A minimal set of glycolytic genes reveals strong redundancies in *S. cerevisiae* central metabolism

Eero Gustafsson, Lauri Honkanen, Martina Huusela

Introduction

- Paralog genes are formed in gene duplication events and can be found in all taxa [1-3]
  - A multiplied gene can be divided into major and minor gene(s) according to the importance of their function
- Purpose
  - provide template DNA for evolutionary mutations, gene dosage effect [4]
- Duplicated genes are lost from the genome with time when new functions do not arise [5]
- *Saccharomyces cerevisiae* is a well-studied model organism and widely used for industrial purposes
- *S. cerevisiae* has multiple conserved regions of paralogues genes [6-9]
- Result from a whole genome duplication events in an ancestor [10]

Aim of the study

- The Embden-Meyerhof-Parnas (EMP) pathway in *S. cerevisiae* is formed from 12 enzyme-catalyzed reactions
- 8 of these are produced by paralog genes
- Previous hypothesis for EMP paralog reservation
  - High glycolytic capacity with or without O_2
- Study the role of the minor paralog genes in the EMP pathway
  - Effects on growth kinetics
  - Intracellular metabolite concentration
  - Gene expression
- Create a ”minimal glycolysis” yeast strain (IMX372) with paralog deletions

Materials &Methods

- A ”minimal glycolytic” strain was constructed by deleting 13 minor paralogs and leaving 14 major ones
- Experiments conducted using minimal strain and control strain
  - Glycolytic flux analysis in several conditions
    - Aerobic and anaerobic
  - Detection of phenotypic changes by transcriptome analysis, metabolite testing and enzyme activity analysis
  - Sequencing of the resulted strains

Materials & Methods

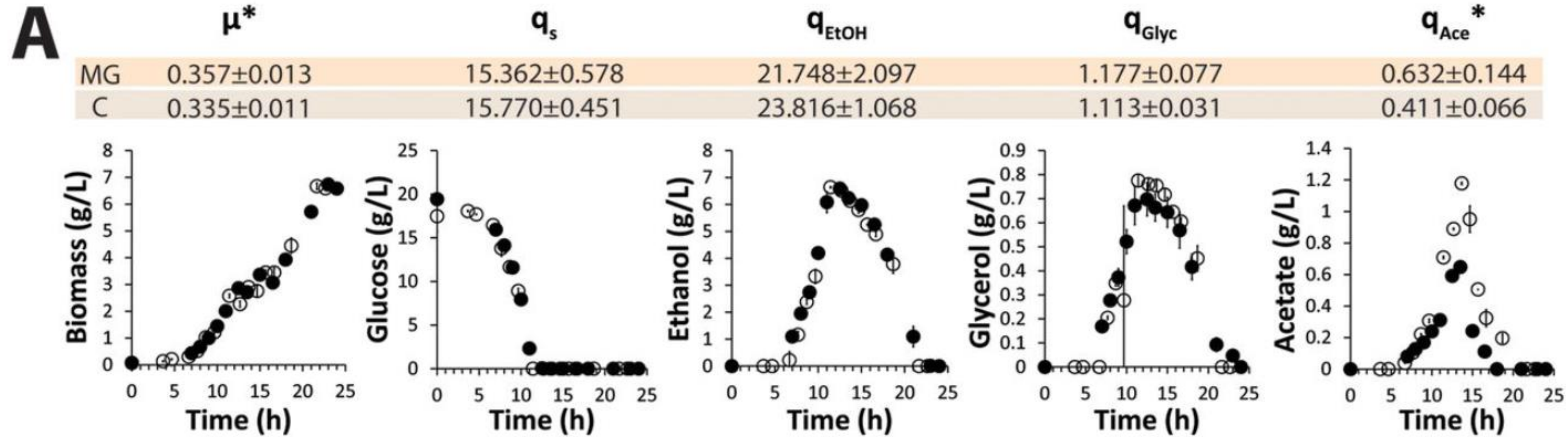
- The experiment used strains from the CEN.PK family
- IMX372, minimal strain, was constructed from CEN.PK102-12A strain
- Genes were deleted using
  - Dominant and auxotrophic markers
  - KlURA3/5-fluoroorotic acid system
- kgnslnlsnglsg

