REFERENCE

1. Whitmore, A., and C. S. Krishnaswami, " An account of the discovery of a hitherto undiscribed infective disease occuring among the population of Rangoon., " Indian. Med. Gaz., 47, 262-267, 1912.

2. Ellison, D. W., H. J. Baker., and M. Mariappan., " Melioidosis in Malaysia I. A method for isolation of P. pseudomallei from soil and surface water, " Am. J. Trop. Med., 18, 694-697, 1969.

3. Struss, J. M., M. G. Grove., M. Mariappan., and D. W. Ellison ., " Melioidosis in Malaysia II. distribution of Pseudomanas pseudomallei in soil and surface water., " Amer. J. Trop. Med., 18, 698-702, 1969.

4. Howe, C, A. Sampath., and M. Spotnitz, " The Pseudomallei Group : A review, " J. Infect. Dis., 124, 598-606, 1971.

5. Kishimoto, R. A., G. L. Brown., E. B. Blair., and D. Wenkheimer., " Melioidosis : Serologic Studies on US Army Personnel Returning from Southeast Asia, " Mitt. Med., Aug. 694-698, 1971.

6. Ashdown, L. R., and R. W. Guard , " The prevalence of human Melioidosis in Northern Queenland, " Am. J. Trop. Med. Hyg., 33, 474-478, 1984.

7. Strauss, J. M., A. D. Alexander, G. Rapmund, E. Gan, and A. E. Doresey, " Melioidosis III. Antibodies to Pseudomallei in human population, " Am. J. Trop. Med., 18, 703-707, 1969.

8. Rubin, H. L., A. D. Alexander, and R. H. Yager, " Melioidosis a military problem ? " Milit.Med., 128, 523-542, 1963.

9. Remington, R. D. " Melioidosis in North Queenland, " Med. J. Aust. 1, 50-53, 1962.

10. Thin, R. N. T., M. Brown, J. B. Stewart, and C. T. Garrett, " Melioidsis : A report of ten cases, " Quart. J. Med., 39, 115-127, 1970.

11. Patamasucon, P., U. B. Schaad, and J. D. Nelson, " Medical progress : Melioidosis, " J. Pediatr., 100, 175-182, 1982.

12. Nigg, C., " Serological studies on subclinical Melioidosis, " J.Immunol., 91, 18-28, 1963.

13. National workshop on Melioidosis organized by the Infectious Disease Association of Thailand at Ambassador Hotel, Bangkok, Thailand. Nov. 23-24, 1985.

14. Piggott, J. A., and L. Hochholzer., " Human Melioidosis, " Arch.Path., 90, 101-111, 1970.

15. Guard, R. W., F. A. Khafagi, M. C. Brigden, and L. R. Ashdown, " Melioidosis in far North Queenland. A clinical and epidemioligical review of twenty cases," Amr. J. Trop. Med. Hyg. 33, 467-473, 1984.

16. Spotniz, M., J. Rudnitzky and J. J., Rambaud , " Melioidosis pneumonitis nine cases of a benign from of Melioidosis, " JAMA., 202, 126, 1967.

17. Nigg, C., R. J. Heckly and M. Colling, " Toxin produced by Malleomyces pseudomallei, " Proc. Soc. Expt. Biol. Med., 89, 17-20, 1955.

18. Rapaport, F. T., J. W. Millar and J. Ruch " Endotoxic properties of Pseudomonas pseudomallei, " Arch. Path. 71,429-436, 1961.

19. Lusby, M ., H. B., Levine " An intracellular Mortality enhancing Material from Malleomyces pseudomallei, " J. Immunol., 80,446-453,1958.

20. Levine, H. B., O. G. Lein and R. L. Maurer., " Motality enhancing polypeptide constituents from Pseudomonas pseudomallei, " J. Immunol., 83, 468-477, 1959.

21. Liu, P. V., " Survey of Hemolysin production among species of pseudomonads, " J. Bacteriol., 74, 717-727, 1957.

22. Heckly, R. J., C. Nigg, " Toxins of Pseudomonas pseudomallei II. Characterization, " J. Bacteriol., 76, 427-436, 1958.

23. Ismail, G., M, N. Embi , O. Omar, J. C. Allen and C. J. Smith, " A competitive immunosorbent assay for detection of Pseudomonas pseudomallei exotoxin, " J.Med.Microbiol., 23, 353-357, 1987.

24. Heckly, R., " Differentiation of Exotoxin and other Biologically Active Substances in Pseudomonas pseudomallei Filtrates, " J. Bacteriol., 88, 1730-1736, 1964.

