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Effect of Salinity and Temperature on the Growth of Diatoms and Green Algae

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ABSTRACT

Salinity and temperature are two of the major factors controlling the growth rate of microalgae. In this study, the effect of salinity and temperature on the growth of marine microalgae; an unidentified *Chlorella* sp. and *Chaetoceros calcitrans* were investigated to optimize the microalgal biomass production. These species were cultured at different salinities (20, 25 and 30‰) and temperatures (20, 25 and 30°C). *C. calcitrans* and *Chlorella* sp. had significantly higher ($p<0.05$) growth rate when cultured at salinities of 30 and 25‰, respectively. In terms of temperature, the highest ($p<0.05$) growth rate was observed in *C. calcitrans* and *Chlorella* sp. cultivated at temperatures of 30 and 25°C, respectively. This study indicated that *C. calcitrans* was suitable to marine condition, whereas *Chlorella* sp. showed optimum growth at lower salinity and temperature.

Key words: Microalgae, diatoms, green algae, *Chaetoceros*, *Chlorella*, salinity, temperature

INTRODUCTION

Marine microalgae are an excellent source of protein, lipid, carbohydrate and vitamins, i.e., A, B, C, E, folic acid and pantothenic acid (Becker, 2007). Their high nutritional values can provide a high quality nutritional package for different stages of aquaculture animals (Banerjee *et al*., 2010; Khatoon *et al*., 2009, 2012). Microalgae that have been found to have good nutritional properties includes *Chaetoceros calcitrans* (a diatom) and a green alga; *Chlorella* sp. (Natrah *et al*., 2007; Goh Jr. *et al*., 2009; Goh *et al*., 2010). Optimizing the algae growth rates by manipulating the environmental parameters could become an effective approach in increasing microalgal biomass production (Mata *et al*., 2010). In fact, marine microalgae have been projected as potential species for various industrial applications due to their fast growth rate and valuable chemical contents (Singh and Gu, 2010).

Salinity and temperature have been shown to induce the characteristic of the nutritional properties in microalgae (Hemaiswarya *et al*., 2011). Moreover, different culture environment such as seasonal fluctuation, effect of low and high temperature and the species origin can also cause variable growth rates of microalgae (Oliveira *et al*., 1999; Thompson *et al*., 1992; Banerjee *et al*., 2011). Previous study on the arctic sea diatom *Chaetoceros* spp. showed that the species tolerate low salinity (Zhang *et al*., 1999).

Variations in salinity also influence several biochemical and physiological mechanisms such as lipid production and growth which are essential in marine organisms (Fava and Martini, 1988). Although temperature is the easiest factor that can be controlled in the practical operation of microalgae cultivation, it is a sensitive factor for algae growth and metabolic processes. Different temperatures have been associated with various quality and composition of microalgae.

There are limited studies on the effect of various salinities and temperatures on phytoplankton especially involving marine *Chlorella* sp. and *C. calcitrans*. Thus, a study on the effect of different salinities and temperatures on the growth of two marine microalgae *C. calcitrans* and *Chlorella* sp. was carried out in order to contribute to better understanding on this matter.

MATERIALS AND METHODS

Algae cultures: *Chaetoceros calcitrans* (UPMC-A0010) and *Chlorella* sp. (UPMC-A0013) were obtained from the Laboratory of Marine Biotechnology, University Putra Malaysia (UPM). Stock cultures were maintained regularly on both liquid and agar plates of Conway medium (Walne, 1966).

Experimental design: Five-day old culture of *Chlorella* sp. and *C. calcitrans* (10^4 cells mL^{-1}) were used as inocula. Ten percent (30 mL v/v) of inocula were inoculated into 500 mL Erlenmeyer flask containing 300 mL of fresh Conway medium with salinities of 20, 25 and 30‰. All treatments were carried out in triplicates. Salinity lower than 30‰ was adjusted by diluting the seawater using Milli-Q water. The cultures were sparged with filter sterilized air at 0.1 min (v/v) and continuously illuminated by fluorescent lamp at a light intensity 120 μmol^{-2} sec^{-1}, with temperature 25±1°C. The experiment was carried out for 14 days.

The analyses of different growth temperatures were carried out by cultivation of 10^4 cells mL^{-1} inoculum in 500 mL Erlenmeyer flask containing 300 mL of fresh Conway medium with salinity 30±2‰. The replicates were cultured in incubators with temperatures of 20, 25 and 30°C under continuous illumination at 600-800 lux light density and sparged with filter-sterilized air by using air pump without additional carbon dioxide.

Analytical methods: Biomass estimation and determination of cell number. One milliliter aliquots of cultures were filtered on fiberglass Whatman GF/F filters using a vacuum pump and washed with a solution of ammonium formate (0.5 M) to remove silicate residue. The filters were dried at 100°C for 4 h to volatilize the ammonium formate. The dry weights of algae biomass were determined gravimetrically and the growths were expressed in terms of dry weight (g DW L^{-1}). Meanwhile, the cell number was determined and counted using haemocytometer.

