7.1 List of Abbreviations

% : Per cent
(nm): Nano meters
@ : At the rate of
°C : Degree Celsius
ActA: Act A protein
AIDS: Acquired Immuno Deficiency Syndrome
ALOA: Ottoviani and Agosti
BHI: Brain Heart Infusion Broth
CAMP: Christie, Atkins, Munch-Petersen
CFU: colony-forming unit
CNS: central nervous system
CPE: cytopathic effect
CSF: cerebrospinal fluid
Csp: cold shock proteins
DIM: differentiation of innocua and monocytogenes
DLABN: DL-alanine β-naphthylamide
DNA: Deoxribonucleic Acid
DRIA: Dominguez Rodriguez isolation agar
DTHIP: Delayed hypersensitivity inducing protein
EDTA: Ethylene Diamine Tetra Acetic Acid
ELISA: Enzyme Linked Immunosorbent Assay
FAT: Fluorescent Antibiotic Technique
FDA: Food and Drug Administration
Fig: Figure
g: Grams
Gm: Gram
hlyA: Listeriolysin O
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td>Hour</td>
</tr>
<tr>
<td>iap</td>
<td>Invasive associated protein</td>
</tr>
<tr>
<td>ICAR</td>
<td>Indian Council of Agriculture Research</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodalton</td>
</tr>
<tr>
<td>LEB</td>
<td>Listeria enrichment broth</td>
</tr>
<tr>
<td>LIPI-1</td>
<td>Listeria Pathogenicity island 1</td>
</tr>
<tr>
<td>LM</td>
<td><em>Listeria</em> monocytogenes</td>
</tr>
<tr>
<td>LM</td>
<td><em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>LRB</td>
<td><em>Listeria</em> Repair Broth</td>
</tr>
<tr>
<td>LRR</td>
<td>Leucine rich repeat</td>
</tr>
<tr>
<td>LSA</td>
<td><em>Listeria Selective Agar</em></td>
</tr>
<tr>
<td>LSAM</td>
<td><em>Listeria</em> selective agar of Domínguez-Rodriguez</td>
</tr>
<tr>
<td>MABs</td>
<td>Monoclonal Antibody</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliters</td>
</tr>
<tr>
<td>MSP</td>
<td>Major secreted Polypeptide</td>
</tr>
<tr>
<td>MTCC</td>
<td>Microbial Type Culture Collection</td>
</tr>
<tr>
<td>NEHI Region</td>
<td>North Eastern Hilly Region</td>
</tr>
<tr>
<td>NGFIS</td>
<td>Netherland Government Food Inspection Service</td>
</tr>
<tr>
<td>NRC Mithun</td>
<td>National Research Center on Mithun</td>
</tr>
<tr>
<td>NTYSysp</td>
<td>Numerical Taxonomy SYStem for personal computer</td>
</tr>
<tr>
<td>PALCAM</td>
<td>Polymixin-acriflavin-lithiumchloride-ceftazidimeaesculin-Mannitol</td>
</tr>
<tr>
<td>PALCAMY</td>
<td>Polymixin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol</td>
</tr>
<tr>
<td></td>
<td>Egg Yolk</td>
</tr>
<tr>
<td>PC-PLC</td>
<td>phosphatidylcholine-phospholipase C</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PI-PLC</td>
<td>phosphatidylinositol-phospholipase C</td>
</tr>
<tr>
<td>plcA</td>
<td>Phosphatidylinositol specific phospholipase C</td>
</tr>
<tr>
<td>plcB</td>
<td>Phospholipase C/lecithinase C</td>
</tr>
<tr>
<td>prfA</td>
<td>Protein of 237 amino acid</td>
</tr>
<tr>
<td>psi</td>
<td>Presure per Square Inch</td>
</tr>
<tr>
<td>RADP</td>
<td>Random Amplified Polymorphic DNA</td>
</tr>
</tbody>
</table>
$RBC$: Red Blood Cell

RNA: Ribonucleic acid
rpm: Revolution per minute
rRNA: Ribosomal RNA
RRTC: Rural Research and Training Center
RT-PCR: Reverse Transcription PCR
$smaC$: sphingomyelinase C
$TAE$: Tris Acetate EDTA

TBE Buffer: Tris Borate EDTA Buffer

UK: United Kingdom

UPGMA: Unweighted Pair Group Method with Arithmetic Mean

USA: United States of America

USDA: United States Department of Agriculture

UV: Ultra violet

UV Light: Ultraviolet

UVM: University Vermont Broth

UVM-I: University of Vermont Medium -1

UVM-II: University of Vermont Medium -2

WHO: World Health Organization

$\mu l$: Micro liter
7.2 Preparation of reagents

Bacteriological Media

1. Brain heart infusion broth:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard formula</td>
<td></td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10.0g</td>
</tr>
<tr>
<td>Calf brain infusion (solid)</td>
<td>12.5g</td>
</tr>
<tr>
<td>Beef heart infusion (solid)</td>
<td>5.0g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.0g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0g</td>
</tr>
<tr>
<td>Di-sodium phosphate</td>
<td>2.5g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

