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Response of cyanobacteria to low atmospheric pressure

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ABSTRACT

Maintaining a low pressure environment in a controlled ecological life support system would reduce the technological complexity and resupply cost in the course of the construction of a future manned lunar base. To estimate the effect of a hypobaric environment in a lunar base on biological components, such as higher plants, microbes, and algae, cyanobacteria was used as the model by determining their response of growth, morphology, and physiology when exposed to half of standard atmospheric pressure for 16 days (brought back to standard atmospheric pressure 30 minutes every two days for sampling). The results indicated that the decrease of atmospheric pressure from 100 kPa to 50 kPa reduced the growth rates of *Microcystis aeruginosa*, *Merismopedia sp.*, *Anabaena sp.* PCC 7120, and *Anabaena flos-aquae*. The ratio of carotenoid to chlorophyll a content in the four tested strains increased under low pressure conditions compared to ambient conditions, resulting from the decrease of chlorophyll a and the increase of carotenoid in the cells. Moreover, low pressure induced the reduction of the phycocyanin content in *Microcystis aeruginosa*, *Anabaena sp.* PCC 7120, and *Anabaena flos-aquae*. The result from the ultrastructure observed using SEM indicated that low pressure promoted the production of more extracellular polymeric substances (EPSs) compared to ambient conditions. The results implied that the low pressure environment of 50 kPa in a future lunar base would induce different effects on biological components in a CELSS, which must be considered during the course of designing a future lunar base. The results will be a reference for exploring the response of other biological components, such as plants, microbes, and animals, living in the life support system of a lunar base.

1. Introduction

A controlled ecological life support system (CELSS) is an effective solution to support human habitation of a future Lunar/Mars base and human deep space exploration. An arti-ecological system can supply oxygen, water and food based on the roles of higher plants, microbe, algae, and so on. Designing a low pressure environment has advantages during the construction of space bases due to the limitations of vessels on the Lunar/Mars surface. A low pressure system not only helps to reduce the requirements of the materials in the base building by decreasing the difference in pressure between inside and outside of the CELSS but also increases the material conservation of the CELSS through minimisation of the gas leakage from the inside to the outside of the CELSS (Chamberlain, 2004; Nangalia and Habershon, 2004; Paul and Ferl, 2006; He et al., 2007). However, the presence of humans in the space restricts the application of low pressure conditions. The compromise proposed by NASA to meet the demands of both engineering and humans was 54 kPa of atmosphere pressure (NASA, 1998, 2004). Even then, the recommendation did not consider the response of other biological components in the CELSS; these other biological components will be subject not only to unnatural environments, such as reduced gravity, but also to the conditions designed mainly to fulfil the requirements for the human environment (Wheeler et al., 2001).

A large amount of research has been conducted by using microbes under simulated Lunar/Mars surface air environments (Olsson-Francis and Cockell, 2010; Thomas et al., 2008; Moeller et al., 2012; Nicholson et al., 2013; Schuerger et al., 2013; Waters et al., 2014; Fajardo et al., 2012) or higher plants under reduced atmospheric pressure for human space missions (Tang et al., 2011; Andre and Massimino, 1992; Arai et al., 2003; Chamberlain, 2004; Corey et al., 1997, 2002; Goto et al., 2002; Guo et al., 2008; He et al., 2007, 2009). These studies all determined the response of the biological components to low pressure stress in space in different fields. And their results indicate that many microbes can survive in space low pressure environments such as in simulated Mars surface conditions and even can resist to vacuum on low Earth orbit. For higher plants, low pressure induces different changes of their growth, photosynthesis, transpiration, gene express and so on.

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Fig. 1. Photograph of the low pressure bacteria culture facility.

In this study, we examined the effects of lower pressure through an alternative method by using cyanobacteria as the model to be exposed to reduced pressure stress, which was chosen based on following aspects. Cyanobacteria are prokaryotes that have the characteristics of aging, wide adaption, photosynthesis and survival under extreme environments, such as high temperatures, low temperatures, saline, and vessels (Friedmann, 1980; Wierzchos et al., 2006). They can even survive in low Earth orbit and simulated Mars surface conditions (Olsson-Francis et al., 2010), enabling them to be seen as the colonisation species of "pioneer biology". Furthermore, cyanobacteria play vital roles in the control of atmospheric composition via photosynthesis and in the in-situ source utilisation via nitrogen fixation and rock damage (Olsson-Francis and Cockell, 2010). These characteristics make cyanobacteria an important model for understanding the response of biological components to the varied environments in a CELSS, and the results will be an important reference for the construction of a Lunar/Mars base.

Thus, we examined the response of cyanobacteria to the environment in a lunar base CELSS by determining the changes of growth, morphology, and photosynthetic pigments under the above-mentioned low pressure condition to evaluate its potential effect on the biological component of a CELSS.

2. Materials and methods

2.1. The cyanobacterial strains

Four cyanobacteria strains with two different morphologies of single cells (*Microcystis aeruginosa*, FACHB-942 and *Merismopedia sp.*, FACHB-1045) and filaments (*Anabaena PCC 7120* and *Anabaena flos-aquae*, FACHB-245) were tested in our study. Among them, *Anabaena PCC 7120* was obtained from the State Key Laboratory of Protein and Plant Genetic Engineering of Peking University, China, and the others were provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences (FACHB).

2.2. Growth conditions

A low-pressure bacteria culture facility (Fig. 1) was used for the experiment. The facility has two low-pressure closed cabins, each with a volume of approximately 50 L (diameter of 20 cm × height of 30 cm) and each made of glass. The pressure and temperature parameters in each closed cabin are measured by sensors and are controlled by an intelligent data acquisition instrument. One millilitre of each cyanobacterial strain during the index growth period was inoculated in 100 ml of BG11 medium (Allen, 1968) contained in a 200-ml Erlenmeyer flask in triplicate. Next, a total of 24 flasks were separated averagely into two halves, one half was placed in a closed cabin to be exposed under low pressure condition (50 ± 1 kPa) with half of the gas contents, such as CO_2, O_2 and so on. The other half was placed in another cabin for exposure to ambient pressure (100 ± 1 kPa). The cultures were illuminated by six fluorescent lights of 30 $\mu mol\, m^{-2}\, s^{-1}$ PAR at $26 \pm 1\,^{\circ}C$ with continuous exposure to light (24 hours/day). All of the strains were cultured statically by manual shaking the flasks once a day and were exposed under two pressure treatments for 16 days from inoculation.

2.3. Measurement index

2.3.1. Growth analysis

From inoculation to 16 days, 3 ml of culture was obtained every two days to measure the OD values using a spectrophotometer (UV2300, Tianmei Instrument Company, China) at wavelengths of 678.6 nm, 685.4 nm, 679 nm and 675.8 nm, and the dry mass concentration was calculated according Eqs. (1) to (4) for *Microcystis aeruginosa*, *Merismopedia sp.*, *Anabaena PCC 7120* and *Anabaena flos-aquae*, respectively. The four equations were calculated by measuring the dry mass concentration of six gradient levels cultures for each cyanobacteria.

$$y(\text{mg DW/L}) = (\text{OD}_{678.6\ \text{nm}} - 0.058)/0.0047 \quad (1)$$

$$y(\text{mg DW/L}) = (\text{OD}_{685.4\ \text{nm}} - 0.0324)/0.0074 \quad (2)$$