J Plant Biotechnol (2017) 44:462-471
DOI:https://doi.org/10.5010/JPB.2017.44.4.462

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Upregulation of thiamine (vitamin B1) biosynthesis gene upon stress application in *Anabaena* sp. and *Nannochloropsis oculata*

Lee Li Fern · Aisamuddin Ardi Zainal Abidin · Zetty Norhana Balia Yusof

Received: 28 August 2017 / Revised: 27 October 2017 / Accepted: 14 November 2017

Abstract Thiamine pyrophosphate (TPP), the active form of thiamine is a cofactor for enzymes involved in central metabolism pathways. However, it is also known to have a role as a stress signaling molecule in response to environmental changes. *Anabaena* sp. and *N. oculata* are microorganisms which are abundantly found in Malaysia's freshwater and marine ecosystem. However, not much studies have been done especially in regards to thiamine biosynthesis. This work aimed to amplify of gene transcripts coding for thiamine biosynthesis enzymes besides looking at the expression of thiamine biosynthesis genes upon stress application. Various stress inducers were applied to the cultures and RNA was extracted at different time points. The first two genes, ThiC and ThiG/Thi4 encoding enzymes of the pyrimidine and thiazole branch respectively in the thiamine biosynthesis pathway were identified and amplified. The expression of the genes were analysed via RT-PCR and the intensity of bands were analysed using ImageJ software. The results showed up to 4-fold increase in the expression of ThiC and ThiG gene transcript as compared to control sample in *Anabaena* sp. ThiC gene in *N. oculata* showed an expression of 6-fold higher as compared to control sample. In conclusion, stresses induced the expression of the gene coding for one of the most important enzymes in thiamine biosynthesis pathway. This is an agreement with the hypothesis that overexpression of thiamine is crucial in assisting plants to combat abiotic stresses.

L. L. Fern · A. A. Z. Abidin · Z. N. B. Yusof (✉)
Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
e-mail: zettynorhana@upm.edu.my

A. A. Z. Abidin · Z. N. B. Yusof
Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Keywords Thiamine, Vitamin B1, Stress, Microalgae, Cyanobacteria, Gene expression

Introduction

In recent years, oxygenic phototrophic microorganism such as cyanobacteria and microalgae have been the center of interest as they are ideal models for many applications. They bloom rapidly with minimal amount of nutrients such as light, sugar, carbon dioxide, nitrogen, potassium and phosphorus (Behera, 2015). As compared to plants, they do not require land to grow and they are fast growing with simple genetic background to manipulate. Prokaryotic cyanobacteria do not have membrane-bound cell organelles and a defined nucleus whereas eukaryotic microalgae are more complex in structure and possess nucleus (Seckbach, 2007).

Thiamine and its active form, thiamine pyrophosphate (TPP) play important roles in human nutrition and central metabolism (Tunc-Ozdemir et al. 2009, Guan et al., 2014). Besides that, in recent studies thiamine and TPP have been reported as crucial stress-response molecules in plant adaptations to counteract different abiotic stress conditions (Tunc-Ozdemir et al. 2009; Rapala-Kozik et al. 2012). It is hypothesized that thiamine enhances tolerance to oxidative stress via salicylic acid (SA) signaling pathway (Ahn et al. 2007).

Presently, cyanobacteria and microalgae are not fully utilised as a source for thiamine production. Knowledge of thiamine biosynthesis pathway will pave the way for manipulation of thiamine biosynthesis for various purposes. Thiamine that are available in the market today are mainly synthetic. Cyanobacteria and microalgae could actually be an alternative source of thiamine but they are under explored. Previous study reported thiamine content in about 29 to 109 μg g^{-1} when extracted on dry weight basis during late exponential phase are *Nannochloropsis* sp., *Pavlova pinguis*, *Stichococcus*

sp. and *Tetraselmis* sp. (Brown et al. 1999).

Either in freshwater or marine ecosystem, cyanobacteria and microalgae are sensitive indicator to environmental changes (Levy et al. 2007). These microorganisms are abundantly found in extreme environmental conditions of temperature, pH, salt and metal (Seckbach, 2007). Nevertheless, rising of water pollution increases the abiotic stresses which can alter the production of thiamine (Sylvander et al. 2013). These microorganisms are able to synthesize thiamine *de novo* whereas higher trophic levels acquire thiamine through grazing and predation (Sylvander et al. 2013).

Salt is a natural component of the aquatic systems. Increasing salinity exceeds 100 mgL^{-1} (Nielsen et al. 2003) will limit growth and productivity of aquatic biota. NaCl can lead to two types of stress, ionic and hyperosmotic stress (Singh et al. 2002). Accumulation of inorganic ions in the cytoplasm will give a toxic effect on cell metabolism. The mechanism to combat the salinity stress involves the prevention of Na^+ influx by pumping out excess Na^+, resulting in salt tolerance (Apte et al. 1998).

Oxidative stress due to the accumulation of metals will cause lipid peroxidation, nucleic acid damage and inhibition of essential metabolism (Huertas et al. 2014). Copper and sodium hypochlorite have been used to generate oxidative stress. Copper can be a form of metal stress. Even though copper is an essential micronutrient to aquatic life, it can be toxic at concentration as low as 1 μgL^{-1} (Levy et al. 2007). Formation of hydroxyl radicals through Haber-Weiss and Fenton reaction are triggered by redox active metal as copper can transform between two oxidative stages (Srivastava et al. 2005; Huertas et al. 2014). Sodium hypochlorite (NaOCl) is a form of a bleaching agent widely used in industry. The mode of action of NaOCl is not yet well clarified but it has been suggested that exposure to NaOCl is similar to hydrogen peroxide (H_2O_2) which will induce the making of reactive oxygen species such as superoxide anions and hydroxyl radicals (Ceragioli et al. 2010).

