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APPENDICES

APPENDIX A
CHEMICAL AND MEDIA PREPARATIONS

1. Ammonium persulphate solution, 10% freshly prepared

	: per millilitre
Ammonium persulphate (PlusOne ; Amersham Biosciences)	0.1 g
Deionized water	1 ml

Mix together and then use immediately

2. Blood Agar

	: per litre
Blood agar base (Merck™ ; Merck KGaA)	40 g
Distilled water	950 ml

Suspend the medium powder and then autoclave at 121°C for 15 min. Cool to 50°C and add 50 ml sterile human blood to take 5% blood in total agar.

3. Nitrocefin, 500 µg/ml

	: per 2 ml
Lyophilized nitrocefin (Oxoid ; Oxoid Ltd)	1 mg
Phosphate buffer (0.1M, pH 7.0) (Oxoid ; Oxoid Ltd)	1.9 ml
Dimethylsulphoxide (Oxoid ; Oxoid Ltd)	0.1 ml

Mix together. Keep the solution away from light and store at -20 °C.

4. Phosphate Buffer, pH 7.0

	: per litre
Monosodium phosphate (AnlalaR [®] ; BDH)	5.84 g
Disodium phosphate (AnlalaR [®] ; BDH)	15.47 g
1 mM EDTA (Vivantis ; Vivantis Technologies)	0.37 g
Deionized water	1 L

Mix together and make total volume up to 1 L with deionized water. Store at 4°C.

5. Phosphate buffer saline (PBS), 10X pH 7.2

	: per litre
Sodium chloride (Merck [™] ; Merck KgaA)	80 g
Potassium chloride (M&B ; May&Baker Ltd, Dagenham, England)	2 g
Disodium hydrogen phosphate (AnlalaR [®] ; BDH, Dorset, England)	14.4 g
Photassium dihydrogen phosphate (AnlalaR [®] ; BDH)	2.4 g

Mix together in 900 ml of distilled water. Adjust to pH 7.2 by using 1 M NaOH and 1 M HCl. Add the distilled water up to 1 L. Store the solution at room temperature.

6. Resolving polyacrylamide gel, 12%

	: per 1 gel
1.5 M Tris - HCL pH 8.8	2.5 ml
40% Acrylamide /Bis (PlusOne; Amersham Biosciences)	3.125 ml
10% (w/v) Sodium dodecyl sulphate	100 µl
10% (w/v) Ammonium persulphate	50 µl
TEMED (USB®; USB Corporation, Cleveland, OH)	3.3 µl
Deionized water	4.225 ml

Mix together and swirl gently to initiate polymerization.

7. SDS electrophoresis buffer, 10X

	: per litre
Tris base (Merck™; Merck KGaA)	30.3 g
Glycine (Merck™; Merck KGaA)	144 g
Sodium dodecyl sulfate (Vivantis ; Vivantis Technologies, Selangor, Malaysia)	10 g
Deionized water	1 L

Mix together in 1 L of distilled water. Do not adjust pH. Store the solution at room temperature.

8. Sodium dodecyl sulphate solution (SDS), 10% w/v

	: per 100 ml
Sodium dodecyl sulphate	10 g
Deionized water	90 ml

Dissolve the sodium dodecyl sulphate in 90 ml of deionized water with gentle stirring and make to 100 ml with deionized water. Store at room temperature.

9. Stacking polyacrylamide gel, 4%

	: per 2 gels
0.5 M Tris-HCL pH 6.8	0.625 ml
40% Acrylamide /Bis	250 μ l
10% (w/v) Sodium dodecyl sulphate	25 μ l
10% (w/v) Ammonium persulphate	12.5 μ l
TEMED	3.3 μ l
Deionized water	1.582 ml

Mix together and swirl gently to initiate polymerization.

10. Tris - HCl buffer, 0.5 M pH 6.8

	: per litre
Tris-base (Merck TM ; Merck KGaA)	60.5 g
Deionized water	900 ml

Mix together and then adjust the solution to pH 6.8 with 5 M HCl. Make total volume up to 1 L with deionized water and store at 4°C.

11. Tris - HCl buffer, 1.5 M pH 8.8

	: per litre
Tris - base	181.5 g
Deionized water	900 ml

Mix together and then adjust the solution to pH 8.8 with 5.0 M HCl. Make total volume up to 1 liter with deionized water and store at 4°C.

12. Urea Agar

	: per litre
Agar powder (BBL™ ; Becton Dickinson, Sparks, MD.)	15 g
Urea agar base (BBL™ ; Becton Dickinson)	29 g
Distilled water	1 L

Dissolve 29 g of the urea agar base powder in 100 mL of purified water. Mix thoroughly. Sterilize by filtration. Suspend 15 g of agar powder in 900 mL of purified water and autoclave at 121°C for 15 minutes. Cool to 50°C and add 100 mL of the sterile Urea Agar Base. Mix thoroughly and dispense aseptically in sterile tubes. Cool tubed medium in a slanted position. Do not remelt the complete medium.

APPENDIX B

LACTOBACILLUS

I. Data of primary antimicrobial screening

Table B1 Primary antimicrobial screening of lactobacilli (batch 1)

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
B309	13	14	11	12	10	10	13	13
B134	10	10	6	6	6	6	8	8
B16	12	13	13	13	11	11	15	15
B277	11	12	12	11	11	11	13	14
B35/2	15	16	13	13	10	11	15	15
B40/3	15	14	13	13	10	10	15	14
B1/4	14	15	11	11	11	11	13	13
B59/8	12	12	10	10	9	9	13	13
B1	16	14	12	12	11	10	12	12
B36/2	15	15	13	13	11	11	13	13
B43/3	14	14	12	14	10	10	13	13
B80/2	16	17	15	12	13	13	14	15
B93/2	13	13	12	11	11	9	13	13
B31/4	16	15	13	13	12	11	14	14
B97/3	12	13	12	11	9	9	11	11
B194	12	13	12	11	9	9	12	12
B16/3	11	10	12	12	11	12	15	14
B42/3	16	15	13	12	11	11	14	15
B19	12	12	6	6	10	10	13	13
B2	12	12	10	10	11	11	13	14
B247	12	12	6	6	11	11	12	12
B12/2	14	13	11	11	10	10	12	13
B8/2	16	16	13	13	12	13	14	15
B189	12	11	6	6	10	10	12	13
B11/4	13	14	13	13	12	12	14	15
B22/2	13	11	6	6	10	9	13	14
B211	11	11	6	6	10	10	13	13
B163	13	13	6	6	9	9	13	14
B18	13	13	12	10	9	10	13	12
B72/8	12	12	11	11	10	10	13	13
B21/8	15	15	14	14	12	13	15	15
B3	15	14	13	13	11	10	14	14
B8/8	12	12	12	12	11	10	12	12
B87/8	12	12	10	10	9	9	11	12
B22	17	17	15	15	13	13	15	15

Table B1 (continued)

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
B6/7	13	12	12	9	10	11	13	12
B8	11	15	14	14	11	11	15	15
B14	15	16	14	15	11	11	13	15
B126	12	11	6	6	6	6	8	8
B282	15	14	11	12	9	9	14	15
B40/8	12	12	9	10	8	8	12	12
B199	13	13	10	11	10	10	12	12
B280	18	18	16	16	13	13	17	16
B146	11	11	6	6	6	6	9	9
B79/3	10	10	6	6	11	11	9	10
B18/3	12	12	9	9	10	10	12	12
B38/3	11	10	6	6	6	6	9	9
B30/3	11	12	9	9	6	6	10	10
B120	10	10	6	6	6	6	7	7
B99/2	13	15	13	13	12	12	15	15
B85	11	10	6	6	6	6	9	9
B41	12	12	11	11	11	12	13	12
B18/9	10	10	7	7	6	6	9	9
B2/7	13	13	12	13	11	11	13	12
B1/9	12	12	12	12	11	12	12	12
B45	14	14	13	13	9	9	12	12
B305	16	17	17	16	13	11	14	14
B184	16	15	15	13	10	10	13	10
B63/8	9	9	9	10	10	9	10	12
B40/2	12	12	11	11	10	11	12	12
B85/4	11	11	6	6	9	9	11	11
B49	15	15	14	13	10	11	13	14
B37/5	14	15	12	12	10	11	12	13
B28/5	14	13	11	11	10	11	12	12
B39/5	15	14	11	12	11	11	12	12
B39/4	16	15	14	14	11	11	13	13
B18/4	15	14	13	13	11	11	13	13
B100/2	16	15	13	12	11	12	13	13
B92/4	14	14	11	11	11	11	13	12
B35/4	14	15	13	12	11	10	14	15
B50	15	14	13	13	12	12	15	15
B20/5	15	15	13	13	11	10	13	14

*Bold numbers : Selected isolates for further study

Table B2 Primary antimicrobial screening of lactobacilli (batch 2)

