DOI号: 10.3724/SP.J.0000.2009.40732

THE CLONING AND PRELIMINARY CHARACTERIZATION OF A C18 :0 Δ9 DESATURASE GENE FROM MARINE MICROALGAE PAVLOVA VIRIDIS

YUAN Dong-Juan[1], ZHOU Ke-Yuan[1], KANG Jing-Xuan[2] and JIANG Li-Ming[1]
(1. *Department of Biochemistry and Molecular Biology, Guangdong Medical College, Zhanjiang* 524023, *P.R. China;*
2. *Department of Medicine, Massachusetts General Hospital, and the Harvard Medical School, Boston, MA* 02114, *USA*)

Abstract: In higher plant, Δ9 desaturase introduces the first double bond into saturated fatty acids, resulting in the corresponding monounsaturated fatty acids. We have cloned gene, designated as *PvfadA*, for C18 0 Δ9 desaturase catalytic activity from *Pavlova viridis* by RT-PCR, RNA ligase mediated RACE (RLM-RACE) and Overlap-PCR strategy. This desaturase, when expressed in *Escherichia coli*, desaturated stearic acid to yield oleic acid, but did not desaturate other fatty acids in *E. coli*. These results indicate that PvFadA is specific to stearic acid. The deduced amino acid sequences of PvFadA show that a putative (D/E) X_2 HX～100 (D/E) X_2 H metal binding motif, which specifically existed in acyl-ACP desaturase. Moreover, Amino acids of PvFadA are similar in part to those of acyl-ACP desaturases from higher plant, such as *Arabidopsis thaliana*, *Oryza sativa* and *Glycine max*, but not to those of acyl-lipid desaturases of *Cyanobacteria*, and acyl-CoA desaturases of higher plant. In addition, the 3D-model structure of PvFadA is composed of 11 helixes, moreover, α3, α4, α6 and α7 form a core 4-helix bundle to regards as an active center in this enzyme, similar as acyl-ACP desaturase of *Ricinus communis* and *Mycobacterium tuberculosis* H37Rv.

Key words: Stearoyl-ACP; Δ9 desaturase; RLM-RACE PCR; *Pavlova viridis*; GC-MS
CLC number: Q176 **Document code:** A **Article ID:** 1000-3207(2009)04-0732-08

C18 0 (Stearic acid) Δ9 desaturase played a key role in the ratio of saturated and unsaturated fatty acids (UFA) of higher plant. The enzymes that catalyze this type of desturation reaction include three types: (1) Δ9 acyl-lipid desaturase, introduce the first double bond at the Δ9 position of a saturated fatty acid that has been esterified to a glycerolipid. And it is bound to plasmatic and thylacoid membranes of cyanobacteria, and endoplasmic reticulum and chloroplast membrane of higher plant. (2) Δ9 acyl-CoA desaturase, fatty acids are desaturated in a CoA-bound form, and which is bound to endoplasmic reticulum and chloroplast membrane of higher plant and animals. (3) Δ9 acyl-ACP desaturase, which is soluble in the stroma of the chloroplast in higher plant, and fatty acid is desaturated in an ACP-bound form, and shows different amino acid sequences compared to the former two[1,2].

Pavlova viridis, Chrysophyta, is a unicell eukaryotic microalga and applied to an essential food organism for early larvae of crustacean, fish and shellfish in aquiculture. The total lipid of *P. viridis* is composed of 46.08% UFA. Thus *P. viridis* always regards as a good source of triacylglycerols, PUFA (polyunsaturated fatty acid) and polar lipids, and attracted increased interest recently because of the possible use of PUFA bioreactor in bioengineer.

Although many acyl-ACP desaturases from higher plant have been cloned, and crystal structures of acyl-

Received date: 2009-01-14; **Accepted date:** 2009-04-22
Foundation item: This work was supported in part by grants from the Guangdong Technology Plan Program (Grant 2005B10401011), Guangdong Science Foundation (Grant 5011595)
Corresponding author: JIANG Li-Ming, E-mail: lmjiang@gdmc.edu.cn

ACP desaturase from *R. communis* and *M. tuberculosis* H37Rv have been elucidated[3,4], searching on the Δ9 desaturase in Genbank, several Δ9 desaturase genes have been isolated from cyanobacteria[5] and *Phaeodactylum tricomutum*[6]. In this report, we cloned a possible acyl-ACP desaturase gene from marine microalgae *P. viridis*.

It is known that both plants and *E. coli* contain type II fatty acid synthases[7,8]. Moreover, the enzymes of higher plant in the biosynthesis of fatty acids are more closely resemble in *E. coli* than in animal. When genes for the Δ6 acyl-ACP desaturase from *Thunbergia alata*[9], Δ9 acyl-ACP desaturase from Sunflower[10] and Δ4 acyl-ACP desaturase from Ivy[11] expressed in *E. coli*, the expressed protein showed a soluble and active form[12]. Therefore, we demonstrate in this paper that an in vivo system for the characterization of plant Δ9 acyl-ACP desaturase activity in *E. coli*.

