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Systems metabolic engineering strategies for the production of amino acids

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ABSTRACT

Systems metabolic engineering is a multidisciplinary area that integrates systems biology, synthetic biology and evolutionary engineering. It is an efficient approach for strain improvement and process optimization, and has been successfully applied in the microbial production of various chemicals including amino acids. In this review, systems metabolic engineering strategies including pathway-focused approaches, systems biology-based approaches, evolutionary approaches and their applications in two major amino acid producing microorganisms: *Corynebacterium glutamicum* and *Escherichia coli*, are summarized.

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1. Introduction

Systems metabolic engineering is an emerging discipline that combines the concepts of systems biology, synthetic biology and evolutionary engineering [1], as defined by Lee [2], it involves the application of omics data and the utilization of omics data for synthetic biology and evolutionary engineering for strain breeding and process improvement. With the development of high-throughput technologies, computational methods and simulation approaches, systems biology has become much more mature and applicable, and has already manifested its giant potential in providing genome-wide information and clues for synthetic biology and evolutionary engineering. The various combinations of systems biology, synthetic biology and evolutionary biology have been successfully applied for metabolic engineering of industrial strains [3–5]. Undoubtedly, systems metabolic engineering could dig out the maximum potentials of microbial cell factories.

The microbial production of amino acids is a large area where systems metabolic engineering strategies have been successfully applied, mainly in two important producing microorganisms: *Corynebacterium glutamicum* and *Escherichia coli*. Since the first discovery of the L-glutamate producing strain *C. glutamicum* in 1957, strain breeding has become a fierce competing spot of leading amino acid manufacturing enterprises with the expanding market demand for amino acids. L-Glutamate is the major bulk amino acid, which covers nearly two thirds of the amino acid market. The market demand of L-lysine ranks just next to L-glutamate, with an current annual production of over 2200000 tons [6]. Various strain breeding approaches have been developed, whilst genetically defined metabolic strategies have gradually taken the place of the conventional random mutagenesis-selection method and become the mainstream. While local metabolic engineering of microorganisms that focuses on the engineering of one or a few specific genes or metabolic pathways generally has the limitation of being not able to take the whole metabolic process into consideration. Systems metabolic engineering tries to overcome this limitation by combined approaches to obtain rationally designed strains.

In this review, systems metabolic engineering strategies and applications for amino acid producing strain improvement are summarized, mainly focusing on the two major industrial production microorganisms: *C. glutamicum* and *E. coli*.

2. Strategies for systems metabolic engineering of microorganisms for amino acids production

According to Lee et al. [7], strategies for systems metabolic engineering could be categorized into two groups, the rational intuitive approaches and the systematic and rational-random approaches. The former group covers the typical metabolic engineering process of the synthetic pathway of a certain product, that from the uptake of carbon source, elimination of byproducts, enrichment of precursors, to the reconstruction of related metabolic pathways, supply of cofactor, and so on [7], when the target genes to be engineered are obvious. The latter group mainly includes omics-based metabolic engineering techniques and various evolution approaches when no obvious target genes are known. Applications of systems metabolic engineering of microorganisms for amino acid production have been increasing, and representative examples are shown in Table 1. In this review, we classified the systems metabolic strategies for amino acid high-producing strains into three categories as illustrated in Fig. 1, which are summarized below.

2.1. Pathway-focused approaches

Pathway-focused approaches usually aim to increase the production ability of certain products by combining local metabolic engineering methods, such as enhancing carbon source utilization and key enzyme expression, removing feedback inhibition and transcriptional attenuation, and blocking bypass pathway etc. A lot of endeavors have been made in the pathway-focused engineering of microorganisms for amino acid production.

2.1.1. Carbon source utilization engineering

The carbon source uptake and utilization process is the first crucial step for the production of amino acids. By enhancing the uptake and utilization of carbon sources, more carbon flux could be provided for the synthesis of amino acids. Generally, there are two types of carbon source transport systems, the phosphotransferase system (PTS) and non-phosphotransferase system. The PTS requires phosphoenolpyruvate (PEP) for the phosphorylation of carbon sources, which is usually an important intermediate for the synthesis of certain amino acids. In that situation, the replacement of the PTS with non-PTS could save more PEP for the following step of amino acid synthesis [8,9]. Other ways of increasing the intracellular PEP have been tried. For example, Tatarko et al. [10] disrupted a global regulatory gene *csrA* encoding Csr (carbon storage regulator) to increase the gluconeogenesis and decrease the glycolysis, which elevated intracellular PEP for the synthesis of phenylalanine.

The enhancement of the expression of *ptsG*, encoding the glucose-specific EII permease of the PTS, could increase the utilization of glucose in *C. glutamicum* and *E. coli*. Except for the direct gene manipulation, the expression of *ptsG* could also be affected by the existence of other carbon sources. For example, the existence of acetate could reduce the expression of *ptsG* to 45% by the SugR-mediated repression of *ptsG*, while, the addition of maltose could increase the *ptsG* expression by counteracting the SugR-mediated repression [11] in the presence of acetate. It has been reported that the addition of maltose increased the glucose utilization of a pyruvate dehydrogenase complex-deficient *C. glutamicum* strain, and thus, improved its L-valine productivity [12]. Recently, Henrich et al. [13] found out that maltose uptake by the novel ABC transporter system MusEFGK2I was the reason that caused increased expression of *ptsG* in *C. glutamicum*.

Besides, to cope with the food crisis all over the world, the utilization of cellulose and hemicellulose derived sugars, such as xylose and arabinose, for the production of amino acids, has become more and more urgent. *E. coli* can grow efficiently on a wide range of carbon substrates including various pentose such as xylose, mannose, arabinose etc [14]. Simultaneous uptake of lignocellulose-based monosaccharides in *E. coli* has been reported [15]. In nature, *C. glutamicum* is not capable of utilizing xylose as a