



Bacillus Cereus Agar Base

M833

Intended use

Bacillus Cereus Agar Base with added supplements is used as a selective medium for the isolation and enumeration of *Bacillus cereus* from food samples.

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Mannitol	10.000
Sodium chloride	2.000
Magnesium sulphate	0.100
Disodium phosphate	2.500
Monopotassium phosphate	0.250
Sodium pyruvate	10.000
Bromo thymol blue	0.120
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.5 grams in 475 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 and aseptically add rehydrated contents of 1 vial of Polymyxin B Selective Supplement (FD003) and 25 ml of sterile Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Bacillus cereus causes food poisoning due to the consumption of contaminated rice (4, 6), eye infections (5) and a wide range of other clinical conditions like abscess formation, meningitis, septicemia and wound infection. *Bacillus cereus* is a known cause of disease mastitis, especially in ewes and heifers among the veterinarians (7). Holbrook and Anderson (1) developed Bacillus Cereus Agar, which is a highly specific and selective medium for the isolation and enumeration of *Bacillus cereus* from foods. It supports the growth of even a small number of *Bacillus cereus* cells and spores in the presence of large number of other food contaminants. The typical colonies of *Bacillus cereus* are crenate, about 5 mm in diameter and have a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour. The bacteria do not ferment mannitol and thus there is no change in colour of the indicator dye around the colonies.

Addition of polymyxin-B sulphate (2, 3) at a final concentration of 100 units per ml of medium is sufficient to make the medium selective for the isolation of *Bacillus cereus*. It suppresses the growth of accompanying bacterial flora. If moulds are suspected in the inoculum, 40 mcg per ml filter-sterilized cycloheximide may be incorporated to suppress the mould contamination. Some strains of *Bacillus cereus* have very weak egg yolk reaction. Moreover, on this medium *Bacillus cereus* is indistinguishable from *Bacillus thuringiensis*.

Peptone provides and sodium pyruvate improve egg yolk precipitation and enhance sporulation. Bromothymol blue acts as pH indicator to detect mannitol fermentation. For the isolation and enumeration of *Bacillus cereus* in foodstuffs the following method is recommended. Distribute 0.1ml of the homogenized specimen diluted in Peptone Water (M028) onto the surface of the medium. Incubate at 37°C under aerobic conditions for 24-48 hours. Possible growth of contaminants is greatly reduced by incubation for 24 hours. Report the results as the number of *Bacillus cereus* colonies per gram weight of the food sample. Confirmatory tests should be carried out before interpretation.

Type of specimen

Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,12). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

Bacillus cereus and *Bacillus thuringiensis* shows identical characteristics and hence difficult to identify.

Identification of *Bacillus cereus* is done by colony characteristics and reaction, however further biochemical characteristics should be carried out for confirmation.

Some strains of *Bacillus cereus* may show poor growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Green coloured clear to slightly opalescent gel. After addition of egg yolk emulsion : Yellowish green coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.1% w/v aqueous solution (basal medium) at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added Polymyxin B Selective Supplement (FD003) and Egg Yolk Emulsion (FD045) after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Egg Yolk Reaction
Cultural Response					
<i>Bacillus cereus</i> ATCC 10876	50-100	good-luxuriant	≥50%	blue	positive, precipitation
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 ³	inhibited	0%		
<i>Proteus vulgaris</i> ATCC 13315	50-100	good-luxuriant	≥50%	green	negative
<i>Serratia marcescens</i> ATCC 8100	50-100	good-luxuriant	≥50%	yellow-light pink (pigment production is enhanced by incubation at 25-30°C)	negative
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	good-luxuriant	≥50%	yellow	positive, clearing

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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