Characterizing and developing yeasts for the fermentation industry

George van der Merwe
Molecular and Cellular Biology
University of Guelph

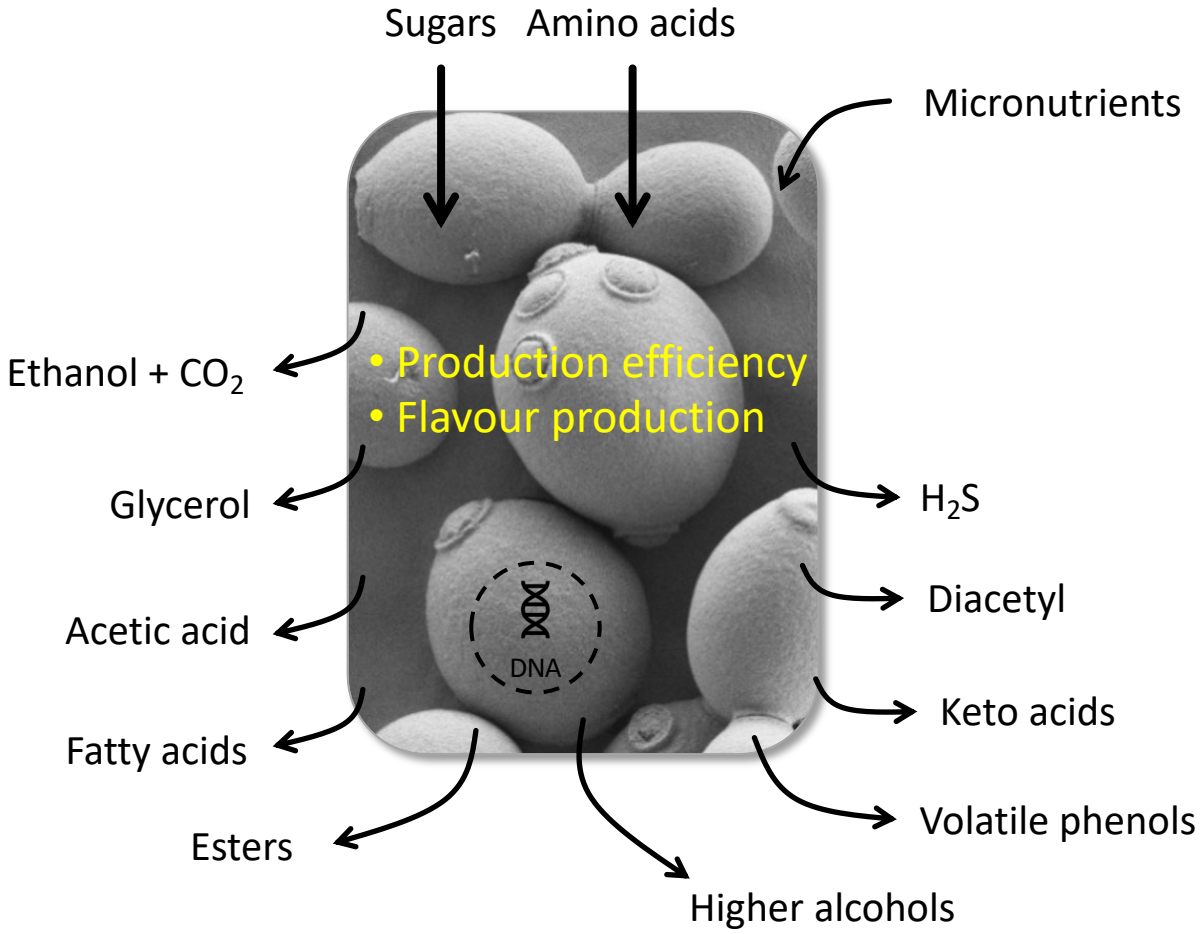
Overview of yeast & fermentation

Metabolite analyses

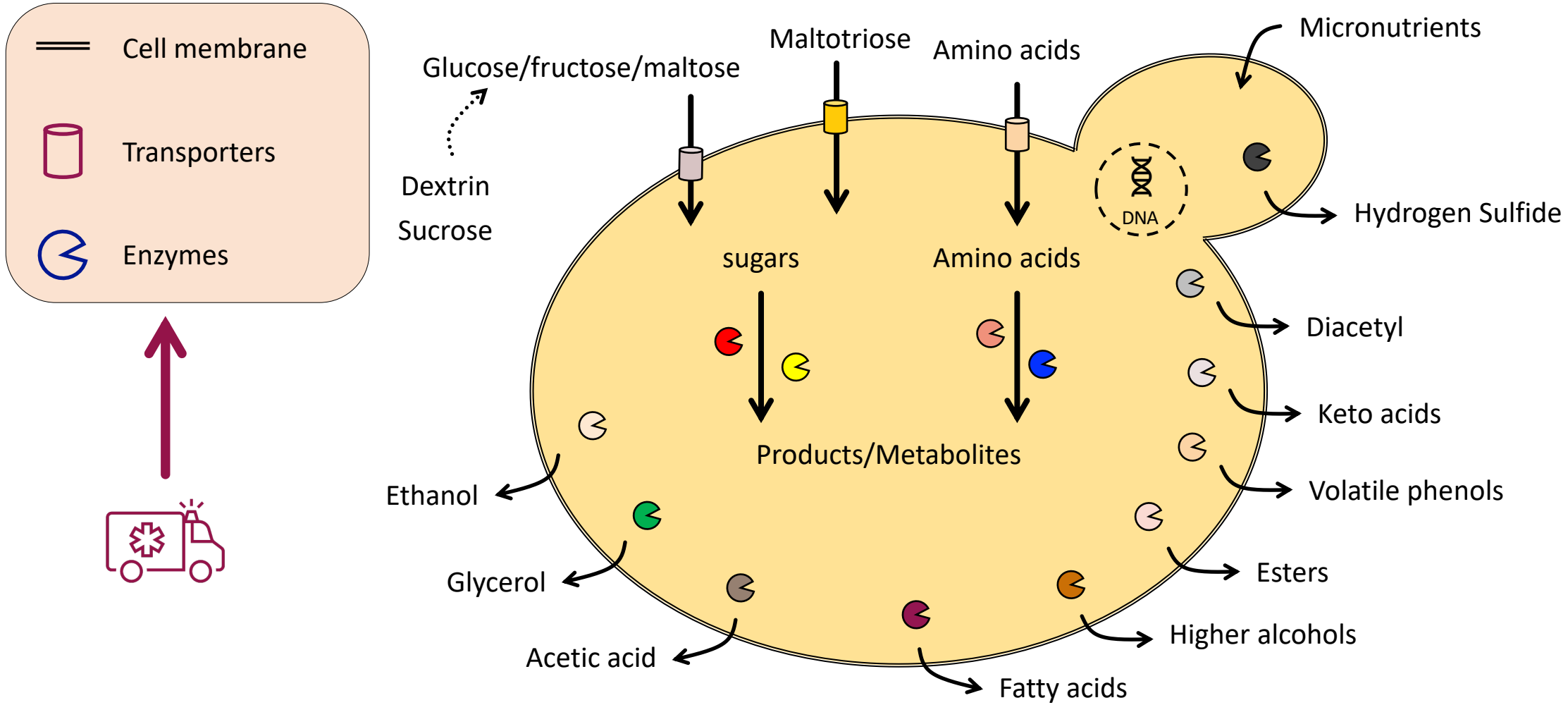
Flavour compounds

Fermentation efficiency

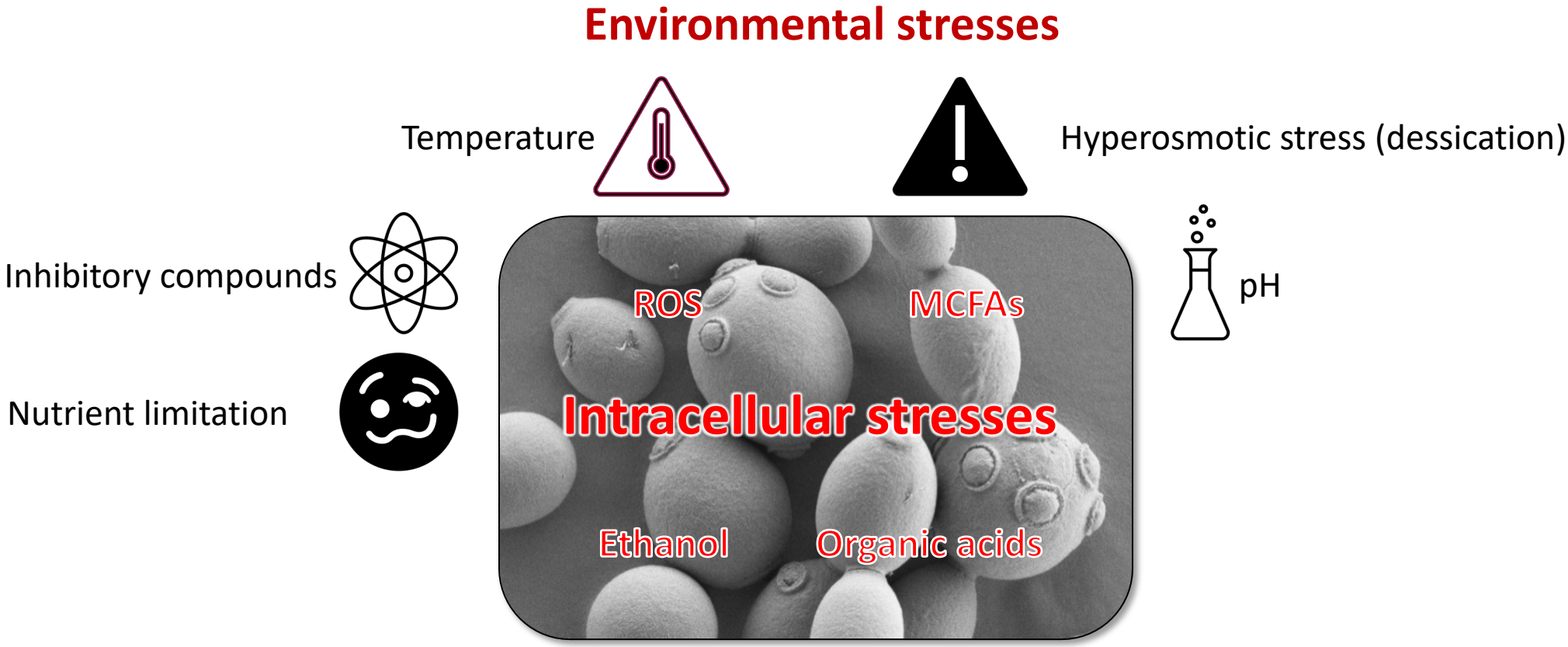
- Sugars
- Alcohol
- Acids



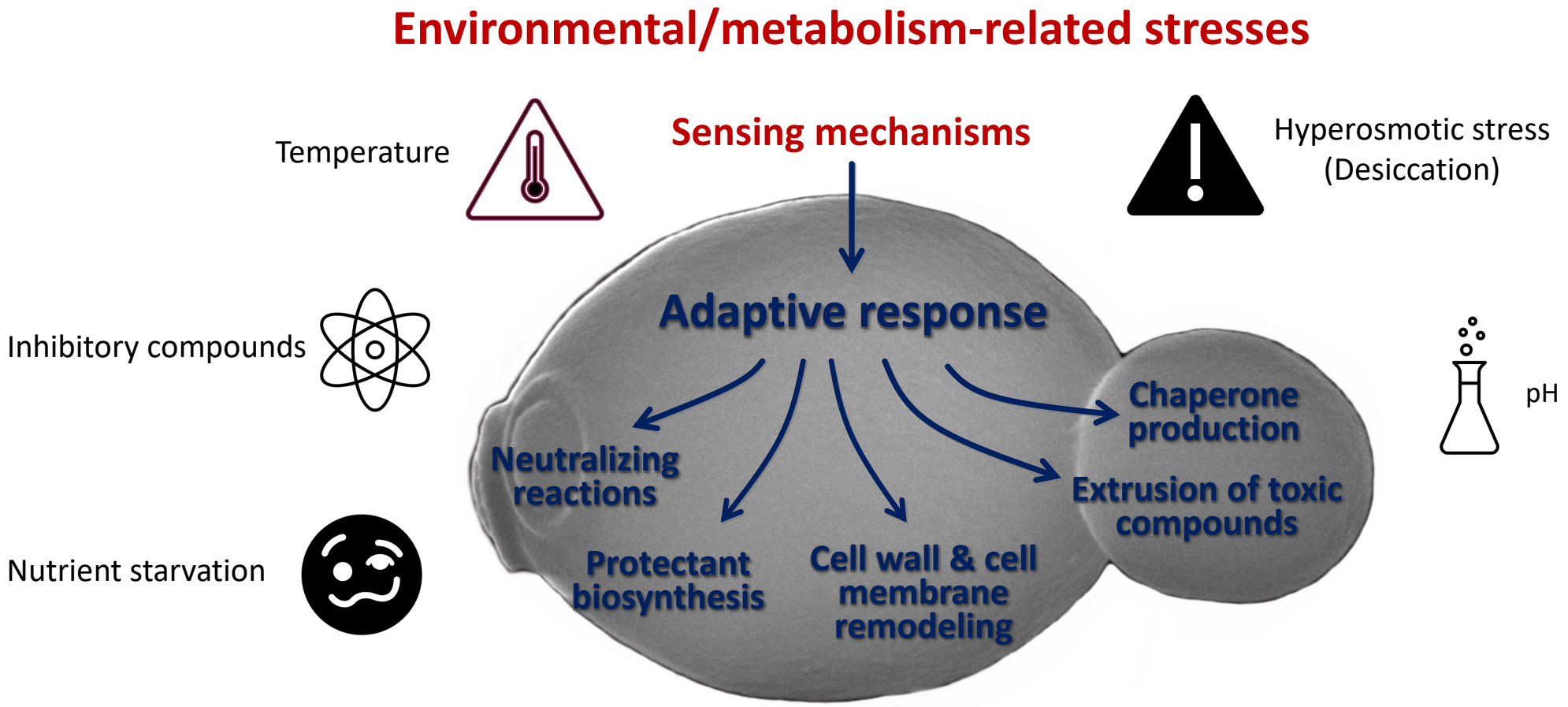
Central yeast components in fermentation



