

Biochemical composition and cyst formation of *Tetraselmis marina* under varied temperature conditions

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Abstract

Tetraselmis marina was grown under varied temperature conditions (7 ± 1 ; 17 ± 1 ; 27 ± 1 and $33\pm1^\circ\text{C}$). Growth rate, dry weight, chlorophyll *a* content, total carbohydrate content, total lipid content and cell ultrastructure were investigated when there was growth, whereas only cell ultrastructure, and survival were investigated when no growth was observed. *Tetraselmis marina* was able to grow under temperature ranged from $7\pm1^\circ\text{C}$ to $27\pm1^\circ\text{C}$, with a relatively stable growth rate (0.69-0.91 division/day) and dry weight (0.188 - 0.241 gL^{-1}) respectively. Decreasing in chlorophyll *a* content and total carbohydrate content were observed under low temperature; this was coupled with increasing in total lipid content. Whereas no growth observed when *Tetraselmis marina* was maintained at $33\pm1^\circ\text{C}$. Transmission electron micrographs illustrate that, *Tetraselmis marina* grown under high temperature conditions ($33\pm1^\circ\text{C}$) forms non-motile cysts, these cysts are capable of rejuvenating when return to suitable growth temperature.

Key words: *Tetraselmis marina*, cysts, behavior, survival, Biochemical composition

Introduction

Marine microalgae have been recognized as promising resources for food supplements, cosmeceuticals, and biofuels (Da Silva *et al.*, 2016), *Tetraselmis* sp. includes many species that basically inhabit marine water. It is also comprising one of the most important phytoplankton that has been used in aquaculture, as a food for larval and juvenile bivalves, the genus has been also used for scientific research purpose. Temperature and salinity are among the most important factors that influence and control the growth, biochemical composition and survival of the phytoplankton (Abu-Rezq *et al.*, 1999; Shah *et al.*, 2018). Although there are several studies have been conducted to understand and illustrate the effect of the important factors (temperature, light, salinity, nutrition limitation, heavy metals and pesticides) on the growth rate and biochemical composition within the possible range of the growth (Ghezelbash *et al.*, 2008), a few studies have examined the effect of those factors on the ultrastructure, behavior and survival of phytoplankton.

The aim of the study

In the present study, we want to investigate and understand: -

- 1- How the ultrastructure, growth and biochemical composition of *Tetraselmis marina* is affected by the fluctuations of the temperature.
- 2-How cells of *Tetraselmis marina* behave in response to the varied growth temperatures.

Material and Methods

Tetraselmis marina was maintained in batch cultures, conical flasks (250 ml) each containing 150 ml working volume. For investigating the influence of fluctuation of the temperature, natural filtered-autoclaved seawater (salinity $28 \pm 1 \text{ gL}^{-1}$), enriched with F/2 medium (Guillard, 1975) as described and modified by Andersen *et al.* (2005), was used as medium, cultures maintained under 24 h light regime at 2500 Lux in an adjustable orbital checker incubator at 100 rpm. Triplicates were implemented at $7 \pm 1^\circ\text{C}$, 17°C , $27 \pm 1^\circ\text{C}$, and $33 \pm 1^\circ\text{C}$.

1- Measuring Growth rate

Growth was daily monitored by direct cell counting, after Lugol solution was added to the sample. cell counting was carried out using Bright lined improved Neubauer Haemocytometer (Germany), and Growth rate calculated according to the equation:

$$\mu = \ln(F1-F0)/t1-t0.$$

Whereas *F1* is the number of cells at *t1* (time1), *F0* is the number of cells at *t0* (time0).

2- chlorophyll *a* content and dry weight

Aliquots were collected when cultures reached the mid exponential growth phase, centrifuged at 3000 rpm for 15 min, the pellet resuspended in distilled water and recentrifuged as described. For extraction of chlorophyll *a*, acetone 90% was added to the pellet, kept in dark for overnight at 2°C , chlorophyll *a* estimated according to Jeffery and Humphrey (1975). The pellet was dried in an oven at 105°C , and weighted for the dry weight.

