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A single desaturase gene from red yeast *Sporidiobolus pararoseus* is responsible for both four- and five-step dehydrogenation of phytoene

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ABSTRACT

Carotenoids are one of the most common classes of natural pigments widely occurring within organisms. These structurally diverse pigments are of great importance in different processes such as nutrition, vision, cellular growth and development. While found in various yeast strains, one of the best-studied carotenoid producer is the pigmented species *Sporidiobolus pararoseus*. However, the precise nature of the genes involved in the biosynthesis of carotenoids in this species remains unclear. Here, we cloned a cDNA copy of the phytoene desaturase gene *crtI* from *Sporidiobolus pararoseus* CGMCC 2.5280. The *crtI* full-length genomic DNA and cDNA are 2330bp and 1683bp, respectively. This gene encodes a 560-amino acid protein with a predicted molecular mass of 62.28 kDa and a pI of 7.27. Functional identification of the gene was performed using heterologous complementation detection in *Escherichia coli*. Our experimental findings indicate that the enzymatic conversion of phytoene to lycopene (fourth step product) and 3,4-didehydrolycopene (fifth step product) is catalyzed by this phytoene desaturase of *S. pararoseus* through consecutive dehydrogenation. Furthermore, our findings suggest that the *crtI* gene of *S. pararoseus* represents an alternative gene source for the reconstruction of carotenogenic pathways vital for the production of engineered carotenoids.

1. Introduction

Carotenoids are a group of structurally diverse natural pigments with considerable value for pharmaceutical, food and feed industries. Carotenoids act as vitamin A precursors, possess antioxidant properties and exhibit properties deemed protective against a range of diseases (Avalos and Carmen Limón, 2015; Frengova and Beshkova, 2009). In addition, they are used as coloring agents for foods such as processed meats and beverages (Gammone et al., 2015; Hussain et al., 2015). Carotenoid production is found in many organisms, ranging from non-photosynthetic prokaryotes to higher plants (Li et al., 2015; Priya and Siva, 2014; Takano et al., 2006). Nevertheless, few carotenoids have so far been successfully produced by way of chemical synthesis, fermentation, or extraction from natural resources.

A number of basidiomycete yeast species produce carotenoids forming pigmented colonies, and are therefore commonly referred to as red yeasts (Mannazzu et al., 2015). In particular, red yeasts of the genera *Phaffia*, *Rhodotorula* and *Sporidibolus* exhibit production of considerable amounts of carotenoids (Davoli et al., 2004; Dias et al., 2016), rendering these yeast strains potential sources for industrial pigment production (Freitas et al., 2014; Yen and Chang, 2015).

Carotenoids biosynthesis has been extensively investigated in red yeasts (Avalos and Carmen Limón, 2015). The general pathway for carotenoid biosynthesis in red yeasts commonly involves several steps: (1) conversion of acetyl-CoA to IPP through the intermediates HMG-CoA and MVA; (2) isomerization of IPP to DMAPP, and conversion to GGPP; (3) condensation of two GGPP molecules to phytoene, which undergoes dehydrogenation to form lycopene or hydrolycopene catalyzed by phytoene desaturase. Lycopene is the major branch point of the carotenogenic pathway, where it acts as precursor of cyclic carotenoids such as γ-carotene, β-carotene, torulene, torularhodin and astaxanthin.

Phytoene desaturase is the key enzyme in the biosynthesis pathway of carotenoid-generating organisms. The phytoene dehydrogenation reaction is thought to be the rate-limiting step in carotenogenic pathway (Sandmann, 2009), and is the target of numerous commercially important herbicides (Windhövel et al., 1994).

Because of its ease of genetic manipulation, the non-carotenogenic heterologous host *E. coli* is being widely used for genetic and biochemical analysis of carotenoids and the genes involved in its production. However, due to the absence of phytoene biosynthetic genes, studying

Abbreviations: crt, carotenogenic gene; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; MS, mass spectrometric; IPTG, isopropyl β-D-1-thio-galactopyranoside; IPP, isopentenyl-pyrophosphate; HMG-CoA, 3-hydroxymethyl-3-glutaryl-CoA; MVA, mevalonic acid; DMAPP, dimethylallyl pyrophosphate; GGPP, geranyl geranyl pyrophosphate.

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carotenoid biosynthesis in *E. coli* requires extension of the general terpenoid pathway with CrtB and CrtE for the production of phytoene, which is a precursor of carotenoids (Seo et al., 2015).

In the majority of carotenoid-producing microorganisms, the colorless phytoene is dehydrogenized by the CrtI-type phytoene desaturase via three, four, five or even six consecutive steps to form neurosporene, lycopene, 3,4-didehydrolycopene or 3,4,3',4'-tetradehydrolycopene, respectively (Sandmann, 2009). For example, in *Rhodobacter capsulatus*, phytoene desaturase is involved in the production of neurosporene via a three-step dehydrogenation of phytoene (Raising et al., 1996). In *Rhodobacter azotoformans*, phytoene desaturase is involved in the production of neurosporene and lycopene via both three- and four-step dehydrogenation, respectively (Zhang et al., 2012). In *Xanthophyllomyces dendrorhous*, phytoene desaturase is involved in the production of lycopene via four-step dehydrogenation (Visser et al., 2003). In *Neurospora crassa*, the phytoene desaturase catalyzes the stepwise introduction of up to five double bonds into phytoene, to produce 3,4-didehydrolycopene, which is subsequently transformed into torulene (Hausmann and Sandmann, 2000). Furthermore, both *Pantoea stewartii* $CrtI_{14}$ and *Rubrivivax gelatinosus* CrtI catalyze the formation of 3,4,3',4'-tetradehydrolycopene from phytoene at specific concentrations of the phytoene desaturase (Kim et al., 2010; Schmidt-Dannert et al., 2000). In addition, CrtI-type phytoene desaturases were identified in other carotenoid-synthesizing bacteria and fungi, apart from green-sulfur bacteria *Chlorobium tepidum* (Frigaard et al., 2004) and cyanobacteria (with the exception of *Gloeobacter violaceus*) (Steiger et al., 2005).

