Chromosome engineering *Escherichia coli* host for the efficient production of *Picrophilus torridus* trehalose synthase

Po Ting Chen,† Chung-Jen Chiang,‡ Yu-Ting Chen,§ Hsien-Chung Lin, ‖ Cheng-Huan Liu, ‖

Yun-Peng Chao,*,⊥ and Jei-Fu Shaw*,#,∇

Abstract

Trehalose is a kind of non-reducing disaccharide. It's widespread in organisms capable of survival in stress condition such as dehydration, heat, cold and radicals. Thus, trehalose is widely applied in cosmetics, pharmaceutials and food industries. The novel production of trehalose is privileging enzymatic biotransformation. *Picrophilus torridus* trehalose synthase (PTTS) can convert maltose into trehalose by singal step. PTTS with high trehalose conversion rate shows high activity under acid and thermal conditions. It had been over expressed in *Escherichia coli* Rossetta-gami B(DE3)/pET23a(+)-PTTS. For the convenience of application in industry, *E. coli* Rossetta-gami B(DE3)/pET23a(+)-PTTS was cultivated without addition of antibiotics. The producing crude PTTS were applied for trehalose production. However, the trehalose converted by crude PTTS was degraded. Besides, the productivity of PTTS was also unstable. Therefore, there may be a problem of plasmid instability. To reduce degradation of trehalose, we tried to knock out trehalase gene in *E. coli* by chromosome engineering. We also tried to enhance the expression of *E. coli* rare codons and ingrate *ptts* into chromosome by chromosome engineering to establish recombinant BT06(DE3)/HK-T7 PTTS in purpose of stable productivity.The results showed knocking out of trehalase gene in *E. coli* reduced trehalose degradation and reached 65% conversion rate by using crude PTTS. Enhancing trehalose expression of *E. coli argU* and *ileX*, the expression of *E. coli* rare codon tRNA of AGG/AGA and AUA was promoted used to replace pRARE. The PTTS productivity was also approved and reached 125 mg/L. by integration *ptts* into chromosome, the recombinant BT06(DE3)/HK-T7PTTS was created and produced PTTS stable with maximum productivity of 155 mg/L PTTS (4.77 fold productivity of Rosetta-gamiB(DE3)/pET23a(+)-PTTS) with the aim of 1 mM lactose induction. In this study, we established a non-plasmid PTTS-producing strain successively. The recombinant can stable produce PTTS without addition of antibiotics that can applied for trehalose production without purification and show high productivity.