Model-based analysis and metabolic design of a cyanobacterium for bio-products synthesis

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Programa de doctorado: Matemática Aplicada
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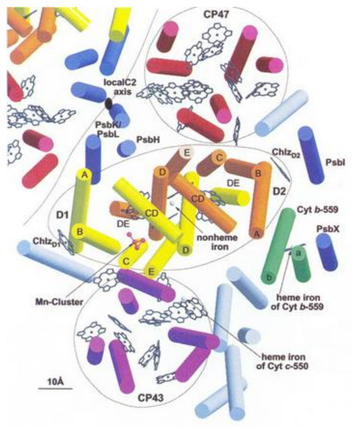
Abstract

The current investigation is aimed at the reconstruction and analysis of genome-scale metabolic models. Specifically, it is focused on the use of mathematical-computational simulations to predict the cellular metabolism behavior towards bio-products production, such as: ethanol, higher alcohols, lipids and hydrogen. The photosynthetic cyanobacterium *Synechococcus elongatus* PCC7942 was studied as biological system.



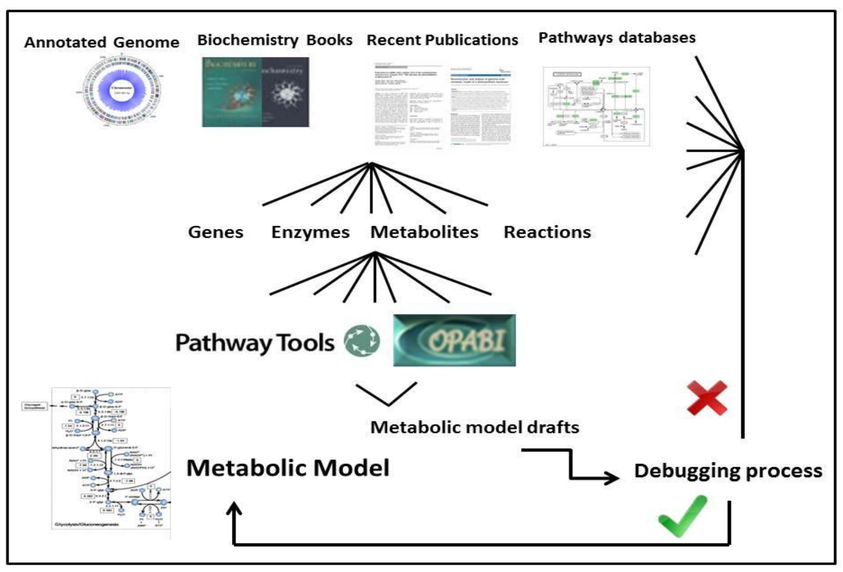
PII of *Synechococcus elongatus*[*]

Main Objectives

1. *Reconstruct a genome-scale metabolic model for Synechococcus elongatus PCC7942.*
2. *Validate reconstructed metabolic models for Synechococcus elongatus PCC7942.*
3. *Analyze environmental and genetic variations imposed on the metabolic network under a systemic perspective.*
4. *Define strategies for the production of substances of socioeconomic importance through metabolic engineering designs.*
5. *Analyze the discrete metabolic phenotypes*
6. *Integrate transcriptomics data into metabolic model*

