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Gene expression profile of marine *Chlorella* strains from different latitudes: stress and recovery under elevated temperatures

Bahram Barati[1,2] · Phaik-Eem Lim[1] · Sook-Yee Gan[3] · Sze-Wan Poong[1] · Siew-Moi Phang[1,4]

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Abstract

Global warming, as a consequence of climate change, poses a critical threat to marine life, including algae. Studies on algal response at the molecular level to temperature stress have been significantly improved by advances in omics technologies. Algae are known to employ various strategies in response to heat stress. For example, algae regulate starch synthesis to provide energy for the cell or rebuild the damaged subunits of photosystems to regain photosynthetic activity. The aim of the present study is to examine the expression of selected photosynthesis-related genes of marine *Chlorella* originating from different latitudes, in response to heat stress and during the recovery period. In this study, marine *Chlorella* strains from the Antarctic, temperate region, and the tropics were grown at their ambient and stress-inducing temperatures. The maximum quantum efficiency (F_v/F_m) photosynthetic parameter was used to assess their stress levels. When subjected to heat stress, the F_v/F_m began to decline and when it reached ~ 0.2, the cultures were transferred to their respective ambient temperature for recovery. Total RNA was isolated from these cultures at F_v/F_m ~ 0.4, 0.2, and when it regained 0.4 during recovery. The expression of four genes including *psbA*, *psaB*, *psbC*, and *rbcL* was analyzed using RT-PCR. The housekeeping gene, histone subunit three (H3) was used for data normalization. Studying the genes involved in the adaptation mechanisms would enhance our knowledge on algal adaptation pathways and pave the way for genetic engineers to develop more tolerant strains.

Keywords Abiotic stress · Photosystem · Photosynthesis · Stress adaptation

Introduction

Global climate change is documented as one of the most serious environmental matters facing the Earth. An average rise of 4 to 5 °C is anticipated by the end of the century based on the report from the Intergovernmental Panel on Climate Change (IPCC) (Stocker et al. 2014). In addition, the severity, duration, and frequency of heatwaves, with unusually high temperatures are also increasing (Robinson 2001; Tripathi et al. 2016). It has been presented that climate variations are altering the base of the food web and consequently the food chain (Smith et al. 2008; Montes-Hugo et al. 2009). On a global scale, species respond to thermal stress with distributional range shifts and phenological alternations that often result in regional extinction (Jueterbock et al. 2014). Algae are omnipresent organisms that contribute to about half of the total primary production at the base of food chains (Behrenfeld et al. 2001; Beardall and Raven 2004; Chapman 2013; Falkowski and Raven 2013). Temperature plays a critical role in growth (Raven and Geider 1988; Singh and Singh 2015) and photosynthesis (Davison 1991; Huner et al. 1993), through changes in the stability of biomolecules and the rates of biochemical and physiological processes (Fujimoto et al. 1994). Fundamentally, optimal temperature increases growth rate, while temperatures beyond the optimal are lethal (Ras et al. 2013).

Algae photosynthesis is recognized as one of the most heat-sensitive processes and it can be entirely suppressed by high temperature earlier than other traits of the stress response are detected (Berry and Bjorkman 1980). Environmental stressors

✉ Phaik-Eem Lim
phaikeem@um.edu.my

[1] Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

[2] Institute of Graduate Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia

[3] School of Pharmacy, International Medical University, 57000 Kuala Lumpur, Malaysia

[4] Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia

such as heat generate an imbalance between consumption and production of energy in photosynthetic algae, which in turn initiates adjustment of the photosynthetic apparatus, light harvesting antenna (Dall'Osto et al. 2015), xanthophyll cycle (Havaux and Tardy 1996), electron transport pathways, and Calvin-Benson cycle (Berry and Bjorkman 1980). This includes photosynthetic temperature acclimation or even inhibition of photosynthesis (Ras et al. 2013). Environmental variations are known to affect the subunit composition of reaction centers, i.e., the photosystems I (PSI) and II (PSII). Among the photosynthetic components PSII is reportedly the most vulnerable to heat stress (Foyer et al. 1994; Allakhverdiev et al. 2008). It is involved in the water-splitting step of photosynthesis where water is transformed into protons and oxygen via the oxygen-evolving complex and the electrons are released and transferred to the PSI complex (Gururani et al. 2015). Therefore, several photosynthesis-related genes particularly those involved in PSI and PSII reaction centers have been rigorously studied to assess photosynthetic responses to various stress conditions. These genes include the *psbC* gene which encodes a PSII chlorophyll-binding protein (Qian et al. 2009b; Chong et al. 2011) and *psaB* which encodes one of the reaction center subunits of PSI (Qian et al. 2009b; Qian et al. 2011). Photosynthesis-related genes are among the many differentially expressed genes involved in various metabolic pathways during an algal response to heat stress as shown in a recent transcriptomic study by Poong et al. (2018).

