Modeling growth kinetics and metabolism of *Clostridium acetobutylicum*/*Clostridium ljungdahlii* co-culture with cell fusion

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Project Goals: The goal of this project is to develop syntrophic *Clostridium* co-culture systems, involving three *Clostridium* organisms, for producing C4-C8-chain length metabolites that can be used as chemicals or serve as biofuels and biofuel precursors. Part of the effort includes modeling the behavior of these triple co-cultures. To build this capability, we first model the binary co-culture of *Clostridium acetobutylicum (C. ac)* with *C. ljungdahlii* (*C. lj*). This first modeling sub-goal then is to develop a method for modeling the co-culture growth kinetics that accounts for novel cell fusion events observed in this binary co-culture. Using the resulting kinetic model in conjunction with a community genome-scale metabolic model and the SteadyCom community modeling framework, we aim to explain the observed co-culture metabolism/redox state, and identify genetic intervention strategies maximizing production of isopropanol and 2,3-butanediol.

Clostridia organisms have been of interest for decades due to their ability to ferment a wide range of carbon sources to useful bioproducts. The metabolic repertoire of these anaerobes has been further expanded in co-cultures due to the diversity of substrates they can consume and unexpected syntrophic behaviors that are still being discovered. One such example lies in the syntrophic co-culture of *Clostridium acetobutyllicum* (*C. ac*) and *C. ljungdahlii* (*C. lj*). In addition to the discovery of an upregulation of *C. lj sadh* and *23bdh* gene expression in the presence of *C. ac* (allowing *C. lj* to convert acetone and acetoin produced by *C. ac* to isopropanol and 2,3-butanediol, respectively) [1], *C. ac* and *C. lj* cells were recently shown to fuse membranes and exchange proteomes. This work aims to characterize the resultant change in growth kinetics due to the observed fusion/protein exchange event using a kinetic model which characterizes both the pure and mixed-proteome *C. ac* and *C. lj* growth rates and the cell fusion/proteome exchange rate. The parameterized kinetic model is used to inform the construction of a community genome-scale metabolic model of pure and mixed-proteome *C. ac* and *C. lj* cells using the SteadyCom framework, and characterize the dynamic shift in co-culture metabolism and redox state related to the observed fusion event required to support the experimentally measured isopropanol and 2,3-butanediol production. Single organism strain design tools (i.e. optKnock, optForce) are being adapted to support the inclusion of multi-organism models in order to understand how the *C. ac* and *C. lj* genomes can be manipulated to maximize the production of fermentation products of interest (i.e isopropanol, 2,3-butanediol) under the newly discovered *C. ac*/*C.lj* co-culture conditions.

References

1. Charubin K, Papoutsakis ET. Direct cell-to-cell exchange of matter in a synthetic Clostridium syntrophy enables CO2 fixation, superior metabolite yields, and an expanded metabolic space. Metab Eng. 2019;52:9-19.

SUPPORTED by the U.S. Department of Energy (Award No. DE-SC0019155).