Growth and primary cell composition of plateau microalgae Desmodesmus sp. QL96 under low temperature

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Abstract. Microalgae Desmodesmus sp. QL96 was newly isolated from Northwest Tibetan Plateau area which featured low temperature and oxygen content. In this work, Desmodesmus sp. QL96 was cultivated at 4 °C, and the algal growth and cell composition was investigated. It turned out that the microalgae has stayed at lag phase for over 10 days post inoculation, with a slight increase in cell numbers in the following 18 days. The cell content composed mainly of proteins with a percent of more than 40%, followed by lipid, though their amount varying over the culture time. The adaptation of this microalgae to extreme environmental conditions deserves a further study.

1. Introduction

Microalgae is a general term for unicellular algae, which needs to be resolved by means of a microscope. Microalgae are rich in protein, fat, vitamins, chlorophyll, carotenoids and various minerals, meeting the requirement of functional food and medical fields. Based on its excellent nutritional and health functions, it is used to promote the growth and development of cells and bodies, delay aging, improve the body's immune system, and effectively prevent target diseases[1]. Therefore, microalgae attract more interesting things around the world, focused on its nutritious and application. At present, most research on energy in chlorella, spirulina, cyanobacteria, etc[2].

In this study, Desmodesmus sp. QL96 is a strain isolated from Bam Co Lake located at Northwest Tibetan Plateau. The average sunshine duration is 11.6 hours and the mean annual temperature is 8 °C, with -21~2 °C in winter and 1~25 °C in summer[3-4]. We have studied the growth and metabolites of this microalgae growing at 25 °C and found that the microalgae produced primarily proteins which could reach up to 79% of cell dry weight, and this is the highest protein producing microalgae ever reported[5]. The adaptability of this microalgae to low temperature is one of the reasons why we chose it as the experimental object.

Low temperature is a common stress factor for most microorganisms, which affects all stages of cell metabolism and growth. At present, there is less research on the growth trend and cell composition of microalgae at low temperature has been found. Therefore, this study is based on the commonly used
medium BG-11 for freshwater culture of microalgae. \( D.s.p.QL96 \) was cultured at 4 \(^\circ\)C. The growth cycle and cell composition of \( D.s.p.QL96 \) at low temperature were investigated. The results will further illustrate our understanding of this microalgae metabolite and relate to future applications. It also provides new ideas for the optimization of microalgae culture.

2. Methods

2.1 Culture of microalgae \( D. \) sp. QL96
The medium used in this research was BG-11, basically consisted of various salts as described in Berges\(^6\). The initial cell number were controlled at \( 5 \times 10^5 \) /ml. Culture conditions were as follows: light intensity 6000 lux, temperature 4 \(^\circ\)C, and photoperiod of 12 h: 12 h (light: dark). The culture was shaken at 150 rpm 3 times per day. All chemicals were of analytical grade purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (China).

2.2 Measurement of growth
Measurements were performed every 3 days (three replicates). The microalgae cells were continuously cultured at 4 \(^\circ\)C for 30 days. The number of cells was counted every three days using an inverted fluorescence microscope (DMI6000B, Leica, Germany).

2.3 Measurement of carbohydrate content
Carbohydrate content was determined according Ho et al\(^7\). 15.0 mg freeze-dried algal cells were resuspended in 1 mL of 72% \( \text{H}_2\text{SO}_4 \) solution in a 2 mL centrifuge tube, and crashed with 0.5 g grinding beads in a crusher for 7 min. The content was incubated at 30 \(^\circ\)C for 1h and then transferred to a 50 ml centrifuge tube. The cell content was separated from the grinding beads and diluted to 4% \( \text{H}_2\text{SO}_4 \) with distilled water. Centrifugation was performed at 6000 rpm for 2 min, and the carbohydrate content in the supernatant was determined by phenol-sulfuric acid method\(^7\).

2.4 Measurement of protein content
15 ml of algae solution was centrifuged at 2000 rpm for 10 min, and the supernatant was discarded, then the algal mud was ground and chilled at -20 \(^\circ\)C. After adding 3 ml of PBS buffer and fully centrifuged for 10 min. 1 ml of the supernatant was added to 3 ml of Coomassie Brilliant Blue solution, and the absorbance at 595 nm was measured. The protein content was determined according Bradford method\(^8\).

2.5 Measurement of lipid content
The lipid content was measured by the method of Ho et al\(^7\). 40 mg of dry algae was resuspended in 8 mL of 0.5M potassium hydroxide/ethanol solution in a 15 mL centrifuge tube and crushed for 7 min using a crusher. The content was saponified under a water bath at 100 \(^\circ\)C for 15 min and cooled to room temperature. Esterification was conducted by adding 8mL of 0.7 mol/L hydrochloric acid, as well as 10mL of 14% boron trifluoride in methanol, and incubated at 100 \(^\circ\)C for 15min. 5 mL of n-hexane and 50 uL of 40 g/L of eicosane was added into the mixture after cooling to the room temperature and mixed well. 2 mL of saturated sodium chloride solution was used to prevent the esterification. The mixture kept still for around 1 h, and the upper layer of n-hexane was drawn out and dehydrated with anhydrous sodium sulfate. The resulted lipid was filtered through a 0.22 um organic membrane and analyzed it gas chromatography.

