Constraint-Based Network Analysis of Sugar Fermentation in *Saccharomyces cerevisiae*

M. Abdul Majeed[1], Joonhoon Kim[2] and Jennifer L. Reed[2]
Khorana Program 2009
[1]*Indian Institute of Technology -Madras,* [2]*University of Wisconsin-Madison*

Abstract

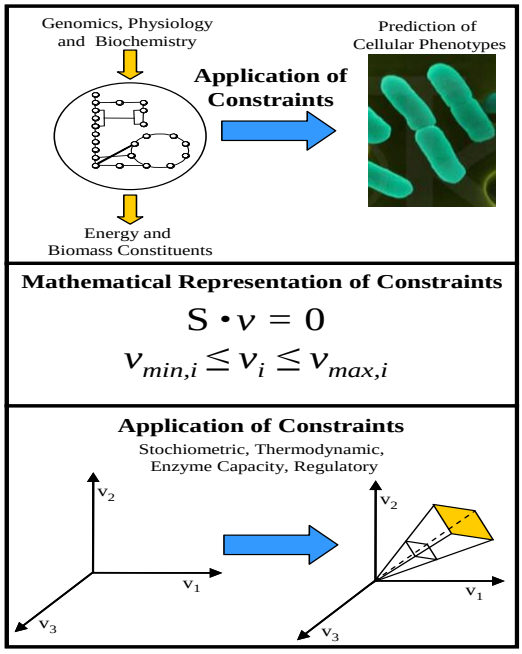
There is considerable interest in recent years in the bioconversion of forestry and agricultural residues into ethanol and value added chemicals. High ethanol yields from lignocellulosic residues are dependent on efficient use of all the available sugars including glucose and xylose. The well-known fermentative yeast Saccharomyces cerevisiae is the preferred microorganism for ethanol production and various genome scale metabolic networks have been reconstructed for S. cerevisiae in the recent years. In this study, S. cerevisiae's network is analyzed by constraint based modeling techniques and the organism's inability to ferment xylose is studied. This inability is due to the cofactor imbalance occurring at the XR and XDH reactions in the xylose uptake pathway. Two genes were found when up-regulated in the regulatory network; xylitol secretion was not predicted. Also, various strains were designed computationally for improving the S. cerevisiae's ethanol yield on glucose fermentation.

Introduction

Diverse datasets, including genomic, transcriptomic, proteomic, and metabolomic data are becoming readily available for a large number of organisms. There is currently a need to integrate these datasets within an *in silico* modeling framework. Constraint-based models of *Saccharomyces cerevisiae* have been developed over the past recent years and have been used to study the organisms metabolism and regulation, and to predict it's phenotypic behavior. These models have also been useful for generating testable hypotheses about network components and interactions, predict behavior of perturbed systems and for metabolic engineering applications. The most comprehensive *Saccharomyces cerevisiae* metabolic and regulatory models to date are (iMM904) and (iMH805) respectively.

What are Constraint Based Models?

Unlike kinetic models which find one solution to a system of equations, constraint-based models use physico-chemical constraints to eliminate solutions, leaving a set of feasible solutions defining the allowable solution space.

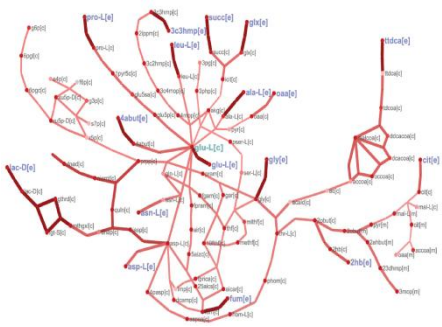


Types of Constraints:

1. Steady-State Mass Balance
   $\sum$Prod. = $\sum$Cons.
   $\Rightarrow \sum_j S_{ij}v_j = 0$
2. Enzyme Capacity
   $V_{j,lb} \leq v_j \leq v_{j,ub}$
3. Thermodynamics
   $v_{irrev} \geq 0$

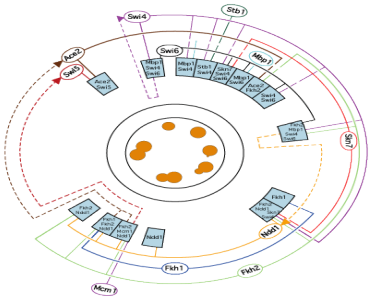
Genome - Scale Network Reconstructions

Metabolic Network:



iMM904 metabolic network used in this analysis accounts for 904 metabolic genes, 1577 metabolic reactions and 1228 metabolites.

