ADDENDA

Addenda

Table 6.1: Biomass (g/l) profiles at various cultivation temperatures after 48 hours.

	Isolates		
Temperature	FM	TapLac	TapSuc
15°C	2.28 ± 0.04	2.32 ± 0.02	2.65 ± 0.03
30°C	2.52 ± 0.04	4.79 ± 0.02	3.99 ± 0.03
37°C	2.96 ± 0.06	5.11 ± 0.03	4.82 ± 0.03
45°C	2.34 ± 0.06	4.90 ± 0.03	4.67 ± 0.01
50°C	1.85± 0.07	1.90 ± 0.03	1.8 ± 0.03

Table 6.2: Shows the end point pH profiles at various cultivation temperatures after 48 hours.

	Isolates		
Temperature	FM	TapLac	TapSuc
15°C	5.83 ± 0.02	4.63 ± 0.02	4.60 ± 0.03
30°C	4.43 ± 0.02	3.63 ± 0.03	3.63 ± 0.02
37°C	4.22 ± 0.02	3.66 ± (0.03	3.64 ± 0.02
45°C	4.22 ± 0.04	3.76 ± 0.04	3.82 ± 0.02
50°C	4.53 ± 0.03	4.93 ± 0.04	4.97 ± 0.04

Table 6.3 Biomass (g/l)profile at various initial starting pH conditions after 48 hours.

	Isolates		
pH	FM	TapLac	TapSuc
4.5	1.76 ± 0.03	3.54 ± 0.03	2.09 ± 0.02
7	2.79 ± 0.06	5.29 ± 0.04	5.16 ± 0.07
9	2.93 ± 0.04	4.36 ± 0.03	2.36 ± 0.08

Table 6.4: End point pH profile at various initial starting pH conditions after 48 hours.

	Isolates		
pH	FM	TapLac	TapSuc
4.5	4.35 ± 0.03	3.54 ± 0.03	3.53 ± 0.06
7	4.21 ± 0.03	3.99 ± 0.07	3.93 ± 0.04
9	4.31 ± 0.02	4.36 ± 0.03	7.8 ± 0.02

Table 6.5 : Biomass (g/l) profile at various NaCl concentrations after 48 hours

	Isolates		
NaCl Concentration (%w/v)	FM	TapLac	TapSuc
1.5	5.31 ± 0.02	6.34 ± 0.03	5.07 ± 0.03
2.5	5.75 ± 0.03	5.85 ± 0.02	3.94 ± 0.03
5	2.01 ± 0.03	2.13 ± 0.04	2.15 ± 0.01
7.5	2.01 ± 0.02	1.92 ± 0.02	1.93 ± 0.03
10	1.86 ± 0.03	1.84 ± 0.03	1.81 ± 0.02

Table 6.6 : End point pH profile at various NaCL concentrations after 48 hours

	Isolates		
NaCl Concentration (%w/v)	FM	TapLac	TapSuc
1.5	4.06 ± 0.03	3.46 ± 0.02	3.46 ± 0.03
2.5	4.09 ± 0.04	3.56 ± 0.03	3.54 ± 0.03
5	4.17 ± 0.04	4.44 ± 0.03	4.42 ± 0.02
7.5	4.45 ± 0.02	6.61 ± 0.01	6.57 ± 0.03
10	6.7 ± 0.04	6.66 ± 0.01	6.74 ± 0.03

Table 6.7: Biomass (g/l) profile during the time course study

	Isolates		
Time (hours)	FM	TapLac	TapSuc
0	0.053 ± 0.02	0.054 ± 0.03	0.072 ± 0.03
6	1.660 ± 0.03	1.113 ± 0.03	1.375 ± 0.03
12	1.742 ± 0.03	2.500 ± 0.04	2.745 ± 0.01
18	1.695 ± 0.02	3.342 ± 0.02	3.040 ± 0.03
24	1.712 ± 0.03	2.892 ± 0.03	3.220 ± 0.02
30	1.603 ±0.04	3.525 ± 0.02	3.285 ± 0.03
36	1.570 ± 0.04	3.482 ± 0.02	3.135 ± 0.03
42	1.613 ± 0.06	3.400 ± 0.03	3.095 ± 0.03
48	1.622 ± 0.03	3.377 ± 0.03	2.895 ± 0.01
54	1.615 ± 0.07	3.235 ± 0.03	3.000 ± 0.03

