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Comparison of the Growth Performance of *Nannochloropsis oceanica* IMET1 and *Nannochloropsis gaditana* CCMP526 under Various Culture Conditions

Yongxue Chi, Yasuyuki Takiguchi*

Department of Life and Environmental Sciences, Chiba Institute of Technology, Chiba, Japan

Email address:
yongxue101@hotmail.com (Yongxue Chi)

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Abstract: We studied the growth performance of *Nannochloropsis oceanica* IMET1 under various culture conditions, including different CO_2 concentrations, temperature, or light intensities compared with that of *N. gaditana* CCMP526. When CO_2 concentrations were changed, the growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 were the highest at a CO_2 concentration of 2 vol%. *N. oceanica* IMET1 had a higher overall growth rate than that of *N. gaditana* CCMP526. The best growth rate occurred at 30°C, and higher growth rates were generally exhibited by *N. oceanica* IMET1 than *N. gaditana* CCMP526. The growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526 when light intensity was changed. These results indicate that *N. oceanica* IMET1 has better growth performance compared with that of *N. gaditana* CCMP526. The optimum growth conditions for *N. oceanica* IMET1 is CO_2 concentration of 2 vol%, light intensity of 53 μmol/m^2/s, and 30°C in modified BG11 medium.

Keywords: *Nannochloropsis oceanica* IMET1, *N. gaditana* CCMP526, Growth Performance, Culture Conditions, Microalgae

1. Introduction

Nannocholopsis sp., which includes marine, fresh, and brackish water algal species, is a potential resource for biofuel production [1–5]. In addition, *Nannocholopsis* sp. is a resource for high quality protein [6], eicosapentaenoic acid [4], and omega-3 fatty acid [7].

Nannochloropsis oceanica IMET1 collected from Eilat, Israel has been maintained at the Institute of Marine and Environmental Technology (IMET) for > 10 years. *N.oceanica* IMET1 has also been distributed to and studied in other laboratories.

Wang *et al.* investigated the effect of bacterial communities isolated from groundwater at different temperatures on biofuel production using *N. oceanica* IMET1 [8]. Xiao *et al*. evaluated the effects of nitrogen deficiency stress on liquid content, fatty acid distribution, and metabolic profiles under CO_2 concentrations of 1.5–2 vol % at 20°C [9]. Dong *et al*. reported that long-term nitrate depletion changes the *N. oceanica* IMET1 proteome using a two-dimensional gel electrophoresis proteomic approach [1]. Ma *et al*. applied heavy-ion irradiation to improve *N. oceanica* IMET1 characteristics for biofuel production [10]. Li *et al*. clarified the triacylglycerol-producing mechanism in *N. oceanica* IMET1 under N-repleted and N-depleted conditions [11].

The growth performance and characteristics of IMET1 under various culture conditions such as different temperatures, CO_2 concentrations, light intensities, and media have not been reported. Understanding growth performance under different conditions is important to determine the optimum conditions for producing biofuel, eicosapentaenoic acid, and omega-3 fatty acid. Therefore, we investigated the effects of CO_2 concentration, temperature, light intensity, and culture media on the growth performance of *N. oceanica*IMET1 and *N. gaditana* CCMP526.

2. Materials and Methods

2.1. Algal Strains and Culture Conditions

*N. oceanica*IMET1 and *N. gaditana*CCMP526 were used

in this study. *N. oceanica* IMET1 was obtained from the IMET, Maryland University Center for Environmental Science. We used *N. gaditana*CCMP526 based on the good growth performance of other CCMP under the same culture conditions. *N. gaditana* CCMP526 was purchased from the National Center for Marine Microalgae and Microbiota.

A modified BG11 (MBG11) medium and artificial seawater (ASW) medium were used to grow both strains.

BG11 [12] is a common culture medium for freshwater algae and contains more nutrients than those in ASW medium. MBG11 medium is BG11 with salinity adjusted to 30 ppm with NaCl. ASW medium was prepared according to the ingredients described by Radakovits *et al*. [13].

