Spirit Blue Agar

M445

Spirit Blue Agar is used for detection and enumeration of lipolytic microorganisms

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
Spirit blue	0.150
Agar	17.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.15 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 50°C and add 30 ml lipase substrate slowly while agitating to obtain an even distribution.

Note :For proper lipase activity, it is recommended to use glass plates instead of disposable plastic plates.

Principle And Interpretation

Lipids, including fats and oils, are highly reduced. When a lipid is catabolized, it has the potential to yield more pairs of electrons per gram, and thus more energy, than either carbohydrates or proteins (1). This process is brought about by the enzyme lipase, and the organisms possessing the enzyme lipase are called lipolytic organisms. Growth of lipase-producing microorganisms can contribute to flavour defects in milk and high fat dairy products. Some of the free fatty acids released by the action of lipolytic enzymes have a low flavour threshold and can impart a rancid flavour at low concentrations.

Spirit Blue Agar is prepared according to the formulation of Starr (2) is recommended by APHA (3) for detection and enumeration of lipolytic microorganisms. It is a basal medium to which lipoidal substrate is added for the detection, enumeration and study of lipolytic microorganisms. Formulations in practice before Starr which included dyes as indicators of lipolysis were sometimes inhibitory to the microorganisms. Starr showed spirit blue to be inert and an ideal indicator of lipolysis, visualized as clear halos around colonies.

Casein enzymic hydrolysate and yeast extract in the medium are sources of carbon, nitrogen, vitamins and minerals. Spirit blue is a dye which acts as an indicator of lipolysis. The lipase reagents recommended as the lipid source are cotton seed meal, cream, olive oil etc. A satisfactory emulsion can be prepared by dissolving 10 gram acacia or 1 ml polysorbate 80 in 400 ml warm distilled water, adding 100 ml cotton seed or olive oil and agitating vigorously to emulsify.

Prepare 1:10 or other suitable dilution of the product to be tested. Spread 0.1 ml of the desired dilutions over the surface of the medium. Incubate at 35-37°C for 24-48 hours. Colonies of lipolytic organisms develop a clear zone and /or a deep blue colour around and under each colony (3).

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% Agar gel.

Colour and Clarity of prepared medium

Basal medium yields blue coloured, clear to slightly opalescent gel. With addition of lipase substrate, lavender coloured slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.22% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M445: Cultural characteristics observed after an incubation at 35-37°C for 48-72 hours with added Lipase substrate .

Organism	Inoculum (CFU)	Growth	Lipase activity
Proteus mirabilis ATCC 25933	50-100	luxuriant	negative, absence of zone around colony
Staphylococcus aureus ATCC 25923	50-100	luxuriant	positive reaction, clear zone around colony
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	positive reaction, clear zone around colony

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

1. Nortan C. F., 1986, Microbiology, 2nd Ed., Addison-Wesley Publishing Company.
2. Starr, 1941, Science, 93:333.3. Marshall R. T., (Ed.) 1993, Standard Methods for the Examination of Dairy Products, 16th Ed, APHA, Washington, D.C.

Revision : 1 / 2011