Chapter 4
Increase of the Lycopene Production in the Recombinant Strains of *Escherichia coli* by Supplementing with Fructose

Tong-Cun Zhang, Wen Li, Xue-Gang Luo, Cui-Xia Feng, Ming-Hui Zhang, Wen Du and De-Yun Ma

Abstract Lycopene is an important carotenoid which has been widely used as functional foods and feed supplements, and pharmaceutical compounds. In the present study, the host strain and the auxiliary carbon source were optimized to enhance the lycopene yield in the recombinant *Escherichia coli*. The results showed that lycopene concentration of the cells could be significantly increased when the recombinant strain was grown on the LB medium supplemented with 6 g l^{-1} fructose, whereas glucose has little effect. Under the optimized conditions, the recombinant bacteria could exhibited a lycopene yield up to 1,050 mg l^{-1} for 24 h in a 250 ml baffled flask. This is the first report of enhancing lycopene production by supplementing with fructose.

Keywords Lycopene · *Eschericiha coli* · Fructose · Glucose

4.1 Introduction

Numerous carotenoids are known to be naturally biosynthesized by plants and microorganisms and their diversity is derived from differences in type and levels of desaturation and other modifications of the C40 backbone [1]. Currently, lycopene,

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an important carotenoid which has striking anti-cancer, anti-inflammatory [2], and anti-oxidative activities, has been widely used as functional food and feed supplements, and pharmaceutical compound [3].

Various strategies for the lycopene production such as natural extraction, chemical synthesis [4], and fermentation [5] have been described in prior reports. Up to now, a large number of reports on the production of lycopene in *Escherichia coli* by using metabolic engineering [6] have been published in the literature. In the previous study, the pACLYCipi plasmid containing crtE, crtB, and crtI, which plays key roles in the lycopene biosynthetic pathway, has been constructed. In this work, we further attempted to enhance the lycopene yield by optimizing of the bacteria strain and culture medium, and the results showed that supplementation with fructose could have stronger effects than glucose, glycerol and L-arabinose on the lycopene production in recombinant *E. coli*.

4.2 Materials and Methods

4.2.1 Bacterial Strains, Plasmids, and Growth Conditions

Diverse strains of *E. coli* K-12 (2T1R), DH5α, JM109, and BL21 (DE3) were used in this study. The pACLYCipi plasmid containing genes of *crtE*, *crtB*, and *crtI* from Pantoeaagglomerans, were constructed in our previous study. All the *E. coli* strains were grown in LB medium (10 g l^{-1}tryptone, 5 g l^{-1}yeast extract, and 10 g l^{-1}NaCl) supplemented or not with additional carbon source at 37 °C with shaking. The media were supplemented with the antibiotics chloramphenicol (50 mg l^{-1}) as needed.

4.2.2 Extraction and Quantification of Lycopene

The lycopene content of recombinant *E. coli* strains was quantified as previously reported [7]. The recombinant *E. coli* cells were harvested by centrifugation at 12,000 rpm for 5 min. The cell pellet was washed with water and then extracted in 1 mL of acetone at 55 °C for 15 min with intermittent vortexing. The mixture was then centrifuged at 12,000 rpm for 10 min, and the acetone supernatant was transferred to a new tube. The absorbance of the resulting extract was measured at 475 nm and converted to lycopene concentration (mg/l) using a standard curve obtained using commercial lycopene (Sigma).

4.2.3 Detection of Lycopene Yield Curve

A single colony of the recombinant *E. coli* was inoculated into a 20 ml test tube containing 5 ml of the growth medium and grown overnight at 37 °C with shaking; then the starter culture was separately diluted into LB, LB supplemented with 6 g l^{-1} glucose, and LB supplemented with 6 g l^{-1} fructose to an OD_{600} of 0.025. Chloramphenicol (50 g/ml) was added to the above medium. Every other 4 h, the absorbance of the resulting extract was measured at 475 nm and the lycopene concentration (mg/l) was calculated using commercial lycopene as a standard (Sigma). Each sample was measured in triplicate.

