Chinese Journal of Oceanology and Limnology
Vol. 34 No. 4, P. 772-780, 2016
http://dx.doi.org/10.1007/s00343-016-4369-x

Production of γ-linolenic acid and stearidonic acid by *Synechococcus* sp. PCC7002 containing cyanobacterial fatty acid desaturase genes*

DONG Xuewei (董学卫)[1], HE Qingfang (何庆芳)[2, 3], PENG Zhenying (彭振英)[2], YU Jinhui (于金慧)[2], BIAN Fei (边斐)[2], LI Youzhi (李有志)[1, **], BI Yuping (毕玉平)[2, **]

[1] *College of Life Science and Technology, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi University, Nanning 530004, China*

[2] *Biotechnology Research Center, Shandong Academy of Agricultural Science, Key Laboratory for Genetic Improvement of Crop, Animal and Poultry of Shandong Province, Key Laboratory of Crop Genetic Improvement and Biotechnology, Huanghuaihai, Ministry of Agriculture, Jinan 250100, China*

[3] *Department of Applied Science, University of Arkansas, Little Rock, Arkansas 72204, US*

Received Dec. 15, 2014; accepted in principle Feb. 25, 2015; accepted for publication May 8, 2015

Abstract Genetic modification is useful for improving the nutritional qualities of cyanobacteria. To increase the total unsaturated fatty acid content, along with the ratio of ω-3/ω-6 fatty acids, genetic engineering can be used to modify fatty acid metabolism. *Synechococcus* sp. PCC7002, a fast-growing cyanobacterium, does not contain a Δ6 desaturase gene and is therefore unable to synthesize γ-linolenic acid (GLA) and stearidonic acid (SDA), which are important in human health. In this work, we constructed recombinant vectors Syd6D, Syd15D and Syd6Dd15D to express the Δ15 desaturase and Δ6 desaturase genes from *Synechocystis* PCC6803 in *Synechococcus* sp. PCC7002, with the aim of expressing polyunsaturated fatty acids. Overexpression of the Δ15 desaturase gene in *Synechococcus* resulted in 5.4 times greater accumulation of α-linolenic acid compared with the wild-type while Δ6 desaturase gene expression produced both GLA and SDA. Co-expression of the two genes resulted in low-level accumulation of GLA but much larger amounts of SDA, accounting for as much to 11.64% of the total fatty acid content.

Keyword: *Synechococcus* sp. PCC7002; *Synechocystis* sp. PCC6803; Δ15 fatty acid desaturase; Δ6 fatty acid desaturase; polyunsaturated fatty acids

1 INTRODUCTION

Polyunsaturated fatty acids (PUFA) are important for human health and nutrition. They are cellular components conferring membrane fluidity, as well as precursors for groups of eicosanoids that regulate physiological processes in mammals (Lee et al., 2009; Lenihan-Geels et al., 2013). An unbalanced intake of PUFA is significantly associated with occurrence and development of cardiovascular disease, cardiac arrest, and mental illness (Khozin-Goldberg et al., 2011; Lazic et al., 2014).

The predominant sources of the PUFA are oils from plants, including borage, evening primrose, black currant and soybean. In addition, some species of microorganisms, such as *Mortierella* and *Mucor* spp. are also used to produce PUFA (Bakowska-Barczak et al., 2009; Harris, 2012; Tasset-Cuevas et al., 2013; Simon et al., 2014). However, these sources cannot meet the growing demand for nutritional sources of PUFA because of resource constraints, fluctuations in their availability, production/purification costs, and safety concerns. Thus, there is increasing interest in developing more reliable and economical alternative sources of γ-linolenic acid

* Supported by the International S&T Cooperation Program of China (No. 2012DFA30450), the National Natural Science Foundation of China (No. 30871541), the Taishan Scholar Foundation of Shandong Province (No. tshw20091014), and the Innovation Program of the University Institutes of Jinan, Shandong Province (No. 201004044)
** Corresponding authors: dyzl@gxu.edu.cn; yupingbi@vip.sina.com