2.6 Measurement of chlorophyll content
The content of chlorophyll of the algal was determined by the method reported by Hansmann et al\(^9\). 5 mg of dry algae was resuspended in 8 mL of 90% acetone in a 15 mL centrifuge tube and crushed for 7 min with a crusher. The contents were centrifuged at 6000 rpm for 2 min, and the supernatant was appropriately diluted with 90% acetone. The values of \( \text{OD}_{665\text{nm}} \), \( \text{OD}_{645\text{nm}} \) and \( \text{OD}_{630\text{nm}} \) were measured.
by ultraviolet spectrophotometer. The mass concentration (ρ) of chlorophyll a and b in algal body was calculated according to formula (1) to (2) respectively.

$$\rho_a = 11.6 \times \text{OD}_{665\text{nm}} - 1.31 \times \text{OD}_{645\text{nm}} - 0.14 \times \text{OD}_{630\text{nm}} \quad (1)$$

$$\rho_b = 20.70 \times \text{OD}_{665\text{nm}} - 4.34 \times \text{OD}_{645\text{nm}} - 4.42 \times \text{OD}_{630\text{nm}} \quad (2)$$

2.7 Measurement of carotenoids content

The carotenoid content was determined using the method of Xie Youping et al [10]. The algal culture was centrifuged at 6000 rpm for 2 min. The supernatant was decanted. The algal cells were washed twice with deionized water and freeze-dried. 10 mg of dry algae was resuspended in 1 mL 60% KOH solution and crushed. The contents were incubated 40 °C for 40 min, chilled to room temperature and supplemented with 2 mL of anhydrous. The carotenoids were extracted with diethyl ether and washed 3 times diethyl ether. Nitrogen was used to blow dry the ethyl ether extract, and 3 mL acetone was added to resuspend the carotenoids. The carotenoid content in the suspension was determined by HPLC.

3. Results and discussion

3.1 Growth of D. sp. QL96

The microalgae D. sp. QL96 was cultured in BG-11 medium and the growth rates were investigated by measuring the cell numbers from day zero to day thirty. As shown in figure 1, the microalgae stayed at lag phase for over 10 days post inoculation, with a slight increase in cell numbers in the following 18 days. Cell numbers increased from the initial 5×10⁵ /ml to around 25×10⁵/ml, with approximately 4 time increase with 30 days.

![Cell number vs. days](image)

Figure 1. Growth curve of microalgae D. sp. QL96 grown at 4 °C

3.2 Production of carbohydrates in D. sp. QL96

The cells of microalgae D. sp. QL96 were harvested and carbohydrates were extracted and measured. The results showed that the production of carbohydrates had kept increase over the culture time, though the increase between days 0-18 was very slight and a sharp increase showing up after day 18. The final carbohydrate content in the cell was around 0.09 mg/mg in the dry cells. The production of carbohydrate was correlated with the growth of the microalgae. At the beginning of the cultivation, the growth of the cells was slow and the production of intracellular carbohydrates was tiny accordingly.
3.3 Production of proteins in D. sp. QL96
The cells of microalgae D. sp. QL96 were harvested and proteins were extracted and measured. It turned out that the production of protein in microalgae D. sp. QL96 had fluctuated over the culture time. The production of proteins peaked on day 12, with an amount of 0.70 mg/mg in the dry cells. One could assume that the microalgal cells were synthesizing organic substances such as proteins to prepare for future growth at the beginning of the low temperature cultivation. Once the microalgal growth entered the stationary phase, the rates of protein synthesis and decomposition got to a balance, lowering the amount of proteins in the microalgal cells\[11\].

3.4 Production of lipids in D. sp. QL96
The cells of microalgae D. sp. QL96 were harvested and lipids were extracted and measured. As can be seen from figure 4, the lipid content in D. sp. QL96 grown under 4 °C was in a range of 0.08–0.15 mg/mg dry cell, with a gradual decrease from day 0-24 and a tiny increase by the end of the culture. It was assumed that the enzymes involved in the metabolism of lipids had been less active at low temperature, resulting an insufficient in the production of lipids\[12\]. Also, the rates of lipid synthesis might be lower than its consumption rates, leading to the reduction of lipid production\[13\].
Figure 4. Production of lipids in D. sp. QL96 grown at 4 °C

3.5 Production of chlorophyll in D. sp. QL96
Chlorophylls were extracted from the microalgal cells and the production was measured through the spectrophotometry. As shown in figure 5, both the chlorophyll a and b presented a reduction in their production though there were some fluctuation. The production of chlorophyll a reached a bottom on day 18 and then peaked on day 24, while the chlorophyll b peaked on both day 12 and 24. However, both the production of chlorophyll a and b were quite low in the microalgae grown at 4 °C. It could be assumed that the algal energy metabolism had consumed most of the nutrition the algae obtained at 4 °C so that less energy was releasing to the cell composition synthesizing[14].

Fig.5. Production of chlorophylls in D. sp. QL96 grown at 4°C (a) chlorophyll a, (b) chlorophyll b

3.6 Production of carotenoid in D. sp. QL96 grown at 4 °C
The production of carotenoid in D. sp. QL96 was quite low, with an amount of 0~0.004 mg/mg dry cells before day 18 post inoculation and became undetectable in the following cultures. Researches indicates that carotenoids are not only important photosynthetic auxiliary pigments, but also have important protective effects on chlorophyll[15]. In this work, both the production of chlorophyll and carotenoid was low and experienced a decrease over the culture period.

Figure 6. Production of carotenoid in D. sp. QL96 grown at 4 °C
4. Conclusion
In this work, the growth and production of various cell compositions of microalgae D. sp. QL96 grown under low temperature were studied. The results demonstrated that the microalga was able to grow at 4 °C though a lag phase of over 10 days post inoculation observed. The cell composition consisted primarily of proteins which took up to 70% by the end of the cultivation, followed by the production of lipids and carbohydrates, with a percentage of approximately 10% over the culture period. The production of chlorophyll and carotenoid were extremely low in the microalga cultured at 4 °C and all experienced a decrease with the extension of the culture time.

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