Previous studies showed that thiamine production was upregulated in *Zea mays* (Rapala-Kozik et al. 2008), *Arabidopsis thaliana* (Rapala-Kozik et al. 2012) and *Elaies guineensis* (Abidin et al. 2016) under salinity and oxidative stress. Research done by Balia Yusof et al. (2015) revealed that *G. boninase* treated oil palm exhibited an upregulation of thiamine biosynthesis genes. Thi1/Thi4 showed an increase up to 70.21% whereas ThiC increased on a smaller scale of up to 54.17% while Wong et al. (2015) described the upregulation of both THIC and THI4 genes expression under osmotic stress in oil palm.

In this study, salinity and oxidative damage were investigated at molecular level in correlation to the production of thiamine. In addition to that, the productions of thiamine in prokaryotic and eukaryotic microorganisms are compared under different abiotic stresses. Generally, this study will give an idea on how abiotic stresses affect the cyanobacteria and microalgae in relation with thiamine production.

Materials and Methods

Culture maintenance

Cyanobacterium culture *Anabaena* sp. (G) was kindly given by Dr. Japareng Lalung of Universiti Sains Malaysia, Pulau Pinang. Microalgae culture *Nannochloropsis oculata* was obtained from Centre of Marine Science (COMAS), UPM. The cyanobacterium was cultivated in nitrate-free BG11 medium supplemented with sodium bicarbonate ($NaHCO_3$) and the microalga was cultivated in TAP/2 seawater medium. 200 ml of media was used to grow cultures in 250 ml conical flasks under continuous illumination of light and shaken at 150 rpm on a shaker incubator at temperature 30°C for cyanobacterium and 24°C for microalga.

Growth analysis

Two growth analyses were conducted in determining the growth of the cultures which were optical density and chlorophyll a analysis. 2 ml of cultures was taken for each analysis. The optical density was measured at 1000 nm (OD 1000) every seven days and two days for *Anabaena* sp. and *N. oculata* respectively using the UV-Spectrophotometer with a 3 ml cuvette and an optical path of 1 cm. Three replicate readings were taken.

For chlorophyll a analysis, 2 ml of the culture was centrifuged at 10 000 ×g for 10 minutes in a 2 ml Eppendorf tube. The supernatant was discarded and 2 ml of methanol was added to the cell pellet. The mixture was homogenised via pipetting and vortexed for 30 seconds. The samples were covered with aluminium foil and incubated overnight at 4°C. The supernatant was then collected by centrifugation at 10 000 ×g for 10 minutes. The spectrophotometer was calibrated using methanol as blank. The absorbance of blank and samples were read at 665 nm and 720 nm. The concentration of the chlorophyll a content was calculated according to formula: Chl_a [μgml^{-1}] = 12.9447 (A_{665}-A_{720}) (Zavřel et al. 2015).

Stress Induction

Three types of stresses were induced on the cultures at mid-exponential phase. Cultures were treated with two different concentrations and no treatment act as control. The samples were collected at four time points namely, day zero, day two, day four and day six. Similar experimental set up were used for all three stresses. The treatments given were 150 mM and 250 mM of sodium chloride (NaCl) (Srivastava et al. 2005), 2.0 ppm and 5.0 ppm of copper (Cu^{2+}), 10 μM and 50 μM of sodium hypochlorite (NaOCl).

Data mining and primer designing

The designing of primer for the amplification of thiamine biosynthesis genes in *Anabaena* sp. was done using protein translated sequence from Uniprot (http://www.uniprot.org/). The primers were designed using the translated sequences using Primer 3 Plus website (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). For *N. oculata*, Multiple Sequence Alignment (MSA) was carried out on various ThiC and Thi4 gene sequences obtained from five closely related plant and microalgae species retrieved from GenBank of NCBI database (Table 1). The sequences were aligned using ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The consensus regions were identified and forward and reverse primers were designed accordingly.

RNA extraction

The total RNA was extracted using the PGTX (Phenol/Glycerol/Triton-X) method as described by Pinto et al., (2009). The PGTX solution has the following composition (final volume of 100 ml): phenol (36.9 g), glycerol (6.9 ml), 8-hydroxyquinoline (0.1 g), EDTA (0.58 g), sodium acetate (0.8 g), guanidine thiocyanate (9.5 g), guanidine hydrochloride (4.6 g) and Triton X-100 (2 ml), with a final pH of 4.2. The PGTX mixture forms a monophasic solution at room temperature. 10 ml of cells were collected by centrifugation at 10 000 ×g for 10 minutes and 1 ml of PGTX was added and vortexed. The sample was incubated at 95°C for 5 minutes before being placed on ice for 5 minutes. 100 μl of bromochloropropane was added and mixed vigorously and incubated at room temperature for 2 to 15 minutes. The extraction mixture was then centrifuged at 12000 ×g at 4°C for 15 minutes to stimulate phase separation and the aqueous phase was then transferred to a new tube and mixed with equal volume of isopropanol. The sample was mixed by inversion and stored for 5 to 10 minutes at room temperature before centrifuging at 12000 ×g at 4°C for 15 minutes. The supernatant was discarded and the RNA pellet was washed with 1 ml 75% ethanol. The sample was then centrifuged at 8 000 ×g at 4°C for 5 minutes. The RNA pellet was air-dried for 5 minutes at room temperature in a laminar flow hood before resuspending it in 20 μl of dH_2O and stored at -80°C until further use.

