Understanding Yeast Strain Differences Through a Genome-Scale Modeling Approach

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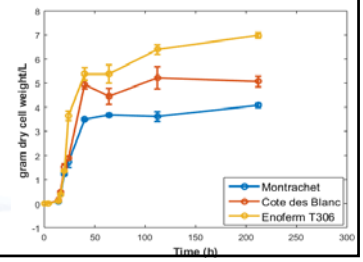
Why is ethanol tolerance important to study?

- Stuck and sluggish fermentations are a chronic problem in the wine industry
- Higher or lower ethanol tolerance in yeast could be useful traits in the wine industry
- "Understanding" will lead to being able to change tolerance—new strains



Commercial Yeast Vary Considerably in Growth

TABLE 2 Fermentation characteristics of *S. cerevisiae* strains used in this study

Strain[a]	UCD accession no.	Mean ± SD: Max OD_{600}	Final °Brix	% (wt/wt) ethanol	End time point (h)
Enoferm T306	2502	5.89 ± 0.16	0.60 ± 0.36	9.93 ± 0.13	312
ICVK1 (V-1116)	2537	5.84 ± 0.42	0.67 ± 0.61	9.83 ± 0.59	264
Sake A18	612	5.76 ± 0.49	0.10 ± 0.17	10.36 ± 0.90	312
Cepage chardonnay	2061	5.64 ± 0.48	0.43 ± 0.45	10.18 ± 0.50	312
FM16-7	V4	5.61 ± 0.14	0.47 ± 0.32	10.67 ± 0.17	264
Enoferm Simi White	2501	5.51 ± 0.32	0.90 ± 0.79	10.67 ± 0.23	312
EC 1118	777	5.49 ± 0.40	0.33 ± 0.58	10.62 ± 0.37	264
Lalvin Rhone L2226	2545	5.20 ± 0.21	0.30 ± 0.30	10.44 ± 0.29	312
ICV D254	2499	5.03 ± 0.15	0.50 ± 0.36	10.34 ± 0.11	312
C0490GP2-B11	V3	4.99 ± 0.32	1.20 ± 0.53	10.57 ± 0.25	312
M2	906	4.97 ± 0.12	0.36 ± 0.02	9.50 ± 0.21	552
Uvaferm 43	2032	4.78 ± 0.51	1.17 ± 1.11	9.08 ± 0.56	312
Lalvin ICVD47	963	4.78 ± 0.14	0.73 ± 0.87	10.10 ± 0.51	312
Cote de Blanc	2031	4.37 ± 0.18	0.30 ± 0.26	10.55 ± 0.16	360
Bread yeast	668	4.20 ± 0.21	0.27 ± 0.46	10.13 ± 0.38	312
Zymaflore VL-1	2074	4.01 ± 0.20	0.27 ± 0.46	9.69 ± 0.28	384
Premier Cuvee	2212	3.21 ± 0.07	1.17 ± 1.00	8.88 ± 0.31	576
FST 40-27*	1427	3.14 ± 0.17	4.40 ± 0.90	7.96 ± 0.44	408
Montrachet*	522	2.98 ± 0.08	3.63 ± 0.32	7.96 ± 0.14	576
Prise de Mousse*	594	2.93 ± 0.06	2.53 ± 0.86	8.57 ± 0.35	432
CY3079*	2497	2.77 ± 0.08	2.60 ± 0.30	8.46 ± 0.20	576
DV10*	2498	2.68 ± 0.07	2.80 ± 0.26	8.33 ± 0.07	576

[a] *, fermentation was stopped due to slow progress.

Why?

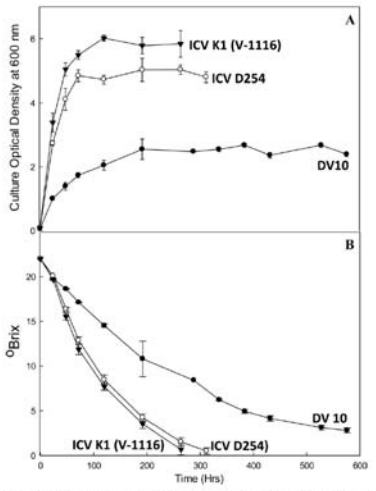


FIG 1 Representative experimental culture optical density at 600 nm (A) and °Brix curves (B) for three industrial yeast strains: DV10 (●), ICV D254 (○), and ICV K1 (V-1116) (▼). Each data point represents the average from fermentations carried out in triplicate.

C. M. Henderson, M. Lozada-Contreras, V. Jiranek, M. Longo, D. E. Block. *Appl. Environ. Microbiol.*, **79**, 91-104, 2013.

