Expression of an exogenous isopentenyl diphosphate isomerase gene enhances isoprenoid biosynthesis in *Escherichia coli*

Susumu KAJIWARA*, Paul D. FRASER†, Keiji KONDO and Norihiko MISAWA‡

Central Laboratories for Key Technology, Kirin Brewery Co. Ltd., 1-13-5, Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa 236, Japan

Escherichia coli expressing the *Erwinia* carotenoid biosynthesis genes, *crtE*, *crtB*, *crtI* and *crtY*, form yellow-coloured colonies due to the presence of β-carotene. This host was used as a visible marker for evaluating regulatory systems operating in isoprenoid biosynthesis of *E. coli*. cDNAs enhancing carotenoid levels were isolated from the yeast *Phaffia rhodozyma* and the green alga *Haematococcus pluvialis*. Nucleotide sequence analysis indicated that they coded for proteins similar to isopentenyl diphosphate (IPP) isomerase of the yeast *Saccharomyces cerevisiae*. Determination of enzymic activity confirmed the identity of the gene products as IPP isomerases. The corresponding gene was isolated from the genomic library of *S. cerevisiae* based on its nucleotide sequence, and was confirmed to have the same effect as the above two IPP isomerase genes when introduced into the *E. coli* transformant accumulating β-carotene. In the three *E. coli* strains carrying the individual exogenous IPP isomerase genes, the increases in carotenoid levels are comparable to the increases in IPP isomerase enzyme activity with reference to control strains possessing the endogenous gene alone. These results imply that IPP isomerase forms an influential step in isoprenoid biosynthesis of the prokaryote *E. coli*, with potential for the efficient production of industrially useful isoprenoids by metabolic engineering.

INTRODUCTION

Carotenoids, sterols, hopanols, dolichols, gibberellin and rubber are all examples of numerous compounds termed isoprenoids. Despite their structural and functional diversity, they are synthesized via a common precursor in all organisms, i.e. the first isoprene isopentenyl diphosphate (IPP; C_5) (Scheme 1). IPP is formed generally from acetyl coenzyme A (acetyl-CoA) via 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) and mevalonate, whereas an alternative pathway appears to exist in several bacteria or green algae [1,2]. IPP is isomerized to dimethylallyl diphosphate (DMAPP) by IPP:DMAPP isomerase (IPP isomerase, EC 5.3.3.2) (Scheme 1) [3]. DMAPP is condensed with IPP to generate geranyl diphosphate (GPP; C_{10}), which undergoes further condensation with IPP to form farnesyl diphosphate (FPP; C_{15}). These sequential elongation reactions are catalysed by FPP synthase (Scheme 1) [4,5]. Sterols, dolichols, hopanols and other various isoprenoids branch at FPP. Geranylgeranyl diphosphate (GGPP; C_{20}) is formed from FPP by GGPP synthase, which enables further addition of IPP (Scheme 1) [6–8]. GGPP is the substrate for the biosynthesis of carotenoids, quinones, gibberellins and other various isoprenoid compounds.

In yeasts and animals, the sterols ergosterol and cholesterol are the abundant isoprenoids formed respectively. Numerous studies have been performed on the regulatory mechanisms involved in the biosynthesis of sterols, especially cholesterol in animals [9]. HMG-CoA reductase is well known as a key enzyme involved in various regulatory systems, in sterol biosynthesis in eukaryotes [9–11]. It has also been reported in yeasts and animals that HMG-CoA synthase and HMG-CoA reductase are subject to feedback regulation by sterols [9,12].

Bacteria such as *Escherichia coli* contain dolichols (sugar carrier lipid), the respiratory quinones ubiquinone and menaquinone, and isopentenyl tRNA isoprenoid compounds [13,14]. In contrast with eukaryotes, little is known about the regulatory mechanisms involved in prokaryotic isoprenoid biosynthesis [5,13–15].

