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Amplification of HMG-CoA Reductase Production Enhances Carotenoid Accumulation in *Neurospora crassa*

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***Neurospora crassa*, a filamentous fungus, naturally produces the carotenoids lycopene and neurosporaxanthin. To increase the carbon flux through the carotenoid biosynthetic pathway, the 1658-bp region of the *HMG1* gene encoding the catalytic domain (*cHMG1*) of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase of *Saccharomyces cerevisiae* was expressed in *N. crassa* under control of the strong, constitutive glyceraldehyde-3-phosphate dehydrogenase (*GPD*) promoter and the inducible alcohol dehydrogenase (*alcA*) promoter. Overexpressing *cHMG1* under control of the *GPD* promoter increased lycopene and neurosporaxanthin production 6- and 1.5-fold, respectively, relative to the wild-type strain. Overexpression of *cHMG1* under control of the *alcA* promoter increased production of lycopene and neurosporaxanthin 3- and 2-fold, respectively.** © 2002 Elsevier Science (USA)

***Key Words:* isoprenoids; lycopene; neurosporaxanthin; *Neurospora crassa*; metabolic engineering.**

INTRODUCTION

Isoprenoids are a diverse class of natural products that are derived from successive addition of an active isoprene (C_5) unit, isopentenyl diphosphate (IPP), followed by enzymatic cyclization, oxidation, reduction, isomerization, and/or hydroxylation (McGarvey and Croteau, 1995). Sterols, quinones, carotenoids, dolichols, and taxol are a few examples under close investigation. Fungal isoprenoids comprise a structurally diverse group of primary and secondary metabolites. Two IPP biosynthetic pathways have been reported in the literature: DXP (or non-mevalonate) and mevalonate. In contrast to some organisms, fungi use only the mevalonate pathway, in which three molecules of acetyl-CoA form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is converted to mevalonate in an irreversible reaction catalyzed by HMG-CoA reductase and ultimately to isopentenyl diphosphate (IPP), which serves as a precursor for isoprenoid biosynthesis (Homann *et al.*, 1996; Britton *et al.*, 1998; Disch and Rohmer, 1998). The reduction of HMG-CoA to mevalonate catalyzed by HMG-CoA reductase is considered a key regulatory point in the isoprenoid pathway (Britton *et al.*, 1998). Overexpression of the catalytic domain of *HMG* (a homologue of *HMG1*) and *HMG1* in engineered yeasts has successfully increased isoprenoid accumulation (Shimada *et al.*, 1998).

Carotenoid biosynthesis has been studied extensively in the filamentous fungus, *Neurospora crassa* (Harding *et al.*, 1969; Harding and Shropshire, 1980; Harding and Turner, 1981; Lauter and Russo, 1991; Lyudnikova *et al.*, 1992; Morelli *et al.*, 1993; Sandmann, 1993; Eberle and Russo, 1994; Oda and Hasunuma, 1994; Li and Schmidhauser, 1995; Cogoni *et al.*, 1996; Li *et al.*, 1997). Four genes are known to be involved in carotenoid biosynthesis in *N. crassa*: the three *albino* genes, *al-1*, *al-2*, and *al-3* and the *yellow-1* (*ylo-1*) gene (Goldie and Subden, 1973; Harding and Turner, 1981; Schmidhauser *et al.*, 1994). The *al-3* product, a trifunctional enzyme encoding geranylgeranyl diphosphate synthetase, geranyltransferase, and farnesyltransferase, is required for the conversion of isopentenyl diphosphate to geranylgeranyl diphosphate (Sandmann *et al.*, 1993; Schmidhauser *et al.*, 1994; Vittorioso *et al.*, 1994; Perkins *et al.*, 2001). The *al-2* gene product, phytoene synthase, catalyzes the condensation of two 20-carbon prephytoene pyrophosphate molecules (Harding and Turner, 1981; Schmidhauser *et al.*, 1994; Perkins *et al.*, 2001) and possibly cyclization of lycopene to β-carotene and torulene (Verdoes *et al.*, 1999). The *al-1* product, phytoene dehydrogenase, catalyzes the stepwise introduction of up to five double bonds into the substrate phytoene, and its major products are 3,4-dihydrolycopene and lycopene (Hausmann and Sandmann, 2000). The *ylo-1* product has been suggested to catalyze either the dehydrogenation of lycopene to 3,4-dehydrolycopene or torulene or the oxidation of γ-carotene to neurosporaxanthin (Perkins *et al.*, 2001).

