Biotechnol Lett (2012) 34:849–852
DOI 10.1007/s10529-011-0839-6

ORIGINAL RESEARCH PAPER

Improved lycopene production by *Blakeslea trispora* with isopentenyl compounds and metabolic precursors

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Received: 1 November 2011 / Accepted: 19 December 2011 / Published online: 30 December 2011

Abstract The highest lycopene production in mated cultures of *Blakeslea trispora* was 578 mg/l by adding 42 mg geraniol/l to the medium after 48 h of growth. The control gave 317 mg/l. Adding isopentenyl alcohol at 40 mg/l, mevalonic acid at 17.5 mg/l or dimethyl allyl alcohol at 150 mg, each after 36 h growth, gave lycopene yields 62, 45 and 47%, respectively, higher than the control.

Keywords *Blakeslea trispora* · Isopentenyl compounds · Lycopene · Metabolic precursors

Introduction

Lycopene is an isoprenoid pigment of industrial and nutritional interest. It can prevent cardiovascular diseases and can regulate the immune system (Larsson and Bergkvist 2010). Lycopene is also an antioxidant and anti-carcinogenic agent (Krinsky and Johnson 2005). A heterothallic zygomycete, *Blakeslea trispora*, has been successfully used to produce lycopene on a semi-industrial scale by introducing inhibitors of lycopene cyclase, such as nicotine or pyridine into the medium (López-Nieto et al. 2004). However, important problems remain to be solved to overproduce lycopene efficiently in fermentation process. One of the important measures is the supply of fermentation accelerants. Lycopene biosynthesis in *B. trispora* could be stimulated by various accelerants, such as trisporic acid (Gessler et al. 2002), non-ionic surfactant (Kim et al. 1997), abscisic acid, as well as vitamin A and antibiotics (Sheetal et al. 2008), oxygen-vectors (Xu et al. 2007), cyclase inhibitors (Mehta and Cerdá-Olmedo 1999), and ergosterol biosynthesis inhibitors (Sun et al. 2007).

Carotenoids, in *B. trispora*, as in most fungi, are derived from mevalonic acid (MVA) with acetyl-CoA as the precursor. MVA is further converted into isopentenyl pyrophosphate (IPP), which is the crucial C_5 terpene precursor. The isomerization of IPP to dimethylallyl pyrophosphate (DMAPP) is the key step in carotenoid biosynthesis. Successive condensations of C_5 intermediates lead to the production of the ubiquitous halfway metabolite precursor of C_{40} carotenoids that are geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). The successive reactions lead to carotenoid synthesis through the intermediates,

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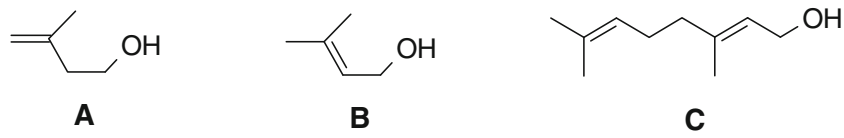

Fig. 1 Formulae of isopentenyl alcohol, dimethyl allyl alcohol, and geraniol. **a** Isopentenyl alcohol. **b** Dimethyl allyl alcohol. **c** Geraniol

phytoene, ζ-carotene, neurosporene, lycopene, and β-carotene. The isoprene side chain of lycopene is produced through the GPP, FPP, and GGPP biosyntheses in which the condensation of IPP and DMAPP is repeated. Theoretically, the addition of MVA, IPP, DMAPP, GPP, or GGPP, may probably increase the carbon flux towards lycopene and later enhance lycopene accumulation. Figure 1 shows that the structure of isopentenyl alcohol is the same as that of the isoprene side of IPP, whereas the dimethyl allyl alcohol is the same as that of the isoprene side of DMAPP. The structure of geraniol is similar to those of the isoprene sides of GPP and GGPP.

In the present study, lycopene production was increased by adding geraniol, isopentenyl alcohol, and dimethyl allyl alcohol during the lycopene biosynthesis by mated cultures of *B. trispora*. MVA was also added to the cultures medium during lycopene production.

Materials and methods

Microorganism and culture medium

Blakeslea trispora ATCC 14271, mating type (+) and ATCC 14272, mating type (−), were grown in a seed medium (40 g starch/l, 50 g soybean meal/l, 1 g KH_2PO_4/l, 0.1 g $MgSO_4 \cdot 7H_2O$/l, pH 6.5) at 28°C for 40 h in 500 ml flasks containing 100 ml medium. The cultures were used for the inoculation of the fermentation medium.

