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The influence of nitrogen and phosphorus on the growth of a diatom *Skeletonema costatum* (Greville) Cleve

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Abstract

Nitrogen and phosphorus requirements of a chain-forming diatom, *Skeletonema costatum* (Greville) Cleve, collected from Yatsushiro Sea, Japan, were investigated in a laboratory culture experiment. Sodium nitrate and sodium glycerophosphate were used as nitrogen and phosphorus sources, respectively. Cultures were grown in modified Provasoli's ASP_2NTA medium (Provasoli *et al.* 1957) at 25±1°C, light intensity 60 $\mu E\ m^{-2}\ sec^{-1}$ and photoperiod 12:12-h, L:D cycle. Optimum growth was observed at nitrate concentrations of 3-10 mgl^{-1} and phosphate concentrations of 1.5-15 mgl^{-1}. Adequate growth was also found at the nitrate concentration of up to as high as 300 mgl^{-1}. Significantly poorer growth was found at lower nitrate (< 3.0 mgl^{-1}) and higher phosphate (> 15 mgl^{-1}) concentrations. From the present study, it is concluded that *S. costatum* can grow well at wide ranges of nitrate concentrations but is sensitive to higher phosphate concentrations.

Key words : Diatom, *Skeletonema costatum*, Nitrogen, Phosphorus

Introduction

Skeletonema costatum is a common diatom (Eppley *et al.* 1971) which is one of the important food items of most copepods. In Taiwan and in the Philippines this species is considered as one of the best algae for feeding prawn larvae (Liao *et al.* 1983). *S. costatum* can serve as a good biological source of proteins and fatty acids (Sanchez *et al.* 1995). This species is also very important due to its potential use as valuable assay organism for examining water quality.

On the other hand, *S. costatum*, in some situations, may become noxious, forms heavy blooms when gets suitable environment due to eutrophication and causes economic losses to aquaculture. This species is known to cause blooms in USA, Rumania, France, Norway, Uruguay, China, Japan and Hongkong (Blanchemain *et al.* 1994, Mingyuan and Jiasheng 1993, Hosaka 1992). Noxious phytoplankton blooms are a serious problem for finfish aquaculture because the alga may kill fish by damaging or clogging their gills or kill fish due to

deoxygenation by forming a layer on the water surface (Anderson 1989), and it is believed that these blooms are increasing all over the world (Smayda 1992).

Growth of phytoplankton in nature is controlled by various environmental factors, such as temperature, salinity, irradiance, nutrients, stratification, water turbulence etc. (Tomas 1978, Uye and Takamatsu 1990). In our another study, it was found that *S. costatum* was extremely euryhaline and tolerable to very low salinities (Khan *et al.* 1998). Optimum growth was observed at salinities of 20-35 ppt, temperatures of 20-25°C, light intensities of 80-120 $\mu E\ m^{-2}\ sec^{-1}$ and pH between 7.5-8.0. Nutrients are very important environmental factors that influence the growth of any alga (Okaichi *et al.* 1989). The nutrient requirements of Yatsushiro Sea's strain of *S. costatum* in nature and laboratory have not yet been studied. The present study describes the effect of nitrogen and phosphorus on the growth of Yatsushiro Sea's strain of *S. costatum*.

Materials and methods

Skeletonema costatum used in this study was isolated in 1991 from Yatsushiro Sea, Japan. Stock cultures were grown in modified Provasoli's ASP_2NTA medium (Provasoli *et al.* 1957) (Table 1) at 25±1°C, light intensity 60 $\mu E\ m^{-2}\ sec^{-1}$ and photoperiod 12:12-h, L:D cycle. Sodium nitrate and sodium glycerophosphate were used as sources of nitrogen and phosphorus, respectively. Growth was determined at eight concentrations of nitrate (0.1, 0.3, 1, 3, 10, 30, 100 and 300 mgl^{-1}) and six concentrations of phosphate (0.1, 0.5, 1.5, 5, 15, and 50 mgl^{-1}). Culture media were autoclaved for 15 min at 121°C, and aged for several days prior to inoculation.

