Technical Information

Bacillus Cereus Agar Base

Product Code: DM 1833

Application: - 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
Peptic digest of animal tissue	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

Principle & Interpretation

Bacillus cereus causes not only food poisoning due to the consumption of contaminated rice but also responsible for a wide range of other clinical conditions like eye infection abscess formation, meningitis, septicemia and wound infection [4, 5, 6]. *Bacillus cereus* is a known cause of disease mastitis, especially in ewes and heifers among the veterinarians [7]. Bacillus Cereus Agar, which is a highly specific and selective medium for the isolation and enumeration of *Bacillus cereus* from foods, was developed by Holbrook and Anderson [1]. 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 at a final concentration of 100 units per ml of medium is sufficient to make the medium selective for the isolation of *Bacillus cereus* [2, 3]. It suppresses the growth of accompanying bacterial flora 40 mcg per ml filter-sterilized cycloheximide may be incorporated to suppress the mould. Some strains of *Bacillus cereus* have very weak egg yolk reaction. However this medium fails to distingnish *Bacillus cereus* from *Bacillus thuringiensis.*

Peptic digest of animal tissue 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 (DM1028) 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.

Methodology

Suspend 20.5 grams of powder media in 475 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Polymyxin B Selective Supplement (MS2003) and 25 ml of sterile Egg Yolk Emulsion (MS2045). Mix well and pour into sterile Petri plates.

Quality Control

Physical 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 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 range 7.00-7.40

Cultural Response/Characteristics

DM1833: Cultural characteristics observed with added Polymyxin B Selective Supplement (MS2003) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Egg Yolk Reaction
Bacillus cereus ATCC10876	50-100	Good-luxuriant	>=50%	Blue	Positive,precipitation
Escherichia coli ATCC25922	$>=10^3$	Inhibited	0%		
Proteus vulgaris ATCC13315	50-100	Good-luxuriant	>=50%>	Green	Negative
Serratia marcescens ATCC8100	50-100	Good-luxuriant	>=50%	Yellow-light pink (pigment production is enhanced by incubation at 25-30^0 C)	Negative
Staphylococcus aureus ATCC 25923	50-100	Good-luxuriant	>=50%	yellow	Positive clearning

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Holbrook R. and Anderson J., 1980, Can. J. Microbiol. 26(7):753-759
2. Donovan K.O., 1958, J. Appl. Bacteriol, 21(1): 100.
3. Mossel D.A.A., Koopman J. and Jongerius E., 1967, J. Appl. Microbiol. 15(3):650-653.
4. Mortimer P.R. and McCann G., 1974, Lancet, 1043-1045.
5. Bouza E., Grant S., Jordan C. et al, 1979, Arch. Ophthalmol. 97:498
6. Wohlgemuth K., Kirkbride, C.A., Bicknell, E. J. and Ellis, R.P., 1972, J. Am. Vet. Med. Ass. 161:1691-1695.
7. Kirnbull P.C., J. Clin. Pathol. 32:289

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