Fermentation conditions

Fermentation was carried out in 500 ml flasks with 100 ml medium and a 10% (v/v) inoculums of a 1:4 (v/v) mixture of ATCC 14271 (+) and ATCC 14272 (−). The fermentation medium (pH 6.5) contained 40 g corn starch/l, 45 g soybean meal/l, 1 g KH_2PO_4/l, and 0.1 g $MgSO_4 \cdot 7H_2O$/l. The flasks were shaken at 220 rpm at 28°C. The cyclase inhibitor, nicotine, was added at 5 mM after 48 h for lycopene production. Cultures were maintained for 120 h, and then the dry cell weight and lycopene contents were determined. All culture experiments and estimations were performed in triplicate.

Analytical methods

Estimation of cell biomass

The cell mass was filtered through muslin, washed thoroughly twice with ethanol and dried under vacuum at 45°C for 48 h.

Extraction and estimation of lycopene

To extract lycopene, the dried cells were cut into small pieces, homogenized in petroleum ether, and shaken until they became colorless. The extract (10 µl) was analyzed by HPLC with a Diamonsil C_{18} column (4.6 × 250 mm, 5 µm) with a C_{18} guard column (4 × 8 mm, 5 µm) at 28°C. The mobile phase was acetonitrile/dichloromethane (3:1, v/v) at 1.5 ml/min. Lycopene was detected at 450 nm. Quantitative analysis was performed by a single-point calibration method using Sudan I as internal standard (Xu et al. 2006).

Results and discussion

Optimization of the adding concentrations of isopentenyl compounds and metabolic precursors

The effect of adding various concentrations of the isopentenyl compounds and metabolic precursors on the cell biomass and lycopene production at 36 h of

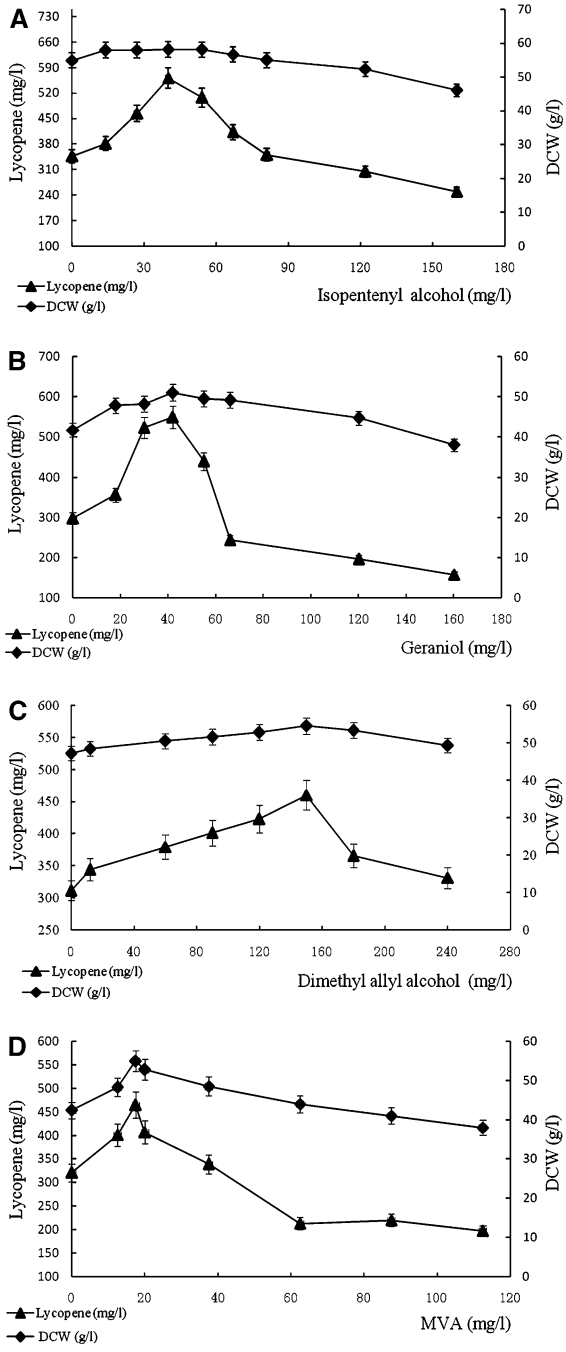

Fig. 2 Effects of various concentrations of the isopentenyl compounds and metabolic precursors on lycopene production and cell biomass by mated cultures of *B. trispora*. **a** Isopentenyl alcohol. **b** Geraniol. **c** Dimethyl allyl alcohol. **d** MVA

