ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Augmentation of the Photoreactivation Gene in *Fremyella diplosiphon* Confers UV-B Tolerance

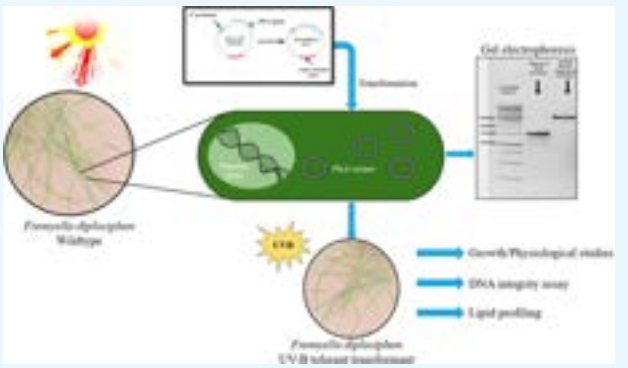
Samson M. Gichuki, Anithachristy S. Arumanayagam, Behnam Tabatabai, Yavuz S. Yalcin, LaDonna Wyatt, and Viji Sitther*

Cite This: *ACS Omega* 2022, 7, 35092−35101

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In spite of the enormous potential of cyanobacteria as a renewable energy source, elevated UV exposure is a major impediment to their commercial viability and productivity. *Fremyella diplosiphon* is a widely explored cyanobacterium with great biofuel capacity due to its high lipid content. To enhance UV stress tolerance in this species, we overexpressed the photoreactivation gene (*phr A*) that encodes for photolyase DNA repair enzyme in the wild type *F. diplosiphon* (B481-WT) by genetic transformation. Our efforts resulted in a transformant (B481-ViAnSa) with a 3808-fold increase in the *phr A* mRNA transcript level and enhanced growth under UV-B stress. Additionally, DNA strand breaks in the transformant were significantly lower after 12 and 16 h of UV radiation, with significantly higher dsDNA recovery in B481-ViAnSa (98.1%) compared to that in B481-WT (81.5%) at 48 h post irradiation. Photosystem II recovery time in the transformant was significantly reduced (48 h) compared to that in the wild type (72 h). Evaluation of high-value fatty acid methyl esters (FAMEs) revealed methyl palmitate, the methyl ester of hexadecenoic acid (C16:0), to be the most dominant component, accounting for 53.43% of the identified FAMEs in the transformant. Results of the study offer a promising approach to enhance UV tolerance in cyanobacteria, thus paving the way to large-scale open or closed pond cultivation for commercial biofuel production.



1. INTRODUCTION

The negative impact of fossil fuels on the environment and human health has sparked enormous interest in the development of biofuels as a renewable energy source. While cyanobacteria are an ideal third-generation feedstock for a variety of fuels including biodiesel, ethanol, and biogas, these photosynthetic organisms face an immense threat due to global climatic changes.[1,2] In recent years, a decrease in the stratospheric ozone layer due to excessive release of air pollutants such as chlorofluorocarbons, organobromides, and reactive nitrogen species has resulted in increased solar UV-B (280−320 nm) reaching the Earth's surface.[3] Several physiological and biochemical processes such as motility, photo-orientation, and CO_2 uptake in cyanobacteria are impaired by UV radiation. In addition, it is known to adversely impact biomolecules in these organisms, with nucleic acids being the primary targets.[4]

Studies by Rastogi et al.[5] and Castenholz and Garcia-Pichel[6] have reported that cyanobacterial genomic function and fidelity are adversely affected by UV-B, as the DNA molecules directly absorb UV-B radiation inducing DNA strand breaks. A variety of mutagenic and cytotoxic DNA lesions including cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4PPs), and their Dewar valence isomers are induced, disrupting genomic integrity. In addition, cyanobacterial UV-B-induced DNA degradation due to thymine dimerization has been confirmed by *Anabaena*, *Nostoc*, and *Scytonema* sp.[7] Additionally, UV-B-induced DNA lesions (CPDs and 6-4PPs) can also cause primary and secondary breaks since they are associated with transcription and replication blockages, leading to the collapse of replication forks in CPD-containing DNA.[8]

To combat the negative effects of radiation stress, cyanobacteria employ a variety of direct and indirect defense strategies that enable tolerance to fluctuating UV levels. The first line of defense employed by most cyanobacterial species is the avoidance by migration through self-shading or mat formation.[7] Cyanobacteria such as *Anabaena* sp. *Nostoc commune* and *Scytonema* sp. have the capacity to produce UV-absorbing compounds mycosporine-like amino acids and scytonemin as a response to UV radiation.[9] Although cyanobacteria use these defense mechanisms to combat UV

Received: June 27, 2022
Accepted: September 12, 2022
Published: September 23, 2022