Methods to Study Complex Yeast Metabolism

- "Omics"
  - Metabolomics
  - Lipidomics
  - Transcriptomics
- Genome Scale Mathematical Modeling

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Omics Approach

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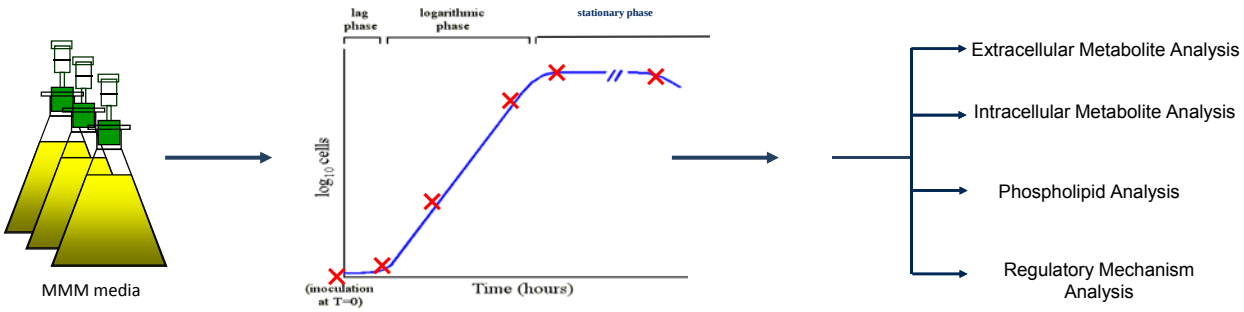
Experimental Design

- 4 yeast strains: Montrachet, Enoferm T306, Uvaferm 43, Cote des blanc
- Anaerobic fermentation at room temperature



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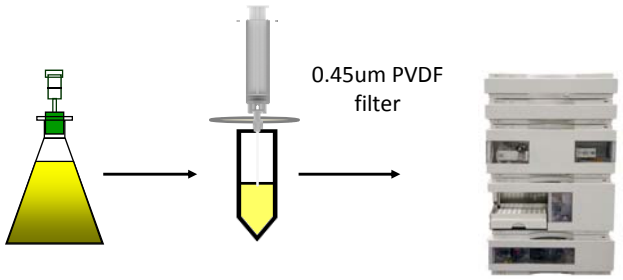
Extracellular Metabolite Analysis



- By Diode Array Detector: Organic acids
- By Refractive Index Detector: Sugars & Ethanol

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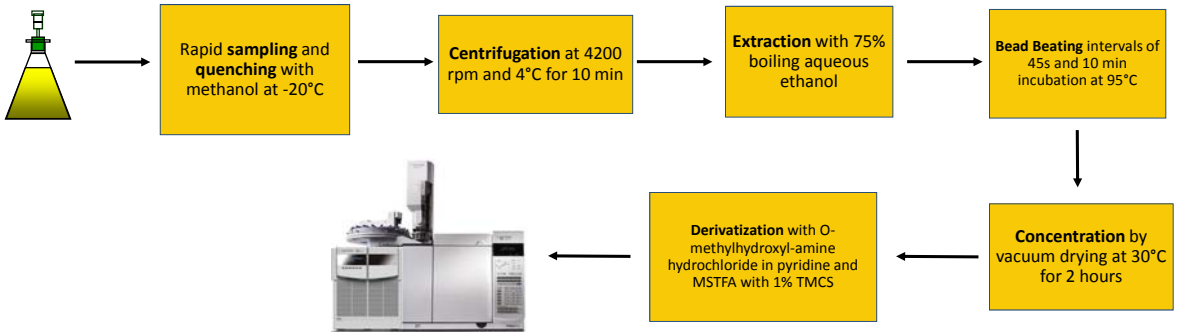
Intracellular metabolite analysis using GC-MS



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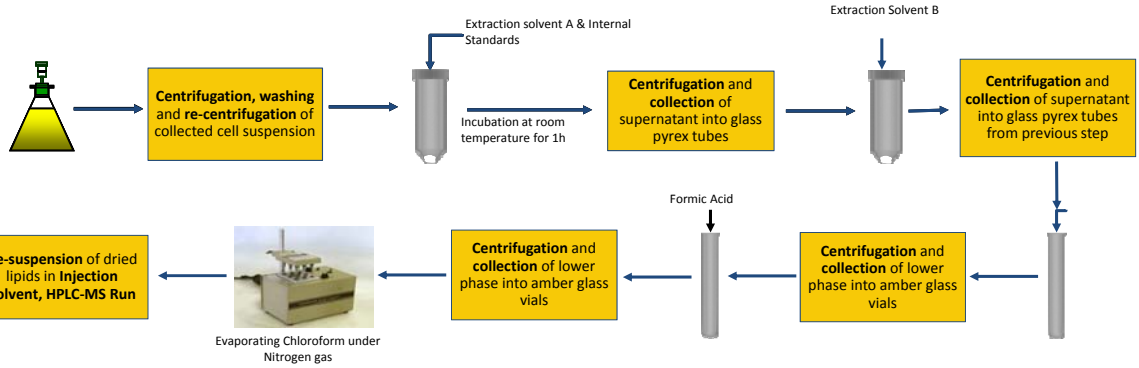
Kind, T., Wohlgemuth, G., Lee, D. Y., Lu, Y., Palazoglu, M., Shahbaz, S., & Fiehn, O. (2009). FiehnLib – mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry.