Fermentation-related stresses



Coping with stress



Overarching goal

Developing new strategies for fermentation industry

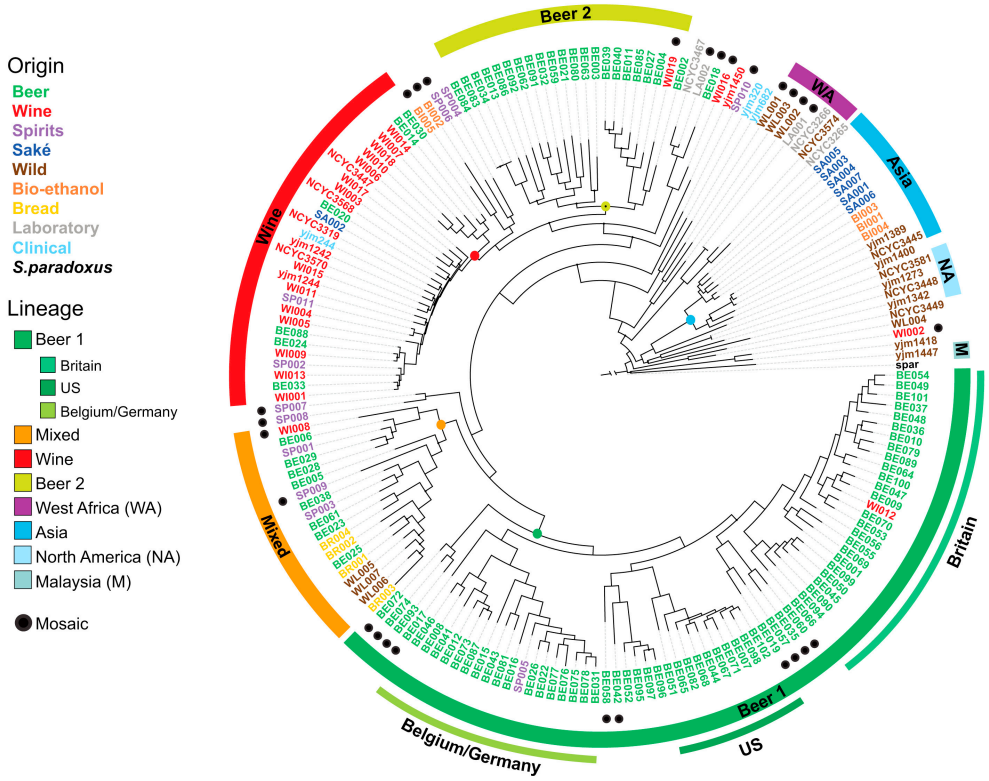
GvdM lab

MOLECULAR and Cellular Biology

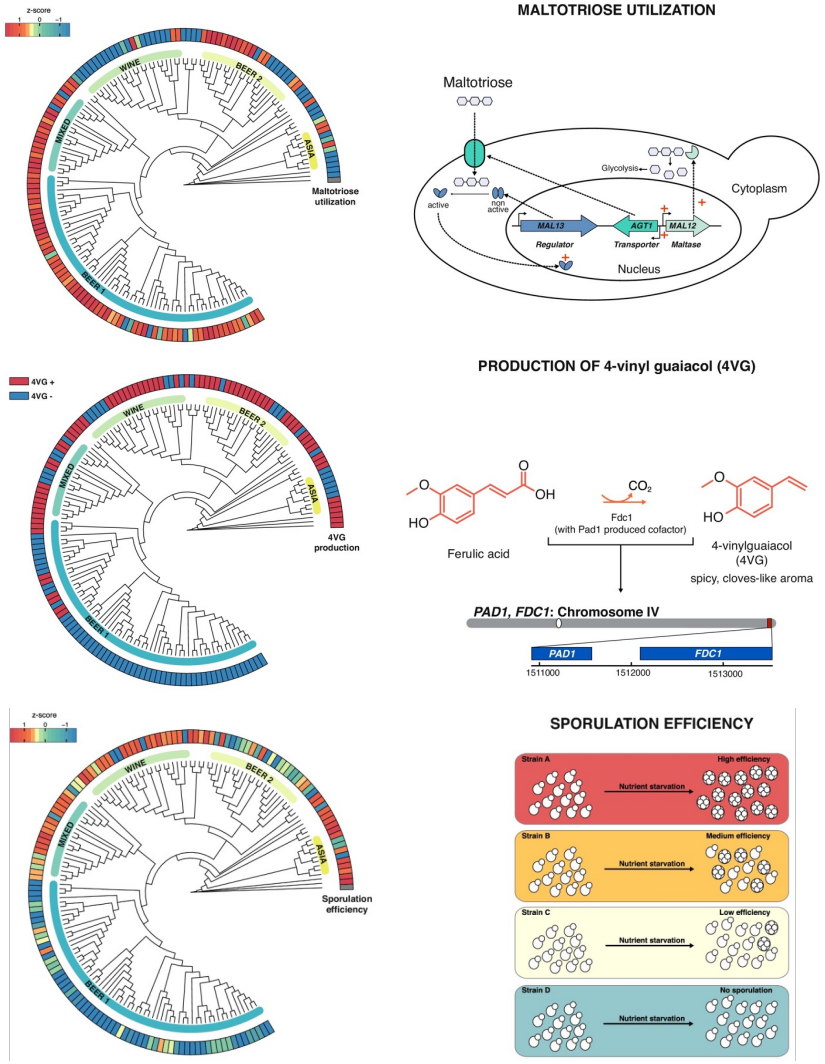
Fermentation industries

Yeast producer

Domestication

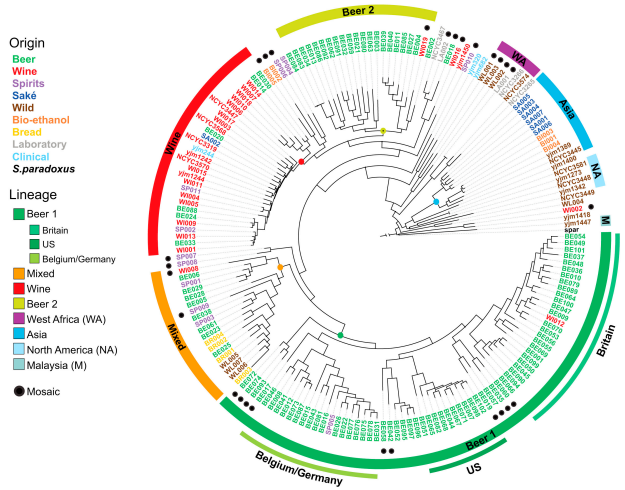


Gallone et al. (2016) *Cell* 1661397-1410.e16DOI: (10.1016/j.cell.2016.08.020)

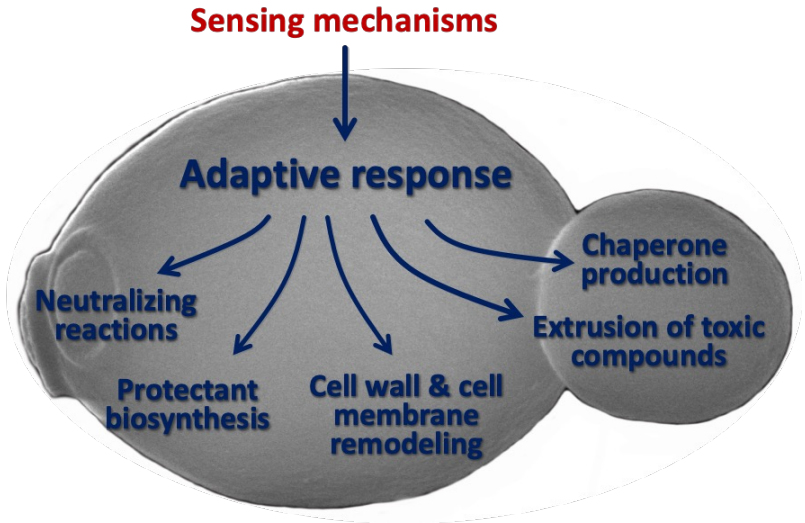


Gallone et al. (2018) *Current Opinion in Biotechnology* 49:148-155

Harnessing stress tolerance to develop novel yeasts



Environmental/metabolism-related stresses



- Domestication and fermentation temperature
  - Can we identify & characterize yeast strains with temperature-dependent traits that could diversify production or quality?
  - How does changes in temperature impact yeast performance?
  - Can omics approaches unravel these specific traits?

