Technische Universität Hamburg-Harburg

BIO PROCESS AND SYSTEMS ENGINEERING TUHH

Integrated Laboratory Evolution and Rational Engineering of GalP/Glk-dependent *Escherichia coli* for Higher Yield and Productivity of L-Tryptophan Biosynthesis

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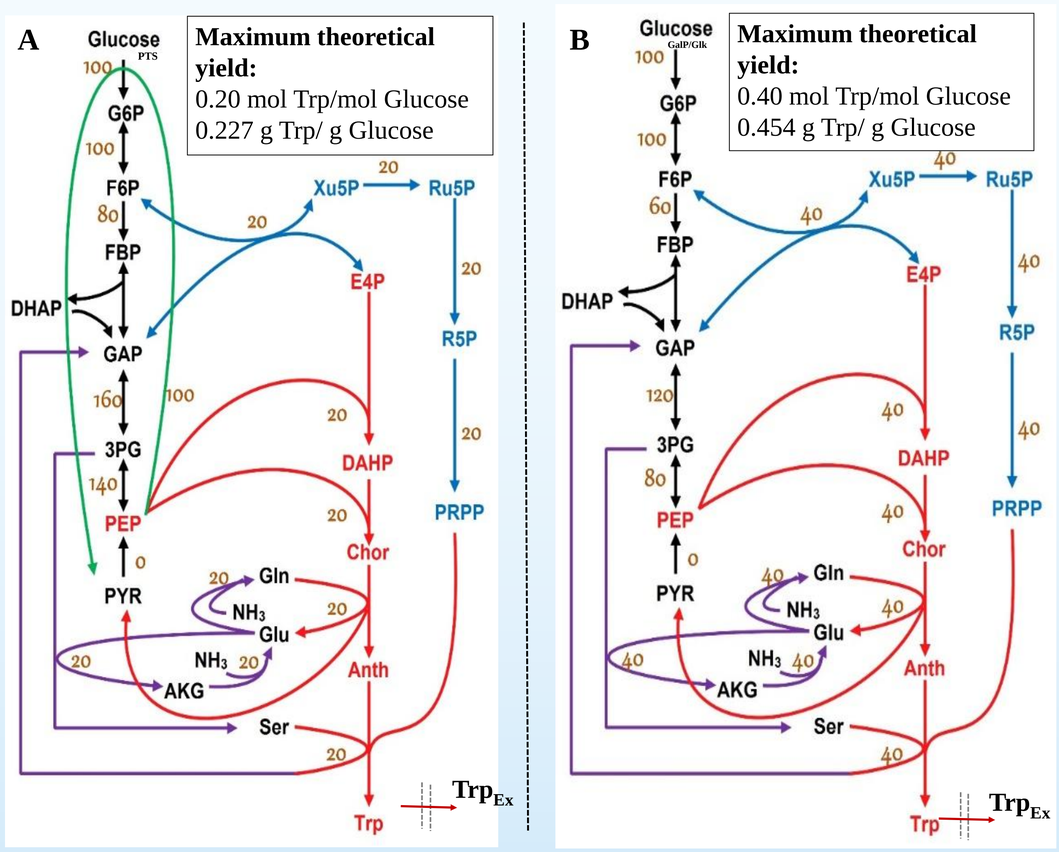

➢ Background

L-Tryptophan (Trp) is a high-value aromatic amino acid with diverse applications in food and pharmaceutical industries. Although production of Trp by engineered *Escherichia coli* has been extensively studied, the need of multiple precursors for its synthesis and the complex regulations of the biosynthetic pathways make the achievement of a high product yield still very challenging. Metabolic flux analysis suggests that the use of a phosphoenolpyruvate:sugar phosphotransferase system (PTS) independent glucose uptake system, i.e. the galactose permease/glucokinase (GalP/Glk) system, can theoretically double the Trp yield from glucose.

Figure 1. Optimal metabolic flux distributions calculated for achieving maximum yield of L-tryptophan (Trp) production in *E. coli*. (A) *E. coli* using the phosphoenolpyruvate:sugar phosphotransferase system (PTS) for glucose uptake. (B) *E. coli* using the GalP/Glk pathway for glucose uptake. G6P glucose-6-phosphate, F6P fructose-6-phosphate, 3PG glyceraldehyde-3-phosphate, Xu5P xylulose-5-phosphate, Ru5P ribulose-5-phosphate, R5P ribose-5-phosphate, E4P erythrose-4-phosphate, PEP phosphoenolpyruvate, PYR pyruvate, AKG α-ketoglutaric acid, DAHP 3-deoxy-arabinoheptulosonate-7-phosphate, Chor chorismate, Anth anthranilate, RPRR 5-phospho-α-D-ribose 1-diphospahte, Ser L-serine, Trp L-tryptophan, Gln glutamine, Glu glutamate.

➢ Continuous evolution of GalP/Glk-dependent *E. coli* in an automatic system

To explore this possibility, a PTS- and GalP/Glk-dependent *E. coli* strain was constructed from a previously rationally developed Trp producer strain S028. To this end, promoter screening for modulated gene expression of GalP/Glk was carried out, following by a batch mode of adaptive laboratory evolution (ALE) which resulted in a strain K3 with a similar Trp yield and concentration as S028. In order to obtain a more efficient Trp producer, a novel continuous ALE system was developed by combining CRISPR/Cas9-facilitated *in vivo* mutagenesis with real-time measurement of cell growth and online monitoring of Trp-mediated fluorescence intensity.

