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SUMARRY OF DOCTORIAL THESIS

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Title: **Molecular biological study of sugar alcohol oxidation systems in thermotolerant *Gluconobacter* strain**

（耐熱性グルコノバクター酢酸菌における糖アルコール酸化系の分子生物学的研究）

Gluconobacter strains have various membrane-bound dehydrogenases which catalyze incomplete oxidation of sugars and sugar alcohols to accumulate the corresponding products in the culture medium. With this character of the microorganism are useful for application in the industrial fermentation of valuable products such as L-sorbose, which is an important intermediate in Vitamin C production. Among membrane-bound dehydrogenases of *Gluconobacter*, two types of D-sorbitol dehydrogenases (SLDH) have been reported and play a main role for L-sorbose production. The previous study, FAD-SLDH was purified and characterized from *G. frateurii* THD32 which was isolated from Thailand. And then the structural gene of FAD-SLDH was cloned with the franking regions. Upstream of the gene for FAD-dependent D-sorbitol dehydrogenase, *sldSLC*, a putative transcriptional regulator, *sbo*R, was found.

In this study as described in chapter 1, the whole of the *sboR* gene and the adjacent gene, *sboA*, were cloned and analyzed. The *sboR* mutation did not affect FAD-SLDH activity in the membrane fractions. The SboA enzyme expressed and purified from the *Escherichia coli* transformant showed NADPH-dependent L-sorbose reductase (NADPH-SR) activity, which was different from the NADPH-SR previously reported from *G. suboxydans* IFO 3291 in molecular size and amino acid sequence. The mutant defective in *sboA* showed significantly reduced growth on L-sorbose, indicating that this enzyme is required for efficient growth on L-sorbose. The *sboR* mutant grew on L-sorbose even better than the wild-type strain, and higher NADPH-SR activity was detected in cytoplasm fractions. RT-PCR experiments indicate that *sboRA* is composed of an operon. These data suggest that *sboR* is involved in the repression of *sboA* but not in the induction of *sldSLC* on D-sorbitol, and another activator is required for the induction of these genes by D-sorbitol or L-sorbose. This is the first report about the regulation of L-sorbose assimilation in the *Gluconobacter* strain,

although the regulation mechanism is still not clear.

It has been previously reported that there are two different membrane-bound enzymes responsible for the oxidation of D-sorbitol in *G. frateurii* THD32: pyroloquinoline quinone-dependent glycerol dehydrogenase (PQQ-GLDH) and FAD-SLDH. Therefore it is interesting to understand their physiological role for D-sorbitol oxidation in this strain. As the results in chapter 2, a mutant defective in both enzymes grew as well as the wild-type strain, indicating that both enzymes are dispensable for growth on D-sorbitol. The strain defective in PQQ-GLDH exhibited delayed L-sorbose production and its lower accumulation, corresponding to decreased oxidase activity for D-sorbitol, although high D-sorbitol dehydrogenase activity was observed. In the mutant strain defective in PQQ-GLDH, oxidase activity with D-sorbitol was much more resistant to cyanide, and the H^+/O ratio was lower than in either the wild-type strain or the mutant strain defective in FAD-SLDH. These results suggested that PQQ-GLDH connects efficiently to cytochrome bo_3 terminal oxidase and plays a main role in L-sorbose production; on the other hand, FAD-SLDH links preferably to thecyanide-insensitive terminal oxidase, CIO. In this study, it is also indicated biochemically for the first time that CIO may have lower ability to generate a proton gradient than BO3. It is suggested that FAD-SLDH is induced by L-sorbose, not by D-sorbitol. It seems that FAD-SLDH works only when an excess amount of D-sorbitol is available, that is, substantial amount of L-sorbose is accumulated by PQQ-GLDH but D-sorbitol still remains. *Gluconobacter* cells are probably willing to accumulate L-sorbose, even though D-sorbitol is a better C-source, to compete with other bacteria. In *P. aeruginosa*, CIO is induced upon entry into the stationary phase; therefore, this also happens in *Gluconobacter* and is consistent with the expression of FAD-SLDH in the late exponential phase. Further study is required to show how electrons from FAD-SLDH transfer to CIO: directly, mediated by ubiquinone, or by another mediator.