Phospholipid extraction and analysis using LC-MS



Henderson CM, Lozada-Contreras M, Jiranek V, Longo ML, Block DE. Ethanol Production and Maximum Cell Growth Are Highly Correlated with Membrane Lipid Composition during Fermentation as Determined by Lipidomic Analysis of 22 Saccharomyces cerevisiae Strains. *Applied and Environmental Microbiology*. 2013;79(1):91-104. doi:10.1128/AEM.02670-12.

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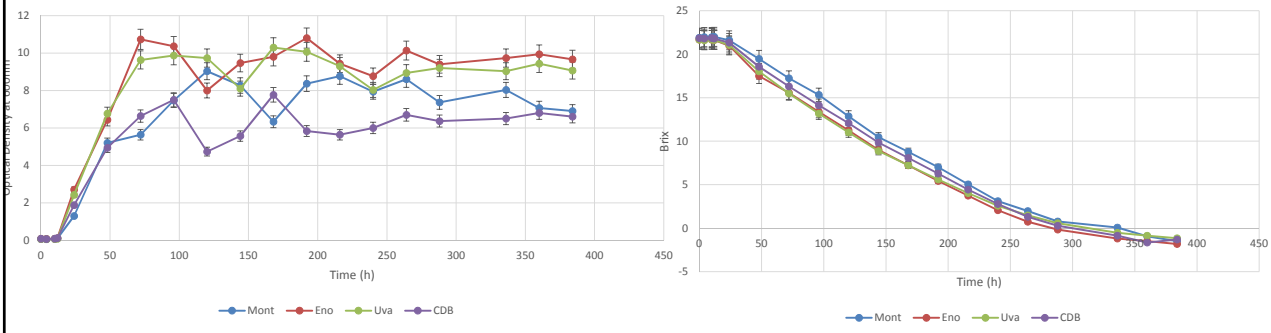
Growth and sugar utilization for representative strains of yeast



