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Molecular Cloning and Characterization of Phytoene Synthase Gene From a Unicellular Green Alga *Haematococcus pluvialis**

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Abstract The unicellular green alga *Haematococcus pluvialis* accumulates a highly valuable ketocarotenoid, i.e. astaxanthin up to 4% dry weight under stress conditions. Phytoene synthase is considered to be the first rate limiting enzyme in carotenoid biosynthesis pathway in *H. pluvialis*. The cDNA and genomic genes of phytoene synthase, i.e. *psy* from *H.pluvialis* were cloned and characterized. Result showed that *psy* had one open reading frame of 1 200 bp encoding a putative polypeptide of 400 amino acids which was interrupted by four introns. Phylogenetic analysis revealed that *psy* from green algae formed a monophyletic clade, and its closer relationship was higher plants. By using genomic walking approach, an approximate 1 kb 5′ flanking region of *psy* gene was cloned and a number of putative *cis*-regulatory elements were revealed. Fusing a 297 bp internal sequence (−297 to −1 bp from the translation initiation codon of *psy*) with the reporter gene, i.e. *lacZ* before attempted introducing the construct into the green alga via particle bombardment resulted in *lacZ* transient expression.

Key words *Haematococcus pluvialis*, phytoene synthase, 5′ flanking region, phylogenetic analysis, promoter

The unicellular green alga *Haematococcus pluvialis* accumulates a highly valuable ketocarotenoid, i.e. astaxanthin up to 4% dry weight under stress conditions[1]. There is growing commercial interest of astaxanthin because of its antioxidative properties [2], anti-cancer bioactivity [3] and immune stimulating effects[4]. There is also increasing need of this most valuable carotenoid in aquaculture used as diet supplements of salmonoids and other marine animals [5~7]. Similar to cyanobacteria and plants, the biosynthesis pathway of carotenoids in green algae starts with condensation of two geranylgeranyl pyrophosphate (GGPP) molecules, thus phytoene synthase is regarded as the first rate limiting enzyme in carotenoid synthesis[8,9]. It was found that blue light can lead to elevated transcript levels of phytoene synthase [10] and that the transcriptional regulation of *psy* was related to astaxanthin accumulation in *H.pluvialis*[11], so far there is still very little information available for transcriptional regulation elements of *psy* in *H.pluvialis*.

In this research, both cDNA and genomic genes of *psy* from *H.pluvialis* were cloned and characterized, and the 1 kb 5′ flanking region including a 297 bp putative promoter sequence was investigated by using reporter gene transient expression technique and computational analysis.

1 Materials and methods

1.1 Strains and growth conditions

Haematococcus pluvialis strain323 kept in our laboratory was grown in a liquid medium described by Boussiba and Vonshak [12] under a light density of 25 μmol photon·m^{-2}·s^{-1} (12 h∶12 h) at (22 ± 1)℃. Cells were collected by centrifugation at 2 500 *g* for 10 min at 22℃ and then frozen immediately in liquid nitrogen.

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1.2 Rapid amplification of 5′ cDNA ends

Total RNAs were extracted using RNeasy Mini kit (Giagen, Hilden, Germany) and used as template for RACE using SMART™ RACE cDNA Amplification Kit (Clontech, California, USA). The reverse transcription reactions were carried out according to manufacturer's instructions. The product was then used as the template for 5′ RACE. The specific primer (5′ CAATCGGCGATGCAAGTCAAT 3′) was designed based on the partial cDNA sequence in GenBank. RACE PCR profile was as following: 94℃ for 5 min; 94℃ for 60 s, 58℃ for 60 s, 72℃ for 3 min (30 cycles) and 72℃ for 10 min. The size of PCR product was determined by agarose gel electrophoresis. The gel band was purified using Gel Purified Kit (Watson, Shanghai, China) before cloning into pMD18-T (TaKaRa, Japan) vector. Ten positive clones were picked and the plasmids were sequenced by Bioasia Company (Shanghai, China).

