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Responses of Different Morphological Cells of *Phaeocystis globosa* to UV-B Radiation

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Abstract: *Phaeocystis globosa* is an important member of the plankton community and was considered to be a typical bloom-forming algae. Its life cycle is variable, comprising both solitary and colony cells. The growth process of *P. globosa* is vulnerable to UV-B radiation. However, the influence of UV-B on photosynthetic activity and the resulting programmed cell death (PCD) process is not entirely understood. Our findings demonstrated that Fv/Fm, rETRmax, Y(II) and α of solitary and colony cells were significantly decreased after UV-B treatment ($p < 0.05$). The colony cells showed a lower damage rate and higher repair rate than solitary cells ($p < 0.05$), suggesting that colony cells have better UV-B radiation resistance. After UV-B radiation, we found the characteristic markers of PCD-phosphatidylserine (PS) externalization and DNA fragmentation were discovered in the two cell morphologies, with increased caspase-3-like activity, proving the onset of PCD. In addition, the reactive oxygen species (ROS) content and antioxidant enzyme activities were examined. The results showed that, the ROS content went up, the solitary cells were significantly greater than colony cells under UV-B radiation ($p < 0.001$). In addition, the superoxide dismutase (SOD) and catalase (CAT) activities increased, and solitary cells always had significantly higher activity than colony cells ($p < 0.05$), but the changing trend in ROS content did not match the changes in CAT and SOD activities. This may have been due to the necrosis of solitary cells. The findings show that, besides PCD, solitary cells also developed necrosis under UV-B radiation. This study provides evidence that different morphological cells of marine microalgae present different reactions to UV-B radiation. It helps to further improve the knowledge of the environmental adaptation mechanism of *P. globosa*.

Keywords: UV-B radiation; bloom; *Phaeocystis globosa*; colony cells; solitary cells; PCD; necrosis

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1. Introduction

Phaeocystis globosa causes harmful algae blooms in tropical, subtropical, and temperate coastal waters worldwide and has a strong influence on the cycling of carbon and sulfur in nearshore waters [1,2]. *P. globosa* blooms in the form of colonies floating on the surface of seawater, which can lead to the discoloration of seawater when many colonies are formed, resulting in marine ecological disasters [3,4]. Blooms can cause severe environmental and human safety problems, including the degradation of water quality, death of aquatic animals, and destruction of aquatic ecosystems [5–7]. *P. globosa* has a complex heterogonic life cycle. It consists of solitary cells, and colonies are made up of numerous cells enclosed in a polysaccharide film [8,9]. This polysaccharide outer membrane can help colony cells resist predators, bacteria, and viruses [10].

As an autotroph, *P. globosa* relies on the photosynthetic oxidative system for energy conversion to accomplish cell reproduction, polysaccharide secretion, and bloom formation [8,11]. When photosynthetic organs absorb sunlight for photosynthesis, they also inevitably absorb UV-B. UV-B radiation is one significant environmental factor affecting

the growth and reproduction of *P. globosa* [12]. With rapid urbanization and industrialization, the ozone layer is being destroyed. This phenomenon has resulted in the increase in the amount of UV-B radiation reaching the Earth's surface [13]. The increased UV exposure's effects on marine algae have attracted considerable attention [14]. In particular, the effect of UV-B on photosynthetic oxidative systems can profoundly affect cellular growth, reproduction, and even cell life history [15]. Scholz et al. reported that the growth rate of intertidal diatoms (*Achnanthes exigua*, *Cocconeis peltoides*, *Diploneis littoralis*, *Navicula digitoradiata*, and *Amphora exigua*) was significantly inhibited under higher-intensity UV-B exposure, and biomass and pigment content were decreased with increasing doses of UV-B exposure [16]. Additionally, UV-B radiation causes photo-inhibition in the marine diatom *Skeletonema costatum* [17]. Similarly, a decrease in the maximum electron transfer rate was observed after 4 d of UV-B treatment in *Isochrysis galbana* [18]. However, the impacts of UV-B radiation on the evolution of *P. globosa* are complicated due to the specialized heterotypic life cycle of this organism. At present, it is unclear how *P. globosa* responds to high doses of UV-B exposure. The polysaccharide outer membrane of colony cells may provide protection against UV-B radiation.

Reports of the effects of UV radiation on marine algae are numerous [19,20]. The majority of UV radiation stress in algae is related to the generation of reactive oxygen species (ROS) [21]. UV-B treatment can have a direct effect on chloroplasts, leading to the inhibition of photosynthesis, which results in high levels of ROS production and accumulation [22]. In addition, UV-B treatment can affect the differentiation, photosynthesis capacity, and pigmentation in algae, leading to many types of cell damage, such as DNA damage and membrane damage, which eventually result in cell death [23–25]. Programmed cell death (PCD) in algae can be induced by UV radiation exposure [26]. PCD is a gene-regulated suicide mechanism and it occurs when the cell is stimulated by environmental factors [27–29]. A number of changes occur, involving phosphatidylserine (PS) externalization, DNA fragmentation, and the initiation of caspase-3-like activity [30,31]. When cells are subjected to stress from the external environment, microalgae may eliminate damaged cells by triggering PCD to protect community cells [32]. PCD detection methods have been widely applied to explore algae responses to UV radiation [33–35]. Nevertheless, the PCD process of *P. globosa* solitary and colony cells under UV-B-induced stress is poorly understood.