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
B2/22	17	16	18	16	13	14	16	16
G8/22	15	15	14	15	13	14	15	16
D6/22	15	14	15	15	13	14	17	16
B6/22	14	14	16	15	13	14	17	17
E1/22	15	14	14	14	13	13	17	16
G4/22	13	14	15	14	14	13	15	15
B4/22	14	14	18	17	14	14	17	17
B8/22	15	15	18	17	13	14	18	19
B6/6	9	10	14	13	9	9	14	14
H9/22	14	15	14	15	11	12	17	18
B9/22	15	16	16	15	12	12	18	18
F9/22	12	14	15	14	13	13	14	15
E3/22	14	15	18	18	12	14	15	14
D1/22	13	13	15	16	12	12	15	16
C9/22	16	17	16	17	14	14	17	17
G7/22	15	15	16	15	12	12	19	19
E9/22	14	15	17	16	12	12	17	16
I2/22	14	15	16	16	12	13	17	17
A5/6	9	10	10	10	8	9	11	12
H6/22	13	14	15	15	12	12	17	16
I8/22	14	14	16	16	13	13	16	16
B9/6	14	13	16	15	13	12	17	17
A1/6	13	15	15	15	12	13	17	18
A7/22	14	15	15	16	15	15	16	16
D7/22	16	14	14	15	12	12	16	19
A4/22	14	14	16	16	12	12	16	18
E6/22	15	14	16	16	12	13	15	15
B5/22	14	15	16	16	15	14	16	18
F1/22	15	15	16	17	13	11	16	18
A9/22	15	15	16	16	12	11	15	15
C6/22	15	14	15	16	12	11	16	16
A8/22	14	15	16	16	12	12	17	16
D4/22	15	15	18	15	12	12	17	17
I6/22	14	14	15	15	12	12	17	17
C8/22	13	14	16	16	13	12	16	17
A6/22	16	14	16	16	13	13	16	16
C3/22	15	15	15	15	14	14	18	17
D5/22	14	14	19	18	12	13	16	16
G9/22	14	15	14	15	12	13	16	16
A5/22	14	15	16	17	14	14	16	17
A8/6	13	12	11	11	10	10	16	16
F8/22	15	16	17	16	14	15	20	20
A4/6	13	12	15	14	13	13	16	16
E4/22	14	14	14	15	13	13	16	17
I9/22	14	14	16	16	13	13	17	17

Table B2 (continued)

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
B7/6	14	14	15	14	13	13	16	16
G1/22	15	15	17	16	13	13	18	19
F3/22	15	16	15	15	13	13	15	17
G5/22	15	16	16	15	13	13	17	18
G2/22	16	15	16	17	13	13	17	17
E2/22	14	13	15	15	13	13	15	15
F2/22	16	17	15	15	13	13	17	17
F7/22	15	15	16	15	13	13	18	17
E5/22	15	14	15	15	13	13	16	16
F4/22	15	14	15	16	12	13	16	17
B8/6	15	15	13	14	13	12	15	15
C2/22	15	14	15	15	12	12	16	18
F5/22	16	16	16	15	11	11	15	17
A3/6	15	14	17	14	11	12	16	16
D2/22	14	15	15	15	13	13	16	16
A9/6	13	12	14	15	9	10	15	15
A6/6	12	14	11	12	11	9	14	12
B1/6	13	13	16	15	13	13	17	17
H7/22	14	14	16	16	14	13	17	16
H5/22	15	14	16	16	13	13	17	19
I5/22	14	14	15	14	13	14	16	17
B3/6	11	11	14	14	11	10	15	15
H3/22	13	14	16	16	12	13	19	19
I4/22	15	15	15	15	15	15	16	17
B4/6	11	10	13	12	11	10	13	13
B5/6	10	10	12	12	9	8	14	14
A2/6	11	11	13	13	12	11	14	13
A7/6	13	13	13	14	13	13	16	16
C4/22	14	15	16	16	14	14	17	17
B1/22	15	15	17	18	13	13	16	16
B7/22	14	15	19	18	13	13	16	15
G6/22	15	14	16	15	13	12	16	16
C7/22	15	15	16	15	13	13	17	17
D9/22	15	15	16	15	13	14	15	16
D8/22	15	16	16	17	12	13	15	15
C5/22	13	13	15	15	12	12	15	15
D3/22	15	13	14	13	13	11	16	16
B3/22	12	15	15	15	11	12	9	9
B2/6	12	10	12	13	6	6	11	11
A3/22	14	15	15	15	14	14	17	17
F6/22	15	14	15	16	13	14	17	17
G3/22	15	15	15	15	13	14	17	17
C1/22	15	15	15	16	14	14	17	17
E8/22	15	14	15	14	12	13	16	17
E7/22	14	14	15	15	12	13	16	17

Table B2 (continued)

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
A2/22	13	14	14	14	13	13	17	16
A1/22	15	15	15	16	13	12	16	16
B16	15	13	15	15	12	12	16	15
B50	15	15	14	15	12	12	15	15
B309	10	11	13	12	10	9	13	13
B62/8	15	15	15	15	13	12	15	16
B184	14	14	17	15	14	13	17	17
B63/8	14	15	16	16	13	13	17	16
I7/22	15	14	16	16	14	13	16	16
B18	11	11	13	12	6	6	11	13

*Bold numbers : Selected isolates for further study

Table B3 Secondary screening of lactobacilli against 4 standard strains

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
F6/22	19	20	17	17	15	14	16	16
F7/22	16	16	27	27	25	22	17	17
B35/2	24	24	19	20	18	14	16	17
B50	16	16	28	28	26	14	16	16
L541	23	23	28	28	26	19	17	17
SS	19	20	25	25	23	16	17	17
B8	16	16	28	28	26	12	18	18
B14	16	15	26	27	25	17	18	18
B99/2	11	11	18	19	17	12	15	14
G1/22	16	15	24	24	22	14	15	15
F8/22	17	17	22	21	19	17	19	18
B80/2	17	16	25	25	23	14	17	17
G5/22	16	17	26	27	25	13	16	16
B8/2	14	14	20	19	17	11	17	16
LP055	17	17	25	25	23	15	16	16
LCR10	18	17	27	26	24	14	16	17
L1034	22	22	28	28	26	19	17	17
LC309	18	18	21	21	19	15	17	17
B31/4	16	16	24	23	21	14	16	16
G2/22	22	22	25	26	24	18	18	18
B305	19	20	27	27	25	19	18	18
B280	19	18	25	25	23	18	17	17
B16	16	17	23	22	20	19	15	16
B22	16	16	19	19	17	18	15	15
B42/3	14	15	18	18	16	14	16	15
B21/8	20	20	27	26	24	18	17	18
B11/4	16	16	19	18	16	16	16	15

Table B3 (continued)

Isolates	Average diameter of inhibition zone (mm) against							
	<i>S. aureus</i>		<i>S. lutea</i>		<i>E. coli</i>		<i>B. subtilis</i>	
	ATCC 25923		ATCC 9341		ATCC 25922		ATCC 6633	
B39/4	21	21	27	26	24	17	17	16
A5/22	15	14	23	24	22	12	13	13
B4/22	18	18	26	26	24	18	16	16
B49	15	15	18	18	16	15	14	15
B100/2	13	13	17	17	15	11	13	13
B2/22	17	17	27	26	24	13	15	16
B8/22	15	14	25	25	23	14	17	18
G3/22	12	13	25	26	24	13	17	16
C9/22	16	15	26	26	24	16	17	17
C4/22	16	16	24	25	23	13	18	18
C3/22	17	16	26	27	25	16	16	15
F2/22	15	15	25	25	23	13	16	17
B5/22	18	18	24	25	23	11	20	20
D4/22	16	16	27	28	26	14	17	17
F1/22	16	16	26	26	24	14	18	18
I4/22	14	14	25	24	22	15	18	18
H5/22	14	14	23	23	21	15	19	19
C7/22	15	14	22	21	19	14	19	18
B1/22	16	15	26	26	24	14	16	17
B184	16	16	23	23	21	14	17	18
C1/22	15	15	25	26	24	15	18	18
B9/22	16	15	21	20	18	14	15	14

*Bold numbers : Selected isolates for further study

Table B4 Antimicrobial screening of lactobacilli against 6 laboratory strains

Isolates	Average diameter of inhibition zone (mm) against					
	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>A. baumannii</i>	<i>P. vulgaris</i>	<i>E. faecalis</i>	<i>S. agalactiae</i>
F7/22	12	14	13	14	17	16
L541	13	15	14	14	19	16
B14	14	13	15	12	17	12
F8/22	13	17	16	13	18	17
G2/22	13	13	15	17	16	13
B305	14	14	16	17	19	15
B21/8	14	15	15	17	17	14
B39/4	13	12	15	14	19	15
LSS	17	15	14	17	16	13
B4/22	14	15	15	15	15	13
L1034	18	13	15	15	18	13
B280	15	14	15	17	19	15

*Bold numbers : Selected isolates for further study



Table B5 Antimicrobial screening of lactobacilli against 38 clinical strains

Isolates	Average diameter of inhibition zone (mm) against									
	L541	F8/22	L1034	LSS	B305	B280				
U808	12	11	14	11	11	11	12	12	12	12
U842	14	15	14	6	13	13	13	13	13	13
U1228	16	12	18	15	15	16	15	15	15	15
U947	12	12	12	13	14	14	11	11	11	10
U1224	19	16	17	17	16	16	18	18	17	17
U1264	14	15	15	15	12	12	13	13	14	14
U1104	17	17	16	18	18	17	16	17	17	17
U1035	16	15	17	15	14	15	16	16	16	16
U846	11	6	10	11	6	6	6	6	6	6
U978	16	14	15	14	12	13	13	13	12	12
U1242	18	18	18	18	19	18	17	17	16	16
U1266	16	15	18	15	15	15	13	13	13	13
U1202	16	14	16	15	15	15	15	15	15	15
U1226	16	13	16	16	15	15	13	13	13	13
U1220	17	15	18	16	17	17	15	15	15	15
U1210	14	14	14	15	15	14	14	14	14	14
U874	12	12	12	13	12	12	12	12	12	12
U965	14	12	12	12	13	13	14	14	14	14
U849	12	12	11	12	12	12	12	12	12	12
U883	10	10	11	12	11	11	12	12	13	13
U1284	17	15	19	17	16	16	15	15	15	15
U1256	16	17	16	16	16	16	17	17	17	17
U916	12	12	11	12	11	10	13	13	13	13
U940	15	9	11	13	13	12	15	15	14	14
U924	10	10	12	11	8	9	10	10	9	9
U1181	18	16	17	16	20	19	16	16	15	15
U958	11	11	10	10	11	11	8	8	9	9
U891	14	13	15	12	13	12	12	12	12	12

