J. Fish. Sci. Tech. 3(2), 151~155, 2000

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Effects of Nitrogen and Sodium on Growth in *Phaeodactylum tricornutum* (Bacillariophyceae)

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(Received April 2000, Accepted September 2000)

Phaeodactylum tricornutum (Bacillariophyceae) is a marine diatom which has been supplied as a food of bivalves. In this study, growth responses of *P. tricornutum* to some nitrogen sources and sodium were investigated by measuring cell number and contents of chlorophyll *a* in culture. In medium with nitrogen and sodium, brisk cell division occurred and maximum growth rate was respectively found in the medium with 150 mg/ℓ of nitrate and 10 mg/ℓ of ammonium and urea. At 10~500 mg/ℓ ammonium and urea and 200~500 mg/ℓ nitrate, specific growth rate decreased slightly. However, no cell division observed in sodium-deficient medium, regardless of presence or absence of nitrogen. This suggests that sodium is required for the nitrogen uptake of *P. tricornutum*, resulting nitrogen uptake leading to cell division. Also the upper limits of ammonium and nitrate for the growth of *P. tricornutum* seem to be 10 mg/ℓ and 500 mg/ℓ, respectively.

Key words: *Phaeodactylum tricornutum* (Bacillariophyceae), nitrogen, sodium, growth response

Introduction

Nitrogen, phosphorus and iron are major nutrients controlling growth of phytoplanktons (Lobban and Harrison, 1994), although they are variable in both space and time (Falkowski et al., 1998). Among them, nitrogen is often a limiting nutrient in the sea (Codispoti, 1989). In general, natural nitrogen sources are frequently in mixtures of inorganic and organic compounds. Ammonium, nitrate and urea, which are inorganic compounds, are considered as a available main nitrogen source for the growth of phytoplankton (Levasseur et al., 1993).

It has been known that sodium, which is the predominant cation in seawater, is also required for the operation of several nutrient transport systems in unicellular marine algae (Rees et al., 1980; Larson and Rees, 1996). Dickson and Kirst (1987) and Zhao and Brand (1988) have focused it on the cell metabolism in marine microalgae including osmoregulation and photosynthesis. Recently, Larson and Rees (1994) reported that sodium is related with cell division.

In this study, effects of nitrate, ammonium and urea associated with sodium in growth of *Phaeodactylum tricornutum*, which has been used in aquaculture of bivalves as a food, are investigated to improve our knowledge of growth responses in this species.

Material and Methods

Phaeodactylum tricornutum (Korea Marine Microalgae Culture Center, KMCC B-14) was axenically cultured in f/2 medium (Guillard and Ryther, 1962) and modified ASP2 medium (Larson and Rees, 1994) at a temperature of 21 ± 1℃ under continuous illumination by cool-white fluorescent lamps (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$). As a nitrogen source, $NaNO_3$ (>99.0 at %, SIGMA), NH_4Cl (>99.5 at %, SIGMA) and urea (>99.5 at %, SIGMA), were added with 0, 10, 50, 100, 150, 200 and 500 mg/ℓ. Cultured subsamples were daily counted microscopically in cell number using a counting chamber (improved Neubauer hemocytometer) to calculate growth rate. The growth constant, divisions per day and the division

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(generation) time follows the solutions of Guillard's equation (Guillard, 1973). Specific growth rate (day^{-1}) was determined by three of more assays at different times during the exponential growth phase by means of linear regression (Suzuki and Takahashi, 1995).

