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Metabolic engineering for isoprenoids production in *Escherichia coli*

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ABSTRACT

Isopentenyl diphosphate (IPP) is the common, five-carbon building block in the biosynthesis of all isoprenoids. IPP in *Escherichia coli* is synthesized through the non-mevalonate pathway. The first reaction of IPP biosynthesis in *E. coli* is the formation of 1-deoxy-D-xylulose-5-phosphate (DXP), catalyzed by DXP synthase and encoded by *dxs*. The second reaction in the pathway is the reduction of DXP to 2-C-methyl-D-erythritol-4-phosphate, catalyzed by DXP reductoisomerase and encoded by *dxr*. To determine if one or more of the reactions in the non-mevalonate pathway controlled flux to IPP, *dxs* and *dxr* were placed on several expression vectors under the control of three different promoters and transformed into three *E. coli* strains (DH5α, XL1-Blue, and JM101) that had been engineered to produce lycopene, a kind of isoprenoids. Lycopene production was improved significantly in strains transformed with the *dxs* expression vectors. At arabinose concentrations between 0 and 1.33 mM, cells expressing both *dxs* and *dxr* from P_{BAD} on a medium-copy plasmid produced 1.4 - 2.0 times more lycopene than cells expressing *dxs* only. However, at higher arabinose concentrations lycopene production in cells expressing both *dxs* and *dxr* was lower than in cells expressing *dxs* only. A comparison of the three *E. coli* strains transformed with the arabinose-inducible *dxs* on a medium-copy plasmid revealed that lycopene production was highest in XL1-Blue.

INTRODUCTION

Isoprenoids, found in all organisms, play important roles such as steroid hormones in mammals, carotenoids in plants, and ubiquinone or menaquinone in bacteria. All these isoprenoids are synthesized by consecutive condensation of the five-carbon monomer isopentenyl diphosphate (IPP). Recently, carotenoids have been successfully synthesized in non-carotenogenic bacteria and yeast using recombinant DNA techniques (Misawa et al., 1991; Sandmann et al., 1990; Yamano et al., 1994). One of the key factors for high-yield carotenoid production is a sufficient supply of precursors, isopentenyl diphosphate (IPP; C5). It means that the engineered strain for carotenoid production can be applied to production of other useful isoprenoids. Until recently, the biosynthesis of IPP was generally assumed to proceed exclusively from acetyl-CoA via the classical mevalonate pathway (McCaskill and Croteau, 1998). Recent studies have demonstrated that mevalonate is not the biosynthetic precursor of IPP in all living organisms (Horbach et al., 1993; Rohmer et al., 1993). The existence of an alternative, mevalonate-

independent pathway for IPP formation was characterized initially in several species of eubacteria including *E. coli* (Rohmer et al., 1993; Rohmer et al., 1996). The first reaction in the non-mevalonate pathway is the transketolase-type condensation reaction of pyruvate and D-glyceraldehyde-3-phosphate to yield 1-deoxy-D-xylulose-5-phosphate (DXP). A gene (*dxr*), which encodes DXP reductoisomerase responsible for the reduction of DXP to 2-C-methyl-D-erythritol-4-phosphate (MEP), the proposed second step in the nonmevalonate pathway, was cloned from *E. coli* (Takahashi et al., 1998). It may be possible to increase the supply of IPP by enhancing DXP synthase and DXP reductoisomerase enzymatic activities.

MATERIALS AND METHODS

Bacterial strains, and plasmids

E. coli DH5α, XL1-Blue and JM101 were used for lycopene production. Plasmids pTrc99A, pBluescript, and pBAD24 were used as vectors for *dxs* and *dxr* expression studies. Plasmid pAC-LYC04 expresses the *Erwinia herbicola crt*E, *crt*B and *crt*I genes necessary for lycopene biosynthesis in *E. coli,* This plasmid also contained the *Haematococcus pluvialis ipi* gene that encodes IPP isomerase.

