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Effects of Varying Temperature on the Growth Rate of Toxic and Non-Toxic Strains of *Microcystis aeruginosa*

Gbenga Folorunsho Oginni[1*], Lord Setsoafia Dzakpasu[2], Ayo Oisagah Ezekiel[3]

[1]Laboratory for Environmental and Life Sciences, University of Nova Gorica, Nova Gorica, Slovenia
[2]Hvivo Research Centre, Imperial College London, London, UK
[3]Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria
Email: *gbenga.oginni@yahoo.com

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Abstract

Cyanobacterial bloom, also called blue-green algae, all over the world is generating great concern due to the effect of temperature resulting from global warming. *Microcystis aeruginosa* is well known to be the major causative agent of algal bloom. However, there is a need for more critical detection to the relationship between temperature and the growth rate of *Microcystic aeruginosa.* In the present study, we cultivated the batch culture of *Microcystis aeruginosa* (Toxic and Non-toxic strain) at five different temperatures 5˚C, 10˚C, 22˚C, 27˚C, and 38˚C to measure its specific growth rate, pigment contents and cell concentration at each temperature. Our findings indicate that non-toxic strains had higher growth **rate** at high temperature than the toxic strains, particularly at 22˚C. Also, a minimal difference was noticed with the chlorophyll a, carotenoid contents of both strains at varying temperature. The above results indicate the significance of temperature in respect to the two strains of *M. aeruginosa* and could constitute a promising tool in the prediction of algal bloom.

Keywords

Cyanobacterial, *Microcystis aeruginosa,* Temperature, Algal Bloom, Pigment, Global Warming

1. Introduction

The distribution of Cyanobacteria in marine and freshwater habitats induces a prominent bloom in water, with different degree of toxicity [1]. An increase in the rate of blooms was noticed in recent decades [2] [3], which is also expected

to increase further, consequently due to global warming [4]. *Microcystis aeruginosa* often produce the hepatotoxin, which is harmful to human and animal health. It was reported that the ratios of toxic to non-toxic cells are highly variable in field samples [5] [6], but factors favouring growth of toxic and non-toxic strains still need to be critically examined.

Cyanobacteria blooms that harm people, animals, or the environment are called cyanobacteria harmful algal blooms. Harmful cyanobacteria blooms may affect people, animals, or the environment by blocking the sunlight that other organisms need to live. Cyanobacteria blooms can steal the oxygen and nutrients other organisms need to live, also making toxins, called cyanotoxins. Cyanotoxins are among the most powerful natural poisons known. Several factors are responsible for the continuous rise in atmospheric temperature, some of which includes indiscriminate burning of fossil fuels, inappropriate industrial discharge into atmosphere and as such increasing the level of carbon dioxide in atmosphere, a condition which directly increases the temperature of the atmosphere, in a long turn escalating the rate of algal bloom [7].

Global change appears to be disrupting phytoplankton seasonal successions within their communities' structures of "plankton phenology" [8]. Competitive advantage is gained by species growing in aquatic ecosystems with conditions such as, higher temperatures and adapted to low-light conditions [3], which favours Cyanobacteria especially in shallow lakes [9]. Numerous researches reported on advanced onsets of phytoplankton blooms as ecological reaction to global warming and changes in plankton phenology [10] [11] [12] [13]. Toxic cyanobacteria (*M. aeruginosa*) have reported high temperature growth rate at 25˚C. Also, toxin production was also observed to have increased with increase in temperature for photosynthesis and growth [3] [14] [15] [16] [17] [18]. The growth of phytoplankton can be influenced directly by temperature through metabolic system changes and nutrient uptake rates [16] [19] [20].

Several factors as a result of photosynthesis and respiration gave rise to increase in oxygen [21]. Therefore, an inexpensive mechanism which could clearly differentiate between toxic and non-toxic strain with respect to their physiology at a certain bloom state and time is needed. In the past, records demonstrate that ecological examination by cell count exhibits at least some amount of success for toxic strains but its application was seen to take a while and not entirely dependable. Consequently, this study aims to determine the growth of *M. aeruginosa* (Toxic and non-toxic strain) in laboratory batch cultures at various temperatures and to determine the variation in the growth form at different temperature conditions.

