



Amoebophilium *protococcarum* Gromov and Mamkaeva

50289™

Description

Strain designation: X-1

Deposited As: *Amoebophilium protococcarum* Gromov and Mamkaeva

Type strain: Yes

Storage Conditions

Product format: Test tube

Storage conditions: See handling procedure

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

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ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submerged in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:

ATCC Medium 5: Sporulation agar

Temperature: 25°C

Handling Procedures

Handling of Live Culture

This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 5 mL. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.** To assure viability, immediately loosen the test tube cap and incubate on a 15° horizontal slant at 25°C for at least one hour before observing the culture. There should be numerous active

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trophozoites attached to the tube or floating in suspension. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, suspend trophozoites by rubbing the inside surface of the tube with a sterile cotton swab and aseptically transfer a 0.5 mL aliquot to the surface of a 20 x 100 mm Petri plate containing a growing culture of *Scenedesmus obliquus* (i.e., ATCC[®] 11457™ or similar) on 20 mL of ATCC medium 5 agar. Wrap the plate culture with parafilm and incubate upright under a 14 hour light (~50 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ irradiance)/10 hour dark cycle. Alternatively, transfer the 0.5 mL aliquot to a 16 x 125 mm screw-capped test tube containing a growing culture of *Scenedesmus obliquus* in 5 mL of ATCC Medium 5 broth. Incubate the tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 25°C under the same light/dark cycle as specified for a plate culture.

Note: In order for the *Amoebophilidium* to more successfully parasitize the host alga, it may be helpful to use agar media.

Culture maintenance:

1. For a plate culture, transfer cells with an inoculating loop to a plate of fresh agar medium from a growing culture at or near peak density. For a broth culture, inoculate a tube of fresh broth medium with 0.3 to 0.5 mL from a growing culture at or near peak density.
2. Incubate at 25°C under a 14 hour light (~50 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ irradiance)/10 hour dark cycle. In the case of a broth culture, screw tube cap on loosely (loosened one-half turn) and incubate on a 15° horizontal slant.
3. Subculture as necessary (typically every 1-2 wks).

Reagents for cryopreservation: ATCC Medium 1323 (Page's Balanced Saline)

Solution 1 (see below) 500.0 mL

Solution 2 (see below) 500.0 mL

Solution 1

Na_2HPO_4 , 0.142 g

KH_2PO_4 , 0.136 g

Distilled H_2O , 500.0 mL

Solution 2

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4.0 mg

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4.0 mg

NaCl , 0.120 g

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Distilled H₂O, 500.0 mL

Autoclave solutions 1 and 2 separately at 121°C. Combine the two solutions when cooled to room temperature.

Cryopreservation:

1. Allow amoebae to encyst. Harvest cysts from a culture that has recently passed peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cysts to 2×10^6 - 2×10^7 /mL in fresh medium.
3. While cysts are centrifuging prepare a 20% (v/v) solution of sterile DMSO in fresh ATCC medium 1323 (Page's Balanced Saline).
4. Mix the cell preparation and the 20% DMSO solution in equal portions. Thus, the final concentration will be 10^6 - 10^7 cells/mL and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO cryoprotective solution to the beginning of the freezing process should be no less than 15 min and no greater than 60 min.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. 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.)
7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. Vials should not be stored above -55°C.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to the surface of an ATCC medium 5 agar plate containing a growing culture of *Scenedesmus obliquus*. Alternatively, transfer the thawed contents to a 16 x 125 mm screw-capped test tube containing a growing culture of *Scenedesmus obliquus* in 5 mL of ATCC medium

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5 broth.

10. Incubate the culture at 50-100 $\mu\text{Einsteins}/\text{m}^2/\text{s}$ irradiance at 25°C. Maintain under a 14/10h light-dark photoperiod.
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Notes

This strain is an endoparasite and must be fed with live *Scenedesmus obliquus* (i.e., ATCC® 11457™ or similar, not provided). The *Scenedesmus* should be maintained separately and fed to *Amoebophilidium* at regular intervals. The feeding interval will depend on the number of amoebae present and the culture density of the host alga. If the number of amoebae is high, increase the feeding interval or passage the culture. *Amoebophilidium* will form cysts once the host alga population has been sufficiently depleted.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Amoebophilidium protococcarum* Gromov and Mamkaeva (ATCC 50289)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2021-05-19

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