Table 1. Composition of modified Provasoli's ASP_2NTA medium (Provasoli *et al.* 1957)

Additive	Concentration
NaCl	18 gl^{-1}
$MgSO_4, 7H_2O$	5 gl^{-1}
KCl	0.6 gl^{-1}
Ca (as Cl^-)	0.1 gl^{-1}
$NaNO_3$	7 mgl^{-1}
Na_2 glycerophosphate	1 mgl^{-1}
$Na_2SiO_3. 9H_2O$	99 mgl^{-1}
Na_2Co_3	30 mgl^{-1}
Vitamin B_{12}	0.2 gll^{-1}
Vitamin mix. S3*	10 mll^{-1}
P II metals**	30 mll^{-1}
Fe (as Cl^-)	0.5 mgl^{-1}
Tris buffer	1.0 gl^{-1}
Distilled water	to 1 liter

The pH of the medium was 7.8.

** One ml of vitamin mix. S3 contains: thiamine HCl 0.05 mg, nicotinic acid 0.01 mg, Ca pantothenate 0.01 mg, p-aminobenzoic acid 1 µg, biotin 0.1 µg, inositiol 0.5 mg, folic acid 0.2 mg, thymine 0.3 mg.*

*** One ml of P II metals contains: Na_2EDTA 1 mg, Fe (as Cl^-) 0.01 mg, B (as H_3BO_3) 0.2 mg, Mn (as Cl^-) 0.04 mg, Zn (as Cl^-) 5 µg, Co (as Cl^-) 1 µg.*

Before starting the experiment the algae were acclimated to the experimental condition for at least two generations. Cells of mid logarithmic growth phase were used for inoculation to ensure that the cells were nutritionally replete. Sterilized micropipettes were used to transfer the inocula. Individual growth medium was gently shaken once a day for accelerating growth and to avoid settlement of algal cells. All growth studies were done in triplicate. The cell concentration was determined by direct counting by using a Sedgewick-Rafter chamber. Counts were made immediately after inoculation and then each other day up to 10 days. For reducing errors due to possible synchronous divisions counts were made at the same time each day. The average number of cell divisions per day (K) for the 6-day growth period was calculated from :

$$K = \ln \frac{C_t}{C_0} \left(\frac{1}{t \ln 2} \right)$$

where, C_t and C_0 are cell concentrations at times t and 0, respectively (Guillard 1973).

Division rates under different conditions were subjected to Analysis of Variance (ANOVA) (Statview S.E. + Graphics, Abacus. Concepts, Inc.). Significant differences among the means were determined using Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

Results

Growth of *S. costatum* at different sodium nitrate concentrations with the fixed salinity (30 ppt), temperature (25°C) and light intensity (60 µE m^{-2} sec^{-1}) is shown in Figure 1. Cultures reached a maximum cell density of 11.36 x 10^5 cells ml^{-1} on the 6th day at 10 mgl^{-1} sodium nitrate and the cell density remained at 9.70x10^5 cells ml^{-1} up to the 10th day in the same medium. No lag phase exhibited at 3 mgl^{-1} and 10 mgl^{-1}, and at 1 mg^{-1} and 30-300 mg^{-1} the lag phase was not distinct. Poorer cell density was found at low concentrations of nitrate (0.1 and 0.3 mg^{-1}) with a 2 days of lag phase.

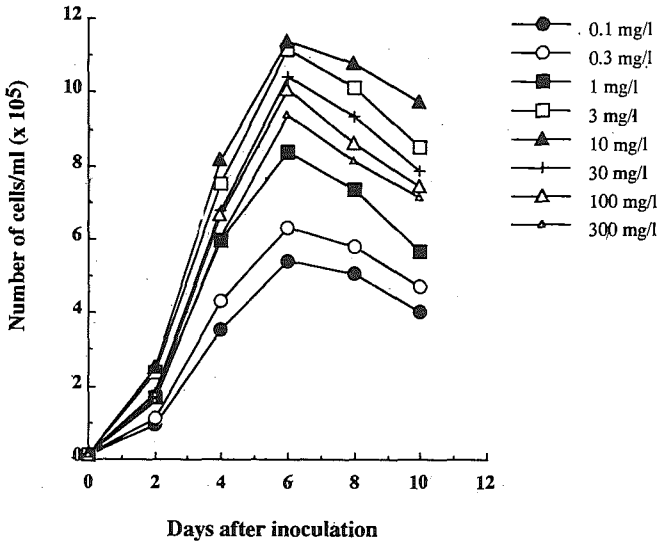

Fig. 1. Growth curves of *S. costatum* at different concentrations of sodium nitrate.

