論文の内容の要旨

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With the increasing concerns about depletion of petroleum supplies, there has been an increasing need in explore alternative energy sources. Plant biomass has been received considerable attention as the most promising alternative energy resource to overcome these problems. Plant biomass must first be converted to fermentable sugars such as glucose by hydrolytic enzymes before being converted to bioethanol. The ascomycete *Trichoderma reesei* is known to produce large amount of hydrolases that convert more efficiently plant biomass. Because of the industrial importance and variety of uses of *T. reesei* hydrolases, several mutants have been developed by classical mutagenesis to improve cellulase productivity. In this work, we investigated by comparative genome analysis single nucleotide polymorphisms (SNPs) that could be involved in high cellulase expression in *T. reesei* mutant. We also analyzed for the first time sugar transporters that are potentially involved in lactose uptake in *T. reesei*.

In Japan, the *T. reesei* mutant PC-3-7 has been developed by several rounds of random mutagenesis. During the screening, we found that this strain exhibited carbon catabolite derepressed phenotype and hyper-cellulase productivity in inducing conditions. To better understand the genetic basis of PC-3-7 phenotype, we sequenced the genome of this strain using Next Generation Sequencing and identified a total of 154 SNPs. Among them, one was found in the *cre1* gene, the carbon catabolite repressor of PC-3-7. Gel shift analysis revealed that the mutant CRE1 was unable to bind to its consensus binding site in the *cbh1* promoter *in vitro*. PC-3-7 strain transformed with the wild-type *cre1* clearly showed a pattern of carbon catabolite repression in inducing and repressing conditions, while PC-3-7 with the SNP in *cre1* exhibited a derepressed phenotype in the same conditions. Cellulase expression was ten to thirty times low when the mutated *cre1* was replaced by the wild-type *cre1* in PC-3-7. These data clearly indicates that the SNP in *cre1* is one of the factors influencing the hyper-cellulolytic phenotype of PC-3-7.

The expression of cellulases and hemicellulases of *T. reesei* are induced by cellulose and oligosaccharides, such as sophorose. However these inducers are still too expensive for economical production of cellulases at the industrial level. Lactose is a cheap alternative inducer found in large amounts as a by-product of cheese and whey production. Cellulase yields produced on lactose are still lower compared to cellulose. Previous studies suggested that lactose must first be internalized to induce cellulase gene expression. To improve cellulase inducibility by lactose, two sugar transporters highly expressed in inducing conditions were investigated. Disruptant

strains of these sugar transporters exhibited a delayed uptake of lactose compared with PC-3-7. Disruption of these two transporters also affected growth and cellulase expression. When cultivated in cellobiose or Avicel culture, no significant differences in cellulase production were observed among these strains. These data clearly indicates that these two transporters are specific for lactose uptake and are important for cellulase expression in the same condition.