Appendix A

1. **Loading dye for agarose gel**
   - Bromophenol blue: 0.5gm
   - Sucrose: 50gm
   - 1M Tris (pH 8.0): 1mL
   - Distilled water: 100mL
   - Ethidium bromide: 10mg/mL

2. **Alkaline lysis solutions for mini-preparation of plasmid DNA**
   **Solution I**
   - 50 mM glucose
   - 25 mM Tris-Cl (pH 8.0)
   - 10 mM EDTA (pH 8.0)

   Solution I can be prepared in batches of approximately 100 ml, autoclaved for 15 minutes at 10 lb / sq. in. on liquid cycle, and stored at 4°C.

3. **Solution II**
   - 0.2 N NaOH (freshly diluted from a 10 N stock)
   - 1% SDS

   **Solution III**
   - 5 M potassium acetate: 60 ml
   - Glacial acetic acid: 11.5 ml
   - H₂O: 28.5 ml
The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

4. Solution III

5 M potassium acetate - 60 ml
Glacial acetic acid - 11.5 ml
H₂O - 28.5 ml

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

5. Phosphate buffered saline (PBS, pH 7.3)

NaCl - 8 gm
KH₂PO₄ - 0.34 gm
K₂HPO₄ - 1.21 gm

Bring to a final volume of 1000 ml.

6. Citrate buffer (pH 4.3)

Stock solution A

0.1 M citric acid

Stock solution B

0.1 M sodium citrate

Mix solution A (31.5 ml) and solution B (18.5 ml). Bring to a final volume of 100 ml.

Bring to a final volume of 1000 ml with distilled water.
8. **TE buffer (pH 8.0)**

1M Tris-Cl (pH 8.0) - 10 ml

0.5M EDTA (pH 8.0) - 2 ml

Bring to a final volume of 1000 ml with sterile water.

9. **5x TBE buffer**

Tris base - 54 gm

Boric acid - 27.5 gm

0.5M EDTA (pH 8.0) - 20 ml

Bring to a final volume of 1000 ml with distilled water.

10. **Difco sporulation medium**

Bacto nutrient broth - 8 gm

KCl - 10 ml

Mg SO4 7H2O - 10 ml

1M Na OH - 1.5 ml

Adjust the volume to 1 L with dd H2O. pH 7.6. Autoclave and allow to cool to 25°C. Just prior to use add the following sterile solution.

1M Ca (NO3)2 - 1ml

0.01M Mn Cl2 - 1ml

1Mn Fe SO4 - 1 ml

11. **MRS Broth**

Proteose peptone - 10gm

Beef extract - 10 gm
Yeast extract  -  5gm
Dextrose       -  20gm
Polysorbate 80 -  1gm
Ammonium citrate -  2gm
Sodium acetate -  5gm
Magnesium Sulphate -  0.10gm
Manganese sulphate -  0.05gm
Dipotassium phosphate -  2gm

Bring to a final volume of 1000 ml with distilled water (pH 6.5± 0.2).

12. Brain Heart Infusion Broth

Calf brain, infusion from - 200 gm
Beef heart, infusion from  - 250gm
Proteose peptone        -  10 gm
Dextrose                -  2gm
Sodium chloride         -  5 gm
Disodium phosphate      -  2.5 gm

Final pH (at 25°C) 7.4±0.2. Suspend 37 grams in 1000 ml distilled water.

13. Nutrient Agar

Peptone       -  5.0 gm
Beef extract/yeast extract -  3.0 gm
Agar          -  15.0 gm
NaCl          -  5.0 gm
Bring to a final volume of 1000 ml with distilled water (pH 7.4 ± 0.2).

14. **Blood Agar**

- Peptone - 5.0 gm
- Beef extract/yeast extract - 3.0 gm
- Agar - 15.0 gm
- NaCl - 5.0 gm
- Sterile fresh sheep blood - 5ml

Bring to a final volume of 1000 ml with distilled water (pH 7.4 ± 0.2).

15. **Egg Yolk Agar**

- Poteose peptone - 40gm
- Di Sodium phosphate - 5gm
- Mono pottassium phosphate - 1gm
- NaCl - 2gm
- Magnesium Sulphate - 100 gm
- Glucose - 2 gm
- Hemin - .005 gm
- Agar - 20 gm

Suspend 75.1 gm in 900 ml distilled water. Heat to dissolve the medium. Autoclave at 121°C for 15 m’. Add sterile egg yolk suspension per 90 ml of sterile medium. Final pH (at 25°C) 7.4±0.2.