Analysis of Variance (ANOVA) showed that the difference in mean daily division rate of the cells at different nitrate concentrations were significant. The best mean daily division rate (0.75 divisions/day) was found at 3 and 10 mgl^{-1} which was not significantly higher than that at concentration of 30 mgl^{-1} (0.74 divisions/day). The mean daily division rates at 0.1-1 mgl^{-1} were significantly lower than at higher concentrations of nitrate. The mean daily division rate of *S. costatum* in relation to different concentrations of nitrate levels showed an increasing trend of growth from 0.1 to 10 mgl^{-1} and then a slow declining trend above 10 mgl^{-1} (Fig. 2).

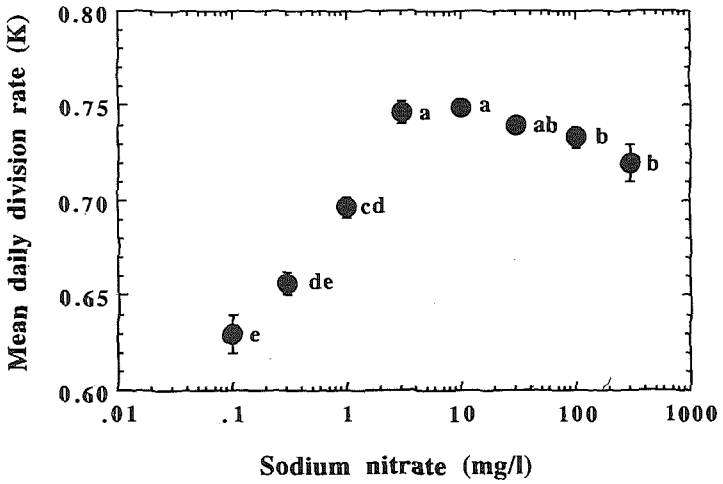

Fig. 2. Mean daily division rate of *S. costatum* at different concentrations of sodium nitrate. Each point and vertical line represent mean ± SD for three replicates. Means with different letters are significantly different ($p < 0.05$).

S. costatum required low amounts of phosphate. It grew well with phosphate concentrations of 1.5-15 mgl^{-1} (Fig. 3). The maximum cell density was found at concentrations of 1.5 mgl^{-1} (10.30×10^5 cells ml^{-1}) and 5 mgl^{-1} (10.27×10^5 cells ml^{-1}). The maximum cell yield at 50 mgl^{-1} sodium glycerophosphate was only 5.01×10^5 cells ml^{-1} on the 6th day of culture which was less than 50% of the maximum cell yield at 1.5 and 5 mgl^{-1}.

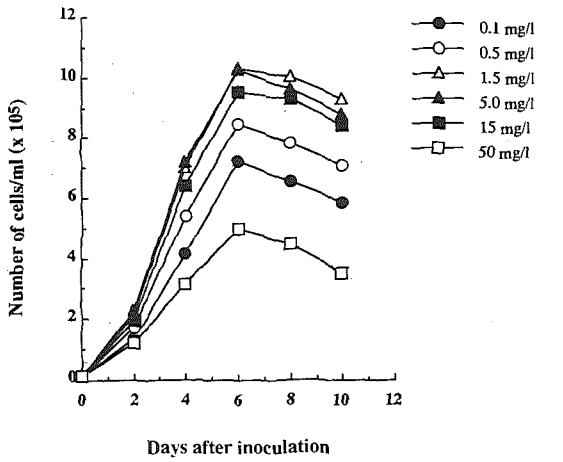

Fig. 3. Growth curves of *S. costatum* at different concentrations of sodium glycerophosphate.

Statistical analysis (ANOVA) indicated that the mean daily division rate of *S. costatum* was significantly different ($p<0.05$) at various glycerophosphate concentrations (Fig. 4). The highest mean daily division rate (0.71 divisions/day) was at 1.5 and 5 mgl^{-1} and no significant differences were observed among the phosphate concentrations of 1.5, 5 and 15 mgl^{-1}. Lower mean daily division rate was found at the concentration of 50 mgl^{-1} with 0.59 divisions/day.

