学 位 論 文 審 査 の 要 旨

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Discovery and functional characterization of multiple hemicellulose-responsive transcriptional regulators in the cellulolytic *Streptomyces* sp. SirexAA-E
(セルロース分解性放線菌における、ヘミセルロース資化関連遺伝子の転写調節因子の探索および機能解析)

His Doctoral thesis is divided into three chapters and comprises 45 pages of main text, 20 figures, 7 tables, and 4 reference literatures. The basic idea of biofuels and biochemical production is on the basis of using the renewable plant materials, which require an efficient enzymatic decomposition of plant biomass. *Streptomyces* sp. SirexAA-E was originally isolated as a symbiont of a wood devastating wood wasp, and it was shown that this bacterium produces abundant extracellular cellulose- and hemicellulose-degrading enzymes, when grown on plant biomass-containing medium. The previous study suggested that *Streptomyces* sp. SirexAA-E can sense the presence of cellulose in the culture medium via cellooligosaccharide-responsive transcriptional regulator, SsCebR. However, how *Streptomyces* sp. SirexAA-E senses other plant materials such as mannan and xylan was not known. Throughout his graduate study, he has focused on discovering the novel transcriptional regulators in *Streptomyces* sp. SirexAA-E, which sense mannan and/or xylan in the growth media to produce various polysaccharide-degrading enzymes.

1) Mannose and mannobiose specific responses of insect associated cellulolytic *Streptomyces*

Streptomyces sp. SirexAA-E was grown in the mannan-containing culture medium, and determined secreted proteins by LC-MS/MS. Both cellulose and mannan-degrading enzymes were found in the culture supernatant, and they were suggested to be transcriptionally regulated. By the genome analysis, a putative mannose and mannobiose responsive regulator, SsManR, was determined together with the potential target DNA motif, and this regulator was overexpressed by *Escherichia coli* heterologous protein expression

method followed by affinity protein purification. The DNA sequence motif was shown to be specifically bound by SsManR using in vitro binding assay. Furthermore, this SsManR-DNA complex was shown to be disrupted by adding the mannose or mannobiose as effector ligand. Thus, SsManR was first proven to be mannose and mannobiose responsive transcriptional regulator. The same effector ligand assay was also performed for the previously reported SsCebR-DNA complex and disruption of this complex by mannobiose was observed. Therefore, it was concluded that *Streptomyces* sp. SirexAA-E senses mannan derivative oligosaccharides in the growth condition via SsCebR and SsManR, and secretes a set of cellulose and mannan-degrading enzymes.

2) Molecular mechanisms of multiple hemicellulose-responsive transcriptional regulators in the cellulolytic *Streptomyces* sp. SirexAA-E

Streptomyces sp. SirexAA-E was grown in the xylan-containing culture medium, and secreted proteins were determined by LC-MS/MS and discovered a suite of xylan-degrading and xylan-utilizing enzymes. By using the pull-down proteomics analysis, three putative xylan-responsive regulators, SACTE_0535p, SACTE_5479p, and SACTE_5759p were determined. They were produced by *E. coli* and purified. The purified proteins were functionally characterized whether they bind specific DNA sequences or not. Results showed that two out of three proteins bound the regulatory sequence elements, upstream from xylan-degrading and xylan utilizing enzymes-coding genes. Thus, SACTE_0535p and SACTE_5479p were further examined by DNA footprinting assay and effector ligand assay. From this study, the xylan responsive transcriptional regulators were determined, which enables *Streptomyces* sp. SirexAA-E to regulate xylan-degrading and xylan-utilizing enzymes upon the presence of xylan in the growth medium.

Overall, therefore, we acknowledge that Mr. Keisuke Ohashi is qualified to be granted the Degree of Doctor of Philosophy in Food Resources from Hokkaido University.