Carotenoids are derived from the condensation of two molecules of GGPP, forming phytoene. Desaturation of this colourless molecule results in the formation of lycopene, which contains a series of conjugated double bonds responsible for its characteristic red coloration. Lycopene is typically cyclized to form β-carotene, having a characteristic yellow colour. The carotenoid biosynthesis gene clusters from the epiphytic bacteria *Erwinia* species [8,16–19], which contain all genes encoding the proteins responsible for the formation of the carotenoids, such as phytoene, lycopene and β-carotene, from FPP, have been the most widely studied and utilized. They have frequently been expressed in *E. coli* in order to produce carotenoids as substrates suitable for subsequent identification of presumptive carotenogenic gene products [20,21], and also been used in plant systems to manipulate carotenoid content [22]. The enzymes encoded by the gene clusters have further been the subject of many biochemical studies [23–25].

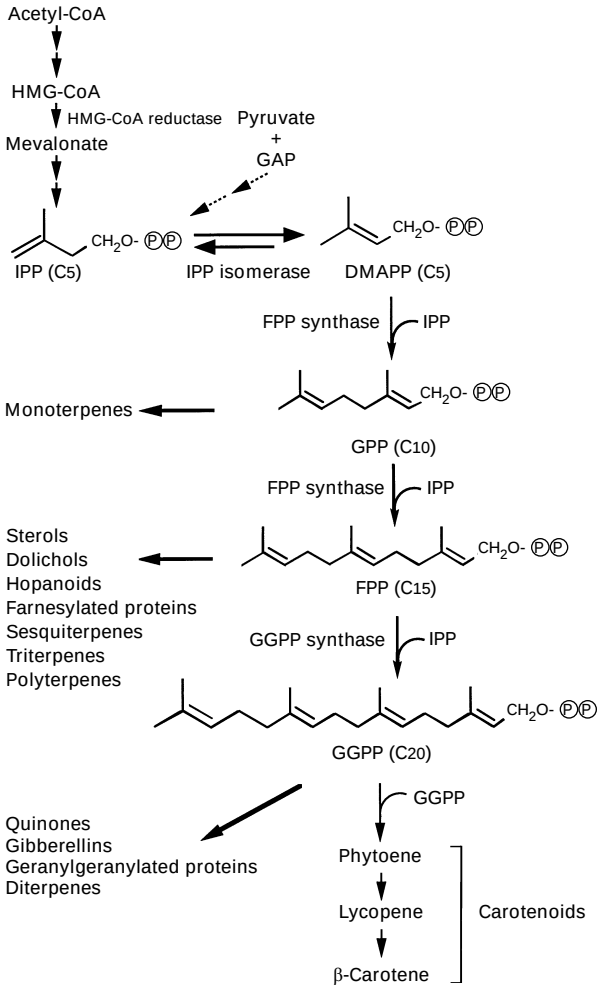
In this article, the *Erwinia* carotenoid biosynthesis gene cluster has been used to generate a visible marker for evaluating regulatory systems operating in isoprenoid biosynthesis in *E.*

Abbreviations used: IPP, isopentenyl diphosphate; acetyl-CoA, acetyl coenzyme A; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate; IPTG, isopropyl-β-D-thiogalactoside; Ap, ampicillin; Cm, chloramphenicol.

* Present address: Department of Chemical Engineering, Tokyo Institute of Technology, 2-12-1, Ohokayama, Meguro-ku, Tokyo 152, Japan.

† On leave from Biochemistry Division, Royal Holloway University of London, Egham, Surrey TW20 OEX, U.K.

‡ To whom correspondence should be addressed.



Scheme 1 Summary of isoprenoid (terpenoid) biosynthetic pathway

A pathway generating IPP, which is considered to be alternative to the mevalonate pathway, is shown with broken arrows. GAP, glyceraldehyde 3-phosphate.

coli. The isolation of cDNAs enhancing carotenoid content in *E. coli* is described, and the function of their gene products is also ascribed.

MATERIALS AND METHODS

Strains and growth conditions

E. coli JM101 [26] was used as a host for construction of a cDNA library and for the production of carotenoids. *E. coli* JM109 [26] was used as the host for plasmid construction and for determination of IPP isomerase activity.