Kveik: Traditional Norwegian Farmhouse Ale yeasts

Western Norway

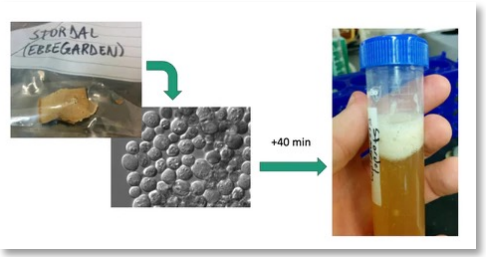
Isolation to purity

Kveik strain bank

Pilot fermentations

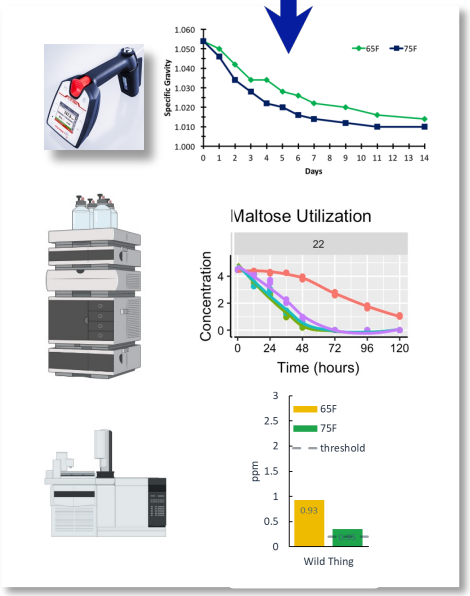
35-37˚C
wort

Desiccation and
cold adaptation



WGS

- Phylogenetic linkages
- Insight into specific traits



Genetic relationships of kveik to domesticated yeasts

Richard Preiss

Caroline Tyrawa

Kristoffer Krogerus

Lars Garshol

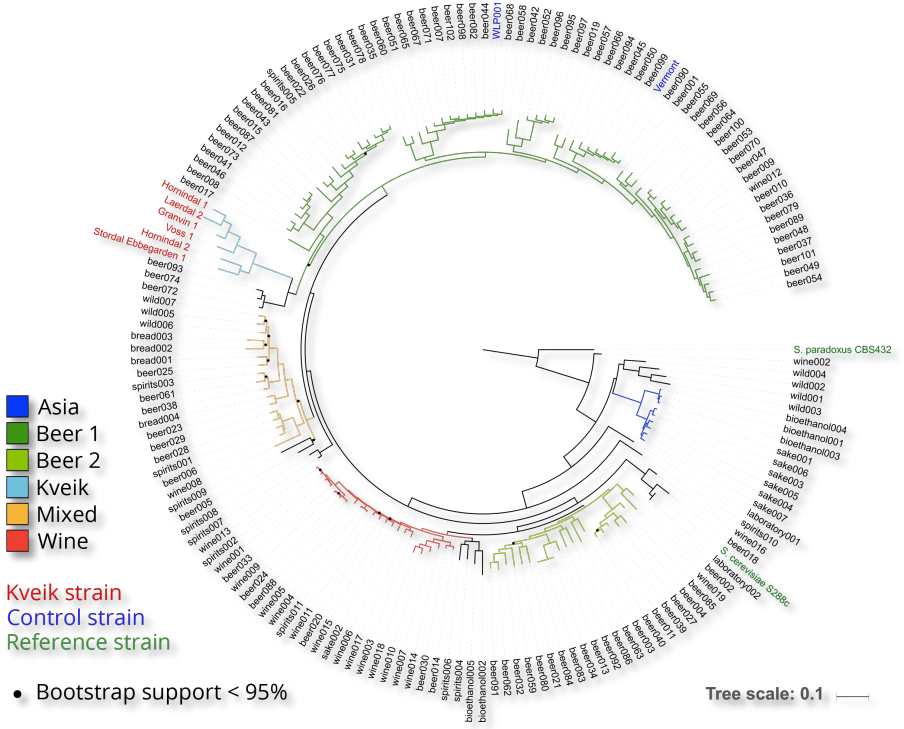
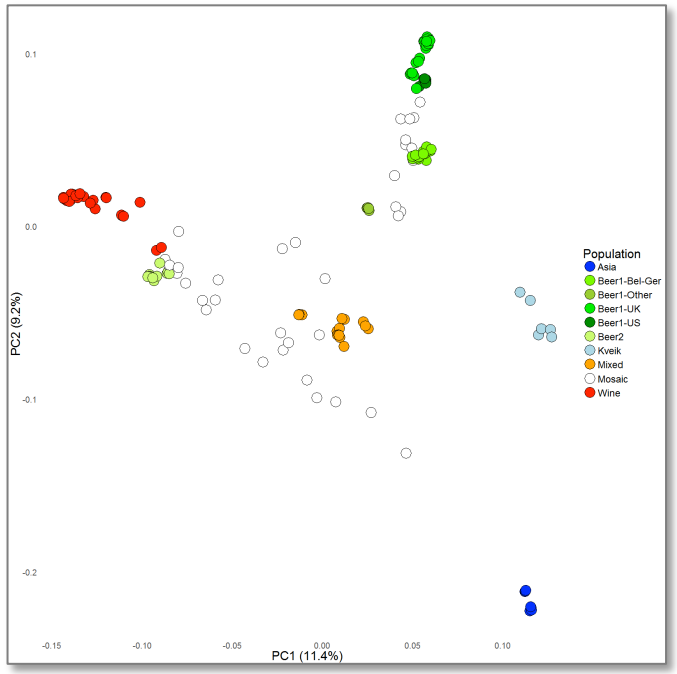


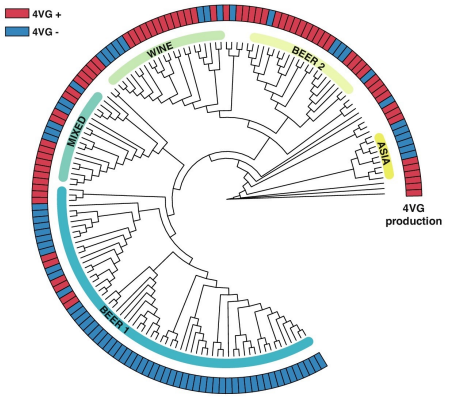
TABLE 2 | Estimated ploidy, spore viability, mean sequencing coverage along *S. cerevisiae* S288c reference genome, and number of heterozygous single nucleotide polymorphisms (SNPs) in the six sequenced kveik strains.

Strain	Estimated ploidy	Spore viability (%)	Sequencing coverage (×)	Heterozygous SNPs
Granvin 1	3.93 (±0.30)	56.5	946	65835
Hornindal 1	3.82 (±0.29)	59.0	1221	67910
Hornindal 2	4.10 (±0.23)	53.3	974	61402
Laerdal 2	4.03 (±0.22)	40.6	472	59090
Stordal Ebbegarden 1	3.92 (±0.23)	5.9	671	54344
Voss 1	3.88 (±0.26)	63.4	1198	64959



Preiss et al. (2018) *Front Microbiol*. 9:2137. doi: 10.3389/fmicb.2018.02137. eCollection.

Shared domestication traits



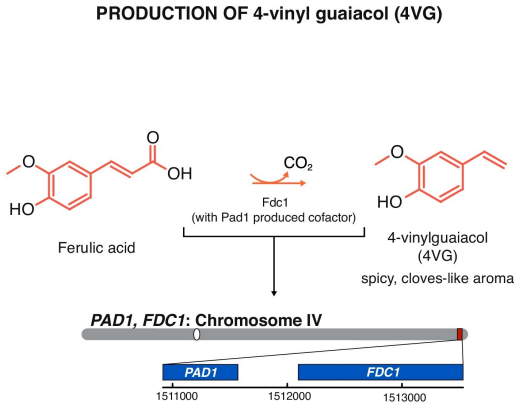
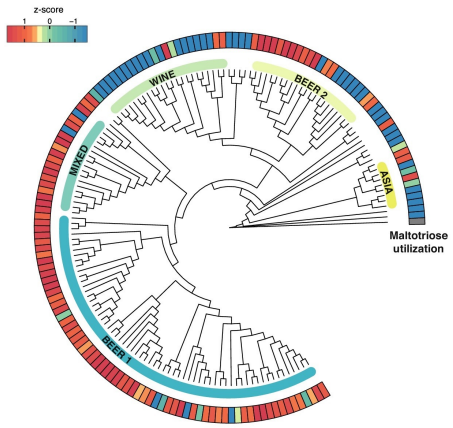
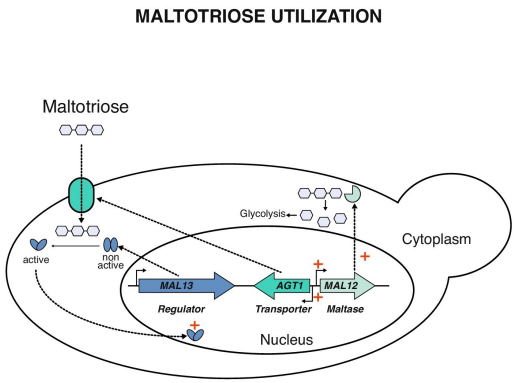


TABLE 5 | Loss-of-function single nucleotide polymorphisms in *PAD1* and *FDC1* in the six sequenced kveik strains.

Strain	*PAD1*	*FDC1*		
	305G>A	232A>T	460C>T	501insA
	Trp102*	Lys78*	Gln154*	Trp168fs
Granvin 1	0/0/0/1		0/0/0/1	1/1/1/1
Hornindal 1	1/1/1/1		1/1/1/1	1/1/1/1
Hornindal 2	1/1/1/1		1/1/1/1	1/1/1/1
Laerdal 2	0/1/1/1		0/0/0/1	1/1/1/1
Stordal Ebbegarden 1	0/0/0/1	0/1/1/1	0/0/0/1	0/0/0/1
Voss 1	1/1/1/1		1/1/1/1	1/1/1/1

*, *premature stop codon; ins, insertion; fs, frameshift.*



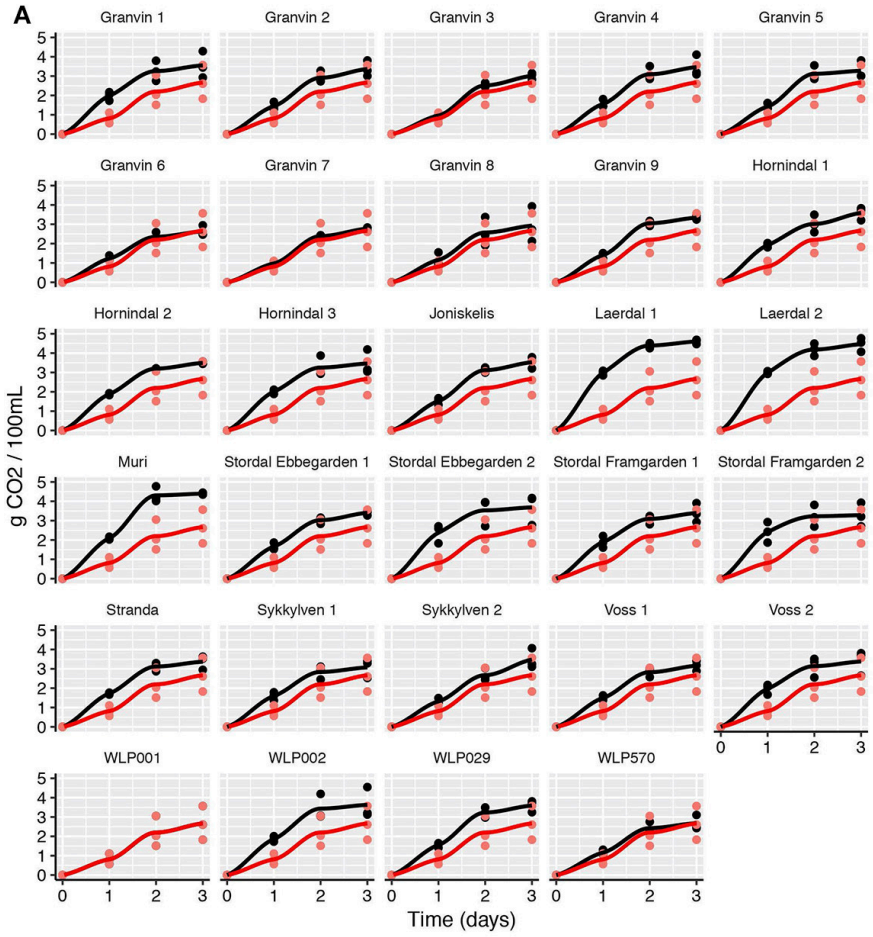


Strain	*MAL1x*	*MAL3x*	*IMA2*	*MPH3*
Granvin 1	2	14	5	4
Hornindal 1	5	14	5	4
Hornindal 2	6	14	6	4
Laerdal 2	4	11	6	4
Stordal Ebbegarden 1	5	11	6	4
Voss 1	2	17	7	4

Preiss et al. (2018)

Increased fermentation rate & flavour diversity

Wort fermentation at 30°C



	Ethyl Acetate	Ethyl Caproate	Ethyl Caprylate	Ethyl Decanoate	Ethyl Nonanoate
Granvin 1	1.715	0.156	2.512	0.494	0.161
Granvin 2	3.118	0.366	4.555	0.455	0.197
Granvin 3	1.492	0.122	1.159	0.013	0.143
Granvin 4	1.195	0.059	0.232	0.012	0.025
Granvin 5	2.231	0.116	1.666	0.08	0.149
Granvin 6	3.2	0.365	5.005	0.88	0.238
Granvin 7	1.564	0.128	1.712	0.056	0.155
Granvin 8	1.229	0.056	0.299	0.026	0.028
Granvin 9	1.537	0.085	1.188	0.076	0.109
Hornindal 1	3.408	0.193	3.58	1.39	0.164
Hornindal 2	2.257	0.084	1.271	0.247	0.091
Hornindal 3	2.505	0.236	4.151	1.412	0.155
Joniskelis	1.495	0.117	2.301	1.277	0.151
Laerdal 1	1.838	0.315	4.124	0.891	0.204
Laerdal 2	1.849	0.102	1.8	0.554	0.159
Muri	2.713	0.224	2.005	1.078	0.188
Stordal Ebbegarden 1	2.103	0.083	0.811	0.272	0.053
Stordal Ebbegarden 2	2.542	0.089	0.619	0.341	0.041
Stordal Framgarden 1	2.395	0.168	2.975	0.772	0.158
Stordal Framgarden 2	2.654	0.44	4.112	0.753	0.176
Stranda	2.393	0.168	2.818	1.035	0.157
Sykkylven 1	2.046	0.101	1.306	0.427	0.08
Sykkylven 2	1.668	0.102	1.392	0.675	0.079
Voss 1	2.156	0.209	3.317	0.618	0.145
Voss 2	2.364	0.307	3.059	0.347	0.157
WLP001	2.064	0.192	0.241	0.105	0.196
WLP002	0.735	0.076	0.537	0.047	0.101
WLP029	3.22	0.348	4.142	0.99	0.292
WLP570	5.734	0.806	8.586	1.583	0.424
Threshold (ppm)	30	0.21	0.9	0.2	0.85

Preiss et al. (2018)