Table B5 (continued)

Isolates	Average diameter of inhibition zone (mm) against									
	L541*	F8/22	L1034	LSS*	B305	B280				
U863	12	13	14	14	12	13				
U944	10	6	10	11	12	13				
U981	12	12	12	14	13	14				
U1003	12	12	12	12	11	11				
U1092	17	16	17	15	17	16				
U1027	18	17	18	18	19	18				
U817	12	10	10	11	10	10				
U885	13	13	14	14	15	12				
U1249	17	18	17	18	18	18				
U1043	17	16	17	17	18	15				

* Selected isolates for further study

II. Data of biochemical identification and characterization of supernatant.

Table B6 Biochemical tests of 2 standard lactobacillus strains by using API50 CHL kit

Biochemical tests	Strains	
	<i>L. casei</i> TISTR 390	<i>L. rhamnosus</i> TISTR 108
CTRL	-	-
GLY	-	-
ERY	-	-
DARA	-	-
LARA	-	-
RIB	+	+
DXYL	-	-
LXYL	-	-
ADO	-	-
MDX	-	-
GAL	+	+
GLU	+	+
FRU	+	+
MNE	+	+
SBE	+	+
RHA	-	+
DUL	-	-
INO	-	-
MAN	+	+
SOR	+	+
MDM	-	-
MDG	-	+
NAG	+	+
AMY	±	+
ARB	±	+
ESC	+	+
SAL	±	+
CEL	±	+
MAL	±	+
LAC	+	+
MEL	-	-
SAC	+	+
TRE	+	+
INU	+	-
MLZ	+	+
RAF	-	-
AMD	-	-
GLYG	-	-
XLT	-	-
GEN	-	-
TUR	+	+

Table B6 (continued)

Biochemical tests	Strains	
	<i>L. casei</i> TISTR 390	<i>L. rhamnosus</i> TISTR 108
LYX	-	-
TAG	+	+
DFUC	-	-
LFUC	-	-
DARL	-	-
LARL	+	-
GNT	-	-
2KG	-	-
5KG	-	-

+, positive reaction; -, negative reaction; ±, doubtful

The species identification was accepted at 80% identity by database.

CTRL, control; GLY, glycerol; ERY, erythritol; DARA, D - arabinose; LARA, L - arabinose; RIB, D - ribose; DXYL, D - xylose; LXYL, L - xylose; ADO, D - adonitol; MDX, methyl - β D - xylopyranoside; GAL, D - galactose; GLU, D - glucose; FRU, D - fructose; MNE, D - mannose, SBE, L - sorbose; RHA, L - rhamnose; DUL, dulcitol; INO, inositol; MAN, D - mannitol; SOR, D - sorbitol; MDM, methyl - α D - mannopyranoside; MDG, methyl - α D - glucopyranoside; NAG, N - acetylglucosamine; AMY, amygdalin; ARB, arbutin; ESC, esculin ferric citrate; SAL, salicin; CEL, D - cellobiose; MAL, D - maltose; LAC, D - lactose (bovine origin); MEL, D - melibiose; SAC, D - saccharose (sucrose); TRE, D - trehalose; INU, inulin; MLZ, D - melezitose; RAF, D - raffinose; AMD, amidon (starch); GLYG, glycogen; XLT, xylitol; GEN, gentiobiose; TUR, D - turanose; LYX, D - lyxose; TAG, D - tagatose; DFUC, D - fucose; LFUC, L - fucose; DARL, D - arabitol; GNT, potassium gluconate; 2KG, potassium 2 - ketogluconate; 5KG, potassium 5 - ketogluconate

Table B7 Biochemical tests of 2 potent antimicrobial producing *Lactobacillus*

Biochemical tests	Strains	
	L541	LSS
CTRL	-	-
GLY	-	+
ERY	-	-
DARA	-	-
LARA	-	+
RIB	+	+
DXYL	-	+
LXYL	-	-
ADO	-	-
MDX	-	-
GAL	+	+
GLU	+	+
FRU	+	+
MNE	+	+

Table B7 (continued)

Biochemical tests	Strains	
	L541	LSS
SBE	-	-
RHA	-	+
DUL	-	+
INO	-	-
MAN	+	+
SOR	+	+
MDM	-	-
MDG	-	+
NAG	+	+
AMY	+	+
ARB	+	+
ESC	+	+
SAL	+	+
CEL	+	+
MAL	+	+
LAC	+	+
MEL	+	+
SAC	+	+
TRE	+	+
INU	-	-
MLZ	-	+
RAF	+	+
AMD	-	-
GLYG	-	-
XLT	-	-
GEN	+	+
TUR	-	±
LYX	-	-
TAG	-	-
DFUC	-	-
LFUC	-	±
DARL	±	+
LARL	-	-
GNT	±	±
2KG	-	-
5KG	-	-

+, positive reaction; -, negative reaction; ±, doubtful

The species identification was accepted at 80% identity by database.

Table B8 The cut - off concentration of hydrogen peroxide

Concentration (mmol/l)	Average diameter of inhibition zone (mm) against							
	<i>E. coli</i> ATCC 25922		U1249		U1104		U874	
10	13	13	10	10	10	9	12	11
9	12	11	11	10	11	11	11	13
8	11	12	9	9	11	11	12	11
7	11	10	10	9	9	9	11	12
6	10	10	10	10	9	9	10	11
5	10	10	9	9	9	8	11	11
4	10	9	9	9	-	-	11	10
3	9	9	9	9	-	-	10	10
2	8	9	-	-	-	-	10	9
1	-	-	-	-	-	-	9	9

-, No zone detected

Table B9 Hydrogen peroxide standard curve

Concentration (μmol/l)	OD ₅₉₅ 1	OD ₅₉₅ 2	OD ₅₉₅ (average)	OD ₅₉₅ test-blank
0.00	0.284	0.282	0.283	0.000
1.95	0.307	0.302	0.305	0.022
3.91	0.327	0.328	0.328	0.045
7.81	0.363	0.43	0.397	0.114
15.62	0.471	0.466	0.469	0.186
31.25	0.67	0.672	0.671	0.388

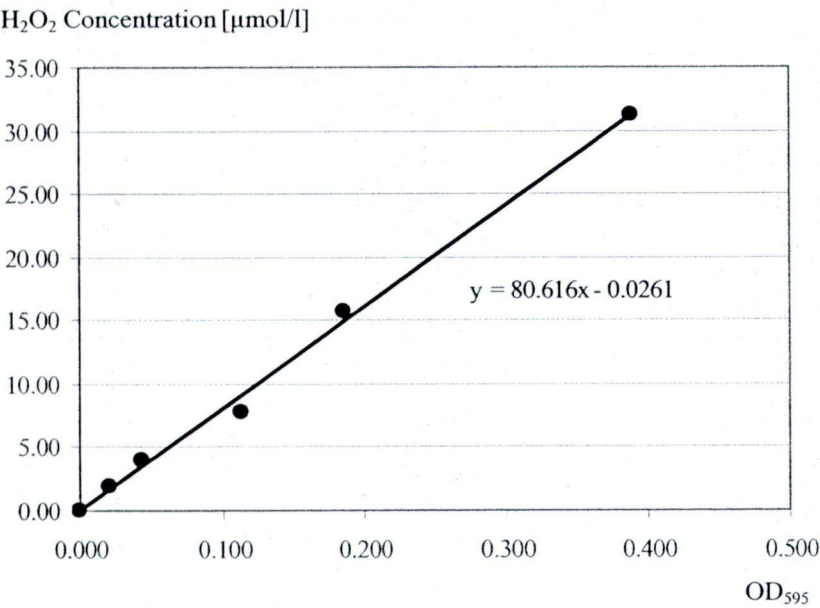


Figure B1 Standard curve of hydrogen peroxide determination

Table B10 Hydrogen peroxide determination of cell - free supernatants

Test	OD ₅₉₅ 1	OD ₅₉₅ 2	OD ₅₉₅ (average)
LSS	0.356	0.350	0.353
L541	0.359	0.357	0.358
MRS	0.304	0.302	0.303

Table B11 The cut - off concentration of lactic acid

Concentration (mmol/l)	Average diameter of inhibition zone (mm) against							
	<i>E. coli</i> ATCC 25922		U1249		U1104		U874	
500	20	21	23	20	17	17	18	15
250	18	17	16	15	12	11	14	12
200	16	16	15	14	11	10	13	12
175	14	15	12	12	10	11	11	10
150	12	13	11	11	9	8	10	10
125	11	11	10	9	9	8	11	9
100	11	10	11	11	-	-	10	9
75	8	9	9	9	-	-	9	8
50	-	-	-	-	-	-	-	-

-, Not zone detected

Table B12 Lactic acid standard curve

Concentration (mmol/l)	OD ₃₄₀ Af	OD ₃₄₀ Ai	OD ₃₄₀ Af-Ai	OD ₃₄₀ test-blank
0.000	0.320	0.281	0.039	0.000
0.019	0.314	0.274	0.040	0.010
0.038	0.344	0.267	0.077	0.039
0.075	0.471	0.272	0.199	0.160
0.150	0.498	0.246	0.252	0.214
0.300	0.766	0.263	0.504	0.465

