



## Survival of desert algae *Chlorella* exposed to Mars-like near space environment



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### ABSTRACT

Desert was considered terrestrial analogues of Mars. In this study, dried cells of desert green algae *Chlorella* were exposed to Mars-like near-space environment using high-altitude scientific balloons. We found that while a majority of *Chlorella* cells survived, they exhibited considerable damage, such as low photosynthetic activity, reduced cell growth, increased cell mortality rate, and altered chloroplast and mitochondrial ultrastructure. Additionally, transcriptome analysis of near space-exposed *Chlorella* cells revealed 3292 differentially expressed genes compared to cells in the control ground group, including heat shock proteins, antioxidant enzymes, DNA repair systems, as well as proteins related to the PSII apparatus and ribosomes. These data shed light on the possible survival strategy of desert algae to near space environments. Our results indicated that Mars-like near space conditions represent an extreme environment for desert algae in terms of temperature, pressure, and radiations. The survival strategy of *Chlorella* in response to near space will help gain insights into the possibility of extremophile colonization on the surface of Mars and in similar extraterrestrial habitats.

### 1. Introduction

Exploration of Mars and utilization of its resources is possible owing to the advances in space science and technology in recent years, and has immense strategic significance and scientific implications in future missions. Mars is generally regarded as the most Earth-like planet in the solar system. High cost and inadequate technology impede the exploration of this planet. The near space environment is similar to the environment present on the surface of Mars, especially with respect to rarified air, high ultraviolet (UV) radiation, sub-zero temperatures, and extreme desiccation (Smith, 2013; Schuerger, 2016). The pressure of the thin and dry stratospheric air at 25–38 km above sea level is roughly equivalent to the pressure on the surface of Mars (0.5–1 kPa) (Khodadad et al., 2017). Relative humidity levels of stratosphere at 36 km above sea level can drop below 10%, and temperatures varies from –60 °C to 0 °C (Smith, 2017), which are similar to the environment on the surface of Mars. Most UV values of stratosphere came from model estimates (Khodadad et al., 2017; Smith et al., 2011), researchers predict the UVB and UVC intensity in the stratosphere between 20 km and 50 km is about 10 and 3 W/m<sup>2</sup>, which is similar to the UVB and UVC intensity in Mars as calculated by Schuerger et al., (2003). Therefore, the near space is

perfect place to simulate Mars environment for studying space biology related to Mars exploration. NASA conducted a study called Exposing Microorganisms in the Stratosphere (E-MIST) to measure survival and DNA damage of stratosphere-exposed biological samples in a high-altitude balloon (Khodadad et al., 2017). European Space Agency (ESA) also launched the Biology and Mars Experiment (BIOMEX) space mission (Biology and Mars Experiment) to investigate the resistance and stability of biomolecules in space and Mars, and to explore the limits of tolerance of terrestrial extremophiles in space (de Vera et al., 2012). DasSarma et al. (2016) assessed the survival of halophilic Archaea in Earth's cold stratosphere by genome-wide transcriptome analysis. However, there is a lack of multi-level systematic studies on physiology and gene expression of organisms exposed to Mars-like environment, thereby elucidating the mechanisms of survival strategies.

Considering technology and cost to conduct biological experiments, high-altitude balloon became the best means of carrying samples to Mars-like environment, near space's environment of Earth's stratosphere. Several biological discoveries can be attributed to studies involving high-altitude balloons, such as the presence of viable cells were detected and collected from the stratosphere (Narlikar et al., 2003; Corte et al., 2014; Griffin, 2004; Deleon-Rodriguez et al., 2013). Part of

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the high-altitude balloons payload usually consists of a small insulated container housing biology experiment. The scientists have sent human cells (Galleri et al., 2003; Wiese et al., 1988), seeds (Li et al., 1997; Jin guo et al., 1999; Beck-Winchatz and Bramble, 2014), yeasts (Pulschen et al., 2018), and bacterial spores (Smith et al., 2014) into near space for astrobiology. Extremophiles, especially desert algae which thriving in hostile environments considered as planetary field analogues for Mars can provide clues for searching exoplanets and the limits of life (Martins et al., 2017). Hence, understanding of their degradation and adaption under different extraterrestrial conditions especially simulated space environment is a key feature for future missions to recognize life beyond Earth (Gómez and Parro, 2012). In recent years, desert cyanobacteria *Chroococcidiopsis* (Billi et al., 2011; Baque et al., 2014) have reported carried out astrobiology experiments, but few about desert green algae. As one of the most famous examples of green eukaryotic microalgae, *Chlorella* is able to adapt to a range of extreme and harsh conditions (Rath, 2012). Studies have shown that *Chlorella* is able to resist intense ultraviolet (UV) radiation and gamma rays (Malanga and Puntarulo, 2010; Cheng et al., 2013). *Chlorella* sp. isolated from desert sand crusts has shown resistance to excessive light intensive (Treves et al., 2013), which is associated with the unique features of photosystem II function (Treves et al., 2020). *Chlorella ohadii* can minimize the formation of singlet oxygen ( $^1\text{O}_2$ ) and avoid oxidative damage (Treves et al., 2016; Ananyev et al., 2017). Besides, *Chlorella* also has the characteristics of fast growth and high photosynthetic efficiency. The study of response of *Chlorella* with strong radiation tolerance to near space environment is of great significance for future deep space exploration and Mars colonization. In this experiment, we selected a *Chlorella* sp. isolated from Tengger Desert (China), which region is characterized by dryness, high radiation, high temperature and large temperature shift. Extreme habitat is expected to give *Chlorella* higher resistance to the environment in near space.

