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The resistance to UV-B radiation in desert microalgae was used to assess potential candidate for inoculating on desertified soils

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Abstract

The adaption of microalgal species to intense UV-B radiation is important for them to survive harsh environments in desert region after inoculating onto unconsolidated sands. In this study, the different responses of photosynthesis, electron transport rate (ETR), reactive oxygen species (ROS) generation and DNA strand breaks to UV-B radiation of four microalgae isolated from biological soil crusts (BSCs) were investigated. It found that less dose of UV-B could easily inhibit photosynthetic activity and induce serious DNA damage of *Chlorella vulgaris*. *Microcoleus vaginatus* only tolerated moderate dose of UV-B by its moderate tolerance and repairing ability, while *Nostoc* could endure high dose of UV-B by its less level of reactive oxygen species (ROS) generation and DNA damage, high UV absorption, strong tolerance and repairing ability in PSII and DNA damage. However, *Scytonema javanicum* owned different strategies to survive UV-B irradiance, which included the closure of PSII when ROS generation increased rapidly, high repairing ability in PSII and DNA damage. The results suggested that these microalgae had different resistance and mechanism in response to UV-B irradiance. Based on above advantages, *Nostoc* sp. is assumed to a preferred species for the primary colonization in desertified areas to form artificial algal crusts.

Keywords UV-B radiation • Biological soil crusts • Photosynthetic efficiency • Tolerance • Repairing ability

Introduction

Biological soil crusts are the notable features in the arid and semiarid regions (Belnap et al. 2001). Generally, these crusts are formed by cyanobacteria, algae, bacteria, moss, lichen, fungi and the matrix of extracellular polymeric substances (EPS) and soil particles (Johansen 1993). Many studies have revealed that those desert microalgae do essential functions in primary colonization and succession, and stabilization of desert soil surface in BSCs (Painter 1993; Mazor et al. 1996; Hu et al. 2002) and for water holding to the residing microbial community (Rossi et al. 2012). Microalgae in BSCs can also effectively accelerate N and C cycles by fixation of nitrogen and increase biomass to desert soil (Acea et al. 2003; Hawkes 2003). Some works have suggested that well-developed BSCs contain more green algae and display stratifications in the vertical structure (Bowker et al. 2002; Hu et al. 2003; Wang et al., 2009), where microalgae species with higher concentrations of UV-shielding pigmentations in the upper layers can effectively protect species with higher chlorophyll *a* pigments in the lower portions (Bowker et al. 2002; Quesada et al. 1999).

Terrestrial microalgae growing in desert regions often subject to intense UV-B irradiance (Chen et al. 2009). The biologically effective doses of UV-B radiation can penetrate deep into surface soil and caused ROS generation in autotrophic organisms and thus may affect species composition and vertical distribution in BSCs (Bowker et al. 2002; Hu et al. 2003). These increased ROS can easily destroy proteins, DNA and other biological molecules, and subsequently affect growth and reproduction, survival,

photosynthetic energy harvesting enzymes, and the content of photosynthetic pigments (He and Häder 2002; Apel and Hirt 2004; Holzinger and Lütz 2006). However, microalgae have also developed adaptation strategies to counteract the damaging effects of UV irradiance, which include avoidance, scavenging, screening, repair and programmed cell death (Quesada et al. 1999; Chen et al. 2009; Singh et al. 2010; Ma et al. 2012), or even the genes are involved in photosynthesis tolerance (Starkenburg et al. 2011). Desert microalgae distribute in the BSCs within a depth of 5 mm (Hu et al. 2003), where the biologically effective doses of UV-B radiation can reach easily and affect metabolic activity of them.