In addition to PCD, necrosis occurs in algae under UV radiation [36]. Necrosis is a passive death mode that occurs simultaneously with apoptosis or independently [37]. UV-B radiation strongly inhibits the activities of algae. El-Sheekh et al. reported that after 7 h of exposure to UV-B radiation, cyanobacteria showed necrosis [38]. Moreover, necrosis and apoptosis were observed in the UV-induced death of *Chlamydomonas reinhardtii* [39]. In fact, the extent of cellular damage is correlated with the level of environmental stress [40]. Necrosis occurs when cells are stressed beyond their ability to cope with environmental stress [41].

Few reports have focused on UV-B radiation-induced PCD in *P. globosa* or on differences between phenotypes (solitary and colony) in response to UV-B radiation. Exploring the process of cell death in different types of *P. globosa* cells under heavy environmental stress provides a valuable way to understand how cells change to adapt and survive in a dynamic environment. In this study, *P. globosa* colony and solitary cells were selected to determine their photosynthetic activity, antioxidant enzyme activities, caspase-3-like activity, PS externalization, and DNA fragmentation after UV-B radiation. The responses of *P. globosa* solitary and colony cells to UV-B radiation stress are discussed from a PCD point of view. Furthermore, by comparing the differences between PCD in colony and solitary cells, new insights into the adaptation of *P. globosa* to UV-B radiation can be obtained. These results highlight the importance of switching between different cell types in determining the fate of *P. globosa* under UV irradiation stress. The study also provides insight into the potential survival strategies and different death mechanisms of microalgal cells in high-pressure environments.

2. Materials and Methods

2.1. Algal Culture and Treatment with UV-B Radiation

P. globosa cells were separated from the nearshore waters of Beibu Gulf and preserved in the Microalgae Resource Centre of Guangxi Academy of Science in 2016. This strain was cultivated using f/2 medium at a temperature of 20 °C [42]. The light intensity for the culture was 50 μmol photons·m^{-2}·s^{-1} and the light–dark cycle was 12 h:12 h. *P. globosa* cells were collected in the period of exponential growth and were filtered by a 10 μm mesh to separate the solitary cells from the colony cells. UV-B radiation was obtained from a UV lamp (Philips, Amsterdam, The Netherlands) and radiation intensity was 485 μw·cm^{-2}. *P. globosa* cells were radiated by UV-B for 2, 4 h. Cultures without UV-B exposure were used as the control group (0 h). Before the experiment, the UV lamp was left on for 8 h to ensure that the radiation level was stable during the experiments. Both the control group and experimental group had three replicates.

2.2. Photosynthetic Activity

P. globosa solitary and colony cells were measured for their photosynthetic activity via a Phyto-PAM fluorometer (Walz, Effeltrich, Germany). The measurement was performed based on a method reported by Zhuang et al. [43]. The maximum photosynthetic efficiency (Fv/Fm), actual photosynthetic efficiency (Y(II)), light energy utilization (α), and relative maximum electron transport rate (rETRmax) of the samples were recorded.

2.3. Damage Rate (k, min^{-1}) and Repair Rate (r, min^{-1}) of PSII

The Fv/Fm values of *P. globosa* solitary and colony cells were measured at 0, 60, 120, 180, and 240 min during UV-B radiation and at 300, 360, 420, and 480 min during the recovery period (5 μmol photons·m^{-2}·s^{-1}). The damage rate and repair rate of *P. globosa* were calculated according to the Kok model [44]. The variation in the maximum photosynthetic efficiency of cells with radiation time was calculated according to Formula (1):

$$P/P_{initial} = a + b * exp^{(-c*t)} \quad (1)$$

$$a = r/(r+k) \quad (2)$$

$$b = k/(r+k) \quad (3)$$

$$c = r + k \quad (4)$$

where P indicates the Fv/Fm at the corresponding time, and $P_{initial}$ indicates the initial Fv/Fm. The values of a, b, and c can be calculated by fitting an exponential equation, and the values of r and k can be calculated by linking (2), (3), and (4).