Understanding the biological principles and regulatory networks by which algae live can aid in explaining their successful survival, reproduction, and distribution. In addition, knowledge on their responses toward stress is valuable if algae are potentially used to meet the increasing need for feed, food, and biomass (Moreno-Risueno et al. 2010). There are numerous studies on the influences of abiotic stress on algae at the molecular level in order to determine the roles and functions of stress-related genes and proteins. In recent years, progression in omics technologies such as transcriptomics and genomics have contributed to profound insights in the system biology of algae (Nouri et al. 2015), thereby increasing the feasibility of engineering strains with greater yields. Transcript profiling techniques allow the simultaneous analysis of numerous genes and can be employed to study alterations in gene expression. Among the transcriptomics tools, real-time quantitative reverse transcription PCR (qRT-PCR) is widely accepted as the gold standard for the analysis of gene expression (Pabinger et al. 2014).

Chlorella is a well-studied phototrophic eukaryotic microalga (Krienitz et al. 2004; Wong et al. 2015) that can be found in various environments. *Chlorella* is also an ideal organism used for the study of biochemical and physiological characteristics of green algae with potential applications in biotechnology (Phang and Chu 2004; Safi et al. 2014; Krienitz et al. 2015). It is recognized that the influence of global warming on species varies geographically (Deutsch et al. 2008). As algae inhabiting regions of diverse latitudes are adapted and acclimated to various temperature regimes (Teoh et al. 2013), therefore the geographical source of the algae has been proposed to influence their response and adaptation to temperature stress. Hence, the current study aims to further investigate how elevated temperatures affect photosynthesis in *Chlorella* from diverse latitudes during stress and recovery via gene expression profiling of several photosynthesis-related genes.

Material and methods

Culture maintenance Three *Chlorella* species from diverse latitudes were obtained as follows: tropical (UMACC 245) and Antarctic (UMACC 250) species from the University of Malaya Algae Culture Collection while the temperate species, originally isolated from Loch Linnhe, Argyll, Scotland (CCAP 211/75 or UMACC 373), was from the Culture Collection of Algae and Protozoa. The stock cultures were grown in Provasoli (Prov) medium (Phang and Chu 1999) and maintained in an incubator illuminated with cool white fluorescent lamps (40 μmol photons m^{-2} s^{-1} on a 12:12 light-dark cycle) at the following respective ambient temperature: 4 °C (Antarctic), 18 °C (temperate), and 28 °C (tropical). For the simplicity of reference in this manuscript, the selected strains will be referred to as *Chlorella*-Ant (UMACC 250), *Chlorella*-Trop (UMACC 245), and *Chlorella*-Temp (UMACC 373).

Stress and recovery treatment In an earlier study by Barati et al. (2018), stress was induced in both *Chlorella*-Ant and *Chlorella*-Temp at 38 °C, while *Chlorella*-Trop showed stress response at 40 °C. Throughout the stress treatment, the cultures were incubated at their respective stress-inducing temperatures and the decline in F_v/F_m was carefully monitored to assess their levels of stress. When the F_v/F_m value decreased to approximately 0.2, the cultures were returned to their ambient temperature for recovery ("recovery" period). The cultures were considered recovered when the F_v/F_m increased to approximately 0.4. Total RNA was isolated from the cells when F_v/F_m decreased to approximately 0.4 (first stress level), 0.2 (second stress level), and upon recovery.

Measurement of maximum quantum yield (F_v/F_m) Chlorophyll *a* variable fluorescence parameters were measured using a pulse amplitude modulated fluorometer (Water PAM; Heinz Walz, Germany) and rapid light curves (RLCs) were achieved under software control (Win Control, Walz) (Ralph and Gademann 2005). The samples were dark-acclimated for at least 15 min before the onset of measurement (Wong et al. 2015; Cao et al. 2016; Wang and Xu 2016). RLCs

were obtained by exposing the samples to eight increasing red actinic irradiances (48, 105, 158, 233, 358, 530, 812, and 1216 μmol photons m^{-2} s^{-1}) for a duration of 10 s, each separated by a 0.8-s saturating flash (2000 μmol photons m^{-2} s^{-1}). The maximum quantum efficiency (F_v/F_m), was calculated as $F_v/F_m = (F_m - F_0)/F_m$, where F_m is the maximum fluorescence, F_0 is the minimum fluorescence, and F_v is the variable fluorescence. The relative electron transport rate (rETR) was calculated by multiplying the quantum yield by irradiance measured at the end of each light interval (Harbinson et al. 1989). Alpha (α), defined as the initial slope of the rETR vs irradiance curve, is used as a measure of light harvesting efficiency. Values for the maximum relative electron transport rate ($rETR_{max}$) and α were computed by fitting the data from RLCs to an exponential function using a multiple non-linear regression (Platt et al. 1980). As all treatments were processed in the same way, the relative changes in electron transport presumably reflect alterations to cell performance in relation to temperature.

RNA extraction, purification, and cDNA synthesis RNA extraction followed the protocol by Poong et al. (2017). All RNA extractions were performed in triplicate using approximately 300 mg fresh weight of cells. Genomic DNA (gDNA) was eliminated using the TURBO DNA-free kit (Ambion) according to the manufacturer's protocol. NanoDrop 2000c UV-Vis spectrophotometer (Thermo Fisher Scientific) was used to determine the yield and purity of the RNA samples. Then, cDNA synthesis was performed with a total of 2000 ng RNA using the High Capacity RNA-to-cDNA kit (Applied Biosystems).

