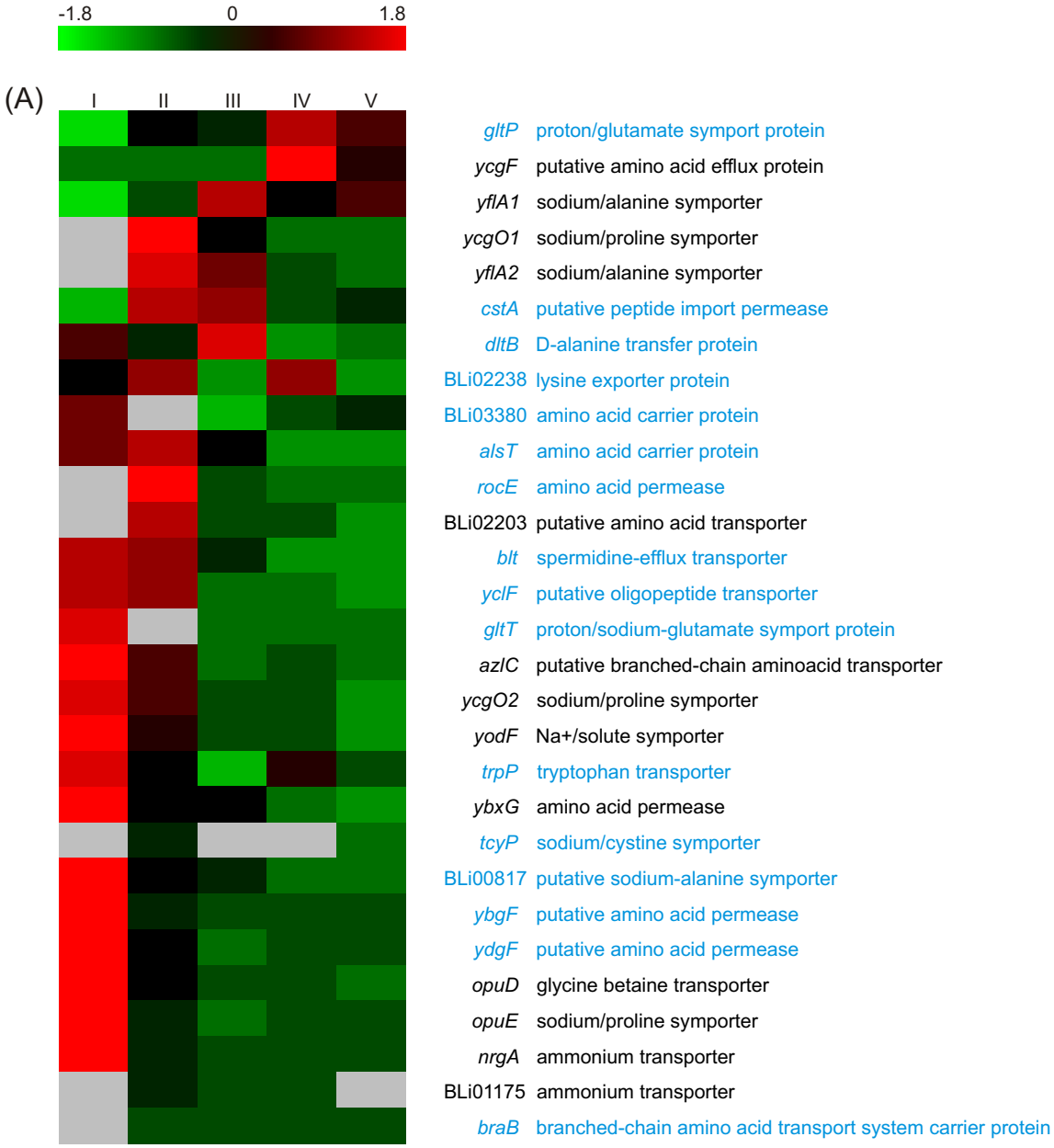


Figure S3 Proteome of the amino acid metabolism – Part II. Heat map representation of Z-score transformed protein spot volumes of proteins involved in amino acid metabolism. In cases where a specific protein is assigned to more than one spot, the particular spots are indicated by an underscore followed, by an ordering letter. Statistically not significant values are indicated by light grey boxes. Yellow frames indicate reactions with multiple assigned enzymes of which only one is strictly necessary. Pyr: Pyruvate, Oxo: 2-Oxoglutarate. For the corresponding transcriptome data please refer to Figure 6.


-1.8
0
1.8
(A)
I
II
III
IV
V
gltP proton/glutamate symport protein
ycgF putative amino acid efflux protein
yflA1 sodium/alanine symporter
ycgO1 sodium/proline symporter
yflA2 sodium/alanine symporter
cstA putative peptide import permease
dltB D-alanine transfer protein
BLi02238 lysine exporter protein
BLi03380 amino acid carrier protein
alsT amino acid carrier protein
rocE amino acid permease
BLi02203 putative amino acid transporter
blt spermidine-efflux transporter
yclF putative oligopeptide transporter
gltT proton/sodium-glutamate symport protein
azlC putative branched-chain aminoacid transporter
ycgO2 sodium/proline symporter
yodF Na+/solute symporter
trpP tryptophan transporter
ybxG amino acid permease
tcyP sodium/cystine symporter
BLi00817 putative sodium-alanine symporter
ybgF putative amino acid permease
ydgF putative amino acid permease
opuD glycine betaine transporter
opuE sodium/proline symporter
nrgA ammonium transporter
BLi01175 ammonium transporter
braB branched-chain amino acid transport system carrier protein