To examine the effects of nitrogen and sodium deficiency, other subsamples were grown in nitrogen- and sodium-deficient medium by harvesting and washing for 5 days prior to the start of the experiments. Nitrogen and sodium (in 10 mℓ distilled water) were added to cultures as follows: (i) water only, (ii) 10 mM KNO_3 or NH_4Cl, (iii) 50 mM NaCl, and (iv) 10 mM KNO_3 or NH_4Cl+50 mM NaCl. The initial condition for the experiments was set in modified ASP2 medium, and 10 mℓ of exponentially growing cell suspension (approximately 6×10^6 cells) was harvested aseptically by centrifugation at 1500 g for 7 min. Then it was washed three times, and inoculated into Erlenmeyer flasks containing 100 mℓ nitrogen-deficient medium in the presence or absence of sodium to give an initial cell density of about 2.35×10^4 cells·mℓ$^{-1}$. The growth of *Phaeodactylum tricornutum* was determined by cell number and chlorophyll *a* contents. Samples for chlorophyll *a* analysis were collected by filtering 2 mℓ of culture onto cellulose-nitrate filters (0.45 μm pore size, Whatman), which were stored at -20℃ until analysis. The filters were extracted by sonication in 5 mℓ extraction solvent (3 : 1 90% acetone : dimethylsulphoxide) (Shoaf and Lium, 1976). Absorbance was measured on a CE 599 (Cecil Inst. Ltd., U.K.) UV/Vis spectrophotometer and chlorophyll *a* content was calculated using the equations of Jeffrey and Humphrey (1975). Data were analysed statistically by one-way analysis of variance (ANOVA).

Results and Discussion

The growth of *Phaeodactylum tricornutum* in f/2 medium showed the typical sigmoid curve. The active growth was not found for the first 3 days (0~0.26 div./day), but thereafter cell number was rapidly increased and peaked at the 11th day (2.47 div./day, 1.69×10^5 cells/mℓ).

The specific growth rate of *Phaeodactylum tricornutum* was dependent on nitrogen concentration (Fig. 1). It increased gradually at 10~150 mg/ℓ nitrate, but slightly decreased at 200~500 mg/ℓ (Fig. 1a). The maximum value of the specific growth rate was 0.85 at 150 mg/ℓ nitrate. However, as ammonium was added as a nitrogen source, the specific growth rate exhibited a peak at 10 mg/ℓ, and then rapidly decreased at 10~500 mg/ℓ (Fig. 1b). Its maximum value was observed as 0.39/day. As the nitrogen source was changed to urea, growth pattern of *P. tricornutum* was similar to that in ammonium but more gradually decreased at 10~500 mg/ℓ (Fig. 1c). The maximum specific growth rate was 0.61/day.

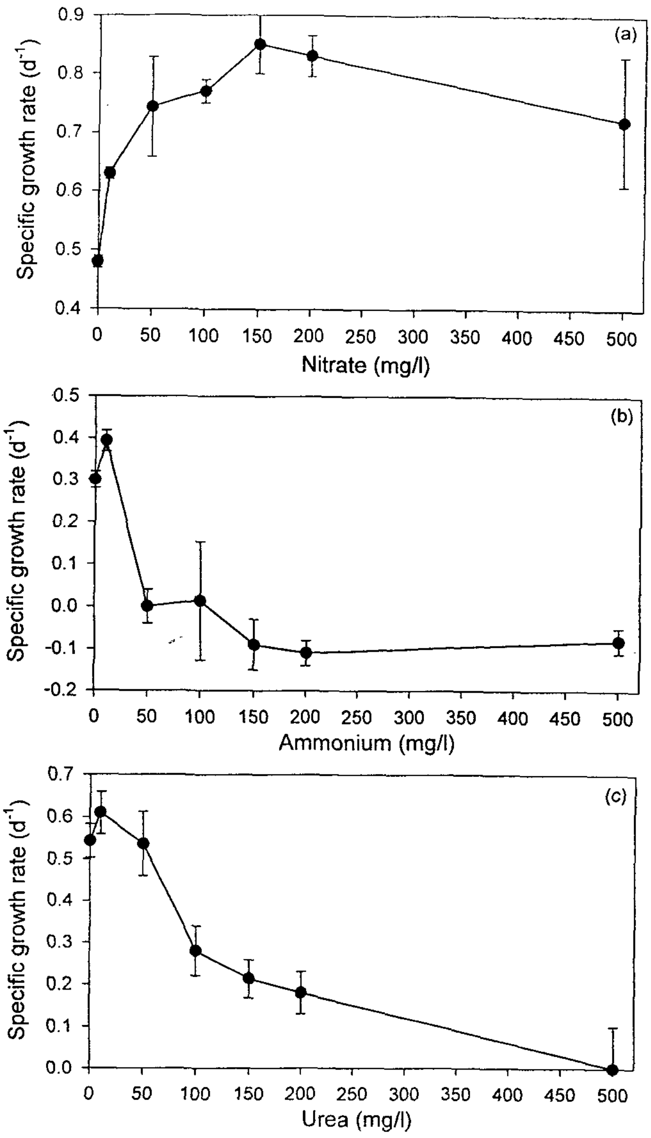

Fig. 1. Specific growth rate of *Phaeodactylum tricornutum* to nitrogen (a), ammonium (b) and urea (c).

