Role of the Cell Surface Oxidation System in the Cell Growth of Acetic Acid Bacteria

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Research objective

Acetic acid bacteria have a unique metabolic system. In acetic acid fermentation, the oxidation reaction from ethanol to acetic acid, the essence of acetic acid fermentation, occurs in the periplasm, which is called the cell surface. The resulting reducing power is used to reduce oxygen to water via ubiquinone on the cell membrane (Fig. 1). Therefore, the oxidation reaction on the cell surface is connected to the respiratory chain electron transport system, obtains energy in the form of a proton driving force, and is used for ATP synthesis (1). As described above, cell surface oxidation only oxidizes substances; therefore, it contributes only to energy production. In contrast, the anabolic metabolism necessary for cell division is performed separately inside the cell. In summary, during the growth of acetic acid bacteria, this cell surface oxidation metabolism provides energy for the intracellular metabolism that assimilates carbon sources (1). The physiological significance of cell surface metabolism has not yet been validated experimentally; therefore, it was investigated in this study.

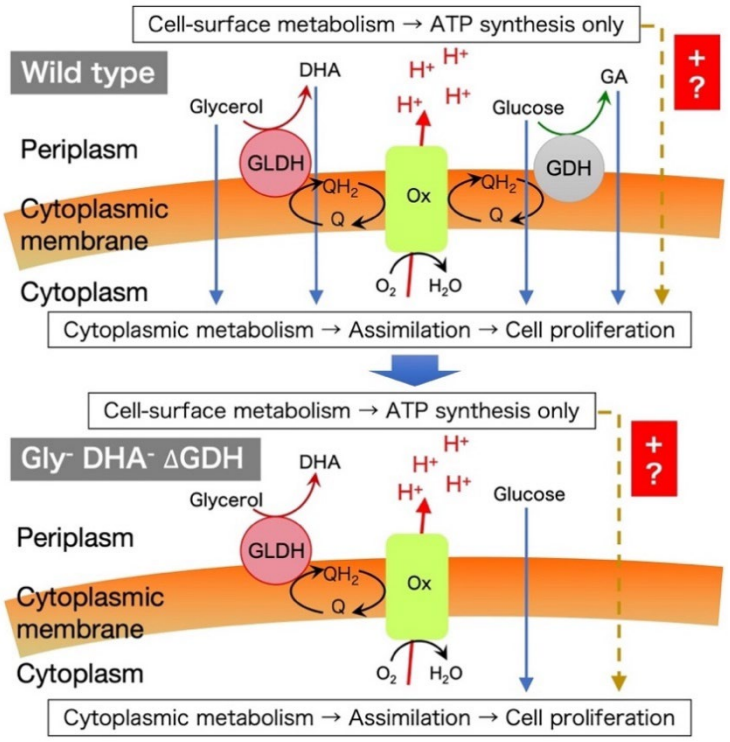

Figure 1. Overview of glycerol and glucose metabolism in the wild-type and mutant strains of *Gluconobacter thailandicus*, and the strategy used in this study.
It is not easy to separately examine the cell surface and intracellular metabolism in wild-type strain (top). Using a mutant strain (Gly$^-$ DHA$^-$ ΔGDH, bottom) that blocks the intracellular metabolism of glycerol and dihydroxyacetone (DHA) as well as the cell surface metabolism of glucose (Gly$^-$ DHA$^-$ ΔGDH), the two metabolic systems can be separately examined.

It is difficult to separate and analyze the two metabolic systems of acetic

acid bacteria, namely the cell surface and intracellular metabolic systems. Glycerol dehydrogenase (GLDH) oxidizes glycerol to produce dihydroxyacetone (DHA), which is a cell surface metabolism, but it requires a small amount of glycerol or DHA and uses it for cell growth (Fig. 1). Therefore, we blocked the intracellular metabolism of glycerol and DHA and constructed a mutant strain (Gly^- DHA^-) in which glycerol underwent only cell surface oxidation (2).

Based on this Gly^- DHA^- mutant strain, we introduced mutations that allowed us to examine the growth of substances that undergo only intracellular metabolism, without cell surface metabolism. We focused on glucose, which is presumably the most well-understood intracellular metabolite of *Gluconobacter* sp. Glucose is oxidized on the cell surface by glucose dehydrogenase (GDH) in the wild-type strain. Even GDH-disrupted strains of *Gluconobacter* sp. take up glucose, metabolize it, and grow. Therefore, in this study, we disrupted the GDH gene (*gdhM*) in Gly^- DHA^- mutant to create a Gly^- DHA^- ΔGDH mutant.

Methods

We used the *Gluconobacter thailandicus* strain (NBRC3255) and Gly^- DHA^- mutant strain (KAO-7), in which the glycerol kinase gene (*glpK*) and two DHA kinase genes (*dhaK* and *derK*) were eliminated (2). This mutant strain produced DHA from glycerol, but could not grow on glycerol medium. We disrupted the *gdhM* gene of the Gly^- DHA^- mutant using homologous recombination to generate the Gly^- DHA^- ΔGDH mutant. In addition, GLDH gene (*sldBA*) of the Gly^- DHA^- mutant was disrupted via homologous recombination to generate the Gly^- DHA^- ΔGLDH mutant.