S. pararoseus is a potential candidate organism for the study of the regulation of the carotenoids biosynthetic pathway, due to their production of the primary carotenoid β-carotene and torulene as well as the secondary carotenoids γ-carotene and torularhodin (Mannazzu et al., 2015; Mata-Gomez et al., 2014). However, despite a considerable number of reports on carotenoids bioproduction by *S. pararoseus*, little is known about the presence of carotenogenic genes, nor the molecular mechanisms underlying carotenoid biosynthesis in *S. pararoseus* (Baffi et al., 2013; Cabral et al., 2011; Han et al., 2012; Shi et al., 2013).

Here, we report the presence of a phytoene desaturase in *S. pararoseus*, an enzyme that is characterized by catalyzing both four- and five-step dehydrogenation of phytoene. Together, our insights into the *crtI* gene of *S. pararoseus* should facilitate a more detailed understanding of the carotenoid biosynthetic pathway in *S. pararoseus*, and advance the development of genetic or metabolic engineering for the biosynthesis of carotenoids.

2. Materials and methods

2.1. Strains and growth conditions

S. pararoseus CGMCC 2.5280 was isolated from strawberry fruit and cultured in flask cultures of 50 mL yeast extract-peptone-dextrose (YPD) medium (yeast extract 10 g/L, peptone 20 g/L, dextrose 20 g/L). Flasks were incubated for 72 h at 28°C, 200rpm. *E. coli* strain DH5α and BL21 (DE3) were used for constructing, propagating and expressing plasmids. *E. coli* strains were grown in LB medium at 37°C, 200rpm with 50 μg/L antibiotics as required.

Table 1
Primers used in this study.

Primers	Sequences	Enzyme site
crtI-F	CGGGATCCATGTCTAATACTGAGAAGGCTTA	*Bam*HI
crtI-R	CGGAATTCTCAGTTCCTTGCGTAGGTGA	*Eco*RI
crtI-ori-F	CGGAATTCATGAAAAAAACCGTTGTGA	*Eco*RI
crtI-ori-R	CCGCTCGAGTCAGGCTGGCGGTGGCTTT	*Xho*I

Underlining indicates restriction enzyme sites. All primers were synthesized by Shanghai Sangon Inc.

Table 2
Plasmid used in this study and their main characteristics.

Plasmids	Relevant characteristics	Source or referance
pET30a	Kan^r	Novagen company
pAC-LYC	*crtE,crtI,crtB*,Cm^r	Cunningham et al. (1994)
pAC-NEUR	*crtE,crtI,crtB*,Cm^r	Cunningham et al. (1994)
pAC-LYCΔ*crtI*	*crtE,crtB*,Cm^r	This study
pET30a-*crtI*-ori	*crtI*,Kna^r	This study
pET30a-*crtI*	*crtI*,Kna^r	This study

**crtE* geranylgeranyl pyrophosphate synthase gene, *crtB* phytoene synthase gene, *crtI* phytoene desaturase gene, Cm^r chloramphenicol resistant, Kan^r kanamycin resistant.

2.2. Genomic DNA and RNA isolation from S. pararoseus and cDNA synthesis

S. pararoseus cells were harvested by centrifugation (7000×g, 4°C, 10 min), the Rapid Yeast Genomic DNA Isolation Kit (Sangon, China) was used for genomic DNA extraction. Subsequently, total RNA was extracted using the UNlQ-10 Column Trizol Total RNA Isolation Kit (Sangon, China) and cDNA was synthesized using the M-MLV RTase cDNA Synthesis Kit (Takara, Japan) according to the manufacturer's instructions. Each 20 μL reaction mixture contained poly (A^+) RNA 2 μg, 5×1st Strand Synthesis Buffer 4 μL, dNTP Mixture 1 μL, Rnase Inhibitor 1 μL, Random primer 2 μL, RTase (M-MLV) 1 μL, RNase-free H_2O up to 20 μL. The reaction mixture was pretreated at 25°C for 10 min, incubated at 42°C for 60 min and then heated at 65°C for 10 min, and then, immediately cooled in ice water for 2 min. The cDNA was stored at -20°C until further use for the cloning of *crtI* gene.