In nitrate/sodium-deficient culture, no changes in the cell number and chlorophyll *a* contents were observed (Figs. 2a, 3a). However, in culture with nitrate, the growth, such as cell number and chlorophyll *a* contents, was influenced on the presence or absence of sodium. In the presence of sodium, the cells divided swiftly to reach a density of 2.60×10^5 cells·mℓ$^{-1}$ by day 5 (Fig. 2a). Contents of chlorophyll *a* was also rapidly increased (Fig. 3 a). By contrast, there was no change in cell number and chlorophyll *a* contents in the sodium-deficient culture (Figs. 2a, 3a).

In ammonium/sodium culture, similar results were obtained and the cells reached to a maximum density of 1.28×10^5 cells·mℓ$^{-1}$ by day 3 (Fig. 2b). However, unlike in nitrate, cell number and chlorophyll *a* content immediately reduced in culture only with ammonium (in sodium-deficient culture) (Figs. 2b, 3b).

Nitrogen deficiency affects algal growth (McCarthy and Goldman, 1979), nitrogen uptake (Syrett, 1981) and assimilation of nitrogenous compound (Syrett and Peplinska, 1988). In some dinoflagellates and diatoms, sexual reproduction and encystment can be also induced by nitrogen deficiency (Brawley and Johnson, 1992; McQuoid and Hobson, 1996). Among these, decline in nitrogenous photosynthetic pigments (chlorophylls, phycobilins) is one of the most obvious effects of the algal nitrogen-deficiency (Turpin, 1991). In this study, it was also observed that chlorophyll *a* contents and growth rate of *Phaeodactylum tricornutum* are limited by nitrogen deficiency or insufficient nitrogen supply. They were also inhibited by sodium deficiency, regardless of presence or absence of nitrogen in medium, as mentioned above. It has been known that unicellular marine algae demand sodium for successful nitrogen uptake (Rees et al., 1980). According to Larson and Rees (1994), an electrochemical gradient of sodium ions drives transport systems. Also they reported that sodium may be associated with the protein synthesis in cell membrane or act as an intracellular signal in different stages of the cell cycle. However, control mechanism of cell division has not been clearly revealed yet. Even though direct role of sodium on cell division cannot be examined, these results suggest that sodium is required for the nitrogen uptake of this species, resulting nitrogen

