**Description**

**Strain Designation:** Arabia Mountain  
**Deposited Name:** Acanthamoeba polyphaga (Puschkarew) Page  
**Depositor:** WS Jenkins  
**Isolation:** soil, Arabia Mountain, DeKalb County, GA, 1972

**Propagation**

**Growth Conditions**  
**Temperature:** 25.0°C  
**Duration:** grown with Enterobacter aerogenes ATCC 13048

**Protocol:** ATCCNO: 30011 SPEC: This strain is distributed as a dried preparation. See the general procedures for opening a dried vial. Aseptically add 1 ml of sterile distilled water to the inner shell vial, remove the filter paper aseptically with a pair of forceps, and place it in the center of an agar plate of ATCC medium 997. Add the liquid remaining in the vial to the plate and spread it evenly over the surface of the plate. Incubate the plate at 25°C. Trophozoites (amebae) should be evident within 2-3 days.

**Medium**  
ATCC® Medium 711: PYB

**Instructions for Complete Medium**  
ATCC Medium 711

**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 several times.  
   *NOTE:* If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
5. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be at least 10⁶ 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.
6. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)
8. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
9. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.

10. 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 and other information relating to this product are available online at www.atcc.org.

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