1 Material and Methods

1.1 Material Marine microalgae *P. viridis* from the Algae Culture Collection of the Institute of Aquatic, Guangdong Ocean University, were grown photoautotrophically at 25℃ in Zhanjiang aquatic 107—118 (Chen, 1998) with shaking several times every day. In the exponential phase of growth, cells were harvested by centrifugation at 2, 500 × g for 10min at 4℃ and vacuum Freeze-Drying at.

Other reagents were of analytical grade or higher. All DNA manipulations and *E. coli* transformations were performed using standard procedures as described[13]. All the assays were repeated three times.

1.2 Cloning of 3′coding sequence PvfadA from P. Viridis Total RNA was extracted from *P. viridis* with Trizol reagent (Invitrogen, USA). First-strand cDNA was synthesized by reverse-transcript with Oligo (dT) primer using SuperScript III Reverse Transcriptase (Invitrogen, USA). 3′end cDNA was amplified using Oligo (dT) primer and gene specific primer (GSP) (5′GTGGAGAATGACGAGTCCTGGCA 3′or 5′GTGGAGAATGACGAGTCATGGCA 3′) derived from GenBank according to conserved sequence of fatty acid desaturase genes. 3′RACE PCR profile was as following: 94℃ for 2 min; 94℃ for 30s, 55℃ for 30s, 68℃ for 2 min (30 cycles) and 68℃ for 10 min. A 1490bp PCR product was determined by agarose gel electrophoresis and cloned into pTOPO TA vector (Invitrogen, USA) and sequenced.

1.3 Rapid amplification of 5′cDNA end For full-length fragment, RNA ligase-mediated rapid amplification of 5′cDNA end (RLM-RACE) was carried out to determine the 5′nucleotide sequence of the desaturase using GeneRacer Kit (Invitrogen, USA). Total RNA was treated with CIP (Calf intestine Alkaline Phosphatase), TAP (tobacco acid pyrophosphatase) and ligated with GeneRacer RNA Oligo at the 5′end of the mRNA using T4 RNA ligase following manufacture's instructions. And then first-strand cDNA synthesis was performed by SuperScript III Reverse Transcriptase (Invitrogen, USA). A first PCR was conducted on first-strand cDNA using reverse GSP1 (5′GGCCCGTCAGGTAGAGGAACTTA-3′) and GeneRacer 5′ Primer. Nested PCR to amplify 5′end cDNA was then performed using GeneRacer nested 5′Primer and reverse nested GSP2 (5′GTGTTCAACAGCGTCTGGTAGGT-3′). Fragment generated was gel-purified using the S.N.A.P. Gel Purification Kit (Invitrogen, USA), cloned into pTOPO TA vector and sequenced.

1.4 Overlap-PCR to ligate 5′and 3′sequence of PvfadA The templates for chimera construction were the plasmids pTOPO TA-3 and pTOPO TA-5 containing 5′and 3′coding sequences, respectively. The fusion primers were used to generate 5′and 3′coding PCR fragments contained a complementary 180bp region superposition located at the 3′end of 5′coding sequence and 5′end of 3′coding sequence, respectively. After purification of agarose gels using S.N.A.P. Gel Purification Kit (Invitrogen, USA), these fragments served as template in a subsequent PCR. The extension of the overlap using pfu polymerase (Stratagen, USA) yielded a recombinant molecule, which was amplified using the outlying primers (sense primer 5′ACGTGGATCCATGCGGGAGCACA-3′ and antisense primer 5′CAGTAAGCTTGTTAGTGCACGCGC-3′, contained *Bam*H I and *Hin*d III restriction sites (underlined)) to yield a corresponding completed cds of *PvfadA* gene. The temperature profile for a fusion PCR was as following: 94℃ for 2 min, 37℃ for 10 min, 68℃ for 2

min; and then 94℃ for 45 s; 55℃ for 30 s, 68℃ for 2 min (30 cycles) and 68℃ for 10 min. The resultant product was subcloned into pET32a + (Novagen) with *Bam*H I and *Hin*d III and the accuracy of chimeric constructs was confirmed by DNA sequencing.

1.5 Expression of recombinant PvFadA The *E. coli* BL21 (DE3) cells was transformed with *PvfadA* gene (pET-32a + -*PvfadA*) and grown at 37℃ induced with IPTG (isopropylthio-β-galactopyranoside) at a final concentration of 0.5mM until cell density reached 0.5 OD600 (optical density at 600 nm). In addition, exogenous fatty acids stearic acid were added to a final culture concentration of 0.05mmol/L to increase the basal level of C18 ∶0 and Tergitol NP-40 (Sigma) at final concentration of 0.2% (v/v), respectively. BL21 cells containing the empty plasmid vector pET-32a (+) was used as a negative control. For assessment of desaturase activity, recombinant *E. coli* cells were carried at 37℃ with shaking (200 rpm) for 2 h and harvested after induced with IPTG.

