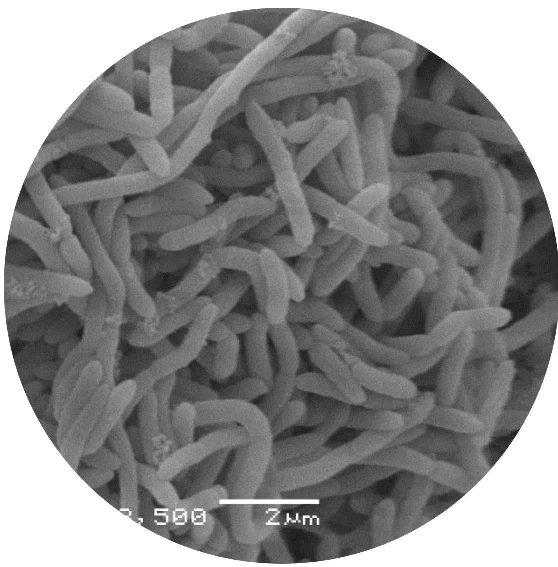
Analysis of the Transcriptional Response to Butanol Challenges during Acidogenic Continuous Culturing of *Clostridium acetobutylicum*

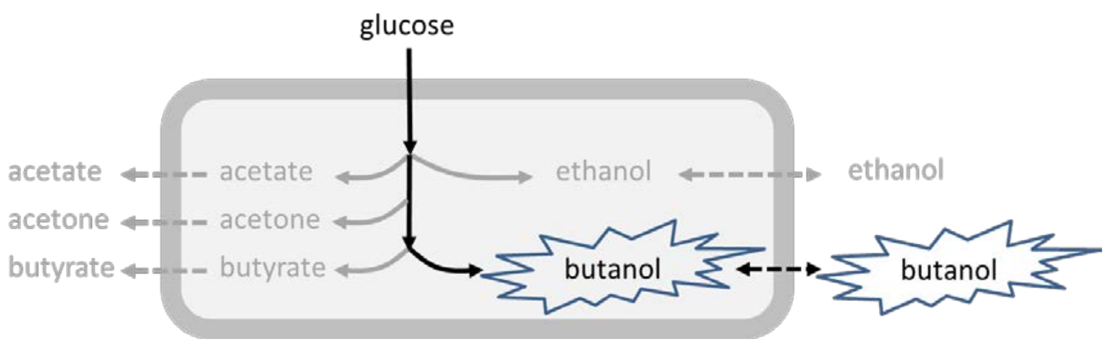
September 12, 2012, Servé Kengen



WAGENINGEN UNIVERSITY
WAGENINGEN UR

Butanol toxicity

- Intolerance to butanol is major factor that hampering cost-effective production



- Chaotropic effects → increase fluidity → ion leakage
- Inhibition of transport of nutrients, sugars, amino acids, ATPase activity

Tackle toxicity:

- Improving *in situ* butanol removal (DSP techniques)
- Raising the butanol tolerance
  - Classical screening for tolerant strains
  - Metabolic engineering (random/rational approaches)

We need knowledge about the genes involved in butanol tolerance and their regulation.

Previous research

2003 Tomas et al.: overexpression of groESL operon caused higher tolerance
2004 Tomas et al.: transcriptional analysis of WT and mutant (0.25% and 0.75% butanol)
2004 Alsaker et al.: transcriptional analysis of spo0A modified strains
2007 Borden et al: random genomic library approach
2010 Alsaker et al.: gene expression under butanol, acetate, butyrate
2010 Janssen et al.: proteomics and transcriptomics of acidogenic/solventogenic steady states
2010 Mao et al.: membrane proteome of WT and butanol tolerant strain
2011 Grimmler et al.: transcriptomic analysis of the acidogenic/solventogenic swith
2011 Mao et al.: proteomics of WT and butanol tolerant strain
2012 Jia et al.: two adjacent genes found to affect butanol tolerance (CAC1493/1494 ortholog)
2012 Mann et al.: overexpression of *groESL*, *grpE*, *htpG* improved tolerance

Previous research

2003 Tomas et al.: overexpression of groESL operon caused higher tolerance
2004 Tomas et al.: transcriptional analysis of WT and mutant (0.25% and 0.75% butanol)
2004 Alsaker et al.: transcriptional analysis of spo0A modified strains
2007 Borden et al: random genomic library approach
2010 A
2010 Ja
2010 M
2011 G
2011 M
2012 Ji
2012 M

- Overexpression of general stress proteins encoding genes
- Overexpression of solventogenic genes / butyrate forming genes
- Repression of fatty acid biosynthesis /glycolysis / sporulation genes
- Glycerol-3-P related genes upregulated
- Overexpression of genes gave higher tolerance (CAC1869/CAC0003)(CAC1493/1494)

Butanol stress response is complex.
Multiple synergistic effects are responsible for the improved solvent tolerance

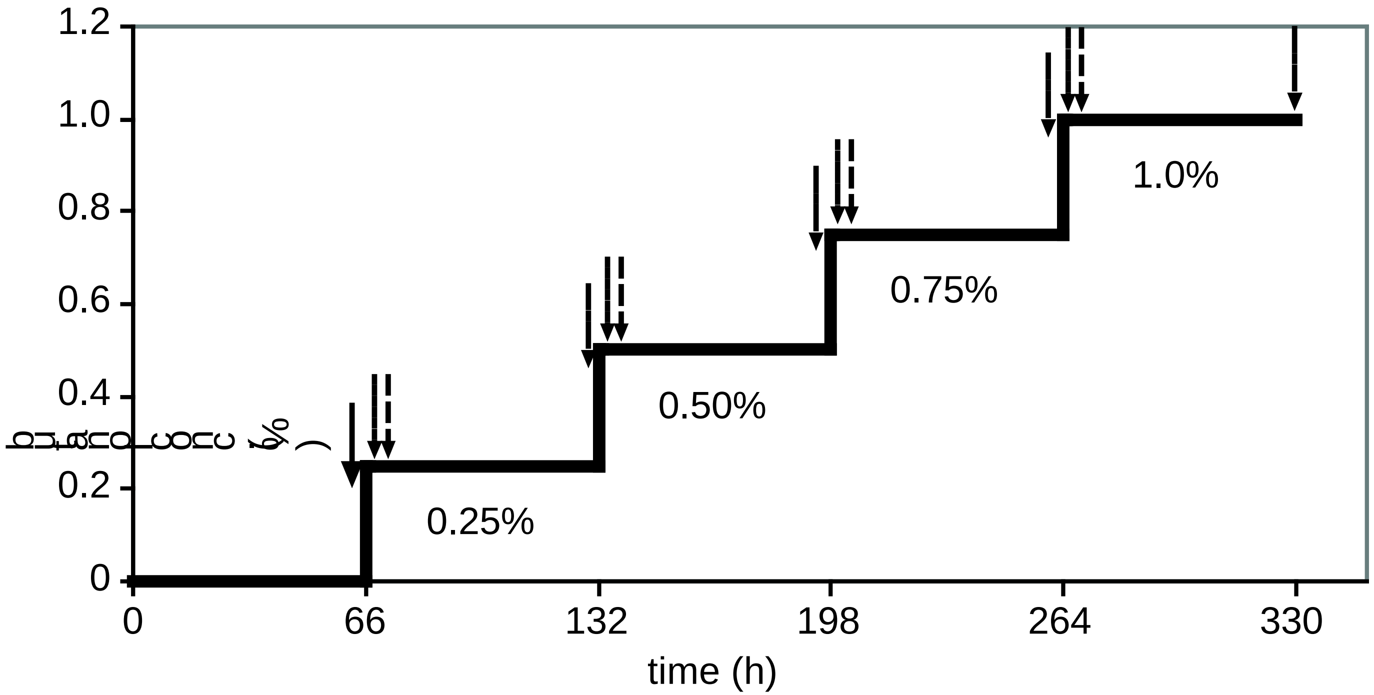
New setup

COSMIC consortium (SySMo):
All partners used the same pH-controlled phosphate-limited chemostat system and the same *Clostridium acetobutylicum* strain ATCC824 (COSMIC strain)

pH 6.0
D= 0.075 h^{-1}
37°C
4% glucose
minimal medium
[Pi] = 0.5 mM

Enabled us to analyse the specific butanol response, with minimum background butanol formation, and uncoupled from solvent formation which occurs at low pH

