Methanohalophilus mahii Paterek and Smith

35705™

Description

Strain designation: SLP
Deposited As: *Methanohalophilus mahii* Paterek and Smith
Type strain: Yes

Storage Conditions

Product format: Freeze-dried

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 2485: MH-OCM Medium
Temperature: 37°C
Atmosphere: 80% H_2, 20% CO_2

Handling Procedures

1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flaming the top.

2. If needed exchange the gas in the test tube for 80% H_2-20% CO_2

3. If the medium is pink (see discussion about resazurin) add 0.1 ml of reducing agent (3% cysteine, stock solution) per each 10 ml of medium. Let the medium sit at room temperature for 30 minutes, until the resazurin becomes colorless, before

inoculating.

4. Allow the frozen vial to thaw under anaerobic conditions. Once thawed, take a gassed 1.0 ml syringe tipped with 22-gauge needle and withdraw the entire contents of the thawed vial and immediately transfer it to a #2485 broth tube.

5. Plate 0.1 ml on a non-selective medium to check for aerobic and anaerobic contamination.

6. Incubate tubes and one plate under an anaerobic atmosphere at 37ºC. Incubate non-selective plate aerobically at 37ºC to check for purity.

7. In 72 to 96 hours, growth should be evident by light turbidity in the broth. No growth should occur on the non-selective plate incubated aerobically or anaerobically. No growth should occur on blood agar plates.

ANAEROBIC CONDITIONS:

a. Balch tubes (available from Bellco Glass, Vineland, NJ) are specially designed for anaerobic work and use an aluminum crimp cap to hold a rubber stopper in place. Needles can easily be inserted through the stopper, and the tubes can be pressurized to 2 atm. Alternatively, serum vials may be used, or screw cap tubes with butyl rubber stoppers, in the latter case the stopper may be removed and the tube placed under a cannula system that dispenses sterile, oxygen free gas for addition of reducing agents or inoculation.

b. Resazurin is a commonly used redox indicator that is pink when the redox potential is above 50 mv, and colorless when the redox potential is below 110 mv. i.e. highly reducing. Most strict anaerobes require this low redox potential for optimum growth.

c. To obtain a fully reduced medium, it is necessary that the medium be anoxic and that a reducing agent be added. Common reducing agents are sodium sulfide, cysteine, dithiothreitol, and titanium citrate.

Notes

Within 96 hours of incubation, there should be turbidity in the broth. Cell morphology is irregular coccoid cells with no motility. This organism can utilize 20

mM of trimethylamine as a secondary substrate, which would promote growth.

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

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Methanohalophilus mahii* Paterek and Smith (ATCC 35705)

References

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

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