1.6 Western-blotting analysis 15ug of total protein were electrophoresed on an 11% SDS-PAGE and then transferred to PVDF membrane. Proteins were detected using anti-His antibody (Invitrogen, USA) and antibody conjugated with horseradish peroxidase (Zhongshan, China) and chemiluminescent agent (enhanced chemiluminescence; Zhongshan, China).

1.7 Fatty acid analysis Total fatty acids of *P. viridis* and *E. coli* cells were extracted and methylated as Kang's one-step method[14] and methyl C17 ∶0 and C23 ∶0 as an internal standard, respectively. Fatty acid methyl esters (FAMEs) were analyzed with GC-17A and MS-QP5000 fitted a 30m ×0.25mm (inner diameter) DB-WAX (Supelco, USA) column. Oven temperature was raised from 100℃ at 15℃/min to 220℃ after a 1 min hold and then at 10℃/min to 250℃ after a 13min hold, then holds 15min. Most FAMEs were identified by comparison of retention times to a 37 FAME mix (Supelco). Double bond positions of monounsaturated fatty acid methyl esters were determined by GC-MS (gas chromatography-mass spectrometry).

1.8 Sequence analysis and characterization of PvfadA gene Sequence analysis, signal peptide and secondary-structure prediction were conducted with BLAST program (http://www.ncbi.nlm.nih.gov/) and Anteprot software, respectively. Amino acids sequences of front-end Δ9 acyl-ACP desaturases, acyl-lipid desaturases and acyl-CoA desaturases of different plants were extracted from GenBank, and multiple alignments of representative desaturase genes sequences of these three fatty acid desaturase families were preformed using CLUSTAL X[15].

1.9 Molecular modeling of PvFadA Crystal structure of *R. communis* Δ9 Acyl-ACP desaturase (PDB code: 1afr_A)[16] showed 41% identity with deduced amino acid sequences of *PvfadA* gene. The NCBI blast analysis showed that PvFadA belongs to a protein of ferritin-like superfamily, with E-value of 3.1e-93. Therefore, prediction 3D-model structure of PvFadA was performed by 3D-JIGSAW servers using 1afr_A as model (http://www.bmm.icnet.uk/servers/3djigsaw/)[17—21].

2 Results

2.1 Cloning and sequence analysis of PvfadA gene

Fatty acid profiling of six species of marine microalgae (Zhanjiang, Guangdong, China) have shown that *P. viridis* is a good candidate to discover genes involved in the production and storage of PUFA (data not shown). Fatty acid content of *P. viridis* reached to 5.77% in dry sample. UFA, EPA and DHA content reached to 46.08%, 23.95% and 4.83% of total lipids in *P. viridis*, respectively (Tab. 1).

Tab. 1 GC-MS results of Fatty acid contents in *P. viridis* (%)

Sample	C14 ∶0	C16 ∶0	C16 ∶1	C18 ∶0	C18 ∶1	C18 ∶2	C18 ∶3 (n-6)	C18 ∶3 (n-3)	C20 ∶4	C20 ∶5	C22 ∶6
Pavlova viridis	18.00 ± 1.02	16.34 ± 1.43	12.87 ± 1.52	1.29 ± 0.04	5.42 ± 0.41	1.21 ± 0.03	2.93 ± 0.17	9.94 ± 0.81	3.22 ± 0.11	23.95 ± 3.86	4.83 ± 0.52

Note: Results were averaged and expressed as ±SE (n = 3)

To clone and characterize the *PvfadA* present in *P. viridis*, total RNA was isolated from *P. viridis* and

used for first-strand cDNA synthesis. A 1490bp 3′end cDNA was amplified by 3′-RACE PCR using Oligo (dT) primer and GSP primer designed for desaturase gene. Then, the remainder of a 490bp 5′ORF was obtained by RLM-RACE PCR and nested PCR. And 5′ and 3′-end cDNA were ligated by Overlap-PCR using outlying primers. Thus, a complete cds of Δ9 fad (designated as *PvfadA*, GenBank accession number EU000382) gene was obtained. *PvfadA* was 1700bp, including a putative ORF of 1161bp and a signal peptide of 22bp with a cleavage site located at G22 and S23 by Anteprot[22,23]. The putative protein contains a 387 amino acid residues and isoelectric point 5.795. The deduced amino acids sequence of PvFadA showed a putative specific (D/E) X_2 HX～100 (D/E) X_2 H metal binding motif at residues 160 to 196, which particularly conserved at stearoyl-ACP desaturase family (Fig. 1), whereas no three typical HRX_3 HR, HX_2HH, HX_2 HH clusters of histidine residues, which specifically existed at acyl-lipid desaturase and acyl-CoA desaturase, were found in PvFadA[24] (Fig. 1).