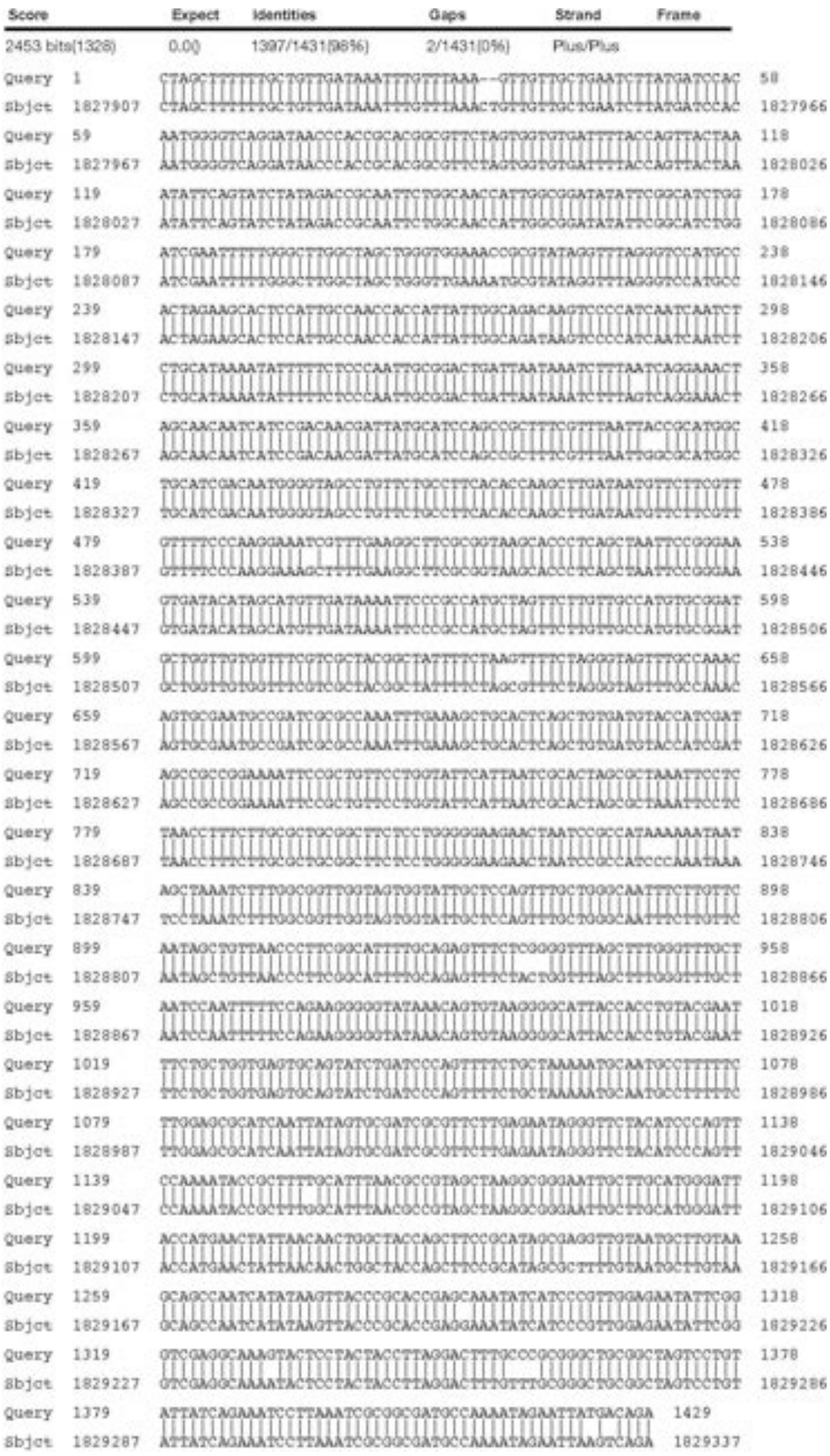


Figure 1. Basic local alignment search tool analysis of *F. diplosiphon* B481-WT photolyase gene on the National Center for Biotechnology Information (NCBI) showing a 97.82% similarity.

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

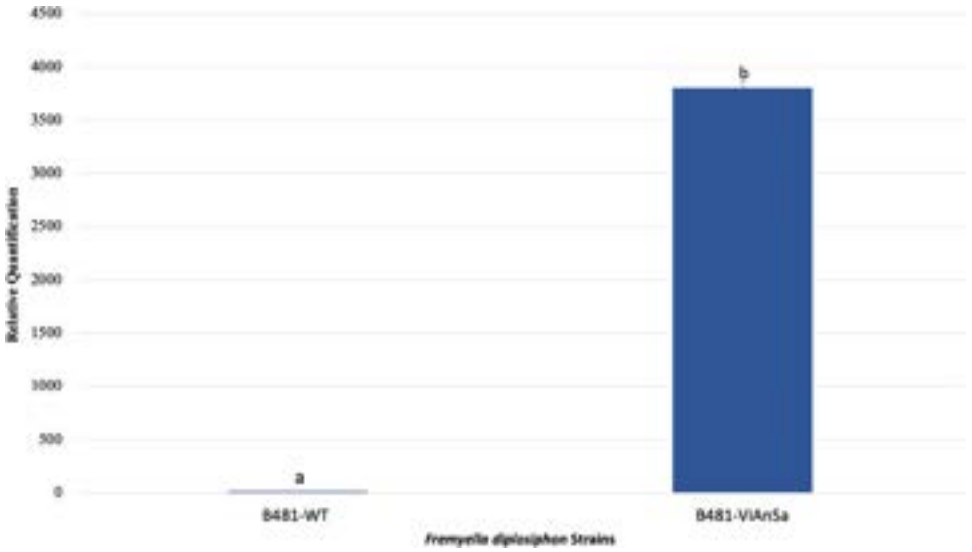


Figure 2. Quantification of photolyase transcript levels in *F. diplosiphon* wild type (B481-WT) and transformant (B481-ViAnSa). Error bars indicate ΔC_t values at a 95% confidence interval across four replicates.

stress, these repair systems can be rapidly overwhelmed by sustained UV radiation.[10] However, some species employ photoreactivation, a process in which photolyase is activated by the blue wavelength of solar light, to reverse and modify nitrogenous bases to their normal state followed by thymine dimer formation caused by UV radiation.[11]

Fremyella diplosiphon is a well-studied cyanobacterial species that has great potential as a third-generation biofuel agent due to its fatty acid methyl esters.[12] In addition to growth in varying light intensities by modifications of its light-harvesting complexes, the organism is extremely amenable to genetic transformation.[13] Efforts to enhance value-added traits such as halotolerance and cellular lipid content in this species have enabled unique environmental applications.[14,15] A report by Vass et al.[16] has indicated that the *Phr A* gene plays a role in the DNA repair mechanism of *Synechocystis* sp. PCC 6803 and mutant cells lacking the gene were highly susceptible to UV-B damage. To the best of our knowledge, there are no reports to augment UV-B tolerance in this organism. The development of a UV-B-tolerant strain would be invaluable to maximize its potential for biofuel production in scale-up systems. The objective of the present study was to overexpress the photoreactivation (*phr A*) gene in *F. diplosiphon* B481-WT to enhance UV-B tolerance. Successful transformation was confirmed using RT-qPCR and fluorometric analysis of DNA unwinding assays and photosynthetic efficiency, which is known to be adversely impacted by UV-B, evaluated as a measure of photosystem II functionality. Additionally, the fatty acid methyl ester (FAME) profile of the transformant was compared to the wild type to determine its biofuel efficacy.

2. RESULTS AND DISCUSSION

2.1. Identification, Cloning, and Expression of Photolyase *phr A* Gene in *F. diplosiphon*. In this study, *F. diplosiphon* UV-B tolerance was enhanced by taking advantage of the gene expression system of a plasmid vector containing the photolyase gene. Gel electrophoresis of the double-digested vector construct revealed bands at the expected sizes of ∼1500 and ∼3000 bp for *Phr A* gene and pGEM-7Zf plasmid, respectively (Figure S1). The high similarity of 97.82% to the *phr A* gene from B481-WT indicated homology to the photolyase gene in *Nostoc* sp. (Figure 1). Quantification of the *phr A* gene transcript levels in the transformant revealed a 3808-fold increase ($p < 0.05$) compared to that of the wild-type strain (Figure 2). The *phr A*-overexpressing *F. diplosiphon* strain was designated as B481-ViAnSa, and the gene sequence was deposited in NCBI Genbank with the accession number MW357071. DNA photolyase homologues have been reported to be the major factors for UV resistance in the cyanobacterium *Synechocystis* sp. PCC 6803.[16] As observed in our study, enhanced gene transcript levels indicated enhanced UV-B tolerance via *phr A* gene overexpression. The photolyase-deficient *Synechocystis* sp. mutants incurred a significant amount of 70% damaged DNA compared to 30% in the wild type following UV-B radiation and were incapable of repairing UV-B-induced DNA damages. In addition, upregulation of the *phr A* gene by 5.19- and 9.98-fold after 30 and 60 min of rewetting dried biofilms in the desert cyanobacterium *Chroococcidiopsis* exposed to Mars-like UV flux has been reported.[17]

Given that cyanobacteria use solar energy for essential energy-dependent processes, harmful UV-B radiation affects several physiological and biochemical processes such as photosynthesis, growth, survival, cell differentiation, genome integrity, and total lipid profiles.[7] Therefore, we evaluated the efficacy of the transformant under simulated UV-B conditions at an intensity of 3.0 W m^{-2} at the surface of the cell culture. Our results revealed significantly high UV-B tolerance in the transformant radiated for 20−160 min. While a significant reduction ($p < 0.05$) in the growth of B481-WT was observed even at 20 min of UV-B exposure, the transformant (B481-ViAnSa) showed no significant reduction of growth at an exposure time of 40−160 min (Table S1). Interestingly, we observed a significantly rapid growth recovery of the B481-ViAnSa strain compared to that of B481-WT (Figure 3). Furthermore, irradiation of B481-ViAnSa for 20 min significantly ($p < 0.05$) increased the growth rate over a 14 day period compared to the nonirradiated transformant, indicating exceptional growth performance under UV stress. These results correlate to the report of Sinha et al.,[18] where 30 min of UV-B irradiation impaired 50% cell growth in *Anabaena*