Both *N. oceanica* IMET1 and *N. gaditana* CCMP526 were routinely maintained in flasks containing ASW. The log-phase cultures were harvested, and the pellets were washed (6,000 × g, 10 min) with the respective medium to remove wastes. The algal pellets were re-suspended in either ASW or MBG11 medium. One ml of each culture was added to one well of a 48-well Costar plate (Corning Glass, Corning, NY, USA), and six replicates were performed for each culture. The microplates containing the algal cultures were placed inside a GasPak^{TM} bag (Becton Dickinson, Parsippany, NJ, USA).

2.2. Specific Growth Rate

Cell density ($O.D._{600}$) was determined at an absorbance of 600 nm using a multi-mode microplate reader (Spectra Max M5; Molecular Devices, Sunnyvale, CA, USA). The microplate was taken from the GasPak^{TM} bag to read absorbance and was then placed back into the GasPak^{TM} bag.

Cell density on day 0 was set to an $O.D._{600}$ of 0.35. Cell density was measured every 2 days at the media change time. All $O.D._{600}$ data indicated the average value of n = 6 with the standard deviation.

Specific growth rate μ was calculated from the $O.D._{600}$ value after 2 days of culture (OD_2) and the $O.D._{600}$ value after 8 days of culture (OD_8) using the following equation

$$\mu = \ln(OD_8/OD_2)/6$$

The specific growth rate was the average value of n = 6 with the standard deviation.

2.3. Growth Conditions

The effect of CO_2 concentration on growth rate was investigated under the following conditions. The CO_2 concentration (vol%) was set by blending pure CO_2 and air using a gas mixture device with two gas flow meters [14]. The microplates containing the algal cultures were placed in a GasPak^{TM} bag and charged with 0, 2, 10, 15, and 20 vol %. The algal cultures were incubated at 23°C with a light intensity of 53 μmol/m^2/s and a 12 h/12 h light-dark cycle.

The effect of light intensity on growth rate was investigated under the following conditions. Light intensities were set to 13, 53, 82, and 132 μmol/m^2/s, respectively. The algal cultures in the microplates were incubated under a CO_2 concentration of 2 vol% at 23°C with a 12 h/12 h light-dark cycle. Each treatment contained six replicates.

The effect of temperature on growth rate was investigated under the following conditions. Temperature was set to 10, 23, and 30°C, respectively. The algal cultures in the microplates were incubated under a 2 vol % CO_2 concentration, light intensity of 53 μmol/m^2/s, and a 12 h/12 h light-dark cycle. Each treatment contained six replicates.

3. Results and Discussion

3.1. Growth Performance under Different CO_2 Concentrations

Fig. 1 shows the culture time and $O.D._{600}$ values for *N. oceanica* IMET1 and *N. gaditana* CCMP526 in ASW or MBG11 under various CO_2 concentrations. Fig. 2 shows the comparison of growth rate in ASW or MBG11 under various CO_2 concentrations.

The growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 were the highest at a CO_2 concentration of 2 vol%. Moreover, the growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 were higher in MBG11 medium than those in ASW medium. Growth of both strains was inhibited at higher CO_2 concentrations (Figs. 1, 2).

N. oceanica IMET1 maintained relatively higher growth rates compared with those of *N. gaditana* CCMP526 at CO_2 concentrations of 10, 15, and 20 vol%. The growth rate of *N. oceanica* IMET1 outperformed the growth rate of *N. gaditana* CCMP526 in both ASW and MBG11 culture media at the higher CO_2 concentrations. At a CO_2 concentration of 20 vol%, in MBG11 medium, *N. gaditana*CCMP526 nearly ceased growing ($\mu = 0.002$ d^{-1}); however, the growth of *N. oceanica* IMET1 only slowed down at ~35 vol% compared to the highest growth rate at the 2 vol% CO_2 concentration. Both strains seemed to grow better in MBG11 medium than in ASW medium, particularly under the higher CO_2 concentrations. The growth rate of *N. oceanica* IMET1 outperformed that of *N. gaditana* CCMP526 at all tested CO_2 concentrations in ASW medium.

