Lees Multidifferential Agar

M1333

Lees Multidifferential Agar is used in the brewing industry for the cultivation and identification of brewing bacteria including fastidious type.

Composition**

Ingredients	Gms / Litre
Tomato Juice broth	41.000
Peptonized milk	20.000
Calcium pantothenate	2.000
Citric acid	1.100
Calcium carbonate	5.000
Polysorbate 80	0.500
Bromo cresol green	0.022
Cycloheximide	0.007
Agar	15.000
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 84.63 grams in 1000 ml distilled water. Heat the medium just to boiling. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. AVOID OVERHEATING. Stir the medium while dispensing to prevent settling of calcium carbonate. Note: Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

Principle And Interpretation

Lee's Multidifferential Agar is a nutrient medium that detects most organisms commonly found in the brewery. Beer is not a very appropriate medium for the development of bacteria due to its characteristics, such as the low quantity of available nutrients, the presence of alcohol, carbon dioxide and sulphur dioxide, as well as low conservation temperatures. Beer filtration and pasteurization phases also contribute to the stabilization of the product against microorganisms(1).

Lee's Multidifferential Agar contains Tomato juice broth which provides nutrients and acid environment for the growth of acidophilic bacteria. Peptonized milk provides lactose as an energy source. The low pH of the medium inhibits bacteria other than acidophilic bacteria. Polysorbate 80 serves as a source of fatty acids. Bromo cresol green acts as a pH indicator. Acid producing bacteria produce a clear yellow halo around the colonies. Other bacteria produce colonies in colours ranging from colourless to yellow green and blue depending on species and strain. Further tests should be carried out for their identification.

Lactic and acetic acid bacteria are differentiated from non-acid producers by giving a yellow colour to the medium and producing a clear halo zone; Lactobacilli appear translucent to greenish white with dark green centre. All lactobacilli have a well-developed halo zone; *Pediococcus* generally produces tiny greenish colonies surrounded by a narrow halo zone; *Acetobacter* produces a weak halo zone, *Acetomonas* produces a substantial halo zone; non-acid producers such as *Flavobacterium* , *Zymomonas* and *Enterobacter*) do not produce halo zone or yellow colour in the medium around colonies(2).

Quality Control

Appearance

Ligth yellow to light green homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Green to light blue coloured opaque gel forms in Petri plates.

Reaction

Reaction of 8.5% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH
5.30-5.70

Cultural Response
M1333: Cultural characteristics observed after an incubation at 25-30°C for 48-72 hours.

Organism	Growth Cultural Response
Acinetobacter calcoaceticus ATCC 23055	none-poor
Lactobacillus acidophilus ATCC 4356	luxuriant with clear yellow halo
Lactobacillus fermentum ATCC 9338	luxuriant with clear yellow halo
Lactobacillus leichmannii ATCC 4797	luxuriant with clear yellow halo
Lactobacillus plantarum ATCC 8014	luxuriant with clear yellow halo
Proteus vulgaris ATCC 13315	inhibited

Storage and Shelf Life
Store below 8°C in tightly closed container and the prepared medium at 2-8°C.Use before expiry date on the label.

Reference
1. Mar 1976 DT Journal Article AU Lee, S. Y.; Jangaard, N. O.; Coors, J. H.; Hsu, W. P.; Fuchs, C. M.; Brenner.
2. M. W. PY 1975 AD Adolph Coors Co., Golden, Colorado 80401, USA SO Proceedings. American Society of Brewing Chemists 33 (1).

Revision : 2 / 2015