Amplification of Gene Transcripts

Reverse transcription was performed using Qpcrbio cDNA Synthesis Kit (PCR Biosystems, London). Complementary-DNA (cDNA) was prepared by mixing 1 μg of total RNA, 2 μl of 5× cDNA synthesis mix, 0.5 μl of 20 × RTase (with RNAse inhibitor) and topped up with RNase-free H_2O to make up to 10 μl. The mixture was mixed gently by pipetting. After that, the mixture was incubated at 42°C for

Table 1 Data selected from different plant and microalgae GenBank and their accession number for ThiC and ThiG/Thi4 primer designing

Gene	Data from GenBank	Accession no.
ThiC	LOC4333719 phosphomethylpyrimidine synthase, chloroplastic [*Oryza sativa* Japonica Group (Japanese rice)]	NM_001057432.1
	THIC phosphomethylpyrimidine synthase [*Arabidopsis thaliana* (thale cress)]	NM_001202705.1
	THICb|THICa hydroxymethylpyrimidine phosphate synthase [*Chlamydomonas reinhardtii*]	XM_001697703
	CHLNCDRAFT_58425 hypothetical protein [*Chlorella variabilis*]	XM_005845784.1
	Coccomyxa subellipsoidea C-169,whole genome shotgun sequence	XM_005645335.1
Thi4	THI1 thiazole biosynthetic enzyme [*Arabidopsis thaliana* (thale cress)]	NM_124858.3
	ThI4b|THI4a THI4 regulatory protein [*Chlamydomonas reinhardtii*]	XM_001698620.1
	COCSUDRAFT_14398 thiazole biosynthesis enzyme [*Coccomyxa subellipsoidea* C-169]	XM_005648872.1
	thi1 thiamine biosynthesis 1 [*Zea mays*]	NM_001112226.1
	LOC4343443 thiamine thiazole synthase 2, chloroplastic [*Oryza sativa* Japonica Group (Japanese rice)]	NM_001066376.1

30 minutes followed an incubation at 85°C for 10 minutes. The mixture was then immediately put on ice and stored at -20°C until further use.

PCR was performed by using MyTaq™ Red Mix (Bioline, USA). A 10 µl reaction was prepared by mixing with 1 µl of cDNA Template, 0.5 µl of 10 mM forward primer, 0.5 µl of 10 mM reverse primer, 5 µl of MyTaq™ Red Mix, 2 × and 3 µl nuclease-free water. The mixture was vortexed and spun briefly. The reactions were placed in a thermocycler and the PCR cycling conditions included the initial denaturation step at 95°C for 1 minute 30 seconds for 1 cycle, followed by 35 repetitive cycles of denaturation step at 95°C for 20 seconds, annealing step at 52°C for 20 seconds and extension step at 72°C for 15 seconds. The final extension step was set at 72°C for 5 minutes for 1 cycle and then held at 4°C. The PCR product was then kept at -20°C until further use.

Analysis of gene expression

The intensity of the bands were analysed using ImageJ software (http://rsb.info.nih.gov/ij/index.html). ImageJ is a public domain, Java-based image processing and analysis program established by National Institutes of Health (NIH). A rectangle was drawn around the first band. 'Select First Lane' was selected to fix the rectangle in place. The following bands were done similarly. 'Plot Lanes' were selected to draw a profile plot for each band. A straight line was drawn to enclose the base of the peak. The peaks were clicked with a 'Wand' tool. 'Label Peaks' were selected to calculate the percentage of the total size of the enclosed peaks. Data analysis was done by calculating the relative density. Relative density is the ratio of the area of treated culture over the area of control culture as a reference.

$$\text{Relative Density} = \frac{Area\ of\ Treated\ Culture}{Area\ of\ Control\ Culture}$$

DNA Sequencing

Purified PCR products were sequenced using the 1st Base DNA sequencing service (1st BASE, Singapore). The sequencing results were analysed using Basic Local Alignment Search Tool (BLAST) to search for sequence similarity with what is available in the GenBank.

Results

Growth analysis on treated Anabaena sp. and N. oculata

The growth on treated organisms were analysed by OD 1000 and chlorophyll a analysis at four time points, day zero, day two, day four and day six. Four time points were selected after the exponential phase for both organisms were identified. Exponential phase is a stage of cell growth where cells replicate rapidly accompanied by multiple rounds of DNA replication and cell division. It is the best period to study cell behaviour as cell most active and appropriate amounts of metabolites are produced (Watanabe et al. 2015).

Growth analysis on salt treated organisms

The effect of salinity on the growth of *Anabaena* sp. and *N. oculata* were demonstrated at concentration of 150 mM and 250 mM NaCl. Based on Figure 1 and Figure 2, the growth and chlorophyll a of control samples increased gradually. The *Anabaena* sp. treated with 150 mM NaCl was decreased initially and started to adapt on day four whereas in 250 mM the growth of treated cultures decreased

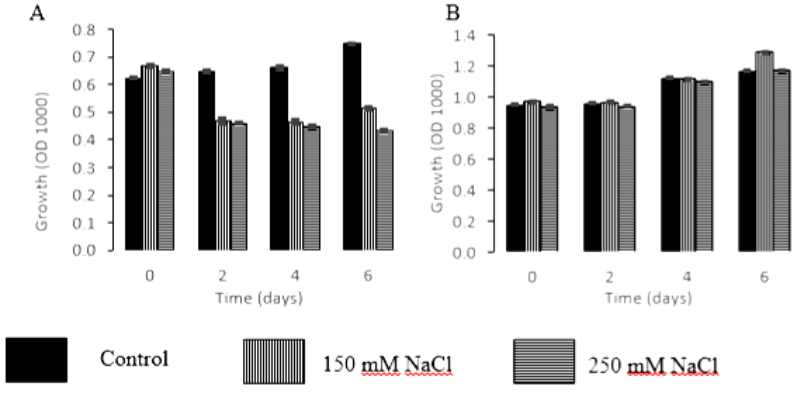

Fig. 1 Optical density growth analysis of (A) *Anabaena sp.* and (B) *N. oculata* under salinity stress (150 mM NaCl and 250 mM NaCl) along with a non-treated culture as control. Values represent mean ± SE of three replicates