Ai , initial absorbance; Af , final absorbance

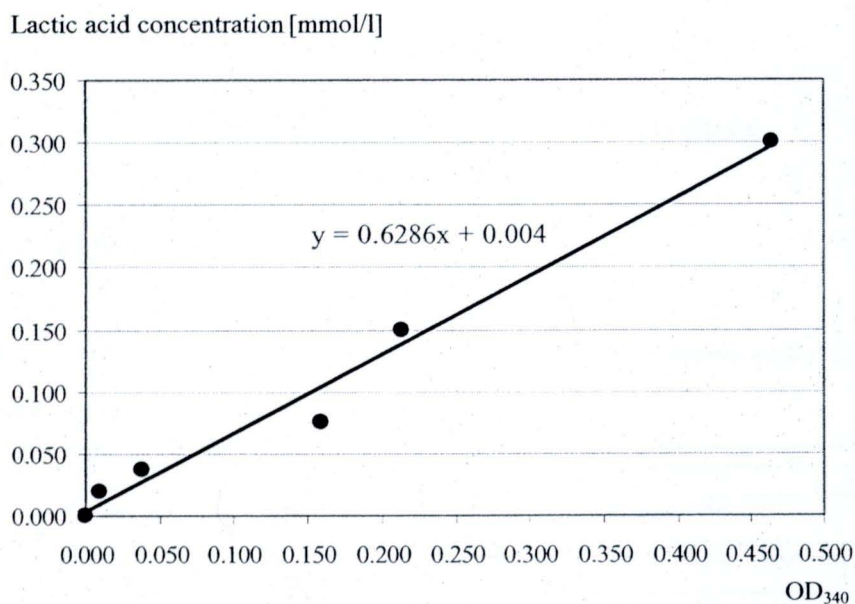


Figure B2 Standard curve of lactic acid determination

Table B13 Lactic acid determination of cell - free supernatants

Test	OD ₃₄₀ Af	OD ₃₄₀ Ai	OD ₃₄₀ Af - Ai*
LSS	1.418	0.353	1.065
L541	1.789	0.331	1.458
MRS	0.972	0.705	0.267

Ai, initial absorbance; Af, final absorbance

* 1/10 dilution

Table B14 Total protein determination of cell - free supernatants

Test	Concentration - read (µg/ml)	Concentration - calculation (µg/ml)	Dilution of sample
LSS	18.5	1,850	undiluted
L541	19.3	1,930	undiluted
B120	20.0	2,000	undiluted
MRS	14.6	1,460	undiluted

Table B15 Total protein determination of crude bacteriocins

Test	Concentration - read (µg/ml)	Concentration - calculation (µg/ml)	Dilution of sample
LSS	4.05	405	1:10
L541	6.86	686	1:10
B120	7.55	755	1:10
MRS	18.6	1860	1:2

APPENDIX C

UROPATHOGENIC ENTEROBACTERIA

II. Data of antimicrobial susceptibility test

Table C1 Antimicrobial susceptibility test of 149 bacterial isolates toward the antibiotic drugs in group A and B antibiotics

Isolates	Average diameter of inhibition zone (mm) due to												
	Group A*			Group B**									
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX
u1/1	-	-	15	21	17	-	13	22	22	28	-	13	-
u1/2	-	-	17	23	15	-	12	17	14	30	-	11	-
u1/3	-	17	16	24	17	35	25	29	27	25	16	-	-
u1/4	-	-	-	23	10	-	-	17	10	27	-	10	-
u1/5	-	14	21	21	19	25	32	32	23	30	18	15	-
u1/6	-	-	17	20	26	-	17	29	22	28	15	10	14
u1/7	-	-	-	24	15	-	-	12	9	30	-	-	-
u1/8	-	-	-	21	14	-	-	15	24	28	-	10	-
u1/9	-	-	18	22	16	-	14	15	24	28	-	-	-
u1/10	-	-	-	20	23	18	25	29	21	29	18	14	29
u1/11	-	-	-	20	18	-	25	31	22	27	19	17	-
u1/12	-	-	19	25	18	-	25	13	13	30	-	-	21
u1/13	-	-	-	22	15	30	11	17	24	29	-	10	-
u1/14	-	18	22	22	18	12	31	33	24	29	25	18	-
u1/15	-	15	24	24	21	-	34	34	24	28	17	14	27
u1/16	-	-	-	23	23	8	-	14	22	33	-	9	-
u1/17	-	-	-	16	16	-	-	15	19	29	-	-	-
u1/18	-	17	22	22	18	33	31	35	26	28	26	19	-
u1/19	-	-	-	21	17	-	14	21	22	31	-	13	-
u1/20	-	-	-	19	14	-	13	18	15	30	-	12	-
u1/21	-	17	20	23	19	10	33	30	25	28	25	16	19
u1/22	-	-	-	20	14	-	-	11	23	29	-	-	14
u1/23	-	-	-	19	13	-	-	11	20	28	-	-	19
u1/24	-	-	-	18	13	8	13	11	20	24	-	-	-
u1/25	-	-	-	20	13	-	-	12	20	29	-	-	20
u1/26	-	-	-	20	13	-	12	15	20	28	-	-	-
u1/27	-	-	-	18	10	-	-	-	8	26	-	-	-
u1/28	-	-	18	19	9	29	22	30	9	24	15	11	-
u1/29	15	-	16	20	10	32	25	30	9	24	21	20	27
u1/30	-	-	-	-	20	-	10	16	8	30	-	-	-
u2/1	-	11	-	20	17	-	30	30	18	27	16	10	-
u2/2	-	-	7	16	13	-	-	11	20	26	-	7	-
u2/3	-	-	7	15	13	-	-	11	12	24	-	8	-

Table C1 (continued)

Isolates	Average diameter of inhibition zone (mm) due to												
	Group A*			Group B**									
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX
u2/4	-	-	-	20	18	-	-	18	11	25	-	-	-
u2/5	-	-	13	18	17	-	15	14	22	27	-	18	-
u2/6	-	-	-	22	18	10	-	11	20	27	-	-	-
u2/7	-	-	-	19	11	-	14	27	7	27	8	13	-
u2/8	-	-	-	20	21	-	-	13	21	27	-	-	-
u3/1	-	-	-	15	14	-	13	18	15	30	-	10	-
u3/2	-	-	17	22	17	-	13	20	23	35	10	12	28
u3/3	-	16	-	16	20	-	26	25	22	26	20	12	-
u3/4	-	-	18	17	17	-	23	26	10	27	12	11	22
u3/5	-	-	-	18	14	-	14	18	15	25	-	11	-
u3/6	-	16	-	19	20	9	26	29	26	28	20	13	-
u3/7	-	17	17	18	20	8	27	30	25	29	21	15	-
u3/8	-	-	-	20	21	-	15	19	27	30	-	-	-
u3/9	-	-	-	20	18	8	-	13	20	28	-	-	-
u3/10	-	11	16	20	17	35	26	28	23	29	17	15	-
u3/11	-	13	-	18	18	-	28	30	26	28	20	13	25
u3/12	-	11	-	20	16	21	26	26	21	26	15	10	-
u3/13	-	-	17	17	13	-	-	-	22	25	-	-	-
u3/14	-	-	-	15	14	-	11	13	21	25	-	-	-
u3/15	-	-	8	17	13	-	10	14	19	27	-	-	-
u4/1	-	15	18	20	18	32	27	28	24	27	20	12	30
u4/2	-	12	-	21	19	-	28	27	25	23	21	12	12
u4/3	-	8	8	20	21	30	21	27	24	26	12	18	-
u4/4	-	-	16	15	10	-	15	21	-	23	-	10	-
u4/5	-	-	7	16	17	-	14	18	22	25	-	10	-
u4/6	-	-	12	21	17	-	-	9	15	31	-	-	22
u4/7	-	-	19	19	18	20	-	12	25	26	-	-	26
u4/8	17	15	8	20	21	8	32	32	26	30	28	26	-
u4/9	-	14	-	20	18	-	30	32	20	27	21	11	-
u4/10	-	-	-	15	13	-	-	-	19	27	-	-	-
u4/11	-	16	12	20	20	12	28	26	25	27	23	17	-
u4/12	-	10	-	19	15	-	26	25	17	29	17	13	-
u4/13	-	16	10	18	18	10	26	26	21	27	21	16	-
u4/14	-	-	-	18	14	-	-	10	16	26	-	-	-
u4/15	-	15	23	22	17	23	28	30	25	28	20	10	-
u4/16	-	20	11	22	23	11	27	30	22	29	25	20	-
u4/17	-	-	21	13	17	21	-	13	22	28	-	-	-
u4/18	-	-	15	17	8	-	-	18	-	25	-	10	-
u5/1	-	8	17	15	13	-	20	22	23	25	10	12	-
u5/2	-	14	20	20	21	31	26	25	17	26	18	18	-
u5/3	-	-	-	19	13	16	-	11	19	24	-	-	-
u5/4	-	-	18	20	15	35	10	15	23	28	-	-	19
u5/5	-	-	-	22	10	-	11	16	-	22	-	-	-
u5/6	-	20	16	16	21	30	29	28	25	25	21	21	25

Table C1 (continued)

