Technical Information

Lees Agar

Product Code: DM1602

Application: - Lees Agar is used for differential enumerations of yoghurt starter bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus).

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Yoghurt is a fermented milk 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 produce defects in the yoghurt Lees Agar, described by Lee et al [(1)] is used for the differential enumeration of yoghurt starter bacteria. This medium is also recommended by APHA for the same purpose [(2)]. Lees Agar contains sucrose, which most L. bulgaricus strains will not ferment, but S. thermophilus will, where as lactose is utilize by both species using a suitable combination of sucrose and lactose, the rate of acid production by S. therm ophilus 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 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 [(3)].

Casein enzymic hydrolysate 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.

Methodology

Suspend 51.52 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. 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.

Quality Control

Physical 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
Reactionof 5.15% w/v aqueous solution at 25°C.pH:-7.0±0.2

pH range 6.80-7.20

Cultural Response/ characteristices
DM 1602: 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	50-100	luxuriant	>=50 %	white
Streptococcus therm ophilus ATCC 14485	50-100	luxuriant	>=50 %	yellow

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^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Lee S. Y., Vedamuthu E. R., Washam C. J. and Reinbold G. W., 1974, J . Milk Food Technol., 37: 272
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
3. Davis J. G., Ashton T. F. and MaCaskill M., 1971, Dairy Ind., 36:569.

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