Technical Information

Iron Sulphite Agar

Product Code: DM 1868

Application: - Iron Sulphite Agar is recommended for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Sodium sulphite	0.500
Iron (III) citrate	0.500
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Iron Sulphite Agar is a modification of Cameron Sulphite Agar coined by the National Canners Association of America [1]. Beerens [2] found that 0.1% sulphite concentration in the original formula was inhibitory to some strains of *Clostridium sporogenes.* This observation was later confirmed by Mossel et al [3], who showed that 0.05% sulphite concentration was not inhibitory to the organisms. Most clostridia have sulfite reductase in their cytoplasm but fail to expel them out to the exterior. So when H_2S is produced from sulfite, the colony becomes dark due to the formation of precipitates of iron sulfide from citrate.

Casein enzymic hydrolysate provides nitrogen and other nutrients necessary to support bacterial growth. Sulphite-reducing bacteria usually produce black colonies as a result of the reduction of sulphite to sulphide, which reacts with the iron (III) salt.

For the detection of organisms causing sulphide spoilage, two methods can be followed:

a) Deep-Shake Culture Method: Take 10 ml of medium in each tube. Inoculate the sample when the medium is at about 50°C. Allow to meet the medium and incubate at 55°C for 24-48 hours.

Typical thermophilic species - *Desulfotomaculum nigrificans,* produces distinct black spherical colonies deep in the medium.

Attenborough and Scarr [4] Method: In this method, diluted samples of sugar or any other food are filtered through membrane filters. These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite Agar (at 50°C) to cover them. The medium is allowed to set and then incubated at 55-56°C for 24-48 hours. After incubation, the number of black colonies on the membrane filter is counted. Confirmation tests are further carried out to identify the organism growing in the medium. This membrane filter technique is quicker, more accurate and permits the examination of larger samples. The blackening reaction is only presumptive evidence of clostridial growth. Confirmation test must be carried out for identification. There are many gram-negative bacteria that are able to reduce sulfite with iron sulfide production in this medium, but in these cases the enzymes are extra cellular and the entire medium becomes dark, rendering their enumeration impossible.

Methodology

Suspend 26 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 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured, slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.6% w/v aqueous solution at 25°C pH : 7.1±0.2

pH Range 6.90-7.30

Cultural Response/ characteristices
DM1868: Cultural characteristics observed under anaerobic condotions,after an incubation at 55-56°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Clostridium botulinum ATCC 25763	50-100	luxuriant	>=50%	black
Clostridium butyricum ATCC 13732	50-100	luxuriant	>=50%	black
Clostridium sporogenes ATCC 19404	50-100	luxuriant	>=50%	black
Desulfotomaculum nigrificans ATCC 19998	50-100	luxuriant	>=50%	black
Escherichia coli ATCC 25922	50-100	good	40-50%	no blackening

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. Tanner F. W., 1944, "The Microbiology of Foods", 2nd Ed., Garrard Press, Illinois, P. 1127.
2. Beerens H., 1958, DSIR, Proc. 2nd Internat. Sym. Food Microbiol., 1957, HMSO, London, P. 235.
3. Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S., 1959, J. Path. Bacteriol., 78:290.
4. Attenborough J. and Scarr M., 1957, J. Appl. Bacteriol., 20: 460.

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