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Research Article

Effect of Vitamin-B_1 and Vitamin-B_{12} on the Growth and Carotenoid Content of *Haematococcus pluvialis* CH-1

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Abstract: An economic microalgae *Haematococcus pluvialis* CH-1 was used as experimental material. An experiment of adding six grades of concentrations of Vitamin-B_1 and Vitamin-B_{12} respectively was conducted. Cell density, carotenoid content was measured. The results showed that the growth of *H. pluvialis* was accelerated significantly by adding of Vitamin-B_1 and Vitamin-B_{12} respectively. The optimal adding concentration of Vitamin-B_1 and Vitamin-B_{12} respectively for *H. pluvialis* was 10000 and 50 μg/L. Under the optimal concentration for *H. pluvialis*, cells density, carotenoid content were enhanced with Vitamin-B_1: 20.1 and 21.3%; Vitamin-B_{12}: 29.5 and 24.4% higher than the blank respectively. On the mass culture of motile cells of *H. pluvialis*, properly adding Vitamin-B_1 and Vitamin-B_{12} respectively was effective for increase cells density and carotenoid content.

Keywords: Carotenoid; *Haematococcus pluvialis*; vitamin-B_1; vitamin-B_{12}

INTRODUCTION

Haematococcus pluvialis that belongs to Chlorophata, Chlorophyceae, Volvocales, is a single economic microalgae and accumulates remarkable amounts of natural carotenoid, of which the astaxanthin content is accounting for 80% (Chen and Jiang, 1999; Grung *et al*., 1992; Lee and Soh, 1991; Lorenz and Cysewski, 2000). The astaxanthin is an excellent feed additive, food colorant and a potential medicine (Miki, 1991; Fukuzawa *et al*., 1998; Martin *et al*., 2003; Bjerkeng and Johnsen, 1995). The Chlorophyte alga *Haematococcus pluvialis* is believed to accumulate the highest levels of astaxanthin in nature (Martin *et al*., 2003).

It was reported; adding VB_1, VB_{12} into culture medium can effectively promote the growth of microalgae. Ford (1958) proved there is a close relationship between adding concentration of VB_{12} and cell division rate of *Isochrysis galbana*. Liu *et al*. (2002) pointed out that growth promotion effect is the best for the transgenic Anabaena while adding VB_1, VB_{12} and VH together. However, it is unknown how to influence on cell density and carotenoid content content of *H. pluvialis* when respectively adding two kinds of Vitamin-B_1 and Vitamin-B_{12}. In this study, the effect of different concentrations of VB_1 and VB_{12} on cell density, chlorophyll-a and carotenoid content of *H. pluvialis* was studied and the results may provide the support of theory and technology for the high density cultivation of *H. pluvialis*

MATERIALS AND METHODS

Algal strain, medium and cultivation conditions: *Haematococcus pluvialis* CH-1 used in the present work was obtained from the Research Center of Hydrobiology of Jinan University (Guangzhou, China), Stock culture of *H. pluvialis* CH-1 was grown photoautotrophically in BBM medium (Lorenz and Cysewski, 2000) at 24±1°C under 12:12 h photoperiod (60 μmol/ (m^{-2}.sec) in 25 mL flask. The cells of logarithmic growth phase were inoculated into 150 mL culture medium; inoculation density is 5-10×10^3 cells/mL.

Experimental design: The experiment was conducted in the two groups, added VB_1 groups and added VB_{12} groups, each group was divided into 6 concentrations grades treatment, including 3 replicates per treatment (Table 1). The concentration grade of 0 treatments was without any vitamin, which was the blank.

Measure indexes and methods:
Cell density: The sample was measured every 48 h, using hemocytometer counting.

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Table 1: Treatments on three kinds of vitamin with six concentration grades (μg/L)

	Concentration grades					
Treatment	0	1	2	3	4	5
VB_1	0	10	100	1000	10000	100000
VB_{12}	0	0.05	0.5	5	50	500

Chlorophyll-a: According to Bochiroc's method (Jing and Ding, 1981), extraction and measure every 6 days.

Carotenoid content: According to Bochiroc's (Jing and Ding, 1981) method for the extraction and measure of carotenoid.

Data analysis: Analyses were done using the EXCEL program (Microsoft) and the SPSS software package version 11.5 of SPSS Inc. (Chicago, IL, USA.)

RESULTS AND DISCUSSION

Cell density: The results showed that, adding VB_1 and VB_{12} respectively could obviously increase the cell density of *H. pluvialis* (Fig. 1). In the adding VB_1 test group, the cell density increased with the increase of the adding concentration of VB_1 in 10-10000 μg/L (Fig. 1a). When adding concentration was 10000 μg/L, the cell density were higher than other treatments in the late culture period (12-18 day) and reached 4.48×10^5 cells/mL in the 18th day, enhanced 20.1% than the blank ($p<0.05$). When adding the concentration was 100000 μg/L, the cell density was lower than the blank.

