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APPENDICES

APPENDIX A

CULTURE MEDIA AND CHEMICAL PREPARATION

1. Culture media

1.1 Nutrient broth

Beef extract	3	g
Peptone	5	g
Distilled water	1000	ml

Sterile by autoclave at 121 °C 15 min

1.2 Nutrient agar

Beef extract	3	g
Peptone	5	g
Agar	20	g
Distilled water	1000	ml

Sterile by autoclave at 121 °C 15 min

1.3 Nutrient broth and nutrient agar with salt

Prepared as 1.1 and 1.2 and add 3% NaCl before sterile.

1.4 Yeast Malt borth

Malt extract	3	g
Yeast extract	3	g
Peptone	5	g
Glucose	10	g

Distilled water	1000	ml

Sterile by autoclave at 121 °C 15 min

1.5 Yeast Malt agar

Malt extract	3	g
Yeast extract	3	g
Peptone	5	g
Glucose	10	g
Agar	20	g
Distilled water	1000	ml

Sterile by autoclave at 121 °C 15 min

2. Chemical preparation

2.1 Mixed indicator

A : methyl red 0.001 g dissolved in 10 ml of ethyl alcohol

B : bromocresol green 0.001 g dissolved in 10 ml of ethyl alcohol

Mixed indicator = A : B as 1 : 1

2.2 Pepstatin A

1 mg of Pepstatin A was dissolved in 1 ml of ethanol / acetic acid (9 : 1) as a stock solution. The stock solution was stored at – 70 °C until use.

2.3 Bacitracin

500 units of Bacitracin was dissolved in 1 ml of sterile distilled water as a stock solution and stored at – 70 °C until use.

2.4 Colistin

10,000 units of Colitin was dissolved in 1 ml of sterile distilled water as a stock solution and stored at – 70 °C until use.

2.5 Ketokonazole

0.1 mg of Ketokonazole was dissolved in 1 ml of sterile distilled water as a stock solution and stored at – 70 °C until use.

2.6 Mobile phase for HPLC

Solution A : Dissolved 900 ml of acetonitrile and 100 ml of 1% trifluoroacetic acid and sonicate for 30 min before use.

Solution B : 1000 ml of 0.1% trifluoroacetic acid and sonicate for 30 min before use.

APPENDIX B

PROTEIN DETERMINATION BY BCA PROTEIN ASSAY KIT

Protein determination by microplate procedure

1. Pipette 25 µl of each protein standard or unknown sample replicate into a microplate well (working range = 20-2,000 µg/ml).
2. Add 200 µl of the working reagent* to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37 °C for 30 min
4. Cool plate to room temperature
5. Measure the absorbance at 540 nm on a plate reader for blank, protein standard and unknown sample
6. Prepare a standard curve by plotting the blank-corrected measurement for each protein standard with its concentration in µg/ml. Use the standard curve (figure B1) to determine the protein concentration of each unknown sample.

* working reagent

- BCA reagent A, containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide.
- BCA reagent B containing 4% cupric sulfate.
- Add 1 part of "B" to 50 part of "A" and mix before use.

