Dehydrated Culture Media
Bases / Media Supplements

Technical Information

KG Agar Base

Product Code: DM 1658

Application: - Kim-Goepfert (KG) Agar with added supplements is used for promoting fast and free spore formation which helps in distinguishing *Bacillus cereus* from *Bacillus thuringiensis.*

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	1.000
Yeast extract	0.500
Phenol red	0.025
Agar	18.000
Final pH (25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & 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 devised by Kim and Goepfert [(1)] and recommended by APHA [(2)] 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.

Peptic digest of animal tissue 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.

Methodology

Suspend 19.52 grams of powder media in 900 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 100 ml sterile, Egg Yolk Emulsion (MS2045) and sterile contents of 2 vials of reconstituted Polymyxin B Selective Supplement (MS2003). Mix well and pour into sterile Petri plates.

Quality Control

Physical 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 Range 6.60-7.00

Cultural Response/ characteristices
DM 1658: Cultural characteristics observed with added sterile Egg Yolk Emulsion (MS2045) and Polymyxin B Selective Supplement (MS2003), 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	50-100	none-poor	<=10%	negative

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° in sealable plastic bags for 2-5 days.

Further Reading

1. Kim H. V. and Goepfert J. M., 1971, Appl. Microbiol., 22:58 1.
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

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