IX. APPENDICES

APPENDIX A

CHEMICAL AND MEDIA PREPARATIONS

1. Acetate buffer, 25 mM

	: per litre
Sodium acetate (Merck™; Merck KGaA)	3.4 g
Distilled water	900 ml

Adjust to pH 5.0 with glacial acetic acid and make the volume up to 1 liter with distilled water. Filter through a 0.45 µm membrane filter (Minisart®; Sartorius AG) and store at 4°C.

2. Acetic acid – methanol solution

	: per litre
Methanol (Merck™; Merck KGaA)	400 ml
Glacial acetic acid (Merck™; Merck KGaA)	100 ml
Distilled water	500 ml

Mix together and then store the solution at room temperature.

3. Acrylamide/Bis (30% T, 2.67% C)

	: per litre
Acrylamide (BIO-RAD®; BIO-RAD Laboratory)	292 g
N′N′-bis-methylene-acrylamide (BIO-RAD®; BIO-RAD Laboratory)	8 g
Distilled water	1 L

Mix together. Filter through a 0.45 µm membrane filter and store at 4°C in the dark condition.

4. Agarose sealing solution, 0.5%

	: per 100 ml
Agarose powder (BIO-RAD®; BIO-RAD Laboratory)	5 g
10X SDS electrophoresis buffer	10 ml
Bromophenol blue (PlusOne™; Amersham biosciences)	a few grains
Deionized water	90 ml

Suspend all ingredients in deionized water. Heat by a microwave oven on low power until the agarose is completely dissolved.

5. Ammonium persulfate solution, 10% freshly prepared

	: per 1 ml
Ammonium persulfate (BIO-RAD®; BIO-RAD Laboratory)	0.1 g
Deionized water	1 ml

Mix together

6. Anaerobic blood agar

: per litre

6.1 Prepared lysed-red blood cells supplemented with menadione

Menadione solution (Konakion®; Roche: Basel, Switzerland)	1 g
Packed red cells	40 ml
Sterilized distilled water	40 ml

Add the packed red cells into the sterilized distilled water.

6.2 Melted blood agar

Blood agar base (Merck™; Merck KGaA)	40 g
Distilled water	920 ml

Suspend the agar base in distilled water. Autoclave at 121°C for 15 min. Cool to 45-50°C and then add lysed red blood cells.

7. Brain-Heart Infusion broth (BHI)

	: per litre
Brain-Heart Infusion (Bacto™; Becton Dickinson)	37 g
Distilled water	1 L

Suspend the medium powder in distilled water. Autoclave at 121°C for 15 min.

8. Bromphenol blue solution, 0.1% w/v

	: per 10 ml
Bromphenol blue (Merck™; Merck KGaA)	0.1 g
Distilled water	10 ml

Mix together and store the solution at room temperature.

9. Coomassie Brillient Blue G250 stock solution, 5% w/v

	: per litre
Coomassie Brillient Blue G250 (Coomassie™; Amersham Biosciences)	50 g
Distilled water	1 L

Mix the solution for a few minutes to disperse the dye. Store the solution at room temperature.

10. Coomassie Brillient Blue G250 working solution, 0.1% w/v

	: per litre
5% w/v Coomassie Brillient Blue G250 stock solution	10 ml
Distilled water	990 ml

Mix together and store at room temperature.

11. Coomassie Brillient Blue R250 stock solution, 0.1% w/v

	: per litre
Coomassie Brillient Blue R250 (BIO-RAD®; BIO-RAD Laboratory)	1 g
Acetic acid – methanol solution	1 L

Mix the solution for a few minutes to disperse the dye. Store the solution at room temperature.

12. Equilibration buffer, pH 8.3

	: per litre
Urea (PlusOne™; Amersham Biosciences)	360 g
Sodium dodecyl sulfate (AMRESCO®; AMRESCO)	31.25 g
Glycerol (PlusOne™; Amersham biosciences)	150 ml
0.05 M Tris-HCl buffer, pH 8.8	16.7 ml
Deionized water	1 L

Mix together and store the solution at room temperature. Urea and SDS in final concentration were 6M and 2% w/v, respectively.

13. De Mann-Rogosa-Sharpe agar (MRS)

	: per litre
De Mann-Rogosa-Sharpe agar (Bacto™; Becton Dickinson)	70 g
Distilled water	1 L

Suspend the medium powder in 1 liter of distilled water. Autoclave at 121°C for 15 min.

14. De Mann-Rogosa-Sharpe broth (MRS)

	: per litre
De Mann-Rogosa-Sharpe broth (Merck™; Merck KGaA)	55 g
Distilled water	1 L

Suspend the medium powder in 1 liter of distilled water. Autoclave at 121°C for 15 min.

15. MRS-Glycerol medium

	: per litre
De Mann-Rogosa-Sharpe broth	55 g
Distilled water	1 L
Glycerol (Merck™; Merck KGaA)	100 ml

Suspend the medium powder in 1 liter of distilled water. Heat to boiling until the granulated agar was completely dissolved. Add 100 ml of glycerol and autoclave at 121°C for 15 min.

16. Modified MRS broth (mMRS)

	: per litre
Yeast extract (BBL™; Becton Dickinson)	5 g
Dextrose (Merck™; Merck KGaA)	20 g
Tween 80 (Tween®80; Fisher Scientific: Fair Lawn, NJ, USA)	1 g
Ammonium citrate (Redel-de HaenAG; Seelze, Germany)	2 g
Sodium acetate (Merck™; Merck KGaA)	5 g
Magnesium sulfate (Redel-de HaenAG)	0.1 g
Manganese sulfate (Redel-de HaenAG)	0.05 g
Dipotassium phosphate (Merck™; Merck KGaA)	15 g

Mix with order in 900 ml of distilled water until all chemicals are completely dissolved. Add the distilled water up to 1 liter. Autoclave at 121°C for 15 min.

17. Phosphate buffer saline (PBS), 10X

	: per litre
Sodium chloride (Merck™; Merck KGaA)	80 g
Potassium chloride (Merck™; Merck KGaA)	2 g
Disodium hydrogen phosphate (Anla R®; BDH: Dorset, London, England)	14.4 g
Potassium dihydrogen phosphate (Merck™; Merck KGaA)	2.4 g
Distilled water	1 L

Mix together in 900 ml of distilled water. Adjust to pH 7.2 by using 1M NaOH or 1 M HCl. Add the distilled water up to 1 liter. Filter through a 0.45 μm membrane filter. Store the solution at room temperature.

18. Phosphate buffer saline (PBS), 1X

	: per litre
10X Phosphate buffer saline	100 ml
Distilled water	900 ml

Store the solution at room temperature.

19. Rehydration buffer, pH 8.3

	: per litre
Urea (PlusOne™; Amersham Biosciences)	750 g
CHAPS (PlusOne™; Amersham Biosciences)	31.25 g
IPG buffer (Immobiline™ IPG buffer; Amersham Biosciences)	500 µl
Bromphenol blue (PlusOne™; Amersham Biosciences)	a few grains
Deionized water	16 ml

Mix together, divide the solution into 2.5 ml aliquots and then store at -20°C.

Rehydration buffer kept in aliquot	2.5 ml
10 mM Dithiothreitol (DTT; PlusOne™ Amersham Biosciences)	7 mg

Mix together and freshly prepare. In conclusion, urea, CHAPS and IPG buffer were 8M, 2% (w/v) and 2% (v/v) concentration, respectively.

20. Resolving polyacrylamide gel, 12%

	: per 2 gels
1.5 M Tris-HCl pH 8.8	2.5 ml
30% acrylamide /Bis (BIO-RAD®; BIO-RAD Laboratory)	4 ml
10% (w/v) Sodium dodecyl sulfate	50 µl
10% (w/v) Ammonium persulphate	50 µl
TEMED (BIO-RAD®; BIO-RAD Laboratory)	5 µl
Deionized water	3.4 ml

Mix together and swirl gently to initiate polymerization.

21. Rogosa SL agar

	: per litre
Rogosa SL agar (Bacto™; Becton Dickinson)	75 g
Distilled water	1 L
Glacial acetic acid (Merck™; Merck KGaA)	1.3 ml

Suspend the medium powder in 1 liter of distilled water. Heat to boiling until the granulated agar is completely dissolved. Adjust pH to 5.4 by glacial acetic acid 1.3 ml. Do not autoclave.

22. SDS electrophoresis buffer, 10X

	: per litre
Tris base (Merck™; Merck KGaA)	30.3 g
Glycine (Merck™; Merck KGaA)	144 g
Sodium dodecyl sulfate (AMRESCO®; AMRESCO)	10 g
Deionized water	1 L

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

23. SDS electrophoresis buffer, 1X

	: per litre
10X SDS electrophoresis buffer	100 ml
Deionized water	900 ml

Mix together and freshly prepare for each electrophoresis run.

24. SDS-reducing buffer, 5X

	: per 15 ml
10% (w/v) Sodium dodecyl sulfate	2 ml
Glycerol (Merck™; Merck KGaA)	4 ml
0.1 % (w/v) Bromphenol blue	120 µl
1 M Tris-HCl, pH 6.8	3 ml
β-Mercaptoethanol (BIO-RAD®; BIO-RAD Laboratory)	1.2 ml
Deionized water	4 ml

Mix together and store at room temperature. Dilute the sample at least 1:2 with SDS-reducing buffer and heat at 95°C for 10 min.

25. Skim milk agar, 4%

	: per litre
25.1 Skim milk solution, 8%	
Skim milk powder (Bacto™; Becton Dickinson)	40 g
Distilled water	500 ml

Suspend the milk powder in 500 ml of distilled water. Autoclave at 110°C for 15 min.

25.2 Melted agar, 2.4%

Agar powder (Bacto™ Agar; Becton Dickinson)	12 g
Distilled water	500 ml

Suspend the agar powder in 500 ml of distilled water. Autoclave at 121°C for 15 min and then cool to 45-50°C. Add the sterilized skim milk solution.

26. Sodium dodecyl sulfate solution (SDS), 10% w/v

	: per litre
Sodium dodecyl sulfate (AMRESCO®; AMRESCO)	100 g
Deionized water	900 ml

Dissolve the sodium dodecyl sulphate in 900 ml of deionized water with gentle stirring and make to 1,000 ml with deionized water. Store at room temperature.

27. Stacking polyacrylamide gel, 4%

	: per 2 gels
0.5 M Tris-HCl pH 6.8	1.25 ml
30% acrylamide /Bis (BIO-RAD®; BIO-RAD Laboratory)	0.65 ml
10% (w/v) Sodium dodecyl sulfate	50 µl
10% (w/v) Ammonium persulphate	50 µl
TEMED (BIO-RAD®; BIO-RAD Laboratory)	5 µl
Deionized water	3.05 ml

Mix together, swirl gently to initiate polymerization.

28. Tris-HCl buffer, 0.05 M pH 8.8

	: per litre
Tris base (Merck™; Merck KGaA)	6.05 g
Deionized water	900 ml

Mix together, and then adjust the solution to pH 8.8 with 5N HCl. Make total volume up to 1 liter with deionized water and store at 4°C.

29. Tris-HCl buffer, 0.5 M pH 6.8

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

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

30. Tris-HCl buffer, 1.0 M pH 6.8

	: per litre
Tris base (Merck™; Merck KGaA)	121 g
Deionized water	900 ml

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

31. Tris-HCl buffer, 1.5 M pH 8.8

	: per litre
Tris base (Merck™; Merck KGaA)	181.5 g
Deionized water	900 ml

Mix together, and then adjust the solution to pH 8.8 with 5N HCl. Make total volume up to 1 liter with deionized water and store at 4°C.

32. Tris-HCl buffer, 20 mM pH 8.0

	: per litre
Tris base (Merck™; Merck KGaA)	2.42 g
Reverse osmosis water	900 ml

Mix together, and then adjust the solution was adjusted to pH 8.0 with 5N HCl. Make total volume up to 1 liter with reverse osmosis water. Filter through a 0.45 µm membrane filter before store at 4°C.

33. Tris-HCl buffer, 20 mM pH 8.0 containing 1.0 M NaCl

	: per litre
Tris base (Merck™; Merck KGaA)	2.42 g
NaCl (Merck™; Merck KGaA)	58.44 g
Reverse osmosis water	900 ml

Mix together, and then adjust the solution to pH 8.0 with 5N HCl. Make total volume up to 1 liter with reverse osmosis water. Filter through a 0.45 μm membrane filter before store at 4°C.

34. Tryptic soy broth (TSB)

	: per litre
Tryptic soy broth (Bacto™; Becton Dickinson)	30 g
Distilled water	1 L

Suspend the medium powder in 1 liter of distilled water. Autoclave at 121°C for 15 min.