2. Materials and Methods

2.1. Cyanobacterial Culture Growth

Axenic culture of *Microcystis aeruginosa* non-toxic (PCC 7005) and toxic (PCC 7806) strains were obtained from Institute Pasteur (Paris, France). Five ml of

pre-cultured organisms were grown for two weeks at 28˚C and constant rocking (130 rpm) in 250 ml flasks until they reached the concentration of 10^8 cells/ml and then used to inoculate 50 ml of sterilized Blue Green (BG-11) medium. Cultures were grown for 6 - 8 weeks under continuous agitation (130 rpm) and 12 h light/dark cycles until the concentration reached 2×10^7 cells/ml. Cultures were irradiated with cool white fluorescent tubes with a photon flux density of 30 μmol·m^{-2}·sec^{-1}. Subcultures were prepared by aseptically inoculating 50 ml of fresh BG-11 medium with 5 ml of *M. aeruginosa* from 6 - 8 weeks culture above. [22]

2.2. Growth Experimental Setup

In this experiment, *M. aeruginosa* (toxic strain PCC 7806) and (non-toxic strain 7005) cells were subjected to varying temperature conditions ranging from 5˚C, 10˚C, 22˚C, 27˚C, 37˚C (degree Celsius).Cultures were placed in the refrigerator to achieve the temperature 5˚C and 10˚C, While 22˚C, 27˚C and 37˚C were monitored in the presence of a thermometer in an incubator. The culture was made to undergo shaking twice a day. This was done for all the experimental temperature conditions. Cultures were irradiated with cool white fluorescent tubes with a photon flux density of 30 μmol·m^{-2}·sec^{-1} at 12 h light/dark cycles. At exponential stage of growth, the cells were used in the estimation of the growth rate, pigmentation and Cell concentration [23].

2.3. Pigment Analysis

Chlorophyll a was examined at three- and six-days intervals under varying temperatures with light intensity remaining constant at 30 μmol·m^{-2}·sec^{-1} at 12 h light/dark cycles. Measurements were taken by harvesting cells through centrifugation at 5000×g for 20 minutes, followed by resuspending in 80% methanol and kept in the dark at 4 degree Celsius overnight. The resulting suspension was centrifuged again and the supernatant was used to measure the absorbance at 620, 650, and 680 nm using a Cary-50 UV-spectrophotometer. Chlorophyll a content was determined with the absorbance value from the UV-spectrophotometer. Carotenoid concentrations were estimated using the same extract but with absorbance measured at 480 nm [24].

2.4. Cell Concentration

Cell number of *M. aeruginosa* (PCC 7806 and PCC 7005) were estimated by counting with Neubauer hematocytometer under Olympus IX81 inverted epi-fluorescence microscope equipped with a 300 W Xenon lamp at ×40 magnification. To determine *Microcystis aeruginosa* cell concentration, 10 μl of *M aeruginosa* culture were loaded in a Levy Hemocytometer (Hausser Scientific 100 MM deep, USA) cell counting chamber. Cells in major quadrants were counted under the microscope and the cell numbers from 4 major quadrants was averaged. The cell number per ml was calculated applying the formula [24].

Cell concentration/ml = average cell number × 10,000 × dilution factor.

3. Results and Discussion

The growth rate indicated that toxic strain (PCC 7806) shows growth at both 5˚C and 10˚C but the growth was seen to be slow compare to the Non-toxic strain (PCC 7005) where growth was a little faster, this growth response could be due to differences in their photosynthetic and respiratory abilities. Optimum growth for both strains was seen at 22˚C as seen in **Figure 1(a)** and **Figure 1(b)**, this is also in line with the results obtained for chlorophyll a content and carotenoids contents below, which are photosynthetic pigments supporting growth, this agrees with the work described by [24] in his study on the interdependence between light and the growth of *Microcystis aeruginosa* (PCC 7806).

The cell concentration in relation to temperature change for PCC 7005 (Non-toxic) strain and PCC 7806 (toxic strain) indicated that this algal species were

