WALAILAK JOURNAL
http://wjst.wu.ac.th Agricultural Technology and Biological Sciences

Cloning of a C-phycocyanin Alpha Subunit from *Thermosynechococcus* sp. TUBT-T01 and Prediction of Its Properties

Plaipol DEDVISITSAKUL[1,*], Sichon HUADRAKSASAT[2], Supenya CHITTAPUN[2], Theppanya CHAROENRAT[2] and Chanitchote PIYAPITTAYANUN[2]

[1]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*Biotechnology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Prathum Thani 12120, Thailand*

([*]Corresponding author's e-mail: plaipol@mfu.ac.th)

Received: 3 March 2017, Revised: 23 November 2017, Accepted: 22 December 2017

Abstract

C-Phycocyanin, a blue-colored and water soluble protein, is a class of phycobiliproteins that are the major light-harvesting pigments of a photosynthetic system in cyanobacteria. C-phycocyanins are utilized in many industries, including as natural colorants in food and cosmetics and as antioxidant compounds. However, the uses of C-phycocyanins have been limited due to their vulnerability to high temperatures. Therefore, the objective of this study was to identify and analyze the C-phycocyanin gene isolated from *Thermosynechococcus* sp. TUBT-T01, living in a hot spring in Surat Thani province, in the hope that this C-phycocyanin exhibited thermostable properties and that their applications could be expanded over a wide range of industries. In the present study, the polymerase chain reaction of the gene encoding alpha subunits of C-phycocyanin (*cpcA*) was performed, using primers designed based upon the sequence alignments of *cpcA* from *Thermosynechococcus* sp. available in the GenBank database. The putative *cpcA*, with an approximate size of 500 base pairs, was detected on an agarose gel. The DNA sequencing analysis indicated that the *cpcA* was 489 base pairs in length, and its nucleotide sequence was 94 % identical to those of thermophilic *Thermosynechococcus* sp. NK55, *T. elongatus* BP-1, and *Synechococcus vulcanus*. The deduced amino acid sequence was very similar to those of *Thermosynechococcus* sp. NK55, *T. elongatus* BP-1, and *S. vulcanus*. The data derived from the homologous model revealed that the presence of Asp28, Lys32, and Ser72 in the alpha subunit of C-phycocyanin from *Thermosynechococcus* sp. TUBT-T01 could provide the high thermostability property of this protein.

Keywords: Cyanobacteria, hot spring, alpha subunit, C-phycocyanin gene, Thermostability, *Thermosynechococcus* sp. TUBT-T01

Introduction

Cyanobacteria are prokaryotic blue-green algae in the division Cyanophyta. They are photoautotrophs, which are the primary producers in ecosystems because of the presence of chlorophyll *a* and various accessory pigments which conduct oxygenic photosynthesis. There are more than 7,500 species of cyanobacteria found in the world. They are found in freshwater, marine, and terrestrial environments, as well as in extreme environments such as hot springs, hypersaline water, deserts, and freezing habitats [1-4]. Phycobiliproteins are accessory protein pigments that function in light harvesting and in passing energy to photosynthetic reactions of cyanobacteria and red algae [5]. C-phycocyanin (Cpc), a blue-colored phycobiliprotein in cyanobacteria, has maximum absorption and emission at 620 nm and 650 nm respectively [6]. C-phycocyanin is a protein complex that contains an

alpha and a beta subunit, which each attach to 1 and 2 chromophores respectively. Association of the C-phycocyanin alpha and beta subunits is considered to be a one monomer (αβ) structure but, in nature, C-phycocyanin complex is present as a trimer $(\alpha\beta)_3$ and hexamer $(\alpha\beta)_6$ [7].

The C-phycocyanin α and β subunits encoded by *cpcA* and *cpcB* gene belonging to C-phycocyanin operon, are highly conserved in cyanobacteria [8]. The C-phycocyanin operon comprises 6-7 genes from the 5' to 3'region respectively. Complete genome analysis indicates that *T. elongatus* BP-1 has complete sets of *cpcB-cpcA-cpcC-cpcD-cpcE-cpcF-cpcG* [9], while *Anabaena* PCC7120 contains only *cpcB-cpcA-cpcC-cpcD-cpcE-cpcF* in the operon [10].

Because of its non-toxic properties, C-phycocyanin has potential applications in food and cosmetic coloring, such as chewing gum, ice cream, candy, jelly, lipstick, and eye-liner [11-15]. In pharmaceutical and medical applications, it has been used to inhibit proinflammatory cytokines which cause inflammation, fever, and tissue destruction [16]. There are also some reports for the utilization of C-phycocyanin for nociception [17]. In addition, C-phycocyanin has antioxidant properties. It is able to scavenge several oxygen radicals, for example alkoxyl, hydroxyl, and peroxyl radicals [18].

