別紙様式１１

（課程博士・論文博士共通）

論　文　要　旨

専攻名 （又は推薦専攻名）	地域イノベーション学専攻	氏　名（ふりがな）	市川　俊輔（いちかわ　しゅんすけ）　㊞

学位論文題目　Engineering of a Cellulolytic Bacterium and a Lignocellulose-degrading Enzyme for Utilization of Cellulosic Biomass

（英訳又は和訳：　セルロース系バイオマス利用に向けたセルロース系バイオマス分解細菌とその分解酵素の改変　）

Heavy dependence on products derived from petroleum has generated concerns about global warming and the depletion of fossil resources. Biomass is a sustainable resource, and its utilization has been described as carbon neutral because it does not contribute to global warming. Cellulosic biomass is a particularly attractive candidate sustainable resource material because of its abundance. Numerous studies have examined the use of cellulosic biomass in biorefinery processes for the production of petroleum products, including fuel compounds. **The development of economic processes for biofuel production from cellulosic biomass will require significant cost reductions, however**. Some synthetic biology approaches for biofuel production have utilized microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* to produce fuel compounds from sugars. Lignocellulose-degrading enzymes are used to convert cellulosic biomass into fermentable sugars; however, the efficiency of the enzymatic degradation process is low (Figure). **To improve the efficiency of the enzymatic degradation process, lignocellulose-degrading enzymes were characterized and engineered in this thesis**.

Simplified “consolidated bioprocessing” strategies have been reported, in which microorganisms secrete enzymes for producing fermentable sugars from cellulosic biomass and simultaneously ferment them into fuel compounds. **The consolidated bioprocessing approach is promising because it eliminates the need to add lignocellulose-degrading enzymes, which significantly increases the cost of biofuel production**. Lignocellulose-degrading enzymes such as cellulases can be expressed in engineered strains of *E. coli* or *S. cerevisiae*, but their lignocellulose-degradation activity is low. The efficient production of lignocellulose-degrading enzymes by non-cellulolytic microorganisms remains a challenging task.

The clostridia are anaerobic soil bacteria, and some species can degrade cellulosic biomass without chemical pretreatment or the supplementation of lignocellulose-degrading enzymes. These microorganisms are promising for use in consolidated bioprocessing applications because of their ability to degrade cellulosic biomass and produce ethanol. Despite their high lignocellulose-degrading ability, only a few studies have examined biofuel production using genetically engineered cellulolytic clostridia.

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To collect water-soluble fuel compounds such as ethanol and butanol from culture broth, a distillation step is required, which also significantly increases the cost of biofuel production (Figure). Furthermore, ethanol and butanol are toxic and repress growth of the microorganisms. To produce higher concentrations of ethanol, researchers have attempted to enhance the ethanol tolerance of *Clostridium thermocellum*. In contrast to these alcohols, water-insoluble compounds such as aliphatic hydrocarbons are less toxic to *C. thermocellum*. Additionally, due to their insolubility in water, it is expected that these compounds could be more easily collected from the culture broth by phase separation.

It has been reported that the expression of just two enzyme genes, fatty acyl-acyl carrier protein (ACP) reductase (ACR) and aldehyde-deformylating oxygenase (ADO), are sufficient to engineer *E. coli* strain to produce higher alcohols and hydrocarbons. It is expected that introducing the genes encoding ACR and ADO into *C. thermocellum* would enable the organism to produce higher alcohols and hydrocarbons. **Because wild type *C. thermocellum* can utilize cellulosic biomass, the engineered *C. thermocellum* might be able to convert it into higher alcohols and hydrocarbons directly, without the supplementation of cellulases**. Additionally, **the higher alcohols and hydrocarbons could potentially be collected without distillation because these compounds are water-insoluble** (Figure).

To verify this hypothesis, *C. thermocellum* was engineered with ACR and ADO genes to produce water-insoluble fuel compounds from cellulose. Expression of ADO gene was clearly detected, whereas only slight expression of ACR gene was detected. Cells expressing ACR and ADO accumulated fatty aldehydes (higher alcohol precursors). After cultivation with cellulose, the higher alcohols, decanol and dodecanol, were detected in the organic solvent phase of the culture broth, indicating that the strain secreted the higher alcohols. These results suggest that **the engineered *C. thermocellum* strain, expressing ACR and ADO genes, directly produces and secretes higher alcohols from cellulose without the supplementation of cellulases. The higher alcohols can be collected by phase separation** (Figure).

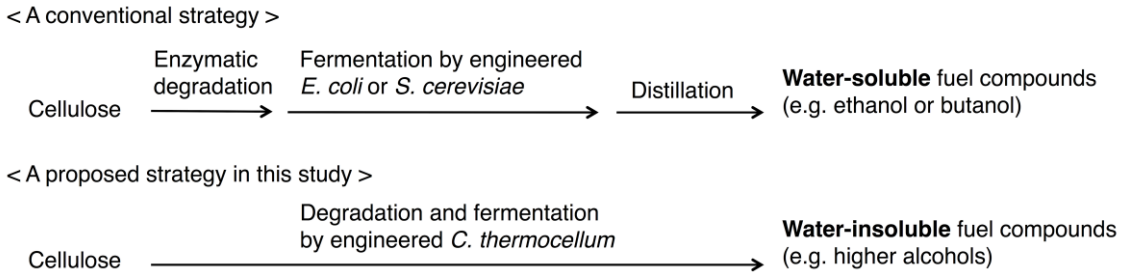

Figure: Conventional and proposed strategies for biofuel production.
Conventional strategies include expensive steps, such as enzymatic degradation of cellulosic biomass and distillation of water-soluble fuel compounds. In this study, a cellulolytic bacterium, *Clostridium thermocellum*, was engineered to produce and secrete water-insoluble fuel compounds in order to avoid the costly steps in the conventional process.