Desertification is a serious environmental problem all over the world, especially in China where it has occupied 18.9% of land mass (Yang et al. 2004). Since cyanobacterial inoculation would be a suitable and effective technique to recover BSCs and restore the desert ecological system in desertified areas, more and more attentions have been paid to it (Chen et al. 2006; Navarro-González and Rainey 2003; Wang et al. 2009). However, the survival rate of inoculums is lower (Chen et al. 2006). The aims of this investigation were to resolve these issues via comparing the different response of photosynthetic activity, ROS generation and DNA damage between these crusted microalgae, so as to reveal the surviving mechanisms of them to UV-B radiation and choose the best candidate for inoculating onto desertified soil. Three cyanobacteria, *Microcoleus vaginatus*, *Nostoc* sp., *Scytonema javanicum* and one green alga, *Chlorella vulgaris* growing in BSCs were used.

Materials and methods

Microcoleus vaginatus Gom., *Nostoc* sp., *Scytonema javanicum* and *Chlorella vulgaris* were isolated from the biological soil crusts of Shapotou, Zhongwei County, Ningxia Autonomous Region, China (37°27′N, 104°57′E). Inoculums were grown in BG-11 medium, at 25±1°C and illuminated with cool white fluorescent light at 50 $\mu Em^{-2}s^{-1}$.

Radiation sources and irradiated samples

Exponentially growing cultures ($2\text{-}5\times10^4$ cells/ml) were placed in Petri dishes. The cultures growing in Petri dishes with quartz lids were placed on a magnetic shaker to reduce cell aggregation and sedimentation, and then treated with UV-B radiation using a Spectronics Ultraviolet-BLE-1T158 tube (New York, USA) with the main output at 312 nm and a cellulose acetate filter to screen out UV-C, and measured with a spectroradiometer (Spectronics, XF-1000, BLE-6T312, USA). The cultures were irradiated for 0, 10, 30, 60, 120 or 240 min with different levels of UV-B by adjusting the distance of the lamp or recovered for 16 h after removed UV-B away.

Phyto-PAM fluorometry of photosynthetic efficiency and electron transport rate (ETRmax)

In vivo chlorophyll *a* fluorescence and the maximum of electron transport rate (ETRmax) of the cultures of these microalgae were measured with a Phyto-PAM fluorometer (Walz, Germany). Cells were dark-adapted for at least 15 min before measuring the fluorescence parameter *Fv/Fm* (PSII efficiency). The excitation light intensity was about 1500 $\mu E\ m^{-2}s^{-1}$ and the recording time was 5 s. The ratio of variable fluorescence and maximum fluorescence (*Fv/Fm*) of the dark-adapted

chlorophyll a fluorescence parameters indicates the extent and functional consequences of changes in the maximum photochemical efficiency of photosystem II (PSII) reaction centers. The different levels of light intensity were given by this machine itself.

Absorbance spectrum

Cultures were harvested by centrifugation (10,000 g, 10 min). The pellets were oven dried (50°C, 8 h) and then ground with 100% acetone in the mortar. The solution was placed overnight at 4°C in the darkness. Before experiment, the solutions were centrifuged at 10,000 g for 10 min. The supernants were used for analyzing absorbance spectrum with a UV-visible spectrophotometer (Pharmacia, 2000) according to Garcia-Pichel and Castenholz (1991).

The detection of reactive oxygen species (ROS) using DCFH-DA

ROS was analyzed by using the DCFH-DA method according to He and Häder (2002). DCFH-DA was immediately added to the irradiated cultures (final concentration 5 mM) and incubated on a shaker at room temperature in the dark for 1 h. The fluorescence of the samples was measured with a spectrofluorometer (F-4500, Hitachi, Japan) at room temperature, with an excitation wavelength of 485nm and an emission band between 500 and 600 nm. The fluorescence intensity at 520 nm normalized to the protein content was used to determine the relative ROS production.

Analysis of DNA strand breaks

DNA strand breaks were determined by fluorometric analysis of DNA unwinding (FADU) as described by He and Häder (2002), and modified by Chen et al. (2009).

