Technical Information

Tributyrin Agar Base w/o Tributyrin

Product Code: DM 1157

Application: Tributyrin Agar Base w/o Tributyrin is used for detection of lipolytic microorganisms.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	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

Principle & 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 many bacteria, yeasts and moulds produce lipolytic enzymes that are capable of causing both hydrolytic and oxidative deterioration of fats when present in food samples [1].

Lipolytic enzymatic activities of microorganisms are one of the most important causes for food spoilage and a limited shelf life. Tributyrin Agar was originally devised by Anderson [2] for the detection and enumeration of lipolytic microorganisms such as *staphylococci* [3], *clostridia* [4], marine *Flavobacteria* and *Pseudomonas* [5] 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 [6, 7].

Peptic digest of animal tissue 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 make the medium transparent by converting the fat to water soluble butyric acid [8]. The medium should have a uniform turbid emulsion for the effectiveness of the assay [9].

Methodology

Suspend 23 grams of powder media in 990 ml distilled water. Add 10 ml of Tributyrin (MS2081). Mix well and heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (12 1°C) for 15 minutes. Shake the flask and individual plate so as to maintain uniform turbidity.

Quality Control

Physical 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 range 7.30-7.70

Cultural Response/Characteristics
DM 1157: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours with added Tributyrin (MS2081) (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 ATCC 6633	50-100	luxuriant	positive, clear zone around colony
Escherichia coli ATCC 25922	50-100	luxuriant	negative, absence of clear zone around colony
Staphylococcus aureus ATCC 25923	50-100	luxuriant	positive, clear zone around colony

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: 2-8^0 in sealable plastic bags for 2-5 days

Further Reading

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2. Anderson J. A., 1939, Ber, IIIrd Int. Mikrobiol. Kongress, 3 : 726
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4. Willis A. T., 1960, J. Path. Bacteriol., 80 (2): 379
5. Hayes P. R., 1963, J. Gen. Microbiol., 30: 1
6. Alford J. A., and Steinle E. E., 1967, J. Appl. Bacteriol., 30: 488
7. Frayer T. T., Lawrence R. C., Reiter B, 1967, J. Dairy Sci., 50: 477.
8. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

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