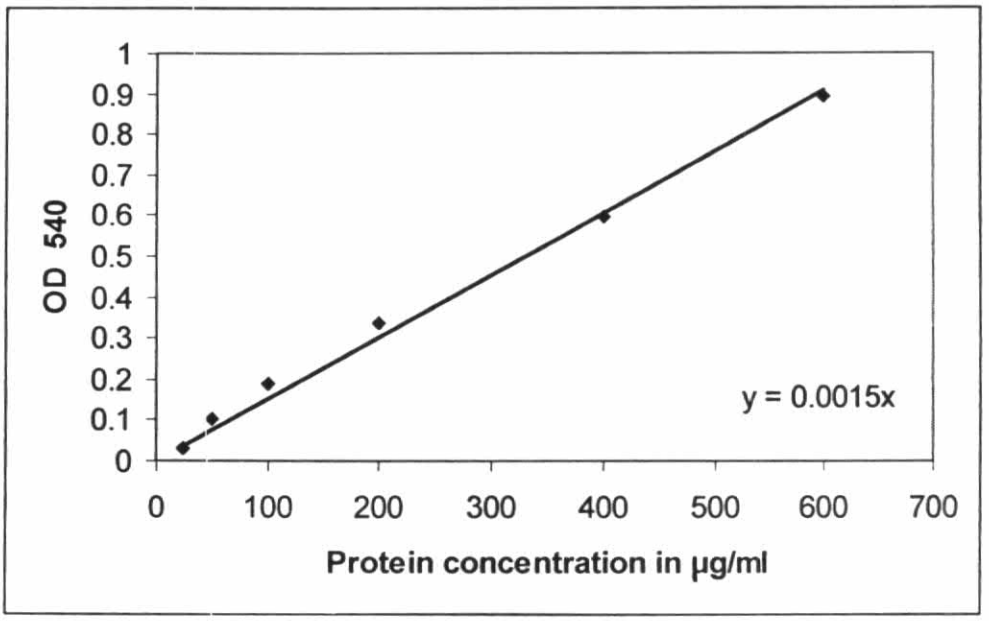


Figure B1 Standard curve of protein determination

APPENDIX C

ANTIMICROBIAL ACTIVITY BY PAPER DISC METHOD

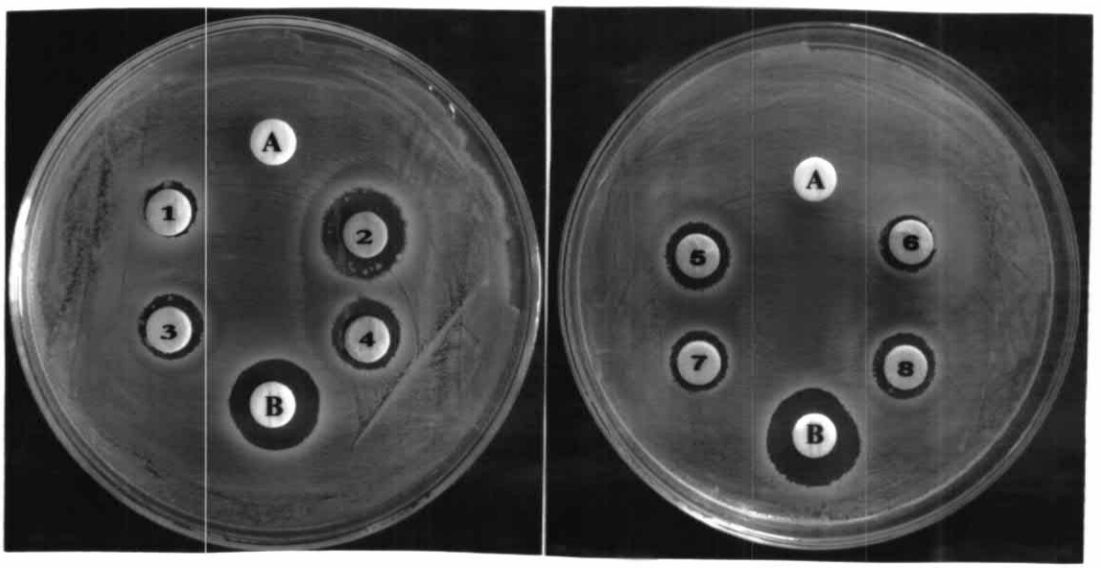


Figure C1 Antimicrobial activity of crude extract from non-challenged and challenged sandworms against *B. subtilis* (thick disc, ∅ 0.8 cm)

A = 0.1% acetic acid

B = bacitracin 0.45 units/disc

1 = control (non-challenged)