Briefly, cells were harvested by centrifugation (12,000 g, 10 min). The pellet was washed with TE buffer and resuspended in solution A (50 mM Tris, pH 8.0; 50 mM Na_2EDTA; 1 M NaCl). Sarkosyl solution (10% N-lauroyl sarcosine, 10 mM Tris-HCl, pH 8.0; 20 mM EDTA) was added to the samples (final concentration 0.1%) and kept at 4°C for 2 h. After centrifugation (7000 g, 10 min), the pellet was resuspended in solution B (50 mM Tris, pH 8.0; 50 mM Na_2EDTA; 25% sucrose) up to a final volume of 184 μL. Then, 20 μL of 160 g L^{-1} lysozyme was added to the suspension to destroy the cell walls completely. The following steps were operated as the method of He and Häder (2002).

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) and values were shown the means of 3 replicates.

Results

Effect of light intensity on photosynthetic efficiency (*Fv/Fm*)

As shown in Fig.1, *C.vulgaris* contained the highest photosynthetic efficiency at less light intensity, and the follows were *Nostoc*, *S.javanicum* and *M.vaginatus*. *Fv/Fm* of *C.vulgaris*, *Nostoc* and *M.vaginatus* decreased with the increase of light intensity duration. However, the optimum light intensity of *S.javanicum* was 200 $\mu Em^{-2}s^{-1}$. *Fv/Fm* of *C.vulgaris* decreased rapidly with the increase of light intensity, while that of *Nostoc* sp. and *S.javanicum* decreased slowly and was partly inhibited, and that of *M.vaginatus* didn't have a significant change even at 700 $\mu Em^{-2}s^{-1}$.

Effect of UV-B on photosynthetic efficiency and ETRmax

As illustrated in Fig.2, *Fv/Fm* of four microalgae decreased obviously after irradiated with different doses of UV-B. *Fv/Fm* of *C.vulgaris* was sensitive to UV-B irradiance, and it decreased significantly after treated with 1 W/m^2 UV-B and restored partly after recovered for 16 h; while that of *M.vaginatus* and *Nostoc* decreased slowly and restored to the normal after recovered for 16 h. When treated with 2 or 4 W/m^2 UV-B, *Fv/Fm* of *C.vulgaris* decreased rapidly and was not restored any more, while that of *M.vaginatus* and *Nostoc* sp. was partly inhibited and could partly restore to the normal level after recovered for 16 h. Especially, *Fv/Fm* of *S.javanicum* decreased very rapidly under different doses of UV-B, and it was not measured after treated with for 4 h at 2 or 4 W/m^2 UV-B, but it restored to the normal level after recovered for 16 h.

The maximum electron transport rate (ETR_{max}) was also measured with Phyto-PAM. It was found that ETR_{max} of *C.vulgaris* decreased very quickly ($p<0.01$), and that of *Nostoc* and *S.javanicum* also decreased significantly ($p<0.05$) after irradiated with $2W/m^2$ UV-B (Table 1), while that of *M.vaginatus* were not sensitive to UV-B radiation. After recovered for 16 h, ETR_{max} of *C.vulgaris* was still not measured, while that of *S.javanicum* and *M.vaginatus* restored to the normal level.

Effect of UV-B on Absorbance spectrum

The absorbance spectrum of *S.javanicum* and *Nostoc* had higher absorption values in UV-B region of the spectrum (280-320nm), while that of *M.vaginatus* was not obviously (Fig.3). *S.javanicum* also had higher absorption in UV-A region of the spectrum (320-400 nm). After irradiated for 4 h with 2 W/m^2 UV-B and 16 h recovery, the absorbance spectrum of *Nostoc* didn't have a significant change, while that of

M.vaginatus and *S.javanicum* increased obviously in the region of 280-320 nm compared with un-irradiated cells. However, the absorbance spectrum of *C.vulgarisc* didn't have an obvious absorption peak even after 16 h recovery.