25. Heckly, R. J., " Toxin of Pseudomonas " Microbial Toxin ,vol.III, ed. Samual. J. Ajl. et. al. ,p. 473-490 ,Academic press N.Y. ,London, 1970

26. Morihara, K., " Pseudomanas Proteinase I. Purification and general properties, " Biochim. Biophys. Acta., 73, 113-124, 1963.

27. Lyerly, D., and A. Kreger, " Purification and characterization of

a Serrata marcescens metalloprotease, " Infect. Immun., 24, 411-421, 1979.

28. Miroshi, N., C., Shimizu, S. Miyoshi., " Purification and characterization of Vibrio vulnificus Protease, " Microbiol. Immunol., 31(1), 13-25, 1987.

29. Rosenfeld, J. S., et. al., " A protease from Legionella pneumophila with cytotoxic and dermal ulcerative, " FEMS microbiol. Lett., 37, 51-58, 1986.

30. Holder, I. A., C. G. Haidaris, " Experimental studies of the pathogenesis of infection due to Pseudomonas aeruginosa : extracellular protease and elastase as in vivo virulence factors," Can. J. Microbiol., 25, 593-599, 1979.

31. Wretlind, B., and O. R. Pavlovkis, " The Role of Proteases and Exotoxin A in the Pathogenicity of Pseudomonas aeruginosa Infections," Scan. J. Infect. Dis. Suppl., 29, 13-19, 1981.

32. Kamata, R., T.Yamamoto, K. Matsumoto and H. Maeda., " A serratial protease causes vascular permeability reaction by activation of the Hageman factor-dependent pathway in guinea pigs, " Infect. Immun., 48, 747-753, 1985.

33. Miyoshi, S., and S. Shinoda, " Role of the Protease in the permeability Enhancement by Vibrio vulnificus, " Microbiol. Immunol., 32(10), 1025-1032, 1988.

34. Matumoto, K.,T. Yamamoto, R. Kamata and H. Maeda, " Pathogenesis of serratial infection : activation of the Hageman factor-prekallikrein cascade by serratial protease ", J. Biochem., 96, 739-749, 1984.

35. Finkelstein, R. A., M. B. Filkelstein, and R. Holt " Vibrio cholerae hemagglutinin /lectin/protease/hydrolyzes fibronectin and ovomucin : F. M. Burnet revisited," Proc. Natl. Acad. Sci.USA., 80, 1092-1095, 1983.

36. Finkelstein, R. A., and L. F. Hanne., " Purification and Characterization of soluble Hemagglutinin (Cholera lectin) Produced by Vibrio cholerae, " Infect. Immun., 36, 1199-1208, 1982.

37. Crowther, R. S., N. W. Roomi, R. E. F. Fahim and J. F. Foortner " Vibrio cholerae metalloprotease degrades intestinal mucin and facilitates enterotoxin induced secretion from rat Intestine, " Biochim. Biophy. Acta., 924, 393-402, 1987.

38. Riepe, S. P., J. Goldstein and D. H. Alpers " Effect of Secreted Bacteriodes protease on human Intertinal Brush Border Hydrolase, " J. Clin. Invest., 66, 314-322, 1980.

39. Redfearn, M. S. and N. J. Palleroni, R. Y. Stainer., " A comparative study of Pseudomonas pseudomallei and Bacillus mallei, " J. Gen. Microbiol., 43, 293-313, 1966.

40. Haynes, W. C and H. Burkhdder, Bergey's Manual of Determinative Bacteriology :Breed R. S., Murray E. G. D.,and Smith, N. R., (Eds) p100, The Williams and Wilkins Co., Baltimore, 7 editions, 1957.

41. Nigg, C., and M. M. Johnton, " Complement fixation test in experimental clinical and subclinical melioidosis. " J. Bacteriol., 82, 159-168, 1961.

42. Rice, C. E., H. Konst, and R. C. Duthie, " Studies by complement fixation methods of malleins produced in broth and synthetic media I. relative immunizing activities in horses and Rabbit." Canadian J. of Compar Med., 15, 284-291, 1951.

43. Alexander, A. D., D. L. Huxsoll, and A. R. Warner.," Serological diagnosis of Human melioidosis with indirect hemagglutination and complement fixation test, " Appl. Microbiol., 20, 825-832, 1970.

44. Suwangool, Pa., Po. Suwangool, and S. Reinprayoon., : Proc. National workshop on Melioidosis, Nov 23-24 Bangkok, Thailand. p37-34, 1985.

