Acanthamoeba sp., Strain CDC:V538

Catalog No. NR-46474

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Acanthamoebidae*, *Acanthamoeba*
Species: *Acanthamoeba* sp.
Strain: CDC:V538
Original Source: *Acanthamoeba* sp., strain CDC:V538 is a clinical isolate collected in 2003 from the lung of a 61-year-old female patient in Pennsylvania, USA.[1]

Amoebae belonging to the genus *Acanthamoeba* inhabit a wide variety of environmental niches worldwide and have been isolated from soil, freshwater, air, humans, and animals, both domestic and feral, and are able to exist both as free-living amoebae and as parasitic pathogens.[2] In healthy humans, *Acanthamoeba* is the causative agent of *Acanthamoeba* keratitis, an increasingly-prevalent sight-threatening eye disease among contact lens wearers. In immunocompromised individuals, *Acanthamoeba* can cause disseminated infections of other tissues and, in severe cases, the fatal disease granulomatous amebic encephalitis.[2-5]

Acanthamoeba are currently classified by twelve sequence types (T1 to T12) based on nuclear small ribosomal subunit RNA genotyping and divided into three morphological groups: Group I (T7, T8, T9), Group II (T3, T4, T11) and Group III (T1, T2, T5, T6, T10, T12).[5] Identification of *Acanthamoeba* on the genus level is based on spiny surface projections (acanthopodia) present on the surface of trophozoites.[2] Highly-specific PCR methods for subgeneric identification of isolates have been developed for both clinical and environmental applications.[6]

Material Provided:

Each vial of NR-46474 contains approximately 0.5 mL of cells in cryopreservative (7.5% DMSO). Please refer to the Certificate of Analysis for the specific culture media used for each lot and refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-46474 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:

Peptone Yeast Glucose medium (PYG; ATCC® medium 712) (Appendix II)
Incubation:
Temperature: 30°C
Note: *Acanthamoeba* are usually cultured at 25°C. However, during production, it was discovered that strain CDC:V538 demonstrates optimal growth at 30°C.
Atmosphere: Aerobic
Propagation:

1. Place the frozen vial in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the contents of the vial to a T-25 tissue culture flask containing 5 to 10 mL PYG medium.
3. Screw the cap on tightly and incubate the tube or flask at 30°C.

Maintenance:

1. When the culture is at or near peak density, vigorously agitate or scrape the surface of the flask to detach adherent cells.
2. Transfer approximately 0.25 mL to a fresh flask containing 5 to 10 mL fresh PYG.
3. Screw the caps on tightly and incubate at 30°C.
4. The amoeba will form an almost continuous sheet of cells on the bottom surface of the flask. Repeat steps 1 through 3 every 10 to 14 days.

Please refer to Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Acanthamoeba* sp., Strain CDC:V538, NR-46474."

Biosafety Level: 2

Appropriate safety procedures should always be used with this

material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Visvesvara, G. S., Personal Communication.
2. Marciano-Cabral, F. and G. Cabral. "*Acanthamoeba* spp. as Agents of Disease in Humans." Clin. Microbiol. Rev. 16 (2003): 273-307. PubMed: 12692099.
3. Visvesvara, G. S. "Amebic Meningoencephalitides and Keratitis: Challenges in Diagnosis and Treatment." Curr. Opin. Infect. Dis. 23 (2010): 590-594. PubMed: 20802332.
4. Clarke, D. W. and J. Y. Niederkorn. "The Pathophysiology of *Acanthamoeba* Keratitis." Trends Parasitol. 22 (2006): 175-180. PubMed: 16500148.
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6. Schroeder, J. M., et al. "Use of Subgenic 18S Ribosomal DNA PCR and Sequencing for Genus and Genotype Identification of Acanthamoebae from Humans with Keratitis and from Sewage Sludge." J. Clin. Microbiol. 39 (2001): 1903-1911. PubMed: 11326011.

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APPENDIX I: CRYOPRESERVATION

1. Harvest *Acanthamoeba* from multiple agar plates by scraping the surface of the flask with a cell scraper to detach adhering trophozoites.
2. Transfer the cell suspensions to 15 mL or 50 mL plastic centrifuge tubes.
3. Adjust the cell concentration to 1×10^6 to 2×10^7 cells/mL with fresh PYG medium.
   Note: If the concentration of cells is too low, centrifuge at 1300 × g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired cell concentration.
4. Mix equal volumes of cell suspension and fresh medium containing 15% DMSO to yield a final concentration of 1×10^6 to 2×10^7 cells/mL in 7.5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell suspension.
5. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. Place the vials in a controlled rate freezing unit. From room temperature cool the vials 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 this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: Peptone Yeast Glucose (PYG) MEDIUM (ATCC® MEDIUM 712)

1. Prepare the Basal medium (see recipe below), autoclave for 20 minutes at 121°C, and allow to cool.
2. Prepare each of the five inorganic stock solutions (listed below), autoclave for 20 minutes at 121°C, and allow to cool.
3. Prepare the 2 M glucose stock solution (see recipe below) and filter sterilize.

Basal Medium	
Proteose Peptone	20.0 g
Yeast Extract	1.0 g
Distilled water	900.0 L

Inorganic Stock Solutions
0.4 M $MgSO_4 \cdot 7H_2O$
0.05 M $CaCl_2$
0.005 M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$
0.25 M $Na_2HPO_4 \cdot 7H_2O$
0.25 M KH_2PO_4

2 M Glucose Stock Solution	
Glucose	18.0 g
Sodium Citrate • $2H_2O$	1.0 g
Distilled water	50.0 mL

4. Aseptically prepare the PYG medium by adding the components listed below to the Basal medium in the following order:

Basal medium	900.0 mL
0.4 M $MgSO_4 \cdot 7H_2O$	10.0 mL
0.05 M $CaCl_2$	8.0 mL
0.005 M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	10.0 mL
0.25 M $Na_2HPO_4 \cdot 7H_2O$	10.0 mL
0.25 M KH_2PO_4	10.0 mL
2 M glucose stock solution	50.0 mL

5. Adjust the pH of the complete medium to 6.5 with sterile solutions of 1 N HCl or 1 N NaOH.
6. Bring the final volume up to 1 L with sterile distilled water.

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