IX. APPENDIX

Useful recipes

Culture Media

1. Sabouraud Dextrose Agar (SDA)

Dehydrated SDA agar (Becton Dickinson)	65	gm
Distilled water	1000	ml

Suspend 65 gm of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Autoclave at 121 °C 15lbs for 15 min.

2. Littman Oxgall Agar (LOA)

Dehydrated LOA agar (Difco)	55	gm
Distilled water	1000	ml

Suspend 55 gm in a liter of distilled water and heat to boiling to dissolve completely. Sterilize in the autoclave at 121 °C 15lbs for 15 min. Cool to 56 °C. Add 30 streptomycin per ml of medium. Dispense into sterilize Petri dishes.

3. Caffeic acid slant

Dextrose	2.5	gm
Ammonium sulfate	2.5	gm
Yeast extract	1.0	gm
Potassium Phosphate	0.4	gm
Magnesium sulfate	0.35	gm
Caffeic acid	0.09	gm
2% Ferric citrate solution	0.05	ml
Agar	7.5	gm
Distilled water	500	ml

Suspend all ingredients in 500 ml of distilled water and heat to boiling to dissolve completely. Sterilize in the autoclave at 121°C 15lbs for 12 min. Dispense 2 ml of medium into each 13x100 mm sterilize test tubes in a slanted position.

4. 2% Ferric citrate solution

Ferric citrate	0.2	gm
Distilled water	10.0	ml

Stir to dissolve ferric citrate in distilled water and keep the solution in 4°C.

5. Assimilation and fermentation

Basal medium

Bromcresol purple (1.6%)	0.2	ml
0.1 N Sodium Hydroxide	1.0	ml
Agar	2.0	gm
Distilled water	90.0	ml

Heat to dissolve

Stock carbohydrate solution

Carbohydrate	1.00	gm
(if using raffinose)	2.00	gm
Yeast nitrogen base	0.67	gm
Distilled water	10.0	ml

Mix to dissolve; gently heat if necessary.

Preparation

Add the stock carbohydrate solution to the method agar base. Mix well. Adjust to pH 7.0. Dispense in 5-ml amounts in 16x125-mm screw-cap tubes. Sterilize by autoclaving at 110 °C 15lbs for 10 min. Allow in solidity in a slanted position. Store in refrigerator at 4 °C.

6. Urea agar

Solution A

Urea agar base (Christensen)	29	gm
Distilled water	100	ml

Dissolve powder in water and sterilize by filtration.

Solution B

Agar	15	gm
Distilled water	900	ml

Dissolve agar in water and sterilize by autoclaving at 121 °C 15lbs for 15 min. Cool agar to approximately 50 °C. Add the 100 ml of sterilize urea agar base. Mix well; dispense aseptically into sterilize tubes. Allow to cool in slanted position to form butt about 1 inch deep and slant approximately 1.5 inches long.

Reagents and buffers

1. Phosphate buffer saline (PBS) pH 7.2

Sodium Chloride	8.0	gm
Potassium chloride	0.20	gm
Na2HPO4	1.15	gm
KH2PO4	0.20	gm
Distilled water	1000	ml

Adjust pH to 7.2 with 1 N HCl

2. 500 mM Ethylene diaminetetra acetic acid (EDTA)

EDTA (sodium salt, dehydrate)	18.61	gm
Distilled water	100	ml

3. 0.85% Normal saline solution

Sodium Chloride	0.85	gm
Distilled water	100	ml

Dissolve and autoclave at 121 °C 15lbs for 15 min.

4. Phenol-chloroform-isoamyl mixture

Phenol	25.0	ml
Chloroform	24.0	ml
Amyl alcohol	1.0	ml

Mix thoroughly and store at 4 °C in the dark glass bottle.

5. 10% Sodium dodecyl sulfate (SDS)

SDS	1.0	gm
Distilled water	10	ml

Store at room temperature and should be prepared fresh weekly.

6. 10X Tris EDTA (TE) buffer

1M Tris-Cl (pH 8.0)	100	ml
0.5M EDTA	20	ml
Distilled water	1000	ml

7. 1M Tris-Cl

Tris-Cl	121.1	gm
Distilled water	1000	ml

Dissolve 121.1 gm of Tris base in 800 ml of distilled water. Adjust the pH to 8.0. Adjust the volume of the solution to 1 liter with distilled water. Sterilize by autoclaving.

8. 50X Tris-Acetate-EDTA (TAE) electrophoresis buffer

2M Tris-base	243.0	gm
0.1M EDTA	37.2	gm
1M Acetic acid	60.0	ml
Distilled water	1000	ml

Dissolve and adjust the pH to 8.0. Store in refrigerator at 4 °C.

9. Gel of electrophoresis preparation

Seakem ME agarose	2	gm
Nuseive GTG agarose	0.1	gm
0.5X TAE buffer	100	ml

Stir to dissolve Seakem ME and Nuseive GTG agarose in 0.5X TAE buffer. Melt and cool gel to approximately 50 °C.

Direction

1. Place the gel maker stand in a horizontal position.
2. Install the Gel Maker Plate on the gel maker stand.
3. Set the comb.
4. Allow the molten agarose to cool to below 70 °C, to prevent Gel Maker Plate deformation.
5. Pour the dissolved agarose into Gel Maker Plate.
   - For 4 mm thick gels, about 25 ml of agarose solution is required for the large Gel Maker Plate and 15 ml for small Gel Maker Plate. The gel thickness can be altered as needed.
   - If air is trapped between the Gel Maker stand and the plate, the plate may tilt. To dislodge air, push the Gel Maker Plate using or press down on the vertical portion of the plate. Ensure that no air is clinging to the gel surface.
6. After the gel is hardened, place buffer solution to a level just above the gel surface and gently draw out the comb.

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