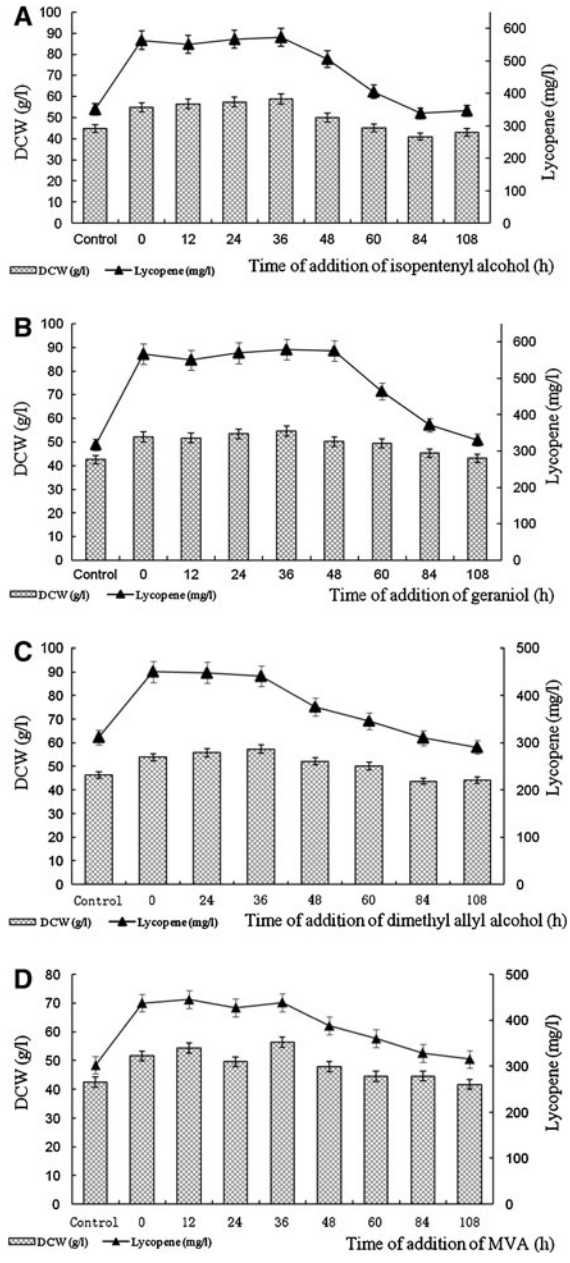

Fig. 3 Effects of the adding time of the isopentenyl compounds and metabolic precursors on lycopene production and cell biomass by mated cultures of *B. trispora*. **a** Isopentenyl alcohol. **b** Geraniol. **c** Dimethyl allyl alcohol. **d** MVA

fermentation are shown in Fig. 2. The addition of 40 mg isopentenyl alcohol/l gave 560 ± 6.25 mg lycopene/l, 62% higher than that of the control (346 ± 4.3 mg/l). Addition of geraniol, dimethyl allyl alcohol and MVA also demonstrated the same trend as that of isopentenyl alcohol (see Fig. 2b–d) with respective increases of 85, 47 or 45% higher than that of the control.

Optimization of the adding time of isopentenyl compounds and metabolic precursors

The time of adding isopentenyl compounds and metabolic precursors was examined at 12 h intervals up to 120 h at the optimal concentrations (Fig. 3), but this did not significantly change the biosynthesis of lycopene up to 36 h. Additions after this time decreased lycopene production. However, the addition of 40 mg isopentenyl alcohol/l to the culture before 36 h of fermentation significantly increased the lycopene production at 57–63% higher than the control (350 ± 3.4 mg/l). The optimal time for adding dimethyl allyl alcohol and MVA was before 36 h, identical to that of the isopentenyl alcohol (Fig. 3c, d), but with geraniol this was up to 48 h and gave the highest improvement in lycopene production (Fig. 3b).

Conclusions

The addition of optimal concentrations of isopentenyl alcohol, dimethyl allyl alcohol, geraniol, and MVA at the initial fermentation stage significantly enhanced lycopene accumulation. The direct metabolic precursors (MVA, IPP, DMAPP, GPP, or GGPP), have unstable chemical properties and are not readily available in contrast to the stable and affordable isopentenyl alcohol, dimethyl allyl alcohol, and geraniol. The latter compounds can increase yield, reduce the costs and are suitable for industrial production. The results from the present study suggest that the addition of the isopentenyl compounds is an effective tool in enhancing lycopene production.

Acknowledgements The authors would like to acknowledge the National Natural Science Foundation of China (Nos. 20776009 and 20976009) and PHR (IHLB) for financial support.

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