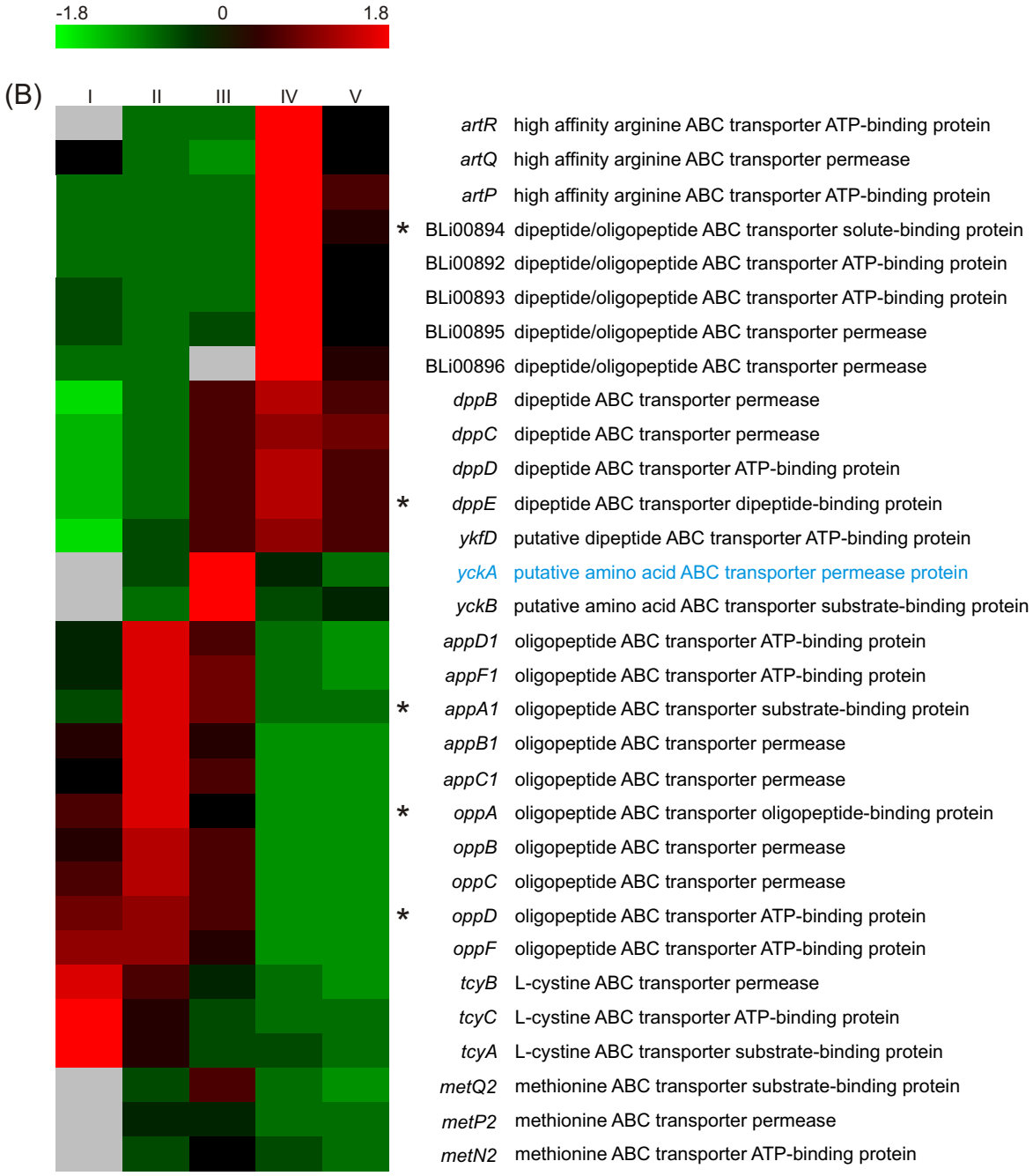


Figure S4 Amino acid transport. Heat map representation of Z-score transformed NPKM values. The depicted genes have been annotated as **(A)** amino acid or nitrogen transporters and **(B)** amino acid ABC transporter components. Please note that the figure does not give a complete list of genes involved in amino acid transport. Genes with an assigned antisense RNA [1] are marked in blue, asterisks indicate a detected protein spot for the respective gene (Additional File 2: Table S3) and statistically not significant values are marked by grey boxes.
Transporter genes with high transcript abundances during the early stages of the fermentation process are for example encoding a tryptophan transporter (trp), cystine (tcyABC) and methionine (metNPQ) ABC transporters and diverse proteins for uptake of alanine or unspecific amino acids. Additionally, the transcript of the ammonium transporter NrgA, especially required for ammonium transport at low ammonium concentrations in *B. subtilis* [2], is highly abundant at sampling point I. The only operon besides the dipeptide ABC transporters mentioned in the main text with distinct transcript abundance at the later sampling points encodes a high-affinity arginine ABC transporter (artPQR).

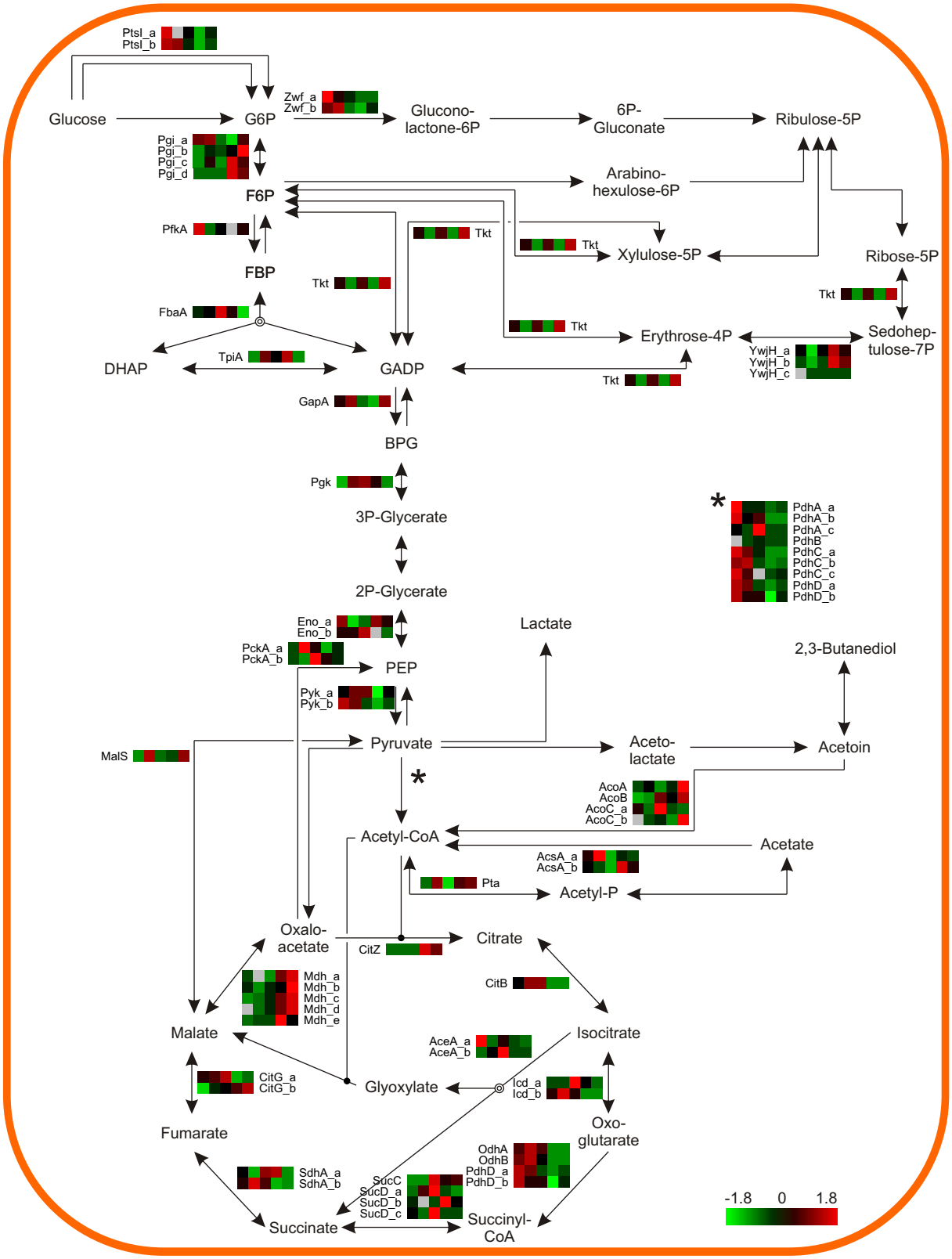
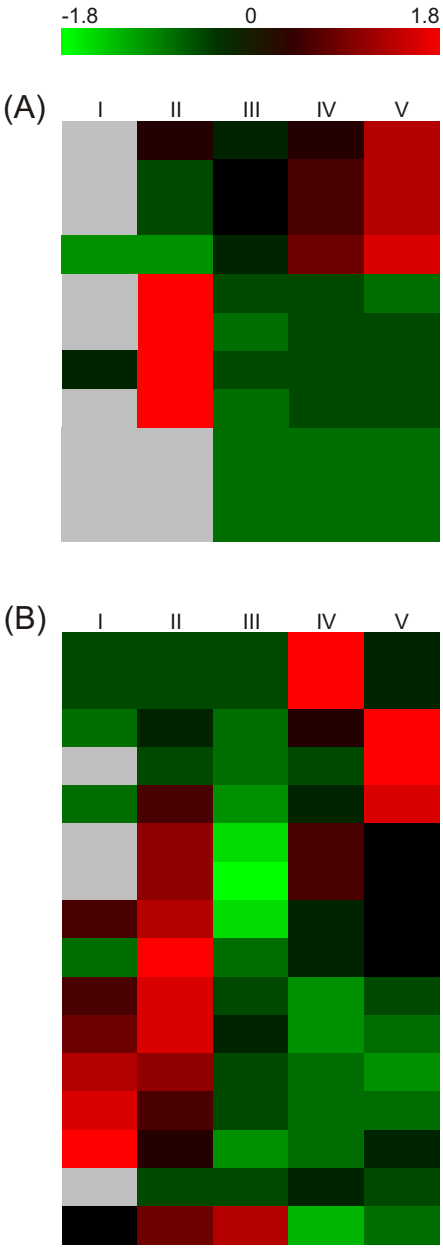


Figure S5 Proteome of the central carbon metabolism. Heat map representation of Z-score transformed protein spot volumes of proteins involved in central carbon metabolism. In cases where a specific protein is assigned to more than one spot, the particular spots are indicated by an ordering letter. Statistically not significant values are indicated by light grey boxes. For the corresponding transcriptome data please refer to Figure 7.


-1.8
0
1.8
(A)
I
II
III
IV
V
BLi00444 multiple monosaccharide ABC transporter membrane protein
* BLi00442 monosaccharide ABC transporter substrate-binding protein
BLi00443 multiple monosaccharide ABC transporter ATP-binding protein
ytcQ1 carbohydrate ABC transporter substrate-binding protein
msmX carbohydrate ABC transporter ATP-binding protein
amyD carbohydrate ABC transporter permease
msmE carbohydrate ABC transporter substrate-binding protein
amyC carbohydrate ABC transporter permease
ganQ carbohydrate ABC transporter permease
ganP carbohydrate ABC transporter permease
cycB carbohydrate ABC transporter ATP-binding protein
(B)
I
II
III
IV
V
BLi02505 putative lactose/cellobiose-specific phosphotransferase system EIIC comp.
BLi02506 putative lactose/cellobiose-specific phosphotransferase system EIIB comp.
murP N-acetyl muramic acid-specific phosphotransferase system EIIBC comp.
* ptsG trigger enzyme glucose-specific phosphotransferase system EIICBA comp.
nagP N-acetylglucosamine-specific phosphotransferase system EIICB comp.
ulaA ascorbate-specific phosphotransferase system EIIC comp.
BLi00493 putative ascorbate-specific phosphotransferase system EIIB comp.
licB trigger enzyme lichenan-specific phosphotransferase system EIIB comp.
licC lichenan-specific phosphotransferase system EIIC comp.
licA lichenan-specific phosphotransferase system EIIA comp.
BLi03866 sorbitol-specific phosphotransferase system EIIC comp.
BLi03865 sorbitol-specific phosphotransferase system EIIBC comp.
BLi03864 putative sorbitol-specific phosphotransferase system EIIA comp.
fruA fructose-specific phosphotransferase system EIIABC comp.
bglP trigger enzyme beta-glucoside-specific phosphotransferase system EIIBCA comp.
* ypqE putative phosphotransferase system EIIA comp.