1.3 Cloning and analysis of *psy* cDNA and genomic sequence

The full length cDNA and genomic sequence of *psy* were amplified from *H. pluvialis* first strand cDNA and genomic DNA, respectively, using sense primer: 5′ ATCAACTCTGACAATGCAGACAACA 3′ and anti-sense primer: 5′ GGTGCCATTCTTTCATCAC-TTACA 3′ corresponding to the ends of cDNA sequence. The following PCR parameters were used: 94℃ for 5 min; 94℃ for 60 s, 55℃ for 60s, 72℃ for 3 min (30 cycles) and 72℃ for 10 min. The PCR products were cloned as described above. Multiple protein sequence alignment was performed using BioEdit program with the implanted ClustalW [13,14]. Other PSY orthologous sequences were obtained from GenBank. A phylogenetic tree was reconstructed using neighbor-joining method [15] as implemented in the program MEGA 2.1 [16]. The reliability of the tree was evaluated by the bootstrap method with 1 000 replications.

1.4 Cloning of *psy* 5′ -flanking region and analysis

According to manufacturer's instructions of Universal GenomeWalker (Clontech, California, USA), total genomic DNA was digested with four types of restriction endonucleases (*Dra* Ⅰ, *Eco*R Ⅴ, *Pvu* Ⅱ, *Stu* Ⅰ) respectively, then ligated with a GenomeWalker adaptor before used as templates for nested PCR. The adaptor primer AP1 provided by the kit and a gene-specific primer GSP1 (5′ TCAACTGCGGCTGTTTAGGAACCACCTGG 3′) based on cDNA sequence were used in the primary PCR. Moreover, another nested adaptor primer AP2 and a nested gene-specific primer GSP2 (5′ GAACCACCTGGCTGGATGCTATGGACCAG 3′) based on the same cDNA sequence as above were used in the secondary PCR amplification. Touchdown PCR was used with the annealing temperature from 70℃ to 65℃. The 5′ flanking sequence was analyzed using both the Web Signal Scan Program and the PlantCare database[17].

1.5 Promoter investigation

The plasmid vector pβ-gal-Basic harboring a promoter-lacking *lacZ* (encoding β-galactosidase) reporter gene was purchased from Clontech Laboratories Inc.(Palo Alto. CA). The –297 bp to –1 bp 5′ flanking region of *psy* containing putative TATA-box and CAAT-box was amplified by PCR. The forward primer (5′ CATGGTACCCAGCTCA-CAGCTGCAAT 3′) contains a *Kpn* Ⅰ restriction site, and the reverse primer (5′ TACAAGCTTGTATA-TCACAGGAGTC 3′) contains a *Hin*d Ⅲ restriction site. The PCR product was double-digested with *Kpn* Ⅰ and *Hin*d Ⅲ and then inserted into *Kpn* Ⅰ - Hind Ⅲ site of the above vector to get p PSY-*lacZ* before attempted introducing into *H. pluvialis* cells using micro-particle bombardment as described by Teng *et al.* [18]. The transient assays were performed 48 h after transformation using the histo-chemical staining for *lacZ* activity. Blank controls were not bombarded cells, while negative controls were bombarded cells by using pβ-gal-Basic vector without promoter as donor DNA.

2 Results

2.1 Isolation of *psy* cDNA and genomic genes from *H. pluvialis*

By RACE and RT-PCR, *psy* cDNA was synthesized. The genomic DNA of *H. pluvialis* was used as template for polymerase chain reaction (PCR) to obtain *psy* genomic gene. The sequences of *H. pluvials* phytoene synthase gene and cDNA in this study were released in GenBank with accession number AY835634 and DQ057355, respectively. To elucidate the genomic organization of *psy*, the genomic DNA sequence and cDNA sequence of *psy* were aligned with software CLUSTAL W [14]. The result indicated that *psy* consisted five extons and four introns (Figure 1). The sizes of the exons and introns

are summarized in Table 1.

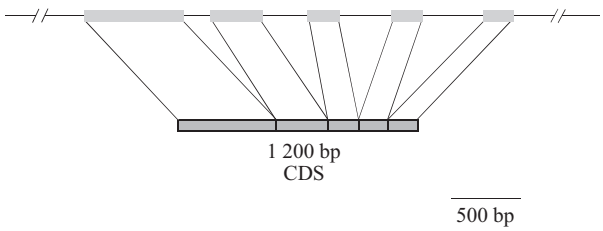

Fig. 1 Gene structure of phytoene synthase gene of *H. pluvialis*

Five exons and four introns are indicated.

