The compositions of media used for the growth and differentiation of *Candida* species are as follows:

**COMPOSITION OF DIFFERENT MEDIA**

**CORN MEAL AGAR (H-Media)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Meal</td>
<td>50gm</td>
</tr>
<tr>
<td>Agar</td>
<td>15gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Corn meal agar media was mixed in 1000 ml of distilled water and sterilized by autoclaving at 121°C for 15 min.

**SABOURAUD’S DEXTROSE AGAR (SDA) (H-Media)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>40gm</td>
</tr>
<tr>
<td>Peptone</td>
<td>10gm</td>
</tr>
<tr>
<td>Agar</td>
<td>15 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>5.5</td>
</tr>
</tbody>
</table>

The components were dissolved in 1L of distilled water and sterilized by autoclaving at 121°C for 15 min.

**HICROME *Candida* DIFFERENTIAL AGAR**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone special</td>
<td>15gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>4gm</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>1gm</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>7.22gm</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.50gm</td>
</tr>
<tr>
<td>Agar</td>
<td>15gm</td>
</tr>
</tbody>
</table>

The constituents were suspended in 1L of distilled water and boiled to dissolve.

**SABOURAUD’S DEXTROSE BROTH**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>40gm</td>
</tr>
<tr>
<td>Peptone</td>
<td>10gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>5.5</td>
</tr>
</tbody>
</table>

The specified amounts of glucose and peptone were added in 1000ml of distilled water and sterilized by autoclaving at 121°C for 15 min.

**YEAST PHOSPHATE DEXTROSE BROTH (YPD) (Hi-Media)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto peptone</td>
<td>10gm</td>
</tr>
<tr>
<td>Glucose</td>
<td>20gm</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>10gm</td>
</tr>
</tbody>
</table>
Distilled water 1000ml
The components were dissolved to 1000ml of distilled water and sterilized at 121°C for 15 min.

**RPMI 1640 Media (with L Glutamine without Sodium bicarbonate) (Hi-Media)**

RPMI 1640 (10.4g) was added to 900ml distilled water and MOPS buffer (Final conc. 0.165mol/l) was then added and contents stirred until dissolved, pH adjusted to 7.0 using 1mol/l NaOH. This medium is filter sterilized and stored at 4°C until used.

**YEAST NITROGEN BASE (YNB) (H-Media)**

- Ammonium sulphate 5.00
- L-Histidine hydrochloride 0.01
- P-Amino benzoic acid (PABA) 0.0002
- DL-Methionine 0.02
- Pyridoxine hydrochloride 0.0004
- DL-Tryptophan 0.02
- Copper sulphate 0.00004
- Biotin 0.000002
- Ferric chloride 0.0002
- Calcium panthothenate 0.004
- Manganese sulphate 0.0004
- Folic acid 0.000002
- Zinc sulphate 0.0002
- Insositol 0.002
- Calcium chloride 0.10
- Niacin 0.0004
- Riboflavin (Vitamin B2) 0.0002
- Thiamine hydrochloride 0.0004
- Boric acid 0.0005
- Potassium iodide 0.0001
- Monopotassium phosphate 1.00
- Magnesium sulphate 0.50
- Sodium chloride 0.10
Final pH (at 25°C) 5.4±0.2

Added 6.7gm of media to 1000ml of distilled water and sterilized by filtration.

**MUELLER-HINTON AGAR (Hi-Media)**

- Beef infusion 300ml
- Casein hydrolysate 17.5g
- Starch 1.5g
- Agar 23-30g
- Distilled water 1000ml

All the ingredients are added to 1000ml of distilled water and autoclave at 121°C for 15 min.

**EGG YOLK AGAR**

- SDA 13g NaCl 11.7g
- CaCl2 0.11g Egg yolk 10%
Distilled water 1000ml
All the above ingredients except egg yolk are added to 900ml of distilled water and autoclaved at 121°C for 15 min separately. 10% egg yolk in sterile 100ml distilled water is then added followed by mixing of both.

**SKIMMED MILK AGAR**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDA</td>
<td>13g</td>
</tr>
<tr>
<td>Skimmed milk</td>
<td>20g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

SDA in 900ml of distilled water and autoclaved at 121°C for 15 min separately. 20g in sterile 100ml distilled water is then added followed by mixing.

**GELATIN AGAR**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDA</td>
<td>13g</td>
</tr>
<tr>
<td>Gelatin</td>
<td>10g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

All the ingredients are added in 1000ml of distilled water and autoclaved at 115°C for 15 min.

