Technical Information

Phenylalanine Agar

Product Code: DM 1281

Application: Phenylalanine Agar is used for the differentiation of Proteus and Providencia group of organisms from other members of Enterobacteriaceae on the basis of their ability to form phenyl pyruvic acid from phenylalanine.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Sodium chloride	5.000
DL-Phenylalanine	2.000
Disodium phosphate	1.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The ability of *Proteus* species to convert phenylalanine to phenylpyruvic acid is an important reaction in the differentiation of members of *Enterobacteriaceae* [1, 2]. Based on this principle, Buttiaux developed Phenylalanine Agar for differentiation of *Proteus* and *Providencia* group from other members of *Enterobacteriaceae* [3, 4] by the ability to deaminate phenylalanine. Phenylalanine Agar is the modification of the medium originally developed by Ewing et al [5].

Yeast extract in the medium supports the growth of the organisms. Sodium chloride maintains osmotic equilibrium. The phenylalanine serves as the substrate for enzymes, which are able to deaminate it to form phenylpyruvic acid. As per standard method inoculate the slant surface with plenty of inoculum and incubate it for 12-16 hours. After incubation, add 0.2 ml of 10% ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 ml of concentrated HCl per 100 ml of reagent) so that the solution floods all over the growth to. Observe for the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color. Interpret the results within 5 minutes upon addition of reagent as the green colour fades quickly [1, 4].

Methodology

Suspend 26 grams of powder media in 1000 ml distilled water. Shake well and heat to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to cool in a slanting position.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Light amber coloured slightly opalescent gel forms in tubes as slants
Reaction
Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.3±0.2
pH Range 7.10-7.50
Cultural Response/ characteristices
DM 1281: Cultural characteristics observed after an incubation at 35-37°C for 12-16 hours

Organism	Inoculum (CFU)	Growth	Phenylalanine deaminase
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	negative reaction
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
Proteus vulgaris ATCC 13315	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
Providencia alcalifaciens ATCC 9886	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride

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. Singer J. and Volcani B. E., 1955, J. Bacteriol., 69:303.
2. Henrikson S. D., 1950, J. Bacteriol., 60:225.
3. Buttiaux R., Osteux R., Fresnoy R. and Moriamez J., 1954, Ann. Inst. Pasteur Lille., 87:375.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
5. Ewing W. H., Davis B. R. and Reavis R. W., 1957, Public Health Lab., 15:153.

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