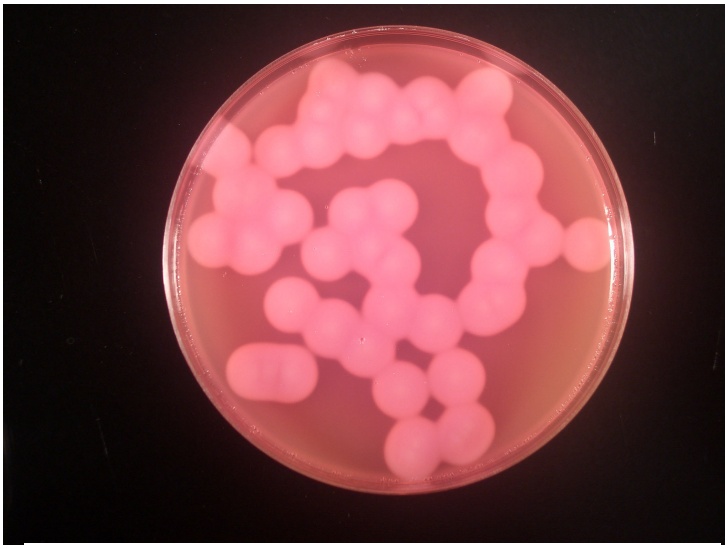
BACILLUS CEREUS SELECTIVE AGAR BASE - MYP
BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT
BACILLUS CEREUS SELECTIVE AGAR -MYP

Basal medium, selective supplement and ready to use plates and flasks for the enumeration of *B. cereus* in foodstuffs and other samples (ISO 7932 and ISO 21871)



MYP: typical *Bacillus cereus* colonies

TYPICAL FORMULAS

Bacillus Cereus Agar Base (MYP) (g/L)

Beef Extract	1.000
Peptone	10.000
D-mannitol	10.000
Sodium Chloride	10.000
Phenol Red	0.0250
Agar	12.000

Bacillus Cereus Selective Supplement (vial contents)

Polymyxin B Sulphate	50,000 IU

Bacillus Cereus Selective Agar (MYP) – ready to use plates (g/L)

Bacillus Cereus Agar Base (MYP)	900 mL
Egg Yolk Emulsion	100 mL
Polymyxin B Sulphate	100,000 IU

DIRECTIONS FOR POWDERED MEDIUM

Suspend 21.5 of Bacillus Cereus Agar Base MYP in 450 mL of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Cool to 45- 50°C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (Ref. 4240001) with 5 mL of sterile distilled water, add to the base medium and mix. Add 50 mL of Egg Yolk Emulsion, (Ref. 42111601) mix and distribute into sterile Petri dishes.
Final pH 7.2 ± 0.2

DIRECTIONS FOR READY TO USE MEDIUM IN FLASKS

Heat to boiling the ready to use medium in flasks and cool to 45-50°C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (Code 4240001) with 5mL of sterile distilled water, add 1 mL to the base medium and mix. Add 10 mL of Egg Yolk Emulsion (Code 42111601) mix and distribute into sterile Petri dishes.

DESCRIPTION

Bacillus cereus is responsible for food poisoning, provoked by two toxins, one being heat stable and emetic and the other being thermolabile the cause of diarrhoea. The infection is caused by ingestion of foods such as meat, rice and vegetables that are contaminated with *B. cereus,* and have been left at room temperature after cooking. The minimum infective dose is 100 cells/g of food. The diagnosis must be supported by the isolation of *B. cereus* from foodstuffs and faeces using quantitative cultures. The MYP medium (Mannitol Yolk Polymyxin Agar) corresponds to the formulation recommended by FDA BAM for the enumeration of *B.cereus* in foods.

TECHNIQUE

For the isolation and enumeration of *B. cereus* in foodstuffs according ISO 7932 the following method is recommended:

1. Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned.
2. Distribute 0.1mL of test sample if the product is liquid, or of the initial suspension if solid onto the surface of two agar plates (90mm). Repeat the procedure using further decimal dilutions.
3. If low number of *B.cereus* is expected, distribute 1mL of test sample if the product is liquid or 1mL of the initial suspension if solid to each of two agar plates (140mm) or over the surface of three 90mm plates.
4. Incubate at 30°C in aerobic conditions for 18-24 hours. If colonies are not visible incubate the plates for further 24 hours before counting.
5. Count the presumptive *B. cereus* colonies in the plates with less than 150 colonies, that have the following characteristics: large, pink (indicating that mannitol fermentation has not occurred) and generally surrounded by a zone of precipitation (indicating the production of lecithinase).
   Some strains of B. cereus produce only little or no lecithinase. Colonies of these strains will not be surrounded by a precipitation zone. These colonies should also be subjected to confirmation tests.
6. Select five presumptive colonies from each plate and streak the selected colonies onto the surface of sheep blood agar in a manner which allows good interpretation of the haemolysis reaction. Incubate at 30 °C for 24 h ± 2 h and interpret the haemolysis reaction.

Confirmation tests (not included in ISO 7932):

✓ Microscopic observation (large Gram-positive bacilli in short-to-long chains; spores are ellipsoidal, central to sub-terminal, and do not swell the sporangium)
✓ Glucose fermentation (+)
✓ Voges Proskauer Reaction (+)
✓ Nitrate reduction (+)

The following tests allow to differentiate between *B.cereus* and other correlated bacilli :

✓ Haemolysis on blood agar plates (*B.cereus*: +)
✓ Colour of the colonies on Blood Agar plates (*B.cereus* colonies are green)
✓ Motility (*B.cereus*: +)
✓ Presence of parasporal granules (*B.cereus* is negative)

USER QUALITY ASSURANCE (24-48 h /30°C)

Productivity control
B.cereus ATCC 11778: good growth, pink colonies with opaque halo
Selectivity control
E.coli ATCC 25922: inhibited
Specificity control
B,subtilis ATCC 6633: growth , colonies without halo

STORAGE

Dehydrated media: 10-30°C
Selective Supplement: 2-8°C
Ready to use plates and flasks: 2-8°C
User prepared plates: up to 4 days at 2-8°C

REFERENCES

- ISO7932 :2004 - Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of presumptive Bacillus cereus -- Colony-count technique at 30 degrees C
- ISO 21871:2005 - Microbiology of food and animal feeding stuffs -- Horizontal method for the determination of low numbers of presumptive Bacillus cereus -- Most probable number technique and detection method
- Holbrook, A. and Anderson, J.M. (1980), Can. J. Microbial, **26**,753-759
- Weasel, D.A.A., Kaapman. M.J. and Jongerius, E. (1967), App. Microb. **15**, 650
- FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.

PACKAGING

4011112	**Bacillus Cereus Selective Agar Base (MYP),**	**500 g (11.6 L)**
4240001	**Bacillus Cereus Antimicrobic Supplement**	**10 vials, each for 500 mL of medium**
42111601	**Egg Yolk Emulsion**	**50 mL**
5111112	**Bacillus Cereus Agar Base (MYP),**	**6 x 90 mL ready to use flasks**
541112M	**Bacillus Cereus Selective Agar (MYP),**	**20 ready to use plates**