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Effects of Temperature and pH on Seasonal Changes and Growth Characteristics of a Bloom Forming *Mallomonas elongata* (Synurophyceae)

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The growth characteristic of a predominant planktonic blooming species, *Mallomonas elongata* in a small shallow eutrophic pond was investigated in the field (from October 2004 to September 2005) and laboratory. Dense blooming (max. 17,600 cells mL^{-1}) of this silica-scaled chrysophytes was observed for a short time period in early spring (water temperature 12-18°C and pH 8.4-9.5). The growth characteristics of *M. elongata* isolated from this pond was investigated at various temperatures and pH under batch culture. The unialgal culture of *M. elongata* showed maximum growth rate (μmax) at 15°C similar to the natural conditions. However, the optimal pH of the isolated batch culture was lower than the pond water pH at which *M. elongata* appeared in large population density.

Key words : planktonic bloom, *Mallomonas elongata*, unialgal culture, growth rate

INTRODUCTION

Silica-scaled chrysophytes (Synurophyceae) have been known as a phytoplankton group primarily restricted to cold and oligotrophic waterbodies (Kristiansen, 1975; Kristiansen and Takahashi, 1982). Recently rich silica-scaled chrysophyte flora however, has been found in various ecological conditions worldwide (Kristiansen, 1988; Cronberg, 1989; Saha and Wujek, 1990; Siver, 1995), and several species often caused dense water blooms (Kristiansen, 1971; Clasen and Bernhardt, 1982; Hoffmann and Wille, 1992; Kiss and Kristiansen, 1994; Kim and Hwang, 2001). Although the chrysophytes occupy an important group of the biomass in freshwater reservoirs and lakes, little is known about chrysophytes in Korea due to difficulties of identification and examination using electron microscopy (EM) is necessary for a reliable identification (Lee *et al*., 1994; Kim, 1995, 1997; Kim *et al*., 1995; Kim and Takamura, 2001).

The distribution and periodicity of chrysophytes are mainly controlled by water temperature, pH, light intensity, and nutrient status (Takahashi, 1978; Siver and Chock, 1986; Siver and Hamer, 1989). There are studies concerning the ecological characteristics that control the occurrence and seasonal abundance of this group in natural waters. However, few comprehensive studies were done on the growth characteristics for the individual taxa (Kristiansen, 1986; Hartmann and Steinberg, 1989). Furthermore, comparatively little is known about the environmental factors that affect the distribution and population change of many members of this algal group. Up to now, *in vitro* cultures of chrysophytes have been rarely successful in revealing the effects of the physicochemical factors on the population growth of this algal group (Healey, 1983; Wee *et al*., 1991; Saxby-Rouen *et al*., 1997).

In the present study, a field investigation was carried out to elucidate a seasonality of *Mallo-*

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monas elongata which has water blooms in a shallow eutrophic pond. In addition, unialgal culture experiments were carried out in a laboratory to examine optimum temperature and pH for the growth of this blooming species. The natural and laboratory physico-chemical conditions for the optimal growth of the chrysophytes are compared in this paper. This is the first report of a unialgal culture experiment of *M. elongata* while there have been a few reports regarding other synurophyceae including Synura petersenii cultures (Munch, 1972; Knowles and Zingmark, 1978; Martin-Wagemann and Gutowski, 1995; Saxby-Rouen *et al*., 1997).

MATERIAL AND METHODS

1. Field experiments

In order to examine the physicochemical factors that affect a planktonic bloom, field investigations were carried out from October 20, 2004 to September 22, 2005 in a small shallow eutrophic pond, located at the campus of Kyungpook National University, South Korea. The surface area was about 200 m^2 and the mean depth was 0.7 m. Phytoplankton samples were collected from the surface layer (20 cm in depth) during afternoons (2-3 pm) at intervals ranging from 2 days to 2 weeks, except for the period when the pond surface was covered with ice. Samples were fixed in Lugol's solution and the cell number of phytoplankton counted by the Utermöhl technique (Lund *et al*., 1958) using an inverted microscope with a magnification of 400×. Samples for scanning electron microscopy (SEM) were placed on a coverglass, air dried, and coated with platinum. Scanning electron micrographies were taken by Hitachi S-570.

Water temperature, pH and electrical conductivity were measured using an oxygen electrode (YSI 58), pH meter (MP120) and EC meter (MC126), respectively. Total nitrogen and phosphorus amounts were measured according to the standard methods (APHA, 1992).

