

Technical Information

Rose Bengal Agar Base

Product Code: DM 1842

Application: - Rose Bengal Agar Base is used 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

Principle & Interpretation

Rose Bengal Agar Base 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 used 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 acts as energy source. Monopotassium phosphate provides buffering capability. Magnesium sulphate supplies 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 (MS 2033) 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).

Methodology

Suspend 31.55 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & 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 (MS 2033) for each 500 ml of medium. Shake well and pour into sterile Petri plates.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

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 Range

7.00-7.40

Cultural Response

DM 1842: Cultural characteristics observed after an incubation at 20-25°C for 5 days with added Chloramphenicol Selective Supplement (MS 2033).

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

*Key: Formerly known as *Aspergillus niger*

Storage and Shelf Life

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

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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5. Jarvis B., 1973, J. Appl. Bacteriol., 36:723.
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Dehydrated Culture Media
Bases / Media Supplements

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9. Martin J. P., 1950, Soil Sci. 69:215.
10. Banks J. G., Board R. G., and Paton J., 1985, Lett. Appl. Microbiol., 1:7.
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12. Smith M. R. and Dawson V. T., 1944, Soil Sci. 58:467.
13. Tyner L. E., 1944, Soil Sci. 57:271.
14. Waksman S. A., 1922, J. Bacteriol., 7:339.

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