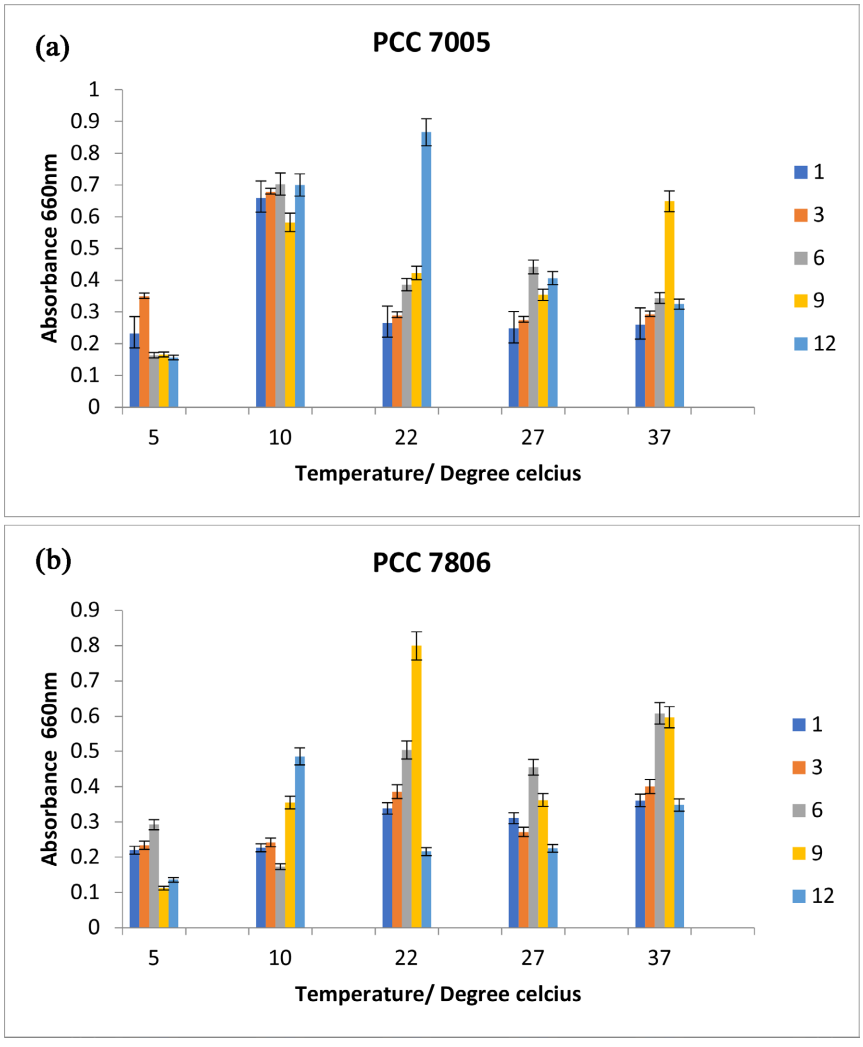


Figure 1. (a) Growth measurement of non-toxic strain of *Microcystis aeruginosa* (PCC 7005) plotted against temperature in degree Celsius; (b) Growth measurement of toxic strain of *Microcystis aeruginosa* (PCC 7806) plotted against temperature in degree Celsius.

able to increase in cell number over a broad temperature range of 5˚C and 37˚C, with lower and higher temperature effecting the cell production, the cell concentration was found to be highest at 22˚C, a similar result was obtained on fourteenth day culture at 22˚C, the result therefore affirm that temperature had much effect on cell concentration than time duration **Figure 2(a)** and **Figure 2(b)**. Not surprising, all showed a strong relationship to temperature this is similar to the report of [25] where he investigated the comparison of cyanobacterial and green algal growth rates at different temperatures, showing that the optimum growth temperature for *P. agardhii* is around 27˚C. Thus these strains are able to increase in cell concentration at much lower temperatures than previously suggested by the literature, this therefore means influence of environmental conditions might have resulted to PCC 7005 and PCC 7806 adapting to

