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Comparison of heat and cold stress induced responses in yeast cells

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The heat shock response represents universal cellular protective behavior which involves reduction in the synthesis of normal cell proteins and induction of another specific set of proteins. These proteins have been assigned to play an important role in the survival and recovery of cells.

Saccharomyces cerevisiae cells cultured normally at 30°C when shifted to 43°C (2hrs) exhibit altered protein profile which three proteins (70, 83 and 90kD) were consistently observed on SDS-Poly Acrylamide Gels. However when these cells were exposed to cold shock at 10°C (2hrs), 35kD proteins appeared. Phase contrast microscopy of these cells revealed the structural adaptations of vacuoles to heat as well as cold stress. In the former case the dilated vacuoles were observed whereas in the latter they shrink/split. Nevertheless, both of these biochemical as well as structural adaptations observed during heat and cold stress were completely reversible on the transferring of cells to normal temperature. Physiological significance of those adaptive response is yet to be worked out.

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Screening of Microbes Utilizing *Haematococcus pluvialis* Cell Wall

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A unicellular green alga, *Haematococcus pluvialis* synthesizes astaxanthin as a major oxygenated carotenoid in the cell. Astaxanthin has been focused not only as pigmentation source for fish aquaculture but also as an antioxidant more powerful than vitamin E. *Haematococcus*, however, doesn't possess any favorable characteristics like salt tolerance and vigorous growth for easy handling against microbial contamination in open pond culture. Protoplast fusion between *Haematococcus* and *Chlamydomonas* species, some of which has the above characters would be an appropriate choice of the breeding for *Haematococcus*. Thus in order to carry out the protoplast formation, we have tried to isolate lytic enzyme sources for *Haematococcus* cell wall.

In the present study, *Haematococcus pluvialis* NIES-144 was mixotrophically cultivated in an acetate containing medium at 20°C under 12L/12D illumination cycle (1.5 klux). Several microorganisms from soil capable of degrading *Haematococcus* cell wall were screened on the selection plate composed of the intact algal cell, 2-3 g/l, yeast extract, 1 g/l, and several minerals as clear zone formers. Lytic activity was assayed for the cell number decrease after incubation for 2 hrs at 30°C, in the reaction mixture composed of the intact *Haematococcus* cells, Tris-acetate buffer solution pH 7, culture fluid and 2-mercaptoethanol.

The cultivation of the isolated strains in liquid medium were carried out using French-pressed or the intact algal cells as carbon source at 28°C for 4-5 days with shaking.

One of the strain, strain no. DP-1 showed the highest lytic activity, and lysed *Haematococcus* cells in the logarithmic growth phase (3 days culture) more effectively than those in the stationary phase (5 days culture).

The ability of strain DP-1 to lyse algal cells was about 40 % of the total cell suspension. In combination with proteinase K which has an ability to remove gelatin layer around the cells, the lytic activity was able to be enhanced to more than 60 %.