The % G+C contents of the four introns were 55.73%, 51.27%, 57.75% and 54.52%, respectively. These introns began with the sequence GT and ended with AG, confirming the consensus 5′ and 3′ intron splice sites for mRNA. The 3′ acceptor splice sites were also preceded by pyrimidine-rich sequences as are a common feature of mRNA 3′ splice sites. A comparison of the splice junction nucleotide sequences from other eukaryotic genes[19] shows these sequences are very similar suggesting that *psy* in *Haematococcus* exhibits a strong sequence preference at its exon/intron boundaries. Comparing to *Dunaliella*, the first exons are more variable than other exons. However, on average, the exons are 75% identical in their sequences and therefore they are more conserved than the introns.

Table 1 The organization of exons and introns in *psy*

No.	Exon size /bp	5′ Splice donor	3′ Splice acceptor	Intron size /bp
1	480	CCCAAGgtacgc	gcttag GCCCTG	131
2	261	TACAAGgtaggc	ctgtagGGACCC	236
3	156	CAAGATgtgagt	tgacagGTGCTG	258
4	152	TGTCTGgtgagt	atgcagGTCTGC	299
5	154			

"5′ splice donor" shows exon/intron boundaries and "3′ splice acceptor" shows intron/exon boundaries in which exon sequences are shown in uppercase letters and intron sequences are shown in lowercase letters.

2.2 Sequence analysis of 5′ flanking region and promoter activity assay

The 5′ flanking region of *psy* was first investigated by genome-walking method in *H. pluvialis* instead of screening from the genomic library. A sequence of approximate 1 kb had been released to GenBank DQ152009. Internet tool (http://oberon.rug.ac.be:8080/PlantCARE/index.html)[17] was used to perform sequence analysis including prediction of possible transcriptional elements. The putative TATA box is located between nucleotides −256 to −249 (TATAAA), two presumed CAAT boxes occur at −284 to −280 and −338 to −334 in the 5′ flanking region. Several potential *cis*-regulatory elements such as C-repeat/DRE, ABRE (abscisic acid regulated expression), ERE (ethylene-regulated expression) and other potential elements responsive to stress conditions are shown in 5′-flanking region of *psy*. Figure 2 shows

```
−1027 CTGAGACTGC GAGATAAGCC ACCAAAGGCC CAGCAGCAGC AACAGCAGCC TGCTGAGGCA CAGTAGTGTG TCTCCCCCCA TCTGAGCTGT CATCAGCTTA
                                                                                    TCCmotif
−927  CAATTAGTGC ATGTTTTGTC ATGCCAAGTC TACACGATAG CCCTAACGGG TCAGGCAATA ATGTAGGTGG TAGACGGCTC ATGTGCCATG TGCACTGGGT

−827  TTACTCCACA CAACCCAATA ACACAATATG AGTCGGGCCA CTATTGCCCA CTAGTATATT CCCGCTAGTG GCTGCTAGTG GCTACTACTG GTCCGGCTCA
                  MRE
−727  AATGGAGGCC CTAGTGAGGG ACAGGGGGGC TGTGTCCAAG TTCTGTTCGT GCAGCCGCTT TCACCACTGA CATGTCGCAG CACCCGGGCG CCGCCAAGGC
                          GC-box                                                                  GC-box      GC-box
−627  CAAGGCCCCA GCCGGTGCCA GACACTTGCC GGGCTGGGGG CCATGAAAGC TGGAGGCGGA GGTCGGGCAG TGACAATTGT CGTCACCCAT TTGTGCAATT
        GC-box                              GC-box                          C-repeat/DRE
−527  GTCTCACCCG TACCGCAACT TTGACCATTG CAACTTTGTT TGGCAGATAG GGCACGACCG GCCTTGTTAC ATAGGCTTAG TCGCAAATGT CGTTCTCTGT
                                                  I-box
−427  CGCAAATGCA AAGGATATGT TAACGCTTTG TCTTGATTAC GTGCAATGCC AGCACAAGGG TGTTTCGCCA TTTTCTCATC AAGTTGTTAC AATTGCGCCC
                                          ABRE                                                                CAAT box
−327  TGGTTGGCAT AGCATAACTG CCATAGTATA CAGCTCACAG CTGCAATGAC TGCCCAGGTG CTTGGCAAGT GTATAAACTT TGTGACTTCT TCCTTTATGT
                                          CAAT box                                  TATA box
−227  TTTCACTGCA ATTGGCGCCT GATGGACATC TGATTAGAAG CCTGCATAAC CCATTTAGAA GCCTGCAGAC GCTCTGCATC TCGAGGCAAA ATGCTTGAGA

−127  CGCTTTCCAT ATGCGGGCCG GCACGAGGAT CTGAAATGCG CGATTGGCAA TTGGTAGAAG CAATGCAGCA AGGCTGAAGT GTGTTGCTCG TTTGACTCGC
                                    ERE
−27   TGGCCTCACT CCGACTCTGT GATATACATG
                                  M
```