2.4. ROS Content and Antioxidant Enzyme Activity

The ROS contents in *P. globosa* solitary and colony cells were measured by 2′,7′-dichlorodihydrofluorescein diacetate (Nanjing Jiancheng, Nanjing, China). *P. globosa* cells were collected via centrifugation and incubated with 10 μM DCFH-DA in darkness for 20 min at 37 °C. The fluorescence of the cells was measured with a multifunctional enzyme labeler system (Tecan, Männedorf, Switzerland).

The superoxide dismutase (SOD) and catalase (CAT) activities in *P. globosa* solitary and colony cells were measured via a SOD assay kit (Nanjing Jiancheng, Nanjing, China) and a CAT assay kit (Nanjing Jiancheng, Nanjing, China), respectively. The cells were collected by centrifugation. The collected cells were transferred to a new tube and then subjected to ultrasonic processing at low temperature by an ultrasonic pulverizer (Xinzhi, Ningbo, China) at 200 W for 5 min (ultrasonication 2 s; rest 3 s). The supernatants were obtained by centrifugation and used to analyze the activities of SOD and CAT following the instructions provided by the test kit manufacturers.

2.5. Caspase-3-like Activity

The caspase-3-like activities of *P. globosa* solitary and colony cells were measured via a caspase-3-like activity assay kit (Solarbio, Beijing, China). The cells were collected by centrifugation and were subsequently lysed at low temperature for 15 min with 100 μL of lysis buffer. The supernatants were obtained via centrifugation and measured following the kit manufacturer's instructions. The caspase-3-like activity of *P. globosa* solitary and colony cells was determined by measuring the absorbance at 405 nm with an enzyme labeler instrument (Thermo, Waltham, MA, USA).

2.6. PS Detected by Annexin V-FITC

PS externalization of *P. globosa* cells was detected via an Annexin V-FITC detection kit (Nanjing Jiancheng, Nanjing, China). Cells were collected by centrifugation. The supernatant was removed, and the residue was retained. The residue was incubated with 500 μL of binding solution and 5 μL of Annexin V-FITC in the dark for 10 min at 20 °C. The samples were subsequently washed with PBS three times. Cells were collected in low light and were analyzed via fluorescence microscopy (Nikon, Tokyo, Japan).

2.7. TUNEL Detected by Fluorescence Microscopy

The DNA fragmentation in *P. globosa* cells was detected with a TUNEL assay kit (Beyotime, Shanghai, China). The cells were collected by centrifugation. The supernatant was removed, and the residue was retained. The residue was treated for 30 min with paraformaldehyde (Beyotime, Shanghai, China) and kept for 5 min at 20 °C in cell lysis buffer supplemented with 0.3% Triton X-100 (Biosharp, Hefei, China). After permeabilization, the cells were added to 50 μL of TUNEL assay solution, then kept at 37 °C in low light for 1 h. Subsequently, the cells were washed with PBS three times. The sample cells were collected in the dark and were analyzed via fluorescence microscopy.

2.8. Statistical Analysis

Data on the photosynthetic activity, PSII damage rate and repair rate, caspase-3-like activity, SOD and CAT activities, and ROS content of *P. globosa* solitary and colony cells are presented as the means ± standard deviations (SDs). All data analyses were performed via the software SPSS 20.0. The differences between solitary and colony cells were analyzed via a *t*-test, with a significance level of 5%.

3. Result

3.1. Photosynthetic Activity

Compared to 0 h, the Fv/Fm in *P. globosa* solitary and colony cells decreased gradually within 2, 4 h of UV-B radiation ($p < 0.01$). Initial Fv/Fm values of *P. globosa* solitary and colony cells were not significantly different ($p > 0.05$, Figure 1A). Under 2, 4 h of UV-B radiation, the Fv/Fm of solitary cells decreased by 42.6% and 73.7%, respectively; the Fv/Fm of colony cells decreased by 32.0% and 49.5%, respectively. These findings indicated that the Fv/Fm in the solitary cells was inhibited to a greater extent than that in the colony cells. The Y(II) of *P. globosa* solitary and colony cells significantly decreased with longer exposure time to UV-B ($p < 0.05$). The initial value of Y(II) did not differ between the solitary and colony cells. After 2 h of UV-B radiation, the Y(II) of solitary cells was significantly lower than that of colony cells ($p < 0.05$; Figure 1B). Initially, the α and rETRmax of solitary *P. globosa* cells did not differ from those of colony cells. After 2, 4 h of UV-B radiation, the α, rETRmax values in *P. globosa* solitary and colony cells decreased significantly ($p < 0.01$) and continued to decrease with time. During 2 h, 4 h of UV-B radiation, the α and rETRmax values in *P. globosa* solitary were significantly lower than those of colony cells ($p < 0.01$; Figure 1C,D).