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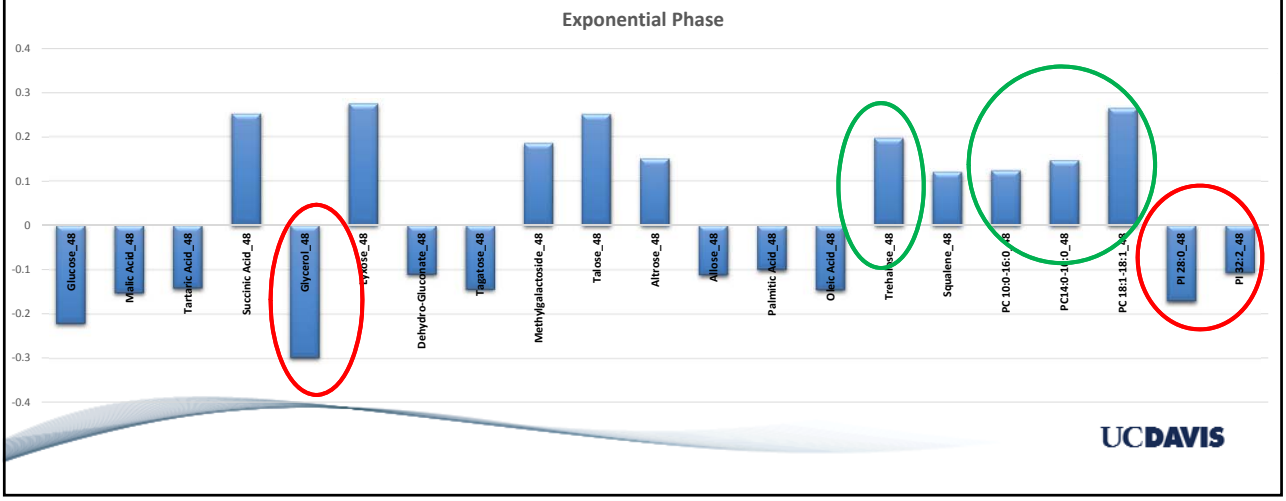
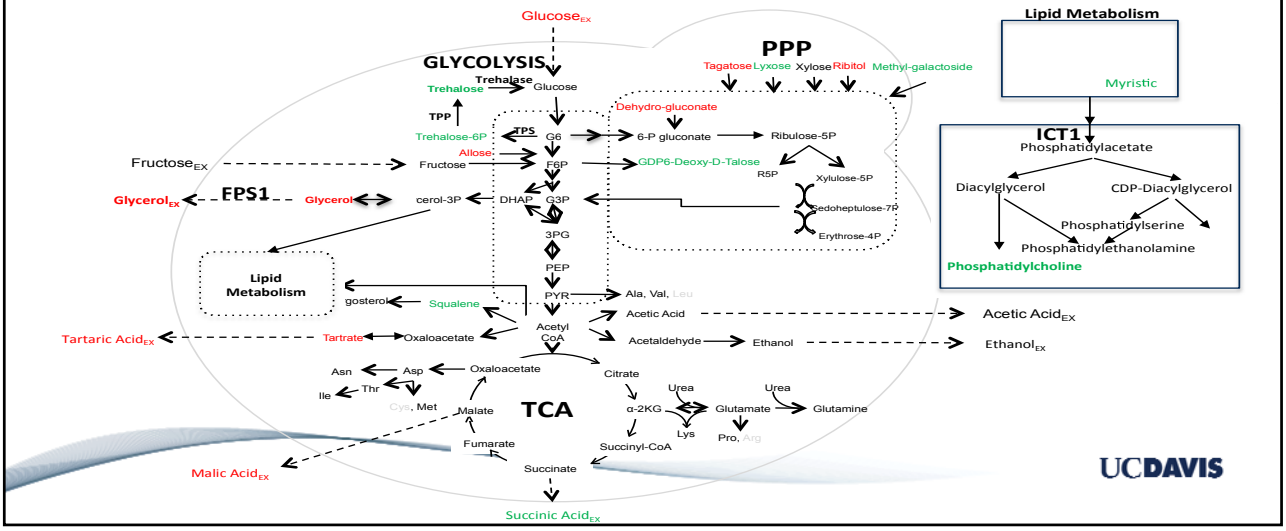
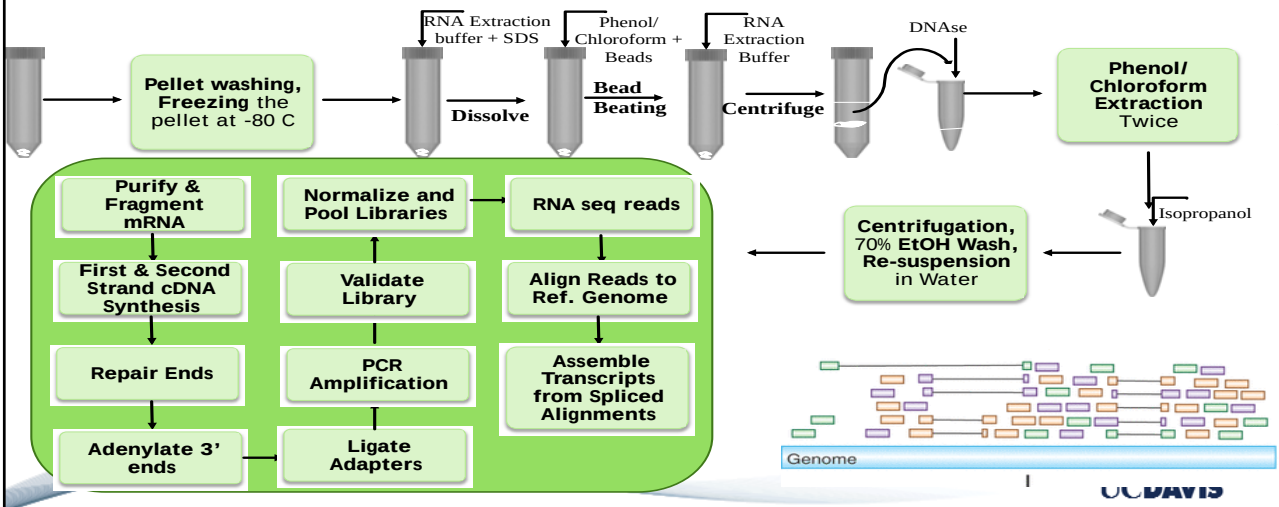
PLS highlights metabolites and lipids correlated with high nutrient utilization efficiency



What part of metabolism is related to nutrient utilization efficiency?



