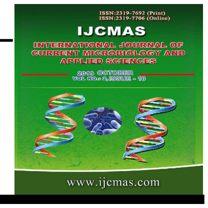
Int.J.Curr.Microbiol.App.Sci **(2013) 2(10): 325-328**

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 325-328**
http://www.ijcmas.com



Original Research Article

Optimization of growth conditions for carotenoid production from *Spirulina platensis* (Geitler)

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A B S T R A C T

Keywords

Spirulina; salinity; nitrogen starvation; carotenoids; β-carotene.

Carotenoid production by *Spirulina platensis* was enhanced under different stress conditions such as higher salinity and nitrogen starvation. Exposure of *S. platensis* to high saline levels and low levels of nitrogen caused an immediate cessation of growth and a decrease in biomass. The carotenoids and β-carotene content was found to be increased at high saline levels and nitrogen starvation.The increase in β-carotene content in starved cells may be attributed to excessive formation of free radicals under the stress, produced in order to protect the cells and to continue their growth.

Introduction

Microalgae are able to enhance the nutritional content of conventional food preparations and hence, to positively affect the health of humans and animals due to their chemical composition. Consequently, there has been increasing interest in the production of microalgae for commercial use due to its source of biochemicals, lipids, polysaccharides and colorants (Moller *et al.*, 2000). Carotenoids are naturally occurring pigments in algae that are involved in the light harvesting reaction and protection of algal organelle cells against singlet oxygen induced damage. *Spirulina* contain a higher content of β-carotene, cryptoxanthin and zeaxanthin compared to most other natural source. Also, *Spirulina* offer a wide scope for production of large quantities of natural carotenoids directly in an edible state. The nutritional and therapeutic relevance of certain carotenoids is due to their pro-vitamin A action and antioxidant activity. This activity comes from carotenes ability to deactivate the free radicals responsible for the cell regeneration that promotes cancer (Pisal and Lele, 2005).

The primary objective of this study is to increase the carotenoid and β-carotene content of *S. platenis* under stress conditions like nitrogen depletion and high salinity.

Materials and Methods

Spirulina platensis was isolated from the alkaline lake samples of Kodaikanal (Tamil Nadu, India) using Zarrouk's medium (Zarrouks 1966).

In order to evaluate the effect of different $NaNO_3$ levels, *S. platensis* (10%) was added to 250 ml Zarrouks medium with different $NaNO_3$ concentrations viz., 0.01 M (0.835 g) , 0.02 M (1.045 g), 0.03 M (1.265 g),0.04 M (1.465 g)and control (0.625 g). The flasks were kept in shade net under diurnal natural light conditions. In order to evaluate the effect of NaCl levels, *S. platensis* (10%) was added to 250ml Zarrouks medium with different NaCl concentrations *viz*., 0.1 M, 0.2 M, 0.3 M, 0.4 M and control. The flasks were kept in shade net under diurnal natural light conditions. Cultures were analyzed for their growth and biochemical (nutrient and pigment) constituents on 30th day after inoculation (DAI).

For biomass content, a known volume of *Spirulina* sample was filtered in a pre-weighed filter paper and oven dried at 75°C for 4 to 6 h, cooled and then again weighed. The difference between the initial and final weight were taken as the dry weight.

Total carotenoids were extracted by cell lysis (sonication) and homogenization in 90 per cent acetone as described by Rafiqul *et al*., (2003). The final extract was measured at A_{450} nm against acetone as blank. As for β-carotene, the cells were homogenised using acetone and petroleum ether and the extracts were pooled and measured at A_{453} nm against petroleum ether as blank(Govindaraju *et al*., 2001)

Results and Discussion

The results revealed that the growth of *S. platensis* was significantly inhibited in salt stress compared to control. Exposure of *S.platensis* to high NaCl concentrations results in an immediate cessation of growth and a decrease in biomass (Fig 1). An initial lag phase was observed before a new steady state growth was established. This lag period is associated with the decline in chlorophyll and biomass content due to inhibition of photosynthetic and respiratory system after exposed to high salt concentration(Vonshak, 1997). It has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting reduced photosynthesis and reduced growth.In regarding carotenogeneis, at higher NaCl concentration the grown cells contained higher amount of total carotenoids and β-carotene content (Fig. 1) with similar to previous studies. Pisal and Lele (2005) reported that the β-carotene is a secondary metabolite and these molecules are produced by the cells in stress condition as cell protecting mechanism. Since there an increase in total carotenoids and β-carotene content at higher saline conditions.

Nitrogen is the major form of nitrogen commonly used for assimilation by non-nitrogen fixing cyanobacterium under laboratory conditions. The assimilatory reduction of nitrate is a fundamental biological process in which a highly oxidised form of organic form of nitrogen is reduced to nitrite and then to ammonia (Bhattacharya and Shivaprakash, 2006).