2 = after challenge 24 hr

3 = after challenge 24 hr + 12 hr

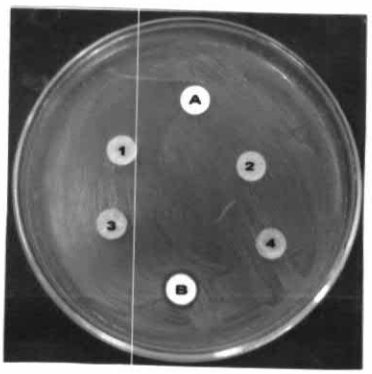
4 = after challenge 24 hr + 24 hr

5 = after challenge 24 hr + 48 hr

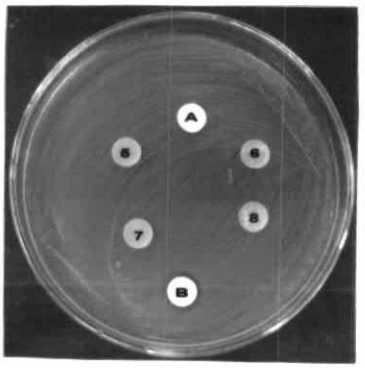
6 = after challenge 24 hr + 72 hr

7 = after challenge 24 hr + 96 hr

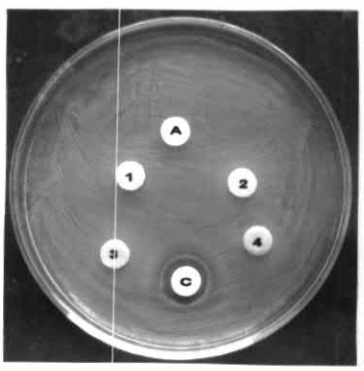
8 = after challenge 24 hr + 120 hr



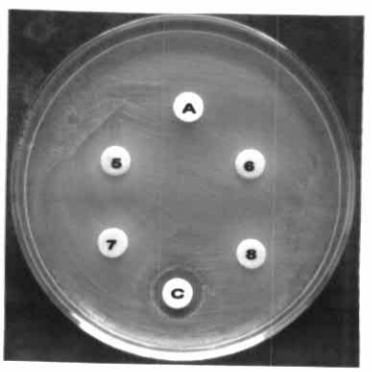
S. aureus



S. aureus



E.coli



E. coli

Figure C2 Antimicrobial activity of crude extract from non-challenged and challenged sandworms against *S. aureus* and *E. coli* (thick disc, ∅ 0.8 cm)

A = 0.1% acetic acid

B = bacitracin 0.45 units/disc

C = colistin 5000 units/disc

1 = control (non-challenged)