Fig. 2 Nucleotide sequences of the 5′-flanking region of *psy* gene

Nucleotide positions are shown relative to the translation start site which is indicated by a vertical arrow. The putative TATA and CAAT boxes as well as potential *cis*-elements are underlined. The translation initiation codon, ATG, is underlined.

non exhaustive list of some of these elements, previously shown to be involved in various regulatory processes.

To assay the promoter activity of 5′ -flanking region, the histo-chemical staining for *lacZ* activity was detected 48h after transformation. No blue cell appeared in blank and negative control (Figure 3a). However, some blue cells were found in transformed cells (Figure 3b). These results indicated that the 300bp proximal region containing TATA box and CAAT box was sufficient to drive the expression of the reporter gene.

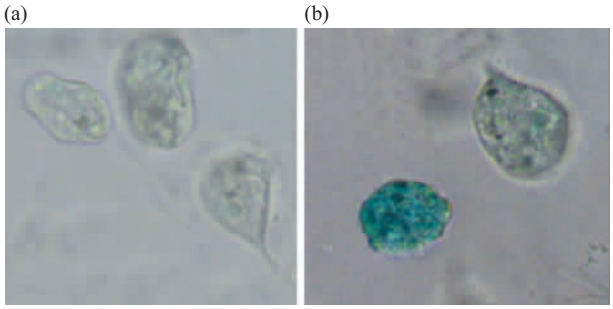

Fig. 3 Transient expression of *lacZ* in *H. pluvialis* driven by 5′-flanking region of *psy*

(a) Negative controls. (b) *lacZ* expression driven by 5′-flanking region of *psy* to drive.

2.3 Sequence alignment and phylogenetic analysis of *psy*

psy genes derived from green algae, higher plants, cyanobacteria, proteobacteia, chlorobi, and some other bacteria were used to construct phylogeny to discern the evolutionary history of *psy* genes. Different methods (NJ, MP) were used to construct phylogenetic trees, which exhibit quite similar topologies (Data not