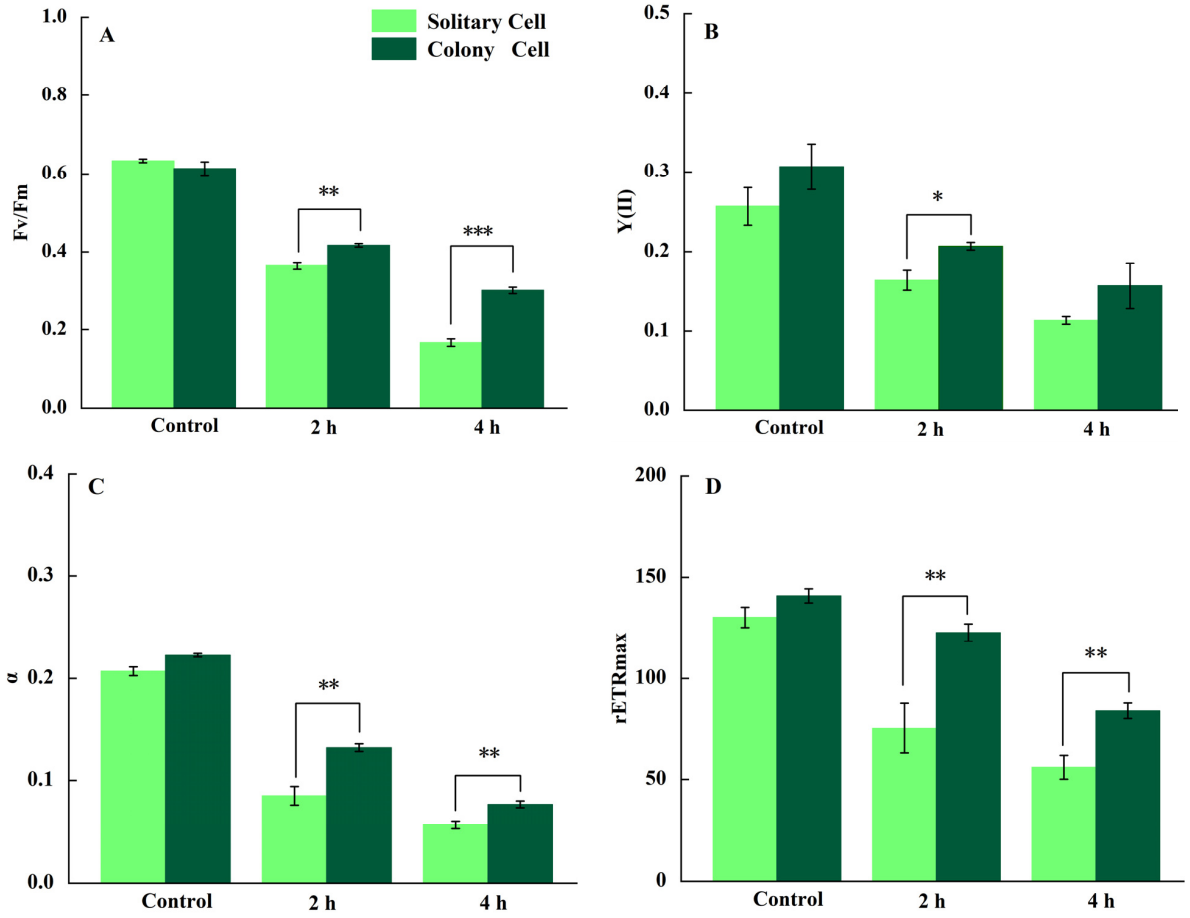

Figure 1. Effect of UV-B on the photosynthetic activity of solitary and colony cells of *P. globosa*. Note: (**A**) maximum photosynthetic reaction rate; (**B**) actual photosynthetic reaction rate; (**C**) utilization of light energy by photosynthetic organs; (**D**) relative maximum electron transfer rate. * indicates statistically significant differences in the data. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2. Damage Rate and Repair Rate of PSII

Table 1 shows the damage rates and repair rates of *P. globosa* solitary and colony cells under UV-B radiation. The damage rates of *P. globosa* solitary and colony cells were 10.87 ± 0.252 min^{-1} and 7.74 ± 0.157 min^{-1}, respectively, and the damage rate of solitary cells was significantly greater than that of colony cells ($p < 0.05$). Following 4 h of recovery in poor light, *P. globosa* solitary cells presented a lower repair rate (5.62 ± 0.42 min^{-1}) than did colony cells (16.10 ± 1.00 min$^{-1)}$. Compared with solitary cells, colony cells had a significantly greater repair rate ($p < 0.001$).

Table 1. The damage rate (k) repair rate (r) in PSII for solitary and colony cells of *P. globosa*.

Cell Morphology	r ($\times 10^{-3}$ min^{-1})	k ($\times 10^{-3}$ min^{-1})
Solitary cell	5.62 ± 0.42	10.87 ± 0.252
Colony cell	16.10 ± 1.00 ***	7.74 ± 0.157 *

Note: * indicates statistically significant differences in the data. * $p < 0.05$; *** $p < 0.001$.