Haematococcus pluvialis Flotow NIES-144 and *Phaffia rhodozyma* ATCC24230 were obtained from the National Institute for Environmental Studies (NIES) and from American Type Culture Collection respectively. *Saccharomyces cerevisiae* S288C was also used. The growth conditions for *H. pluvialis* have been previously described [27]. *P. rhodozyma* and *S. cerevisiae* were cultured in YM medium [0.3 % (w/v) yeast extract/0.3 % (w/v) maltose extract/0.5 % (w/v) tryptone/1 % (w/v) glucose] and YPD medium [1 % yeast extract/2 % (w/v) peptone/2 % glucose] at 20 °C and 30 °C respectively.

Recombinant DNA techniques

Recombinant DNA techniques were performed using standard methods [26] or as instructed by suppliers.

Plasmids and cDNA libraries

Plasmids used, which confer carotenoid-biosynthesizing ability on *E. coli*, were pACCRT-EB, pACCRT-EIB and pACCAR-16ΔcrtX, which carried the *crtE* (GGPP synthase) and *crtB* (phytoene synthase) genes for phytoene synthesis, the *crtE*, *crtB* and *crtI* (phytoene desaturase) genes for lycopene synthesis, and the *crtE*, *crtB*, *crtI* and *crtY* (lycopene cyclase) genes for β-carotene synthesis respectively [19].

mRNAs with poly(A) were isolated from the cyst cells of *H. pluvialis* as described [27].

The exponential-phase cells of *P. rhodozyma* were frozen with liquid N_2, thawed and suspended in ANE buffer composed of 10 mM sodium acetate/100 mM NaCl/1 mM EDTA (pH 6.0) containing 1 % (w/v) SDS. Glass beads and prewarmed phenol were added to the suspension, and kept at 65 °C for 5 min. After

mixing, the suspension was rapidly cooled down to room temperature, and centrifuged at 15000 ***g*** for 10 min. The aqueous phase was transferred and extracted further with phenol, then phenol/chloroform and finally chloroform. The RNAs in the aqueous phase were precipitated with ethanol. The pellet containing total RNAs was washed with 70 % (v/v) ethanol and dissolved in 10 mM Tris/HCl/1 mM EDTA, pH 8 buffer. mRNAs with poly(A) were isolated from this solution using Oligotex-dT 30 Super (Takara). cDNAs were synthesized from the poly(A)-RNAs that were prepared from *H. pluvialis* and *P. rhodozyma* with SUPERSCRIPT® Plasmid System for cDNA synthesis and plasmid cloning (Gibco BRL). The synthesized cDNAs were ligated into the *Sal*I and *Not*I site of vector pSPORT1 (Gibco BRL), and transformed *E. coli* JM101 (pACCAR16ΔcrtX) that accumulated β-carotene. The transformants were cultured on Luria broth plates [26] including 50 μg/ml ampicillin (Ap), 30 μg/ml chloramphenicol (Cm) and 1 mM isopropyl-β-D-thiogalactoside (IPTG) at 20 °C for 3 days.

PCR amplification

Based on the nucleotide sequence of the IPP isomerase gene of *S. cerevisiae* [3], the following primers were synthesized: 5′-TCGATGGGGGTTGCCTTTCTTTTTCGG-3′, 5′-CGCGTTGTTATAGCATTCTATGAATTTGCC-3′. The primers were designed to have the *Taq*I site upstream and the *Acc*II site downstream. PCR (30 cycles) was performed with *Pyrococcus furiosus* DNA polymerase (Stratagene) and with genomic DNA, that was prepared from *S. cerevisiae* as described [28].

DNA sequencing

Each cDNA or genomic DNA fragment was subcloned into the vector pBluescript II KS$^+$ or pBluescript II SK$^+$ (Stratagene), and plasmids containing a nested series of deletions of the cDNA or genomic fragments were constructed using a Kilo-sequence Deletion Kit (Takara) according to the supplier's instructions. Double-stranded DNAs were sequenced using a *Taq* Dye Primer Cycle Sequencing Kit (Applied Biosystems) on an ABI 373A DNA sequencer.

