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PRODUCTION OF PHOTOSYNTHESIS PIGMENTS BY *SPIRULINA PLATENSIS* UNDER DIFFERENT NACL CONCENTRATIONS

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Abstract

Blue green alga *Spirulina platensis* was subjected to different concentrations of NaCl, *i.e.* (0.1, 0.5, 5 and 10g/l) by using 1g/l (as stock salt) that considered to be the control of the experiments according to their concentration in Zarrouk medium, in order to study the effect of NaCl concentrations on the photosynthesis pigments which are chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phycocyanin, allophycocyanin and phycobiliproteins.

Sodium chloride caused different effects on photosynthetic pigments of the studied algae. In 10g/l NaCl, the maximum reduction of chlorophyll a, chlorophyll b, and total chlorophyll were 2.540, 0.808, and 3.349mg/1, respectively, while maximum reduction of phycocyanin, allophycocynin, and phycobiliproteins were 0.303, 0.232, and 0.535mg/1, respectively. Moreover, carotenoids induced to reach 1.182mg/l in 10g/l NaCl.

Rising NaCl concentration up to 5 and 10g/l caused the induction of carotenoids as well as the inhibition of chlorophyll a, chlorophyll b, total chlorophyll, phycocyanin, allophycocyanin and phycobiliproteins and vice versa happen when NaCl concentration decreased to 0.1 and 0.5g/l.

Key words: Blue green algae, *Spirulina platensis*, photosynthesis pigments, NaCl.

Introduction

Algae have the capability to manipulate its growth rate as well as its biochemical composition under varying physiochemical conditions (Al-Qasmi *et al*., 2012) with salinity as one of the most infuential parameters. Salinity refers primarily to sodium chloride concentration unless otherwise specified. Exposing algae to lower or higher salinity levels than their natural (or adapted) levels can change, growth rate and alter the biochemical composition (Fabregas *et al*., 1984; Zhila *et al*., 2011).

Cultivation of microalgae under salt stress can limit contaminants, invasive organisms, and competing microorganisms in microalgal systems, which presents another advantage. However, too high salinity introduced can also inhibit the cell growth and change the shape and structure of microalgal cells, due to the water pressure between media and cells. Thus, an optimal range for salinity level is supposed to be determined (Zhu *et al*., 2016). Among the important non-specific changes of microalgal cells to salt stress are: (i) increased rates of biopolymers and lipid catabolism; (ii) changes in the rates of energy-yielding processes; (iii) change of membrane permeability with interruption of ion homeostasis (Alyabyev *et al*., 2011).

Arthrospira (*Spirulina platensis*) is a multicellular filamentous, spiral-shaped blue-green microalgae. It can exist in various types of habitats; namely sand, soil, marshes and different kinds of aquatic media like fresh water, sea water and brackish water as well as waters of industrial and domestic uses (Khan *et al*., 2005; Charpy *et al*., 2012). WHO, (1998) and Gutiérrez-Salmean *et al*., (2015) emphasized that *Spirulina* is not toxic and can be safely used as food. So that, this alga is considered as an excellent food, lacking toxicity and having corrective properties against viral attacks, anemia, tumor growth and malnutrition (Hernandez-Corona *et al*., 2002; Mendes *et al*., 2003). Also, it has a high phytonutrient value and pigments which have applications in healthy food, animal feed, therapeutics and diagnostics (Becker, 1994; Vonshak and Tomaselli, 2000). Additionally, researchers have investigated the feasibility of energy recovery from ubiquitous *Spirulina platensis* microalgae in the form of biodiesel and biogas (El-Mashad, 2013; Sumprasit *et al*., 2017).

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Fig. 1: Kit of blue-green alga, *Spirulina platensis*

Materials and Methods

Algal Strain

Kit of blue-green alga, *Spirulina platensis*, was purchased from Algae Research and supply (Carlsbad, CA USA) (Fig. 1).

