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

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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

Methods
Phenotype MicroArray
Each well contains:
1. Bacteria strain (C. sedlakii)
2. MOPS (minimal media)
3. [C, N, P] substrate

Figure 2. Process Growth Curves. 96 well plates are run on instrument (right) for 32hrs. OD600nm 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

Figure 3. 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.

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

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.

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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