APPENDIX B

LACTIC ACID BACTERIA

Table B1 The antimicrobial activity of the potent antimicrobial producing lactic acid bacteria against various tested bacteria. Results were expressed as the average diameter of inhibition zone in mm (Data from Formerly Special Problem Report of Ms. Thidarat Suksangpleng)

Tested stains	Diameter of inhibition zone (mm) against tested bacteria		
	B85/4	**B282**	**B63/8**
S. aureus ATCC 25923	8.0	9.5	9.5
S. lutea ATCC 9341	13.5	14.0	11.0
E. coli ATCC 25922	9.0	8.0	9.5
B. subtilis ATCC 6633	13.0	14.0	10.0
Ps. aeruginosa ATCC 27853	14.5	12.0	13.5
S. epidermidis	11.0	14.5	9.0
K. pneumoniae	9.0	10.5	9.0
B. cereus	13.0	13.0	13.5
E. faecalis	9.5	11.5	9.5
S. mitis	14.0	15.5	20.0
S. mutans	17.5	18.5	16.0
P. gingivalis W50	25.0	24.5	18.5

Table B2 Biochemical identification table of some lactobacilli according to API 50 CHL V5.0 biochemical identification software

Biochemical tests	Strains			
	L. fermentum	*L. paracasei* subsp. *paracasei*	*L. plantarum*	*L. rhamnosus*
CTRL	0	0	0	0
GLY	0	20	1	42
ERY	0	0	0	0
DARA	0	1	0	9
LARA	31	0	74	8
RIB	95	100	92	100
DXYL	50	0	2	0
LXYL	0	0	0	0
ADO	0	13	0	0
MDX	1	0	0	0
GAL	92	100	92	100
GLU	100	100	100	100
FRU	80	100	100	100
MNE	50	100	100	100
SBE	0	53	2	92
RHA	0	1	33	100
DUL	0	13	0	14
INO	0	6	0	42
MAN	0	100	99	100
SOR	0	86	78	100
MDM	0	0	55	7
MDG	6	46	33	85
NAG	6	100	100	100
AMY	2	98	94	99
ARB	0	100	99	100
ESC	5	99	99	85
SAL	0	100	99	100
CEL	1	93	99	100
MAL	95	99	100	99
LAC	87	99	99	100
MEL	90	0	94	9
SAC	86	93	88	71
TRE	16	99	96	99
INU	0	26	0	0
MLZ	0	93	92	99
RAF	76	0	74	7
AMD	1	0	7	0
GLYG	0	6	7	7
XLT	0	0	0	0
GEN	1	80	98	85

Table B2 (continued)

Biochemical tests	Strains			
	L. fermentum	*L. paracasei* subsp. *paracasei*	*L. plantarum*	*L. rhamnosus*
TUR	4	80	62	92
LYX	0	20	0	42
TAG	0	100	7	99
DFUC	0	0	0	0
LFUC	0	1	0	7
DARL	0	0	36	0
LARL	0	40	0	7
GNT	73	93	62	85
2KG	1	0	0	0
5KG	1	0	0	0

The number represent % of positive reactions after 48 hours at 37°C

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-ßD-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-αD-mannopyranoside; MDG, methyl-αD-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 B3 Biochemical reactions of 3 potent antimicrobial producing lactic acid bacteria compared with 4 standard strains of lactobacilli according to API 50 CHL kit

Biochemical tests	Strains						
	L. fermentum **TISTR 055**	***L. casei*** **TISTR 330**	***L. plantarum*** **TISTR 541**	***L. rhamnosus*** **TISTR 108**	***L. paracasei*** **subsp. *paracasei* B85/4**	***L. paracasei*** **subsp. *paracasei* B282**	***L. paracasei*** **subsp. *paracasei* B63/8**
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	±	-	+	-	±	±	±

Table B3 (continued)

Biochemical tests	Strains						
	L. fermentum TISTR 055	*L. casei* TISTR 330	*L. plantarum* TISTR 541	*L. rhamnosus* TISTR 108	*L. paracasei* subsp. *paracasei* B85/4	*L. paracasei* subsp. *paracasei* B282	*L. paracasei* subsp. *paracasei* B63/8
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 B4 Biochemical reactions of 3 potent antimicrobial producing lactic acid bacteria compared with 4 standard strains of lactobacilli according to BBL Crystal ID kit

Biochemical tests	Strains					
	L. casei TISTR 390	*L. fermentum* TISTR 055	*L. rhamnosus* TISTR 108	*L. paracasei* subsp. *paracasei* B85/4	*L. paracasei* subsp. *paracasei* B282	*L. paracasei* subsp. *paracasei* B63/8
FCT	-	-	-	-	-	-
FAR	+	+	-	+	+	+
FHI	+	+	-	+	+	-
FAM	-	-	-	-	-	-
FSE	+	-	+	+	+	+
FIS	+	-	+	+	+	+
FBM	-	-	-	-	-	-
FGL	+	-	+	+	+	+
FAL	+	+	+	+	+	+
FGA	-	-	-	-	-	-
FPY	+	-	+	+	+	+
FLY	+	+	+	+	+	+
FME	+	+	+	+	+	+
FCE	-	-	-	-	-	-
FXY	-	-	-	-	-	-
FPH	+	+	+	+	+	+
FLE	+	+	+	+	+	+
FSC	-	-	-	-	-	-
DIS	-	+	-	+	+	+
FUR	+	+	+	+	+	+
PYO	+	+	+	+	+	+
AGA	-	+	-	-	-	-
NPG	+	+	+	+	+	+
PHO	-	-	-	-	-	-
AGL	-	+	+	-	+	+
NAG	+	-	-	+	+	+
PRO	-	-	-	+	-	-
AFU	-	-	-	+	+	-
BGL	+	-	+	+	+	+
ALA	+	-	+	+	+	+

+, positive reaction; -, negative reaction

FCL, fluorescent negative control; FAR, L-arginine-AMC; FHI, L-histidine-AMC; FAM, 4MU-α-D-mannoside; FSE, L-serine-AMC; FIS, L-isoleucine-AMC; FBM, 4MU-ß-D-mannoside; FGL, glycine-AMC; FAL, L-alanine-AMC; FGA, 4MU-N-acetyl-ß-D-galactosaminide; FPY, L-pyroglutamic acid-AMC; FLY, L-lysine-AMC; FME, L-methionine-AMC; FCE, 4MU-ß-D-cellobiopyranoside; FXY, 4MU-ß-D-xylosideFPH, L-phenylalanine-AMC; FLE, L-leucine-AMC; FSC, escosyl; DIS, disaccharide; FUR, furanose; PYO, pyranose; AGA, p-nitrophenyl-α-D-galactoside; NPG, p-nitrophenyl-ß-D-galactoside; PHO, p-nitrophenyl-phosphate; AGL, p-nitrophenyl-α-D-glucoside; NAG, p-nitrophenyl-N-acetyl-N-acetyl-glucosaminide; PRO, L-proline-p-nitroanilide; AFU, p-nitrophenyl-α-L-fucoside; BGL, p-nitrophenyl-ß-D-glucoside; ALA, L-alanyl-L-alanine-p-nitroanilide

Table B5 The standard zone diameters of *S. aureus* ATCC 25923 provided by Oxoid®

Antimicrobial agents	Disc content (µg)	Zone diameter (mm)		
		R	I	S
Penicillin				
Penicillin G	10	≤ 26	27-36	≤ 37
Ampicillin	10	≤ 27	28-34	≤ 35
Cephalosporins				
Cephalothin	30	≤ 29	30-36	≤ 37
Ceftazidime	30	≤ 16	17-19	≤ 20
Aminoglycosides				
Gentamicin	10	≤ 19	20-26	≤ 27
Macrolides				
Erythromycin	15	≤ 22	23-29	≤ 30
Quinolones				
Norfloxacin	10	≤ 17	18-27	≤ 28
Others				
Chloramphenicol	30	≤ 19	20-25	≤ 26
Clindamycin	2	≤ 24	25-29	≤ 30

R, resistant; I, intermediate; S, susceptible

Table B6 The zone diameters in millimeter of the potent antimicrobial producing lactobacilli to antimicrobial agents

Antibiotics	*Lactobacillus paracasei* subsp. *paracasei*		
	L.paracasei subsp. *paracasei* B85/4	*L.paracasei* subsp. *paracasei* B282	*L.paracasei* subsp. *paracasei* B63/8
Penicillin G	26	26	26
Ampicillin	23	23	23
Cephalothin	17	19	18
Ceptazidime	14	16	13
Gentamicin	15	15	17
Erythromycin	31	35	32
Norfloxacin	18	20	18
Chloramphenicol	24	20	28
Clindamycin	24	27	30

Table B7 The calculations of growth curve of *L. paracasei* subsp. *paracasei* B85/4 according to their OD_{600} and viable counts in duplicate

Incubation time (hr)	OD600	Mean	Count (dilution)	CFU/ml	Mean
0	0.006 0.006	0.006	9, 7 (10^{-2}) 10, 8 (10^{-2})	4.00×10^4 4.55×10^4	4.25×10^4
1	0.004 0.006	0.005	ND	ND	ND
2	0.010 0.008	0.009	ND	ND	ND
3	0.006 0.010	0.008	ND	ND	ND
4	0.012 0.010	0.011	ND	ND	ND
5	0.017 0.016	0.0165	ND	ND	ND
6	0.015 0.015	0.015	ND	ND	ND
7	0.026 0.024	0.025	ND	ND	ND
8	0.024 0.024	0.024	ND	ND	ND
9	0.031 0.028	0.029	ND	ND	ND
10	0.031 0.030	0.0305	ND	ND	ND
11	0.047 0.046	0.0465	ND	ND	ND
12	0.055 0.055	0.055	ND	ND	ND
13	0.064 0.061	0.062	ND	ND	ND
14	0.136 0.130	0.013	ND	ND	ND
15	0.234 0.229	0.232	ND	ND	ND
16	0.526 0.531	0.523	ND	ND	ND
17	0.726 0.758	0.742	ND	ND	ND
18	1.088 1.084	1.086	33, 37 (10^{-7}) 22, 35 (10^{-7})	1.75×10^{10} 1.425×10^{10}	1.58×10^{10}
19	1.486 1.492	1.489	ND	ND	ND
20	1.672 1.652	1.662	ND	ND	ND
21	1.692 1.693	1.6925	49, 63 (10^{-9}) 53, 54 (10^{-9})	2.80×10^{12} 2.67×10^{12}	2.73×10^{12}
22	1.680 1.681	1.6805	43, 44 (10^{-9}) 49, 51 (10^{-9})	2.17×10^{12} 2.50×10^{12}	2.33×10^{12}
23	1.468 1.544	1.506	53, 51 (10^{-9}) 54, 62 (10^{-9})	2.60×10^{12} 2.90×10^{12}	2.75×10^{12}
24	1.400 1.452	1.426	ND	ND	ND

ND, not done

Table B7 (continued)

Incubation time (hr)	OD600	Mean	Count (dilution)	CFU/ml	Mean
25	1.414 1.401	1.4075	ND	ND	ND
26	1.476 1.474	1.475	ND	ND	ND
27	1.420 1.419	1.495	ND	ND	ND
28	1.430 1.428	1.429	ND	ND	ND
29	1.416 1.404	1.410	ND	ND	ND
30	1.422 1.428	1.426	ND	ND	ND
32	1.384 1.244	1.314	ND	ND	ND
34	0.874 0.826	0.850	72, 68 (10^{-6}) 69, 71 (10^{-6})	3.50×10^9 3.50×10^9	3.50×10^9
36	0.836 0.834	0.835	ND	ND	ND
38	0.752 0.700	0.726	ND	ND	ND
40	0.316 0.501	0.408	ND	ND	ND

ND, not done

Table B8 The calculations of growth curve of *L. paracasei* subsp. *paracasei* B282 according to their OD_{600} and viable counts in duplicate

Incubation time (hr)	OD600	Mean	Count (dilution)	CFU/ml	Mean
0	0.008 0.008	0.008	5, 3 (10^{-2}) 4, 3 (10^{-2})	2.00×10^4 1.75×10^4	1.875×10^4
1	0.010 0.014	0.012	ND	ND	ND
2	0.012 0.012	0.012	ND	ND	ND
3	0.014 0.010	0.012	ND	ND	ND
4	0.013 0.012	0.0125	ND	ND	ND
5	0.012 0.012	0.012	ND	ND	ND
6	0.014 0.022	0.018	ND	ND	ND
7	0.022 0.022	0.022	ND	ND	ND
8	0.022 0.022	0.022	ND	ND	ND
9	0.023 0.025	0.024	ND	ND	ND
10	0.023 0.021	0.022	ND	ND	ND
11	0.028 0.026	0.027	ND	ND	ND
12	0.030 0.037	0.033	ND	ND	ND
13	0.038 0.038	0.038	ND	ND	ND
14	0.112 0.008	0.060	25, 27 (10^{-3}) 37, 29 (10^{-3})	1.30×10^6 1.65×10^6	1.47×10^6
15	0.230 0.222	0.226	ND	ND	ND
16	0.558 0.557	0.5575	ND	ND	ND
17	0.886 0.863	0.874	ND	ND	ND
18	1.086 1.151	1.118	57, 74 (10^{-6}) 72, 62 (10^{-6})	3.27×10^9 3.35×10^9	3.31×10^9
19	1.536 1.536	1.536	ND	ND	ND
20	1.608 1.599	1.603	ND	ND	ND
21	1.620 1.618	1.619	ND	ND	ND
22	1.732 1.734	1.733	28, 24 (10^{-8}) 35, 33 (10^{-8})	1.30×10^{11} 1.70×10^{11}	1.50×10^{11}
23	1.742 1.742	1.742	14, 11 (10^{-8}) 26, 19 (10^{-8})	6.25×10^{10} 1.12×10^{11}	8.75×10^{10}
24	1.700 1.700	1.700	53, 44 (10^{-7}) 64, 51 (10^{-7})	2.42×10^{10} 2.87×10^{10}	2.64×10^{10}