2. Laboratory experiments

In order to investigate specific physico-chemical requirements of this algal strain, *M. elongata* was isolated from the pond and cultured in a DY III medium (Lehman, 1976) and in a raw water medium, which was filtered and autoclaved the pond water. The strains are available from the personal culture collection of the authors.

The unialgal stock culture was maintained at 15±1°C and at 100 μmol m^{-2} s^{-1} of cool white fluorescent light on a 14 : 10 h light-dark cycle in the DY III medium buffered to pH 7. The initial cell density was adjusted to ca. 500 cells mL^{-1}, and triplicate samples were used in each culture experiment. The experiments were carried out in Erlenmeyer flasks at temperatures of 9-21°C and pH of 4-9, under continuous white fluorescent illumination of about 100 μmol m^{-2} s^{-1} of light intensity. The samples were fixed with Lugol's solution and the cell number was counted in a Sedgwick Rafter chamber. The number of cells was enumerated at 5 day intervals, and counts were continued until the stationary phase, typically for 25 to 62 days. The specific growth rates (μ) for periods of exponential growth were calculated using the regression of the logarithm of the count against time over the period exponential growth (Martin-Wagenmann and Gutowski, 1995).

RESULTS

During the study period, the water temperature varied in the range of 2.9-30°C, and pH was ranged between 6.9 and 10.1 (Fig. 1). The total nitrogen and phosporus varied from 3.714 to 8.702 mg L^{-1} and 0.026 to 0.049 mg L^{-1}, respectively (Table 1). Based o the nutrient concentration the pond was considered as an eutrophic.

The dominant species that caused planktonic blooms were identified as *M. elongata*. The sea-

Table 1. Physico-chemical conditions of the studied pond. Physico-chemical factors are presented as maximum, minimum and mean values from samples collected during the study period.

Variable	Min. - Max.	Mean
Water temperature (°C)	2.9 - 30	18.5
pH	6.9 - 10.1	8.5
Total phosphorus (spring) (mg L^{-1})	0.026 - 0.049	0.036
Total nitrogen (spring) (mg L^{-1})	3.714 - 8.702	4.599
Nitrate nitrogen (NO_3-N) (mg L^{-1})	0.526 - 2.106	1.315
Ammonia nitrogen (NH_4-N) (mg L^{-1})	1.330 - 3.837	2.564

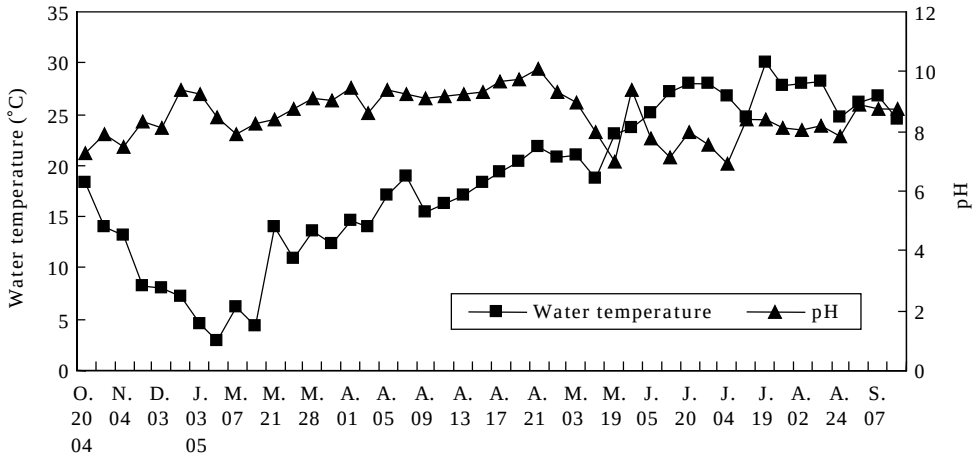

Fig. 1. Seasonal variations of water temperature and pH between October 2004 and September 2005 in a small shallow eutrophic pond.