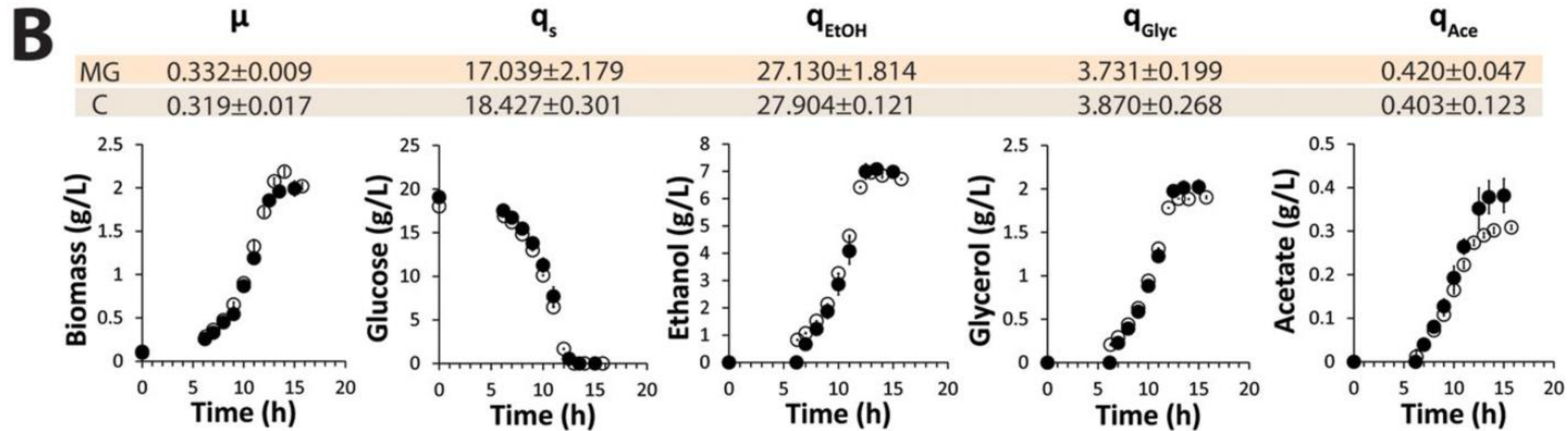
Gene deletions

GLK1	HXK1	TDH1, TDH2	GPM2 , GPM3	ENO1	PYK2	PDC5, PDC6	ADH2, ADH5, ADH4
GLUCOKINASE	HEXOKINASE	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROG.	PHOSPHOCLYCERATE MUTASE	ENOLASE	PYRUVATE KINASE	PYRUVCATE DECARBOXYLASE	ALCOHOL DEHYDROGENASE

Achievements of this work

- Successful creation of a MG (minimal glycolysis) strain
  - Deletion of 13 minor paralog glycolytic genes from the reference strain
  - GLK1, HXK1, TDH1,TDH2, GPM2, GPM3, ENO1, PYK2,PDC5, PDC6, ADH2, ADH5 and ADH4
  - Sequence data NCBI: PRJNA269221
- Results suggest that under the various test conditions, these paralog glycolytic genes have little-to-no effect on the metabolic or transcriptive activity of the yeast
- There is still a mystery on why these paralog genes have survived through evolutive pressure, suggesting that there is indeed a niche environment of where the yeast benefits from these paralogs

