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学 位 論 文 要 旨
Dissertation Abstract

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Study on the enzymes involved in the later steps of degradation pathway of ulvan extracted from green algae *Ulva* sp.
（緑藻ウルバ属から抽出したウルバン分解経路における後期反応をつかさどる酵素についての研究）

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Ulvan is a complex sulfated polysaccharide in the cell wall of green algae of genus *Ulva* (Chlorophyta). It is composed of L-rhamnose-3-sulfate (Rha3S), glucuronic acid (GluA), iduronic acid (IduA), and D-xylose (Xyl) distributed in three repetition moieties. Ulvan polysaccharide and depolymerized oligo-ulvans can be used in various food, feed, biomedical, paint, and textile industries. The conversion of polymers into fermentable oligomers requires chemical or enzymatic breakdown. While a chemical breakdown is effective, enzymatic hydrolysis is seemed to be less energy consumption. Ulvan-utilizing bacteria can degrade ulvan into monosaccharide, rhamnose, using ulvan-degrading enzymes. The first step is depolymerization of ulvan outside cells with ulvan lyases catalyzing the cleavage of glycosidic bonds between Rha3S and uronic acids by elimination mechanism producing an unsaturated uronyl residue. The oligo-ulvans are imported into inside cells and further degraded to monosaccharide. The objective of this study is to investigate the characteristics of the enzymes for the later steps of ulvan degradation inside cells.

The laboratory bacterial stock contains ulvan-utilizing marine bacteria, which use ulvan extracted from either *Ulva ohnoi* or *Ulva meridionalis* as a sole carbon source. The draft genome sequences of 4 *U. ohnoi* ulvan utilizers and 7 *U. meridionalis* ulvan utilizers were determined. I found multiple ulvan lyase genes on the genomes. *ullA* encodes a long-type ulvan lyase belonging to PL (polysaccharide lyase) 24 family, which is known to be a major ulvan lyase reacting with ulvan outside cells. *ullB* and *ullC* encode short-type PL24 ulvan lyase and PL25 ulvan lyase, respectively. Genes responsible for polysaccharide degradation are usually clustered in the region polysaccharide utilization locus (PUL) on the genome. I found several ulvan degrading genes, unsaturated glucuronyl hydrolase, rhamnosidases, and sulfatases,

surrounding ulvan lyase genes.
Besides the main ulvan lyases, long-type PL24 UllA, ulvan-utilizing bacteria contain multiple ulvan lyase genes, *ullB*, and *ullC*. I would like to elucidate how these ulvan lyases work in the ulvan degradation pathway. An *Alteromonas* KUL106 strain contains two long-type PL24 *ullA* genes, three short-type PL24 *ullB* genes, and one PL25 *ullC* gene. Truncated *ullA* deleting C-terminal half, three *ullB* genes and *ullC* were cloned in pET21a with His-tag on the C-terminal end and heterologously expressed in *Escherichia coli* BL21(DE3). Overexpressed proteins were purified with a nickel column to homogeneity. All the purified proteins showed ulvan lyase activities, although the activities of UllB2 and UllB3 were weak. Kinetic parameters for ulvan and oligo-ulvans were measured with UllA, UllB, and UllC. UllA reacted much more with ulvan than oligo-ulvans. UllB reacted with ulvan and oligo-ulvans in a similar way. UllC reacted with oligo-ulvans higher than ulvan. The affinities of all enzymes were higher to ulvan than oligo-ulvans. Secreted proteins from KUL106 and soluble proteins in KUL106 were partially separated using a Q Sepharose anion-exchange chromatography, and proteins with ulvan lyase activity were detected. The long-type PL24 ulvan lyase UllA was detected only in the secreted proteins and the short-type UllB was detected only in the soluble fraction. Altogether, I concluded that while secreted UllA mainly reacted with ulvan, UllB was a major ulvan lyase reacted with oligo-ulvans inside cells. Since UllC was not detected from KUL106 cells, the function of UllC is still unclear.

Ulvan lyase cleaves the β -glycosidic bond between Rha3S and GluA/IduA through a β -elimination mechanism to produce oligo-ulvans with unsaturated 4-deoxy-L-threo-hex-4-enopyranosiduronate (Δ) at the non-reducing end. PUL was identified between two ulvan lyase genes belonging to PL24 and PL25 in the genome of a ulvan-utilizing bacterium *Glaciecola* KUL10 strain. The PUL contains many genes responsible for oligo-ulvan degradation. Among them, I demonstrated that both KUL10_26540 and KUL10_26770 had an unsaturated β-glucuronyl hydrolase activity to produce Rha3S and oligosaccharides, such as Rha3S-GluA-Rha3S, Rha3S-IduA-Rha3S and, Rha3S-Xyl-Rha3S, by releasing 5-dehydro-4-deoxy-D-glucuronate. KUL10_26540 showed much higher activity than KUL10_26770 and was more active on disaccharide than tetrasaccharide. I also found a rhamnosidase activity on four KUL10 gene products, although they could not react on the sulfated rhamnose.