Hu and Gao reported that biomass yield while supplying air with 2,800 μl CO_2 l^{-1} is higher than that supplying air with 350 μl CO_2 l^{-1}, when *Nannochloropsis* sp. (PP983) is used [15]. Chiu *et al.* reported that biomass concentration and growth rate at a CO_2 concentration of 2 vol% were higher than those at other CO_2 concentrations because *N. oculata* utilizes CO_2 in response to adding CO_2 [16]. We also found the highest growth rate at a CO_2 concentration of 2 vol%. Chiu *et al.* also reported that biomass concentration and growth rate decreased when the CO_2 concentration was > 5 vol%, which correlates with our result. Although growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 decreased when CO_2 concentration was >10 vol%, the growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526. The growth performance of *Nannochloropsis* spp. was not clear; therefore, it is difficult to understand the reasons for the difference in growth rate over CO_2 concentration of 10 vol% between *N. oceanica* IMET1 and

N. gaditana CCMP526. This may have resulted from the different genetic backgrounds between *N. oceanica* IMET1 and *N. gaditana* CCMP526, which are not closely related [13]. In addition, *N. oceanica*IMET1 has different genes in the Kennedy pathway from those of *C. reinhardtii* [11].

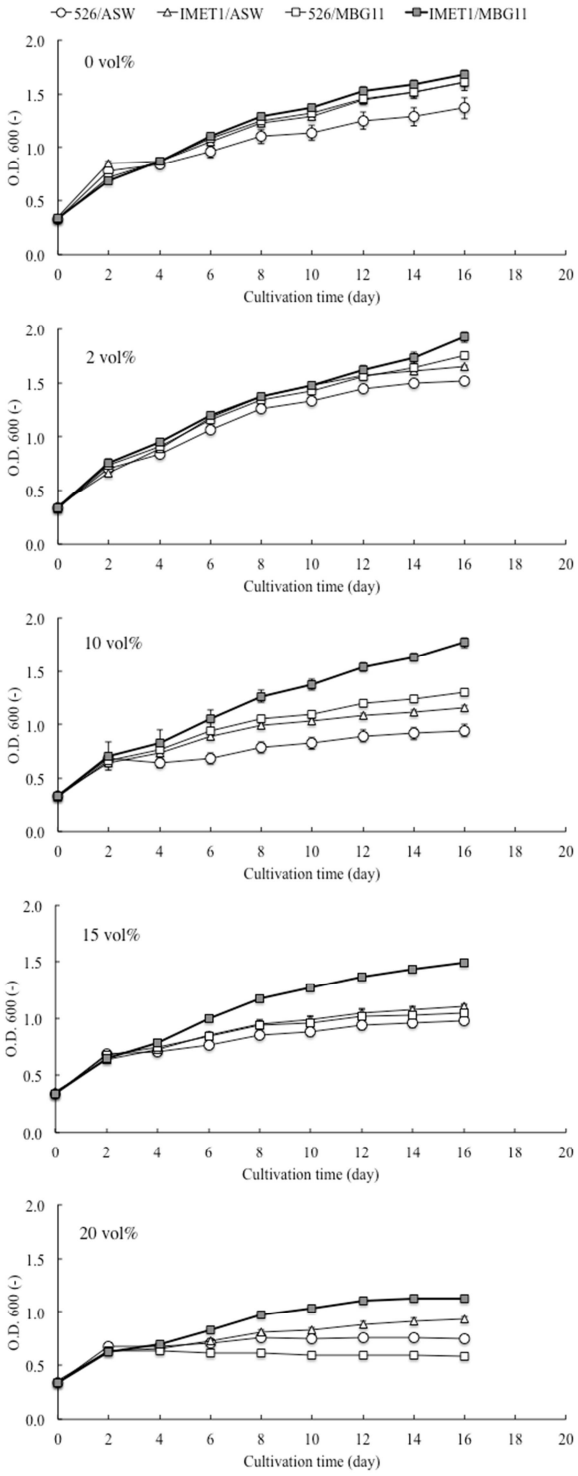

Figure 1. *Growth curves for Nannochloropsis gaditana CCMP526 and N. oceanica IMET1 cultured at CO_2 concentrations of 0, 2, 10, 15, and 20 vol%. All cultures were maintained at 23°C with 53 µmol/m^2/s light intensity and a 12 h/12 h light/dark (LED source) cycle. The two culture media (MBG11 and ASW) were compared. Each data point is the average of six replicates (n = 6), and error bars represent standard deviations (n = 6), p < 0.05.*

