**Description**

**Strain Designation:** 31-B [AC-005]  
**Deposited Name:** Acanthamoeba jacobsi Sawyer et al.  
**Depositor:** TK Sawyer  
**Isolation:** marine sediment, New York Bight Apex, 1974

**Growth Conditions**

**Temperature:** 25°C  
**Medium**  
ATCC® Medium 997: Fresh water ameba medium  
ATCC® Medium 711: PYB

**Instructions for Complete Medium**

- **Media:** ATCC medium 997 optionally inoculated with Klebsiella pneumoniae subsp. pneumoniae (ATCC® 700631™) or Enterobacter aerogenes (ATCC® 13048™).
- **Alternate media:** ATCC medium 711 optionally inoculated with bacteria as above. (Note that ATCC medium 711 is a richer formula than ATCC medium 997 and will produce denser and faster bacterial growth. Excess bacterial growth can inhibit growth of amoebae.)

**Protocols**

**Storage and Culture Initiation**

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
2. Immediately after thawing, aseptically transfer contents to a plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C. Trophozoites should be seen within 5-7 d.

**Culture Maintenance**

1. Streak an ATCC medium 711 plate with Enterobacter aerogenes (ATCC® 13048) and incubate at 35°C overnight.
2. Remove an agar block (~5 mm²), with trophozoites or cysts, from the edge of an agar plate culture and invert the block at the edge of the freshly bacterized plate.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.
4. Repeat steps 1-3 at 10-14 d intervals.

Note: a monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

**Cryopreservation**

1. Allow the cells to encyst. To detach cysts from the plate flush the surface with 5 ml fresh ATCC medium 1323 (Page's Balanced Salt Solution). Rub the surface of the plate with a spread bar to detach adhering cysts.
2. Transfer the liquid medium to a sterile centrifuge tube.
3. If the cyst concentration does not exceed 2 x 10⁶ cysts/ml adjust the suspension to that concentration. To adjust the concentration, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield 2 x 10⁶.
4. While cells are centrifuging prepare a 15% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube
Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be at least 10^6 cysts/ml and 7.5% (v/v) DMSO. The equilibration time (the time between addition of DMSO and the start of the cooling cycle) should be no less than 15 min and no longer than 30 min.

Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryo (special plastic vials for cryopreservation).

To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immediately after thawing, aseptically remove the contents of the ampule and distribute to the center of a fresh plate of ATCC medium 711. Distribute the material evenly over the plate using a spread bar. Incubate at 25°C.

References

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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