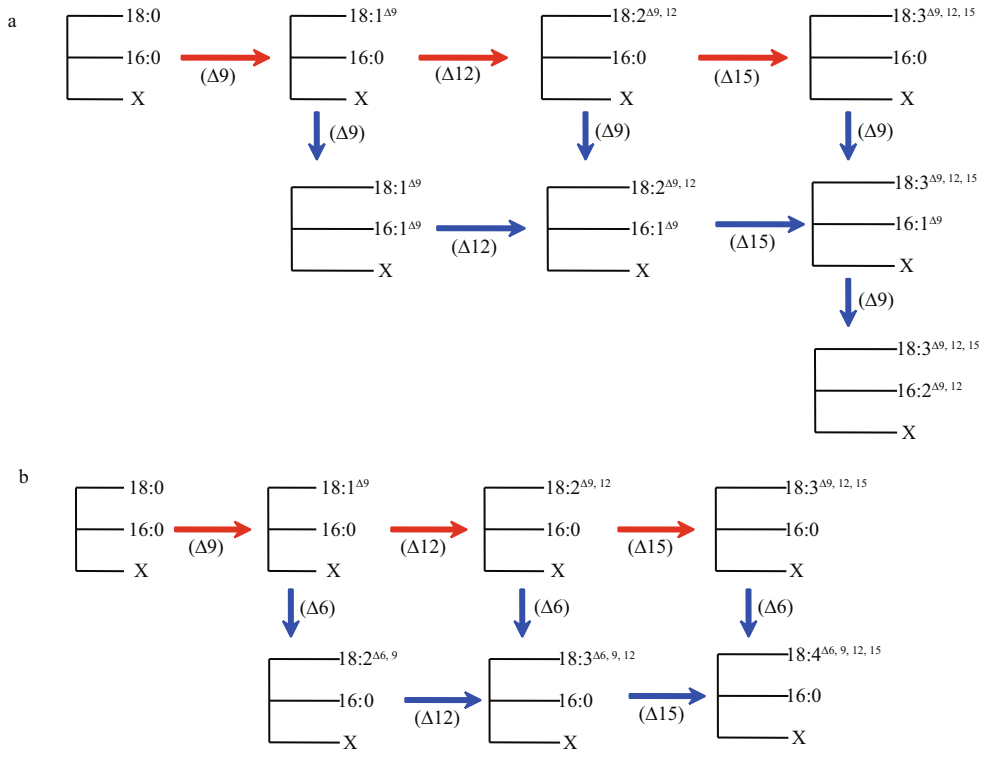

Fig.1 Acyl-lipid desaturation of fatty acids in *Synechococcus* sp. PCC7002 (a) and *Synechocystis* sp. PCC6803; (b) red arrows: desaturation occurring in monogalactosyldiacylglycerol (MGDG), sulfoquinovosyl diacylglycerol, and propylene glycol; blue arrows, desaturation occurring only in MGDG

Numbers in parentheses indicate the positions at which a double bond is introduced (Murata and Wada, 1995).

(GLA) and other PUFA. In particular, modification of microbial fatty acid biosynthetic pathway (especially in cyanobacteria) by genetic manipulation is a promising alternative (Graham et al., 2007; Meesapyodsuk and Qiu, 2012).

Cyanobacteria have a simple internal structure lacking a nucleus and membrane-bound organelles such as mitochondria. They grow faster than food crops, but their lipid content is low (ranging from 5%–13%) (Becker et al., 1994). Unicellular species of cyanobacteria are generally deficient in PUFA, although some species, such as *Spirulina platensis*, *Synechocystis* sp. PCC6803, and *Tolypothrix tenuis*, contain low levels of α-linolenic acid (ALA) and stearidonic acid (SDA) (Murata et al., 1996; Maslova et al., 2004; Chen et al., 2014). In most cyanobacteria, the primary biosynthetic product of fatty acid metabolism is the monounsaturated, 18-carbon fatty acid, oleic acid (OA). Desaturation steps at the Δ12 and Δ15 positions, or at the Δ6, Δ12, and Δ15 positions, which are required for the production of GLA and SDA from OA, respectively, are shown in Fig.1 (Murata and Wada, 1995).

In the past decades, researchers have rationally designed and engineered strains of cyanobacteria for the synthesis of these important fatty acids (Takeyama et al., 1997; Liu et al., 2011; Chen et al., 2014). Chen et al. (2014) found that transgenic expression of Δ6 and Δ15 fatty acid desaturases enhanced accumulation of specific ω-3 PUFA in *Synechocystis* sp. PCC6803 (up to 8.9 mg/L of ALA and 4.1 mg/L of SDA). Takeyama et al. (1997) isolated the eicosapentaenoic acid (EPA)-synthesis gene cluster from *Shewanella putrefaciens* and expressed it in *Synechococcus* sp. The EPA yield was further improved to 0.64 mg/g dry weight by incubation of cyanobacterial transconjugants harbouring pJRDEPA for 1 day at 17°C. Liu et al. (2011) applied a fatty acid uncoupling strategy in *Synechocystis* sp. PCC6803 to overproduce and secrete free fatty acids. In this case, the fatty acid secretion yield was increased to 197 mg/L.

Although some cyanobacteria and microalgae are rich in PUFA, slow growth and relatively low cell densities have limited their industrial application (Khozin-Goldberg et al., 2011). *Synechococcus* sp. PCC7002 (previously known as *Agmenellum quadruplicatum* PR-6) (Möllers et al., 2014), a euryhaline cyanobacterium, can grow over a wide

Table 1 Primers used for amplifying the *rbcL* promoter, Δ15 desaturase gene (U36389.1*) and Δ6 desaturase gene (L11421.1*)

Primer set	Primer name	Sequence (5′→3′)
A	PrbcL-1	GACAGCACCCAAGAGGCA
	PrbcL-2	GCGGTTTTCCTCCAGCAAAAATGCT
B	DesB-1	TGCTGGAGGAAAACCGCATGCAAAGTACAGTCCG
	DesB-flag	TTACTTATCGTCGTCATCCTTGTAATCTTTTTTCGGCTGGTAAT
C	DesD-1	TAAGGAATTATAACCAAATGCTAACAGCGGAAAG
	DesD-his	TCAATGATGATGATGATGATGCGATGCTTTGCCCAT
D	DesB-2	TTGGTTATAATTCCTTATTATTTTTTCGGCTGGTAAT
	DesD-rbcl	TGCTGGAGGAAAACCGCATGCTAACAGCGGAAAG
	DesD-his	TCAATGATGATGATGATGATGCGATGCTTTGCCCAT

*GenBank accession number.

range of NaCl concentrations and can tolerate high-light irradiation (Bernstein et al., 2014; Therien et al., 2014). As a naturally transformable cyanobacterium, it has been extensively used to generate a range of commercially significant products, including biosensors, hydrogen, limonene, and mannitol (Boyanapalli et al., 2007; McNeely et al., 2010; Davies et al., 2014; Jacobsen and Frigaard, 2014). In *Synechococcus* sp. PCC7002, linoleic acid (LA) and ALA, precursors of most PUFA, can be synthesized from OA via the action of two enzymes, Δ12 and Δ15-desaturases. However, it cannot convert LA and ALA to GLA and SDA, respectively, because it does not contain Δ6 desaturase. In this work, we describe the expression of cyanobacterial desaturase genes in *Synechococcus* sp. PCC7002 to modify the C18 desaturation pathway towards GLA and SDA accumulation, with the aim of obtaining nutritionally superior cyanobacterial oil. In addition, the Δ6 and Δ15 desaturase-expressing strain can be used as a platform to express other genes involved in PUFA biosynthesis.