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The goal of metabolic engineering is to produce desirable molecules with superior yields and productivity by using recombinant DNA technology through modification of metabolic networks in living cells (Bailey, 1991; Stephanopoulos, 1994). Based on this principle, some organisms, primarily bacteria and food yeasts, have been used to produce molecules of commercial and pharmaceutical value (Misawa and Shimada, 1998; Shimada *et al*., 1998; Kennedy *et al*., 1999; Barkovich and Liao, 2001). Filamentous fungi, a relatively understudied class of organisms with respect to metabolic engineering, produce an array of secondary metabolites, some of which are valuable antibiotics. Metabolic engineering of filamentous fungi for secondary metabolite production could take advantage of the native pathways. In addition, filamentous fungi may serve as valuable hosts for the production of some valuable secondary metabolites from plants and other eukaryotic organisms. In this paper, we report the metabolic engineering of IPP biosynthesis in *N. crassa* by overexpression of the catalytic domain of *HMG1* encoding 3-hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase from *Saccharomyces cerevisiae* for increased production of carotenoids.

MATERIALS AND METHODS

Strains and Media

Escherichia coli strain DH5α (F ϕ80d*lacZΔM15 recA1 endA1 gyrA96 thi-1 hsdR17*($r_k^- m_k^+$) *supE44 relA1 deoR Δ*(*lacZYA-argF*)U169) (Bethesda Research Laboratories, Gaithersburg, MD) was used for routine DNA manipulation. *N. crassa* strain CJ44 (*Δ het-c arg-5; pan-2 A*) was used for transformation (Wu and Glass, 2001). All *N. crassa* strains were grown on Vogel's vegetative growth medium (VM) at 30°C unless otherwise specified (Vogel, 1964).

Construction of Expression Vectors

Plasmids pOAHT and pOGHT, used for overexpression of the *cHMG1* gene, were constructed as follows. The alcohol dehydrogenase (*alcA*) promoter was removed from pCN2 (a gift from Dr. Nancy Keller) with restriction enzymes *Bam*HI and *Sac* I and cloned into pOKE102 (Fungal Genetics Stock Center); the resulting plasmid was named pOA. The oligonucleotides GPD-F (5′-CCCAAGCTTGAATTCCAAAAGTCAGACGGCG TAAC-3′) and GPD-R (5′-GGGATGGATCCCGGGT-GATGTCTGCTCAAGCGG-3′) were used as PCR primers to amplify the glyceraldehyde-3-phosphate dehydrogenase (*GPD*) promoter using pAN-1Not (a gift from Dr. Peter Punt, TNO Nutrition and Food Research Institute) as a template. The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide (EtBr). The band containing the *GPD* promoter was excised and DNA extracted using QIAquick Gel extraction Kit (QIAGEN). The resulting DNA was digested with *EcoR* I and *Sma* I. The restriction products were cleaned with the QIAquick PCR purification Kit (QIAGEN) and cloned into pOKE102 to give pOG. The *trpC* terminator was amplified using oligonucleotides trpC-F (5′-CCCAAGCTTCGTTGGTGTCGATGTCAG-3′) and trpC-R (5′-CCCAAGCTTCAGTACACGAGGACT-TCTAG-3′). The PCR fragment containing the *trpC* terminator was digested with *Hind* III and cloned into pOA and pOG to give new plasmids, pOAT and pOGT, respectively. The catalytic domain of the *HMG1* gene from *S. cerevisiae* was amplified from pBHR-1, which contains the full-length cDNA of the gene, using oligonucleotides HMG-F (5′-CGGGATCCCGGGATGGAC CAATTGG TGAAAACTGA AG-3′) and HMG-R (5′-CGGAATTCTC GAGAGTAAAT ATTTTACGTA ACACATG-3′). The primer HMG-F corresponds to the sequence between +1701 and +1722, and primer HMG-R corresponds to the sequence between +3336 and +3359. The PCR fragment encoding the catalytic domain of the *HMG1* gene was digested with *BamH* I and cloned into pOAT and pOGT to give two new expression plasmids, pOAHT and pOGHT, respectively. The correct orientations of the *trpC* terminator and *HMG1* catalytic domain in pOAHT and pOGHT were confirmed by DNA sequencing.

