Hypsibius dujardini collection notes and culture protocol from Bob McNuff May 2007

The original strain of *Hypsibius dujardini* was obtained and isolated by Robert McNuff (Sciento) on 13/11/87 from a pond in Darcy Lever, Bolton, Lancashire (British National Grid Ref. SD741078). The pond is typical of many in this reclaimed wetland area surrounded by dense shrubs i.e. *Corylus, Erica* and marginal mosses, i.e. *Polytrichum, Sphagnum, Rhytidiadelphus spp*. The tardigrade was found in a benthic sample.

Culture Protocol

Hypsibius dujardini ingests the cytoplasm of many unicellular algae genera i.e *Chlorococcum, Botrydiopsis, Chlorella, Ankistrodesmus* including some filamentous types e.g. *Chaetophora*. For ease of culturing and stability of the strain we have maintained our stock of *H. dujardini* on *Chlorococcum spp*. The alga is easily cultured in simple algae media i.e. Bolds Basal Medium and retains its nutritional capacity for several months after harvesting.

For long term maintenance and viability we routinely culture tardigrades in 250ml Erlenmeyer flasks containing approx 150ml of simple mineral medium (Chalkley's Medium) plus soil extract and *Chlorococcum* algal food. Sub-culturing intervals are subject to temperature, algal food inoculum and density of tardigrades. Generally cultures peak between 4-6 weeks when most of the algae is grazed over and sub-culturing within this time scale is best. Microscopical observation of cultures for tardigrades and eggs will reveal the best time for setting up and inoculating fresh culture media.

We find cultures of *H.dujardini* are best kept between 10-18°C for optimum production of egg clusters (cuticles). Tardigrades will tolerate temperatures from freezing to 30°C , however both extremes of temperature range will inhibit fecundity.

Flask Cultures are exposed to a normal photoperiod rhythm of approx L:D 14-10 in a shaded situation. Cultures can also be kept in the dark without any apparent loss of vitality. Old cultures can be kept in reserve if necessary by concentrating and storing in the refrigerator +2°C where the tardigrades will survive for many months in an inactive or lethargic state.

Methods, Materials & Media

Culture of Hypsibius

Pour 150ml of Chalkley's Medium plus Soil extract into a sterile 250ml Pyrex Erlenmeyer flask. Add approx. 3-5ml of concentrated *Chlorococcum* cells to each flask and inoculate with tardigrades or eggs. To prevent contamination from fungal spores and mites seal each flask with clingfilm or parafilm. Place in a cool shaded situation, circa 10-20°C and sub-cultures every 4-6 weeks as necessary

Culture of Chlorococcum

Add 150ml of Bold's Basal Medium plus Soil extract to each of several 250ml Pyrex Erlenmeyer flasks, stopper with non-absorbent cotton wool and bacofoil. Autoclave for 20mins at 15psi and allow flasks to stand for 24 hours before inoculating each with <2ml of unicellular algae (i.e. *Chlorococcum, Botrydiopsis,Chlorella*).

Illuminate under fluorescent lighting (40Watt Tubes) either continuously or controlled to give a photoperiod of L:D 14:10.
At room temperature or incubation between 15-25°C the flasks can be harvested for algae after 4-6 weeks for feeding to tardigrades.
The algae is non-motile and settles the bottom of the flask where the concentrated cells can be pipetted, or centrifuged down.

Culture Media Recipes

Chalkley's Medium plus Soil extract

Stock Solutions in 100ml Distilled water

1. NaCl 2.0 g
2. KCl 0.08 g
3. $CaCl_2$ 0.12 g

Final Medium (made up to 1 litre with Distilled water)
5ml of each Stock solution
20ml Soil Extract

Preparation of Soil Extract
Obtain a quantity of fertile soil i.e. humus enriched soil and air dry.
Add 1 part soil to 2 parts tapwater by volume and autoclave.
Allow to settle over a few days and decant the supernatant soil extract.

Bold's Basal Medium

Stock Solutions in 400ml Distilled water

1. $NaNO_3$ 10.0 g
2. $MgSO_4.7H_20$ 3.0 g
3. NaCl 1.0 g
4. K_2HPO_4 3.0 g
5. KH_2PO_4 7.0 g
6. $CaCl_2.2H_20$ 1.0 g

Trace Element Solutions in 1 litre Distilled water

7. $ZnSO_4.7H_20$ 8.82 g
   $MnCl_2.4H_20$ 1.44 g
   MoO_3 0.71 g
   $CuSO_4.5H_20$ 1.57 g

$CoNO_3.6H_2O$ 0.49 g
(Autoclave to disssolve)

8. H_3BO_3 11.42 g
9. EDTA – KOH Solution
   EDTA 50.0 g
   KOH 31.0 g
10. $FeSO_4.7H_2O$ 4.98 g
    H_2SO_4 (conc) 1.0 ml

Final Medium (made up to 1 litre with Distilled water)
Stock Solutions 1-6 10.0ml each
Trace Element Solutions 7-10 1.0ml each
Soil Extract 250ml

Culture in petri plates
Gabriel & Goldstein 2007

For collecting embryos in the laboratory, small cultures of tardigrades were kept in 60mm glass Petri dishes in commercial bottled spring water (Crystal Geyser or Deer Park) at room temperature in a shaded location. These cultures were fed *Chlorococcum* sp. algae, adding about 1 vol algal culture to 4 vol tardigrade culture. Algae and water were changed once every ten days by allowing animals to settle and pouring out most of the water and algae and adding back bottled water, 4-5 times, then adding back fresh algal culture. Cultures were kept in a shaded location. Hundreds of tardigrades per Petri dish can be reared continuously in the lab at room temperature in this manner.