HEAT STRESS RESPONSE IN *SKELETONEMA COSTATUM*

A. PENNA[1], V. SCOCCIANTI[2] AND M. MAGNANI[1]

[1] Centro Biologia Ambientale, [2] Istituto di Botanica
University of Urbino, 61029 Urbino, Italy

Skeletonema costatum (Grev.) Cleve is a cosmopolitan marine diatom. Its main distribution is in coastal waters where it occurs frequently and often as the dominant species. Because of its ubiquitous nature and its ability to reach bloom densities, *S. costatum* has been subject of many physiological and ecological investigations. In the present paper we investigated the *in vitro* responses of *S. costatum* under conditions of temperature stress. Among the several cellular responses to stress conditions in plants and animal cells there is the synthesis of heat-shock proteins (HSPs) (NAGAO *et al.*, 1986), the activation of ATP and ubiquitin dependent degradation of damaged or unfolded proteins (RECHSTEINER, 1988), and the early changes in polyamine content (GALSTON, 1989). The results here reported show that *S. costatum* is able to activate all these responses. Furthermore, due to the importance of the production of extracellular polysaccharide in the Adriatic sea and since *S. costatum* accumulates large amounts of β-1,3-D-glucan (VÅRUM and MYKLESTAD, 1984), we also investigated the variation of polysaccharide content in *S. costatum* under temperature stress conditions.

S. costatum was cultured in the f/2 medium of Guillard and Ryther at 18°C, in a day:night cycle of 12h:12h. Polysaccharide content and polyamine content were determined according to DUBOIS *et al.* (1956) and SMITH and BES (1977) respectively. Protein pattern was analyzed by SDS-PAGE, the presence of ubiquitin and ubiquitin conjugates was revealed by western blotting. Ornithine and arginine decarboxylase activities were determined by measuring the $^{14}CO_2$ evolution from L-[1-14C] ornithine and DL-[1-14C] arginine as described by TORRIGIANI *et al.*, 1987.

S. costatum cells, usually growth at 18°C, were exposed to higher temperatures for 40 min. In cells at 25°C, 30°C and 35°C respectively, total cellular and extracellular carbohydrate content was determined. No variations in total carbohydrate content were observed; however extracellular carbohydrates increased after 40 min of 35°C stress. This production is probably not due to new synthesis but to cell disruption as supported by a corresponding decrease of the intracellular carbohydrates. At 35°C in fact a drastic decrease in cell number and viability was observed.

The temperature shift from 18°C to 30°C induced the appearance of new polypeptides typical of the heat shock response. At 35°C the protein electrophoretic pattern was similar to that observed at 18°C, suggesting that the cell protein machinery is no longer active at this temperature. Western blotting experiments performed with an anti-ubiquitin antibody revealed that *S. costatum* cells contained free ubiquitin and ubiquitin conjugates. The heat stress increased the number of ubiquitin conjugate, particularly those of high molecular weight. Thus the heat stress in *S. costatum* cells is associated with the conjugation of ubiquitin to endogenous proteins.

The polyamines putrescine, spermidine and spermine were present in *S. costatum* cells. Heat shock (40 min of 30°C stress) caused a marked increase in free putrescine and spermidine and in putrescine biosynthetic enzymes activities. A possible role for these different metabolic events in the adaptive response of *S. costatum* to rapid temperature shifts has been hypothized.

REFERENCES

BONIN D.J., DROPP M.R., MAESTRINI S.Y. and BONIN M.C., 1986. Physiological features of six micro-algal to be use as indicators of seawater quality. *Cryptogamie, Algologie*, 7 : 23.

DUBOIS M., GILLES K.A., HAMILTON J.K., REBERS P.A. and SMITH F., 1956. Colorimetric method of determination of sugars and related substances. *Analyt. Chem.* 18 : 350-356.

GALSTON A.W., 1989. Polyamines and plant response to stress. *In* : Bachrach, U., Heimer, Y.M. (eds.) The Physiology of Polyamines. CRC Press, Boca Raton, Florida.

NAGAO R.T, KIMPEL J.A., VIERLING E. and KEY J.L., 1986. The heat shock response: a comparative analysis. In Miflin, B.J. (ed.) Oxford surveys of plant molecular and cell biology. Oxford Univ. Press, Oxford.

RECHSTEINER M., 1988. Ubiquitin, Plenum Press, New York.

SMITH T. A. and BEST G.R., 1977. Polyamines in barley seedlings. *Phytochemistry*, 16 : 841-843.

TORRIGIANI P., SERAFINI-FRACASSINI D. and BAGNI N., 1987. Polyamines biosynthesis and effect of dicyclohexylamine during the cell cycle of *Helianthus tuberosus tuber*. *Plant. Physiol.*, 84 : 148-152.

VÅRUM K.M. and MYKLESTAD S.,1984. Effect of light, salinity and nutrient limitation on the production of β-1,3-D-glucan and exo-D-glucanase activity in *Skeletonema costatum* (Grev.) Cleve. *J. exp. mar. Biol. Ecol.*, 83 : 13-25.