Dynamic modeling of a synthetic *Clostridia* triple co-culture for producing medium-chain fatty acids from glucose

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Project Goals: To construct and integrate a *Clostridium kluyveri* genome scale metabolic model into dynamic modeling framework with a novel cell fusion/growth model and hybrid *C. acetobutylicum* and *C. ljungdahlii* metabolic models, and deploy the resulting community model with existing strain design algorithms to identify engineering targets across the triple syntrophic culture to enhance diesel and aviation fuel precursor production.

Clostridial co-cultures have the potential to improve fermentation yields for value-added bioproducts through mutualistic cross-feeding which improves carbon conversion efficiency. In addition to 100% substrate conversion, *C. acetobutylicum* (*Cac*) and *C. ljungdahlii* (*Clj*) in syntrophic culture were shown to fuse membranes and exchange cytosolic contents, adding to their bioproduction potential. Hybrid cells with significant shifts in gene expression caused higher-than-expected fermentation yields, and *Clj* persisted at high abundance thanks to its acquisition of *Cac*-native metabolic enzymes during cell fusion. By introducing a dynamic genome-scale metabolic modeling framework with hybrid *Cac* and *Clj* metabolic models, we offer a mechanistic explanation for how cell fusion alters the growth phenotype and panel of metabolites produced by this binary community. Inclusion of hybrid metabolic models and a novel growth model accounting for cell fusion/proteome swap enables quantitative agreement with fermentation profiles that is beyond the scope of dynamic simulations in the absence of

such detail. We now seek to add value to this system by incorporating *C. kluyveri* (*Ckl*) to produce medium-chain fatty acids from ethanol and acetate excreted by *Cac* and *Clj*. To this end, we first assemble a genome-scale reconstruction of *Ckl* (iCKL841) containing 1989 reactions corresponding to 841 gene products inferred through gene homology analysis and gene-protein-reaction mapping from six existing *Clostridia* genome scale models. We then merge iCKL841 into a dynamic simulation of the triple synthetic co-culture which includes *Ckl* together with hybrid metabolic models of *Cac* and *Clj*. We are integrating this system with optKnock and optForce strain design algorithms to identify perturbation strategies for maximizing medium-chain fatty acid production from glucose.

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