3.3. Determination of ROS and Antioxidant Enzyme Activities

After 2, 4 h of UV-B radiation, the increase in ROS content in *P. globosa* solitary cells was significant compared with that at 0 h ($p < 0.01$). In *P. globosa* solitary cells, the ROS contents after 2, 4 h of UV-B radiation were 3.5 and 3.1 times greater than those at 0 h, respectively. In *P. globosa* colony cells, the ROS contents after 2, 4 h of UV-B radiation were 1.4 and 4.1 times greater than those in the control group, respectively. The ROS of solitary cells

were significantly greater than those of colony cells after being exposed to UV-B radiation for 2 h ($p < 0.001$), whereas the ROS of colony cells were significantly greater than those of solitary cells after being exposed to UV-B radiation for 4 h ($p < 0.001$; Figure 2A).

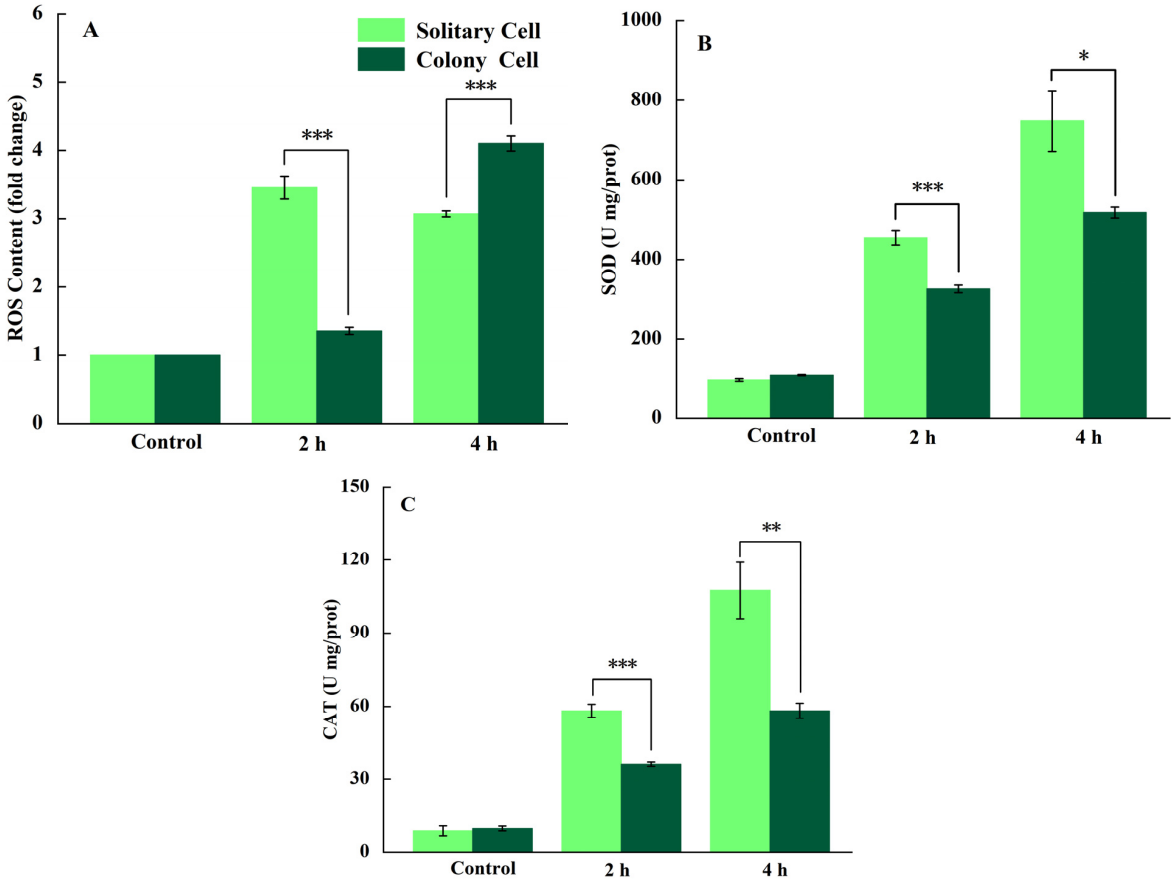

Figure 2. Effect of UV-B on the ROS content and antioxidant enzyme activity of solitary and colony cells of *P. globosa*. Note: (**A**) ROS content; (**B**) SOD activity; (**C**) CAT activity. * indicates statistically significant differences in the data. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

When *P. globosa* solitary cells were radiated with UV-B for 2 h, the SOD activity rapidly increased by 3.7 times and then increased by 6.9 times at 4 h compared with 0 h. A rapid increase in the SOD activity was also detected in *P. globosa* colony cells with UV-B radiation, but the level was significantly ($p < 0.05$) lower than that in solitary cells. Compared with 0 h, the SOD activity of *P. globosa* colony cells increased 2.0 and 3.8 times with 2, 4 h of UV-B radiation, respectively (Figure 2B). Compared with 0 h, the CAT activity in *P. globosa* solitary and colony cells significantly increased by 6.1 and 2.8 times, respectively ($p < 0.01$), after radiation with UV-B for 2 h; it significantly ($p < 0.001$) increased by 11.9 and 5.1 times, respectively, after radiation with UV-B for 4 h (Figure 2B). The CAT activity of *P. globosa* solitary cells was significantly higher than that of colony cells under UV-B radiation at 2, 4 h ($p < 0.01$; Figure 2C).