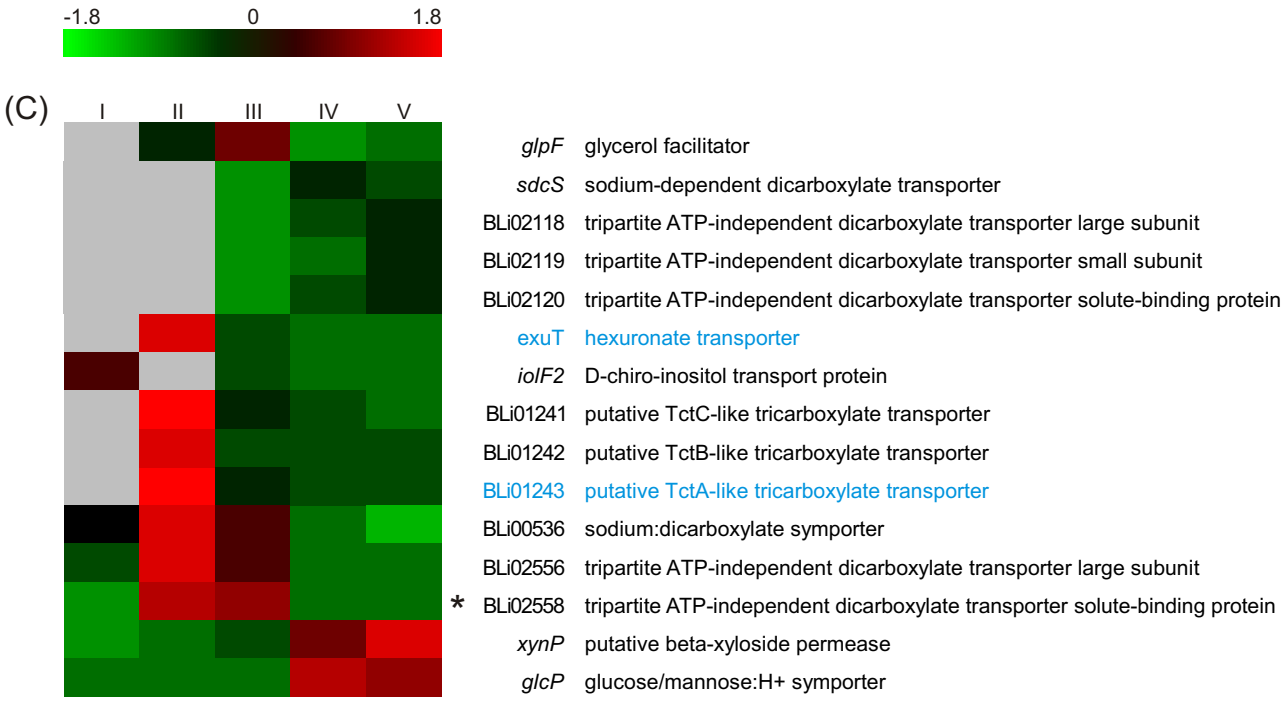


Figure S6 Carbohydrate transport. Heat map representation of Z-score transformed NPKM values. The depicted genes have been annotated as **(A)** carbohydrate ABC transporter components, **(B)** phosphotransferase system EII components and **(C)** further carbohydrate transporters. Please note that the figure does not give a complete list of genes involved in carbohydrate transport. Genes with an assigned antisense RNA [1] are marked in blue, asterisks indicate a detected protein spot for the respective gene (Additional File 2: Table S3) and statistically not significant values are marked by grey boxes.

At sampling point II, the transcript abundances indicate the consumption of the previously synthesized acetate (Figure 7). These results are supported by RNA abundance shifts of associated transporters. The transcript of a tripartite ATP-independent periplasmic dicarboxylate transporter (TRAP) of the TAXI type (BLi02556 and BLi02558), which is suggested as capable of acetate transport [3], is highly abundant.

During the late production stages, transcripts of transporters of diverse sugars (GlcP, XynP, BglP, SdcS) and cell wall components (MurP, NagP) are abundant. It is likely that this reaction can be accounted to the availability of such compounds due to cell lysis, as for example described at the onset of sporulation in *B. subtilis* [4], or shear effects in the fermenter.

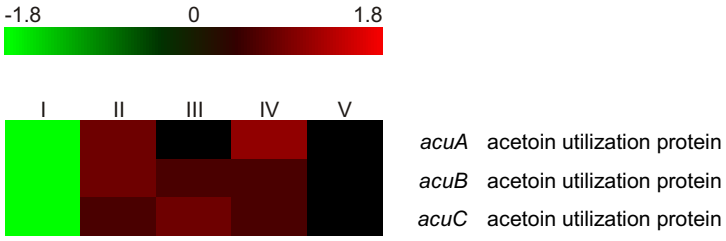


Figure S7 Acetoin utilization operon *acuABC*. Heat map representation of Z-score transformed NPKM values. The depicted genes are annotated as acetoin utilization operon *acuABC*.

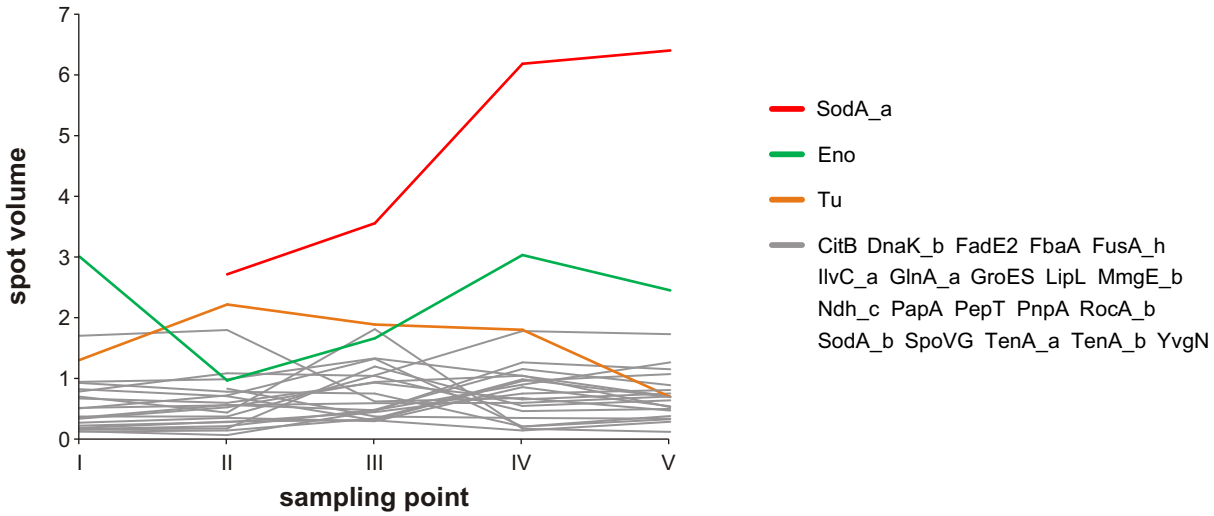


Figure S8 Most abundant proteins. Mean spot volumes of the most abundant proteins are plotted against sampling points (see also Additional File 2: Table S3). Grey lines represent protein spot volumes either higher than 0.8% at one sampling point or higher than 0.5% at all sampling points. Lines colored green, red and orange indicate the three most abundant protein spots Eno, Tu and SodA_a. In case a specific protein can be assigned to more than one spot, the particular spot is indicated by an ordering letter. Please note that statistically not significant values are not shown.

Highly abundant transcripts (Figure 2) for which predominant proteins could also be observed are coding for SodA, Tu and SpoVG. Other strongly synthesized proteins are, for example, the enolase Eno and proteins involved in carbon and amino acid metabolism or cofactor synthesis. They also comprise peptidases, the heat shock proteins GroES and DnaK and elongation factor G. Protein spots corresponding to the strongly transcribed genes *lanA1* and *lanA2* could not be identified. This was expected, as these proteins are probably exported by ABC transporters [5] and thus cannot be detected by proteome analysis of cytoplasmic proteins. The findings of highly abundant proteins match the results for *B. licheniformis* shown by Voigt et al. [6]. The only major exception is the absence of the flagellin protein Hag, which is also not highly abundant on transcript level. This effect is due to repression of *hag* gene expression in the presence of amino acids [7].