Transformation Assays

N. crassa spheroplasts were prepared as described by Schweizer *et al*. (1981). Strain CJ-44 was used as a recipient for the pOGHT, pOAHT, and pOKE102 (FGCS) vectors. Transformation was performed according to Wu and Glass (2001). Fifty microliters of spheroplasts were thawed on ice and subsequently given a heat shock by incubating at 48°C for 5 min, followed by a 30 s incubation on ice. The spheroplasts were then placed at room temperature for 10 min before DNA addition. One microgram of each plasmid DNA construct prepared using the QIAprep Spin Miniprep Kit was used for each 50 μl of spheroplasts. The mixture of spheroplasts and DNA was incubated at room temperature for another 10 min and then added to 1 ml of 40% polyethylene glycol (mol. wt. 3350), 10 mM MOPS (morpholine propanesulfonic acid, pH 6.3), and 50 mM $CaCl_2$. After 10 min additional incubation at room temperature, 6 ml of top agar warmed to 48°C was added to the mixture, mixed and poured onto warmed minimal plates with arginine, but without pantothenic acid, at 30°C.

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Transformants were incubated at 30°C for 3–4 days. Transformants with the phenotype of interest were transferred to slants and plates for further analysis. Integration of the expression cassettes into the genome was confirmed using PCR with the corresponding primers described above.

Carotenoid Induction and Extraction

For plate assays, transformants with the phenotype of interest were inoculated at 2×10^6 conidia/ml into minimal medium containing 23 μM arginine as a supplement and 10 mM threonine as an inducer for transformants containing the *alcA* promoter (Creaser *et al.*, 1985). After 48 h incubation at 30°C, plates were induced in constant light at room temperature for 4 h. After imaging the plates, the mycelia were peeled off and extracted for carotenoid analysis. Alternatively, conidia of transformants were inoculated into 250 ml flasks containing appropriate supplements and butan-2-one as an inducer for the *alcA* promoter (Creaser *et al.*, 1985). The flasks were incubated at 30°C with shaking at 100 rpm for 48 h. The resulting mycelia were harvested and transferred evenly onto a wetted sterile filter paper with liquid media in a petri dish. After 4 h induction in light, mycelia were dried on a paper towel and the mycelial mat lyophilized overnight in a microcentrifuge tube. The dried mycelia were ground in a microcentrifuge tube containing a small stirring bar by vortexing at maximum speed for 5 min. The carotenoids in the resulting mycelial powder were extracted by adding 0.5 ml tetrahydofuran (HPLC grade) to the tube, mixing completely, incubating at 55°C for 10 min, and centrifuging at maximum speed for 10 min. The supernatant was transferred to a new tube. The extraction was repeated once and the supernatant from the second extraction was pooled with the first supernatant in the new tube. The resulting supernatant was vaporized under a stream of N_2 until completely dry. The residue containing carotenoids was dissolved in acetone (HPLC grade), centrifuged at $16,000 \times g$ for 15 min, and the resulting supernatant analyzed by liquid chromatography–mass spectrometry (LC–MS). All the extraction procedures were performed under dim light or in complete darkness.

RNA and Northern Blot Analysis

Total RNA was extracted as described in Arpaia *et al.* (1995) and the Northern blot analysis performed using procedures described by Wang *et al.* (2001). Hybridization was carried out using the 1.658 kb cDNA fragment of *cHMG1* as a probe. The fragment was labeled with ^{32}P using the Random Primed DNA Labeling Kit (Roche). Total RNA samples (10 μg) prepared from *N. crassa* mycelia were denatured, resolved on a 1% agarose gel containing 20% (w/v) formaldehyde, and transferred to a Hybond N^+ nylon membrane (Amersham Pharmacia Biotech). The RNA was fixed to the membrane by heating (80°C) in an oven, prehybridized in a solution containing 50% formamide, 5 × SSC, 0.05 M phosphate buffer, 5 × Denhardt's solution, 0.1% (w/v) SDS, 0.01% (w/v) sodium pyrophosphate ($NaPP_i$), and 200 μg/ml denatured salmon sperm DNA at 42°C for 3 h, and then hybridized in a solution containing 50% formamide, 5 × SSC, 0.02 M phosphate buffer, pH 7.2, 1 × Denhardt's solution, 0.1% (w/v) SDS, 0.01% $NaPP_i$, 200 μg/ml denatured salmon sperm DNA at 42°C, and a [^{32}P]-labeled full-length *cHMG1* probe (1×10^6 cpm/ml) at 42°C for 16 h. The membrane was washed sequentially in a solution containing 2 × SSC and 0.1% SDS at room temperature for 15 min, a solution containing 1 × SSC and 0.1% SDS at 40°C for 10 min, and finally in a solution of 0.5 × SSC and 0.1% SDS at 40°C for 10 min. Quantitative analysis of the radioactive bands was carried out with a Variable Mode Imager-Typhoon 8600 (Amersham Pharmacia Biotech).

