

Predicting multiple gene targets for optimal oil production in acetic acid metabolism of *Yarrowia lipolytica* by ensemble modeling

Po-Wei Chen¹, Gregory Stephanopoulos² and James C. Liao¹

¹Department of Chemical and Biomolecular Engineering, University of California, Los Angeles

²Department of Chemical Engineering, Massachusetts Institute of Technology

Abstract

Ensemble modeling (EM) has been applied on lipids overproduction of glucose feeding and acetic acid feeding metabolic pathways in *Yarrowia lipolytica*. Since the optimization of glucose feeding strain has been well explored, our team has focused on identifying gene targets to further increase lipids yield in acetic acid feeding pathway system. EM based strain optimization simulation has provided five gene overexpression (OE) and knockdown (KD) target, respectively. The five overexpression targets are, oxoglutarate dehydrogenase, acetyl-CoA carboxylase, isocitrate dehydrogenase, ATP transport, adenylate kinase, respiration; the five knockdown targets are, isocitrate lyase, malate synthase, succinate dehydrogenase, fumarase, malic enzyme NADH mi and pyruvate transport. Among these single gene targets, overexpressing oxoglutarate dehydrogenase can boost lipids yield to 8% more than the reference state. Recently, a new EM strategy has been applied to further increase lipids yield through multiple gene manipulation/control. All possible combination of double and triple gene OE/KD selected from top 3 single OE and KD targets mentioned above has been simulated and compared with single gene OE/KD results. The result suggested that the following multiple gene control can further increase lipids yield to around 10% above the reference state.

- Overexpress oxoglutarate dehydrogenase + knockdown malate synthase
- Overexpress oxoglutarate dehydrogenase + knockdown isocitrate lyase
- Overexpress oxoglutarate dehydrogenase + knockdown isocitrate lyase & malate synthase

Interestingly, among all the targets including changing one, two and three and four gene activities at a time, the strategies of changing two genes shown highest yield. The quadruple gene control strategy: OE oxoglutarate dehydrogenase + OE acetyl-CoA carboxylase + KD isocitrate lyase + KD malate synthase shown similar yield to OE oxoglutarate dehydrogenase. The triple gene control strategy: OE acetyl-CoA carboxylase + KD isocitrate lyase + KD malate synthase even shown lower lipids yield as 7%, which is lower than the best single gene control strategy. Overall, the EM predicted result not only shown the next potential acetic acid feeding strain(s), but also demonstrated the value for aiding designs of sophisticated multiple gene manipulation.