Primer design and qRT-PCR As shown in Table 1, five sets of primers were used to amplify the selected genes, namely, *psbA*, *psaB*, *psbC*, *rbcL*, and the endogenous control gene, histone protein subunit (*H3*). The primers were either obtained from literature or designed using Primer Express (Applied Biosystems). The qRT-PCR assays were conducted using PowerUP SYBR green Master Mix (Applied Biosystems) and the ABI 7500 Fast real-time PCR system (Applied Biosystems) with the following cycling steps: initial denaturation for 2 min at 95 °C and 40 cycles of 95 °C for 15 s and 60 °C for 60 s. No template control (NTC) consisted of all the reaction components except for the sample.

Data analysis For the qRT-PCR analysis, delta cycle threshold (ΔCt) values were calculated by deducting the Ct of the reference gene from the Cts of the genes of interest. Delta delta Ct (ΔΔCt) values were calculated by deducting the ΔCts from the control and stressed samples, and fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). All calculations were done using Microsoft Excel 2016.

Results

Maximum quantum yield (F_v/F_m) Incubation of *Chlorella*-Trop at the stress-inducing temperature (40 °C) caused a decline of F_v/F_m from 0.637 to about 0.4 and 0.2 at 48 and 72 h respectively, while it took 36 h to regain an F_v/F_m ~ 0.4 (Fig. 1a). For *Chlorella*-Temp, temperature stress at 38 °C decreased F_v/F_m from 0.520 to approximately 0.4 and 0.2, at 6 and 24 h respectively, while the time taken to regain F_v/F_m ~ 0.4 was 52 h (Fig. 1b). On the other hand, under temperature stress at 38 °C, the F_v/F_m of *Chlorella*-Ant declined from 0.649 to approximately 0.4 and 0.2 at 48 and 72 h respectively. The F_v/F_m continued to decline even after being transferred to the ambient temperature and it took approximately 96 h to regain F_v/F_m ~ 0.4 (Fig. 1c).

Light harvesting efficiency, alpha (α) The value α of *Chlorella*-Trop at control temperature (28 °C) fluctuated between 0.537 and 0.425. Meanwhile at the stress-inducing temperature (40 °C), it initially declined and reached 0.366 when the F_v/F_m was around 0.2. During the "recovery" period, it showed an increasing trend and reached as high as the control (0.464) (Fig. 2a). In *Chlorella*-Temp, α began to decline from 0.494 to 0.307 during stress and was not able to recover during the "recovery" period, but continued to decline for more than

Table 1 List of genes and primers

Gene name	Forward and reverse primers	Function	Amplicon size	Reference
psbA	F:GGTGGTCCTTACCAACTTATCGTTTG R:GGTCCTTACCAACTTATCGTTTG	D1 synthesis	98 bp	This study
psbC	F: GAACATCACCACCACCAGGA R: CGGTGCTTGGCTTTTAGTTTG	PSII subunit	79 bp	Qian et al. 2009b
psaB	F:CATGATTTTGAAAGTCATGATGGC R:TGATTTTGAAAGTCATGATGGC	PSI subunit	91 bp	This study
rbcL	F: CTTCCAGGAGCGCGCCACCAA R: ATCTGCTTGCGCATCATGTC	Carbon fixation	163 bp	This study
H3	F:GAGATCCGCAAGTACCAGAAG R:GGTCTTGAAGTCCTGGGC	Endogenous control	93 bp	This study