2 = after challenge 24 hr

3 = after challenge 24 hr + 12 hr

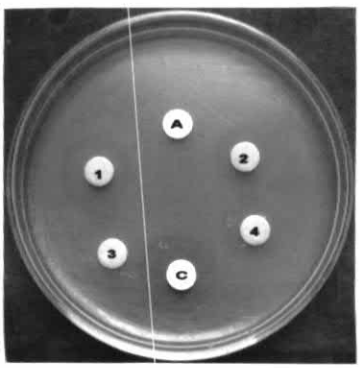
4 = after challenge 24 hr + 24 hr

5 = after challenge 24 hr + 48 hr

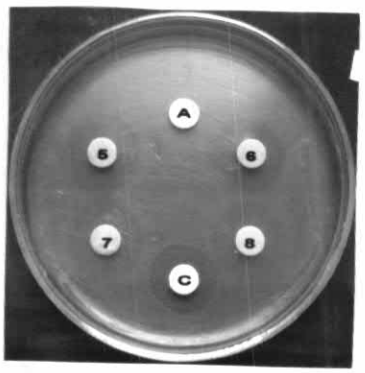
6 = after challenge 24 hr + 72 hr

7 = after challenge 24 hr + 96 hr

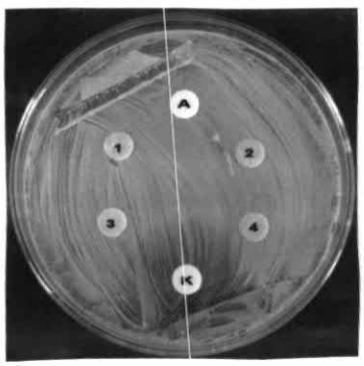
8 = after challenge 24 hr + 120 hr



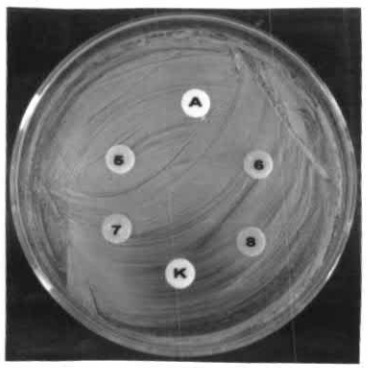
V. harvevi



V. harvevi



C. albicans



C. albicans

Figure C3 Antimicrobial activity of crude extract from non-challenged and challenged sandworms against *V. harveyi* and *C. alibicans* (thick disc, ∅ 0.8 cm)

A = 0.1% acetic acid
C = colistin 5000 units/disc
K = ketoconazole 0.1 mg/disc
1 = control (non-challenged)
2 = after challenge 24 hr
3 = after challenge 24 hr + 12 hr
4 = after challenge 24 hr + 24 hr
5 = after challenge 24 hr + 48 hr
6 = after challenge 24 hr + 72 hr
7 = after challenge 24 hr + 96 hr
8 = after challenge 24 hr + 120 hr

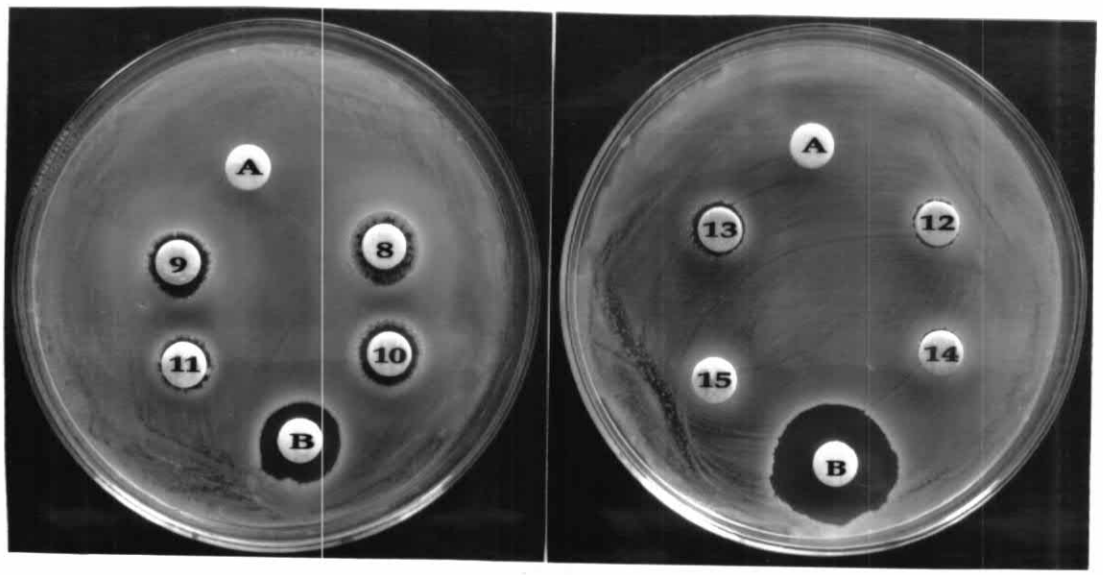


Figure C4 Antimicrobial activity of peptide from gel-filtration fraction (each fraction) against *B. subtilis* (thick disc, ∅ 0.8 cm)

