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Original article

The response of carbohydrate metabolism to the fluctuation of relative humidity (RH) in the desert soil cyanobacterium *Phormidium tenue*

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ABSTRACT

The excreting of exopolysaccharides (EPS) is thought as one of the main protection ways for cyanobacteria in desert algal crusts to survive desiccation. But how cyanobacteria adjust their carbohydrate metabolism to survive this stress is not elucidated. In this study, we treated *Phormidium tenue*, a cyanobacterium isolated from biological soil crusts with the changes of relative humidity (RH) to simulate different levels of desiccation and investigated its carbohydrate metabolism. It was found that photosynthetic activity (*Fv/Fm*) and cellular total carbohydrates production decreased significantly at low RH. But the production of EPS, reducing sugar, sucrose and the activity of sucrose phosphate synthase (SPS) increased significantly at low RH and reached maximum at 75% RH. Low RH could also cause the enhanced production of reactive oxygen species (ROS) generation, malondialdehyde (MDA) production and DNA strand breaks. However, when pretreated with exogenous 100 mg/L EPS, *Fv/Fm* and carbohydrate production were improved significantly, while ROS generation, MDA production and DNA strand breaks decreased significantly at various levels of RH in *P. tenue*. These results indicated that *P. tenue* in arid regions could enhance desiccation tolerance by adjusting the carbohydrate metabolism to eliminate ROS and decrease oxidative damage.

1. Introduction

Cyanobacteria living on the top soil in desert biological crusts have to suffer from water deficit, low relative humidity and diurnal desiccation/rehydration cycles in arid semiarid regions [25,28]. As the results of prolonged desiccation, drying of cells leads to the crowding of cytoplasmic components, the condensation of nucleoid and the imposition of stress upon cell walls [27]. Desiccation also causes serious oxidative damage in the early drying period. It can induce electron flow through the photosystems transferred from photo-excited chlorophyll pigments to O_2, which causes the increase of reactive oxygen species (ROS) in photosynthetic organisms [13]. The increase of ROS can break the balance of producing and scavenging of ROS, and results in the impairing of various biomolecules through metal-dependent Fenton reaction [1,19,27,32].

Cyanobacteria have developed both simple and complex interactions at the cell structure, physiological and molecular levels to enhance desiccation tolerance [2,3]. Autotrophic organisms depends on the production of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) to eliminate activated oxygen molecules to defend themselves against reactive oxygen species (ROS) during desiccation [11]. *Nostoc* has the ability to reversibly activate metabolism and grow in the short periods when water is available, while to retard metabolic activity during dehydration in desert algal crust [14]. One aspect of desiccation tolerance for cyanobacteria is the extrusion of EPS, which can fulfil a protective role by reducing the water exchange with environment to prevent water loss, and absorbing moisture directly from the atmosphere [4,6]. EPS could also prevent cell damage caused by desiccation and rewetting processes, protect the intact of cell walls, thylakoid membranes and inhibit the fusion of membrane vesicles during desiccation in *Nostoc commune* [10,16,30]. Some works also reported that EPS could effectively eliminate ROS and enhance UV tolerance in *Microcoleus vaginatus* [8]. But few works have revealed the relationship between oxidative damage induced by desiccation and carbohydrate metabolism in cyanobacteria [9].

As one of sheathed cyanobacterial strains, *Phormidium tenue* excretes EPS under stress, which can combine sand granules with filaments in desert region to form biological soil crusts in desert area [6,10,33]. However, the excreting of EPS in the cultivation process was released into culture medium instead of surrounding

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the cells in desert soil [7], which might cause the high death probability of inoculums on sand dune because of the lack of protection from EPS [6,16]. Since cyanobacterial inoculation to form biological soil crusts would be a suitable and effective technique in desertification areas in China [6,33], the present work is aimed to simulate the change of RH to investigate the adjustment and functions of carbohydrate metabolism of *P. tenue* during the water loss process, in order to assess the possibility of using this cyanobacterium as inoculum on sand dunes and develop the new sand-fixing technology.

2. Materials and methods

2.1. Cultures and materials preparation

P. tenue (*Menegh.*) Gom. was isolated from desert algal crust of Shapotou, Zhongwei County, Ningxia Autonomous Region, China (37°27′N, 104°57′E). Inoculations were gently dispersed with a glass homogenizer and grown in BG-11 medium [29], at 25 °C and illuminated with white fluorescent light at 40 μE $m^{-2}s^{-1}$. Cells were collected on the sieve membrane by filtration after cultured for 14 d, and resuspended in fresh medium or fresh medium containing 100 mg/L EPS for 30 min. The cultures resuspended in 50 ml fresh medium were prepared as control 1, and cells resuspended in 50 ml fresh medium contained 100 mg/L EPS were prepared as control 2. The resuspended cells were filtrated and washed with distilled water for three times and then transferred to 5 cm watch glass.