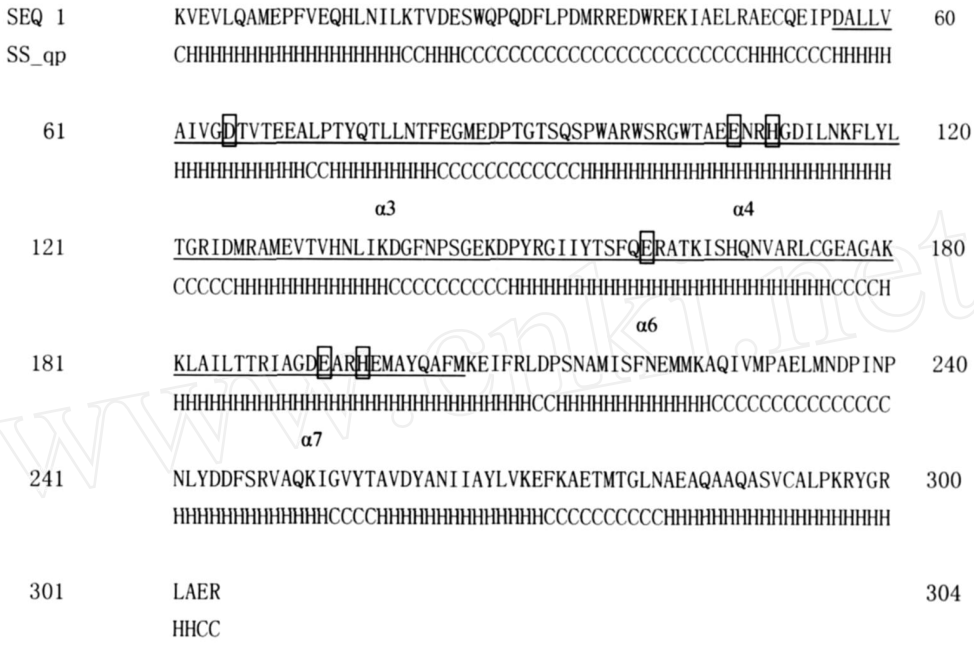

Fig. 1　Prediction of secondary structure of PvFadA

Deduced amino acids sequence (SEQ) and predicted secondary structure (SS_qp) for *PvfadA* gene from residues 1—304. A putative (D/E) X_2HX～100 (D/E) X_2 H metal binding motif in the acyl-ACP desaturase was marked with square. Prediction Ferritin-liked domain was marked with underline. *C* coiled region; *H* α-helix. α3, α4, α6 and α7 composed a prediction bimetal-ion bundle

2.2　Expression of PvFadA in E. coli

Fig. 2A and Fig. 2B showed the PvFadA accumulates to high levels in solution in *E. coli* after centrifugation and identification by SDS-PAGE. PvFadA expression in a soluble and active form in *E. coli* and further assessed by Western-blotting (Fig. 2C). The content of C18 ∶1 Δ9 in PvFadA carrying cells was increased 6.85 folds compared with control, but the contents of 12 ∶0, 14 ∶0, 16 ∶0, 18 ∶1 Δ11 was slightly reduced (Tab. 2). Thus, the PvFadA represents a specific Δ9 acyl-ACP desaturase that is amenable to biochemical investigation. It also provides a system to perform comparative structure-function studies on Δ9 desaturase to gain insights into enzymatic control of regiospecifity with respect to saturated substrates.

2.3　Sequence analysis and characterization of PvFadA

PvFadA were sequenced and shown to be homologous, with a 40.4% identity, to Δ9 acyl-ACP desaturase from *R. communi*. Phylogenetic analysis of 25 species from higher plant contained these three groups of Δ9 desaturase families by Clust X 1.81 showed that PvFadA contained a closer evolution position to acyl-ACP desaturase than acyl-lipid desaturase and acyl-CoA

desaturase (Fig. 3). The Conserved Domain Database (CDD) (http://www.ncbi.nlm.nih.gov/sites/entrez?db=cdd) result of PvFadA showed two putative conserved domains: one belongs to acyl-ACP desaturase family (residues 1—304) and another belongs to a ferritin-like family (residues 56—204) (Fig. 1)[25].