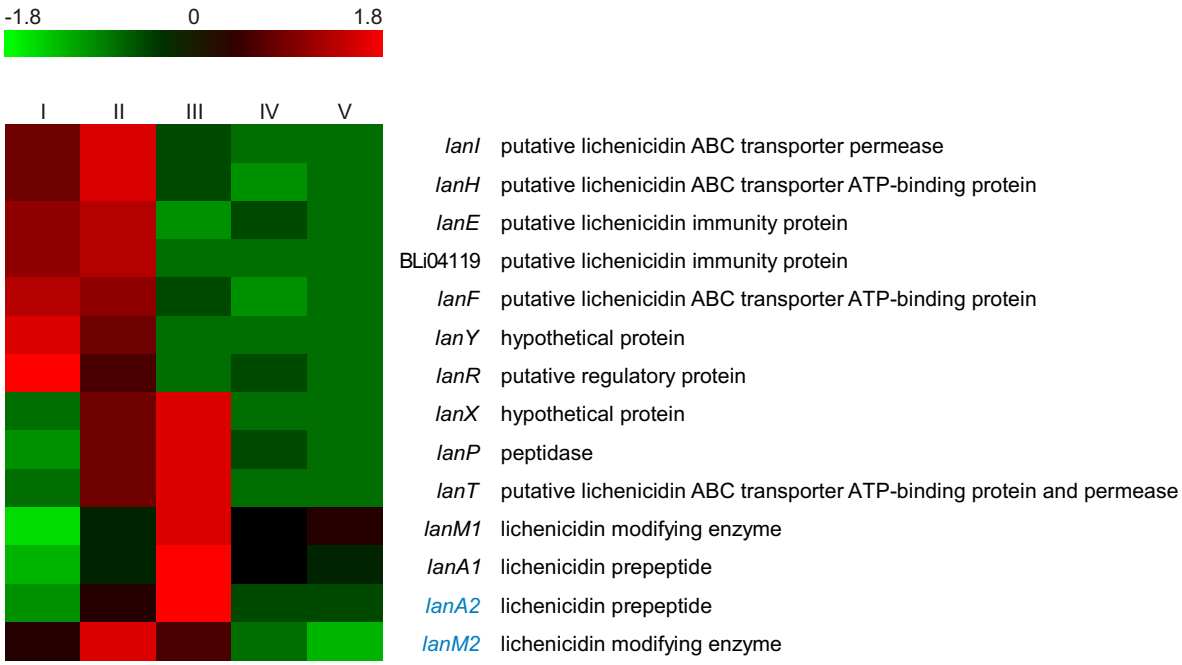
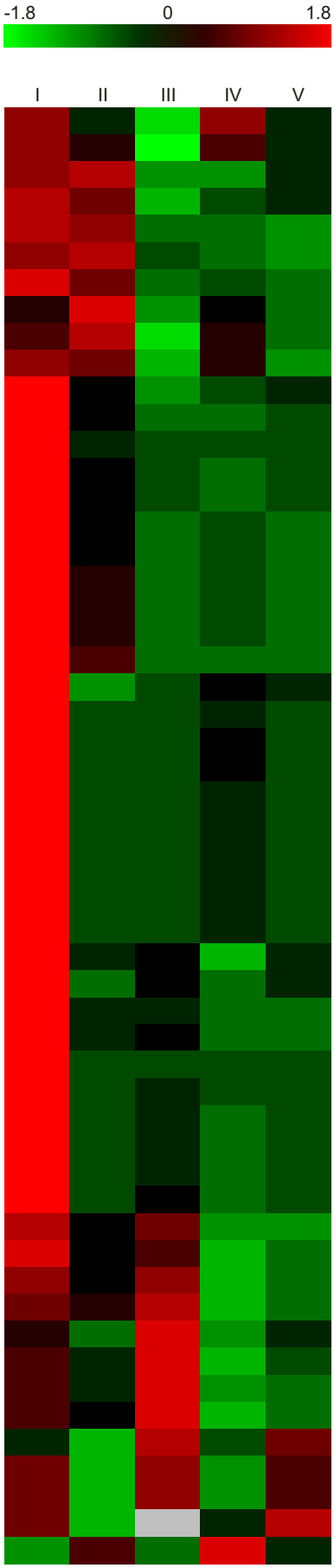
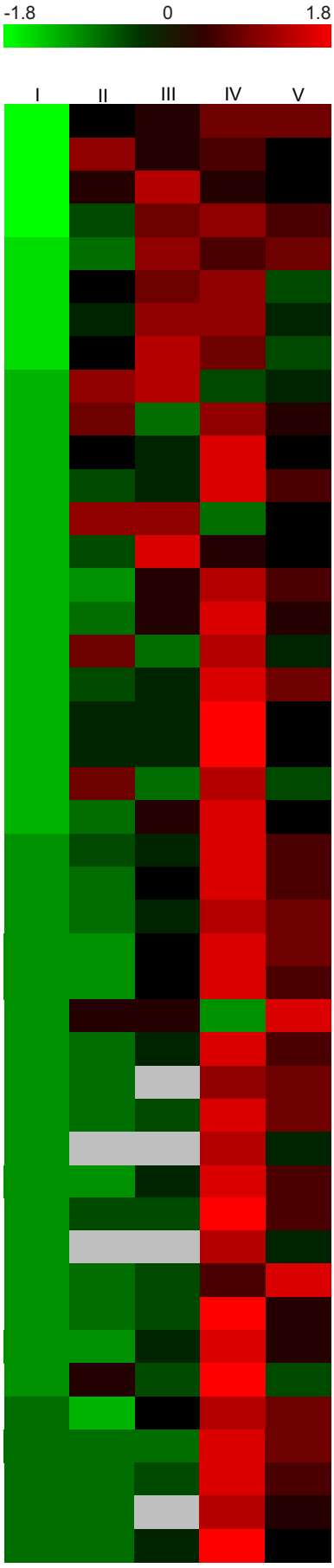


Figure S9 Lichenicidin gene cluster. Heat map representation of Z-score transformed NPKM values. The depicted genes have been identified as two-peptide lantibiotic lichenicidin-processing gene cluster Lan in *B. licheniformis* [5, 8]. Genes with an assigned antisense RNA [1] are marked in blue.

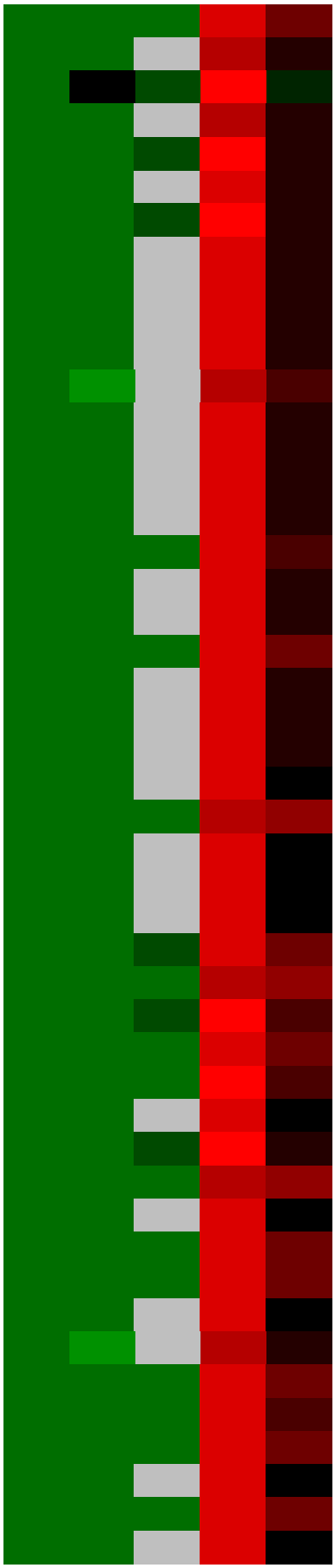


	ydaH	putative transmembrane protein
	yqjL	hypothetical protein L
	yrhH	methyltransferase
	spx	transcriptional regulator
	BLi00514	putative extracellular protein
	ltaS2	polyglycerolphosphate lipoteichoic acid synthase
	bcrC	undecaprenyl-diphosphatase
	yhdL	putative anti-sigma-M factor
	yhdK	putative anti-sigma-M factor
	ywtF	putative cell envelope transcriptional attenuator
	yjbC	hypothetical protein
	sigW	RNA polymerase sigma factor
	yceC	putative stress protein
*	*yceD*	putative stress protein
*	*yceE*	putative stress protein
	liaI	transmembrane protein
	liaH	hypothetical protein
	liaG	hypothetical protein
	liaF	LiaR negative effector
	liaS	two-component sensor histidine kinase
	liaR	two-component response regulator
	pyrR	transcriptional antiterminator
*	*pyrC*	dihydroorotase
	pyrB	aspartate carbamoyltransferase
	pyrAA	carbamoyl-phosphate synthase pyrimidine-specific small subunit
	pyrD	dihydroorotate dehydrogenase catalytic subunit
	pyrP	uracil permease
	pyrK	dihydroorotate dehydrogenase electron transfer subunit
	pyrF	orotidine 5'-phosphate decarboxylase
	pyrAB	carbamoyl-phosphate synthase pyrimidine-specific large subunit
	pyrE	orotate phosphoribosyltransferase
	rsiW	anti-sigma-W factor
	BLi02027	putative Band 7 protein
	ydbT	cytosolic protein
	azoR1	FMN-dependent NADH-azoreductase
	yvlA	transmembrane protein
	yozO	hypothetical protein
	ydbS	cytosolic protein
	yvlB	hypothetical protein
	yvlC	transmembrane protein
	yvlD	DUF360 transmembrane protein
	dltC	D-alanine carrier protein
	yknW	antimicrobial peptide ABC exporter permease
	dltD	D-alanine transfer protein
	yknX	antimicrobial peptide ABC exporter permease
*	*dltA*	D-alanyl-D-alanine carrier protein ligase
	yknY	antimicrobial peptide ABC exporter ATP-binding protein
	dltB	D-alanine transfer protein
	yknZ	antimicrobial peptide ABC exporter permease
	pbpE1	penicillin-binding protein 4
	BLi04079	hypothetical protein
	BLi00343	hypothetical protein
	yocK	general stress protein
	sigM	RNA polymerase sigma factor