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

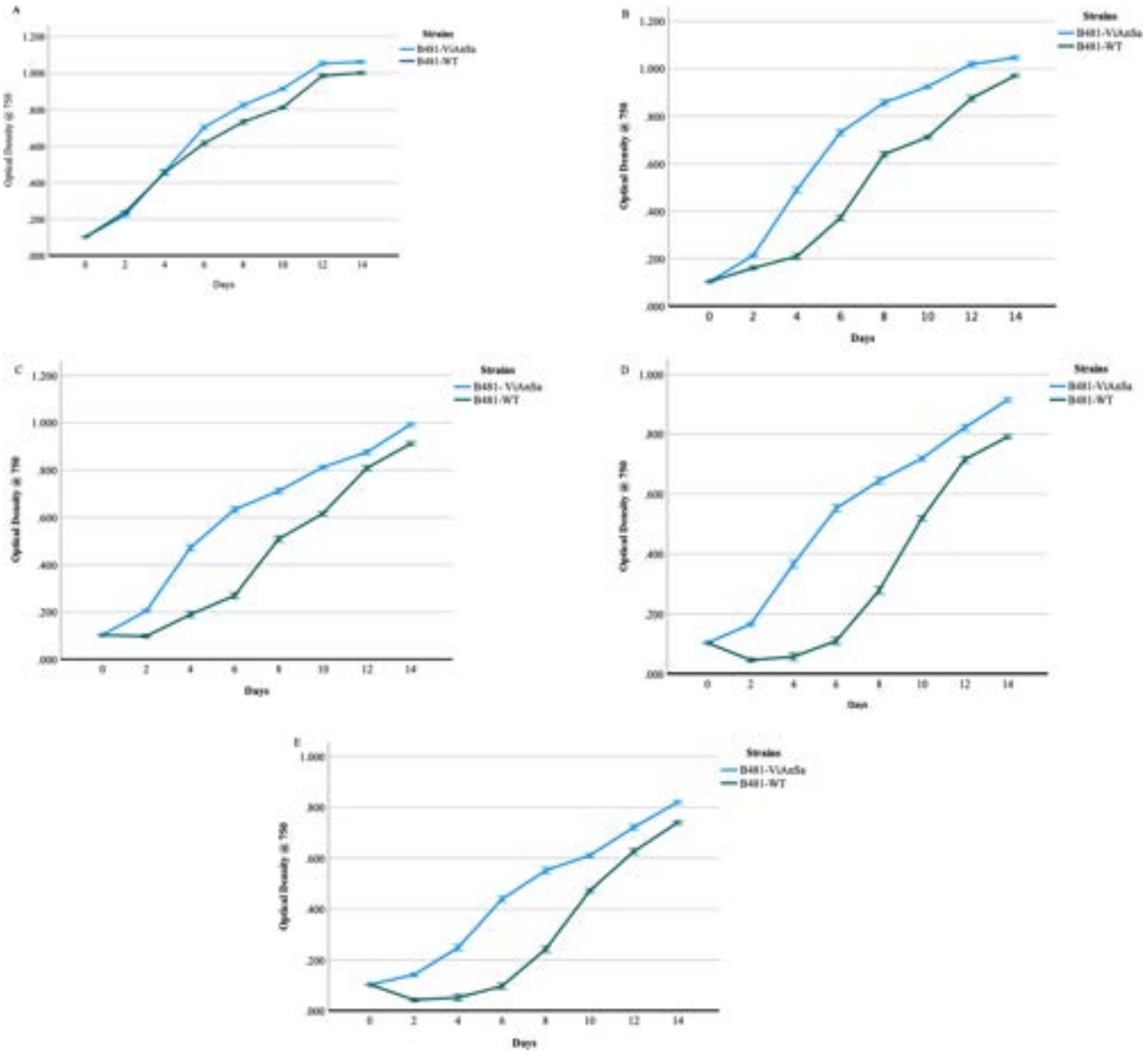


Figure 3. Growth of *F. diplosiphon* wild-type (B481-WT) and transformant (B481-ViAnSa) strains irradiated under UV-B (3.0 W m^{-2}) for 0−160 min and grown in BG11/HEPES media. Growth comparisons of strains at UV-B treatments for 0, 20, 40, 80, and 160 min exposure are shown in panels (A−E). Different letters above the final time point indicate significance among treatment means ($p < 0.05$).

sp., *Nostoc* sp., *Nostoc carmium*, *N. commune*, and *Scytonema* sp., and *Anabaena* sp. with no recuperation even after 120 min. We observed a reduction in the growth of B481-WT and B481-ViAnSa strains in a dose-dependent manner, which is in accordance to a previous report in which UV-B inhibited growth in *Nostoc muscorum*, *Pediastrum boryanum*, and *Aphanothece* sp.[19] It is known that thymine−thymine dimer photoproducts, which are biologically the most relevant UV-B-induced lesions, account for ∼75−80% of all UV-B-induced damage, resulting in cyanobacterial growth reduction.[11,16] In addition, an increase in the frequency of thymine dimers was reported in *Anabaena* sp., *Nostoc* sp., and *Scytonema* sp., and *Anabaena variabilis* PCC 7937 when exposed to UV-B.[5,7]

2.2. Comparison of DNA Strand Breakages in the Transformant and Wild-Type *F. diplosiphon* Strains. FADU assay, an accurate and powerful method for the quantitative analysis of DNA damage, was used to measure DNA strand breaks in the transformant engineered with the *phr A* (B481-ViAnSa). In prior studies, this technique has accurately measured UV-B-induced strand breakage in the cyanobacterium *A. variabilis* PCC 7937.[5] Quantification of dsDNA damage detected by fluorescence analysis of the fluorochrome-bound DNA revealed maximal fluorescence at 450 nm, while it was lower in ssDNA (Figure 4). The difference between the upper and lower fluorescence limits of ds- and ss-samples provided a more reliable analysis of DNA strand breaks in UVt samples since the amount of DNA damage in treated cells is expressed by the difference in fluorescence intensities. Using this assay, we detected significantly higher ($p < 0.05$) DNA damage in both B481-WT and B481-ViAnSa strains exposed to UV-B at 12 and 16 h (Figure 5) compared to the untreated control (sample-ds).

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

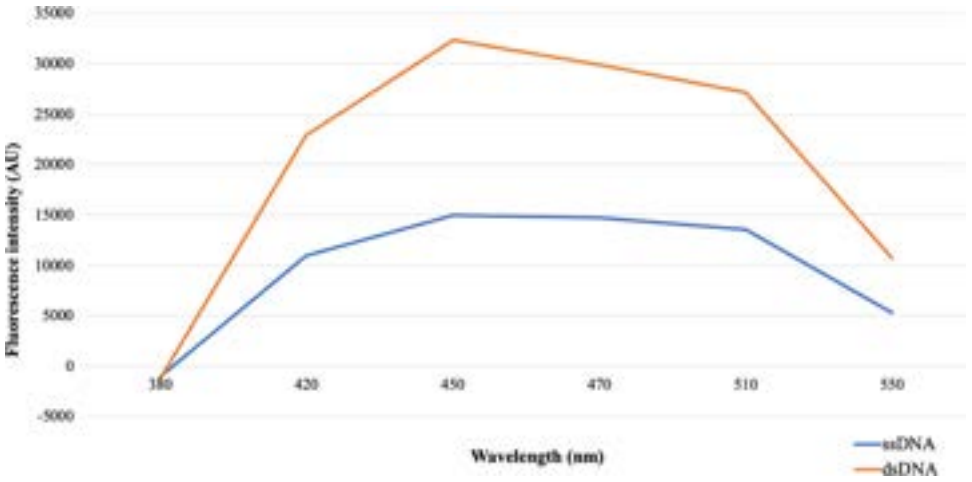


Figure 4. Fluorescence excitation of *F. diplosiphon* DNA-bound Hoechst 33258. Emission data (emission peak 450 nm) were obtained using the maximum wavelength of the excitation peak at 343 nm. The double-stranded (ds) DNA was not subjected to alkaline unwinding, while the single-stranded (ss) DNA was subjected to complete alkaline unwinding.