Transcriptomic analysis using RNA-Seq



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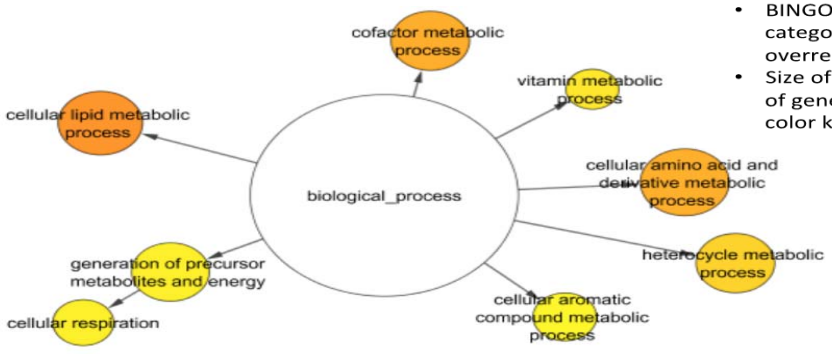
BINGO analysis identifies classes of genes most associated with nutrient utilization efficiency



- BINGO determines which gene ontology categories are statistically overrepresented in a set of genes.
- Size of the circles represent the number of genes involved in the process, and the color key represents the p-value

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Genome Scale Mathematical Modeling Approach

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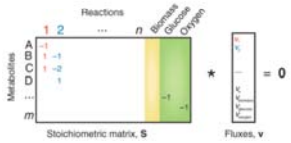
Reconstruction of a GSMM for S. cerevisiae: Yeast 8.3.1

Mathematical representation of a metabolism

A ↔ B + C Reaction 1
B + 2C → D Reaction 2
…
Reaction *n*



Jeffrey D Orth, Ines Thiele & Bernhard Ø Palsson, Nature Biotechnology (2010)

The consensus GEM of *S. cerevisiae* was introduced in 2008 (Herrgard et al., Nature Biotechnology, 2008) → Iterative improvements → Yeast 7.6 (Aung et al., 2013)

Yeast 8.3.1

Taxonomy	Template Model	Reactions	Metabolites	Genes
Saccharomyces cerevisiae	Yeast 7.6	3949	2680	922

Division of Systems and Synthetic Biology, Department of Biology and Biological Engineering, Chalmers University of Technology

Modification of Yeast 8.3.1 to fit enological conditions

- No O_2 uptake
- Allow unrestricted uptake of sterols
- Remove the requirement of heme a in the biomass equation
- Block oxaloacetate-malate shuttle
- Block glycerol dehydrogenase (only acts in microaerobic conditions)
- Block 2-oxoglutarate + L-glutamine → 2 L-glutamate

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Use of dFBA to predict *S. cerevisiae* growth under enological conditions

$$v_G = \frac{v_{max,G}\cdot G}{\left(1+\frac{F}{K_{iF}}\right)\cdot\left(K_G\cdot\left(1+\frac{F}{K_F}\right)+G\right)}$$

$$v_F = \frac{v_{max,F}\cdot F}{\left(1+\frac{F}{K_{iF}}\right)\cdot\left(K_F\cdot\left(1+\frac{G}{K_G}\right)+F\right)}$$

$$v_N = \frac{v_{N,max}\cdot N}{(K_N+N)}$$

Set fructose, glucose, and nitrogen lower and upper bounds

$$v_{by-product,i} = f_i\cdot(v_G+v_F)$$

Set by-product lower bounds

Flux boundaries

$$\text{Min}\{-v_j\}$$
subject to
$$T\cdot v = 0$$
$$v_i^{LB} \le v_i \le v_i^{UB}$$

Fluxes = v

$$\frac{dX}{dt} = \mu\cdot X$$
$$\frac{dC_{extracellular}}{dt} = v_{EX}\cdot X$$

$C_{extracellular}$

Media

COBRA Toolbox, (Hierendt et al., 2013) using Gurobi optimizer (http://www.gurobi.com, 2016)

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Testing dFBA predictions: Experimental methods

3 strains

MMM

UV-vis spectrophotometer

$OD_{600\ nm}$ for cell growth

Gallery machine

Enzymatic assay for yeast assimilable nitrogen (YAN)

