Lee's Agar

M602

Intended Use:

Recommended for differential enumeration of yoghurt starter bacteria (*Lactobacillus bulgaricus, Streptococcus thermophilus*).

Composition**

Ingredients	Gms / Litre
Tryptone#	10.000
Yeast extract	10.000
Lactose	5.000
Sucrose	5.000
Calcium carbonate	3.000
Dipotassium hydrogen phosphate	0.500
Bromocresol purple	0.020
Agar	18.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Casein enzymic hydrolysate

Directions

Suspend 51.52 grams in 1000 ml purified/distilled water. Heat just to boiling and sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Cool to 45-50°C. While dispensing, mix carefully to suspend calcium carbonate evenly. Pour into sterile Petri plates to obtain 4-5 mm thick gel.
Note: Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

Principle And Interpretation

Yoghurt is a fermented milk product in which *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the essential microbial species that are active in a symbiotic relationship. To obtain optimum consistency, flavour and odour, the two species should be present in about equal numbers in the culture. Dominance by either species can cause defects. Lees Agar, described by Lee et al (5) is used for the differential enumeration of yoghurt starter bacteria. This medium is also recommended by APHA for the same purpose (6). Lees Agar contains sucrose, which most *L. bulgaricus* strains will not ferment, but *S. thermophilus* will, and lactose, which both species utilize. With a suitable combination of sucrose and lactose, the rate of acid production by *S. thermophilus* is enhanced and that of *L. bulgaricus* restricted. Therefore, Streptococci grow first and produce a creamy, buttery aroma from diacetyl and similar metabolites. The redox potential is also thus, lowered by Streptococci, which enables Lactobacilli to grow, thereby growth stimulatory products for Streptococci are synthesized by Lactobacilli. Hence the typical sharp acetaldehyde flavour of mature yoghurt is formed (2).
Tryptone and yeast extract provide the essential nitrogenous nutrients to the yoghurt (lactic) starter bacteria. Lactose and sucrose are the fermentable carbohydrates. Calcium carbonate along with dipotassium phosphate is added to buffer the medium and avoid the drastic drop in pH due to lactic acid formation. Bromocresol purple is the pH indicator, which turns yellow in acidic condition and imparts yellow colour to the colony. It is recommended to dry the media plates for 18-24 hours prior to use. Refer appropriate references for standard procedures.

Type of specimen

Pure isolate from dairy sample- Yoghurt sample

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,7). 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. It is recommended to dry the media plates for 18-24 hours prior to use.
2. Some strains may show poor growth due to nutitional variations.

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 grey homogeneous free flowing powder
Gelling
Firm, comparable with 1.8% Agar gel.
Colour and Clarity of prepared medium
Purple coloured, opaque gel forms in Petri plates
Reaction

Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.0±0.2
pH
6.80-7.20
Cultural Response
Cultural characteristics observed in presence of Carbon dioxide (CO_2),after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Lactobacillus bulgaricus ATCC 11842 (00102*)	50-100	luxuriant	>=50%	white
Streptococcus thermophilus ATCC 14485	50-100	luxuriant	>=50%	yellow

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 (3,4).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Davis J. G., Ashton T. F. and MaCaskill M., 1971, Dairy Ind., 36:569.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Lee S. Y., Vedamuthu E. R., Washam C. J. and Reinbold G. W., 1974, J . Milk Food Technol., 37: 272
6. 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.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

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