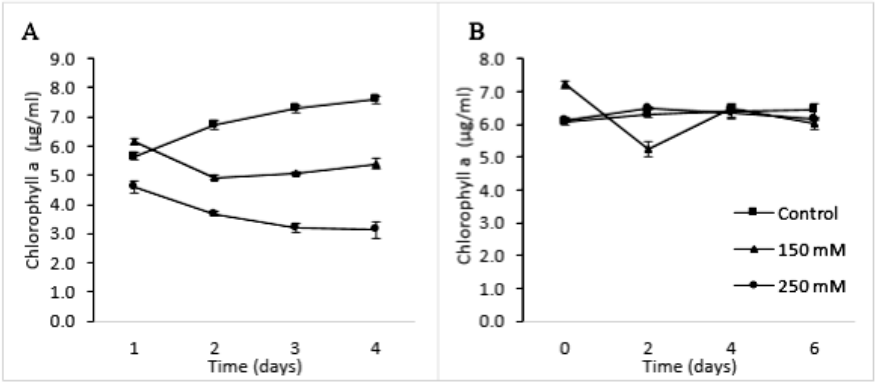

Fig. 2 Chlorophyll a analysis of (A) *Anabaena sp.* and (B) *N. oculata* under salinity stress (150 mM NaCl and 250 mM NaCl) along with a non-treated culture as control. Values represent mean ± SE of three replicates

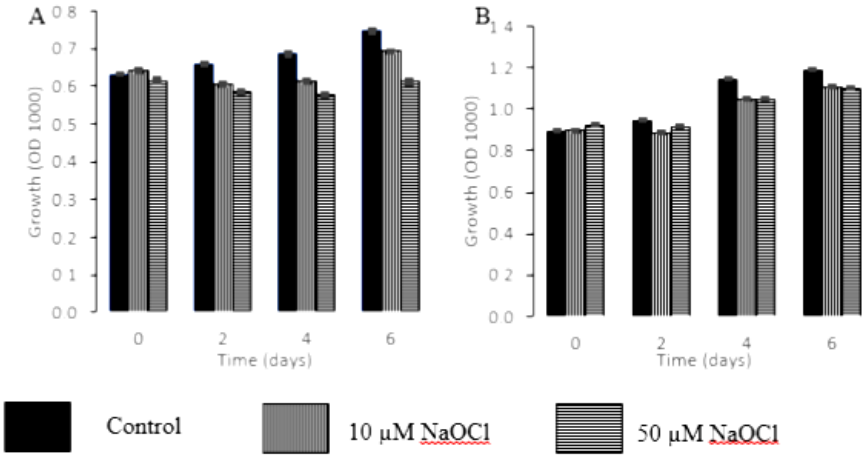

Fig. 3 Optical density analysis of (A) *Anabaena* sp. and (B) *N. oculata* under sodium hypochlorite oxidative stress (10 µM and 50 µM) along with a non-treated culture as control. Values represent mean ± SE of three replicates

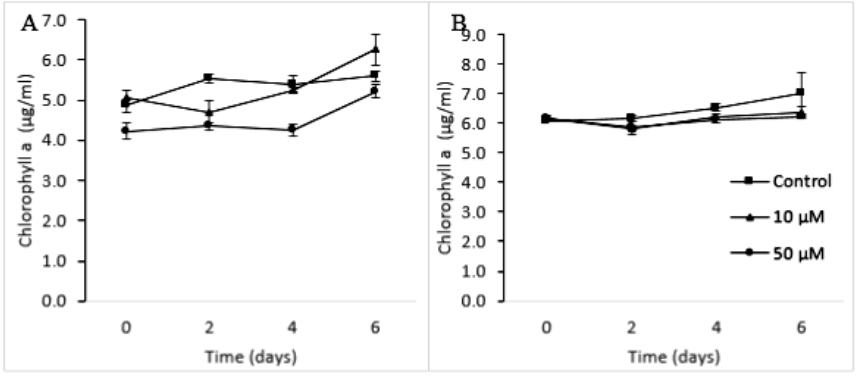

Fig. 4 Chlorophyll a analysis of (A) *Anabaena* sp. and (B) *N. oculata* under sodium hypochlorite oxidative stress (10 µM and 50 µM) along with a non-treated culture as control. Values represent mean ± SE of three replicates

until day six. At both concentrations of NaCl, *N. oculata* started to adapt after day two. One of the mechanisms that allow *Anabaena* sp. and *N. oculata* to acclimate to salt stress is the fatty acids on the membrane desaturate and thus activate the Na^+/H^+ antiporter (Singh et al. 2002).

Growth analysis on sodium hypochlorite treated organisms

The effect of sodium hypochlorite on the growth of *Anabaena* sp. and *N. oculata* were analysed at concentrations of 10 µM and 50 µM NaOCl. Based on Figure 3 and Figure 4, the optical density and chlorophyll a of control cultures

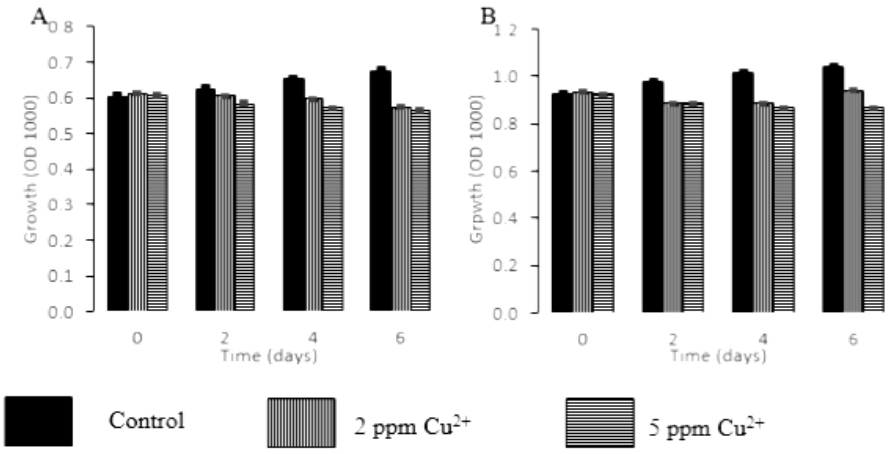

Fig. 5 Optical density analysis of (A) *Anabaena* sp. and (B) *N. oculata* under copper induced oxidative stress (2 ppm and 5 ppm) along with a non-treated culture as control. Values represent mean ± SE of three replicates