## 2. Material and methods

### 2.1. Algal strains, culture conditions and sample preparation

*Chlorella* sp. was obtained from FACHB collection (Freshwater Algae Culture Collection of Institute of Hydrobiology, The Chinese Academy of Sciences), which was originally isolated from by Dr. Haijian Yang from the desert crust of the Tengger Desert, Ningxia Hui Autonomous Region of China. *Chlorella* sp. was grown under routine conditions at 25 °C with BG11 medium. Sample preparation refers to the method of Billi et al., (2019a), Billi et al., (2019b). Cell pellets obtained from cultures in their exponential growth phase were resuspended at a density of approximately  $10^9$  cells/mL, and 200  $\mu\text{L}$  of the cell suspension was plated onto 1.5% (w/v) BG11 agar medium in Petri dishes placed in the sample boxes. Next, cells were air-dried under a laminar flow hood for 24 h in sterile and dark conditions and transported to flight site for flight experiments. After the flight test, each treatment group was divided into 4 equal parts and eluted with BG11 culture medium respectively. Three of them were removed immediately after elution, and then fixed with liquid nitrogen and sent to the transcriptome for sequencing. The remaining one was divided into 3 subparts for related physiological parameters tests.

### 2.2. Exposure to near space and ground references

Biological Exposure payload was launched on a high-altitude scientific balloon. Four treatment groups were set up in the experiment, namely, flight light group (FL group), flight dark group (FD group), ground light group (GL group), and ground dark group (GD group). Fresh *Chlorella* that was not dried was referred to as the CK (control check) group. GD group was used as the control group for the flight test. Due to the precious location of the biological payload placed sample box, the flight group had only one sample per treatment group. Main

material of the sample box is polycarbonate, which air leakage rate  $Q \leq 10^{-4} \text{ Pam}^3/\text{s}$  at 0.05 atmospheric pressure. The sample boxes (Fig. 1A) of light groups were equipped with a fused silica glass (JGS2) window, which could pass through 93% ultraviolet radiation, while the sample boxes of dark groups possessed a black aluminum cover (Fig. 1B) which prevented transmittance of UV radiation. Mixture of *Chlorella* sp. and agar were inoculated on the substrate.

The balloon was launched from the Wulate airport (Inner Mongolia, China). Environmental data during the flight were recorded from sensors and radiation intensity was detected by a radiochromic film detector (MD-55-2 Gafchromice film; International Specialty Products, Wayne, NJ). The balloon travelled until an altitude of 31 km above sea level and maintained a steady altitude. After that, the cover of Biological Exposure payload was opened (Supplementary file, Fig. S1) and the sample exposure experiment was conducted for about 3 h. Meanwhile, corresponding ground reference experiments were carried out and environmental parameters at the launch site were monitored in real time. All samples were stored immediately in a dark icebox (3–6 °C) after the flight, and transported to Wuhan laboratory within 12 h for further analysis.

### 2.3. Measurement of photosynthetic activity

The photosynthetic quantum yield ( $F_v/F_m$ ) and chlorophyll a fluorescence kinetics (OJIP curve) were determined as a measure of the photosynthetic activity of *Chlorella*. The algal cell suspensions were prepared immediately after the flight with BG11 medium, thereafter  $F_v/F_m$  and OJIP curve were measured at 25 °C using Handy PEA (Hansatech Instruments, Norfolk, UK).

