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学 位 論 文 要 旨
(Summary of the Doctoral Dissertation)

学位論文題目 (Dissertation Title)	Cell surface and intracellular metabolism of *Gluconobacter* spp. （グルコノバクター属酢酸菌の細胞表層および細胞内代謝）
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This dissertation was contributed to the understanding of metabolism in acetic acid bacteria, specifically *Gluconobacter* sp. It is organized in five chapters. The chapter 1 is general introduction whereby I described fundamental understanding of *Gluconobacter* sp, and expressed rationale of carrying out this research. The following three chapters are corresponding with three studies respectively. They are the metabolism of intermediate oxidation products (Chapters 2 and 4) and characterization of cryptic membrane bound dehydrogenase (Chapter 3) of *Gluconobacter* sp. The last section is general discussions and conclusions whereby the main research findings are discussed and concluded as well as restating significances of this research.

The first study is characterization of 5-keto-fructose reductase. *Gluconobacter* sp. strain CHM43 has mannitol dehydrogenase (quinoprotein glycerol dehydrogenase)(GLDH) and flavoprotein D-fructose dehydrogenase (FDH) on the outer surface of the membrane. In the periplasmic space, GLDH oxidize mannitol to fructose and then FDH does fructose to 5-keto-D-fructose (5KF). NADPH-dependent 5KF reductase was found in the soluble fraction of *Gluconobacter* spp., 5KF might be transported into the cytoplasm and metabolized. This study identified the *GLF_2050* gene as the *kfr* gene encoding 5KF reductase (KFR). A mutant strain devoid of the *kfr* gene showed lower KFR activity and less 5KF consumption than the wild-type strain. The crystal structure revealed that KFR is similar to $NADP^+$ -dependent shikimate dehydrogenase (SDH), which catalyzes the reversible $NADP^+$-dependent oxidation of shikimate to 3-dehydroshikimate. This study found that several amino acid residues in the putative substrate-binding site of KFR were different from those of SDH. Phylogenetic analyses revealed that only a subclass in the SDH family including KFR conserved such a unique substrate-binding site. We constructed KFR derivatives with amino acid substitutions, including replacement of Asn21 in the substrate-binding site with Ser that is found in SDH. The KFR-N21S derivative showed a strong increase in the Michaelis constant for 5KF, but a higher shikimate oxidation activity than wild-type KFR, suggesting that Asn21 is important for 5KF binding. In addition, the conserved catalytic dyad Lys72 and Asp108 were individually substituted for Asn. The K72N and D108N derivatives showed only negligible activities without a dramatic change in the Michaelis constant for 5KF, suggesting a similar catalytic mechanism to that of SDH. Taken together, we suggest that KFR is a new member of the SDH family.

The second study is about the orphan PQQ-dependent dehydrogenase 9 (PQQ-DH9) in *Gluconobacter* sp. strain CHM43 is a homolog of membrane-bound glycerol dehydrogenase (GLDH; also referred to as polyol dehydrogenase or sorbitol dehydrogenase). However, the functions of PQQ-DH9 remain

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unknown. PQQ-DH9 consists of two gene products—GLF_2583 and GLF_2584—which are similar to the transmembrane subunit SldB and the catalytic subunit SldA of GLDH, respectively. The GLF_2583 and GLF_2584 proteins have amino acid identities of 97% and 82% to their counterparts SldB (GLF_2777) and SldA (GLF_2776) of the CHM43 strain, respectively. We used a plasmid construct to express PQQ-DH9. The expression host was a derivative strain of CHM43, which lacked the genes for GLDH and the membrane-bound alcohol dehydrogenase and consequently had minimal ability to oxidize primary and secondary alcohols. The membranes of the transformant exhibited considerable D-arabitol dehydrogenase activity, whereas the reference strain did not, even if it had PQQ-DH9-encoding genes in the chromosome and harbored the empty vector. This suggests that PQQ-DH9 is not expressed in the genome. The activities of the membranes containing PQQ-DH9 and GLDH suggested that similar to GLDH, PQQ-DH9 oxidized a wide variety of secondary alcohols but had higher Michaelis constants than GLDH with regard to linear substrates such as glycerol. Cyclic substrates such as *cis*-1,2-cyclohexanediol were readily oxidized by PQQ-DH9.

The third study focuses on the three DHA kinases in DHA metabolism of *G. thailandicus* NBRC 3255. The NBRC3255 strain has two genes annotated with DHA kinase [*NBRC3255_2003* (*dhaK*) and *NBRC3255_3084* (*derK*)] whereas 621H strain has one gene (*GOX2222*). The NBRC3255 Δ*dhaK* Δ*derK* strain showed DHA kinase activity similar to wild type so that this study purified DHA kinase in the Δ*dhaK* Δ*derK* cells. N-terminal amino acid sequence of the purified DHA kinase was determined to match with that of glycerol kinase (*NBRC3255_0651*: *glpK*). The NBRC3255 Δ*glpK* strain remained 10% DHA kinase activity of wild type. Several combinations of deletions in *dhaK*, *derK* and *glpK* were constructed. These mutant strains lacked of both *derK* and *glpK*, lost DHA kinase activity and did not grow in glycerol medium. However, the Δ*derK* Δ*glpK* strain consumed DHA in the later growth phase in YPGD medium. Triple mutants lost DHA kinase activity, did not grow in glycerol medium and accumulated DHA in YPGD medium. Therefore, DhaK, DerK and GlpK are involved in DHA metabolism in *G. thailandicus* NBRC3255.

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学位論文審査の結果及び最終試験の結果報告書

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【論文審査の結果及び最終試験の結果】

NGUYEN MINH THUY 君による学位論文「Cell surface and intracellular metabolism of *Gluconobacter* spp.（グルコノバクター属酢酸菌の細胞表層および細胞内代謝）」について，その論文審査と口頭発表による最終試験を行った。本学位論文は，グルコノバクター属酢酸菌が行う酸化発酵と呼ばれる特徴的な物質生産系に関わる代謝を，遺伝子工学，分子生物学，分子系統解析，生化学的なアプローチで解析したものである。酸化発酵は，物質を細胞の表層で酸化し所望化合物を得る過程（正の過程）と所望化合物を細胞内で分解する過程（負の過程）の和と考えることができる。物質生産系としては，正の過程をより強く，負の過程をより弱くすることが肝要となる。本論文は背景説明と考察の２章を含めた５章構成となっており，第２章と第４章は，負の過程と言える細胞内代謝に関わる酸化還元酵素とリン酸化酵素に関するもので，第３章は，正の過程の細胞表層の酸化酵素に関するものである。詳細は割愛するが，低カロリー甘味料となり得る5-ケトフルクトース（第２章），日焼け剤として利用されるジヒドロキシアセトン（第４章），新規な物質酸化系（第３章），それぞれが抱える問題に取り組み，解決の糸口をつかんだ。

学位論文について審査委員による審査が行われ，研究内容の説明が口頭により行われた。その後，審査委員ならびに出席者からの質問を受け，それらに対して的確に回答した。これらの結果から，本論文が高度な内容を有していること，また本人が十分に本研究内容を理解して主体的に本研究を推進したことが明らかになった。また，本研究にはいくつかの独創的な内容が含まれており，しかも，それらの多くは，本人の主体的な発想と研究によって産みだされたものと判断された。

以上のことより，NGUYEN MINH THUY 君による本研究は十分に博士号を与えるにふさわしい内容を有するものと判定された。