Heat & ethanol tolerance

	Heat treatment			Ethanol Treatment		
Strain	*40ºC*	*42ºC*	*43ºC*	*12%*	*14%*	*16%*
WLP001	0.14	0.13	0.12	0.48	0.34	0.14
WLP570	1.80	0.51	0.39	0.50	0.41	0.37
Horn1	1.76	0.41	0.35	0.48	0.30	0.27
Horn2	1.67	0.33	0.26	0.40	0.32	0.22
Laerdal2	1.21	0.45	0.33	0.47	0.39	0.24
StorEbb1	1.41	0.36	0.29	0.47	0.47	0.34
Granvin1	1.53	0.42	0.35	0.42	0.10	0.10
Voss1	1.84	0.70	0.30	0.56	0.39	0.22

Preiss et al. (2018)

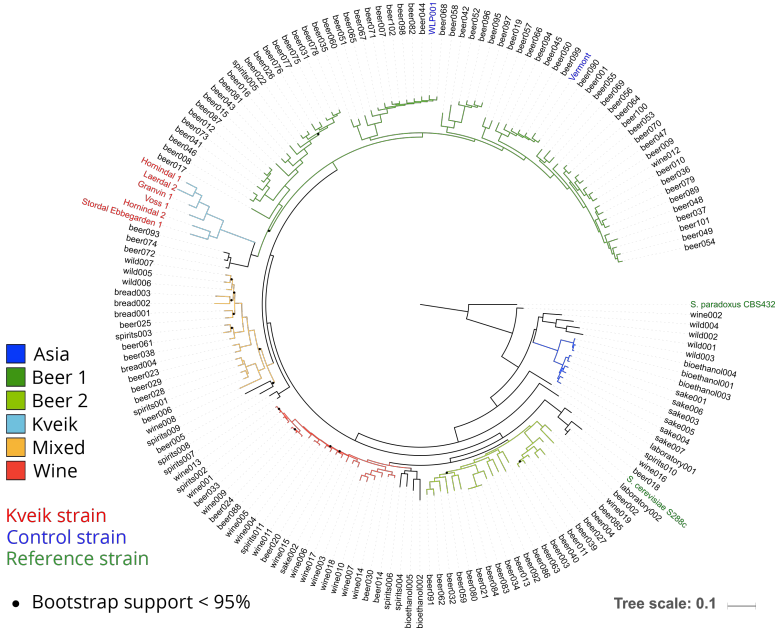
Kveik: Stress tolerant yeast


Richard Preiss


Caroline Tyrawa


Kristoffer Krogerus

Lars Garshol



- Genetically distinct farmhouse ale yeasts
  - Sub-population of Beer 1
- Share domestication traits
  - POF negative
  - Multiple copies of maltose metabolic genes
  - Gained flocculation trait
- Temperature tolerant

- How do they perform at a broader fermentation temperature range?
  - Tested six Kveik, three Beer 1 & one Beer 2 strain at 8 temperatures


Barret Foster


Emine Ozsahin

Preiss et al. (2018)

Testing fermentation temperature range of kveiks

Strain	Family
Cali Ale	Beer 1 American
Vermont	Beer 1 British
Kolsch	Beer 1 Belgian/German
St. Lucifer	Beer 2 Belgian
Granvin 1	Kveik
Hornindal 1	Kveik
Hornindal 2	Kveik
Laerdal 2	Kveik
Stordal Ebbegarden 1	Kveik
Voss 1	Kveik

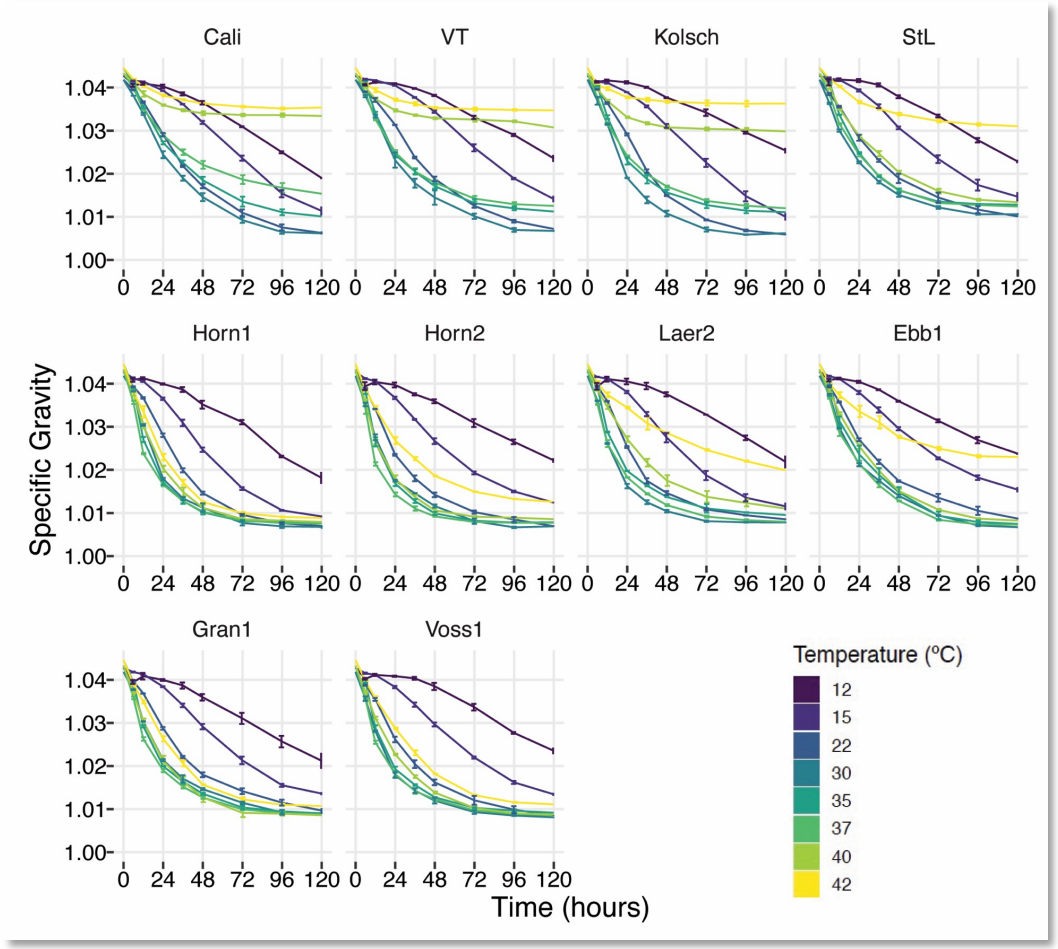
Seed Rate: 10 million cells/ml

Wort: SPA (SG = 1.0425)

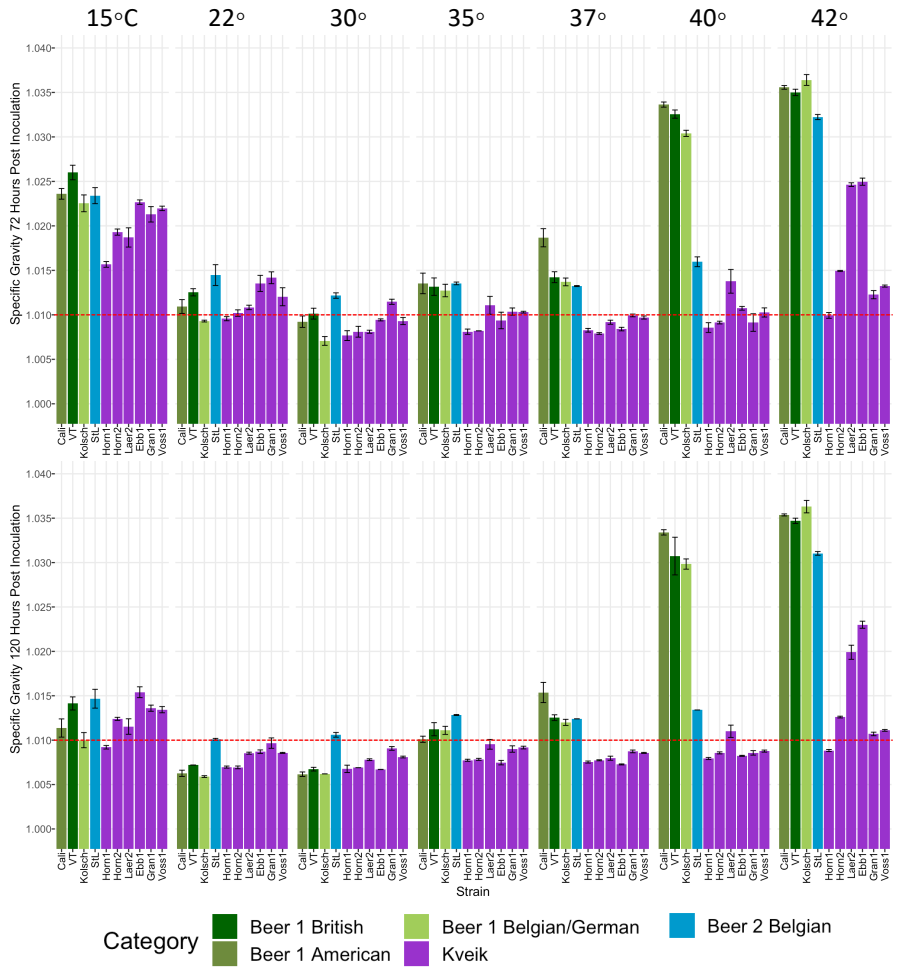
Temperatures: 12°C, 15°C, 22°C, 30°C, 35°C, 37°C, 40°C, 42°C

Timepoints: 6h, 12h, 24h, 48h, 72h, 96h, and 120h

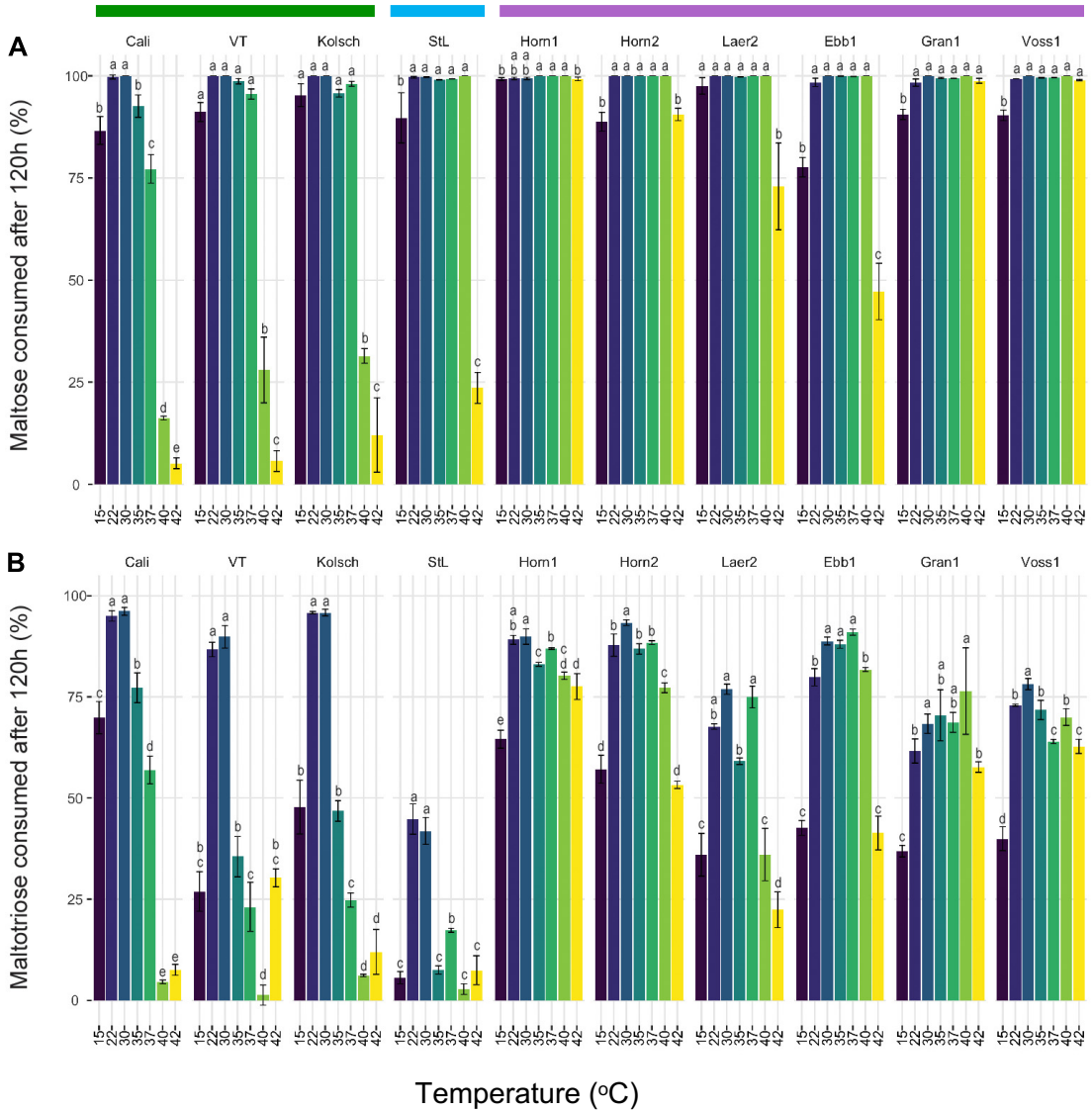
Kveik fermentation at broad temperature range



