Strain Designation: T66
Depositor: I Aasen
Isolation: Marine sediment and seawater, Southcoast of Madeira, Portugal, 2001

Growth Conditions
Temperature: 25°C
Culture System: Axenic

Medium
ATCC® Medium 2673: Thraustochytrid medium
ATCC® Medium 2348: Freshwater Diplophrys medium
ATCC® Medium 790: By+ medium

Instructions for Complete Medium
Media:
ATCC Medium 2673 should be diluted in ATCC medium 2348 in a 2:1 ratio (2 parts ATCC Medium 2673, 1 part ATCC Medium 2348)
Alternate Media:
ATCC Medium 790 should be diluted in ATCC medium 2348 in a 2:1 ratio (2 parts ATCC Medium 790, 1 part ATCC Medium 2348)

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules may be stored at or below -70°C for approximately one week. Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C). Storage of frozen material at this temperature will result in the death of the culture.

1. 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.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask or 16 x 125 mm test tube containing 5 mL of a 2:1 mixture of ATCC Medium 2673 and ATCC Medium 2348.
3. Screw the cap on tightly and incubate the tube or flask at 25°C.

Culture Maintenance
1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.25 mL to a fresh tube or flask containing 5 mL of a 2:1 mixture of ATCC Medium 2673 and ATCC Medium 2348.
3. Screw the caps on tightly and incubate at 25°C (incubate test tubes at a 15° horizontal slant).
4. Thraustochytrids will eventually form an almost continuous sheet of cells on the bottom surface of the flask or test tube, with other cells floating in the liquid column and collecting at the fluid meniscus.
5. Repeat steps 1-3 at 2-3 week intervals.

Cryopreservation

Harvest and Preservation
1. To achieve the best results set up cultures with several different inocula (e.g. 0.25 mL, 0.5 mL, 1.0 mL). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between 2 x 10^6 and 2 x 10^5 cells/mL with fresh medium. If the concentration is too low, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 20% (v/v) solution of sterile DMSO as follows: Add the required
Please read this FIRST

Storage Temp.
Frozen: -70°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Protocols Section

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: Aurantiochytrium sp. (ATCC® PRA-276™)

Protocols Section

Live Culture:

- Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.
- Incubate at 25°C.

Freeze-Dried:

- Place the ampules into a freezer at -80°C for 1.5 to 2 hours. If an alternative freezing apparatus is used, 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 ampules 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.)
- Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 5 mL of a 2:1 mixture of ATCC Medium 2673 and ATCC Medium 2348 in a T-25 tissue culture flask or 16 x 125 mm screw-capped test tube. Incubate at 25°C.

Freeze-Dried Products

- 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.)
- The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
- Place the vials in a water bath set at 35°C (2-3 min).
- Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 5 mL of a 2:1 mixture of ATCC Medium 2673 and ATCC Medium 2348 in a T-25 tissue culture flask or 16 x 125 mm screw-capped test tube. Incubate at 25°C.

Additional Information

- Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10⁶ and 10⁷ cells/mL and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 30 min.
- Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryotubes (special plastic vials for cryopreservation).

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.

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