### 2.4. Cell population density measurement

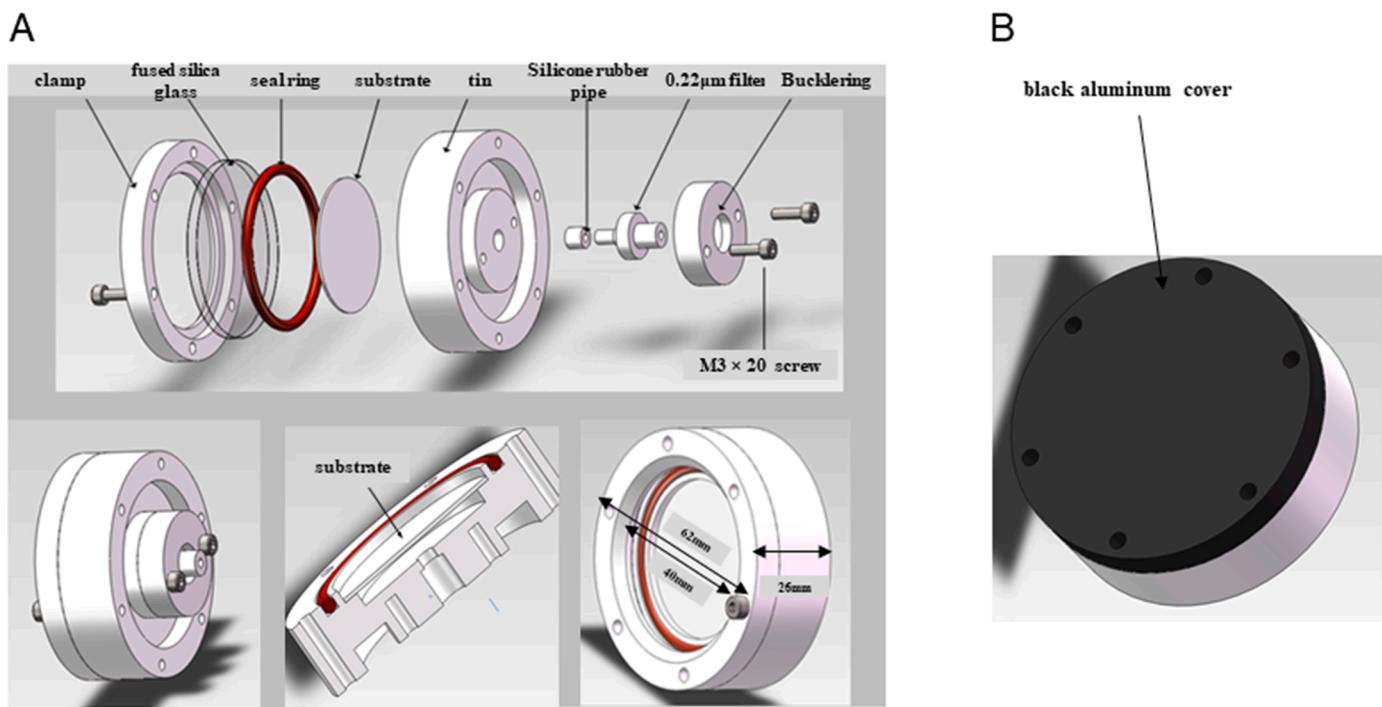
After flight, we eluted the samples of each treatment and then cultured with BG11 medium under low light ( $15 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). Every few days, 1 mL of algal fluid was taken out under sterile conditions and the changes in cell density were observed. The cell density, a parameter of growth, was measured by counting cells with a blood cell counting chamber (0.1 mm, 1/400 mm<sup>2</sup>), under an ordinary optical microscope.

### 2.5. Cell mortality

Flow cytometry with a single 488-nm argon laser was used to measure the cell mortality rate after staining with vital dyes. A flow cytometer (FACS AriaIII, BD, USA) coupled to a propidium iodide (PI) dye (BS078A; Biosharp, China) was used to determine the cell mortality. PI is a non-fluorescent nuclear DNA staining reagent that is often used for cell apoptosis detection. When it combines with DNA, it can emit a 615 nm red light under a 535 nm wavelength excitation light. It can pass through damaged cell membranes and combine with nuclear DNA, but cannot penetrate the membranes of living cells.

### 2.6. Transmission electron microscopy

Cells were harvested by centrifugation at  $10,000 \times g$  for 3 min and washed twice with phosphate buffered saline (PBS; pH = 7.4). Cell pellet was fixed with 2.5% glutaraldehyde for 12 h, and subsequently the supernatant was removed by centrifugation and cell were washed several times with PBS. Osmium tetroxide (1%) was added and cells were fixed for 2 h, and washed four times with PBS. Then, cells were dehydrated in a gradient ethanol series, infiltrated, embedded in the Spurr resin, and hardened at 37 °C, 45 °C, and 65 °C, respectively for 24 h. The sections were sliced at 50–70 nm and mounted on a copper grid, and stained with 2% uranyl acetate. A Hitachi HT7700 transmission electron microscope (Tokyo, Japan) was used for observation and photography.