Foster et al. (2022) *Front Microbiol*. 13:747546. doi:10.3389/fmicb.2022.747546. eCollection



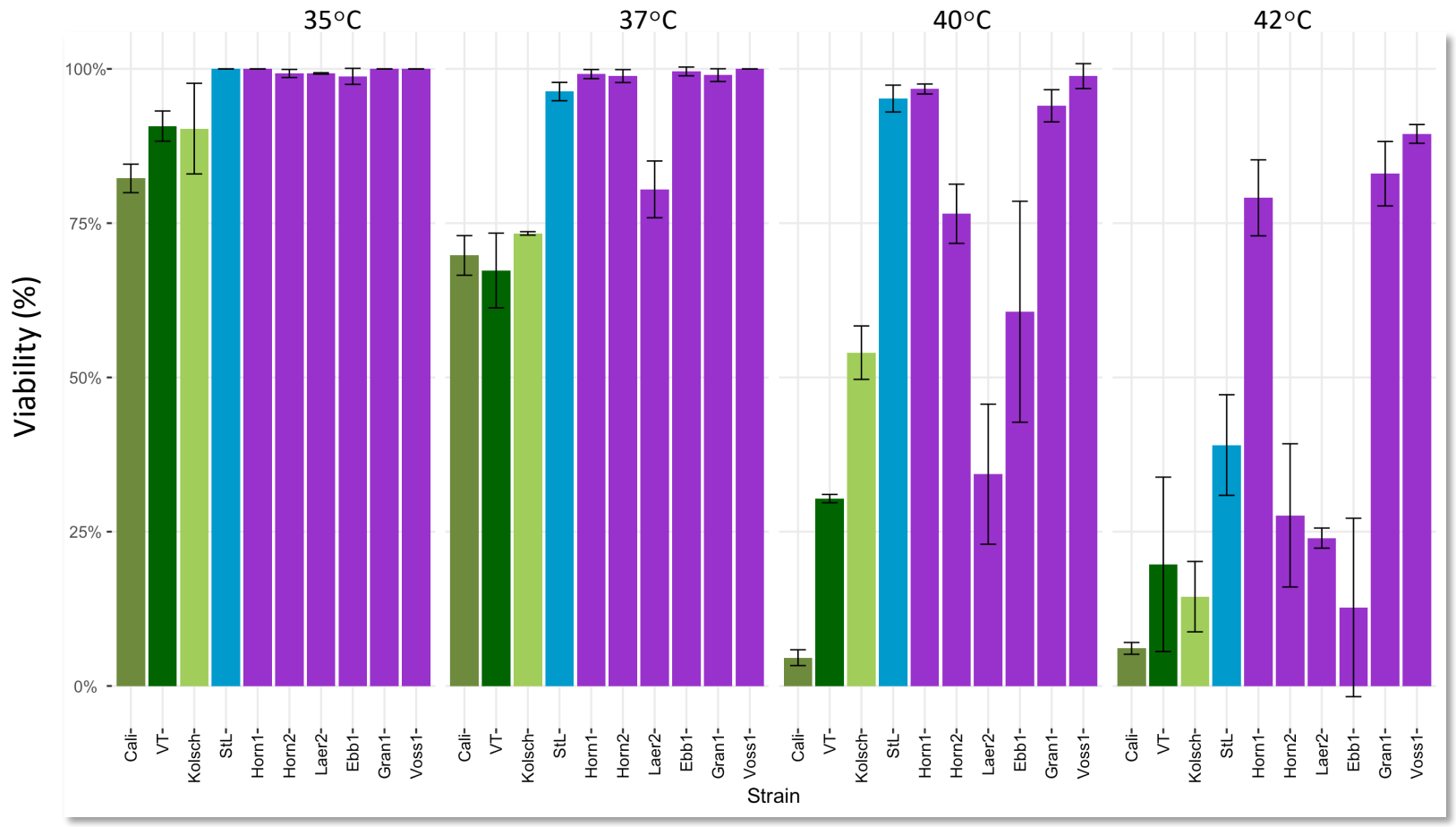
Sugar consumption at higher temperatures



- Efficient consumption of maltose by kveik
- Control yeasts have strong maltotriose consumption in a defined temperature range
- Variability among kveiks in maltotriose consumption at higher temperatures

Foster et al. (2022)

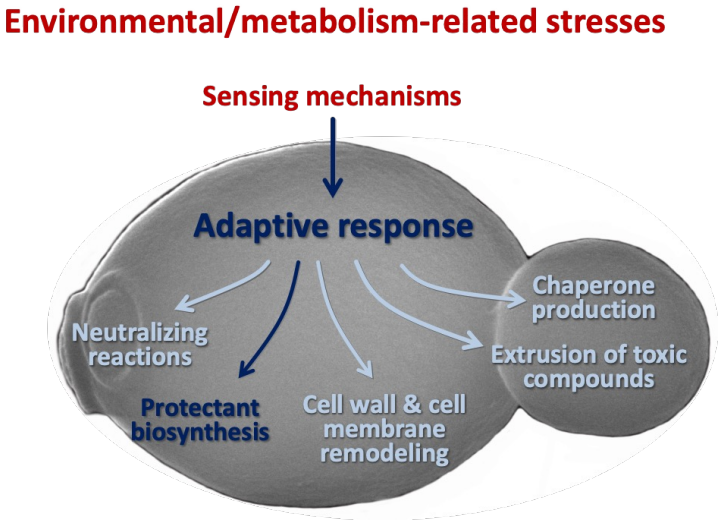
Kveik viability at higher temperatures



- Kveiks have increased cell viability at higher temperatures
- Variability in thermotolerance among kveiks

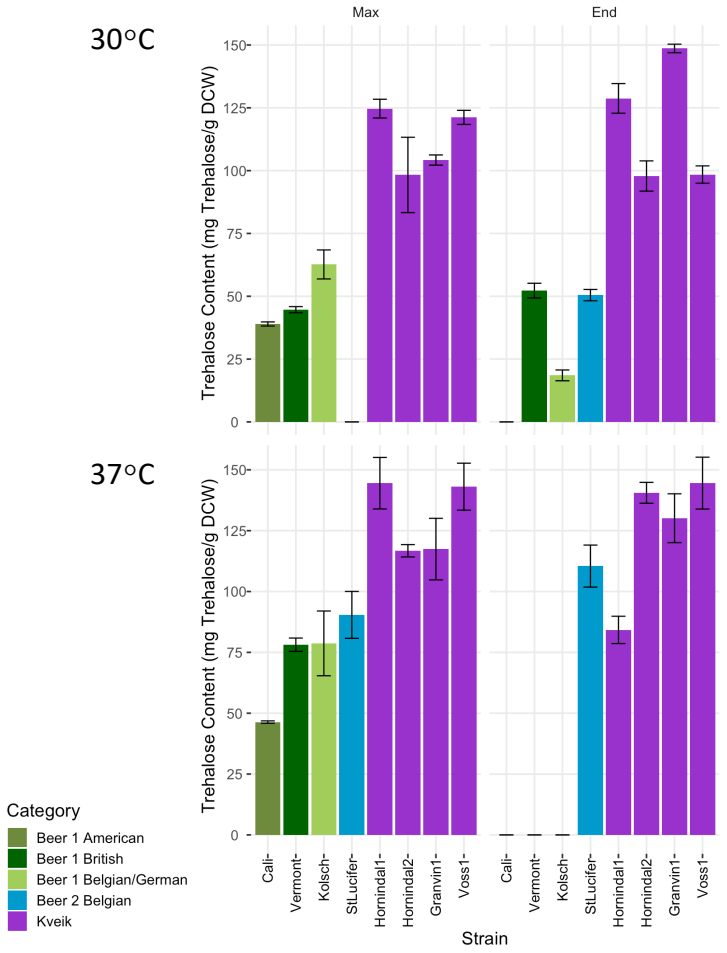
Foster et al. (2022)

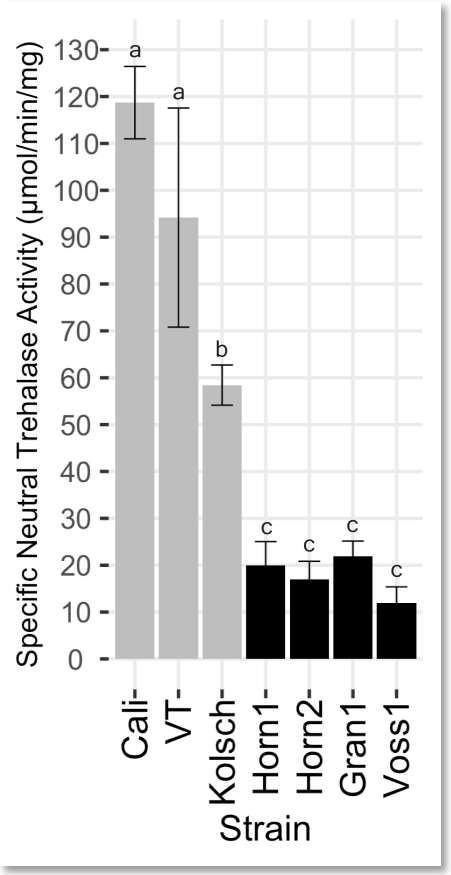
Increased trehalose production at higher temps



- Trehalose = disaccharide (glucose); cellular protectant
  - Stabilizes membranes; Assist in protein (transporter & enzyme) folding and stability
  - Accumulates intracellularly
  - Induced by stress

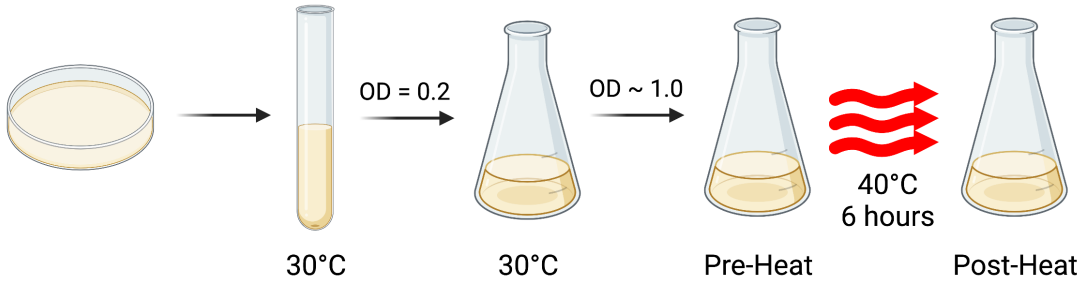
- Kveiks have increased intracellular trehalose
- Deficient in neutral trehalase activity
  - WGS revealed heterozygous SNPs close to catalytic domain in *NTH1*



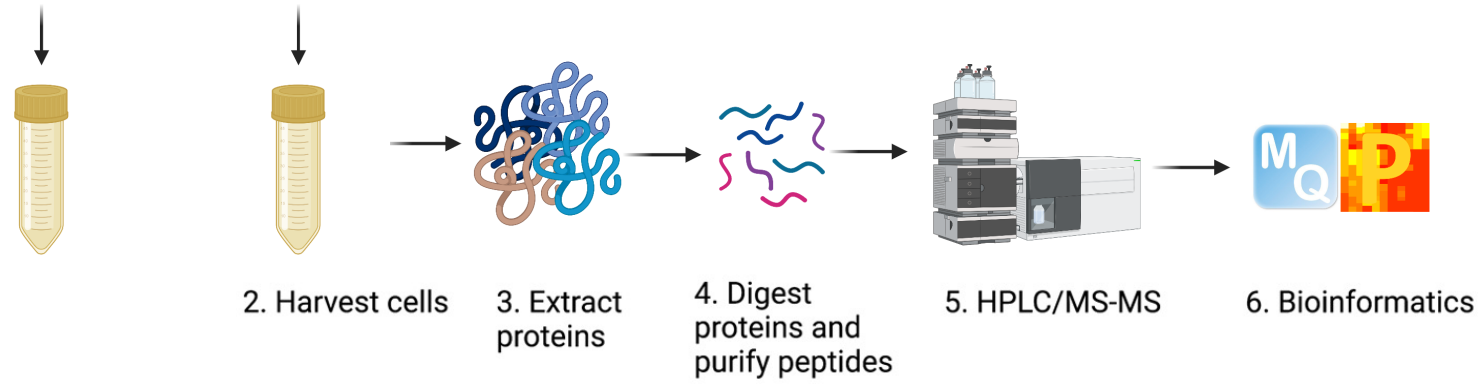


Foster et al. (2022)

