Algae
Volume 19(2): 145-148, 2004

[Note]

Optimal Growth Conditions for the Two Euryhaline Cyanobacterial Clones, *Anabaena* sp. CB-MAL21 and CB-MAL22 Isolated from Mankyeong Estuary, Korea

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As a result of the 2-year monthly monitoring of the phytoplankton community at 3 stations in Mankyeong Estuary, Korea, we learned that cyanobacterial species of the genus *Anabaena* occurred at most sampling points with huge salinity differences (0.1-32.5 psu). We isolated several clones of *Anabaena* spp. from the monitoring stations, and screen out two euryhaline and nitrogen-fixing *Anabaena* clones, CB-MAL21 and CB-MAL22. The two clones were grown under various environmental gradients such as temperature (20, 30, 35 and 40°C), salinity (0, 2, 5, 15 and 30 psu), and PO_4^{3-}-P concentration (0, 1.6, 8.0, 40 and 200 μM). Growth of CB-MAL21 and CB-MAL22 was measured by daily monitoring of chlorophyll fluorescence from each experimental culture for more than three serial transfers. Both the two experimental clones did not grow at 0 psu. Maximal growth rates of the two clones were markedly reduced at lower PO_4^{3-}-P concentrations showing negligible growth at 0 and 1.6 μM. However, growth of CB-MAL21 was not affected by low NO_3^--N concentration in culture media, showing the nitrogen-fixing ability. Maximum biomass yields of the two clones decreased dramatically at 35 and 40°C. Optimal growth conditions for the two experimental clones were determined to be 20-30°C, 40 μM PO_4^{3-}-P, and wide salinity range from 5.0 to over 30 psu. Best growth of CB-MAL21 was shown at (20°C-15 psu), which is less saline and cooler condition than those (i.e., 30°C-30 psu) for the best growth of CB-MAL22. The euryhaline and nitrogen-fixing CB-MAL21 strain thus can be a candidate laboratory culture for the future cyanobacterial marine biotechnology in temperate coastal waters.

Key Words: cyanobacterial clone, euryhaline, Mankyeong Estuary, nitrogen fixing, optimal growth

INTRODUCTION

Korean Saemankeum Reclamation Project to build up ca. 40,000-ha farming land at southern west coast of Korean peninsula within 10-15 years was launched in 1998, and evoked social concerns about the potential eutrophication and cyanobacterial bloom of the newly formed artificial lake (Herath 1997; Havens *et al.* 2003). Even before the completion of the estuarine dams, cyanobacterial blooms by *Anabaena, Aphanizomenon, Microcystis* were repeatedly observed at the low salinity region of the Mankyeong Estuary. Among the three major genera for cyanobacterial blooms in the Mankyeong Estuary, we learned that *Anabaena* spp. occurred at many samples with huge salinity differences (0.1-32.5 psu). Several clones of the *Anabaena* spp. were established as laboratory strains, and *Anabaena* sp. CB-MAL21 and *Anabaena* CB-MAL22 were euryhaline and nitrogen-fixing strains. Filamentous N_2-fixing cyanobacterium, like *Anabaena* sp., can compete out the non-nitrogen-fixers at low N/P environments (Havens *et al.* 2003), and can be an ideal target creature for cyanobacterial biotechnology (Moreno *et al.* 2003), if the strain is very refractory to extreme temperature and salinity conditions (Valvarde *et al.* 2001; Moreno *et al.* 2003). Thus, we aimed to find out optimal growth condition for the two clons of *Anabaena* species, CB-MAL21 and CB-MAL22, from Mankyeong Estuary by measuring the growth rate and biomass yield along the gradients of experimental culture conditions (i.e. temperature, salinity, NO_3^--N, and PO_4^{3-}-P). The results could provide the scientific basis to apply the strains for cyanobacterial biotechnology as well as to mitigate *Anabaena* blooms at the new artificial lake within Saemankeum Reclamation Region in near future.

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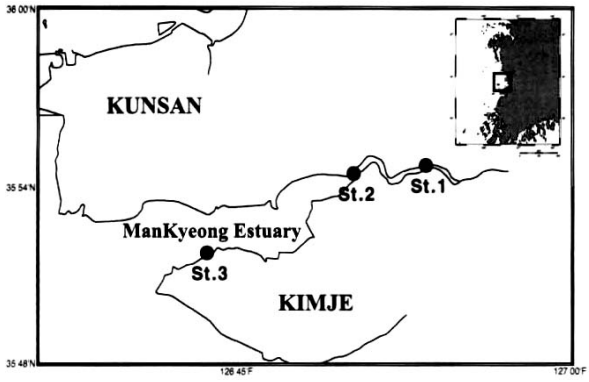

Fig. 1. Sampling stations at Mankyeong Estuary, Korea.

