



Neosporioccum *excentricum* Deason and Bold

40335™

Description

Strain designation: HS1 [B]

Deposited As: *Neosporioccum excentricum* Deason and Bold

Type strain: No

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Patent number:

5,437,997

Technical information: ATCC Technical Services does not have technical information on patent deposits that are not produced or characterized by ATCC. Additional information can be found in the corresponding patent available from the patent holder or with the U.S. and/or international patent office.

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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

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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 2001: CYU 2%

Temperature: 25-35°C

Handling Procedures

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 ampules 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 in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing 5 mL of ATCC Medium 2001 broth. Incubate the tube on a 15° horizontal slant with the cap screwed on loosely (loosened one half turn) at 25-35°C under a 14 hour light (~50 $\mu\text{Einstein}/\text{m}^2/\text{s}$ irradiance)/10 hour dark cycle. **NOTE:** Do not distribute the thawed material to a larger volume of medium. It is essential to first establish the culture in a small volume.

Culture maintenance:

1. Screw the cap on tightly and vigorously agitate the culture.
2. Aseptically transfer a 0.1 mL aliquot to 5 mL of fresh medium in a 16 x 125 mm screw-capped test tube.
3. Screw caps on loosely (loosened one-half turn) and incubate on a 15° horizontal slant at 25-35°C under a 14 hour light (~50 $\mu\text{Einstein}/\text{m}^2/\text{s}$ irradiance)/10 hour dark cycle.
4. Subculture every 5-7 days.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cells to 2×10^6 - 2×10^7 /mL in fresh medium.
3. While cells are centrifuging prepare a solution of 14% (v/v) sterile DMSO

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solution in fresh medium.

4. Mix the cell preparation and the cryoprotective solution in equal portions. Thus, the final concentration will be 10^6 - 10^7 cells/mL and 7% (v/v) DMSO. The time from the mixing of the cell preparation and cryoprotective stock solution to the start of the cooling cycle should be no less than 5 min and no greater than 15 min.
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 the vials in a controlled rate freezing unit. From room temperature cool at $-1^\circ\text{C}/\text{min}$ to -40°C . If the freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ 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 $-1^\circ\text{C}/\text{min}$.)
7. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. Vials should not be stored above -55°C .
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C . Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to a centrifuge tube containing 5 mL of ATCC medium 2001.
10. Incubate the culture at 50 - $100 \mu\text{Einsteins}/\text{m}^2/\text{s}$ irradiance at 25 - 35°C . Maintain under a 14/10h light-dark photoperiod.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Neosporangiococcum excentricum* Deason and Bold (ATCC 40335)

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 2022-11-07

Contact Information

***Neosporiobacterium excentricum* Deason and Bold**
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ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
