The effects of malate dehydrogenase gene deletion on lysine production in *Corynebacterium glutamicum*

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*Corynebacterium glutamicum* is widely used for industrial amino acids production, such as glutamate and lysine. We are attempting to improve lysine productivity of *C. glutamicum* by enhancing a precursor supply including oxaloacetate (OAA). To increase OAA supply, we have focused on three mutations; deletion of pyruvate kinase gene (ΔPYK), feedback-inhibition-resistance of phosphoenolpyruvate carboxylase (PEPC<sup>fr</sup>), reduced activity of citrate synthase (CS<sub>low</sub>). It has been revealed that these mutations have a synergistic effect on an improvement of lysine production (1). In this study, we investigated an effect of a new mutation; deletion of malate dehydrogenase gene (ΔMDH). Because MDH catalyzes the formation of malate from OAA, we hypothesized that ΔMDH might prevent OAA from flowing out to malate, and improve lysine productivity.

Aspartokinase (AK) is a key enzyme for lysine fermentation as AK is inhibited by lysine plus threonine allosterically. As a control lysine-producing strain, strain P having a feedback-inhibition-resistant-AK (AK<sup>fr</sup>) was used. Two mutants were constructed; strain M (AK<sup>fr</sup>/ΔMDH) and strain DRLM (ΔPYK/PEPC<sup>fr</sup>/AK<sup>fr</sup>/CS<sub>low</sub>/ΔMDH) from strain P (AK<sup>fr</sup>) and strain DRL (ΔPYK/PEPC<sup>fr</sup>/AK<sup>fr</sup>/CS<sub>low</sub>) as the parent strains, respectively. Lysine productivity in a jar fermentor culture was evaluated, and the enzyme activities and the respiration rate were measured.

Contrary to our expectation, lysine productivities of strain M and strain DRLM were comparable to that of their parent strains. In addition, their respiration rates, which seemed to be affected by MDH activity, were not changed. Accordingly, carbon flux from OAA to malate was considered to be very limited. In strain M, lysine productivity was slightly decreased, and phosphoenolpyruvate carboxykinase (PEPCK) activity was higher relative to strain P. Increased PEPCK activity might cause a decrease in OAA supply. This indicates that anaplerotic pathway is more important in OAA metabolism than MDH reaction. We are planning to carry out metabolome analysis in order to measure the carbon flow around OAA precisely and to find out a next promising target.

(1) Yanase M., Aikoh T., Sawada K., Ogura K., Hagiwara T., Imai K., Wada M., Yokota A., Pyruvate kinase deletion as an effective phenotype to enhance lysine production in *Corynebacterium glutamicum* ATCC13032: Redirecting the carbon flow to a precursor metabolite, *Journal of Bioscience and Bioengineering*, 122(2) 160-167, 2016