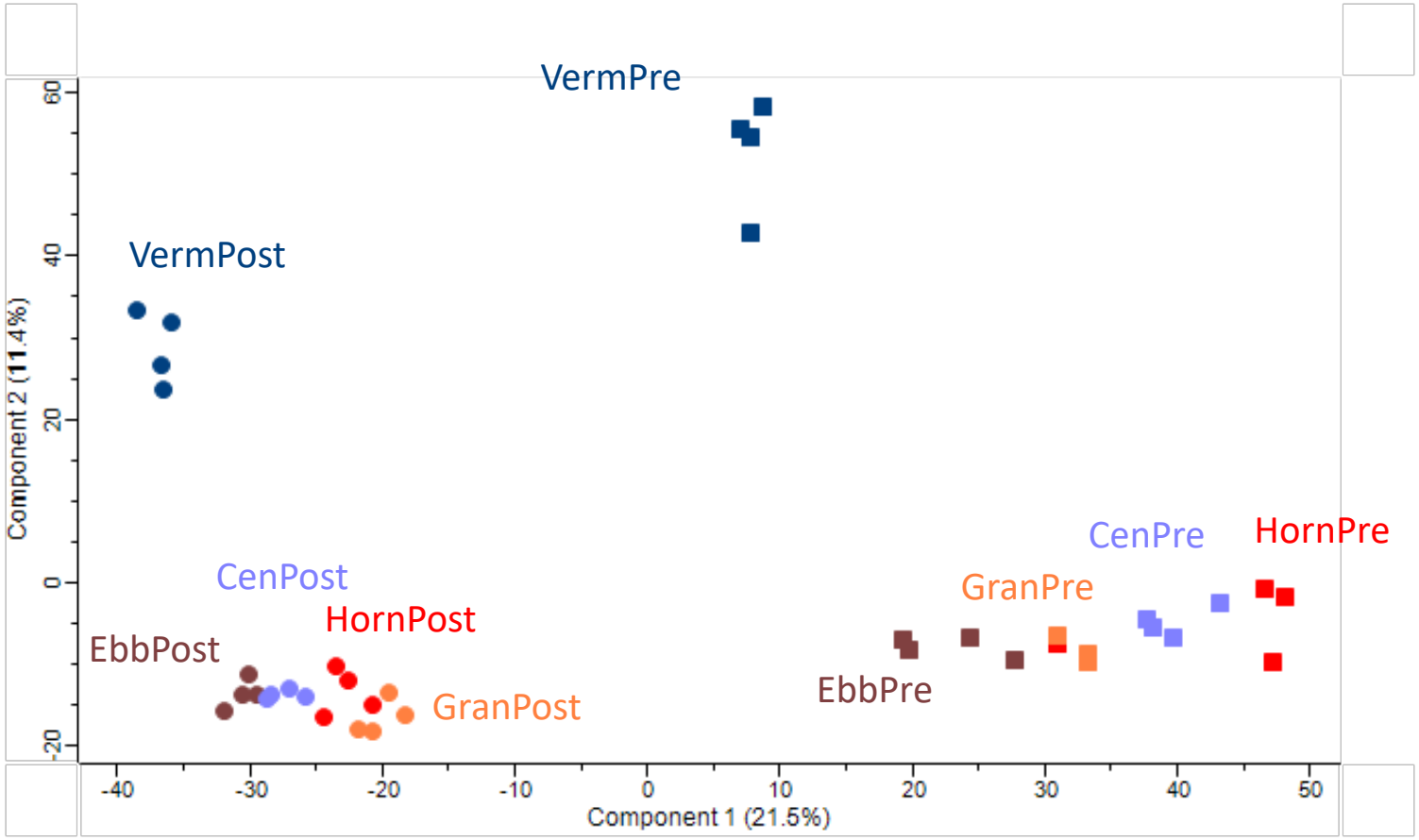
Heat treatment & proteomics workflow



Strain	Family
CEN.PK 113-7D	Lab strain
Vermont	Beer 1 British
Hornindal1	Kveik
Granvin1	Kveik
Ebbegarden1	Kveik



Principle Component Analysis



Pre-Heat

Post-Heat

Vermont

CEN.PK

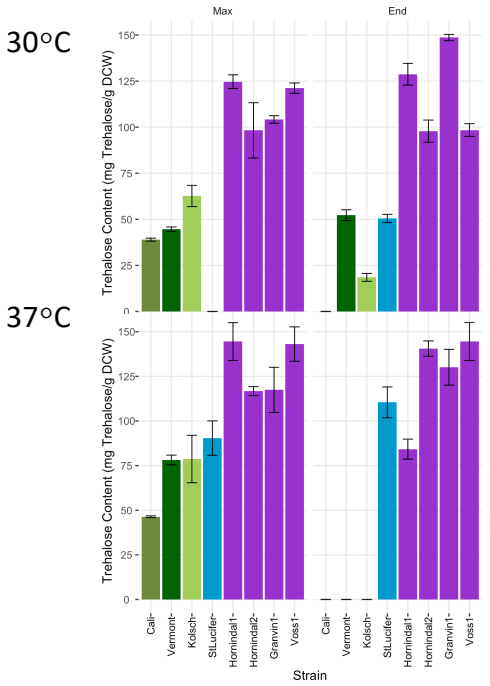
Granvin1

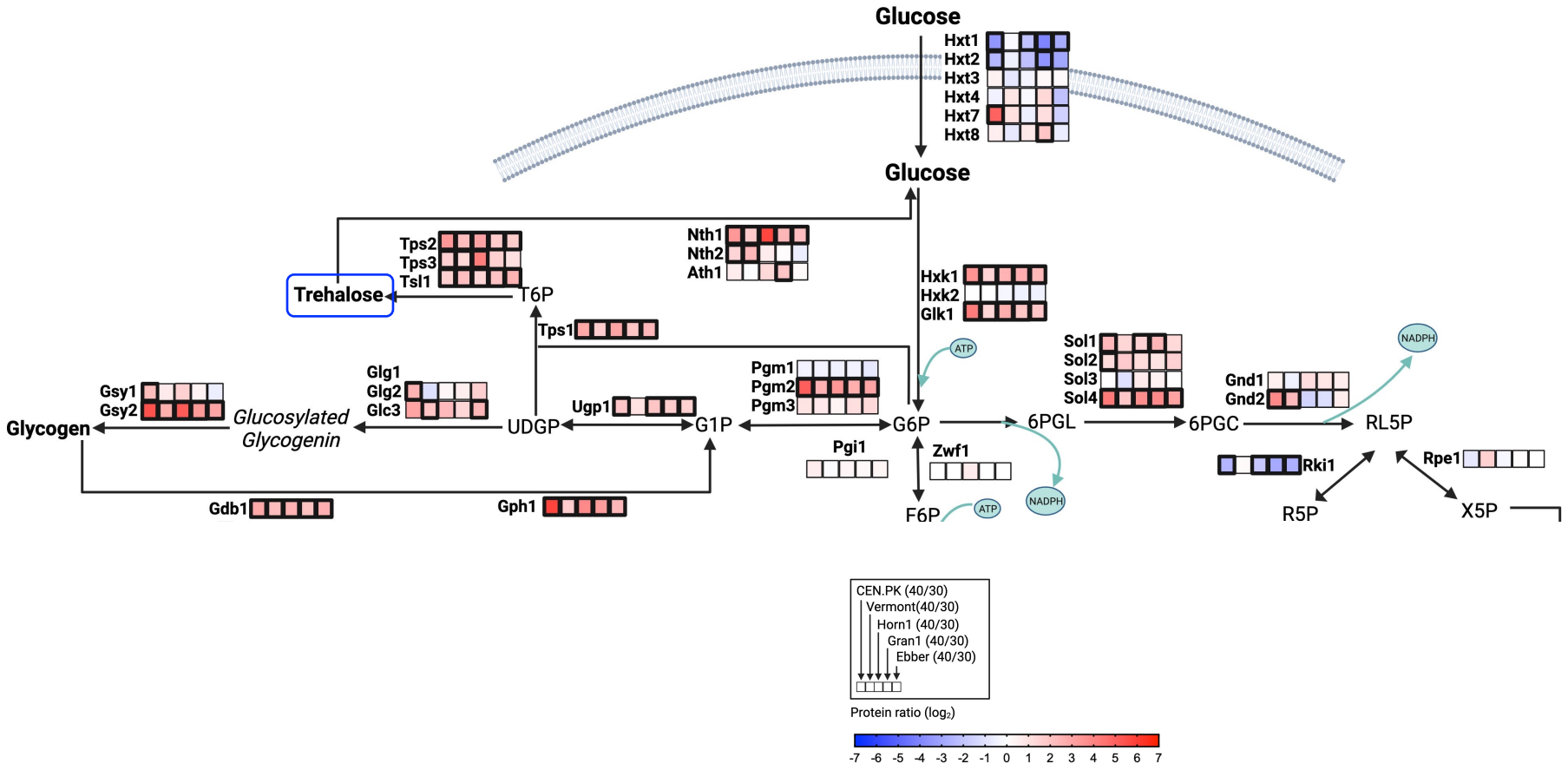
Hornindal1

Ebbegarden

Foster et al. (in preparation)

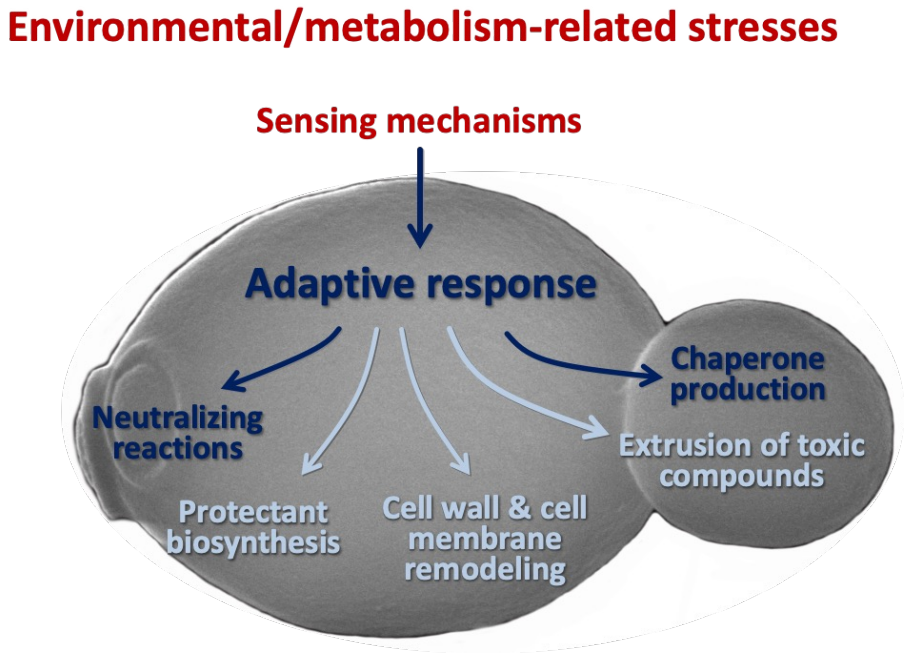
Trehalose enzymes upregulated after temperature shift

