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

M. Abdul Majeed[1], Joonhoon Kim[2] and Jennifer L. Reed[2]

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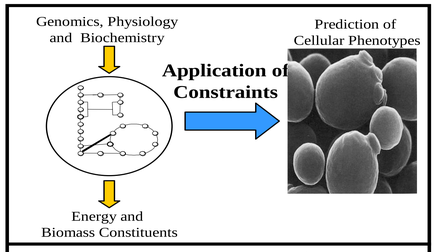
[1]*Indian Institute of Technology -Madras,* [2]*University of Wisconsin-Madison*

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.



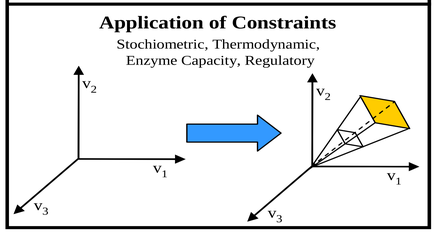
Mathematical Representation of Constraints

$$S \cdot v = 0$$

$$v_{min,i} \le v_i \le v_{max,i}$$

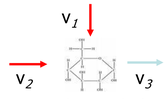
Application of Constraints

Stochiometric, Thermodynamic, Enzyme Capacity, Regulatory



Types of Constraints

1. Steady-state mass balance

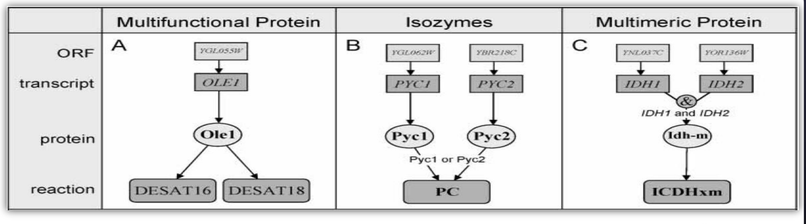


For each metabolite:

$\Sigma s_{ij} \cdot v_{produce} = \Sigma - s_{ij} \cdot v_{consume}$

2. Thermodynamic
3. Enzyme capacity

Gene - Protein - Reaction Associations



Rxn(DESAT16) = Pro(OLE1)
Rxn(DESAT18) = Pro(OLE1)
Pro(OLE1) = Trans(OLE1)
Trans(OLE1) = ORF(YGL055W)

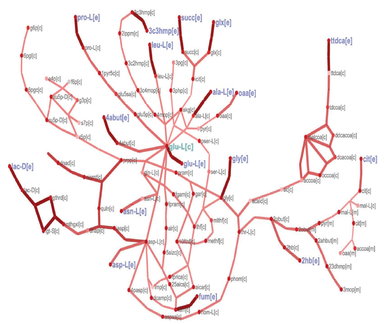
Rxn(PC) = Pro(Pyc1) or Pro(Pyc2)
Pro(Pyc1) = Trans(PYC1)
Pro(Pyc2) = Trans(PYC2)
Trans(PYC1) = ORF(YGL062W)
Trans(PYC2) = ORF(YBR218C)

Rxn(ICDHxm) = Pro(Idh-m)
Pro(Idh-m) = Trans(IDH1) and Trans(IDH2)
Trans(IDH1) = ORF(YNL037C)
Trans(IDH2) = ORF(YOR136W)

Natalie C. Duarte, Markus J. Herrgård and Bernhard Ø. Palsson (Genome Res. 2004 14: 1298-1309)

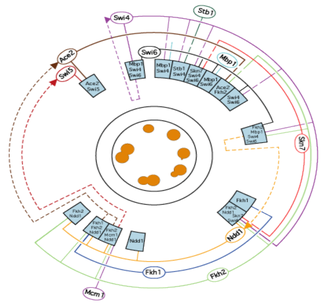
Genome-Scale Network Reconstructions

iMM904: Metabolism (904 Genes)



Monica L Mo, Bernhard Palsson and Markus J Herrgård (BMC Systems Biology 2009, 3:37 doi:10.1186/1752-0509-3-37) (2009)

iMH805: Regulation (837 Regulatory Interactions)



Simon et al - Cell.;106(6):697-708 (2001)

Metabolic Network: 904 metabolic genes, 1577 metabolic reactions, 1228 metabolites (iMM904)(*Mo et al - BMC Systems Biology,doi:10.1186/1752-0509-3-37)(2009*)

Transcriptional Regulatory Network: 92 TF interactions, 745 Target interactions, regulation of 805 metabolic genes (iMH805) (*Herrgard et al - Genome Research,* 16(5):627-35)*(2006*)

Strategies for improving yeast on Xylose

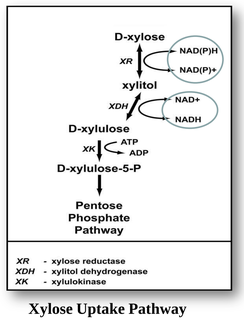
Issue:

Yeast does not have a transhydrogenase reaction

NADH + NADP ⇎ NAD + NADPH

i.e. a Cofactor Imbalance

Xylitol secretion



Xylose Uptake Pathway

Solution:

2 genes are up-regulated; when Xylitol secretion did not occur.

How?

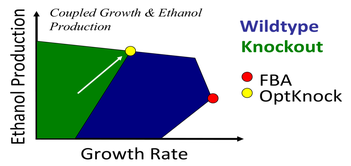
- Xylitol secretion is not predicted by the metabolic model.
- But, when the regulatory model is introduced, Xylitol secretion is observed.
- Upon comparing the 2 simulations; 2 genes where found which when up-regulated in the regulatory model; Xylitol secretion is not seen.
- Hence, Xylitol secretion caused by the cofactor imbalance can be attributed to the down-regulation of the 2 genes.

***not included in the Poster:**

Integration of expression data to the metabolic model.

Strain Design for Ethanol Production

Optknock: Optimal Reaction Deletion

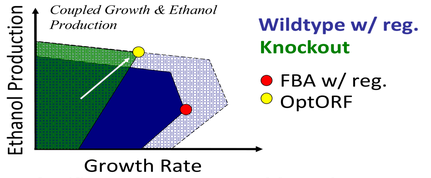


Identifies reactions, whose removal forces the **coupling between growth** rate and **metabolite production.**

To **achieve the maximum growth rate the** corresponding knockout mutant must also **secrete a metabolite of interest.**

Burgard AP, Pharkya P, Maranas CD. Biotech & Bioeng. 84(6):647-657 (2003)

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.

Kim J, Reed J. L.(2009) in preparation

Computational Design of Ethanologenic *S.cerevisiae* Strains

- **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

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

- **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

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)

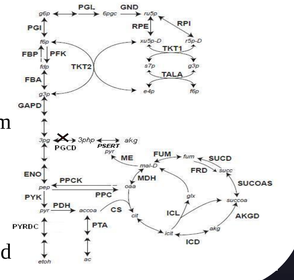
Simple illustration:

- 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 fig.

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.



Acknowledgments

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