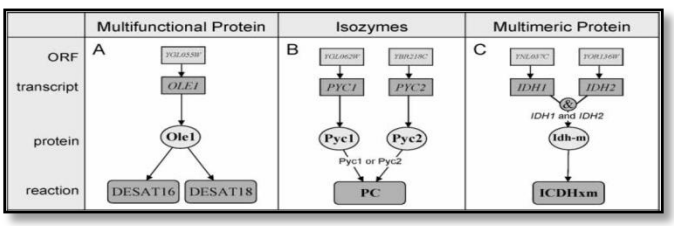
Transcriptional Regulatory Network:



iMH805 regulatory network used in this analysis accounts for 92 TF interactions, 745 Target interactions and regulation of 805 metabolic genes.

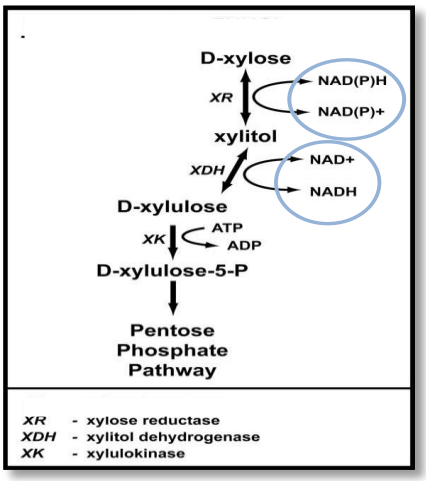
Gene - Protein - Reaction Associations:
Not all genes have a one-to-one relationship with their corresponding enzymes or reactions. So, a systematic generation of the GPR associations is needed in order to link the genes to the reactions/enzymes.

For instance,



Strategies for improving yeast on Xylose

Issue: *Redox imbalance from xylose reductase (XR) and Xylitol dehydrogenase (XDH).*



The differencing cofactor specificities result in the accumulation of NADP and NADH during these oxidoreduction reactions.

NADPH can be regenerated via fructose-6-P in the PPP and the NADP-dependent enzymes isocitrate dehydrogenase and aldehyde dehydrogenase, which maximize the accumulation of NADP. However insufficient quantities of NAD are recycled for the XDH reaction, resulting in an overabundance of NADPH relative to NAD for the XR and XDH catalyzed reactions, respectively. The electron transfer system is unable to re-oxidize NADH to NAD by respiration due to low O2 levels, and ethanol fermentation does not produce a net yield of NAD.

This cofactor-recycling imbalance occurs under conditions of reduced respiration, and results in inhibition of XDH activity, which in xylose-fermenting yeasts significantly contributes to increased xylitol formation and decreased ethanol production.

Possible Solution: *Up-regulation of 2 genes.*

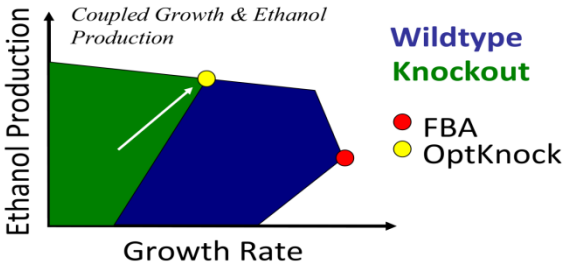
The metabolic model alone did not predict any Xylitol secretion. But, when the regulatory model is introduced, Xylitol secretion was observed. Upon further analysis, i.e. comparing the 2 simulations, 2 genes were

found which when up-regulated in the regulatory model, xylitol secretion was not predicted.

Hence, xylitol secretion caused by the cofactor imbalance can be attributed to the down-regulation of the 2 genes.

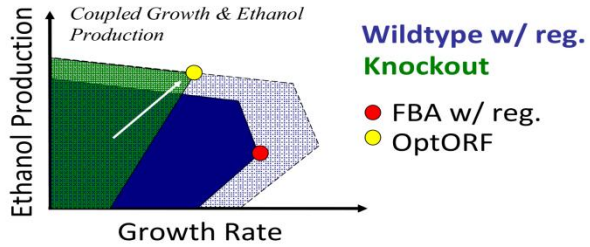
Strain Design for Ethanol Production

Optknock: Optimal Reaction Deletion:



- **Identifies reactions**, whose removal **forces the coupling between growth** rate and **metabolite production.**
- To achieve the maximum growth rate the corresponding knockout mutant must also secrete a metabolite of interest.

OptORF : Optimal Gene Deletion (metabolic and/or regulatory genes)



- **Identifies genes**, whose removal **forces the coupling between growth** rate and **metabolite production.**
- Gene-protein-reaction associations and transcriptional regulations are systematically formulated as constraints and accounted for in the strain designs.