ND, not done

Table B8 (continued)

Incubation time (hr)	OD600	Mean	Count (dilution)	CFU/ml	Mean
25	1.734 1.736	1.735	ND	ND	ND
26	1.722 1.722	1.722	ND	ND	ND
27	1.736 1.721	1.728	ND	ND	ND
28	1.740 1.741	1.7405	ND	ND	ND
29	1.730 1.740	1.735	ND	ND	ND
30	1.720 1.731	1.723	ND	ND	ND
32	1.681 1.720	1.700	ND	ND	ND
34	0.058 0.069	0.063	80, 65 (10^{-5}) 73, 85 (10^{-5})	3.62×10^{8} 3.95×10^{8}	3.78×10^{8}
36	0.038 0.054	0.046	ND	ND	ND
38	0.034 0.024	0.029	ND	ND	ND
40	0.020 0.019	0.0195	ND	ND	ND

ND, not done

Table B9 The calculations of growth curve of *L. paracasei* subsp. *paracasei* B63/8 according to their OD_{600} and viable counts in duplicate

Incubation time (hr)	OD600	Mean	Count (dilution)	CFU/ml	Mean
0	0.010 0.006	0.008	11, 15 (10^{-2}) 4, 8 (10^{-2})	6.50×10^4 3.00×10^4	4.75×10^4
1	0.005 0.005	0.005	ND	ND	ND
2	0.005 0.005	0.005	ND	ND	ND
3	0.011 0.006	0.008	ND	ND	ND
4	0.009 0.010	0.0095	ND	ND	ND
5	0.009 0.006	0.0075	ND	ND	ND
6	0.016 0.010	0.013	ND	ND	ND
7	0.020 0.017	0.0185	ND	ND	ND
8	0.018 0.018	0.018	ND	ND	ND
9	0.020 0.022	0.021	ND	ND	ND
10	0.033 0.029	0.031	ND	ND	ND
11	0.035 0.040	0.0375	ND	ND	ND
12	0.035 0.041	0.038	ND	ND	ND
13	0.050 0.050	0.050	ND	ND	ND
14	0.084 0.078	0.081	ND	ND	ND
15	0.134 0.138	0.136	ND	ND	ND
16	0.332 0.348	0.340	ND	ND	ND
17	0.550 0.541	0.545	ND	ND	ND
18	0.744 0.824	0.784	68, 65 (10^{-5}) 61, 72 (10^{-5})	3.32×10^8 3.32×10^8	3.32×10^8
19	1.088 0.996	1.024	ND	ND	ND
20	1.180 1.145	1.162	ND	ND	ND
21	1.390 1.405	1.397	ND	ND	ND
22	1.418 1.424	1.421	28, 24 (10^{-8}) 35, 33 (10^{-8})	1.30×10^{10} 1.70×10^{10}	1.50×10^{10}
23	1.354 1.400	1.377	14, 11 (10^{-8}) 26, 19 (10^{-8})	6.25×10^{10} 1.125×10^{11}	8.75×10^{10}
24	1.360 1.377	1.368	53, 44 (10^{-7}) 64, 51 (10^{-7})	2.42×10^{10} 2.87×10^{10}	2.64×10^{10}

ND, not done

Table B9 (continued)

Incubation time (hr)	OD600	Mean	Count (dilution)	CFU/ml	Mean
25	1.360 1.352	1.356	ND	ND	ND
26	1.382 1.342	1.362	ND	ND	ND
27	1.374 1.361	1.367	ND	ND	ND
28	1.370 1.361	1.365	ND	ND	ND
29	1.374 1.374	1.374	ND	ND	ND
30	1.376 1.361	1.368	ND	ND	ND
32	1.344 1.350	1.347	ND	ND	ND
34	0.366 0.761	0.563	53, 47 (10^{-3}) 54, 51 (10^{-3})	2.50×10^6 2.62×10^6	2.56×10^6
36	0.340 0.572	0.456	ND	ND	ND
38	0.236 0.464	0.350	ND	ND	ND
40	0.196 0.284	0.240	ND	ND	ND

ND, not done

APPENDIX C

CHARACTERIZATION OF CELL-FREE SUPERNATANTS

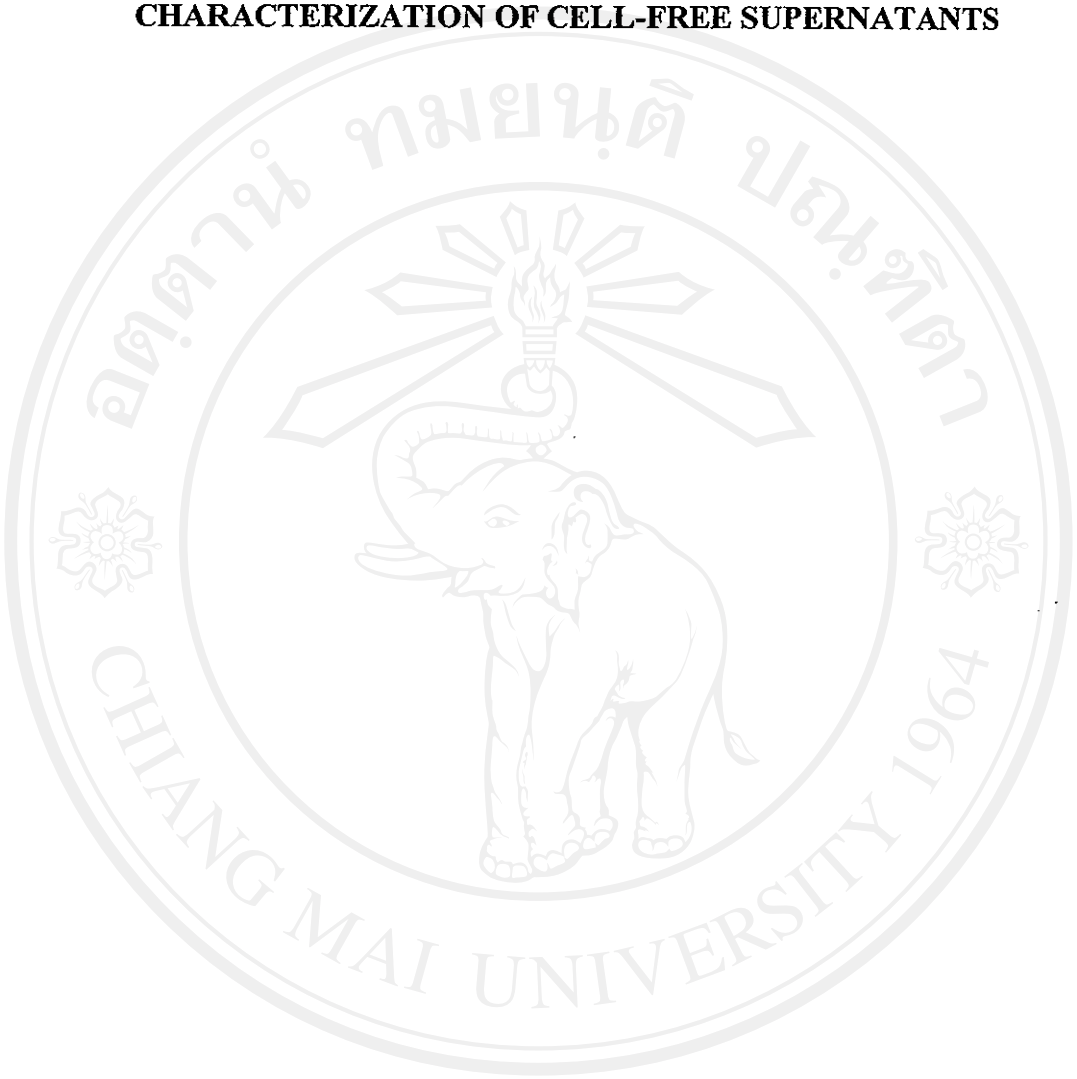


Table C1 The effect of pH on the antimicrobial activity of the potent antimicrobial producing lactobacilli B85/4 isolate and MRS broth against *P. gingivalis* W50

Sample	Zone (mm)	Mean	% Residual activity	Mean ± SD
Cell-free supernatant (pH 4.3)	17, 17 18, 17	17.0 17.5	100.0 100.0	100.0 ± 0
B85/4 supernatant treated to pH 1	17, 17 16, 18	17.0 17.0	100.0 95.6	94.1 ± 2.63
pH 2	17, 16 19, 16	16.5 17.5	95.4 99.9	92.2 ± 5.35
pH 3	16, 18 17, 18	17.0 17.5	100.0 99.9	94.1 ± 2.63
pH 4	17, 16 17, 17	16.5 17.0	95.4 95.6	94.1 ± 2.92
pH 5	15, 17 16, 15	16.0 15.5	95.4 91.2	82.3 ± 3.25
pH 6	16, 15 15, 16	15.5 15.5	86.4 86.9	80.4 ± 0.53
pH 7	12, 11 12, 10	11.5 11.0	50.0 43.5	41.1 ± 1.62
pH 8	10, 10 8, 9	10.0 8.5	36.4 21.7	29.4 ± 1.95
pH 9	10, 10 8, 8	10.0 8.0	36.4 17.4	33.4 ± 3.71
pH 10	9, 9 7, 9	9.0 8.0	27.3 17.4	23.5 ± 0.66
pH 11	8, 8 7, 7	8.0 7.0	18.2 8.7	19.7 ± 6.10
pH 12	8, 7 7, 6	7.5 6.5	13.6 4.3	15.8 ± 5.99
pH 13	7, 8 7, 7	7.5 7.0	13.6 8.7	11.8 ± 0.33
pH 14	8, 7 8, 6	7.5 7.0	13.6 8.7	9.8 ± 2.50

Table C1 (continued)

Sample	Zone (mm)	Mean	% Residual activity	Mean ± SD
MRS broth	7, 7	7.0	9.1	8.9 ± 0.28
	7, 7	7.0	8.7	
MRS broth treated to				
pH 1	7, 7	7.0	9.1	8.9 ± 0.28
	7, 7	7.0	8.7	
pH 2	7, 6	6.5	4.5	6.6 ± 2.93
	6, 8	7.0	8.7	
pH 3	7, 7	7.0	9.1	6.7 ± 3.36
	7, 6	6.5	4.3	
pH 4	6, 7	6.5	4.5	6.6 ± 2.93
	7, 7	7.0	8.7	
pH 5	7, 6	6.5	4.5	6.6 ± 2.93
	7, 7	7.0	8.7	
pH 6	7, 7	7.0	9.1	8.9 ± 0.28
	7, 7	7.0	8.7	
pH 7	7, 6	6.5	4.5	6.6 ± 2.93
	7, 7	7.0	8.7	
pH 8	7, 6	6.5	4.5	6.6 ± 2.93
	7, 7	7.0	8.7	
pH 9	7, 7	7.0	9.1	6.7 ± 3.36
	7, 6	6.5	4.3	
pH 10	7, 7	7.0	9.1	8.9 ± 0.28
	7, 7	7.0	8.7	
pH 11	8, 6	7.0	9.1	8.9 ± 0.28
	7, 7	7.0	8.7	
pH 12	8, 7	7.5	13.6	9.0 ± 6.57
	7, 6	6.5	4.3	
pH 13	6, 8	7.0	9.1	6.7 ± 3.36
	7, 6	6.5	4.3	
pH 14	7, 8	7.5	13.6	9.0 ± 6.57
	7, 6	6.5	4.3	

Table C2 The effect of pH on the antimicrobial activity of the potent antimicrobial producing lactobacilli B282 isolate and MRS broth against *P. gingivalis* W50