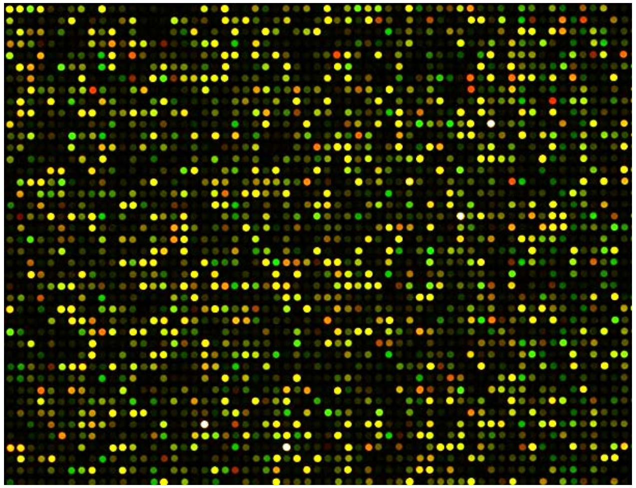
New setup



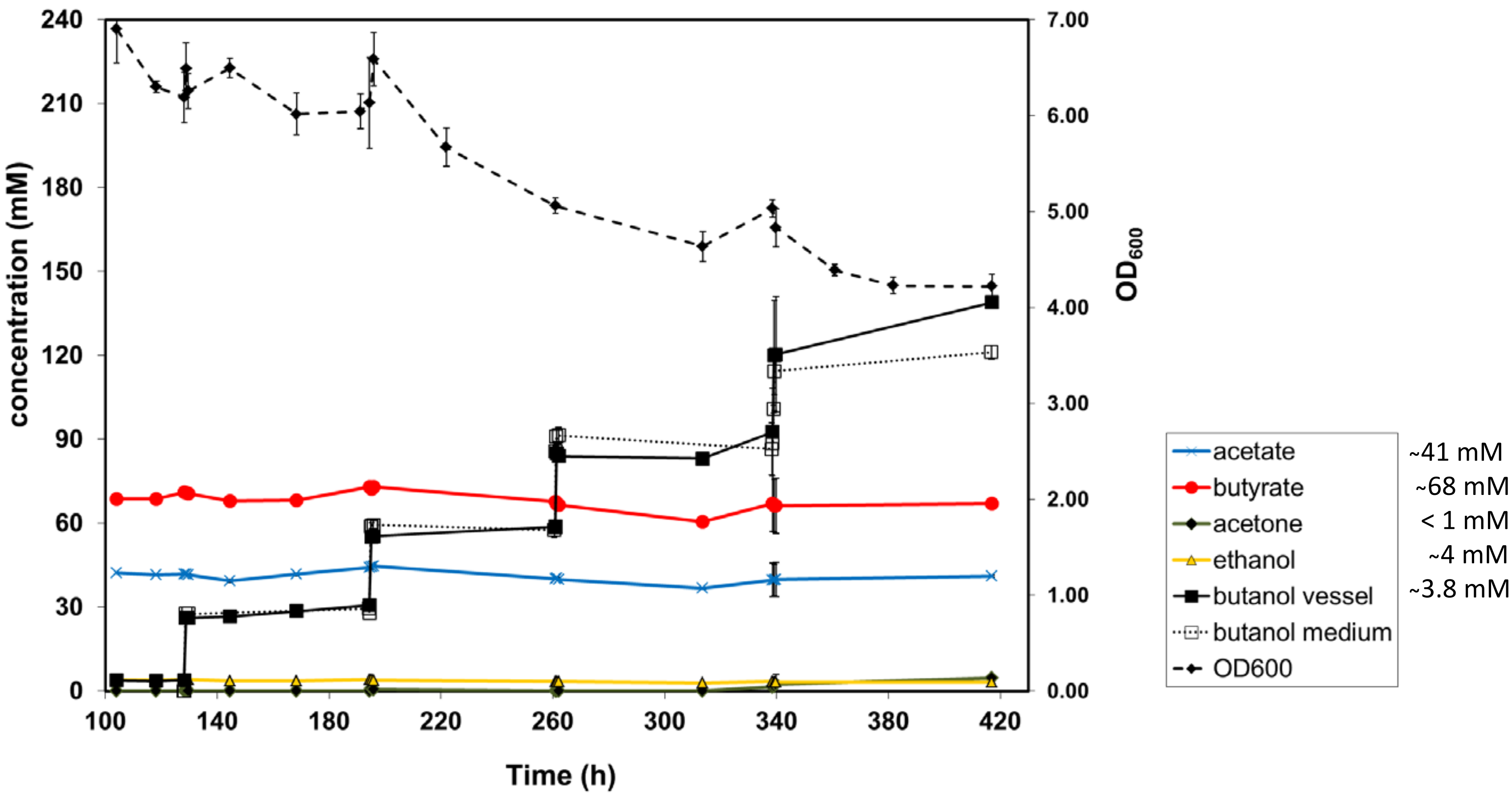
- Stepwise addition of butanol (0.25%, 0.50%, 0.75%, 1.0%)
- Samples for analysis of fermentation and RNA taken after 0.25h, 1 h and 66h
- Growth followed by OD_{600} measurements

Microarrays

- Two independent biological replicates
- Common reference design (0% butanol as common reference)
- Significant up- or downregulation: expression ratio >3 or < 0.33, respectively



Results – chemostat culturing



No significant changes in fermentation pattern

Culture remains in acidogenic phase

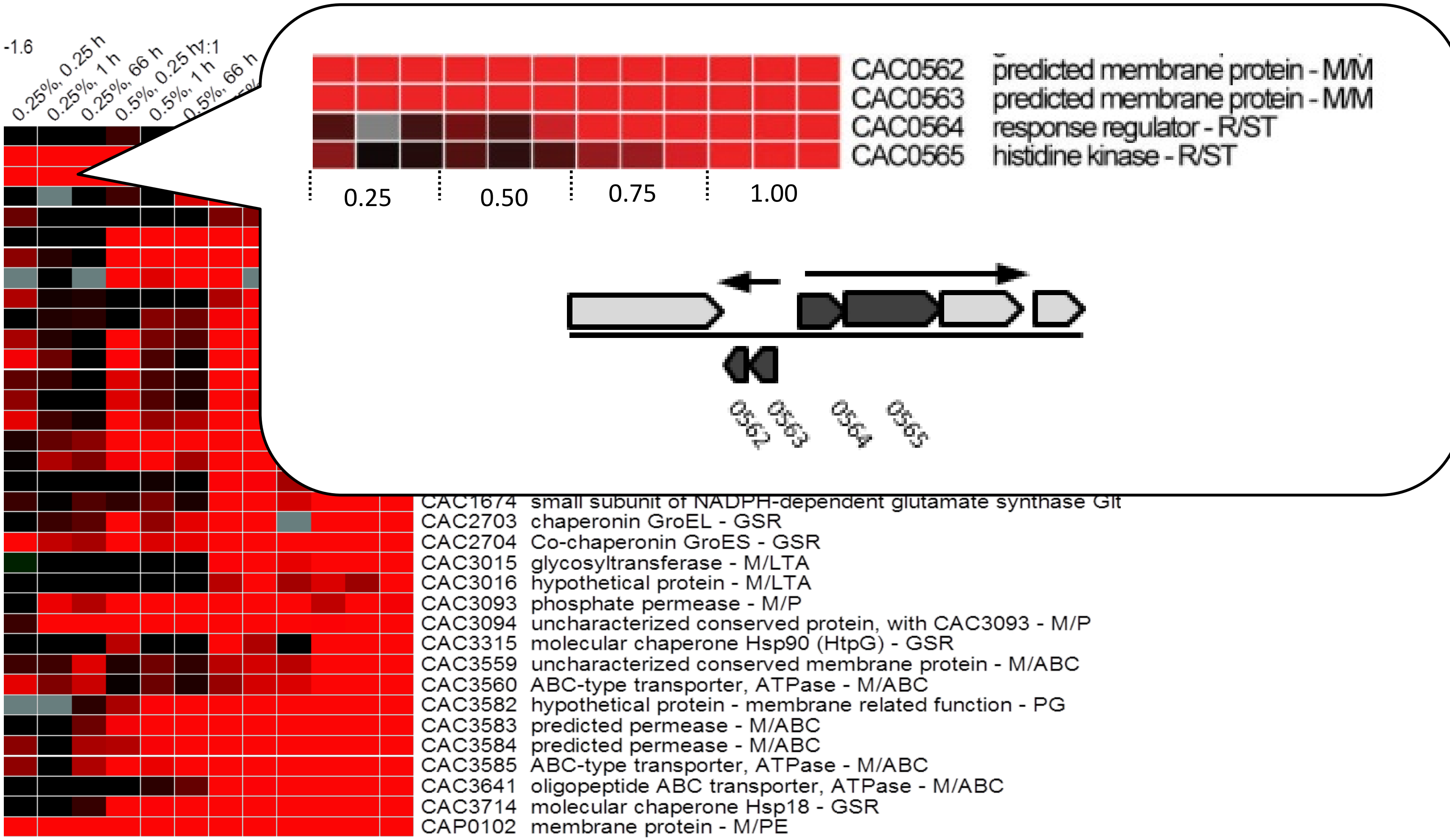
OD_{600} decreases → ATP used for dealing with stress