Growth analysis: Growth was expressed in terms of growth rates using the following equation:

$$\text{Growth rate } (\mu)\ (\text{day}^{-1}) = \frac{\ln (F_1/F_0)}{t_1 - t_0}$$

where, F_1 is the biomass at time of harvest (t_1) and F_0 is biomass at time of zero (t_0) (Guillard, 1973).

Statistical analysis: Statistical package for social sciences (SPSS 15.0) were applied for one way ANOVA tests in the evaluation of differences in the mean values. Significance was tested at 95% level.

RESULTS

Effect of salinity on growth: The best growth of *C. calcitrans* occurred at salinity of 30‰, with the highest growth rate of 0.28±0.03 μ day^{-1} ($p<0.05$) and maximum cell count of 2.7×10^6 cells mL^{-1} on the 12th day of the culture period (Fig. 1 and 2a). Meanwhile, *Chlorella* sp. reached the maximum cell count of 3.1 x 10^6 cells mL^{-1} on 12th day in culture grown at salinity of 25‰ with a growth rate of 0.37±0.01 μ day^{-1} (Fig. 1 and 2b, $p<0.05$). The maximum biomass ($p<0.05$) obtained from *C. calcitrans* and *Chlorella* sp. were 1.52±0.2 and 1.34±0.4 g DW L^{-1}, respectively (Fig. 3).

Effect of temperature on growth: In *C. calcitrans*, the highest growth rate was observed in cultures at 30°C (0.27±0.02 μ day^{-1}) with maximum cell count ($p<0.05$) of 2.1×10^6 cells mL^{-1} on day 10 (Fig. 4, 5a). However, there were no significant changes ($p>0.05$) in growth rates in cultures grown at 20 and 25°C. Meanwhile, *Chlorella* sp. showed the best growth at 25°C with the highest growth rate of 0.35±0.04 μ day^{-1} and cell count of 2.9×10^6 cells mL^{-1} on day 10th (Fig. 4 and 5b, $p<0.05$). *Chaetoceros calcitrans* and *Chlorella* sp. reached the maximum biomass ($p<0.05$) of 1.44±0.5 and 1.42±0.3 g DW L^{-1}, respectively (Fig. 6).

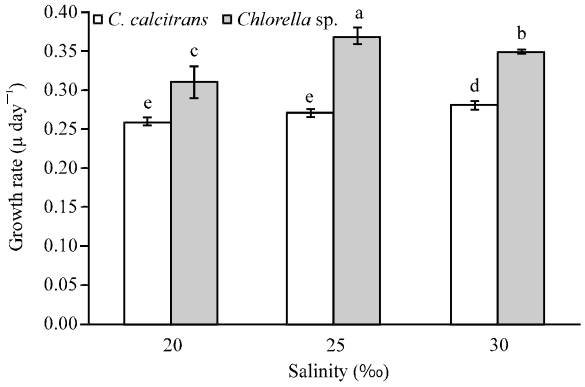

Fig. 1: Growth rates of *Chaetoceros calcitrans* and *Chlorella* sp. in response to different salinities, Vertical bars are means±SE (n = 3). Means with different letters are significantly different at $p<0.05$

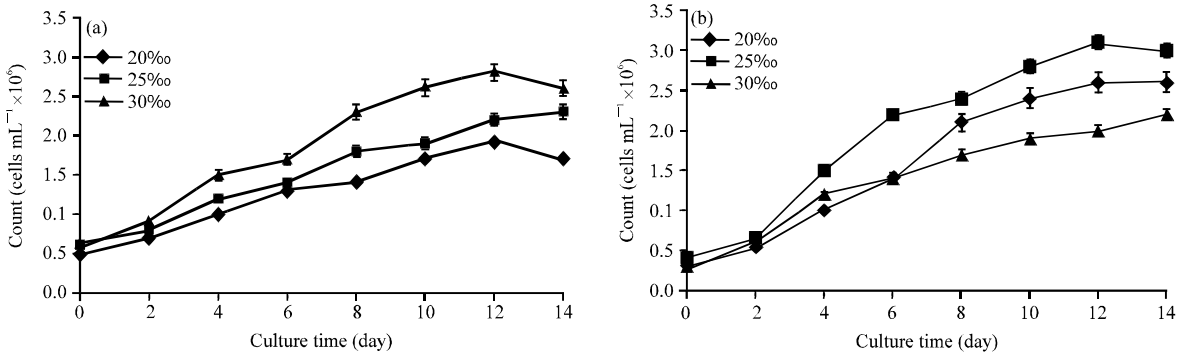

Fig. 2(a-b): Changes of mean densities (n =3) of (a) *Chaetoceros calcitrans* and (b) *Chlorella* sp. under different salinities during the culture period