Due to its scavenging properties toward the oxygen radical species, it may prevent some diseases which are associated with free radicals, including atherosclerosis, Alzheimer's disease, cancer, diabetes, and rheumatoid arthritis. Furthermore, C-phycocyanin could also be used as a fluorescent probe in varieties of molecular researches [19-24]. Although several applications of C-phycocyanin have been reported, the uses of C-phycocyanins are still limited at the industrial level because it has poor stability at the high temperatures required in some industrial processes. Some studies showed that cyanobacteria living in high temperature habitat could produce thermostable C-phycocyanin [25-27]. However, there are a limited number of reports on C-phycocyanins from thermophilic cyanobacteria.

Recently, a new rod-shape cyanobacterium strain *Thermosynechococcus* sp. TUBT-T01, has been isolated from a hot spring, Surat Thani province, Thailand. This strain has been successfully cultured in BG-11 liquid medium supplemented with $NaNO_3$ 3 g L^{-1} at 50 °C [28].

This work aimed to isolate and clone the *cpcA* gene from *Thermosynechococcus* sp. TUBT-T01 and evaluate the thermostability properties of the alpha subunit of phycocyanin protein by molecular sequence analysis. This study should provide fundamental information for the further development of thermotolerant phycocyanin production and hopes that its applications could be applied over a wide range of industries.

Materials and methods

Cyanobacterium strain and culture

Thermosynechococcus sp. TUBT-T01 was isolated from Borkrung hot spring in Surat Thani province, Thailand. The average temperature of the water in this hot spring is approximately 56 °C [28]. The isolate was cultured in BG-11 medium supplemented with $NaNO_3$ 3 g L^{-1} and incubated for 30 days at 50 °C with continuous lighting and shaking at 100 rpm.

Genomic DNA extraction

The cyanobacterium was collected by centrifugation at 4,000 rpm at 4 °C for 30 min, and resuspended in TE buffer. The genomic DNA was extracted by TIANamp Bacteria DNA Kit (Tiangen Biotech, China), following the manufacturer's instructions, then stored at −20 °C until use.

Primer design and amplification of *cpcA* gene from *Thermosynechococcus* sp. TUBT-T01

Primers corresponding to the 5' and 3' end of *cpcA* gene were designed from the closely related species of cyanobacterium available in the GenBank database using CLC sequence viewer 7 (CLC bio, QIAGEN) and the Primer3plus program [29]. The genomic DNA of *Thermosynechococcus* sp. TUBT-T01was used as a template for the PCR amplification of *cpcA* using *TaKaRa Ex Taq*™ DNA polymerase (5 U/µl) under the following condition: a primary denaturation at 94 °C for 30 sec, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec, extension at 72 °C for 30 sec, and final

extension at 72 °C for 2 min. Gel electrophoresis was performed to visualize the PCR product on 1.5 % agarose.

Cloning of α subunit of phycocyanin

The PCR product was purified with a Gel/PCR Fragments Extraction Kit (RBC Bioscience). The purified DNA (25 ng/µl) was then ligated into pGEM-T Easy cloning vector (50 ng) (Promega, Madison, WI) and transformed into *E. coli* DH5α competent cells by the heat shock method. The bacterial cells were grown in LB agar containing 100 µg/ml of ampicillin, and the recombinant clones were selected by blue-white colony selection. Plasmid extraction was performed from the recombinant clones using TIANpure Midi Plasmid Kit (Tiangen Biotech, China), following the manufacturer's instructions, and further used for DNA sequencing.

Nucleotide and deduced amino acid sequence analysis

The recombinant DNA of the *cpcA* from *Thermosynechococcus* sp.TUBT-T01 was submitted for nucleotide sequencing at Macrogen, Korea. Nucleotide and deduced amino acid sequence similarity searches were performed using BLASTn and BLASTp [30,31]. Maximum Likelihood phylogenetic analysis of CpcA amino acid sequences was constructed using Mega 7 software [32]. The physicochemical parameters of CpcA, including molecular weight, isoelectric point (pI), instability index, and hydropathicity index, were calculated using the ProtParam program [33]. The secondary structure of CpcA was predicted using the GOR4 program [34]. Finally, the comparison of the CpcA amino acid sequences from 2 thermophilic cyanobacteria, *T. elongatus* BP-1 and *Synechococcus vulcanus*, and 4 mesophilic cyanobacteria, *Synechococcus* sp. PCC7002, *Synechocystis* sp. PCC 6803, *Fremyella diplosiphon*, and *Arthrospira platensis*, was performed by CLUSTAL OMEGA [35]. These sequences were selected from phycocyanin proteins in which their structures had already been reported [27,36-39].

Results and discussion

Cloning and nucleotide sequencing of *cpcA*

Thermosynechococcus sp. TUBT-T01 was collected and isolated from Borkrung hot spring in Surat Thani province, Thailand, at a temperature of approximately 56 °C, suggesting its nature as a thermophilic cyanobacterium [28].