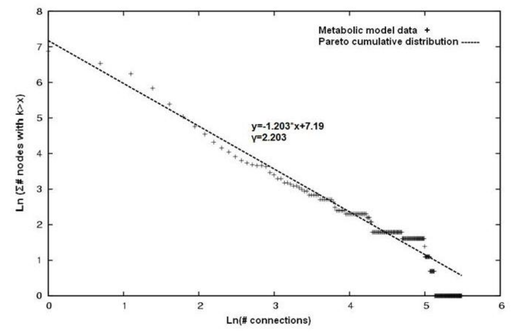
Main Steps

1. Genome-Scale Metabolic Model Reconstructions and Constraint-Based Analysis

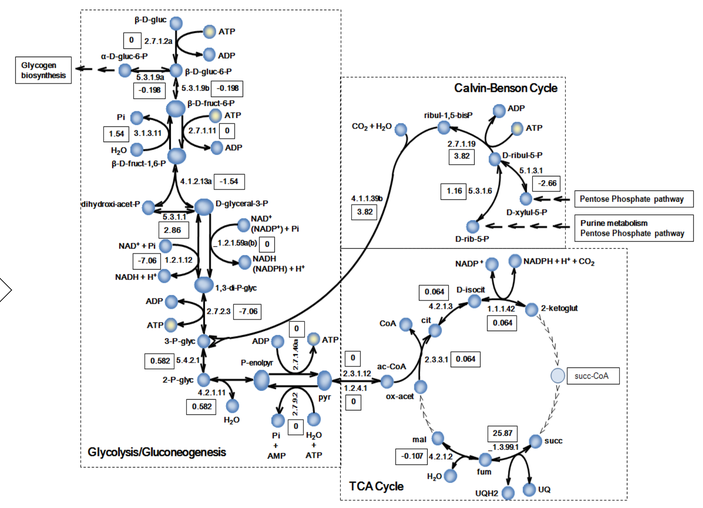


Reconstruction Procedure

Draft file from Pathways Tools	Number of reactions
Genes	672
Enzymes	540
Multimeric enzymes	98
Reactions	898
***i*Syf714 metabolic model**	
Number of genes	714
Number of metabolic reactions	849
Number of metabolites	838
Enzymes	529
Multimeric enzymes and complexes	79
Reactions overview	
Reversible reactions	325
Irreversible reactions	524
Reactions with assigned genes	734
Enzymatic conversion	709
Protein-mediated transport	25
Reactions with no cognate genes	115
Non-enzymatic conversion	13
Transport reactions	16
EC reactions not annotated	76
Unassigned reactions	11



Connectivity Distribution Analysis

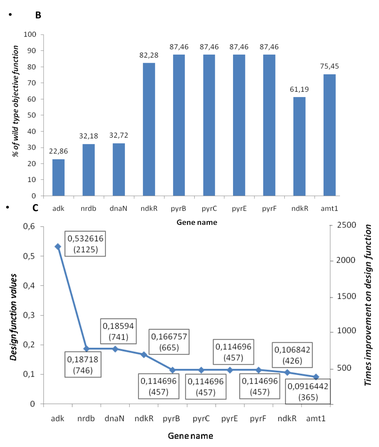


Flux Balance Analysis
Variability and Robustness Analysis

2. Assessment of Metabolic Capabilities

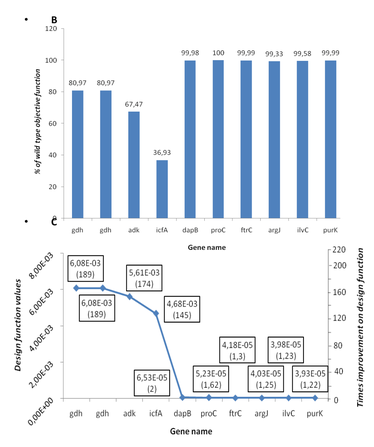
Single, double and triple knock-out strategies for the bio-products synthesis, simulated by Minimization of Metabolic Adjustment (MOMA) algorithm

Reaction in model	Gene
2.7.4.3a	*adk*
1.17.4.1c	*nrdb*
2.7.7.7b	*dnaN*
2.7.4.6d	*ndkR*
2.1.3.2	*pyrB*
3.5.2.3	*pyrC*
2.4.2.10	*pyrE*
4.1.1.23	*pyrF*
2.7.4.6a	*ndkR*
Ammonia H3N TRANS-RXN59G-178	*amt1*