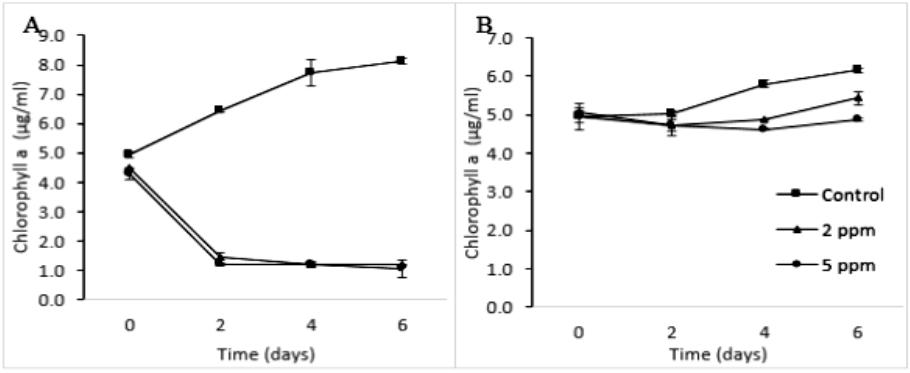

Fig. 6 Chlorophyll a analysis of (A) *Anabaena* sp. and (B) *N. oculata* under copper induced oxidative stress (2 ppm and 5 ppm) along with a non-treated culture as control. Values represent mean ± SE of three replicates

rose steadily. Growth of both NaOCl treated organisms decreased until the day two and started to increase in cell density after that until day six. From this observation, the concentration given may be insufficient to give stress effect on the organism.

Growth analysis on copper treated organisms

The effect of copper on the growth of *Anabaena* sp. and *N. oculata* were analysed with 2 ppm and 5 ppm of copper. Based on Figure 5 and Figure 6, the optical density and chlorophyll a of control cultures increased moderately. In copper treated *Anabaena* sp., it showed an inhibition in growth. Copper showed some degree of toxicity as proven in the drastic drop in chlorophyll a content. A negative relationship were observed as the chlorophyll a decreased with the increase in copper concentration. Heavy metal inhibits the biosynthesis of photosynthetic pigments because it contributes a great impact on the PSII reaction centre. Due to high irradiance, copper ion embed directly into the PSII and cause a damage (Dey et al. 2014). Electron transport reaction is affected as a decline in energy transfer from the antennae to the reaction centre (Ralph and Burchett, 1998). In addition to that, other consequences of copper toxicity are the modification of PSII membrane fluidity and the alteration of thylakoid membrane as a result of lipid peroxidation (Yruela, 2005). *N. oculata* showed less effect of stress as compared to *Anabaena* sp.

Data mining and primer designing

Five pairs of primer were successfully designed by using different approaches. The primer sequences were as shown in Table 2. Primer 3 Plus software was used for *Anabaena* sp. whereas MSA was applied on *N. oculata*. Based on a previous study, 16S gene was selected for *Anabaena* sp. as housekeeping gene. 16S was classified as the most stable gene that can be used for normalisation in all tested condition (Pinto et al. 2012).

Table 2 Primers design for the amplification of ThiC, ThiG/Thi4 and 16S gene transcripts

Organism	Target gene	Sequence
Anabaena sp.	16S F	5'-CAC ACT GGG ACT GAG ACA-3'
	16S R	5'-CTG CTG GCA CGG AGT TAG-3'
	ThiC F	5'-TCT CTG TCT CTT GC ACCT GG-3'
	ThiC R	5'-ACA GGT GAT GGT AGA AGG GC-3'
	ThiG F	5'-GCT TCT TCT GCG GTT TGA CA-3'
	ThiG R	5'-TAT TGG TGG CGG CGT TAT TG-3'
N. oculata	ThiC F	5'-GCG CAG TTT GCA GAG CTG AAG-3'
	ThiC R	5'-CTT GAC ATC GTC CCG GTT TGG-3'
	Thi4 F	5'-GAG ACC GAC GTC GTC ATC G-3'
	Thi4 R	5'-CGT TGA ACA GCT TCA CGT TGG-3'

