論　文　の　要　旨

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論　文　題　目　Development of the genetic engineering system in *Moorella thermoacetica* and application to production of useful materials.
（*Moorella thermoacetica* における遺伝子組換え技術の開発及び有用物質生産への応用）

Chapter 1 General introduction

Syngas fermentation is a biological process to produce useful metabolites using chemolithotrophs that catabolize the mixed gas of H_2, CO, and CO_2 generated by gasification of various organic substances. In syngas fermentation, whole biomass including non-degradable components such as lignin that cannot be used in conventional sugar fermentation can be used as substrates via gasification. Furthermore, the syngas fermentation offers several advantages over chemical conversion with metal catalysts such as its high specificity, no requirement of a highly specific H_2 plus CO or CO_2 ratio, and no contamination by metal poisoning.

Acetogens are known to be bacteria that grow on H_2 and CO as electron donors and CO_2 as a carbon source. In mesophilic acetogens such as *Clostridium ljungdahlii* and *Clostridium autoethanogenum*, ethanol production from syngas has been reported. Thermophilic acetogens such as *Moorella* sp. HUC22-1 have been also investigated for ethanol production from H_2-CO_2. The use of thermophilic acetogens such as *Moorella thermoacetica* for microbial production platforms based on syngas and H_2-CO_2 has several advantages. Thermophilic acetogens have high growth and metabolism rates and low risk of microbial contamination due to their high growth temperatures. In addition, the use of thermophilic acetogens will enable the continuous distillation of alcohols such as ethanol because aqueous alcohols will readily vaporize at temperatures over 50°C. Thus, thermophilic acetogens should be promising candidates for syngas fermentation rather than mesophilic bacteria. However, ethanol yield of H_2-CO_2 fermentation using *Moorella* sp. HUC22-1 was a slight amount because *Moorella* mainly produced acetate. Hence, for efficient production of useful materials such as ethanol except acetate, modification of metabolic pathway by genetic transformation is needed. Thus, the genetic transformation system of *M. thermoacetica* was developed and production of useful materials by genetic modification was investigated in this study.

Chapter 2 Development of genetic transformation and heterologous expression system in carboxydotrophic thermophilic acetogen *Moorella thermoacetica*

To develop a microbial production platform based on hydrogen and carbon dioxide, a genetic transformation system was developed for the thermophilic acetogen *M. thermoacetica* ATCC39073. The uracil auxotrophic strain dpyrF was constructed by

disrupting *pyrF* gene cording orotate monophosphate decarboxylase. The transformation plasmids were methylated by restriction methylases of *M. thermoacetica* to avoid the decomposition of introduced plasmids by restriction-modification system. Reintroduction of native *pyrF* into the mutant by homologous recombination ensured recovery from uracil auxotrophy. To test heterologous gene expression in dpyrF, the lactate dehydrogenase (LDH) gene (*T-ldh*) from *Thermoanaerobacter pseudethanolicus* ATCC33223 was introduced into dpyrF with a promoter of the glyceraldehyde-3-phosphate dehydrogenase (G3PD) gene of *M. thermoacetica* ATCC39073. The resulting transformant (C31) successfully transcribed *T-ldh* and exhibited higher LDH activity than ATCC39073 and dpyrF, yielding 6.8 mM of lactate from fructose, whereas ATCC39073 did not produce lactate.

Chapter 3 Transformation of thermophilic acetogen *Moorella thermoacetica* ATCC39073 using thermostable kanamycin resistance gene

A transformation system for *M. thermoacetica* ATCC39073 was developed using thermostable kanamycin resistance gene (*kanR*) derived from the plasmid that *Streptococcus faecalis* harbored. When the *kanR* with its native promoter was introduced into uracil auxotrophic mutant of *M. thermoacetica* ATCC39073 together with *pyrF* gene to complement the uracil auxotrophy as a selection marker, it did not give kanamycin resistance due to a poor transcription level of *kanR*. On the other hand, the use of the G3PD promoter significantly improved the transcription level of *kanR* and resulted in the cell growth in the presence of more than 150 μg/ml kanamycin. It was also demonstrated that *kanR* with the G3PD promoter can be used as the selection marker for transformation of wild-type strain of *M. thermoacetica* ATCC39073.

Chapter 4 Homo lactic acid fermentation by the genetically engineered thermophilic homoacetogen, *Moorella thermoacetica* ATCC39073

For efficient production of target metabolites from carbohydrates, syngas or H_2-CO_2 by genetically engineered *M. thermoacetica*, the control of acetate production, a main metabolite of *M. thermoacetica*, is desired. Although propanediol utilization protein (PduL) was predicted to be a phosphotransacetylase (PTA) involved in acetate production in *M. thermoacetica*, it has not been confirmed so far. The results here directly demonstrate that two putative PduL proteins, encoded by Moth_0864 (*pduL1*) and Moth_1181 (*pduL2*), are involved in acetate formation as PTAs. To disrupt those genes, each gene was substituted by lactate dehydrogenase gene from *Thermoanaerobacter pseudethanolicus* ATCC33223 (*T-ldh*). Acetate production from fructose by the *pduL1* deletion mutant was almost the same as that of wild-type strain while the disruption of *pduL2* significantly decreased acetate yield to approximately one third that of the wild-type strain. The double-deletion (both *pduL* genes) mutant did not produce acetate, but solely produced lactate as the end product from fructose. This suggests that both *pduL* genes are associated with acetate formation via acetyl-CoA and that their disruption enables a shift of the homo acetic pathway from fructose to the genetically synthesized

homo lactic pathway.

Chapter 5 Conclusion

In this study, I successfully developed the genetic engineering system in *M. thermoacetica*, in which the uracil auxotrophic strain (dpyrF) and *pyrF* gene were used as the host and the selection marker, respectively. I also found that the thermostable kanamycin resistance gene (*kanR*) controlled by the G3PD promoter from *M. thermoacetica* was used as the alternative selection marker to transform a wild-type strain of *M. thermoacetica*. By using the developed genetic engineering system, I identified that PduL1 and PduL2 functioned as phosphotransacetylase for acetate production of *M. thermoacetica* ATCC39073. Furthermore, I demonstrated that the genetically engineered *M. thermoacetica* with the disruption of *pduL1* and *pduL2,* and the introduction of *T-ldh* solely produced lactate from fructose without production of acetate. This is the first report on efficient production of useful metabolite except acetate from fructose by using genetically engineered *M. thermoacetica*.