Densitometer

°Brix for total sugar

HPLC

Determine extracellular metabolite concentrations

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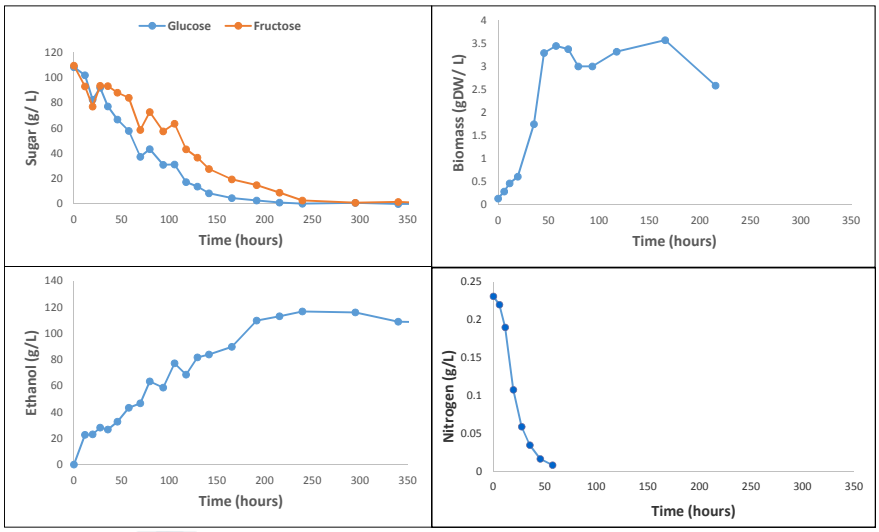
Growth of *S. cerevisiae* in Nitrogen limited grape juice media



Nitrogen is the limiting nutrient

Cramer, 2002

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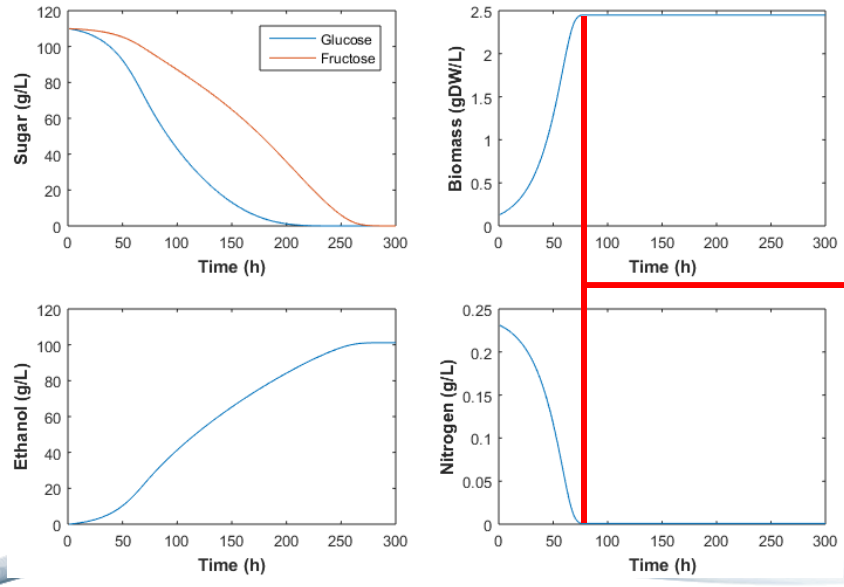
Simulation results: dFBA can predict a nitrogen limited wine fermentation



Nitrogen runs out when cells reach their maximum density

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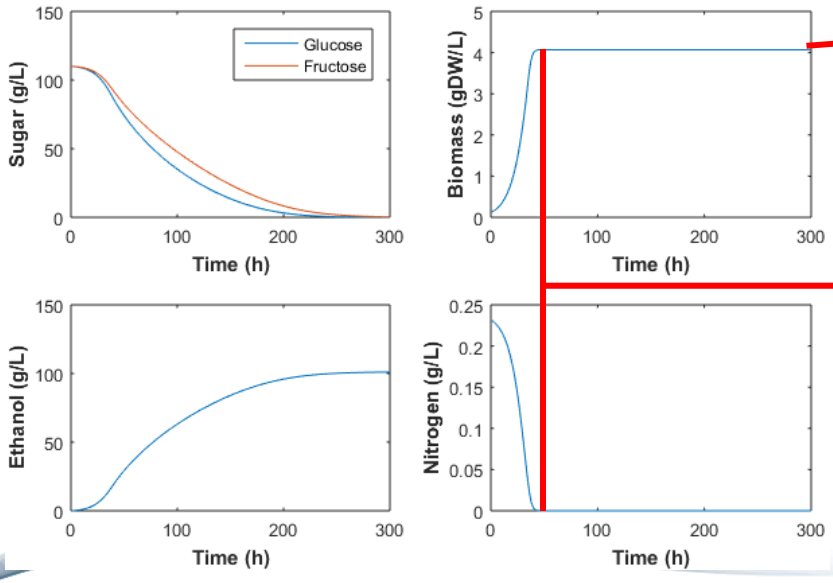
Simulation results: dFBA predictions improve with changing the biomass composition



Biomass composition changes with changing limiting substrate (Schulze 1995): Better predictions

