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Designer Host for Cellulosic Ethanol Production via Synthetic Biology

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Abstract

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.

Biography

Associate Research Fellow, Department of Medical Research, China Medical University Hospital, Taiwan (2013)

Postdoctoral Fellow, Genomics Research Center, Academia Sinica, Taipei, Taiwan (2008-2013)

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