Results – transcriptome analysis

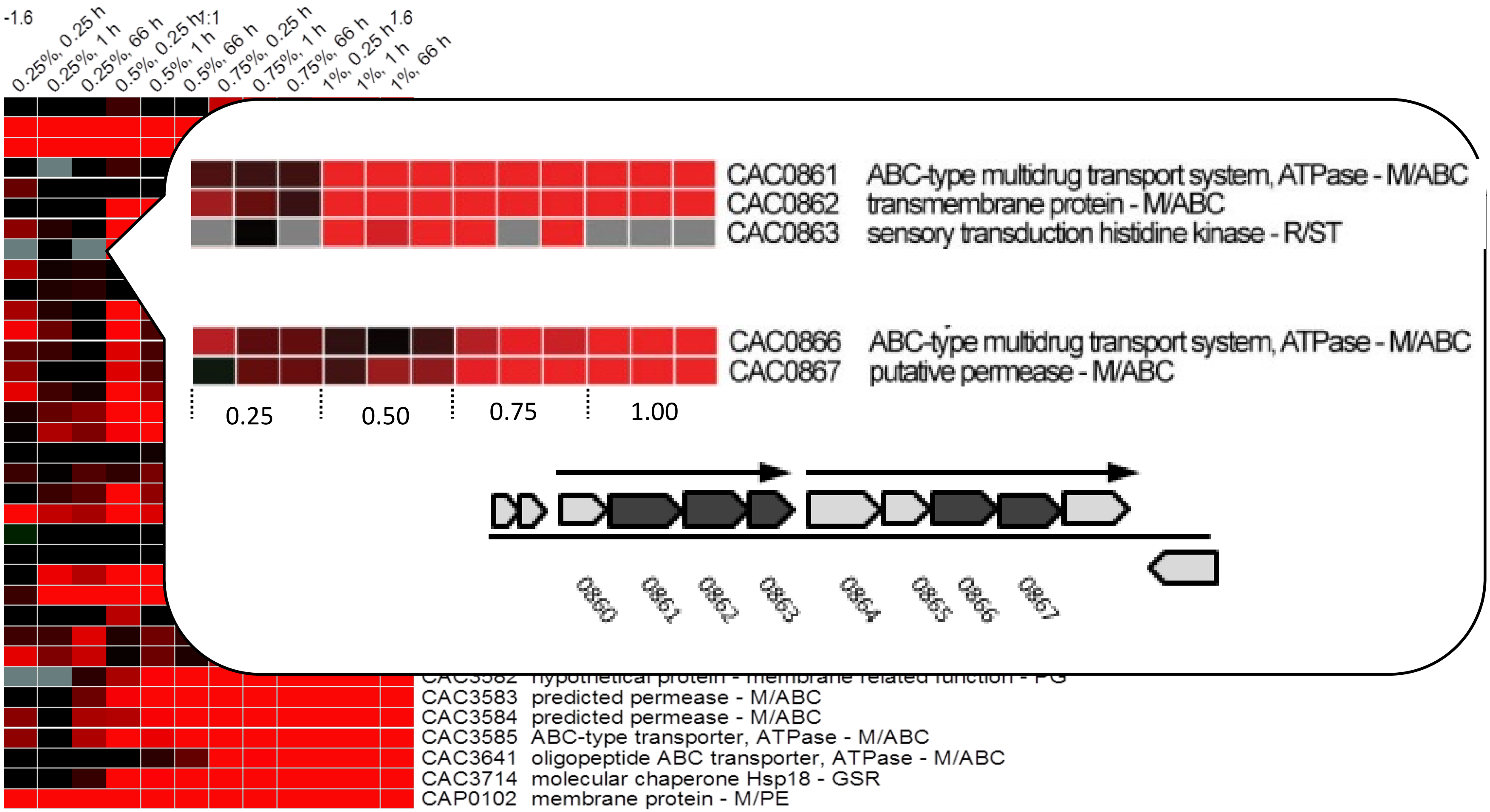
- 132 genes (35 up; 97 down) considered significantly up- or downregulated
- Only 13 were located on het pSOL1 plasmid (1 up; 12 repressed)
- Spread over the genome; often clustered in putative transcriptional units (Paredes)
- Genes grouped according to function:

functional group		upregulated genes	repressed genes
membrane associated	M	20	36
general stress response	GSR	8	-
regulation	R	4	4
glutamate/glutamine metabolism	GG	2	-
hypothetical proteins	HP	-	14
hydrolases	H	-	11
oxidoreductases	OR	-	7
cofactor synthesis	CF	-	6
carbohydrate metabolism	C	-	5
defense mechanism	D	-	5
peptidoglycan	PG	1	3
bacterial cyclic lipopeptide metabolism	BCL	-	2
sporulation	S	-	2
nucleotide metabolism	N	-	1
polyketide metabolism	PK	-	1

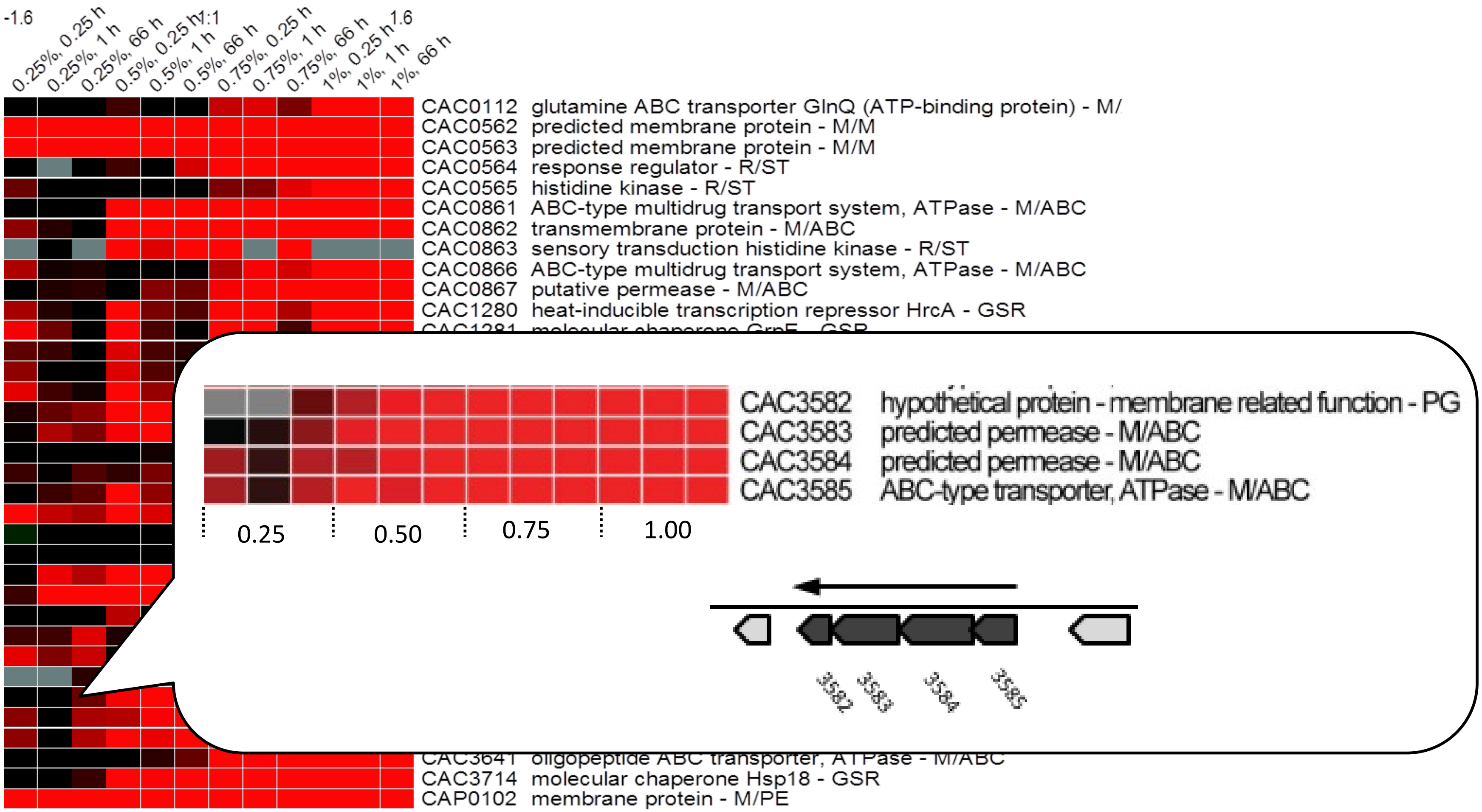
Results – transcriptome - upregulated genes



