Deoxycholate Lactose Agar

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone, special</td>
<td>10.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>0.500</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.53 grams in 1000 ml purified / distilled water. Mix well and heat to boiling to dissolve the medium completely. The medium requires no autoclaving if it is to be used at once. If the medium is to be stored, it should be sterilized 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

Deoxycholate Lactose Agar is a modification of Deoxycholate Agar as described by Leifson (5) and prepared according to formula specified in Standard Methods for Examination of Dairy Products (6) Water and Waste Water (2) and Food (7) for the detection of coliform bacilli. It differs from Deoxycholate Agar (M030) by its decreased concentration of sodium deoxycholate. Pour plate method is carried out using suitable dilutions. A thin layer of additional agar can be poured over the solidified pour plates to facilitate enumeration.

Deoxycholate Lactose Agar is selective against gram-positive organisms which are inhibited by optimum concentration of sodium deoxycholate and sodium citrate in the medium. It helps to differentiate between lactose fermenting and nonfermenting enteric bacilli. Peptone special provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential nutrients. Lactose helps in differentiating enteric bacilli, as lactose fermenters produce red colonies while lactose non-fermenters produce colourless colonies. Coliform bacteria, if present form pink colonies on this medium. The degradation of lactose causes acidification of the medium surrounding the relevant colonies and the pH indicator neutral red changes its colour to red. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid due to acidification of the medium. Sodium deoxycholate combines with neutral red in an acidic environment, causing the dye to go out of the solution with the subsequent precipitation of deoxycholate (5).

Type of specimen

Dairy samples; Water samples

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

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. Certain *Salmonella* species are lactose non-fermentors and certain species are non H₂S producers.
2. This medium is a selective medium and needs to be run in parallel with other media for confirmation.
3. Other biochemical and serological tests must be carried out for confirmation.

**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**
Light yellow to pink homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Reddish orange coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.25% w/v aqueous solution at 25°C. pH : 7.1±0.2

**pH**
6.90-7.30

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis subsp. spizizenii</em> ATCC 6633 (00003*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td></td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pink w/bile precipitate</td>
</tr>
<tr>
<td># <em>Klebsiella aerogenes</em> ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>good - luxuriant&gt;=50%</td>
<td></td>
<td>pink</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC &gt;=10⁴</td>
<td></td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.
(#) Formerly known as *Enterobacter aerogenes*

**Storage and Shelf Life**
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order 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 (3,4).
Reference


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