In this study, we compared *cpcA* gene sequences from several cyanobacteria available in the NCBI database. However, there was limited information on *cpcA* gene sequences from thermophilic cyanobacteria. To gain deeper insight into c-phycocyanin from *Thermosynechococcus* TUBT-T01, the *cpcA* gene was isolated from *Thermosynechococcus* TUBT-T01, and the nucleotide sequences of the *cpcA* gene were analyzed. The genomic DNA was extracted from the 50 °C-cultured *Thermosynechococcus* sp. TUBT-T01 and used as a template for PCR amplification reaction with a pair of primers: 5'-ATGAAAACCCCGATTACTGAAGC-3' and 5'-CTAGCTGAGGGCGTTGATGG-3', which were designed specifically for *cpcA* genes of other *Thermosynechococcus* available in the NCBI database. The primers were targeted to cover the full-length *cpcA* open reading frame (ORF). After the amplification, a single band of approximately 500 bp PCR product was observed by agarose gel electrophoresis (**Figure 1**). The PCR product was subsequently cloned into pGEM-T easy vector and transformed into *E. coli* DH5α, and the recombinant plasmid was submitted for DNA sequencing.

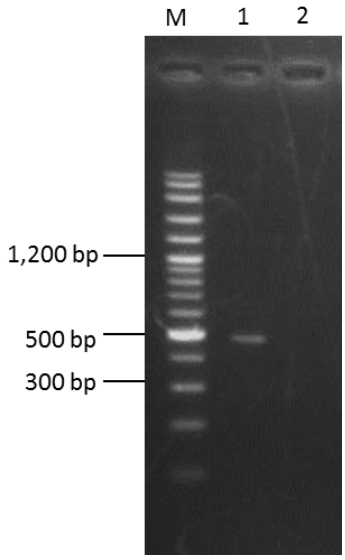

Figure 1 PCR amplification of *cpc A* gene from *Thermosynechococcus* sp. TUBT-T01 Lane M: 100 bp ladder (Biolabs, UK), Lane 1: PCR product of *cpcA* from *Thermosynechococcus* sp. TUBT-T01 and Lane 2: Negative control (water as a template).

The nucleotide sequence homology analysis indicated that the PCR product was a C-phycocyanin alpha subunit (*cpcA*) 94 % identical to the *cpcA* gene from *T. elongatus* BP-1 (BA000039), *Thermosynechococcus* sp. NK55 (CP006735), and *S. vulcanus* (AF333175). All of these cyanobacterial strains were reported as thermophiles [9,27,40-42]. From BLASTP analysis, the deduced amino acid sequence was 99 % identical to *T. elongatus* BP-1, *Thermosynechococcus* sp. NK55a, and *S. vulcanus.* Phylogenetic analysis revealed that the CpcA of *Thermosynechococcus* sp. TUBT-T01 was clustered with *T. elongates* BP-1, *Thermosynechococcus* sp. NK55a and *T. vulcanus* with 97 % confidence, all of which are thermophilic cyanobacteria (**Figure 2**). It was obvious that the CpcA protein was conserved among thermophilic cyanobacteria and was quite distant from the other groups of cyanobacteria, including mesophilic cyanobacteria. The amino acid sequences of *Thermosynechococcus* sp.TUBT-T01 was very similar to those of *T. elongatus* BP-1 and *S. vulcanus* with only 2 amino acid differences in which, at position 95, it was valine in *Thermosynechococcus* sp.TUBT-T01 and *T. elongatus* BP-1, but was isoleucine in *S. vulcanus* while, at position 130, it was also valine in *Thermosynechococcus* sp.TUBT-T01 but was isoleucine in both *T. elongatus* BP-1 and *S. vulcanus*.