A = 0.1% acetic acid

B = bacitracin 0.45 units/disc

Number = fraction number

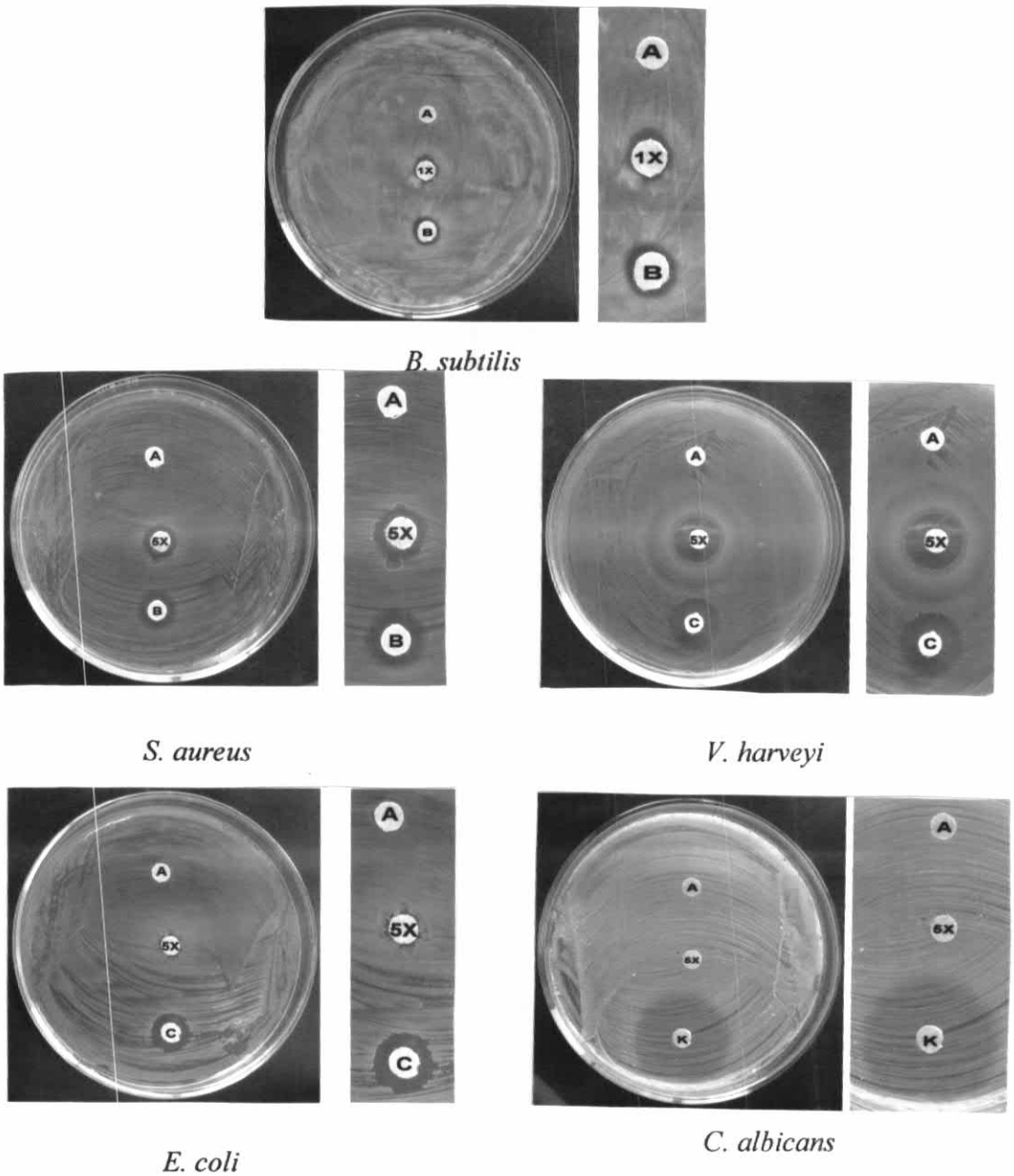


Figure C5 Antimicrobial activity of peptide from concentrated gel-filtration fraction against *B. subtilis* (1X), *S. aureus*, *E. coli*, *V. harveyi* and *C. albican* (5X) (thin disc, ∅ 0.6 cm)