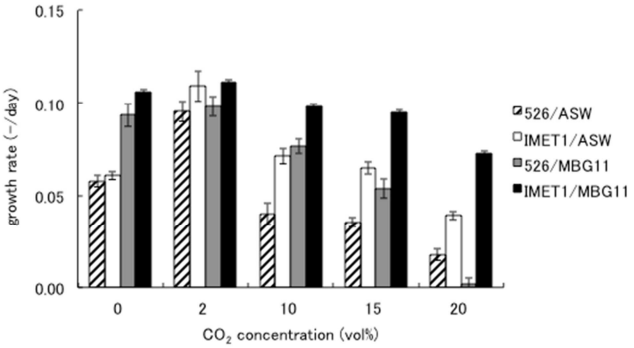

Figure 2. *Specific growth rates of Nannochloropsis oceanica IMET1 and N. gaditana CCMP526 under different CO_2 concentrations. Both strains were cultured at 23°C with a light intensity of 53 µmol/m^2/s and a 12 h/12 h light/dark (LED source) cycle. Each data point is the average of six replicates, and error bars represent standard deviations (n = 6), p < 0.05.*

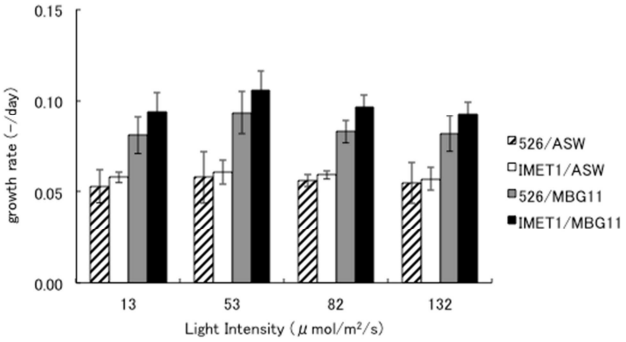

Figure 3. *Specific growth rates of Nannochloropsis oceanica IMET1 and N. gaditana CCMP526, cultured under different light intensities (13, 53, 82, and 133 µmol/m^2/s), at 23°C with a 12 h/12 h light/dark cycle. Each data point is the average of six replicates, and error bars represent standard deviations (n = 6), p < 0.05.*

3.2. Growth Performance under Four Light Intensities

Fig. 3 shows the comparison of growth rates between *N. oceanica* IMET1 and *N. gaditana* CCMP526 irradiated under light intensities of 13, 53, 82, and 132 µmol/m^2/s. The growth rate of *N. oceanica* IMET1 in ASW was nearly the same as the growth rate of *N. gaditana* CCMP526 in ASW at the various light intensities. The growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 in MBG11 medium were higher than those in ASW. The growth rate of *N. oceanica* IMET1 in MBG11 was maximum at a light intensity of 53 µmol/m^2/s and was higher than that of *N. gaditana* CCMP526 regardless of light intensity. These results indicate that light intensity affects the growth rate of *Nannochloropsis* in MBG11. In addition, the growth rate of *N. oceanica* IMET1 in MBG11 was the highest at a light intensity of 53 µmol/m^2/s.

Optimal growth of both strains occurred at 53 µmol/m^2/s. Because the experiments were conducted in 48-well microplates, the optimal light intensity was generally lower than the optimal light intensity of a flask-based culture system. Growth of both *N. oceanica* IMET1 and *N. gaditana* CCMP526 was photoinhibited to some extent at a light intensity of 132 µmol/m^2/s. *N. oceanica* IMET1 and *N.*

gaditana CCMP526 again preferred MBG11 medium to ASW medium. Dipasmita also reported the same result in a light intensity experiment in which the growth rate of *Nannochloropsis* sp. declined under high light intensity in 13 g/L NaCl [18].

