Orange Serum Agar

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.

Composition**

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

**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 (6). Orange Serum Broth is used to initiate growth of saprophytic, pathogenic fungi in small samples (5).

Tryptone provides essential nitrogenous, carbonaceous compounds, long chain amino acids and 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.

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)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Aspergillus brasiliensis ATCC 16404 (00053*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td></td>
</tr>
<tr>
<td>Candida albicans ATCC 10231 (00054*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Lactobacillus acidophilus ATCC 4356</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Lactobacillus fermentum ATCC 9338</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides ATCC 12291</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae ATCC 9763 (00058*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
</tbody>
</table>

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. Use before expiry date on the label.

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