3.4. Measurement of Caspase-3-like Activity

After UV-B radiation for 2 h, the caspase-3-like activities of *P. globosa* solitary and colony cells significantly increased, with increases of 5.3 and 3.8 times, respectively ($p < 0.01$). The caspase-3-like activity of *P. globosa* solitary cells was significantly higher than that of colony cells after being exposed to UV-B radiation for 2 h ($p < 0.05$). After 4 h of UV-B radiation, caspase-3-like activity in *P. globosa* solitary and colony cells decreased, but both groups had higher values than at 0 h (Figure 3).

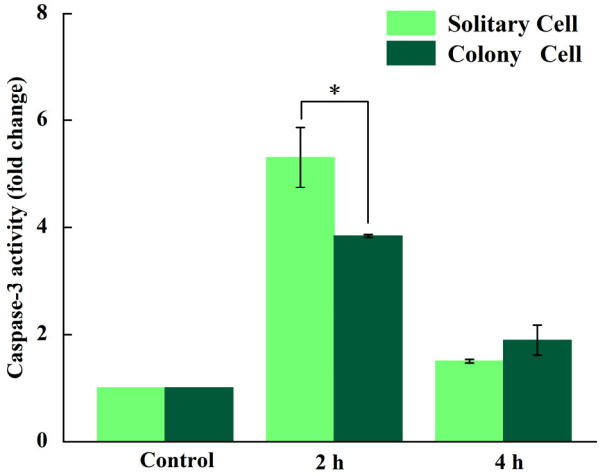

Figure 3. Effect of UV-B on the caspase-3-like activity of *P. globosa*'s solitary and colony cells. Note: * indicates statistically significant differences in the data. * $p < 0.05$.

3.5. Determination of PS Externalization and DNA Fragmentation

PS externalization, as revealed by Annexin V-FITC staining, was monitored in *P. globosa* solitary and colony cells. *P. globosa* solitary and colony cells radiated by UV-B presented bright green fluorescence, which indicated positive PS externalization at 2, 4 h (Figure 4G,H,M,N). In contrast, the control group exhibited bright red fluorescence (Figure 4A,B). Detection of DNA fragmentation by TUNEL assay. Unlike the red fluorescence found in the control group (Figure 4C,D), the experimental group presented bright green fluorescence, indicating that high levels of DNA fragmentation occurred in *P. globosa* solitary and colony cells (Figure 4I,J,O,P). In addition, *P. globosa* solitary and colony cells were observed under a microscope. After 4 h of exposure to UV-B, both solitary and colony cells were complete (Figure 4K,L). However, the solitary cells lysed after UV-B radiation for 4 h (Figure 4Q). This phenomenon was not detected in the colony cells (Figure 4R). These findings suggested that under the same UV-B dose, solitary cells suffered more damage than colony cells.