Nitrogen runs out when cells reach their maximum density

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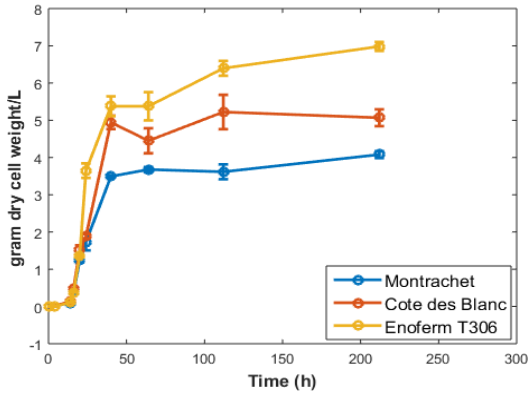
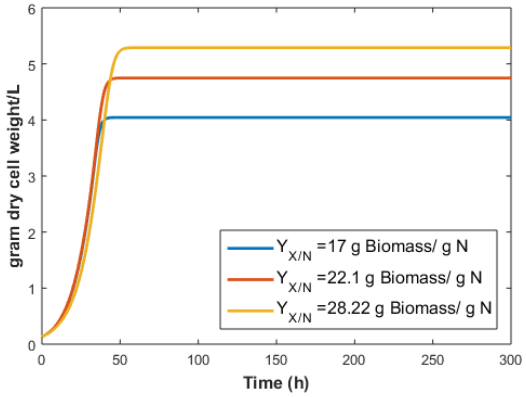
Simulation results: Different strain performances can be predicted

Using different yield coefficients for the nitrogen source can predict differences in strain performances.

Experiment



Genome scale model prediction



These curves have potential to simulate 3 different strains

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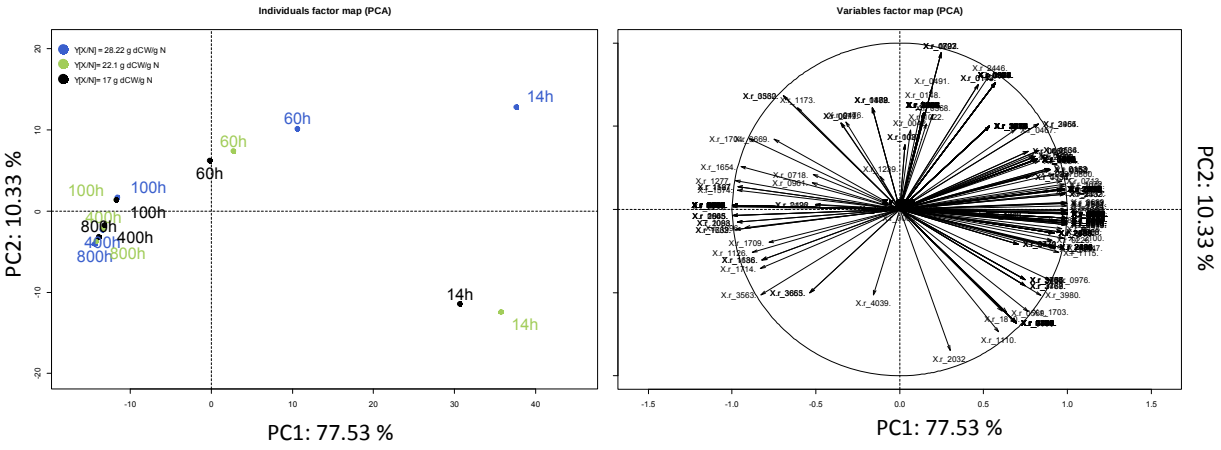
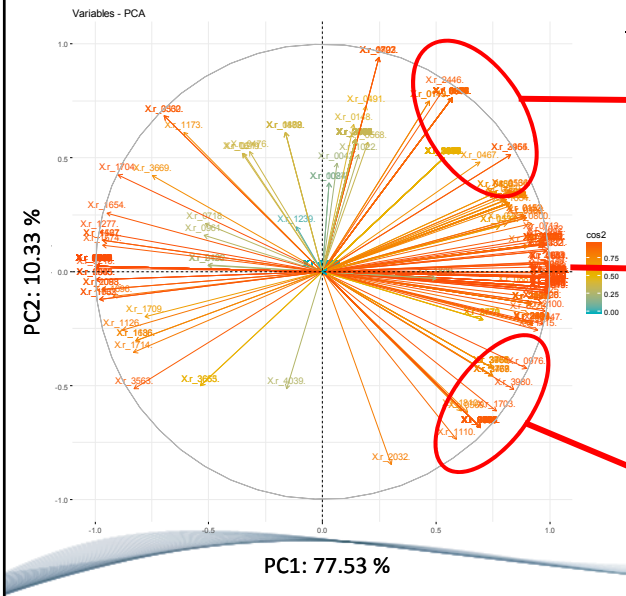
Analysis of metabolic pathways: Comparing strains with PCA



