Development of a Clostridia Cell-free Platform Facilitating Accelerated Engineering of Clostridia Strains

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Project Goals: We are addressing the complex challenge of designing, building, and optimizing biosynthetic pathways in cells in a new interdisciplinary clostridia Foundry for Biosystems Design (cBioFAB) with government and industry partners. Working both in vitro and in vivo, the goal is to expand the set of platform organisms that meet DOE bioenergy goals by utilizing and advancing state-of-the-art cell-free technologies, omics measurements, systems-biology analyses, computational modeling, and genome editing. cBioFAB will (i) reconceive how we engineer complex biological systems by linking pathway design, prospecting, validation, and production in an integrated framework, (ii) enable systems-level analysis of the David T. Jones collection, one of the largest collections of clostridia strains in the world, to uncover novel metabolic pathways, regulatory networks, and genome editing machinery, and (iii) realize more efficient clostridia strain engineering for the synthesis of biofuels and bioproducts.

Modern world challenges like rapid population growth, rising global living standards and its accompanying increase in energy demand and waste generation necessitate the production of low-cost fuels and high-value compounds from sustainable resources. Microbes can be engineered to produce biofuels, chemicals, materials, and therapeutics. Particularly attractive engineering targets are gas- and food waste-fermenting anaerobes like clostridia strains. Unfortunately, designing, building, and optimizing biosynthetic pathways in clostridia for manufacturing applications remain complex challenges. Cell-free protein synthesis (CFPS) platforms have the potential to accelerate biological design by speeding up design-build-test cycle. Here, we present the development of a clostridia CFPS system. We demonstrate its potential to facilitate rapid studying of metabolic pathway performance and prototyping in vitro. We expect that our new cell-free platform will accelerate engineering clostridia strains that efficiently convert wastes into high-value products.

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