Tributyrin Agar Base w/o Tributyrin

M157

Intended Use:

Recommended for detection of lipolytic microorganisms.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	3.000
Agar	15.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23 grams in 990 ml purified / distilled water. Add 10 ml of Tributyrin (FD081). Mix and heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Note : For proper lipase activity, it is recommended to use glass plates instead of disposable plates. Hence USE ONLY GLASS PLATES. DO NOT USE PLASTIC PLATES.

Principle And Interpretation

Many foods contain significant amount of fats that may be susceptible to hydrolysis. The free fatty acids (FFA) liberated by hydrolysis of fat can be responsible for unpleasant flavous or they may oxidize to compounds with undesirable flavour notes. Many of the problems of fat breakdowns in foods are non-microbial in origin, but numerous bacteria, yeasts and moulds produce lipolytic enzymes that are capable of causing both hydrolytic and oxidative deterioration of fats when present in food samples (12).

Lipolytic enzymatic activities of microorganisms are one of the most important causes for food spoilage and a limited shelf life. Tributyrin Agar was originally formulated by Anderson (3) for the detection and enumeration of lipolytic microorganisms such as Staphylococci (7),Clostridia (13), marine Flavobacteria and Pseudomonas (6) and moulds in foodstuffs and other materials.

Tributyrin is the simplest triglyceride occurring in natural fats and oils. It is hydrolyzed by some microorganisms that do not hydrolyze other triglycerides or fats containing longer chain fatty acids. However, for screening purposes, to enumerate lipolytic microorganisms of potential importance in foods, it is the substrate of choice (1,5).

Peptone and yeast extract in the medium provide nutrients to the organisms. Tributyrin degradation by the microorganisms is indicated by clear zones surrounding the lipolytic colonies in the otherwise turbid culture medium. Lipolytic organisms render the medium transparent by converting the fat to water soluble butyric acid (4). The medium should have a uniform turbid emulsion for the effectiveness of the assay (10).

Type of specimen

Food and dairy samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,11,14).
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.This medium is general purpose medium and may not support the growth of fastidious organisms.

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 with oil droplets in Petri plates.

Reaction

Reaction of 2.3% w/v aqueous solution containing 1% Tributyrin at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours with added Tributyrin (FD081) (under appropriate conditions).

Organism	Inoculum (CFU)	Growth	Lipase activity
Clostridium perfringens ATCC 12924	50-100	luxuriant	negative, absence of clear zone around colony
Clostridium sporogenes ATCC 11437	50-100	luxuriant	positive, clear zone around colony
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50-100	luxuriant	positive, clear zone around colony
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	negative, absence of zone around colony
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	positive, clear zone around colony

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. Use before expiry date on the label.
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 (8,9).

Reference

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