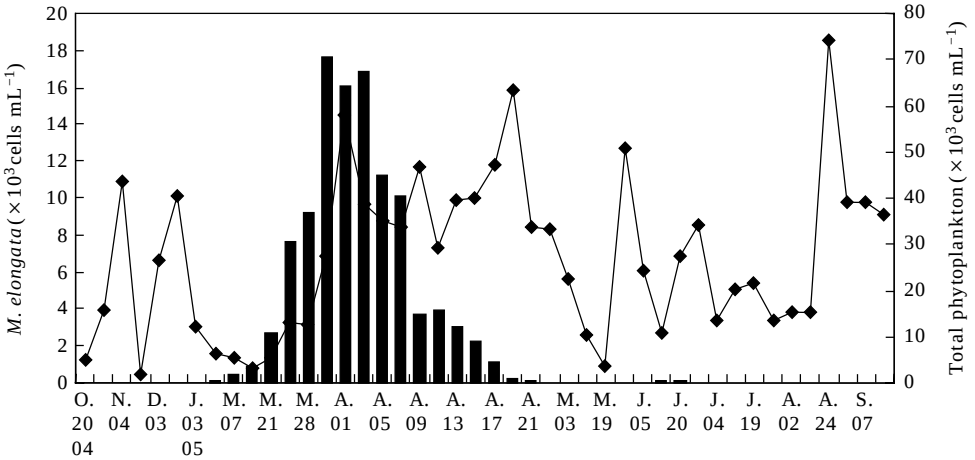

Fig. 2. Seasonal variations of total number of phytoplankton cells and the number of cells of *M. elongata* (filled bars) between October 2004 and September 2005 in a small shallow eutrophic pond.

sonal changes of the total phytoplankton and *M. elongata* are shown in Fig. 2. The population of *M. elongata* appeared in early spring and maintained a high population density between March 21 and April 7 when water temperature ranged between 11 and 18.9°C. And the cell number peaked (17,600 cells mL^{-1}) in March 30, 2005. At this time, this species accounted for 27.6–65.5% of the total phytoplankton population. The population density gradually decreased as the water temperature increased in April.

Although several other *Mallomonas* species showed normal growth response in a DY III medium, *M. elongata* isolated did not grow. We do not know why it happened (data not shown). Thus, a raw water medium was used to determine the growth rate of *M. elongata*.

Nutrient influx and efflux might not be critical factors in this case since the studied pond was already eutrophic. Thus, we focused our physico-chemical study on water temperature and pH. The population growth of the isolated *M. elongata* as a unialgal culture was examined at various temperatures and pH levels (Fig. 3). *M. elongata* did not show significant growth at 9°C and 18°C (Fig. 3A). The maximum growth rate (μmax) was observed at 15°C, and the growth rate (μ) increased as the temperature increased from 9°C

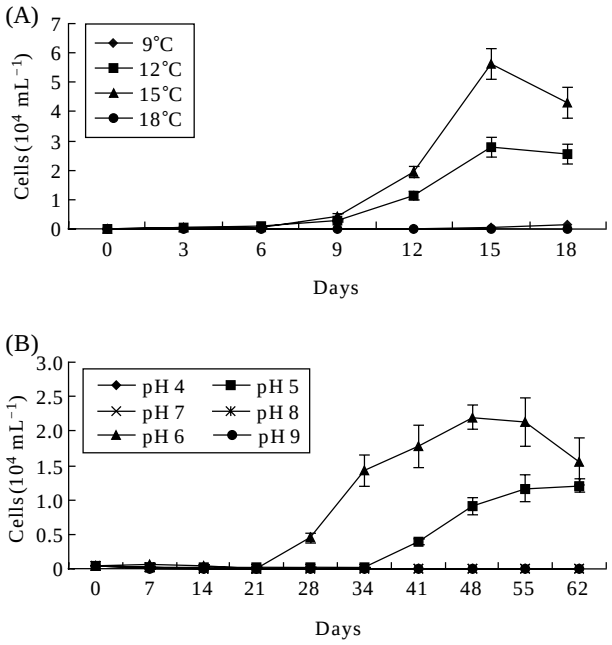

Fig. 3. The growth response curves of *M. elongata* over the range of incubation temperatures (A), and pH levels under an optimum growth temperature (B). Error bars provide maximum and minimum values recorded at each tested temperature and pH. Each data indicate the average of triplicate culturres.