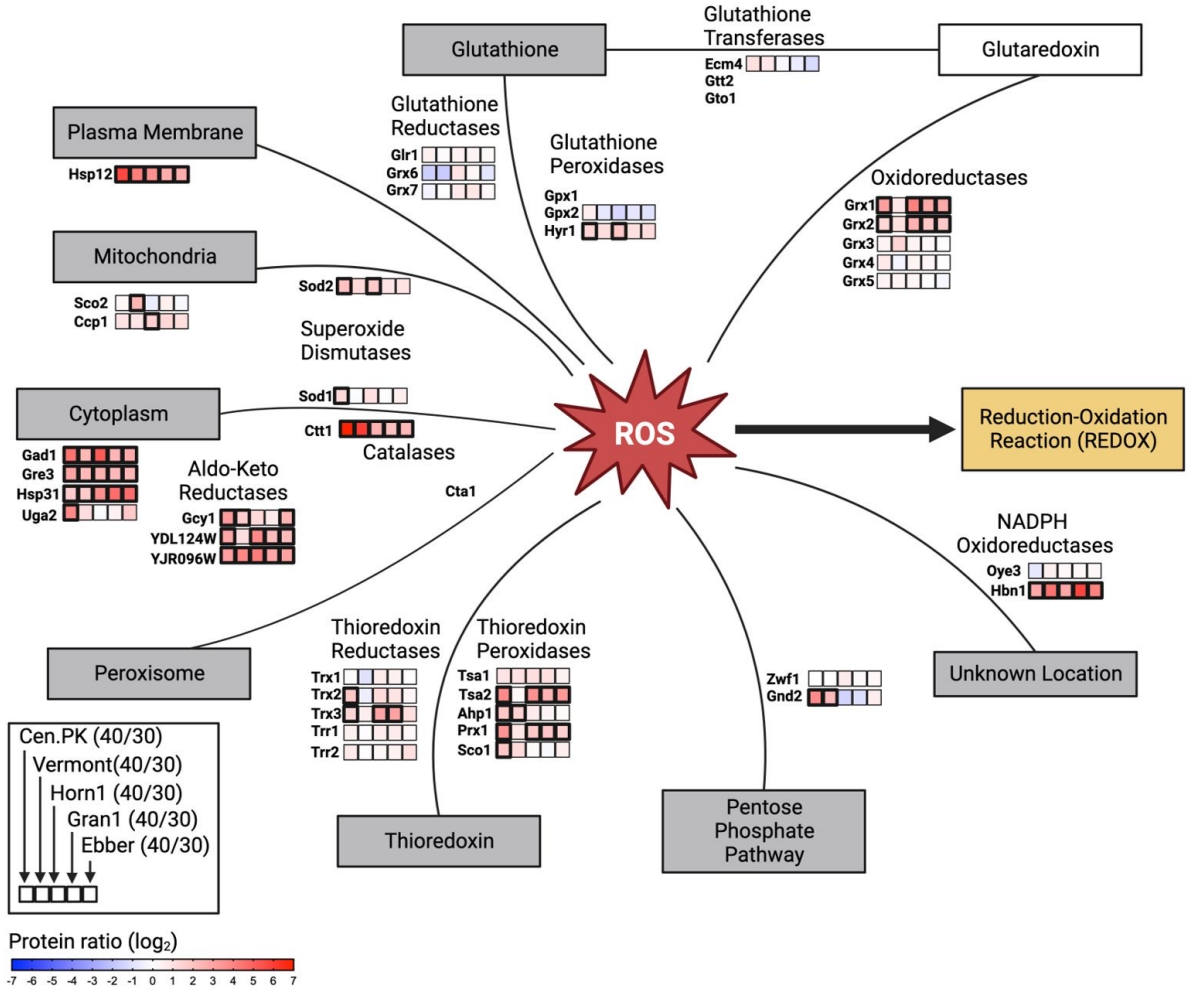




Foster et al. (in preparation)

ROS response upregulated after temperature shift





Foster et al. (in preparation)

Kveik & breeding thermotolerant yeast strains

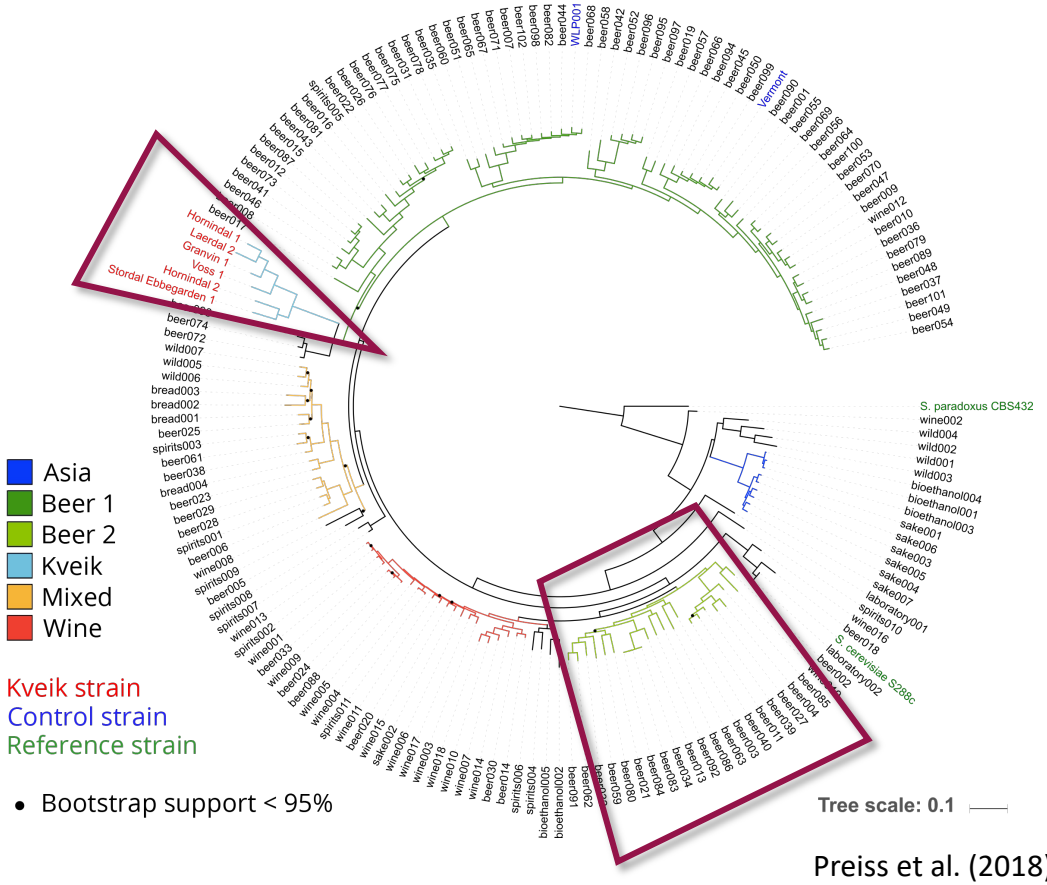
- Hybridization
  - Kveik and Beer 2 strains
    - Kveik x Saison
      - Kveik #1 x Saison = NorthSea
      - Kveik #2 x Saison = Mjolnir


Caroline Tyrawa


Barret Foster

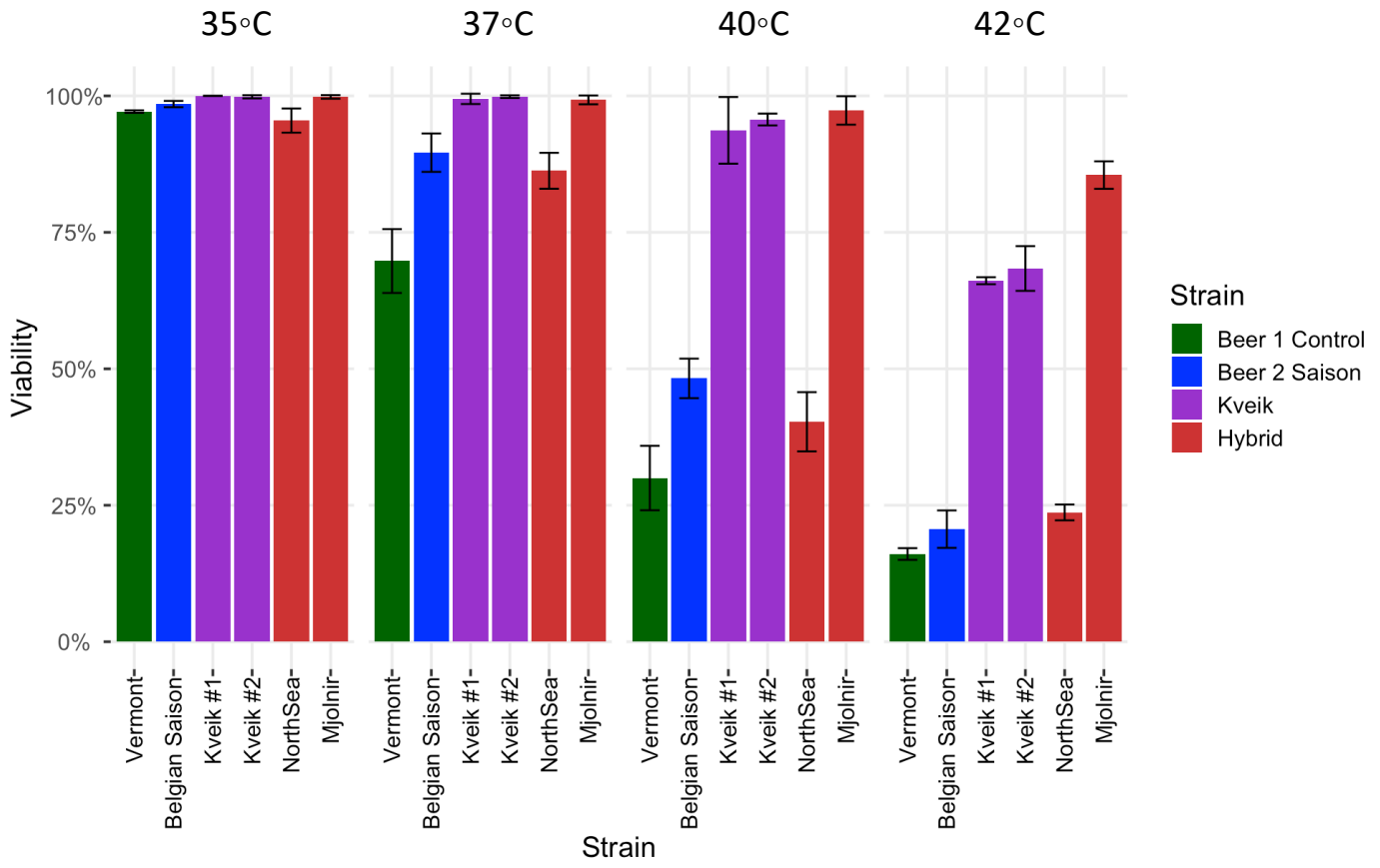

Bryan Chalk


Hollie Rowlands



Preiss et al. (2018)