Isolates	Average diameter of inhibition zone (mm) due to												
	Group A*			Group B**									
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX
u5/7	-	-	-	18	13	-	-	-	17	25	-	-	-
u5/8	-	17	19	17	20	11	30	26	24	25	22	12	-
u5/9	-	17	9	20	18	-	25	24	24	29	20	14	-
u5/10	-	15	17	16	19	-	28	25	24	22	20	14	-
u5/11	-	-	-	18	17	-	8	-	17	27	-	-	-
u5/12	-	15	-	16	20	-	27	26	23	27	19	12	-
u5/13	-	18	20	20	21	-	34	34	26	32	22	20	-
u5/14	13	11	17	17	20	-	25	26	15	27	20	21	24
u5/15	-	15	18	18	16	38	34	30	26	27	15	11	14
u5/16	-	16	18	16	19	-	25	25	22	25	20	12	-
u5/17	-	11	15	14	17	24	25	23	25	24	13	-	23
u5/18	-	12	19	17	16	-	27	26	20	26	18	14	8
u5/19	-	12	16	17	17	24	25	23	24	25	12	10	26
u5/20	-	-	10	19	17	-	-	-	22	23	-	-	-
u5/21	-	15	8	18	18	27	29	27	24	22	18	12	24
u5/22	-	-	-	17	17	20	-	10	21	25	-	-	-
u5/23	-	-	-	18	11	-	-	-	9	28	-	-	-
u6/1	15	14	18	20	19	-	26	28	22	24	22	25	27
u6/2	-	15	-	22	20	-	21	27	20	30	17	16	-
u6/3	-	14	17	20	16	-	31	28	23	25	17	11	-
u6/4	-	-	-	22	18	-	14	17	18	27	-	-	-
u6/5	-	10	20	21	15	20	28	30	25	27	13	-	21
u6/6	-	12	20	21	18	-	28	26	21	27	18	15	-
u6/7	-	-	11	20	16	-	8	10	21	27	-	-	-
u6/8	-	-	10	19	11	-	-	10	21	25	-	-	-
u6/9	-	-	-	20	18	-	-	10	22	26	-	-	-
u6/10	-	-	9	21	17	-	-	8	21	18	-	-	-
u6/11	-	-	-	20	16	23	15	20	22	26	-	-	-
u6/12	-	-	18	19	19	26	20	23	21	25	12	11	-
u6/13	-	-	8	21	15	-	-	9	20	25	-	-	-
u7/1	-	-	20	21	17	-	-	12	20	27	-	-	24
u7/2	-	-	-	16	17	-	8	13	21	26	-	-	-
u7/3	-	-	17	17	20	-	14	15	24	22	-	8	21
u7/4	-	-	19	22	18	-	14	15	24	26	-	8	-
u7/5	-	-	10	22	18	30	11	11	11	23	-	-	-
u7/6	-	-	-	21	15	-	-	10	21	26	-	-	-
u7/7	-	-	-	18	15	-	8	11	23	28	-	-	-
u7/8	-	-	17	18	20	-	-	12	24	26	-	-	23
u7/9	-	-	-	16	14	-	-	-	15	16	-	-	-
u7/10	-	-	-	17	14	-	10	14	23	25	-	-	-
u7/11	-	-	8	20	18	-	-	10	23	25	-	-	-
u7/12	-	-	21	21	18	-	9	12	21	27	-	-	26
u7/13	-	-	-	17	16	-	9	13	20	22	-	-	-
u7/14	-	-	8	18	12	-	16	22	17	25	-	-	-

Table C1 (continued)

Isolates	Average diameter of inhibition zone (mm) due to												
	Group A*			Group B**									
	AM	CF	GM	AK	AMC	CIP	CTX	FEP	FOX	IPM	MA	PRL	STX
u7/15	-	-	13	17	20	11	15	22	20	25	22	12	-
u7/16	-	-	9	19	14	-	13	14	19	26	-	-	-
u7/17	-	-	20	20	10	-	-	17	-	23	-	-	25
u7/18	-	-	10	21	10	-	-	10	-	25	-	-	-
u7/19	14	-	15	19	10	28	26	26	-	21	20	21	24
u7/20	-	-	20	20	-	30	-	16	9	21	-	-	27
u8/1	-	14	18	16	19	30	28	23	21	22	17	12	-
u8/2	-	16	18	17	17	30	26	26	21	22	21	12	-
u8/3	-	-	-	18	16	-	12	17	21	25	-	-	-
u8/4	-	-	-	20	14	-	-	12	17	14	-	-	-
u8/5	-	11	19	18	17	-	28	27	17	27	12	10	-
u8/6	-	10	-	15	14	-	25	20	14	25	14	10	15
u8/7	-	-	16	18	8	-	21	26	8	22	11	17	-
u8/8	19	12	-	16	22	23	27	30	21	25	25	25	13
u8/9	-	-	16	17	9	22	18	26	12	21	8	-	-
u8/10	-	-	-	17	16	-	11	18	23	25	-	-	-
u8/11	-	-	19	18	17	-	8	15	15	25	-	10	-
u8/12	-	-	17	17	17	12	13	16	22	25	-	8	10
u8/13	-	13	16	19	14	-	28	25	22	25	14	-	-
u8/14	-	-	-	17	11	-	-	9	14	26	-	-	-
u8/15	-	-	8	23	21	-	16	22	28	29	-	10	20
u8/16	-	-	-	18	12	-	-	9	21	24	-	-	-
u8/17	-	-	22	22	20	12	29	20	22	25	-	13	21
u8/18	-	-	-	18	12	-	-	9	19	23	-	-	-
u8/19	-	-	-	16	15	-	27	23	20	25	-	-	-
u9/1	-	25	18	19	21	25	25	25	24	23	26	21	25
u9/2	8	21	16	18	13	26	28	25	25	25	25	20	25
u9/3	-	21	16	17	20	30	28	27	21	26	21	20	25

Bold numbers: Selected isolates for further study

-, No zone detected

*Group A antibiotic; Primary test and Report: AM, Ampicillin 10 µg; CF, Cephalothin 30 µg; GM, Gentamicin 10 µg

**Group B antibiotic; Primary test Report selectively: AK, Amikacin 30 µg; AMC, Amoxicillin/clavulanic acid 30 µg; CIP, Ciprofloxacin 5 µg; CTX, Cefotaxime 30 µg; FEP, Cefepime 30 µg; FOX, Cefoxitin 30 µg; IPM, Imipenem 10 µg; MA, Cefamandole 30 µg; PRL, Piperacillin 100 µg; STX, Trimethoprim/sulfamethoxazole 25 µg

Table C2 Antimicrobial susceptibility test of 149 bacterial isolates toward antibiotic drug in group C and U antibiotics

Isolates	Average diameter of inhibition zone (mm) due to									
	Group C*						Group U**			
	C	CAZ	K	NET	TE	TOB	CAR	F/M	NOR	TMP
u1/1	24	22	19	18	-	18	-	20	-	-
u1/2	-	19	-	19	-	20	-	18	-	-
u1/3	24	25	17	19	24	20	-	18	36	-
u1/4	-	13	-	15	-	13	-	20	-	-
u1/5	28	29	22	26	-	21	-	22	20	-
u1/6	21	18	19	19	-	18	-	20	-	19
u1/7	8	12	-	14	-	13	-	17	-	-
u1/8	21	16	17	13	-	10	-	17	-	-
u1/9	11	21	20	20	-	22	-	17	-	-
u1/10	25	26	-	15	23	15	-	20	-	25
u1/11	-	24	17	14	-	14	-	18	-	-
u1/12	-	24	-	26	-	21	-	20	-	17
u1/13	8	23	18	19	-	12.5	-	17	26	-
u1/14	25	28	21	24	-	18	-	19	10	-
u1/15	27	31	25	25	-	20	-	22	-	28
u1/16	-	18	-	22	-	15	-	25	-	-
u1/17	20	21	-	18	-	8	-	17	-	-
u1/18	27	30	-	28	22	20	-	21	28	-
u1/19	25	13	16	20	-	11	-	25	-	-
u1/20	8	23	10	14	-	10	-	22	-	-
u1/21	26	28	20	25	-	20	-	20	-	25
u1/22	11	-	12	13	-	9	-	12	-	15
u1/23	12	-	12	11	-	8	-	12	-	16
u1/24	26	12	12	14	-	13	-	9	-	-
u1/25	10	-	12	14	-	9	-	13	-	16
u1/26	18	9	13	15	-	12	-	-	-	-
u1/27	-	-	-	8	-	-	-	9	-	-
u1/28	-	24	18	20	21	19	-	15	25	-
u1/29	25	26	20	20	23	19	-	14	23	21
u1/30	22	-	-	-	17	-	-	12	-	18
u2/1	-	26	-	-	18	12	-	14	-	-
u2/2	24	13	11	12	-	9	-	15	-	-
u2/3	22	9	11	19	-	9	-	20	-	-
u2/4	22	7	18	-	17	12	-	18	-	-
u2/5	13	15	14	13	-	13	-	21	-	-
u2/6	-	11	8	17	-	11	-	14	-	-
u2/7	-	15	11	15	-	10	-	23	-	-
u2/8	-	15	15	14	-	11	-	20	-	-
u3/1	-	21	-	15	-	11	-	20	-	-
u3/2	22	23	20	21	-	15	-	20	-	24
u3/3	-	25	14	13	-	11	-	18	-	-
u3/4	14	23	18	20	-	18	-	21	-	20
u3/5	8	20	-	14	-	11	-	20	-	-

Table C2 (continued)

