Protocol

Desiccation of *Hypsibius exemplaris*

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Many species of tardigrades can survive severe water loss, but different species tolerate different desiccation conditions. *Hypsibius exemplaris* is able to survive desiccation after an initial period of slow drying, as described here. This protocol will likely work for other tardigrade species as well. Drying of tardigrades can be used for probing the mechanistic underpinnings of desiccation tolerance, as well as for practical purposes such as shipping and long-term storage of the animals.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Agar
Bottled spring water (e.g., Deer Park)
Glycerol
Tardigrades (*Hypsibius exemplaris*)
See Protocol: *Laboratory Culture of Hypsibius exemplaris* (McNuff 2018).

Equipment

Desiccator with Drierite or silica gel
Dissecting microscope
Glass watch dish
Hot plate at 100°C
Hygrometer
Micropipette
Plastic Petri dishes (35 mm, with lids)
Small plastic box (~5 inch × 5 inch × 10 inch, with lid)

METHOD

Prepare Tardigrades

Food should be absent from the guts of the tardigrades before desiccation. Perform these steps on the day before desiccation.

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1. Pour ~2 mL of spring water into a new 35-mm plastic Petri dish.
2. Isolate tardigrades from food by transferring them to the new dish containing spring water. Starve the tardigrades by leaving them in the dish overnight at room temperature.

Prepare a Humidified Chamber

Perform these steps on the day before desiccation.

3. Place a glass watch dish containing ~5 mL of a 10% glycerol solution (1 part glycerol, 9 parts spring water) in a small plastic box with a lid.
4. Leave the box overnight (~16 h) at room temperature.
5. Use a hygrometer to check the humidity in the chamber.

The relative humidity should be >90% after ~16 h. See Troubleshooting.

Prepare Agar-Coated Petri Dish Lids

Prepare agar-coated lids on the day of use. Do not store or reuse coated lids.

6. Prepare a stock of a 2% agar solution using bottled spring water, and boil it.
7. Pipette 300 μ L of the boiling agar solution into the lid of a 35-mm Petri dish.
Discard the dish itself; only the lid will be used.
8. Working quickly, use the tip of the pipette to spread the agar solution evenly around the entire lid. Minimize excess agar and uneven distribution.
9. Tilt the lid on its side so that excess agar pools to one side. Remove and discard excess agar from the side of the lid with a pipette.
10. Allow the thin agar coating to solidify and dry for 5–10 min.

Use the coated lids immediately.

Desiccate Tardigrades

11. Transfer starved tardigrades from Step 2 to the agar-coated dish lid from Step 10 using a micropipette. Minimize the amount of water that is transferred with the tardigrades.
12. Remove excess water from the dish lid using a micropipette.
13. Place the agar-coated lid containing the tardigrades in the humidified chamber from Step 5, and incubate overnight (~16 h).
14. Remove the lid from the humidified chamber, and inspect the dried samples using a dissecting microscope.
Specimens should have adopted a rounded tun-like appearance. See Troubleshooting.
15. Keep tardigrades at ambient humidity (~40% relative humidity) for 24 h, and then store uncovered in a desiccator at room temperature.

Rehydrate Tardigrades

16. Pipette 2 mL of bottled spring water into dish lid.

When observed under a dissecting microscope, tardigrades that have been stored dry for 24 h begin to exit their tun state within 10 min. Coordinated movement is typically observed within 30–60 min. Survival is typically ~80% and is judged by scoring for coordinated movement and response to stimuli (touching with a micropipette tip).

TROUBLESHOOTING

Problem (Step 5): The relative humidity is <90%.

Solution: Remake the 10% glycerol solution and repeat Steps 3–5.

Problem (Step 14): The animals are still active.

Solution: Ensure that the agar coating made during Steps 7–9 is thin and evenly distributed. A thin and even distribution of agar is essential for proper desiccation of specimens. Work quickly during Steps 7–9 to ensure proper distribution, and discard lids that are not optimal.

Problem (Step 14): The animals are dried but do not have a tun-like appearance.

Solution: Check the relative humidity of the chamber and ensure it is >90%.

DISCUSSION

Many species of tardigrades can survive severe water loss. The rate at which different species of tardigrades can desiccate and survive varies (Wright 1989). *H. exemplaris* is able to survive desiccation after an initial period of slow drying (Wright 1989; Kondo et al. 2015; Boothby et al. 2017), as described in this protocol. This protocol will likely work for other species as well. The need for initially slow desiccation in many species is likely linked to their need to up-regulate production of molecules that will serve to protect them from the harmful effects of drying (Kondo et al. 2015; Boothby et al. 2017).

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REFERENCES

- Boothby TC, Tapia H, Brozena AH, Piszkiwicz S, Smith AE, Giovannini I, Rebecchi L, Pielak GJ, Koshland D, Goldstein B. 2017. Tardigrades use intrinsically disordered proteins to survive desiccation. *Mol Cell* 65: 975–984.
- Kondo K, Kubo T, Kunieda T. 2015. Suggested involvement of PP1/PP2A activity and de novo gene expression in anhydrobiotic survival in a tardigrade, *Hypsibius dujardini*, by chemical genetic approach. *PLoS One* 10: e0144803.
- McNuff R. 2018. Laboratory culture of *Hypsibius exemplaris*. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot102319.
- Wright JC. 1989. Desiccation tolerance and water-retentive mechanisms in tardigrades. *J Exp Biol* 142: 267–292.

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