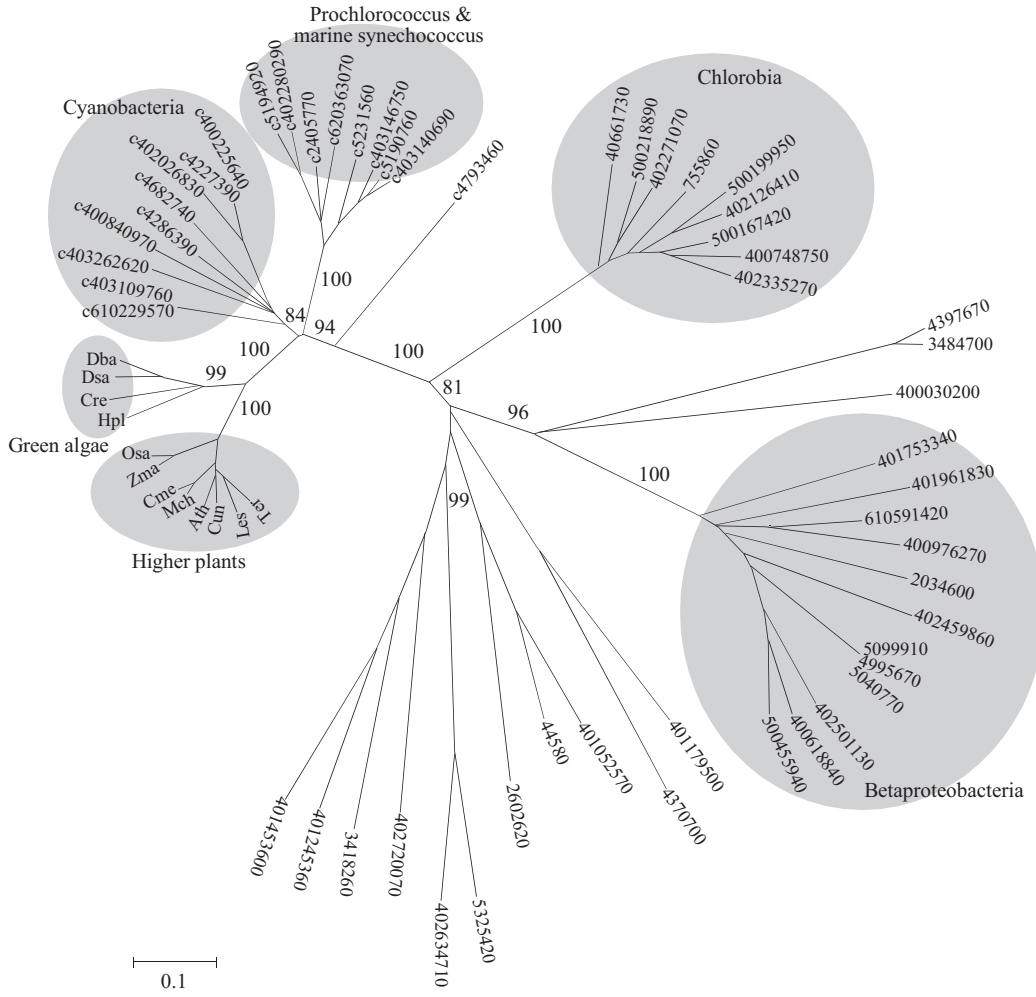

Fig. 4 Phylogenetic tree of PSY sequences from various organisms

Unrooted neighbor-joining tree inferred from poission-corrected evolutionary distances. The numbers on the tree show the levels of bootstrap support based on the 1000 replications. The sequences are as followed: Hpl: *H. pluvialis* AAY53806; Dba: *D. bardawil* T10702; Dsa: *D. salina* AAT28184; Cre: *C. reinhardtii* AAT38475; Osa: *O. sativa* AAK07735; Zma: *Z. mays* AAQ91837; Cun: *C. unshiu* BAB18514; Mch: *M. charantia* AAR86104; Ath: *A. thaliana* AAB65697; Les: *L. esculentum* CAA42969. Besides above abbreviations, other sequence names are obtained from IMG database (http://img.jgi.doe.gov) according to their Gene Object Identifier number.

shown). As shown in Figure 4, all the green algae (Hpl, Cre, Dsa, Dba) form a monophyletic clade with strong bootstrap support (99%), and its closer counterpart is higher plants (Osa, Zma, Cun, Mch, Ath, Les, Ter). Moreover, as anticipated, the sequences from cyanobacteria, betaproteobacteria and chlorobia also fell into distinct branches of the tree (Figure 4). Interestingly, *psy* genes derived from marine *Synechococcus* and *Prochlorococcus* fell into a separate group, outside of the cyanobacterial clade, indicating the closer relationship between these two species and different evolutionary patterns from other cyanobacteria.

Amino acid (aa) sequences were deduced from the DNA sequences. The deduced size of PSY is 400aa, and the putative molecular mass is 45.17 ku. Alignments at the protein level with other green aglae and plants showed the sequences were poorly conserved at the N terminus (Figure 5). The variable sequences were detected in the portions of the signal peptides responsible for the localization of these enzymes in chloroplasts and chromoplasts [20]. The C terminus showing a high intraspecies sequence similarity may be involved in the catalytic activity or carotenoid-recognization/binding[21].