2 MATERIAL AND METHOD

2.1 Cyanobacteria materials

For cloning of the *rbcL* promoter (PrbcL) and the Δ15 desaturase gene (GenBank accession number: U36389.1), as well as for transformation, *Synechococcus* sp. PCC7002 was cultured in 150 mL of autoclaved Medium A^+ described by Stevens et al. (1973) in 250-mL glass flasks at 30±1°C with a 16:8 photoperiods. The cultures were bubbled with air under a light intensity of 60.5 μmol photons/(m^2·s). Cell growth was monitored by measuring optical density at 750 nm (OD_{750}) using a UV-visible spectrophotometer UV-723 (Shanghai Spectrum Instruments Co., Shanghai, China). Both wild-type and transgenic *Synechococcus* sp. PCC7002 strains were cultured in triplicate. *Synechocystis* sp. PCC6803, grown in BG11 culture medium described by Stanier et al. (1971) at 30±1°C with continuous illumination under a light intensity of 40 μmol photons/(m^2·s) was used to the clone the Δ6 desaturase gene (GenBank accession number: L11421.1).

2.2 Vector construction

Synechococcus and *Synechocystis* in logarithmic growth phase were collected by centrifugation at 6 000×*g* for 5 min and used for extraction of total genomic DNA with phenol:chloroform (1:1, v/v) described by Nakamura et al. (1998). Overlap extension PCR was used to construct the PrbcL/DesB, PrbcL/DesD, and PrbcL/DesB/DesD cassettes using the primer sets given in Table 1. Taking the construction of vector Syd6Dd15D as an example, the *rbcL* promoter and the Δ15 desaturase gene (*desB*), from *Synechococcus*, along with the Δ6 desaturase gene (*desB*) from *Synechocystis*, were amplified by PCR from their respective genomic DNA templates. PCRs were performed in a volume of 25 μL containing 2.5 μL 10× PCR buffer, 5 U of *Taq* DNA polymerase (TaKaRa Bio), approximately 25 ng of genomic DNA, 2.5 mmol/L dNTP mixture, and 25 pmol of specific primers (primer sets shown in Table 1) in sterile distilled water. The thermal cycler parameters were: 94°C for 6 min, 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final extension of 72°C for 10 min.

The resulting PrbcL and DesB amplicons were mixed in a molar ratio of 1:1 for overlap extension PCR with primers PrbcL-1 and DesB-2. The overlap

extension PCR protocol was similar to that in the Δ6 desaturase gene amplified except that it used the *LA Taq* polymerase (TaKaRa, Dalian, China) instead of *Taq* polymerase and amplified by two-step PCR. The first step without primers, thermal cycler parameters were: 94°C for 5 min, 10 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 5 min, and a final extension of 72°C for 10 min. The second step with primers, thermal cycler parameters were: 94°C for 5 min, 25 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 3.5 min, and a final extension of 72°C for 10 min. The resulting PrbcL/DesB fragment was then mixed 1:1 with the DesD amplicon and subjected to overlap extension PCR with primers PrbcL-1 and DesD-his, the overlap extension PCR protocol was the same to above. The PrbcL/DesB/DesD product was cloned into T-vector pMDl8-T (TaKaRa Bio, Dalian, China) following the manufacturer's instructions and confirmed by sequencing at the Biotechnology Research Center, Shandong Academy of Agricultural Sciences (Jinan, China). The *luxAB*-Ω cassette, excised by *Dra*I digestion from pRL57 (Bernstein et al., 2014), was then ligated into the plasmids, which had been digested with *Xho*I and *Sma*I and blunted with T4 DNA polymerase. Similar approaches were used to construct Syd6D and Syd6Dd15D.

2.3 Transformation and screening of transformants

The vectors were transformed into *Synechococcus* cells as described by Chen et al. (2014), and positive colonies were selected on Medium A^+ agar plates containing spectinomycin (12.5 μg/mL). The resulting colonies were continuously transferred onto selective agar plates containing high concentrations of spectinomycin (up to 50 μg/mL). The transformants were then cultured in liquid medium containing 50 μg/mL spectinomycin for analysis.

2.4 PCR analysis of transgenic *Synechococcus*

Total DNA was isolated from the wild-type and transgenic *Synechococcus* strains according to the method of Chen et al. (2014), and RNA was removed using RNase A (TransGen Biotech, Beijing, China). The PCR protocol was the same with that of the Δ6 desaturase gene amplified as we mentioned in 2.2. PCR products were verified on a 1% agarose gel.

2.5 Fatty acid extraction and analysis

Cyanobacterial cells were harvested by centrifugation at 6 000×*g* for 5 min, washed twice with distilled water, and then dried at 40°C for 24 h. Oil was extracted from the cyanobacteria using a previously described method (Bligh and Dyer, 1959). Briefly, the dried cell powder was resuspended in 4 mL chloroform/methanol (1:10 v/v) and then sonicated at 70 Hz using a Transonic model 460/H sonicator (Elma, Singen, Germany) at room temperature. The cell debris was pelleted by centrifugation at 2 000×*g* for 10 min. The supernatant was then mixed with 5 mL of 7% KOH-methanol solution and incubated for 10 min at room temperature. The samples were then centrifuged at 10 000×*g* for 10 min prior to analysis.

Fatty acid methyl esters were analyzed by gas chromatography (Perkin Elmer Clarus 500) coupled to a flame ionization detector. Samples were injected into a TG-WAXMS polyethylene glycol capillary column (30 m×0.32 mm inner diameter; 250 nm film thickness; Thermo Scientific, USA) at an oven temperature of 140°C which was held for 5 min. Immediately after injection, the temperature was increased at 4°C/min to 240°C, which was held for 15 min. Nitrogen was used as the carrier gas. The proportion of each fatty acid was calculated based on the integrated peak area of the corresponding fatty acid methyl ester as a percentage relative to the sum of integrated peaks by TotalChrom Ver 6.3.1 Chromatography Data System (PerkinElmer, MA, US).