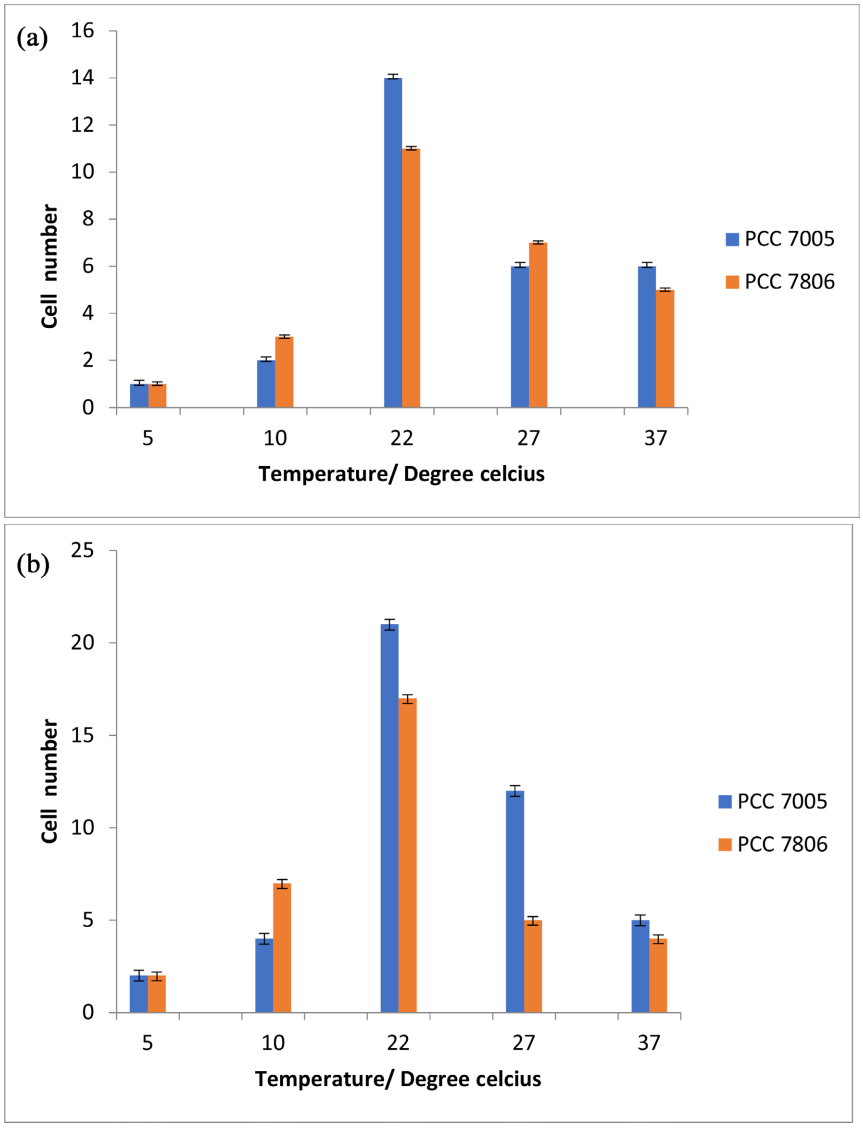


Figure 2. (a) Mean cell concentration at Nine days culture of *M. aeruginosa* strain PCC 7806 and 7005 plotted against temperature; (b) Mean cell concentration of fourteenth day culture of *M. aeruginosa* strain PCC 7806 and 7005 plotted against temperature.

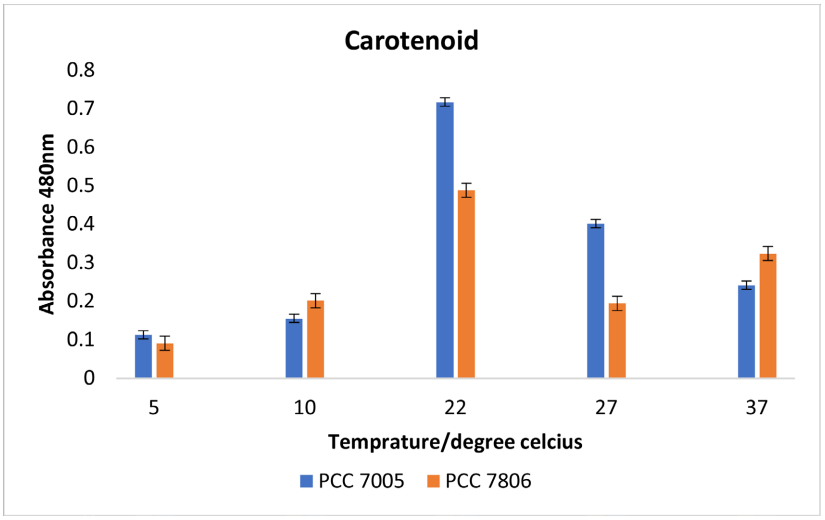
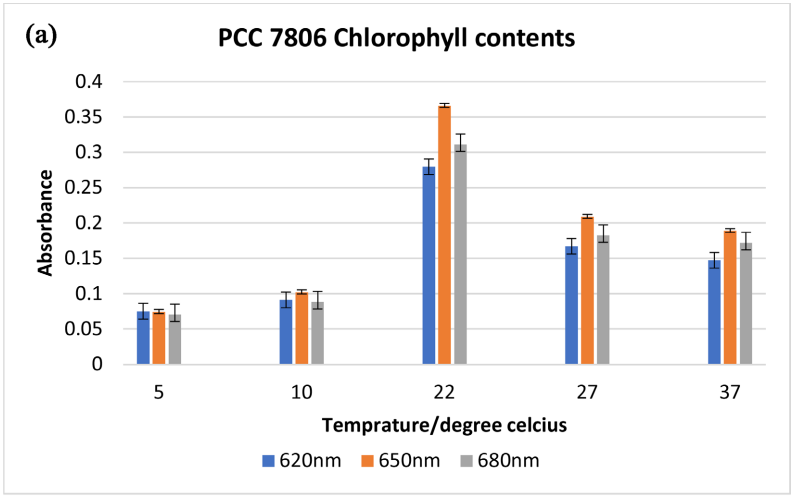