How to see which fluxes are more important? Fluxes with higher squared loadings (cos2) values

Analysis of metabolic pathways: Fluxes (variables) that cause strain differences



Reactions with Significant Contribution

Glucose-6P-isomerase
Adenylate kinase
PE 18:1-18:1 transport from vacuolar memb to ER memb
PS 18:1-18:1 transport to ER memb

→ Associated with higher nutrient utilization efficiency

Ergosterol exchange
Phosphate exchange
Succinate exchange
Sulphate exchange
Oleate exchange

→ Exchange reactions

ergosteryl palmitoleate [er membrane] SLIME rxn
palmitoleate [cytoplasm] SLIME rxn
PE (1-16:1, 2-16:1) [er membrane] SLIME rxn
triglyceride (1-16:1, 2-16:1, 3-16:1) [er membrane] SLIME rxn

→ Lipid reactions

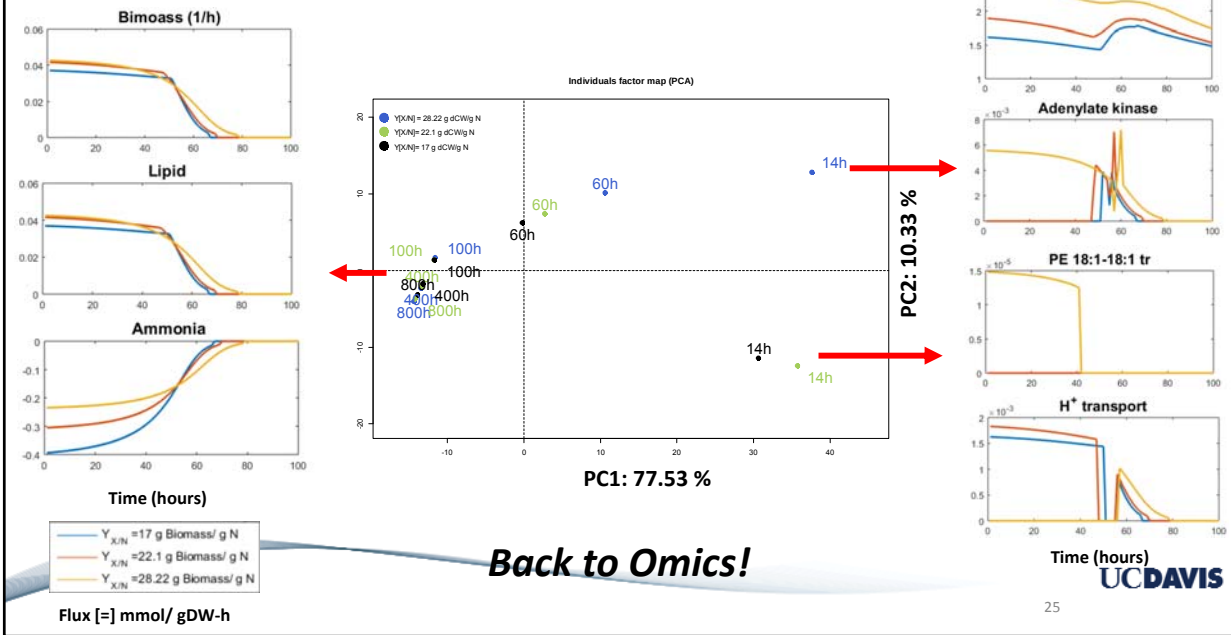
biomass pseudo-reaction
protein pseudo-reaction
carbohydrate pseudo-reaction
RNA pseudo-reaction
DNA pseudo-reaction
lipid pseudo-reaction – merge

→ Biomass component reactions

ADP/ATP Transport
Cytidylate kinase (CMP)
Ribonucleotide reductase
H^+ transport from mitochondria

→ Associated with lower nutrient utilization efficiency

Analysis of metabolic pathways: Comparing fluxes over time



Back to Omics!

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Summary and Future Work

- Metabolism of wine yeast is crucial for nutrient utilization efficiency and ethanol tolerance
- dFBA is a practical way to simulate wine fermentations
- Using a GSMM combined with dFBA offers a useful approach to understand metabolic differences in commercial wine strains
- Apply to other yeast traits like aroma production

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- Systems Biology Research Group of Chalmers University, Sweden
- UC Davis Pilot Winery
  Charles Brenneman, Eric Hanson
- Ernest Gallo Endowed Chair in Viticulture and Enology
- American Vineyard Foundation

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