Orange Serum Agar

M933

Intended use

Recommended for cultivation and enumeration of microorganisms associated with the spoilage of citrus products, cultivation of Lactobacilli, other aciduric organisms and pathogenic fungi from food samples.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	3.000
Dextrose (Glucose)	4.000
Dipotassium hydrogen phosphate	2.500
Orange serum (Solids from 200 ml)	9.000
Agar	17.000
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.5 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Fruit juices are generally acidic, with pH values ranging from approximately 2.4 for lemon juice, to 4.2 for tomato juice. The low pH of these foods is selective for yeast, moulds and a few groups of aciduric bacteria. The microorganisms of greatest significance in citrus juices are the lactic acid bacteria, primarily species of *Lactobacillus* and *Leuconostoc*, yeast and moulds. Microbial spoilage of these citrus fruit juices are most commonly due to aciduric microbes such as lactic acid bacteria and yeast. The lactic acid bacteria include *Lactobacillus fermentum, L.plantarum*, and *Leuconostoc mesenteroides*. Orange Serum Agar is recommended by APHA (1) for cultivation of Lactobacilli and other aciduric organisms. Orange Serum Agar was originally developed by Murdock et al (2) and Hays (3) for examining citrus concentrates. Hays and Reister further used this medium for studying the spoilage of orange juice (4). Dehydrated agar medium containing orange serum was reported by Stevens (5). Orange Serum Broth is used to initiate growth of saprophytic, pathogenic fungi in small samples (6). Tryptone provides essential nitrogenous, carbonaceous compounds, long chain amino acidsand other essential nutrients. Dextrose (Glucose) serves as the fermentable carbohydrate and energy source. Yeast extract supplies B- complex vitamins, which stimulate growth. Orange serum provides an optimal environment for the recovery of acid tolerant microorganisms from citrus fruit products.

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some strains may show poor growth due to nutritional variations.
2. Further biochemical and serological testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

Medium to dark amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.55% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH

5.30-5.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.(Fungal species are incubated at 25-30°C)

Organism	Inoculum (CFU)	Growth	Recovery
# *Aspergillus brasiliensis* ATCC 16404 (00053*)	50-100	good-luxuriant	
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%
Lactobacillus acidophilus ATCC 4356	50-100	good-luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	good-luxuriant	>=50%
Leuconostoc mesentoroides ATCC 12291	50-100	good-luxuriant	>=50%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good-luxuriant	>=50%

Key : (#) - Formerly known as *Aspergillus niger* (*) - Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

2.Murdock P. I., Folinazzo J. F., and Troy V. S., 1951, Food Technol., 6:181.

3.Hays G. L., 1951, Proc. Florida State Hortic. Soc., 54:135.

4.Hays G. L. and Reister D. W., 1952, Food Technol., 6:186.
5.Stevens J. W., 1954, Food Technol., 8:88.
6.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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