2.3. PCR amplification and expression plasmids construction

Based on the complete phytoene desaturase gene sequence of *S. pararoseus* resulting from transcriptome sequencing, two oligonucleotide primers (forward primer *crtI*-F and reverse primer *crtI*-R, listed in Table 1), were designed for specific amplification of *crtI* with restriction enzymes sites for *Bam*HI and *Eco*RI. The carotenogenic gene *crtI* was amplified from genomic DNA or cDNA of *S. pararoseus* by using KOD DNA polymerase (Toyobo, Japan) under the following conditions: one

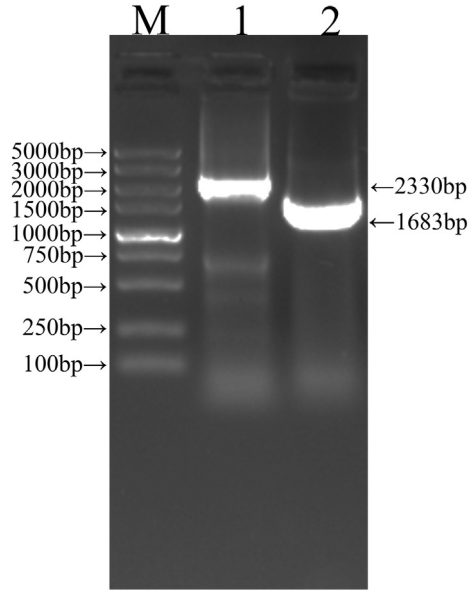

Fig. 1. Agarose-gel electrophoresis of *crtI* PCR products obtained from genomic DNA and cDNA of *Sporidiobolus pararoseus* CGMCC 2.5280. Gels were stained with ethidium bromide and visualized using UV light. M: 5,000 bp marker (Takara, Japan); 1: PCR product of *crtI* genomic DNA; 2: PCR product of *crtI* cDNA.

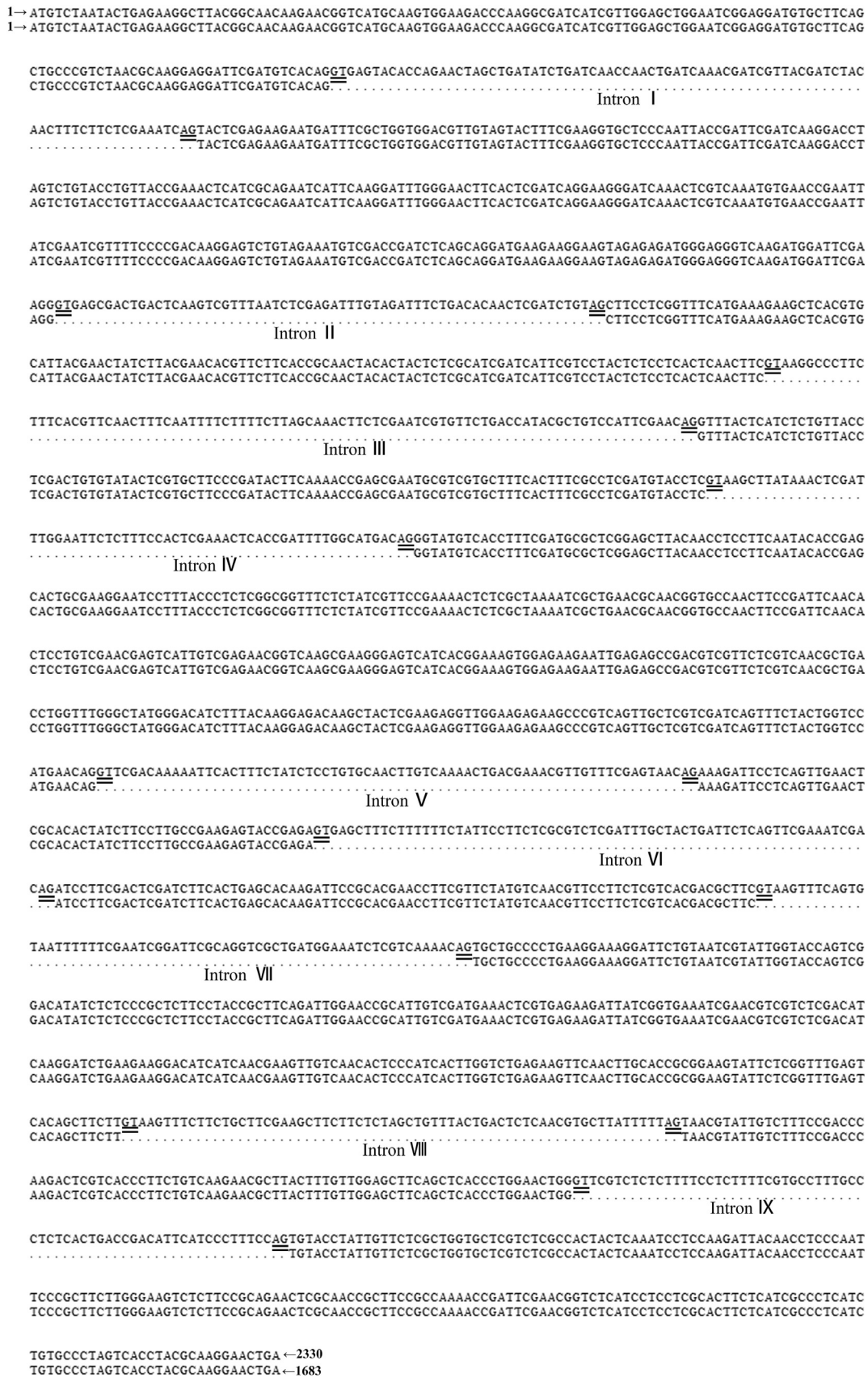

Fig. 2. Nucleotide sequence comparison of *Sporidiobolus pararoseus* CGMCC 2.5280 *crtI* genomic DNA with its cDNA.

cycle at 94°C for 5 min, 30 cycles at 98°C for 10 s, 53°C for 30 s, and 68°C for 2 min, final extraction cycle at 68°C for 7 min. The PCR products were then linked into cloning vector pTA2 (Toyobo, Japan) and then digested with *Bam*HI and *Eco*RI. Expression vector pET30a was digested with same restriction enzymes. Products were ligated with T4 DNA ligase (Takara, Japan) and then transformed into *E. coli* DH5α competent cell, and the positive expression vector named as pET30a-*crtI*.

The plasmid pAC-LYC carries three *Erwinia herbicola* carotenogenic genes, *crtE*, *crtB* and *crtI*. The plasmid pAC-NEUR carries *crtE*, *crtB* (from *E. herbicola*) and *crtI* (from *Rhodobacter capsulatus*). Both plasmids pAC-LYC and pAC-NEUR were constructed according to a previously described method (Cunningham et al., 1994). The construction of the plasmid pAC-LYCΔ (*crtI*) carrying *crtE* and *crtB* was performed through deletion of adjacent *Bgl*II-*Stu*I fragments and insertion of *Bgl*II-*Stu*I non-function fragments based on the plasmid pAC-LYC. All three overexpression systems were used to measure the activity of the putative phytoene desaturase of *S. pararoseus*. Plasmids used in this study are listed in Table 2.

2.4. Functional analysis of putative phytoene desaturase from S. pararoseus

The following protocol was used for functional analysis of the phytoene desaturase. pET30a-*crtI* was transformed into the phytoene accumulating strain of *E. coli* BL21 (DE3) carrying the reconstructed

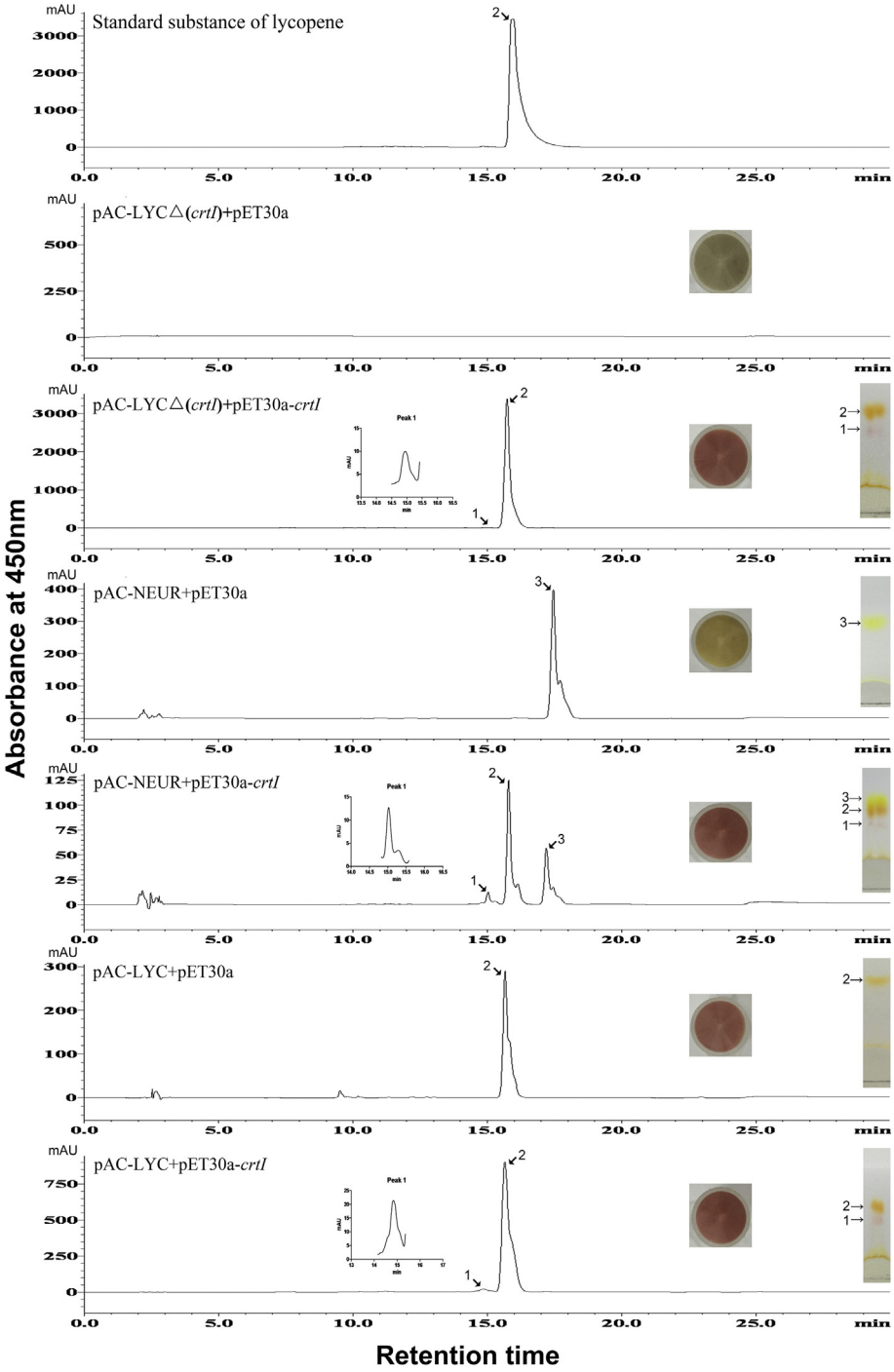

Fig. 3. HPLC and TLC analysis of carotenoid extracted from *E. coli* BL21 (DE3) with pAC-LYCΔ (*crtI*), pAC-NEUR or pAC-LYC and with pET30a-*crtI* or pET30a as a control. Pigment detection was performed by monitoring absorption at 450 nm. Peak 1: 3, 4-didehydrolycopene; Peak 2: lycopene; Peak 3: neurosporene.

plasmid pAC-LYCΔ (*crtI*); plasmid pET30a-*crtI* was transformed into the neurosporene accumulating strain of *E. coli* BL21 (DE3) carrying the plasmid pAC-NEUR; pET30a-*crtI* was transformed into the lycopene accumulating strain of *E. coli* BL21 (DE3) carrying the plasmid pAC-LYC. The expression vector pET30a lacking *crtI* was used for the three control strains: (1) BL21 (DE3) carrying both plasmid pAC-LYCΔ (*crtI*) and pET30a;(2) BL21 (DE3) carrying both plasmid pAC-NEUR and pET30a;(3) BL21 (DE3) carrying both plasmid pAC-LYC and pET30a. The recombinant *E. coli* BL21 (DE3) strains were cultured in LB medium at 37°C to OD_{600} 0.5 and induced with 1mol/L IPTG. Lastly, pigments were identified using TLC, HPLC and MS analysis.

2.5. Carotenoids extraction and detection

A 0.5 mL overnight culture was used to inoculate 50 mL of LB culture medium containing 50 μg/mL kanamycin and 50 μg/mL chloramphenicol in a 250 mL Erlenmeyer flask. The culture was shaken at 37°C until the OD_{600} reached 0.4–0.6, then induced with 1 mol/L IPTG until the OD_{600} reached 0.8. Cultures were grown for 24 h in the dark, and cells were collected by centrifugation.

Following freeze-drying of cells, carotenoids were resuspended in DMSO-acetone (1/3, v/v) and extracted at 65°C for 30 min (Taskin et al., 2011). TLC analysis was performed using a 95% cyclohexane and 5% ethyl acetate solvent system and 3,4-didehydrolycopene (peak 1) was isolated by TLC on activated silica plates (Silica Gel HSAF254, 200×200 mm, Yantai Chemical Industry Research Institute, China). The reverse-phase column (C18, 5 μm, 250×4.6 mm, Dikma Diamonsil (2), USA) was used with acetonitrile/H_2O (9/1, v/v) and ethylacetate as the mobile phase for gradient elution at a column temperature of 32°C. HPLC analysis of carotenoids was performed on a CBM-20A system equipped with SPD-M20A diode array detector (Shimadzu, Japan). HPLC detection conditions were as follows, remained flow velocity at 1 mL/min; injection volume was 20 μL; determine wave length was 450 nm; determine time was 30 min, gradient elution system: solvent A= acetonitrile/water 9:1 (v/v); solvent B=ethyl acetate; program 0–6 min, 20%–60% B; 6–15 min, 60% B; 15–20 min, 60%–100% B; 20–25 min, 100%–20% B; 25–30 min, 20% B isocratic step.

A ~100 μg sample was subjected to mass spectrometry on a Thermo Q Exactive high-resolution mass spectrometer (Thermo Scientific, USA) in positive mode under the following conditions: capillary temperature 320°C, spray voltage 3.8 kV, positive ionization, scan range 400–800 *m/z*. Lycopene standard substance was bought from Dalian Meilun Biotech Co., Ltd (China).

3. Results and discussion

3.1. Cloning and sequence analysis of putative phytoene desaturase gene

In order to clone the *crtI* gene from *S. pararoseus*, we designed oligonucleotide primers *crtI*-F and *crtI*-R on the basis of transcriptome sequencing. As shown in Fig. 1, we obtained a full-length genomic DNA and cDNA clone of the phytoene desaturase gene, with lengths of 2330bp and 1683bp, respectively. Comparison of the two sequences showed that the genomic DNA contains nine introns, with all splicing sites following the GT-AG rule (as shown in Fig. 2). The cDNA sequences and deduced amino acids of *crtI* reported in this paper are available in the GenBank databases under the accession number: KR108014.

3.2. Construction of the plasmid pAC-LYCΔ (crtI)

In order to detect the activity of putative phytoene desaturase from *S. pararoseus*, we constructed the plasmid pAC-LYCΔ (*crtI*). To test whether the plasmid pAC-LYCΔ (*crtI*) was successfully constructed, we cloned the *crtI* gene of *Erwinia herbicola* and then inserted *crtI* into the expression vector pET30a, which was named pET30a-*crtI*-ori. Primers were designed (listed in Table 1) for cloning gene *crtI* of *E. herbicola* based on GenBank accession number M87280. *E. coli* BL21 (DE3) cells carrying pAC-LYCΔ (*crtI*) were transformed with pET30a-*crtI*-ori plasmid, with successfully transformed colonies displaying a color change from white to pink. Control carrying the insert-free plasmid pET30a showed no change in color. HPLC analysis showed that the accumulating pigments of the recombinant strain carrying pAC-LYCΔ (*crtI*) transformed with pET30a-*crtI*-ori plasmid are identical with the accumulating pigments of the recombination strain carrying the plasmid pAC-LYC (data not shown). Together, these results indicate that the plasmid pAC-LYCΔ (*crtI*) was fully functional in *E. coli*.