**BLOOD AGAR**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDA</td>
<td>1.3g</td>
</tr>
<tr>
<td>Human blood</td>
<td>5-6ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100ml</td>
</tr>
</tbody>
</table>

SDA is mixed in 95ml of distilled water and autoclave at 121°C for 15 min separately. Human blood (5-6ml) is then added and mixed thoroughly.
ANNEXURE- II

The details of chemicals and the reagent used in the study are as follows:

**BUFFERS AND SOLUTIONS**

**0.5 MacFarland standard**
BaCl\(_2\) Barium chloride in a vol. of 0.5ml 0.048mol/l (1.175g of BaCl\(_2\).2H\(_2\)O in 100ml of distilled water) is added to 99.5ml of 0.18mol/l H\(_2\)SO\(_4\) one ml of H\(_2\)SO\(_4\) in 99ml of distilled water).

**1Mol NaOH**
40g of NaOH pallets are dissolved in 1000ml of distilled water.

**Normal saline**
Normal saline is prepared by adding 0.85 gm of NaCl in 100 ml of water.

**70% Ethanol**
70% Ethanol is prepared by adding 70.0ml absolute ethanol in 30 ml of distilled water.

**10mM Phosphate buffer saline (PBS)**
KH\(_2\)PO\(_4\) 0.26g, Na\(_2\)HPO\(_4\)-7H\(_2\)O- 2.17g and NaCl-8.7g are added in 800ml distilled water, pH adjusted to 7.4 and final volume made to 1000ml with distilled water.

**Fetal calf serum (Hi-Media)**
**Sugars (Hi-Media)** Glucose, Sucrose, Lactose, Maltose, Celbiose, Xlose, Rafinose, Galactose, Dulcitol, Ionositol, Melbiose and Trehalose

**0.8% Agarose**
Agarose was prepared by adding 0.8 gm of agarose in 100 ml of water.

**MOPS buffer (H-Media)**

**PCR REAGENTS**

**Lysis buffer**
Lysis buffer is prepared by adding 1% sodium dodecyl sulfate, 2.5 Mm EDTA, 25Mm sodium acetate and 267ug/ml proteinase K in 100 ml of distilled water.

**TE buffer**
TE buffer is prepared by adding 0.12 g of 10mM Tris and 0.037 g of 1mM EDTA in 90 ml distilled water. The pH is adjusted to 7.5 with dil. HCl. The final volume is made to 100 ml.

**Phenol chloroform isoamyl alcohol mix**
Phenol:chloroform:isoamyl alcohol mixture is prepared by adding 25.0ml phenol, 24.0ml chloroform and 1.0ml of isoamyl alcohol.

**3M Sodium acetate**
3M anhydrous sodium acetate is prepared by adding 24.6gm sodium acetate to 50 ml of distilled water and adjusting the pH to 4.8 using glacial acetic acid. Distilled water is then added to make the final volume 100 ml.

**TBE Buffer 5X**

TBE Buffer (5x) is prepared by adding 27.0 gm Tris base, 13.7gm Boric acid, and 10.0ml of 0.5 M EDTA in 500ml of distilled water.

**Ethidium bromide solution**

Ethidium bromide solution is prepared by adding 10mg ethidium bromide to one ml distilled water and stored in a dark and cool place.

**Molecular grade water (Gbiosciences, India).**

2mM each dNTP (Gbioscience, India).

**Taq DNA polymerase (3unit) (Banglore Genei, India)**

**10X PCR buffer (Banglore Genei, India)**

**1kb marker** (DNA Ladder (1kb) 100 Loads, G biosciences, India.

**Bromophenol Blue 0.25% (w/v) - 25mg**

**Primers for ERG11 gene (Eurofins Genomics India pvt Ltd).**

Forward 5'--GTT GAA ACT GTC ATT GAT GG -3

Reverse 5'-TCA GAA CAC TGA ATC GAA AG-3
ANNEXTURE-III

The details of equipment and glassware used in the study are as follows:

**EQUIPMENT**

- Incubator (Instruments and chemicals pvt. Ltd (INCO), India)
- Laminar air flow (Instruments and chemicals pvt. Ltd (INCO), India)
- Autoclave (Instruments and chemicals pvt. Ltd (INCO), India)
- Weighing balance (Kerro), Thermocycler (Genaxy), Gel documentation system (Alpha innotech), UV. Transilluminater (Cleaver Scientific), ELISA plate reader (Easys), Refrigerator (Godrej, India), Deep freezer (New Brunswick Scientific), Centrifuge (Eppendorf), Vacuum pump, Filtration assembly (Hi-Media), Digital colony counter (Instruments and chemicals pvt. Ltd (INCO), India).

**GLASSWARE**

- Petri plates (Genaxy India), Test tubes (Borosil), Conical flasks (100ml, 250ml and 500ml, Borosil), Measuring cylinders, Beakers (100ml, 250ml and 500ml, Borosil), Six well microtiter plates (Corning)

**MISCELLANEOUS**

- Micropipette (Adarsh, India), Inoculating loops (Hi-Media), Non absorbent cotton, Filter paper, Aluminium foil, Microtips (Genaxy), Paper discs (Hi-Media), Microtiter plates (Genaxy India).