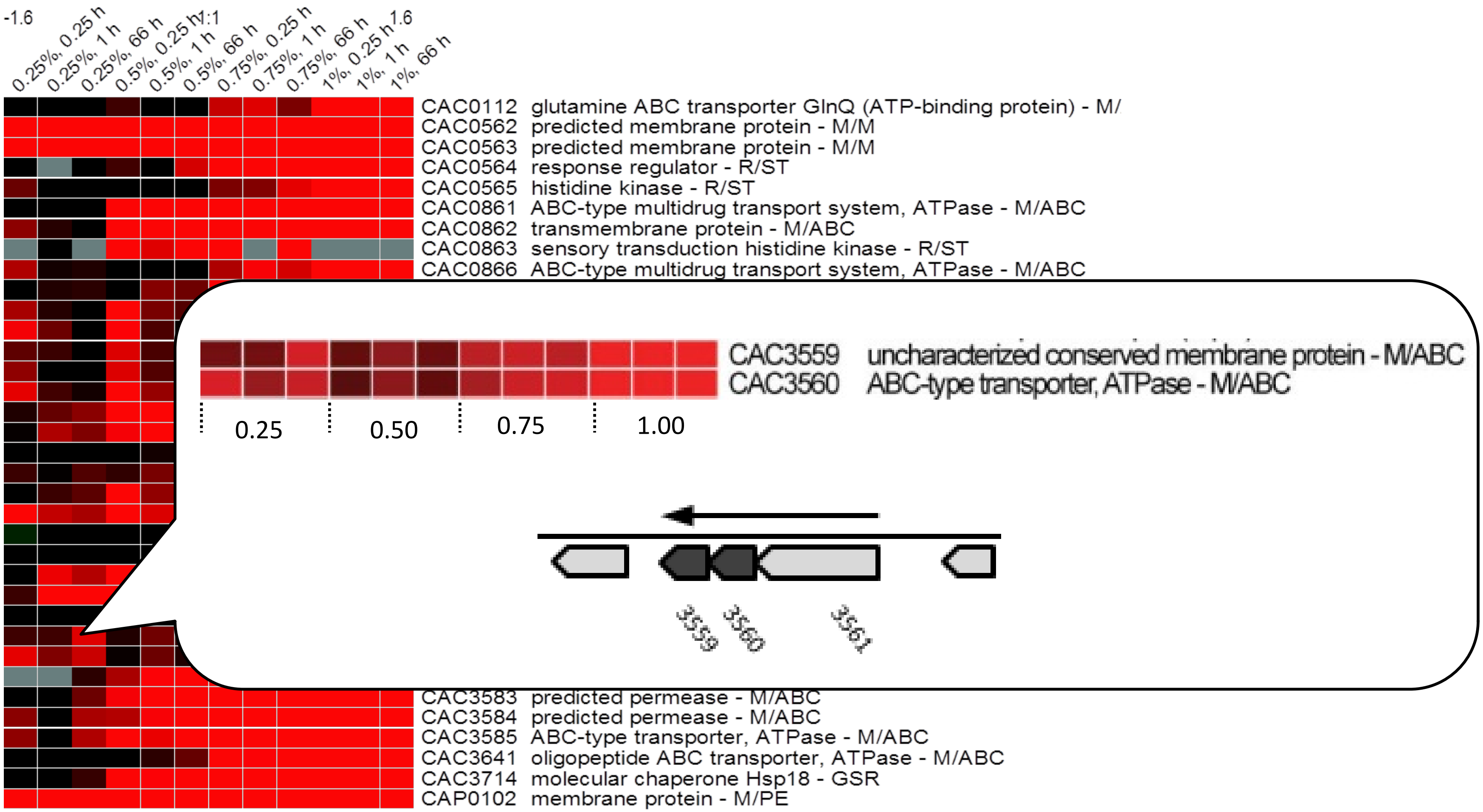
Results – transcriptome - upregulated genes



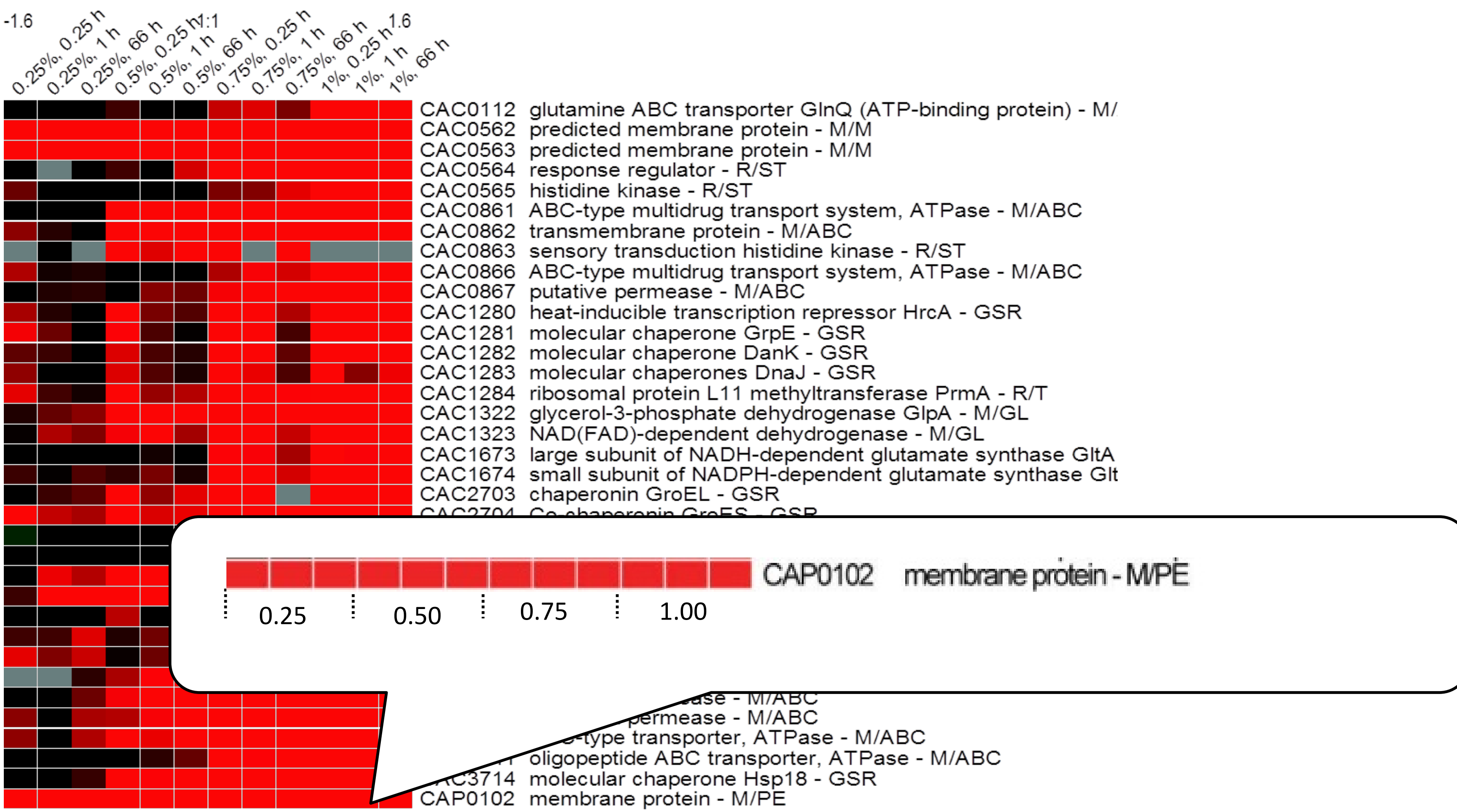
Results – transcriptome - upregulated genes