Quantification of carotenoids accumulated in *E. coli*

E. coli JM101, carrying two distinct plasmids, was cultured in 2 × YT medium [1.6 % tryptone/1 % yeast extract/0.5 % (w/v) NaCl] including 150 μg/ml Ap, 30 μg/ml Cm and 0.1 mM IPTG at 28 °C. Carotenoid pigments were extracted from their cells with acetone. Lycopene and β-carotene amounts were quantified by optical absorbance at 474 nm and 454 nm with molar absorption coefficients of 185.0 $mM^{-1} \cdot cm^{-1}$ and 134.4 $mM^{-1} \cdot cm^{-1}$ respectively, using a JASCO UVIDEC-220B [29]. In order to measure phytoene, the acetone extract was dried *in vacuo*, further extracted with petroleum ether, and quantified by optical absorbance at 286 nm with a molar absorption coefficient of 41.2 $mM^{-1} \cdot cm^{-1}$ [29]. These carotenoids were also subjected to HPLC; the column [3.9 mm × 300 mm; Nova-pak HR 6 μ C_{18} (Waters)] was developed with acetonitrile/methanol/2-propanol (90:6:4, by vol.) at a flow rate of 1 ml/min, and on-line spectra recorded with Waters photodiode array detector 996. HPLC analysis confirmed that the lycopene and β-carotene synthesized were the only pigments detectable at the absorbances of 474 nm and 454 nm respectively, and 70 % of the absorbance at 286 nm is due to the phytoene synthesized.

Measurement of IPP isomerase activity in *E. coli*

E. coli JM109 strains transformed with plasmids were cultured in 2 × YT medium containing 150 μg/ml Ap and 0.1 mM IPTG at 30 °C until mid-exponential phase. Cells (0.5 g wet wt.) were separated from the medium (100 ml) by centrifugation at 6000 ***g*** for 10 min. A solution of 0.1 M Tris/HCl (pH 8.0) containing 1 mM dithiothreitol and a protease inhibitor cocktail [0.1 mM PMSF/leupeptin (1 μg/ml)/pepstatin (1 μg/ml)] was used to resuspend the cells at a 1:1 (w/v) ratio. Cell-free extracts were prepared by passage through a French press cell at an internal pressure of 35.7×10^5 Pa. DNase (50 μg) was added to the broken extract and the mixture was incubated on ice for 30 min. The homogenate was centrifuged at 10000 ***g***, and the supernatant was used as the source of respective IPP isomerases. In all cases this fraction contained virtually all the total activity. Determination of IPP isomerase activity was made by incubating the cell-free extract (100 μl) with [^{14}C]IPP (0.25 μCi, 4.7 nmol; Amersham) buffered in 0.4 M Tris/HCl (pH 8.0) containing 1 mM dithiothreitol, 3 mM ATP, 5 mM $MnCl_2$, 5 mM $MgSO_4$ and 0.1 % (v/v) Tween 60. The final volume of the reaction mixture was 200 μl. Incubations were shaken (140 strokes/min) in the dark at 28 °C over a 0–3 h period. Reactions were terminated with methanol (200 μl). IPP is resistant to acid hydrolysis but subsequent reaction products are susceptible. Therefore determination of IPP isomerase activity and conversion into other prenyl phosphate products was carried out by acid hydrolysis. This procedure was performed by adding HCl at a final concentration of 1.5 M to the suspension, incubating for 30 min at 37 °C while agitating (140 strokes/min). Before extraction, the pH was adjusted to > 8 with ammonia solution. The isoprenyl alcohols formed were extracted by mixing with diethyl ether (600 μl), and a partition was carried out by centrifugation at 2500 ***g*** and 4 °C for 5 min. Identical re-extraction was performed twice and the pooled organic phases were taken to dryness under N_2. The residue was resuspended in diethyl ether (1 ml) and an aliquot removed (1/100) was subjected to quantification by liquid-scintillation counting. Controls were performed with boiled (3 min) extracts as well as in the absence of cell-free extracts. HPLC analysis, as described in [30], was also performed to confirm the identity of the diethyl ether-extractable products following acid hydrolysis.

