High-throughput phenotype profiling for bacterial flux-balance model optimization

Daniel A Cuevas[1], Daniel Garza[4], Savannah E Sanchez[2], Jason Rostron[2], Chris Henry[3], Anca Segall[2], Forest Rohwer[2], and Robert A Edwards[1,2,3]

Computational Science Research Center[1], Departments of Biology[2], San Diego State University, CA; Argonne National Lab[3], IL; Evandro Chagas Institute[4], Brazil

Introduction

Current bacterial models are built from gene annotations, where gene function is deduced through homology-based algorithms and software, 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 phenotype microarrays (**PMs**) provide a high-throughput, large-scale technique for profiling bacterial phenotypes upon a variety of growth conditions

Citrobacter sedlakii genome was sequenced using next-gen sequencing and assayed on PMs

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

Experimental Design

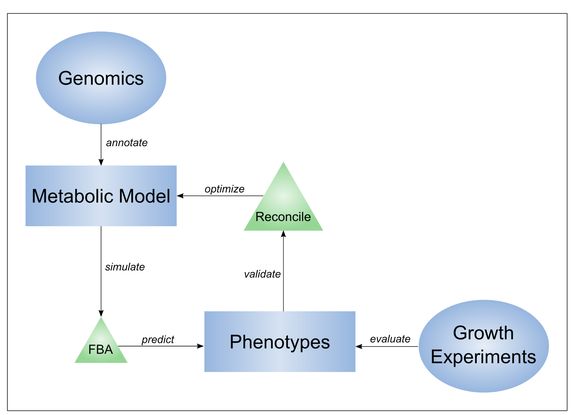


Figure 1. Analysis Overview. Combine genomics and phenomics in order to create, test, and reconcile bacteria metabolic models.

Methods

Phenotype MicroArray

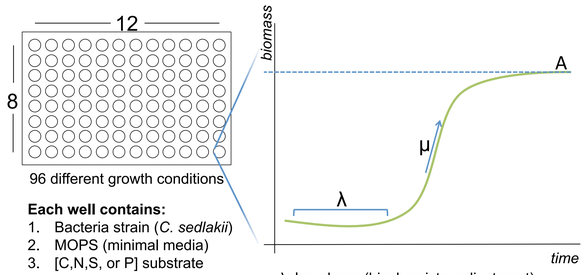


Figure 2. Process Growth Curves. 96 well plates are run on instrument (right) for 32hrs. $OD600_{nm}$ is recorded every 30mins to produce growth curve (above). Parameters are captured to fit to a logistic model, which is then used to determine growth level.

Genomics

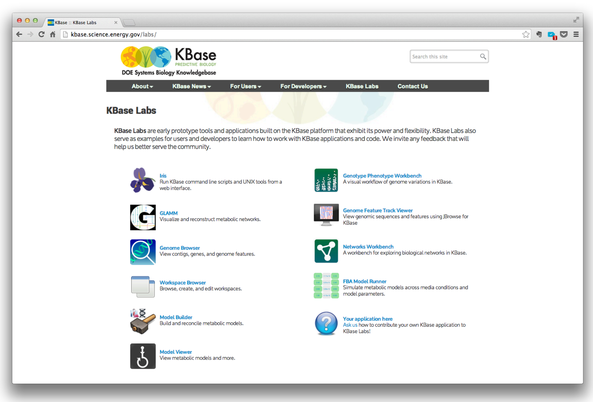
Citrobacter sedlakii

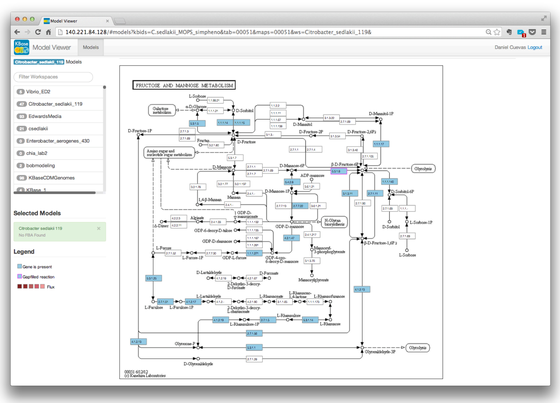
Size	4,604,104 bp
Contigs	320
Subsystems	
Total Subsystems	536
Hypothetical Proteins	811 (19%)

Figure 3. Metabolic Models. Next-generation sequencing platforms are used to sequence the *C. sedlakii* genome. Sequences are uploaded to RAST to obtain gene function annotations.

Flux-Balance Analysis

KBase PREDICTIVE BIOLOGY
DOE Systems Biology Knowledgebase





KBase Features

Import model | FBA | PM simulation | Gapfill (+ reactions)
View model | Inspect pathways | Gapgen (- reactions)

Figure 4. KBase Modeling Software. KBase supplies online tools and features (left) where a metabolic model can be imported and used in FBA, growth predictions, PM simulations, and other modeling procedures (bottom). KBase includes methods to view model reaction composition and biochemical pathways (right).

Results

FBA Prediction

		Growth	No Growth
PM Experiment	G	44	0
	NG	10	36

Table 1. A comparison between experimental results and FBA prediction. After using *gapfilling* on KBase, 80 cases (89%) were in agreement with the PM results. 10 cases did not match the PM experiments.

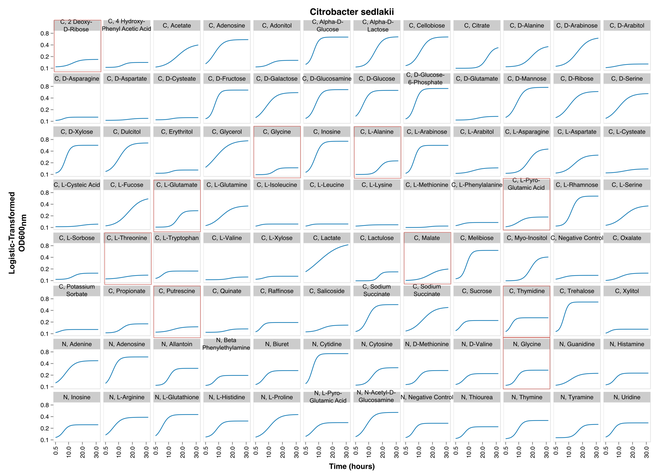


Figure 5. *C. sedlakii* Growth Curves. A logistic model is fitted to the growth curve to extract phenotype parameters. Red boxes highlight the 10 cases where FBA predicted growth and PM modeling resulted in no growth. The letter preceding the substrate name identifies it as a carbon or nitrogen source.

Before Gapfilling
1,367 reactions

After Gapfilling
1,399 reactions

32 reactions total added to model
13 out of 32 are metabolite transporters
12 existing reactions made reversible

Conclusion

Model optimization for over 90 growth conditions can be completed quickly

Gapfilling still requires manual execution (run for each growth condition)

Why were these genes missing from model?

Increase number of growth conditions and bacteria strains

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Further information: contact presenter at dcuevas08@gmail.com