Sample	Zone (mm)	Mean	% Residual activity	Mean ± SD
Cell-free supernatant (pH 4.3)	20, 17	18.5	100.0	100.0 ± 0
	17, 17	17.0	100.0	
B282 supernatant treated to				
pH 1	18, 16	17.0	88.0	87.2 ± 1.16
	16, 15	15.5	86.4	
pH 2	15, 16	15.5	76.4	78.9 ± 4.11
	15, 15	15.0	81.8	
pH 3	15, 14	14.5	68.0	68.1 ± 0.12
	14, 13	13.5	68.2	
pH 4	14, 15	14.5	68.0	70.4 ± 3.34
	15, 13	14.0	72.7	
pH 5	13, 11	12.0	48.0	49.0 ± 1.41
	11, 12	11.5	50.0	
pH 6	10, 10	10.0	32.0	34.2 ± 3.08
	11, 9	10.0	36.4	
pH 7	10, 10	10.0	32.0	31.9 ± 0.13
	10, 9	9.5	31.8	
pH 8	8, 8	8.0	16.0	19.4 ± 4.76
	8, 9	8.5	22.7	
pH 9	8, 8	8.0	16.0	17.1 ± 1.54
	8, 8	8.0	18.2	
pH 10	9, 8	8.5	20.0	21.4 ± 1.93
	8, 9	8.5	22.7	
pH 11	8, 8	8.0	16.0	17.1 ± 1.54
	8, 8	8.0	18.2	
pH 12	8, 7	7.5	12.0	10.5 ± 2.06
	8, 6	7.0	9.1	
pH 13	7, 8	7.5	12.0	12.8 ± 1.16
	7, 8	7.5	13.6	
pH 14	8, 7	7.5	12.0	15.1 ± 4.37
	8, 8	8.0	18.6	

Table C2 (continued)

Sample	Zone (mm)	Mean	% Residual activity	Mean ± SD
MRS broth	7, 7	7.0	8.0	8.5 ± 0.77
	7, 7	7.0	9.1	
MRS broth treated to				
pH 1	7, 7	7.0	8.0	8.5 ± 0.77
	7, 7	7.0	9.1	
pH 2	7, 6	6.5	4.0	3.6 ± 3.60
	6, 8	7.0	9.1	
pH 3	7, 7	7.0	8.0	6.3 ± 2.44
	7, 6	6.5	4.5	
pH 4	6, 7	6.5	4.0	6.5 ± 3.60
	7, 7	7.0	9.1	
pH 5	7, 6	6.5	4.0	6.5 ± 3.60
	7, 7	7.0	9.1	
pH 6	7, 7	7.0	8.0	8.5 ± 0.77
	7, 7	7.0	9.1	
pH 7	7, 6	6.5	4.0	6.5 ± 3.60
	7, 7	7.0	9.1	
pH 8	7, 6	6.5	4.0	6.5 ± 3.60
	7, 7	7.0	9.1	
pH 9	7, 7	7.0	8.0	6.3 ± 2.44
	7, 6	6.5	4.5	
pH 10	7, 7	7.0	8.0	8.5 ± 0.77
	7, 7	7.0	9.1	
pH 11	8, 6	7.0	8.0	8.5 ± 0.77
	7, 7	7.0	9.1	
pH 12	8, 7	7.5	12.0	8.3 ± 5.27
	7, 6	6.5	4.5	
pH 13	6, 8	7.0	8.0	6.3 ± 2.44
	7, 6	6.5	4.5	
pH 14	7, 8	7.5	12.0	8.3 ± 5.27
	7, 6	6.5	4.5	

Table C3 The effect of pH on the antimicrobial activity of the potent antimicrobial producing lactobacilli B63/8 isolate and MRS broth against *P. gingivalis* W50

Sample	Zone (mm)	Mean	% Residual activity	Mean ± SD
Cell-free supernatant (pH 4.3)	20, 18	19.0	100.0	100.0 ± 0
	18, 19	18.5	100.0	
B63/8 supernatant treated to				
pH 1	19, 17	18.0	92.3	94.1 ± 2.63
	17, 19	18.0	96.0	
pH 2	17, 18	17.5	88.4	92.2 ± 5.35
	18, 18	18.0	96.0	
pH 3	18, 18	18.0	92.3	94.1 ± 2.63
	19, 17	18.0	96.0	
pH 4	19, 18	18.5	96.1	94.1 ± 2.92
	15, 20	17.5	92.0	
pH 5	17, 17	17.0	84.5	82.3 ± 3.25
	17, 15	16.0	90.0	
pH 6	16, 17	16.5	80.7	80.4 ± 0.53
	17, 15	16.0	80.0	
pH 7	12, 11	11.5	42.3	41.1 ± 1.62
	12, 10	11.0	40.0	
pH 8	10, 10	10.0	30.8	29.4 ± 1.95
	10, 9	9.5	28.0	
pH 9	10, 10	10.0	30.8	33.4 ± 3.71
	10, 11	10.5	36.0	
pH 10	9, 9	9.0	23.1	23.5 ± 0.66
	9, 9	9.0	24.0	
pH 11	8, 8	8.0	15.4	19.7 ± 6.10
	9, 9	9.0	24.0	
pH 12	8, 7	7.5	11.5	15.8 ± 5.99
	9, 8	8.5	20.0	
pH 13	7, 8	7.5	11.5	11.8 ± 0.33
	8, 7	7.5	12.0	
pH 14	8, 7	7.5	11.5	9.8 ± 2.50
	8, 6	7.0	8.0	

Table C3 (continued)

Sample	Zone (mm)	Mean	% Residual activity	Mean ± SD
MRS broth	7, 7	7.0	7.7	7.8 ± 0.22
	7, 7	7.0	8.0	
MRS broth treated to				
pH 1	7, 7	7.0	7.7	7.8 ± 0.22
	7, 7	7.0	8.0	
pH 2	7, 6	6.5	3.8	5.9 ± 2.94
	6, 8	7.0	8.0	
pH 3	7, 7	7.0	7.7	5.8 ± 2.61
	7, 6	6.5	4.0	
pH 4	6, 7	6.5	3.8	5.9 ± 2.94
	7, 7	7.0	8.0	
pH 5	7, 6	6.5	3.8	5.9 ± 2.94
	7, 7	7.0	8.0	
pH 6	7, 7	7.0	7.7	7.8 ± 0.22
	7, 7	7.0	8.0	
pH 7	7, 6	6.5	3.8	5.9 ± 2.94
	7, 7	7.0	8.0	
pH 8	7, 6	6.5	3.8	5.9 ± 2.94
	7, 7	7.0	8.0	
pH 9	7, 7	7.0	7.7	5.8 ± 2.61
	7, 6	6.5	4.0	
pH 10	7, 7	7.0	7.7	7.8 ± 0.22
	7, 7	7.0	8.0	
pH 11	8, 6	7.0	7.7	7.8 ± 0.22
	7, 7	7.0	8.0	
pH 12	8, 7	7.5	11.5	7.8 ± 5.33
	7, 6	6.5	4.0	
pH 13	6, 8	7.0	7.7	5.8 ± 2.61
	7, 6	6.5	4.0	
pH 14	7, 8	7.5	11.5	7.8 ± 5.33
	7, 6	6.5	4.0	

Table C4 The effect of heat on the antimicrobial activity of the potent antimicrobial producing lactobacilli B85/4 isolate and MRS broth against *P. gingivalis* W50

Isolate	Temperature (°C)	Zone (mm)	Mean	% residual activity	Mean ± SD
B85/4	Cell-free supernatant	17, 17	17.0	100.0	100.0 ± 0
		18, 16	17.0	100.0	
	Supernatant heated with 60°C, 30 min	17, 17	17.0	100.0	97.7 ± 3.22
		17, 16	16.5	95.4	
	80°C, 30 min	15, 15	15.0	81.8	79.5 ± 3.21
		14, 15	14.5	77.3	
	100°C, 30 min	10, 10	10.0	36.4	34.1 ± 3.25
		9, 10	9.5	31.8	
	121°C, 15 min	10, 8	9.0	31.8	27.3 ± 6.43
		8, 9	8.5	22.7	
MRS broth	MRS broth	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	MRS broth heated with 60°C, 30 min	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	80°C, 30 min	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	100°C, 30 min	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	121°C, 15 min	7, 7	7.0	7.4	7.4 ± 0
		7, 7	7.0	7.4	

Table C5 The effect of heat on the antimicrobial activity of the potent antimicrobial producing lactobacilli B282 isolate and MRS broth against *P. gingivalis* W50

Isolate	Temperature (°C)	Zone (mm)	Mean	% residual activity	Mean ± SD
B282	Cell-free supernatant	20, 17	18.5	100.0	100.0 ± 0
		17, 17	17.0	100.0	
	Supernatant heated with 60°C, 30 min	17, 17	17.0	88.0	87.2 ± 1.16
		16, 16	16.0	86.4	
	80°C, 30 min	15, 16	15.5	76.0	78.9 ± 4.11
		15, 15	15.0	81.8	
	100°C, 30 min	15, 16	15.5	76.0	76.6 ± 0.89
		15, 14	14.5	77.3	
	121°C, 15 min	14, 12	13.0	56.0	55.3 ± 1.03
		12, 12	12.0	54.5	
MRS broth	MRS broth	7, 7	7.0	8.0	8.5 ± 0.77
		7, 7	7.0	9.1	
	MRS broth heated with 60°C, 30 min	7, 7	7.0	8.0	8.5 ± 0.77
		7, 7	7.0	9.1	
	80°C, 30 min	7, 7	7.0	8.0	8.5 ± 0.77
		7, 7	7.0	9.1	
	100°C, 30 min	7, 7	7.0	8.0	8.5 ± 0.77
		7, 7	7.0	9.1	
	121°C, 15 min	7, 7	7.0	8.0	8.5 ± 0.77
		7, 7	7.0	9.1	

Table C6 The effect of heat on the antimicrobial activity of the potent antimicrobial producing lactobacilli B63/8 isolate and MRS broth against *P. gingivalis* W50

Isolate	Temperature (°C)	Zone (mm)	Mean	% residual activity	Mean ± SD
B63/8	Cell-free supernatant	20, 18	19.0	100.0	100.0 ± 0
		18, 19	18.5	100.0	
	Supernatant heated with 60°C, 30 min	17, 15	16.0	76.9	80.5 ± 5.02
		17, 16	16.5	84.0	
	80°C, 30 min	15, 16	16.5	73.1	72.5 ± 0.75
		15, 15	15.0	72.0	
	100°C, 30 min	10, 11	10.5	34.6	37.3 ± 3.81
		12, 10	11.0	40.0	
	121°C, 15 min	9, 8	8.5	19.2	17.6 ± 2.28
		8, 8	8.0	16.0	
MRS broth	MRS broth	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	MRS broth treated with 60°C, 30 min	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	80°C, 30 min	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	100°C, 30 min	7, 7	7.0	9.1	9.1 ± 0
		7, 7	7.0	9.1	
	121°C, 15 min	7, 7	7.0	7.4	7.4 ± 0
		7, 7	7.0	7.4	

Table C7 The effect of proteolytic enzyme on the antimicrobial activity of the potent antimicrobial producing lactobacilli B85/4 isolate and MRS broth against *P. gingivalis* W50

Isolate	Supernatant	Enzyme concentration (g/l)	Zone (mm)	Mean	% residual activity	Mean ± SD
B85/4	Cell-free supernatant	-	17, 17	17.0	100.0	100.0 ± 0
			18, 16	17.0	100.0	
	Supernatant treated with	Trypsin				
		0.125	16, 15	15.5	90.9	93.2 ± 3.21
			17, 17	17.0	95.4	
		0.250	13, 14	13.5	72.7	75.0 ± 3.21
			15, 15	15.0	77.3	
		0.500	12, 12	12.0	54.5	56.8 ± 3.21
			12, 13	12.5	59.1	
		Pepsin				
		0.125	13, 13	13.0	63.6	61.4 ± 3.21
			11, 13	12.0	59.1	
		0.250	10, 12	11.0	50.0	47.7 ± 3.21
			11, 11	11.0	45.5	
		0.500	10, 9	9.5	31.8	31.8 ± 0
			9, 10	9.5	31.8	
MRS broth	MRS broth	-	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
	MRS broth treated with	Trypsin				
		0.125	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
		0.250	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
		0.500	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
		Pepsin				
		0.125	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
		0.250	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
		0.500	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	

Table C8 The effect of proteolytic enzyme on the antimicrobial activity of the potent antimicrobial producing lactobacilli 282 isolate and MRS broth against *P. gingivalis* W50