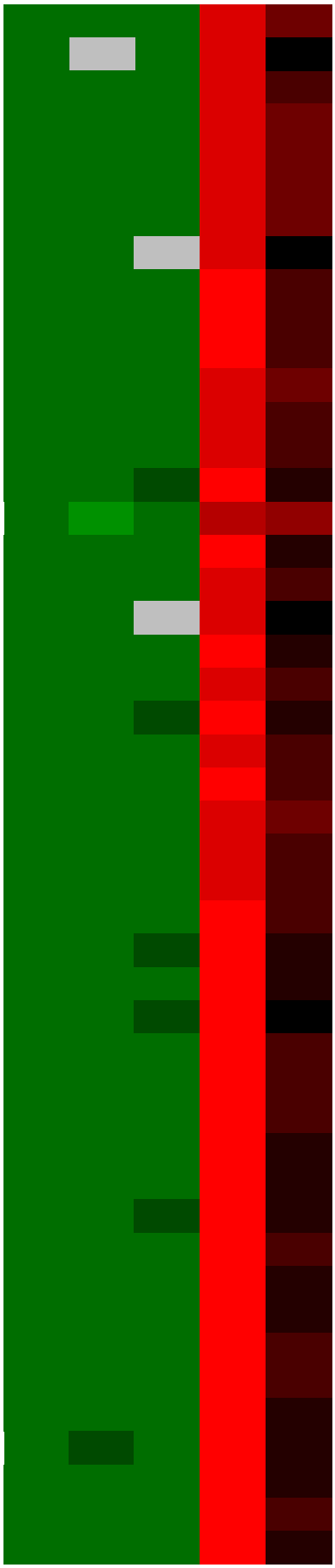
Figure S10 Cell envelope stress response. Heat map representation of Z-score transformed NPKM values. The depicted genes have been identified as marker genes for the *B. licheniformis* cell envelope stress response by Wecke et al. [9]. Genes with an assigned antisense RNA [1] are marked in blue, asterisks indicate a detected protein spot for the respective gene (Additional File 2: Table S3) and statistically not significant values are marked by grey boxes. The genes *yvnB*, *sigY*, *pbpX* and *yxlCDEFG* are not shown due to lacking transcript abundances (NPKM values <10).



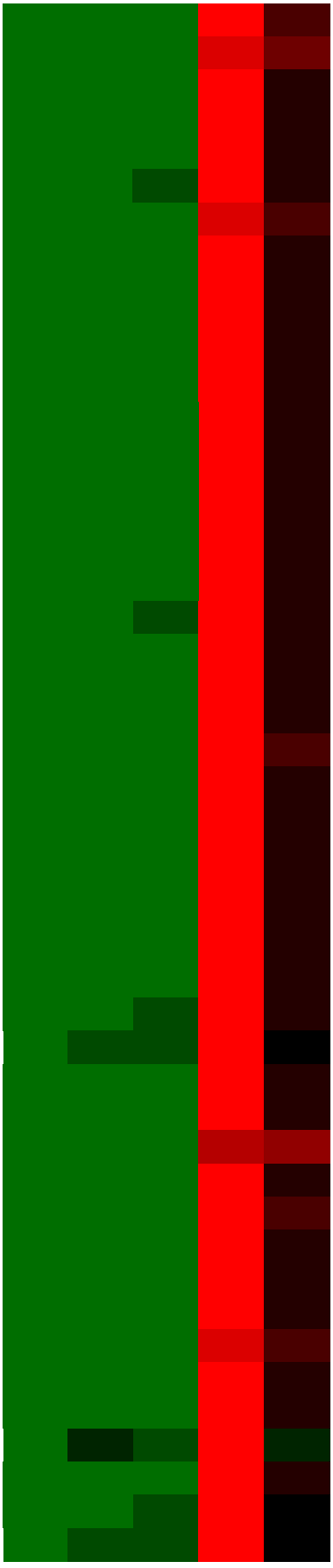
ynfE	hypothetical protein
yteT	oxidoreductase
spoVG	putative septation protein
divIC	cell division initiation protein
sspH	small acid-soluble spore protein
dapG	aspartokinase
yusW	hypothetical protein
dapA1	dihydrodipicolinate synthase
bdbD	thiol-disulfide oxidoreductase
ylmC	hypothetical protein
cgeE	N-acetyltransferase involved in spore outer layer maturation
ykwB	putative N-acetyltransferase
bdbC	thiol-disulfide oxidoreductase
yumB	putative NADH dehydrogenase
sigF	RNA polymerase sigma factor
spoIIAB	anti-sigma-F factor
spoVS	stage V sporulation protein
yoaW	hypothetical protein
asd	aspartate-semialdehyde dehydrogenase
yabR	putative S1 RNA-binding domain protein
lysA	diaminopimelate decarboxylase
lipC	spore germination lipase
yflN	putative beta-lactamase-like protein
spoIIAA	anti-sigma-F factor antagonist
ydcA	putative transmembrane protein
yodL	hypothetical protein
spoIIB	stage II sporulation protein
ctaA	heme A synthase
ylbB	hypothetical protein
spoIIR	stage II sporulation protein
spoVK	stage V sporulation protein
cotJB	spore coat peptide assembly protein
yqhP	hypothetical protein
ylbC	hypothetical protein
cotJA	spore coat associated protein
ybaK	hypothetical protein
glgP	glycogen phosphorylase
sigG	RNA polymerase sigma factor
ypeP	putative ribonuclease
yttP	transcriptional regulator
yngK	hypothetical protein
ytvB	transmembrane protein
spoIIIAB	stage III sporulation protein
yabQ	spore protein



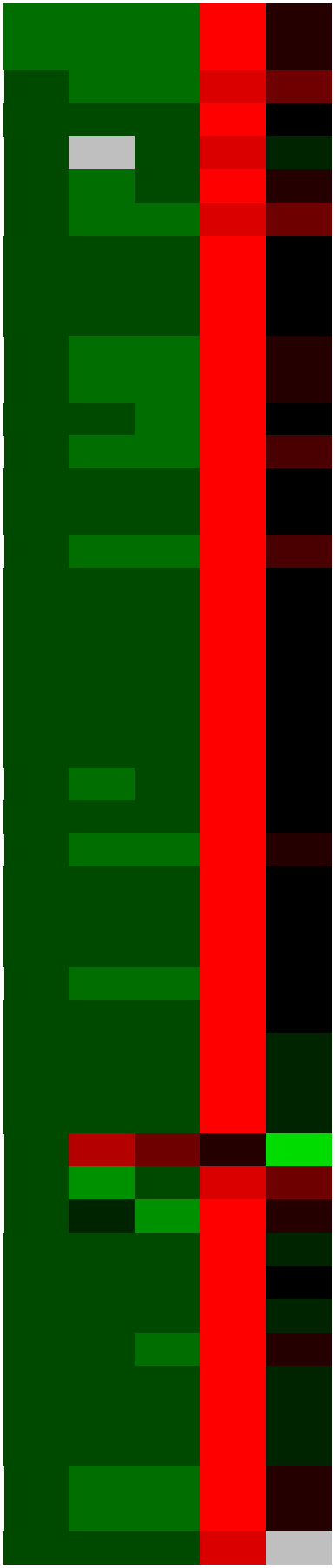
yhdB	hypothetical protein
spoIIID	stage III sporulation protein
ypfB	hypothetical protein
spoIVA	stage IV sporulation protein
spoVD	stage V sporulation protein
yyaC	sporulation protein
yqfC	sporulation protein
spoIIIAG	stage III sporulation protein
yabT	putative serine/threonine-protein kinase
yqfD	SigE-dependent sporulation gene
yabS	hypothetical protein
yozD	hypothetical protein
yqxA	hypothetical protein
yngHB	biotin/lipoyl attachment protein
yhxC	putative oxidoreductase
yaaH	glycoside hydrolase family protein
yhaL	hypothetical protein
spoIIIAH	stage III sporulation protein
yodQ	4-acetamidobutyryl-CoA deacetylase
yuzC	sporulation protein
ydcC	putative sporulation protein
yngE	methylcrotonoyl-CoA carboxylase
ysxE	positive modulator
sqhC	squalene--hopene cyclase
ymfJ	hypothetical protein
spoIIP	cell wall hydrolase
prkA	serine protein kinase
spoIIGA	sigma-E factor-processing peptidase
yqhO	putative acyl hydrolase/lysophospholipase
ytfI	DUF2953 family protein
spoIISA	stage II sporulation protein
spoVAC	stage V sporulation protein
yhaX	hypothetical protein
spoIIIAF	stage III sporulation protein
glgA	glycogen synthase
BLi03524	putative small acid-soluble spore protein
kamA	L-lysine 2,3-aminomutase
yhcN	forespore-specific protein
yhcV	CBS domain protein
yngG	3-hydroxy-3-methylglutaryl-coenzyme A lyase
yteV	sporulation protein
ykzD	hypothetical protein
yjbA	hypothetical protein
BLi01360	hypothetical protein
spoIIIAC	stage III sporulation protein
sspA	small acid-soluble spore protein
yngJ	putative acyl-CoA dehydrogenase



