Heat Shock Treatment of *Chlamydomonas reinhardtii* and *Chlorella* Cells

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[Abstract] The protocol is very reliable and simple for inducing heat shock in unicellular green algae cells. The main purpose was to compare cellular response of three *Chlorella* species, isolated from different habitats: *Chlorella vulgaris* 8/1- thermophilic, *Chlorella kesslery*- mesophilic and *C. vulgaris*- extremophilic. Species were isolated from different habitats and differ in their temperature preferences and tolerance. Temperature induced stress response was measured as cell survival, induction of chloroplast HSP70B and DSBs induction and rejoining.

Materials and Reagents

1. Species:
   Three *Chlorella* species were used: *C. vulgaris*, isolated from soil samples of Livingston Island, the South Shetland Archipelago, Antarctic; *C. vulgaris* strain 8/1, isolated in 1968 from thermal springs in the region of Rupite, Bulgaria, and cultivated in our laboratory since 1975 and *Chlorella kesslery* a mesophile, from the Trebon collection.
   Cultivation: *Chlorella* species were cultivated on Tris Acetate Phosphate (TAP) medium under continuous light of 60 μmol $m^{-2}s^{-1}$ and a temperature of 23 °C ± 0.1 °C in a Phytotron GC 400 growth chamber. The species were cultivated at this temperature because it is well known, that eurythermal algae, could be grown at a wide range of temperatures.
2. TAP medium (see Recipes)
3. Sager–Granick medium (see Recipes)

Equipment

1. Phytotron GC 400 growth chamber (NUVE Ankara/Turkey 2009)
2. Microscope
3. Bürker chamber
4. Circulating water bath

Procedure

A. Cultivation

1. Cultivate for 4-5 days algae strains or species on TAP medium (Harris, 1989) under continuous light of 60 µmol/m^2/s and a temperature of 23 °C ± 0.1 °C in a Phytotron GC 400 growth chamber to the end of exponential and early stationary phase of growth.
2. Check under the microscope whether cell culture is not contaminated.
3. Count cell number under the microscope using Bürker chamber.
4. Centrifuge certain volume (depending on the cell density, counted in step A-3) of cell suspension at 1,200 *x g* by RT, resuspend in Sager–Granick medium or other appropriate medium, so to get 10 ml of cell suspension with a density of 1 x 10^6 cells/ml for every sample.

B. Temperature treatment

1. Keep 10 ml cell culture with a density 1 x 10^6 cells/ml in an incubator under continuous shaking, at different temperatures:

   t = 39 °C for 30 min

   t = 42 °C for 5 min

   t = 45 °C for 5 min

   Notes:

   a. *The same heating procedure could be done in a circulating water bath at the same three temperatures: t = 39 °C for 30 min, t = 42 °C for 5 min, and t = 45 °C for 5 min.*

   b. *Use 50 ml flasks to comply with the requirement that volume of the cell culture must be not more than 1/3 of the volume of the flask.*
2. Place on ice to stop the heating process.
3. For HSP70B analysis keep cells for 2 and 4 h after the step B-2 at t = 23 °C ± 0.1 °C to allow cells to recover.
4. Centrifuge 10 ml cell suspension at 1,200 *x g* for 5 min.

Recipes

1. TAP medium

   Stock solution for 1 L of TAP media

1 M Tris base	20 ml
Phosphate Buffer II (see 1-a)	1.0 ml
Solution A (see 1-b)	10.0 ml
Hutner's trace elements (see 1-c)	1.0 ml

Glacial acetic acid (pH to 7.0)	1.0 ml

a. Phosphate buffer II (Stock solution)

Component (For 100 ml)

K_2HPO_4	10.8 g
KH_2PO_4	5.6 g

b. Solution A

Component (For 500 ml)

NH_4Cl	20 g
$MgSO_4 \cdot 7H_2O$	5.0 g
$CaCl_2 \cdot 2H_2O$	2.5 g

c. Hutner's trace elements

i. Dissolve 50 g of acid free EDTA in 250 ml of deionized. Heat to dissolve.

ii. Dissolve the following one by one in order. Heating to approximately 100 °C in 500 ml deionized H_2O.

Component	**Quantity**
H_3BO_3	11.4 g
$ZnSO_4 \cdot 7H_2O$	22.0 g
$MnCl_2 \cdot 4H_2O$	5.06 g
$FeSO_4 \cdot 7H_2O$	4.99 g
$CoCl_2 \cdot 6H_2O$	1.61 g
$CuSO_4 \cdot 5H_2O$	1.57 g
$Mo_7O_{24}(NH_4)_6 \cdot 4H_2O$	1.1 g

iii. Mix the two solutions together. The resulting solution should be blue-green.

iv. Heat to 100 °C. Cool slightly, but don't let the temperature drop below 80 °C-90 °C.

v. Adjust pH to 6.5-6.8 with 20% KOH (approximately 83 ml).

2. Sager and Granick medium **(**adjust pH 6.8-7.0)

Component	In 1 L stock	For 1 L media
Trace elements*	--	1 ml
$NaCitrate \cdot 2H_2O$	100 g	5 ml
$FeCl_3 \cdot 6H_2O$	10 g	1 ml
$CaCl_2 \cdot 6H_2O$	58 g	1 ml
$MgSO_4 \cdot 7H_2O$	100 g	3 ml
NH_4NO_3	100 g	3 ml
KH_2PO_4	100 g	1 ml

K_2HPO_4	100 g	1 ml
*Trace Elements		
Component	In 1 L stock	
H_3BO_3	1.0 g	
$ZnSO_4 \cdot 7H_2O$	1.0 g	
$MnSO_4.H_2O$	0.303 g	
$CoCl_2 \cdot 6H_2O$	0.2 g	
$Na_2MoO_4 \cdot 2H_2O$	0.2 g	
$CuSO_4 \cdot 5H_2O$	0.063 g	

References

1. Chankova, S. G., Yurina, N. P., Dimova, E. G., Ermohina, O. V., Oleskina, Y. P., Dimitrova, M. T. and Bryant, P. E. (2009). Pretreatment with heat does not affect double-strand breaks DNA rejoining in *Chlamydomonas reinhardtii.* *J Thermal Biol* 34(7): 332-336.
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