Technical Information

Cooke Rose Bengal Agar Base

Product Code: DM 1499

Application: - Cooke Rose Bengal Agar is used for selective cultivation and isolation of fungi.

Composition**

Ingredients	Gms / Litre
Papaic digest of soyabean meal	5.000
Dextrose	10.000
Monopotassium phosphate	1.000
Magnesium sulphate	0.500
Rose Bengal	0.035
Agar	20.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Cooke [1, 2] used the Waksman medium without adjustment for isolation of fungi from sewage. It was discovered that papaic digest of soyabean meal was particularly suitable for use in this medium and that the combination of chlortetracycline, or oxytetracycline, with rose bengal increased the selectivity of the medium.

Smith and Dawson [5] used rose bengal for the inhibition of bacteria in media which has almost neutral reaction concerned with retardation of the development of fungi. Martin [6] used 1: 30,000 Rose bengal and 30µg Streptomycin per ml and found that a wide variety of bacteria are inhibited at reactions between pH 5.5 to 6.5 without inhibiting fungi.

The Kingdom Fungi includes some of the most important organisms, both in terms of their ecological and economic roles. By breaking down dead organic material into simpler forms, they continue the cycle of elements through ecosystems. In addition, most vascular plants could not grow without a symbiotic association with fungi, or mycorrhizae, that inhabit their roots and supply essential nutrients. Other fungi provide numerous drugs (such as penicillin and other antibiotics), foods like mushrooms, truffles and morels, and the bubbles in bread, champagne, and beer [3]. Waksman [4] described an acid medium consisting of peptone, dextrose, inorganic salts and agar for the isolation of fungi from soil.

The medium should not be exposed to light as photo-degradation of rose bengal yields compounds that are toxic to fungi [7, 8].

Microscopic examination coupled with biochemical testing using pure cultures is recommended for complete identification. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or partially inhibited.

Papaic digest of soyabean meal provides nitrogen, carbon and vitamins. Dextrose is an energy source. Rose bengal and chlortetracycline selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing moulds. Monopotassium phosphate provides buffering capability. Magnesium sulfate is a source of divalent cations.

Methodology

Suspend 36.54 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. To increase the selectivity of the medium, add 35µg chlortetracycline per ml of the medium. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel

Colour and Clarity of prepared medium

Pink-red coloured, slightly opalescent forms in Petri plates

Reaction

Reaction of 3.65% w/v aqueous solution at 25°C. pH : 6.0±0.2

pH range 5.80-6.20

Cultural Response

DM 1499: Cultural characteristics observed after an incubation at 25-30°C for 1-4 days.

Organism	Inoculum (CFU)	Growth	Recovery (Plain)	Growth with chlortetracycline	Recovery with chlortetracycline
Candida albi cans ATCC 10231	50-100	luxuriant	>=50%	luxuriant	>=50%
Saccharomyces cerevisiae ATCC 9763	50-100	luxuriant	>=50%	luxuriant	>=50%
Aspergillus brasiliensis ATCC 16404	50-100	good		good	
Escherichia coli ATCC ATCC 25922	50-100	luxuriant	>=50%	luxuriant	0%
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	inhibited	0%

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° in sealable plastic bags for 2-5 days.

Further Reading

1. Cooke, 1954, Antibiot. Chemother., 4:657.
2. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A. E., (Eds.) , 2005, Standard Methods for the Examination of Water and Wastewater, 21 st Ed., APHA, Washington, D.C.
3. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D. C.
4. Waksman, 1922, J. Bacteriol., 7:339.
5. Smith and Dawson, 1944, Soil Sci., 58:467.
6. Martin, 1950, Ibid., 69:215.
7. Banks, Board and Paton, 1985, Lett. Appl. Microbiol., 1:7.
8. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

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