Effect of UV-B on ROS generation

Time courses of ROS generation exposed to 2 W/m^2 UV-B as measured by DCF fluorescence of four microalgae were investigated. ROS generation of these microalgae increased quickly with the increase of irradiance time, and reached steady after irradiated for 4 h with 2 W/m^2 UV-B (Fig.3). The production of DCF fluorescence of *S.javanicum*, *M.vaginatus* and *Nostoc* were decreased one by one at the same time, and suggested a significant difference ($p<0.05$) between them when irradiated for only 1 h. Especially, the production of DCF fluorescence of *S.javanicum* increased very rapidly, which suggested the quick increase of ROS generation. The production of DCF fluorescence of *C.vulgaris* was the lowest at the first, but it increased very rapidly and then kept steady even irradiated for 2 h.

Effect of UV-B on DNA strand breaks

As shown in Table 2, significant decreases ($p<0.05$) in dsDNA (%) of these microalgae were detected after irradiated for 4 h with 2W/m^2 UV-B, indicating significant DNA strand breaks happened. It was also found that dsDNA (%) of *Nostoc* was significant higher ($p<0.05$) than other three microalgae after treated for 4 h with 2 W/m^2 UV-B, indicating lower DNA damage occurred in it. After 16 h recovery, the dsDNA (%) of *S.javanicum* and *Nostoc* recovered significantly ($p<0.05$), wile that of *M.vaginatus* was partly recovered, and that of *C.vulgaris* still kept the lowest level.

Discussion

BSCs play an important role in stabilizing sandy areas and affect the vegetation composition in arid and semiarid region (Belnap et al. 2001). Well developed biological soil crusts tend to display stratification with dark-pigmented *Nostoc* sp. and *S.javanicum* restricted to the soil surface, motile filamentous *M.vaginatus* extending as deep as 5 mm below the soil surface and C.vulgaris dominated the lower portions (Garcia-Pichel and Belnap 1996; Bowker et al. 2002; Hu et al., 2003). Therefore, desert cyanobacteria and green algae are exposed to high level of UV radiation on desert soil surface. The effect of solar light on photosynthesis is critical for the survival of algae on the surface of desert crust (Belnap 2001). High level of solar PAR (photosynthetically active radiation) usually results in most of the inhibition of PSII (Xu and Gao 2007). In our results, *C.vulgaris* had a higher photosynthetic efficiency at low PAR and was sensitive at high PAR, which suggested that it was more favorable less intensity of PAR and dominated in the lower portion with less PAR, *M.vaginatus* was more favorable higher PAR, while *Nostoc* and *S.javanicum* were not sensitive to PAR and dominated in the upper layer (Fig. 1).

There is increasing evidence that UV-B radiation affects normal metabolism in all organisms, especially desert cyanobacteria, which are exposed to high level UV radiation on desert soil surface (He and Häder 2002; Chen et al. 2009, Wang et al. 2008). Excessive ROS production can destroy D1 protein, and affect photon absorption, electron transport and the PSII reaction center (Xia et al. 2004), thus inhibiting photosynthesis in the photosystem (Xiong et al. 2001), and finally

destroying photosynthetic apparatus, enzymes and DNA (He and Häder 2002; Kumar et al. 2004; Wang et al. 2008). ROS generation was considered to be cumulative dose-dependent to destroy photosynthetic apparatus (Sinha 2001). PSII activity of *C.vulgaris* was very susceptible to UV-B radiation, and that of *M.vaginatus* could endure moderate UV-B radiation, while that of *Nostoc* could tolerate a higher UV-B radiation (Fig.2 and Table 1). The decreased PSII activity suggested a rapid degradation of its core proteins of PSII (Niyogi 1999). The production of DCF fluorescence (ROS generation) of *S.javanicum*, *M.vaginatus*, *Nostoc* and *C.vulgaris* showed obvious difference after irradiated with UV-B, which showed the different ROS eliminate capacity in cyanobacteria and led to different level of DNA damage in different species (Fig. 4; Table 2). Therefore, *C.vulgaris* was sensitive to UV-B irradiance, while *M.vaginatus* could stand moderate UV-B and *Nostoc* sp. had a good tolerance to UV-B.