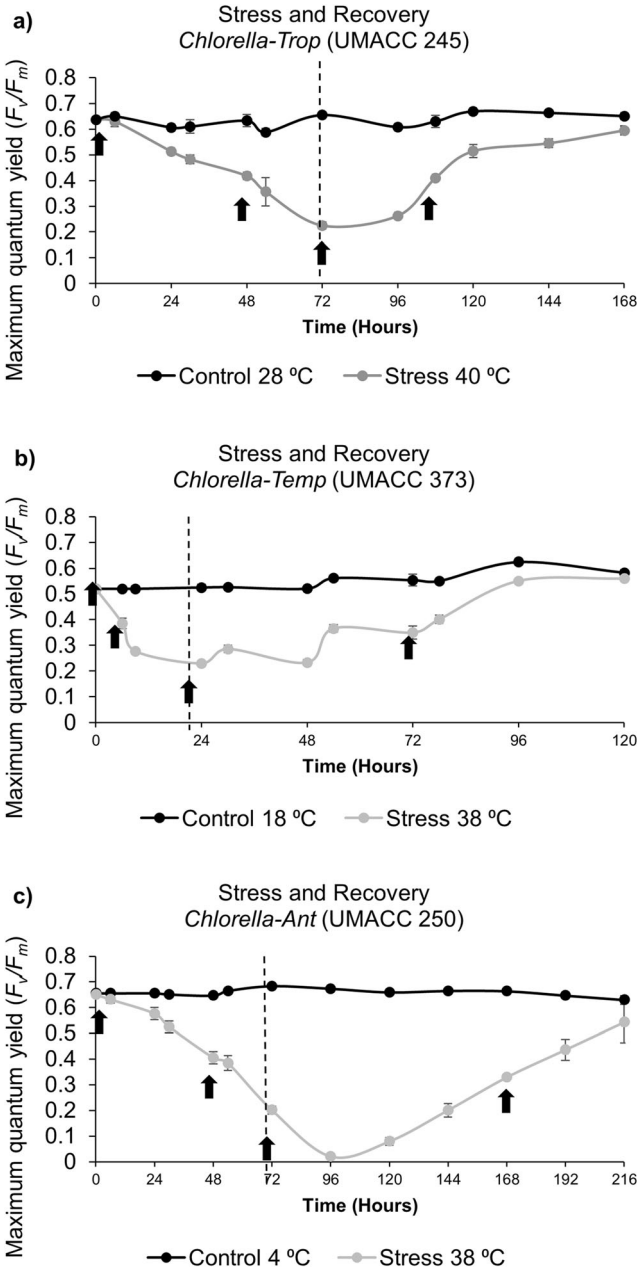

Fig. 1 Maximum quantum yield (F_v/F_m) of **a** *Chlorella*-Trop (UMACC 245), **b** *Chlorella*-Temp (UMACC 373), and **c** *Chlorella*-Ant (UMACC 250). The dash lines indicate the time point of transfer to ambient temperature for recovery. The black arrows indicate the time points of biomass harvesting for RNA extraction

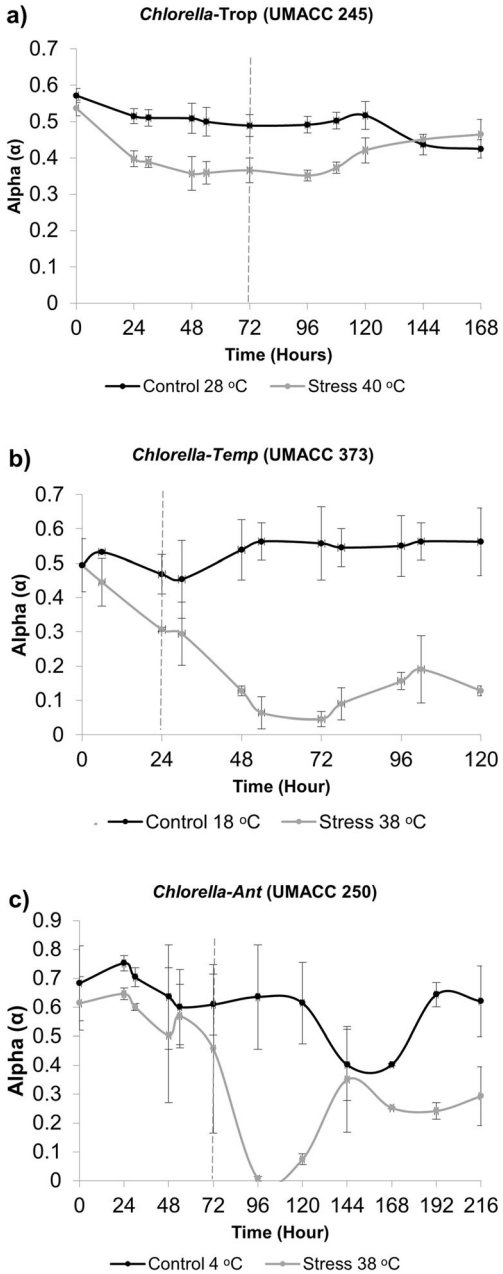

Fig. 2 Light harvesting efficiency, α of **a** *Chlorella*-Trop, **b** *Chlorella*-Temp, and **c** *Chlorella*-Ant during stress and recovery. The dash lines indicate the time point of transfer to ambient temperature for recovery. Data represent the mean value of triplicates and error bars are standard deviations

48 h reaching 0.070 before it began to increase until 0.127 at the end of the experiment (Fig. 2b). In *Chlorella*-Ant, α decreased from 0.613 to 0.475 during stress as F_v/F_m declined to 0.2. Although α continued to decline during the "recovery" period and reached zero, it recovered partially to 0.293 (around 47% of the initial value) (Fig. 2c).

Capacity of photosynthesis (rETR$_{max}$) The rETR$_{max}$ of *Chlorella*-Trop showed a decreasing trend similar to F_v/F_m during stress and reached 51 μmol e m^{-2} s^{-1}. During the "recovery" period, the rETR$_{max}$ continued to decrease from 51 to 39 μmol e m^{-2} s^{-1} within the first 24 h and then increased sharply for the next 24 h reaching 129 μmol e m^{-2} s^{-1} (Fig. 3a). In *Chlorella*-Temp, rETR$_{max}$ declined sharply during stress and reached 14 μmol e m^{-2} s^{-1}. It did not increase during the first 48 h of the "recovery" period, but later increased steadily and exceeded the value of the control at 81 μmol e m^{-2} s^{-1} at the end of the experiment (Fig. 3b). In *Chlorella*-Ant, rETR$_{max}$ declined and was totally inhibited upon stress. It showed increment only after 72 h from the start