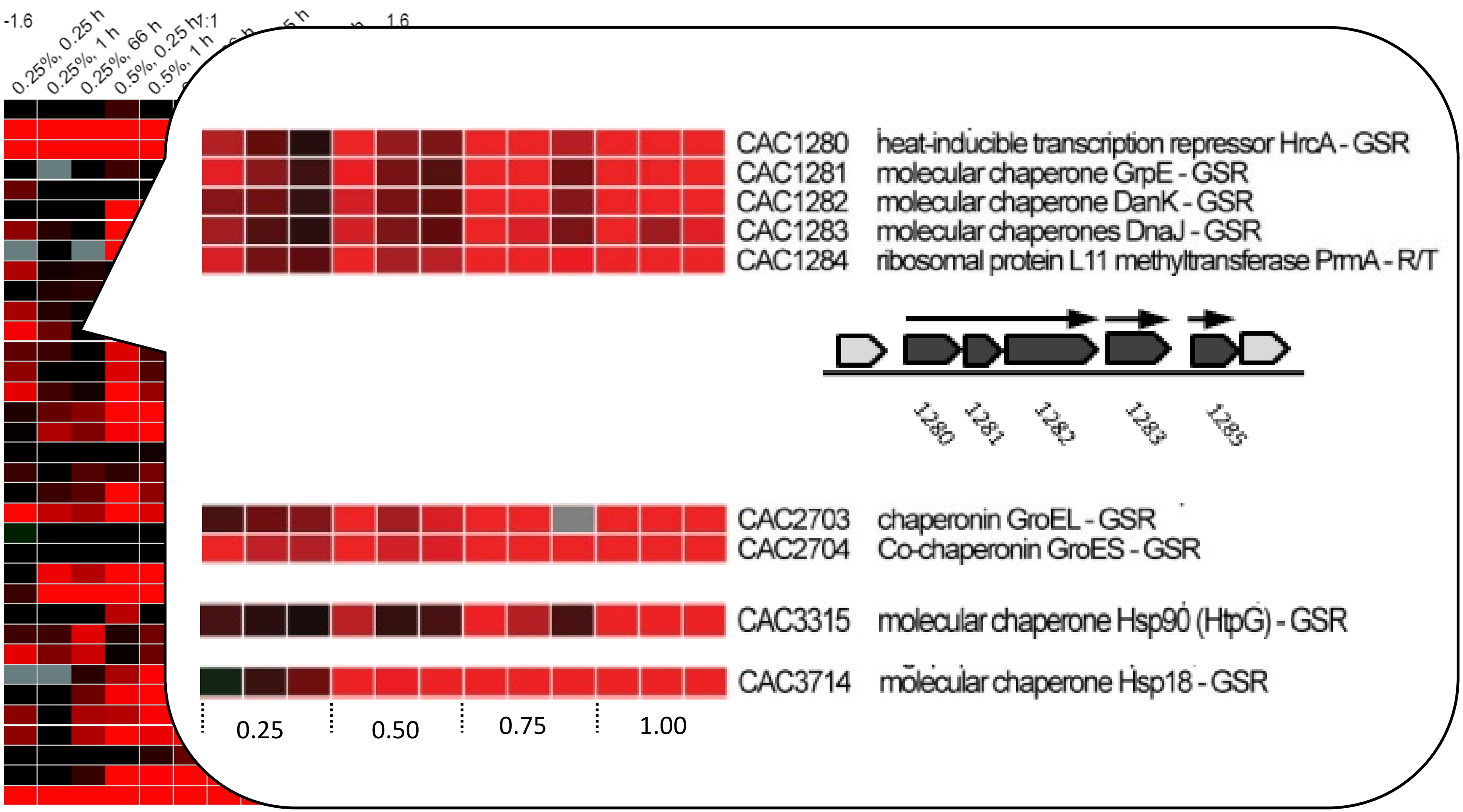
Results – transcriptome - upregulated genes



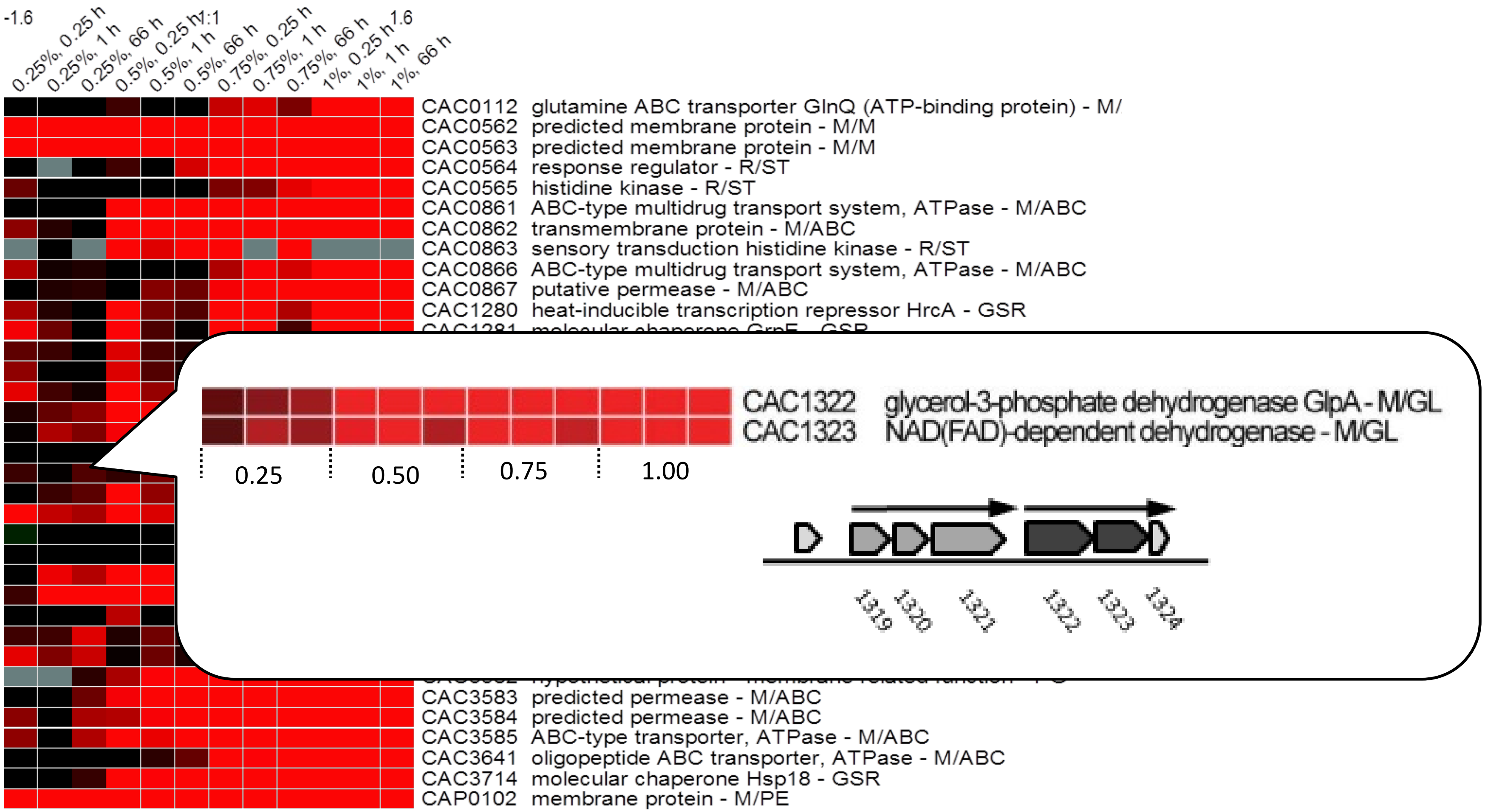
Results – transcriptome - upregulated genes