In the present study, various $NaNO_3$ concentrations in Zarrouks medium were

Figure.1 Effect of different NaCl concentrations on growth and carotenoid contents of *Spirulina platensis*

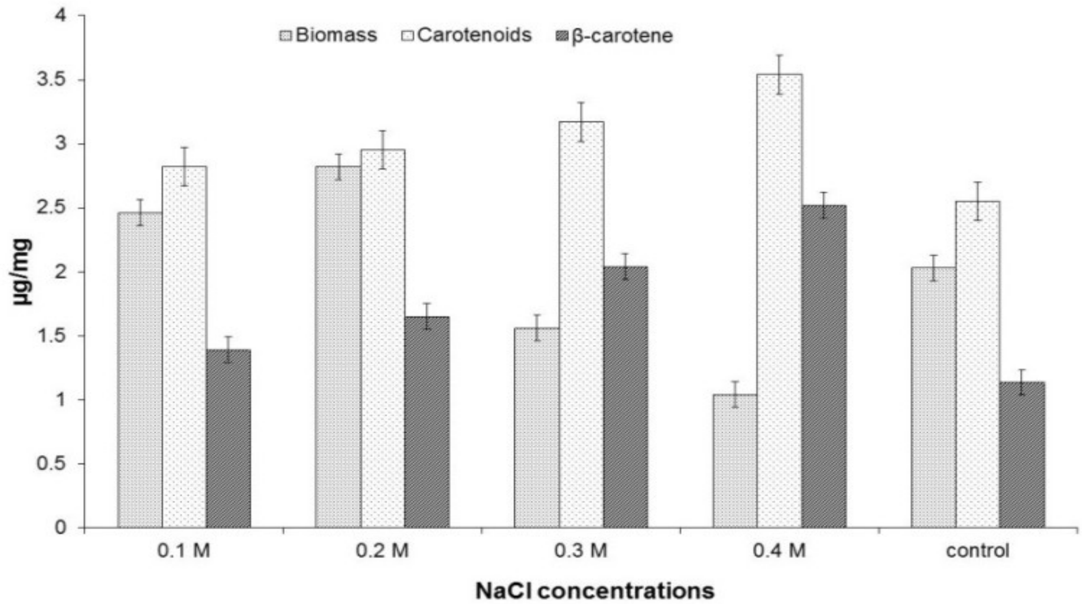
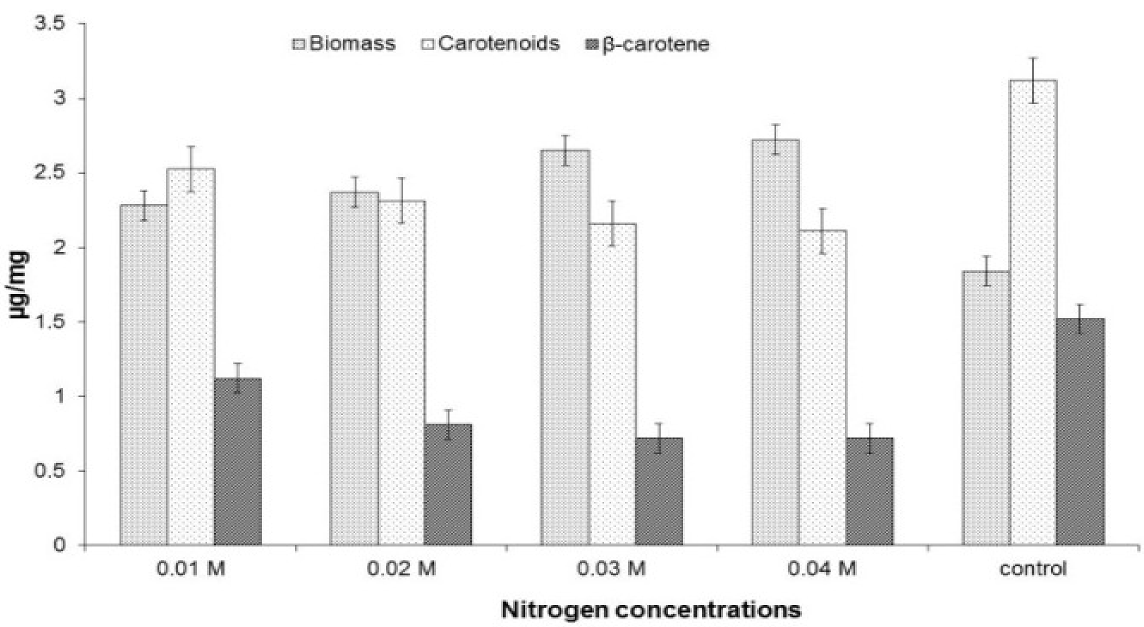


Figure.2 Effect of different nitrogen concentrations on growth and carotenoid content Of *Spirulina platensis*



tried for the optimal growth and chemical composition of *S. platensis* and the results revealed that increase in the nitrogen concentration upto 0.04 M recorded relatively higher growth (Fig 1) in terms of biomass (2.72 mg/ml) followed by 0.03 M (2.65 mg/ml). Filali *et al.,* (1997) reported that *S. platensis* is able to use numerous mineral and organic nitrogen sources for growth. Among them nitrate is the more convenient because it may be provided to the culture at higher concentrations (up to 100 mM), that delay the appearance of nitrogen limitation and therefore allow long term cultures.

With regard to carotenogenesis (Fig. 2), the total carotenoids (3.124 µg/mg) were found to be increased with decrease in the nitrogen concentration. El-Baz *et al.*, (2002) stated that the mechanism suggested for the acclimation of carotenoids in *Chlorella* and *Dunaliella* to grow under stress conditions also applies to *Spirulina.* It seems that the division of algae cells grown under nitrogen starvation is blocked, while photosynthesis continues, leading to storage of carotenoids, carbohydrates and triglycerides as they do not require nitrogen for their synthesis. The accumulation of carotenoids grown under nitrogen starvation may be due to stimulation of lipolysis process which produced a large amount of acetyl-CoA, which serves as a precursor for synthesis of carotenoids. β-carotene has anti-oxidant properties that quench excessive free radicals, restoring the physiological balance. The increase in β-carotene (Fig. 2) content of control (1.520 µg/mg) in nitrogen starved cells may be attributed to excessive formation of free radicals under the stress. Additional β-carotene is produced in order to protect the cells and to continue their growth (Moller *et al.*, 2000). Hence β-carotene production is markedly increased in nitrogen starvation.

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