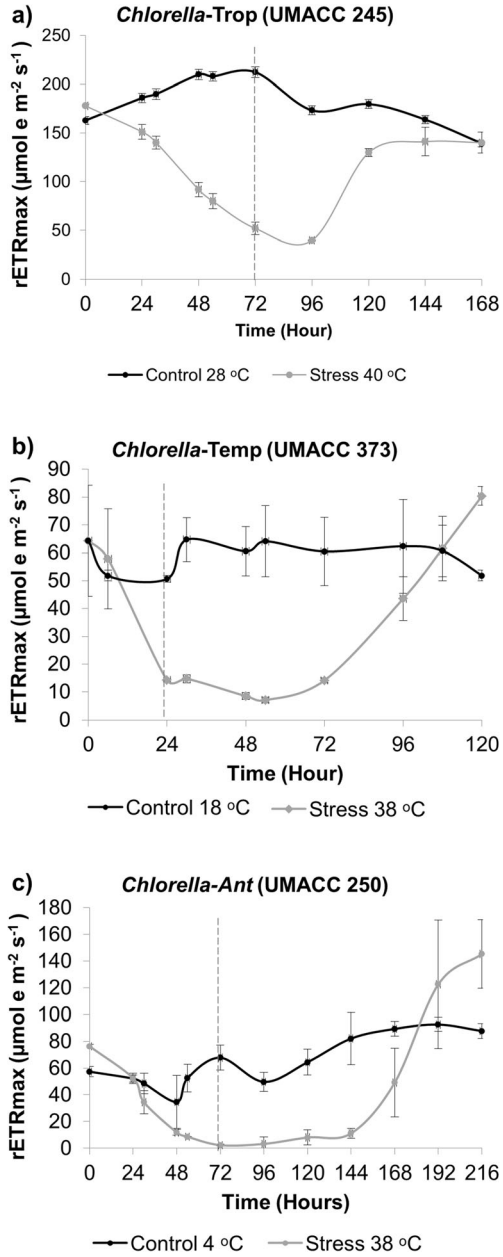

Fig. 3 $rETR_{max}$ of **a** *Chlorella*-Trop, **b** *Chlorella*-Temp, and **c** *Chlorella*-Ant during stress and recovery. The dash lines indicate the time point of transfer to ambient temperature for recovery. Data represents the mean value of triplicates and error bars are standard deviations

of the "recovery" period and eventually reached 145 µmol e m^{-2} s^{-1} which was higher than the control (Fig. 3c).

Gene expression The expression of *psbA* in *Chlorella*-Trop was not affected by the temperature stress at 40 °C. In *Chlorella*-Temp, the expression of *psbA* was down-regulated by 0.12- and 0.77-fold when F_v/F_m dropped to about 0.4 and 0.2, respectively. Nevertheless expression of *psbA* was up-regulated by 7-fold after being transferred to ambient temperature. In *Chlorella*-Ant, the transcript abundance of *psbA* increased by 1.6- and 3.3-fold when the F_v/F_m dropped to about 0.4 and 0.2, respectively. However, during recovery its abundance decreased to values similar to the control (Fig. 4a). The expression of *psaB* in *Chlorella*-Trop did not show any obvious changes when F_v/F_m was around 0.4, while further stress ($F_v/F_m \sim 0.2$) up-regulated its expression by 1.68-fold, nevertheless it was later down-regulated by 0.5-fold during recovery. In *Chlorella*-Temp, the expression of *psaB* was considerably inhibited during stress and reached nearly 0.15-fold; it was then up-regulated by 3.4-fold upon "recovery" at its ambient temperature. In *Chlorella*-Ant, *psaB* transcript abundance increased to 2.1- and 4.6-fold when the F_v/F_m declined to about 0.4 and 0.2, respectively. Although the transcript abundance declined after the culture was transferred to ambient temperature, its abundance remained higher than that of the control by 1.5-fold (Fig. 4b). The expression of *psbC* in *Chlorella*-Trop was down-regulated by 0.3-fold when the F_v/F_m decreased to 0.4, but with further stress ($F_v/F_m \sim 0.2$), it was up-regulated by 2.5-fold, and during recovery, it increased to 7.6-fold compared to the control. In *Chlorella*-Temp, the expression of *psbC* was inhibited during stress; however, during recovery, the expression of *psbC* increased slightly although it remained lower than the control. In *Chlorella*-Ant, expression of *psbC* increased during stress by 24.8-fold when F_v/F_m dropped to around 0.2, and although the transcript abundance decreased after the transfer to ambient temperature, it remained up-regulated by 6.1-fold (Fig. 4c). In *Chlorella*-Trop, the expression of *rbcL* was initially inhibited and declined by 0.34-fold, but as the stress intensified ($F_v/F_m \sim 0.2$), it was up-regulated by 1.5-fold. In *Chlorella*-Temp, *rbcL* expression was down-regulated by 0.2- and 0.6-fold when F_v/F_m declined to around 0.4 and 0.2 respectively, while during recovery, it was up-regulated by 15.6-fold. In *Chlorella*-Ant, *rbcL* transcript abundance decreased to 0.6-fold when F_v/F_m declined to around 0.4, but it increased to 1.9-fold with further stress ($F_v/F_m \sim 0.2$). However, during recovery, it was unexpectedly down-regulated by 0.2-fold (Fig. 4d).