3- Biochemical composition

Total carbohydrates content extracted in 2M HCl at 90°C for 1 hour, and determined using Phenol-sulfuric acid method as described by Laurens (2012), glucose solution was used as standard. Total lipids were extracted in Methanol: Chloroform: Water 10:5:4, and estimated gravimetrically according to Bligh and Dyer as described by Sato (2021)

4- Motility and survival

Samples were collected twice daily, from cultures maintained at maintained at $33 \pm 1^\circ\text{C}$, examined under the light microscope, for motility monitoring (no Lugol solution added). For survival 1.5 ml was collected from each culture maintained under the conditions mentioned above, and transferred into conical flasks 100 ml, each contains 50 ml natural filtered-autoclaved seawater, enriched with F/2 medium, these flasks were maintained at $25 \pm 1^\circ\text{C}$, 12 h:12 h light/dark regime under 2500 lux. These cultures examined twice daily for observing the ability of cells to rejuvenate and revitalize.

5- Transmission Electron Microscope

Aliquots were collected of each culture, centrifuged at 2000 rpm for 15 min, the pellet was resuspended in distilled water and centrifuged again, the supernatant discarded and the pellet fixed using 1% Osmium tetroxide buffer (0.1 M, PH 7.2) for 1 h. Dehydration was carried out using graded Ethanol concentrations, followed by infiltration in spurr's resin: acetone (1:1) for 1 h, specimens transferred to pure spurr's resin for overnight.

Statistical analysis

The obtained data were analyzed using one way analysis of variance (ANOVA), Tukey's test at significant level 0.05.

Results and Discussion

Obtained results are summarized in Table 1. Maintaining *Tetraselmis marina* at temperature $27 \pm 1^\circ\text{C}$, resulted in the highest growth rate, chlorophyll *a* content and dry weight (0.91 d/day^{-1} , $2.85 \mu\text{g/ml}^{-1}$ and 0.241 gL^{-1}) respectively; but these were not significantly ($P > 0.05$) different compare to those maintained at $17 \pm 1^\circ\text{C}$ (Table 1). This indicates that, *Tetraselmis marina* can tolerate fluctuation in temperature from $17 \pm 1^\circ\text{C}$ to $27 \pm 1^\circ\text{C}$, with no remarkable effects on any of the previous investigated

parameters. On the other hand, growth rate, chlorophyll *a* content and dry weight were dramatically ($P<0.05$) decreased when the species grown at $7\pm1^{\circ}\text{C}$. Decreasing in photosynthetic pigments under low temperature has been reported (Christov, et al. 2001), this decrease reflects that cells were suffering of unfavorable conditions. Previous study (Murata, Takahashi *et al.*, 2007) reported that low temperature along with other factors such as CO_2 limitation, high concentrations of NaCl suppress the synthesis of proteins *de novo*, which is required for the repair of PSII. Depending on the obtained results, and in agreement with Develi *et al.* (2006), using the chlorophyll *a* content as a parameter to measure the growth of the microalgae, definitely will result in inaccurate results. Total lipid content significantly ($P<0.05$) increased, while total carbohydrate content significantly ($P<0.05$) decreased when *Tetraselmis marina* cultured at $7\pm1^{\circ}\text{C}$, compare to those maintained at $17\pm1^{\circ}\text{C}$ and $27\pm1^{\circ}\text{C}$; this might be attributed to the tendency of cells, to accumulate lipids as growth slows down, under low temperature conditions. The recorded decreasing in total carbohydrate content at ($7\pm1^{\circ}\text{C}$), might ascribed to the reduce in enzymes activities under low temperature conditions, it can also be considered as a consequence to the drop in chlorophyll *a* content, that caused by low temperature conditions. It has been reported that, there was no a consistent pattern of lipid production when microalgae are grown under varied growth temperature conditions (Renaud *et al.* 1995), increasing in total lipid content under low and high temperatures has been reported (Hanhua & Kunshan 2006; Ming-Li *et al* 2004).

Table (1): Growth rate, chlorophyll *a* content, dry weight, total lipids content and total carbohydrates content under different temperature conditions.

	Temperature ($\pm 1^{\circ}\text{C}$)			
	7°C	17°C	27°C	33°C
Growth rate (division/day⁻¹)	0.69 ^a	0.86	0.91	0.0
Chlorophyll <i>a</i> content ($\mu\text{g}/\text{ml}^{-1}$)	1.59 ^a	2.18	2.85 ^b	-
Dry weight (g/L^{-1})	0.188 ^a	0.226	0.241 ^b	-
Total Lipids content (%)	18.87 ^a	13.40	12.86	-
Total carbohydrates content (%)	08.8 ^a	11.33	12.98	-

* Total Lipids content and total carbohydrates content (%) of the dry weight.