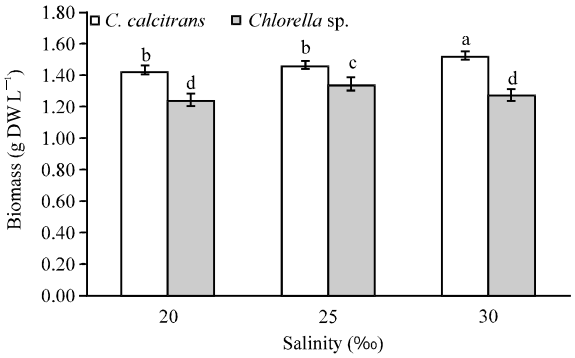

Fig. 3: Biomass for *Chaetoceros calcitrans* and *Chlorella* sp. under different salinities, Vertical bars are means±SE (n = 3). Means with different letters are significantly different at $p<0.05$

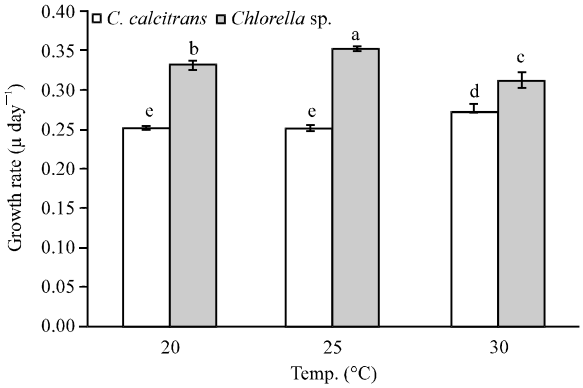

Fig. 4: Growth rates of *Chaetoceros calcitrans* and *Chlorella* sp. in response to different growth temperatures, Vertical bars are means±SE (n = 3). Means with different letters are significantly different at $p<0.05$

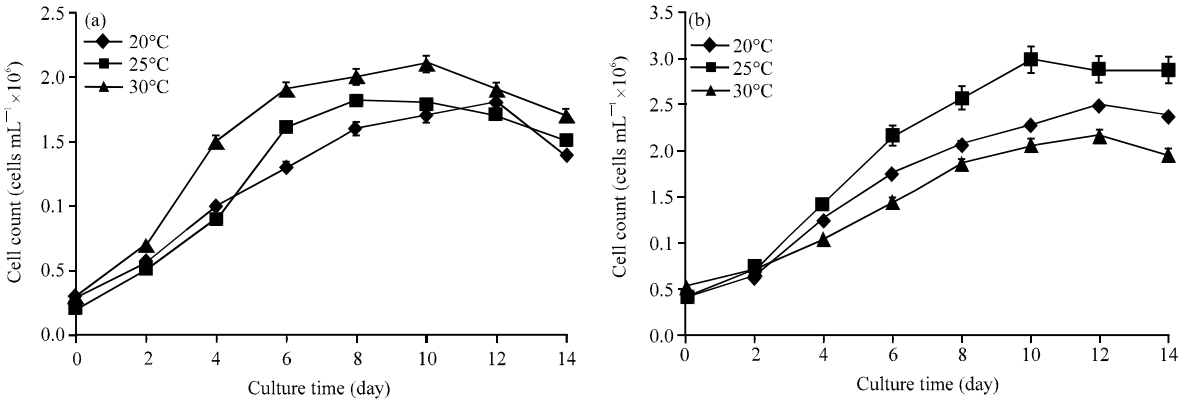

Fig. 5(a-b): Changes in mean cell densities (n = 3) of (a) *Chaetoceros calcitrans* and (b) *Chlorella* sp. under different temperatures during the culture period

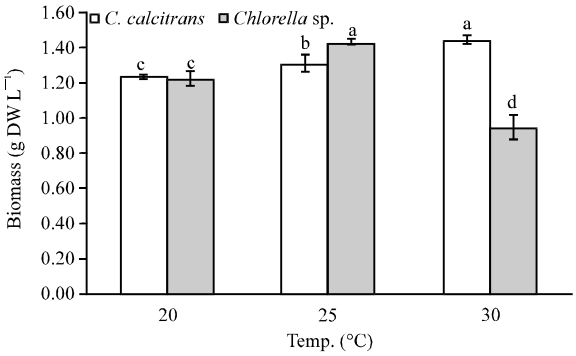

Fig. 6: Biomass of *Chaetoceros calcitrans* and *Chlorella* sp. at different temperatures, Vertical bars are means±SE (n = 3). Means with different letters are significantly different at $p<0.05$

DISCUSSION

Microalgae growth is highly dependent on the environmental conditions where the variables that affect the growth rates are different from one species to another. However, the most studied variables are salinity, pH, temperature, light as well as the nutrition (Banerjee *et al*., 2011; Liang *et al*., 2009; Raghavan *et al*., 2008; Ji and Sherrell, 2008). In the present study, *C. calcitrans* and *Chlorella* sp. showed that the growth rate increased with increasing salinity. In the present study, *C. calcitrans* and *Chlorella* sp. reached the highest growth rates of 0.28±0.03 and 0.37±0.01 μ day^{-1}, respectively at the optimum salinity. In fact, the biomass production of *C. calcitrans* and *Chlorella* sp. achieved in this study was higher than the values reported in previous studies (<1.0 g DW L^{-1}) (Araujo and Garcia, 2005).

