



Bacillus cereus Selective Agar Base (MYP) (In accordance with ISO 7932)

This medium is used for isolation and identification of *Bacillus* species and pathogenic Staphylococci. The composition and performance criteria of this medium are as per the specification laid down in ISO 7932:2004.

Composition**

Ingredients	Gms / Litre
Enzymatic digest of casein	10.000
Beef extract	1.000
D-Mannitol	10.000
Sodium chloride	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.03 grams in 900 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 2 vials of sterile Polymyxin B Sulphate (FD003) solution and 100 ml sterile Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Bacillus cereus is ubiquitously present in soil, vegetation water and dust. It has been isolated from a large variety of foods, including vegetables, meat, cereals, pasteurized fresh milk and powdered milk (1, 2, 6) and processed foods. Under favourable conditions, the organism multiplies and causes gastrointestinal illness (6). It is implicated in two different forms of food poisoning; an emetic illness and a diarrhoeal illness. The emetic illness is mediated by a highly stable toxin that survives high temperature, exposure to trypsin, pepsin and pH extremes. The diarrhoeal illness is mediated by a heat and acid labile enterotoxin.

Lecithinase activity is the key reaction in the differential identification of *B.cereus*, the most commonly encountered and important species in clinical laboratories, from the majority of the other *Bacillus* species. If unknown isolate produces lecithinase, *Bacillus cereus* can be presumptively identified by also observing colonial morphology, hemolytic reactivity and motility tests. When present in large numbers in certain foodstuffs, *B. cereus* can produce metabolites responsible for the clinical symptoms of food poisoning (3). This medium differentiates *B.cereus* from other bacteria based on the basis of lecithinase activity, mannitol fermentation and resistance to polymyxin (FD003) (4, 5). *Bacillus cereus* Selective Agar Base is recommended by the ISO committee for the enumeration of *Bacillus cereus* (9).

It contains enzymatic digest of casein and beef extract, which provide nitrogen source. Mannitol fermentation can be detected by phenol red, which yields yellow colour to the mannitol fermenting colonies due to acid production. Added egg yolk emulsion helps in differentiation of lecithinase producing colonies, which are surrounded by a zone of white precipitate. Addition of Polymyxin B Sulphate (FD003) helps to restrict growth of gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. These differentiating media allow differentiation of *B.cereus* from other *Bacillus* species by its inability to ferment mannitol and poor sporulation. *B.cereus* dissimilates egg yolk and gives rise to typical bacilli form colonies with reddish zones and white halos. Acid produced by organisms other than *B.cereus* often diffuse through the medium, making it difficult to distinguish between mannitol fermenters and non-fermenters. So it is advised to transfer the suspected colonies to a fresh medium to visualize the true reaction.

Quality Control

Appearance

Light yellow to pinkish purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Red coloured clear to slightly opalescent gel. After Addition of Egg Yolk Emulsion (FD045) : Light orange coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.0-7.40

Cultural Response

Cultural characteristics observed with added Egg Yolk Emulsion (FD045) and Polymyxin B Sulphate (FD003) after an incubation at 30±2°C for 18-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase activity
<i>Bacillus cereus</i> ATCC 10876	50-100	luxuriant	≥50%	red	positive, opaque zone around the colony
<i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	≥50%	yellow	negative
<i>Escherichia coli</i> ATCC 25922	50-100	none-poor	≤10%		Negative
<i>Proteus mirabilis</i> ATCC 25933	50-100	luxuriant	≥50%	red	negative
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	none-poor	≤10%		Negative
<i>Staphylococcus aureus</i> ATCC 25923	50-100	luxuriant	≥50%	yellow	positive, opaque zone around the colony

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

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