These results are corroborated by previous studies in which DNA lesions and strand breaks were reported in the cyanobacteria *A. variabilis* PCC 7937 and *Synechocystis* sp., PCC 6803 exposed to UV-B.[5,16] While our results indicated a significant reduction ($p < 0.05$) in the dsDNA of both strains exposed to UV-B, the transformant exhibited significantly less dsDNA breakages compared to wild type. In addition, a significantly high ($p < 0.05$) dsDNA of 60.3, 70.2, and 98.1% were observed in B481-ViAnSa compared to that of B481-WT (50.4, 55.6, and 81.5%) at 0, 24, and 48 h post UV-B irradiation. Interestingly, we noted significantly higher ($p < 0.05$) dsDNA recovery in B481-ViAnSa (98.1%) compared to that in B481-WT (81.5%) after 48 h (Figure 5B).

Based on these results, we hypothesize that higher photolyase activity could have resulted in more efficient DNA repair in the transformant. In addition, this strain exhibited a significantly higher ($p < 0.05$) percentage of dsDNA at 16 h of UV-B radiation. Comparison of gene transcription in the transformant and DNA damage showed an inverse correlation. While the *phr A* gene overexpression in the transformant was significantly high ($p < 0.05$) compared to that in the wild type, DNA damage as indicated by FADU assay was low. These results indicate that the overexpression of the photolyase gene could have reduced thymine dimers caused by UV-B. Our findings are consistent with a report by Chen et al.[20] where UV-B radiation of *Anabaena* sp. and *Microcystis viridis* significantly decreased ($p < 0.05$) the percentage of dsDNA due to ROS-induced damage, elucidating a correlation between oxidative stress and DNA damage. It is known that ROS generated under UV radiation stress damages cyanobacterial DNA by reacting with sugars, purines, and pyrimidines.[8] Further, ROS-induced damage can indirectly activate Ca^{2+}-dependent endonucleases in response to increasing intracellular free Ca^{2+} and inhibiting enzymes involved in DNA replication. Consequently, DNA strand breakage is common in cells subjected to oxidative stress linked to UV-B-induced double-strand breaks.

2.3. Evaluation of Photosystem II Activity and Chlorophyll *a* Content in the Transformant B481-ViAnSa. The ratio of variable and maximum fluorescence (F_v/F_m) of the dark-adapted chlorophyll *a* fluorescence parameter was used to measure the photochemical efficiency of photosystem II reaction centers. Comparison of photosystem II activity and chlorophyll *a* content between the wild-type and transformant strains did not reveal significant differences (Figure 6). However, we noticed a significant difference ($p < 0.05$) in the photosystem II (PSII) recovery rate of the UV-treated transformant compared to that of the wild type. While the transformant PSII recovered in 48 h following UV-B radiation at an intensity of 3.0 W m^{-2} for 1 h, the wild-type strain took 72 h, indicating enhanced photolyase gene activity in the transformant contributing to UV stress tolerance. Our results were consistent with the findings of Vass et al.[16] where the *phr A*-deficient mutant *Synechocystis* sp., PCC 6803, lacked the capacity to restore PSII activity following UV-B irradiation. The study reported a 70% loss of PSII activity in the mutant *Synechocystis* sp., PCC 6803, and only 30% in the wild type. UV-B radiation is known to affect cyanobacterial photosynthetic performances by causing disassembly of the phycobilisome complexes and photobleaching of critical solar harvesting pigments, which include chlorophyll *a*, carotenoids, and phycobiliproteins.[21] In *Synechocystis* sp., UV-B was reported to interfere with cyanobacterial solar energy harvesting phycobilisomes leading to their disintegration and potential cell death.[22]

2.4. Fatty Acid Methyl Ester Composition in UV-B-Irradiated and Nonirradiated *F. diplosiphon* Strains, B481-ViAnSa and B481-WT. Previously, researchers have reported *F. diplosiphon* to possess valuable biodiesel qualities, which can maximize biofuel production.[19,23] Hence, we compared the high-value saturated and unsaturated FAMEs in the transformant to the wild-type strain. Our results showed methyl palmitate, the methyl ester of hexadecenoic acid (C16:0), to be the most dominant FAME component, accounting for 53.43 and 51.69% in B481-ViAnSa and B481-WT, respectively. Methyl octadecenoate (C18:0), the second abundant FAME, accounted for 30.12 and 33.02% in B481-ViAnSa and B481-WT, respectively. This was followed by methyl octadecenoate (C18:1), which accounted for 22% in B481-ViAnSa and 23.02% in B481-WT. Additionally, we detected methyl tetradecanoate (C14:1), methyl hexadecanoate (C16:1), and methyl octadecadienoate (C18:2) in both strains (Table 1). The FAMEs identified in the present study corroborate with previous studies in *F. diplosiphon* treated with