Preparation of Media and Alga Cultivation for Biomass

It is essential to transfer algal strain into specific growth media to enhance the growth and the enrichment of it. For the cultivations, Zarrouk medium was used and its constituents are shown in table 1 (Zarrouk, 1966). The solutions with the respective salts were sterilized separately by autoclaving at 121°C, for 15 minutes and mixed afterwards to achieve the final medium (Walter *et al*., 2011).

The alga *S. platensis* cells were inoculated at a concentration of 10% ($V_{inoculation}/V_{media}$) in 500ml Erlenmeyer flasks incubated in chemically defined Zarrouk Medium (Fig. 2). The experiment was carried out in triplicates at 32 ± 1°C, pH 9, under 135µEm^2 s^{-1} irradiance using cool white fluorescent lamps with a photoperiod cycle of 12:12 h light/dark and daily shaking by hand (Sarpal *et al*., 2016).

Fig. 2: Cultivation of *S. platensis*

Table 1: Compositions of Zarrouk media

Ingredients	Concentration (g/l)
NaCl	1.0
$CaCl_2.2H_2O$	0.04
$NaNO_3$	2.5
$FeSO_4.7H_2O$	0.01
EDTA (Na)	0.08
K_2SO_4	1.0
$NaHCO_3$	16.8
K_2HPO_4	0.5
$MgSO_4.7H_2O$	0.2
A5 micronutrient (H_3BO_3 (2.86g), $MnCl_2.4H_2O$ (1.810g), $ZnSO_4.4H_2O$ (0.222g), Na_2MoO_4 (0.390g), $CuSO_4.5H_2O$ (0.079g)	1 ml

Experimental Design

To study the effect of sodium chloride stress on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phycocyanin, allophycocyanin and phycobiliproteins, the stationary phase cultures were subjected to different concentrations of NaCl, *i.e.* (0.1, 0.5, 5 and 10g/l) by using 1g/l as control according to their concentration in Zarrouk medium (Table 1).

Estimation of Chlorophyll

The estimation of chlorophyll was done by the method of Arnonn, (1948). Algal cells were collected and resuspended in 1ml of 80% acetone. After centrifugation, the chlorophyll content of the supernatant was measured according to optical absorbance at 663nm and 645nm by using a UV-VIS spectrophotometer. The chlorophyll content was determined by the following Equations (1)–(3):

$$\text{Chlorophyll } a \text{ (mg / l)} = (12.7 \times A663)-(2.698 \times A645) \quad (1)$$

$$\text{Chlorophyll } b \text{ (mg / l)} = (22.9 \times A645)-(4.68 \times A663) \quad (2)$$

$$\text{Total chlorophyll (mg / l)} = \text{chlorophyll } a + \text{chlorophyll } b = (20.2 \times A645) + (8.02 \times A663) \quad (3)$$

Estimation of Carotenoids

An aliquot (5ml) of *S. platensis* cell suspension was taken and subjected to centrifugation (4000r\min for 10 mins.). Discarded the supernatant and washed the pellet 2-3 times with distilled water to remove traces of adhering salts. To the pellet, added 2-3 ml of acetone (80%) and vortex mixed until a white precipitate appeared (which, generally required 1min). The cell membrane gets ruptured because of organic solvent (acetone). Acetone extract

separated from cell debris by centrifugation it at 3000 r\min for 10min, After centrifugation, the carotenoids content of the supernatant was measured according to the equation reported by Lichtenthaler and Welburn, (1985) as follows:

Total carotenoids (mg / l) = 1000 A470 – 2.860 Ca – 129.2 Cb /245

(Ca = chlorophyll a, Cb = chlorophyll b)

Estimation of Phycobiliproteins

The estimation of phycobiliproteins was done by the method of Bennett and Bogorad, (1973). An aliquot of 10ml of the sample was centrifuged at the rate of 4500 r/min for 20min, and then the supernatant was decanted. The pellets were washed with distilled water, suspended in 10ml phosphate buffer (0.05 M, pH 7.0) and homogenized, then the contents were freeze thawed, repeated and centrifuged at 4500 r/min for 10min. The absorbance of the supernatant was determined at the wavelengths of 652, 615, and 562nm, using phosphate buffer as a blank. The concentrations of phycocyanin (PC), allophycocyanin (APC), and Phycobiliproteins were calculated according to (4) to (6), respectively (Devanathan and Ramanathan, 2012).

