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Modification of tryptophan transport system and its impact on production of L-tryptophan in *Escherichia coli*

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ABSTRACT

The production of L-tryptophan through chemical synthesis, direct fermentation, bioconversion and enzymatic conversion has been reported. However, the role of transport system for aromatic amino acids in L-tryptophan producing strains has not been fully explored. In this study, the fact was revealed that L-tryptophan production and cell growth were affected by the modification of transport systems based on YddG functioning as aromatic amino acid excretion and AroP functioning as general aromatic amino acid permease. Through comparing glucose conversion rates of recombinant strains such as *Escherichia coli* TRTH Δ*aroP*, *E. coli* TRTH-Y, and *E. coli* TRTH Δ*aroP*-Y, the moderate modification of transport system resulted in the metabolic flux redistribution of L-tryptophan biosynthesis pathway. In the fed-batch fermentation by *E. coli* TRTH and *E. coli* TRTH-Y in 30-liter fermentor, the final production of L-tryptophan fermented by *E. coli* TRTH-Y was 36.3 g/L, which was 12.6% higher than fermentation by *E. coli* TRTH.

1. Introduction

L-tryptophan as the third limiting amino acid is widely used as the common feedstocks (Hinman, 1991). Due to its commercial importance, a number of researchers are being devoted to explore L-tryptophan production. The traditional methods for the production of L-tryptophan are composed of chemical synthesis, direct fermentation bioconversion, and enzymatic conversion of precursors (Leuchtenberger et al., 2005; Aiba et al., 1980; Tribe and Pittard, 1979; Zhao et al., 2010). Microbial fermentation allows the production of L-tryptophan from cheap and renewable carbon source such as sucrose or glucose (Sprenger, 2007) and, therefore it is usually more favorable than biotransformation processes. In normal conditions, the strategy of L-tryptophan-producing strains requires first alleviation of all control levels in the biosynthesis pathway, such as repression, attenuation and feedback, which made it difficult to redirect the carbon flux towards L-tryptophan (Ikeda and Katsumata, 1994), to identify and remove rate-limiting steps by the appropriate overproduction of enzymes of the general aromatic amino acid pathway (Dell and Frost, 1993; Ikeda, 2003), and then to reduce competing pathways and to improve and balance precursor supply both in the common pathway as well as in the specific branch (Ikeda, 2003, 2006). However, the biosynthesis pathway of L-tryptophan is subjected to multiple regulations at several steps (Berry, 1996), thus it is not easy to remove completely all regulatory controls existing in the pathway. In recent years, a new type of producer with a different production mechanism has been developed in which feedback control does not operate when an amino acid is overproduced because of the low level of intracellular amino acid concentration. Some of these strains with altered transport systems are distinguished from classical regulatory mutants. The well-known examples are the secretion of glutamate and threonine by *Corynebacterium glutamicum* and *Escherichia coli*, respectively (Gourdon and Lindley, 1999; Clément and Lanéelle, 1986; Livshits et al., 2003; Gosset, 2009). In the biosynthesis of L-tryptophan, on the one hand, transport and re-uptake of products and cause unwanted futile cycles (Krämer, 1994b); on the other hand, efflux of L-tryptophan from producing cells is generally assumed to proceed via simple diffusion as its hydrophobicity, while an involvement of excretion mechanisms cannot be ruled out yet. Therefore, more detailed studies of the transport systems of L-tryptophan are needed.

It has been reported that *E. coli* possesses three permeases including AroP, TnaB, and Mtr, which play different roles in accumulation of intracellular tryptophan (Yanofsky et al., 1991), as shown in supporting information (SI) Fig. 1 Fig. 1. AroP, a general aromatic amino acid permease, encoding the *aroP* gene, can transport phenylalanine and tyrosine with high affinity (Brown, 1970; Honoré and Cole, 1990; Sarsero et al., 1991). However, Mtr and TnaB are specific for L-tryptophan. TnaB is a low-affinity transporter encoded in the tryptophanase operon together with the *tnaA* gene (Koyanagi et al., 2004); Mtr, a high-affinity tryptophan

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