

## Statistical Optimization of *Chaetoceros neogracile* for the production of anti-freezing protein

Sung-Eun Jung, Sung-Ho Kang<sup>2</sup> and Choul-Gyun Lee<sup>1</sup>

Marine Biotechnology Laboratory, Department of Biotechnology, Inha University

TEL: +82-32-860-7518, FAX: +82-32-872-4046

In antarctic environment, many organisms are exposed to freezing temperatures. And interestingly, the adaptation of microbial organisms at subzero temperatures leads to the elaboration of proteins that have an ability to bind with ices and to modify the normal growth of ice crystals.<sup>1)</sup> So we optimized culture medium of *Chaetoceros neogracile* using a statistical method in order to maximize anti-freezing protein.<sup>2)</sup>

In the first, the chlorophyll concentration after 18 days of incubation were measured to determine a medium that is good for propagation of *C. neogracile*. These results showed 1.58mg/L in Kalle medium, 1.30mg/L in MBL, 1.18mg/L in Millero. Therefore, the best one is Kalle medium for the growth of *C. neogracile* and selected to carry out fractional factorial design (FFD) and central composite design (CCD).

The 250 mL Erlenmeyer flasks containing 120 mL Kalle medium were inoculated with  $5 \times 10^5$  cell/mL of *C. neogracile* for FFD and incubated for 8 days at 125 rpm and 4°C. The results of fractional factorial design showed that the salinity, nitrate and phosphate concentration, and light intensity were significant factors. In CCD, the central values of the concentration of sodium chloride related on salinity, the nitrate and phosphate concentration were 30g/L, 150mg/L, and 9mg/L, respectively. The central value of light intensity was  $25 \text{mEm}^{-2} \text{s}^{-1}$ . Finally, the maximum cell concentration of *C. neogracile* increased up to 50% and the activity of anti-freezing protein improved more than 45% after the optimization of the culture medium comparing with the control.

### References

1. Jonh Barrett, Thermal hysteresis proteins(2001), *The International Journal of Biochemistry & Cell Biology*, 33, 105-117.
2. Park E. K., M. W. Seo, C. -G. Lee, Effects of medium composition for the growth and the astaxanthin production of *Haematococcus pluvialis*(2001), *Kor. J. Appl. Microbial. Biotechnol.*, 29; 227-233.