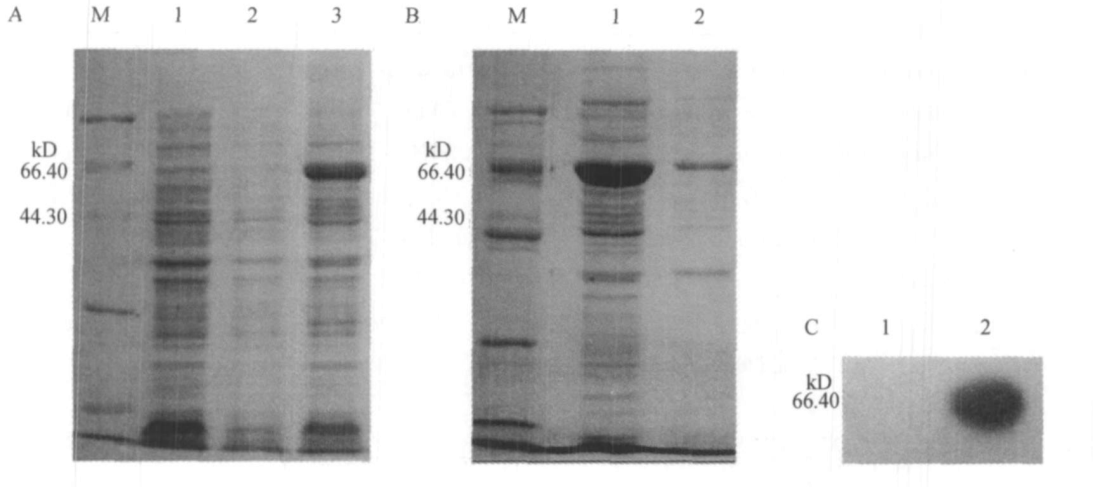

Fig. 2 SDS-PAGE picture of fused expression of PvFadA

1. pET32a+, 2. Non-induced of PvFadA, 3. Induced of PvFadA [A: SDS-PAGE result of PvFadA (66.40kD)]; 1. Supernatant of PvFadA; 2. Sediment of PvFadA [B: SDS-PAGE result for soluble of PvFadA (66.40kD)]; 1. Non-induced of PvFadA; 2. Induced of PvFadA (C: western-blotting result)

Tab. 2 GC-MS results of fatty acid composition in *E. coli* **(nmol)**

	C12:0	C14:0	C16:0	C16:1	C16:2	C18:0	C18:1Δ11	C18:1Δ9
pET32a+	14.69±1.20	25.09±2.41	105.43±6.74	38.97±4.02	4.79±0.36	26.86±3.67	89.53±5.63	2.20±0.25
pET32a-PvFadA	15.01±1.48	24.57±2.89	103.56±4.16	36.43±3.27	4.32±0.60	23.05±3.98	88.37±6.58	15.08±1.47

Note: Results were averaged and expressed as ±SE (n=3)

After searching Swiss-Model program, PvFadA was homologous to 3D-structure (1afr_A) of *R. communi* Δ9 acyl-ACP desaturase, and then, a 3D-model structure of PvFadA, which included 11 α-helixes from N-terminal to C-terminal to form a big helix domain, was simulated by comparative method of 3D-JIGSAW. Nine α-helixes (α3-α11) form a reverse direction parallel α-helical bundle and α3, α4, α6 and α7 form a four-helical bundle structure which is the predicted substrate-combined center after superposition with 1afr_A[26,27] (Fig. 4).

3 Discussion

Fatty acid profiling of *P. viridis* showed it is a good candidate to discover genes involved in the production and storage of PUFA. Although many genes coding for C18:0 Δ9 acyl-ACP desaturase have been cloned from higher plants, such as *Jatropha curcas*, *Glycine max* and *Oryza sativa*, etc[28]. However, there is little known of fatty acid desaturases from *P. viridis* and this is the first report of identification of functional Δ9 desaturase in *P. viridis*. In the present study, we preliminarily characterized the *PvFadA* gene using an expression system under the control of T7 RNA polymerase in *E. coli*. The level of C18:0 decreased and that of C18:1$^{\Delta 9}$ increased 6-fold in the cells that had been transformed with pET32a-*PvFadA*. And there were no significant differences between the C12:0, C14:0, C16:0, C16:1, C16:2, C18:1Δ11 compositions in *E. coli* cells that had been transformed with pET32a+ and pET32a-*PvFadA*. Therefore, it could confirmed that *PvFadA* gene demonstrate a C18:0 Δ9 acyl-ACP desaturase activity specifically.

PvFadA contains an 1161bp putative ORF, corresponding to 387 amino acid residues. A putative (D/E) X_2 HX ~ 100 (D/E) X_2 H metal binding motif (D124, E166, H169, E219, E252 and H255), specifically conserved in acyl-ACP desaturase, which ap-

peared in the amino acid sequence of PvFadA, indicates that PvFadA possibly belongs to soluble acyl-ACP desaturase, but not to transmembrane acyl-lipid desaturase and acyl-CoA desaturase (Fig. 1)[16]. Phylogenetic analysis of Δ9 desaturase further indicated PvFadA contained a closer evolution relationship with Δ9 desaturase of higher plant than acyl-lipid desaturase of cyanobacteria and acyl-CoA desaturase of *B. taurus*.