Figure 3. Measurement of carotenoid contents at 480 nm plotted against temperature for *M aeruginosa* strain PCC 7806 and 7005.



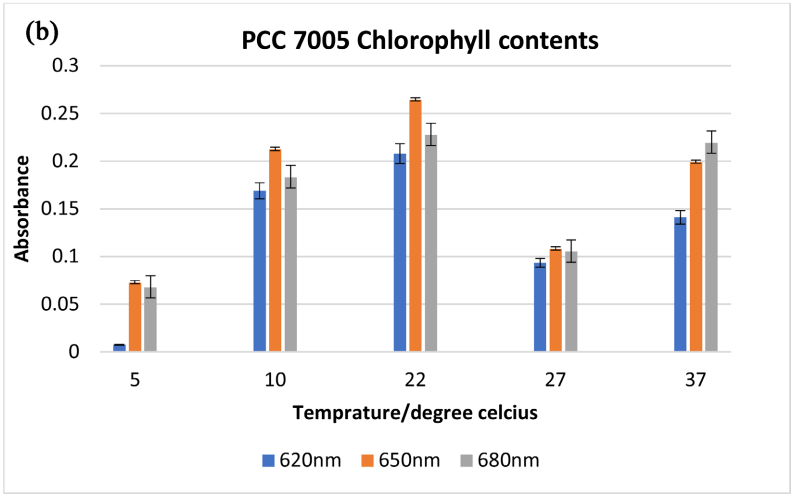


Figure 4. (a) Measurement of Chlorophyll a content plotted against temperature at 620,650 and 680 nm for *M. aeruginosa* PCC 7806; (b) Measurement of Chlorophyll a content plotted against temperature at 620,650 and 680 nm for *M. aeruginosa* PCC 7005.

optimal temperature growth close to 22˚C.

The carotenoid content was examined at 480 nm for both strains. The highest values of carotenoid were seen at 22˚C for PCC 7806 and PCC 7005, PCC 7005 had the highest carotenoid content. However, the lowest value of carotenoid content was observed at 5˚C for both strains **Figure 3**. The results indicated a relationship between carotenoid content and temperature, the optimum temperature for increase in carotenoid content was found to be around 22˚C for PCC 7005 and PCC 7806 strains, therefore during photosynthesis, PCC 7005 and PCC 7806 strains may have ability to prevent harmful photodynamic reaction at a temperature of 22˚C. This is similar to the work of [26] who studied chlorophyll and carotenoids analysis using spectrophotometer.

The chlorophyll a content was checked at three wavelength (620 nm, 650 nm and 680 nm) the highest values for both strains were obtained at 650 nm and PCC 7005 had highest values, the chlorophyll a contents values were higher at a temperature of 22˚C for both PCC 7005 and PCC 7806 strains this is illustrated in **Figure 4**. This is an indication that wavelength and temperature are important in the anabolic processes of PCC 7005 and PCC 7806 strains which in turn indicates a higher light absorbing potentials at 650 nm and 22˚C, this is similar to the study of [27] [28] who stated that some *strains Microcystis aeruginosa* UTEX 2388 and CYA 228 have associated microcystin production and chlorophyll *a* content in relation to temperature.

4. Conclusion

Measurements were conducted to investigate the effects of temperatures on *M. aeruginosa* strains PCC 7806 and PCC 7005 growth rate, variation in chlorophyll and carotenoids contents and cell concentrations, both strains were found to have optimum temperature to be 22˚C, with non-toxic strain of *Microcystis aeruginosa* (PCC 7005) having stronger affinity. This is an indication that growth and development of toxic and non-toxic strains of *M. aeruginosa* are temperature dependent. The results from this study could serve as a guide in the monitoring and control of algal bloom particularly caused by *M. aeruginosa*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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