A = 0.1% acetic acid

B = bacitracin 0.045 units/disc in *B. subtilis* and 0.250 units/disc in *S. aureus*

C = colistin 500 units/disc

K = ketoconazole 0.01 mg/disc

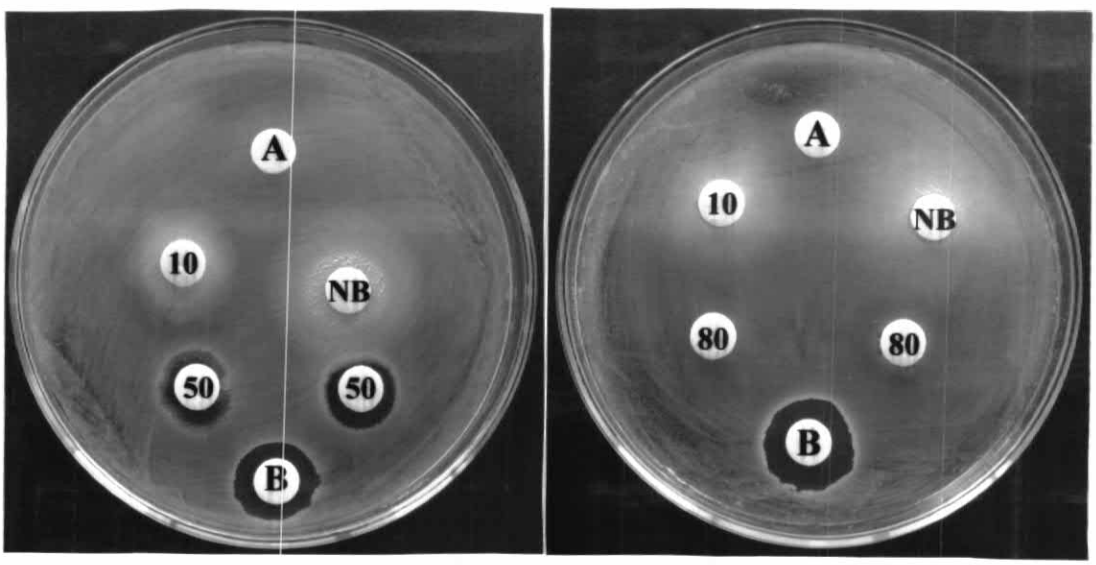


Figure C6 Antimicrobial activity of peptide from SPE C18 against *B. subtilis* (thick disc, ∅ 0.8 cm)