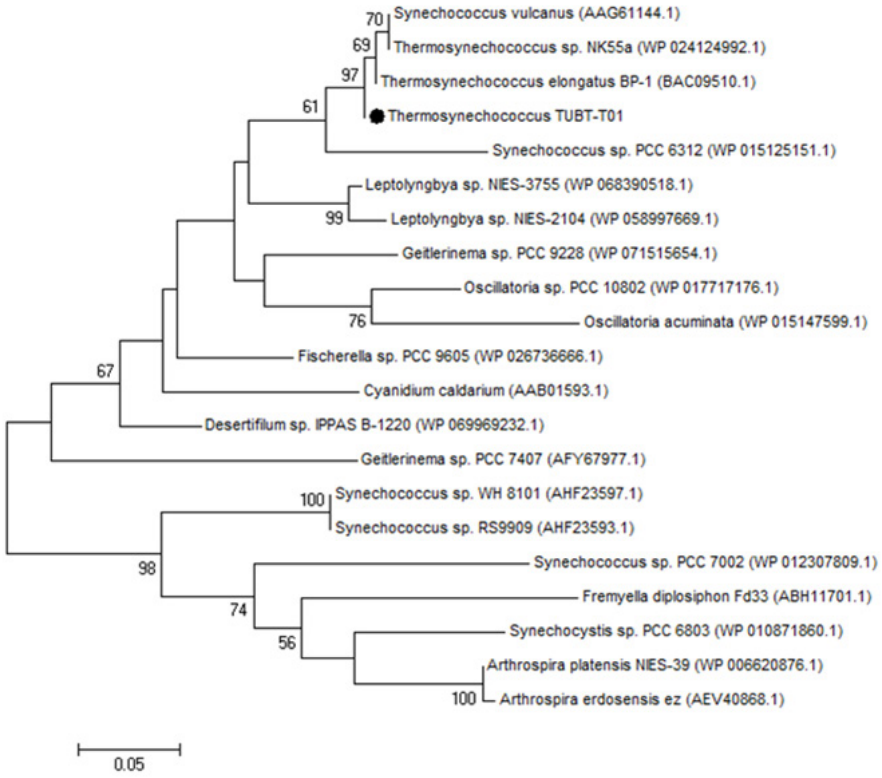

Figure 2 The phylogenetic analysis of *cpcA* was constructed by Maximum Likelihood method with 1,000 bootstrap values.

Structural analysis of CpcA

The physicochemical parameters of the CpcA from *Thermosynechococcus* sp.TUBT-T01 were calculated by the ProtParam program [33]. It was revealed that the molecular weight of CpcA protein was 17.43 kDa, pI was 5.36, instability index was 24, and hydropathicity index was –0.110 (**Table 1**). The pI value can be useful for buffer and column selection in protein purification steps. An instability index of less than 40 indicates that this protein could be stable in the solution [43]. A more negative value of hydropathicity index indicates that the protein was highly hydrophilic [44]; therefore, this result suggested that CpcA was a hydrophilic protein.

Table 1 Physicochemical parameters of CpcA protein from *Thermosynechococcus* sp. TUBT-T01.

Physicochemical parameters	CpcA protein
Length	162 residues
Molecular weight	17.43 kDa
Isoelectric point	5.36
Instability index	24.16
Hydropathicity index	-0.110

The predicted secondary structure of CpcA using the GOR4 program showed that the main structure, accounting for 56.79 % of the protein sequences, were α-helix, while 12.96 % of the extended strands were found in the middle and C-terminal region and 30.25 % of random coil regions were generally distributed throughout the CpcA peptide chain and were probably loop and turn structures. From amino acid sequence analysis (**Figure 3**), the CpcA from *Thermosynechococcus* sp. TUBT-T01 had cysteine at position 84 (Cys84) that was conserved in *T. elongatus* BP-1, *Thermosynechococcus* sp. NK55a, and *S. vulcanus.* The crystal structure of C-phycocyanin from *S. vulcanus* showed that Cys84 was a binding site of chromophore [27], and it was in the α-helix region of the CpcA protein sequence. In addition, it has been reported that several tyrosine residues in the alpha subunit of phycocyanin showed interaction with cysteine at position 84 [45].

Thermal stability implication of CpcA

Inoue *et al.* reported on the thermostability of phycocyanin from *S. vulcanus* by high-temperature-induced denaturation of C-phycocyanin. They found that the absorbance at 620 nm (photosystem II complex) remained unchanged up to 70 °C, which suggested that the activity of phycocyanin from *S. vulcanus* did not decrease at high temperature [46]. Interestingly, the deduced amino acid sequences of CpcA from *Thermosynechococcus* sp. TUBT-T01 was highly conserved with that of *S. vulcanus,* except for only 2 positions in which isoleucine and valine at positions 95 and 130 of *S. vulcanus* changed to valine and isoleucine in *Thermosynechococcus* sp.TUBT-T01, respectively. However, these 2 amino acid differences should not alter the protein structure and its thermostability due to their common physicochemical properties of both valine and isoleucine side chain (non-polar uncharged). Therefore, the thermostability of CpcA from *S. vulcanus* and that of *Thermosynechococcus* sp. TUBT-T01 should be conserved.

The alpha subunit of C-phycocyanin, the CpcA protein from *Thermosynechococcus* sp.TUBT-T01, was compared to other CpcA, in which their crystal structures had been previously determined (2 thermophilic cyanobacteria: *T. elongatus* BP-1(Te; WP_011057793), *S. vulcanus* (Sv; AAG61144), and 4 mesophilic cyanobacteria: *Synechococcus* sp PCC7002 (S7; ACB00191.1), *Synechocystis* sp. PCC 6803 (S6803; 4F0T_A), *F. diplosiphon* (Fd33; 1CPC_A), and *Arthrospira platensis* (Ap; ABD64608.1) (**Figure 3**) [27,36-39]. One interesting observation was the amino acid at position 28 of the *Thermosynechococcus* sp.TUBT-T01. The CpcA alpha subunit was an aspartate (αAsp28); it has been previously shown that, in the monomer form, αAsp28 interacted with asparagine at position 35 (βAsn35) and a chromophore of a C-phycocyanin beta subunit, resulting in the enhanced stability of the C-phycocyanin monomer structure. In the trimer form, the amino acid at position 72 of the *Thermosynechococcus* sp.TUBT-T01 CpcA alpha subunit was a serine (αSer72) (**Figure 3**). The αSer72 was previously reported to have interacted with glutamine at position 68 (βGlu68) of the C-phycocyanin beta subunit in an adjacent monomer, resulting in the increased stability of the C-phycocyanin trimer structure [27]. Due to the lack of αSer72, these interactions could not be found in the mesophilic cyanobacterial species. In the hexamer form, αAsp28 also provided interaction with lysine at position 32 (αLys32) of the C-phycocyanin alpha subunit in one of the monomers of an adjacent trimer [27]. Both αAsp28 and αLys32 were found to be conserved among thermophilic cyanobacteria *S. vulcanus* (Sv), and *T. elongatus* (Te), but not in mesophilic cyanobacteria *F. diplosiphon* (Fd33) and *Synechococcus* sp PCC7002 (S7), *Synechocystis* sp. PCC 6803 (S6803), and *A. platensis* (Ap) (**Figure 3**).

