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学位論文要旨

専攻名　生物圏生命科学

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題目　Studies on characterization of a novel hemicellulase Abf62A-Axe6A from *Ruminiclostridium josui* and development of its host-vector system
（*Ruminiclostridium josui* の新規ヘミセルラーゼ Abf62A-Axe6A の酵素特性と宿主・ベクター系の開発に関する研究）

From the viewpoint of reduction of the global warming, the utilization and application of the renewable energies is required. Among various renewable energies, plant biomass is one of the most important energy resources. In the current process of bioconversion of plant biomass to value-added products, plant biomass is hydrolyzed by cellulolytic enzymes from fungi such as *Trichoderma reesei*, followed by fermentation of the resultant fermentable monosaccharides to biofuels etc. by yeasts or bacteria. On the other hand, "consolidated bioprocessing" (CBP) is widely recognized as the efficient strategy for simultaneous hydrolysis and fermentation of biomass using cellulolytic anaerobic bacteria. In this study, a novel hemicellulase Abf62A-Axe6A from *Ruminiclostridium josui* was biochemically characterized and its host-vector system was established for better use of this bacterium for CBP.

R. josui Abf62A-Axe6A is a modular enzyme consisting of an N-terminal signal peptide, a catalytic module of glycoside hydrolase family 62 (GH62), a family 6 carbohydrate binding module (CBM6), a dockerin module and another catalytic module of carbohydrate esterase family 6 (CE6) in order from the N-terminus. Therefore, three recombinant enzymes, RjAbf62A-Axe6A consisting of 4 modules devoid of the signal peptide, RjAbf62A-CBM6 consisting of GH62 and CBM6, and RjAxe6 consisting of CE6 only, were produced by *Escherichia coli* recombinants and biochemically characterized. RjAbf62A-Axe6 was highly active toward arabinoxylan and moderately active toward sugar beet arabinan, releasing mainly

arabinose. Analysis of the hydrolysis products of arabinoxylooligosaccharides indicated that RjAbf62A-Axe6 released α-1,2- and α-1,3-linked arabinofuranoses from both singly and doubly substituted xylosyl residues. RjAbf62A-Axe6A efficiently hydrolyzed A^3X to release arabinose, and the activity toward A^3X was stronger than that toward XA^3XX, A^2XX and $A^{2,3}XX$, suggesting that RjAbf62A-Axe6A prefers α-1,3-linked Ara*f* residues on singly substituted xylosyl residues located at non-reducing end to α-1,2-linked Ara*f* residues on singly substituted xylosyl residues and 1,3-linked Ara*f* residues in doubly substituted xylosyl residues. The enzyme showed a weak activity toward linear 1,5-α-L-arabinan, indicating that it had an exo-α-1,5-L-arabinofuranosidase activity. Surprisingly, RjAbf62A-Axe6 demonstrated an endoxylanase activity toward birchwood and beechwood xylans and xylooligosaccharides. RjAbf62A-Axe6 is the first GH62 enzyme having arabinofuranosidase and endoxylanase activities. Both RjAbf62A-Axe6 and RjAxe6 had an acetylxylan esterase activity toward insoluble wheat arabinoxylan.

R. josui host-vector system had not been established because of the existence of a restriction and modification system in *R. josui*. A restriction endonuclease *Rjo*I was purified from *R. josui* cell extract using column chromatography and characterized. The results showed that *Rjo*I is an isoschizomer of *Dpn*I, recognizing the sequence 5′-G^{met}ATC-3′, where the A nucleotide is Dam-methylated. *Rjo*I cleaved the recognition sequence between the A and T nucleotides, producing blunt ends. Plasmids prepared from *E. coli* C2925 (*dam*$^-$/*dcm*$^-$) could be introduced into *R. josui* by electroporation. The highest transformation efficiency of 6.6 × 10^3 transformants/μg-DNA was obtained using a square wave pulse (750 V, 1 ms). When the *R. josui cel48A* gene devoid of the dockerin-encoding region was introduced into *R. josui* using a newly developed plasmid pKKM801 as a vector, a truncated form of Cel48A, RjCel48AΔdoc, was detected in the culture supernatant but not in the intracellular fraction. This is the first study on the establishment of fundamental technology for molecular breeding of *R. josui*.

（備考）日本語（2000 字以内）または英語（500 ワード以内）にまとめて記載してください。