RESULTS

Isolation of genes enhancing carotenoid levels

E. coli JM101 carrying pACCAR16ΔcrtX shows yellow colour due to the accumulation of β-carotene. The cDNA expression libraries of *P. rhodozyma* and *H. pluvialis* were constructed using this strain, JM101 (pACCAR16ΔcrtX), as a host. About 200000 and 40000 colonies were generated on the plates of the above libraries respectively. Five and 10 colonies, displaying a deep yellow (near to orange) phenotype in comparison, appeared in the respective libraries. Plasmid DNAs were isolated from each of the deep yellow colonies. Common restriction fragments were found among the cDNAs from each library. One plasmid, containing the *P. rhodozyma* cDNA, was named pRH1 (Figure 1), and one, containing the *H. pluvialis* cDNA, was designated pHP1. pHP11 (Figure 1) was created by inserting the 1.07-kb *Sal*I–*Not*I cDNA fragment digested from pHP1 into the *Sal*I and *Not*I site of pBluescript II KS$^+$.

A 1.06-kb *Taq*I–*Acc*II fragment amplified by PCR, which is expected to carry the *S. cerevisiae* IPP isomerase (*ipi*) gene, was inserted into the *Cla*I and *Sma*I site of the vector pBluescript II

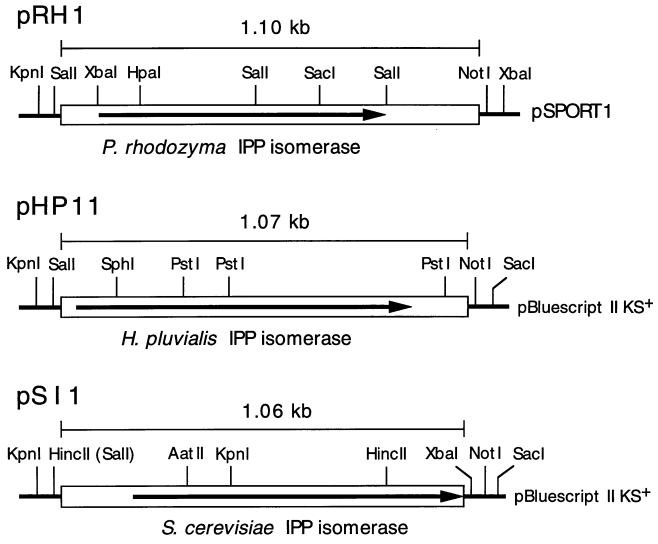


Figure 1 Structure of plasmids pRH1, pHP11 and pSI1

Each cDNA is inserted in the same direction as the *lac* promoter of the vector pSPORT1 or pBluescript II KS+. Each cDNA was proven to code for IPP isomerase as described later.