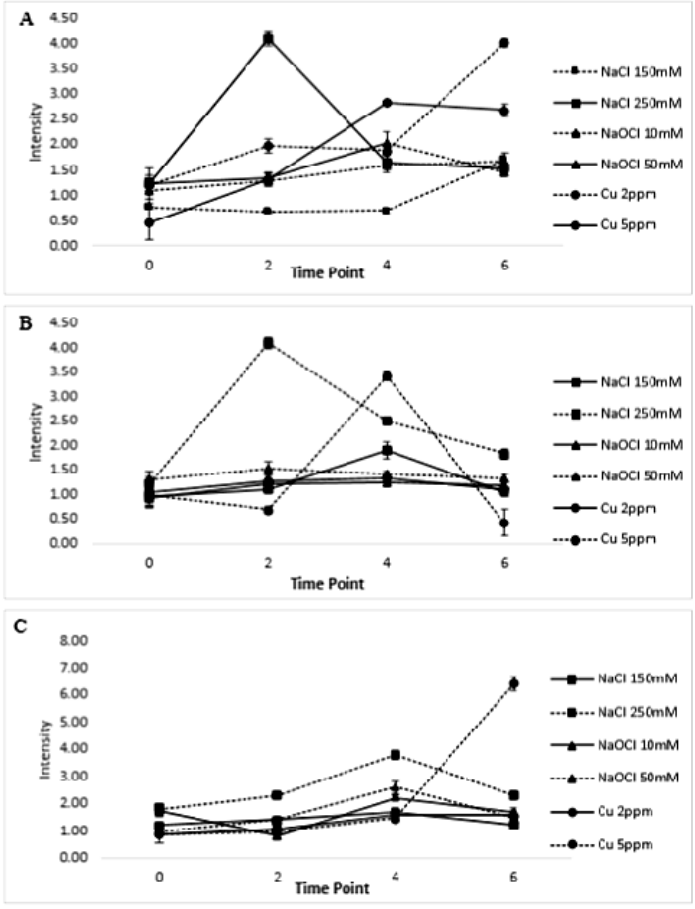

Fig. 7 Expression of (A) ThiC, (B) ThiG in treated *Anabaena* sp. and (C) Thi4 in treated *N. oculata*. Values represent mean ± SE of three replicates

Amplification and quantification of gene of interest

The upregulation of biosynthesis gene, ThiC and ThiG/Thi4 were studied through gene expression. The intensity of the bands appeared brighter in treated sample as shown in Figure 7. Gene expression profiling revealed the information about the behaviour of gene to adapt under different conditions. The PCR products on the agarose gel gave a quick visual effect on the result. A next level of interpretation was done via ImageJ.

ImageJ was used to compare the intensity of the bands as shown in Figure 7. The area and pixel value statistics were calculated on the density histogram and line profile plots. Overall, there was an increase in ThiC, ThiG/Thi4 expression in stress-induced samples. However, different organisms behave differently towards different stresses and concentrations. A similar trend could be spotted on *Anabaena* sp. under 250 mM NaCl, both ThiC and ThiG showed the

highest expression on day two with about four fold increase as compared to control sample. In some treated samples, the gene expression decreased along with the time point. A few samples decreased back to same level or lower than control. This may be due to the fact that thiamine comes into play to help the organism adapt to the environmental stress. Thiamine triggers defence responses via salicylic acid pathway by preventing oxidising effect on *salicylic acid induction-deficient 1* mutant (Tunc-Ozdemir et al. 2009). However, the metabolism and aspects of biochemistry still remain unclear. Previous study illustrated a threefold increase in total thiamine content in hydrogen peroxide treated maize seedlings (Goyer, 2010). Expression of pathogenesis-related (PR) proteins is induced by exogenous thiamine was proven in tobacco, cucumber and *Arabidopsis* (Al-Hakimi and Hamada, 2011). In order to develop new adaptation strategy and to overcome the effect of stress, the organisms increase the expression of thiamine as stress-response molecules. In *N. oculata*, it showed the highest expression of Thi4 when subjected to 5 ppm Cu^{2+} at day six. The expression was 6 fold higher as compared to control. As a comparison, *Anabaena* sp. is faster in responding to stresses than *N. oculata* because the expression of thiamine biosynthesis genes was higher at early stage of stress application. It could be due to the dosage of stresses induced had less effect on *N. oculata*. The nature of habitat may be a factor contributing to this phenomenon. *N. oculata* lives at seawater whereas *Anabaena* sp. uses freshwater to survive. The seawater itself has higher salinity and mineral content. Therefore, the stresses did not give much effect to *N. oculata*.

Discussion

In this study, cyanobacterium *Anabaena* sp. and microalga *N. oculata* were subjected to various abiotic stress inducers, namely NaCl, NaOCl and Cu. Samples were taken at various time points and satisfactory quality and quantity of total RNA were successfully extracted with PGTX method for *Anabaena* sp. and *N. oculata* with A_{260}/A_{280} ratio of 1.8 and above. Four thiamine biosynthesis genes and one housekeeping gene primers were designed to study the gene expression of ThiC and ThiG/Thi4 under abiotic stress. RT-PCR was performed to study the level of gene expression in control and treated cultures. The level of gene expression was analysed through ImageJ software. Generally, all treated samples showed an increase in expression upon stress application. The expression was up to 400% higher as compared to control sample of *Anabaena* sp. for ThiC and ThiG gene. ThiC gene in *N. oculata* showed an expression of 600% higher as compared to control. To sum up, the objectives were successfully achieved together with the hypothesis. Stress induced the expression of thiamine biosynthesis gene in both cyanobacterium and microalga. Different type of stresses affected the expression of thiamine biosynthesis gene differently. In general, copper upregulated thiamine biosynthesis gene most efficiently whereas sodium hypochlorite has the least effect.

For future study, quantification of thiamine and its intermediates should be carried out in order to see if the upregulation of genes actually affect the biosynthesis of thiamine overall. Since thiamine biosynthesis gene for *Anabaena* sp. and *N. oculata* have been successfully identified, manipulation of the thiamine biosynthesis pathway could be utilised for commercial thiamine production.