Results – transcriptome - upregulated genes

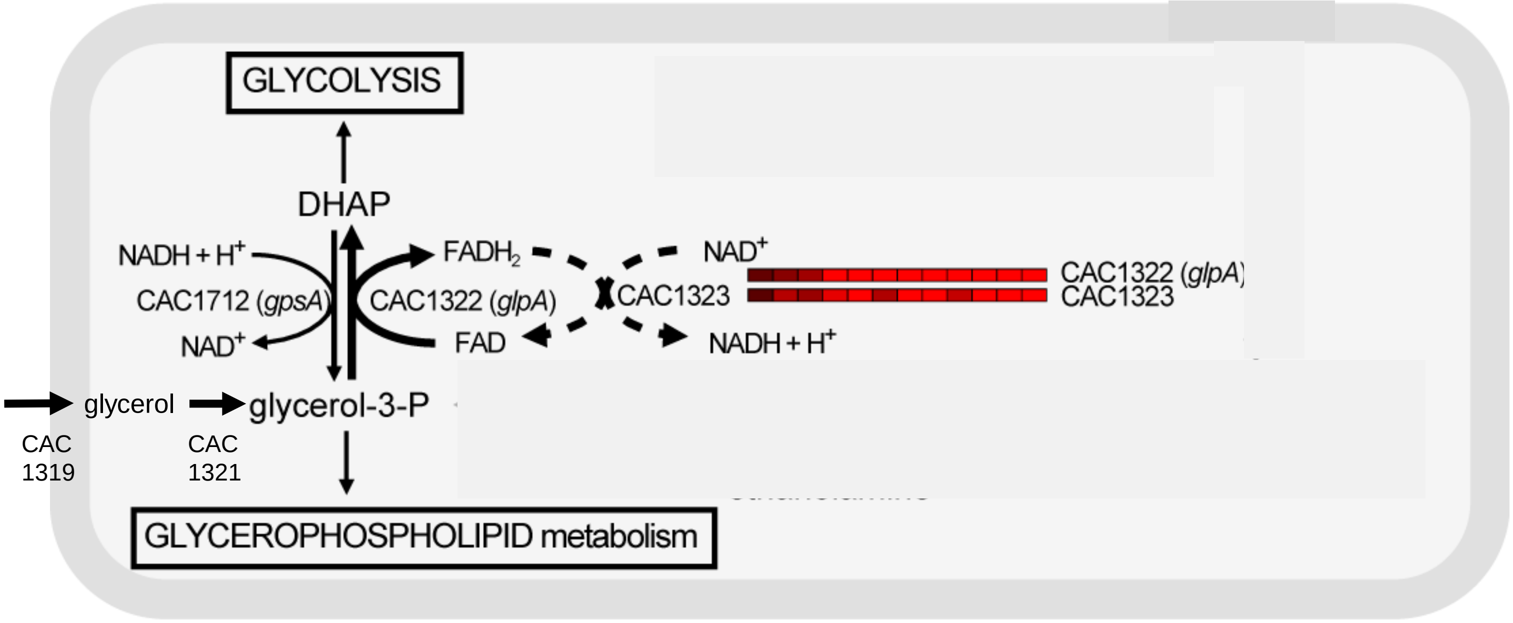


Results – transcriptome - upregulated genes

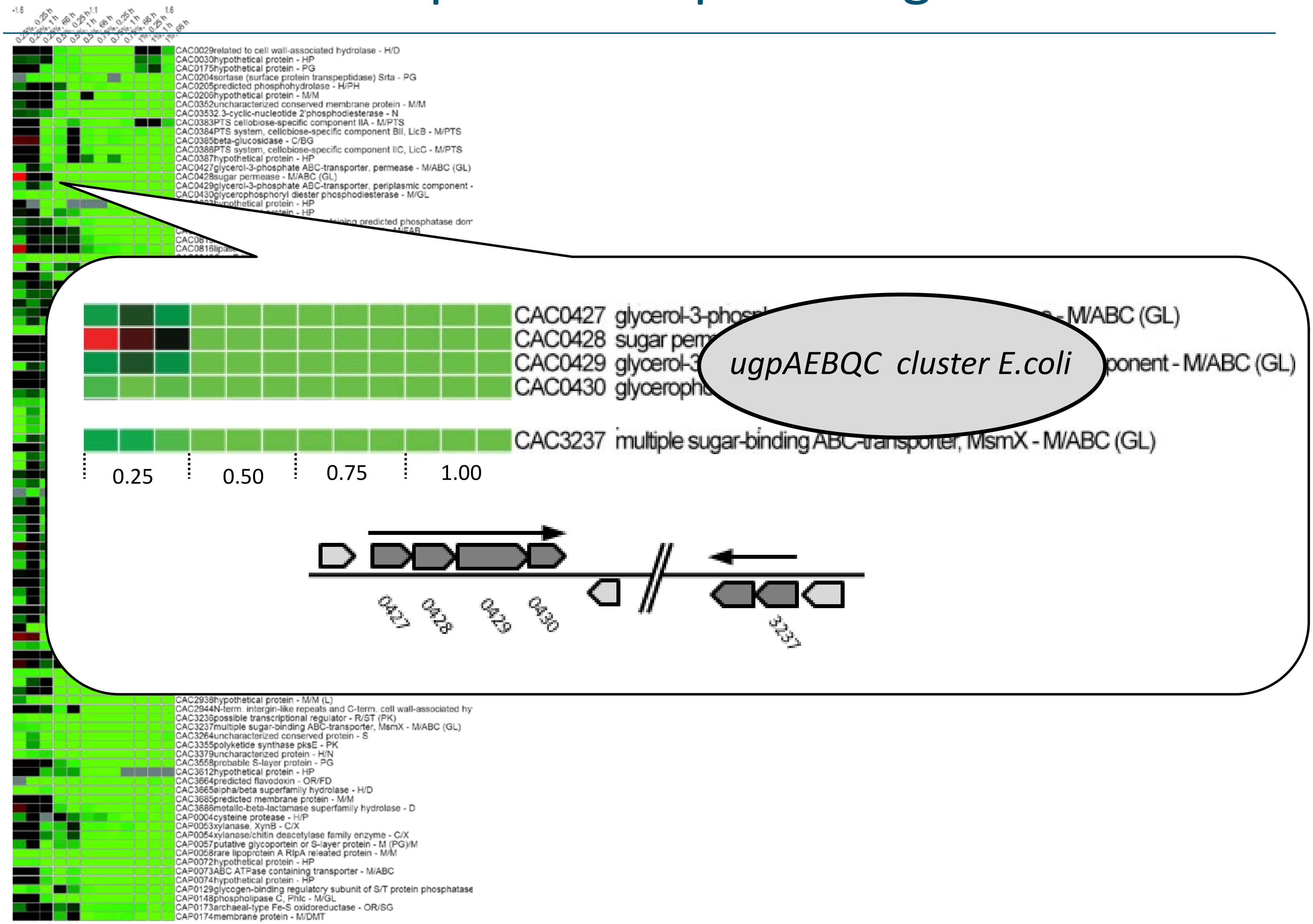


Results – transcriptome – Glycerol-3-P metabolism