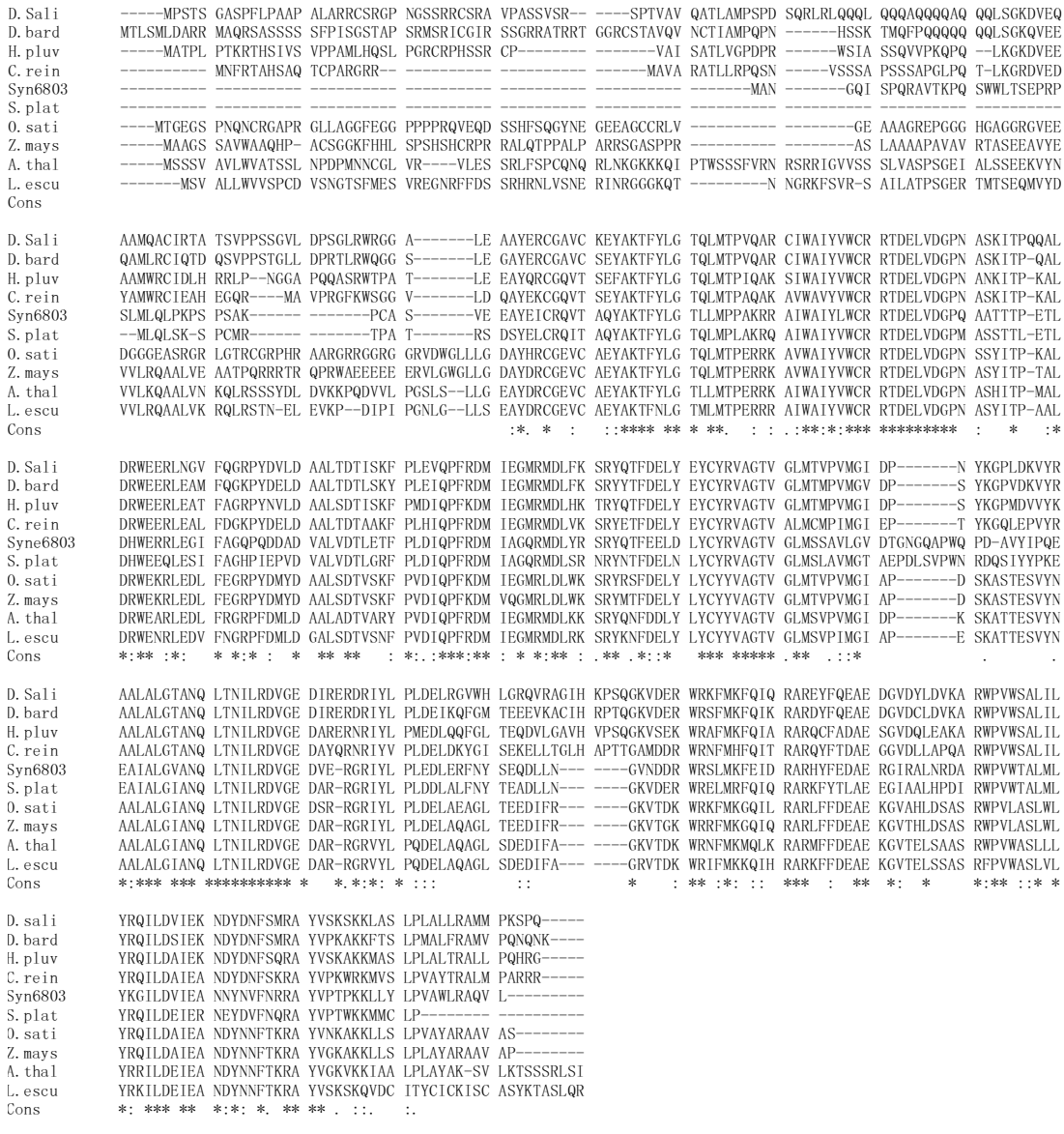

Fig. 5 Multiple alignment of different phytoene synthase

Sequences were obtained from GenBank. Species and Accession numbers are: D.Sali: *D. salina*, AAT28184; D.bard: *D. bardawil* T10702; C.rein: *C. reinhardtii* AAT38475; Syn6803: *Synechocystis* sp. PCC 6803 BAA17848; S.plat: *S. platensis* BAA20384; O.sati: *O. sativa* AAK07735; *Z. mays* AAQ91837; L.escu: *L. esculentum* CAA42969; A.thal: *A. thaliana* AAB65697. Identical amino acid residues among species are indicated by *. Two or one point below residues indicates decreasing amino acid similarity.

3 Discussion

The enzyme phytoene synthase (PSY) catalyzes the first reaction specifically devoted to carotenoid formation and has been considered as a stress responsive gene[10, 11] for a long time. However, little information is available on its molecular regulatory mechanisms of *Haematococcus pluvialis*. Information obtained on *psy* gene and promoter region in this work will help us get more insight into the regulatory mechanisms.

Sequence analysis of the 5′ flanking region showed several potential *cis*-regulatory such as C-reapteat/DRE[22, 23], ABRE (abscisic acid regulated expression)[24,25], ERE(ethylene-regulated expression)[26] and MRE(MYB-core sequence)[27] which were known as the potential elements responsive to stress conditions are shown in 5′ -flanking region of *psy*. In addition, I-box, GC-box and TCCC box involving in light response presented in the 5′ -flanking region. C-repeat/ DRE had been reported to be induced at a transcriptional level under stressed environment such as high salt, lack of water or lower temperature[22, 23]. Some of these *cis*-elements were also found in the 5′ flanking region of *pds*[28] and *bkt*[29] implying that these gene may be regulated by the same environmental factors.

