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学位論文の概要及び要旨

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題　　目 Studies on the structural analysis and physiological properties of exopolysaccharides (EPS) secreted by a newly isolated *Pseudomonas stutzeri* BL58
（新規に単離されたPseudomonas stutzeri BL58が分泌する菌体外多糖（EPS）の構造及び生理的特徴の研究

学位論文の概要及び要旨

Synthetic polymers are important materials in our lives, and they have contributed to the development of novel technologies in many fields. However, environmental problems have resulted from the large-scale distribution and disposal of synthetic polymers that are not biodegradable. To avoid environmental problems, some biodegradable polymers produced by microorganisms have been developed, and they show many unique physical and chemical properties for foods, cosmetics and pharmaceuticals. The objective of my thesis research was to study extracellular polysaccharides produced by microbes that assimilate ethanol under abnormal environmental conditions and to investigate these polysaccharides for their unique properties.

In Chapter 2, an ethanol-assimilating BL58 strain was isolated from soils and grown in liquid culture medium including ethanol as a carbon source under alkaline conditions. The BL58 strain produced exopolysaccharides which have very high viscosity and were identified to be *Pseudomonas stutzeri* based on the sequence of 16S rDNA.

In Chapter 3 and 4, the optimum culture conditions for the BL58 strain to produce the exopolysaccharides, such as initial pH, nitrogen source, carbon source and ethanol concentration, was investigated, and the largest production was observed when the BL58 strain was cultured in medium including peptone or polypeptone as a nitrogen source and 5% ethanol as a carbon source under alkaline conditions at pH 10. Under the optimal conditions, the BL58 strain produced 12.5 g/L of BL58 polymer during cultivation for 72 hours with periodic feeding of 1% ethanol.

In Chapter 5, 6 and 7, purification methods for the BL58 polymer were evaluated, and an effective purification method was developed by combining adsorption with activated charcoal, filtration with Hyflo super-cell, and dialysis with UF membrane. BL58 polymer purified using this developed method was analyzed for its physical and rheological properties. The purified polymer had a mean molecular weight of approximately 1,800 kDa, and the viscosity and pH of a 1% (w/v) solution were 160,000-250,000 cp and 6.3, respectively. This viscosity was 10 times higher than the same concentration of xanthan gum solution that is one of the most widely used microbial polysaccharides. The 1% polymer solution was more viscoelastic at less than 40 ºC and at pH 5 through 12.

In Chapter 8, the chemical structure of BL58 polymer was investigated by TLC and HPLC analysis of the hydrolysate of the polymer, by NMR analysis of native and partially hydrolyzed

BL58 polymer, and by GC-mass analysis of alditol-acetate of the hydrolysate and methylated alditol-acetate derivative. The ^{1}H-NMR analysis of native and partially hydrolyzed BL58 polymer indicated that BL58 polymer has a different structure from the already known polymers produced by *P. stutzeri* ATCC 17588 and *Pseudomonas sp*. OX1, and, therefore, it is a novel polymer. TLC analysis of the completely hydrolyzed products suggested that glucose, mannose and rhamnose were the main components of the BL58 polymer. Alditol-acetate analysis of BL58 polymer supported the results of the GC-MS analysis, and the ratio of these sugars was estimated as D-rhamnitol hexaacetate: D-mannitol hexaacetate: D-glucitol hexaacetate were equaled to 0.07: 0.6: 1.0. In addition to the peaks of the three monosaccharides, an unknown peak was observed in the alditol-acetate analysis. Alditol-acetate analysis of methylated non-hydrolyzed and partially-hydrolyzed BL58 polymer using GC-MS showed four peaks in both spectra; three peaks were observed at the same retention time, and one peak increased remarkably with the decrease of another peak, suggesting the BL58 polymer has a branched chain or substituted group of organic acid. The results from all structural analyses in the Chapter 8 indicated that the BL58 polymer is comprised of four types of monosaccharide units: a "-4)-glucose-(1-" unit, a "-4)-mannose-(1-" unit, a "-4)-glucose-(1-" unit with a branch at the 3 position and 3-*O*-carboxyethyl rhamnose unit, and the proposed structure for BL58 polymer differs from the previous microbial polysaccharides. This structure contributes to the rheological properties such as pseudo-plastic behavior, high elasticity, good mechanical strength and stability with high-absorption ability that is expected to apply to food and cosmetic additives.

In Chapter 9, further safety testing of BL58 polymer as cosmetic materials and bioactivity testing as pharmaceutical materials were evaluated. The acute toxicity test, eye irritancy test, rabbit and human skin irritation tests, and mutagenicity test were performed and the results were negative. The antibacterial and antiviral activities were tested and showed that the polymer exhibited anti-bacterial effects against *Escherichia coli* cells and concentration-dependent inhibition of viral infection. The antigenicity test was evaluated and suggested that BL58 polymer has weak antigenicity. The anti-proliferation tests of human acute promyelocytic leukemia cells (HL60) and human monocytoid leukemia cells (U-937) were evaluated and showed HL60 and U-937 classified as the same leukemia cell type have different susceptibility to the BL58 polymer. The anti-proliferative test of human liver carcinoma cells (HepG2) and human normal pulmonic cells (MRC5) were also evaluated and indicated that BL58 polymer has interesting anti-proliferative physiological properties suggesting potential applications in the pharmaceutical field.

In conclusion, the *Pseudomonas stutzeri* BL58 strain is non-pathogenic, and the developed production and purification methods for the BL58 polymer are inexpensive. Therefore, the commercial production and application of this biopolymer to various industries is very possible. The BL58 polymer was also novel, and it is anticipated that it could be used for as a new thickener of foods and cosmetics due to its non-toxic nature and beneficial physical properties.