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学位論文題目	Studies on enzymatic properties of *Trichoderma reesei* β-glucosidases and their role in cellulase induction (*Trichoderma reesei* 由来 β-グルコシダーゼの酵素化学的性質とセルラーゼ誘導における役割に関する研究)
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論　文　内　容　の　要　旨

Improved production of cellulose-degrading enzymes is important for promoting efficient biomass conversion. *Trichoderma reesei* has been developed into one of the most prolific cellulase producers at an industrial scale. The *Trichoderma reesei* genome contains 10 different genes encoding β-glucosidases, which catalyzes the release of glucose from cello-oligosaccharides and glycosides. Moreover, β-glucosidase produces isomeric glucosides including sophorose via a transglycosylation reaction and cellulase in *Trichoderma reesei* are potently induced by a precious disaccharide, sophorose (2-O-β-D-glucosyl-D-glucose). However, the properties of β-glucosidases, especially those responsible for sophorose synthesis have rarely been investigated. Elucidating the functions of β-glucosidases, including their transglycosylation, would be of interest for understanding its role in cellulase induction and production, bringing efficient decomposition of cellulose.

Chapter 1 provides the background for this research and my overall research objective.

Chapter 2 describes the identification of 4 of 10 β-glucosidases (Cel1A, Cel3A, Cel3B and Cel3E), which were capable of catalyzing transglycosylation reactions and the formation of cellotriose and isomeric glucobioses, including sophorose. Interestingly, Cel1A and Cel3A showed a high rate of conversion of cellobiose into sophorose, exhibiting 10% and 3.2% conversion ratio, respectively, with 10% cellobiose as substrate. Therefore, both β-glucosidases were suspected to be involved in sophorose synthesis followed to *in vivo* cellulase induction.

Chapter 3 describes the construction of two mutant strains, which were deleted *cel1a* and *cel3a* genes, respectively. Their influences were detected based on their contribution to the expression of cellulase genes on cultivation supplemented with cellulose. These results suggested that compared to the wild-type *Trichoderma reesei*, the mutant strain lacking *cel3a* showed a decrease in cellulase production during the later phases of the cultivation, that were considered owing to the lack of a major extracellular β-glucosidase Cel3A, might result in cellobiose accumulation. However, the deletion of *cel1a* gene resulted in a serious lag in cellulase induction, shifting the cellulase expressions from 12 to 96 h. Futher, addition of sophorose to Avicel growth medium of the mutant led to complete recovery of the transcription of the cellulase genes and

transcription regulator genes (*xyr1* and *cre1*) as the same level as it in the WT strain. The SDS-PAGE analyses of the secreted protein in the medium with sophorose clearly indicated the elimination of the long lag phase of appearance of extracellular proteins. The results of the transcriptional analysis also supported that the absence of *cel1a* resulted in the loss of early induction of cellulase genes in the wild-type strain. As mentioned above, Cel1A is the best sophorose synthesizer in *Trichoderma reesei*, although sophorose synthesis *in vivo* provided insufficient evidence, the lag in cellulase induction owing to a deletion of *cel1a* might be involved in the lack of sophorose synthesis. Thus Cel1A might responsible for the expressions of cellulase genes in the early culture phase.

Chapter 4 describes the creation of a highly functional Cel1A mutant by mutagenesis of only 2 amino acid residues. β-glucosidase not only produces glucose but also plays an important role in the release of product inhibition of cellulases by 4erd09degradation of cellobiose. However, most β-glucosidases are strongly inhibited by glucose, decreasing hydrolysis and transglycosylation activities. The Cel1A mutant (L167W/P172L) showed high glucose tolerance (50% of inhibitory concentration = 650 mM), even glucose stimulation (2.0 fold at 50 mM glucose) to the wild-type Cel1A. Kinetic studies also revealed that the glucose stimulation was caused by resolved in substrate inhibition which in particular shown in Cel1A mutant.

To conclusion, based on comparison of β-glucosidases transglycosylation ability, Cel1A and Cel3A were targeted in high conversion of capable of synthesis sophorose. *In vivo*, deletion of *cel1a* gene particular present in cellulase induction defect, suggesting Cel1A might play a role in sophorose synthesis. Last, a high functional Cel1A mutant was constructed, expecting to offer great advantages in formation of sophorose as well as the degradation of cellulosic biomass together with cellulases.