KG Agar Base

M658

Intended Use:

Recommended for promoting fast and free sporulation to distinguish between *Bacillus cereus* and *Bacillus thuringiensis.*

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Yeast extract	0.500
Phenol red	0.025
Agar	18.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 19.53 grams in 900 ml purified/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 100 ml sterile, Egg Yolk Emulsion (FD045) and sterile contents of 2 vials of reconstituted Polymyxin B Selective Supplement (FD003). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Bacillus cereus, a gram-positive rod shaped and beta haemolytic bacteria, is widely distributed in nature and can be isolated from a variety of foods. *B. cereus* is classified as a large-celled species of Group I bacilli (species with a cell width greater than 0.9µm and whose spores do not appreciably swell the sporangium). KG Agar Base formulated by Kim and Goepfert (3) and recommended by APHA (4) is used to promote free spore formation of *B.cereus, Bacillus thuringiensis* within an incubation period of 20-24 hours. This feature allows a) direct confirmation of zone forming organisms as Group I bacilli by means of microscopic examination and b) immediate differentiation of *B. cereus* from *B.thuringiensis* by visualization of the endotoxin crystal in sporulated cells of the latter organism. Additionally Group 2 bacilli such as *Bacillus polymyxa*, which produce lecithinase, are unable to form lecithinase under the rather nutritionally poor conditions imposed by KG Agar Base.

Peptone and yeast extract in the medium supports the growth of *B.cereus, B.thuringiensis*. Lecithinase activity is observed as an opaque zone surrounding the individual colony. *B. cereus* is resistant to Polymyxin B, which restricts gram-negative organisms. *B.cereus* and *B.thuringiensis* can be distinguished by means of microscopic examination of stained cells. *B.thuringiensis* shows endotoxin crystals in sporulated cells.

Type of specimen

Isolated Microorgansim from food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4). 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 :

1. Well isolated colonies must be used for differentiation on this media.

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

Light yellow to light pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.8% Agar gel.

Colour and Clarity of prepared medium
Basal medium : Orange coloured clear to slightly opalescent gel After addition of Egg Yolk Emulsion : Light orange coloured opaque gel forms in Petri plates

Reaction
Reaction of 1.95% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed with added sterile Egg Yolk Emulsion (FD045) and Polymyxin B Selective Supplement (FD003), after an incubation at 30-35°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Lecithinase
Bacillus cereus ATCC 14579	50-100	good-luxuriant	>=50%	positive,opaque zone around the colony
Bacillus thuringiensis ATCC 10792	50-100	good	40-50%	positive,opaque zone around the colony
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	<=10%	negative

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Kim H. V. and Goepfert J. M., 1971, Appl. Microbiol., 22:581.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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