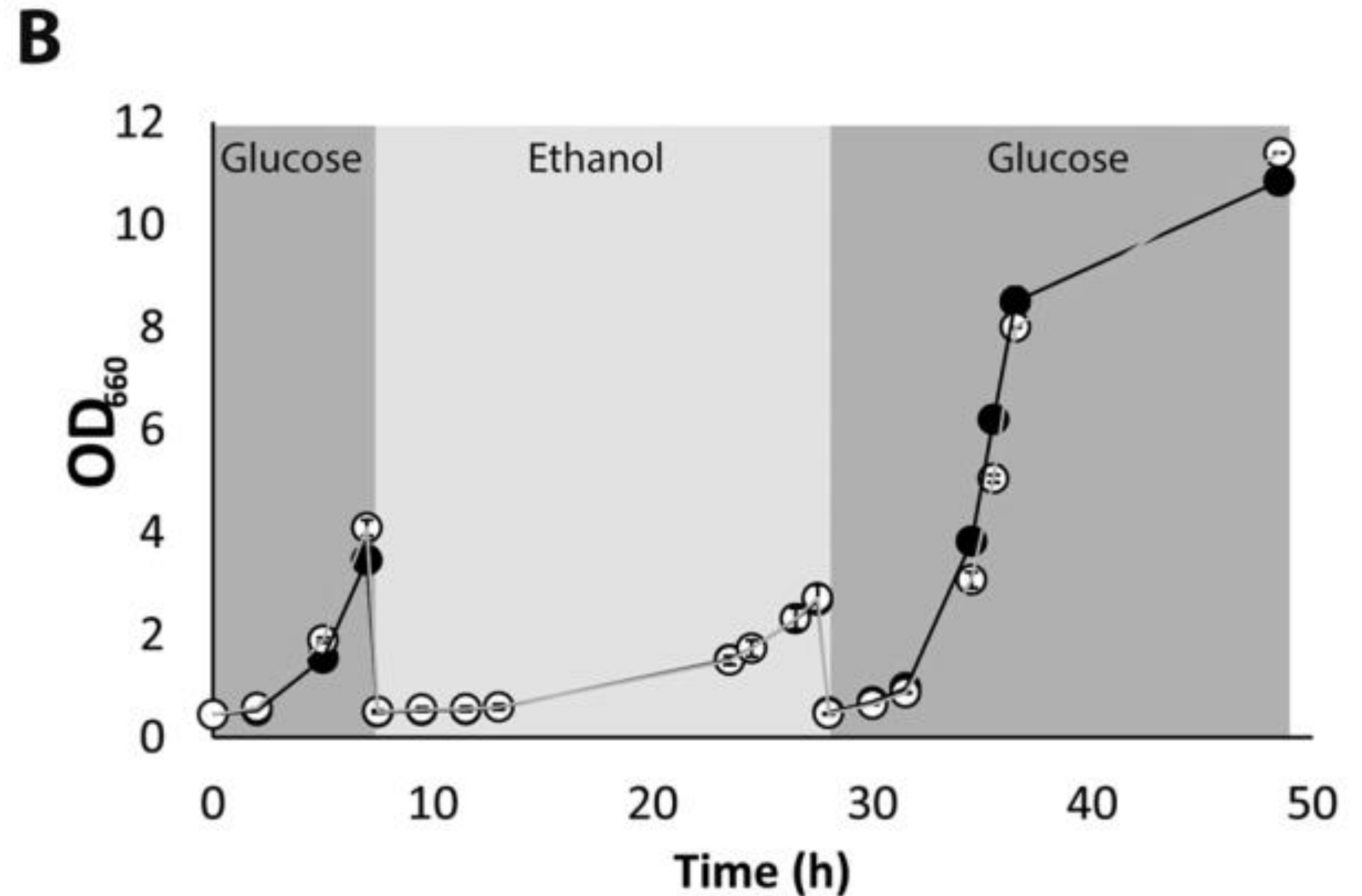




Picture: DOI:https://doi.org/10.1128/EC.00064-15

Figure illustrating the growth of the MG strain and the unmodified reference strain in shake flasks in different conditions (Optical density of the culture vs. time in hours)

The MG-strain is marked with white dots, whereas the reference strain is marked with black dots



Picture: DOI:https://doi.org/10.1128/EC.00064-15

Relevancy of this work

- Figuring out the relevancy of the genes in *S. cerevisiae* helps us understand and engineer the organism better
- Metabolic and glycolytic engineering of yeast has tremendous importance and potential in the field of synthetic biology
- Getting rid of redundancies allows for more effective digitalization and mathematization of the metabolic pathways in yeast
- This article has been used as a reference in many research papers investigating metabolic engineering highlighting the relevancy, such as:
  - Efficient protein production by yeast requires global tuning of metabolism (2017)
  - Coupling gene regulatory patterns to bioprocess conditions to optimize synthetic metabolic modules for improved sesquiterpene production in yeast (2017)

References – Minimal set of glycolytic genes reveals strong redundancies in *S. cerevisiae* central metabolism

1. Fothergill-Gilmore LA, Michels PAM. 1993. Evolution of glycolysis. *Prog Biophys Mol Biol* 59:105–235.

2. Dandekar T, Schuster S, Snel B, Huynen M, Bork P. 1999. Pathway alignment: application to the comparative analysis of glycolytic enzymes. *Biochem J* 343:115–124

3. Steinke D, Hoegg S, Brinkmann H, Meyer A. 2006. Three rounds (1R/2R/3R) of genome duplications and the evolution of the glycolytic pathway in vertebrates. *BMC Biol* 4:16.