Table 6.8: pH profile during the time course study

	Isolates		
Time (hours)	FM	TapLac	TapSuc
0	6.84 ± 0.02	6.83 ± .02	6.94 ± 0.02
6	4.84 ± 0.02	5.29 ± 0.03	4.88 ± 0.03
12	4.45 ± 0.02	4.00 ± 0.03	3.43 ± 0.01
18	4.29 ± 0.03	3.52 ± 0.01	3.03 ± 0.03
24	4.15 ± 0.04	3.43 ± 0.01	2.94 ± 0.02
30	4.10 ± 0.03	3.39 ± 0.04	2.94 ± 0.03
36	4.04 ± 0.04	3.32 ± 0.03	2.80 ± 0.03
42	4.06 ± 0.04	3.37 ± 0.02	2.79 ± 0.02
48	4.02 ± 0.02	3.58 ± 0.02	2.88 ± 0.03
54	4.25 ± 0.04	3.45 ± 0.03	2.89 ± 0.03

APPENDICES

Appendix A

Appendix A.1 MRS Broth (DeMan *et al.*, 1960)

Composition per litre:

Glucose	20.0g
Peptone	10.0g
Beef extract	8.0g
Sodium acetate.$3H_2O$	5.0g
Yeast Extract	4.0g
K_2HPO_4	2.0g
Triammonium citrate	2.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.4H_2O$	0.03g
Sorbitan Monooleate (Tween 80)	1ml

All of the components except glucose were dissolved in 900ml distilled water. The glucose component of the media was dissolved in 100ml of distilled water. Both were autoclaved for 15 minutes at 121°C. After cooling, both were mixed together.

Appendix A.2 MRS Agar (DeMan *et al.*, 1960)

Composition per litre:

Glucose	20.0g
Peptone	10.0g
Agar	10.0g
Beef extract	8.0g
Sodium acetate.$3H_2O$	5.0g
Yeast Extract	4.0g
K_2HPO_4	2.0g
Triammonium citrate	2.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.4H_2O$	0.03g
Sorbitan Monooleate (Tween 80)	1ml

All of the components except glucose were dissolved in 900ml distilled water. The glucose component of the media was dissolved in 100ml of distilled water. The pH of the media was adjusted to pH 6.5 with 4N NaOH. Both were autoclaved for 15 minutes at 121°C. After cooling, both were mixed together.

Appendix A.3 Nutrient Gelatine (Gibson & Ab-del Malek, 1941)

Composition per litre:

Gelatine	200.0g
Peptone	5.0g
Yeast Extract	3.0g
Glucose	5.0g

All of the components except glucose were dissolved in 900ml distilled water. The glucose component of the media was dissolved in 100ml of distilled water. Both were autoclaved for 15 minutes at 121°C. After cooling, both were mixed together.

Appendix A.4 Sterile Agar

Composition per litre:

Agar	15g

The agar was dissolved in 1000ml of distiiled water and was autoclaved for 15 minutes at 121°C

Appendix A.5 Basal MRS broth with Bromocreosol Purple (modified from DeMan *et al.*,1960)

Composition per litre:

Glucose	20.0g
Peptone	10.0g
Sodium acetate.$3H_2O$	5.0g
Yeast Extract	4.0g
K_2HPO_4	2.0g
Triammonium citrate	2.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.4H_2O$	0.03g
Sorbitan Monooleate (Tween 80)	1ml
Bromocreosol Purple	0.17g

All of the components except glucose were dissolved in 900ml distilled water. The glucose component of the media was dissolved in 100ml of distilled water. The pH of the medium was adjusted to 7 with 4N NaOH. Both were autoclaved for 15 minutes at 121°C. After cooling, both were mixed together.

