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Strategies to Modify Central Metabolism for Improved Fermentation in Amino Acid- & Nucleotide-production Strains

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Metabolic flux analyses using NMR spectroscopy or metabolite balancing have recently led to large progress in *in vivo* quantification of carbon fluxes. These methodologies, however, do not necessarily teach us which reactions should be engineered and how, to achieve maximum product yield, especially in classically derived industrial strains.

Here I will highlight the pentose phosphate pathway of the typical amino acid- and nucleotide-producing organisms *Corynebacterium glutamicum* and *Corynebacterium ammoniagenes*, respectively, to illustrate how we have approached toward the goal of further improvement of industrial strains. As the introduction, the recent advances in the development of amino acid producers will be summarized focusing on new strategies and trends of strain improvement in the last decade.

The pentose phosphate pathway in coryneform bacteria

The pentose phosphate pathway is responsible for supplying two important precursors, ribose 5-phosphate (R-5-P) for biosynthesis of nucleotide and histidine, and erythrose 4-phosphate (E-4-P) for biosynthesis of the aromatic amino acids. Therefore, metabolism within the pathway is of key importance for improving availability of each precursor. The pathway forms a cycle and consists of an oxidative route and a nonoxidative route (Fig. 1). The oxidative route is one-way in the clockwise direction during glucose metabolism while carbon on the nonoxidative route can flow to both directions since the constituent enzymes are all reversible. So far, little information has been available about the contribution of each route to the precursor synthesis during fermentation. For this investigation, we focused on reversible transketolase (TK), a possible key enzyme in the nonoxidative route, and examined how the enzyme was involved in the precursor supply during fermentation.

The pentose pathway gene cluster in coryneform bacteria

To rationally modify the TK activity, the gene (*tkt*) was isolated from each wild-type genome of *C. glutamicum* and *C. ammoniagenes*. Enzymatic and molecular analyses revealed that *tkt* was clustered with other pentose pathway genes, *tal* and *zwf*, encoding transaldolase (TAL) and glucose 6-phosphate dehydrogenase (G6PD), respectively. Using these cloned genes, we conducted the following metabolic engineering.

Effect of TK modifications on E-4-P availability

Firstly, a TK-deficient mutant was isolated from *C. glutamicum*. The mutant required the aromatic amino acids for growth, indicating that the activity was indispensable for the synthesis of E-4-P, a precursor of aromatic biosynthesis. On the other hand, the presence of additional copies of *tkt* in aromatic producers of *C. glutamicum* has led to increased yields in proportion to the copy numbers. This indicates that the overexpressed activity functions in the direction of the E-4-P formation *in vivo* in

aromatic amino acid-producing cells and contributes to an increased availability of the precursor for aromatic production (Fig. 1).

Effect of TK modifications on R-5-P availability

Secondly, *tkt*-amplified and disrupted strains were constructed from a nucleotide (5í-xanthylic acid)-producing *C. ammoniagenes* strain as well as a histidine-producing *C. glutamicum* strain. Production analysis showed that the presence of additional copies of *tkt* led to decreased yields of both products while better yields were achieved by its disruption. These findings have shown that the reversible TK normally shunts R-5-P back into glycolysis in both production strains (Fig. 1) and interception of this shunt allows cells to redirect carbon flow through the oxidative route from the intermediate towards the nucleotide or histidine pathway.

Oxidative versus nonoxidative routes.

Lastly, we disrupted the *zwf* gene of each of the 5í-xanthylic acid-producing *C. ammoniagenes* and the phenylalanine-producing *C. glutamicum* to examine the impact of interception of the oxidative route

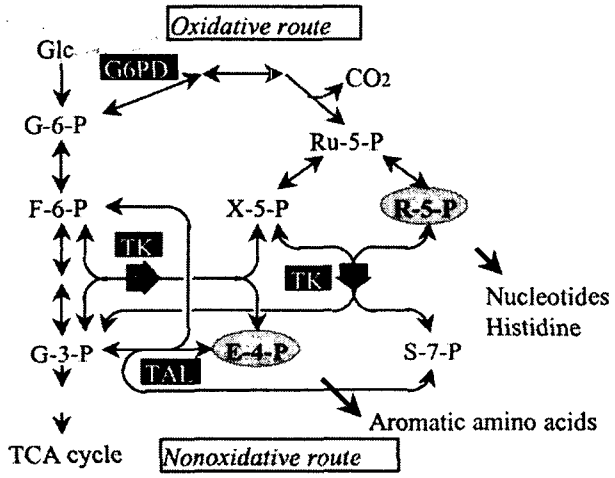

Fig. 1. The pentose phosphate.
Thick arrows in TK reactions indicate directions of carbons flux *in vivo*.

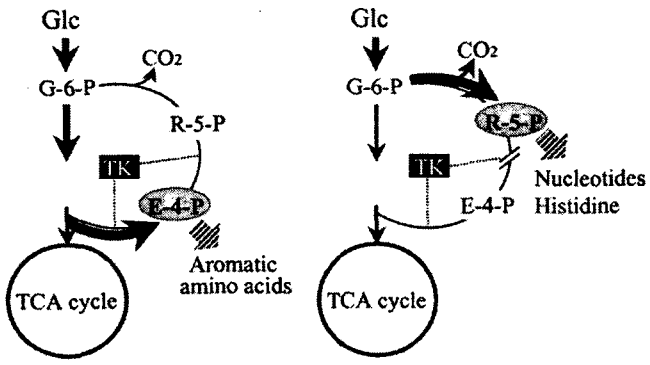

Fig. 2. Strategies for improved fermentation

on production. Elimination of G6PD activity resulted in no decrease in phenylalanine production but a large decrease in the nucleotide production with no obvious phenotype during growth on glucose. These results have shown that the counter-clockwise flux and the clockwise flux within the pentose phosphate cycle are of importance to increase availability of E-4-P and R-5-P, respectively, in coryneform bacteria (Fig. 2).

Taking all of these findings into consideration, we can propose the strategies useful for increasing carbon flow to the aromatic amino acids, histidine, and nucleotides as follow. For aromatic production, amplification of *tkt* could be effective for enhancing the potential of producers to supply E-4-P through the nonoxidative route. On the other hand, for histidine and nucleotide production, better yields could be achieved by decreased activity or deficiency of TK.