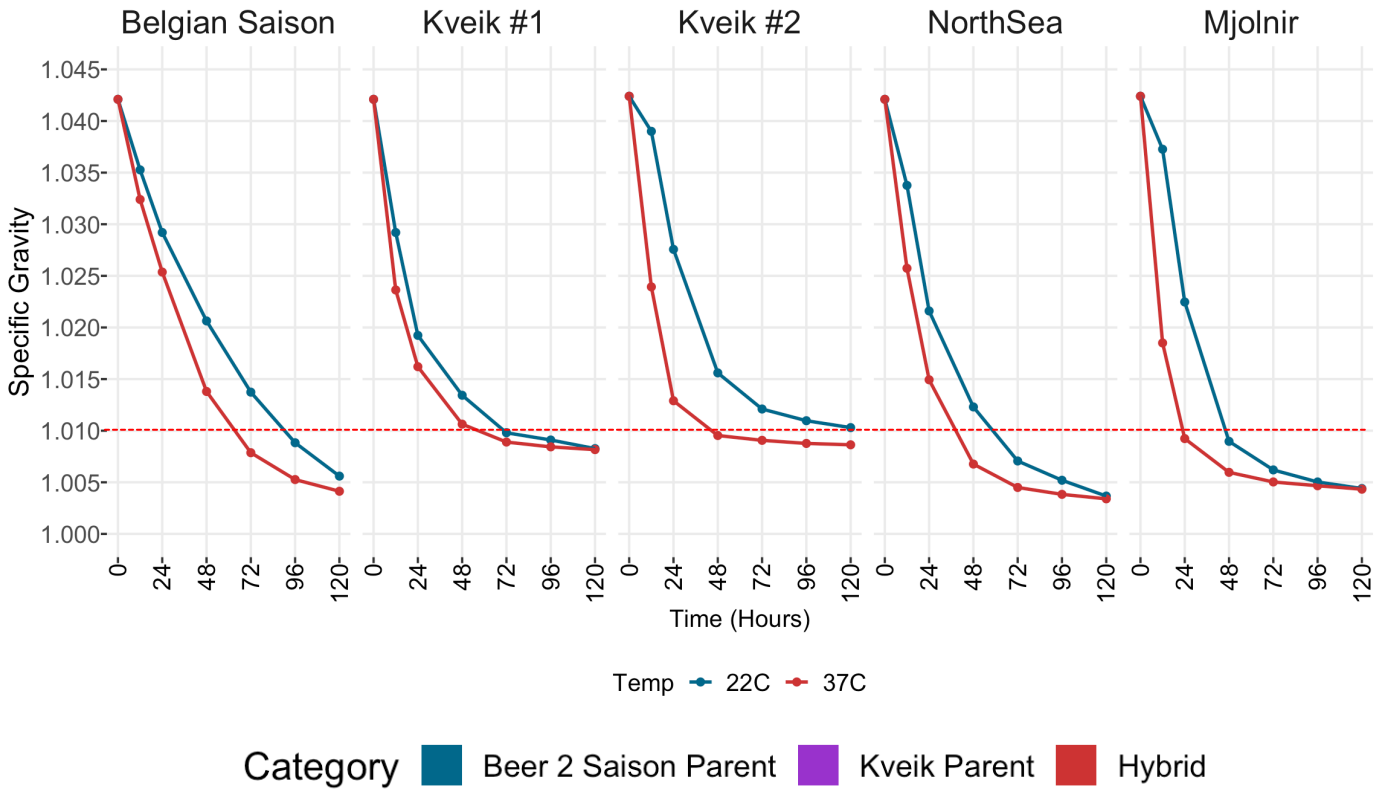
Viability of hybrid strains

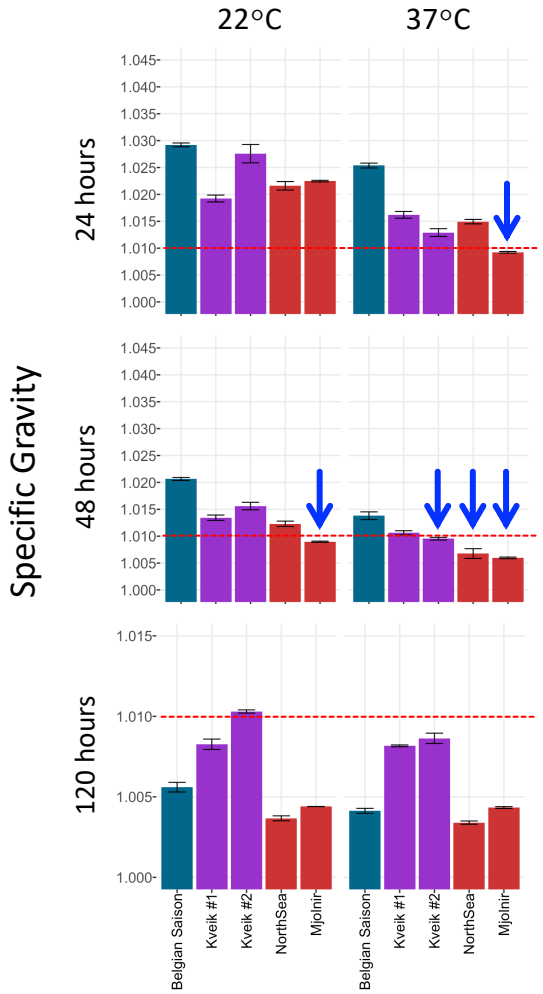


- Mjolnir has strong thermotolerance
- NorthSea has temperature sensitivity similar to Saison

Foster et al. (in preparation)

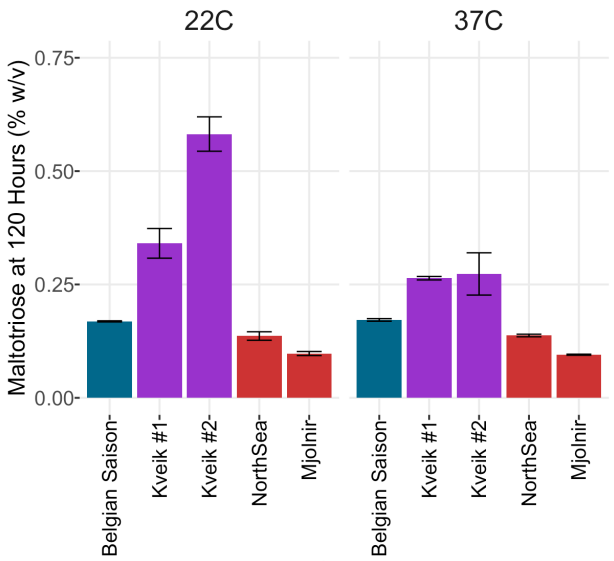
Rapid attenuation by kveik hybrids





Foster et al. (in preparation)

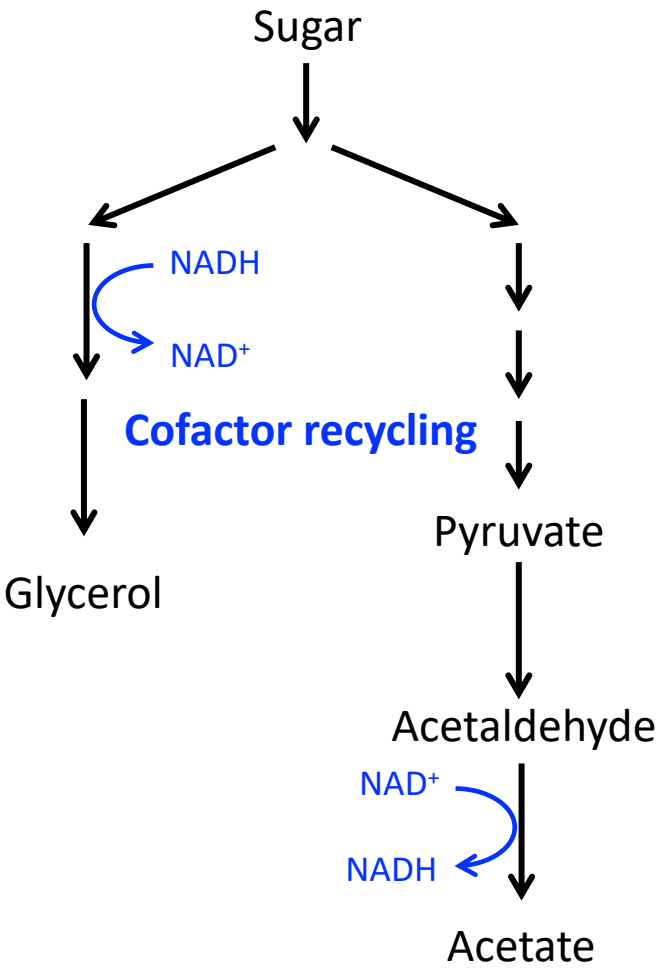
Carbohydrate consumption

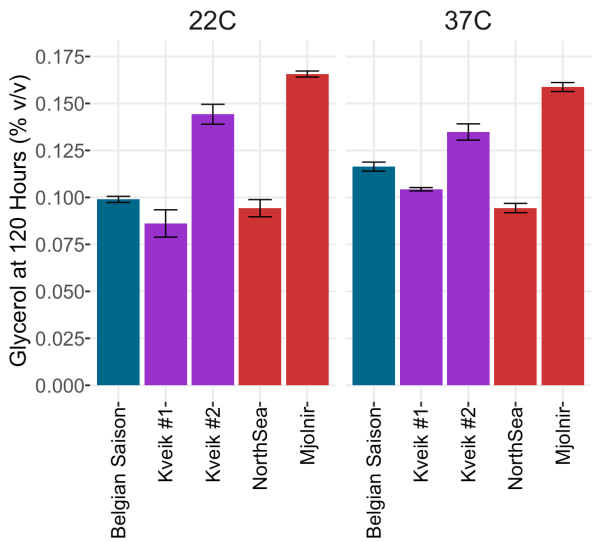


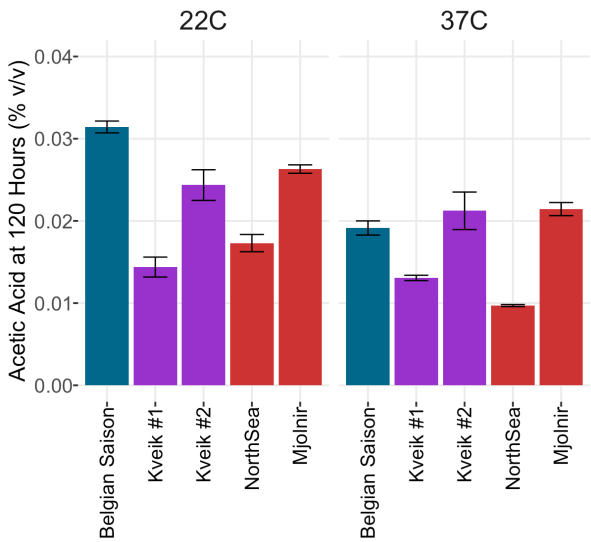
- Kveik maltotriose consumption improves with temperature
- Hybrids most efficient maltotriose metabolism

Foster et al. (in preparation)

Acetate & Glycerol production







- Kveik #2 & Mjolnir produced most glycerol
- Saison produces most acetic acid (22^{o}C), while kveiks and hybrids produced less
- Overall acetic acid production decreased at 37^{o}C
- Kveik #1/NorthSea generally produce less glycerol and acetic acid than Kveik #2/Mjolnir

Foster et al. (in preparation)

Summary

- Several cellular mechanisms at play to convey increased thermal tolerance in kveik yeasts
  - Responses vary among kveik strains

- Kveik-Beer 2 hybrids showed increased temperature tolerance
  - Offer improved fermentation efficiencies compared to parents
  - Produce higher glycerol, but lower acetic acid levels compare to parents

- Not all kveik strains make for suitable breeding partners
  - Some strains more efficient than others

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Research Team


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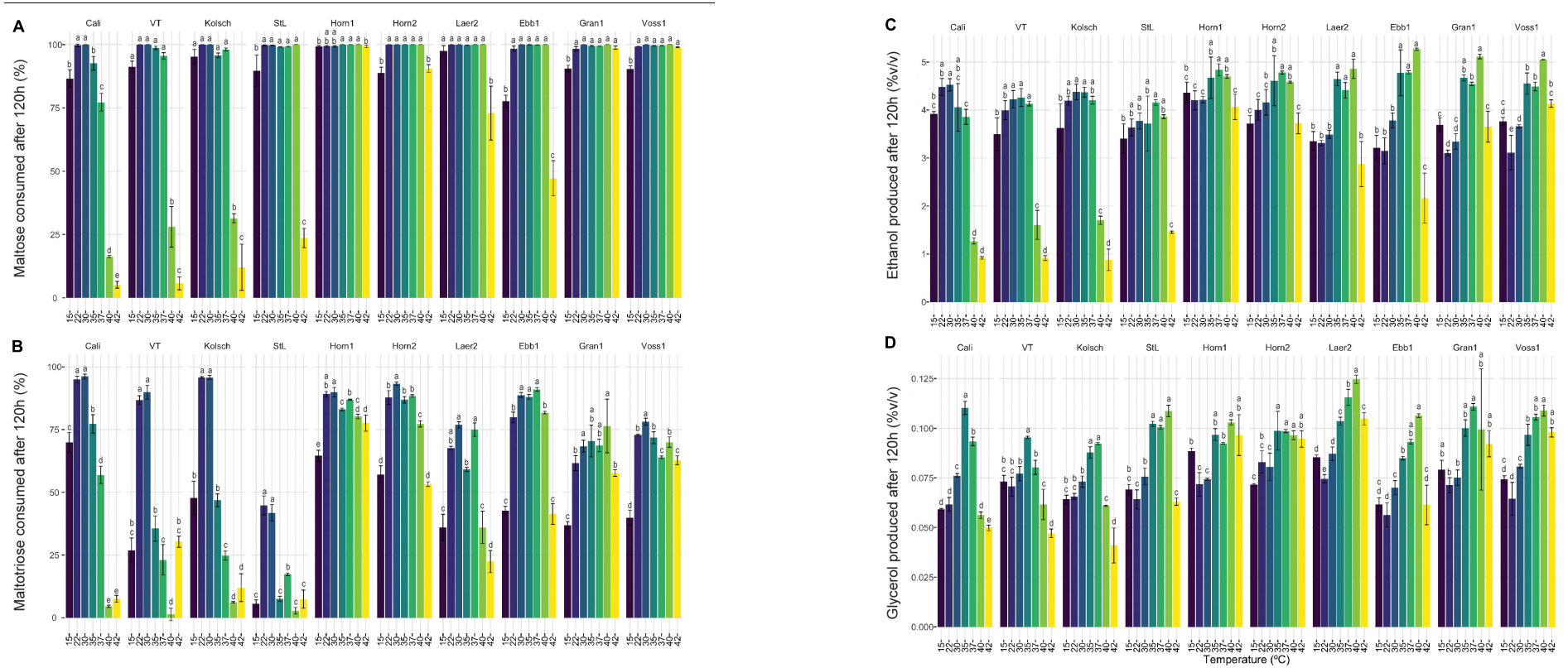
ESCARPMENT LABORATORIES

Carbon metabolism



Wort Sugar	SPA	Upside
Glucose	1.1%	2.1%
Fructose	0.24%	0.27%
Maltose	4.5%	6%
Maltotriose	1.5%	2%
Total Consumable	7.4%	10.3%
Dextrin	2.75%	4.75%