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

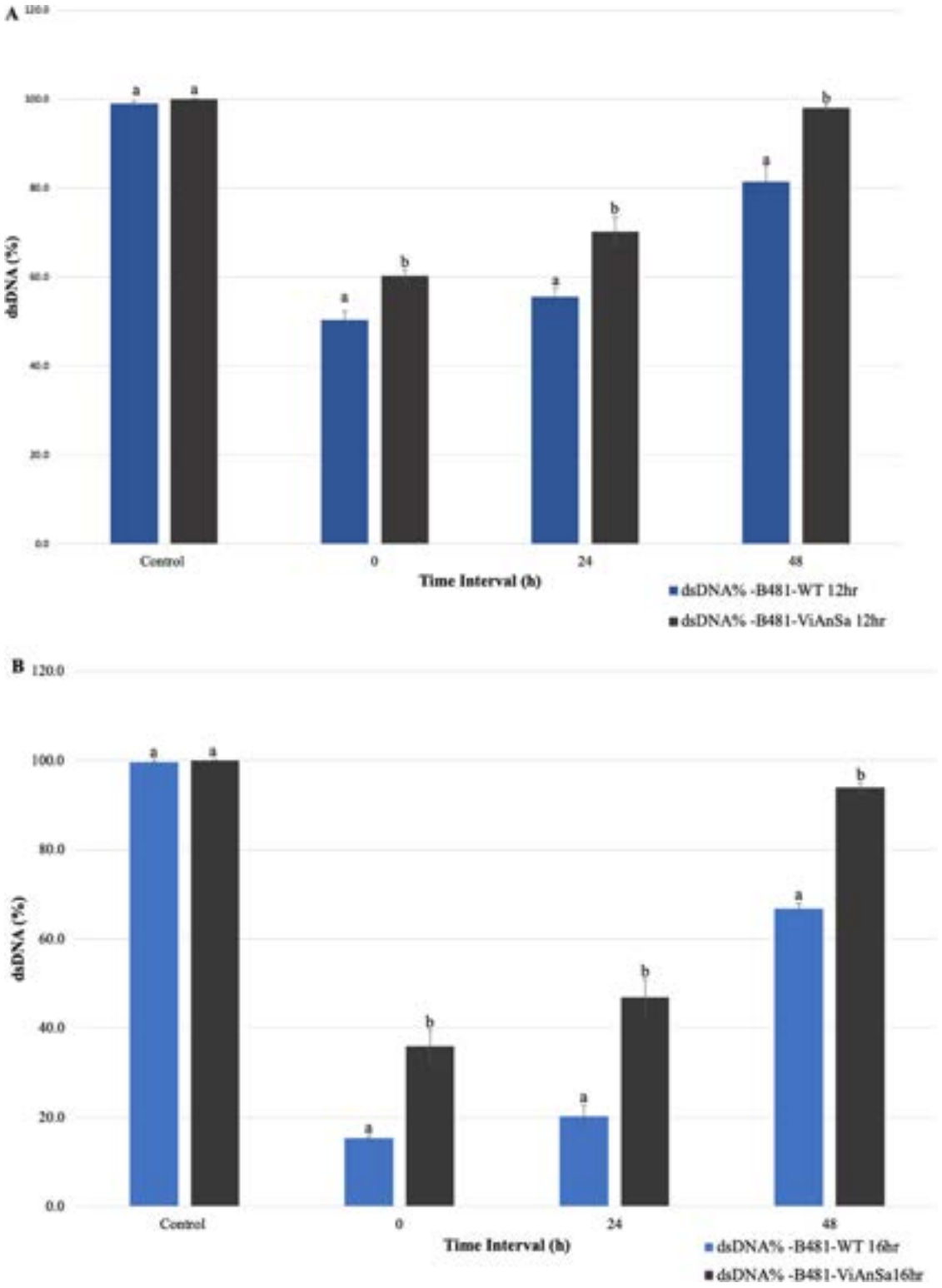


Figure 5. Percentage double-stranded (ds) DNA in *F. diplosiphon* wild-type (B481-WT) and transformant (B481-ViAnSa) strains after exposure to UV-B radiation (3.0 W m^{-2} (~8.0 μmol m^{-2} s^{-1})) for 12 h (A) and 16 h (B). Different letters above the standard error bars indicate significance between percentages ($p < 0.05$).

gold and iron nanoparticles.[15,23] Interestingly, UV-B radiation significantly reduced ($p < 0.05$) the percentage of all FAME components, including methyl palmitate, which was reduced by 20.51 and 19.25% in B481-ViAnSa and B481-WT, respectively (Table 1). This observation has also been reported by Kumar et al.,[24] in which UV-B radiation resulted in a decline in the FAME content of the microalgae *Chlorella sorokiniana*. Due to the exposure of cultures to simulated UV-B radiation for 4 continuous h, a reduction of FAMEs is expected. However, we observed significantly higher ($p < 0.05$) amounts of saturated FAMEs in both strains irradiated with UV-B when compared to those of the untreated control. Our

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

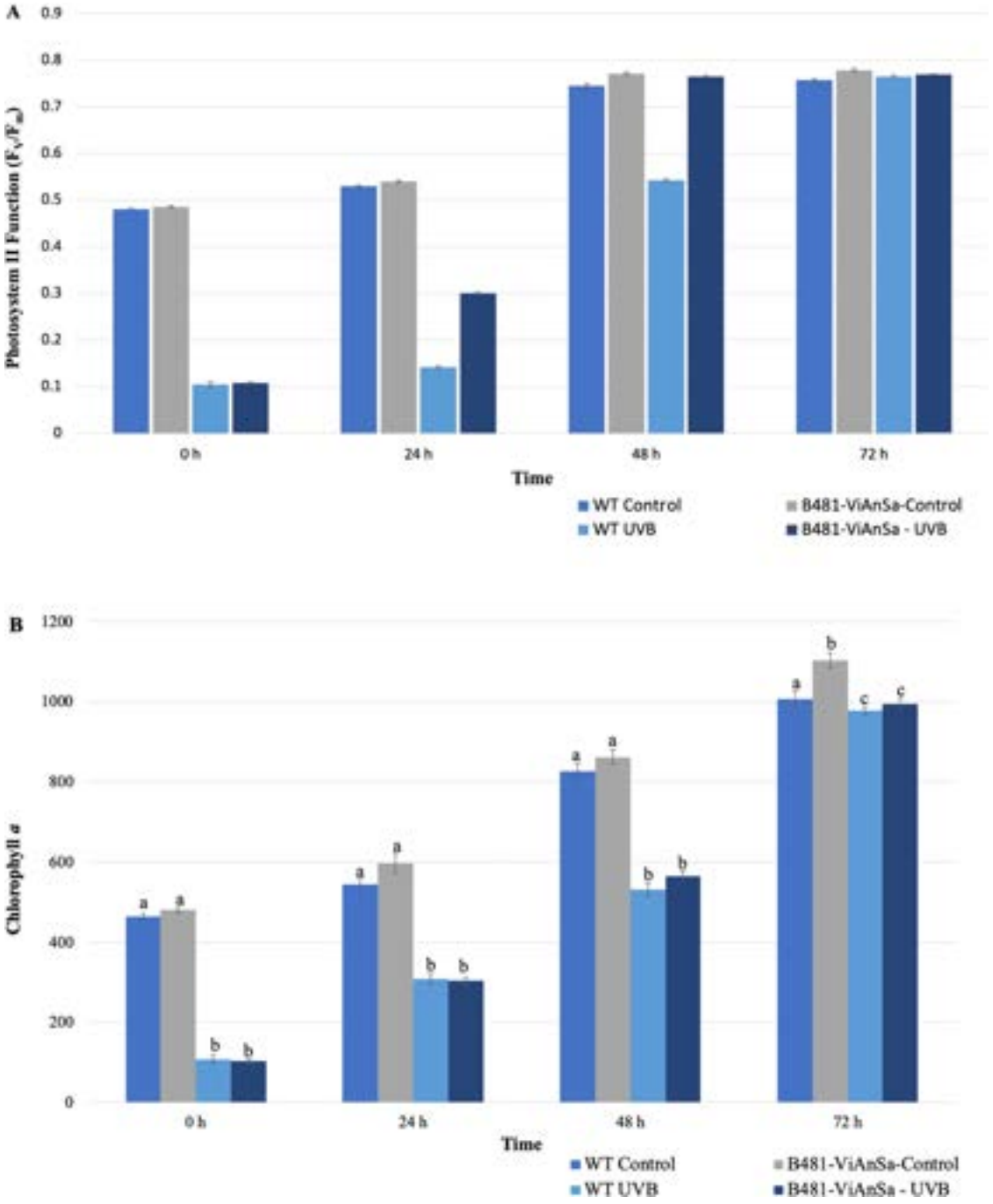


Figure 6. Evaluation of photosystem II activity (A) and chlorophyll *a* (B) content in *F. diplosiphon* B481-WT and B481-ViAnSa strains after 12 h UV-B radiation. Different letters above the error bars indicate significant differences ($p < 0.05$).

results are consistent with a previous report study in *Lyngbya purpurem*, where the saturated FAMEs and lipid saturation index were significantly greater ($p < 0.05$) in UV-B-exposed cultures compared to those in UVA or PAR.[25]

In summary, our results indicate that overexpression of the *phr A* gene enhanced *F. diplosiphon* UV stress tolerance, with enhanced PSII reversal rate and no negative impact on lipids. Considering future projections of increased UV-B radiation reaching the Earth's surface due to environmental pollution and depletion of the ozone layer,[25] this study has paved the way for cultivating *F. diplosiphon* in large-scale outdoor systems. Future studies will aim toward screening of diverse protective sunscreen compounds in the UV-tolerant transformant, which lead to the production of environment-friendly sunscreen and moisturizers.