Isolate	Supernatant	Enzyme concentration (g/l)	Zone (mm)	Mean	% residual activity	Mean ± SD
B282	Cell-free supernatant	-	20, 17	18.5	100.0	100.0 ± 0
			17, 17	17.0	100.0	
	Supernatant treated with	Trypsin				
		0.125	15, 16	15.5	92.0	93.7 ± 2.44
			18, 15	16.5	95.4	
		0.250	15, 16	15.5	80.0	83.2 ± 4.49
			16, 15	15.5	86.4	
		0.500	15, 12	13.5	60.0	64.1 ± 5.78
			14, 13	13.5	68.2	
		Pepsin				
		0.125	14, 13	13.5	68.0	70.4 ± 3.34
			14, 14	14.0	72.7	
		0.250	11, 10	10.5	36.0	36.2 ± 0.28
			11, 11	11.0	36.4	
		0.500	10, 9	10.0	28.0	29.9 ± 2.70
			9, 10	9.5	31.8	
MRS broth	MRS broth	-	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
	MRS broth treated with	Trypsin				
		0.125	7, 7	7.0	8.0	8.5 ± 0.77
			7, 7	7.0	9.1	
		0.250	7, 7	7.0	8.0	8.5 ± 0.77
			7, 7	7.0	9.1	
		0.500	7, 7	7.0	8.0	8.5 ± 0.77
			7, 7	7.0	9.1	
		Pepsin				
		0.125	7, 7	7.0	8.0	8.5 ± 0.77
			7, 7	7.0	9.1	
		0.250	7, 7	7.0	8.0	8.5 ± 0.77
			7, 7	7.0	9.1	
		0.500	7, 7	7.0	8.0	8.5 ± 0.77
			7, 7	7.0	9.1	

Table C9 The effect of proteolytic enzyme on the antimicrobial activity of the potent antimicrobial producing lactobacilli B63/8 isolate and MRS broth against *P. gingivalis* W50

Isolate	Supernatant	Enzyme concentration (g/l)	Zone (mm)	Mean	% residual activity	Mean ± SD
B63/8	Cell-free supernatant	-	20, 18	19.0	100.0	100.0 ± 0
			18, 19	18.5	100.0	
	Supernatant treated with	trypsin				
		0.125	14, 16	15.0	76.9	78.5 ± 2.19
			16, 16	16.0	80.0	
		0.250	14, 14	14.0	69.2	70.6 ± 1.97
			16, 14	15.0	72.0	
		0.500	12, 10	11.0	38.5	39.2 ± 1.1
			12, 10	11.0	40.0	
		pepsin				
		0.125	15, 15	15.0	69.2	68.6 ± 0.86
			15, 14	14.5	68.0	
		0.250	13, 12	12.5	50.0	51.0 ± 1.42
			13, 12	12.5	52.0	
		0.500	11, 10	10.5	34.6	33.3 ± 1.84
			10, 10	10.0	32.0	
MRS broth	MRS broth	-	7, 7	7.0	9.1	9.1 ± 0
			7, 7	7.0	9.1	
	MRS broth treated with	trypsin				
		0.125	7, 7	7.0	7.7	7.8 ± 0.22
			7, 7	7.0	8.0	
		0.250	7, 7	7.0	7.7	7.8 ± 0.22
			7, 7	7.0	8.0	
		0.500	7, 7	7.0	7.7	7.8 ± 0.22
			7, 7	7.0	8.0	
		pepsin				
		0.125	7, 7	7.0	7.7	7.8 ± 0.22
			7, 7	7.0	8.0	
		0.250	7, 7	7.0	7.7	7.8 ± 0.22
			7, 7	7.0	8.0	
		0.500	7, 7	7.0	7.7	7.8 ± 0.22
			7, 7	7.0	8.0	

Table C10 Total protein concentration and protein increasing folds of 3 isolates of the potent antimicrobial producing lactobacillus

Isolate	Ammonium sulphate concentration	Total protein concentration (mg/ml)	Protein increasing folds
B85/4	0%	2.9	1
	20%	5.3	1.83
	40%	39.7	13.69
	60%	40.3	13.90
	80%	45.6	15.72
B282	0%	3.5	1
	20%	16.9	4.83
	40%	26.9	7.68
	60%	27.5	7.86
	80%	53.7	15.34
B63/8	0%	6.4	1
	20%	16.4	2.56
	40%	25.7	4.01
	60%	26.3	4.11
	80%	43.8	6.84
MRS broth	0%	4.7	1
	20%	2.3	0.49
	40%	5.3	1.13
	60%	7.0	1.49
	80%	8.2	1.74

Table C11 The absorbance at 590 nm of standard BSA

Final BSA concentration (µg/ml)	Absorbance	Mean	Blank-corrected measurement
0 (Blank)	0.115 0.114	0.1145	0
30	0.125 0.124	0.1245	0.0100
60	0.130 0.126	0.128	0.0135
125	0.138 0.132	0.135	0.0205
250	0.160 0.157	0.1585	0.0440
500	0.185 0.185	0.185	0.0705
1,000	0.236 0.233	0.2345	0.1200
2,000	0.271 0.272	0.2715	0.1570

The 2,000 µg/ml of standard BSA was diluted to the serial dilutions with deionized water.

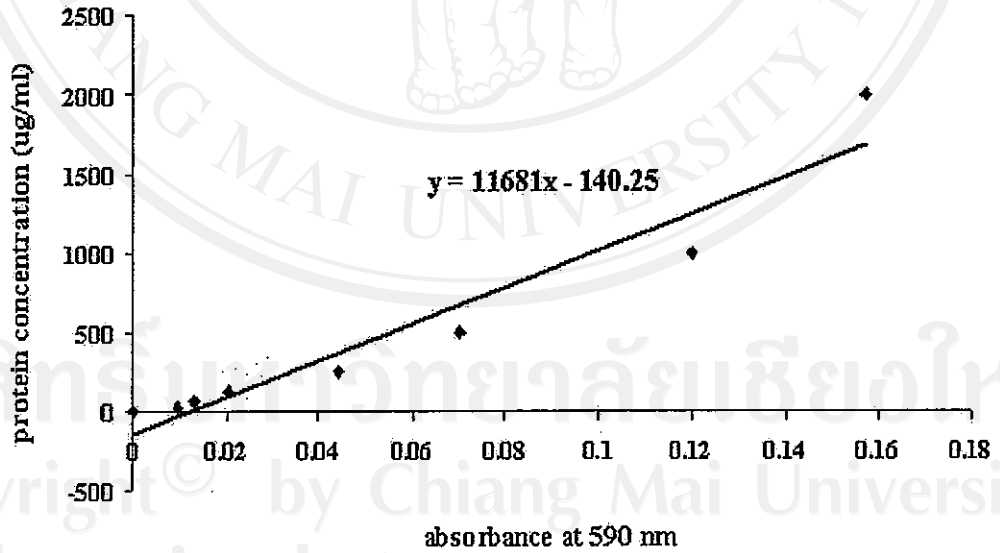


Figure C1 The standard curve of BSA from the BCA protein assay, the X-axis represented as absorbance at 590 nm and the Y-axis was represented as protein concentration in µg/ml

Table C12 The absorbance at 590 nm and total protein calculation of each crude bacteriocin and MRS broth

Isolate	Absorbance	Mean	Calculation	Blank-corrected calculation	Dilution 10^{-2}	Concentration (mg/ml)
Blank	0.115 0.114	0.1145	1,197.2	0	0	0
B85/4	0.146 0.143	0.1445	1,547.7	350.4	35,043.0	35.0
B282	0.138 0.137	0.1375	1,465.9	268.6	26,866.3	26.9
B63/8	0.139 0.139	0.139	1,483.4	286.2	28,620.0	28.6
MRS broth	0.122 0.118	0.120	1,261.5	64.2	6,420.0	6.4

The calculation was performed with the equation; y = 11,681x– 140.25

APPENDIX D

PARTIAL PURIFICATION OF CRUDE BACTERIOCINS

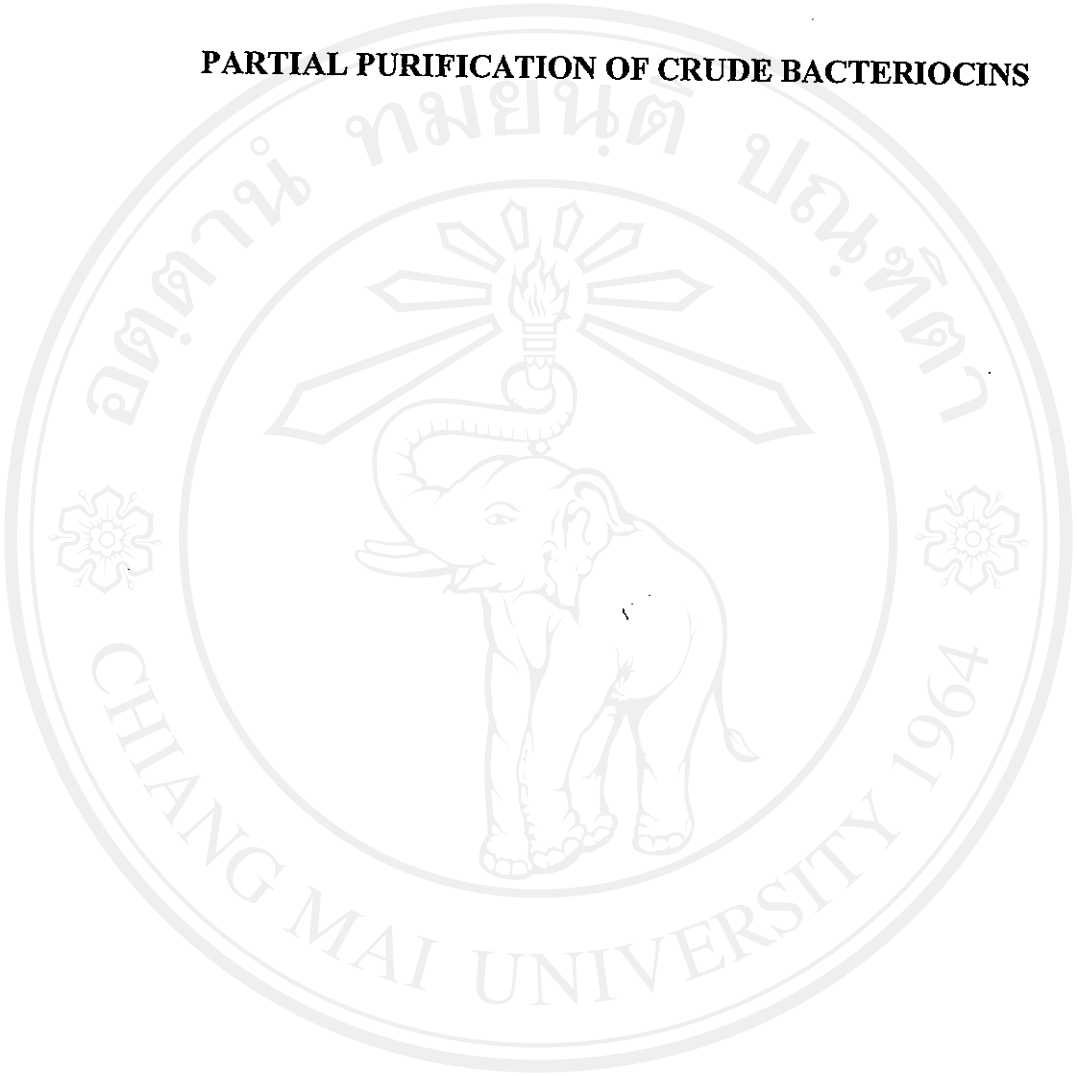


Table D1 The elution profiles of B85/4's crude bacteriocins by using anion exchange column chromatography

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
F	1	0.00	-0.65	ND
A1	1	0.00	0.54	ND
A2	1	0.00	3524.47	
A3	2	0.00	4923.90	15.9
A4	3	0.00	211.03	
A5	4	0.00	38.57	ND
A6	5	0.00	24.70	ND
A7	6	0.00	18.55	ND
A8	7	0.00	15.08	ND
A9	8	0.00	12.75	ND
A10	9	0.00	11.19	ND
A11	10	0.00	10.02	ND
A12	11	0.00	9.07	ND
B1	12	0.00	8.31	ND
B2	13	0.00	7.74	ND
B3	14	0.00	7.28	ND
B4	15	0.00	6.90	ND
B5	16	0.00	6.56	ND
B6	17	0.00	6.32	ND
B7	18	0.00	6.11	ND
B8	19	0.00	5.93	ND
B10	20	0.00	5.90	ND
B11	21	0.00	5.73	ND
B12	22	35.00	9.53	ND
C1	23	35.80	1034.85	
C2	24	36.60	3296.71	
C3	25	37.50	1776.61	84.1
C4	26	38.30	1090.74	
C5	27	39.10	772.28	
C6	28	40.00	571.51	
C7	29	40.80	429.46	
C8	30	41.60	325.32	22.7
C9	31	42.40	251.81	
C10	32	43.30	204.11	
C11	33	44.10	174.51	ND
C12	34	44.90	153.11	ND
D1	35	45.80	136.81	ND
D2	36	46.60	125.47	ND
D3	37	47.40	119.23	ND
D4	38	48.20	113.71	ND
D5	39	49.10	110.03	ND
D6	40	49.90	108.67	ND
D7	41	50.70	108.75	ND
D8	42	51.60	109.09	ND
D9	43	52.40	109.00	ND

ND, not done

Table D1 (continued)

