Bacillus Cereus HiCynth™ Agar Base

MCD833

Bacillus Cereus HiCynth™ Agar Base is used as a selective medium for the isolation, detection and enumeration of *Bacillus cereus* from food samples.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.2*	1.000
Mannitol	10.000
Sodium chloride	2.000
Magnesium sulphate	0.100
Disodium hydrogen phosphate	2.500
Potassium dihydrogen phosphate	0.250
Sodium pyruvate	1.000
Bromo thymol blue	0.120
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

* Chemically defined peptone

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. Bacillus Cereus HiCynth™ Agar is a modification of Bacillus Cereus Agar wherein animal based peptones are replaced with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones. 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* .

HiCynth™ peptone No.2 provides nitrogen and carbon source, long chain amino acids, vitamins and other growth factors. Sodium pyruvate improves 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.

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					
Bacillus cereus ATCC 10876	50-100	good-luxuriant	>=50%	blue	positive,precipitation
Escherichia coli ATCC 25922	>=10^3	inhibited	0%		
Proteus vulgaris ATCC 13315	50-100	good-luxuriant	>=50%	green	negative
Serratia marcescens ATCC 8100	50-100	good-luxuriant	>=50%	yellow-light pink(pigment production is enhanced by incubation at 25-30°C)	negative
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=50%	yellow	positive, clearing

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. 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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