Rose Bengal Agar Base, Granulated

GM842

Rose Bengal Agar Base, granulated is recommended for the selective isolation and enumeration of yeasts and moulds from environmental materials and foodstuffs.

Composition**

Ingredients	Gms / Litre
Papaic digest of soyabean meal	5.000
Dextrose	10.000
Monopotassium phosphate	1.000
Magnesium sulphate	0.500
Rose bengal	0.050
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.55 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 40-45°C and add 2 ml of rehydrated Chloramphenicol Selective Supplement (FD033) for each 500 ml of medium. Mix thoroughly and pour into sterile Petri plates.

Principle And Interpretation

Rose Bengal Agar is a selective medium to detect and enumerate yeasts and moulds in food samples. The use of media with an acidic pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed (6, 1, 14). Neutral pH media with antibiotics is advantageous for fungal growth compared to acidified media as the later may inhibit fungal growth or fail to inhibit bacterial growth (7, 9) and may restrict the size of mould colonies (13). Smith and Dawson (12) used rose bengal in a neutral pH medium for the selective isolation of fungi from soil samples. Chloramphenicol, streptomycin, oxytetracycline and chlortetracycline have been used for the improved, selective isolation and enumeration of yeasts and moulds from soil, sewage and foodstuffs (4, 5, 9, 11).

Rose Bengal Agar Base supplemented with chloramphenicol is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis (5). Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum (8). Rose Bengal Agar is recommended in standard methods for the enumeration of yeasts and moulds from foodstuffs and water (2, 3, 8).

Papaic digest of soyabean meal provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source. Monopotassium phosphate provides buffering capability. Magnesium sulphate provides necessary trace elements. Rose bengal is a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing moulds. Rose bengal is taken up by yeast and mould colonies, thereby facilitating their recognition and enumeration. Chloramphenicol Selective Supplement (FD033) inhibit bacteria.

Add 1 ml aliquots of a suitable series of dilution to Petri plates. Pour the cooled medium, mix well and incubate for upto 5 days at 25°C. Calculate the number of yeasts or moulds per 1 gram or 1 ml by multiplying the number of colonies by dilution factor. Colonies of yeast appear pink due to uptake of rose bengal.

Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may grow poorly or fail to grow on the complete medium; similarly, some strains of bacteria may also not inhibited or only partially inhibited.

Care should be taken not to expose this medium to light, since photodegradation of rose bengal yields compounds that are toxic to fungi (10, 2).

Quality Control

Appearance
Light yellow to pink coloured granular medium

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Deep pink coloured clear to very slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.16% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 20-25°C for 5 days with added Chloramphenicol Selective Supplement (FD033).

Organism	Inoculum(CFU)	Growth	Recovery
**Aspergillus brasiliensis ATCC 16404*	50-100	good	
Candida albicans ATCC 10231	50-100	good	>=50%
Escherichia coli ATCC 25922	>=10^3	inhibited	0%
Micrococcus luteus ATCC 10240	>=10^3	inhibited	0%
Saccharomyces cerevisiae ATCC 9763	50-100	good	>=50%

**Key: Formerly known as Aspergillus niger*

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
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