Acknowledgements

This work was carried out with the support of "Geran Putra Universiti Putra Malaysia (Project No.: 9425900)", Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

References

Abed RMM, Kohls K, Schoon R, Scherf A, Schacht M, Palinska KA, Al-Hassani H, Hamza W, Rullkotter J, Golubic S (2008) Lipid biomarkers, pigments and cyanobacterial diversity of microbial mats across intertidal flats of the arid coast of the Arabian Gulf (Abu Dhabi, UAE). Federation of European Microbiological Societies 65:449-462

Abidin AAZ, Wong SY, Rahman NSA, Idris ZHC, Balia Yusof ZN. (2016) Osmotic, oxidative and salinity stresses upregulate the expressions of thiamine (Vitamin B1) biosynthesis genes (THIC and THI1/THI4) in oil palm (*Elaies guineensis*). J. Oil Palm Res. 28(3):308-319

Ahn I, Kim S, Lee Y (2005) Vitamin B1 functions as an activator of plant disease resistance. Physiol Plant 138:1505-1515

Ahn IP, Kim S, Lee YH, Suh SC (2007) Vitamin B1- induced priming in dependent on hydrogen peroxide and the NPRI gene in *Arabidopsis*. Physiol Plant 143:838-848

Al-Hakim AM, Hamada AM (2011) Ascorbic acid, thiamine or salicylic acid induced changes in some physiological parameters I wheat grown under copper stress. Plant Protect Sci 47: 92-108

Allen MM (1968) Simple conditions for growth of unicellular blue-green algae. J General Microb 51:199-202

Apte SK, Fernandes T, Badran H, Ballal A (1998) Expression and possible role of stress-responsive proteins in *Anabaena*. J Biosci 23:399-406

Balia Yusof ZN, Borhan FP, Mohamad FA Rusli MH. (2015). The effect of *Ganoderma boninense* infection on the expressions of thiamine (vitamin B1) biosynthesis genes in oil palm. J. Oil Palm Res. 27, 12-18

Behera S, Singh R, Arora R, Sharma NK, Shukla M, Kumar S (2015) Scope of algae as third generation biofuels. Frontiers in Bioengineering and Biotechnology, 2. Doi: 10.3389/fbioe. 2014.00090

Beversdorf LJ, Miller TR, McMahon KD (2013) The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperature, eutrophic lake. PloS ONE, 8. Doi:10.1371/journal.pone. 0056103

Brown MR, Mular M, Miller I, Farmer C, Trenerry C (1999) The vitamin content of microalgae used in aquaculture. J Appl Phycol 11: 247-255

Cao S, Zhang X, Ye N, Fan X, Mou S, Xu D, Liang C, Wang Y, Wang X (2012) Evaluation of putative internal reference genes for gene expression normalization in *Nannochloropsis* sp. by quantitative real-time RT-PCR. Biochemical and Biophysical Research 424:118-123

Ceragioli M, Mols M, Moezelaar R., Ghelardi E, Senesi S, Abee T (2010) Comparative transcriptomic and phenotypic analysis of the responses of *Bacillus cereus* to various disinfectant treatments. Appl Environment Microb 76:3352-3360

Chen M, Li J, Zhang L, Chang S, Liu C, Wang J, Li S (2014) Auto-flotation of heterocyst enables the efficient production of renewable energy in cyanobacteria. Scientific reports, 4. Doi: 10.1038/srep03998

Chiu S, Tsai, M, Kao C, Ong S, Lin C (2009) The air-lift photobioreactors with flow patterning for high-density cultures of microalgae and carbon dioxide removal. Engineering in Life Sciences 9:254-260

Croft MT, Moulin M, Webb ME, Smith AG (2007) Thiamine biosynthesis in algae is regulated by riboswitches. Proceedings of the National Academy of Sciences 104:20770-20775

Dey S, Mazumder PB, Paul SB (2014) Effect of copper on growth and chlorophyll content in tea plants (Camellia sinesis (L.) O. Kuntze). Impact Journals 2:223-230

Fernandes TA, Iyer V, Apte SK (1993) Differential responses of nitrogen-fixing cyanobacteria to salinity and osmotic stresses. Appl and Environmental Microb 59:899-904

Gao Y, Tahmasebi A, Dou J, Yu J (2016) Combustion characteristics and air pollutant formation during oxy-fuel co-combustion of microalgae and lignite. Bioresource Technol 207:276-284

Gorman DS, Levine RP (1966) Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences USA 54:1665-1669

Goyer A (2010) Thiamine in plants: aspects of its metabolism and functions. Phytochemistry 27:1615-1624

Guan R, Qu Y, Guo Y, Yu L, Liu Y, Jiang J, Chen J, Ren Y, Liu G, Tian J, Jin L, Liu Z, Hong H, Chang R, Gilliham M, Qiu L (2014) Salinity tolerance in soybean is modulated by natural variation in GmSALT3. The Plant Journal 80:937-950

Gupta V, Ratha SK, Sood A, Chaudhary V, Prasanna R (2013) New insights into the biodiversity and applications of cyanobacteria (blue-green algae) - prospects and challenges. Algal Research 2:79-97

Haas RH (1988) Thiamine and the brain. Annual reviews 8:483-515

Huertas MJ, López-Maury L, Giner-Lamia J, Sánchez-Riego M, Florencio FJ (2014) Metals in cyanobacteria: analysis of the copper, nickel, cobalt and arsenic homeostasis mechanisms. Life 4:865-886

Hughes EO, Gorham PR, Zehnder A (1958) Toxicity of a unialgal culture of *Microcystis aeruginosa*. Canadian J Microb 4:225-236

Kagan, ML, Matulka, RA (2015) Safety assessment of the microalgae *Nannochloropsis oculata*. Toxicology report 2:617-623

Kaneko T, Nakamura Y, Wolk CP, Kuritz T, Sasammoto S, Watanabe A, Iriguchi M, Ishikawa A, Kawashima K, Kimura T, Kishida Y, Kohara M, Matsumoto M, Matsuno A, Muraki A, Nakazaki N, Shimpo S, Sugimoto M, Takazawa M, Yamada M, Yasuda M, Tabata S (2001) Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. DNA research 8:205-213

Kehr JC, Picchi DG, Dittmann E (2011) Natural product biosynthesis in cyanobacteria: a treasure trove if unique enzymes. Beilstein J Organic Chem 7: 1622-1635

Kim B, Ramanan R, Cho D, Choi G, La H, Ahn C, Oh H, Kim H (2012) Simple, rapid and cost-effective method for high quality nucleic acids extraction from different strains of *Botryococcus braunii*. PloS ONE, 7. Doi:10.1371/journal. pone.0037770