2.6 Statistical analysis

All results are reported as the mean±standard deviation. All data were assessed by one-way ANOVA (analysis of variance) and Duncan's multiple range test ($P<0.05$) using SPSS version 10 software (SPSS, Chicago, IL, USA). Significant differences were identified using ANOVA with 95% confidence (probability limit of $P<0.05$).

3 RESULT

3.1 Construction of expression vectors

The C18 fatty acid profiles showed that the *Synechococcus* sp. PCC7002 had no detectable GLA although ALA was discernible. However, distinct peaks for GLA and SDA were observed from the fatty acid extracts of *Synechocystis* sp. PCC6803 (Fig.2). As Δ15 fatty acid desaturase can increase levels of ω-3 fatty acids (Wang et al., 2014), we constructed the Syd6D, Syd15D, and Syd6Dd15D expression cassettes containing the Δ6 desaturase gene from

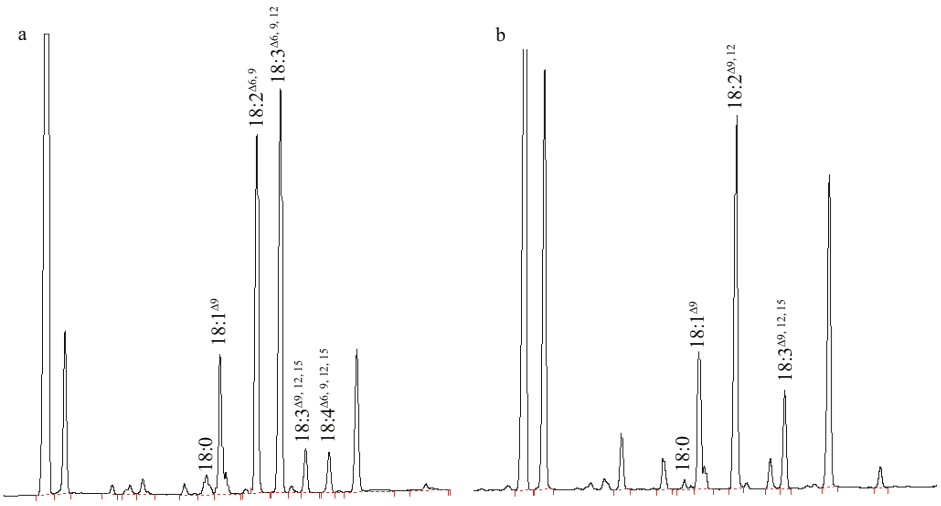

Fig.2 Fatty acid profiles of wild-type of *Synechocystis* sp. PCC6803 (a) and *Synechococcus* sp. PCC7002 (b)

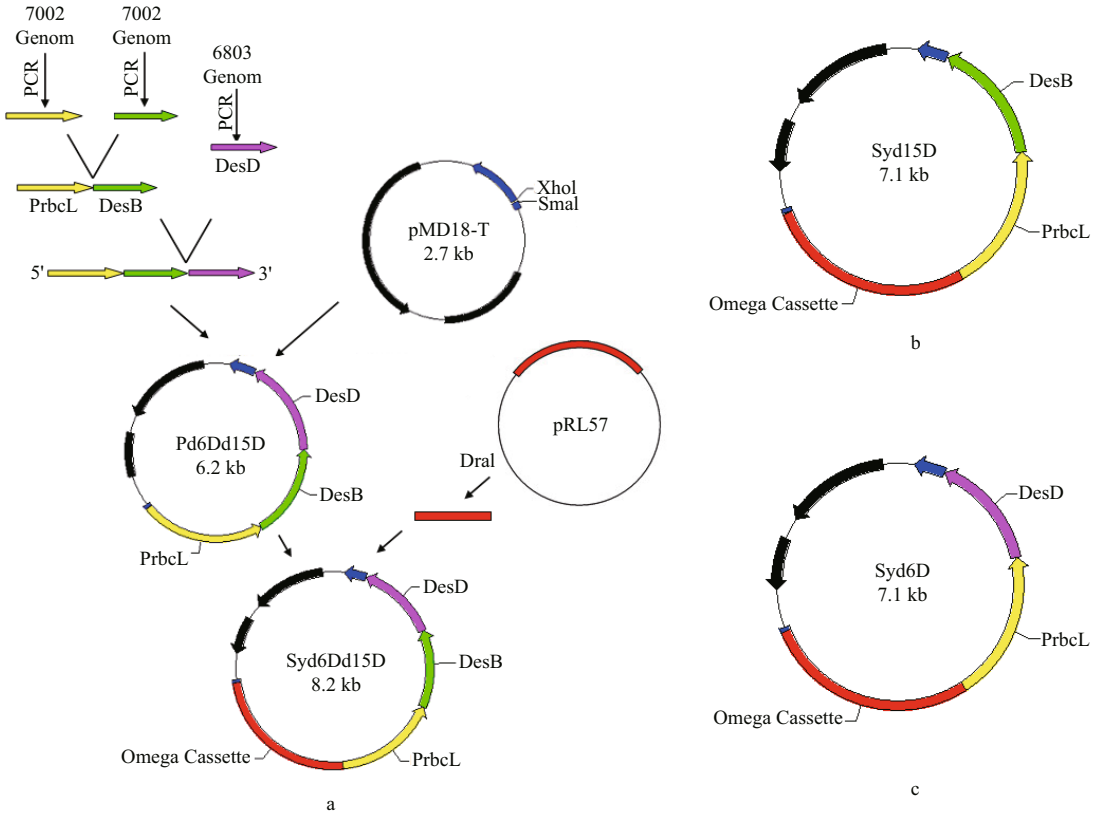

Fig.3 Schematic diagram showing the construction of plasmids used in the expression of the Δ6 and Δ15 desaturase genes in *Synechococcus* sp. PCC7002

The stem-loop structure of the omega cassette, which is also a spectinomycin-resistance marker, represents the shorter inverted repeat sequences where transcription can be terminated.

Synechocystis and the Δ15 desaturase gene from *Synechococcus*. These genes were under the control of PrbcL, and were expressed in *Synechococcus* sp. PCC7002 (Fig.3).