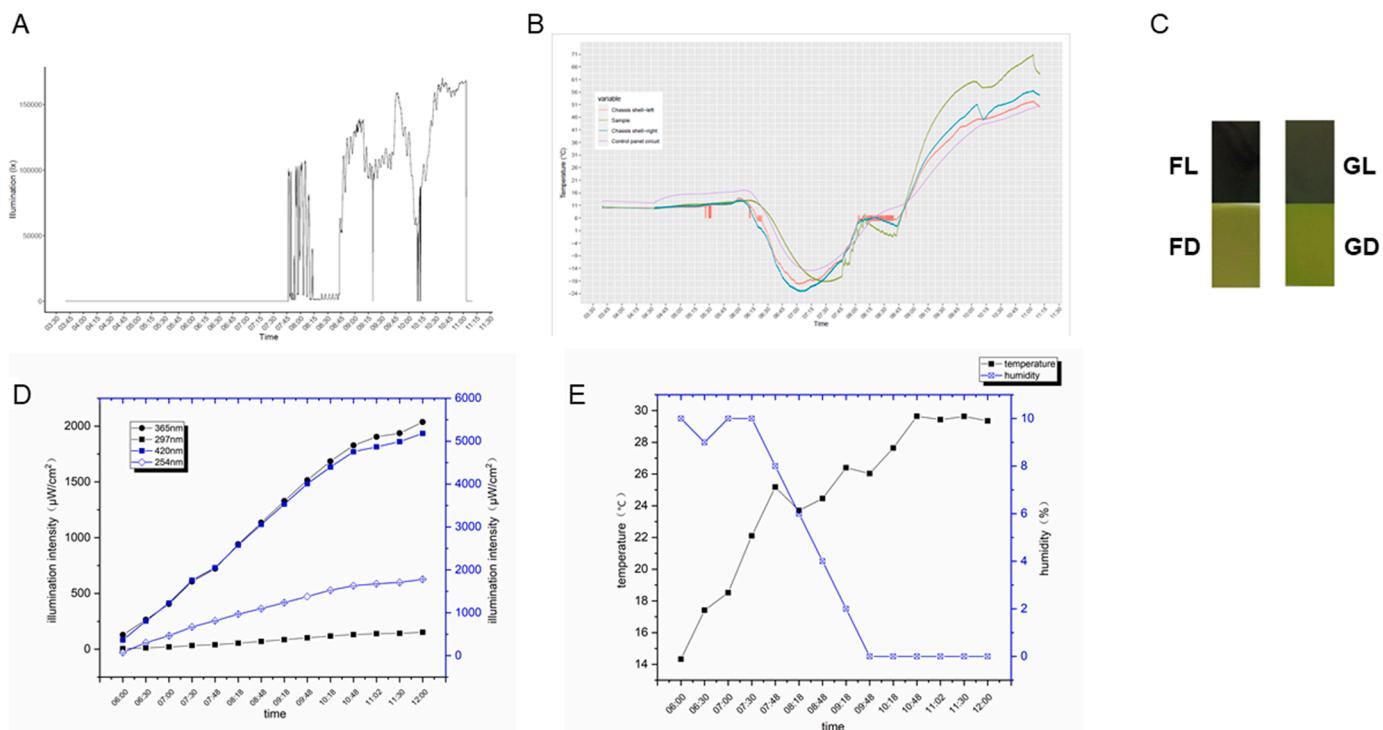


**Fig. 1.** Schematic diagram of the sample box structure. (A) light group; (B) dark group.

### 2.7. Transcriptome sequencing

Transcriptome analysis was carried out in four groups (GD, FL, FD, GL) samples. Total RNA of *Chlorella* was extracted using TRIzol® Reagent according the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA) and genomic DNA was removed using DNase I (TaKara). RNA quality was assessed using a 2100 Expert Bioanalyzer (Agilent). Library

construction and sequencing were performed using Illumina Novaseq 6000 of Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China). To visualize the transcriptional abundance of DEGs, heatmaps were generated using TBtools (Chen et al., 2020), and other plots were generated by using the free online platform Majorbio Cloud Platform ([www.majorbio.com](http://www.majorbio.com)).



**Fig. 2.** Changes of environmental parameters during near-space flight. (A) Flight illumination intensity; (B) Flight temperature; (C) Radiation intensity of each groups; (D) Ground illumination intensity; (E) Ground temperature and humidity.

## 2.8. Statistical analysis and experimental repeats

Data were evaluated using one-way analysis of variance and Tukey's honest significant test with the help of SPSS 13.0 software. Three technical repeats or three biological repeats were performed for each experiment.

## 3. Results

### 3.1. Flight and ground control environmental parameters

The flight illuminance was kept at a stable state except when the cover was opened and closed (Fig. 2A). Due to uncontrollable factors, the flight samples experienced variable temperature ranging from  $-19^{\circ}\text{C}$  to  $71^{\circ}\text{C}$  (Fig. 2B). The color of radiation detection film of FL group and GL group both changed (Fig. 2C). The ground illumination increased over time (Fig. 2D) with the temperature varying from  $14^{\circ}\text{C}$  to  $29^{\circ}\text{C}$  and air humidity lower than 10% (Fig. 2E). A comparison of environmental parameters on the Martian surface, during the flight, and on the ground is shown in Table S1.

### 3.2. Near space environment increased cell mortality rate and inhibited algal growth

In order to evaluate the effect of near space environment on cell survival, we measured the cell mortality rate of each treatment groups, and cultured the samples after flight experiment to observe the change of cell density. Our results showed near space environment increased cell mortality rate and inhibited cell growth of *Chlorella* sp.. Flow cytometry analysis showed that FL, GL, and FD groups showed increased cell mortality rate compared GD group (Fig. 3A). Cell density of FL group consistently lower than other groups during recovery culture. The FD and GL groups displayed lower cell density than the GD group in the early stages of growth recovery and did not show any significant difference after the ninth day (Fig. 3B).