Crystal structures and site-directed mutagenesis of higher plant acyl-ACP desaturases have provided insight into the structure-function characterization of PvFadA. Detailed comparative analysis and computer modeling

Fig. 3 Phylogenetic relationship of three kinds of Δ9 desaturase inferred from parsimony analysis. Determined using CLUSTAL X 1.81 The length of branches is proportional to the inferred evolutionary distance between desaturases. The Genbank accession number, identity and subcellular location of each desaturase are indicated. 1 is acyl-ACP desaturase, 2 is acyl-CoA desaturase, 3 is acyl-lipid desaturase. *P. viridis*: ABS20117 *Pavlova viridis*; *G. max* 1: AAX86049 *Glycine max*; *A. thaliana* 1: NP186911 *Arabidopsis thaliana*; *M. truncatula* 1: AAY43331 *Medicago truncatula*; *J. curcas* 1: AAY86086 *Jatropha curcas*; *L. comiculatus* 1: AAY78547 *Lotus comiculatus* var. japonicus; *M. unguis* 1: AAC05293 *Macfadyena unguis-cati*; *R. communis* 1: CAA39859 *Ricinus communis*; *H. annuus* 1: AAB65144 *Helianthus annuus*; *O. sativa* 1: BAA07631 *Oryza sativa* (Japonica cultivar-group); *C. reinhardtii* 1: EDP04705 *Chlamydomonas reinhardtii*; *G. hirsutum*: CAA65232 *Gossypium hirsutum*; *T. alata*: AAA61560 *Thunbergia alata* (black-eyed Susan vine); *T. pseudonana* 1: EED86327 *Thalassiosira pseudonana* CCMP1335; *P. tricornutum* 1: XP_002177417 *Phaeodactylum tricornutum* CCAP 1055; *H. sapiens* 2: NP005054 *Homo sapiens*; *D. rerio* 2: NP942110 *Danio rerio*; *A. aegypti* 2: XP001658226 *Aedes aegypti*; *A. thaliana* 2: NP172098 *Arabidopsis thaliana*; *B. Taurus* 2: NP776384 *Bos Taurus*; *P. tricornutum* 2: AAW70158 *Phaeodactylum tricornutum*; *M. musculus* 2: AAR06950 *Mus musculus*; *C. carpio* 2: AAB03857 *Cyprinus carpio*; *A. thaliana* 3: BAA25181 *Arabidopsis thaliana*; *A. variabilis* 3: BAA03434 *Anabaena variabilis*; *Nostoc* sp. 3: CAF18426; *P. hollandica* 3: AAG16761 *Prochlorothrix hollandica*; *S. vulcanus* 3: AAD00699 *Synechococcus vulcanus*; *Synechocystis* sp. PCC 6803 3: BAA03982

www.cnki.net

of 3D-model of PvFadA further demonstrate that a putative ferritin-like domain exists at residues 56—204, α3, α4, α6 and α7 form a typical 4-helical bundle structure with left-handed twist and 1 crossover connection (Fig. 1 and Fig. 4). Moreover, alignment results of PvFadA with stearoyl-ACP desaturase of higher plant indicated another conserved site, WX_2WX_3WTAEE at residues 155 to 162, which localized at α4 domain of metal binding motif in stearoyl-ACP desaturase[16, 29]. Based on the structural superposition of PvFadA with crystal structure of *R. communi*, W103 of WX_2WX_3 WTAEE participates to consist of the substrate channel of PvFadA. Of course, the current work highlights the fact that desaturase function, in terms of regioselectivity, cannot solely be based on prediction from primary amino acid sequence identity. The structure and function characters of PvFadA need further experiments, such as site-directed mutagenesis, to further confirm the prediction.

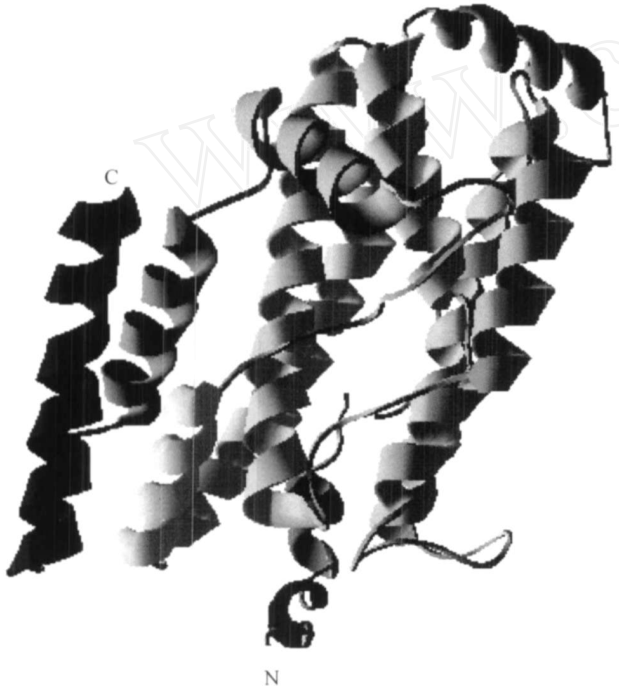

Fig. 4 Proposed structural models for PvFadA
Ribbon diagram of the 3D modeling structure of PvFadA, generated using Swiss-Pdb Viewer