Knoop H, Gründel M, Zilliges Y, Lehmann R, Hoffmann S, Lockau W, Steuer, R (2013) Flux balance analysis of cyanobacterial metabolism: the metabolism network of *Synechocystis* sp. PCC 6803. PloS Computational Biology, 9. Doi:10.1371/journal. pcbi.1003081

Latifi A, Ruiz M, Zhang, C (2008) Oxidative stress in cyanobacteria. Federation of European Microbiological Societies 33:258-278

Levy B, Stauber, JL, Jolley DF (2007) Sensitivity of marine microalgae to copper: the effect of biotic factors on copper adsorption and toxicity. Science of the Total Environment 387:141-154

Méjean A, Paci G, Gautier V, Ploux O (2014) Biosynthesis of anatoxin-a and analogues (anatoxins) in cyanobacteria. Toxicon 91:15-22

Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V (2015) Microalgae biofuels as an alternative to fossil fuel for power generation. Renewable and Sustainable Energy Reviews 58:180-197

Neilsen DL, Brock MA, Rees GN, Baldwin DS (2003) Effect of increasing salinity on freshwater ecosystems in Australia. Australian Journal of Botany 51:655-665

Pade N, Hagemann M (2014) Salt acclimation of cyanobacteria and their application in biotechnology. Life 5:25-49

Pinto E, Pedersen M, Snoeijs P, Nieuwerburgh LV, Colepicolo P (2002) Simultaneous detection of thiamine and its phosphate esters from microalgae by HPLC. Biochemical and biophysical research communications 291:344-348

Pinto FL, Thapper A, Sontheim W, Lindblad, P (2009) Analysis of

current and alternative phenol based RNA extraction methodologies for cyanobacteria. BioMed Central (BMC) Molecular Biology, 10. DOI: 10.1186/1471-2199-10-79

Rajaram H, Chaurasia AK, Apte, SK (2014) Cyanobacterial heat-shock response: role and regulation of molecular chaperones. Microbiology, 160:647-658

Ralph PJ, Burchett, MD (1998) Photosynthetic response of *Halophila ovalis* to heavy metal stress. Environmental Pollution, 103:91-101

Rapala-Kozik M, Kowalska E, Ostrowska K (2008) Modulation of thiamine metabolism in *Zea mays* seedlings under conditions of abiotic stress. Journal of Experimental Botany 59:4133-4143

Rapala-Kozik M, Wolak N, Kujda M, Banas AK (2012) The upregulation of thiamine (vitamin B_1) biosynthesis in *Arabidopsis thaliana* seedlings under salt and osmotic stress condition is mediated by abscisic acid at the early stages of their stress response. BioMed Central Plant Biology, 12. DOI: 10.1186/1471-2229-12-2

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Seckbach J (2007) Algae and cyanobacteria in extreme environments. Netherlands: Springer

Singh SC, Sinha RP, Hader D (2002) Role of lipids and fatty acids in stress tolerance in cyanobacteria. Acta Protozoologica 41:297-308

Solimeno A, Samso R, Garcia, J (2016) Parameter sensitivity analysis of a mechanistic model to simulate microalgae growth. Algal Research 15:217-223

Spolaore P, Joannis-Cassan C, Duran E, Isambert, A (2006) Commercial application of microalgae. J biosci and bioeng 101:87-96

Srivastava AK, Rai S, Pandey S, Singh PK, Agrawal C, Rai LC (2013) Understanding the mechanisms of abiotic stress management in cyanobacteria with special reference to proteomics. In: Srivastava AK, Rai AN, Neilan BA(ed) Stress biology of cyanobacteria molecular mechanisms to cellular responses, CRC Press, New York, pp 93-112

Srivastava AK. Bhargava P, Rai LC (2005) Salinity and copper-induced oxidative damage and changes in the antioxidative defence systems of *Anabaena doliolum*. World J Microb and Biotechnol 21:1291-1298

Sukarni Sudjito, Hamidi N, Yanuhar U, Wardana ING (2014) Potential and properties of marine microalgae *Nannochloropsis oculata* as biomass fuel feedstock. International Journal of Energy and Environmental 5:279-290

Sylvander P, Haubner N. Snoeijs, P (2012) The thiamine content of phytoplankton cells is affected by abiotic stress and growth rate. Microbiology of Aquatic Systems 65:566-577

Tunc-Ozdemir M, Miller G, Song L, Kim J, Sodek A, Koussevitzky S, Misra AN, Mittler R, Shintani, D (2009) Thiamine confers enhanced tolerance to oxidative stress in Arabidopsis. Physiol Plant 151:421-432

Watanabe S, Ohbayashi R, Kanesaki Y, Saito N, Chibazakura T, Soga T, Yoshikawa H (2015) Intensive DNA replication and metabolism during the lag phase in cyanobacteria. PloS One, 10. Doi:10.1371/journal.pone.0136800

Watrbury JB (2006) The cyanobacteria-isolation, purification and identification. Prokaryotes 4:1053-1073

Wong SY, Aziz SDA, Balia Yusof ZN (2016) Osmotic stress upregulates the expression of thiamine (Vitamin B1) biosynthesis genes (THIC and THI4) in oil palm (*Elaies guineensis*). African Journal of Biotechnology, 15(29): 1566-1574

Xu H, Cai H, Yu G, Jiang H (2013) Insights into extracellular polymeric substances of cyanobacterium *Microcystis aeruginosa* using fractionation procedure and parallel factor analysis. Water Research 47:2005-2014

Yruela I (2005) Copper in plants. Brazilian J Physiol Plant 17:145-156

Zavřel T, Sinetova MA, Červený J (2015) Measurement of chlorophyll a and carotenoids concentration in cyanobacteria. Bio-protocol 5. Doi: e1467. http://www.bio-protocol.org/e1467