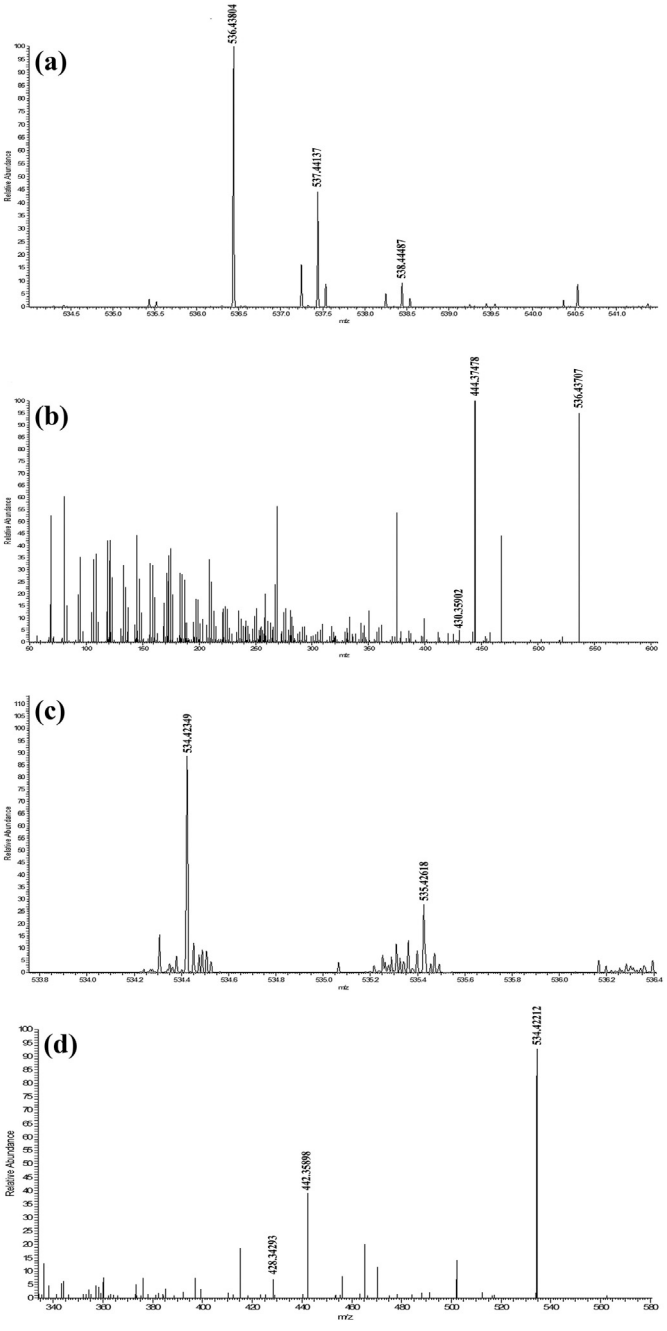

Fig. 4. Mass spectrometric analysis of lycopene standard substance and 3, 4-didehydrolycopene from *E. coli* BL21 (DE3) with pAC-LYCΔ (*crtI*), pAC-NEUR and pAC-LYC co-transformed with pET30a-*crtI*. (a) Lycopene standard substance analyzed at scan range 400–800 *m/z* mass spectra; (b) MS/MS spectrometric analysis of lycopene standard substance from the fragmentation of the *m/z* = 536.43804 ion; (c) 3, 4-didehydrolycopene analyzed at scan range 400–800 *m/z* mass spectra; (d) MS/MS spectrometric analysis of 3, 4-didehydrolycopene from the fragmentation of the *m/z* = 534.42349 ion. Bottom panel: mass spectra signal of the major peak.