### 3.3. The near space environment affected the photosynthetic system of *Chlorella* sp

$Fv/Fm$  represents maximum photochemical yield of PSII, which value is not varying under normal conditions, but decreases under stress

as it reflects a reduction of PSII activity (Ranglova et al., 2019). The  $Fv/Fm$  of FL group samples reduced approximately 36.8% compared to GD samples (Fig. 4A). The chlorophyll a fluorescence (Fig. 4B) showed the same trend as  $Fv/Fm$ , with FL group displaying lowest fluorescence, followed by GL group. Although the photosynthetic activity of the FD group was lower than GD group, there was no significant difference in photosynthetic activity between the FD and GD groups. These data indicate that radiation emanating both from sunlight on the ground and near space damaged the photosynthetic system of *Chlorella*, but other factors in the near space environment have less impact.

### 3.4. Alteration in structure of chloroplast and mitochondria upon exposure to solar irradiation and near space environment

Ultrastructural study helps to evaluate the effect of near space environment on cell structures. Transmission electron micrographs (Fig. 5) showed that the chloroplast thylakoids of *Chlorella* of samples in CK group and GD group are arranged like a comb, whereas FL group, FD group and GL group chloroplast thylakoids appear abnormal. An unusual swollen morphology of mitochondria was observed in the cells of FL group, but not in the other groups.

### 3.5. Transcriptome analysis of desert algae *Chlorella* sp. upon exposure to near space environment

Transcriptome sequencing, also known as RNA-seq, which quickly and comprehensively obtain the transcription status of biological samples at a specific time by using high-throughput sequencing technology. The transcriptome sequencing of the samples from each treatment group is helpful for us to understand the influence of near space environment on *Chlorella* at the gene level. Assembly assessment of sequencing data was provided in supplementary materials (Supplementary file, table S2, S3, S4). PCA analysis showed a good correlation between the repeated samples in each group (Supplementary file, Fig. S2a, 2b). Differentially expressed genes (DEGs) in the FL, FD, and GL groups included a majority of upregulated genes. There were 3292 DEGs in the FL group. To further understand the function of DEGs, Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed. Upregulated and downregulated DEGs in FL group were classified into three GO categories as shown in Fig. S3. The Venn diagram in Fig. 6A showed that 2487 genes were specific to the FL group