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
D10	44	53.20	109.29	ND
D12	45	54.10	109.43	ND
E1	46	54.90	111.90	ND
E2	47	55.00	112.31	
E3	48	59.04	132.34	
E4	49	64.00	204.98	11.4
E5	50	68.70	251.91	
E6	51	73.30	161.81	
E7	52	78.00	76.18	
E8	53	82.70	43.48	ND
E9	54	87.30	31.44	ND
E10	55	92.00	27.20	ND
E11	56	96.70	26.45	ND
E12	57	99.80	25.33	ND
F1	58	100.00	22.78	ND

ND, not done

Table D2 The elution profiles of B282's crude bacteriocins by using anion exchange column chromatography

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
F	1	0.00	1.57	ND
A1	1	0.00	1.23	ND
A2	1	0.00	3210.91	
A3	2	0.00	4924.78	13.8
A4	3	0.00	1278.81	
A5	4	0.00	120.42	ND
A6	5	0.00	53.15	ND
A7	6	0.00	37.34	ND
A8	7	0.00	31.06	ND
A9	8	0.00	26.95	ND
A10	9	0.00	23.81	ND
A11	10	0.00	21.26	ND
A12	11	0.00	19.20	ND
B1	12	0.00	17.47	ND
B2	13	0.00	15.97	ND
B3	14	0.00	14.78	ND
B4	15	0.00	13.81	ND
B5	16	0.00	12.97	ND
B6	17	0.00	12.25	ND
B7	18	0.00	13.72	ND
B8	19	0.00	11.16	ND
B10	20	0.00	11.12	ND
B11	21	0.00	10.63	ND
B12	22	35.00	13.84	ND
C1	23	35.80	1411.99	
C2	24	36.60	4838.86	
C3	25	37.50	2544.16	60.8
C4	26	38.30	1650.91	
C5	27	39.10	1238.81	
C6	28	40.00	950.09	
C7	29	40.80	721.01	
C8	30	41.60	561.37	17.7
C9	31	42.40	452.18	
C10	32	43.30	372.64	
C11	33	44.10	314.11	ND
C12	34	44.90	272.22	ND
D1	35	45.80	241.41	ND
D2	36	46.60	218.37	ND
D3	37	47.40	200.29	ND
D4	38	48.30	185.37	ND
D5	39	49.10	175.23	ND
D6	40	49.90	168.18	ND
D7	41	50.70	163.68	ND
D8	42	51.90	161.93	ND
D9	43	52.40	162.53	ND

ND, not done

Table D2 (continued)

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
D10	44	53.20	164.31	ND
D12	45	54.10	163.99	ND
E1	46	54.90	165.57	ND
E2	47	55.00	167.20	
E3	48	59.04	203.30	
E4	49	64.00	325.68	7.8
E5	50	68.70	398.28	
E6	51	73.40	255.44	
E7	52	78.10	133.07	
E8	53	82.70	81.29	ND
E9	54	87.40	59.01	ND
E10	55	92.00	50.04	ND
E11	56	96.70	48.58	ND
E12	57	99.80	40.23	ND
F1	58	100.00	35.11	ND

ND, not done

Table D3 The elution profiles of B63/8's crude bacteriocins by using anion exchange column chromatography

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
F	1	0.00	1.27	ND
A1	1	0.00	1.12	ND
A2	1	0.00	2796.34	
A3	2	0.00	4908.19	9.8
A4	3	0.00	1462.46	
A5	4	0.00	94.26	ND
A6	5	0.00	37.19	ND
A7	6	0.00	23.76	ND
A8	7	0.00	17.76	ND
A9	8	0.00	14.37	ND
A10	9	0.00	12.45	ND
A11	10	0.00	11.21	ND
A12	11	0.00	10.19	ND
B1	12	0.00	9.47	ND
B2	13	0.00	8.83	ND
B3	14	0.00	8.34	ND
B4	15	0.00	7.92	ND
B5	16	0.00	7.56	ND
B6	17	0.00	7.23	ND
B7	18	0.00	6.96	ND
B8	19	0.00	6.73	ND
B10	20	0.00	6.72	ND
B11	21	0.00	6.47	ND
B12	22	35.00	10.16	ND
C1	23	35.80	1131.82	
C2	24	36.60	3225.55	
C3	25	37.50	1671.64	70.6
C4	26	38.30	1047.62	
C5	27	39.10	766.48	
C6	28	39.90	570.20	
C7	29	40.80	430.41	
C8	30	41.60	332.76	11.8
C9	31	42.40	263.39	
C10	32	43.20	215.57	
C11	33	44.10	185.39	ND
C12	34	44.90	166.66	ND
D1	35	45.70	152.65	ND
D2	36	46.60	143.15	ND
D3	37	47.40	136.86	ND
D4	38	48.20	132.96	ND
D5	39	49.10	131.50	ND
D6	40	49.90	133.25	ND
D7	41	50.80	137.27	ND
D8	42	51.60	143.25	ND
D9	43	52.40	148.50	ND

ND, not done

Table D3 (continued)

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
D10	44	53.20	156.53	ND
D12	45	54.10	157.88	ND
E1	46	54.90	165.79	ND
E2	47	55.00	171.63	
E3	48	59.04	216.93	
E4	49	64.10	366.58	7.8
E5	50	68.70	475.96	
E6	51	73.40	312.20	
E7	52	78.10	149.01	
E8	53	82.70	77.41	ND
E9	54	87.40	54.63	ND
E10	55	92.00	45.59	ND
E11	56	96.60	44.86	ND
E12	57	99.80	44.01	ND
F1	58	100.00	42.18	ND

ND, not done

Table D4 The elution profiles of MRS's crude proteins by using anion exchange column chromatography

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
F	1	0.00	-0.01	ND
A1	1	0.00	0.40	ND
A2	1	0.00	53.07	ND
A3	2	0.00	9.22	ND
A4	3	0.00	5.99	ND
A5	4	0.00	4.81	ND
A6	5	0.00	4.05	ND
A7	6	0.00	3.40	ND
A8	7	0.00	2.87	ND
A9	8	0.00	2.48	ND
A10	9	0.00	2.15	ND
A11	10	0.00	2.00	ND
B1	11	0.00	1.96	ND
B2	12	0.00	1.74	ND
B3	13	35.00	1.88	ND
B4	14	35.80	65.21	ND
B5	15	36.60	100.72	ND
B6	16	37.40	74.50	ND
B7	17	38.20	47.13	ND
B8	18	39.10	27.59	ND
B9	19	39.90	15.94	ND
B10	20	40.70	9.83	ND
B11	21	41.50	6.03	ND
B12	22	42.40	4.08	ND
C1	23	43.20	2.85	ND
C2	24	44.10	2.11	ND
C3	25	44.90	1.63	ND
C4	26	45.70	1.34	ND
C5	27	46.60	1.15	ND
C6	28	47.40	1.05	ND
C7	29	48.20	1.00	ND
C8	30	49.00	0.99	ND
C9	31	49.90	1.01	ND
C10	32	50.70	1.08	ND
C11	33	51.50	1.14	ND
C12	34	52.30	1.22	ND
D2	35	53.20	1.32	ND
D3	36	54.00	1.35	ND
D4	37	54.90	1.44	ND
D5	38	55.00	1.52	ND
D6	39	59.00	1.97	ND
D7	40	63.90	3.67	ND
D8	41	68.90	4.01	ND
D9	42	73.30	1.64	ND
D10	43	77.90	-0.24	ND

ND, not done

Table D4 (continued)

Fractions	ml	NaCl concentration in buffer B (%)	Absorbance at 280 nm (mAU)	% Residual activity
D11	44	82.50	-1.21	ND
D12	45	87.20	-1.73	ND
E1	46	91.90	-1.97	ND
E2	47	96.60	-2.03	ND
E3	48	100.00	-2.52	ND

ND, not done

Table D5 The inhibition zone of each purified fractions which obtained from an anion exchange column chromatography

Isolate	Fractions	Zone (mm)	Mean	% Residual activity	Mean ± SD
B85/4	Cell-free supernatant	17, 17	17.0	100.0	100.0 ± 0
		18, 16	17.0	100.0	
	Unbound fraction	8, 8	8.0	18.2	15.9 ± 3.21
		8, 7	7.5	13.6	
	pooled 23-27	16, 15	15.5	86.4	84.1 ± 3.21
		15, 15	15.0	81.8	
	pooled 28-32	8, 9	8.5	22.7	22.7 ± 0
		9, 8	8.5	22.7	
	pooled 47-52	7, 8	7.5	13.6	11.4 ± 3.21
		7, 7	7.0	9.1	
B282	Cell-free supernatant	20, 17	18.5	100.0	100.0 ± 0
		17, 17	17.0	100.0	
	Unbound fraction	8, 8	8.0	16.0	13.8 ± 3.16
		7, 8	7.5	11.5	
	Pooled 23-27	15, 12	13.5	60.0	60.8 ± 1.07
		15, 13	14.0	61.5	
	Pooled 28-32	9, 8	8.5	20.0	17.7 ± 3.27
		8, 8	8.0	15.4	
	Pooled 47-52	7, 7	7.0	8.0	7.8 ± 0.22
		7, 7	7.0	7.7	
B63/8	Cell-free supernatant	20, 18	19.0	100.0	100.0 ± 0
		18, 19	18.5	100.0	
	Unbound fraction	8, 7	7.5	11.5	9.8 ± 2.50
		7, 7	7.0	8.0	
	pooled 23-27	15, 15	15.0	69.2	70.6 ± 1.97
		16, 14	15.0	72.0	
	pooled 28-32	8, 7	7.5	11.5	11.8 ± 0.33
		8, 7	7.5	12.0	
	pooled 47-52	7, 7	7.0	7.7	7.8 ± 0.22
		7, 7	7.0	8.0	

Table D6 Total protein concentration of each step of the purification of bacteriocin

Isolate	Fractions	Absorbance*	Mean	Calculation**	Blank-corrected measurement	Dilution 10^{-2}	Concentration (mg/ml)
B85/4	blank	0.115 0.114	0.1145	1,197.2	0	0	0
	Cell-free supernatant	0.122 0.112	0.117	1,226.4	29.2	2,922.7	2.9
	Crude bacteriocins	0.146 0.143	0.1445	1,547.7	350.4	35,043.0	35.0
	Unbound fraction	0.124 0.132	0.128	1,354.9	157.7	15,770.0	15.8
	Pooled 23-27	0.129 0.129	0.129	1,366.6	169.4	1,694.0	16.9
	Pooled 28-32	0.116 0.114	0.115	1,203.1	5.8	580.0	0.6
	Pooled 47-52	0.109 0.109	0.109	1132.9	-64.24	-6,424.0	-6.4
B282	Cell-free supernatant	0.113 0.122	0.117	1,232.3	35.1	3506.7	3.5
	Crude bacteriocins	0.138 0.137	0.1375	1,465.9	268.6	26,866.3	26.9
	Unbound fraction	0.129 0.128	0.1295	1,360.7	163.5	16350	16.4
	Pooled 23-27	0.126 0.125	0.1255	1,325.7	128.5	12,850.0	12.8
	Pooled 28-32	0.119 0.116	0.118	1,232.2	35.0	3500.0	3.5
	Pooled 47-52	0.115 0.114	0.1145	1,197.2	0	0	0
B63/8	Cell-free supernatant	0.125 0.115	0.120	1,261.5	64.3	6,427.0	6.4
	Crude bacteriocins	0.139 0.139	0.139	1,483.4	286.2	28,620.0	28.6
	Unbound fraction	0.134 0.123	0.129	1,360.7	163.5	16,350.0	16.4
	Pooled 23-27	0.128 0.123	0.126	1,325.7	128.5	12,850.0	12.8
	Pooled 28-32	0.104 0.111	0.108	1,115.5	-81.7	-8,170.0	-8.2
	Pooled 47-52	0.113 0.113	0.113	1,179.7	-17.5	-1,750.0	-1.8
MRS broth	Cell-free supernatant	0.122 0.115	0.118	1,243.9	46.7	4,674.8	4.7
	Crude proteins	0.122 0.118	0.120	1,261.5	64.2	6,420.0	6.4
	Unbound fraction	0.115 0.113	0.114	1,191.4	-5.8	-580.0	-0.6
	Pooled 16-21	0.104 0.105	0.1045	1,080.4	-116.8	-11,680.0	-11.7

*BCA assay was performed to measure the protein by detecting at 590 nm

**The calculation was performed with the equation, $y = 11{,}681x - 140.25$

Table D7 The inhibition zone demonstrated by the partially purified bacteriocin of B85/4 in each solvent