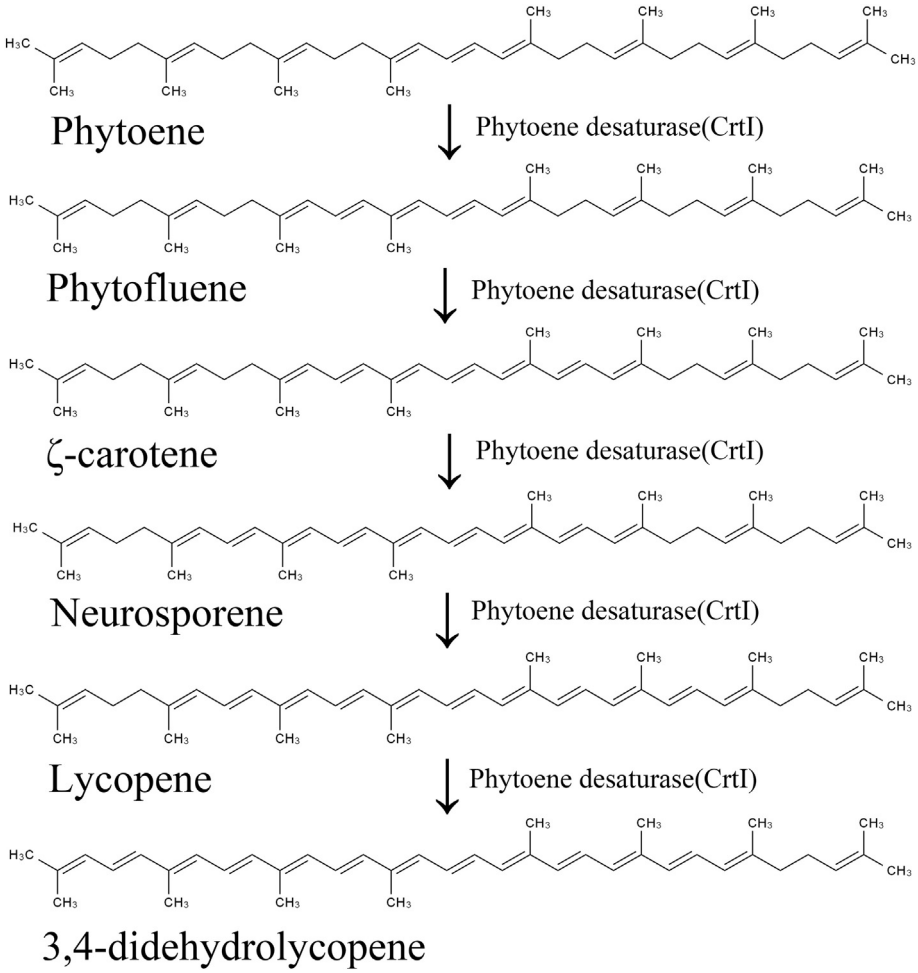

Fig. 5. Biosynthesis pathway for carotenoids of *Sporidiobolus pararoseus* CGMCC 2.5280 involved in phytoene desaturase.

3.3. Functional analysis of phytoene desaturase gene from S. pararoseus

Functional analysis of the pET30a-*crtI* expression product was performed via complementation experiments in *E. coli* BL21 (DE3) carrying the plasmid pAC-LYCΔ (*crtI*). As shown in Fig. 3, the color of thalli of strains carrying pAC-LYCΔ (*crtI*) transformed with pET30a-*crtI* plasmid turned from colorless to red, while control transformants carrying the plasmid pET30a lacking the *crtI* insert remained white; In order to investigate whether the phytoene desaturase of *S. pararoseus* has neurosporene dehydrogenation activity, we also transformed the plasmid pET30a-*crtI* into *E. coli* BL21 (DE3) carrying pAC-NEUR. As shown in Fig. 3, the color of the thallus turned from faint yellow to red, while control transformants carrying the plasmid pET30a without the *crtI* insert remained faint yellow; In order to assess whether the phytoene desaturase of *S. pararoseus* has lycopene dehydrogenation activity, plasmid pET30-*crtI* was transformed into *E. coli* cells BL21 (DE3) carrying pAC-LYC. As shown in Fig. 3, the thalli of these strains changed from pink to red, whereas in control strains carrying the plasmid pET30a without the *crtI* insert, they remained pink.

To assess the nature of the phytoene desaturase of *S. pararoseus*, we performed TLC, HPLC and MS analysis. As shown in Fig. 3 (TLC and HPLC results) and Fig. 4 (MS results), the expression product of *crtI* appeared to be essential for phytoene conversion to lycopene and 3,4-didehydrolycopene. We therefore concluded that the phytoene desaturase from *S. pararoseus* catalyzes *cis*-phytoene through ζ-carotene, neurosporene-generated lycopene and lycopene-generated 3,4-didehydrolycopene (as shown in Fig. 5). The phytoene desaturase of *S. pararoseus* appears to possess functions similar to that of the phytoene desaturase of *N. crassa* that is involved in the biosynthesis pathway of β-carotene and torulene (Hausmann and Sandmann, 2000).

The *crtI* gene of *Phaffia rhodozyma* (its teleomorph *Xanthophyllomyces dendrorhous*) is the best-studied representative among red yeasts (Lodato et al., 2003; Shi et al., 2014). The phytoene dehydrogenase of *R. diobovatum* with a four-step dehydrogenation activity of the enzyme was recently isolated (Guo et al., 2015). However, it remains unclear whether *Sporidiobolus crtI* gene codes for a phytoene desaturase (Mannazzu et al., 2015). The clone and characterization of *crtI* gene described here should provide much-needed insights into the molecular basis underlying carotenoid biosynthesis in *S. pararoseus*.

3.4. Comparative sequence analysis and phylogenetic tree construction

Phylogenetic profiling analysis was performed to compare the *S. pararoseus* CrtI to other phytoene desaturase related proteins. As shown in Fig. 6, we constructed the phylogenetic tree by the neighbor-joining method. Our analysis showed that the amino acid sequence of the phytoene desaturase of *S. pararoseus*, CrtI, exhibits significant homologies with that of *Rhodotorula* toruloides (GeneBank accession: CDR36131.1), *Rhodotorula* sp. (GeneBank accession: KWU44829.1), *Rhodosporidium diobovatum* (GeneBank accession: AHB14354.1) and *Rhodotorula graminis* (GeneBank accession: KPV74225.1). The N-terminus of these phytoene desaturases of proteins contains a conserved domain (pfam13450) belonging to the NAD (P)-biding Rossmann-like domain of the superfamily cl21454. Most members of this family, part of the larger pfam01593 family, which also contains amino oxidases, are CrtI itself; it is likely that all members catalyze either