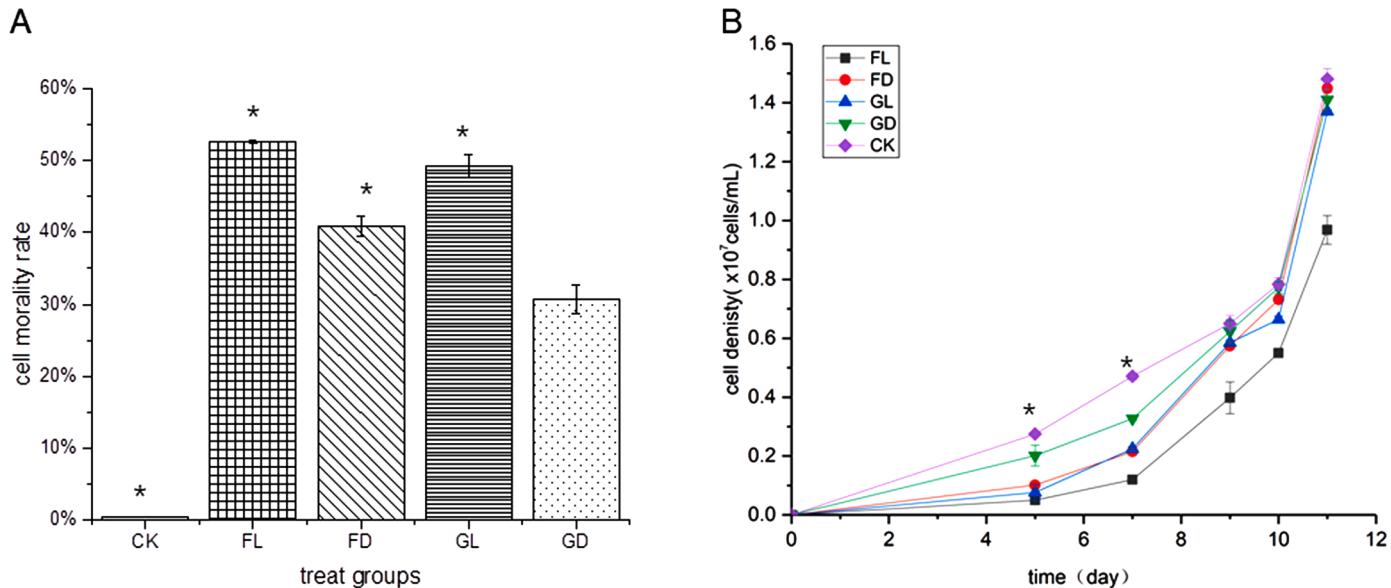
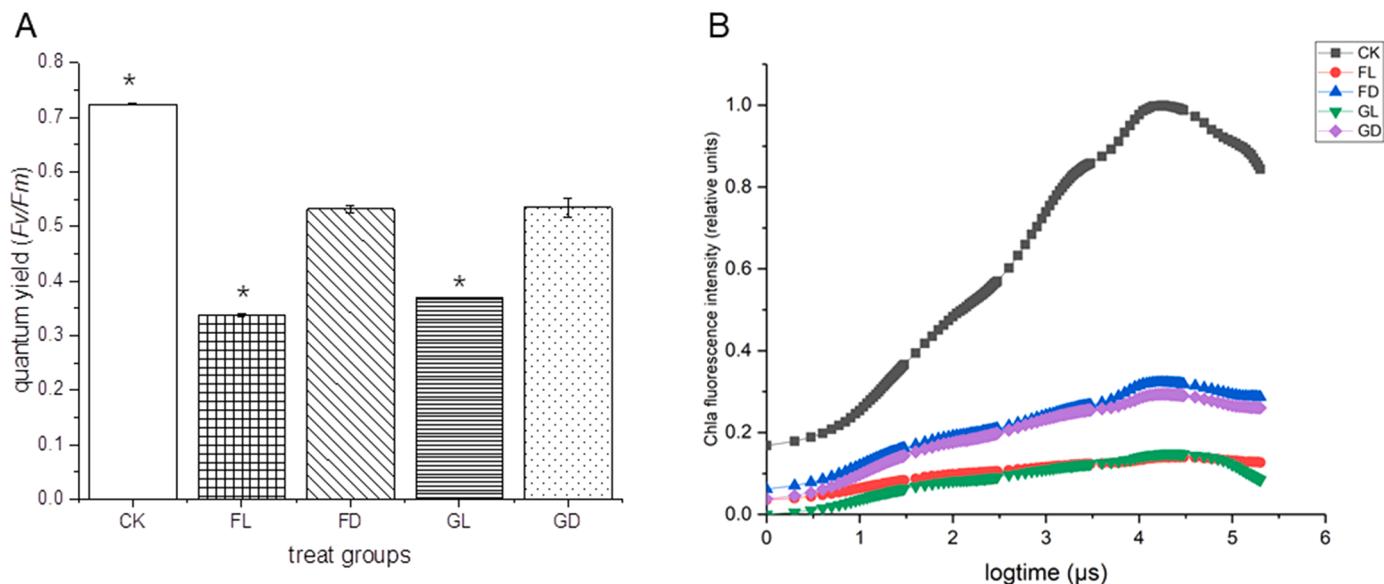
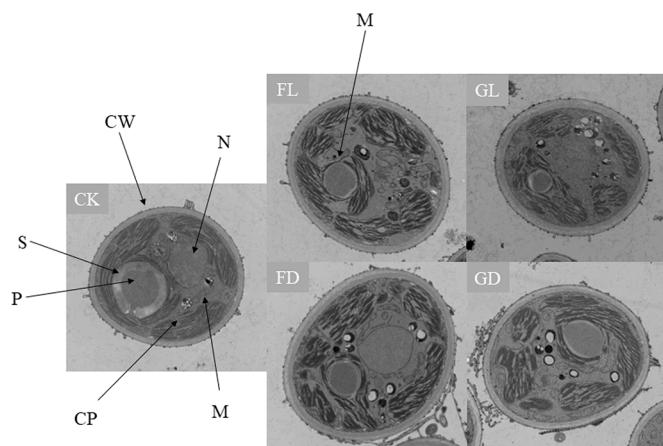


Fig. 3. The growth of *Chlorella* sp. in each treat group. (A) cell mortality rate; (B) cell density. \* Indicates that differences between the other treat groups and the GD group were significant at  $P < 0.05$ .



**Fig. 4.** Photosynthetic system parameters of *Chlorella* sp. in each treat group. (A) quantum yield ( $F_v/F_m$ , B): Chla fluorescence intensity. \* Indicates that differences between the other treat groups and the GD group were significant at  $P < 0.05$ .



**Fig. 5.** The ultrastructure of *Chlorella* sp. in each treat group. P: pyrenoid; S: starch sheath; CP: chloroplast; M: mitochondria; N: nucleus; CW: cell wall.

(not FD group) suggesting their importance in solar radiation exposure in near space. A total of 805 DEGs showed overlap in FD group and FL group which are related to non-radiation factors in near space environment such as temperature. Furthermore, we also analyzed DEGs of FL group related to antioxidant enzymes, DNA repair, photosynthesis, ribosome proteins and heat shock proteins (Fig. 6B). As visualized in the heat map, most transcripts of related this were upregulated in the FL group.

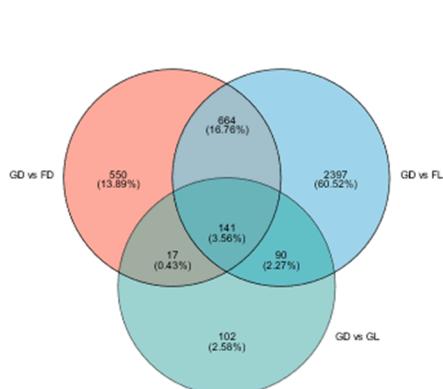
#### 4. Discussion

It is essential to elucidate the survival strategy of organisms exposed to Mars-like extreme environments in order to study the possibility of microbial colonization on the surface of Mars. With the support of HH-19-2 flight mission of SENES in 2019, we investigated the survival of desert green algae under Mars-like near space environment. We found that a majority of *Chlorella* cells survived following the near space flight, however, significant physiological damage was noted in the cells. These results indicated that desert algae *Chlorella* sp. has the ability to survive under Mars-like environment and potentially colonize the surface of Mars.

