Project Goals: This project aims to understand and model the stress response of Clostridium acetobutylicum ATCC 824 to two important toxic metabolites: butanol and butyrate, using a regulated genome scale model.

Clostridia are anaerobic Gram-positive Firmicutes with the ability to use varied substrates to produce a range of industrial compounds. In particular, Clostridium acetobutylicum has been used to produce butanol on an industrial scale through acetone-butanol-ethanol (ABE) fermentation. A genome-scale metabolic (GSM) model is a powerful tool to understand the metabolic capacities of an organism and develop metabolic engineering strategies for strain development. The GSM model can be integrated with stress-related specific transcriptomics information to elucidate the nexus points of regulation, which underlie cellular response to the stressors.

We describe here the construction and validation of a GSM model for C. acetobutylicum ATCC 824, iCac802. iCac802 spans 802 genes and includes 1,137 metabolites and 1,462 reactions, along with gene-protein-reaction associations. Both 13C-MFA and gene deletion data in the ABE fermentation pathway were used to test the predicted flux ranges allowed by the model. We also describe the CoreReg method to integrate transcriptomic data and identify core sets of reactions that, when their flux was selectively restricted, reproduced flux and biomass-formation ranges seen under all regulatory constraints. CoreReg was used in response to butanol and butyrate stress to tighten bounds for 50 reactions within the iCac802 model. The model, incorporating the regulatory restrictions from CoreReg under chemical stress, exhibited an approximate 70% reduction in biomass yield for most stress conditions.

CoreReg regulated the model for the two stresses and identified differences in their respective responses, including distinct core sets and the restriction of biomass production similar to experimental observations. Given the core sets predicted by the CoreReg method, remedial actions can be taken to counteract the effect of stress on metabolism. In the case of lesser-known systems, countermeasures such as plausible regulatory loops can be suggested around the affected metabolic reactions, and the hypotheses can be tested experimentally.

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