MATERIALS & METHODS

The phytoplankton community was monitored at 3 stations in Mankyeong Estuary, Korea monthly from August 1999 to October 2000 (Fig. 1). The two clones of *Anabaena* species, CB-MAL21 and CB-MAL22, were established by single cell isolation method (Guillard, 1975) from samples collected at St. 2 and St. 3, respectively (Fig. 2). Experimental clones were grown under various environmental gradients such as temperature (20, 30, 35, and 40°C), salinity (0, 2, 5, 15, and 30 psu), NO_3^--N concentration (0, 0.04, 1.0, 5.0 and 10 mM), and PO_4^{3-}-P concentration (0, 1.6, 8.0, 40 and 200 μM). Continuous illumination of 110 $\mu E \cdot m^{-2} \cdot s^{-1}$ by cool-white fluorescent lamps was applied for the N-P experiments, and 160 $\mu E \cdot m^{-2} \cdot s^{-1}$ for the temperature-salinity experiments. Daily monitoring of *in vivo* chlorophyll fluorescence of the experimental cultures was performed at the same time of a day using Turner Designs Fluorometer (Model 10-AU) during for more than three serial transfers. Daily growth rate (rm, divisions per day) was calculated by the changes in *in vivo* chlorophyll fluorescence (F): $rm = (\ln F - \ln F_0) / [(t - t_0) \times (\ln 2)]$, where F_0, chlorophyll fluorescence at t_0; F, chlorophyll fluorescence at t; t_0, time zero; and t, time t.

RESULTS & DISCUSSION

CB-MAL21 and CB-MAL22 showed no growth at 0 psu under all the 4 experimental temperatures (Fig. 3, 4). The growth response of CB-MAL21 and CB-MAL22 to temperature gradient was quite similar, showing highest biomass yields at 20°C or 30°C with remarkably reduced growth above 30°C. Both of the two clones are

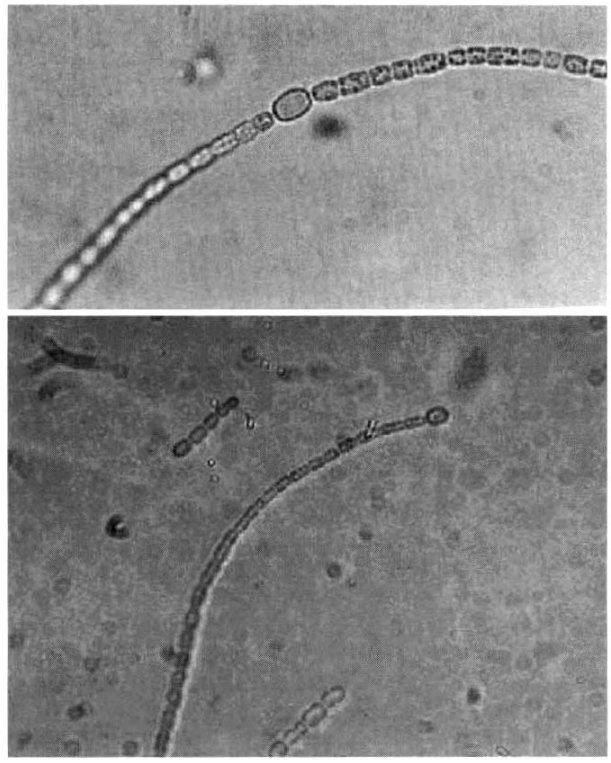

Fig. 2. The cyanobacterial strains, *Anabaena* sp. CB-MAL21 (×1,000, upper) and *Anabaena* sp. CB-MAL22 (×400, lower) from Mankyeong Estuary.

euryhaline, with considerable growth all along 2-30 psu salinity range (Figs 3, 4). Best growth (i.e. in growth rate as well as in biomass yield) of CB-MAL21 was found at 20°C-15 psu while that of CB-MAL22 was at 30°C-30 psu. Therefore, CB-MAL21 seems to prefer relatively less-saline and cooler conditions than those for CB-MAL22. Under 20°C, growth rates of CB-MAL21 and CB-MAL22 were sharply reduced by low PO_4^{3-}-P concentration (Fig. 6) but not by NO_3^--N concentration (Fig. 5), to demonstrate that CB-MAL21 is a nitrogen-fixer. Under 20°C, controlling PO_4^{3-}-P concentration below 1.6 μM could be a method to mitigate the algal blooms of two clones in the field (Fig. 6).

