APPENDIX-I

All media were sterilized at 121\(^{0}\)C for 20 minutes unless otherwise stated. Antibiotics & B-Vitamins were sterilized by filtration.

1. Yeast Extract Malt Extract agar (YEME) medium:
   - Yeast Extract: 4.0g
   - Malt Extract: 10.0g
   - Dextrose: 4.0g
   - Agar: 20.0g
   - Distilled Water: 1000ml
   - pH: 7.3

2. Glucose-aspargine agar medium:
   - Glucose: 10.0g
   - L-aspargine: 0.5g
   - \(K_2HPO_4\): 0.5g
   - Agar: 20.0g
   - Distilled Water: 1000ml

3. Nutrient Agar medium (NAM):
   - Peptone: 5.0g
   - Beef extract: 3.0g
   - NaCl: 5.0g
   - Agar: 20.0g
   - Distilled Water: 1000ml
   - pH: 7.0-7.5
4. Half-strength nutrient agar medium:

- Peptone : 2.5g
- Beef Extract : 1.5g
- NaCl : 2.5g
- Agar : 10.0g
- Distilled Water : 1000ml

5. Gelatin Agar medium:

- Peptone : 5.0g
- Beef Extract : 3.0g
- Gelatin : 5.0g
- Agar : 20.0g
- Distilled Water : 1.0L
- pH : 7.0

6. Potato Dextrose Agar medium (PDA):

- Peeled Potatoes : 20.0-30.0g
- Dextrose : 20.0g
- Agar : 20.0g
- Distilled Water : 1000ml
- pH : 7.2-7.4

Peeled potatoes are steamed for 20mins. Glucose was dissolved in the extract and water was then added to make up the volume.
7. Oat meal agar medium:

- Oat meal : 20.0g
- Agar : 18.0g

20g Oatmeal was cooked or steamed in 1000ml of distilled water for 20 minutes filtered through cheese cloth. Distilled water was added to restore volume of filtrate to 1000ml.

- Trace salts solution : 1000ml
- Agar : 18.0g
- pH : 7.2

8. Trace salts solution:

- FeSO$_4$.7H$_2$O : 0.1g
- MnCl$_2$.5H$_2$O : 0.1g
- ZnSO$_4$.7H$_2$O : 0.1g
- Distilled Water : 1000ml

9. L.C Agar medium:

- Tryptone : 10.0g
- Yeast Extract : 5.0g
- NaCl : 5.0g
- Agar : 20.0g
- Distilled Water : 900ml
- pH : 7.3

After sterilization, 10% pasteurized milk was added.

10. Starch Agar medium:

- Beef Extract : 3.0g
- Starch : 2.0g
- Agar : 20.0g
- Distilled Water : 1000ml
11. Inorganic Salts- Starch Agar (ISP- medium 4):

Solution I: 1.0 gm of soluble starch was made into a paste with a small amount of cold water and then the volume was made up to 50ml with distilled water.

Solution II:

\[
\begin{align*}
K_2HPO_4 & : 0.1g \\
MgSO_4.7H_2O & : 0.1g \\
NaCl & : 0.1g \\
(NH_4)_2SO_4 & : 0.2g \\
CaCO_3 & : 0.2g \\
Distilled Water & : 50ml \\
Trace salt solution & : 0.1ml \\
pH & : 7.0-7.4
\end{align*}
\]

Solutions I and II were mixed and 2.0g of agar was added.

12. Starch Casein Agar medium:

\[
\begin{align*}
\text{Soluble Starch} & : 10.0g \\
\text{Casein} & : 3.0g \\
KNO_3 & : 2.0g \\
NaCl & : 2.0g \\
K_2HPO_4 & : 2.0g \\
MgSO_4.7H_2O & : 0.05g \\
CaCO_3 & : 0.02g \\
FeSO_4.7H_2O & : 0.01g \\
\text{Agar} & : 20.0g \\
\text{Distilled Water} & : 1000ml
\end{align*}
\]
13. Skimmed milk agar medium:

- Skim milk powder : 1.0g
- Glucose monohydrate : 1.0g
- Casein enzymic hydrolysate : 5.0g
- Yeast extract : 2.5g
- Distilled Water : 1000ml
- pH : 7.0

14. Glycerol- Aspargine agar medium:

- L-aspargine : 1.0g
- Glycerol : 10.0g
- K$_2$HPO$_4$ : 1.0g
- Trace salts solution : 1.0g
- Agar : 20.0g
- Distilled Water : 1000ml
- pH : 7.0-7.4

15. Jowar Starch agar medium:

- Beef Extract : 3.0g
- Tryptone : 2.0g
- Jowar Starch : 2.0g
- Agar : 20.0g
- Distilled Water : 1000ml
- pH : 7.6
16. Peptone-yeast extract-iron agar medium:

Bacto-Peptone iron agar, dehydrated (Difco) : 36.0g
Bacto-Yeast extract (Difco) : 1.0g
Distilled Water : 1000ml
pH : 7.0-7.2

Bacto-Peptone-iron agar dehydrated contained the following ingredients. When reconstituted as 36.58 g/L of water:

Bacto-Peptone : 15.0g
Protease Peptone (Difco) : 5.0g
Ferric ammonium citrate : 0.5g
Di Potassium phosphate : 1.0g
Sodium thiosulphate : 0.08g
Bacto-Agar : 12.0g

17. Milk casein agar medium:

Peptone : 1.0g
Agar : 20.0g
Sterile skimmed milk (10%) : 100ml
Distilled Water : 1000ml
pH : 7.6

18. Bennett’s agar medium:

Yeast Extract : 1.0g
Beef extract : 1.0g
Nz-amine A : 2.0g
Agar : 15.0g
Glucose : 10.0g
Distilled Water : 1000ml
pH : 7.3

19. Carbon utilization medium:

A) Sterile Carbon source

B) Pridham and Gottlieb trace salts (only 1ml of this solution was used per liter of final medium)

- CuSO$_4$.7H$_2$O : 0.64g
- FeSO$_4$.7H$_2$O : 0.11g
- MnCl$_2$.4H$_2$O : 0.79g
- ZnSO$_4$.7H$_2$O : 0.15g
- Distilled Water : 1000ml

C) Basal mineral salts agar (analytical reagent grade chemicals were used)

- (NH$_4$)$_2$SO$_4$ : 2.64g
- KH$_2$PO$_4$ (anhydrous) : 2.38g
- K$_2$HPO$_4$.3H$_2$O : 5.65g
- MgSO$_4$.7H$_2$O : 1.0g
- Pridham and Gottlieb trace salts (B) : 1ml
- Distilled Water : 1000ml
- pH : 6.8-7.0
- Agar (Difco) : 15.0g

D) Complete medium

The sterile basal agar medium (C) was cooled to 60$^\circ$C and sterile carbon source was aseptically added to give a concentration of approximately 1%.