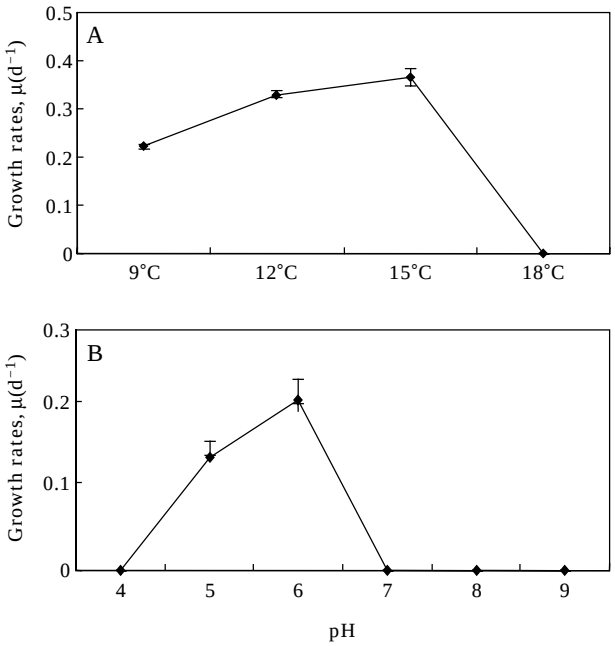

Fig. 4. The growth rates of *M. elongata* under various conditions of temperature (A) and pH (B).

to 15°C (Fig. 4A). *M. elongata* showed apparent population growth at a pH level of 5 and 6, and the optimum growth was observed at pH 6 (Fig. 3B and Fig. 4B). By contrast to growth responses shown in various temperatures (Fig. 3A), there was long lag phase in cultures at various pH (Fig. 3B).

DISCUSSION

Although various environmental parameters were considered to affect the growth of silica-scaled chrysophytes in natural water systems, it was well known that water temperature and pH are the most important limiting factors (Smol, 1986; Dixit *et al*., 1988; Siver, 1989). In our field investigations, *M. elongata* appeared at wider temperature and pH ranges (between 2.9 and 28 °C and pH 6.9-10.1) than those found in previous research (collective data in Siver, 1991). Maximum growth was observed at a narrow but relatively higher temperature range (14-18°C) than in previous reports (Siver, 1991, maximum growth was mostly below 9°C). In contrast to Siver's categorization of *M. elongata* as a 'pH indifferent species with alkaliphilic tendences', the unialgal *M. elongata* culture preferred week acidic conditions for its growth in our analysis. The pH of the pond water was between 7.9 and 9.8 during the period in which *M. elongata* occurrenced. This pH environment correlates with many previous reports which stated that this taxon preferred alkali water for its growth (Asmund and Hilliard, 1961; Takahashi, 1978). However, in contrast to previous reports and our field data, unialgal *M. elongata* culture preferred slightly acidic conditions. Based on our data, water temperature seems to be an important factor that can control the growth of *M. elongata*. The differences of lag phase in Fig. 3A and 3B were presumably due to the differences of nutrient's concentration and composition at each collecting time of raw water that had been used for culture media. As a result, further research on the pH effect in combination with various nutrient and light conditions will be necessary.

Our results showed that *M. elongata* preferred cold and acidic water for growth, and that its distribution and population growth may be controlled mainly by water temperature and pH levels. However, further ecological studies on

populations from natural waters and more culture experiments are needed to understand the growth characteristics of this species under various environmental conditions including nutrients and light intensities.

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＜**국문적요**＞

수화를 형성하는 *Mallomonas elongata* (Synurophyceae)의 계절적 변동과 증식 특성에 대한 온도와 pH의 영향

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작은 부영양 저수지에서 수화를 형성하는 *Mallomonas elongata*의 계절적 변동(2004년 10월–2005년 9월)과 온도와 pH에 대한 증식 특성을 실험실 배양을 통해 조사하였다. 수온 12–18°C, pH 8.4–9.5의 범위를 나타낸 3월 말에서 4월 초의 짧은 기간 동안 *M. elongata*의 심한 bloom (최고 17,600 cells mL^{-1})이 발생하였다. 이 저수지로부터 분리한 *M. elongata*의 batch culture를 통한 다양한 온도에 대한 성장 반응은 bloom을 형성 하였을 때의 저수지 수온과 유사한 15°C에서 최대 성장률을 나타내었다. 반면 pH에 대한 증식 특성은 bloom 형성기의 저수지 pH 범위 보다 낮은 pH 6에서 최대 성장률을 나타내었다.