Computational Design of Ethanologenic S.cerevisiae Strains:

1. Optknock (Glucose Oxygen Limited)

Strain Description	No: of genes to be Knocked out	Growth Rate (1/hr)	Ethanol yield (%theor.)
Wild type	-	0.342	83.8
G3PD1ir ; GHMT2r ; HSDxi ; PC	5	0.1528	91.8
ATPS3m ; GLUK ; HEX1	6	0.163	91.7

2. Optknock (Glucose Anaerobic)

Strain Description	No: of genes to be Knocked out	Growth Rate (1/hr)	Ethanol yield (%theor.)
Wild type	-	0.298	83.2
AMPDA ; MDH ; MDHm ; PPA	4	0.212	91.1
MDH ; MDHm ; PPA	3	0.229	90.1

3. OptORF (Glucose Oxygen Limited)

Strain Description	Growth Rate (1/hr)	Ethanol yield (%theor.)
Wild type	0.340	83.9
YEL024W ; YJL121C ; YML004C	0.250	87.9
Q0080 ; YCR032W ; YGR183C ; YLR058C ; YKL174C	0.265	87.6

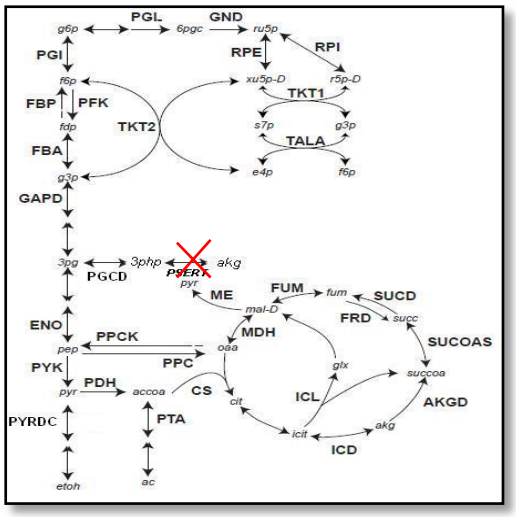
4. OptORF (Glucose Anaerobic)

Strain Description	Growth Rate (1/hr)	Ethanol yield (%theor.)
Wild type	0.273	85.3
YAL054C ; YBR011C ; YML035C ; YGR193C	0.177	90.0
YBR011C;YML035C;YGR193C	0.183	89.6

Theoretical Yield = 0.51 g ethanol/g glucose (2 mol ethanol / mol glucose)

Simple illustration:

(Optknock Glucose Anaerobic)
Rxn knocked out: PGCD {μ= 0.29; etoh= 87.3%} (Phosphoglycerate Dehydrogenase)
Knockout of PGCD pushes flux through the downstream pathway as shown in the figure below.



While some of the suggested deletion strategies are straightforward and involve competing reaction pathways, many others suggest complex and non-intuitive mechanisms of compensating for the removed functionalities.

Integration of gene-expression data to the metabolic model

Using the tissue specific modeling algorithm from Shlomi paper; integration of gene expression data to the metabolic model was done in order to constraint the model further. But even on the various modifications to the original algorithm (introducing a threshold for low expression set and varying the threshold values); one could not see xytitol secretion prediction.

Acknowledgments

Thanks to Department of Biotechnology (DBT) – Government of India, the Indo-US Science and Technology Forum (IUSSTF), University of Wisconsin-Madison (UW) and the Great Lakes Bioenergy Research Center (GLBRC) for making this possible.

Contact:

Email: m.abdul.majeed1@gmail.com

References:

- Connecting extracellular metabolomic measurements to intracellular flux states in yeast (Monica L Mo, Bernhard Ø Palsson and Markus J Herrgård) (BMC Systems Biology 2009, 3:37 doi:10.1186/1752-0509-3-37)
- Integrated analysis of regulatory and metabolic networks reveals novel regulatory mechanisms in Saccharomyces cerevisiae (Markus J. Herrgård, Baek-Seok Lee, Vasiliy Portnoy, and Bernhard Ø. Palsson) (Genome Research, 16(5):627-35)(2006))
- Gene Protein Reaction figure - Natalie C. Duarte, Markus J. Herrgård and Bernhard Ø. Palsson (Genome Res. 2004 14: 1298-1309)
- Regulatory model figure - Simon et al - Cell.;106(6):697-708 (2001)
- OptKnock: A Bilevel Programming Framework for Identifying Gene Knockout strategies for Microbial Strain Optimization (Anthony P. Burgard, Priti Pharkya, Costas D. Maranas) (Biotechnology and Bioengineering;Volume 84 Issue 6, Pages 647 – 657; 2003)
- Network-based prediction of human tissue-specific metabolism (Tomer Shlomi, Moran N Cabili1, Markus J Herrgard, Bernhard Ø Palsson & Eytan Ruppin)(Nature biotechnology volume 26; number 9; doi:10.1038/nbt.1487;2008)