Solvents	Samples	Zone (mm)	Mean	% Residual activity	Mean ± SD
-	Cell-free supernatant	17, 17 18, 16	17.0 17.0	100.0 100.0	100.0 ± 0
Tris-HCl pH 8.0	Control	7, 7 8, 7	7.0 7.5	ND ND	ND
	Unbound fraction	10, 10 10, 9	10.0 9.5	36.4 31.8	34.1 ± 3.21
	Pooled fraction 23-27	12, 12 12, 11	12.0 11.5	63.6 63.8	63.7 ± 0
Sodium acetate buffer pH 5.0	Control	8, 7 8, 7	7.5 7.5	ND ND	ND
	Unbound fraction	10, 9 11, 8	9.5 9.5	31.8 31.8	31.8 ± 0
	Pooled fraction 23-27	13, 12 11, 12	12.5 11.5	59.1 50.0	54.5 ± 6.43
MRS broth	Control	7, 7 7, 7	7.0 7.0	ND ND	ND
	Unbound fraction	9, 9 11, 9	9.0 10.0	27.3 36.4	31.8 ± 6.43
	Pooled fraction 23-27	12, 11 12, 12	11.5 12.0	50.0 54.5	52.3 ± 3.21
mMRS broth	Control	8, 7 7, 7	7.5 7.0	ND ND	ND
	Unbound fraction	10, 9 8, 10	9.5 9.0	31.8 27.3	29.5 ± 3.21
	Pooled fraction 23-27	13, 13 14, 13	13.0 13.5	63.6 68.2	65.9 ± 3.21

ND, not determined

Table D8 The inhibition zone demonstrated by the partially purified bacteriocin of B282 in each solvent

Solvents	Samples	Zone (mm)	Mean	% Residual activity	Mean ± SD
-	Cell-free supernatant	20, 17 17, 17	18.5 17.0	100.0 100.0	100.0 ± 0
Tris-HCl pH 8.0	diluent control	7, 7 8, 7	7.0 7.5	ND ND	ND
	unbound fraction	11, 9 10, 10	10.0 10.0	32.0 36.4	34.2 ± 3.08
	pooled fraction 23-27	11, 13 14, 11	12.0 12.5	48.0 59.1	53.5 ± 7.84
Sodium acetate buffer pH 5.0	diluent control	8, 7 8, 7	7.5 7.5	ND ND	ND
	unbound fraction	9, 9 9, 10	9.0 9.5	24.0 31.8	27.9 ± 5.53
	pooled fraction 23-27	11, 14 12, 12	12.5 12.0	52.0 54.5	53.3 ± 1.80
MRS broth	diluent control	7, 7 7, 7	7.0 7.0	ND ND	ND
	unbound fraction	10, 10 11, 10	10.0 10.5	32.0 40.9	36.5 ± 6.30
	pooled fraction 23-27	14, 12 13, 12	13.0 12.5	56.0 59.1	57.5 ± 2.18
mMRS broth	diluent control	8, 7 7, 7	7.5 7.0	ND ND	ND
	unbound fraction	10, 11 9, 11	10.5 10.0	36.0 36.4	36.2 ± 0.25
	pooled fraction 23-27	13, 12 12, 13	12.5 12.5	52.0 59.1	55.5 ± 5.01

ND, not determined

Table D9 The inhibition zone demonstrated by the partially purified bacteriocin of B63/8 in each solvent

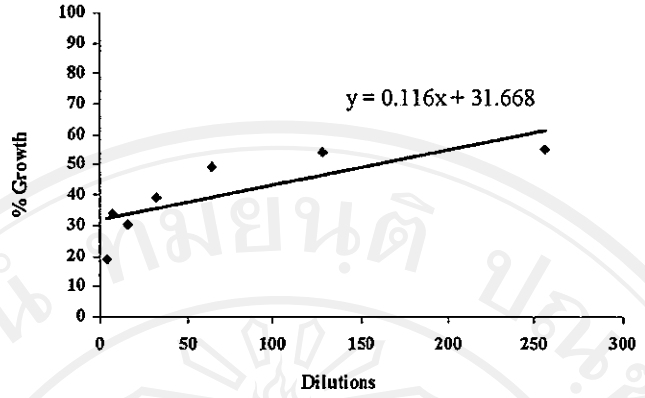
Solvents	Samples	Zone (mm)	Mean	% Residual activity	Mean ± SD
-	Cell-free supernatant	20, 18	19.0	100.0	100.0 ± 0
		18, 19	18.5	100.0	
Tris-HCl pH 8.0	Control	7, 7	7.0	ND	ND
		8, 7	7.5	ND	
	Unbound fraction	10, 9	9.5	26.9	29.5 ± 3.60
		11, 9	10.0	32.0	
	Pooled fraction 23-27	12, 12	12.0	46.1	49.1 ± 4.14
		13, 12	12.5	52.0	
Sodium acetate buffer pH 5.0	Control	8, 7	7.5	ND	ND
		8, 7	7.5	ND	
	Unbound fraction	10, 9	9.5	26.9	27.5 ± 0.77
		10, 19	9.5	28.0	
	Pooled fraction 23-27	11, 11	11.0	38.5	39.2 ± 1.10
		12, 10	11.0	40.0	
MRS broth	Control	7, 7	7.0	ND	ND
		7, 7	7.0	ND	
	Unbound fraction	9, 10	9.5	26.9	29.5 ± 3.60
		10, 10	10.0	32.0	
	Pooled fraction 23-27	16, 15	15.5	73.1	70.5 ± 3.57
		14, 15	14.5	68.0	
mMRS broth	Control	8, 7	7.5	ND	ND
		7, 7	7.0	ND	
	Unbound fraction	8, 10	9.0	23.1	21.5 ± 2.17
		8, 9	8.5	20.0	
	Pooled fraction 23-27	14, 12	13.0	53.8	54.9 ± 1.53
		13, 13	13.0	56.0	

ND, not determined

Table D10 Bacteriocin unit of each sample of B85/4 against *P. gingivalis* W50

Sample	Fold-dilution	Turbidity at 590 nm	Mean	Blank-corrected turbidity	% growth	Titer of 50% growth	Bacteriocin unit (BU/ml)
Blank (mMRS broth+BHI broth)	ND	0.059 0.059	0.059	0	0	0	0
P. gingivalis culture	ND	0.762 0.758	0.760	0.701	100	ND	ND
Supernatant	4	0.186 0.274	0.190	0.131	18.62	158	6,320.0
	8	0.256 0.393	0.297	0.238	33.88		
	16	0.279 0.267	0.273	0.223	30.527		
	32	0.316 0.351	0.334	0.275	39.157		
	64	0.402 0.409	0.406	0.347	49.428		
	128	0.395 0.482	0.439	0.380	54.136		
	256	0.348 0.439	0.444	0.385	54.849		
Ammonium sulphate precipitate	4	0.225 0.246	0.236	0.177	25.18	55	2,200.0
	8	0.317 0.269	0.293	0.234	33.38		
	16	0.344 0.376	0.360	0.301	42.94		
	32	0.399 0.426	0.413	0.354	50.43		
	64	0.584 0.422	0.503	0.444	63.34		
	128	0.654 0.567	0.611	0.552	78.67		
	256	0.654 0.607	0.631	0.572	81.52		
Pooled fraction 25-29	4	0.236 0.314	0.275	0.216	30.81	485	19,400.0
	8	0.230 0.274	0.252	0.193	27.53		
	16	0.296 0.328	0.312	0.253	36.09		
	32	0.281 0.291	0.286	0.227	32.38		
	64	0.283 0.294	0.289	0.230	32.74		
	128	0.348 0.296	0.322	0.263	37.52		
	256	0.358 0.327	0.343	0.284	40.44		

The titer of 50% growth calculation was performed by equilibration; supernatant, y=0.116x+31.668; ammonium sulphate precipitate, y=0.2062x+38.672; pooled fraction 23-27 y=0.0623x+36.844



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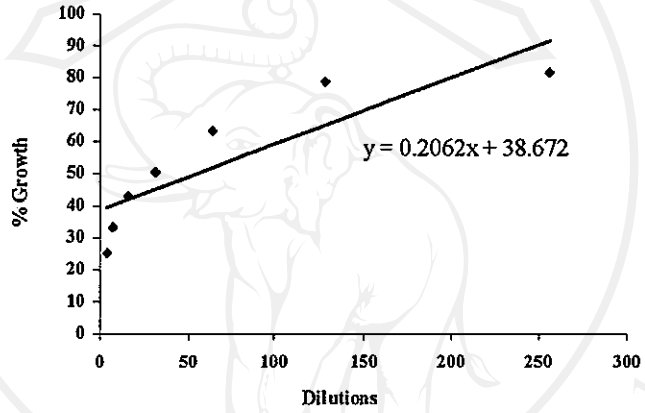

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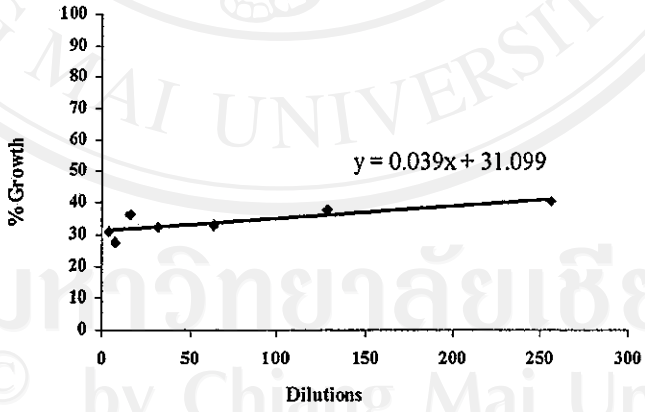


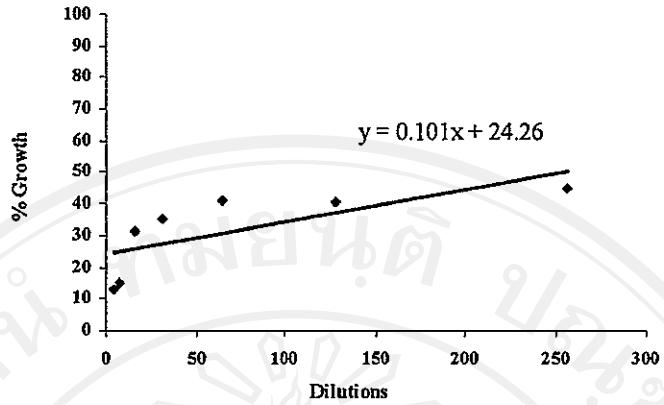
(c)

Figure D1 Bacteriocin curve of each sample of B85/4 against *P. gingivalis* W50, the x axis was represented as two-fold dilution titer of sample in the appropriate diluent and the y axis was represented as the percent growth of *P. gingivalis*; (a) supernatant, (b) ammonium sulphate precipitate, (c) pooled fraction 23-27)

Table D11 Bacteriocin unit of each sample of B282 against *P. gingivalis* W50

Samples	Fold-dilution	Turbidity at 590 nm	Mean	Blank-corrected turbidity	% growth	Titer of 50% growth	Bacteriocin unit (BU/ml)
Blank (MRS broth+BHI broth)	ND	0.049 0.053	0.051	0	0	0	0
P. gingivalis culture	ND	0.753 0.751	0.752	0.701	100	ND	ND
Supernatant	4	0.167 0.117	0.142	0.091	12.98	255	10,200.0
	8	0.146 0.163	0.155	0.104	14.76		
	16	0.263 0.281	0.272	0.221	31.53		
	32	0.288 0.307	0.298	0.247	35.16		
	64	0.353 0.325	0.339	0.288	41.08		
	128	0.309 0.362	0.336	0.285	40.58		
	256	0.326 0.407	0.367	0.316	45.00		
Ammonium sulphate precipitate	4	0.278 0.290	0.284	0.233	33.24	3	120.0
	8	0.415 0.368	0.392	0.341	48.57		
	16	0.433 0.461	0.447	0.396	56.49		
	32	0.361 0.395	0.378	0.327	46.65		
	64	0.530 0.542	0.536	0.485	69.18		
	128	0.483 0.583	0.533	0.482	68.76		
	256	0.361 0.528	0.445	0.394	56.13		
Pooled fraction 25-29	4	0.179 0.225	0.202	0.151	21.54	363	14,520.0
	8	0.287 0.267	0.277	0.226	32.24		
	16	0.274 0.295	0.285	0.234	33.31		
	32	0.361 0.303	0.332	0.281	40.08		
	64	0.306 0.348	0.327	0.276	39.37		
	128	0.326 0.315	0.321	0.270	38.44		
	256	0.388 0.312	0.350	0.299	42.65		

The titer of 50% growth calculation was performed by equilibration; supernatant, y=0.101x+24.26; ammonium sulphate precipitate, y=0.0591x+49.836; pooled fraction 23-27, y=0.0504x+31.717



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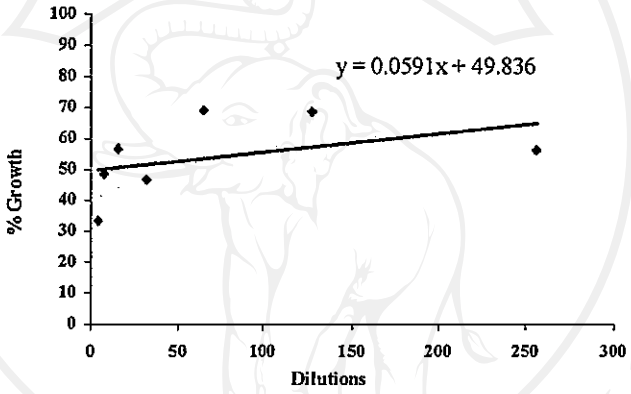

