Technical Information

MP-5 Medium

Product Code: DM 1596

Application: - MP-5 Medium is recommended for the detection of pectinolytic microorganisms especially those producing poly galacturonase.

Composition**

Ingredients	Gms / Litre
Pectin	5.000
Monopotassium phosphate	4.000
Disodium phosphate	6.000
Ammonium sulphate	2.000
Yeast extract	1.000
Ferrous sulphate	0.001
Magnesium sulphate	0.200
Calcium chloride	0.001
Boric acid	0.00001
Manganese sulphate	0.00001
Zinc sulphate	0.00007
Copper sulphate	0.00005
Molybdenum trioxide	0.00001
Agar	15.000
Final pH (at 25°C)	5.5±0.5

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

MP-5 Medium is recommended for the detection of pectinolytic organisms especially those producing polygalactouronase. MP-5 medium is used by APHA for detecting pectinolytic organisms (1).

Pectin is an important cell wall component of higher plants that helps in cementing plant cells together. Most pectin-degrading organisms are associated with raw agricultural products and with soil. Detection of pectinolytic activity of an organism is carried out either by observing depression in the gel around the colony where the substrate has been degraded or by flooding the plate with a precipitant solution.

Detection of polygalactouronase by plate assay is generally done by lowering the pH of the medium, designed for detection of pectate lyase, (i.e. MP-7 Medium) to 6 or below so that polygalactouronase will be active and pectate lyases will be inactive (2, 3). Thus the acidic pH of MP-5 Medium is the main parameter used to distinguish polygalactouronase producers from pectate lyase producers. A 1.0% aqueous solution of hexadecyltrimethyl ammonium bromide (4) is used to detect pectinolytic activity. After incubating the plates for 2-3 days at 30-35°C, the polysaccharride precipitant is poured over the surface of the plate taking care not to dislodge the colonies. Zones of pectin hydrolysis will be visible usually within few minutes and can be best viewed against dark background. The reagent precipitates the intact pectin in the medium whereas pectinolytic growth is surrounded by a clear halo in an opaque medium. High phosphate level in the medium is required to observe pectinolytic activity.

Methodology

Suspend 33.2 grams of dehydrated powder media in 1000 ml. distilled water. Mix thoroughly & heat to boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121oC) for 15 minutes. Mix well and pour into sterile Petri plates.

Note: Due to presence of various inorganic salts, slight precipitate may develop upon heating. Shake well before pouring into sterile Petri plates.

Polysaccharide precipitant solution: Dissolve 1.0 gm of hexadecyltrimethyl ammonium bromide in 100 ml of water and the solution is sterilized by autoclaving if desired.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.32% w/v aqueous solution at 25°C. pH : 5.5±0.5

pH Range
5.00-6.00

Cultural Response
DM 1596: Cultural characteristics observed after an incubation at 35-37°C for 2-3 days.

Organism	Polygalacturonase production
**Aspergillus brasiliensis ATCC 16404*	positive, clear halo around the colony when flooded with 1% polysaccharide precipitant.
Fusarium moniliforme	positive, clear halo around the colony when flooded with 1% polysaccharide precipitant.

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

1. Downes F. P. and Ito K. (Eds.), 2001, Compendium for the Microbiological Examination of Foods, 4th Ed. APHA, Washington, D.C.
2. Hankin L. and Anagnostakis S. L., 1975, Mycologia 67:597.
3. Vaughn R. H., Balatsouras G. D., York G. K. II and Nagel C. W., 1957, Food Res. 22:597.
4. Jayasankar N. P. and Graham P. H., 1970, Can J. Microbiol., 16:1023.

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