For growth experiments, preculture was performed in a medium containing both glucose and glycerol. The preculture was transferred to a medium containing only glucose, only glycerol, or both glucose and glycerol, and cultured with shaking at 30 °C.

Results

Growth behavior of the mutant strains was investigated (Fig. 2). Gly^- DHA^- mutant did not grow on the glycerol medium. Since glucose metabolism is healthy, it grew on the glucose medium. The addition of glycerol improved its growth (Fig. 2A). This improvement is thought to be due to the energy obtained from the cell surface metabolism of glycerol by GLDH. To test this hypothesis, we compared the growth on glucose–glycerol medium with the Gly^- DHA^- ΔGLDH mutant and found that the growth was worse than that of the Gly^- DHA^- mutant (Fig. 2B). This decrease is thought to be derived from the energy obtained by cell surface metabolism of glycerol by GLDH, supporting the hypothesis that cell surface metabolism helps growth.

Finally, we obtained results that indicated the importance of cell surface metabolism

in the Gly$^-$ DHA$^-$ ΔGDH mutant strain. This strain could not grow on the glucose or glycerol medium, but grew on the glucose–glycerol medium (Fig. 2C). These results indicate that this strain cannot grow by cytoplasmic metabolism alone, and requires energy from cell surface metabolism. These results strongly support the hypothesis of this study. Cell surface metabolism supports intracellular metabolism and promotes cell proliferation.

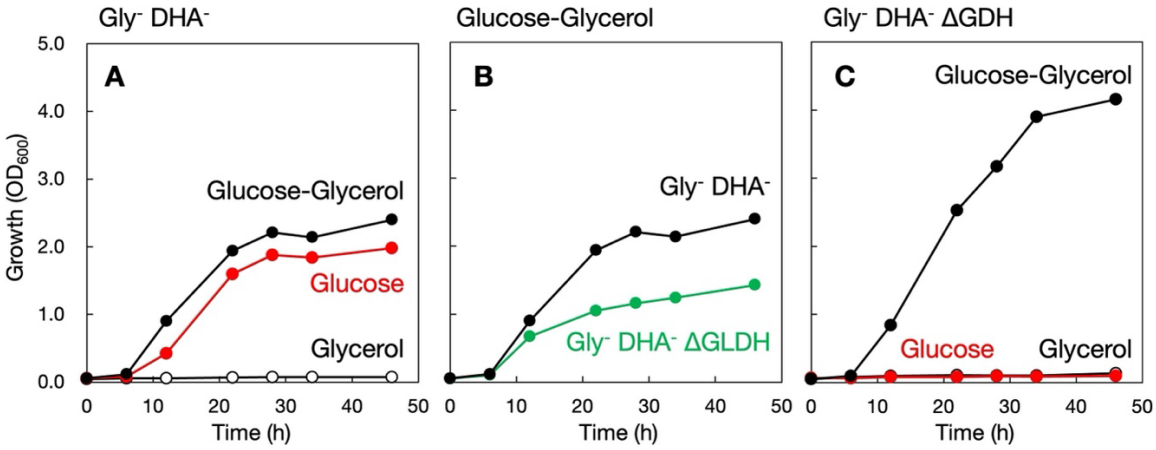

Figure 2. Growth of *Gluconobacter thailandicus* mutants on the glucose-only medium, glycerol-only medium, and medium containing both glucose and glycerol. Preculture was performed in a glucose–glycerol medium. A, Growth of the Gly$^-$ DHA$^-$ mutant on glucose medium (red), glycerol medium (white), and glucose–glycerol medium (black). B, Growth comparison of Gly$^-$ DHA$^-$ mutant (black) and Gly$^-$ DHA$^-$ ΔGLDH mutant (green) on glucose–glycerol medium. C, Growth of the Gly$^-$ DHA$^-$ ΔGDH mutant strain (The symbols are the same as those used in A).

Conclusion

In acetic acid bacteria, cell surface oxidation supplies the energy required for anabolic metabolism and cell proliferation.

References

1) Matsushita, K., Toyama, H., and Adachi, O. (1994) Respiratory chains and bioenergetics of acetic acid bacteria. *Adv. Microb. Physiol.* **36:** 247-301.

2) Kataoka, N., Hirata, K., Matsutani, M., Ano, Y., Nguyen, T. M., Adachi, O., Matsushita, K., and Yakushi, T. (2021) Three ATP-dependent phosphorylating enzymes in the first committed step of dihydroxyacetone metabolism in *Gluconobacter thailandicus* NBRC3255. *Appl. Microbiol. Biotechnol.* **105:** 1227-1236.