Salinity is the main factor for the process of giving life to plants and can cause retardation of central metabolic activities such as photosynthesis (Liska *et al*., 2004). Microalgae differ in their adaptability to salinity and based on their tolerance as they are grouped as halophilic and halotolerant (Rao *et al*., 2007). In natural condition or marine waters, active multiplication normally started at day 5 or 7 of the growth phase. The culture can last for 2 or 3 weeks depending on the microalgae species. In this study, *C. calcitrans* culture increased progressively from the beginning of the culture period culminating in maximum densities and biomass on day 12 in all treatments. There were significant differences ($p<0.05$) in the growth rate amongst different salinities indicating that salinity was an important factor to be considered when culturing *C. calcitrans* on a large scale. Similarly, *Chlorella* sp. showed similar growth pattern with the highest production on the 12th day. However, the effect of salinity on *Chlorella* sp. growth was significantly different compared to *C. calcitrans* as the growth response was slowest at high salinity (Fig. 1). Different algae species response varies to different salinities as have been reported in previous studies (Huang *et al*., 2011; Hu and Gao, 2006; Takagi *et al*., 2006; Zhang *et al*., 1999). Sudhir and Murthy (2004) illustrated that although high salt content influence physiological process in microorganism, each species differs in the growth response to salinity.

Saros and Fritz (2000) showed that diatom physiology can be affected directly or indirectly via interaction with other growth factors such as the ion composition in the saline system. In a few species of diatoms, low salinity can result in decrease of cell dimension (Lynn *et al*., 2000). However, other diatom species such as *Thalassionema eccentrica* and *Pseudo-nitzschia seriata* are able to

survive salinity up to 150‰ (Nagasathya and Thajuddin, 2008). Although both marine microalgae are capable of growing and photosynthesizing in salinities ranging from 20 to 30‰, significant decrease ($p<0.05$) in growth patterns as salinity decreases was observed in *C. calcitrans*. This trend might be associated with the limitation of nutritional factors in sea water after dilution (Raghavan *et al*., 2008).

In the present study, both microalgae species were able to tolerate growth temperature range from 20 to 30°C. *Chaetoceros calcitrans* performed best at 30°C (0.27±0.01 μ day^{-1}) and this finding was within the range (27-30°C) reported by Renaud *et al*. (2002). At its optimum temperature, the cell density of *C. calcitrans* observed in this study was significantly higher than the values (1.6×10^5 cells mL^{-1}) reported by Phatarpekar *et al*. (2000). Meanwhile, lower growth temperatures reduced the cell density of this diatom. Sheehan (1998) illustrated that increasing the growth temperature induced the cell proliferation, probably due to the changes of cell metabolic activities in response to the environmental stress.

The green microalgae *Chlorella* sp. growth appeared to be at optimum when cultured at 25°C (0.35 μ day^{-1}). At this temperature, the growth rate was 40% higher than value reported in *C. vulgaris* (Converti *et al*., 2009). Thus, this study illustrated that *Chlorella* sp. proliferated at a maximum rate at low temperature, an observation which was also reported in *Chlorella sorokiniana* (Franco *et al*., 2012). Although *Chlorella* sp. cultured at high temperature (30°C) resulted in significant decrease ($p<0.05$) in growth rate, the values obtained in this study were two times higher than those reported for *C. vulgaris* cultured at a similar temperature (Converti *et al*., 2009). This finding illustrated that tolerance to temperature changes is species specific (Oliveira *et al*., 1999). The ability of *Chlorella* sp. and *C. calcitrans* in adapting to different temperatures indicated that they are versatile candidates for outdoor mass culture.

CONCLUSION

This study illustrated that the salinity of 30‰ and temperature of 25-30°C induced optimum cell proliferation in *C. calcitrans*. Meanwhile, *Chlorella* sp. required a lower salinity of 25‰ and temperature of 25°C for optimum growth. It can be concluded that both marine microalgae had different ranges of tolerance and adaptability to environmental changes. In this study, *Chlorella* sp. and *C. calcitrans* were demonstrated as fast growing microalgae and they can be considered as suitable species for large outdoor microalgal cultivation.

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