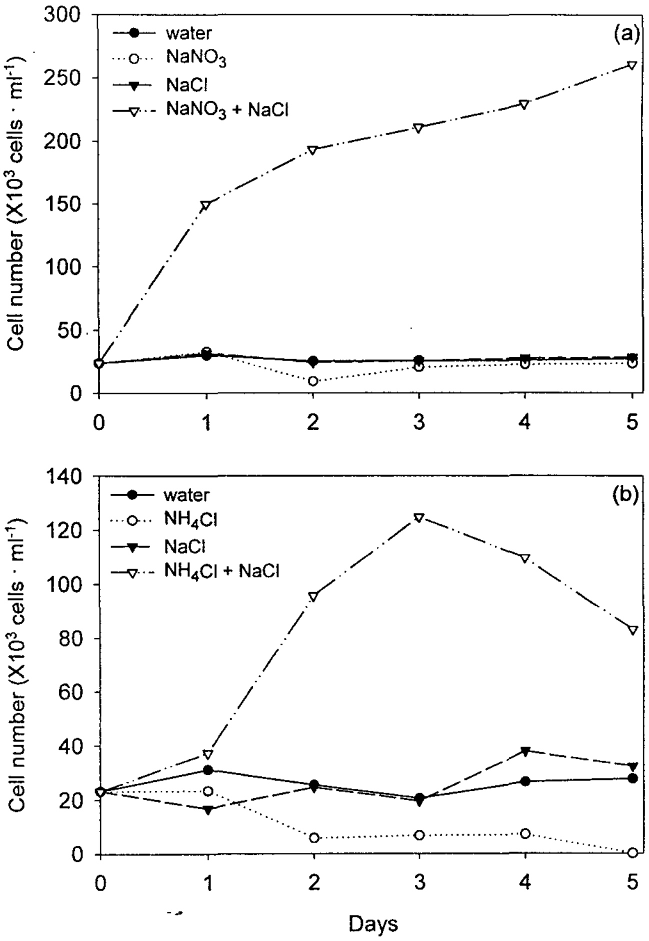

Fig. 2. Response of cell number to nitrogen and sodium for *Phaeodactylum tricornutum* grown with nitrate (a) or ammonium (b).

uptake leading to cell division.

High nitrogen concentration, which is over optimum range, also inhibits algal growth (Achiha and Iwasaki, 1990; Iwasaki et al., 1990). Particularly growth-inhibiting effects of ammonium for microalgae have been reported by many authors (Thomas et al., 1980; Carpenter and Dunham, 1985; Hillebrand and Sommer, 1996), even though ammonium is preferentially taken up as a more readily utilizable form (Dortch, 1990). Its upper limiting concentration is various for each species (ZoBell, 1935; Thomas et al., 1980; Bates et al., 1993). Iwasaki (1967) documented general upper limit of ammonium for the growth of *Phaeodactylum tricornutum* was around 0.7 mg/ℓ. Terry et al. (1985), however, cultured it successfully in 3.4~8.5 mg/ℓ ammonium. In this study, its upper limit exhibited similar range to that of Terry et al. (1985), but differed from

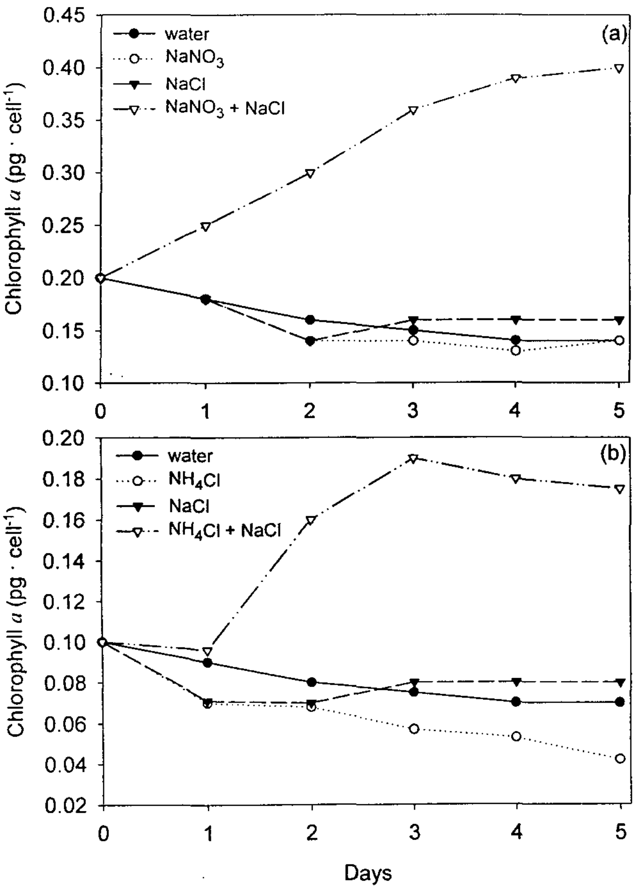

Fig. 3. Response of chlorophyll *a* contents to nitrogen and sodium for *Phaeodactylum tricornutum* grown with nitrate (a) or ammonium (b).

Iwasaki's report (1967). This may be due to different strain of the species.

In culture, *Phaeodactylum tricornutum* shows the three typical morphological types, triradiate, fusiform or oval form (Lewin et al., 1958). The former two forms were found only in this study. These forms seem to be depended on the culture condition, as mentioned by Marsot and Houle (1989) and Gutenbrunner et al. (1994).

Acknowledgements

This work was supported in part by a research grant from Seafood & Marine Resources Development (1999) Center, Pukyong National University.

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