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学 位 論 文 要 旨
SUMMARY OF DOCTORAL THESIS

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題目 Title: **Genetic Study of Pellicle Polysaccharide Synthesis in *Acetobacter tropicalis***
（アセトバクター・トロピカリスの菌膜多糖合成系の遺伝子解析）

Acetic acid bacteria are known to produce a relatively large amount of polysaccharide. In contrast to *Gluconacetobacter xylinus*, which produces cellulose, other acetic acid bacteria have been shown to produce heteropolysaccharide as their pellicle attached to the cell surface. *Acetobacter tropicalis* SKU1100 produces a novel polysaccharide composed of galactose, glucose and rhamnose at a molar ratio of 1:1:1. In this study, I have isolated several genes invloved in pellicle polysaccharide synthesis in this bacterium. Some gene disruption mutants have been shown to produce polysaccharides with a different composition or property compared to that of the wild type. These novel polysaccharides are expected to be a new biomaterial useful for various kinds of application.

In the first chapter, I showed that *A. tropicalis* SKU1100, like in several *Acetobacter* species, formed two different types of colony on agar plate: a rough-surfaced colony (R strain) and a smooth-surfaced colony (S strain). R and S strains were isolated and characterized; R strain formed a pellicle, which is a polysaccharide attached to the cells, on the medium surface in static culture, while S strain did not. A mutant having smooth-surfaced colony and a defect in pellicle formation as in the case of S strain, was isolated by random gene disruption using transposon mutagenesis. The mutant could not produce cell-attached polysaccharide, but instead extracellularly secreted into the culture medium. A gene cluster designated *polABCDE* was identified at or near the transposon insertion site, in which the transposon was found to locate in *polE*. The deduced amino acid sequences of *polABCD* showed a high homology to *rfbBACD*, that is involved in dTDP-rhamnose synthesis, respectively. Whereas PolE had a relatively low homology to genes encoded glycosyltransferases. *PolB* disruption was also prepared, which showed to completely defect in polysaccharide production. Plasmid harboring *polE* or *polB* gene could restore pellicle formation in the transposon mutant and S strain, or in *polB* mutant, respectively.

Thus both *polE* and *polB* are evidently involved in pellicle formation, most likely by anchoring polysaccharide to the cell surface and through the production of dTDP-rhamnose, respectively. The transposon and *polB* mutants were unable to grow under static culture and became more sensitive to acetic acid due to the loss of pellicle formation. Additionally, this study identified the mutation sites of several S strains which were spontaneously isolated from their original culture, and found them to be concentrated in a seven repetitive C sequence in the coding sequence of *polE,* with the deletion or addition of a single C nucleotide.

In the second chapter, I focused on the syntheseis of galactose that is one of sugar component presented in the pellicle polysaccharide produced by *A. tropicalis,* because genes responsible for the biosynthesis of UDP-galactose have not been found at *polABCDE* genes cluster. In most of Gram-negative bacteria, galactose utilization occurs by the Leloir pathway that involves enzymes encoded by the *galKTE* operon (Boel *et al*., 2001; Metzger *et al*., 1994; Prieson and Carlson, 1996). The *galE* gene product, UDP-galactose 4-epimerase, catalyzes the conversion of UDP-galactose to UDP-glucose and the reverse reaction. In this study, I identified *galE* gene encoding galactose-4-epimerase, involved in UDP-galactose biosynthesis, from *A. tropicalis* SKU1100 by PCR. The *galE* mutant did not produce CPS and thus could not form pellicle under static condition, but secreted EPS consisting of glucose and rhamnose into the environment.

In the third chapter, as shown in Chapter I, the *polABCD* genes show a high sequence homology to rhamnose biosynthetic genes, which are *rfbB, rfbA, rfbC*, and *rfbD*, respectively. In this study, I tried to investigate the role of *polABCD* genes in *A. tropicalis* SKU1100 by gene disruption and complementation analysis. Disruption of *polABCD* in the R strain resulted in mutants that formed smooth-surfaced colony on agar plate and exhibited a complete defect in polysaccharide synthesis, neither CPS on the cell surface or EPS in the medium. These results indicated that *polABCD* genes play an essential role on pellicle polysaccharide synthesis in this bacterium. Complementation analysis confirmed that there was no polar effect during the gene disruptions. To address the function of *polABCD*, we performed gene complementation with *rfbBDAC* genes from *E. coli* VW187, which the function has been reported. Pellicle formation of *polB, polC* and *polD* mutants with *E. coli* genes could be complemented, supporting that PolB is RfbA, which is glucose-1-phosphate thymidylyl transferase, PolC is RfbC, which and PolD is RfbD, which is dTDP-L-rhamnose synthase. However, complementation of *polA* mutant with *E. coli rfb* genes were not succeeded, which might due to the low expression of gene.