ATCC

Product Sheet

Trichosphaerium sp. (ATCC® 40318™)

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® 40318™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: Am-I-7 wt (Amoeba-I-7 Wild Type)
Deposited Name: *Trichosphaerium* sp.
Depositor: M Polne-Fuller
Isolation:
stipes of Sargassum muticum, Alegria Beach, Hollister Ranch, Santa Barbara Co., CA, 1984

Propagation

Growth Conditions
Max Temperature: 25.0°C
Min Temperature: 20.0°C
Protocol: ATCCNO: 40318 SPEC: 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 20-25C. 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 25C. 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 60C 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 25C under 50-75 uE of light for 7 days. 10. The heat-killed algae can be stored at 4C for up to one year.

Medium
ATCC® Medium 1405: HESNW medium

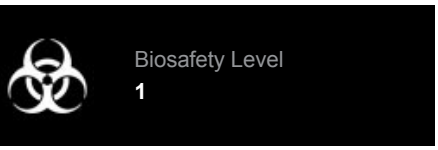
Instructions for Complete Medium
ATCC Medium 1405 inoculated with heat-killed *Dunaliella tertiolecta* ATCC® 30929 as a food source* (see below and in the product sheet for this strain for cultivation details).
*Note: Since this strain is cultivated with heat-killed *Dunaliella tertiolecta* ATCC® 30929 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 µEinsteins/m^2/s irradiance 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.

ATCC

Product Sheet

Trichosphaerium sp. (ATCC® 40318™)

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® 40318™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

6. 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.
7. Place bottle prepared in step 6 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.
8. As a check for viable cells, add 3 drops of the cell suspension prepared in step 7 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 µEinsteins/m^2/s irradiance for 7 days.
9. The heat-killed algae can be stored at 4°C for up to one year.

Culture Maintenance

1. To transfer the culture, vigorously agitate a culture at or near peak density and aseptically transfer a 0.1 ml aliquot to a T-25 tissue culture flask containing 10 ml of fresh ATCC medium 1405 and 0.5 ml of heat-killed *Dunaliella tertiolecta* ATCC® 30929.
2. Incubate in a 25°C incubator with the cap screwed on tightly. Subculture every 4-6 weeks.

Cryopreservation

Cryoprotective Solution

DMSO	1.5 ml
Fresh growth medium	8.5 ml

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest the culture by agitating the contents of each flask. A sterile cotton swab or cell scraper may be rubbed over the bottom surface of each flask to detach any adhering amoebae. Transfer the cell suspensions to 15 ml plastic centrifuge tubes.
2. Spin the cell suspensions at approximately 500 x g for 5 min.
3. Pool the cell pellets and adjust the concentration to 2.0 - 4.0 x 10^7 cells/ml with a fresh ATCC medium 1405.

*If the concentration is too low centrifuge at 500 x g for 5 min and resuspend in the volume of ATCC medium 1405 required to yield the desired concentration.

4. Mix the cell preparation and 15% (v/v) DMSO in equal portions. The final concentration will be 1.0 - 2.0 x 10^7 cells/ml and 7.5% DMSO. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 30 min.
5. Dispense in 0.5 ml aliquots to 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 2.5 to 3 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately
-1°C/min.)
7. Store in either the vapor or liquid phase of a nitrogen refrigerator.
8. 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.
9. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing 10 ml ATCC medium 1405.
10. Incubate in a 25°C incubator with the cap screwed on tightly.
11. Once a healthy culture has been established, aseptically add 0.5 ml of heat-killed *Dunaliella tertiolecta* ATCC® 30929 (delayed addition of the food organism following the initial thaw seems to be beneficial).
12. Follow the protocol for maintenance of culture.

References

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

Biosafety Level: 1

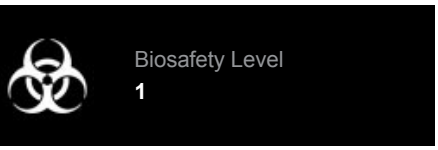
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National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

ATCC

Product Sheet

Trichosphaerium sp. (ATCC® 40318™)

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® 40318™)

for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium 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.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor