

Acclimation of *Saccharomyces cerevisiae* to Low Temperature: A Chemostat-based Transcriptome Analysis

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Outline

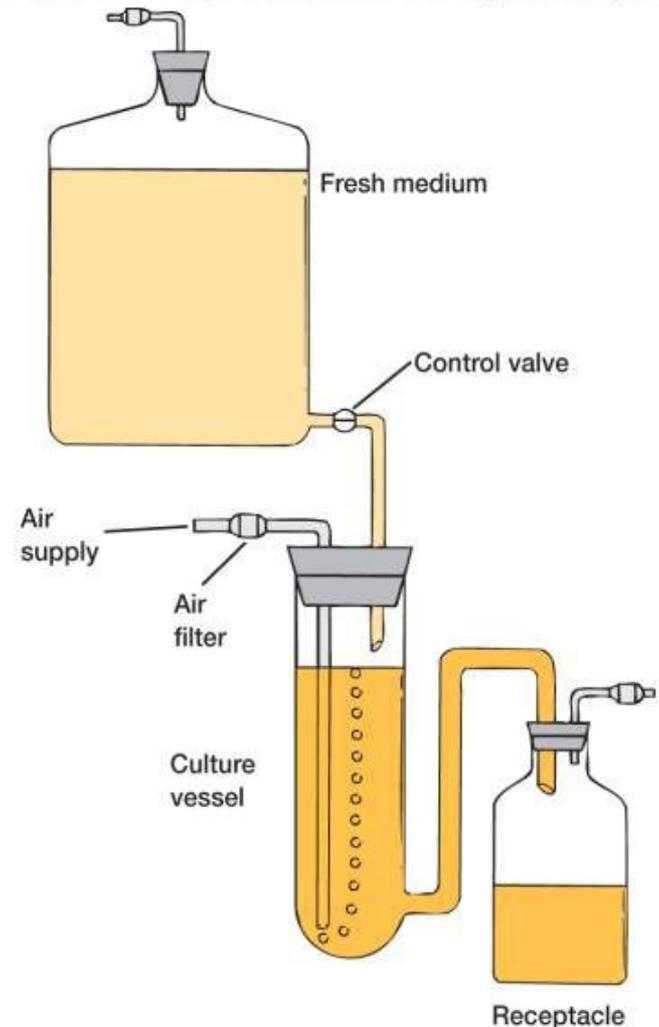
- **Limitations of previous studies led to development of experiment using chemostat culture and low temperature acclimation.**
- ***S. cerevisiae* was grown in four different experimental conditions.**
- **DNA microarray analysis was used to analyze growth limitations applied to *S. cerevisiae*.**
- ***S. cerevisiae* stores carbohydrates during chemostat culture for unknown reasons.**
- **Promoter analysis reveals regulation trends in gene regulation.**
- **Temperature response genes overlap in expression.**
- **The genes that are responsible for adaptation and acclimation are expressed separately.**
- **Environmental stimulation responses differed in batch vs. chemostat cultures.**

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Chemostat culture allows for fixed experimental conditions

- Chemostat culture involves the continuous addition of fresh medium.
- Allows for control of growth factors.
- Specific growth rate can be measured accurately.
- Batch culture is a closed system which does not allow for accurate measurement of specific growth rate.



Low-temperature acclimation and cold shock have differing results

- **Cold shock entails a rapid decrease to near freezing temperatures.**
- **Low-temperature acclimation is the biological response of cells to steady decreases to near freezing temperatures.**
- **Trehalose is required for transcription in low temperature situations.**
 - **Not present in low temperature acclimation results.**

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S. Cerevisiae grown in varying conditions of temperature and nutrient content

Limiting nutrient	Growth temperature (°C)	$Y_{Glu/X}$ ($g_{DW} \cdot g_{glucose}^{-1}$)	q_{Glu}^a	q_{EtOH}^a	$q_{CO_2}^a$	Carbon recovery (%)	Residual glucose (mM)	Residual ammonia (mM)
Glucose	12	0.07 ± 0.01	-2.5 ± 0.2	3.8 ± 0.3	4.4 ± 0.3	100 ± 3	2.8 ± 1.1	65.2 ± 2.2
Glucose	30	0.07 ± 0.00	-2.3 ± 0.0	3.5 ± 0.0	3.8 ± 0.2	95 ± 1	0.3 ± 0.1	61.3 ± 4.5
Ammonium	12	0.05 ± 0.00	-3.6 ± 0.2	6.1 ± 0.3	6.0 ± 0.6	97 ± 4	90.0 ± 9.8	1.5 ± 0.2
Ammonium	30	0.04 ± 0.00	-4.0 ± 0.1	6.8 ± 0.2	7.4 ± 0.2	97 ± 2	85.1 ± 8.2	0.2 ± 0.1

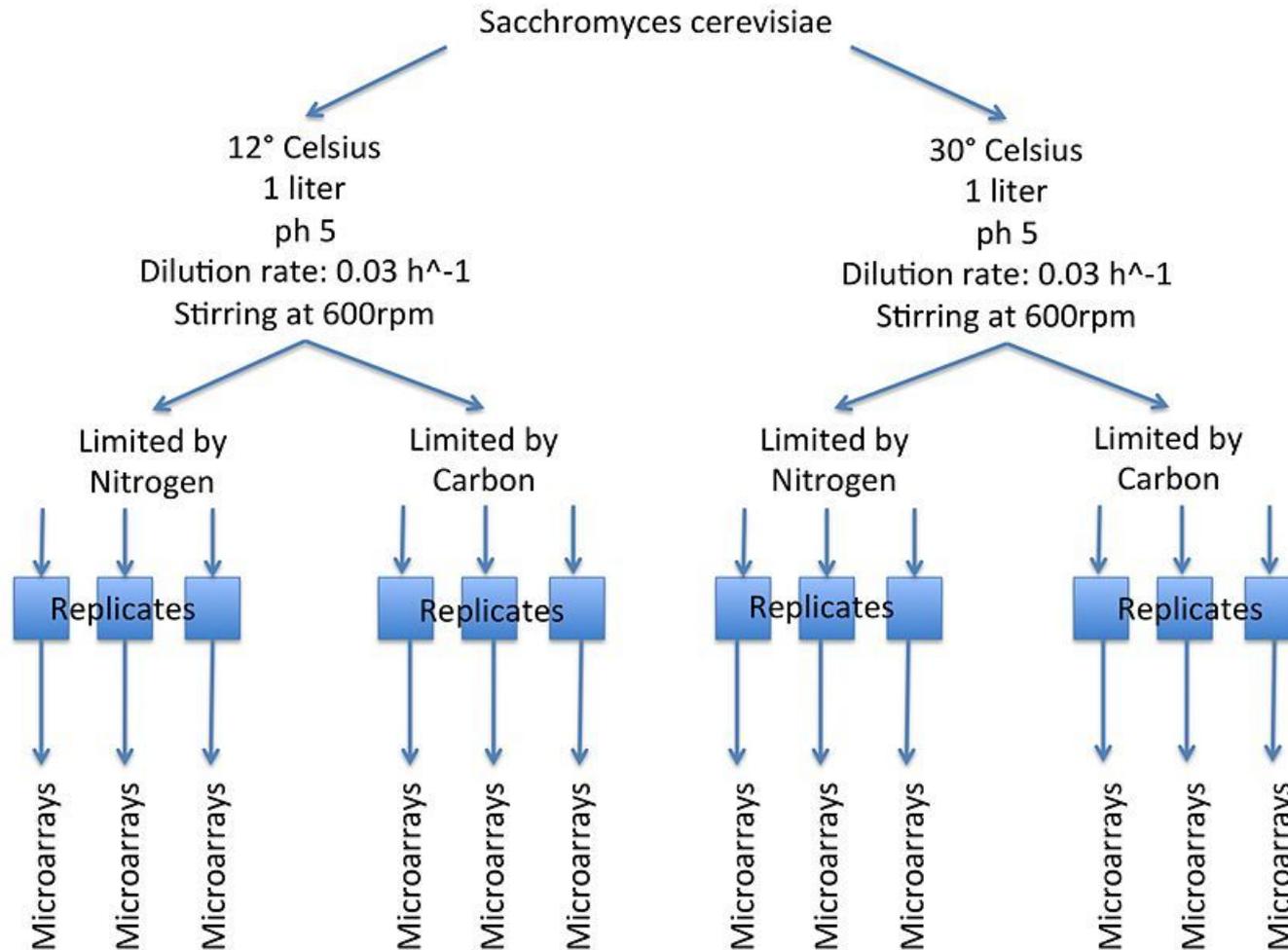
Cultures were grown at 30 and 12°C ($D = 0.03 \text{ h}^{-1}$). Values represent the mean \pm SD of data from three independent steady-state chemostat cultivations. $Y_{Glu/X}$, biomass yield on glucose; DW, dry weight.

^a Values expressed as $\text{mmol} \cdot g_{DW}^{-1} \cdot \text{h}^{-1}$.

Table 1

- *S. Cerevisiae* is minimally affected by variation in temperature.
- Higher temperatures correlated with lesser amounts of a limiting nutrient corresponding residual residue.
 - Larger amounts of residual glucose at 12°C connected to possible contamination of gene sets.

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Temperature and nutrient limitations were analyzed using DNA microarray analysis

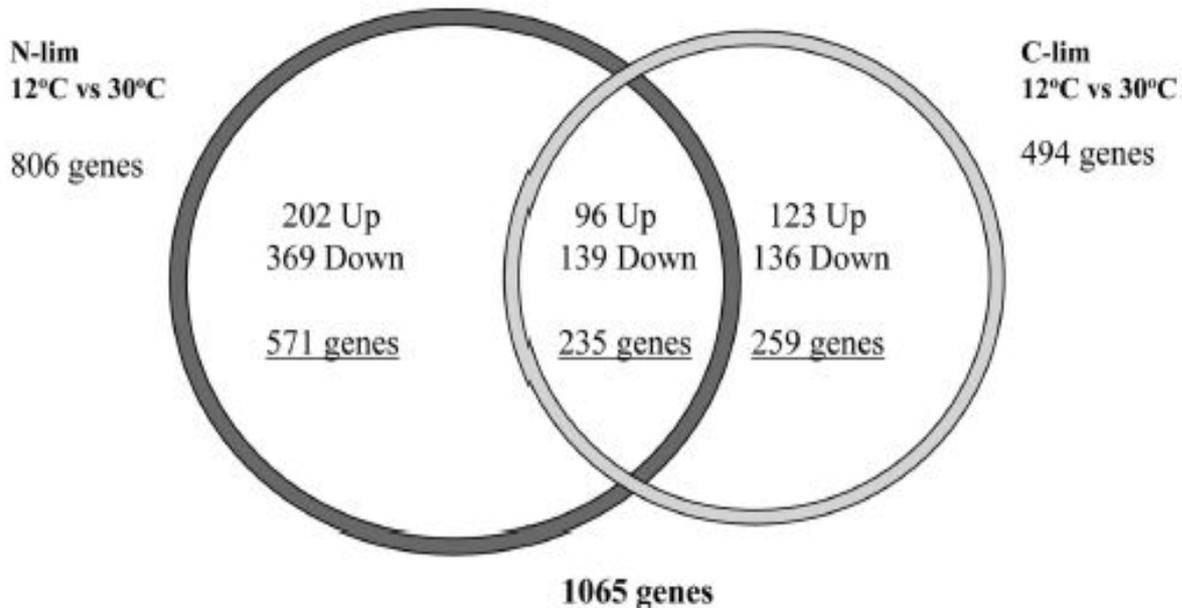


Figure 1

- **1065** represents the number of temperature responsive genes in *S. cerevisiae* genome.

Heat mapping was used to identify transcription level of temperature responsive genes

C-10h 30°C
C-10h 15°C
C-10h 10°C
N-10h 15°C
N-10h 10°C



- Goal was to identify regulatory networks involved in acclimation of *S. cerevisiae* to low temperature.
- Observed changes in
 - Transporters of limiting nutrient
 - Translational machinery
- NCR genes and catabolite repression

Figure 2

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S. cerevisiae stores protein and carbohydrate in chemostat culture

Limiting nutrient	Growth temperature (°C)	Biomass dry weight (g _{DW} · l ⁻¹)	Whole cell protein (g _{protein} · g _{DW} ⁻¹)	Biomass nitrogen content (mg _{nitrogen} · g _{DW} ⁻¹)	Trehalose (g _{equivalent glucose} · g _{DW} ⁻¹)	Glycogen (g _{equivalent glucose} · g _{DW} ⁻¹)
Glucose	12	1.71 ± 0.09	0.40 ± 0.01	nd	<0.005	0.06 ± 0.01
Glucose	30	1.89 ± 0.06	0.43 ± 0.01	nd	0.02 ± 0.00	0.04 ± 0.00
Ammonium	12	2.27 ± 0.05	0.47 ± 0.03	63 ± 3	<0.005	0.02 ± 0.00
Ammonium	30	3.53 ± 0.01	0.34 ± 0.01	41 ± 2	0.04 ± 0.00	0.05 ± 0.01

Cultures were grown at 30 and 12°C (D = 0.03 h⁻¹). Values represent the mean ± SD of data from three independent steady-state chemostat cultivations. DW, dry weight; nd, not determined.

Table 2

- Trehalose and glycogen biosynthesis occurs under stress conditions.
 - Biosynthesis varies with temperature.
- Reason for storage of carbohydrates in nonfreezing temperature is unclear.
 - Trehalose was not produced in *tps1* and *tps2* strains, yet did not affect viability.

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Promoter Analysis Reveals Regulation Trends in Gene Regulation

(A) 5' upstream cis-regulatory motif

Regulatory cluster	Motif name	Putative-binding protein	Promoter element	occ ^a	Expected occ ^b	occ E ^c
Low Temperature C-lim Up	—	—	—	—	—	—
Low Temperature C-lim Up	—	—	—	—	—	—
Low Temperature N-lim Up	—	—	TGAAAAA	206	113.04	2.30E-11
	PAC	—	CGATGAG	57	17.49	6.1E-14
	—	—	TGAGATG	49	16.3	4.1E-07
Low Temperature N-lim Down	GATAA	Gln3/Gat1/Dal80/Gz3	AGATAAG	203	102.57	3.1E-15
	STRE	Msn2/Msn4	ACCCCTT	29	8.73	1.6E-03
Low Temperature C- and N-lim Up	PAC	—	CGATGAG	30	8.39	5.0E-05
Low Temperature C- and N-lim Down	—	—	CGTCCAC	13	2.85	7.8E-03

(B) Overrepresentation of transcription factors (TF) binding targets

Regulatory cluster	Factor	p value	K ^d	P ^e
Low Temperature C-lim Up	Mbp1p	1.6E-03	10	65
Low Temperature C-lim Down	Hap2-Hap1	3.9E-05	3	4
	Hap3-Hap1	9.9E-06	3	3
Low Temperature N-lim Up	Fhl1p	3.4E-05	19	203
	Sfp1p	1.3E-03	7	51
Low Temperature N-lim Down	Gln3p	2.1E-07	20	92
	Gln3-Dal82	5.5E-07	8	15
	Hap2-Dal82	6.8E-05	5	9
Low Temperature C- and N-lim Up	Aft2p	7.5E-04	10	34
Low Temperature C- and N-lim Down	Hsf1p	3.0E-08	16	133
	Nrg1p	7.6E-07	14	128
	Phd1p	3.3E-04	9	99
	Rcs1p	1.1E-04	9	86
	Rox1p	6.0E-05	8	62
	Sok2p	5.7E-05	9	79
	Nrg1-Aft2	6.0E-05	5	20
	Phd1-Nrg1	1.4E-05	7	37
	Rox1-Phd1	7.8E-05	5	21
	Sok2-Nrg1	4.3E-07	8	33

(A) Significantly overrepresented cis-regulatory binding motifs in 5' upstream regions. (B) Significantly overrepresented promoter elements that bind known transcription factors (TF) or TF pairs according to ChIP-on-chip analysis (Harbison *et al.*, 2004). C-Lim, glucose-limited; N-Lim, ammonium-limited.

^a The number of occurrences of promoter element in the regulatory cluster.

^b Expected number of occurrences of the promoter element in a randomly chosen cluster of genes of the same cluster size.

^c The probability of finding the number of patterns with the same level of overrepresentation, which would be expected by chance alone.

^d Number of genes in category in cluster.

^e Number of genes in category in genome.

- STRE elements exist in promoters of downregulated genes in N-limited cultures
- Upregulation in both conditions show PAC regulatory motifs in promoters
- Common promoter sequences show possible regulation relationships

Table 3

Genes Common to All Researchers Were Categorized According to Regulation

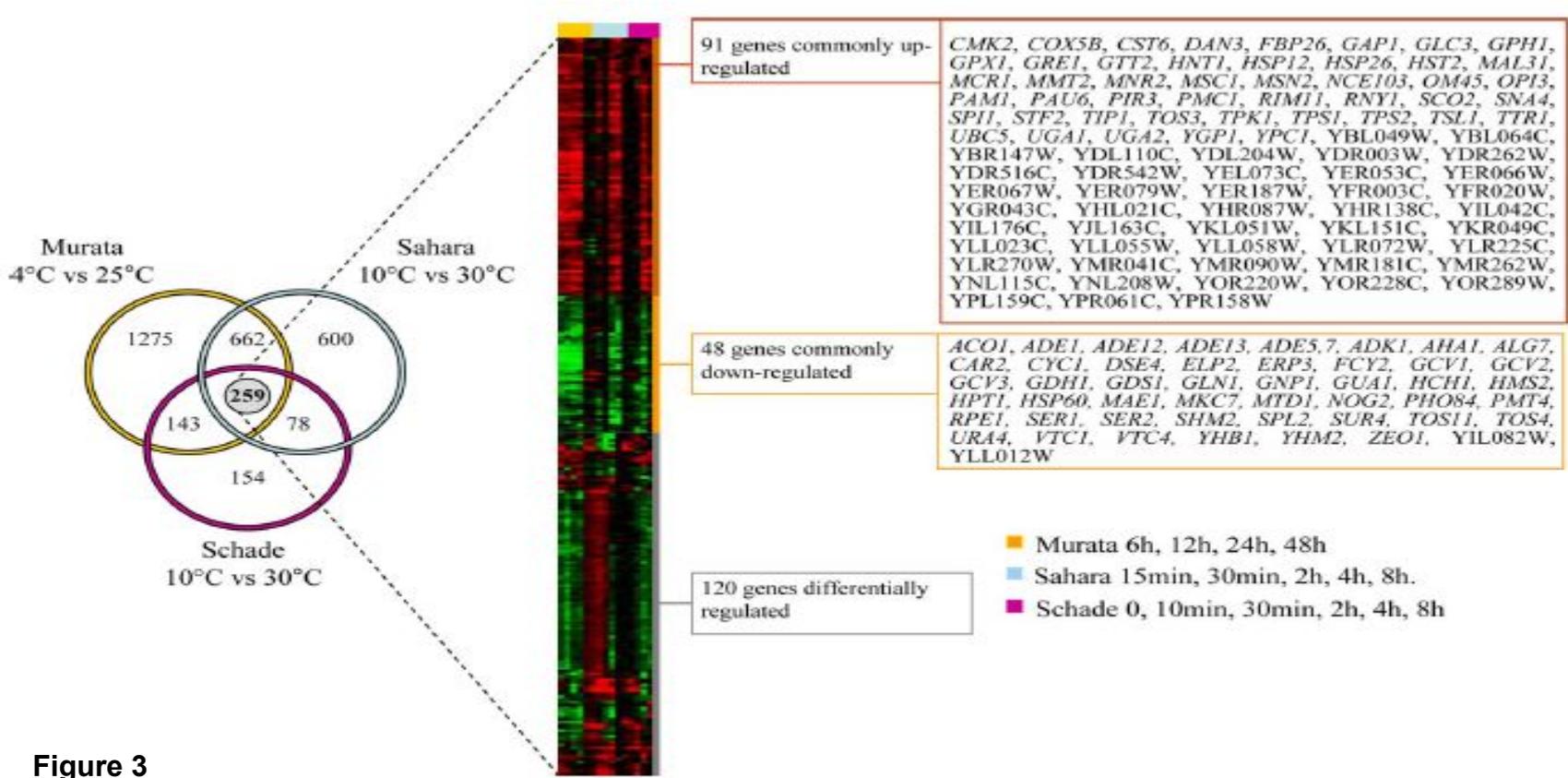


Figure 3

- Sahara and Schade tested cold shock, dropping temperature to between 10 and 20 degrees Celsius
- Murata tested cold shock at temperatures below 10 degrees Celsius, which caused cell growth to cease.

