ATCC

Product Sheet

Tokophrya lemnarum (ATCC® 50033™)

Please read this FIRST

Biosafety Level
1

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: *Tokophrya lemnarum* (ATCC® 50033™)

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
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Or contact your local distributor

Description

Strain Designation: Oneonta
Deposited Name: *Tokophrya lemnarum* (Stein) Entz
Depositor: LA Colgin-Bukovsan
Isolation:
wastewater treatment plant, Oneonta, NY, 1982

Notes

This strain must be fed with live *Paramecium* (i.e., ATCC® 30567 or similar, not provided). The *Paramecium* should be maintained separately and fed to *Tokophrya* at regular intervals. Overfeeding of *Tokophrya* may result in monster formation. Attempt to maintain a ratio of 2-3 prey organisms per each suctorian. If the number of abnormal suctorians is high, reduce the feeding interval or passage the culture.
This strain of *Tokophrya lemnarum* is mating type I. The culture is polyxenic and contains mixed bacterial flora.

Propagation

Growth Conditions
Temperature: 25.0°C
Protocol: ATCCNO: 50032 SPEC: Food source, Paramecium tetraurelia ATCC 30567, not supplied.

Medium
Medium 1323: Page's balanced salt solution (PBS)

Instructions for Complete Medium
ATCC® Medium 1323

Culture Maintenance
Periodically add prey organisms as follows:
1. Maintain growing cultures of *Paramecium* separately at 25°C in T-25 tissue culture flasks containing 10 ml ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
2. Prepare washed *Paramecium* as follows: Remove 5-10 ml from a culture at or near peak density, centrifuge at 300 x g for 5 min, quickly remove most of the supernatant (leaving approx. 1 ml), then resuspend cells in 10 ml ATCC medium 1323. Centrifuge and resuspend cells again as above. Repeat this washing step at least twice.
3. When the *Tokophrya* have consumed all prey *Paramecium*, add 0.5-2 ml of washed *Paramecium* prepared in step 2. The feeding interval will depend on the number of suctorians present and the culture density of the washed prey.
4. The *Tokophrya* may be passaged to a new petri plate or T-25 flask by gently rubbing the agar surface with a spread bar to dislodge attached suctorians, then transferring 0.5 to 2 ml to a fresh petri plate or T-25 flask containing a bed of non-nutrient agar (ATCC medium 919) and 10 ml ATCC medium 1323. Incubate the culture at 20-25°C, feeding periodically with washed *Paramecium*.

Cryopreservation

Cryoprotective Solution

DMSO	2.0 ml
Fresh growth medium w/o bacteria	8.0 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 *Tokophrya* cells from a culture that has recently passed peak density by centrifugation at 250-300 x g for 5 min.
3. Adjust the concentration of cells to at least 2 x 10^4/ml in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions by adding the cryoprotective solution to the cell suspension in 3 equal aliquots at 2 min. intervals.
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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing

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unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules 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. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and transfer to a petri plate or T-25 tissue culture flask containing a bed of non-nutrient agar (ATCC medium 919) and 10 ml ATCC medium 1323.
9. Aseptically transfer 0.5-2.0 ml of washed *Paramecium* to the petri plate or T-25 flask (see section on MAINTENANCE OF CULTURE). Incubate the culture at 20-25°C.

Once the culture is established, 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 for Health.

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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.

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