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学位論文内容の要旨

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学 位 論 文 題 目

Studies on alginate lyases from an alginolytic bacterium *Hydrogenophaga* sp. strain UMI-18 that produces poly(3-hydroxybutylate)

（ポリヒドロキシ酪酸合成アルギン酸資化細菌 *Hydrogenophaga* sp. UMI-18 株のアルギン酸リアーゼに関する研究）

Alginate is a major structural polysaccharide from brown seaweeds, which is composed of β-D-mannuronate (M) and α-L-guluronate (G). These uronic acid units build up poly(M) block, poly(G) block and mixed M and G block (poly(MG) block) of the alginate polymer. Alginate has been widely used as a viscosifier and gelling agent in food, pharmaceutical and biotechnological industries and it is recognized as a viable biomass to produce biofuels and biomaterials. Recently, a novel alginolytic bacterium *Hydrogenophaga* sp. strain UMI-18 has been isolated, and it was shown to produce poly(3-hydroxybutylate) (PHB) using alginate as its sole carbon source. While the PHB synthesizing capability of strain UMI-18 have been studied to some extent, there is still a need to understand how this bacterium breakdown the alginate substrate to produce an unsaturated monosaccharide 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH), which is used as a carbon source for PHB synthesis.

In Chapter 1, the significance of PHAs as an alternative to petrochemically derived plastics is introduced along with bacteria that can use seaweeds as a fermentable biomass. There are only a few strains described that utilize seaweed carbohydrates in PHA production. To date, strain UMI-18 is the only alginolytic and PHB-producing bacterium reported. The importance of understanding the mechanism of alginate degradation of this strain and its connection to its PHB-synthesizing capability have also been described in this chapter. Furthermore, the alginate lyases of strain UMI-18 can also be used as a tool to produce oligoalginates which have many important applications.

In Chapter 2, the characteristics in growth and the PHB production of strain UMI-18 were investigated. This strain produces PHB in the alginate mineral salt (AMS) medium containing 1% (*w/v*) sodium alginate as its sole carbon source. PHB yield by strain UMI-18 was 1.1 ± 0.15 g/L of AMS and 58 ± 4% (*w/w*) of the dried cell pellet. Sugars like glucose, fructose, galactose, mannose, mannitol, and sucrose were also utilized by this strain for PHB production. PHB yield in 1% (*w/v*) carbohydrate media reached 2.03 – 2.24 g/L for glucose and fructose, 0.75 – 1.64 g/L for alginate, galactose, mannitol and sucrose, and ~0.15 g/L for lactose. The PHB produced by this strain had a glass-transition temperature (*Tg*) at 4 °C, melting temperature at 175 °C and an average molecular mass of 860 kDa. Draft genome analysis of strain UMI-18 revealed the co-occurrence of alginate-assimilating and PHB-synthesizing gene clusters. Four alginate lyase genes, HyAly-I – HyAly-IV were annotated in the alginate-assimilating gene cluster.

In Chapter 3, primary structures of the alginate lyases encoded by HyAly-I – HyAly-IV were characterized. Based on the primary structures of HyAly-I, HyAly-II, HyAly-III and HyAly-IV, the four alginate lyases were classified to PL-17, PL-7, PL-15 and PL-5, respectively. HyAly-I and HyAly-III are considered exolytic alginate lyases; whereas, HyAly-II and HyAly-IV are endolytic lyases. The two endolytic alginate lyases contain signal peptides suggesting their localization in the periplasmic space of the cell, while the two exolytic alginate lyases are thought to be localized in the cytosol. Out of the four alginate lyases, HyAly-I, HyAly-III and HyAly-IV were subjected to the expression in *E. coli*. However, only PL-17 recHyAly-I was successfully produced. The purified recHyAly-I showed a major band of 80 kDa on SDS-PAGE which was well consistent with the molecular mass predicted from the primary structure.

In Chapter 4, biochemical properties of the recHyAly-I were investigated. recHyAly-I showed optimal temperature and pH at 40 °C and pH 7.2, respectively, while it was unstable at temperature higher than 30 °C and pH lower than 6.0 and higher than 7.0. recHyAly-I directly released DEH from polymer substrates and showed the highest activity toward poly(M) and poly(MG) blocks of alginate. The degradation products of recHyAly-I were analyzed by TLC and ESI-MS. Interestingly, recHyAly-I was found to produce dimeric DEH (diDEH), a novel unsaturated disaccharide, along with monomeric DEH. Production of diDEH was prominent in

the degradation of trisaccharides. This indicates that diDEH is produced in the final phase of the exolytic degradation of alginate.

In conclusion, *Hydrogenophaga* sp. strain UMI-18 is a promising bacterium that can be used as a tool to explore brown seaweeds as a viable fermentable biomass in bioplastic production. Strain UMI-18 contains alginate-assimilating and PHB-synthesizing genes that can serve as a template to allow researchers to design new metabolic pathways of strains that can utilize seaweed biomass to produce high value materials. recHyAly-I was found to produce a novel dimeric DEH directly from poly(M) and alginate trisaccharides.