4. Innan H, Kondrashov F. 2010. The evolution of gene duplications: classifying and distinguishing between models. *Nat Rev Genet* 11:97–108.

5. Ohno S. 1970. *Evolution by gene duplication*. Springer-Verlag, New York, NY.

6. Dean EJ, Davis JC, Davis RW, Petrov DA. 2008. Pervasive and persistent redundancy among duplicated genes in yeast. *PLoS Genet* 4:e1000113.

7. DeLuna A, Vetsigian K, Shoresh N, Hegreness M, Colon-Gonzalez M, Chao S, Kishony R. 2008. Exposing the fitness contribution of duplicated genes. *Nat Genet* 40:676–681

8. Gu X, Zhang ZQ, Huang W. 2005. Rapid evolution of expression and regulatory divergences after yeast gene duplication. *Proc Natl Acad Sci U S A* 102:707–712

9. Musso G, Costanzo M, Huangfu MQ, Smith AM, Paw J, Luis BJS, Boone C, Giaever G, Nislow C, Emili A, Zhang ZL. 2008. The extensive and condition-dependent nature of epistasis among whole-genome duplicates in yeast. *Genome Res* 18:1092–1099.

10. Wolfe KH, Shields DC. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* 387:708–713.

Pathway swapping: Toward modular engineering of essential cellular processes

Introduction and aim of the study

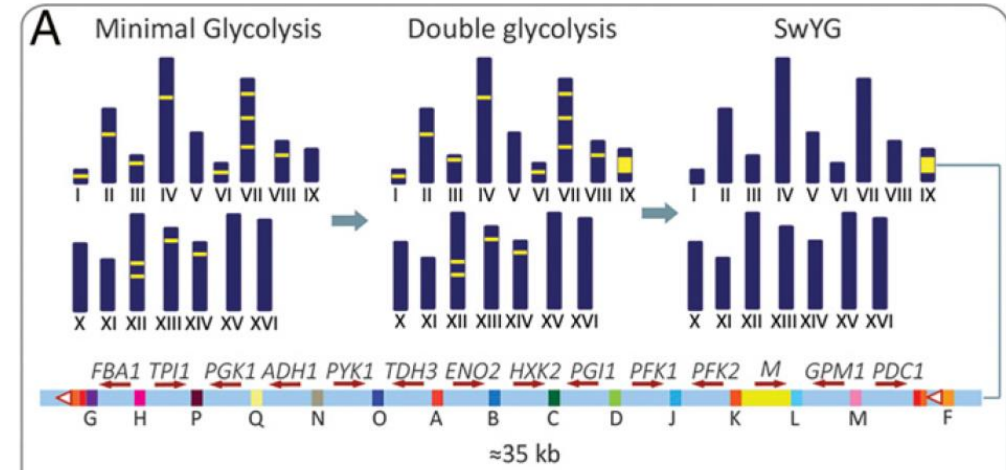
- In contrast to single gene modifications, modification of entire genetic processes in wild-type organisms is laborious
    - Genes related to process are typically scattered across the genome
- Easy improvement, modification and introduction of essential cellular processes to an organism is one of the aims of synthetic biology

Aim of the study

- Building on the results of the previous study, the first aim of this study was to construct a *S.cerevisiae* strain with all the minimal glycolytic genes arranged to a single chromosomal locus
- The second aim was to replace this native glycolytic "module" by a different set of glycolytic genes
  - Module containing *S.kudriavzevii* promoters and glycolytic genes
  - A mosaic module containing glycolytic genes from *S.cerevisiae, S.kudriavzevii* and *Homo sapiens*