Increased UV-B radiation affects the normal metabolism in all organisms (Zeeshan et al. 2009; Wang et al. 2012). However, autotrophic organisms have developed a number of repair and tolerance mechanisms to counteract the damaging effects of UV radiation (Quesada et al. 1997; Singh et al. 2010). The UV-absorbing/screening compounds, including mycosporine-like amino acids (MAAs) and scytonemins, are an important mechanism to prevent UV-induced photodamage (Sinha and Häder 2008). In this study, *Nostoc*, *S.javanicum* and *M.vaginatus* had high absorbance spectrum at UV-B region (Fig. 3), which were UV protectants in UV photoprotection in cyanobacteria (Soule et al. 2007). Photoreactivation with the help

of photolyase is one of the most important and frequently repair mechanisms in organisms (Häder and Sinha 2005). In this study, dsDNA content restored from 62.9% to 83.1% in *S.javanicum*, 73.7% to 83.6% in *Nostoc* and 61.4% to 64.7% in *M.vaginatus* (Table 2). The results suggests that *S.javanicum* and *Nostoc* had better repair capacity than others, which might have been due to the photoreactivation reaction in PSII, which was associated with a strong recovery capacity related to the D1 turnover-mediated repair cycle in both cyanobacteria (Xiong 2001; Van de Poll et al. 2001; Häder and Sinha 2005). Photoreactivation also plays an important role in DNA repair with the help of a number of glycosylases and polymerases, which was due to irreversible DNA damage by UV-B radiation in both cyanobacteria (Mitchell and Karentz 1993). Thus, the cyanobacteria repaired UV-B induced oxidative damage through the effective repair ability of DNA and the photosynthetic system.

UV-B tolerance of photosynthetic apparatus in microalgae is also associated with a strong recovery capacity related to the D1 turnover-mediated repair cycle in PSII system (Xiong 2001, Starkenburg et al. 2011). The de novo synthesis of the D1 protein of PSII center is rapidly degraded under photoinhibitory conditions, which is essential for repair of PSII from photodamage (Harel et al. 2004). Photosynthetic activity of *S.javanicum* decreased very rapidly after irradiated with UV-B, and completely restored in recovery process (Fig. 1), which showed that photosynthetic apparatus of *S.javanicum* might be own an different strategy to endure high level of UV-B, including closure its photosystem after exposing to UV-B, and high tureover of D1 protein and high repairing DNA strand breaks (Adir et al. 2003; Harel et al. 2004).

The increased ROS in *S.javanicum* were considered to be a general alarm signal to alert the metabolism and activate gene expression for possible modifications (Vranova et al. 2002), and regulate the signal transduction such as the antioxidant defense response and increase D1 protein synthesis or decrease Rubisco large subunit synthesis (Irihimovitch and Shapira 2000). All these results implied that UV-B sensitivity/tolerance of the photosynthetic apparatus was not merely determined by damaging process but also by the ongoing repair process, i.e. by the ratio of damage to repair (Xiong 2001).

In conclusion, with the comparison of the damage and recovery process of photosynthetic activity, pigmentation and DNA strand breaks, we concluded that *S.javanicum* endured intense UV irradiance by its sunscreen pigment and repairing capacity of photosynthetic apparatus and DNA damage, and *Nostoc* sp. by its sunscreen pigment, well tolerance and repairing capacity. However, motile *M.vaginatus* had moderate tolerance and sunscreen pigments and dominated the middle layer of BSCs, where UV radiation was not intense. *C.vulgaris* had high photosynthesis efficiency and dominated the bottom layer of BSCs, where UV irradiance is less. Based on above advantages, the four microalgae in well-developed BSCS had different adaption strategies to UV irradiance. However, *Nostoc* sp. is assumed to a preferred species for the primary colonization.