3. MATERIALS AND METHODS

3.1. Strains and Culture Conditions. *F. diplosiphon* strain (B481-WT) obtained from the UTEX algal repository (Austin) was grown in liquid BG11 medium containing 20 mM HEPES (hereafter termed as BG11/HEPES) to an exponential growth phase (optical density at 750 nm of ∼0.6). Cultures were grown under continuous shaking at 170 rpm and 28 °C in an Innova 44R incubator shaker (Eppendorf, Germany). The photosynthetic light in the shaker had peak wavelengths at 437

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

Table 1. Quantitative Composition of Fatty Acid Methyl Esters in Transesterified Lipids of Nonirradiated and UV-B-Radiated *F. diplosiphon* Wild-Type (WT) and Transformant (B481-ViAnSa) Strains

	nonirradiated (%)		UV-B-irradiated (%)	
FAME type	B481-ViAnSa	B481-WT	B481-ViAnSa	B481-WT
methyl palmitate (C16:0)	53.43	51.69	42.47	41.74
methyl octadecanoate (C18:0)	30.12	33.02	23.33	24.18
methyl octadecanoate (C18:1)	22	23.02	15.99	17.05
methyl tetradecanoate (C14:1)	5.19	5.07	1.18	2.07
methyl hexadecanoate (C16:1)	4.76	4.59	0.87	0.81
methyl octadecadienoate (C18:2)	3.01	3.13	0.91	0.78

and 600−650 nm with an intensity adjusted to 30 μmol m^{-2} s^{-1} using the model LI-190SA quantum sensor (Li-Cor).

3.2. RNA Isolation and Complementary DNA Synthesis. Total RNA was extracted from *F. diplosiphon* grown to an exponential phase (7 days) using Tri Reagent (Molecular Research Center, Inc.) according to the manufacturer's protocol with modifications. The concentration and purity of the extracted RNA were tested on an agarose gel, and $A_{260/280}$ absorbance ratio was measured using a Nanodrop 2000 (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized using the high-capacity RNA to cDNA kit (Life Technologies). A 20 μL reaction mixture containing 1000 ng of RNA, 2× reverse transcription buffer (RT), and 10× RT random primers was incubated at 37 °C for 60 min followed by 95 °C for 5 min. Synthesized cDNA was aliquoted and stored at −20 °C.

3.3. Identification and Cloning of the Photoreactivation Gene. To identify homologs of the *phr A* gene in B481-WT, the forward (5′-AAGCTTTATGTGGCACACGACTGTACC-3′) and reverse (5′-GGATCCGGTTATTTGACCAATTGATAAC-3′) primers with *EcoR1* and *BamHI* restriction sites were designed (sequence ID: AP018233). Complementary DNA synthesized as described above was used as a template for *phr A* gene amplification. PCR conditions were set in a C1000 Touch thermocycler (Bio-Rad) as follows: 95 °C for 2 min; 40 cycles at 95 °C for 30 s and an annealing temperature of 51.4 °C for 30 s, followed by a final elongation step at 72 °C for 45 s. Amplified products were electrophoresed on a 1.5% agarose gel, bands were excised at the expected size ranges, and DNA was extracted using the gel recovery mini pre-kit (Zymo Research).

The amplified gene products and pGEM-7Zf vector containing T7 promoter were double-digested with *EcoR1* and *BamHI* restriction enzymes (Promega) and purified using Zymo DNA clean and concentrator kit. Inserts were ligated into the vector at the digested restriction sites with T4 DNA ligase (New England BioLabs), and pGEM-7Zf-*phr A* expression plasmid was constructed to overexpress the photolyase gene. The ligated plasmids were transformed into *Escherichia coli* FB5α competent cells via heat shock at 42 °C for 20 s followed by incubation on ice for 5 min. The transformed cells were plated on Luria Bertani (LB) agar plates containing 80 mg L^{-1} ampicillin and incubated for 16 h at 37 °C. Twenty resistant single colonies were randomly selected, transferred to liquid LB medium containing 80 mg L^{-1} ampicillin, and grown at 37 °C for 16 h. Plasmids were extracted using the Zyppy plasmid miniprep kit (Zymo Research), and the insert was confirmed by PCR and Sanger sequencing.

3.4. Electroporation-Mediated Transformation of the *phr A* Gene in *F. diplosiphon*. Expression plasmid containing the *phr A* gene was transformed into *F. diplosiphon* B481-WT according to parameters described by Tabatabai et al.[14] Competent cells (40 μL) were mixed with ligated purified plasmid DNA and electroporated using a GenePulser Xcell with CE module (Bio-Rad) at 200 Ω resistance, 1.0 kV, and 25 μF capacitance. After incubation on ice for 20 min, the transformant was grown in BG11/HEPES liquid medium for 16 h and plated on LB agar containing 80 mg L^{-1} ampicillin. To verify the insertion of the *phr A* gene, PCR was performed using gene-specific primers as mentioned in Section 2.3, and products were visualized on a 1.5% agarose gel.

3.5. Quantitation of Gene Overexpression in the Transformant by Reverse Transcription-Quantitative PCR (RT-qPCR). Total RNA from the wild type and the transformant was extracted, cDNA was synthesized as mentioned in Section 2.2, and RT-qPCR was performed to quantify gene overexpression. Gene-specific primers for the *phr A* gene were designed, and real-time amplifications were performed using SYBR green master mix (Applied Biosystems) in a Thermal Cycler CFX96 Real-Time machine (Bio-Rad). Amplifications were performed under the following conditions: 95 °C for 20 s; and 40 cycles at 50.9 °C for 30 s. Four replicates were maintained for each treatment type, and the experiment was repeated. Relative quantification (RQ) data of the transformant was analyzed using the ΔC_t method with CFX Manager 3.1 (Bio-Rad) with the B481-WT with pGEM-7Zf vector as a control. The 16S rRNA was used as the internal control, and fold-change values were calculated.

3.6. Detection of DNA Breakages Using Fluorometric Analysis of DNA Unwinding Assay. Fluorometric analysis of DNA unwinding (FADU) assay was performed to determine DNA breakages, as described previously by Rastogi et al.[5] *F. diplosiphon* wild-type and transformant strains were grown to logarithmic phase under culture conditions described in Section 2.1. Cultures were diluted to an $OD_{750\,nm}$ of 0.3 and 30 mL of culture exposed to 12 and 16 h of UV-B radiation in an open Petri dish. Three samples were tested in this assay: UVt-sample (exposed to UV), ds-sample (double-stranded sample; not UV-treated or subjected to alkaline condition), and ss-sample (subjected to alkaline condition to fully unwind the DNA).

After centrifuging 1.0 mL of each sample at 3000*g* for 10 min, the pellets were washed with 1 mL of TE buffer (tris−HCl 10 mM, EDTA 1 mM), followed by 20 μL of 0.5 M EDTA and 164 μL of TE buffer. The cell suspension was centrifuged at 3000*g* for 10 min, and 16 μL of lysozyme (50 mg mL^{-1}) was added to the pellet and incubated at 37 °C for 90 min to lyse the cell walls. To the suspension, 15 μL of 10% SDS, 10 μL of 4M NaCl, 15 μL of proteinase K (6 mg mL^{-1}), and 60 μL of TE buffer were added and incubated at 60 °C for 30 min for complete cell lysis. Finally, 300 μL of 0.1 M NaOH was added to each sample and subjected to different unwinding protocols as described below.