4.3 Result

4.3.1 Optimization of the Host Strain for Lycopene Production

To select an optimal host strain of recombinant strains for lycopene production, *E. coli* K-12, DH5α, JM109, and BL21 (DE3) were transformed with the pACLY-Cipi plasmid respectively, and then cultured in a 250 ml baffled flask in LB medium which is popular with bacteriologists because it permits fast growth and good growth yields for many species [8] (Fig. 4.1). As shown in Fig. 4.1, *E. coli* K-12 (pACLYCipi) exhibited the highest cell mass and lycopene content among the used strains. The final concentration of lycopene produced by the used strains followed the order K-12(pACLYCipi) > DH5α (pACLYCipi) > JM109 (pACLYCipi) > BL21 (pACLYCipi). Thus, *E. coli* K-12 strain was selected as the optimal host for lycopene production.

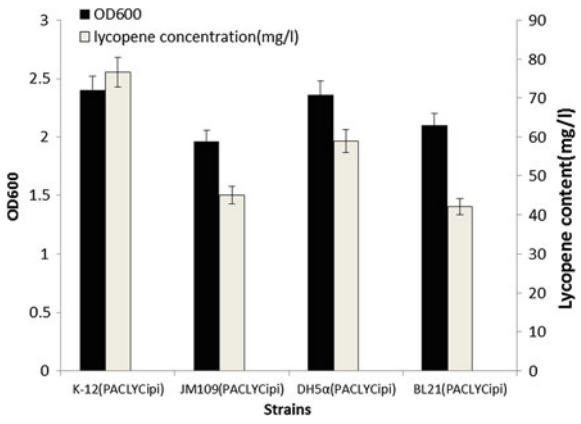

Fig. 4.1 Lycopene yield (mg/l) and cell concentration by various *E. coli* strains as hosts in a 250 ml baffled flask

4.3.2 Optimization of the Auxiliary Carbon Source for Lycopene Production

When LB was used as main medium for the recombinant strains, both the cell mass and the lycopene production were very low, and the lycopene yield was below 80 mg l^{-1} (Fig. 4.1). Since LB medium contains little carbohydrate, in the present study, an auxiliary carbon source, including of glucose, fructose, glycerol, arabinose, was supplemented respectively to overcome this limitation (Fig. 4.2). The auxiliary carbon source of were all supplemented at 6 g l^{-1} into the LB medium. Fructose supplementation could exhibited a lycopene yield up to 1,050 mg l^{-1} for 24 h in a 250 ml baffled flask. After 24 h, the culture by fructose supplementation was the most effective for increases of the lycopene yield, which were 14.9, 5.1, 13.9 and 11.9 fold higher than the culture by control, glycerol, glucose, arabinose respectively. As a result, supplementation with fructose exhibited the highest lycopene production and the cell mass, whereas supplementation with glucose showed the lowest cell mass and the lycopene production, suggesting that fructose was the optimal auxiliary carbon source for lycopene production.

Furthermore, the detailed influence of fructose on lycopene production was evaluated with initial concentrations of 4, 5, 6, 7, and 8 g l^{-1} (Fig. 4.3). After 24 h, the final lycopene concentrations and the cell mass of the cultures with fructose supplementation for all initial concentrations were higher than the culture with no fructose supplementation. In addition, Fig. 4.3 showed that there was a clear positive relationship between cell growth and lycopene yield in both cases. The maximal lycopene content of 1,050 mg l^{-1} was observed at an initial fructose concentration of 6 g l^{-1}. Thus, 6 g l^{-1} was considered as the optimal initial concentration of fructose for lycopene production.

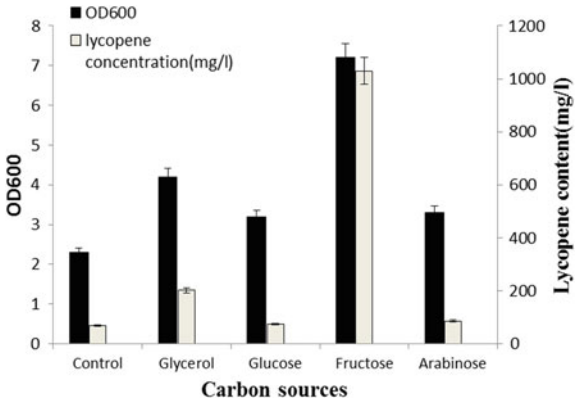

Fig. 4.2 Effects of different auxiliary carbon sources on lycopene yield (mg/l) and cell concentration (OD600) in a 250 ml baffled flask

