Phenotyping Diverse Bacteria for Metabolic Network Reconstruction

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Introduction

Current bacterial models are built from gene annotations, where gene function is deduced through homology-based algorithms and tools, such as RAST (Rapid Annotation using Subsystem Technology)

Novel functional roles are left undiscovered when they cannot be extrapolated from current annotation software

Using flux-balance analysis (**FBA**) software, metabolic models can be used for *in silico* prediction of growth rates and biomass yield upon a variety of growth conditions

Recent developments using multi-phenotype assay plates (**MAPs**) provide a high-throughput technique for profiling bacterial phenotypes upon a variety of growth conditions

Coupling PM experiments with FBA software, metabolic models can be reconciled and optimized to best predict bacteria response and yield

Experimental Design

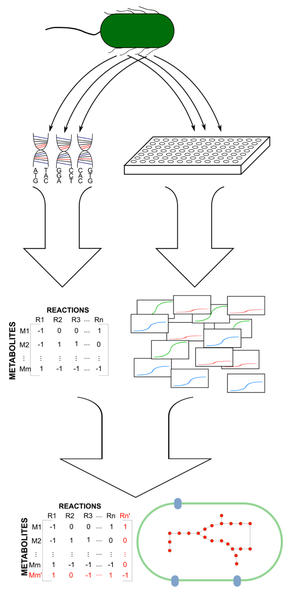


Figure 1. Model reconciliation workflow. Combine genomics and phenomics in order to create, test, and reconcile bacteria metabolic models.

Methods

Genomics

RAST Gene Annotations

Membrane Transport | K, S, P, N Metabolism
Polyamine Synthesis and Degradation | Respiration

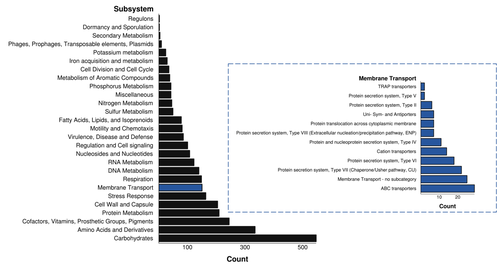
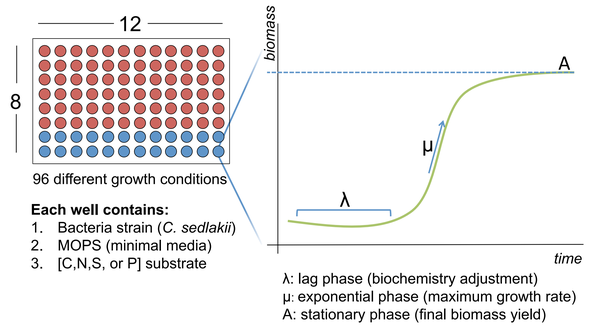


Figure 2. Genomic annotations. Next-generation sequencing platforms are used to sequence the *Citrobacter sedlakii* genome. Sequences are uploaded to RAST to obtain gene function annotations.

Phenomics



Growth curve model

$$\hat{y} = y_1 + \frac{A - y_1}{1 + \exp\left[\left(\frac{\mu_{max}}{A}\right)(\lambda - t_i)\right] + 2}$$

Figure 3. Modeling growth curves. Bacteria are grown on 96-well plates over time. OD 600nm is recorded to produce growth curves. Model parameters are automatically determined using an optimization method to produce a best fit logistic curve. Growth is computed based on model parameters.

Genome-Scale Metabolic Modeling

KBase
PREDICTIVE BIOLOGY
DOE Systems Biology Knowledgebase

KBase Features

Import model | FBA | MAP simulation | Gap-fill (+ reactions)
View model | Inspect pathways | Gap-gen (- reactions)

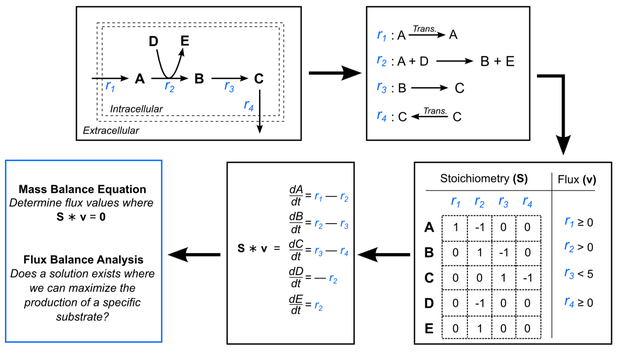


Figure 4. Constraint-based model. KBase supplies the framework where a metabolic model can be imported as a stoichiometric matrix of metabolites and reactions. Included in the model are several constraints including thermodynamics, reaction flux rate, and mass balancing. Linear programming maximizes biomass production to answer the question: does the model predict growth?

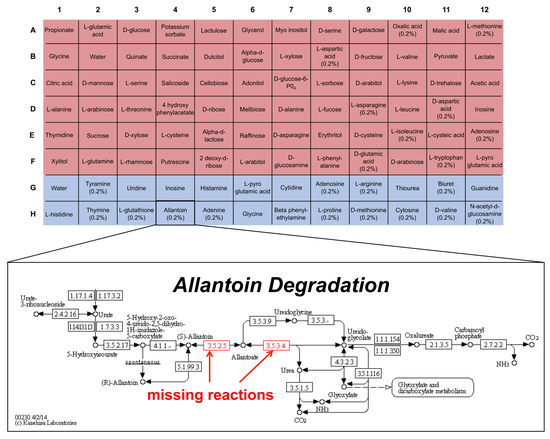


Figure 5. Gap-filling. Given a model that incorrectly predicts no growth, Kbase includes the capability to identify reaction gaps in the model that allow the bacterial model to correctly exhibit growth. Above is an example of two missing reactions in the *C. sedlakii* model involved in the allantoin degradation pathway.

Results

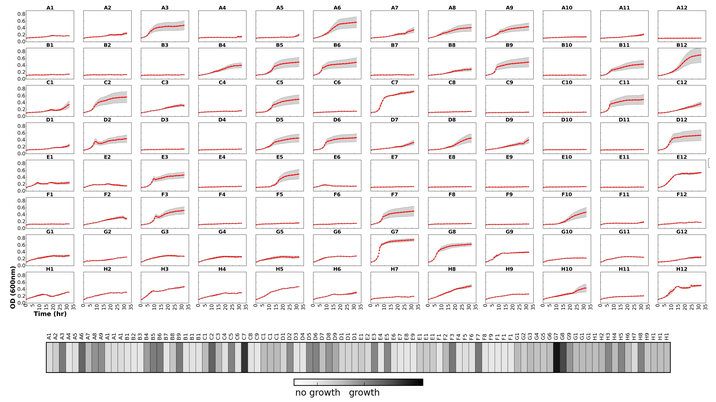


Figure 6. *C. sedlakii* growth curves. The automated analysis pipeline creates growth curve plots with standard error bars. Growth levels are quickly assessed in the grey-scale bar.

FBA Prediction

Phenotypic Result		G	NG
	G	48	0
	NG	6	36

Table 1. A comparison between experimental results and FBA prediction. After using *gap-filling* on KBase, 84 cases (93%) were in agreement with the MAPs results. 6 cases did not match the MAPs experiments.

# of Bacteria	
Sequenced	22
MAPs	49

Table 2. Current data collection.

Questions

Why were these reactions missing from model?

Continue to model, sequence, and assay a broad and diverse set of bacteria – can we improve annotations?

Additional Information

https://vdm.sdsu.edu/pmanalyzer
https://edwards.sdsu.edu/dbbp

PMAnalyzer

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