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Recent advances in amino acid production by microbial cells

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Amino acids have been utilized for the production of foods, animal feeds and pharmaceuticals. After the discovery of the glutamic acid-producing bacterium *Corynebacterium glutamicum* by Japanese researchers, the production of amino acids, which are primary metabolites, has been achieved using various microbial cells as hosts. Recently, metabolic engineering studies on the rational design of amino acid-producing microbial cells have been successfully conducted. Moreover, the technology of systems biology has been applied to metabolic engineering for the creation of amino acid-producing microbial cells. Currently, new technologies including synthetic biology, single-cell analysis, and evolutionary engineering have been utilized to create amino acid-producing microbial cells. In addition, useful compounds from amino acids have been produced by microbial cells. Here, current researches into the metabolic engineering of microbial cells toward production of amino acids and amino acid-related compounds are reviewed.

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Introduction

Amino acids have been utilized for the production of foods, feed, cosmetics and pharmaceuticals. Amino acids are primary metabolites, which constitute proteins in cells, and therefore it was commonly believed that they could not be produced by microbial cells However, in 1956 Japanese researchers Drs. Shukuo Kinosita and Shigezo Udaka at Kyowa Hakko Kogyo Co., Ltd. (currently Kyowa Hakko Bio Co., Ltd.) isolated a bacterium that produced a significant amount of glutamic acid, namely *Corynebacterium glutamicum* [1,2]. Since the isolation of *C. glutamicum*, it has been realized that amino acids can actually be produced by microbial cells. To date, a number of amino acids have been produced by microbial cells and the size of the market for amino acids has dramatically increased. For example, the market for glutamic acid is more than 2.5 million tons per year.

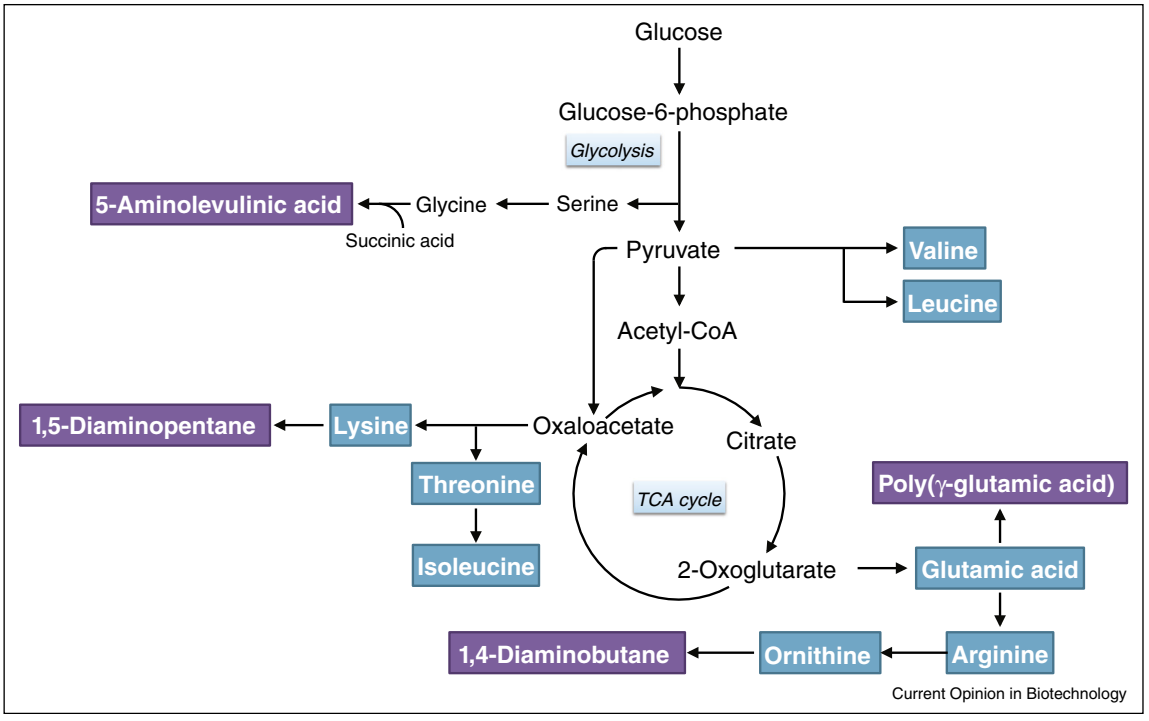
Various researches related to the metabolic engineering and rational design of microbial cells toward amino acid production have been reported. Recently, systems metabolic engineering, in which the concepts and techniques of systems biology are applied to metabolic engineering, has been used in the design of microbial cells toward amino acid production [3]. Moreover, new technologies for generating amino acid-producing microbial cells, including single cell analysis, synthetic biology, and evolutionary engineering, have been used. In addition, production of useful compounds from amino acids has been also carried out. In this review, recent advances in metabolic engineering researches (Figure 1) and the application of new technologies for the design of microbial strains toward production of amino acid and amino acid-related compounds are reviewed.