LC–MS Analysis

Carotenoids were analyzed by reverse phase liquid chromatography (LC) on a Hewlett-Packard 1100 system equipped with a diode-array (DOD) detector and mass selective (MS) detector (Hewlett-Packard model G1946A). All separations were performed at a flow rate of 1.1 ml/min on Hewlett-Packard Eclipse XDB-C8 column (150 mm × 4.6 mm i.d., 5 μm particle size, 80 Å pore size) maintained at 40°C. Carotenoids were detected at 425 nm. All chromatographic data were acquired and processed over a wavelength range of 300–600 nm using the Chemstation software system (Hewlett-Packard). Eluent A (60:40 methanol:water), Eluent B (acetonitrile), and Eluent C (methanol) were used as the solvent systems. Methanol, acetonitrile and acetone (all HPLC grade) were filtered through a 0.22-μm nylon filter before use. Nanopure water was filtered through a 0.22-μm cellulose acetate filter prior to being mixed with methanol. A 30-μl sample was injected into the system. The samples were eluted using a three-solvent gradient system (minutes; % solvent A, % solvent B, % solvent C): (0; 75, 23, 2,), (20; 3.5, 32.5, 64), and (33; 3.5, 32.5, 64). The column was then reconditioned for 5 min. The LC–MS was equipped with an atmospheric pressure chemical ionization interface (APCI) in the positive ion mode. The drying gas (nitrogen) flow was set to 5 L/min, the nebulizer pressure was 60 psi, the fragmentor voltage was chosen to be at 10 V, and the drying gas and vaporizer temperatures were 350°C and 325°C, respectively. The corona discharge voltage was set to

4000 V with a current of 4 μA. Parameters for the identification of carotenoids were compiled by Liaaen-Jessen (1995).

RESULTS

Characterization of N. crassa Transformants

N. crassa strain CJ-44 (*arg-5; pan-2*) was transformed with pOKE103, pOAMT and pOGMT. The transformants were plated onto minimal medium supplemented with arginine and transferred to minimal slants. After incubating at 30°C in the dark for 48h, the transformants on the slants were then moved to the light for induction of carotenoid production. The phenotype of red-pink color was used to select transformants for further characterization. The presence of expression cassettes was confirmed using PCR with primers described above. Among 10 red-pink transformants, four contained the 1.658-kb catalytic domain of *HMG1* and the correct expression cassettes in the genome (data not shown). Also, the growth rate of transformants was virtually the same as that of control (data not shown).

Effect of Overexpression of cHMG1 on Carotenoid Production

Transformants of *N. crassa* were inoculated onto Vogel's minimal plates with appropriate supplement(s), and the plates were incubated in the dark at 30°C for 48 h. The plates were then induced in the light for 4 h. Mycelia of the transformants CJ44A and CJ44G were more pigmented than the controls, CJ44P and CJ44; transformant CJ44G, which contained *cHMG1* under the control of the *GPD* promoter, was more pigmented than transformant CJ44A, which contained *cHMG1* under control of the *alcA* promoter (Fig. 1). The reason that CJ44G produced more carotenoids than CJ44A was possibly that the *GPD* promoter was stronger than that of the *alcA* promoter with threonine as an inducer. Since the *GPD* promoter is a constitutive promoter, it is difficult to compare the strength of the inducible *alcA* promoter with 10 mM threonine as an inducer to that of the *GPD* promoter. However, threonine, reported as poor inducer for the *alcA* promoter, resulted in only 35% of the induction level compared to butan-2-one, the best inducer reported so far for the *alcA* promoter (Creaser *et al.*, 1985).

