L. S. Differential Medium Base

M582

Intended Use:

Recommended for differentiation of Lactobacilli and Streptococci on the basis of colonial morphology, TTC reduction and casein reaction.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Soya peptone	5.000
HM peptone B #	5.000
Yeast extract	5.000
Dextrose (Glucose)	20.000
Sodium chloride	5.000
L-Cysteine hydrochloride	0.300
Agar	15.000
Final pH (at 25°C)	6.1±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 65.3 grams in 1000 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 the following sterile solutions previously kept warm at 50°C just prior to use; (1) 100 ml of 10% w/v aqueous solution of antibiotic-free skim milk powder sterilized at 15 lbs pressure (121°C) for 5 minutes. (2) 10 ml of 2, 3, 5-Triphenyl-Tetrazolium Chloride (T.T.C.) (FD057) Solution. Mix well and pour into sterile Petri plates.

Principle And Interpretation

L. S. (Lactobacillus Streptococcus) Differential Medium is used to differentiate *Lactobacillus* and *Streptococcus*. L. S. Medium is prepared according to the formulation of Eloy and Lacrosse (3) and is a selective medium that supports good growth and differentiation of thermophilic lactobacilli and streptococci in yoghurt products (7). Yoghurt is a fermented milk product in which *Streptococcus thermophillus* and *Lactobacillus bulgaricus* are the essential microbial species and are active in a symbiotic relationship. A ratio of 1:1 is recommended by various workers (6,8,9). The reduction of triphenyl tetrazolium chloride in connection with the casein reaction allows differentiation between lactobacilli and streptococci by means of colony morphology (2).

The medium contains Soya peptone, L-cysteine hydrochloride, Tryptone, HM peptone B and yeast extract as sources of carbon, nitrogen, vitamins and minerals. Sodium chloride helps in maintaining osmotic balance.

Test samples of yoghurt or starter cultures are added to melted and cooled L.S. Differential Medium Base. These are mixed thoroughly and plates are poured. The plates are incubated at 43°C for 48 hours.

Type of specimen

Dairy sample.

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,10). 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. Further biochemical and serological testing is required for complete identification.
2. Some strains may show poor growth due to nutritional 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

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

Reaction

Reaction of 6.53% w/v aqueous solution at 25°C. pH : 6.1±0.2

pH

5.90-6.30

Cultural Response

Cultural characteristics observed with added antibiotic free skim milk powder and 1% T.T.C.(FD057), after an incubation at 43-45°C for 48 hours.

Organism	Colony characteristics
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 11842 (00102*)	red,rhizoidal, surrounded by opaque zone
Streptococcus thermophilus ATCC 14485	red, smooth, surrounded by clear zone

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 (4,5).

Reference

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