Discussion

Stress and recovery A reduction in F_v/F_m (Fig. 1) was observed for all three (tropical, temperate, and Antarctic) strains subjected to temperature stress. This indicates a loss in the efficiency of primary photochemistry in the stressed cells or possible damage to the PSII system (Ralph and Gademann 2005). The *Chlorella* strains were able to recover their photosynthetic activity after being transferred to their ambient temperatures, despite the variations in the period of recovery. *Chlorella*-Trop was able to regain its photosynthetic activity faster than the other two strains, while *Chlorella*-Ant took the longest time to regain its activity. Despite the relatively slower recovery rate shown by *Chlorella*-Ant, its tolerance to a wide

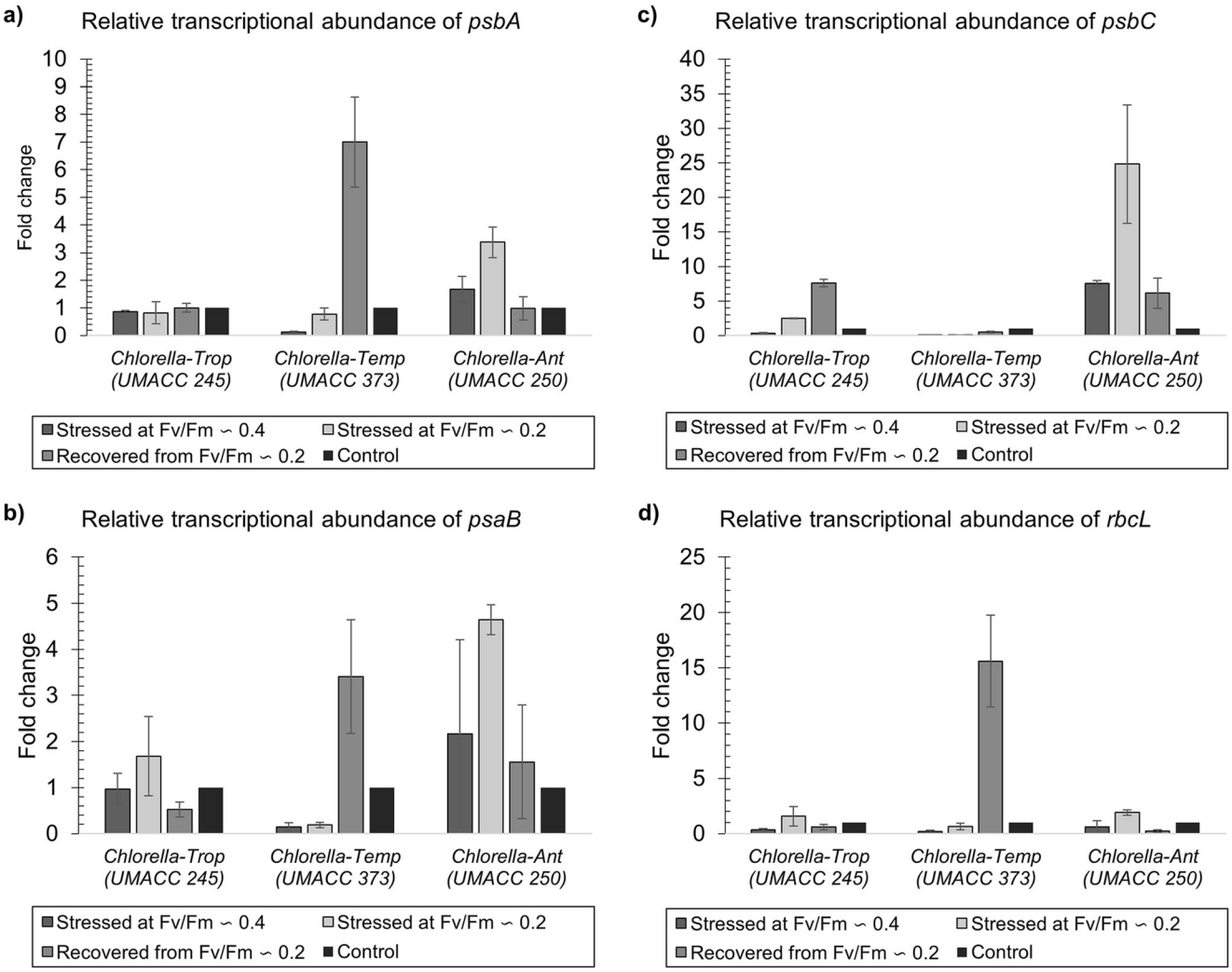

Fig. 4 Relative transcriptional abundance of **a** *psbA*, **b** *psaB*, **c** *psbC*, and **d** *rbcL* in *Chlorella*-Trop, *Chlorella*-Temp, and *Chlorella*-Ant during stress and recovery. Data are presented as mean ± standard error of the mean (SEM)