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Table 1 The variance of ETR_{max} of *C.vulgaris*, *M.vaginatus*, *Nostoc* sp. and *S.javanicum* in response to exposure to 2 W/m^2 UV-B for 4 h and following 16 h recovery. Values represent mean ± S.D. (CK showed untreated cells)

ETR_{max}	*C.vulgaris*	*M.vaginatus*	*Nostoc* sp.	*S.javanicum*
CK	110.2±7.1	78.8±8.2	84.7±8.3	47.9±5.3
UV	37.6±6.2**	75.3±5.4	46.6±7.2*	31.3±2.7*
Recovery	0**	76.5±4.8	79.3±6.4	46.3±3.1

Note: *showed that ETRmax decreased significantly *($p<0.05$) and **($p<0.01$)compared with CK.

Table 2 The variance of DNA strand breaks of *C.vulgaris*, *M.vaginatus*, *Nostoc* sp. and *S.javanicum* in response to exposure to 2 W/m^2 UV-B for 4 h and following 16 h recovery. Values represent mean ± S.D. (CK showed untreated cells)

Species	CK (%)	UV (%)	Recovery (%)
S.javanicum	95.7±2.9	62.9±2.2ab	83.1±2.6a
Nostoc sp.	96.8±3.1	73.7±1.9a	87.6±2.5a
M.vaginatus	96.2±3.0	61.4±1.7ab	64.7±1.9ac
C.vulgaris	96.0±2.8	47.8±2.0ab	43.2±3.2ac

Note: “a” showed that dsDNA decreased significantly ($p<0.05$) compared with CK, and “ab” significantly compared with *Nostoc* (UV), and “ac” significantly with *Nostoc* or *S.javanicum*.

Figure caption s:

Fig. 1. The variances of *Fv/Fm* of these microalgae in response to white light intensity measured by Phyto-PAM. Values are means of three replicates ±SD

Fig. 2. Time courses of the variances of *Fv/Fm* of these microalgae after irradiated with different levels of UV-B radiation. Values are means of three replicates ±SD

Fig. 3. The variances of absorption spectrum of these microalgae after irradiated with 2W/m^2 UV-B for 4 h and recovered for 16 h. Values are means of three replicates ±SD

Fig. 4. Time course of ROS generation in these microalgae after irradiated with 2 W/m^2 UV-B. Values are means of three replicates ±SD

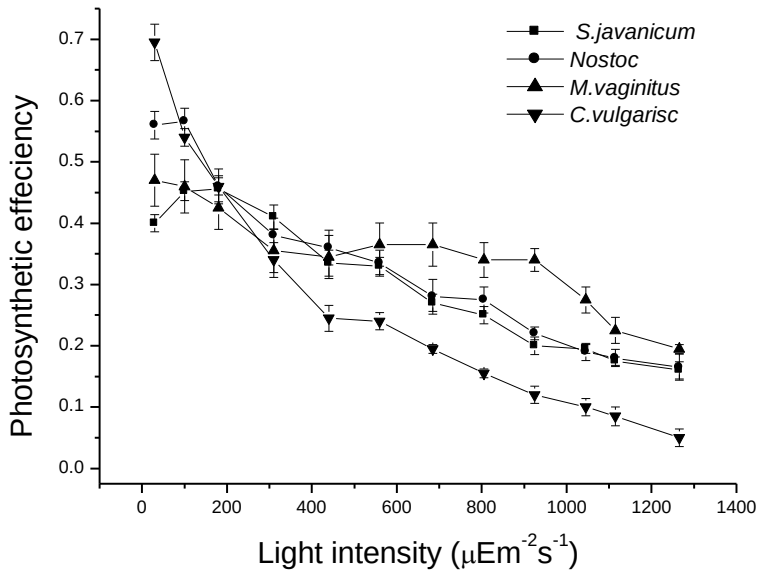
S.javanicum
Nostoc
M.vaginitus
C.vulgarisc
Photosynthetic effeciency
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
200
400
600
800
1000
1200
1400
Light intensity (μEm-2s-1)

