Engineering clostridia for *n*-butanol production from lignocellulosic biomass

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Butanol is an important industrial solvent and a better transportation fuel than ethanol. However, biobutanol produced from corn starch or sugarcane molasses by conventional acetone-butanol-ethanol (ABE) fermentation is expensive due to the co-production of acetone, which results in a relatively low butanol yield of <0.25 g/g, and the biphasic fermentation kinetics that is difficult to control and prone to acid crash in industrial operation. We have engineered the acidogenic *Clostridium tyrobutyricum* to overexpress a heterologous alcohol/aldehyde dehydrogenase for biobutanol production from various sugar-containing biomass feedstocks, including sugarcane bagasse and cotton stalk. The fermentation with the engineered *C. tyrobutyricum* produced *n*-butanol as the main product from biomass hydrolysates with a high yield of >0.3 g/g sugar fermented, providing an economically superior process for biobutanol production form abundant, inexpensive, and renewable lignocellulosic biomass. However, enzymatic hydrolysis of cellulose in lignocellulosic biomass into fermentable sugars is expensive. It is thus desirable to engineer cellulolytic clostridia for biobutanol production directly from lignocellulosic biomass in a consolidated bioprocess without requiring prior enzymatic hydrolysis. For this purpose, we have also engineered the acidogenic *Clostridium cellulovorans* to directly convert cellulose to *n*-butanol. The engineered *C. cellulovorans* strain with optimized carbon flux toward C4 metabolites (mainly butyrate and *n*-butanol) was able to produce *n*-butanol at a high yield of >0.32 g/g cellulose. To further increase butanol production and reduce cost and CO_2 emission, we have also engineered a carboxydotrophic acetogen (*Clostridium carboxidivorans*) to convert CO_2 and H_2 co-produced in cellulose/sugar fermentation to acetate, ethanol, and butanol, which has the potential to further increase butanol yield by ~30% to >0.4 g/g. Key technical issues and challenges in engineering clostridia will be discussed in this talk. Metabolic and process engineering strategies to increase biobutanol production for industrial application will also be presented.

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Research Interests:

Biofuels and biobased chemicals production from renewable biomass; Metaboli engineering

Selected publications:

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2. M. Yu, Y. Zhang, I.C. Tang, and S.T. Yang, Metabolic engineering of *Clostridium tyrobutyricum* for n-butanol production, Metab. Eng., 13: 373-382 (2011).
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