16. **LB Agar**

- Yeast extract - 5gm
- Tryptone - 10gm
NaCl                                          - 10g m
Agar                                           - 15.0 gm

Bring to a final volume of 1000 ml with distilled water (pH 7 ± 0.2).

17. Muller Hinton Agar

Acid casein peptone                        - 17.5 gm
Beef Infusion                              - 2 gm
Starch Agar                                - 1.5 gm
Bacteriological Agar                       - 17 gm

Suspend 38 gm in one liter of distilled water. (Final pH 7 ± 0.2).

18. Brain Heart Infusion Agar

Calf brain, infusion from                  - 200 gm
Beef heart, infusion from                  - 250 gm
Proteose peptone                          - 10 gm
Dextrose                                   - 2 gm
Sodium chloride                           - 5 gm
Disodium phosphate                        - 2.5 gm
Agar                                       - 20 gm

Final pH (at 25°C) 7.4±0.2 Suspend 37 grams in 1000 ml distilled water.

19. Nutrient Broth

Peptone                                    - 5.0 gm
Beef extract/yeast extract                - 3.0 gm
NaCl                                       - 5.0 gm
Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

20. Phosphate buffered saline (PBS, pH 7.3)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8 gm</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.34 gm</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>1.21 gm</td>
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</table>

Bring to a final volume of 1000 ml.

21. LB Broth

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>5gm</td>
</tr>
<tr>
<td>Tryptone</td>
<td>10gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>10gm</td>
</tr>
</tbody>
</table>

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

22. Hepes Hank Buffer (HH Buffer)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8gm</td>
</tr>
<tr>
<td>KCl</td>
<td>0.4gm</td>
</tr>
<tr>
<td>CaCl2</td>
<td>0.14gm</td>
</tr>
<tr>
<td>MgSO4.7H2O</td>
<td>0.2gm</td>
</tr>
<tr>
<td>Na2HPO4</td>
<td>0.05gm</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.35gm</td>
</tr>
<tr>
<td>Hepes</td>
<td>2.6gm</td>
</tr>
</tbody>
</table>

Bring to a final volume of 1000 ml distilled water
23. Bott and Wilson salts

K2HPO4 - 1.24%,
H2PO4 - 0.76%,
trisodium citrate - 0.1%,
(NH4)2SO4 - 0.6%,

24. Simulated Intestinal fluid

Pancreatin - 0.1%
Oxgall bile salt - 0.15%

Bring to a final volume of 1000 ml with distilled water (pH 8 adjusted with 5mol/L NaOH).

25. Simulated Gastric fluid

NaCl - 125 m mol/L
KCL - 7 m mol/L
Na HCO3 - 45 m mol/L
Pepsin - 3 gm

Bring to a final volume of 50 ml gastric fluid. (Required pH was adjusted with 1 N NaOH and 1 N HCl).

26. Manittol Fermentation Media

Manittol - 5.0 gm
Beef Extract - 1.0gm
Peptone - 10.0gm
NaCl - 5.0gm
Bromocresol purple - 0.015gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).
27. Simmon’s citrate agar medium

Sodium citrate - 2 gm  
Mg SO₄ - 2 gm  
Ammonium di hydrogen phosphatate - 1 gm  
Bromo thymol blue - 0.08 gm  
Agar - 15 gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

28. Indol tryptophan broth medium

Tryptone - 10 gm  
Distilled water - 1 litre

29. Kovac’s reagent:

p-dimethylaminobenzaldehyde - 15 gm  
Isoamyl alcohol - 150 ml  
HCL - 75 ml

30. MRVP broth

Peptone - 7.0 gm  
Glucose - 5.0 gm  
Pottasium phosphate - 5.0 gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

31. Barritt’s reagent

VP I reagent (5% a-naphthol dissolved in absolute alcohol)
VP II reagent (40% KOH solution)

32. Skim Milk Agar

Casein peptone - 5.0gm
Yeast extract - 2.5gm
Skim milk powder (inhibitor-free) - 1.0gm
D(+)Glucose - 1.0gm
Agar - 10.5gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

33. Tributyrin Agar

Peptone from meat - 2.5 gm
Peptone from casein - 2.5gm
Yeast extract - 3.0gm
Agar - 12.0gm
Tributyrin - 10 ml

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2). To this add 10 ml neutral tributyrin/litre, mix uniformly.