3.6.1. ss-Sample. The cell extract was sonicated for 2 min, incubated at 20 °C for 30 min, neutralized by adding 300 μL of 0.1 M HCl, and sonicated for 15 s to fully unwind dsDNA, and the lowest level of background fluorescence was estimated.

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

3.6.2. ds-Sample. To estimate total fluorescence, cell extract was neutralized by adding 300 μL of 0.1 M HCl, incubated at 20 °C for 30 min, and sonicated for 15 s to prevent the unwinding of dsDNA.

3.6.3. UVt-Sample. Cell extract was incubated at 20 °C for 30 min under alkaline conditions, neutralized by adding 300 μL of 0.1 M HCl, and sonicated for 15 s to diminish the single- and double-stranded DNA regions. This sample set was used to estimate the UV-induced DNA breaks.

After processing each sample as mentioned above, 20 μL of 20 mM Hoechst 33258 (bisbenzimide) DNA probe in 0.6 M phosphate buffer (pH 7.6) was added and centrifuged at 10 000*g* for 5 min. Fluorescence intensity of 200 μL supernatant was measured using a microplate reader (Agilent BioTek Synergy H1 Hybrid) at 343 nm with emissions between 380 and 550 nm. The percentage fraction (% *F*) of dsDNA was calculated using the formula, $F = (\mathrm{UVt} - \mathrm{ss})/(\mathrm{ds} - \mathrm{ss}) \times 100$, where ss, ds, and UVt corresponded to fluorescence intensities of ss, ds, and UVt samples, respectively.

3.7. Physiological Evaluation of the Transformant (B481-ViAnSa) Exposed to UV-B. *3.7.1. Evaluation of Growth and Stability.* The wild-type and transformant strains were grown in liquid BG11/HEPES media to logarithmic phase under culture conditions described in Section 2.1. Cultures were adjusted to an OD_{750} of 0.1 with BG11/HEPES media and irradiated under UV-B (3.0 W m^{-2}) for 0−160 min in a UV cross-linker (Fisher Scientific). Three biological replicates were maintained, and cells not irradiated with UV-B served as control. Growth of the strains at $OD_{750\,nm}$ was measured for a period of 14 days. The stability of the transformant was tested on BG11/HEPES plates containing 80 mg L^{-1} ampicillin for a 10 day period under culture conditions described above and exposed to UV-B for 30 min/day. Stability and presence of the gene were confirmed by RT-qPCR after 25 generations of subculture.

3.7.2. Evaluation of Photosynthetic Pigment Levels. Photosynthetic efficacy of the wild type and transformant was quantified as a measure of PSII activity and chlorophyll *a* content, which provides an estimate of the well-being of photosynthetic cells. To allow maximal irradiation and avoid cell shadowing, cultures grown to an $OD_{750\,nm}$ of 0.3 were placed in open Petri dishes and irradiated in a UV-B cross-linker (Fisher Scientific) for 60 min. Cultures were grown under conditions mentioned in Section 2.1 for 3 days to allow cell recovery, and PSII functionality was measured after 24, 48, and 72 h using a MINI-PAM (Walz, Effeltrich, Germany) to measure the minimal and maximal fluorescence yields (F_o and F_m). Based on these parameters PSII quantum yield (F_v/F_o) was calculated using the equation $F_v/F_m = (F_m - F_o)/F_m$.[26] In addition, chlorophyll a (chl *a*) was measured at an excitation of 420 nm and an emission of 680 nm using a microplate reader (Agilent BioTek Synergy H1 Hybrid), and the photosynthetic efficacy was compared.

3.8. Characterization of Lipids in the Wild Type and Transformant *F. diplosiphon* Grown under Simulated UV-B Conditions. The lipid profile of the wild type and transformant exposed to simulated UV-B conditions (Omaykey UV-B lamps) was compared using GC-MS. Cultures adjusted to 0.1 at OD_{750} were grown in 5 × 7 × 6 in. containers and exposed to UV-B for 4 h each day for 15 days to simulate the sun's UV-B radiation effects. Three replicated treatments were maintained, OD_{750} was measured every 3 days for a period of 12 days, and the growth rate was calculated. Simultaneous lipid extraction and transesterification were performed as described previously by Tabatabai et al.,[14] and fatty acid composition was analyzed at the Mass Spectrometry Facility at Johns Hopkins University (Baltimore, MD) using the Shimadzu GC17A/QP5050A GC-MS systems (Shimadzu Instruments). Identification of FAMEs was accomplished by comparing each GC/MS mass spectrum to the Lipid Web archived FAME spectra.

3.9. Statistical Analysis. Statistical significance was determined using one-way analysis of variance (ANOVA) and Tukey's honest significant differences post hoc test at 95% confidence intervals ($p < 0.05$). The single-factor, fixed-effect ANOVA model, $Y_{ij} = \mu + \alpha G_i + \varepsilon_{ij}$, was used, where *Y* is the variable being measured in strain i and biological replicate j. The μ represents mean growth with adjustments from the effects of strain (αG), and ε_{ij} is the experimental error from strain i and biological replicate j.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03938.

> Polymerase chain reaction of amplification product of double-digested photoreactivation gene (*Phr A*) from the extracted vector in lane 2 and pGEM-7Zf vector in lane 3; the *Phr A* gene was observed at the expected band size of ∼1500 bp; and pairwise comparison of mean growth differences between the wild-type (B481-WT) and transformant (B481-ViAnSa) *F. diplosiphon* strains (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Viji Sitther − *Department of Biology, Morgan State University, Baltimore, Maryland 21251, United States;* orcid.org/0000-0003-0096-569X; Email: viji.sitther@morgan.edu

Authors

Samson M. Gichuki − *Department of Biology, Morgan State University, Baltimore, Maryland 21251, United States*

Anithachristy S. Arumanayagam − *Department of Pathology and Genomic Medicine, Houston Methodist Hospital Research Institute, Houston, Texas 77030, United States*

Behnam Tabatabai − *Department of Biology, Morgan State University, Baltimore, Maryland 21251, United States*

Yavuz S. Yalcin − *Department of Biology, Morgan State University, Baltimore, Maryland 21251, United States*

LaDonna Wyatt − *Department of Biology, Morgan State University, Baltimore, Maryland 21251, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03938

Author Contributions

S.M.G. designed and performed the experiments, analyzed data, and wrote a draft of the manuscript. A.S.A. assisted with experimental troubleshooting, design modifications, and manuscript editing. B.T. and Y.S.Y. contributed to data analysis and manuscript writing. L.W. assisted with experimental setup and data collection. V.S. designed and conceived the overall study, edited the manuscript, and obtained funding. All of the authors have read and approved the final version of the manuscript.

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101

Notes

The authors declare no competing financial interest.
All contributing authors have read and approved the final manuscript. The authors have mutually agreed to submit the original work to Environmental Science Technology & Engineering. These findings have not been previously submitted to any other journal.

■ ACKNOWLEDGMENTS

This research was supported by the National Science Foundation's Nanoscale Interactions Program grant under award number 1900966 and cosupported by the Excellence in Research. The authors are grateful for the core support facilities partially provided by the National Institute of General Medical Sciences (5UL1GM118973) and the National Institute on Minority Health and Health Disparities (5U54MD013376) grants.