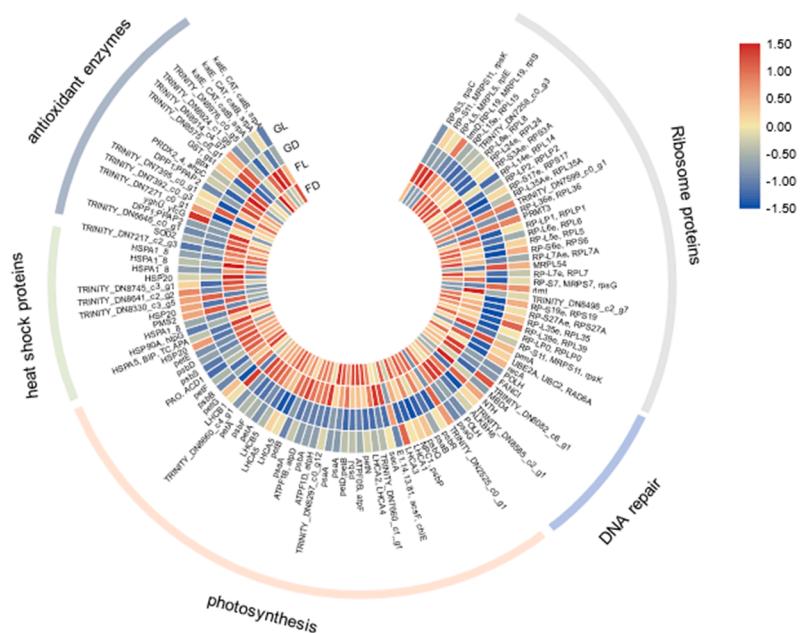
As Mars-like near space environment contains complex environment factors, including high ultraviolet light levels, low pressure, rarified air, sub-zero temperatures, and extreme dryness, it is necessary to identify the predominant determinant affecting organisms. Data from the radiochromic film detector indicated that samples of FL group experienced stronger radiation than the ground control group. Subsequent physiological tests also revealed more damage to the FL group. Hence, UV irradiation may be the major determinant for growth in Mars-like near space environment, these results echoed a previous study (Horneck et al., 2010). Therefore, cellular response and adaptation to radiation is considered an important aspect of microbial endurance.

In this study, we found that  $F_v/F_m$  and chlorophyll a fluorescence in FL group were the lowest compared with GD group, followed by GL group, and there was no significant difference between FD group and GD group.  $F_v/F_m$  reflects the PS II reaction center intrinsic light energy conversion efficiency. The chlorophyll a fluorescence kinetics uncovers the structure and function changes of photosynthetic organs under different environmental conditions (Hermans et al., 2003; Heerden et al., 2004). Our data revealed UV damage to the PSII of *Chlorella* and impact structure and function of photosynthetic organs, while other environmental factors in near space have less effect on the photosynthetic system of *Chlorella* cells. Some reports showed UVB decreased chlorophyll content, disrupted photosynthesis systems, and inhibited growth (Ma et al., 2012), along with degradation of D1 protein and D2 protein, which in turn led to a decline in PS II function (Frisoet al., 1994; Lidon et al., 2012). From our genome-wide transcriptome analysis, upregulation of photosynthesis-related genes in the FL group and GL group was observed, indicating a strategy for the organism to cope with radiation and accelerate the repair of photosynthetic apparatus. PsbA gene encode D1 protein involved in PSII repair (Segovia et al., 2015). Park et al. reported upregulation of photoprotection and PSII -repair genes in the unicellular green algae *Dunaliella salina* under irradiance (Park et al., 2006). Altogether, psb genes, photosystem II oxygen-evolving complex protein, light harvesting complex I protein, and chlorophyll a/b-binding proteins have roles in photosynthetic system repair in *Chlorella*, as a response to near space environment. Solar UV radiation is a deterrent for growth. Growth of FL group samples upon rehydration was significantly decreased during culture recovery period. *Staphylococcus aureus* also showed intense growth inhibition in response to UV from the stratosphere (Dagmar et al., 2015). The ultrastructure of chloroplasts and mitochondria of the FL group samples were

A



B



**Fig. 6.** Differentially expressed genes (DEGs) and expression levels in different groups. (A) Venn diagram of DEGs; (B) Heatmaps of DEGs.

significantly altered, which may be attributed to UVB exposure. Mitochondria of *Dunaliella salina* have also been observed to become irregular and bigger after UVB irradiation, which will further encumber photosynthetic autotrophy mode (Tian and Yu, 2009).