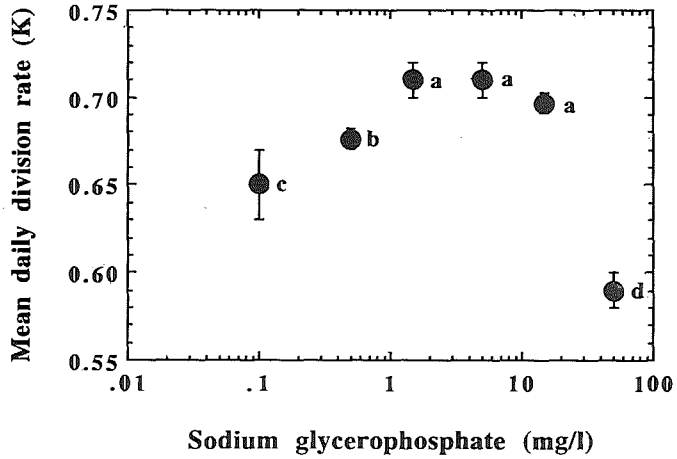

Fig. 4. Mean daily division rate of *S. costatum* at different concentrations of sodium glycerophosphate. Each point and vertical line represent mean ± SD for three replicates. Means with different letters are significantly different ($p < 0.05$).

The division rate of the plankton in relation to different concentrations of sodium glycerophosphate showed an increasing trend of growth from 0.1 to 1.5 mgl^{-1} and a slight declining trend above 1.5 mgl^{-1}. A much declining trend was found from 15 mgl^{-1}.

Discussion

Nitrogen and phosphorus are two important macronutrients which are essential for the growth of phytoplankton. In this experiment, it was found that the growth rates and final cell yields of *S. costatum* were dependent on the nitrate and phosphate concentrations in the media and the optimum requirements of nitrate and phosphate were 3-30 mgl^{-1} and 1.5-15 mgl^{-1}, respectively. Venkataraman (1969) reported about the requirement of a high level of nitrogen (13.6 mgl^{-1}) as compared to phosphorus (0.45 mgl^{-1}) for maximum growth of *Coccochloris peniocystiss.* Similar observations were found for *Selenasturm westii* (N/P ratio of 22.6/1) and *Kirchneriella subsolitaria* (N/P ratio of 15/1).

It has been known that ecological and physiological parameters of phytoplankton may vary for different species (Khan *et al.* 1996). *S. costatum* can tolerate a wide range of nitrate concentration. In the present study, *S. costatum* grew at nitrate concentrations from 0.1 to 300 mgl^{-1} with the optimum at 3 to 10 mgl^{-1}. These optimum nitrate concentrations are similar to that of a previously

reported red-tide-producing phytoflagellate *Chattonella antiqua* (Nishijima and Hata 1986). In all cultures there was a clear decrease in growth rate at lower nitrate concentrations which are similar to the findings of Nishijima and Hata (1986). Adequate growth of *S. costatum* was also found at nitrate concentrations of as high as 300 mgl^{-1}. Similar results were found by Venkataraman (1969) for *Nitzschia closterium*. On the other hand, growth of many algae were found to be strongly inhibited at higher concentrations of nitrate. A concentration greater than 31.5 mgl^{-1} of nitrogen was found to inhibit the growth of *Chlorella vulguris* (Venkataraman 1969).

The optimum phosphorus concentration for the growth of phytoplankton varies with different species. Nishijima and Hata (1986), while studying the effect of glycerophosphate in batch culture of *C. antiqua* using a phosphate concentration range from 0.03 to 8.9 mgl^{-1}, observed that the growth of this plankton was found to be optimum between 1-8.9 mgl^{-1}, was poor at 0.3 mgl^{-1} and was affected at 0.03 to 0.1 mgl^{-1}. Similar observations were found by Venkataraman (1969) for *Anacystis montana* which required a surprisingly low concentration of phosphorus. In our study, the optimum glycerophosphate concentration for the growth of *S. costatum* was found to be between 1.5 to 15 mgl^{-1}. It is noteworthy that, though *S. costatum* required a surprisingly low concentration of phosphate phosphorus for their optimum growth, some phytoplankton require much less concentration of phosphate. The best growth of *Ankistrodesmus fatcatus* and *Acenedesmus quadricauda* were observed at 1.0 mgl^{-1} and *Asterionella formosa* was at the concentration of only 0.002 mgl^{-1} (Venkataraman 1969). The growth of *S. costatum* was found to be inhibited by higher concentration of phosphate.

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