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# Factors Affecting Growth and β-carotene Content of *Chlorosarcinopsis* sp. (PSU/CHL20) in Batch Culture

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## ABSTRACT

The effect of light intensity, potassium nitrate concentrations and temperature on the growth and β-carotene content of *Chlorosarcinopsis* sp. (PSU/CHL20) were determined. In the first experiment, algal cells were grown on a shaker in Erlenmeyer flasks containing 150 ml of NSIII medium and at a shaking speed of 150 rpm. Potassium nitrate concentrations were 0, 0.1 and 0.2 g $l^{-1}$. Light intensities used were 60, 120 and 180 μmol$m^{-2}s^{-1}$. The light / dark cycle was 16:8 h and the temperature was controlled at 25°C. The optimum light intensity and potassium nitrate concentration for algal growth and β-carotene content was 120 μmol$m^{-2}s^{-1}$ and 0.1 g $l^{-1}$ which produced a maximum cell number of $138\times10^5$ cell $ml^{-1}$and a β-carotene content of 0.133 mg $l^{-1}$, (0.012 pg $cell^{-1}$) respectively. In the second experiment, the effect of temperature on the growth and β-carotene content was investigated. In this experiment the light intensity was 120 mmol$m^{-2}s^{-1}$ and the potassium nitrate concentration was 0.1g $l^{-1}$, at temperatures of 25°C, 30°C and 35°C. Although statistically insignificant ($P>0.05$), the algal growth increased slightly with increasing temperature. The highest cell numbers of $148x10^5$ cell $ml^{-1}$ were obtained at 30°C on day 14 of cultivation. The maximum β-carotene content of 0.543 mg $l^{-1}$ (0.052 pg $cell^{-1}$) was achieved at 30°C and this was significantly higher than that of the culture grown at 25°C ($P<0.05$).


**Key words:** *Chlorosarcinopsis* sp., β-carotene, batch culture

## INTRODUCTION

Carotenoids are yellow-orange pigments found in plants, animals and micro-organisms. They are classified into two groups as carotenes and xanthophylls. The carotenoid functions primarily as a photoprotective agent and an accessory pigment in the photosystems. In commercial applications, carotenoids are used as natural food colourings, feed additives to enhance the colour of fish flesh and egg yolks and to improve the health and fertility of cattle. An environmental stress such as high light intensities, high NaCl concentrations, extreme temperatures, extreme pH values or a nutrient deficiency was an important factor for regulating the β-carotene content in the cells of various microorganisms. (Borowitzka and Borowitzka, 1988). Some species of green algae such as *Dunaliella salina* accumulate large amounts of β-carotene when grown under conditions of stress.

*Chlorosarcinopsis* sp. is a unicellular


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freshwater green alga. Under laboratory conditions, cells changed colour from green to orange when nitrogen was limited. This study was therefore designed to examine the effects of light intensities, nitrogen concentrations and temperatures on the growth and $\beta$-carotene content of *Chlorosarcinopsis* sp.

## MATERIALS AND METHODS

Stock cultures of *Chlorosarcinopsis* sp. (PSU/CHL20) (Chlorophyceae) were obtained from Prince of Songkla University Culture Collection and grown in NSIII medium (Hosakul, 1972). Stock cultures were grown in 250 ml Erlenmeyer flasks at 25°C, with a light intensity of 60 $\mu molm^{-2}s^{-1}$, a light-dark cycle of 16/8 h and a shaking speed of 150 rpm.

The first experiment was to monitor the effect of light intensity and potassium nitrate concentrations on *Chlorosarcinopsis* sp. growth and $\beta$-carotene content. Algal cells were harvested in the logarithmic growth phase by filtering through a nylon sheath of 10 µm mesh size. The cells were then washed with sterile water and resuspended in NSIII medium with and without supplementation of 0.1 or 0.2 g $l^{-1}$ potassium nitrate. The cells were grown in 250 ml Erlenmeyer flasks with 150 ml of nutrient medium for 20 days under light intensities of 60, 120 and 180 µmol $m^{-2}s^{-1}$. The initial cell number was $2\times10^6$ cells $ml^{-1}$. Other conditions were the same as those of the stock culture. Each variable was studied in triplicate.

The second experiment was conducted under the optimum light intensity of 120 µmol $m^{-2}s^{-1}$ and a potassium nitrate concentration of 0.1 g $l^{-1}$ but the temperature was varied at 25°C, 30°C and 35°C. The method and other conditions were the same as in the previous experiment. Cell concentrations of samples were counted every 2 days using a hemacytometer. The specific growth rate ($\mu$) was calculated from the growth curve using a fitting program for the data that were obtained during the logarithmic growth phase. $\mu$ was computed from the following formula: $\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$, where $X_1$ and $X_2$ are cell concentrations at time $t_1$ and $t_2$ (Vonshak, 1986). Algal cells were collected every 4 days for the $\beta$-carotene analysis. In order to determine the $\beta$-carotene content, the samples were extracted with 90% acetone and analysed by the reverse phase HPLC (Modified from Vonshak and Borowitzka, 1991). $\beta$-carotene was identified by its retention time and quantified by the absorbance spectra in comparison with a standard. The statistical analyses were carried out using two-way ANOVA. The mean differences were tested by DMRT (Duncan's new multiple range test).