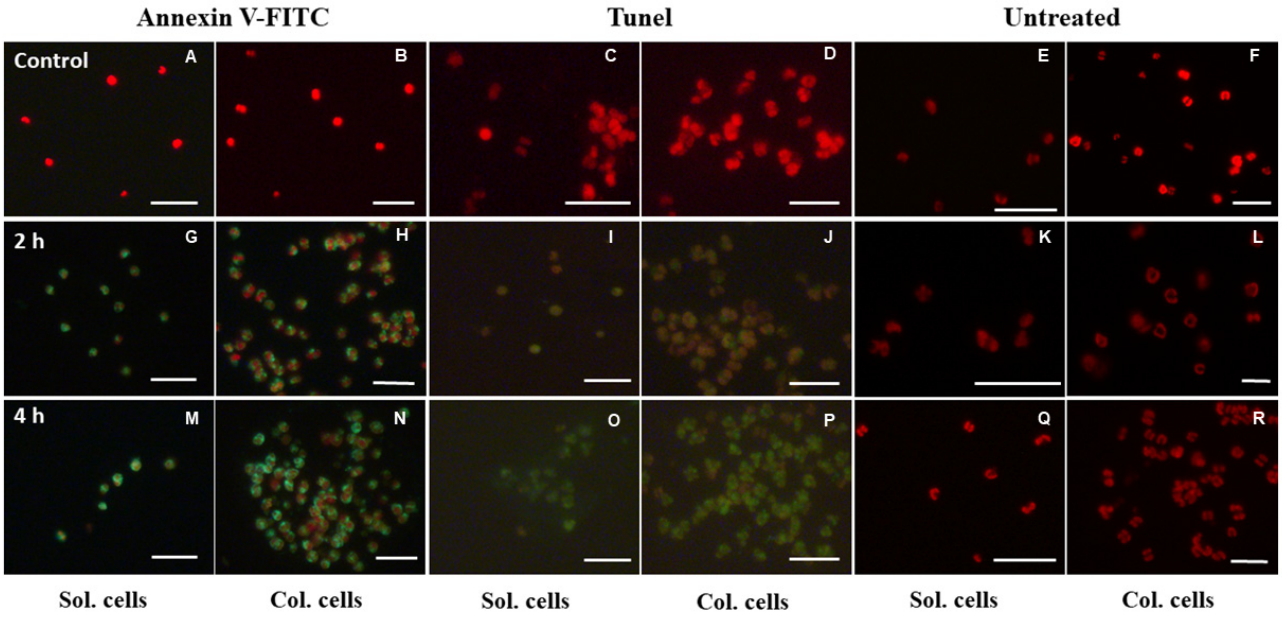

Figure 4. Effect of UV-B on the TUNEL assay of *P. globosa*'s solitary and colony cells. Note: control group (**A**–**F**); UV-B radiation at 2 h (**G**–**L**); UV-B radiation at 4 h (**M**–**R**). Red fluorescence indicates chlorophyll and green fluorescence indicates positive cells. The scale in the picture is 10 μm.

4. Discussion

4.1. Effect of UV-B on the Photosynthesis of P. globosa

One of the most basic and vital life activities in marine algae is photosynthesis [45,46]. PSII is a key component of photosynthesis and is responsible for light energy absorption and electron transfer [47,48]. UV-B radiation attacks the D1 and D2 proteins, which in turn causes PSII damage and hinders electron transfer [49,50]. Energy utilization in PSII can be quickly and effectively monitored via chlorophyll fluorescence analysis to characterize the photosynthetic activity of cells.

The Fv/Fm, Y(II) of *P. globosa* solitary and colony cells significantly declined under UV-B exposure ($p < 0.05$; Figure 1A,B), indicating that UV-B radiation reduced the Fv/Fm, Y(II) of *P. globosa* solitary and colony cells. Similarly, α and rETRmax decreased, indicating that the light capture capability and the efficiency of relative electron transfer in *P. globosa* solitary and colony cells were impaired under UV-B radiation (Figure 1C,D). According to earlier research, UV-B radiation disrupts photosynthesis in marine algae [51]. For example, the Fv/Fm and Y(II) of *Tussaudia salina* decreased dramatically when algae were radiated by UV-B [52]. Similar results were reported in *Trichodesmium erythraeum*, in which the Y(II) of the cells significantly decreased under brief exposure to UV-B [24]. The decrease in the efficiency of energy absorption, transfer, and conversion in PSII of *P. globosa* solitary and colony cells under exposure to UV-B resulted in a decrease in the potential and actual photosynthetic capacity, inhibiting photosynthetic activity.

Notably, the decrease in the Fv/Fm of *P. globosa* solitary cells was significantly greater than that in colony cells ($p < 0.01$), indicating better UV-B radiation tolerance in the colony cells. In addition, according to the damage and repair rate of PSII in *P. globosa* solitary and colony cells, colony cells presented greater UV-B radiation tolerance and greater recovery than solitary cells (Table 1). A prior study found a similar result: *Microcystis aeruginosa* solitary and colony cells radiated with UV-B radiation were allowed to recover under low light for 240 min, and under these conditions, the Fv/Fm of *M. aeruginosa* colony cells was higher compared to that of solitary cells [53]. UV-B radiation can trigger the synthesis of substances like MAAs and Car (carotenoids) that have the ability to absorb UV-B [50,54]. The polysaccharide outer membrane of the colony likely plays a protective role against UV-B radiation. A study revealed that *Phaeocystis* spp. colonies contain a variety of MAAs that can absorb UV radiation [55]. In addition, previous studies confirmed that *Phaeocystis pouchetii* solitary cells are significantly deficient in UV-absorbing substances compared with *P. pouchetii* colony cells [56]. The greater tolerance of *P. globosa* colony cells to UV-B exposure may be attributed to the UV-absorbing substances contained in the polysaccharide outer membrane.

In short, *P. globosa* solitary and colony cells' photosynthetic activity was not hampered to the same extent by UV-B radiation. Compared with solitary cells, colony cells suffered less damage to photosynthetic activity, which may have been caused by protection from the polysaccharide outer membrane.

