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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Studies on Ulvan-Degrading Enzymes from *Alteromonas* sp.
Title of Dissertation （アルテロモナス属由来ウルバン分解酵素に関する研究）

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Ulvans are complex sulfated polysaccharides in the cell walls of green algae belonging to the genus *Ulva*. The production of mono- and oligosaccharides from this polysaccharide could motivate its use to an industrial scale. Conversion from polymers into fermentable oligomers, requires chemical or biological break down. However, the process of chemical conversion could be very expensive and not worth-while to use for an economical large-scale production. The enzymatic hydrolysis has been suggested as a more environmentally and economically treatment due to the low energy requirement, less byproduct production. Thus, identification of ulvan-degrading microorganisms and of the corresponding enzymes is necessary to develop protocols for the bioconversion of ulvan. Thus far, only a few ulvan-degrading enzymes have been isolated. The objective of this study is to discover novel ulvan-degrading bacteria, which can produce ulvan-degrading enzymes and contribute to potential use of ulvan as oligomers. In this study, two ulvan lyases were identified from two ulvan-utilizing *Alteromonas* sp., heterologously expressed in *E. col*i, and characterized. A simple method for efficient renaturation of inclusion body proteins denatured by SDS were developed.

Long-type ulvan lyases in two *Alteromonas* sp. are major enzymes involved in degradation of ulvan extracted from *Ulva ohnoi*.

Ulvan is one of the sulfated polysaccharide residing in the cell walls of green algae,

Ulva. Until now, not many bacterial enzymes that degrade ulvan have been studied. In order to utilize ulvan effectively by breaking down to oligosaccharides and monosaccharide, we have studied ulvan-degrading enzymes. In this study, two ulvan-utilizing *Alteromonas* sp. with high ulvan degrading activity, KUL17 and KUL42, were isolated from feces of small marine animals. We identified ulvan lyases with ca. 55 kDa in size from the secreted proteins of two strains and cloned the genes for them. The predicted molecular weight of the long-type ulvan lyases is 110 kDa. The catalytic domain is located on the N-terminal half and the C-terminus was turned out to be deleted. Although both strains contain two ulvan lyases, long and short, long ulvan lyase was demonstrated to be the major lyase for ulvan degradation.

Efficient renaturation of inclusion body proteins denatured by SDS

Inclusion bodies are often formed when the foreign protein is over expressed in *Escherichia coli*. Since proteins in inclusion bodies are inactive, denaturing and refolding of inclusion body proteins are necessary to obtain the active form. Instead of the conventional denaturants, urea and guanidine hydrochloride, a strong anionic detergent SDS was used to solubilize C-terminal His-tag form of ulvan lyase in the inclusion bodies. Solution containing SDS-solubilized enzyme were kept on ice to precipitate SDS, followed by SDS-KCl insoluble crystal formation to remove SDS completely. After removing the precipitate by centrifugation, the supernatant was applied to Ni-NTA column to purify His-tagged ulvan lyase. The purified protein showed a dimeric form and ulvan lyase activity, demonstrating that SDS-denatured protein was renatured and recovered enzyme activity. This simple method could be useful for refolding other inclusion body proteins.