## RESULTS AND DISCUSSION

In order to determine the effect of light and nitrogen concentrations on the growth and $\beta$-carotene content of *Chlorosarcinopsis* sp., cells were cultivated under various combinations of nitrogen concentrations and irradiances. A nitrogen-free medium resulted in marked decline in cell numbers and there was a change in color from light green to pale green with an increase of light intensity. A similar testing process carried out with *Haematococcus pluvialis*, showed that cultivation in media deficient in nitrogen, severely limited algal growth and greatly stimulated astaxanthin synthesis (Harker *et al*., 1996; Po-Fung *et al*., 2004). The results indicated that the light energy absorbed in the photosynthetic system was not being utilized effectively and an excess of energy might accelerate chlorophyll degradation through photooxidation (Rise *et al*.,1994). The growth curves of algal cells in NSIII medium with potassium nitrate under various light intensities was shown in Figure 1. Optimal growth was obtained at a combination of 0.1 g $l^{-1}$ potassium nitrate and a light intensity of 120 $\mu molm^{-2}s^{-1}$

producing cell numbers of $138\times10^5$ cells $ml^{-1}$ at a specific growth rate of 0.151 $d^{-1}$. That cell numbers increased with the increase of light intensity, was similar to the finding in *Chlorella zofingisensis*, in which the growth was enhanced by a high light intensity of 150 $\mu molm^{-2}s^{-1}$ (Rise *et al.*, 1994).

The highest $\beta$-carotene content was achieved at the early stationary phase on day 16 of cultivation corresponding to a loss of Chl a in parallel with an increase of nonphotochemically active carotenoid pigments (Geider *et al.*, 1993). The lower nitrate concentration (0.1 g $l^{-1}$) favoured the production of $\beta$-carotene in the *Chlorosarcinopsis* sp. culture (Figure 2). The maximum $\beta$-carotene content of 0.133 mg $l^{-1}$ (0.012 pg $cell^{-1}$) was obtained at the light intensity of 120 $\mu molm^{-2}s^{-1}$ and 0.1 g $l^{-1}$ of potassium nitrate.

In spite of these variations overall the $\beta$-carotene content of cells under different culture conditions was not statistically different ($P>0.05$). However under high irradiance (180 $\mu molm^{-2}s^{-1}$), the level of $\beta$-carotene decreased slightly from that at 120 $\mu molm^{-2}s^{-1}$. This could be due to an increase of secondary carotenoids such as canthaxanthin and astaxanthin as it has been previously shown (Donkin, 1976; Rise *et al.*, 1994) that their production was enhanced by raising the irradiance and by nitrogen starvation. In addition, the results of Fan *et al.*(1994) supported the finding that higher irradiation levels usually yielded higher carotenoid concentrations.

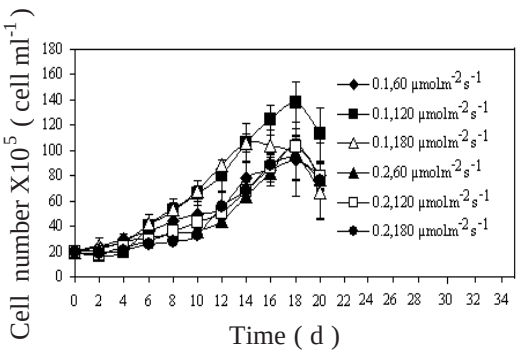


**Figure 1** Growth curves of *Chlorosarcinopsis* sp. grown in NS III medium under various light intensities and potassium nitrate concentrations. Error bars represent one standard error of mean (n=3).

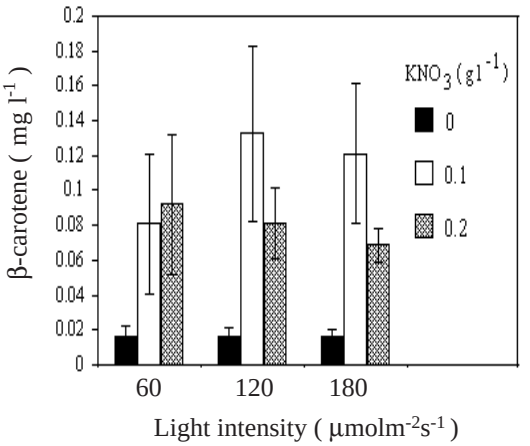


**Figure 2** Effect of light intensities and potassium nitrate concentrations on $\beta$-carotene content in *Chlorosarcinopsis* sp. on day 16 of cultivation. Error bars represent one standard error of mean (n=3).