3.2 PCR analysis of *Synechococcus* transformants

Spectinomycin-resistant colonies showed single amplification products with the expected sizes

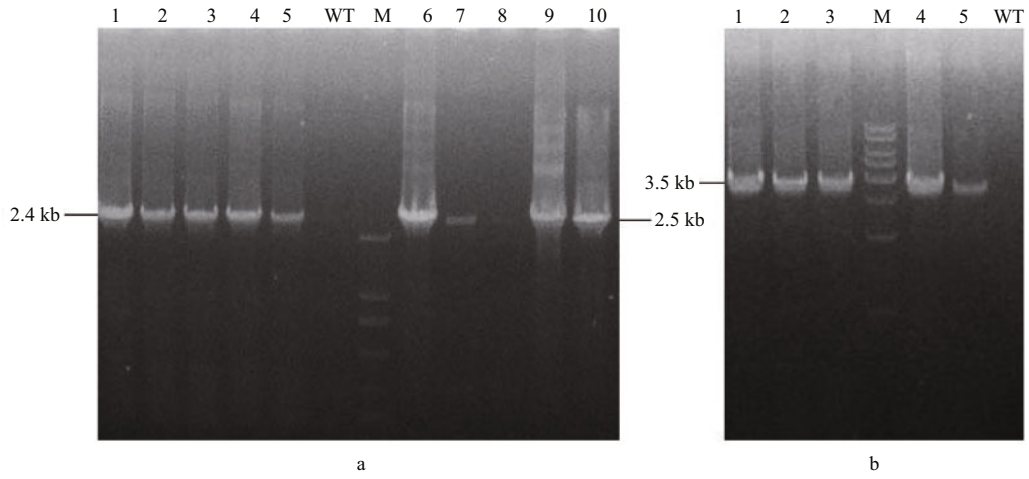

Fig.4 Results of PCR analysis of transgenic *Synechococcus*

a. lanes 1–5: transformants containing Syd15D; WT: wild-type; M: trans2K marker; lanes 6–10: transformants containing Syd6D; b. lanes 1–5: transformants containing Syd15Dd6D; WT: wild-type; M: trans15K marker.

Table 2 Fatty acid content and composition of wild-type and transgenic *Synechococcus* sp. PCC7002

Fatty acid	Syd6Dd15D (%)	Syd6D (%)	Syd15D (%)	WT (%)
16:0	55.03±1.6^{d}	47.31±7.3^{c}	37.60±3.0^{a}	40.38±1.01^{b}
16:1	13.60±0.3^{a}	19.95±2.1^{b}	21.57±5.8^{c}	22.45±3.4^{d}
18:0	2.02±1.2^{c}	2.18±0.15^{c}	0.97±0.01^{a}	1.46±0.2^{b}
18:1	8.06±0.90^{b}	4.14±0.4^{a}	8.63±0.7^{c}	8.83±0.2^{c}
18:2 (6, 9)	2.36±0.4^{b}	5.90±0.3^{c}	ND	ND
18:2 (9, 12)	0.51±0.19^{a}	5.71±0.34^{c}	2.90±0.57^{b}	21.65±3.2^{d}
18:3 (9, 12, 15)	6.57±0.7^{c}	2.19±0.13^{a}	28.33±2.3^{d}	5.24±0.3^{b}
18:3 (6, 9, 12)	0.22±0.04^{b}	11.36±1.8^{c}	ND	ND
18:4 (6, 9, 12, 15)	11.64±1.55^{b}	1.25±0.32^{c}	ND	ND

Each value is the means±SD of six independent experiments; ND: not detected. Different letters indicate the statistic difference at a level of $P<0.05$.

(~2.4 kb, ~2.5 kb, and 3.5 kb), whereas no product was obtained in the untransformed control (Fig.4). These results confirmed the presence of Syd15D, Syd6D, and Syd6Dd15D, respectively, in the transformed cyanobacterial strains.

3.3 Analysis of growth and fatty acid compositions of wild-type and transgenic *Synechococcus*

The growth curves and lipid compositions of the wild-type and transformants are shown in Fig.5 and 6 and Table 2. As shown in Fig.5, the specific growth rates for the Syd6D, Syd15D, and Syd6Dd15D transformants and the wild-type *Synechococcus* strains were 0.48, 0.49, 0.50, and 0.46 OD_{750nm}/d, respectively. The transgenic *Synechococcus* strains had growth rates comparable with the wild-type,

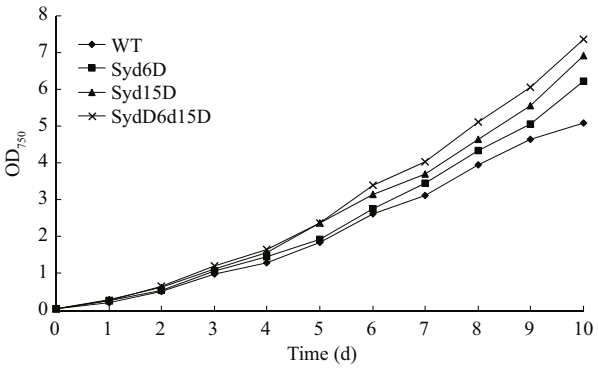

Fig.5 Growth curves of wild-type (WT) and transgenic *Synechococcus* strains

Cultures were grown in Medium A^{+} and bubbled with air under an illumination of 60.5 μmol photons/(m^2·s). The optical density of cells at 750 nm was measured at the indicated time points.

indicating that PrbcL did not affect the growth of the transgenic *Synechococcus*. Results showed that there was a major reduction in the LA content of the transformants containing Syd15D compared with the wild-type. In the Syd6D transformants, the overall LA content was also significantly reduced, but increased amounts of fatty acids GLA and SDA were produced, accounting for 11.36% and 1.25% of the total fatty acids, respectively. In these transformants, the ω-6 series of fatty acids made up a smaller proportion of the total fatty acids, with the ω-3 series of fatty acids accounting for a much larger proportion. The overall amounts of LA and GLA in the Syd6Dd15D transformants were very low (0.51% and 0.22% of total fatty acids), whereas SDA accounted for 11.64% of the total fatty acids in these transformants.