Phycocyanin (PC) = OD615-0.474 (OD652)/5.34 (4)

Allophycocyanin (APC) = OD652-0.208(OD615)/5.09(5)

Phycobiliproteins = PC + APC (6)

Statistical Analysis

Data were analyzed by using Statistical package for social sciences (SPSS) program to study the effect of different NaCl concentration on the chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phycocyanin, allophycocyanin and phycobiliproteins content of *Spirulina platensis*. Least significant difference (LSD) was used to compare the significant difference between means at $p<0.05$.

Results and Discussion

Comparisons with a control, which supported 3.291mg/l Chlorophyll a, 1.174mg/l chlorophyll b, 4.466mg/l total chlorophyll, 0.392mg/l phycocyanin, 0.325mg/l allophycocyanin, 0.717mg/l phycobiliproteins and 0.919mg/l carotenoids, showed that increasing NaCl concentration to 5 and 10g/l affected *Spirulina platensis* by the inhibition of chlorophyll a, chlorophyll b, total chlorophyll, phycocyanin, allophycocyanin, and phycobiliproteins as well as induction of carotenoids. Maximum reduction of chlorophyll a, chlorophyll b, total chlorophyll, phycocyanin, allophycocyanin and phycobiliproteins was 2.540, 0.808, 3.349, 0.303, 0.232 and 0.535mg/l, respectively in the presence of 10g/l NaCl. Moreover, carotenoids increased along with increasing NaCl concentrations and the highest content was 1.182mg/l in 10g/l NaCl. In addition to, decreasing NaCl concentrations to 0.1 and 0.5g/l showed the highest reduction of the carotenoids which was 0.23mg/l and the top rise of the chlorophyll a, chlorophyll b, total chlorophyll, and phycocyanin, was 8.801, 3.627, 12.429, 0.457mg/l at 0.1g/l of NaCl, while maximum allophycocyanin and phycobiliproteins were 0.402, and 0.838mg/l, respectively at 0.5g/l (Fig. 3, Fig. 4, Fig. 5).

Chlorophyll is the primary target to salt toxicity.

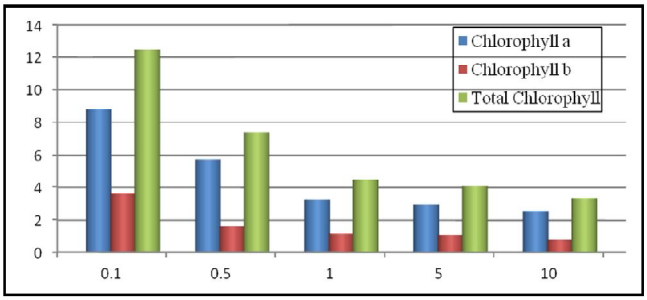

Fig. 3: Chlorophyll a, chlorophyll b and total chlorophyll content of *S. platensis* at different NaCl concentrations. ($p<0.05$)* Significant differences between control and all treatments except 5g/l in chlorophyll a, and total chlorophyll.

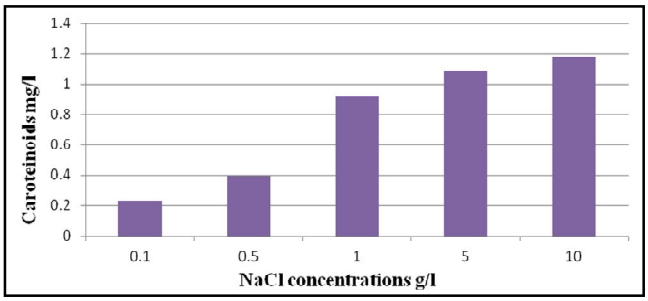

Fig. 4: Carotenoids content of *S. platensis* at different NaCl concentrations ($p<0.05$)* Significant differences between control and all treatments except 5g/l.