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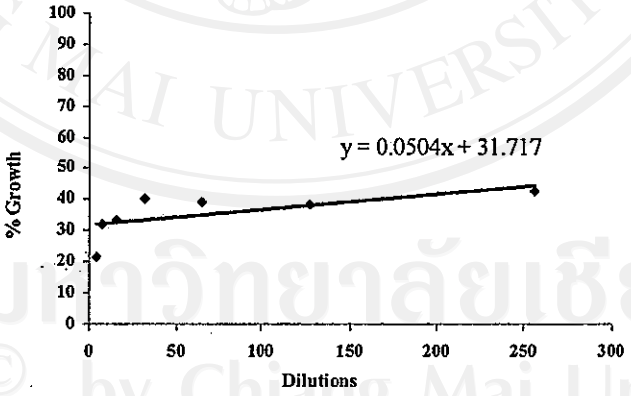


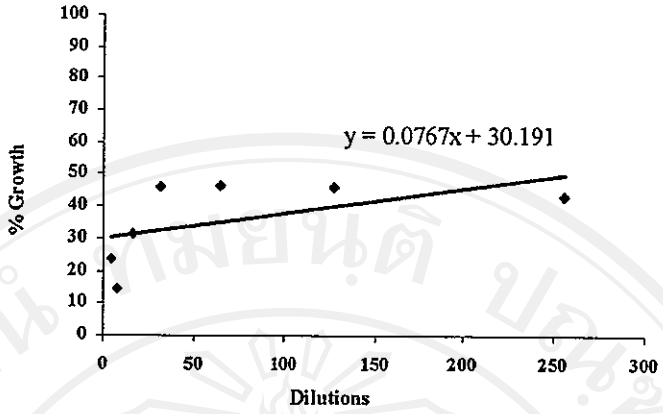
(c)

Figure D2 Bacteriocin curve of each sample of B282 against *P. gingivalis* W50, the x axis was represented as two-fold dilution titer of sample in the appropriate diluent and the y axis was represented as the percent growth of *P. gingivalis*; (a) supernatant, (b) ammonium sulphate precipitate, (c) pooled fraction 23-27

Table D12 Bacteriocin unit of each sample of B63/8 against *P. gingivalis* W50

Sample	Fold-dilution	Turbidity at 590 nm	Mean	Blank-corrected turbidity	% growth	Titer of 50% growth	Bacteriocin unit (BU/ml)
Blank (MRS broth+BHI broth)	ND	0.049 0.053	0.051	0	0	0	0
P. gingivalis culture	ND	0.753 0.751	0.752	0.701	100	ND	ND
Supernatant	4	0.294 0.137	0.216	0.165	23.47	258	10,320.0
	8	0.116 0.189	0.153	0.102	14.48		
	16	0.264 0.275	0.270	0.219	31.17		
	32	0.329 0.419	0.374	0.323	46.08		
	64	0.326 0.423	0.375	0.324	46.15		
	128	0.372 0.373	0.373	0.322	45.86		
	256	0.299 0.407	0.353	0.302	43.08		
Ammonium sulphate precipitate	4	0.253 0.267	0.260	0.209	29.81	14	560.0
	8	0.352 0.439	0.395	0.344	49.07		
	16	0.370 0.432	0.401	0.350	49.93		
	32	0.485 0.455	0.470	0.419	72.82		
	64	0.583 0.540	0.562	0.511	72.82		
	128	0.526 0.660	0.593	0.542	77.32		
	256	0.713 0.649	0.681	0.630	89.87		
Pooled fraction 25-29	4	0.280 0.143	0.212	0.161	22.90	363	14,520.0
	8	0.336 0.339	0.338	0.287	40.87		
	16	0.393 0.334	0.364	0.313	44.58		
	32	0.416 0.329	0.373	0.322	45.86		
	64	0.411 0.328	0.370	0.319	45.43		
	128	0.382 0.327	0.355	0.304	43.29		
	256	0.386 0.338	0.362	0.311	44.36		

The titer of 50% growth calculation was performed by equilibration; supernatant, y=0.0767x+30.191; ammonium sulphate precipitate, y=0.1917x+47.314; pooled fraction 23-27, y=0.0308x+38.806



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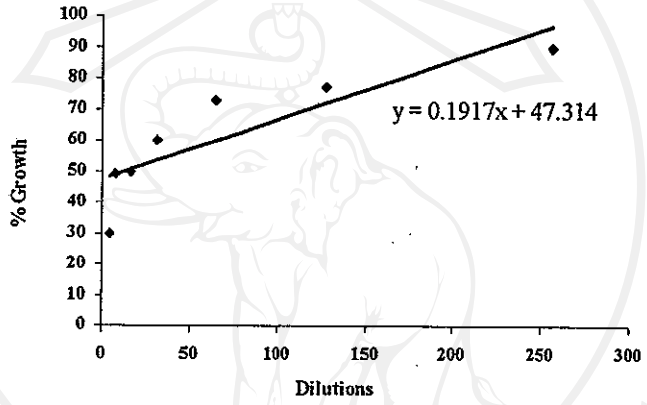

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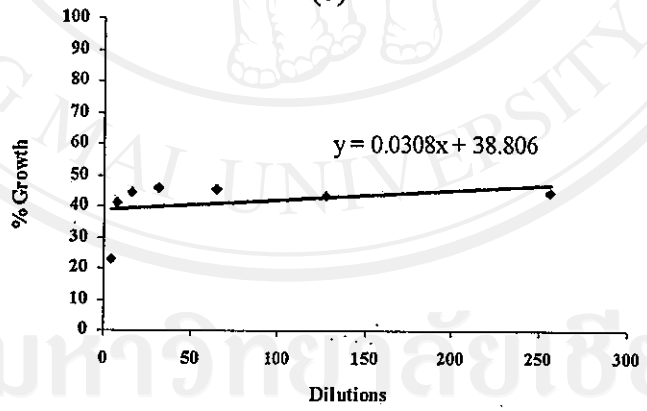

(c)

Figure D3 Bacteriocin curve of each sample of B63/8 against *P. gingivalis* W50, the x axis was represented as two-fold dilution titer of sample in the appropriate diluent and the y axis was represented as the percent growth of *P. gingivalis*; (a) supernatant, (b) ammonium sulphate precipitate, (c) pooled fraction 25-29

Table D13 The growth curve of *P. gingivalis* W50

Time	Count (dilution)	CFU/ml	Mean
0 min	17, 15 (10^{-4}) 15, 11 (10^{-4})	8.0×10^{6} 6.5×10^{6}	7.25×10^{6}
30 mins	10, 12 (10^{-4}) 18, 17 (10^{-4})	5.5×10^{6} 8.75×10^{6}	7.12×10^{6}
1 hr	4, 5 (10^{-4}) 1, 1 (10^{-4})	2.25×10^{6} 5.0×10^{5}	1.37×10^{6}
2 hrs	1, 2 (10^{-4}) 2, 3 (10^{-4})	7.5×10^{5} 1.25×10^{6}	1×10^{6}
3 hrs	4, 6 (10^{-4}) 6, 8 (10^{-4})	2.5×10^{6} 3.5×10^{6}	3×10^{6}
6 hrs	17, 14 (10^{-4}) 23, 15 (10^{-4})	7.75×10^{6} 9.5×10^{6}	8.62×10^{6}
12 hrs	7, 12 (10^{-4}) 15, 11 (10^{-4})	4.75×10^{6} 6.5×10^{6}	5.62×10^{6}

Table D14 The time killing assay against *P. gingivalis* W50 at the MIC of ampicillin

Time	Count (dilution)	CFU/ml	Mean	% Growth inhibition
0 min	17, 15 (10^{-4}) 15, 11 (10^{-4})	8.0×10^{6} 6.5×10^{6}	7.25×10^{6}	0
30 mins	11, 9 (10^{-3}) 4, 12 (10^{-3})	5.0×10^{5} 4.0×10^{5}	4.5×10^{6}	93.68
1 hr	6, 5 (10^{-3}) 5, 10 (10^{-3})	2.75×10^{5} 3.75×10^{5}	3.2×10^{5}	76.36
2 hrs	11, 18 (10^{-2}) 12, 15 (10^{-2})	7.25×10^{4} 6.75×10^{4}	7×10^{4}	93.00
3 hrs	2, 9 (10^{-1}) 4, 10 (10^{-1})	2.75×10^{3} 3.5×10^{3}	3.1×10^{3}	99.90
6 hrs	1, 5 (10^{0}) 2, 7 (10^{0})	1.5×10^{2} 2.25×10^{2}	2×10^{2}	100
12 hrs	10, 8 (10^{0}) 6, 8 (10^{0})	4.5×10^{2} 3.5×10^{2}	4×10^{2}	100

Table D15 The time killing assay against *P. gingivalis* W50 at the MIC of B85/4's supernatant

Time	Count (dilution)	CFU/ml	Mean	% growth inhibition
0 min	17, 15 (10^{-4}) 15, 11 (10^{-4})	8.0×10^{6} 6.5×10^{6}	7.25×10^{6}	0
30 mins	11, 10 (10^{-4}) 7, 11 (10^{-4})	5.25×10^{6} 4.5×10^{6}	4.87×10^{6}	31.58
1 hr	8, 9 (10^{-3}) 9, 10 (10^{-3})	4.25×10^{5} 4.75×10^{5}	4.25×10^{5}	69.09
2 hrs	10, 5 (10^{-3}) 16, 9 (10^{-3})	3.75×10^{5} 3.75×10^{5}	3.75×10^{5}	62.50
3 hrs	6, 10 (10^{-2}) 12, 12 (10^{-2})	4.0×10^{4} 3.5×10^{4}	3.8×10^{4}	98.75
6 hrs	4, 12 (10^{-1}) 6, 8 (10^{-1})	4.0×10^{3} 3.5×10^{3}	3.8×10^{3}	99.57
12 hrs	9, 4 (10^{0}) 16, 10 (10^{0})	3.25×10^{2} 6.5×10^{2}	5×10^{2}	99.90

Table D16 The time killing assay against *P. gingivalis* W50 at the MIC of B85/4's the partially purified bacteriocin

Time	Count (dilution)	CFU/ml	Mean	% Growth inhibition
0 min	17, 15 (10^{-4}) 15, 11 (10^{-4})	8.0×10^{6} 6.5×10^{6}	7.25×10^{6}	0
30 mins	9, 15 (10^{-4}) 11, 4 (10^{-4})	6.0×10^{6} 3.75×10^{6}	4.87×10^{6}	31.58
1 hr	4, 7 (10^{-3}) 13, 13 (10^{-3})	2.75×10^{5} 6.5×10^{5}	4.63×10^{5}	66.36
2 hrs	5, 3 (10^{-3}) 4, 1 (10^{-3})	2.0×10^{5} 1.25×10^{5}	1.63×10^{5}	83.75
3 hrs	13, 14 (10^{-1}) 9, 18 (10^{-1})	6.75×10^{3} 6.75×10^{3}	6.8×10^{3}	99.78
6 hrs	7, 2 (10^{-1}) 6, 5 (10^{-1})	2.25×10^{3} 2.75×10^{3}	2.5×10^{3}	99.97
12 hrs	15, 8 (10^{0}) 7, 10 (10^{0})	5.75×10^{2} 4.25×10^{2}	5×10^{2}	99.99

Table D17 The time killing assay against P. gingivalis W50 at 2-folds MIC of B85/4's the partial purified bacteriocin

Time	Count (dilution)	CFU/ml	Mean	% Growth inhibition
0 min	17, 15 (10^{-4}) 15, 11 (10^{-4})	8.0×10^{6} 6.5×10^{6}	7.25×10^{6}	0
30 mins	8, 12 (10^{-4}) 14, 7 (10^{-4})	5.0×10^{6} 5.25×10^{6}	3.62×10^{6}	49.12
1 hr	12, 14 (10^{-3}) 16, 5 (10^{-3})	6.5×10^{5} 5.25×10^{5}	5.87×10^{5}	57.27
2 hrs	8, 10 (10^{-3}) 11, 9 (10^{-3})	4.5×10^{5} 5.0×10^{5}	4.75×10^{5}	52.50
3 hrs	7, 8 (10^{-1}) 6, 8 (10^{-1})	3.75×10^{3} 3.5×10^{3}	3.6×10^{3}	99.88
6 hrs	5, 4 (10^{-1}) 3, 3 (10^{-1})	2.25×10^{3} 1.5×10^{3}	1.9×10^{3}	99.98
12 hrs	11, 12 (10^{0}) 6, 15 (10^{0})	5.75×10^{2} 5.25×10^{2}	6×10^{2}	99.99

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PUBLICATIONS AND PRESENTATIONS

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