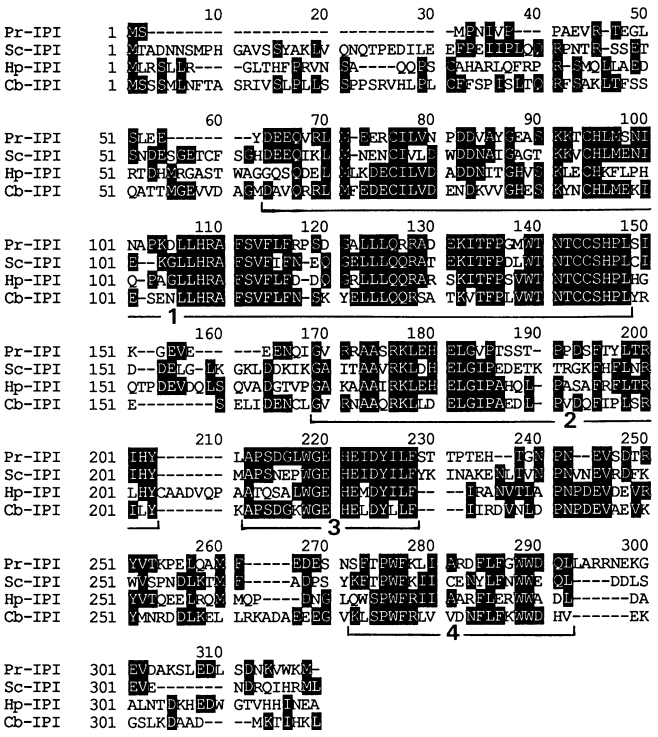


Figure 2 Amino acid sequences of IPP isomerases (IPI) of *Phaffia rhodozyma* (Pr), *Saccharomyces cerevisiae* (Sc), *Haematococcus pluvialis* (Hp) and *Clarkia breweri* (Cb), and amino acid sequence similarity among them

Consensus sequences are shaded. Four highly conserved regions are underlined.

KS+, to form plasmid pSI1 (Figure 1). pSI1 was introduced into *E. coli* JM101 carrying pACCAR16ΔcrtX, and a deep yellow (near to orange) phenotype was observed, similar to that of *E. coli* (pACCAR16ΔcrtX) carrying pRH1 or pHP11 (pHP1).

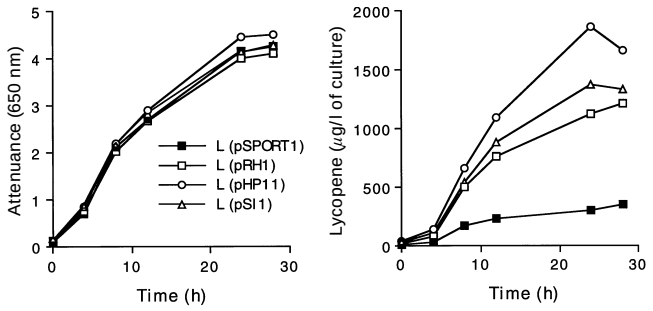


Figure 3 Growth curves of the *E. coli* JM101 transformants carrying the individual IPP isomerase genes in addition to the lycopene biosynthesis genes and production of lycopene by these strains

L represents lycopene-producing *E. coli* JM101 strain due to the presence of plasmid pACCRT-EIB. L (pSPORT1), *E. coli* (pACCRT-EIB, pSPORT1), the control strain; L (pRH1), *E. coli* (pACCRT-EIB, pRH1); L (pHP11), *E. coli* (pACCRT-EIB, pHP11); L (pSI1), *E. coli* (pACCRT-EIB, pSI1).

Sequence analysis

The *P. rhodozyma* cDNA consisted of 1099 bp, and contained an open reading frame that encodes a polypeptide having 251 amino acids with a molecular mass of 28.8 kDa. The *H. pluvialis* cDNA consisted of 1074 bp, and contained an open reading frame that encodes a polypeptide having 293 amino acids with a molecular mass of 33.3 kDa. These polypeptides, derived from *P. rhodozyma* and *H. pluvialis*, showed significant similarity over their entire length not only to each other (47 % identity) but also to IPP isomerases of *S. cerevisiae* [3] (51 % and 44 % identity respectively) and the higher plant *Clarkia breweri* [31] (52 % and 46 % identity respectively) (Figure 2).

The 1.06-kb genomic DNA fragment, which was isolated from plasmid pSI1, was confirmed to have the nucleotide sequence of the *S. cerevisiae ipi* gene [3].