45. Omar, A. R., " Pathology of melioidosis in pig goats and a horse, " J. Comp. Path., 73, 359-372, 1963.

46. Dannanberge, A. M., and E. M., Scott., " Melioidosis : Pathogenesis and Immunity in Mice and Hamsters II. Studies with avirulent strains of Malleomyces pseudomallei. " Amer. J. Patho., 34, 1099-1121, 1958.

47. Dannanberge, A. M., and E. M. Scott., " Melioidosis : Pathogenesis and Immunity in Mice and Hamsters. I.Studies with virulent strains of Malleomyces pseudomallei, " J. Exper. Med., 107, 153-166, 1958.

48. Kishimoto. R. A., and W. C. Evenland., " Interaction between P.pseudomallei and culture rabbit peritoneal macrophage, " Can. J. Microbiol., 22, 1307-1311, 1976.

49. Nigg, C., J. Ruth., E. Scott., and K. Noble., " Enhancement of virulence of Malleomyces pseudomallei, " J. Bacteriol., 71, 39-44, 1961.

50. Miller, R. Jr., and D. R. Clinger., " Melioidosis : Pathogenesis in rabbits : I. In Vivo studies in the rabbit ear chamber, " Arch. Path., 71 : 39-44, 1961.

51. Colling, M., C. Nigg., and R. J. Keckly., " Toxins of Pseudomonas pseudomallei I. production in vitro, " J. Bacteriol., 76, 422-426, 1958.

52. Morihara. K., " Comparative specificity of Microbial proteinase, " Adv. Enzy., ed. Alton Meister vol.41, p. 179-243, An interscience publication, 1974.

53. Apodaca, G., and J. H. Mckerrow. " Purification and Characterization of a 27,000-M_r extracellular proteinase from Trichophyton rubrum , " Infect. immun., 57, 3072-3080, 1989.

54. Hartley. B. S., " Proteolytic enzymes, " Ann. Rev. Biochem. 29, 45-72, 1960.

55. Jensen, S. E., L. Phillippe, J. Teng G. W. Stemke and J. N. Campbell., " Purification and characterization of exocellular proteases produced by a clinical isolate and a laboratory strain of Pseudomonas aeruginosa, " Can. J. Microbiol., 26, 77-86, 1980.

56. Seilfer , G., and E. Happer, " Collagenase " Method. Enz. ed. Gertrude E., Perlmann Laszolo Lorand , vol. 19,p.613-635, Academic Press N.Y. and London, 1970.

57. Schoellman, G., and E. Fisher., " A collagenase from Pseudomonas aeruginosa ", J. Biochim. Biophys. Acta., 122, 557, 1966.

58. Smyth. C. J., and J. P. Arbuthnott., " Properties of Clostridium perfringens (welchii) type-A α- toxin (phospholipase C)

purified by electrofocusing ", J. Med. Micro., 7, 41-66, 1974.

59. Naughton, M. A., and F. Sanger., " Purification and Specificity of Pancreatic Elastase , " Biochem. J. 78, 156-163, 1961.

60. Shotton, D. M., " Elastase " Method. enz. ,ed. Gertrude E. perlmann, Laszolo Lorand, vol.19, p.113-140, Academic Press N.Y. and London, 1970.

61. Wretlind, B., " Purification and properties of a Protease with Elastase Activity from Pseudomonas aeruginosa., " J. Gen. Micro., 103, 319-327, 1977.

62. Kawaharajo, K., J. Y. Homma, Y. Aoyama, and K. Morihara., " In vivo studies on protease and elastase from Pseudomonas areuginosa, " Jpn. J. Exp. Med., 45, 89, 1975.

63. Gray, L. and A. Kreger., " Microscopic characterization of rabbit lung damage produced by Pseudomonas aeruginosa proteases, " Infect. Immun., 23, 150-159, 1979.

64. Kreger, A. S., L. D. Gray., " Purification of Pseudomonas aeruginosa proteases and Microscopic Characterization of Pseudomonal protease Induced Rabbit Corneal Damage ", Infect. Immun., 19, 630-648, 1978.

65. Fisher. E. Jr., and J. H. Allen., " Mechanism of corneal destruction by pseudomonas proteases ", Am. J. opthalmol., 46, 246-255, 1958.

66. Kamata, R., K. Matsumoto, R. Okamura, T. Yamamoto, and H. Maeda, " The serratial 56K protease as a marjor pathogenic factor in serratial keratitis: clinical and experimental study ", Ophthalmology, 92, 1450-1459, 1985.