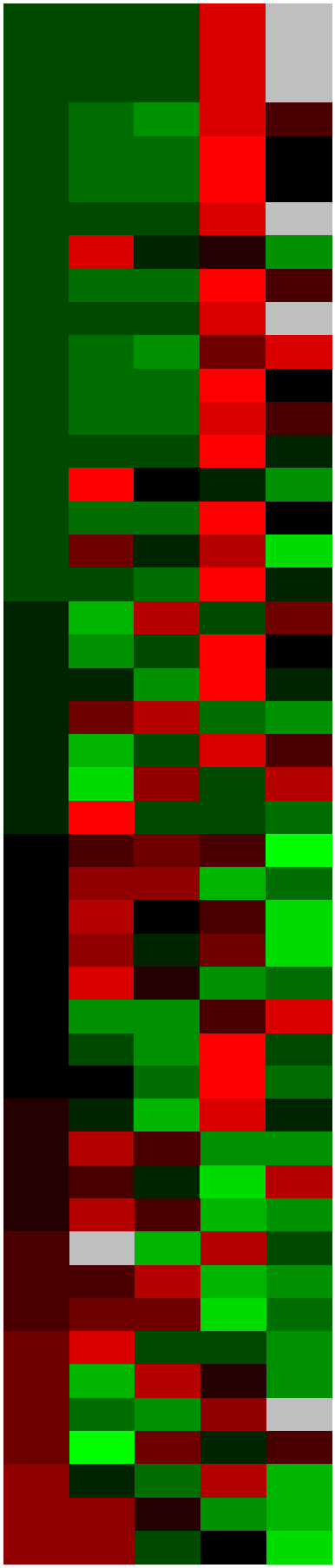
sspO	small acid-soluble spore protein
katX	catalase
sspI	small acid-soluble spore protein
BLi02230	hypothetical protein
sspP	small acid-soluble spore protein
BLi01951	hypothetical protein
yisY	AB hydrolase superfamily protein
yjbE	membrane protein
yngF	short chain enoyl-CoA hydratase
glgD	glycogen biosynthesis protein
spoIIQ	stage II sporulation protein
spoIVB	peptidase S
yqhR	hypothetical protein
yuzA	general stress protein
rsfA	putative transcriptional regulator
yqgO	hypothetical protein
yheD	hypothetical protein
yhcQ	hypothetical protein
yhbH	sporulation protein
yndM	hypothetical protein
yqhG	hypothetical protein
ywcB	DUF485 transmembrane protein
yrrD	forespore-specific sporulation protein
alr2	alanine racemase
azoR2	FMN-dependent NADH-azoreductase
cotO	spore coat morphogenetic protein
sspB	small acid-soluble spore protein
yqhV	hypothetical protein
sodF	putative superoxide dismutase
gerR	putative transcriptional regulator
yhfN	putative metalloprotease
yjaV	hypothetical protein
BLi00840	hypothetical protein
yhfW	putative Rieske 2Fe-2S iron-sulfur protein
dacF	D-alanyl-D-alanine carboxypeptidase
yrzE	transmembrane protein
accC1	acetyl-CoA carboxylase biotin carboxylase subunit
cwlC	N-acetylmuramoyl-L-alanine amidase
spoIIIAD	stage III sporulation protein
yodR	6-acetamido-3-oxohexanoate:acetyl-CoA CoA transferase beta subunit
glgB	1,4-alpha-glucan-branching enzyme
bofC	general stress protein
cotJC	spore coat peptide assembly protein
ywcA	Na+/solute symporter
ytlD	putative anion ABC transporter permease
lonB	Lon-like ATP-dependent protease
spoVAF1	stage V sporulation protein



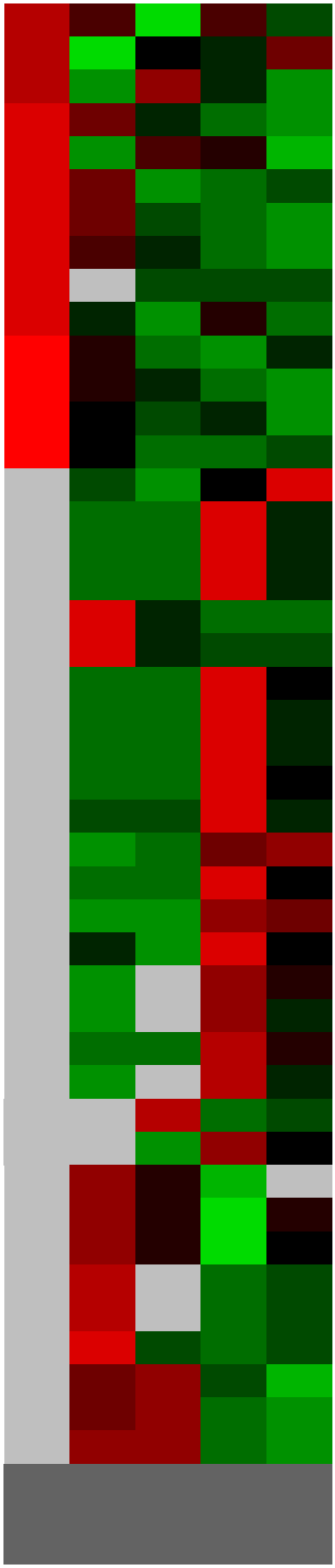
yurZ	putative alkylhydroperoxidase
yoaR	hypothetical protein
spoIVFB	stage IV sporulation protein
sspK	small acid-soluble spore protein
ytrH	sporulation membrane protein
ytrI	sporulation membrane protein
dacB	D-alanyl-D-alanine carboxypeptidase
yutH	spore coat protein
ycgF	putative amino acid efflux protein
spoIIIAE	stage III sporulation protein
spoIIIAA	stage III sporulation protein
ylaJ	hypothetical protein
spoIVFA	stage IV sporulation protein
yusN	putative spore coat protein
spmB	spore maturation protein
yodT	N(6)-acetyl-beta-lysine transaminase precursor
yodP	beta-lysine acetyltransferase
ydhD	putative sporulation-specific glycosylase
pdaB	polysaccharide deacetylase
ytlC	putative anion ABC transporter ATP-binding protein
ylzJ	hypothetical protein
yheC	hypothetical protein
yuiC	sporulation protein
ytlA	putative anion ABC transporter substrate-binding protein
spoVAA	stage V sporulation protein
yodS	6-acetamido-3-oxohexanoate:acetyl-CoA CoA transferase alpha subunit
yknT	putative sporulation protein
asnO	asparagine synthase
cotF	spore coat protein
yqfT	hypothetical protein
glgC	glucose-1-phosphate adenylyltransferase
gin	forespore-specific protein
spoIIM	stage II sporulation protein
pbpG	penicillin-binding protein 2D
sspF	small acid-soluble spore protein
yraD	spore coat protein F-like protein
yteA	sporulation protein
yjcA	sporulation protein
tgl	protein-glutamine gamma-glutamyltransferase
ydfS	UPF0702 transmembrane protein
seaA	spore envelope assembly protein
yokU	hypothetical protein
yitG	putative major facilitator superfamily protein
yqhQ	hypothetical protein
yitD	phosphosulfolactate synthase
ylbJ	putative sporulation integral membrane protein
ytvI	sporulation integral membrane protein



