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論 文 内 容 の 要 旨
Abstract of Dissertation

氏　名 Daranagama Arachchige Nayani Dhanushka Daranagama

Plant biomass is renewable alternative carbon source instead of fossil resource. Glucose derived from degradation of plant biomass can be subjected to fermentation process to produce bioethanol and value-added materials. *Trichoderma reesei* is an industrial used filamentous fungus for plant biomass degrading enzymes. Its secretome contains cellulases, hemicellulases, lipases and proteases. From the view of industrial importance of *T. reesei*, its hydrolytic enzyme system has been subjected to extensive biochemical and genetic analysis. However, cellulases were degraded by proteases in secretome. Due to proteolytic analysis *T. reesei* has been sparse, in this study we aimed to characterize main protease Trichodermapepsin (TrAsP) and elucidate its regulation mechanism with respect to cellulase regulation. This thesis composed of 4 chapters; in chapter 1 background and aims of the study, in chapter 2, role of the TrAsP, in chapter 3, cis acting element analysis of TrAsP and in chapter 4, general conclusions are described.

In this study, substrate specificity of *T. reesei* secretome and its main protease TrAsP was determined up to P1 position using FRETS-25Xaa-libraries. The role of TrAsP was analyzed using *T. reesei* QM9414 and the deletant QMΔ*trasp* in Avicel. Higher activities of CMCase, Avicelase and Xylanase in QMΔ*trasp* compared to that of QM9414 were observed. Saccharification rate of cellulosic biomass increased when using secretome of QMΔ*trasp* but the effect was not significant due to the absence of difference in β-glucosidase activity compared to QM9414. Higher TrAsP was produced when monosaccharides were used as a carbon source, specially galactose, compared to cellulase inducers such as Avicel and α-sophorose. Further, peptone, and acidic pH conditions are preferred for optimal TrAsP expression. However, TrAsP production was low in mixed culture condition.

According to results of this study, TrAsP production depends on carbon and nitrogen sources. Unlike the cellulase mechanism, the regulatory mechanism of TrAsP remains unknown. Therefore, this study aimed to determine the effect of the main cellulase regulator Xyr1 and nitrogen regulator Are1 on *trasp* regulation. Cellulase inducer Avicel and TrAsP inducer galactose were used as carbon sources. qRT-PCR analysis revealed that Xyr1 and Are1 acted as a repressor and an activator for *trasp* expression, respectively. Compared to Avicel, relative expression was higher in galactose. The binding motifs of Xyr1 and Are1 were located in upstream of the *trasp* promoter. From promoter deletant analysis, the area from −870 bp to −670 bp was identified as the only region for positively regulation and there were both binding motifs of Xyr1 and Are1. The results of Beta-glucuronidase activity after site-directed mutagenesis confirmed the downregulation function of Xyr1 and the upregulation function of Are1. Electrophoretic mobility shift assay demonstrated the binding ability of Xyr1 and Are1 to the particular binding motifs and their functionality was confirmed. Further, this study demonstrated that Cre1, Xpp1, and Pac1 downregulate *trasp* expression similar to that in cellulase regulation mechanism. These results

demonstrate that transcriptional regulators of cellulase control *trasp* expression and suggest the possibility of the existence of specific protease regulators in *T. reesei*.