The transient assay showed that the 300 bp proximal region of the 5′ flanking region is sufficient to drive the expression of the reporter gene. This result is in agreement with many other plant promoters where the proximal region of the promoter is generally the main driver of the transcription. Further study on the functions of the *cis*-elements is needed. Alignments at the protein level and nuclear acid level show the *psy* family are conservative over the coding region, more important divergences were observed in upstream region (data were not shown). These observations are in accordance with recent demonstrations that the upstream regulatory regions of genes evolve more rapidly than downstream function of their protein products[30]. With evolutionary time, no new functions are gained, but the *psy* in *H. pluvialis* has different function from the ancestral gene, which maybe result from a subset of promoter elements compared to the ancestral state[31]. The divergences in 5′-flanking regions of PSY family suggest that *psy* has evolved with specific regulatory feature in different organisms.

Fine structure analysis of genomic *psy* gene and its 5′ flanking region will provide the basis for the study on *psy* transcriptional regulation. Work is currently underway to characterization the *cis*-elements and identify trans-acting factors that interact with the putative *cis*-acting motifs. Thus, further study will allow us to investigate molecular regulatory mechanism of astaxanthin biosynthesis in *Haematococcus pluvialis* as response to environmental factors.

References

1 Boussiba S. Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Plant Physiol, 2000, **108** (4): 111～117

2 Lorenz R T, Cysewski G R. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol, 2000, **18** (4): 160～167

3 Chew B P, Park J S, Wong M W. A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice *in vivo*. Anticancer Res, 1999, **19** (3A): 1849～1853

4 Wang X, Willén R, Wadström T. Astaxanthin-rich algal meal and vitamin C inhibit *Helicobacter pylori* infection in Balb/cA mice. Antimicrob Agents Chemother, 2000, **44** (9): 2452～2457

5 Baker R T M, Pfeiffer A M, Schöner F J, *et al*. Pigmenting efficacy of astaxanthin and canthaxanthin in fresh-water reared Atlantic salmon, *Salmo salar*. Anim Feed Sci Tech, 2002, **99** (1): 97～106

6 Guerin M, Huntley M E, Olaizola M. *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol, 2003, **21** (5): 210～216

7 Winston G W, Lemaire D G E, Lee R F. Antioxidants and total oxyradical scavenging capacity during grass shrimp, *Palaemonetes pugio*, embryogenesis. Comp Biochem Physiol C, 2004, **139**(4): 281～288

8 Sandmann G. Carotenoid biosynthesis in microorganisms and plants. Eur J Biochem, 1994, **223** (1): 7～24

9 YanY, Zhu Y H, Jiang J G, *et al*. Cloning and sequence analysis of the phytoene synthase gene from a unicellular chlorophyte, *Dunaliella salina*. J Agric Food Chem, 2005, **53** (5): 1466～1469

10 Bohne F, Linden H. Regulation of carotenoid biosynthesis genes in response to light in *Chlamydomonas reinhardtii*. Biochim Biophys Acta, 2002, **1579** (1): 26～34

11 Steinbrenner J, Linden H. Regulation of two carotenoid biosynthesis genes coding for phytoene synthase and carotenoid hydroxylase during stress-induced astaxanthin formation in the green alga *Haematococcus pluvialis*. Plant Physiol, 2001, **125** (2): 810～817

12 Boussiba S, Vonshak A. Astaxanthin accumulation in the green alga *Haematococcus pluvialis*. Plant Cell Physiol, 1991, **32**: 1077～1082

13 Chenna R, Sugawara H, Koike T, *et al*. Multiple sequences alignment with the clustal series of programs. Nucleic Acids Res, 2003, **31**(13): 3497～3500