range of high temperatures and its recovery capacity were astounding. In the Antarctic region, organisms are adapted to harsh conditions and tend to lower their metabolisms (Piette et al. 2011). Stamenkovic and Hanelt (2013) reported that microalgae exhibited physiological responses that were consistent with their respective climate zones. Basically, there are several target sites for high temperature-induced damage, such as the electron transport chain, photophosphorylation, and the CO_2 fixation system (Allakhverdiev et al. 2008). Also, the damage from the inhibition of Calvin cycle can consequently increase the levels of reactive oxygen species (ROS) (Saibo et al. 2009). The capability of algae to recover from high-temperature instabilities is an essential trait assisting them to survive, mainly in the intertidal ecosystems in which organisms experience extensive variation in temperature (Campbell et al. 2006). Studies on species-specific thermal stress are essential in understanding how temperature variation could result in alterations of the *Chlorella* species composition. The capability of photoautotrophs such as *Chlorella* to survive in high-temperature variations is ecologically vital for marine ecosystem dynamics and primary production (Endres et al. 2016).

Changes in α in response to heat stress were different among the studied strains (Fig. 2). The α value increased during recovery in all studied strains; however, it did not recover completely in all strains. *Chlorella*-Trop was the least affected as the values of α did not decrease below 0.3 even at the higher stress level ($F_v/F_m \sim 0.2$). In *Chlorella*-Temp, α showed a sharp decline during stress but it was not able to fully recover during the "recovery" period and only regained almost 30% of its pre-stress values. This suggested that α was highly affected by heat stress in this strain. In *Chlorella*-Ant, there was a slight decline in α at the initial stage of stress, followed by an abrupt decline to complete inhibition at the 96-h time point. Nevertheless, the α recovered with a lag time which was similar to that observed for F_v/F_m (Fig. 1c), and regained almost 47% of its pre-stress values. This implied a higher capability of *Chlorella*-Ant in regaining its light

harvesting efficacy after experiencing stress, as compared to *Chlorella*-Temp.

Since the $rETR_{max}$ differed among studied strains, it can be suggested that they have different photosynthetic capacity. In *Chlorella*-Trop, the $rETR_{max}$ values fluctuated between 150 and 200, while in *Chlorella*-Temp and *Chlorella*-Ant, the values fluctuated between 60 and 70 on average. The trends of changes were similar to those observed in F_v/F_m during stress, but with slight differences during recovery. In *Chlorella*-Trop, $rETR_{max}$ decreased slightly during the first 24 h of the "recovery" period, and later increased promptly reaching levels as high as the control. This illustrated that this strain was able to protect its electron transport system. In *Chlorella*-Temp, although $rETR_{max}$ was not fully inhibited, it took a while (48 h) to recover; indicating that the damage was moderate. Surprisingly, at the end of the "recovery" period, the $rETR_{max}$ values exceeded those of the control. In *Chlorella*-Ant, the $rETR_{max}$ declined and was totally inhibited (when F_v/F_m was around 0.2). Subsequently, it began to increase and even exceeded the control at the end of the experiment. This showed that heat stress causes effective damages in the electron transport system which takes time to recover. The higher $rETR_{max}$ values in *Chlorella*-Temp and *Chlorella*-Ant compared to the control at the end of the experiment suggest that these strains might have activated alternative mechanisms to intensify electron transport even after stress, probably to anticipate more intense stress.

Gene expression It is believed that the first response of plants to stresses is to protect the cells from possible abiotic damage from the weakening of photosynthesis (Bilgin et al. 2010). The photosynthetic activity of plants was reported to be significantly reduced under diverse stresses including temperature, light, heavy metals, and toxic substances among others (Wang et al. 2014). F_v/F_m is a measure of the efficiency of photons absorbed by PSII being used in photochemistry instead of being quenched (Maxwell and Johnson 2000). However, it is important to note that a decrease in F_v/F_m is not essentially related to damage to the PSII, as the stressed cells may recruit defense mechanisms such as non-photochemical fluorescence quenching (NPQ), causing F_v/F_m to decline (Baker 2008). Heat and drought stresses reportedly caused reduction in the expression of *psbB* and *psbC*, but induced the expression of *psbA* (Bi et al. 2016). Hence, in this study, the gene expression of *psbA*, *psaB*, *psbC*, and *rbcL*, which are the key genes involved in PSI, PSII, and carbon assimilation (CO_2 fixation), was evaluated.

It is reported that the PSII of cyanobacteria, green algae, and higher plants is prone to light-induced inactivation, with the D1 protein being the primary target of such damage. As a consequence, the D1 protein which is encoded by *psbA* is degraded and re-synthesized in a multistep process called PSII repair cycle (Mulo et al. 2012). As shown in Fig. 4a, the abundance of *psbA* in *Chlorella*-Trop was only reduced marginally during stress but returned to near control level during recovery. This may indicate *Chlorella*-Trop's ability to maintain *psbA* expression level during stress and also, its fast recovery rate suggests that the damage was repaired rapidly. In *Chlorella*-Temp, the abundance of *psbA* was decreased during initial stress, but with longer stress, the cells increased the expression of *psbA* to activate their repair mechanisms, and *psbA* increased remarkably during recovery to carry on the repair. For *Chlorella*-Ant, there was a substantial increase in *psbA* transcript abundance which intensified with stress, suggesting an active synthesis of D1 protein (notwithstanding post-transcriptional regulation) during periods of stress.