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Overlapping Genes Showing the Same Temperature Response

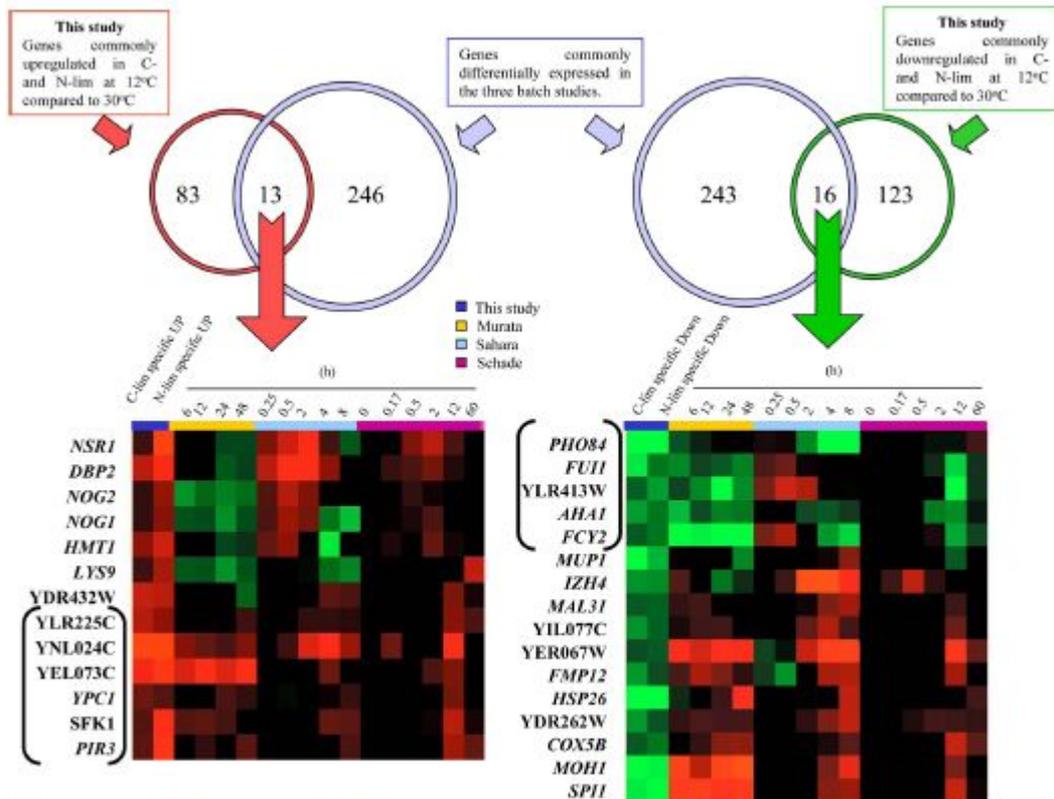


Figure 4

- Chemostat study compared with Sahara et al. 2002, Schade et al. 2004, and Murata et al. 2006
- 29 genes total were in common, only 11 showed consistency

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Few Genes Simultaneously Respond to Temperature and Growth Rate