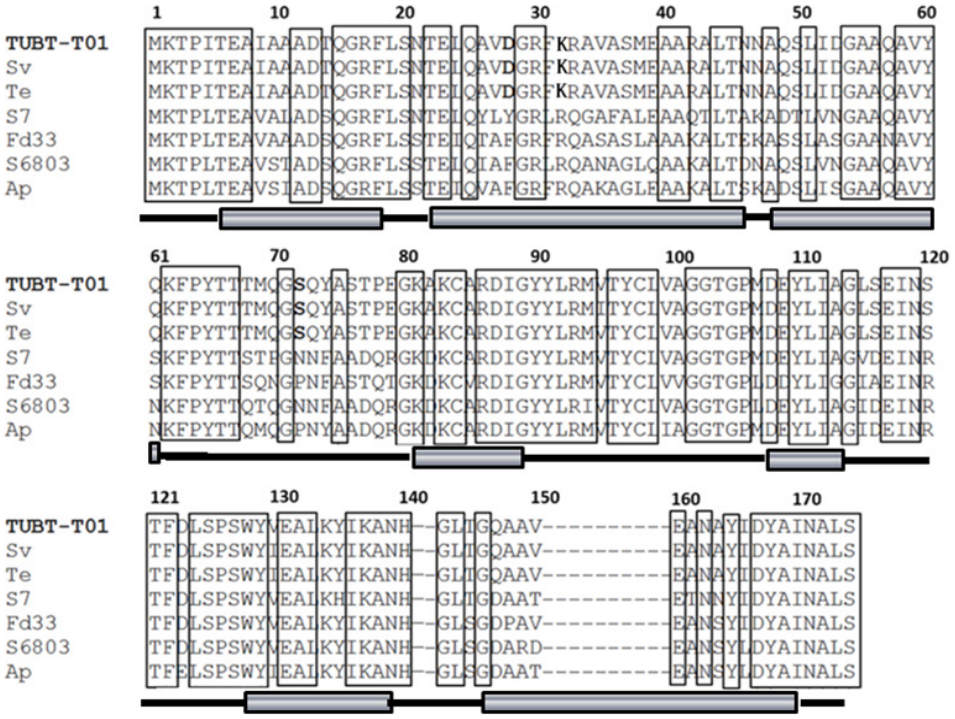

Figure 3 Comparison of deduced amino acid sequences homologous to Cpc A proteins from 3 thermophilic cyanobacteria: *Thermosynechococcus* sp TUBT-T01 (TUBT-T01), *T. elongatus* (Te), and *S. vulcanus* (Sv), and 4 mesophilic cyanobacteria: *Synechococcus* sp PCC7002 (S7), *Synechocystis* sp. PCC 6803 (S6803), *F. diplosiphon* (Fd33), and *A. platensis* (Ap). The sequence ruler for these amino acid sequences including gaps (following Adir *et al*. 2001) are displayed on top of the alignment. Conserved regions are shown in the square boxes, and amino acid residues (D, K, and S), previously reported to have additional interaction within C-phycocyanin in thermophilic cyanobacteria when compared to mesophilic cyanobacteria, are indicated in bold. Helical regions of the alpha subunits of the *Thermosynechococcus* sp. TUBT-T01 are shown as grey boxes. The random coil and extended strand are shown as the connected line.

According to these data, the presence of Asp28, Lys32, and Ser72 could be considered to be important differences between the thermophilic and mesophilic cyanobacteria and, thus, these positions (Asp28, Lys32, and Ser72) are important in enhancing the thermal stability of the protein. Due to the existence of Asp28, Lys32, and Ser72, it is suggested that the CpcA from *Thermosynechococcus* sp. TUBT-T01 should endow thermophilic properties, and could contribute to the thermal stability of its overall complex structure. This study provided more insight into the sequence and structure of alpha phycocyanin from a recently isolated strain of a thermophilic cyanobacteria, *Thermosynechococcus* sp. TUBT-T01 [28]. It is supported that this cyanobacterial strain is possibly a new target for the production of thermostable phycocyanin.