* Values with different superscripts in the same row are significantly different.

Transmission electron micrograph (Figure 1.A), shows a thin section of *Tetraselmis marina* representing cells maintained at $17\pm1^{\circ}\text{C}$ and $27\pm1^{\circ}\text{C}$ (vegetative cells), the cell is laterally compressed, bears four equal flagella that emerge from an anterior pit of the cell. Transmission electron micrographs (Figure 1.B; C) illustrate non-motile (settled) cells, from cultures grown at $17\pm1^{\circ}\text{C}$ and $27\pm1^{\circ}\text{C}$ (respectively). These cells surrounded by a slightly thickened, cast-off theca. Each cell contains two daughter cells (sometimes divide asymmetrically), these dividing cells are still enclosed by parent cell wall. These cells are only observed in cultures maintained at $17\pm1^{\circ}\text{C}$ and $27\pm1^{\circ}\text{C}$. These results are in agreement with previous published results (Arora 2016; Hyung), but with respect to the cell wall, it looks thicker in the present study compared to the previous mentioned study. Transmission electron micrographs (1. D; E) show non-motile (settled) cysts, these cysts observed only in cultures maintained at $33\pm1^{\circ}\text{C}$, where no growth was observed under such this temperature condition, as a result of exposing cultures to this condition, cells gradually lost the capability of movement, became spherical in shape, forming the stage that known as cysts; these transmission electron micrographs also illustrate that, each of those cysts is surrounded by a thin cell wall, ornamented with different outer protuberances (scales), and a thick layer of protoplast situated beneath the cell wall. These cysts are capable of rejuvenating over two weeks of maintaining under these conditions, when returned to suitable growth temperature. The observed thick protoplast layer,

which situated beneath the cell wall, might act as an insulation to protect the inner components (such as the nucleus) of the cell, from the increasing in the external temperature.

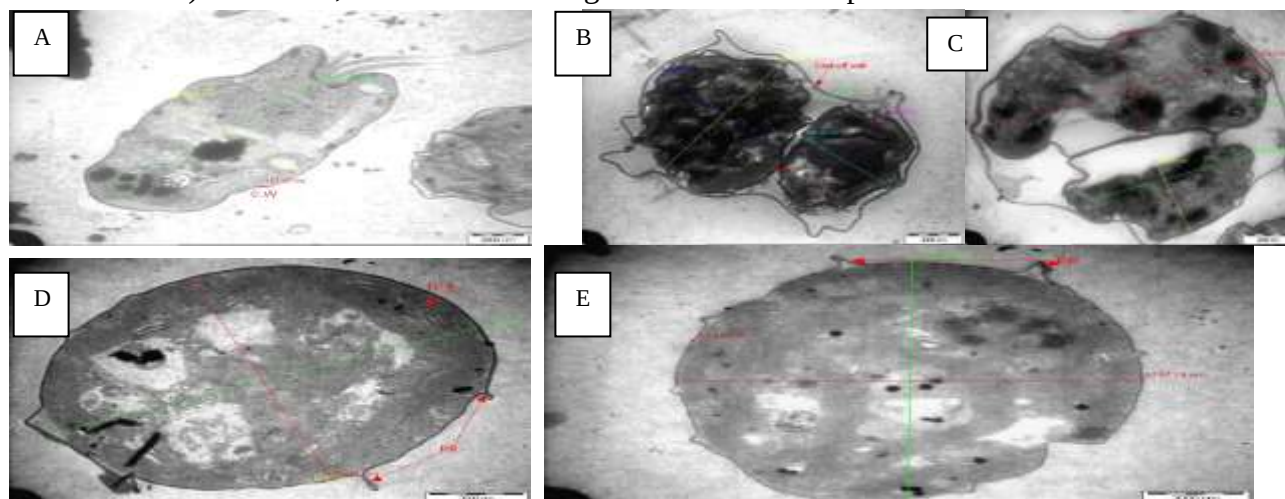


Figure (1): Transmission Electron microscope micrographs for *Tetraselmis marina* (A) vegetative cell at $27\pm 1^\circ\text{C}$; (B, C) cast-off stage at $27\pm 1^\circ\text{C}$ and $17\pm 1^\circ\text{C}$, respectively; (D, E) cyst stage at $33\pm 1^\circ\text{C}$. Abbreviations (C.W) cell wall. (P) protoplast. (PR) protuberances.