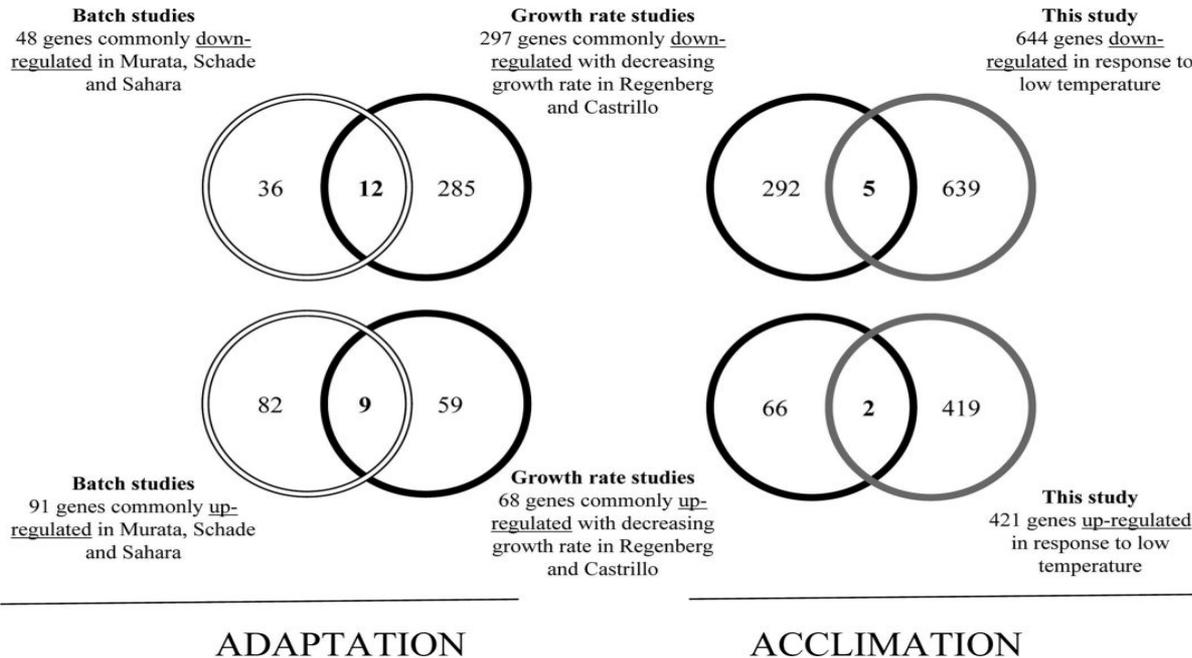


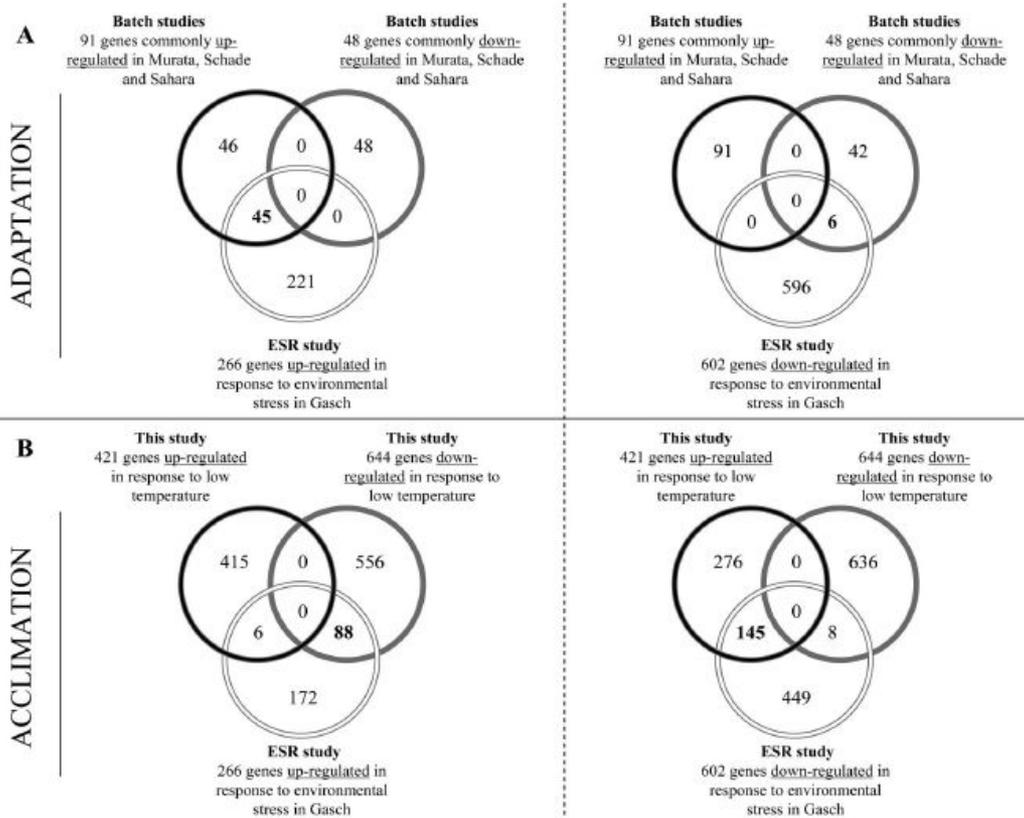
Figure 5

- Studies examined changes in gene regulation in response to a decrease in growth rate compared to both the batch studies and study data for overlap in gene regulation.
- A very small number of genes overlap in response to both temperature decrease and growth rate decrease.

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One Third of Low Temperature Response Genes in the Batch Studies are Attributed to ESR



- **ESR: environmental stress response**
 - **General mechanism responds to multiple stimuli**
- **Batch and chemostat genes compared with Gasch et al. 2000**
- **Some genes showed opposite results in response to low temperatures**

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