Quantification of carotenoid level

E. coli JM101 carrying pACCRT-EIB forms red-coloured colonies due to the accumulation of lycopene. Vector pSPORT1, and plasmids pRH1, pHP11 or pSI1, each containing the new cDNAs, were introduced into this *E. coli* strain, and incubated on Luria broth plates including 150 μg/ml Ap, 30 μg/ml Cm and 1 mM IPTG at 28 °C for 24 h. The three strains, in which the plasmids containing the cDNAs were introduced, showed a deep red colour compared with the control carrying the vector alone. The amount of lycopene produced by the individual strains was quantified in liquid culture. The *E. coli* transformant carrying two distinct plasmids, pACCRT-EIB, and pSPORT1, pRH1, pHP11 or pSI1, was incubated overnight in Luria broth medium carrying 150 μg/ml Ap and 30 μg/ml Cm at 28 °C. Preculture (2 ml) was inoculated with 200 ml of 2 × YT medium carrying 150 μg/ml Ap, 30 μg/ml Cm and 0.1 mM IPTG, and incubated at 28 °C. Figure 3 shows the production rate of lycopene in each *E. coli* transformant and the growth curve. The amount of lycopene accumulated in the three strains, in which the cDNAs are carried, was higher than the *E. coli* control-carrying vector pSPORT1, whereas their growth rate was similar to that of the control. In the 28 h of cultivation corresponding to the stationary phase, the *E. coli* strains carrying the cDNAs produced 3.6–4.5-fold greater levels of lycopene compared with the *E. coli* control (Table 1). In *E. coli* (pACCRT-EIB and pHP11), the yield of lycopene reached 1 mg/g dry wt. (0.1 %). Increases in the

Table 1 Quantification of carotenoids produced in *E. coli* JM101 transformants carrying the individual IPP isomerase genes in addition to the carotenoid biosynthesis genes

Carotenoids were extracted from *E. coli* cells that were harvested after 28 h of culture corresponding to the stationary phase. **E. coli* vector.

E. coli strains	Produced carotenes	Amount (μg of carotene/g dry wt.)	Fold increase
E. coli (pACCRT-EIB, pSPORT1*)	Lycopene	228	1
E. coli (pACCRT-EIB, pRH1)	Lycopene	825	3.6
E. coli (pACCRT-EIB, pHP11)	Lycopene	1029	4.5
E. coli (pACCRT-EIB, pSI1)	Lycopene	859	3.8
E. coli (pACCAR16ΔcrtX, pSPORT1*)	β-Carotene	488	1
E. coli (pACCAR16ΔcrtX, pRH1)	β-Carotene	709	1.5
E. coli (pACCAR16ΔcrtX, pHP11)	β-Carotene	1310	2.7
E. coli (pACCAR16ΔcrtX, pSI1)	β-Carotene	758	1.6
E. coli (pACCRT-EB, pSPORT1*)	Phytoene	246	1
E. coli (pACCRT-EB, pRH1)	Phytoene	413	1.7
E. coli (pACCRT-EB, pHP11)	Phytoene	504	2.1

Table 2 Determination of IPP isomerase activity in *E. coli* JM109 strains carrying the individual IPP isomerase genes and rate of carotenoid synthesis *in vivo* in *E. coli* JM101 transformants carrying the corresponding genes in addition to the carotenoid biosynthesis genes

IPP isomerase activity was determined using the protocol described in the Materials and methods section. The substrate [^{14}C]IPP (0.25 μCi, 4.7 nmol) and protein (150 μg) content of the extract were standardized. Data are means of triplicate determinations (S.E. $\pm$10%) and are representative of typical experiments. The amount of carotenoids used to estimate the rate *in vivo* is an average of phytoene, lycopene and β-carotene.

E. coli strain	IPP isomerase activity (pmol/min)	Fold increase in activity	Rate of carotenoid synthesis *in vivo* (pmol/min)	Fold increase *in vivo*
E. coli	9	1	52.4	1
E. coli (pRH1)	21	2.3	83.0	1.6
E. coli (pHP11)	23	2.6	137.0	2.6
E. coli (pSI1)	21	2.3	95.0	1.8

production level of carotenoids by the isolated cDNAs was further confirmed in the liquid culture of the *E. coli* strains synthesizing β-carotene or phytoene due to the expression of the *Erwinia* carotenogenic genes. In the 28 h of cultivation, the *E. coli* strains carrying the cDNAs produced a 1.5–2.7-fold increase in β-carotene or a 1.7–2.1-fold increase in phytoene, when compared with the respective *E. coli* controls (Table 1).