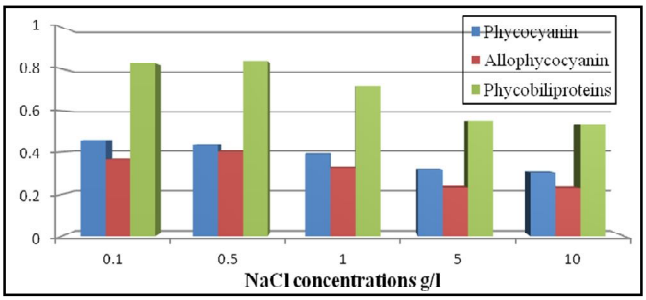

Fig. 5: Phycocyanin, allophycocyanin and total phycobiliproteins content of *S. platensis* at different NaCl concentrations ($p<0.05$) Significant differences between control and all treatments except 0.5g/l in phycocyanin and 0.1g/l in allophycocyanin as well as 0.5 in phycobiliproteins.

Limiting the net assimilation rate and reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress (Moradi and Ismail, 2007). In regards with carotenogeneis, at higher NaCl concentration, the grown cells containing higher amount of total carotenoids, which are produced by the cells in stress condition as cell protecting mechanism decreases (Leema *et al*., 2010; Sujatha and Nagarajan, 2013). Furthermore, according to phycobilli proteins, salinity stress (0.8M NaCl) induced a decrease in oxygen evolution activity, inhibited the electron transport at both donor and acceptor sides of PSII, resulted in damage to phycobilisome and shifted the distribution of excitation energy in favour of PSI so that in these conditions phycocyanin content decreased significantly in *Spirulina* biomass (Lu and Vonshak, 2002).

In agreement with these results Ji *et al*., (2018) demonstrated that NaCl salt could stimulate the accumulation of neutral lipids, and decrease the content of chlorophyll a, b in *Scenedesmus obliquus*. Pisal and Lele, (2005) also reported that with the increase in the salinity chlorophyll amount decreases, while the carotenoid content in the cell increases. This increase in carotenoid with an increase in salinity may be a protective measure of the cell against increased salinity stress (Sunil kumar and Dharmaraj, 2003).

Another study by Sujatha and Nagarajan, (2014) reported that the pigment contents were found to be decreased at high saline concentrations when *Spirulina* was exposed to different concentrations of NaCl ranging from 0.1-0.4M besides control, over a period of 30days. It was found that biomass, total chlorophyll and phycocyanin stimulated at lower concentrations of NaCl (0.1 and 0.2M), but reduced at higher (0.3 and 0.4M) concentrations. Alike results were reported by Hanaa and Abd El-Baky, (2003) who found that increasing NaCl levels at 0.3M and 0.4M led to decrease in the production of phycocyanin and soluble protein contents in *Spirulina platensis*. Decrease in the phycocyanin pigments suggests that the cells may down-regulate their light harvesting capacity to acclimate their low carbon metabolic capacity. Hiremath and Mathad, (2010) too stated that the chlorophyll content of *Chlorella vulgaris* was stimulated in 0.1 and 0.2M NaCl but decreased in 0.3 and 0.4M NaCl, while beta-carotene and carbohydrates increased in 0.3M NaCl.

Moreover, Pasqualetti *et al*., (2011) analyzed the effect of salinity and nitrate concentration on the growth and carotenoid accumulation in a *Dunaliella* in the laboratory. The effects of 14.9% and 22% NaCl (w/v) and 212, 435 and 882µm nitrates were analyzed on the growth and carotenoids production. The results indicated that the highest carotenoids concentration was reported in 22% (w/v) NaCl and 212µm nitrate. Beside that, Tam *et al*., (2012) investigated the effect of different salt concentrations (0.8%, 1.5% and 2.5% NaCl) on the microalga *Haematococcus pluvialis*. The obtained results indicated that the high NaCl concentrations caused an increase in carotenoid content per cell and a decrease in the algal growth.

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