

Also known as

Mannitol-Egg Yolk-Polymyxin Agar; MYP Agar

Specification

Selective solid medium, according to Mossel, for the isolation and identification of *Bacillus cereus* from food samples, according to ISO standard.

Formula * in g/L

Peptone	10.000
Mannitol	10.000
Sodium chloride	10.000
Meat extract	1.000
Phenol red	0.025
Agar	15.000

Final pH 7.2 ± 0.2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 46 g of powder in 900 mL of distilled water. Sterilize in the autoclave at 121°C for 15 minutes. Let it cool to 50°C and then add 100 mL of Egg Yolk Sterile Emulsion (Art. No. FLWR3035) and 2 vials of Polymyxin B Sulfate Selective Supplement (Art. No. DSHB3065). Homogenize well and distribute into plates. Do not reheat or remelt the complete medium.

Description

Mossel's formulation is developed to detect and enumerate *B. cereus* in any food stuff. It is both selective and differential for this microorganism. Polymyxin addition inhibits most of accompanying bacteria, but does not affect the growth of *B. cereus*. This bacterium does not ferment mannitol and thus there is no change in the colour of the indicator around the colonies. The lecithinase activity of *B. cereus* produces a halo or zone of white precipitate around the colonies.

A count of *B. cereus* over 100.000 cells/g of food sample is considered hazardous, since the accumulated phosphorilcholine may cause toxic symptoms in children. For this reason a viable enumeration must be performed to evaluate the real population of cells.

Necessary supplements:

Polymyxin B Sulphate Selective Supplement (Ref.DSHB3065)

Vial Contents:

Necessary amount for 500 ml of complete medium

Polymyxin B sulphate 50000 IU

Excipient 100 mg

Distilled water (solvent)

Technique

According to the authors, dehydrated or dry samples must be treated in the following way: 20 g of sample is mixed with 90 ml of Tryptone Water for a minimum period of 1 hour, à room temperature. Afterwards, add an additional 90 ml of Tryptone Water and homogenize. If necessary dilute 1:10. Proceed to a 1/10 serial dilution bank using Tryptone water as the diluent if necessary. With a Drigalsky loop, spread aliquots of 0.1 ml over the surface of the agar plates and let the agar medium absorb the aliquots. Incubate the plates à 30 °C for 24-48 ± 4 h to allow spore germination before giving definite results.

Suspicious colonies have the following appearance: Irregular borders, pink colour becoming purple in the centre, with a halo of white precipitate. Colonies with yellow halos must be discounted.

Confusion with other colonies of Gram positive bacilli is possible, and hence, confirmation tests must be carried out i.e. glucose fermentation, Gélatine degradation and nitrate reduction.

Quality control

Incubation temperature: 30 °C ± 1

Incubation time: 24 ± 3h / 44 ± 4h

Inoculum: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10⁴-10⁶ CFU (selectivity)/ ≥ 10³ CFU (specificity), according to ISO 11133:2014/Amd 1:2018.

Microorganism

Bacillus subtilis ATCC® 6633
Bacillus cereus ATCC® 11778
Bacillus cereus ATCC® 10876
Escherichia coli ATCC® 25922

Growth

Good (Specificity)
 Productivity > 0.50
 Productivity > 0.50
 Inhibited

Remarks

Yellow colonies, irregular borders. Man + Lec -
 Red colonies, irregular borders. Man - Lec +
 Red colonies, irregular borders. Man - Lec +
 Selectivity

References

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. London.
- CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD. (2003) Handbook of Culture Media for Food Microbiology. Elsevier Sci. B.V. Amsterdam. The Netherlands.
- DOWNES, F.P. & K. ITO (2001) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington DC. USA.
- FIL-IDF 181:1998 Provisional Int. Standard. Dried Milk Products. Enumeration of *Bacillus cereus*.- Most probable number technique.
- ISO 7932 Standard (2004) 3rd ed. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive *Bacillus cereus*. Colony count technique at 30°C.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 21871 Standard (2006) 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.
- MOSSEL, D.A.A., KOOPMAN. M.J. & JONGERIUS, E. (1967) Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. 15:650-653.
- PASCUAL ANDERSON, M^a.R^a (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).