Predicting Gene Targets for Optimizing Lipid Production in Acetic Acid Pathway of *Yarrowia lipolytica* by Ensemble Modeling

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While the opportunity for optimization of Yarrowia lipolytica production of lipids from glucose is well-explored, an acetic acid metabolism based lipid production pathway is investigated by means of the Ensemble Modeling (EM) to predict potential gene targets for maximizing the lipid yield. To build a model for the acetic acid pathway, we have collected information from literatures that the major route for acetic acid metabolism is by the reaction acetyl-CoA synthetase, which converts acetic acid into acetyl-CoA powered by the conversion of ATP to AMP. We used this as the route for acetic acid metabolism in *Yarrowia* for the model. Additionally, we achieved acetyl-CoA transport into the mitochondria via the acyl-carnitine/carnitine translocase system. The glyoxylate cycle is presumed to be active in the mitochondria under acetate conditions, which allows for a net conversion of two acetyl-CoA molecules into a 4-carbon dicarboxylic acid (malate). Malate is then decarboxylated in the mitochondria by malic enzyme which has been noted to be localized there. A pyruvate carrier then moves pyruvate to the cytosol where it can be used for gluconeogenesis.

EM simulation builds large number of models (with randomly sampled but realistic parameter values). We then simulate effects for all single enzyme perturbation. The reference/steady state fluxes, which are the key information for EM simulation, are estimated using publicly available biomass and lipid yield data (this literature will be cited on the poster) and steady state is ensured using linear programming. Estimated results are within 4% of the reported value. In current stage, our EM simulation built 1000 stable models for gene targets prediction, and the perturbation analysis provides 6 knockout and overexpression gene targets for further investigation in the lab.

1. 6 knockout targets: Malate synthase, isocitrate lyase, succinate dehydrogenase, fumarase, malic enzyme NADH (mitochondria) and pyruvate carboxylase. Among them, knocking out malate synthase and isocitrate lyase can increase 5.7% of lipid yield compared to the reference.
2. 6 overexpression targets: Oxoglutarate dehydrogenase, acetyl-CoA carboxylase, isocitrate dehydrogenase, ATP transport, adenylate kinase and respiration. Among these, overexpressing oxoglutarate dehydrogenase increases 8.5% of lipid yield compared to the reference, while the second best, acetyl CoA carboxylase, increases 7%.

This model of *Yarrowia* metabolism under acetic acid feed conditions is a reasonable representation of the system and EM simulation has suggested key gene targets for maximizing lipid yield. Furthermore, since key assumptions about compartmentalization and metabolite flow have been identified, this will allow the model to be adapted to many other conditions, including high density production schemes with low biomass accumulation.