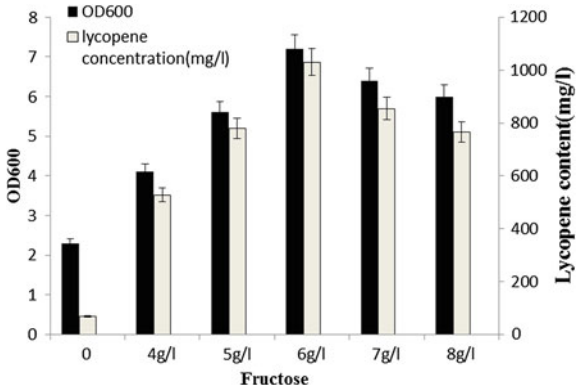

Fig. 4.3 The relationship between the concentration of fructose and the lycopene yield (mg/l) and cell concentration

4.3.3 *Influence of Fructose and Glucose as an Auxiliary Carbon Source on Cell Growth and Lycopene Yield*

Figure 4.2 showed that fructose and glucose supplementation respectively exhibited the highest and the lowest lycopene production and the cell mass. To further investigate the difference between the effect of fructose on lycopene production and that of glucose, the lycopene yield curve was drawn. As shown in Fig. 4.4, apparently, by contrast to the control, it caused little change on lycopene concentration to supplement glucose into LB medium. Meanwhile, there was no significant increase in lycopene production between the culture by glucose supplementation and the culture by LB medium in all growth phases. However, the culture by fructose showed an obvious index growth in lycopene production. And fructose supplementation dramatically stimulated the lycopene production especially when the recombinant *E. coli* was cultured over 12 h, suggesting that *E. coli* K-12 (pACLYCipi) prefer fructose to glucose.

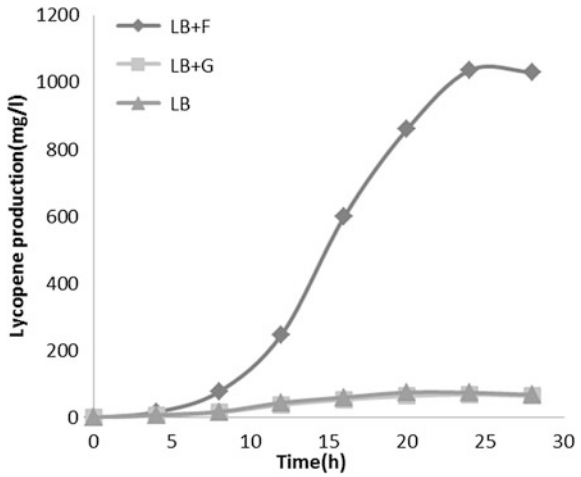

Fig. 4.4 Lycopene yield curve under different concentration of fructose and glucose

4.4 Discussion

As LB medium contains little carbohydrate, peptides and free amino acids [8], a number of carbon sources were added into LB medium to enhance lycopene production in our experiments. Based on the current reports, glycerol, glucose, and L-arabinose have been respectively shown to be the optimal carbon source for lycopene production [9, 10]. However, we have demonstrate that supplementation with fructose as an auxiliary carbon source resulted in the highest lycopene concentration and final cell mass (Fig. 4.2). And the maximum lycopene concentration was harvested when 6 g l^{-1} fructose was supplemented into LB medium (Fig. 4.3). This is the first report of enhancing lycopene production by supplementing with fructose.

Since supplementation with fructose could increase the final cell mass and lycopene concentration (Fig. 4.1), we were interested in evaluating the effect of the lycopene production by supplementation with fructose. Compared to supplementation with or without glucose, it caused a significant change on lycopene concentration by supplementation with fructose especially when the recombinant *E. coli* K-12 (pACLYCipi) was cultured for more than 12 h. By contrast to the previous report, we proposed that the fructose might be a more effective carbon source to stimulated lycopene production.

Besides, our study also found that *E. coli* K-12 showed the highest lycopene productivity among the tested strains. In our opinion, it is likely that *E. coli* K-12 contains a more effective expression of the enzyme relative to the lycopene production.

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