Isolates	Average diameter of inhibition zone (mm) due to									
	Group C*						Group U**			
	C	CAZ	K	NET	TE	TOB	CAR	F/M	NOR	TMP
u3/6	23	25	15	14	-	12	-	20	-	-
u3/7	8	26	18	20	-	19	-	20	-	-
u3/8	20	24	17	15	-	11	-	20	-	-
u3/9	-	16	-	11	-	8	-	16	-	-
u3/10	22	25	19	19	-	18	-	18	26	-
u3/11	23	25	17	15	21	14	-	20	-	25
u3/12	-	22	15	16	-	12	-	17	20	-
u3/13	-	10	10	16	-	8	-	18	-	-
u3/14	12	-	8	12	20	8	-	10	-	-
u3/15	18	21	11	13	-	11	-	-	-	-
u4/1	22	23	18	18	-	17	-	20	26	26
u4/2	8	25	16	17	-	13	-	16	-	15
u4/3	-	21	17	14	23	11	-	20	28	-
u4/4	20	14	10	15	-	9	-	17	-	-
u4/5	-	22	-	16	8	10	-	15	-	-
u4/6	19	11	-	17	10	14	-	16	-	27
u4/7	26	15	20	20	23	17	-	17	18	24
u4/8	21	29	20	19	-	18	23	14	-	-
u4/9	-	26	15	15	-	10	-	19	-	-
u4/10	-	-	10	16	-	10	-	21	-	-
u4/11	26	27	13	21	-	18	-	21	11	-
u4/12	-	25	12	17	-	8	-	18	-	-
u4/13	-	23	19	19	-	13	-	20	8	-
u4/14	20	13	13	16	-	9	-	17	-	24
u4/15	22	22	-	23	-	19	-	16	24	23
u4/16	18	25	25	23	-	19	9	14	11	-
u4/17	12	-	8	12	21	8	-	17	20	-
u4/18	-	-	11	15	-	10	-	12	-	-
u5/1	24	23	11	16	23	8	-	20	-	-
u5/2	-	22	20	21	-	17	-	11	26	-
u5/3	-	9	-	17	-	11	-	16	15	-
u5/4	15	17	-	22	-	18	-	16	32	24
u5/5	23	11	-	18	23	12	-	17	-	-
u5/6	24	28	16	20	22	15	-	20	30	25
u5/7	-	16	13	10	-	-	-	18	-	-
u5/8	25	26	18	17	-	16	-	18	10	-
u5/9	-	24	-	18	-	14	-	22	-	-
u5/10	24	25	18	19	-	16	-	20	-	-
u5/11	-	12	-	27	-	13	-	21	-	-
u5/12	22	25	15	14	-	12	-	19	-	-
u5/13	25	30	21	24	-	18	-	25	-	-
u5/14	11	21	17	17	17	16	20	18	-	19
u5/15	-	26	18	20	25	17	-	21	29	-
u5/16	10	24	19	20	-	16	-	18	-	-

Table C2 (continued)

Isolates	Average diameter of inhibition zone (mm) due to									
	Group C*						Group U**			
	C	CAZ	K	NET	TE	TOB	CAR	F/M	NOR	TMP
u5/17	19	23	17	18	22	15	-	16	20	23
u5/18	-	23	20	20	-	16	-	20	-	-
u5/19	21	22	17	19	22	16	-	18	23	24
u5/20	-	11	-	17	-	13	-	19	-	-
u5/21	23	24	15	16	21	13	-	20	25	22
u5/22	-	9	-	14	-	11	-	13	20	-
u5/23	16	-	-	14	-	9	-	-	-	-
u6/1	24	25	17	18	23	18	22	25	-	26
u6/2	18	25	17	16	-	11	-	21	-	-
u6/3	24	26	18	20	-	17	-	20	-	-
u6/4	-	14	-	16	-	10	-	18	-	-
u6/5	24	28	21	20	-	20	-	21	25	25
u6/6	8	16	20	20	-	18	-	22	-	-
u6/7	12	10	15	18	-	11	-	20	-	-
u6/8	-	10	-	15	-	-	-	21	-	-
u6/9	-	11	-	18	-	14	-	16	-	-
u6/10	21	10	-	18	-	15	-	20	-	-
u6/11	-	22	17	16	-	12	-	16	20	-
u6/12	-	21	19	20	-	15	-	15	21	-
u6/13	-	8	-	20	-	13	-	12	-	-
u7/1	18	10	21	24	-	20	-	19	-	24
u7/2	25	15	-	12	-	-	-	20	-	-
u7/3	-	13	20	20	-	15	-	13	-	25
u7/4	10	21	21	21	-	20	-	18	-	-
u7/5	-	14	20	21	-	15	-	16	26	-
u7/6	-	19	16	13	-	10	-	20	-	-
u7/7	25	11	11	15	-	-	-	21	-	-
u7/8	20	12	18	19	20	17	-	19	-	21
u7/9	-	10	10	13	-	-	-	16	-	-
u7/10	-	14	-	15	-	8	-	20	-	-
u7/11	-	13	17	19	-	13	-	17	-	-
u7/12	14	19	20	22	-	19	-	22	-	24
u7/13	-	13	10	15	21	8	-	18	-	-
u7/14	-	14	17	15	8	14	-	-	12	-
u7/15	-	8	-	14	-	11	-	-	-	15
u7/16	20	10	13	15	-	12	-	-	-	-
u7/17	20	8	19	20	20	17	-	15	-	20
u7/18	-	11	-	18	-	15	-	20	-	-
u7/19	20	24	19	18	-	16	22	15	25	23
u7/20	20	-	21	20	22	17	8	17	25	25
u8/1	-	22	17	21	-	16	-	17	29	-
u8/2	25	21	20	20	-	16	-	18	30	-
u8/3	22	20	-	18	-	11	-	16	-	-
u8/4	-	17	14	13	-	8	-	21	-	-

Table C2 (continued)

Isolates	Average diameter of inhibition zone (mm) due to									
	Group C*						Group U**			
	C	CAZ	K	NET	TE	TOB	CAR	F/M	NOR	TMP
u8/5	17	23	17	21	-	17	-	20	-	-
u8/6	-	23	10	13	-	8	-	18	-	-
u8/7	12	16	11	20	-	11	17	20	-	-
u8/8	22	25	15	16	-	12	23	17	20	-
u8/9	21	16	16	20	21	17	-	16	22	-
u8/10	-	21	-	10	23	10	-	18	-	-
u8/11	20	16	20	21	-	17	-	21	-	-
u8/12	-	23	18	20	-	16	-	20	10	-
u8/13	22	23	18	20	-	15	-	21	-	-
u8/14	-	9	10	15	-	-	-	18	-	-
u8/15	25	25	20	18	-	15	-	23	-	25
u8/16	21	-	11	15	-	8	-	15	-	-
u8/17	8	25	22	24	-	20	-	18	10	-
u8/18	12	7	-	14	-	9	-	-	-	-
u8/19	15	24	10	13	-	-	-	10	-	-
u9/1	24	22	18	20	22	17	8	15	27	21
u9/2	25	24	19	17	21	19	12	16	25	22
u9/3	21	25	18	20	25	19	10	14	25	25

Bold numbers: Selected isolates for further study

-, No zone detected

*Group C antibiotic; Supplementary Report selectively: C, Chloramphenicol 30 µg; CAZ, Ceftazidime 30 µg; K, Kanamycin 30 µg; NET, Netilmicin 30 µg; TE, Tetracycline 30 µg; TOB, Tobramycin 10 µg

**Group U antibiotic; Supplementary for Urine only: CAR, Carbenicillin 100 µg; F/M, Nitrofurantoin 300 µg; NOR, Norfloxacin 10 µg; TMP, Trimethoprim 5 µg

Table C3 ESBL confirmation test of 101 uropathogenic bacterial isolates

Isolates	Average diameter of inhibition zone (mm) due to						
	CAZ/CLA	CAZ	Difference of CAZ		CTX/CLA	CTX	Difference of CTX
			inhibition zone				
u1/1	23	21	2		22	14	8
u1/2	23	20	3		25	12	13
u1/4	20	14	6		17	8	9
u1/7	22	13	9		22	0	22
u1/8	23	15	8		30	8	22
u1/9	25	22	3		27	15	12
u1/11	22	22	0		26	26	0
u1/12	23	23	0		25	25	0
u1/13	23	20	3		25	10	15
u1/16	22	15	7		25	0	25

Table C3 (continued)

Isolates	Average diameter of inhibition zone (mm) due to					
	CAZ/CLA	CAZ	Difference of CAZ inhibition zone	CTX/CLA	CTX	Difference of CTX inhibition zone
u1/17	26	22	4	25	8	17
u1/19	20	14	6	28	14	14
u1/20	23	21	2	23	14	9
u1/22	23	-*	18	25	0	25
u1/23	20	9	11	22	8	14
u1/24	23	13	10	23	14	9
u1/25	19	8	11	20	0	20
u1/26	23	10	13	24	12	12
u1/27	20	-*	15	20	0	20
u1/30	23	10	13	27	11	16
u2/1	29	28	1	30	29	1
u2/2	23	14	9	25	8	17
u2/3	24	9	15	25	0	25
u2/4	22	7	15	23	0	23
u2/6	22	13	9	27	0	27
u2/7	17	16	1	19	15	4
u2/8	23	16	7	25	0	25
u3/1	22	22	0	23	14	9
u3/3	25	24	1	28	26	2
u3/5	21	21	0	22	15	7
u3/6	25	26	-1	25	25	0
u3/8	24	24	0	24	16	8
u3/9	21	15	6	25	0	25
u3/12	22	22	0	24	25	-1
u3/13	20	11	9	22	0	22
u3/14	25	8	17	22	11	11
u3/15	20	20	0	23	12	11
u4/4	22	14	8	17	14	3
u4/5	21	23	-2	22	15	7
u4/6	20	10	10	27	0	27
u4/10	22	-*	17	21	0	21
u4/11	27	27	0	27	28	-1
u4/12	25	25	0	25	25	0
u4/13	24	24	0	27	27	0
u4/14	22	13	9	25	0	25
u4/17	20	-*	15	25	0	25
u4/18	12	-*	7	10	0	10
u5/1	21	21	0	24	21	3
u5/3	19	9	10	20	8	12
u5/5	15	10	5	15	10	5
u5/7	22	15	7	20	0	20
u5/9	26	24	2	25	26	-1
u5/11	23	13	10	22	0	22
u5/12	25	25	0	25	28	-3

Table C3 (continued)