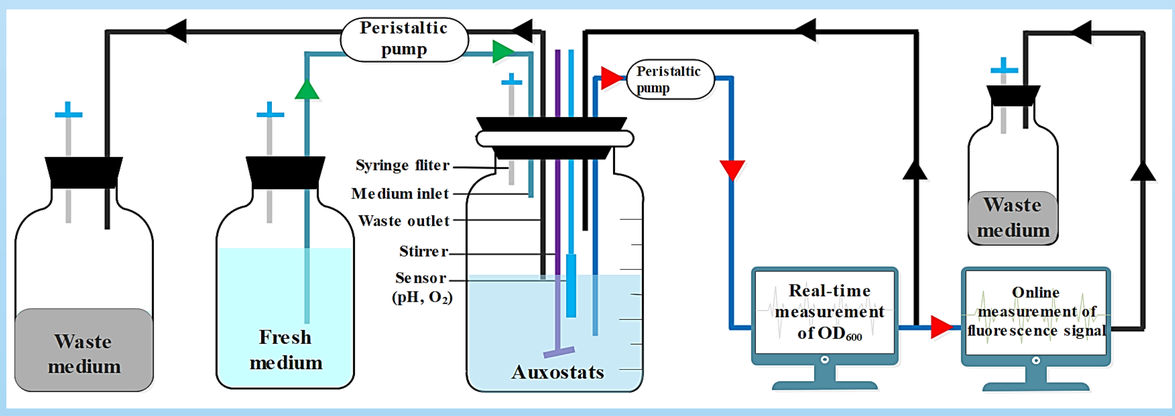

Figure 2: Improvement of GalP/Glk-dependent *E. coli* in an automatic continuous evolutionary system. During the continuous evolution, the flow rates of the fresh medium inlet and the waste culture outlet are dynamically regulated by peristaltic pumps in response to the real-time measured optical density of the culture. The real-time fluorescence intensity monitored by the flow cytometer is used as an indicator for intracellular concentration of Trp.

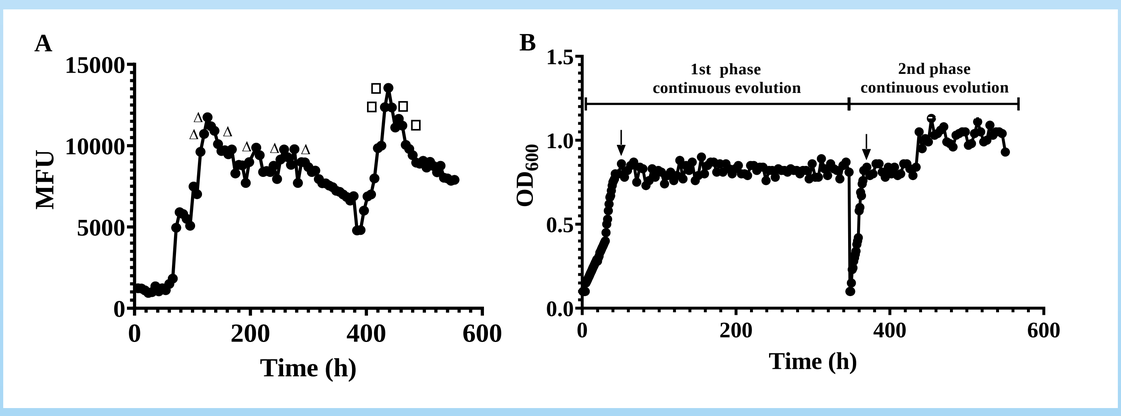

Figure 3: Real-time measurements of fluorescence intensity (A) and OD_{600} (B) of the mutants during the whole process of continuous evolution. The process was performed in two stages, in which the best-characterized mutant from the first phase (0-380h) was forced to the second phase of continuous evolution (381-550h) for further evolution. The data points in (A) marked with open triangles or squares indicate that samples at those time points were selected for off-line characterization. The data points in (B) labeled with arrows indicate the time points for introduction of 0.1% w/v L-arabinose for induction.

➢ Further improvement of Trp production by integration of mutants $AroG^{D6G-D7A}$ and $AnTrpC^{R378F}$

With the aid of this automatic system (auto-CGSS), a promising strain T5 was obtained and fed-batch fermentations showed an increase of Trp yield by 19.71% with this strain compared with that obtained by the strain K3 (0.164 vs. 0.137 g/g). At the same time, the specific production rate was increased by 52.93% (25.28 vs. 16.53 mg/g DCW/h). Two previously engineered enzyme variants $AroG^{D6G-D7A}$ and $AnTrpC^{R378F}$ were integrated into the strain T5, resulting in a highly productive strain T5AA with a Trp yield of 0.195 g/g and a specific production rate of 28.83 mg/g DCW/h.

Table 1: Results of fed-batch fermentations of different Trp-producing strains.

Strains	OD_{600}	DCW (g/L)	L-Trp (g/L)	q_{Trp} (mg/gDCW/h)	Yield (g/g)	Vp (g/L/h)
S028	96.20±0.28	33.67±0.10	39.20±0.78	17.38	0.150	0.59
T5	65.40±0.57	22.89±0.20	38.77±0.02	25.28	0.164	0.58
S028AA	92.20±0.28	32.27±0.10	48.27±0.29	23.74	0.185	0.77
T5AA	66.20±0.84	23.17±0.29	41.49±0.03	28.83	0.195	0.67

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