Carotenoids were extracted from mycelia peeled from the plates and analyzed using LC–MS. Two major carotenoids, neurosporaxanthin and lycopene, were identified in the chromatogram (Fig. 2). Two isomers of neurosporaxanthin had the same MS spectrum and only slightly different UV/Visible spectra (Fig. 3). For quantification purposes, the amounts of the two isomers were added and are reported as a single value. The neurosporaxanthin contents of transformants CJ44A and CJ44G (43.8 μg/g dry mycelial weight and 63.4 μg/g dry mycelial weight, respectively) were about 20% and 70% greater than those of their respective controls (CJ44 and CJ44P); and the contents of lycopene in transformants CJ44A and CJ44G (10.28 μg/g dry mycelial weight and 17.9 μg/g dry mycelial weight, respectively) were about 3- and 5-fold greater than their respective controls (Fig. 4). The absolute amount of neurosporaxanthin was much higher than that of lycopene in both the wild-type and the engineered *N. crassa*, and it was consistent with reports that neurosporaxanthin is a major carotenoid in *N. crassa* (Britton, 1998; Hausmann and Sandmann, 2000). These results indicate that HMG-CoA reductase is a rate-limiting enzyme in the production of carotenoids, and the overexpression of the catalytic domain of the *HMG1* gene under the control of the *GPD* promoter and the *alcA* promoter with threonine

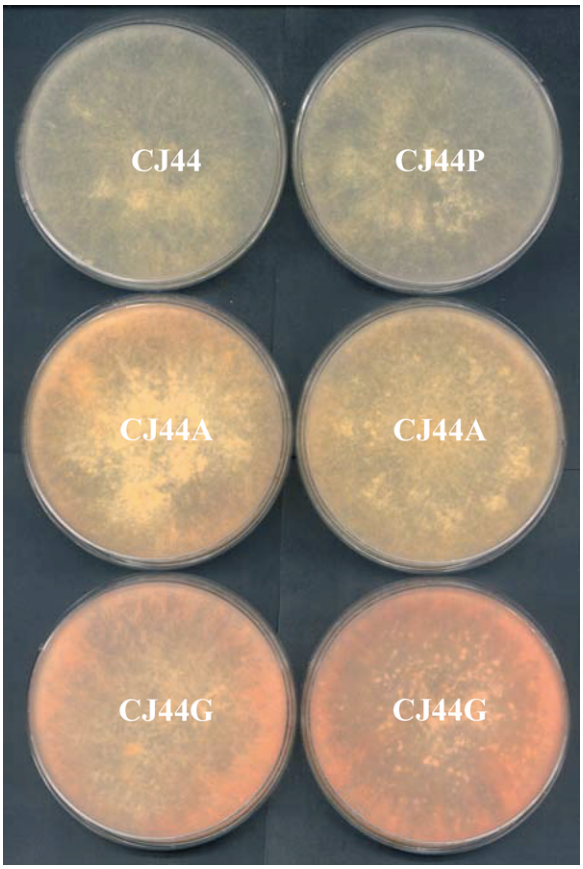

FIG. 1. *N. crassa* strains transformed with various vectors. CJ44, control strain; CJ44P, CJ44 transformed with control vector pOKE102; CJ44A, CJ44 transformed with pOAHT vector; CJ44G, CJ44 transformed with pOGHT vector. The plates with CJ44A contained 10 mM threonine as an inducer.

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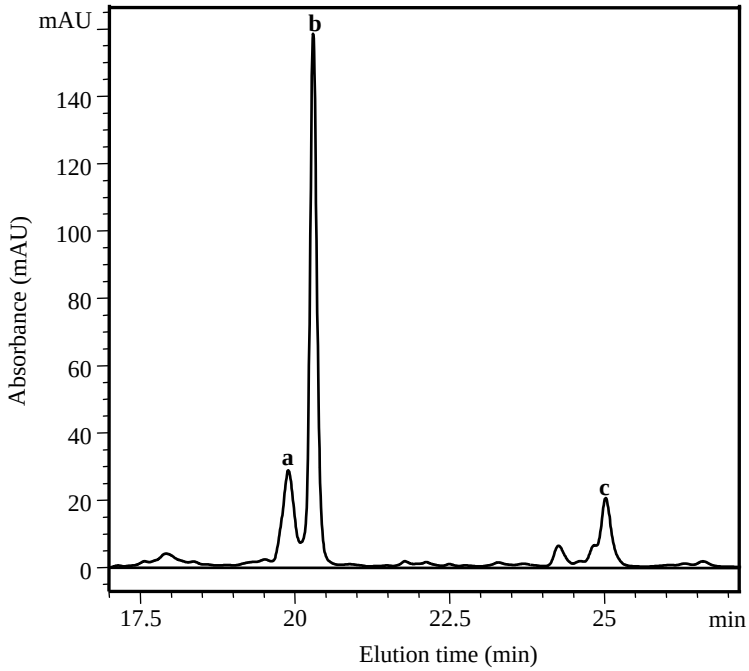