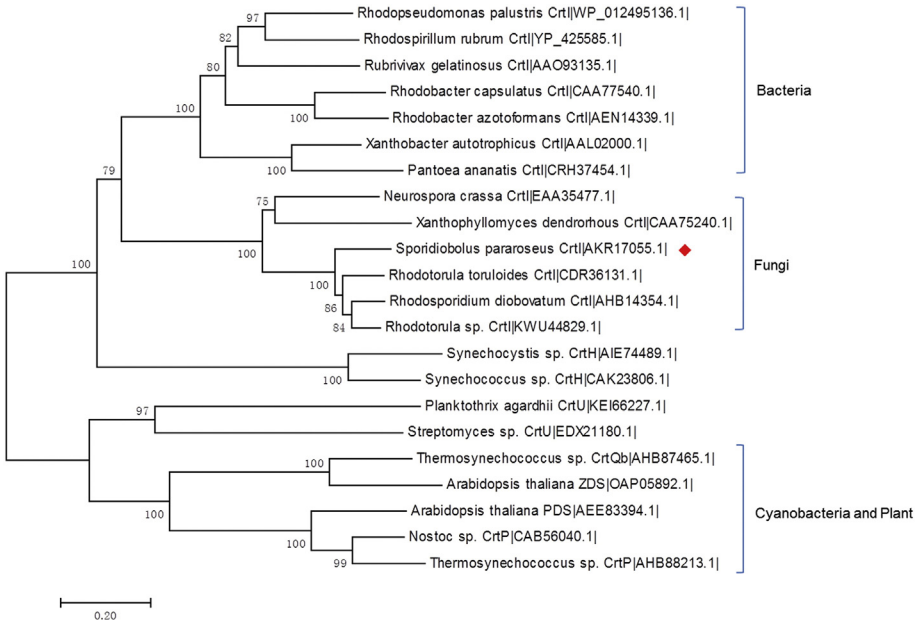

Fig. 6. Phylogenetic tree constructed with available CrtI-type related protein sequences. Bootstrap values, expressed as percentages of 1000 replications, are given at the branching points. MEGA 6.0 software was used to carry out evolutionary analysis.

phytoene or on related compounds such as dehydrosqualene, for carotenoid biosynthesis (Conserved Domain Database) (Marchler-Bauer et al., 2012). Hence, the enzymes of this family are expected to exhibit similar functional characteristics.

Analysis of *S. pararoseus* CrtI protein sequence revealed a homology of approximate 50% with that of the ascomycete's fungi *N. crassa* Al-1, which catalyzes both four- and five-step dehydrogenation reactions of phytoene to lycopene and 3, 4-didehydrolycopene (45.80%; GenBank accession: AAA33555.1). Given the availability of so few phytoene desaturase responsible for the five-step dehydrogenation of phytoene, it remains a challenge to investigate what regions are responsible for the five-step dehydrogenation activity within these proteins of phytoene desaturase.

The biochemistry and evolution of carotene desaturases have been thoroughly studied in microorganisms, algae and higher plants. The functional properties of phytoene desaturase from different organisms display a large degree of functional diversity. The conversion from phytoene to lycopene by dehydrogenation at four sites arranged in two symmetrical pairs is accomplished by a single CrtI-type enzyme in most bacteria and fungi (Sandmann, 2009). In most cyanobacteria, however, a three-unit enzyme containing CrtP, CrtQb and *cis*-to-*trans* isomerase CrtH is essential for the formation of lycopene (Steiger et al., 2005; Zhang et al., 2015). However, in most algae and higher plants, the PDS, ZDS, *cis*-to-*trans* isomerase Z-ISO and CrtISO are responsible for the production of lycopene (Li et al., 2007). The amino acid sequence of CrtP exhibits a higher degree of similarity to the carotene desaturase CrtU of Gram-positive bacteria than to the CrtI-type desaturase, whereas the CrtH is closely related to the CrtI-type of bacterial phytoene desaturases (Sandmann, 2009). This phenomenon supports the hypothesis that the CrtP/CrtQb-type enzyme complex has evolved from CrtU, and that both CrtU and CrtH have evolved from the bacterial CrtI-type phytoene desaturase respectively. Moreover, CrtQb and ZDS are highly homologous to CrtP and PDS, respectively, a fact suggesting that they have evolved by duplication from a common ancestor. Together, these findings strongly support the notion that during evolution of carotene desaturase from bacteria via cyanobacteria to higher plants, a single enzyme system responsible for the entire reaction sequence from phytoene to all-*trans* lycopene transformed into a more complex process.

4. Conclusion

In this study, a phytoene desaturase gene *crtI* was cloned from caroteniod-rich red yeast *S. pararoseus* CGMCC2.5280. This gene encodes a 560-amino acid protein with a predicted molecular mass of 62.28 kDa and a pI of 7.27. Using an *E. coli* complementation assay, we identified the *crtI* regions responsible for enzyme activity essential for four- and five-step dehydrogenation of phytoene.

Further studies are currently in progress in order to illuminate the carotenoid biosynthesis in *S. pararoseus* and then to improve the production of microbial carotenoids through the modification of the related carotenogenic genes of *S. pararoseus*.

Declaration interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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