Microbial Degradation of Plant Lignocellulolytic Biomass without Pretreatment

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ABSTRACT

Biofuel production is a feasible strategy, however it requires significant cost reductions. Here, we propose a new simplified technique including microbial conversion. A lignocellulose degrading bacterium, Clostridium thermocellum, was able to degrade a lignocellulolytic biomass, milled corn hull, without chemical pretreatment. Metabolic engineering studies which modify C. thermocellum to produce water insoluble fuel chemicals will achieve significant cost reductions of biofuel production.

INTRODUCTION

Nobody doubts that we depend on petroleum strongly. The price will increase four-fold over a decade (Agency for Natural Resources and Energy, Japan., 2004). The one reason of the price increase is rapid decrease of potential amount of petroleum. One prediction showed that petroleum will deplete 50 years later without developing renewal petroleum field which includes political and technological problems (BP public limited company, 2005). One strategy to overcome this problem is replacement of petroleum compounds by other sustainable material. In this strategy, we must produce petroleum compounds from inexpensive materials, because cheaper price of petroleum compounds is essential for our life. One of the candidate materials is biomass, because of its abundant amount on the earth. Especially, lignocellulolytic biomass is useful because it is the most abundant material all over the world and its content is relatively pure which makes the conversion process simple (The Japan Institute of Energy, 2008). Thus, conversion of lignocellulolytic biomass into petroleum compounds is an attractive and feasible strategy.

We consume a large amount of petroleum compounds as liquid fuels. Japanese government showed the plan to establish bioethanol technology. It said that we should decrease the cost three times to establish bioethanol tequonology (Ministry of Economy, Trade and Industry, Japan, 2012). However, it is unhopeful because the conventional biofuel production technique is an expensive and complicated process (Fig. 1).

Here we propose a new simplified technique to produce biofuel: microbial conversion in which microorganisms degrade lignocellulolytic biomass and ferment it into oil simultaneously. Clostridium thermocellum is a promising bacterium because of its high biomass degradation ability (McBEE, R.H., 1954). In this study, we show a key step, efficient degradation of lignocellulolytic biomass by C. thermocellum.

Fig. 1: The procedures for biofuel production.
The conventional technique includes expensive steps, chemical pretreatment, enzymatic degradation and distillation of ethanol. We propose a new simplified technique: microbial conversion in which a bacterium, *Clostridium thermocellum*, degrade lignocellulolytic biomass and ferment it into insoluble oil simultaneously.

**MATERIALS AND METHODS**

**Strain and its stock condition:**
*C. thermocellum* strain 132 was isolated in our lab. Stock cultures were maintained in GS medium (Garcia-Martinez, D.V., *et al.*, 1980). Ten gram per one liter of ball milled cellulose was used as the carbon source (Taya, M., *et al.*, 1978).

**Biomass degradation analysis:**
*C. thermocellum* was cultured in 5 mL of GS medium containing 1% cellobiose. When its growth achieve an exponential phase, the optical density at 600nm is 2.0, 50 µL of culture solution was transferred to 100 mL of GS medium containing 1% milled corn hull. It was cultured at 60 °C for 9 days. The amount of residual lignocellulolytic biomass was determined as neutral detergent fiber content (Seeley, R.C., 1969). The experiment was conducted duplicates.

**Result:**
Enzymatic degradation in the conventional technique requires chemical pretreatment of milled lignocellulolytic biomass (Chundawat, S.P.S., *et al.*, 2011) (Fig. 1). On the other hand, *C. thermocellum* degraded 70 % of lignocellulolytic biomass, milled corn hull, for 9 days without any chemical pretreatments (Fig. 2). The amount of residual lignocellulolytic biomass was determined as neutral detergent fiber, which contains cellulose, hemicellulose and lignin. Only cellulose and hemicellulose are fermentable. Few microorganisms, e.g. white-rot fungi, can degrade lignin, but bacteria can not. Thus, lignin amount in residual lignocellulolytic biomass may not change. Because lignin content of corn hull is ca. 5%, cellulose and hemicellulose remained less than 25 % after 9 days after inoculation (Hromádková, Z. and A. Ebringerova, 1995). This result indicates that microbial degradation of corn hull by *C. thermocellum* does not require chemical pretreatment step (Fig. 1).

![Fig. 2](image)

**Discussion:**
Each step in the conventional bioethanol production technique is too expensive to establish its technology. Japanese government estimated that the price of bioethanol should be 40 Japanese yen to replace petroleum oils. The price of biomass degrading enzyme solution has decreased dramatically, however it is ca. 50 Japanese yen in 2012 (Ministry of Economy, Trade and Industry, Japan, 2012). Significant improvement of biomass degradation efficiency by the enzyme solution have not achieved, although many scientist try to overcome this problem for 30 years (Himmel, M.E., *et al.*, 2007). For efficient biomass degradation, the chemical pretreatment step is necessary, however it costs ca. 40 yen (Chundawat, S.P.S., *et al.*, 2011). Here, we showed *C. thermocellum* degraded a lignocellulolytic biomass, milled corn hull, without chemical pretreatment, indicating that *C. thermocellum* does not require expensive chemical pretreatment and enzymatic solutions (Fig. 2, Table 1).

In the conventional technique, products from degraded biomass are fermented into ethanol by yeast. By contrast, we try to degrade and ferment biomass simultaneously, make whole process simple (Fig. 1). However, *C. thermocellum* produces slight amount of ethanol. Thus we propose genetic modification strategy of *C. thermocellum* for efficient biofuel production. Ethanol is not attractive chemical as biofuel, because of high energy consumption of distillation step (Fig. 1). We can collect insoluble fuel chemicals without distillation e.g.
hydrocarbon, fatty alcohol, fatty acid ethyl ester, etc. Some cyanobacteria produce hydrocarbon. One metabolic engineering study showed that *Escherichia coli* harboring only two hydrocarbon synthesis genes from a cyanobacterium, *Synechococcus elongatus*, secreted hydrocarbon (Schirmer, A., et al., 2010). It is expected that transformed *C. thermocellum* by hydrocarbon synthesis genes from thermophile cyanobacteria secretes hydrocarbon from lignocellulolytic biomass. Further genetic studies of *C. thermocellum* for efficient biomass degradation and insoluble fuel chemical production will establish the new biofuel production strategy proposed in this study.

Table 1: Summary of improvement in the new technique.

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<th>Problems in conventional technique</th>
<th>Improvements in the new technique</th>
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REFERENCES