In VB_{12} group, cell density increased with the increase of the adding concentration of VB_{12}. When adding the concentration was 50 μg/L, the cell density was the maximum (Fig. 1b) and was significantly higher than other treatments. It reached 4.96×10^5 cells/mL in the 18th day, enhanced 29.5% than the blank ($p<0.01$). But when the highest concentration (500 μg/L) was adding, the cell density was lower but still higher than the blank. The optimal concentration of adding VB_{12} was 50 μg/L.

Carotenoid content: Respectively adding VB_1 and VB_{12} made the changes of the carotenoid content of *H. pluvialis* (Fig. 2). In VB_1 group, the carotenoid content increased with the increase of VB_1 When adding concentration was 10000 μg/L, the carotenoid content was highest in all treatments, achieved 4.43 mg/L, enhanced 21.3% than the blank ($p<0.05$). When adding the concentration was 100000 μg/L, the carotenoid content was nearly same as the blank (Fig. 2a).

In VB_{12} group, the carotenoid content increased with the increase of the adding concentration, reached maximum 4.53 mg/L at the adding concentration 50 μg/L, enhanced 24.4% than the blank (Fig. 2b). But when the highest concentration (500 μg/L) of VB_{12} was added, the carotenoid content was nearly equal to the blank.

H. pluvialis has a complex life-cycle involving several stages from motile flagellated zooids through to

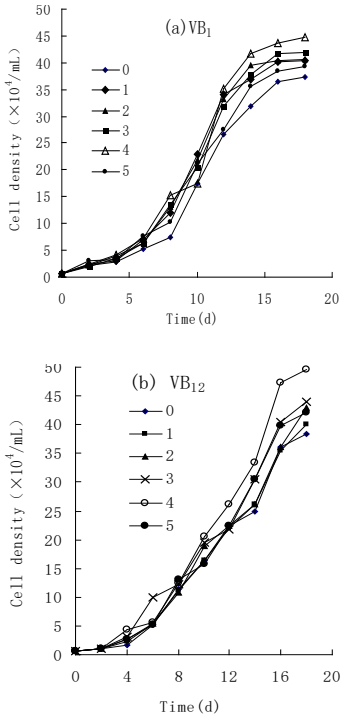

Fig. 1: Effects of vitamin-B_1, vitamin-B_{12} on cell density of the *H. pluvialis*

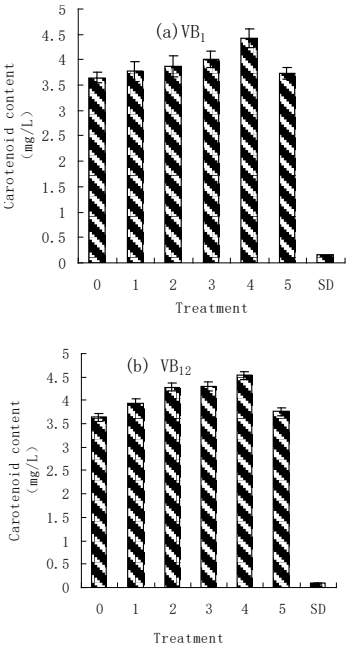

Fig. 2: Effects of vitamin-B_1, vitamin-B_{12} on carotenoid content of the *H. pluvialis*

palmella and encysted stages. The conditions for the carotenoid production are known to be considerably different from those for the growth of *H. pluvialis*. Therefore, to obtain high productivity of carotenoid-hyper-accumulated *H. pluvialis* biomass, a two-stage culture system is likely to be more effective (Choi *et al*., 2002). The first stage is for high-rate growth of green motile cells under optimum conditions, carotenoid is completely absent from the cells. The second stage is for the hyper-accumulation of carotenoid in red cells upon exposure of the cells to growth-limiting conditions, where a morphological and biochemical transformation occurs from green motile cells into inert red cysts. Various factors and methods promoting carotenoid formation have been suggested: high irradiation, nitrogen deficiency, phosphate deficiency, magnesium deficiency, acetate addition, ferrous ion addition and salt addition or high temperature (Harker *et al*., 1996).

CONCLUSION

In BBM medium added VB_1 and VB_{12} can obviously increase the growth of the *H. pluvialis* motile cell respectively. Adding VB_{12} can significantly improve the cell density and carotenoid content. The suitable concentration of VB_{12} and VB_1 is different, the optimal adding concentration of Vitamin-B_{12} and Vitamin-B_1 respectively for *H. pluvialis* was 50 and 10000 μg/L. Consider the cost of Vitamin and carotenoid content, adding suitable concentration of VB_{12} is more suitable for the commercial production process of natural astaxanthin of *H. pluvialis*.

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