The cultures on the 5 cm watch glass were wetted with 1 ml fresh medium or fresh medium contained 100 mg/L EPS to prevent cells drying, and then placed in different chambers (112 cm^3), which contained 30 ml of distilled water (100% relative humidity; RH), saturated solutions of NaCl (75% RH), $KHCO_3$ (43% RH) or NaOH (6.5%) respectively to produce different levels of desiccation stress according to the method of Ong et al. [23]. All containers with watch glass were placed in incubator at 25 °C and illuminated with white fluorescent light at 40 μE $m^{-2}s^{-1}$.

EPS were prepared according to the methods of Huang et al. [17]. The mass cultivation of *P. tenue* were centrifuged and passed through glass fibre membrane. The supernatants were operated to prepare the EPS as the steps of Chen et al. [7].

2.2. Chlorophyll a fluorescence

Chlorophyll *a* fluorescence was measured with a Plant Efficiency Analyzer (PEA, Hansatech®, U. K.) after dark-adapted for 15 min. The maximum excitation light intensity was about 1500 μE $m^{-2}s^{-1}$, and the record time was 5 s. The fluorescence parameter *Fv*/*Fm* (Photosystem II activity) is the ratio of the variable fluorescence was used for assessing the physiological status of the cells.

2.3. Carbohydrate analysis

The treated cultures on the watch glass were washed three times with distilled water, and resuspended in the distilled water to the end volume of 50 ml and then the cells were centrifuged and freeze-dried and stored in refrigerator for future use. The supernatants were used to analyze EPS production.

Cellular total carbohydrates and reducing sugar were prepared according to method of Chen et al. [5], and quantified according to the phenol-sulphuric acid method of Dubios et al. [12], using glucose as standard. The EPS was quantified according to the methods of Huang et al. [17], and normalized to dry weight. The 1 ml medium (contained 100 mg/L EPS) added to wet the cultures on the watch glass and the EPS in the medium of the control were also subtracted from the production of EPS.

Sucrose was extracted and quantified according to the method of Tang [31], using sucrose as standard. SS and SPS activity were analyzed according to the method of Tang [31], and modified according to the method of Chen et al. [5].

2.4. Malonyldialdeide (MDA) analysis

Cultures were homogenized with 10 ml 10% TCA and centrifuged (4000 *g*, 15 min). The supernatant (2 ml) was added to 2 ml 0.6% (w/v) thiobarbituric acid (TBA), and incubated in boiling water for 15 min. The reaction stopped by placing tubes in ice bath, then centrifuged (10,000 *g*, 10 min). The supernatant was measured at 450 nm, 532 nm and 600 nm with a spectrophotometer, and calculated according to the method of [31].

2.5. Analysis of ROS and DNA strand breaks

Reactive oxygen evolution (ROS) production was detected by using DCFH-DA [15]. The fluorescence of the samples was measured with a fluorescence spectrophotometer (F-4500, Hitachi, Japan) at room temperature, with an excitation wavelength of 485 nm and an emission band between 500 and 600 nm. The fluorescence intensity at 535 nm normalized to the protein content was used to determine the relative ROS production.

DNA strand breaks were determined by fluorometric analysis of DNA unwinding (FADU). Briefly, cells were harvested by centrifugation (5000 rpm, 8 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 in 4 °C for 2 h. After centrifuged (7000 *g*, 8 min), the pellet was washed twice with TE buffer. Subsequently, 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 mg/ml lysozyme was added to the suspension, and the mixture was incubated for 40 min at 37 °C to destroy the cell walls completely. A 30 μl sample of 10% SDS, 10 μl of 4 M NaCl and 47 μl of TE buffer were added to a total volume of 291 μl and incubated for 60 min at 37 °C. Finally, 9 μl of 10 mg/ml proteinase K was added to a final volume of 300 μl and incubated for 60 min at 37 °C to lyse the cells. The following steps were operated according to the method of Chen et al. [8].

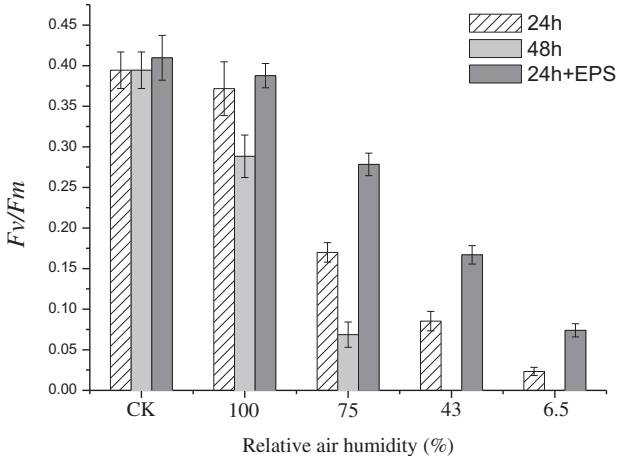

Fig. 1. Various levels of RHs and exogenous 100 mg/L EPS on the effects of PSII activity (*Fv*/*Fm*) of *P. tenue*. Bars indicated the standard deviation ($n = 3$).