Glutamic acid production by microbial cells and its molecular mechanisms

In 1956, *C. glutamicum* was identified as a glutamic acid-overproducing bacterium [1,2]. Glutamic acid production by *C. glutamicum* can be induced by biotin limitation [4], Tween 40 addition [5] and penicillin addition [6]. During glutamic acid production, intracellular metabolism is regulated at the 2-oxoglutarate node in the TCA cycle to increase the metabolic flux toward glutamic acid production [7]. Moreover, it was recently reported that the mechanosensitive channel NCgl1221 was responsible for glutamic acid excretion by *C. glutamicum* [8–11].

In the glutamic acid production process using *C. glutamicum*, the decrease in the pH in the culture is problematic. Therefore, the host microorganisms which can produce glutamic acid under the acid conditions have been desired. In Ajinomoto Co., a novel bacterium *Pantoea ananatis* AJ13355 which can grow and shows tolerance to high concentration of glutamic acid was isolated [12]. By using this microorganism, glutamic acid production under the acidic conditions has been successfully established and metabolic engineering studies has been also carried out [13].

Figure 1



Overview of the metabolic pathways utilized for production of amino acids and amino acid-related compounds in microorganisms, as discussed in this review.

Metabolic engineering of microbial cells for the rational design of amino acid-producing strains

Since the discovery of *C. glutamicum*, the production of primary metabolites such as amino acids and nucleotides by microbial cells has been established [14,15]. Recent reports have described metabolic engineering methods for the rational design of amino acid-producing host cells. Common strategies for the design of amino acid-producing strains are (i) amplification of biosynthesis pathway enzymes for the target amino acids, (ii) reduction of by-products formation, (iii) release of feedback regulation of key enzymes by the target amino acid, (iv) increased supply of reducing equivalents such as NADPH, (v) reduction of metabolic fluxes to the TCA cycle, because most target amino acids are produced from intermediate metabolites in the glycolysis and the pentose phosphate pathways, and (vi) increased export of target amino acids out of the cells (Figure 2). Here, the rational design of microbial cells for the production of amino acids is summarized; the metabolic engineering studies described in this review are summarized in Table 1.

Metabolic engineering for lysine production

Lysine is one of the essential amino acids, and it has been utilized as a feed additive. The market size of lysine has been reaching around 1 million tons per year. The lysine biosynthetic pathways include two NADPH-dependent reactions that are catalyzed by aspartic semialdehyde dehydrogenase and dihydrodipicolinate reductase. In order to produce 1 mol of lysine, 4 mol of reducing equivalent NADPH is required, and therefore, metabolic engineering of microbial cells has been performed to fulfill the NADPH requirement for lysine production. Generally, NADPH is produced via the pentose phosphate pathway. Recently, a number of metabolic engineering studies have been described that increase the NADPH supply via the glycolytic pathway other than the pentose phosphate pathway. For example, Takeno *et al.* replaced NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in *C. glutamicum* with $NADP^+$-dependent GAPDH from *Streptococcus mutans*, which catalyzes the conversion of glyceraldehyde-3-phosphate to 3-phosphoglycerate together with the reduction of $NADP^+$ to NADPH [16]. Despite the fact that the engineered strain showed a growth defect on glucose, a suppressor mutant was isolated, which showed increased growth. Furthermore, lysine production in the engineered suppressor mutant strain was higher compared to that in the reference strain. Bommareddy *et al.* examined the effect of altering the cofactor specificity of endogenous NAD^+-dependent GAPDH of *C. glutamicum* to $NADP^+$-dependent one by rational protein design, and found that lysine production in the mutant strain was increased by around 60% [17••].