In conclusion, CB-MAL21 is a euryhaline and nitrogen-fixing cyanobacterium, and grows best under the environmental condition of lower salinity (15 psu), cooler temperature (20°C) than that for CB-MAL22. Clone CB-MAL21 can serve as supplies of organic nitrogen for aquatic microorganisms by fixing atmospheric nitrogen into ammonia-containing substances (Hori *et al.* 2002) at low N/P environments (Havens *et al.* 2003). Therefore, CB-MAL21 is a candidate laboratory clone for the future cyanobacterial

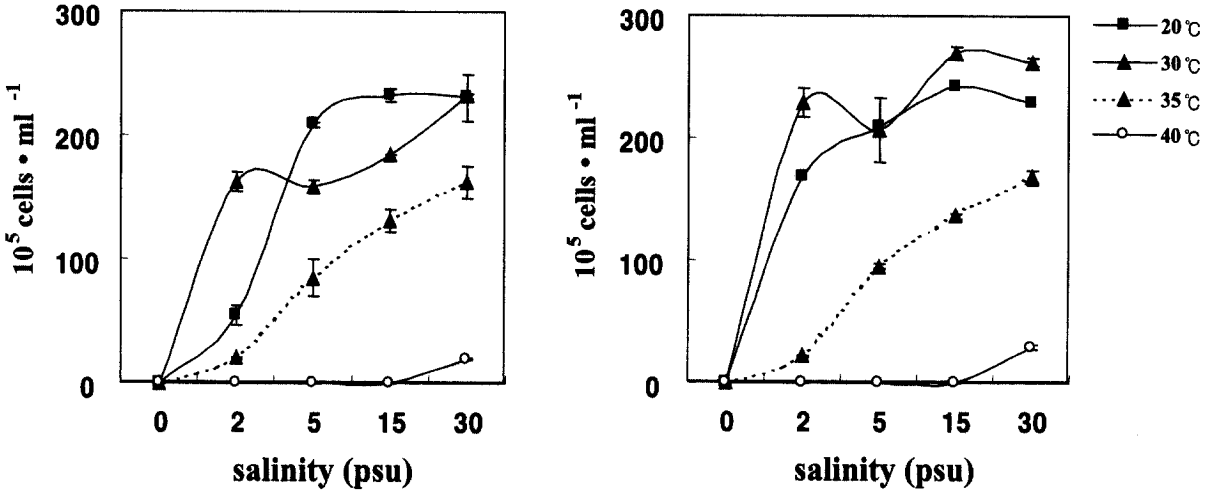

Fig. 3. Maximum biomass yield of *Anabaena* sp. CB-MAL21 (left) and *Anabaena* sp. CB-MAL22 (right) at different temperature-salinity conditions under continuous illumination of 160 $\mu E \cdot m^{-2} \cdot s^{-1}$.