34. Urea broth medium

Urea - 20gm
Yeast extract - 0.1gm
KH2PO4 - 9.0gm
K2HPO4 - 9.5gm
Phenol red - 0.01gm

Bring to a final volume of 1000 ml distilled water (pH 6.8± 0.2).

35. Nitrate broth

KNO$_3$ - 1gm
Peptone - 5gm
Beef extract - 3gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

36. Starch Agar

Starch - 20 gm
Peptone - 5gm
Beef extract - 3gm
Agar - 15gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

37. Gelatin Agar

Gelatin - 120gm
Peptone - 5 gm
Beef extract - 3 gm
Agar - 15 gm

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

38. Erythrocyte lysis buffer (pH 7.2)

0.6M of NH$_4$Cl - 90 mL
0.17 TrisHCl - 10 ml

39. MTT

MTT was dissolved in RPMI-1640 at 5 mg/ mL and filter through 0.22 µm filters

40. Preparation of basal culture medium (RPMI-1640)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI 1640</td>
<td>16.4 gm</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>2.10 gm</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>1.101 mg</td>
</tr>
<tr>
<td>HEPES</td>
<td>5.96 g (50 mM)</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>2.93 gm</td>
</tr>
<tr>
<td>Pencillin</td>
<td>61 mg (100 IU/ mL)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>100 mg (100 µg/ mL)</td>
</tr>
</tbody>
</table>

Bring to a final volume of 1000 ml distilled water (pH 7 ± 0.2).

41. NBT

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBT</td>
<td>0.1 gm</td>
</tr>
<tr>
<td>HBSS</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

42. Balanced salt solutions (Hank’s)

a) Phenol red indicator (0.4%)

Dissolve 1 g phenol red in minimal volume of 0.05 N NaOH and then bring the volume to 250 ml by adding distilled water.
b) Stock solution A

- NaCl - 160gm
- KCl - 8 gm
- MgSO₄·7H₂O - 2 gm
- MgCl₂·6H₂O - 2 gm
- H₂O - 800ml
- 2CaCl₂ - 2.8gm
- H₂O - 10ml

Mix these 2 solution slowly adjust the volume to 1000 ml with H₂O. Add 2ml chloroform and store in polythene bottle at 4°C.

c) Stock solution B

- Na₂HPO₄·12H₂O - 3.4gm
- KH₂PO₄ - 1.2gm
- Glucose - 20gm
- H₂O - 800ml

When dissolved add 100 ml of phenol red and make up to 1000 ml with water. Add 2ml chloroform and store in polythene bottle at 4°C.

d) Sodium bicarbonate solution

- NaHCO₃ - 1.4 gm
- H₂O - 100 ml

Sterilize by autoclaving for 10 minutes at 115°C. Hank’s solution is prepared by adding 1 volume of stock A and 1 volume of stock B to 18 ml of distilled
water. It is sterilized by steaming for one and a half hours immediately before use. Add 0.5 ml of 1.4% of NaHCO₃ to each 20 ml of Hank's solution.

43. Trypan Blue

Trypan blue stain - 0.4 gm
PBS - 100 ml

Dissolve 0.4 g trypan blue in 80ml PBS. Bring to a slow boiling. Cool to room temperature. Make up to 100 ml with PBS.

44. Alsever's solution

Dextrose - 2.05gm (D glucose)
Sodium citrate - .80gm
NaCl - 0.42gm

Mix in 100ml Double distilled Water. Adjust the pH to 6.1 using 10% citric acid. Autoclave and use.
Appendix B

16S-rDNA partial sequences for candidate probiotic

MBTUPBBM1 (GenBank: JN873913)

1  aatgcaaaa ctgggtcccc gatagttatc ggcgga cggg tgagtaacac gtgggtaacc
61  tgcctgtaag actgggataa ctccgggaaa ccggggc taa taccggatgg ttgtttgaac
121  cgcataagtc aaacataaaa ggtgacctcg gtacacactt acagatggac cgcggcgca
181  tttagctagtt ggtgaggtaa cggctcaca aaagcaagat gcgtacccga caatgagagg
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