Isolates	Average diameter of inhibition zone (mm) due to					
	CAZ/CLA	CAZ	Difference of CAZ inhibition zone	CTX/CLA	CTX	Difference of CTX inhibition zone
u5/16	25	24	1	25	24	1
u5/18	24	24	0	26	27	-1
u5/20	23	11	12	25	0	25
u5/22	22	8	14	25	0	25
u5/23	20	-*	15	20	0	20
u6/2	23	24	-1	26	20	6
u6/4	21	13	8	25	14	11
u6/6	22	15	7	27	28	-1
u6/7	20	11	9	21	9	12
u6/8	24	9	15	24	0	24
u6/9	23	12	11	24	8	16
u6/10	21	11	10	26	0	26
u6/11	25	23	2	26	16	10
u6/13	21	8	13	22	0	22
u7/1	23	10	13	27	0	27
u7/2	23	15	8	24	10	14
u7/3	20	13	7	25	15	10
u7/4	23	22	1	23	15	8
u7/5	21	14	7	26	12	14
u7/6	23	18	5	24	8	16
u7/7	22	11	11	24	0	24
u7/8	22	13	9	23	8	15
u7/9	21	11	10	22	0	22
u7/10	20	14	6	26	9	17
u7/11	20	12	8	22	0	22
u7/12	22	17	5	20	10	10
u7/13	21	12	9	23	9	14
u7/14	15	15	0	17	16	1
u7/15	21	10	11	27	15	12
u7/16	25	10	15	27	15	12
u7/17	11	-*	6	11	0	11
u7/18	17	10	7	17	8	9
u7/20	11	-*	6	10	0	10
u8/3	27	21	6	27	13	14
u8/4	23	18	5	23	0	23
u8/5	22	22	0	22	26	-4
u8/6	22	22	0	23	24	-1
u8/7	16	16	0	20	20	0
u8/10	22	22	0	22	14	8
u8/11	21	15	6	21	9	12
u8/12	26	23	3	25	14	11
u8/13	21	21	0	24	27	-3
u8/14	23	10	13	25	0	25
u8/16	20	7	13	24	8	16

Table C3 (continued)

Isolates	Average diameter of inhibition zone (mm) due to					
	CAZ/CLA	CAZ	Difference of CAZ	CTX/CLA	CTX	Difference of CTX
			inhibition zone			inhibition zone
u8/17	25	26	-1	28	28	0
u8/18	21	8	13	22	0	22
u8/19	25	25	0	26	26	0

Bold numbers: Selected isolates for further study

-, No zone detected; *, Diameter of antibiotic disc = 5 mm.

Table C4 Classification of 101 uropathogenic bacterial isolates to resistant groups

Isolates	Average diameter of inhibition zone (mm) due to					
	AMC	CAZ	CDP	CTX	FEP	FOX
u1/1	18	22	-	14	20	25
u1/2	15	21	-	13	16	13
u1/4	10	14	-	-	14	10
u1/7	17	13	-	-	11	10
u1/8	15	15	-	9	15	25
u1/9	17	23	-	15	18	23
u1/11	19	23	23	25	30	20
u1/12	19	25	24	26	15	15
u1/13	16	20	-	-	15	24
u1/16	18	16	-	-	12	20
u1/17	17	21	-	-	13	18
u1/19	15	13	-	13	20	22
u1/20	16	22	-	13	17	14
u1/22	15	-	-	-	-	22
u1/23	14	8	-	-	13	20
u1/24	14	12	-	13	10	21
u1/25	15	-	-	-	10	20
u1/26	14	10	-	11	13	19
u1/27	11	-	-	-	-	10
u1/30	21	10	-	11	17	10
u2/1	18	27	22	29	30	19
u2/2	15	14	-	-	13	22
u2/3	14	9	-	-	10	13
u2/4	17	-	-	-	16	12
u2/6	17	12	-	-	12	22
u2/7	13	16	-	14	26	-
u2/8	20	16	-	-	14	22
u3/1	15	22	-	13	20	14
u3/3	18	24	22	25	26	23
u3/5	15	20	-	14	19	14
u3/6	20	27	22	26	28	25
u3/8	22	24	-	15	20	27
u3/9	17	15	-	-	13	21

Table C4 (continued)

Isolates	Average diameter of inhibition zone (mm) due to					
	AMC	CAZ	CDP	CTX	FEP	FOX
u3/13	13	11	-	-	10	21
u3/14	13	-	-	10	12	20
u3/15	13	20	-	11	13	18
u4/4	11	15	-	16	20	-
u4/5	19	23	-	14	19	23
u4/6	19	10	-	-	9	14
u4/10	14	-	-	-	-	18
u4/11	19	26	23	28	27	24
u4/12	15	25	20	25	26	18
u4/13	17	23	21	26	26	20
u4/14	15	13	-	-	10	14
u4/17	17	-	-	-	12	22
u4/18	10	-	-	-	16	-
u5/1	14	22	12	21	23	24
u5/3	12	8	-	-	11	20
u5/5	9	12	-	11	17	-
u5/7	14	15	-	-	-	17
u5/9	18	24	22	25	25	22
u5/11	18	11	-	-	-	18
u5/12	19	25	23	28	28	25
u5/16	18	24	22	23	25	22
u5/18	16	24	20	28	26	22
u5/20	17	11	-	-	-	21
u5/22	15	8	-	-	11	22
u5/23	11	-	-	-	-	10
u6/2	20	25	20	19	27	20
u6/4	17	13	-	13	18	19
u6/6	17	16	22	27	25	21
u6/7	16	9	-	8	12	20
u6/8	11	9	-	-	9	20
u6/9	17	12	-	-	12	20
u6/10	18	11	-	-	10	22
u6/11	17	23	-	15	21	23
u6/13	15	-	-	-	10	21
u7/1	16	11	-	-	13	20
u7/2	16	14	-	10	14	25
u7/3	20	21	-	14	16	23
u7/4	20	21	8	15	15	25
u7/5	18	14	-	12	12	12
u7/6	16	18	-	-	11	22
u7/7	13	11	-	-	11	23
u7/8	19	13	-	-	13	24
u7/9	13	10	-	-	-	14
u7/10	15	15	-	10	15	24
u7/11	17	12	-	-	10	20
u7/12	17	18	-	8	12	20

Table C4 (continued)

Isolates	Average diameter of inhibition zone (mm) due to					
	AMC	CAZ	CDP	CTX	FEP	FOX
u7/13	17	13	-	10	13	23
u7/14	11	14	-	15	22	18
u7/15	20	9	8	14	22	19
u7/16	13	10	-	13	15	18
u7/17	10	-	-	-	17	-
u7/18	10	10	-	-	10	8
u7/20	-	-	-	-	15	10
u8/3	16	22	-	12	15	20
u8/4	15	19	-	-	10	18
u8/5	19	23	20	26	28	18
u8/6	15	23	18	25	20	15
u8/7	9	16	-	20	27	10
u8/10	17	21	-	14	18	24
u8/11	18	16	-	9	15	14
u8/12	19	24	-	14	17	24
u8/13	16	22	22	27	26	24
u8/14	13	10	-	-	10	14
u8/16	13	-	-	-	10	21
u8/17	22	25	25	30	20	22
u8/18	14	8	-	-	10	19
u8/19	20	24	24	27	25	22

Bold numbers: Selected isolates for further study

-, No detected zone

AMC, Amoxicillin/ clavulanic acid 30 µg; CAZ, Ceftazidime 30 µg; CDP, Cepodoxim 10 µg; CTX, Cefotaxime 30 µg; FEP, Cefepime 30 µg; FOX, Cefoxitin 30 µg

Group 1, derepressed AmpC producers (resistant or intermediate susceptible to FOX and resistant to CTX), had a negative FOX - CTX antagonist test and negative ESBL;

Group 2, partially derepressed AmpC isolates were resistant or intermediate susceptible to FOX and resistant to CTX, had a positive FOX - CTX antagonist test and negative ESBL;

Group 3, (partly) derepressed AmpC producers with ESBL production were resistant to FOX and CTX, had a negative FOX - CTX antagonist test and produce ESBL;

Group 4, strains were susceptible to FOX (18) and had a positive ESBL production, a keyhole phenomenon in the double disc synergy test to at least one of the broad - spectrum cepharosporins surrounding the amoxillin/clavulonic acid disc;

Group 5, inducible AmpC producers were susceptible to FOX (18 mm), had a positive FOX - CTX antagonist test and negative ESBL;

NG, No group classified

Table C5 Determination of MIC of the 61 bacterial isolates toward CAZ

Isolates	Titer	MICs of CAZ for each bacterial isolates (mg/ml)
u1/4	4	25.00
u1/7	4	25.00
u1/8	64	1.56
u1/16	>1024	> 0.10
u1/19	>1024	> 0.10
u1/22	4	25.00
u1/23	2	50.00
u1/24	8	12.50
u1/25	16	6.25
u1/26	8	12.50
u1/27	512	0.20
u1/30	128	0.78
u2/2	16	6.25
u2/3	16	6.25
u2/4	2	50.00
u2/6	4	25.00
u2/8	16	6.25
u3/9	16	6.25
u3/13	4	25.00
u3/14	2	50.00
u4/4	64	1.56
u4/6	4	25.00
u4/8	16	6.25
u4/10	4	25.00
u4/14	8	6.25
u4/17	32	3.13
u5/3	4	25.00
u5/5	64	1.56
u5/7	64	1.56
u5/11	32	3.13
u5/20	512	0.20
u5/22	8	12.50
u5/23	8	12.50
u6/4	512	0.20
u6/7	64	1.56
u6/8	2	50.00
u6/9	8	12.50
u6/10	256	0.39
u6/13	16	6.25
u7/1	8	12.50
u7/2	256	0.39
u7/3	>1024	> 0.10
u7/5	>1024	> 0.10
u7/6	512	0.20
u7/7	32	3.13
u7/8	8	12.50
u7/9	8	12.50