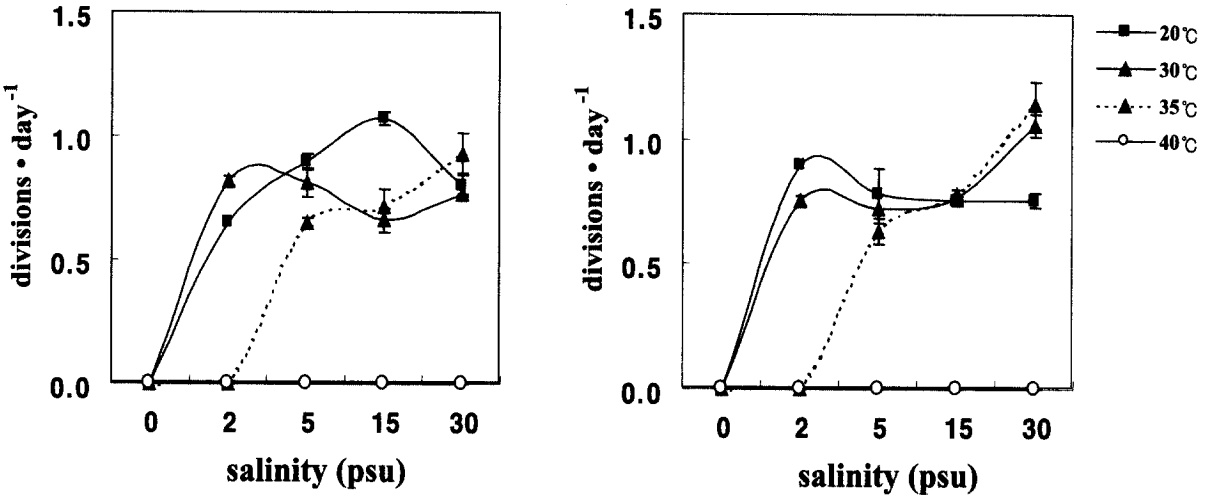

Fig. 4. Maximum growth rate of *Anabaena* sp. CB-MAL21 (left) and *Anabaena* sp. CB-MAL22 (right) at different temperature-salinity conditions under continuous illumination of 160 $\mu E \cdot m^{-2} \cdot s^{-1}$.

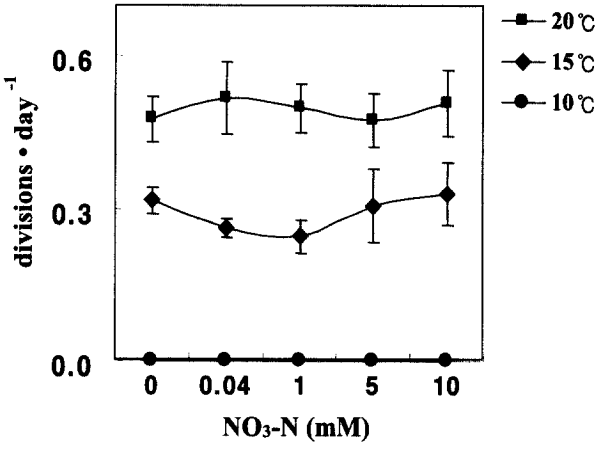

Fig. 5. Maximum growth rate of *Anabaena* sp. CB-MAL21 at different nitrate-N concentrations and temperatures under continuous illumination of 110 $\mu E \cdot m^{-2} \cdot s^{-1}$.

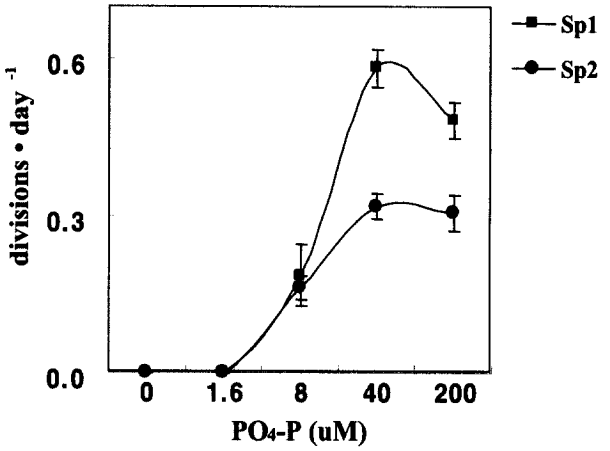

Fig. 6. Maximum growth rate of *Anabaena* sp. CB-MAL21 (■) and *Anabaena* sp.2 CB-MAL22 (●) at different phosphate-P concentrations under 20°C and continuous illumination of 110 $\mu E \cdot m^{-2} \cdot s^{-1}$.

biotechnology in water bodies with high PO_4^{3-}/NO_3^- ratio (Lunn *et al.* 1999; Havens *et al.* 2003) at temperate coastal regions (i.e., with less-saline and cooler water than in the tropical regions). Present results could be applied to mitigate possible *Anabaena* blooms at the new artificial lake within Saemankeum Reclamation Region (Herath 1997; Hori *et al.* 2002). The experimental clones had been originated from the native estuarine water, and thus can be fed into the future Saemankeum Lake as endemic species.

ACKNOWLEDGEMENTS

This work was funded by 2000 Korea Sea Grant Program and 2003 KNU (Kunsan National University) Regional Research Center, Saemankeum Environmental Research Center. We are very grateful to the two anonymous reviewers who greatly helped us revise earlier manuscript.

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Received 23 March 2004
Accepted 29 May 2004