Prototheca wickerhamii Tubaki and Soneda

16522™

Description

Strain designation: PR-53 [UTEX 1441]
Deposited As: *Prototheca wickerhamii* Tubaki and Soneda
Type strain: No

Storage Conditions

Product format: Freeze-dried
Storage conditions: 2°C to 8°C

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.

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 submersed 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 submersed 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 28: Emmons' modification of Sabouraud's agar/broth
Instructions for complete medium: ATCC Medium 28
Temperature: 20-25°C
Culture system: Axenic

Handling Procedures

Establishing Cultures from Dried State

This strain is distributed as a freeze-dried preparation. If the culture will not be rehydrated immediately upon arrival, store at 5°C until processed.

1. To rehydrate an ampule, aseptically add 0.5 ml of ice-cold medium 28 broth containing 12% sucrose to the freeze-dried inner shell vial.
2. Once completely rehydrated, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing a slant of ATCC Medium 28 or distribute over the surface of an agar plate of ATCC medium 28.
3. If a test tube culture has been established, incubate upright at 20-25°C with the cap screwed on loosely. If a plate culture has been established, wrap the plate with parafilm and incubate inverted at 20-25°C.

Culture maintenance:

1. Transfer cells with an inoculating loop to a tube or plate of fresh agar medium from a growing culture at or near peak density.
2. Incubate as described in step 3 under the section for establishing a culture.

Cryopreservation:

1. Harvest cells from a culture which is at or near peak density by adding 3.0-5.0 ml fresh ATCC medium 28 broth to the slant or plate and washing cells into suspension. It may be helpful to rub the surface of the agar with a spread bar or inoculating loop to detach adhering cells.
2. Adjust the concentration of cells to 2 x 10^7/ml with fresh broth medium, then dilute to half this concentration by adding an equal amount of a 20% (v/v) sterile solution of either DMSO or glycerol in fresh ATCC medium 28 broth.
3. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell preparation and the cryoprotective solution to the start of the cooling cycle should be no less than 15 min and no greater than 30 min.
4. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing 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.)
5. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stabile indefinitely. Those stored at temperatures above -130°C are progressively less stabile as the storage temperature is elevated. Vials can be stored between -80 and -70°C for no longer than one week.
6. To establish a culture from the frozen state place an ampule in a water bath

set at 35°C until thawed (2-3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.

7. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to a fresh slant of ATCC medium 28 or the surface of an agar plate of ATCC medium 28.
8. Maintain as described above.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Prototheca wickerhamii* Tubaki and Soneda (ATCC 16522)

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 2024-12-07

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