Mostly, C-phycocyanin was extracted directly from cyanobacteria [36-39]. However, low amounts of proteins were produced from thermophiles at high temperature due to their slow growth rate when compared to that of mesophiles [46]. An alternative to increase the protein yield is to use recombinant DNA technology [47]. According to our survey, the C-phycocyanin gene isolated from some mesophilic cyanobacteria has been successfully cloned and expressed in a bacterial system; however, we have not

found any publication on the production of C-phycocyanin from thermophilic cyanobacteria by recombinant DNA technology [48-50].

The antioxidant potential of C-phycocyanin isolated from several cyanobacterial species has been reported earlier [48,51-54]. Previously, it was believed that phycocyanin exhibited antioxidant activity against free radicals solely by the action of bilin chromophore in holo-phycocyanin [52-54]. However, recently, Cherdkiatikul and Yaneenart (2014) have reported the antioxidant activity of solely either alpha- or beta phycocyanin subunits from *S. platensis* without bilin chromophore [51]. We expected that the alpha subunit of phycocyanin from *Thermosynechococcus* sp. TUBT-T01 should provide antioxidant activity as well. However, the antioxidant activity of C-phycocyanin from *Thermosynechococcus* sp. TUBT-T01 has not yet been explored. We are planning to investigate the antioxidant properties of the recombinant CpcA in the near future, and hope this protein could be used for further developments and applications in the food, cosmetic, and pharmaceutical industries [11].

Conclusions

Cloning and investigation of an alpha subunit of C-phycocyanin encoding gene from *Thermosynechococcus* sp. TUBT-T01 has been performed in this study. The presence of Asp28, Lys32, and Ser72 in a CpcA subunit from *Thermosynechococcus* sp. TUBT-T01 was only conserved among thermophilic cyanobacteria. These amino acids have been previously reported to involve the enhanced stability of C-phycocyanin of *S. vulcanus*. This suggested that the structure of the C-phycocyanin alpha subunit from *Thermosynechococcus* sp. TUBT-T01 has a high possibility to provide thermostable properties to its complex structure of the C-phycocyanin protein. The measurement of photosynthesis, fluorescence, and antioxidant activities of the phycocyanin at high temperature is required to confirm its properties, and the investigation of thermostable potential of other subunits of the C-phycocyanin from *Thermosynechococcus* sp. TUBT-T01 would be valuable to understand the characteristics of the whole complex of the C-phycocyanin protein from *Thermosynechococcus* sp. TUBT-T01.

Acknowledgements

The authors would like to thank Mae Fah Luang University for providing the research fund (Grant number: 58101020061) and facility support at the Scientific and Technological Instruments Center (STIC).

References

[1] WF Vincent. *Cyanobacteria*. Laval University, Quebec City, Canada, Elsevier Inc, 2009, p. 226-32.
[2] WR Hess. Cyanobacterial genomics for ecology and biotechnology. *Curr. Opin. Microbiol.* 2011; **14**, 608-14.
[3] GE Fogg, WDP Stewart, P Fay and AE Walsby. *The Blue-Green Algae*. Academic Press, London and New York, 1973, p. 459
[4] N Thajuddin and G Subramanian. Cyanobacterial biodiversity and potential applications in biotechnology. *Curr. Sci.* 2005; **89**, 47-57.
[5] Govindjee and D Shevela. Adventures with cyanobacteria: A personal perspective. *Front Plant Sci.* 2011; **6**, 2-28.
[6] KM Minkova, AA Tchernov, MI Tchorbadjieva, ST Fournadjieva, RE Antova and MC Busheva. Purification of C-phycocyanin from *Spirulina* (Arthrospira) fusiformis. *J. Biotechnol.* 2003; **102**, 55-9.
[7] R MacColl. Cyanobacterial phycobilisomes. *J. Struct. Biol.* 1998; **124**, 311-34.
[8] SR Kalla, LK Lind, J Lidholm and P Gustaffson. Transcriptional organization of the phycocyanin subunit gene clusters of the cyanobacerium Anacystis nidulans UTEX 625. *J. Bacteriol.* 1998; **170**, 2961-70.
[9] Y Nakamura, T Kaneko, S Sato, M Ikeuchi, H Katoh, S Sasamoto, A Watanabe, M Iriguchi, K. Kawashima, T Kimura, Y Kishida, C Kiyokawa, M Kohara, M Matsumoto, A Matsuno, N

