



Product Sheet

Trichosphaerium sp. (ATCC® 40319™)

Please read this **FIRST**



Intended Use

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Trichosphaerium sp.* (ATCC® 40319™)

Description

Strain Designation: Am-I-7PL (Amoeba-I-7 Plastic)

Deposited Name: *Trichosphaerium sp.*

Depositor: M Polne-Fuller

Isolation: Mutant derived from ATCC 40318, Santa Barbara Co., CA, ?

Propagation

Growth Conditions

Temperature: 20°C

Protocol: This strain is shipped as a test tube culture (10 ml of culture in a 20 x 125-mm screw-capped test tube). Upon arrival, rub the internal surfaces of the tube with a sterile cotton swab to dislodge any adhering amoebae. Aseptically divide the entire contents into 2 equal portions and distribute to T-25 tissue culture flasks. Add 5 ml of fresh ATCC medium 1405 and 0.5 ml of heat-killed *Dunaliella tertiolecta* ATCC 30929 to each flask. Screw the caps on tightly and incubate at 20-25°C. Transfer every 4-6 weeks. Rub the surface of the flask to be subcultured with a sterile cotton swab, agitate and aseptically transfer a 0.25-0.5 ml aliquot to a fresh flask containing 10 ml of ATCC medium 1405 supplemented with 0.5 ml of heat-killed algal suspension. Incubate as above. Note: Since this strain is cultivated with heat-killed *Dunaliella tertiolecta* ATCC 30929 as a food source, the food should be prepared before the strain is ordered. The food is prepared as follows: 1. Establish a culture of ATCC 30929 in 5 ml of ATCC medium 1194 broth in a 16 x 125-mm screw-capped test tube. Incubate with the cap on loose under 50-75 (Einsteins/m²/s) of light at 25°C. 2. When the culture reaches early stationary phase, aseptically transfer 0.25-ml aliquots to each of 10 250-ml cotton-plugged Erlenmeyer flasks containing 100 ml of ATCC medium 1194 broth. Incubate as above. 3. When the cultures reach early stationary phase (approximately 10-14 days), harvest as follows: Aseptically transfer algal suspensions to 50-ml plastic centrifuge tubes. Centrifuge at 400-500 x g for 5 minutes. 4. Decant supernatant and resuspend each pellet in approximately one ml of ATCC medium 1405. Pool all suspensions in a single centrifuge tube and centrifuge as in step 3. The cell pellet should be approximately 5 ml. 5. Resuspend the cell pellet to a final volume of approximately 14 ml with ATCC medium 1405 and transfer to a 15-ml plastic centrifuge tube. Centrifuge as in step 3. Discard the supernatant. 6. Resuspend the cell pellet to a final volume of approximately 14 ml with ATCC medium 1405 and centrifuge as in step 3. Discard the supernatant. 7. Resuspend the final cell pellet to a final volume of 10 ml with ATCC medium 1405. Transfer the cell suspension to a sterile 125-ml screw-capped bottle and aseptically add 40 ml of ATCC medium 1405 bringing the final volume to 50 ml. WARN: Last ATCC#: 40318, unprocessed line: 8. Place bottle prepared in step 7 in a 60°C water bath to a level such that the liquid level of the water bath is above that of the suspension in the bottle. Incubate for a total of 30 minutes, swirling the bottle at 10-minute intervals. Allow the bottle to cool to room temperature. This treatment will kill all algal cells. 9. As a check for viable cells, add 3 drops of the cell suspension prepared in step 10 to the edge of a 100-mm petri plate containing ATCC medium 1194 agar. Hold the plate vertically to allow the drops to move to the opposite edge. Incubate plate at 25°C under 50-75 μ E of light for 7 days. 10. The heat-killed algae can be stored at 4°C for up to one year.

Medium

ATCC® Medium 1405: HESNW medium

References

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

Biosafety Level: 1

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.

ATCC Warranty

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1

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formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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