DNA is one of the most UV-sensitive biomolecules (Karsten and Holzinger, 2014). Besides, UV radiation also causes oxidative stress through the formation of reactive oxygen species (ROS), which affect many cellular functions by damaging nucleic acids, oxidizing protein, and causing lipid peroxidation (Foyer and Noctor, 2005). Effective ROS elimination by antioxidative enzymes, DNA repair by photolyase, and de-novo biosynthesis of damaged proteins are well documented protective mechanisms (Bischof et al., 2006). In the present study, we observed an upregulation of antioxidant enzymes in the flight exposure group. In addition, we also observed a significant upregulation of 9 DNA repair transcripts in the flight exposure group such RECA and ALKB. Homologous recombination repair mediated by recA is the most typical DNA damage repair system (Rowan et al., 2010). DNA damage can induce levels of chloroplast-targeted recA-mRNA in *Chlamydomonas reinhardtii* (Nakazato et al., 2003). ALKB and its homologs are widely found in bacteria, metazoans, plants and viruses, catalyzing oxidative demethylation reactions (Müller and Hausinger, 2015). Ribosome proteins have recently been reported to be involved in UV stress. A large number of ribosomal protein genes were upregulated in FL group and GL group. *RPL10* genes are involved in development and translation under UV-B stress (falcone ferreyra et al., 2010). Ribosomal protein S3 is known to be a multifunctional protein involved in DNA repair (Kim et al., 2009), metastasis (Kim and Kim, 2006), and apoptosis (Jang et al., 2004; Kim et al., 2006). In this study, we found 33 transcripts with significantly differentially expressed ribosomal proteins in the FL group, of which 20 were shared with the GL group, and we speculated that these genes were involved in the stress response to UV radiation.

In addition to radiation, temperature and pressure also have an impact on biology. Temperature in the near space is variable, ranging from approximately  $-70^{\circ}\text{C}$  to  $0^{\circ}\text{C}$  (Jacob, 1999; Wallace and Hobbs, 2006). Prior to the experiment, we concluded that low temperature had negligible effect on *Chlorella*, and it could survive at a low temperature of  $-60^{\circ}\text{C}$  for 12 h (supplementary file, Fig. S4). Due to some uncontrollable factors, the sample temperature shifted from  $-60^{\circ}\text{C}$  to  $60^{\circ}\text{C}$  in this flight mission. From the transcriptome data, a large number of genes were up-regulated in the flight group compared with the GD group,

which may be due to the effect of temperature. Ribosomal protein L5 was significantly differentially expressed in the flight group, which may be related to the sub-zero temperature in the near space environment. Plastid ribosomal protein S5 is involved in cold stress tolerance in *Arabidopsis* (Zhang et al., 2016). The production of heat shock protein has been shown to be positively correlated with the organism's tolerance (Joshi et al., 1997; Downs et al., 1998; Preczewski et al., 2000). We found that 11 HSP transcripts in the FL group and 6 transcripts in the FD group were significantly upregulated, indicating that cells experience elevated stress in the near space environment and *Chlorella* was able to adapt to this stress. HSP90 was upregulated in flight group, conceivably attributed to the high temperature. HSP90 is involved in adaptation to heat stress in plants (Samakovli et al., 2020). The persistent high temperatures in subsequent supplementary experiments did have an effect on *Chlorella* cells (supplementary file, Fig. S5), which provided lessons for subsequent flight experiments. Low pressure in space is additional factor to be considered (Billi et al., 2013). Short-term low pressure was proved to have little influence on organisms. In the BOMEX experiment, genomic DNA damage was not identified by PCR in cells after 1 h exposure to space vacuum and Mars atmosphere (Baqué et al., 2013). Our data also showed that 4 h exposure to low pressure has little effect on photosynthesis activity and cell membrane integrity of *Chlorella* (supplementary file, Fig. S4).

We discussed the possible response mechanisms of *Chlorella* to the near space environment in this study, however, there are still some shortcomings worth considering. First, due to the complexity of the near space environment, some results need further validation such as increased the flight exposure time and conducted additional balloon flight. Furthermore, we found that the sample containers heated up during the flight, therefore we must strictly maintain the environmental parameters within the Biological Exposing payload in forthcoming experiments. Last but not least, the sample was not fixed immediately after the flight due to limited technology and there may be recovery in the processing. However, in terms of the measured parameters, there were significant differences among the treatment groups.

## 5. Conclusions

In summary, our experiments showed that desert *Chlorella* sp. can survive short-term exposure to simulated Mars-like near space

environment. *Chlorella* sp. displayed slow growth, inhibition of photosynthesis and PSII apparatus, damaged cell membrane, and altered mitochondrial and chloroplast structures after exposure to near space environment. *Chlorella* also exhibited increased expression of heat shock proteins and ribosomal proteins in order to resist stress. In addition, photoprotection, PSII and DNA repair were also upregulated as strategies to deal with stress in the near space environment. The study of survival strategies of extremophiles in near space is not only beneficial for utilization of near space resources, but also provides new ideas for colonization and reconstruction of Mars.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.lssr.2021.02.003](https://doi.org/10.1016/j.lssr.2021.02.003).

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