Appendix B

Appendix B.1: Gram's Stain (Hucker Modification)(Larone, 1987)

B.1.1 Materials

1) Crystal Violet Solution

a) Crystal Violet, 85% dye	2g
b) Ethyl Alcohol, 95%	10ml

Dissolve the dye in alcohol.

Add distilled water 100ml

c) Ammonium oxalate	4g
d) Distilled water	400ml

Dissolve the ammonium oxalate in water.

Mix the crystal violet-alcohol solution with ammonium oxalate solution.

2) Gram iodine solution

a) Iodine	1g
b) Kalium Iodide	2g

Dissolve iodine and kalium iodide completely in 5ml of distilled water.

c) Add distilled water	240ml
d) Natrium bicarbonate, 5% aqueous solution	60ml

Mix well; stored in amber glass bottle.

Counterstain Solution

a) Safranine O	1g
b) Ethyl alcohol, 95%	40ml

Dissolve the dye in the alcohol

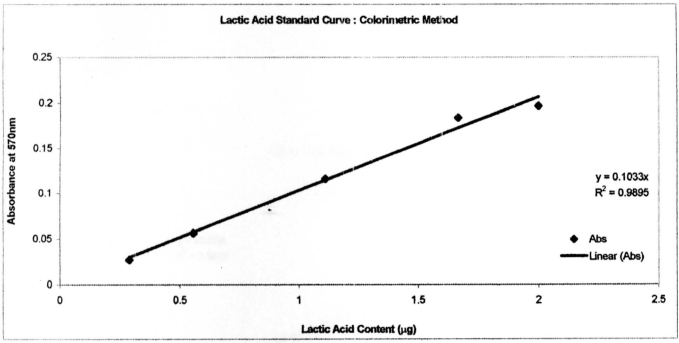
c) Add distilled water	400ml

Mix well.

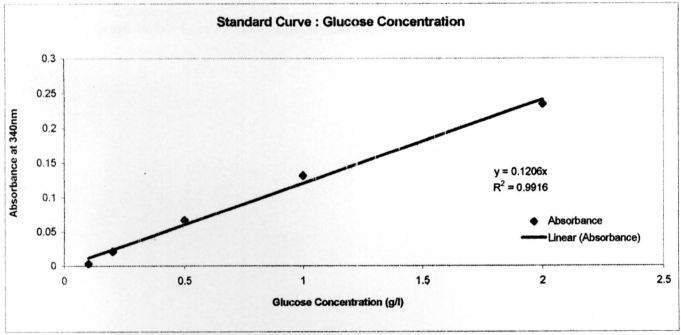
B.1.2 Method

Make a thin smear of bacteria on a glass slide. Fix the smear by passing it over a flame. Place crystal violet solution on the slide for 20s. Rinse off gently with tap water. Apply Gram iodine solution to the smear for 20s. Decolorize quickly in solution of equal parts of acetone and 95% ethanol. Wash gently with tap water. Counter-stain with safranine for 10s. Wash with tap water and blot dry.

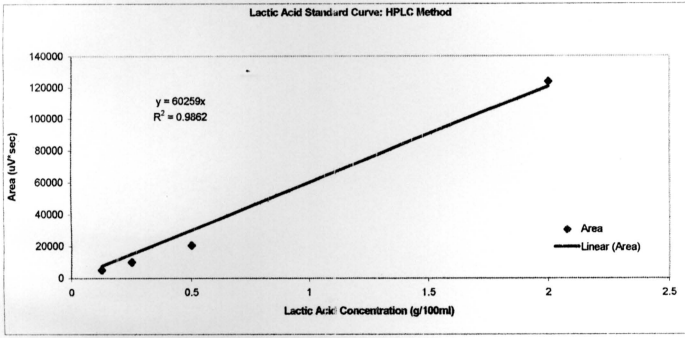
Appendix C: Standard Curve



Appendix C.1 Lactic Acid Content Standard Curve (Colorimetric Method)



Appendix C.2 Glucose Concentration Standard Curve (Enzymatic Method)



Appendix C.3 Lactic Acid Concentration Standard Curve (HPLC Method)