14 Thompson J D, Higgins D C, Gibson T J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight

matrix choice. Nucleic Acids Res, 1994, **22** (22): 4673～4680
15 Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. J Mol Evol, 1987, **4** (4): 406～425
16 Kumar S, Tamura K, Jakobsen I B. MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics, 2001, **17** (12): 1244～1245
17 Lescot M, Dehais P, Thijs G, *et al*. PlantCARE: a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res, 2002, **30** (1): 325～327
18 Teng C Y, Qin S, Liu J G, *et al*. Transient expression of *lacZ* in bombarded unicellar green alga *Haematococcus pluvialis*. J Appl Phycol, 2002, **14** (6): 495～500
19 Zimmer W E, Schloss J A, Silflow C D, *et al*. Structural organization, DNA sequence, and expression of the calmodulin gene. J Biol Chem, 1988, **263** (36):19370～19383
20 Cunningham F X, Gantt E. Genes and enzymes of carotenoid biosynthesis in plants. Annu ReV Plant Physiol, 1998, **49**: 557～583
21 Misawa N, Truesdale M R, Sandmann G, *et al*. Expression of a tomato cDNA coding for phytoene synthase in *Escherichia coli*, phytoene formation *in vivo* and *in vitro*, and functional analysis of various truncated gene products. J Biochem, 1994, **116** (5): 980～985
22 Yamaguchi-Shinozaki K, Shinozaki K. A Novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low temperature, or high-salt stress. Plant Cell, 1994, **6** (2): 251～264
23 Baker S S, Wilhelm K S, Thomashow M F. The 5′-region of *Arabidopsis thaliana* core15a has *cis*-acting elements that confer cold-, frought- and ABA-regulated gene expression. Plant Mol Biol, 1994, **24** (5): 701～703
24 Simpson S D, Nakashima K, Narusaka Y, *et al*. Two different novel *cis*-acting elements of *erd1*, a *clpA* homologous *Arabidopsis* gene function in induction by dehydration stress and dark-induced senescence. Plant J, 2003, **33** (2): 259～270
25 Cowan A K, Rose P D. Abscisic acid metabolism in salt-stressed cells of *Dunaliella salina*. Plant Physiol, 1991, **97** (2): 798～803
26 Jung H W, Lim C W, Hwang B K. Isolation and functional analysis of a pepper lipid transfer protein Ⅲ (CALTP Ⅲ) gene promoter during signaling to pathogen, abiotic and environmental stresses. Plant Sci, 2006, **170** (2): 258～266
27 Urao T, Yamaguchi-shinozaki K, Urao S, *et al*. An Arabidopsis myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell, 1993, **5** (11): 1529～1539
28 Liang C W, Zhao F Q, Meng C X, *et al*. Molecular cloning, characterization and evolutionary analysis of phytoene desaturase (PDS) gene from *Haematococcus pluvialis*. World J Microbial Biotechnol , 2006, **22** (1): 59～64
29 Meng C X, Teng C Y, Jiang P, *et al*. Cloning and characterization of beta-carotene ketolase gene promoter in *Haematococcus pluvialis*. Acta Biochim Biophys Sin, 2005, **37** (4): 270～275
30 Pujade-Renaud V, Sanier C, Cambillau L, *et al*. Molecular characterization of new members of the *Hevea brasilensis* hevein multigene family and analysis of their promoter region in rice. Biochim Biophys Acta, 2005, **1727** (3): 151～161
31 Lynch M, Force A. The probability of the duplicate gene preservation by subfunctionalization. Genetics, 2000, **154** (1): 459～473

雨生红球藻八氢番茄红素合成酶基因的克隆及表征 *

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摘要 雨生红球藻是一种单细胞绿藻，在多种逆境胁迫条件下能够大量合成并迅速积累虾青素，其积累量最高可达细胞干重的 4%，从而成为目前最理想的天然虾青素合成工具. 八氢番茄红素合成酶 (PSY) 是虾青素合成途径中第一个限速酶. 分离了八氢番茄红素合成酶基因 (*psy*) 的全长 cDNA 及基因组 DNA. 其全长 cDNA 包括 1 200 个碱基，编码 400 个氨基酸，基因组 DNA 包括 5 个外显子，4 个内含子. 系统发育分析结果显示，绿藻的八氢番茄红素合成酶基因形成一个进化枝，它们与高等植物的 *psy* 亲缘关系比较近. 通过 Genome Walking 的方法，分离了 *psy* 基因约 1 kb 的 5′侧翼序列. 将含有 TATA-box 和 CAAT-box的 297 bp 的序列与 *LacZ* 报告基因构成嵌合的表达载体，用基因枪法转化雨生红球藻. *lacZ* 的瞬间表达检测结果表明，这段上游序列能够驱动 *lacZ* 表达，具有启动子活性.

关键词 雨生红球藻，八氢番茄红素合成酶， 5′侧翼序列，系统发育分析，启动子
学科分类号 Q78

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