Nakazaki, S Shimpo, M Sugimoto, C Takeuchi, M Yamada and S Tabata. Complete genome structure of the thermophilic cyanobacterium Thermosynechococcus elongatus BP-1. *DNA Res.* 2002; **9**, 123-30.
[10] WR Belknap and R Haselkorn. Cloning and light regulation of expression of the phycocyanin operon of the cyanobacterium Anabaena. *Embo J.* 1987; **6**, 871-84.
[11] M Kuddus, P Singh, G Thomas and A Al-Hazimi. Recent developments in production and biotechnological applications of C-Phycocyanin. *BioMed. Res. Int.* 2013; **2013**, 742859.
[12] T Kato. Blue pigment from Spirulina. *New Food Indust.* 1994; **29**, 17-24
[13] T Hirata, M Tanaka, M Ooike, T Tsunomura and M Sakaguchi. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis. J. Appl. Phycol.* 2000; **12**, 435-9.
[14] L Jespersen, LD Strømdahl and K Olsen. Heat and light stability of three natural blue colorants for use in confectionery and beverages. *Eur. Food Res. Tech.* 2005; **220**, 261-6.
[15] KS Mishra, A Shrivastav and S Mishra. Effect of preservatives for food grade C-PC from *Spirulina platensis. Process Biochem.* 2008; **43**, 339-45.
[16] C Romay, R Delgado, D Remirez, R González and A Rojas. Effects of phycocyanin extract on tumor necrosis factor-alpha and nitrite levels in serum of mice treated with endotoxin. *Arzneimittelforschung* 2001; **51**, 733-6.
[17] CM Shih, SN Cheng, CS Wong, YL Kuo and TC Chou. Antiinflammatory and antihyperalgesic activity of C-phycocyanin. *Anesth Analg.* 2009; **108**, 1303-10.
[18] C Romay, R Gonzalez, N Ledon, D Remirez and Rimbau. C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr. Protein Pept. Sci.* 2003; **4**, 207-16.
[19] K Bagchi and S Puri. Free radicals and antioxidants in health and disease: A review. *Eastern Mediterr. Health J.* 1998; **4**, 350-60.
[20] A Nunomura, R Castellani, X Zhu, P Moreira, G Perry and M Smith. Involvement of oxidative stress in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 2006; **65**, 631-41.
[21] RM Tamimi, SE Hankinson, H Campos, D Spiegelman, S Zhang, GA Colditz, WC Willett and DJ Hunter. Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. *Am. J. Epidemiol.* 2005; **161**, 153-60.
[22] G Davì, A Falco and C Patrono. Lipid peroxidation in diabetes mellitus. *Antioxid. Redox. Sig.* 2005; **7**, 256-68.
[23] C Hitchon and H El-Gabalawy. Oxidation in rheumatoid arthritis. *Arthritis Res. Ther*. 2004; **6**, 265-78.
[24] Richa, VK Kannaujiya, M Kesheri, G Singh and RP Sinha. Biotechnological potentials of phycobiliproteins. *Int. J. Pharm. Bio. Sci.* 2011; **2**, B446-B454.
[25] JY Leu, TH Lin, MJP Selvamani, HC Chen, JZ Liang and KM Pan. Characterization of a novel thermophilic cyanobacterial strain from Taian hot springs in Taiwan for high CO_2 mitigation and C-phycocyanin extraction. *Process Biochem.* 2013; **48**, 41-8.
[26] MR Edwards, C Hauer, RF Stack, LE Eisele and R MacColl. Thermophilic C- phycocyanin: Effect of temperature, monomer stability, and structure. *Biochim. Biophys. Acta* 1997; **1321**, 157-64.
[27] N Adir, Y Dobrovetsky and N Lerner. Structure of c-phycocyanin from the thermophilic cyanobacterium *Synechococcus vulcanus* at 2.5 angstrom: Structural implications for thermal stability in phycobilisome assembly. *J. Mol. Biol.* 2001; **313**, 71-81.
[28] K Suwanmanee, T Charoenrat and S Chittapun. Isolation and cultivation of thermophilic blue green algae from hot springs in Surat thani province. *In*: Proceeding of the 7th National Conference on Algae and Plankton, Narai Hotel, Bangkok, Thailand, 2015. p. 1-12.
[29] A Untergasser, I Cutcutache, T Koressaar, J Ye, BC Faircloth, M Remm and SG Rozen. Primer3-new capabilities and interfaces. *Nucleic. Acids Res.* 2012; **40**, e115.
[30] SF Altschul, W Gish, W Miller, EW Myers and D J Lipman. Basic local alignment search tool. *J. Mol. Biol.* 1990; **215**, 403-10.

[31] SF Altschul, TL Madden, AA Schäffer, J Zhang, Z Zhang, W Miller and DJ Lipman. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic. Acids Res.* 1997; **25**, 3389-402.
[32] S Kumar, G Stecher and K Tamura. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 2016; **33**, 1870-4.
[33] E Gasteiger, C Hoogland, A Gattiker, S Duvaud, MR Wilkins, RD Appel and A Bairoch. *Protein Identification and Analysis Tools on the ExPASy Server*. The Proteomics Protocols Handbook, Humana Press, 2005, p. 571-607.
[34] J Garnier, JF Gibrat and B Robson. GOR method for predicting protein secondary structure from amino acid sequence. *Meth. Enzymol.* 1996; **266**, 540-53.
[35] F Sievers, A Wilm, D Dineen, TJ Gibson, K Karplus, W Li, R Lopez, H McWilliam, M Remmert, J Söding, JD Thompson and DG Higgins. Fast scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol*. 2011; **7**, 539.
[36] A Marx and N Adir. Allophycocyanin and phycocyanin crystal structures reveal facets of phycobilisome assembly. *Biochim. Biophys. Acta* 2013; **1827**, 311-8.
[37] M Duerring, GB Schmidt and R Huber. Isolation, crystallization, crystal structure analysis and refinement of constitutive C-phycocyanin from the chromatically adapting cyanobacterium *Fremyella diplosiphon* at 1.66 A resolution. *J. Mol. Biol.* 1991; **217**, 577-92.
[38] K Brejc, R Ficner, R Huber and S Steinbacher. Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the cyanobacterium *Spirulina platensis* at 2.3 A resolution. *J. Mol. Biol.* 1995; **249**, 424-40.
[39] T Schirmer, W Bode and R Huber. Refined three-dimensional structures of two cyanobacterial C-phycocyanins at 2.1 and 2.5 A resolution: A common principle of phycobilin-protein Interaction. *J. Mol. Biol.* 1987; **196**, 677-95.
[40] C Ohto, C Ishida, H Nakane, M Muramatsu, T Nishino and S Obata. A thermophilic cyanobacterium *Synechococcus elongatus* has three different class I prenyltransferase genes. *Plant Mol. Biol.* 1999; **40**, 307-21.
[41] R Prabha, DP Singh and A Rai. Looking into the genome of *Thermosynechococcus elongatus* (thermophilic cyanobacteria) with codon selection and usage perspective. *Int. J. Bioinform. Res. Appl.* 2015; **11**, 130-41.
[42] S Stolyar, Z Liu, V Thiel, LP Tomsho, N Pinel, WC Nelson and JK Fredrickson. Genome sequence of the thermophilic cyanobacterium *Thermosynechococcus* sp. strain NK55a. *Genome Announc.* 2014; **2**, e01060-e010113.
[43] K Guruprasad, BV Reddy and MW Pandit. Correlation between stability of a protein and its dipeptide composition: A novel approach for predicting in vivo stability of a protein from its primary sequence. *Protein Eng.* 1990; **4**, 155-61.
[44] J Kyte and RF Doolittle. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 1982; **157**, 105-32.
[45] VK Kannaujiya, A Rahman, Adinath, S Sundaram and RP Sinha. Structural and functional dynamics of tyrosine amino acid in phycocyanin of hot-spring cyanobacteria: A possible pathway for internal energy transfer. *Gene Rep.* 2016; **5**, 83-91
[46] N Inoue, T Emi, Y Yamane, Y Kashino, H Koike and K Satoh. Effect of hight-temperature treatments on a thermophilic cyanobacterium *Synechococcus vulcanus. Plant Cell Physiol*. 2000; **41**, 515-22.
[47] P Turner, G Mamo and EN Karlsson. Potential and utilization of thermophiles and thermostable enzymes in biorefining. *Microb. Cell Fact*. 2007; **6**, 9.
[48] XY Guan, WJ Zhang, XW Zhang, YX Li, JF Wang, HZ Lin and S Qin. A potent anti-oxidant property: Fluorescent recombinant α-phycocyanin of Spirulina. *J. Appl. Microbial.* 2009; **106**, 1093-100.

[49] S Qin, Z Tang, F Lin, LA Sung and CK Tseng. Genomic cloning, expression and recombinant protein purification of α and β subunits of the allophycocyanin gene (apc) from the cyanobacterium *Anacystis nidulans* UTEX 625. *J. Appl. Phycol.* 2004; **16**, 483-7.
[50] Z Shoja, HR Memari and MR Ardakani. Cloning and expression of Beta Subunit Gene of Phycocyanin from *Spirulina platensis* in *Escherichia coli. Jundishapur J. Microbiol.* 2015; **8**, e17809.
[51] T Cherdkiatikul and Y Suwanwong. Production of the α and β subunits of *Spirulina* allophycocyanin and C-phycocyanin in *Escherichia coli*: A comparative study of their antioxidant activities. *J. Biomol. Screen* 2014; **19**, 959-65.
[52] MF Hossain, PR Ratnayake, K Meerajini and KL Wasantha Kumara. Antioxidant properties in some selected cyanobacteria isolated from fresh water bodies of Sri Lanka. *Food Sci. Nutr.* 2016; **4**, 753-8.
[53] A Patel, S Mishra and PK Ghosh. Antioxidant potential of C-phycocyanin isolated from cyanobacterial species *Lyngbya*, *Phormidium* and *Spirulina* spp. *Indian J. Biochem. Biol.* 2006; **43**, 25-31.
[54] B Soni, U Trivedi and D Madamwar. A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from Phormidium fragile and its characterization for antioxidant property. *Bioresource Tech.* 2008; **99**, 188-94.