FIG. 2. Liquid chromatographic profiles of *N. crass* strain CJ44 extracts. Peak identification: a, b, isomers of neurosporaxanthin; c, lycopene.

(10 mM) as an inducer was effective in increasing the accumulation of lycopene and neurosporaxanthin.

To test the effect of the *cHMG1* expression level on carotenoid production, the *alcA* promoter was induced with butan-2-one, which is known to be a more effective inducer of the *alcA* promoter than threonine. CJ44A, the transformant carrying *cHMG1* under control of the *alcA* promoter, was grown in medium containing different concentrations of butan-2-one and the resulting mycelia were extracted for neurosporaxanthin and lycopene. The content of neurosporaxanthin and lycopene increased as the concentration of butan-2-one in liquid medium increased from 5 to 20 mM (Fig. 5). The contents of lycopene from mycelia induced with 10 and 20 mM butan-2-one were about 2- and 3-fold of that of control (uninduced), respectively. The contents of neurosporaxanthin from mycelia induced with the two inducer concentrations were about 1.2- and 2-fold of that of controls; however, there was little difference in the contents of lycopene and neurosporaxanthin from the mycelia of control and mycelia that had been induced with 5 mM butan-2-one (Fig. 5).

To further quantify the induction of *cHMG1*, total RNA from the induced mycelia was extracted and used for Northern analysis. The expression level of *cHMG1* increased as the concentration of buton-2-one increased, and a low basal level of message was detected in the absence of inducer (Fig. 6). Similar to the relative levels of carotenoids resulting from expression of *cHMG1* from various promoters, the level of *cHMG* mRNA produced from the *GPD* promoter was greater than that from the *alcA* promoter, even at the highest inducer level. Nevertheless, the above results indicate that the *A. nidulans alcA* promoter functioned well in *N. crassa*.

DISCUSSION

HMG-CoA reductase (HMGR) catalyzes the reduction of HMG-CoA to mevalonate, which, once formed, has no other use than as a precursor of isoprenoid compounds (Britton *et al.*, 1998). The concentration of mevalonate is tightly controlled through the activity of HMGR, which is a key enzyme of the pathway and rate-determining in isoprenoid biosynthesis (Goldstein and Brown, 1990; Gardner and Hampton, 1999). The amount of HMGR is regulated at several levels. Transcription and translation of HMGR increase when the concentrations of products of the mevalonate pathway are low. The amount of HMGR is also regulated by degradation, in which sequences of the transmembrane domain of HMGR have been identified to play an important role (Hampton *et al.*, 1996; Gardner and Hampton, 1999). Therefore, increasing the copy number of the native HMG-CoA reductase gene in the cell could be countered by another level of control, such that the overall flux through the pathway would not be significantly