■ REFERENCES

(1) Sitther, V.; Tabatabai, B.; Fathabad, S. G.; Gichuki, S.; Chen, H.; Arumanayagam, A. S. *Cyanobacteria as a Biofuel Source: Advances and Applications*, Academic Press, 2020; pp 269−289.
(2) Crnkovic, C. M.; May, D. S.; Orjala, J. The impact of culture conditions on growth and metabolomic profiles of freshwater cyanobacteria. *J. Appl. Phycol.* **2018**, *30*, 375−384.
(3) Williamson, C. E.; Zepp, R. G.; Lucas, R. M.; Madronich, S.; Austin, A. T.; Ballaré, C. L.; Norval, M.; Sulzberger, B.; Bais, A. F.; McKenzie, R. L.; Robinson, S. A.; Häder, D. P.; Paul, N. D.; Bornman, J. F. Solar ultraviolet radiation in a changing climate. *Nat. Clim. Change* **2014**, *4*, 434−441.
(4) Wu, H.; Gao, K.; Villafañe, V. E.; Watanabe, T.; Helbling, E. W. Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis*. *Appl. Environ. Microbiol.* **2005**, *71*, 5004−5013.
(5) Rastogi, R. P.; Singh, S. P.; Haeder, D. P.; Sinha, R. P. Ultraviolet-B-induced DNA damage and photorepair in the cyanobacterium *Anabaena variabilis* PCC 7937. *Environ. Exp. Bot.* **2011**, *74*, 280−288.
(6) Castenholz, R. W.; Garcia-Pichel, F. *Cyanobacterial Responses to UV Radiation BT − Ecology of Cyanobacteria II: Their Diversity in Space and Time* Whitton, B. A., Ed.; Springer Netherlands: Dordrecht, 2012; Vol. *1*, pp 481−499.
(7) Rastogi, R. P.; Sinha, R. P.; Moh, S. H.; Lee, T. K.; Kottuparambil, S.; Kim, Y. J.; Rhee, J. S.; Choi, E. M.; Brown, M. T.; Häder, D. P.; Han, T. Ultraviolet radiation and cyanobacteria. *J. Photochem. Photobiol. B* **2014**, *141*, 154−169.
(8) Rastogi, R. P.; Richa; Kumar, A.; Tyagi, M. B.; Sinha, R. P. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *J. Nucleic Acids* **2010**, *2010*, No. 592980.
(9) Oren, A.; Gunde-Cimerman, N. Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiol. Lett.* **2007**, *269*, 1−10.
(10) Kokabi, M.; Yousefzadi, M.; Soltani, M.; Arman, M. Effects of different UV radiation on photoprotective pigments and antioxidant activity of the hot-spring cyanobacterium, *Leptolyngbya cf. fragilis*. *Phycol. Res.* **2019**, *67*, 215−220.
(11) Pathak, J.; Ahmed, H.; Singh, P. R.; Singh, S. P.; Häder, D. P.; Sinha, R. P. Mechanisms of Photoprotection in Cyanobacteria. In *Cyanobacteria from Basic Science to Applications*, Academic Press, 2019; Chapter 7, pp 145−171.
(12) Tabatabai, B.; Chen, H.; Lu, J.; Giwa-Otusajo, J.; McKenna, A. M.; Shrivastava, A. K.; Sitther, V. *Fremyella diplosiphon* as a biodiesel agent: Identification of fatty acid methyl esters via microwave-assisted direct in situ transesterification. *Bioenergy Res.* **2018**, *11*, 528−537.
(13) Montgomery, B. L. Using *Fremyella diplosiphon* as a model organism for genetics-based laboratory exercises. *Biosci. Educ.* **2011**, *17*, 1−7.
(14) Tabatabai, B.; Arumanayagam, A. S.; Enitan, O.; Mani, A.; Natarajan, S. S.; Sitther, V. Overexpression of *hly*B and *mdh* genes confers halotolerance in *Fremyella diplosiphon*, a freshwater cyanobacterium. *Enzyme Microb. Technol.* **2017**, *103*, 12−17.
(15) Fathabad, S. G.; Arumanayagam, A. S.; Tabatabai, B.; Chen, H.; Lu, J.; Sitther, V. Augmenting *Fremyella diplosiphon* cellular lipid content and unsaturated fatty acid methyl esters via sterol desaturase gene overexpression. *Appl. Biochem. Biotechnol.* **2019**, *189*, 1127−1140.
(16) Vass, I. Z.; Kõs, P. B.; Sass, L.; Nagy, C. I.; Vass, I. The ability of cyanobacterial cells to restore UV-B radiation induced damage to photosystem II is influenced by photolyase dependent DNA repair. *Photochem. Photobiol.* **2013**, *89*, 384−390.
(17) Mosca, C.; Rothschild, L. J.; Napoli, A.; Ferré, F.; Pietrosanto, M.; Fagliarone, C.; Baqué, M.; Rabbow, E.; Rettberg, P.; Billi, D. Over-Expression of UV-Damage DNA repair genes and ribonucleic acid persistence contribute to the resilience of dried biofilms of the desert cyanobacterium *Chroococcidiopsis* exposed to mars-like UV flux and long-term desiccation. *Front. Microbiol.* **2019**, *10*, 2312.
(18) Sinha, R. P.; Klisch, M.; Walter Helbling, E.; Häder, D. P. Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. *J. Photochem. Photobiol. B* **2001**, *60*, 129−135.
(19) Zeeshan, M.; Prasad, S. M. Differential response of growth, photosynthesis, antioxidant enzymes and lipid peroxidation to UV-B radiation in three cyanobacteria. *South Afr. J. Bot.* **2009**, *75*, 466−474.
(20) Chen, L.; Xie, M.; Bi, Y.; Wang, G.; Deng, S.; Liu, Y. The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. *Ecotoxicol. Environ. Saf.* **2012**, *80*, 224−230.
(21) Kumar, D.; Kannaujiya, V. K.; Jaiswal, J.; Sinha, R. P. Effects of Ultraviolet and Photosynthetically Active Radiation on Phycocyanin of Habitat Specific Cyanobacteria. *J. Sci. Res.* **2020**, *64*, 74−79.
(22) Apostolova, E. L.; Pouneva, I.; Rashkov, G.; Dankov, K.; Grigorova, I.; Misra, A. N. Effect of UV-B radiation on photosystem II functions in Antarctic and mesophilic strains of a green alga *chlorella vulgaris* and a cyanobacterium *Synechocystis salina*. *Indian J. Plant Physiol.* **2014**, *19*, 111−118.
(23) Tabatabai, B.; Fathabad, S. G.; Bonyi, E.; Rajini, S.; Aslan, K.; Sitther, V. Nanoparticle-mediated impact on growth and fatty acid methyl ester composition in the cyanobacterium *Fremyella diplosiphon*. *Bioenergy Res.* **2019**, *12*, 409−418.
(24) Kumar, V.; Nanda, M.; Kumar, S.; Chauhan, P. K. The effects of ultraviolet radiation on growth, biomass, lipid accumulation and biodiesel properties of microalgae. *Energy Sources, Part A* **2018**, *40*, 787−793.
(25) Chandra, R.; Pons-Faudoa, F. P.; Saldívar, R. P.; Rittmann, B. E. Effect of ultra-violet exposure on production of mycosporine-like amino acids and lipids by *Lyngbya purpurem*. *Biomass Bioenergy* **2020**, *134*, No. 105475.
(26) Wan, J.; Guo, P.; Peng, X.; Wen, K. Effect of erythromycin exposure on the growth, antioxidant system and photosynthesis of *Microcystis flosaquae*. *J. Hazard. Mater.* **2015**, *283*, 778−786.

https://doi.org/10.1021/acsomega.2c03938
ACS Omega 2022, 7, 35092−35101