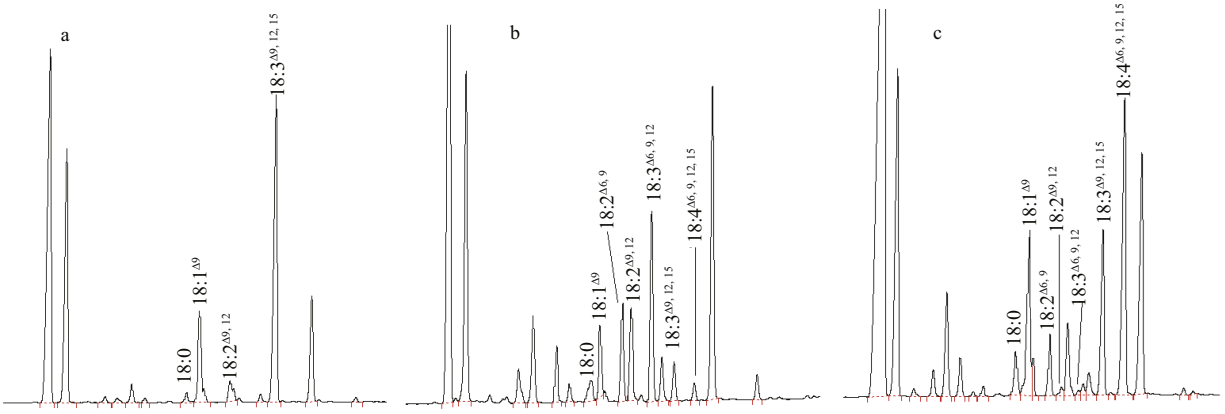

Fig.6 Gas chromatogram traces of fatty acid methyl esters prepared from total lipid extracts of *Synechococcus* sp. PCC7002

a. transformants containing Syd15D; b. transformants containing Syd6D; c. transformants containing Syd6Dd15D.

4 DISCUSSION

Δ15 and Δ6 fatty acid desaturases are the key enzymes in the production of trienoic fatty acids (TFA) (Tan et al., 2011; Davidson, 2013). Genetic engineering of strains to allow the heterologous expression of the Δ15 and Δ6 fatty acid desaturase genes is one of the most effective methods for the modification of TFA. Over the last two decades, many researchers have attempted to improve fatty acid production by metabolic engineering (Eckert et al., 2006; Kim et al., 2014; Gallardo et al., 2015). However, feeding large amounts of substrate to enhance the production of fatty acid always results in low yields.

Our findings show that high levels of GLA and trace amounts of SDA and octadecatetraenoic acid can be produced in *Synechococcus* by expression of *Synechocystis* Δ6 desaturase, which is consistent with findings by sato et al (Sato et al., 2004). Unlike the Δ6 desaturase from *primula* species, the *Synechococcus* Δ6 desaturase shows a preference for LA (Sayanova et al., 2003). The fatty acid synthesis pathway of *Synechococcus* is altered by the introduction of the *Synechocystis* PCC6803 Δ6 desaturase, which introduces double bonds at the Δ6, Δ9, Δ12, and Δ15 (ω-3) positions of C18 acids at the sn-I position (Fig.2) (Murata and Wada, 1995). The first published report of GLA production from the *Synechocystis* Δ6 desaturase gene under the control of a constitutive CaMV 35S promoter was in tobacco (Reddy and Thomas, 1996). The very small amounts of GLA and SDA produced in these transgenic tobacco plants indicated that the prokaryotic Δ6 desaturase did not work well in plants, probably because of a lack of compatible cofactors required for the desaturation reaction. Conjugation of a *Synechocystis* PCC6803 Δ6 gene into *Anabaena* sp. PCC7120 and *Synechococcus* sp. PCC7942 resulted in the gain-of-function expression of GLA and SDA in *Anabaena*, but not in *Synechococcus* (Reddy et al., 1993).

In the transformants containing Syd15D, ALA content dramatically rose to 5.4 times that of the wild-type control. However, Reed et al. (2000) showed that the omega-3 fatty acid desaturase from *Brassica napus* could convert LA to ALA and GLA to SDA in a yeast heterologous expression system, with the former conversion improving product accumulation. Δ15 desaturase overexpression in chloroplasts could alleviate oxidative stress induced by temperature and salt, opening up the possibilities of these transgenic lines for industrial utilization (Zhang et al., 2005; Yu et al., 2009; Wang et al., 2014).

The combined expression of the *Synechococcus* Δ6 and the *Synechococcus* Δ15 desaturases resulted in increased amounts of SDA compared with Δ6 desaturase expression alone in *Synechococcus*. This result was caused by the Δ15 desaturase shifting the fatty acid pool to ALA, and this buildup of the 18:3 substrate for Δ6 desaturase activity led to increased SDA levels. This is consistent with previous reports (Ursin, 2003; Eckert et al., 2006). Co-expression of *B. napus* Δ15 desaturase and *Mortierella alpina* Δ6 and Δ12 desaturases in *B. napus* seeds resulted in production of SDA accounting for 16%–23% of the total cellular fatty acids (Ursin, 2003). Expression of the *Borago officinalis* Δ6 desaturase and *Arabidopsis thaliana* Δ15 desaturase genes in soybean produced a 30% increase in the amount of SDA (Eckert et al., 2006).

To meet the needs of consumption of PUFA consumption, and combat the difficulties encountered in the interconversion of ALA in vivo, alternative sources of PUFA are required (Banz et al., 2012). The genetically modified algae strains developed in this study are rich in SDA, and are therefore suitable candidates for large-scale production of SDA-enriched oil.