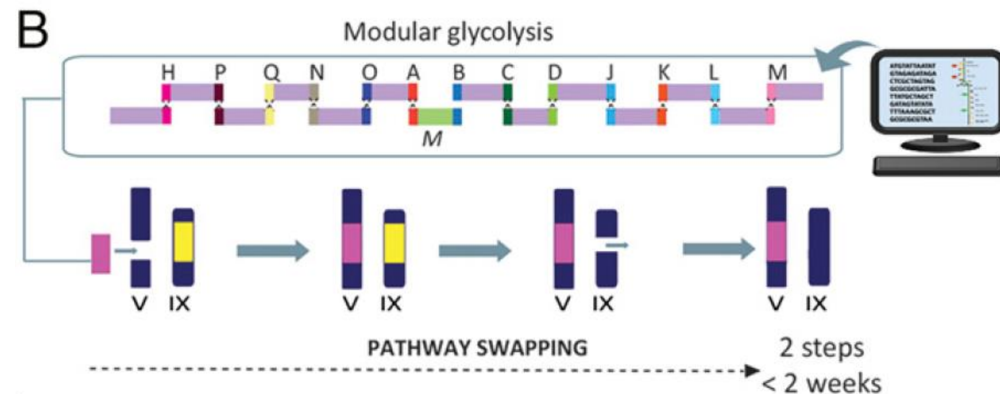
Methods to construct the module

- Construct the chromosomal sequence from the minimal glycolysis genes (previous study)
- The different genes flanked by synthetic homologous regions (SHRs)
- Mutations were screened
- CRISPR-Cas9 to remove the glycolytic genes from their native loci
- Minor alterations (such as synthetic promoter for the gene *ENO2*)



Chromosomal hopping

- CRISPR-Cas9 was used to transfer the module from chromosome IX to its final location in the chromosome V
- The module was finally integrated to the *CAN1* locus of the chromosome V
- The performance of the cluster virtually identical in both loci

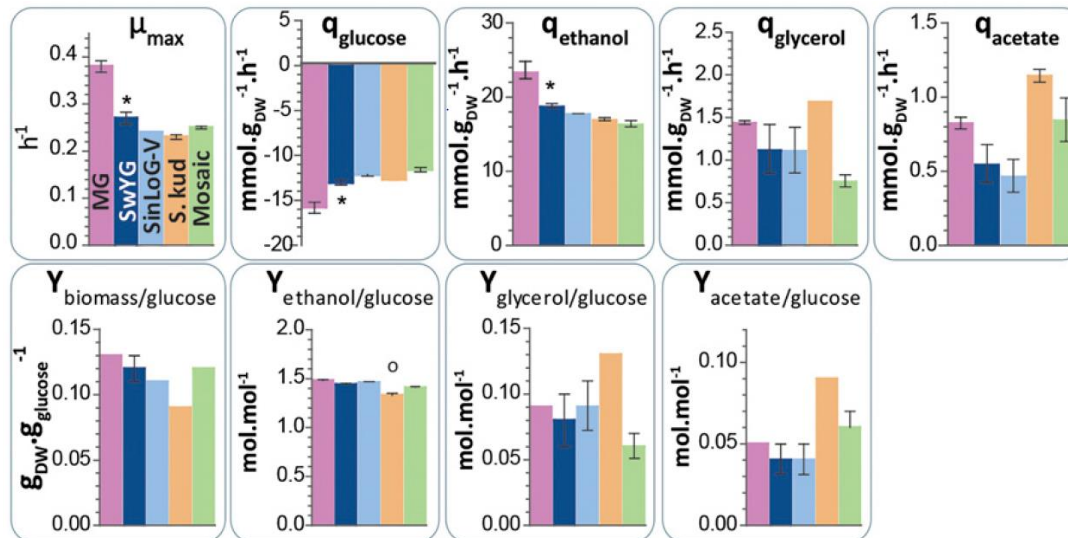


Information about the organisms

- *S. kudriavzevii* is a relative of *S. cerevisiae* adapted to cool climates
- Its genome is sequenced but not well-characterized
  - The putative glycolysis-related genes in *S. kudriavzevii* can be inferred by homology
  - Some difference in the glycolytic gene set between the two organisms
- Many *S. cerevisiae* genes have orthologs in *Homo sapiens*
  - Two gene orthologs used in this study, *HsTPI1* and *HsPGK1*, have been shown to complement null mutations in *S. cerevisiae*
  - *S.cerevisiae* native promoters used for gene expression

Discussion

- Results show that non-native and synthetic glycolytic modules function comparably well in *S. cerevisiae*
- The compact placement of all the genes into a single locus in the chromosome negatively affects the magnitude of metabolic fluxes, but not the product stoichiometry
  - Excellent for research purposes
  - As a production host: module likely needs to be "re-scattered" for strain optimization



Significance of the study

- The results yield potential to study different non-native glycolytic pathways in *S. cerevisiae*
- More generally, potential to study easily different cohesive sets of genes linked to a cellular process in any eukaryotic organism
- The chromosomal locus needs to be carefully considered
  - Vicinity of the genetic module to telomers and centromers lead to gene silencing
  - Proximity to autonomous replicating sequences (ARSs) tends to increase transcription

Thank you !