The *psaB* protein is one of the main constituent of PSI biogenesis and is involved in the formation of the chlorophyll *a*–protein complex I (CPI) that binds most of the pigments and redox cofactors of PSI (Balczun et al. 2005). It also binds a total of about 100 Chl *a* molecules together with *psaA* (Melis 1991). A decrease in *psaB* transcript abundance might result in the reduced activity of PSI (Hihara and Sonoike 2001; Morgan-Kiss et al. 2006). Qian et al. (2009b) reported that the decrease in *psaB* transcript abundance resulted in a decrease in the amount of the corresponding enzyme and its activity, thus preventing normal electron transport in PSI. In the present study, *psaB* transcript abundance in both *Chlorella*-Ant and *Chlorella*-Trop increased during temperature stress and decreased (for *Chlorella*-Trop) during recovery. This indicated that both strains were able to synthesize *psaB* subunits which might aid in the repair of PSI. Although transcript abundance of *psaB* in *Chlorella*-Temp decreased markedly during stress, it increased considerably during recovery. This implied that *Chlorella*-Temp was not able to re-synthesize *psaB* during stress but was able to do so during recovery to rebuild affected PSI. Repression of *psaB* was also reported in *C. vulgaris* when exposed to toxic chemicals (Qian et al. 2011).

Another photosynthesis-related gene, *psbC*, encodes CP43, a PSII chlorophyll-binding protein involved in water-splitting and acting as an oxygen-evolving enzyme of photosynthesis (Qian et al. 2009b). The expression of *psbC* is also affected by harsh conditions such as temperature stress (Chong et al. 2011). As shown in Fig. 4c, *Chlorella* strains from different latitudes responded differently pertaining to *psbC* transcript abundance during temperature stress. In *Chlorella*-Trop, the *psbC* transcript abundance increased during stress and continued to increase even during recovery (reaching almost 8-fold higher than the control). The damage sustained during stress might not be fully repaired, resulting in the continued synthesis during recovery. In contrast, expression of *psbC* in *Chlorella*-Temp was not much affected during stress or recovery. In *Chlorella*-Ant, *psbC* was up-regulated as stress intensified reaching up to 25-fold compared to the control. Both *psaB* and *psbC* are involved in electron transport and their

down-regulation might hinder electron transport in the thylakoid, which in turn results in the accumulation of surplus electron as well as induce oxidative stress (Liu et al. 2015).

Photosynthetic fixation of carbon dioxide is essential for algal growth and development by providing the carbohydrates required for metabolism, structural components, and cellular building blocks (Biswal et al. 2011). Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) plays critical roles in photosynthesis and the expression of genes encoding its subunits including *rbcL* is significantly influenced by various stresses (Qian et al. 2009b; Qian et al. 2012). Expression of *rbcL* is inhibited under high and low salinity conditions, desiccation as well as at temperatures above and below the ambient temperature (Xu et al. 2013). In both *Chlorella*-Ant and *Chlorella*-Trop, *rbcL* transcript abundance increased when F_v/F_m declined to 0.2 during stress. The cells appeared to have up-regulated the expression of *rbcL* to sustain the carbon fixation process. In *Chlorella*-Temp, *rbcL* transcript levels declined slightly during stress but increased remarkably by 16-fold during recovery.

Generally, the present study demonstrated that *Chlorella* strains from different latitudes displayed dissimilar patterns of recovery and different gene expression profiles indicating that they employed different adaptation strategies. Active transcription of the photosynthetic genes in *Chlorella*-Ant (even when it was under stress) suggests that this strain uses a strategy of increasing the expression of photosynthetic genes to compensate for the decrease in photosynthetic activity resulting from the heat-inflicted damage to the photosynthetic components or the lack of translated proteins due to post-transcriptional control. Despite the increased expression of *psbA*, *psaB*, and *psbC* in *Chlorella*-Ant, the lag time of recovery observed for the photosynthetic parameters showed the influence of other photosynthetic components that should be considered for future studies. Meanwhile, the different expression patterns of the four genes observed in *Chlorella*-Trop are not too surprising as genes encoding for proteins with similar functions have been reported with simultaneous up-regulation and down-regulation by the same signal (Hwang et al. 2008; Gierz et al. 2017). Furthermore, the protein product of a certain gene may have other unknown functions i.e. participating in multiple physiologically diverse processes, hence the observed lack of co-regulation with other genes in the same pathway (Hwang et al. 2008; Peng et al. 2016). Expression of the photosynthetic genes in *Chlorella*-Temp was suppressed during stress, but increased considerably during recovery. This is in line with several reports in which different stressors including temperature (Chong et al. 2011), salinity (Kebeish et al. 2014b), and toxins (Qian et al. 2009a; Kebeish et al. 2014a; Liu et al. 2015) inhibited expression of the photosynthetic genes in *Chlorella* strains during stress. The inhibited expression of the four photosynthetic genes in *Chlorella*-Temp during stress may indicate a strategy to conserve existing resources and energy to cope with possible extended periods of stress (Poong et al. 2018). The subsequent up-regulated expression of these genes during recovery is probably a measure to re-synthesize and restore the damaged components.

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