



Nutrient Agar w/ Tyrosine

M561F

Nutrient Agar w/ Tyrosine is used for cultivation and enumeration of *Bacillus cereus* in water and food in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Beef extract	3.000
Peptone	5.000
Agar	15.000
Tyrosine	5.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.00 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense 3.5 ml into sterile tubes with frequent mixing. Keep in slanted position and cool rapidly to prevent separation of tyrosine.

Principle And Interpretation

Bacillus cereus is an aerobic spore-forming bacterium that is commonly found in soil, on vegetables, and in many raw and processed foods. *B. cereus* food poisoning may occur when foods are prepared and held without adequate refrigeration for several hours before serving, with *B. cereus*. Foods incriminated in past outbreaks include cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts. Nutrient Agar w/ Tyrosine is used for cultivation and enumeration of *Bacillus cereus* in water and food in accordance with FDA BAM, 1998(1). The organism can be identified by its ability to hydrolyze tyrosine in the medium. Peptone and beef extract provide essential nutrients for the growth of the organism. Agar acts as the solidifying agent. Tyrosine is a source of amino acid which is hydrolyzed by *Bacillus* species.

Prepare 1:10 dilutions of 50 g of the sample in Butterfield's phosphate-buffered dilution water (R094). Plate count of *B.cereus* can be done on MYP (M636F) agar plates from appropriate dilutions. *B.cereus* gives pink coloured colonies on MYP agar. Suspected colonies are subcultured into Nutrient agar (M561F). Inoculate entire surface of tyrosine agar slant with mm loopful of culture from Nutrient agar. Incubate slants 48 h at 35°C. Positive results are indicated by the zone of clearance in and around the bacterial growth, indicating hydrolysis. Examine negative slants for obvious signs of growth, and incubate for a total of 7 days before considering as negative(1). This media is used in the confirmation of other species of *Bacillus* such as *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. weihenstephanensis*, *B. anthracis* and *B. megaterium* and also for *Streptomyces* and *Nocardia* species (2,3).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared medium

Yellow coloured clear to slightly opalescent gel forms in slants(may shows some white particles after solidification)

Reaction

Reaction of 2.8% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 48hours up to 7days.

Cultural Response

Organism	Growth	Tyrosine hydrolysis
Cultural Response		
<i>Bacillus cereus</i> ATCC 10876	good-luxuriant	positive reaction, clearing of medium in and around the bacterial growth
<i>Bacillus thuringiensis</i> ATCC 10792	good-luxuriant	positive reaction, clearing of medium in and around the bacterial growth
<i>Escherichia coli</i> ATCC 25922	good	negative reaction, no clear zones

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 -8°C. Use before expiry date on the label.

Reference

- 1.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
- 2.Larone. 1995. Medically important fungi: a guide to identification. 3 ed. Washington, D.C: ASM.
- 3.Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Tenover, R. H. 2003. Manual of Clinical Microbiology. 8 ed. Washington, D.C: ASM.

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