5 CONCLUSION

The wild-type *Synechococcus* sp. PCC7002 contains PUFA accounting for nearly 30% of the total fatty acids in the cell. The majority of these are C18 fatty acids, which serve as precursors of EPA and docosahexaenoic acid (DHA) (Kenyon, 1972). In addition, because of its straightforward genetic manipulation, fast growth rate, and light, salt, and thermal tolerances, *Synechococcus* sp. PCC7002 is an ideal platform for large-scale PUFA production. Therefore, genetic modification of *Synechococcus* described here is a cheap and renewable way to meet the growing demand for these fatty acids. At the same time, this system provides the starting material for the synthesis of EPA and DHA.

References

Bakowska-Barczak A M, Schieber A, Kolodziejczyk P. 2009. Characterization of Canadian black currant (*Ribes nigrum* L.) seed oils and residues. *J. Agric. Food Chem*., **57**(24): 11 528-11 536.

Banz W J, Davis J E, Clough R W, Cheatwood J L. 2012. Stearidonic acid: is there a role in the prevention and management of type 2 diabetes mellitus? *J. Nutr*., **142**(3): 635S-640S.

Becker E W. 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge. 293p.

Bernstein H C, Konopka A, Melnicki M R, Hill E A, Kucek L A, Zhang S Y, Shen G Z, Bryant D A, Beliaev A S. 2014. Effect of mono- and dichromatic light quality on growth rates and photosynthetic performance of *Synechococcus* sp. PCC 7002. *Front Microbiol*., **5**: 488.

Bligh E G, Dyer W J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*., **37**(8): 911-917.

Boyanapalli R, Bullerjahn G S, Pohl C, Croot P L, Boyd P W, McKay R M L. 2007. Luminescent whole-cell cyanobacterial bioreporter for measuring Fe availability in diverse marine environments. *Appl. Environ. Microbiol*., **73**(3): 1 019-1 024.

Chen G, Qu S J, Wang Q, Bian F, Peng Z Y, Zhang Y, Ge H T, Yu J H, Xuan N, Bi Y P, He Q F. 2014. Transgenic expression of delta-6 and delta-15 fatty acid desaturases enhances omega-3 polyunsaturated fatty acid accumulation in *Synechocystis* sp. PCC6803. *Biotechnol. Biofuels*, **7**: 32.

Davidson M H. 2013. Omega-3 fatty acids: new insights into the pharmacology and biology of docosahexaenoic acid, docosapentaenoic acid, and eicosapentaenoic acid. *Curr. Opin. Lipidol*., **24**(6): 467-474.

Davies F K, Work V H, Beliaev A S, Posewitz M C. 2014. Engineering limonene and bisabolene production in wild type and a glycogen-deficient mutant of *Synechococcus* sp. PCC 7002. *Front Bioeng. Biotechnol*., **2**: 21.

Eckert H, La Vallee B, Schweiger B J, Kinney A J, Cahoon E B, Clemente T. 2006. Co-expression of the *borage* Δ^6 desaturase and the *Arabidopsis* Δ^{15} desaturase results in high accumulation of stearidonic acid in the seeds of transgenic soybean. *Planta*, **224**(5): 1 050-1 057.

Gallardo M A, Cárcamo J G, Hiller B, Nuernberg G, Nuernberg K, Dannenberger D. 2015. Expression of lipid metabolism related genes in subcutaneous adipose tissue from Chilota lambs grazing on two different pasture types. *Eur. J. Lipid Sci. Technol*., **117**(1): 23-30, http://dx.doi.org/10.1002/ejlt.201400033.

Graham I A, Larson T, Napier J A. 2007. Rational metabolic engineering of transgenic plants for biosynthesis of omega-3 polyunsaturates. *Curr. Opin. Biotechnol*., **18**(2): 142-147.

Harris W S. 2012. Stearidonic acid-enhanced soybean oil: a plant-based source of (n-3) fatty acids for foods. *J. Nutr*., **142**(3): 600S-604S.

Jacobsen J H, Frigaard N U. 2014. Engineering of photosynthetic mannitol biosynthesis from CO_2 in a cyanobacterium. *Metab. Eng*., **21**: 60-70.

Kenyon C N. 1972. Fatty acid composition of unicellular strains of blue-green algae. *J. Bacteriol*., **109**(2): 827-834.

Khozin-Goldberg I, Iskandarov U, Cohen Z. 2011. LC-PUFA from photosynthetic microalgae: occurrence, biosynthesis, and prospects in biotechnology. *Appl. Microbiol. Biotechnol*., **91**(4): 905-915.

Kim S H, Park J S, Kim S Y, Kim J B, Roh K H, Kim H U, Lee K R, Kim J B. 2014. Functional characterization of polyunsaturated fatty acid delta 6-desaturase and elongase genes from the black seabream (*Acanthopagrus schlegelii*). *Cell Biochem. Biophys*., **68**(2): 335-346.

Lazic M, Inzaugarat M E, Povero D, Zhao I C, Chen M, Nalbandian M, Miller Y I, Cherñavsky A C, Feldstein A E, Sears D D. 2014. Reduced dietary omega-6 to omega-3 fatty acid ratio and 12/15-lipoxygenase deficiency are protective against chronic high fat diet-induced steatohepatitis. *PLoS One*, **9**(9): e107658.

Lee J H, O'Keefe J H, Lavie C J, Harris W S. 2009. Omega-3 fatty acids: cardiovascular benefits, sources and sustainability. *Nat. Rev. Cardiol*., **6**(12): 753-758.

Lenihan-Geels G, Bishop K S, Ferguson L R. 2013. Alternative sources of omega-3 fats: can we find a sustainable substitute for fish? *Nutrients*, **5**(4): 1 301-1 315.

Liu X, Sheng J, Curtiss R III. 2011. Fatty acid production in genetically modified cyanobacteria. *Proc. Natl. Acad. Sci. USA*, **108**(17): 6 899-6 904.

Maslova I P, Muradyan E A, Lapina S S, Klyachko-Gurvich G L, Los D A. 2004. Lipid fatty acid composition and thermophilicity of cyanobacteria. *Russ. J. Plant Physiol*., **51**(3): 353-360.

McNeely K, Xu Y, Bennette N, Bryant D A, Dismukes G C. 2010. Redirecting reductant flux into hydrogen production via metabolic engineering of fermentative carbon metabolism in a cyanobacterium. *Appl*. *Environ*. *Microbiol*., **76**(15): 5 032-5 038.

Meesapyodsuk D, Qiu X. 2012. The front-end desaturase: structure, function, evolution and biotechnological use. *Lipids*, **47**(3): 227-237.

Möllers K B, Cannella D, Jørgensen H, Frigaard N U. 2014. Cyanobacterial biomass as carbohydrate and nutrient feedstock for bioethanol production by yeast fermentation. *Biotechnol*. *Biofuels*, **7**: 64.

Murata N, Deshnium P, Tasaka Y. 1996. Biosynthesis of gamma-linolenic acid in the cyanobacterium *Spirulina platensis*, in γ-linolenic acid. *In*: Huang Y S, Milles D E eds. AOCS Press, Champaign. p.22-32.

Murata N, Wada H. 1995. Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochem*. *J*., **308**(1): 1-8.

Nakamura Y, Kaneko T, Hirosawa M, Miyajima N, Tabata S. 1998. CyanoBase, a www database containing the complete nucleotide sequence of the genome of *Synechocystis* sp. strain PCC6803. *Nucl*. *Acids Res*., **26**(1): 63-67.

Reddy A S, Nuccio M L, Gross L M, Thomas T L. 1993. Isolation of a Δ^6-desaturase gene from the cyanobacterium *synechocystis* sp. strain PCC 6803 by gain-of-function expression in *Anabaena* sp. strain PCC 7120. *Plant Mol*. *Biol*., **22**(2): 293-300.

Reddy A S, Thomas T L. 1996. Expression of a cyanobacterial Δ^6-desaturase gene results in γ-linolenic acid production in transgenic plants. *Nat*. *Biotechnol*., **14**(5): 639-642.

Reed D W, Schafer U A, Covello P S. 2000. Characterization of the *Brassica napus* extraplastidial linoleate desaturase by expression in *Saccharomyces cerevisiae*. *Plant Physiol*., **122**(3): 715-720.

Sato S, Xing A Q, Ye X G, Schweiger B, Kinney A, Graef G, Clemente T. 2004. Production of γ-linolenic acid and stearidonic acid in seeds of marker-free transgenic soybean. *Crop Sci*., **44**(2): 646-652.

Sayanova O V, Beaudoin F, Michaelson L V, Shewry P R, Napier J A. 2003. Identification of *primula* fatty acid delta 6-desaturases with n-3 substrate preferences. *FEBS Lett*., **542**(1-3): 100-104.

Simon D, Eng P A, Borelli S, Kägi R, Zimmermann C, Zahner C, Drewe J, Hess L, Ferrari G, Lautenschlager S, Wüthrich B, Schmid-Grendelmeier P. 2014. Gamma-linolenic acid levels correlate with clinical efficacy of evening primrose oil in patients with atopic dermatitis. *Adv*. *Ther*., **31**(2): 180-188.

Stanier R Y, Kunisawa R, Mandel M, Cohen-Bazire G. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol*. *Rev*., **35**(2): 171-205.

Stevens S E Jr, Patterson C O P, Myers J. 1973. The production of hydrogen peroxide by blue-green algae: a survey. *J*. *Phycol*., **9**(4): 427-430.

Takeyama H, Takeda D, Yazawa K, Yamada A, Matsunaga T. 1997. Expression of the eicosapentaenoic acid synthesis gene cluster from *Shewanella* sp. in a transgenic marine cyanobacterium, *Synechococcus* sp. *Microbiology*, **143**(8): 2 725-2 731.

Tan L, Meesapyodsuk D, Qiu X. 2011. Molecular analysis of Δ6 desaturase and Δ6 elongase from *Conidiobolus obscurus* in the biosynthesis of eicosatetraenoic acid, a ω3 fatty acid with nutraceutical potentials. *Appl*. *Microbiol*. *Biotechnol*., **90**(2): 591-601.

Tasset-Cuevas I, Fernández-Bedmar Z, Lozano-Baena M D, Campos-Sánchez J, de Haro-Bailón A, Muñoz-Serrano A, Alonso-Moraga Á. 2013. Protective effect of borage seed oil and gamma linolenic acid on DNA: in vivo and in vitro studies. *PLoS One*, **8**(2): e56986.

Therien J B, Zadvornyy O A, Posewitz M C, Bryant D A, Peters J W. 2014. Growth of *Chlamydomonas reinhardtii* in acetate-free medium when co-cultured with alginate-encapsulated, acetate-producing strains of *Synechococcus* sp. PCC 7002. *Biotechnol*. *Biofuels*, **7**: 154.

Ursin V M. 2003. Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. *J*. *Nutr*., **133**(12): 4 271-4 274.

Wang H S, Yu C, Tang X F, Zhu Z J, Ma N N, Meng Q W. 2014. A tomato endoplasmic reticulum (ER)-type omega-3 fatty acid desaturase (LeFAD3) functions in early seedling tolerance to salinity stress. *Plant Cell Rep*., **33**(1): 131-142.

Yu C H, Wang H S, Yang S, Tang X F, Duan M, Meng Q W. 2009. Overexpression of endoplasmic reticulum omega-3 fatty acid desaturase gene improves chilling tolerance in tomato. *Plant Physiol*. *Biochem*., **47**(11-12): 1 102-1 112.

Zhang M, Barg R, Yin M G, Gueta-Dahan Y, Leikin-Frenkel A, Salts Y, Shabtai S, Ben-Hayyim G. 2005. Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. *Plant J*., **44**(3): 361-371.