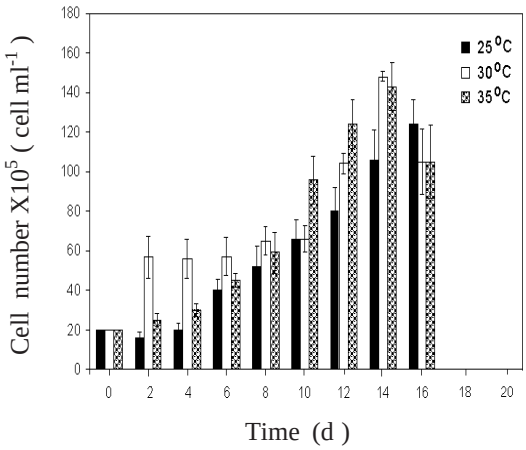


**Figure 3** Effect of temperature on growth of *Chlorosarcinopsis* sp. grown in NS III medium under light intensity of 120 $\mu molm^{-2}s^{-1}$ and potassium nitrate concentration of 0.1 g $l^{-1}$. Error bars represent one standard error of mean (n=3).

The effect of temperature on the growth and β-carotene content of *Chlorosarcinopsis* sp. was also studied. The highest cell number of $148\times10^5$ cells $ml^{-1}$ with a specific growth rate of 0.2 $d^{-1}$ was observed at 30°C on day 14 of cultivation (Figure 3). However, the maximum growth achieved increased slightly with a rise in temperature but the increase was statistically insignificant ($P>0.05$) at the temperatures of 25°C, 30°C and 35°C .

In the present study, the specific growth rate increased with a rise in temperature from 25°C to 35°C whereas Fan *et al.* (1994) found that the specific growth rate of *Haematococcus pluvialis* increased with a rise in temperature from 20°C to 28°C; a further increase in temperature caused a decline in specific growth rate. This study has shown that temperature is a major factor in the synthesis and accumulation of β-carotene in *Chlorosarcinopsis* sp. The maximum β-carotene content of 0.543 mg $l^{-1}$ (0.052 pg $cell^{-1}$) was achieved at 30°C on day 16 of cultivation and was significantly higher than that of the culture at 25°C ($P<0.05$) (Figure 4).

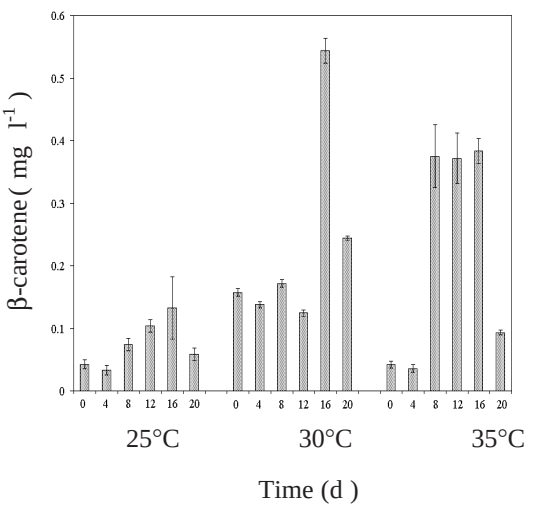


**Figure 4** Effect of temperatures on β-carotene content in *Chlorosarcinopsis* sp. grown in NS III medium under light intensity of 120 $\mu molm^{-2}s^{-1}$ and potassium nitrate concentration of 0.1 g $l^{-1}$. Error bars represent one standard error of mean (n=3).

The same reason was applied by Tjahjono *et al*., (1994), who obtained large amounts of astaxanthin by raising the cultivation temperature of *Haematococcus pluvialis* from 20°C to 30°C. β-carotene is a primary carotenoid and serves as a precursor of the astaxanthin (Johnson and Lewis, 1979; Harker and Young, 1995).

## CONCLUSIONS

*Chlorosarcinopsis* sp. (PSU/CHL20) grew best at 30°C in NSIII medium containing 0.1 g $l^{-1}$ of potassium nitrate and with a light intensity of 120 $mmolm^{-2}s^{-1}$, producing cell numbers of $148\times10^5$ cells $ml^{-1}$ on day 14 of cultivation. The maximum β-carotene content was 0.543 mg $l^{-1}$. This experiment revealed that a low nitrogen level and a high temperature were the major factors in the synthesis and accumulation of β-carotene in *Chlorosarcinopsis* sp. (PSU/CHL20).

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