Fig. 1

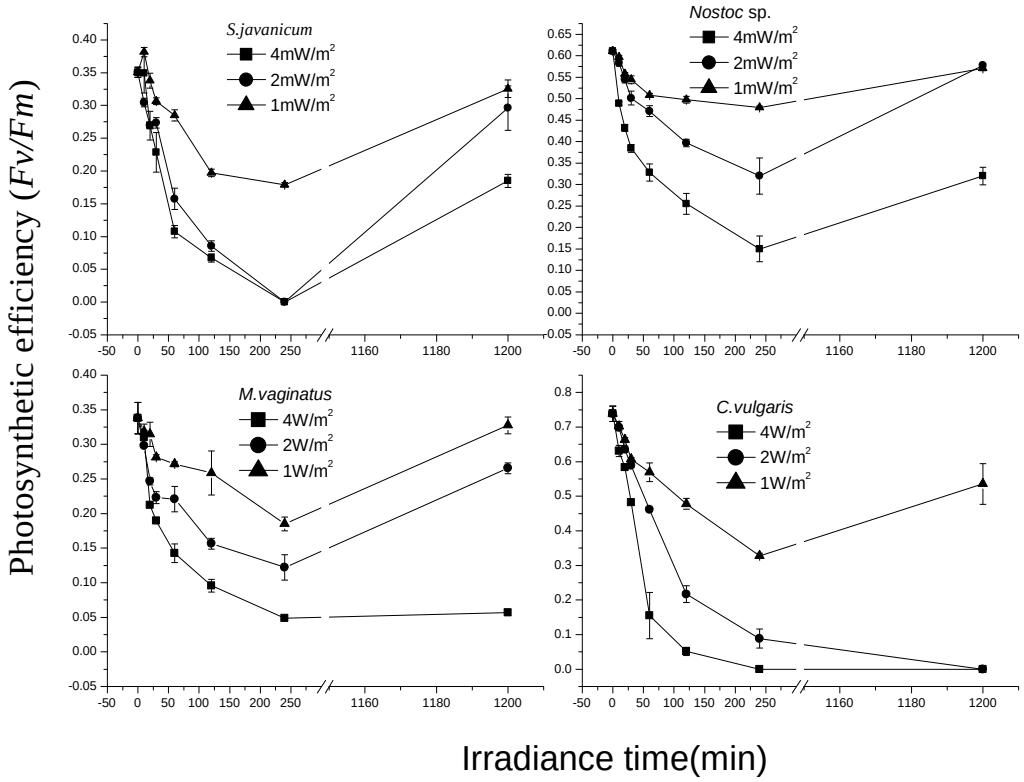
Photosynthetic efficiency (Fv/Fm)
S.javanicum
4mW/m2
2mW/m2
1mW/m2
Nostoc sp.
4mW/m2
2mW/m2
1mW/m2
M.vaginatus
4W/m2
2W/m2
1W/m2
C.vulgaris
4W/m2
2W/m2
1W/m2
Irradiance time(min)