Tetraselmis marina cysts were described first time by Norris *et al.* (1980) using transmission electron microscope, although the given description in the previously mentioned study did not specify the conditions that the cysts were formed under, a cyst with a thick often ornamented cell wall in *Tetraselmis marina* had been described. While Butcher (1959) used the term “cyst” to describe cells which were surrounded by cast-off walls. In the present study, non-motile cells, each contains two daughter cells and surrounded by cast-off walls are only observed when cultures maintained under favorable growth conditions. This is in agreement with previous study (Arora *et al.*, 2015), which described this stage as reproductive stage. It has been reported that *Tetraselmis marina* reproduces by dividing in non-motile stage, and most species dividing once producing two daughter cells. According to the physiological and environmental conditions the daughter cells develop to motile (flagellate) stage or remain in non-motile phase. (Norris *et al.* 1980; Arora M. 2016). In the present study non-divided cysts with thin scaled cell walls were observed only when cultures maintained under unfavorable conditions.

Conclusion; based on the results obtained, *Tetraselmis marina* cells showed ability to grow over a wide range of temperature ($7-27\pm 1^\circ\text{C}$) with a relatively constant growth rate. The Biochemical composition was clearly affected by the fluctuation in temperature ($7\pm 1^\circ\text{C}$). Undivided cysts, with different ultrastructural aspects, were formed when cultures maintained under unfavorable conditions (high temperature $33\pm 1^\circ\text{C}$), the observed cysts showed capability to last and survive under the mentioned temperature, whereas cast-off settled cells (considered as reproductive stage), each divided into two daughter cells, were only observed when the species maintained under favorable growth conditions.

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التركيب البيوكيميائي وتكوين الحويصلات في طحلب *Tetraselmis marina*

النامي تحت درجات حرارة مختلفة

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المخلص:

تم في هذا البحث تنمية الطحلب *Tetraselmis marina* تحت درجات حرارة متباينة ($7\pm 1^\circ\text{C}$; $17\pm 1^\circ\text{C}$; $27\pm 1^\circ\text{C}$)، لدراسة تأثير درجات الحرارة على معدل النمو، الوزن الجاف، محتوى الكلوروفيل أ ، المحتوى الكلي للكربوهيدرات، المحتوى الكلي للدهون، بالإضافة الى التغير في التركيب الدقيق لخلايا الطحلب تحت درجات الحرارة التي لوحظ نمو عندها، أما عند درجات الحرارة التي لم يسجل نمو عندها فقد تم دراسة التغير في التركيب الدقيق للخلايا وقدرتها على تكوين الأبواغ والبقاء فقط. أظهرت خلايا الطحلب قدرة على النمو تحت كل من درجات الحرارة $7\pm 1^\circ\text{C}$; $17\pm 1^\circ\text{C}$; $27\pm 1^\circ\text{C}$ بمعدل نمو يتراوح من 0.69 d/d الى 0.91 d/d ، ووزن جاف من 0.188 الى 0.241 جم/لتر. كما لوحظ انخفاض في محتوى الكلوروفيل أ عند تنمية الطحلب تحت درجة حرارة منخفضة ($7\pm 1^\circ\text{C}$) مصحوب بانخفاض في المحتوى الكلي للكربوهيدرات وارتفاع ملحوظ في المحتوى الكلي للدهون، في حين لم يلاحظ أي نمو عند تنمية الطحلب عند درجة حرارة $33\pm 1^\circ\text{C}$ ، وأوضحت صور المجهر الإلكتروني تكوين الطحلب لأبواغ غير سابحة عند هذه الدرجة، والتي أظهرت القدرة على استعادة الحركة والنمو عند إعادة تنميتها تحت درجات حرارة مناسبة.

الكلمات المفتاحية: *Tetraselmis marina*، الحويصلات، السلوك، البقاء، التركيب الكيميائي الحيوي.