Glycero(phospho)lipid metabolism

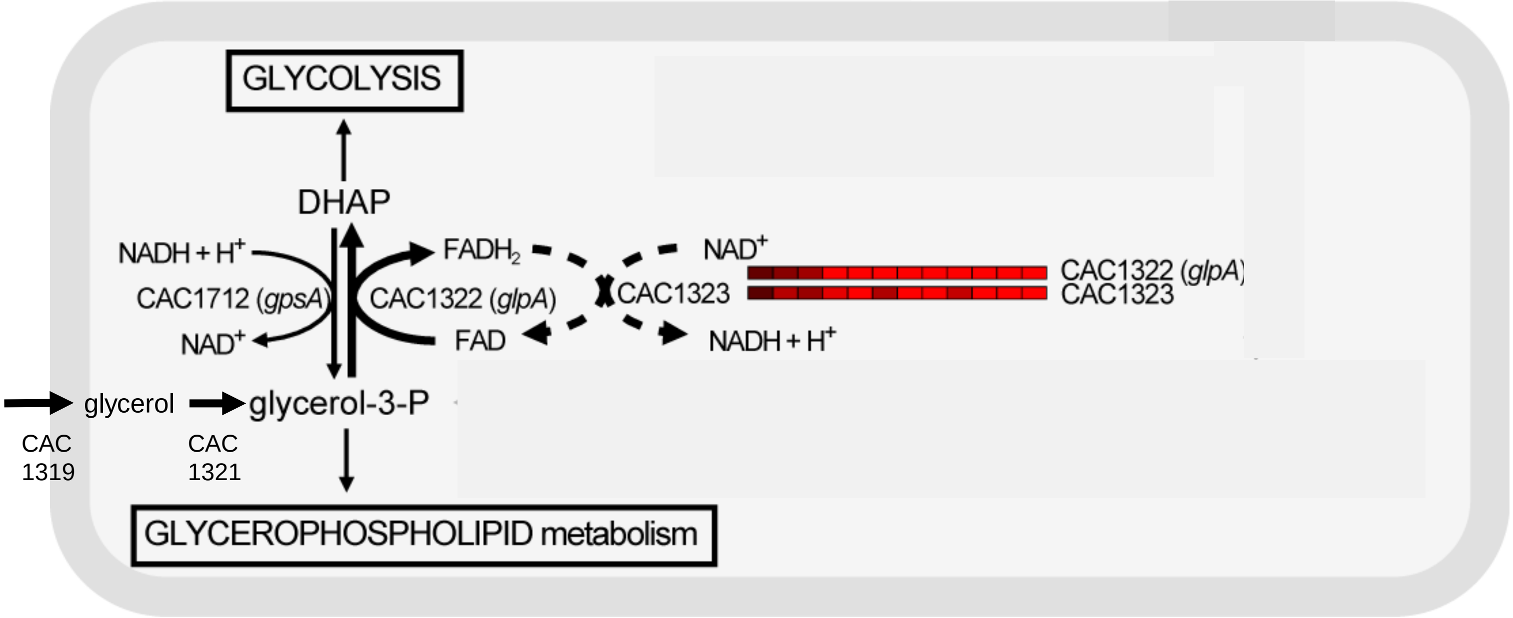


Results – transcriptome - repressed genes



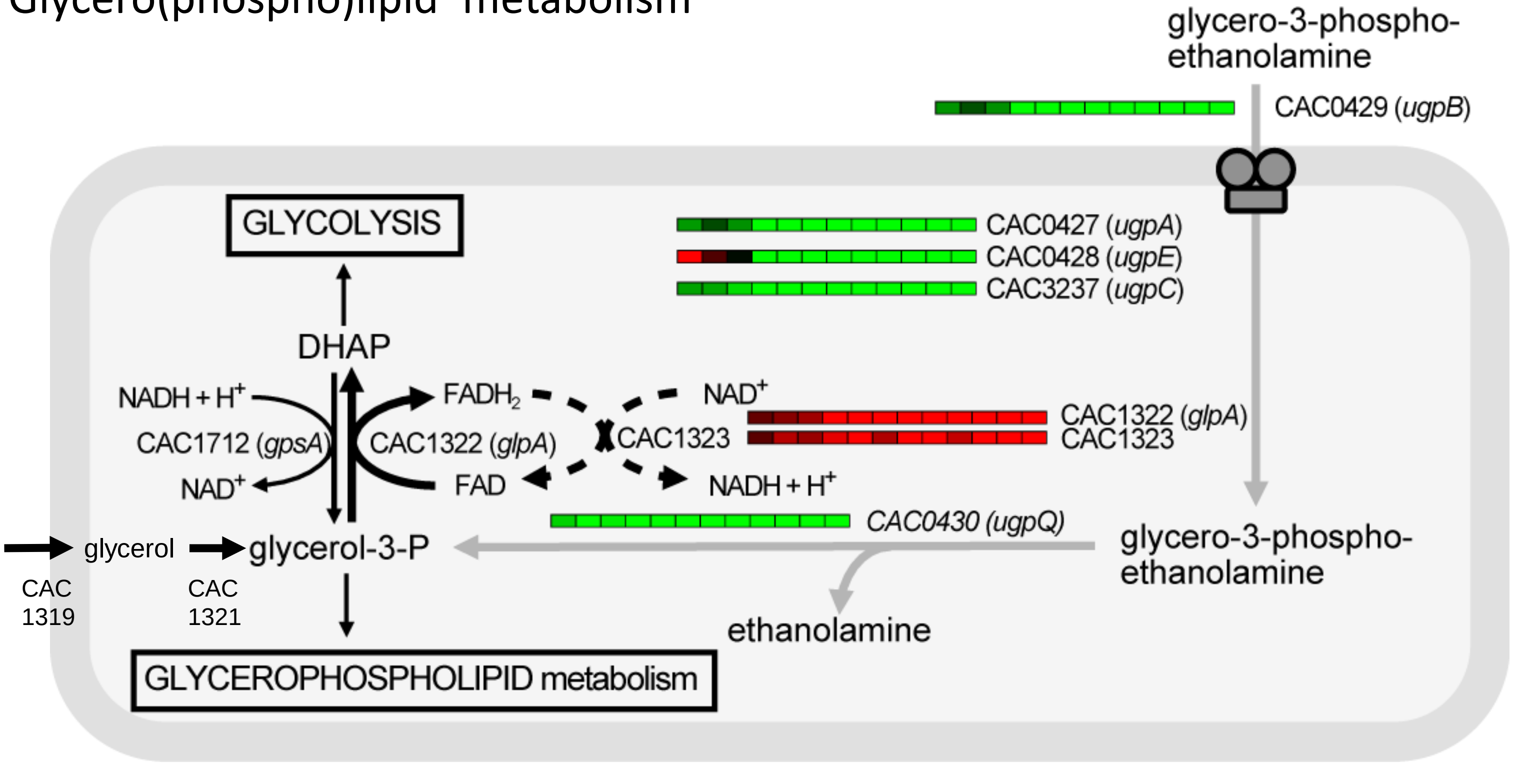
Results – transcriptome – Glycerol-3-P metabolism