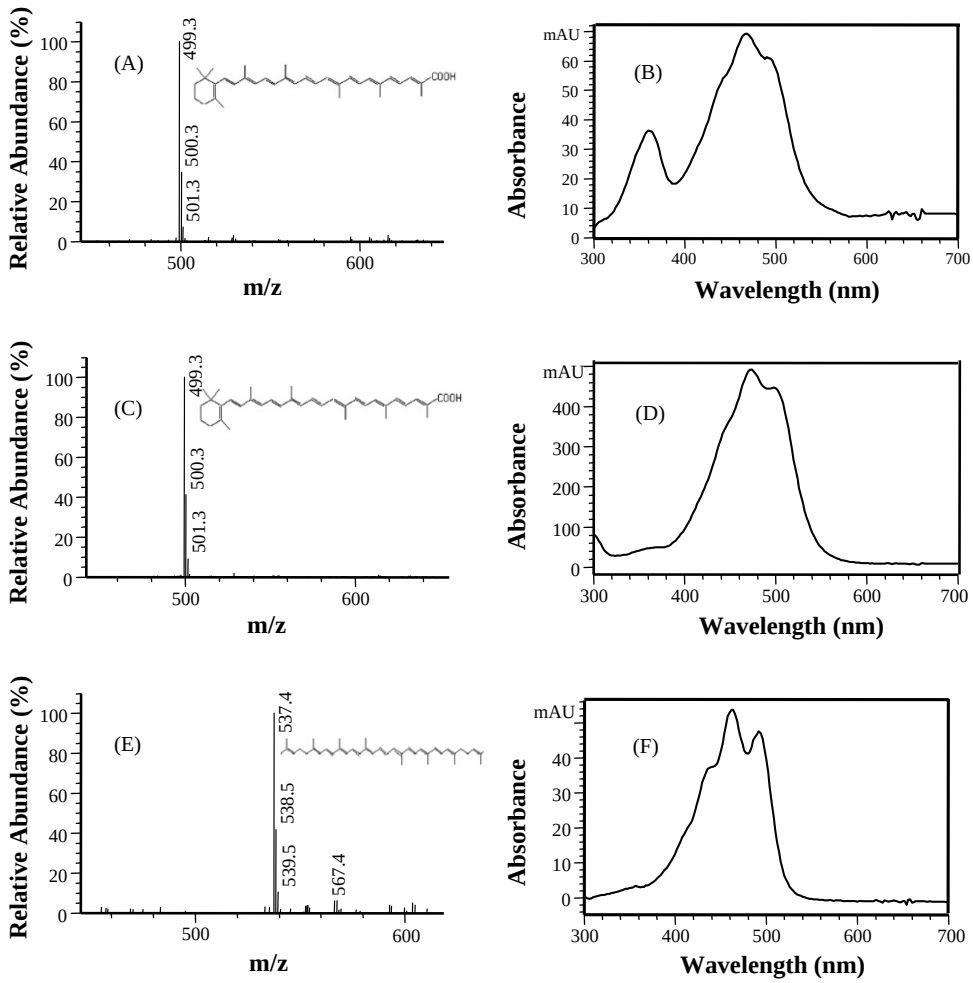

FIG. 3. MS (A, C, E) and UV/visible spectra (B, D, F) for neurosporaxanthin (A–D) and lycopene (E and F) from *N. crassa*.

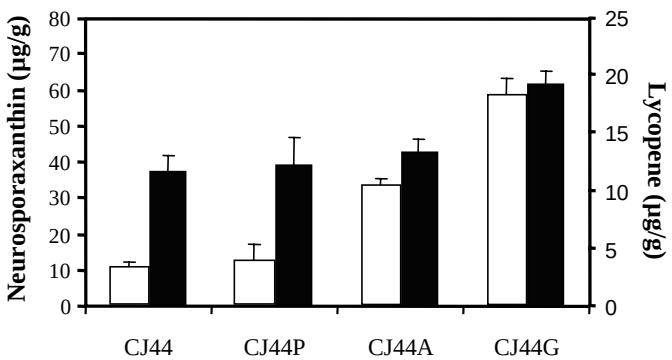

FIG. 4. Neurosporaxanthin and lycopene content of *N. crassa* CJ44 carrying different vectors: Lycopene (open bars); neurosporaxanthin (filled bars). Lycopene and neurosporaxanthin were extracted from mycelia peeled from plates after 4 h induction. The induction condition for the strain CJ44A was the same as indicated in Fig. 1.

changed (Shimada *et al*., 1998). Thus, the coding region of the catalytic domain of HMGR, *cHMG1*, was placed under the control of the *GPD* and *alcA* promoters. Overexpression of *cHMG*1 under control of *GPD* and *alcA* promoters in *N. crassa* increased accumulation of neurosporaxanthin and lycopene. (A blast search using the DNA sequence of *HMG1* against the genome sequence of *N. crassa* did not reveal any homology, but one assumes that *N. crassa* has a functional HMG-CoA reductase.) By comparison with controls, it is reasonable to conclude that the increased content of lycopene and neurosporaxanthin resulted from the overexpression of *cHMG1*. This is consistent with the results obtained when the overexpression of the catalytic domain of *HMG1* in *S. cerevisiae* and its homologue from *C. utilis* in *C. utilis* enhanced isoprenoid accumulation in the two yeasts (Polakowski *et al*., 1998; Shimada *et al*., 1998).