4.2. Response of P. globosa Solitary and Colony Cells to UV-B Radiation

Our study clearly shows that PCD occurred in *P. globosa* solitary and colony cells under UV-B radiation. The typical characteristics of PCD, notably DNA fragmentation, PS externalization and caspase-3-like activity, were observed. PS is on the inner side of the cell membrane under normal conditions, which is altered during the process of PCD, when PS is dislocated to outside of the cell membrane [57,58]. During the early stages of PCD, PS externalization can be detected by staining with Annexin V-FITC. DNA fragmentation is a hallmark of late-stage PCD [59] and occurs in *P. globosa* solitary and colony cells after 2 or 4 h of UV-B radiation (Figure 4). Another important indicator of PCD is the increase of caspase-3-like activity (Figure 3). Caspase-3-like proteins were activated in *P. globosa* in reaction to UV-B radiation. This means that an intrinsic pathway was involved in the PCD process.

UV-B exposure can raise cellular levels of ROS, such as hydroxyl radicals and hydrogen peroxide radicals [60]. ROS attack different targets inside cells [61]. Generally, the overproduction of ROS in cells leads to internal damage, such as overoxidation of proteins and lipids, alterations in membrane permeability, ultimately leading to PCD [62]. In fact, ROS can function as signaling molecules during the early stages of PCD, which has been confirmed in other microalgae, such as *Thalassiosira pseudonana* and *Peridinium gatunense* [63,64]. In our study, excess ROS in *P. globosa* cells was induced by UV-B radiation (Figure 2). Excess ROS can trigger caspase-3-like activity to initiate a series of intracellular cascade reactions and subsequent degradation of intracellular proteins [65,66]. These findings suggest that UV-B exposure causes the accumulation of ROS in *P. globosa*, which subsequently stimulates an increase in capase-3-like activity and ultimately leads to PCD.

Aerobic microorganisms can regulate the level of ROS via CAT and SOD to ensure that the intracellular oxidative system is in balance [61,67]. Thus, the intracellular ROS content is likely to show the same trend as that of CAT and SOD under extrinsic environmental stress conditions. However, activities of CAT and SOD in reaction to UV-B radiation for 2, 4 h did not match the ROS content in the antioxidant system of *P. globosa* cells. After 4 h of UV-B exposure, the CAT and SOD activities of *P. globosa* colony cells were significantly lower than solitary cells ($p < 0.05$, Figure 2). Higher antioxidant enzyme activities are related to higher ROS contents within a normal antioxidant system [68]. As mentioned above, solitary cells suffer more damage from UV-B exposure due to a lack of protection from the polysaccharide outer membrane, which is thought to lead to more ROS accumulation in solitary cells than in colony cells. Our results in this study showed the opposite trend. A reasonable explanation for this finding is that, unlike colony cells, some solitary cells might become necrotic after 4 h of UV-B radiation. Cell lysis due to necrosis allows for the loss of ROS in solitary cells, leading to a lower ROS content in solitary cells than in colony cells (Figure 2A). Moreover, this finding is supported by the results shown in Figure 4. After 4 h of UV-B radiation, lysis was observed via fluorescence microscopy in solitary cells, whereas colony cells were intact. A previous study reported that *Dunaliella viridis* also experienced necrosis after UV-B exposure [36].

The main difference between colony and solitary cells is that the former are protected by a polysaccharide outer membrane, which attenuates UV-B radiation and ensures cellular integrity. In brief, PCD in colony cells is induced by ROS, with the protection of the polysaccharide outer membrane. In contrast, solitary cells exhibited PCD induced by both ROS and necrosis.

5. Conclusions

In this study, we characterized the photosynthetic capacity and PCD process of *P. globosa* colony and solitary cells exposed to UV-B radiation, and found that both colony and solitary cells had PCD characteristics. UV-B radiation causes the overproduction of ROS and activation of caspase-3-like activity. We can conservatively suggest that UV-B radiation could assist in accelerating the death of *P. globosa*. Moreover, the two cell types still showed a difference in responses to UV-B radiation. This study highlights the fact that solitary cells experienced a passive cell death - necrosis compared to colony cells. The colony cells of *P. globosa* are considered to be an evolutionary way to actively accommodate changes in the natural environment. Photosynthetic activity confirms that colony cells have a better recovery ability and are more adaptable to UV-B radiation than solitary cells. It can be speculated that when *P. globosa* is floating on the sea surface, the colony cells defend against UV-B radiation due to the protection of the polysaccharide outer membrane. Colony cells have better growth advantages. In contrast, solitary cells are subjected to more stress from UV-B radiation. This study reveals the lethal mechanism of UV-B radiation on *P. globosa*, which provides a basis for further understanding of the interaction on algae by UV radiation.

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project administration, J.L. (Junxiang Lai); funding acquisition, J.L. (Junxiang Lai). All authors have read and agreed to the published version of the manuscript.

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