Glycero(phospho)lipid metabolism

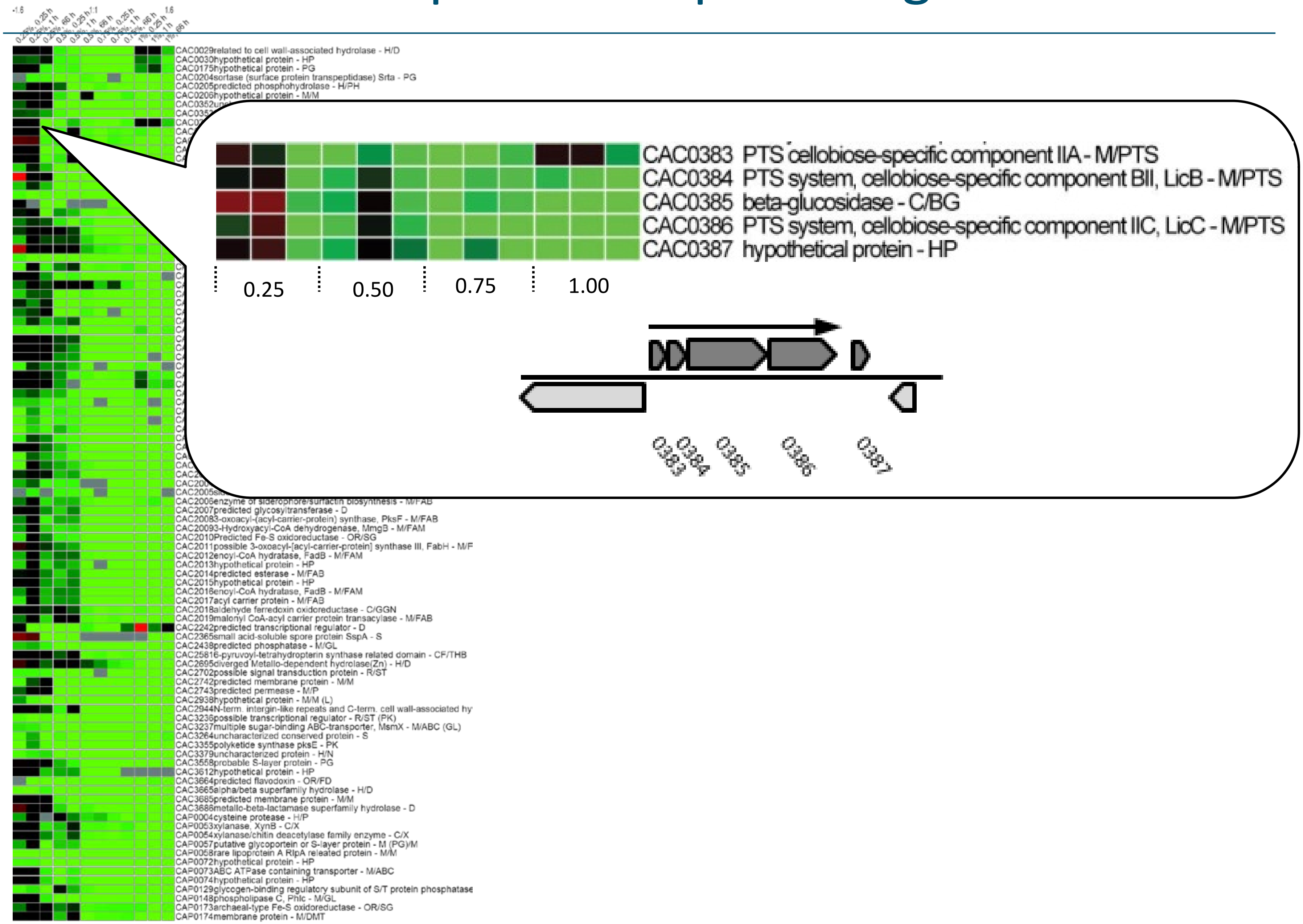


Results – transcriptome – Glycerol-3-P metabolism

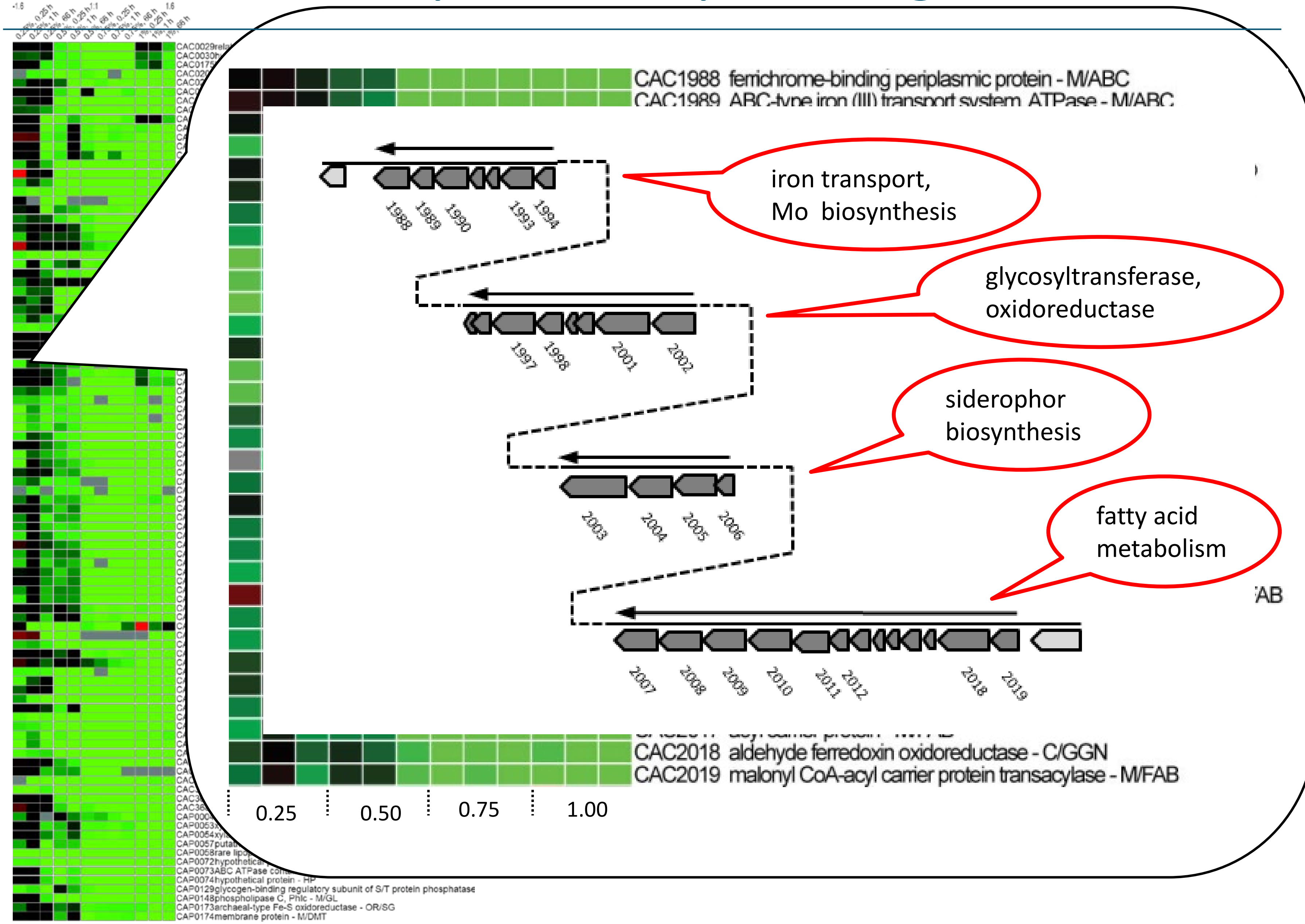
Glycero(phospho)lipid metabolism



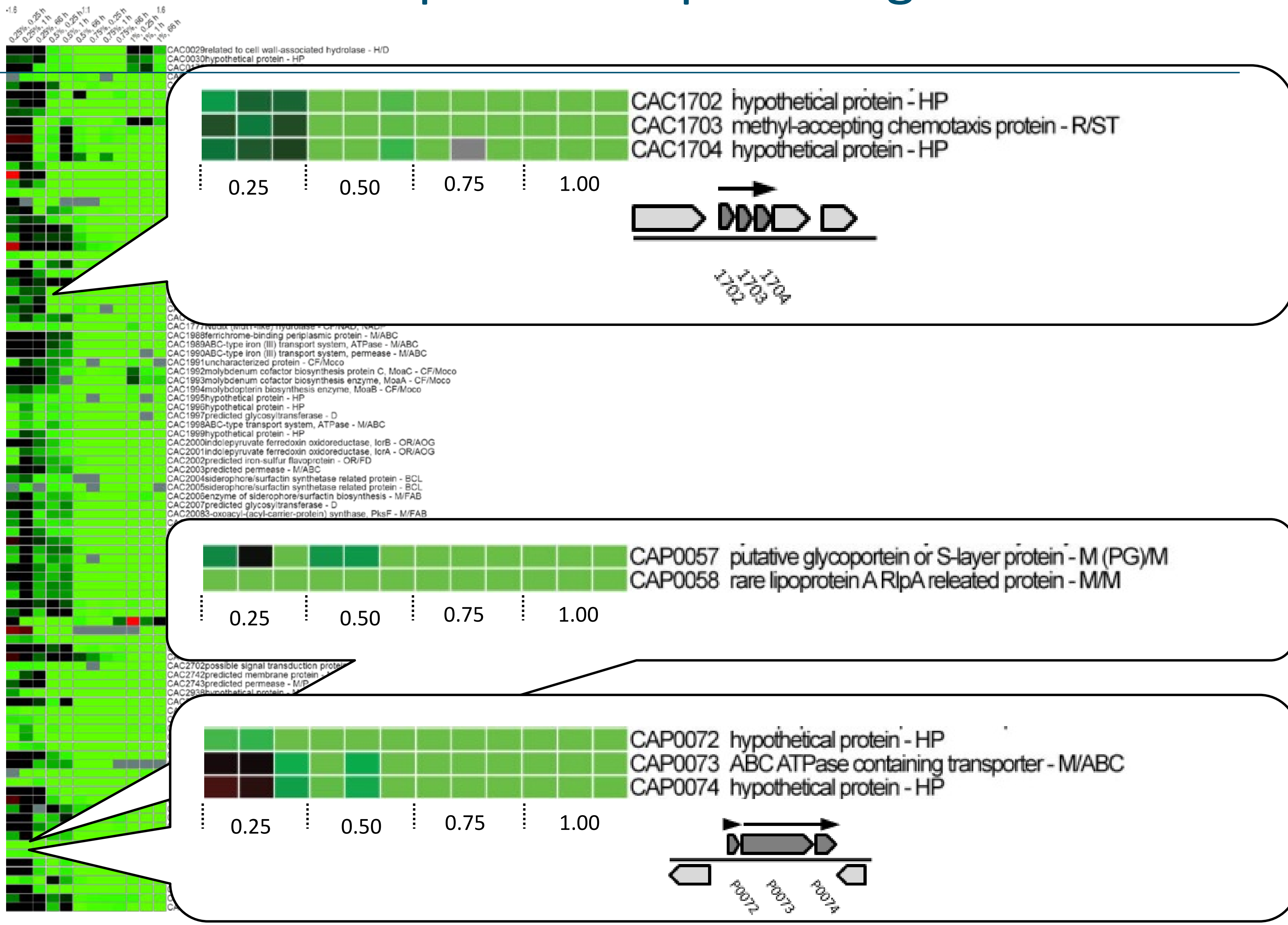
Results – transcriptome - repressed genes



Results – transcriptome - repressed genes



Results – transcriptome - repressed genes



Results – transcriptome – non affected genes

- No significant changes in the glycolytic or solvent genes
- No class IV hsp genes (clp- lon-family proteases, htrA)
- Genes previously shown to increase tolerance were not diff. expressed
  - CAC1869, CAC0003, CAC0977, CAC1463, CAC2495, (Borden *et al.* 2007)
  - CAC1493, CAC1494 (Jia *et al.* 2012)

Results -papers

Journal of Biotechnology 161 (2012) 366–377

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

A transcriptional study of acidogenic chemostat cells of *Clostridium acetobutylicum* – Cellular behavior in adaptation to *n*-butanol

Katrin M. Schwarz[a,b], Wouter Kuit[a,b], Christina Grimmler[c], Armin Ehrenreich[c,d], Servé W.M. Kengen[a,*]

Journal of Biotechnology 161 (2012) 354–365

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

A transcriptional study of acidogenic chemostat cells of *Clostridium acetobutylicum*—Solvent stress caused by a transient *n*-butanol pulse

Holger Janssen[a], Christina Grimmler[b], Armin Ehrenreich[b,c], Hubert Bahl[a], Ralf-Jörg Fischer[a,*]

Conclusions

Butanol does not affect the fermentation pattern under controlled culturing

Butanol does not affect the expression of glycolytic and solvent genes

Growth is decreased, presumably because more ATP is needed in stress response

Upregulation of general stress genes (not class IV)

Upregulation of genes involved in multi drug resistance (efflux pumps)

Genes involved in altering the membrane composition

Response most profound at 0.5% butanol; important treshold

Two-phase response: instant general stress response within 0.25 h
and a long term adaptation response

Acknowledgements

Wageningen University

Katrin Schwarz

Wouter Kuit

Technische Universität München

Christina Grimmler

Armin Ehrenreich

COSMIC consortium

Netherlands Organisation for Scientific Research (NWO)