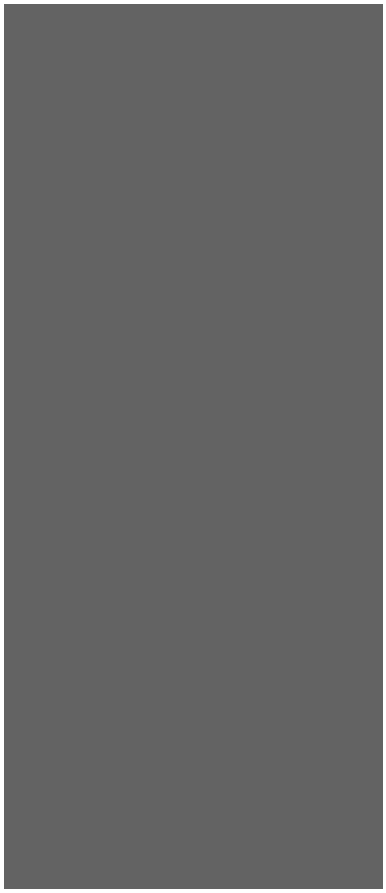
spoVB	stage V sporulation protein
yetF	hypothetical protein
sspN	small acid-soluble spore protein
ykoP	hypothetical protein
cotV	spore coat protein
spmA	spore maturation protein
tlp	small acid-soluble spore protein
yitF	putative mandelate racemase
ytzH	hypothetical protein
ykvI	putative membrane protein
yjaZ	hypothetical protein
tepA	ClpP-like protease
yutG	putative phosphatidylglycerophosphatase
sspD	small acid-soluble spore protein
spoVR	stage V sporulation protein
yqhH	putative ATP-dependent helicase
ykoV	Ku-type ATP-dependent DNA helicase
spoVIF	sporulation-specific transcription factor
ymaF	hypothetical protein
yhjR1	hypothetical protein
cotM	spore coat protein
gerT	spore germination protein
spoVFB	dipicolinate synthase subunit
ypeQ	hypothetical protein
cotA	outer spore coat protein
ytxC	putative sporulation protein
yodH	putative methyltransferase
yabG	sporulation-specific protease
spoVFA	dipicolinate synthase subunit
stoA	thiol-disulfide oxidoreductase
BLi02137	hypothetical protein
spoVAB	stage V sporulation protein
yodN	hypothetical protein
BLi00808	putative pyridoxal phosphate-dependent aminotransferase
murB	UDP-N-acetylenolpyruvoylglucosamine reductase
yybP	hypothetical protein
yhbB	hypothetical protein
yhcO	hypothetical protein
ykoU	ATP-dependent DNA ligase
BLi00804	NAD-dependent epimerase/dehydratase
yabP	spore protein
ypqA	hypothetical protein
yunC	DUF1805 family protein
yndL	hypothetical protein
ywcE	spore morphogenesis/germination protein
yyaD	sporulation protein
glcU	putative l-rhamnose-proton symporter



yitE	UPF0750 membrane protein
ytzC	DUF2524 family protein
lytH	peptidoglycan hydrolase
yphA	putative membrane protein
yueG	spore germination protein
pbpI	sporulation-specific penicillin-binding protein 4b
adhB	alcohol dehydrogenase-like protein
phoR	two-component sensor histidine kinase
cotE	spore coat protein
yutC	putative sporulation lipoprotein
ykzE	hypothetical protein
spoIID	stage II sporulation protein
ykuS	UPF0180 family protein
cotH	inner spore coat protein
ftsK	DNA translocase
yhfA	putative membrane protein
ypjB	sporulation protein
ldt	putative L,D-transpeptidase
clpC	ATP-dependent Clp protease ATP-binding subunit
bofA	pro-sigmaK processing inhibitor
ycgL	hypothetical protein
jag	hypothetical protein
cwlJ	cell wall hydrolase
disA	DNA integrity scanning protein
exuR	HTH-type transcriptional repressor
ylxW	DUF881 family protein
spoIIIJ	membrane protein translocase
divIB	division initiation protein
murG	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase
spo0J	stage 0 sporulation protein
ytkD	nucleoside triphosphatase
yyxA	putative serine protease
spoVE	stage V sporulation protein
mbl	cell-shape determining protein
ispG	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
BLi03803	NAD-dependent epimerase/dehydratase
soj	sporulation initiation inhibitor protein
ykoY	putative membrane protein
BLi03150	hypothetical protein
ftsY	cell division protein
pbpF	penicillin-binding protein 2C
yeaA	hypothetical protein
splA	transcriptional repressor
mcsB	modulator
ytaF	putative sporulation protein
nfo	endonuclease 4
subA	supressor



yrrS	DUF1510 family protein
mcsA	modulator
spoVT	stage V sporulation protein
ylxX	DUF881 family protein
yflJ	hypothetical protein
phoP2	two-component response regulator
sbp	small basic protein
fabL	NAD(P)-binding enoyl-(acyl-carrier-protein) reductase
yqfU	UPF0750 membrane protein
yozE	UPF0346 family protein
BLi04323	putative phage resolvase
rsmA	dimethyladenosine transferase
ytwI	DUF441 transmembrane protein
yrzA	hypothetical protein
ybbC	hypothetical protein
cotZ	spore coat protein
cotY	spore coat protein
cotX	spore coat protein
uxuA1	mannonate dehydratase
exuT	hexuronate transporter
splB	spore photoproduct lyase
ylbD	hypothetical protein
ylbE2	hypothetical protein
ymxH	hypothetical protein
yobW	sporulation membrane protein
ypzA	hypothetical protein
yqfZ	hypothetical protein
yqfX	hypothetical protein
yrzK	hypothetical protein
coxA	sporulation cortex protein
safA	hypothetical protein
ytfJ	sporulation protein
yunB	sporulation protein
uxaA	altronate hydrolase
ctpB	carboxy-terminal processing protease
tuaH	putative teichuronic acid biosynthesis glycosyltransferase
tuaG	putative teichuronic acid biosynthesis glycosyltransferase
tuaF	teichuronic acid biosynthesis protein
mmgC	butyryl-CoA dehydrogenase
mmgB	3-hydroxybutyryl-CoA dehydrogenase
mmgA	acetyl-CoA acetyltransferase
mmgD	2-methylcitrate synthase
mmgE	2-methylcitrate dehydratase
yqiQ	methylisocitrate lyase
BLi02833	hypothetical protein
BLi05032	hypothetical protein
uxaB	altronate oxidoreductase



yaaC	hypothetical protein
ybxH	hypothetical protein
ydfR	DUF421 transmembrane protein
yfhS	hypothetical protein
yfkE	H+/Ca2+ exchanger
yhcT	putative pseudouridine synthase
yhdC	hypothetical protein
yisN	DUF2777 family protein
yjfA	hypothetical protein
ykjA	UPF0702 transmembrane protein
ykoN	glycosyltransferase
ykoQ	putative metallo-dependent phosphatase
ykoS	hypothetical protein
ylyA	hypothetical protein
yngL	putative membrane protein
yppC	hypothetical protein
yqgE	putative major facilitator superfamily protein
yqzG	hypothetical protein
yrbG	DUF421 transmembrane protein
yrrI	UPF0118 transmembrane protein
yveA	aspartate-proton symporter
ywaF	transmembrane protein
ywjE	putative minor cardiolipin synthase

Figure S11 Sporulation. Heat map representation of Z-score transformed NPKM values. The depicted genes were identified as members of the sporulation cascade by reciprocal BLAST analysis [10] between *B. licheniformis* genes [1] and *B. subtilis* genes assigned to sporulation [11]. Genes with an assigned antisense RNA [1] are marked in blue, genes with NPKM values <10 at all sampling points are indicated by dark grey boxes, and statistically not significant values are indicated by light grey boxes.

Figure S12 Iron starvation. Heat map representation of Z-score transformed NPKM values. The depicted genes have been identified as marker genes for *B. licheniformis* iron starvation by Nielsen et al. [12]. Genes with an assigned antisense RNA [1] are marked in blue, genes with NPKM values <20 at all sampling points are indicated by dark grey boxes and statistically not significant values are indicated by light grey boxes.
A well described indicator for iron starvation is the induction of siderophore anabolic genes (*dhbABCEF* and *rhbCDEF*) and siderophore importer genes (*feuABC* and *yclNOPQ*) [12], which are under control of the transcriptional regulator Fur [13]. With exception of the Ycl operon, these transcripts of these genes are not abundant during the fermentation process. This behavior indicates that iron starvation does not occur in any of the examined fermentation phases and corresponds to the observation that the Fur regulon is only induced during growth in minimal medium with restricted iron supply but not in rich medium [12, 14, 15], as used in this study.