Enzyme assay

Table 2 shows the result of an *in vitro* assay of IPP isomerase activity in various *E. coli* JM109 strains. A 2.3–2.6-fold higher IPP isomerase activity than the *E. coli* control was observed in the *E. coli* transformants carrying plasmids pRH1, pHP11 and pSI1. These increases were consistent with respect to incubation time over 3 h, and HPLC analysis of the diethyl ether extractable products following acid hydrolysis confirmed the identity of the prenyl products. These data confirm the functional identity of the cloned gene products as IPP isomerase.

DISCUSSION

Ergosterol in yeasts, cholesterol in animals and phytosterols and carotenoids in plants, are abundant isoprenoids, and have been the subject of many biosynthetic studies [9–11]. On the other hand, numerous bacteria such as *E. coli* do not have such 'lead' isoprenoid compounds. Based on the fact that the *Erwinia* carotenoid biosynthesis genes are efficiently expressed in several bacteria such as *E. coli*, and confer the ability of the carotenoid synthesis from FPP [8,16,32], we expected that the subsequently generated carotenoid pigments would possibly function as a visible marker for evaluating overall isoprenoid compounds in the bacteria. In this study, we have successfully isolated cDNAs enhancing carotenoid levels, by using as a host *E. coli* that accumulates β-carotene due to the presence of the *Erwinia* carotenogenic genes. Based on nucleotide sequence comparison and an *in vitro* enzyme assay, the cDNAs were found to code for IPP isomerases.

IPP isomerase proteins shared significant amino acid sequence similarity among the yeasts, the green alga and higher plants (44–52% identity over their entire length). The alignment of the four IPP isomerase enzymes reveals four highly conserved regions (Figure 2).

The recombinant *E. coli* strains carrying the exogenous IPP isomerase (*ipi*) genes produced elevated levels of lycopene, β-carotene and phytoene, compared with control levels. The level of lycopene or β-carotene was greatest in *E. coli* carrying the *H. pluvialis ipi*, followed by *S. cerevisiae ipi* and *P. rhodozyma ipi* genes respectively (Figure 3 and Table 1). Determinations of IPP isomerase enzyme activity showed consistent quantitative increases relative to the controls, which were comparable to relative *in vivo* elevations (Table 2). Thus the presence of an additional IPP isomerase gene is affected at the level of the enzyme activity in this instance. It is therefore likely that the difference in the level of the carotenoids formed in the *E. coli*

transformants is not due to the nature of the encoded enzymes but a consequence of the IPP isomerase enzyme activity itself.

Without comprehensive metabolic flux control analysis [33] of the overall pathway, a definite rate-limiting step is difficult to attribute. Such a study is not readily applicable to isoprenoid formation, especially in bacteria, since a multi-branched metabolic pathway is formed. However, the results presented here imply that IPP isomerase is an influential enzyme in the isoprenoid biosynthesis of the bacterium *E. coli*. Considering DMAPP is the first substrate for chain elongation reactions by condensation with IPP, it may be reasonable to speculate that isoprenoid metabolism is regulated at the level of DMAPP, although no report has been present stating that IPP isomerases may form the controlling-step in eukaryotes such as yeasts and animals. We have also not detected that the expression of an exogenous IPP isomerase gene in a yeast has any affect on increases in isoprenoid levels (N. Misawa, unpublished work). It is likely that regulatory systems distinct from eukaryotes are present in bacteria.

Carotenoids such as lycopene, β-carotene and astaxanthin have attracted much attention recently, due to their beneficial effect on health [34–37]. The genes responsible for the synthesis of these carotenoids have been isolated from various organisms such as the *Erwinia* species, the marine bacterium *Agrobacterium aurantiacum* and *H. pluvialis* [19,27,38,39]. We have initially succeeded in synthesizing lycopene, β-carotene and astaxanthin in *E. coli* by using the *Erwinia uredovora* and *H. pluvialis* carotenogenic genes [16,27]. In this study, the expression of an exogenous *ipi* gene has been shown to be a useful method for maximizing the production of industrially valuable carotenoids in *E. coli*. This gene could also be utilized in the future for the efficient bacterial production of other isoprenoid compounds.

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