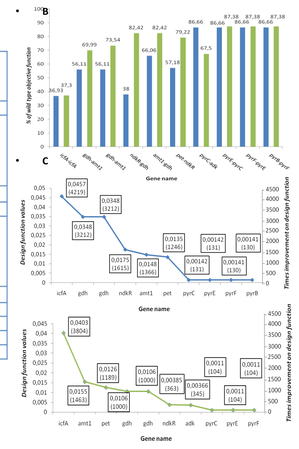
Ethanol synthesis

Reaction in model	Gene
1.1.1.47a	*gdh*
1.1.1.47b	*gdh*
2.7.4.3a	*adk*
4.2.1.1b	*icfA*
_1.3.1.26a	*dapB*
_1.5.1.2a1	*proC*
_1.18.-.-	*ftrC*
2.3.1.35	*argJ*
1.1.1.86a	*ilvC*
6.3.4.18	*purK*



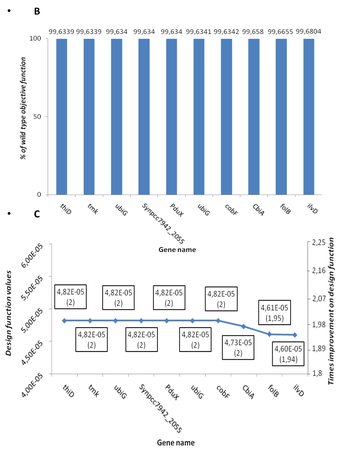
Higher alcohols synthesis

Reaction in model		Gene	
Palmitic acid	***Stearic acid***	***Palmitic acid***	***Stearic acid***
4.2.1.1b	*4.2.1.1b*	*icfA*	*icfA*
1.1.1.47a	*ammonia H3N TRANS-RXN59G-178*	*gdh*	*amt1*
1.1.1.47b	*_1.10.9.1*	*gdh*	*pet*
2.7.4.6d	*1.1.1.47a*	*ndkR*	*gdh*
ammonia H3N TRANS-RXN59G-178	*1.1.1.47b*	*amt1*	*gdh*
_1.10.9.1	*2.7.4.6d*	*pet*	*ndkR*
3.5.2.3	*2.7.4.3a*	*pyrC*	*adk*
2.4.2.10	*3.5.2.3*	*pyrE*	*pyrC*
4.1.1.23	*2.4.2.10*	*pyrF*	*pyrE*
2.1.3.2	*4.1.1.23*	*pyrB*	*pyrF*



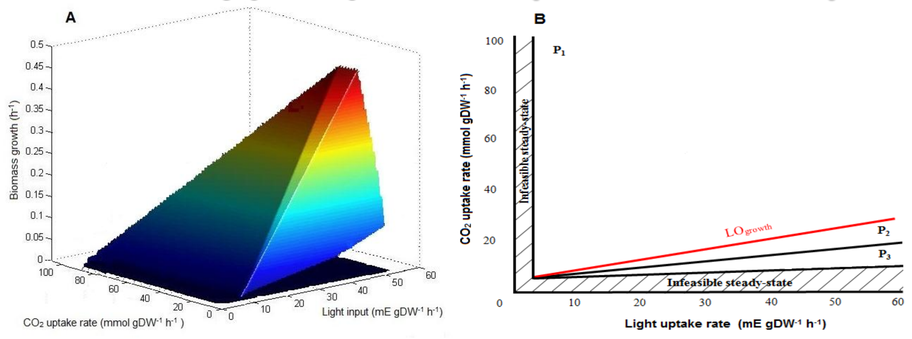
Lipids synthesis

Reaction in model	Gene
2.7.4.7	*thiD*
2.7.4.9a	*tmk*
·2.1.1.64a	*ubiG*
1.14.13.-b	*Synpcc7942_2055*
RXN-8626	*PduX*
·2.1.1.64b	*ubiG*
·2.1.1.152	*cobF*
6.3.5.11	*CbiA*
4.1.2.25	*folB*
4.2.1.9a	*ilvD*



Hydrogen evolution

3. Phenotypic phase plane analysis



CO_2 and light phenotype phase plane for each bio-products

•From Voet D, Voet JG. (2004) Biochemistry. 3rd Ed. John Wiley & Sons, New York.

4. Metabolome dynamic upon Ci** acclimation

Reporter metabolites (yellow points) under High CO_2 / Low CO_2 regime

**Ci: inorganic carbon