A = 0.1% acetic acid

B = bacitracin 0.45 units/disc

NB = Non-bound fraction

10 = eluted 10% ACN fraction

50 = eluted 50% ACN fraction

80 = eluted 8 % ACN fraction

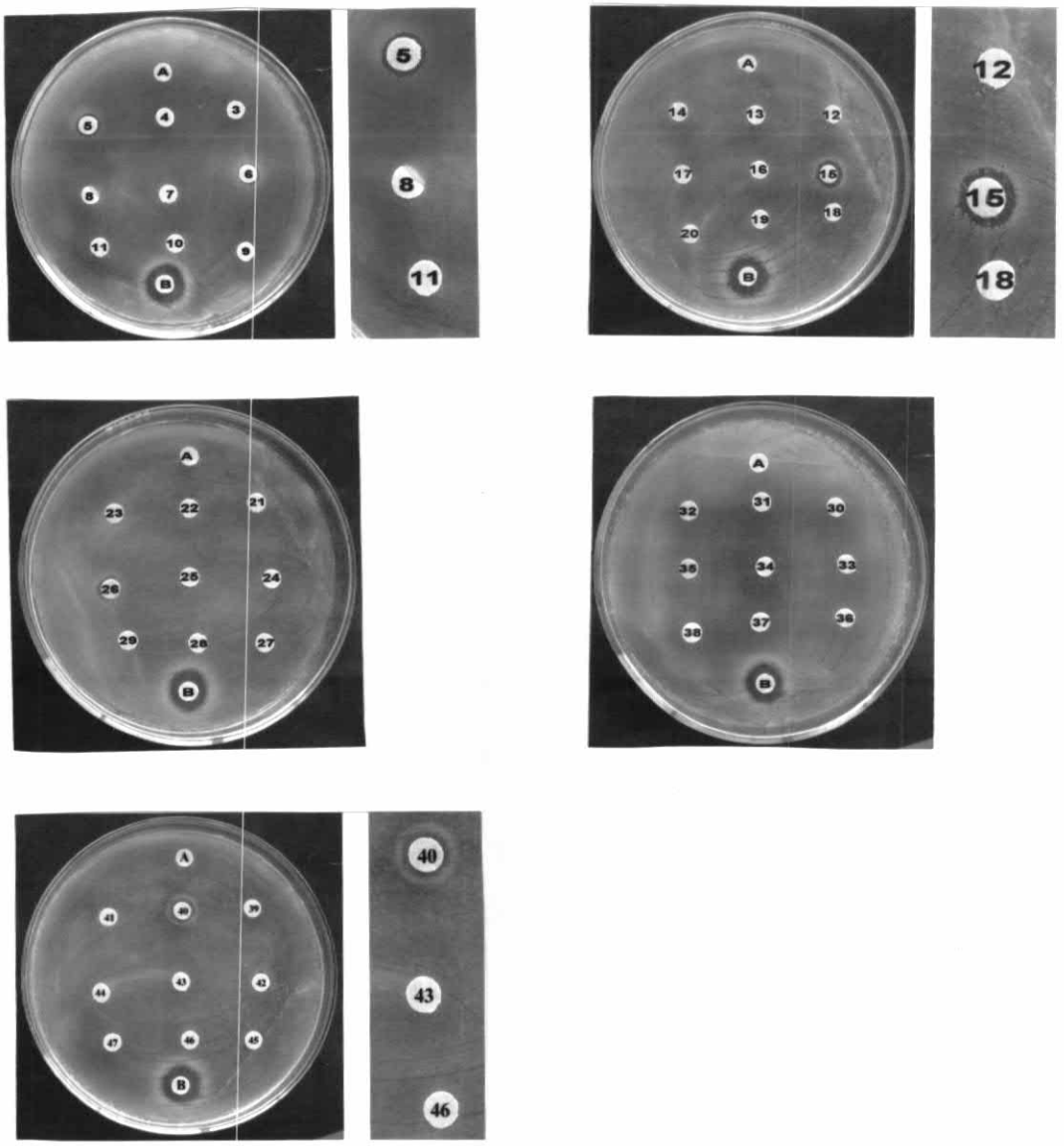


Figure C7 Antimicrobial activity of peptide from semi-preparative RP-HPLC against *B. subtilis* (thin disc, ∅ 0.6 cm)

