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Designer host for cellulosic ethanol production via synthetic biology

Jui-Jen Chang¹, Yueh-Chin Wu¹, Yu-Han Hou¹, Chieh-Chen Huang², Ming-Che Shih¹ and Wen-Hsiung Li¹

¹Academia Sinica, Taiwan

²National Chung Hsing University, Taiwan

To achieve economical biofuel production, such as cellulosic ethanol, a host that can do both cellulosic saccharification and ethanol fermentation is desirable. However, to engineer a non-cellulolytic microbe to be such a host requires synthetic biology techniques to pursue large-scale genomic engineering of the host. We have developed an efficient high-throughput method that can simultaneously introduce many genes into a genome. It is called Promoter-based gene assembly and simultaneous overexpression (PGASO).

PGASO was applied to transform multiple cellulase genes into the genome of *Kluyveromyces maxianus* KY3 with a single selection marker gene. Six genes of different GH families were cloned from the cellulolytic fungi *Trichoderma*, *Aspergillus*, and *Neocallimastix*. The recombinant strain is capable of co-expressing a cellulase cocktail and can directly convert microcrystalline cellulose to ethanol. Our study shows that a designer yeast can be developed to simultaneously express different GH genes, and our enzyme cocktail shows a synergistic effect of these enzymes in digesting cellulose. Thus, PGASO can serve as a platform to study enzyme synergism in a single host and can be used to construct a host for a cell factory for enzyme production.

In addition, KY3 can be co-cultured with bacterial hosts. A designer *Bacillus subtilis* that carries eight cellulosomal genes of *Clostridium thermocellum*, including one scaffolding protein gene, one cell-surface anchor gene, and six cellulase genes, was constructed and employed as a partner of KY3 for cellulosic bioethanol production. A novel dual-microbe co-culture system is developed to improve bioethanol production.

lancecjj@gmail.com