Fig. 2

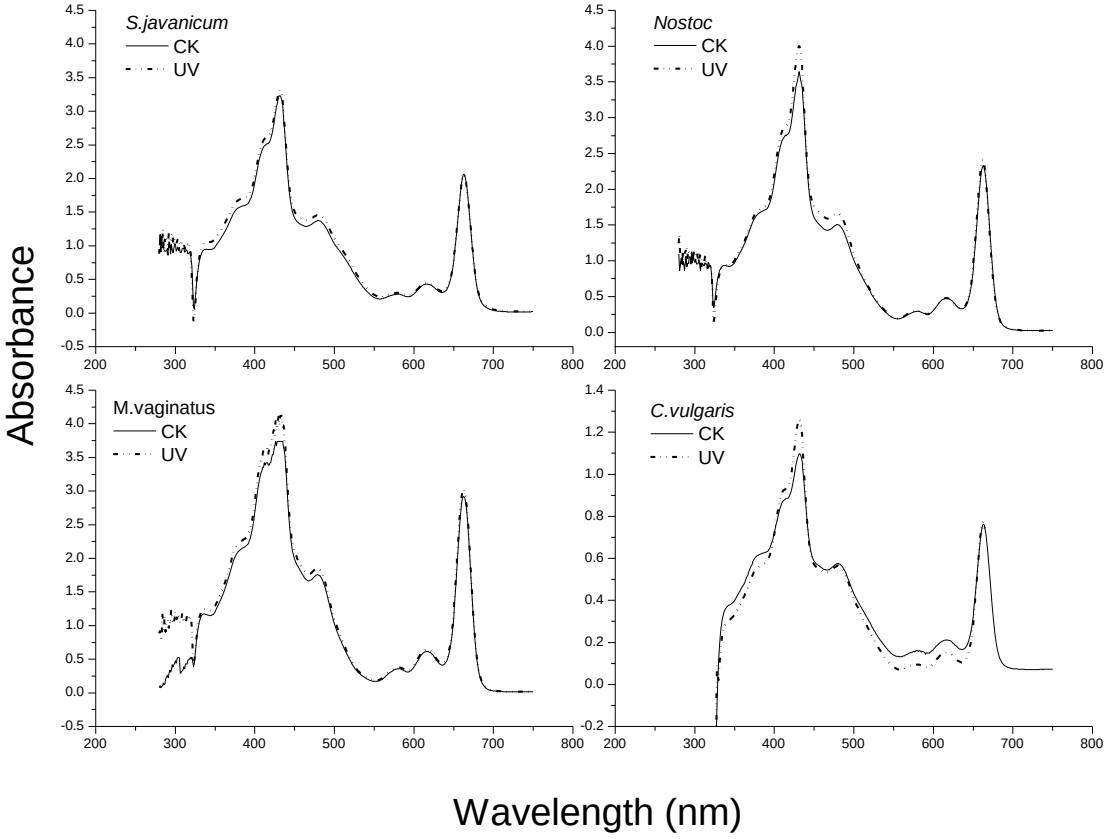
S.javanicum
CK
UV
Nostoc
CK
UV
M.vaginatus
CK
UV
C.vulgaris
CK
UV
Absorbance
Wavelength (nm)

Fig. 3

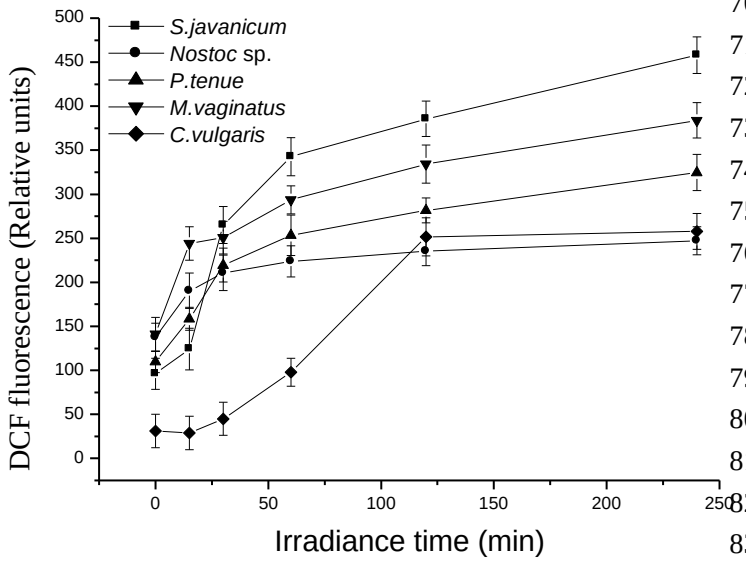
S.javanicum
Nostoc sp.
P.tenue
M.vaginatus
C.vulgaris
DCF fluorescence (Relative units)
Irradiance time (min)
0
50
100
150
200
250
300
350
400
450
500

Fig. 4