Growth and induction experiments

Bacterial growth was determined by measuring the optical density at a wavelength of 600 nm (OD_{600}). A seed culture was made by inoculating cells into 2×YT medium containing 100 μg/ml ampicillin and 50 μg/ml chloramphenicol and growing the cells overnight at 37 °C. An aliquot of the seed culture was inoculated into 5ml of 2×YT medium containing 100 μg/ml ampicillin and 50 μg/ml chloramphenicol to an OD_{600} of 0.1, and incubated at 29°C for 24 h. IPTG and arabinose induction studies were initiated at an OD_{600} of 0.8 (unless otherwise stated).

RESULTS

E. coli cells transformed with pAC-LYC04 are pigmented pink due to the accumulation of lycopene. *E. coli* DH5α engineered to produce lycopene were transformed with either pDdxs, pDdxs/r, or pBAD24 (as a control) to determine the effect of *dxs* and *dxr* overexpression on lycopene biosynthesis. The pBAD24 is a medium-copy-number plasmid containing the arabinose-inducible *araBAD* promoter (P_{BAD}). The amount of lycopene produced by the cells increased with the arabinose concentration (Fig. 1). The cell growth was unaffected by the amount of arabinose added to the culture. When induced with 13.3 mM arabinose, *E. coli* transformed with pDdxs produced 12.3 mg lycopene/l in 24 hours. This lycopene production is four times higher than the control and significantly higher than any other genetic construct. Lycopene production in the absence of inducer was slightly higher in strains harboring pDdxs than those harboring pBAD24, most likely due to leaky expression from P_{BAD}.

Since the amplification of *dxs* expression increased production of lycopene, we postulated that further enhancement of lycopene production could be limited by other reactions in the pathway. To determine if amplification of *dxr* expression could improve lycopene production in strains harboring pDdxs, the *dxr* gene was combined with the *dxs* of pDdxs to obtain pDdxs/r. At arabinose concentrations between 0 and 1.33 mM, cells expressing both *dxs* and *dxr* from P_{BAD} produced 1.4 - 2.0 times more lycopene than cells expressing *dxs* only. However, at higher arabinose concentrations lycopene production in cells expressing both *dxs* and *dxr* was lower than in cells expressing *dxs* only. (Fig. 1). There was no significant difference in the growth of cells harboring pDdxs or pDdxs/r at all arabinose concentrations.

E. coli strains DH5α, XL1-Blue, and JM101 harboring pAC-LYC04 were transformed with either pBAD24 or pDdxs to compare lycopene production in these strains. Lycopene production was highest in XL1-Blue whereas no significant differences in cell growth were observed for all these strains (Fig. 2).

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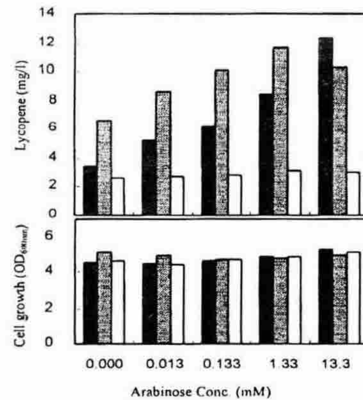

Figure 1. Effect of arabinose concentration on lycopene production and cell growth in *E. coli* DH5α harboring pDdxs (solid bar), pDdxs/r (gray bar), or pBAD24 (open bar).

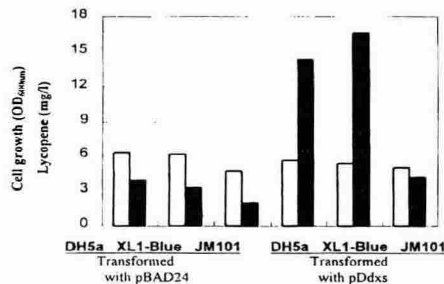

Figure 2. Comparison of lycopene production (solid bars) and growth (open bars) in *E. coli* strains DH5α, XL1-Blue, and JM101 transformed with pBAD24 or pDdxs. Cultivation was carried out for 48 hours and 13.3 mM arabinose was added initially for induction.