Table C5 (continued)

Isolates	Titer	MICs of CAZ for each bacterial isolates (mg/ml)
u7/10	256	0.39
u7/11	16	6.25
u7/12	>1024	> 0.10
u7/13	16	6.25
u7/15	4	25.00
u7/16	16	6.25
u7/17	8	12.50
u7/18	8	12.50
u7/20	32	3.13
u8/4	>1024	> 0.10
u8/11	>1024	> 0.10
u8/14	16	6.25
u8/16	128	0.78
u8/18	4	25.00

Bold numbers : Selected isolates for further study

Table C6 Concentrations of standard β - lactamase and OD at 484 nm by spectrophotometric assay

Final concentrations of standard β - lactamase ($\mu\text{g/ml}$)	Average OD₄₈₄
120	0.490
60	0.487
30	0.465
15	0.461
7.5	0.406
3.75	0.341
1.875	0.284
0.938	0.209
0.469	0.134
0.235	0.087
0.000	0.035

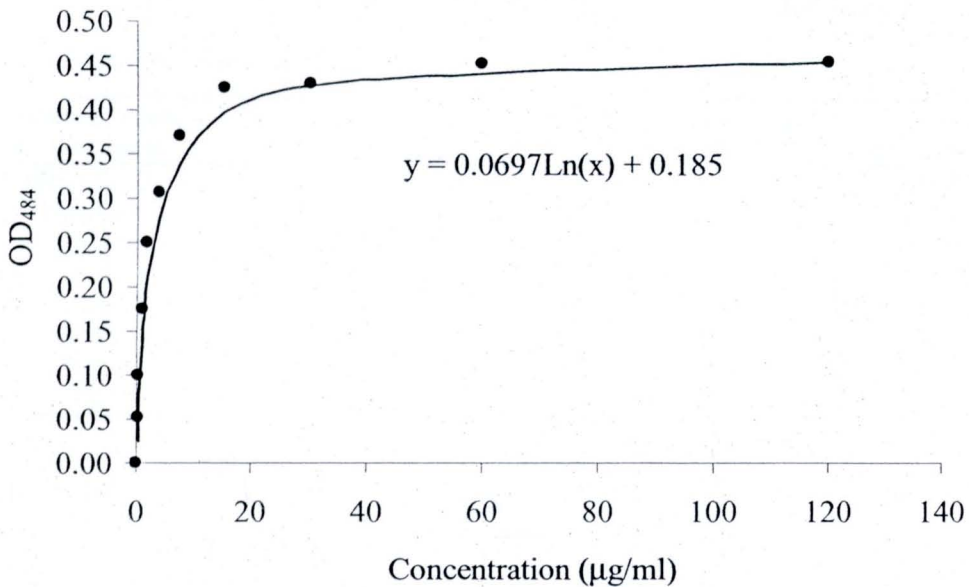


Figure C1 Standard curve of determination of β - lactamase activity

Table C7.1 The minimal concentration of β - lactamase extracted from *E. coli* ATCC 25922 that inhibited the activity of CAZ

Dilution of crude β - lactamase	Average of inhibition zone (mm)		
	Non - induced	CAZ induced	AM induced
PBS pH 7.2	29.0	29.0	29.0
Undilute	29.0	29.0	29.0
d1/2	29.5	29.0	29.0
d1/4	29.0	29.5	29.0
d1/8	29.5	29.0	29.0
d1/16	29.0	29.0	29.5
d1/32	29.0	29.0	29.5
d1/64	29.0	29.5	29.0

Table C7.2 The minimal concentration of β - lactamase extracted from *E. coli* strain u2/4 that inhibited the activity of CAZ

Dilution of crude β - lactamase	Average of inhibition zone (mm)		
	Non - induced	CAZ induced	AM induced
PBS pH 7.2	29.0	29.0	29.0
Undilute	0.0	0.0	0.0
d1/2	0.0	0.0	0.0
d1/4	0.0	10.0	0.0
d1/8	9.0	21.0	8.0
d1/16	20.5	25.0	15.0
d1/32	26.0	26.0	27.5
d1/64	29.0	29.0	29.0

Table C7.3 The minimal concentration of β - lactamase extracted from *E. coli* strain u6/8 that inhibited the activity of CAZ

Dilution of crude β - lactamase	Average of inhibition zone (mm)		
	Non - induced	CAZ induced	AM induced
PBS pH 7.2	29.0	29.0	29.0
Undilute	0.0	0.0	7.0
d1/2	0.0	0.0	22.0
d1/4	7.0	6.5	25.0
d1/8	9.5	19.0	27.0
d1/16	24.0	25.0	28.0
d1/32	25.5	26.0	28.5
d1/64	29.0	29.0	29.0

Table C7.4 The minimal concentration of β - lactamase extracted from *K. pneumoniae* strain u1/23 that inhibited the activity of CAZ

Dilution of crude β - lactamase	Average of inhibition zone (mm)		
	Non - induced	CAZ induced	AM induced
PBS pH 7.2	29.0	29.0	29.0
Undilute	0.0	0.0	0.0
d1/2	25.5	12.0	27.0
d1/4	29.0	27.0	29.0
d1/8	29.0	29.0	29.0
d1/16	29.0	29.0	29.5
d1/32	29.0	29.0	29.0
d1/64	29.0	29.0	29.5

Table C7.5 The minimal concentration of β - lactamase extracted from *K. pneumoniae* strain u3/14 that inhibited the activity of CAZ

Dilution of crude β - lactamase	Average of inhibition zone (mm)		
	Non - induced	CAZ induced	AM induced
PBS pH 7.2	29.0	29.0	29.0
Undilute	0.0	11.0	0.0
d1/2	0.0	25.0	0.0
d1/4	0.0	27.5	21.5
d1/8	7.0	28.0	28.0
d1/16	16.0	29.0	29.0
d1/32	26.0	29.0	29.5
d1/64	29.0	29.5	29.0

APPENDIX D THE EFFECT OF LACTOBACILLUS CULTURES AND THEIR ACTIVE AGENTS

Table D1 Colony count of the uropathogenic bacteria co - cultured with each *Lactobacillus* at 0, 6, 12 hrs of cultured time

Co - culture condition (cells)	Average of the uropathogenic bacteria count (x 10 ³ CFU/ml)																		
	<i>E. coli</i>								<i>K. pneumoniae</i>										
	ATCC 25922				strain u2/4				strain u6/8				strain u1/23				strain u3/14		
	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	
No -	16.5	140	2150	11	235	2000	11000	130	1550	20.5	200	1500	12.5	120	1900				
Lactobacilli																			
L541	18.5	0	0	9.5	0	0	9500	0	0	18.5	0	0	13	0	0				
LSS	26.5	0	0	14	0	0	11000	0	0	9	0	0	11	0	0				
B120	19.5	120	245	19	240	235	13	100	185	16	110	110	12	70	290				

Table D2 Colony count of the uropathogenic bacteria co - cultured with crude bacteriocin of each *Lactobacillus* at 0, 6, 12 hrs of cultured time

Co culture condition (Bacteriocin)	Average of the uropathogenic bacteria count (x 10 ³ CFU/ml)													
	<i>E. coli</i>						<i>K. pneumoniae</i>							
	ATCC 25922						strain u6/8				strain u1/23			
	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	6 hr	12 hr	0 hr	12 hr
No - bacteriocin	20	265	130	15	230	2400	21.5	150	1150	18	300	1300	13	1200
L541	15.5	0	0	20.5	0	0	20.5	0	0	23.5	0	0	23.5	0
LSS	16	0	0	35	0	0	13.5	0	0	20.5	0	0	21.5	0
B120	13	22	21.5	13	290	275	11.5	165	325	23	230	215	180	405
Plantaricin	12	0	0	15.5	0	0	20	0	0	13	0	0	19	0
Imipenem	11.5	0	0	22.5	0	0	18	0	0	24.5	0	0	15	0

Table D3 The optical density inhibition of bacteriocin toward extracted β - lactamase of the uropathogenic bacteria by spectrophotometric assay

Condition	OD ₄₈₆ of inhibition of bacteriocin toward extracted β - lactamase with each induced											
	<i>E. coli</i>						<i>K. pneumoniae</i>					
	ATCC 25922						strain u1/23					
	Non	CAZ	AM	Non	CAZ	AM	Non	CAZ	AM	Non	CAZ	AM
No - bacteriocin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L541	0.001	0.006	0.015	0.025	0.027	0.023	0.014	0.041	0.025	0.001	0.027	0.016
LSS	0.000	0.006	0.013	0.018	0.020	0.013	0.005	0.029	0.012	0.002	0.012	0.017
B120	0.002	0.003	0.002	0.003	0.022	0.000	0.000	0.010	0.016	0.000	0.002	0.003
Plantaricin	0.010	0.011	0.012	0.017	0.054	0.056	0.015	0.046	0.069	0.001	0.041	0.070
Clavulanate	0.012	0.014	0.023	0.039	0.201	0.159	0.026	0.142	0.119	0.004	0.171	0.230

0.005

0.002

0.000

0.035

0.119

CURRICULUM VITAE

NAME Miss. Krittapak Horcharoen

DATE OF BIRTH February 14, 1983



EDUCATION

2005 Bachelor of Science (Medical Technology), Faculty of
Associated Medical Sciences, Chiang Mai University, Chiang
Mai, Thailand

PUBLICATIONS AND PRESENTATION

Horcharoen K, Sookkhee S. Antimicrobial activity of *Lactobacillus* against the urovaginal pathogenic bacteria. In: Noparatnaraporn N, editors. STT.33. Proceedings of the 33rd Congress on Science and Technology of Thailand; Nakhon Si- Thammarat, Thailand; 2007. p B2_B0109

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