3.3. Growth Performance under Different Temperatures

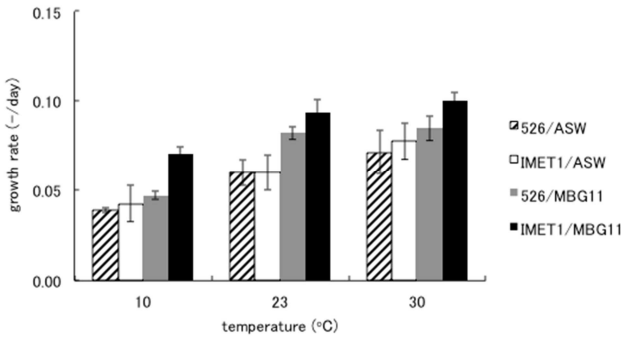

Figure 4. *Specific growth rates of Nannochloropsis oceanica IMET1 and N. gaditana CCMP526 under different temperatures (10, 23, and 30°C). Both strains were cultured at a light intensity of 53 µmol/m²/s with a 12 h /12 h light/dark (LED source) cycle. Each data point is the average of six replicates, and error bars represent standard deviations (n = 6), p < 0.05.*

Fig. 4 shows the comparison of the growth rates between *N. oceanica* IMET1 and *N. gaditana* CCMP526 at 10, 23, and 30°C. The growth rates of *N. oceanica* IMET1 and *N. gaditana* CCMP526 increased with a rise in temperature. The growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526 at all temperatures, regardless of the medium. These results indicate that the growth rate of *N. oceanica* IMET1 at 30°C was the best in MBG11 medium (μ = 0.1 d^{-1}). Growth of *N. oceanica* IMET1 was inhibited at 10°C in a CO_2 concentration of ~25 vol%. Although the growth rates of *N. gaditana* CCMP526 at 23 and 30°C were acceptable, the growth rate of *N. gaditana* CCMP526 at 10°C was inhibited (μ = 0.03 d^{-1}). *N. oceanica*IMET1 and N. *gaditana*CCMP526 grew better in MBG11 medium than that in ASW medium. In general, both strains maintained rapid growth at 23 and 30°C; however, their growth rates significantly decreased when temperature dropped to 10°C. Wu *et al.* reported that the maximum growth rate of *Monoraphidium* sp. SB2 occurred at 30°C [18]. Their result correlated with our result. These results suggest that *N. oceanica* IMET1 had higher growth rates than those of *N. gaditana* CCMP526, regardless of temperature or culture conditions. These results may be because of the activities of five putative genes related to carbon fixation such as carbonic anhydrate mediated carbon-concentrating metabolism and a C4 cycle mechanism in *N. oceanica* IMET1 [11].

In this study, the growth performance of *N. oceanica* IMET1 and *N. gaditana* CCMP526 was compared in 48-well microplates. The microplate culture system is different from other culture systems such as flasks or bioreactors. Therefore, it may be difficult to compare our results with those obtained from large cultivation systems with air bubbling. For example, growth of *N. oculata*NCTU-3 was completely inhibited when cultured at CO_2 concentrations of 5 vol%, 10 vol%, and 15 vol% in flasks [16]. In our experiments, different concentrations of CO_2 were contained in GasPak™ bags. Nevertheless, the growth rates of these two *Nannochloropsis* strains were compared in parallel in this study and they differed greatly in terms of their capability of handling different environmental stressors.

4. Conclusion

We investigated the growth performance of *N. oceanica* IMET1 compared with that of *N. gaditana* CCMP526. The growth performance of *N. oceanica* IMET1 was better than that of *N. gaditana* CCMP526. In particular, the growth rate of *N. oceanica* IMET1 was higher than that of *N. gaditana* CCMP526, regardless of culture medium under CO_2 concentrations > 10 vol%. In addition, growth of *N. oceanica* IMET1 was maintained at a high level under a wide range of light intensities and temperatures in both culture media. The optimum growing conditions for *N. oceanica* IMET1 were a CO_2 concentration of 2 vol%, light intensity of 53 µmol/m²/s, and 30°C when MBG11 was used.

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