学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称： 博 士（食資源学） 氏名 大橋 慧介

学 位 論 文 題 名

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）

Streptomyces sp. SirexAA-E (SirexAA-E) was originally isolated from the pine boring woodwasp, *Sirex noctilio* and its capability to decompose plant biomass was shown to be comparable to the industrial strain and commercial cocktail prepared from a genetically modified fangus, *Trichoderm reesei* RutC-30 (Takasuka *et al.* 2013). In addition, transcriptome and proteome analyses of SirexAA-E showed that SirexAA-E secreted a specialized set of enzymes depending on the carbon sources used to grow the cells, and enzymes can be glycoside hydrolase (GH) 5, 6, 9, 48 families for cellulose degradation and another GH5, 10, 11 families for hemicellulose degradation (Takasuka *et al.* 2013; Book *et al.* 2014, 2016). The knowledge about how the SirexAA-E controls gene expression of the above enzymes was limited except for the well-described cellobiose responsive transcriptional regulator, CebR, in *S. griseus*, and more recently in SirexAA-E (Takasuka *et al.* 2013; Book *et al.* 2016). In the absence of cellobiose, which is the main end-product of cellulose degradation, the CebR binds to a conserved 14 bp palindromic promoter sequence, 5'-TGGGAGCGCTCCCA-3', and blocks RNA polymerase from transcribing the downstream genes encoding cellulases. On the other hands, when cellobiose is available in the growth condition, CebR is released from the DNA, resulting in the expression of downstream genes. However, hemicellulose-specific responses of SirexAA-E are elusive to date.

In this study, I have aimed to understand how the SirexAA-E responds to available carbon

sources and control hemicellulose-degrading enzymes. Especially, I have been focusing on the two major hemicelluloses, mannan and xylan, response of SirexAA-E.

In chapter 2, I sought to examine a poorly understood its response to mannan, one of the major components of the plant cell wall. SirexAA-E grew well with glucose, mannose, cellulose, and galactomannan as a sole carbon source. The proteomic analysis of secreted proteins in each culture supernatant indicated that mannose and two polysaccharides induced the secretion of mannan and cellulose-degrading enzymes. I found two α-1,2-mannosidases that were secreted during the growth on mannose and galactomannan. By genomic analysis, I found a unique 12 bp motif (5'-GACAACGTTGTC-3') at 4 locations in the SirexAA-E genome, two of which were found upstream of genes encoding the above-mentioned α-1,2-mannosidases, along with a putative mannose and mannobiose responsive transcriptional regulator. Using a biochemical approach, the mannose and mannobiose specific regulator, ManR (SACTE_0504), was determined. Furthermore, the previously reported cellobiose-responsive repressor, CebR (SACTE_2285), was determined to also use mannobiose as an effector ligand.

In chapter 3, I sought to determine the already known and potentially new xylan responsive transcriptional regulators in SirexAA-E. Three xylan responsive transcriptional regulators, SACTE_0535p, SACTE_5479p, and SACTE_5759p, were determined and characterized with the corresponding new effector ligands, which likely regulate the downstream genes in a xylooligosaccharide-responsive manner. Furthermore, novel DNA sequence motifs for SACTE_0535p and SACTE_5479p were determined by DNase I footprinting assay.

In conclusion, I identified a set of transcriptional regulators that regulates genes involved in mannan and xylan degradation and metabolism of SirexAA-E. Together with these results, I proposed models for hemicellulose utilization system controlled by multiple transcriptional regulators in the cellulolytic insect-associated *Streptomyces*.