4 Conclusion

The identity of predicted secondary structure, the proposed model, the location of amino acids previously implicated in catalysis and substrate recognition domain, the disposition of the function residues prediction, phylogenetic relationship and NCBI Blast results indicate that 3D-model structure of PvFadA was a kind of a superfamily of acyl-ACP desaturase. GC-MS results showed PvFadA appeared an activity of C18∶0 Δ9 desaturase. Therefore, it could concluded that PvFadA maybe a stearoyl-ACP desaturase in unicell microalgae. PvFadA will extend food-technological applications, and will be useful for studying the biochemical pathway of fatty acid biosynthesis of microalgae.

Acknowledgments

We thank Dr. John Shanklin (Brookhaven National Lab, USA) for helpful suggestions on functional analysis. We are also grateful to Dr. Chun Cai and Lijian Zhang, Guangdong Medical College, for instruction of using GC-MS machine.

References:

[1] Cahoon E B, Ohlrogge J B. Metabolic evidence for the involvement of a [delta]4-palmitoyl-acyl carrier protein desaturase in petroselinic acid synthesis in coriander endosperm and transgenic tobacco cells [J]. *Plant Physiol.* 1994, **104**(3): 827—837

[2] Tocher D R, Leaver M J, Hodgson P A. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases [J]. *Prog Lipid Res*, 1998, **37**: 73—117

[3] Moche M, Shanklin J, Ghoshal A, *et al*. Azide and acetate complexes plus two iron-depleted crystal structures of the di-iron enzyme delta 9 stearoyl-acyl carrier protein desaturase. Implications for oxygen activation and catalytic intermediates [J]. *J Biol Chem.*, 2003, **278**(27): 25072—25080

[4] Dyer D H, Lyle K S, Rayment I, *et al*. X-ray structure of putative acyl-ACP desaturase DesA2 from Mycobacterium tuberculosis H37Rv [J]. *Protein Sci.*, 2005, **14**(6): 1508—1517

[5] Sakamoto T, Wada H, Nishida I, *et al*. Delta 9 Acyl-lipid desaturases of cyanobacteria. Molecular cloning and substrate specificities in terms of fatty acids, sn-positions, and polar head groups [J]. *J Biol Chem.*, 1994, **269**(41): 25576—25580

[6] Domergue F, Spiekermann P, Lerchl J, *et al*. New insight into Phaeodactylum tricornutum fatty acid metabolism. Cloning and functional characterization of plastidial and microsomal delta12-fatty acid desaturases [J]. *Plant Physiol.* 2003, **131**(4): 1648—1660

[7] Magnuson K, Jackowski S, Rock C O, *et al*. Regulation of fatty acid biosynthesis in *Escherichia coli* [J]. *Microbiol Rev.*, 1993, **57**(3): 522—542

[8] Ohlrogge J, Browse J. Lipid biosynthesis [J]. *Plant Cell*, 1995, **7**(7): 957—970

[9] Cahoon E B, Mills L A, Shanklin J. Modification of the fatty acid composition of Escherichia coli by coexpression of a plant ac-

yl-acyl carrier protein desaturase and ferredoxin [J]. *Journal of Bacteriology*, 1996, **178**(3): 936—939

[10] Serrano-Vega M J, Venegas-Calerón M, García R, *et al*. Cloning and expression of fatty acids biosynthesis key enzymes from sunflower (*Helianthus annuus* L.) in *Escherichia coli* [J]. *Journal of Chromatography* B, 2003, **786**(1—2): 221—228

[11] Whittle E, Cahoon E B, Subrahmanyam S, *et al*. A multifunctional acyl-acyl carrier protein desaturase from *Hedera helix* L. (English Ivy) can synthesize 16-and 18-carbon monoene and diene products [J]. *J Biol Chem*., 2005, **280**(31): 28169—28176

[12] Kater M M, Koningstein G M, Nijkamp H J, *et al*. The use of a hybrid genetic system to study the functional relationship between prokaryotic and plant multi-enzyme fatty acid synthetase complexes [J]. *Plant Mol Biol*., 1994, **25**(5): 771—790

[13] Sambrook J, Russell D W. Molecular Cloning: A Laboratory Manual, 3rd ed [M]. New York: Cold Spring Harbor Press. 2001

[14] Kang J X, Wang J. A simplified method for analysis of polyunsaturated fatty acids [J]. *BMC Biochem*, 2005, **6**: 5