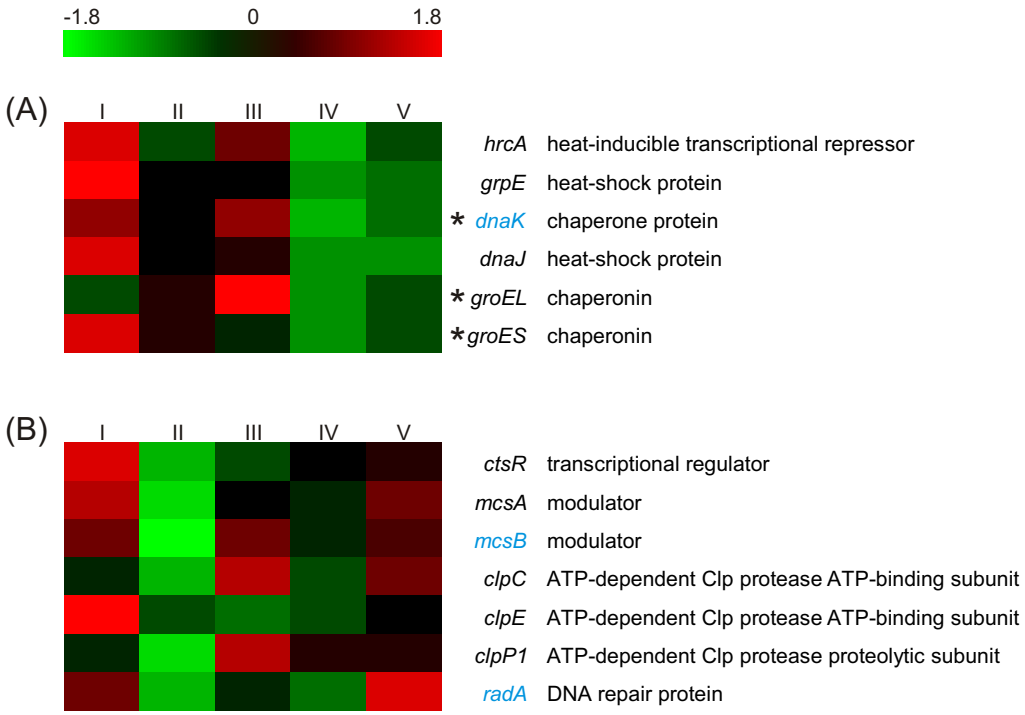


Figure S13 Heat shock response. Heat map representation of Z-score transformed NPKM values. The depicted **(A)** HrcA and **(B)** CtsR regulons have been identified as *B. licheniformis* heat stress markers by Nielsen et al. [12]. Genes with an assigned antisense RNA [1] are marked in blue, asterisks indicate a detected protein spot for the respective gene (Additional File 2: Table S3).

In cases of heat shock in *B. licheniformis*, the HrcA regulon including the *dnaK* and the *groE* operons and the CtsR regulon have been shown to be induced [12]. Furthermore, genes involved in iron and purine metabolism were upregulated, whereas the ABC transporter encoding *ytrABCEF* operon was repressed [12]. Since the here analyzed fermentation was not performed under heat shock conditions, the data did not show a typical heat shock response. Nevertheless, a transcriptional reaction to the fermentation temperature of 39 °C cannot be excluded, as the responses between moderate and severe heat shock can differ remarkably [16]. Despite the constant and moderate process temperature, differential expression of genes involved in a classic heat shock response [12, 17, 18] was observed. The ATP-dependent ClpCP protease shows changes in transcript abundance. However, the enzyme has been reported to be involved in proteolysis of misfolded proteins, which were caused by a variety of different stressors [19, 20] including for example the mentioned oxidative stress [14, 21, 22]. A reaction to non-heat stressors is also known for the chaperonins GroEL and GroES [23–25], which were transcribed with NPKM values >1000 at all sampling points or sampling point I, respectively. Although both chaperonins are encoded by the *groE* operon, the ratio of the transcript levels of the genes is highly variable between sampling points I and III – whereas *groES* declines over time, the *groEL* transcripts seem to be increasing. An explanation might be the processing of the bicistronic transcript into two monocistronic mRNAs. This effect has been described for *Agrobacterium tumefaciens* [26], in which the accumulation of *groEL* over time could also be shown. Furthermore, the determined GroEL protein amount (Additional File 2: Table S3) does not correlate with the amount of *groEL* mRNA, but shows the strongest abundance of protein at sampling point I.

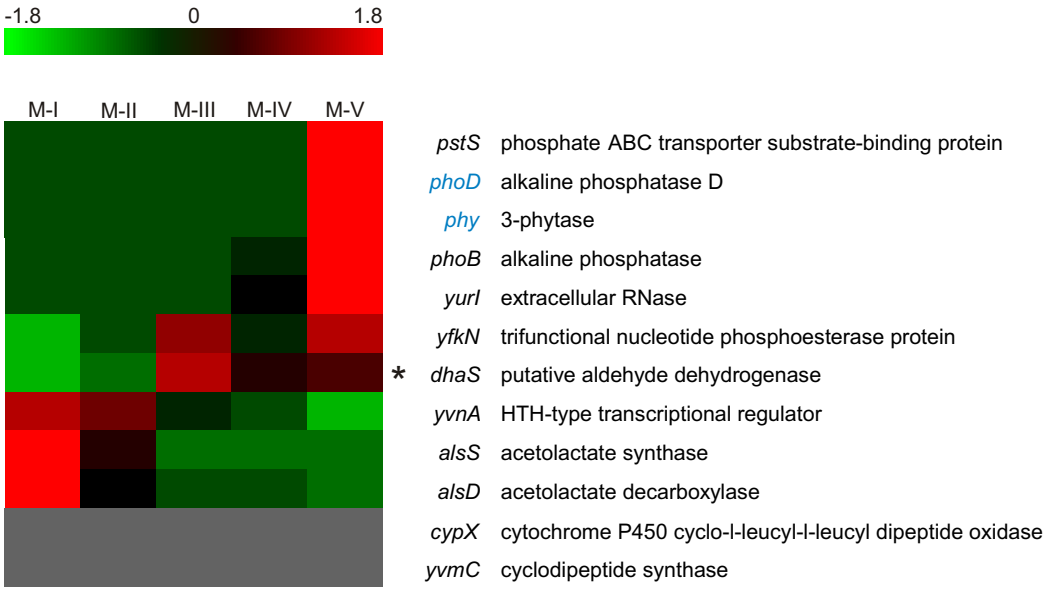


Figure S14 Phosphate starvation response. Heat map representation of Z-score transformed NPKM values. The depicted genes have been identified as marker genes for the *B. licheniformis* phosphate starvation response by Hoi et al. [27]. The figure shows the transcript abundances of those marker genes within the fermentation samples of one replicate (replicate M). Genes with an assigned antisense RNA [1] are marked in blue; asterisks indicate a detected protein spot for the respective gene Additional File 2: Table S3. Genes with NPKM values <10 at all sampling points are indicated by dark grey boxes.

At sampling point V, transcripts of *pstS* and the downstream *pst* operon encoding a phosphate ABC transporter (see also Figure 3), but also of *phy*, *phoB*, *phoD*, and *yfnK* are highly abundant. All of the mentioned genes are members of the Pho regulon, also known to provide a specific phosphate starvation stress response in *B. subtilis* [28, 29]. Furthermore, *yurI* and *dhaS* abundant as described by Hoi et al [27], supporting the inference of a phosphate shortage in this sample. However, no corresponding transcriptional response could be observed for the other fermentation samples (L and R) of sampling point V. This disparity coincides with an increased partial pressure of oxygen, starting ~1 h ahead of sampling point M-V (Additional File 1: Figure S1), which indicates a decreased metabolic activity in this sample at this fermentation stage. At the same time, the genes *yvnA*, *alsD,* and *alsS* show no increased transcript abundance. This is in accordance with the results obtained by Hoi et al. [27], who observed no induction of these genes in the early phases (~2 h) of declining metabolic activity after phosphate exhaustion. In addition, transcripts of genes involved in metabolic pathways, chemotaxis and especially teichoic acid synthesis were lacking, as also shown before [27]. The transcript abundance of the teichuronic acid synthesis operon (*tuaABCDEFG*), which enables the replacement of phosphate-rich teichoic acids, has been described as additional indicator of phosphate starvation in *B. subtilis* [28, 30] and could also be observed in sample M-V (data not shown). In summary, these results indicate that fermentation M is under phosphate limitation during the latest stage of the process. This finding might be a target for bioprocess optimization aiming at the prolongation of the fermentation process.

Figure S15 Putative Tat signal peptide of Subtilisin Carlsberg. (A) Tat signal peptides contain a conserved twin-arginine motif, defined as SRRXFLK, in which the consecutive arginine residues are almost always invariant [31]. Nevertheless, one arginine residue (§), but not both, can be replaced by a lysine residue [32, 33]. The other motif residues occur with a frequency of >50%, whereas the amino acids at the # positions are usually hydrophobic and the amino acid at the X position is polar [31, 34]. **(B)** The signal peptide of Subtilisin Carlsberg matches the Tat model motif with the exception of two hydrophilic residues in the middle positions, a condition not considered an exclusion criterion for a putative Tat signal peptide motif [35, 36]. In Sec signal peptides, helix breaking glycine or proline residues are often found in the middle of the hydrophobic domain following the N-terminal domain [37]. These are not found in *B. subtilis* Tat signal peptides [36] and also not in the signal peptide of Subtilisin Carlsberg.

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