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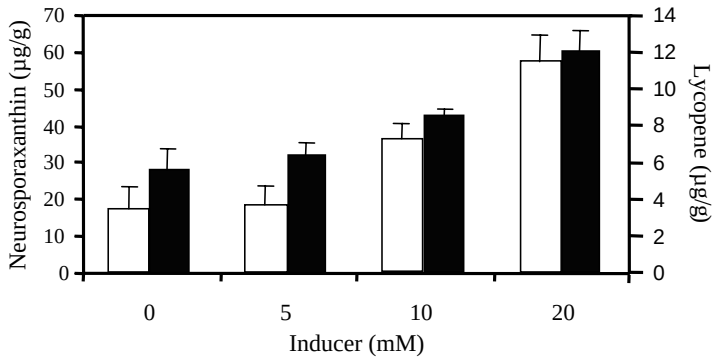

FIG. 5. Effect of butan-2-one concentration on the production of lycopene and neurosporaxanthin by *N. crassa* transformant CJ44A. Lycopene (open bars); neurosporaxanthin (filled bars). Lycopene and neurosporaxanthin were extracted from mycelia grown in liquid culture containing butan-2-one. The resulting mycelia were spread evenly onto a Petri dish for carotenoid production prior to extraction.

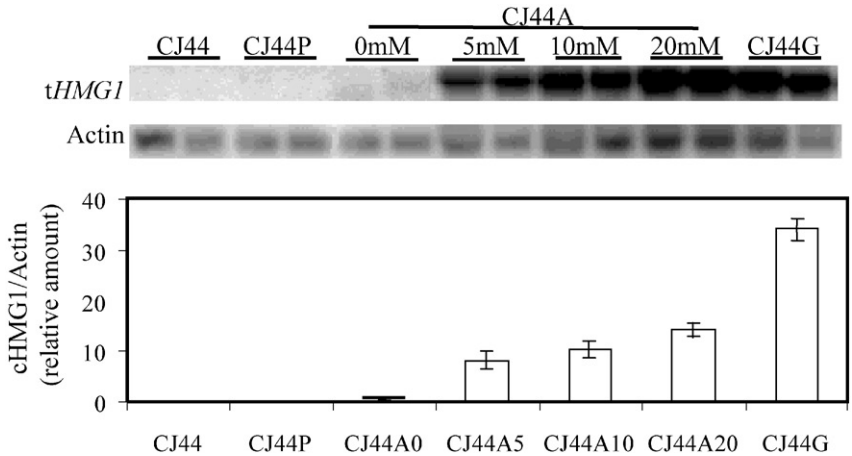

FIG. 6. Northern analysis of *cHMG1* mRNA accumulation under inducible and constitutive conditions. The strain labeling was the same as in Fig. 1.

Although neurosporaxanthin was reported to be a major carotenoid in *N. crassa*, (Britton *et al*., 1998; Hausmann and Sandmann, 2000) a significant amount of lycopene was detected in all mycelia of *N. crassa*. The accumulation of lycopene possibly results from short exposure to light, which limits production of enzymes to catalyze lycopene to neurosporaxanthin; however, extended light exposure seems to decrease the color intensity of the culture (data not shown), possibly due to carotenoid destruction by long period of light exposure. While the mevalonate pool could also be used as a precursor for ergosterol biosynthesis in fungi, disruption of *ERG9* encoding squalene synthase alone was reported to have no effect on the lycopene content in an engineered *C. utilis* strain; the unchanged content of lycopene was ascribed to a second copy of *ERG9* in *C. utilis* (Shimada *et al*., 1998).

Northern blot analysis showed that both *GPD* and *alcA* promoters from *A. nidulans* functioned well in *N. crassa* (Fig. 6). The inducible *alcA* promoter responded to buton-2-one better than to threonine in *A. nidulans* (Creaser *et al*., 1985). Since a limited number of inducers and inducer concentrations were used in this study, more studies are necessary to fully evaluate how the *alcA* promoter functions in *N. crassa*. Indeed, more thorough investigation using a single reporter gene system would help. Nevertheless, this is the first report of using the inducible *alcA* promoter in *N. crassa*.

In addition, this study reported the first successful application of metabolic engineering of carotenoid production in the filamentous fungus *N. crassa*. Given the availability of *N. crassa* strains and its recently sequenced genome, *N. crassa* may be an effective host for the

production of a wide variety of isoprenoids and other secondary metabolites.

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