[15] Thompson J D, Gibson T J, Plewniak F, *et al*. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools [J]. *Nucleic Acids Res*, 1997, **25**: 4876—4882

[16] Lindqvist Y, Huang W, Schneider G, *et al*. Crystal structure of delta 9 stearoyl-acyl carrier protein desaturase from castor seed and its relationship to other di-iron proteins [J]. *EMBO J*, 1996, **15**: 4081—4092

[17] Bryson K, McGuffin L J, Marsden R L, *et al*. Protein structure prediction servers at University College London [J]. *Nucl. Acids Res*, 2005, **33**: W36—38

[18] McGuffin L J, Bryson K, Jones D T. The PSIPRED protein structure prediction server [J]. *Bioinformatics*, 2000, **16**: 404—405

[19] Jones D T. Protein secondary structure prediction based on position-specific scoring matrices [J]. *J. Mol. Biol*, 1999, **292**: 195—202

[20] Jones D T. GenTHREADER: an efficient and reliable protein fold recognition method for genomic sequences [J]. *J. Mol. Biol*, 1999, **287**: 797—815

[21] McGuffin L J, Jones D T. Improvement of the GenTHREADER method for genomic fold recognition [J]. *Bioinformatics*, 2003, **19**: 874—881

[22] Deleage G., Clerc F F, Roux B, *et al*. ANTHEPROT: a package for protein sequence analysis using a microcomputer [J]. *Comput Appl Biosci*, 1988, **4**(3): 351—363

[23] Deleage G, Combet C, Blanchet C, *et al*. ANTHEPROT: an integrated protein sequence analysis software with client/server capabilities [J]. *Computers in Biology and Medicine*, 2001, **31**: 259—267

[24] Shanklin J, Somerville C. Stearoyl-Acyl-Carrier-Protein desaturase from higher plants is structurally unrelated to the animal and fungal homologs [J]. *Proc. Natl. Acad. Sci*. USA, 1991, **88**: 2510—2514

[25] Fox B G, Shanklin J, Somerville C, *et al*. Stearoyl-Acyl carrier protein Δ9 desaturase from ricinus communis is a Diiron-Oxo protein [J]. *Proc. Natl. Acad. Sci*. USA, 1993, **90**: 2486—2490

[26] Cahoon E B, Lindqvist Y, Schneider G, *et al*. Redesign of soluble fatty acid desaturases from plants for altered substrate specificity and double bond position [J]. *Proc. Natl. Acad. Sci*. USA, 1997, **94**: 4872—4877

[27] Edward W, Shanklin J. Engineering Δ9-16 0-Acyl Carrier Protein (ACP) desaturase specificity based on combinatorial saturation mutagenesis and logical redesign of the castor Δ9-18 0-ACP desaturase [J]. *J Biol Chem*, 2001, **276**: 21500—21505

[28] Tong L, Shu-Ming P, Wu-Yuan D, *et al*. Characterization of a new stearoyl-acyl carrier protein desaturase gene from *Jatropha curcas* [J]. *Biotechnology Letter*, 2006, **28**(9): 657—662

[29] Dyer D H, Lyle K S, Rayment I, *et al*. X-ray structure of pupative ayl-ACP desaturase DesA2 from Mycobacterium tuberculosis H37Rv [J]. *Protein Sci*., 2005, **14**(6): 1508—1517

绿色巴夫藻脂肪酸去饱和酶的克隆和初步研究

元冬娟[1] 周克元[1] 康景轩[2] 江黎明[1]

(1. 广东医学院生物化学与分子生物学教研室, 湛江 524023; 2. 哈佛大学医学院麻省总医院, 波士顿 02114)

摘要:在高等植物中,Δ9脂肪酸去饱和酶引入第一个双键到饱和的脂肪酸链中,导致单不饱和脂肪酸的形成。我们通过 RT-PCR、RNA ligase mediated RACE (RLM-RACE) and Overlap-PCR方法从海洋微藻绿色巴夫藻中克隆到一个命名为 *PvfadA*的脂肪酸去饱和酶候选基因。通过将 *PvfadA*基因在大肠杆菌表达系统中成功表达, PvFadA可以特异性地将 C18 0脂肪酸转变成 C18 1脂肪酸。PvFadA的氨基酸序列中存在一个存在于 acyl-ACP去饱和酶的特异性金属离子结合区段 (D/E) X_2 HX～100 (D/E) X_2 H。通过同源模建 PvFadA的 3D结构显示,其包含了 11个 α螺旋,其中 α3、α4、α6和 α7组成了一个 4个螺旋桶的核心结构,预测其可能是酶的活性中心。PvFadA的 3D结构类似于蓖麻和结核分枝杆菌 H37Rv的 acyl-ACP去饱和酶。

关键词:硬脂酰-ACP;Δ9去饱和酶; RLM-RACE PCR;绿色巴夫藻; GC-MS