pH: 7.4 ±0.2 at 25°C

2. Brain heart infusion agar:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard formula</td>
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</tr>
<tr>
<td>Calf brain infusion (solid)</td>
<td>12.5g</td>
</tr>
<tr>
<td>Beef heart infusion (solid)</td>
<td>5.0g</td>
</tr>
<tr>
<td>Protease peptone</td>
<td>10.0g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0g</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.0g</td>
</tr>
<tr>
<td>Di-sodium phosphate</td>
<td>2.5g</td>
</tr>
<tr>
<td>Agar</td>
<td>1.0g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

pH: 7.4 ±0.2 at 25°C

3. Listeria selective agar base (LSA):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard formula</td>
<td></td>
</tr>
<tr>
<td>Columbia blood agar base</td>
<td>39.0g</td>
</tr>
<tr>
<td>Aesculin</td>
<td>1.0g</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.5g</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>15.0g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

pH: 7 ±0.2 at 25°C

4. University of Vermont broth base (UVM):
i) Base

Standarded formula (g/l)
Proteose peptone 5.0 g
Tryptone 5.0 g
Yeast extract 5.0 g
Sodium chloride 20 g
Lab. Lemco Beef extract 5.0 g
Disodium hydrogen phosphate 9.6 g
Potassium dihydrogen phosphate 1.3 g
Aesculin (Optional) 1.0 g
Distilled water 1000 ml
pH: 7.4 ±0.2 at 25°C

ii) Complete medium

For UVM-I

Allow the base to cool and then aseptically add 5 ml filter sterilized 0.25% acriflavin hydrochloride and 10 ml of 0.4% nalidixic acid (filter sterilized).

For UVM-II

To the cool base, add 10 ml of filter sterilized each of 0.25% acriflavin hydrochloride and 0.4% nalidixic acid solutions.

5. Phenol red broth base:

Standarded formula (g/l)
Protease peptone 10.0 g
Beef extracts 1.0 g
Sodium chloride 5.0 g
Phenol red 0.018 g
Distilled water 1000 ml
pH: 7.4 ±0.2 at 25°C

6. Stuart transport medium:

Standarded formula (g/l)
Sodium thioglycollate 1.0 g
Sodium glycerophosphate 10.0 g
Calcium chloride 0.10 g
Methylene blue 0.002 g
Agar 3.0g
Distilled water 1000ml
pH: 7.4 ±0.2 at 25°C

7. Sheep blood agar (SBA):
  Standarded formula (g/l)
  Sheep blood agar base 40.5 g
  Distilled water 1000 ml
  pH: 7.2 ±0.2 at 25°C

8. Nutrient agar:
  Standarded formula (g/l)
  Peptone 10g
  Beef extract 10g
  Sodium chloride 5.0g
  Agar 20g
  Distilled water 1000 ml
  pH: 7.2 ± 0.1 at 25°C

9. Mueller Hinton Agar:
  Standarded formula (g/l)
  Beef, infusion from 300.000
  Casein acid hydrolysate 17.500
  Starch 1.500
  Agar 17.000
  pH: 7.3 ± 0.1 at 25°C

Biochemical reagents

1. Media for sugar fermentation:
i) Peptone water base
   Standarded formula (g/l)
   Protease peptone 10 g
   Sodium chloride 5.0 g
   Meat/beef extract 1.0 g
   Bromocresol purple 0.02 g
   Distilled Water 1000 ml
pH: 6.8 at 25°C

ii) Complete medium

0.5% carbohydrate solution in filter sterilized Distilled water and added in peptone water base @ 1:9.

2. Oxidase reagents:

Standard formula (g/l)
Tetramethyl-phenylenediamine dihydro-chloride 0.1 g
Distilled water 10 ml

Dissolve the chemical in the water. Prepare freshly before use.

Reagent for PCR

1. Reagents for Agarose Gel Electrophoresis:

Tris – borate EDTA (TBE) buffer (5 X stock solution)

Standard formula (g/l)
Tris base/ Tris buffer 54.0 gm
Boric acid 27.5 gm
0.5 M EDTA (pH 8.0) 20 ml
Distilled water 1000 ml

Store at room temperature

2. 6 X loading dye/buffer:

Standard formula (g/l)
Glycerol 3000 μl
10% bromophenol blue 25 μl
Distilled water 675 μl

3. Ethidium bromide solution (10 mg/ml):

Standard formula (g/l)
Ethidium bromide 0.1 gm
Distilled water 10.0 ml

Staining solution

1. Crystal violet solution:

Solution A
Crystal violet 2.5g
Ethanol (95%) 25ml

**Solution B**

Ammonium oxalate 1.0g
Distilled water 100ml

Mix solution A and B, filter and store.

2. **Safranin:**

   **Stock solution**
   
   Safranin 2.5g
   Ethanol (95%) 100ml

   **Working solution**
   
   Dilute stock solution 1:4 with water