A = 0.1% acetic acid

B = bacitracin 0.045 units / disc

Number = fraction number of peptide which eluted from RP-HPLC

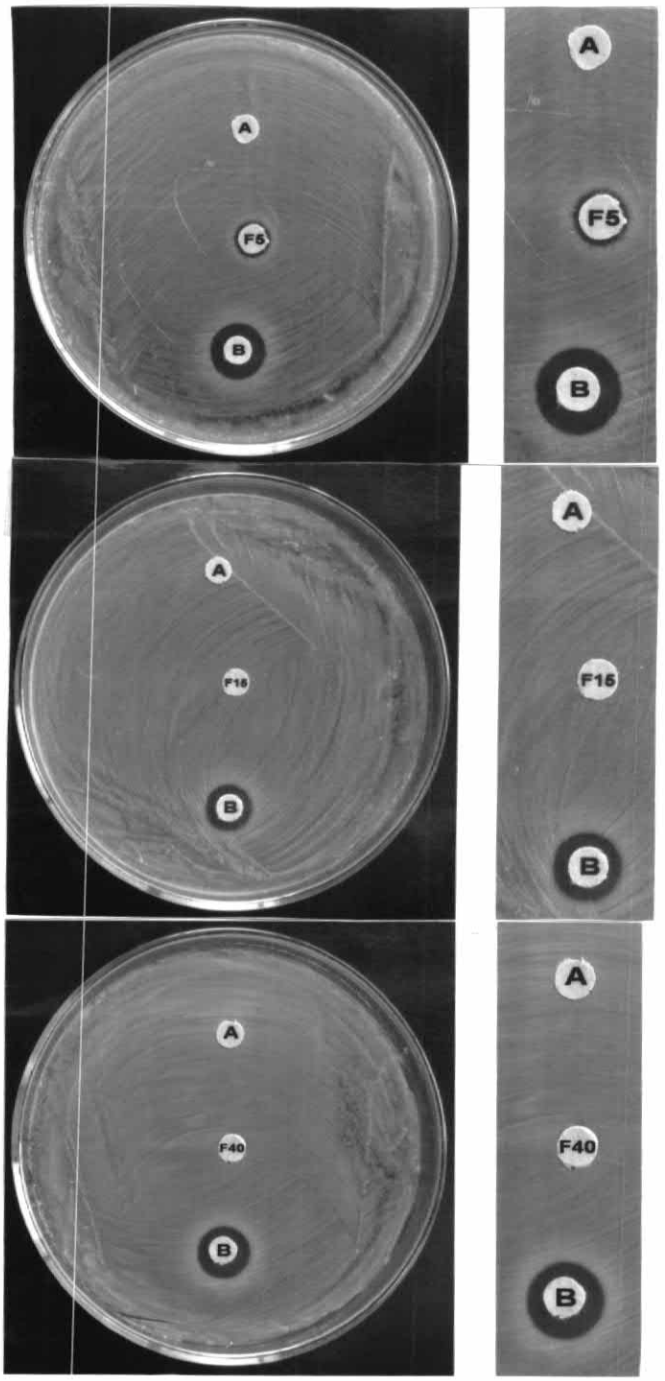


Figure C8 Antimicrobial activity of peptide from AMP - F5(P), AMP - F15(P) and AMP - F40(P) against *B. subtilis* (thin disc, ∅ 0.6 cm)

A = 0.1% acetic acid

B = bacitracin 0.045 units / disc

F5 = AMP - F5(P)

F15 = AMP - F15(P)

F40 = AMP - F40(P)

BIOGRAPHY

Mrs. Supissara Techaprempreecha was born on January 28, 1970 in Bangkok, Thailand. She received Bachelor of Science degree from Department of Biology, Faculty of Science, Silpakorn University in 1991 and Master Degree of Science (Biochemistry) from Chulalongkorn University, Bangkok, Thailand in 1995. She studies for the Degree of Doctor of Philosophy Program in Biotechnology at Biotechnology program, Faculty of Science, Chulalongkorn University since 2003.

Academic presentation :

Techaprempreecha, S., Khongchareonporn, N., Chaicharoenpong, C., Aranyagananda, A., Chunhabandit, S. and Petsom, A. Proximate composition of farmed and wild sandworms *Perinereis nuntia* Savigny. 4th International Greece Biotechnology Forum (4th IGBF). February 2-3, 2008, Athens, Greece.