67. Miyoshi, N., S. Miyoshi, K. Sugiyama, Y. Suzuki, M. Furuta, and S. Shinoda.," Activation of the plasma kallikrein-kinin system by Vibrio vulnificus protease, " Infect. Immun., 55, 1936-1939, 1987.

68. Conlan, A. W., A. Baskerville and A. E. Ashworth, " Separation of Legionella pneumophila proteases and purification of a protease Which produces Lesions like Those of Legionnaires' Disease in Guenea Pig lung ", J. Gen. Micro., 132, 1565-1574, 1986.

69. Schumacher, G. F. B., and W. B. Schill., " Radial Diffusion in gel for Microdetermination of enzyme II. plasminogen activator Elatase and non specific proteases," Anal. Biochem., 48, 9-26, 1972.

70. Honda. T., K. Lertpocasombat., A. Hata and T. Miwatani., " Purification and Characterization of a Protease Produced by Vibrio chalerae Non-o1 and Comparison with a Protease of V. Cholerae 01, " Infect. Immun., 57, 2799-2803, 1989.

71. Sachar. L. A., et, al " Photometric Method for Estimation of Elastase Activity, " Proc.Soc.Exp.Biol. Med. 90, 323-326, 1955.

72. Lowry, O. H., N. J. Rosebrough., A. L. Farr., and R. J. Raudall., " Protein measurement with the Folin Phenol Reagent, " J. Biol. Chem., 193, 265-275, 1951.

73. Reisfeld R. A., U. J. Lewis and D. E. Williams., " Disc electrophoresis of basic proteins and peptides on polyacrylamide gel, " Nature., 195, 281-283, 1962.

74. Davis, B. J., " Disc electrophoresis II. Method and application to human serum proteins, " Ann. N. Y. Acad. Sci., 121, 404-427, 1964.

75. Andrew, P., " Estimation of molecular weights of proteins by Sephadex gel filtration, " Biochem.J., 91, 222-233, 1964.

76. Laemmli, U. K., " Cleavage of structural protein during the assembly of head of Bacteriophage T4, " Nature., 117, 680-685, 1970.

77. Morihara. K, and H. Tsuzuki, " Pseudomonas aeruginosa peptide peptidohydrolase III. some characters as a Ca^{2+}-metallo enzyme, " Biochim. Biophy. Acta., 92, 351-360, 1964.

78. Litchfield, C. A., and J. M. Prescott, " Regulation of proteolytic enzyme production by Aeromonas proteolytica I. Extracellular endopeptidase, " Can. J. Microbiol., 16, 17-22, 1970.

79. Heineken, F. G, and R. J. O. connor, " Continuous Cultures Studies on the Biosynthesis of Alkaline protease, Neutral protease and -Amylase by Bacillus subtilis NRRL-B3411, " J. Gen. Micro., 73, 35-44, 1972.

80. Long, S., M. A. Mothibeli, F. T. Robb and D. R. Woods, " " Regulation of extracellular Alkaline protease Activity by Histidine in collagenolytic Vibrio alginolyticus Strains, " J. Gen. Micro., 127, 193-199, 1981.

81. Sanyal, A. K., S. K. Das, and A. B. Banerjee, " Purification and partial characterization of an exocellular proteinase from Trichophyton rubrum, " Sabouraudia., 23, 165-178, 1985.

J. Gen. Microbiol., 73, 35-44, 1972.

82. Lindberg, R. A., L. D. Eirich, J. R. Price, L. Wolfinbarger, Jr., and H. Drucker, " Alkaline protease from Neurospora crassa: purification and partial characterization ", J. Biol. Chem., 256, 811-814, 1981

83. Auld, D. S., " Use of chelating agents to inhibit enzyme, " Methods in Enzy.,ed. Jame F. Riordan, Bert L. Vallee, vol. 158, p.110-114, Academic Press N.Y. London, 1988.

84. Mandl, I., " Collagenase and Elastase " , Adv. enz., 23, 163-264,1961

85. Oakley, C. L., " Relative toxicities and assay systems, " Microbial. toxins., ed. Samuel J. Ajl, et al. Vol.1, p.353-387, Academic Press N.Y. London,1970.

APPENDIX I

REAGENTS AND INSTRUMENTS

A. REAGENTS

Acrylamide (N,N-methylenebisacrylamide) (LKB, Sweden)

Ammonium sulfate (E.Merck, Darmstadt, W. Germany)

Ammonium persulfate (LKB, Sweden)

Azocasein (Sigma,Mo. U.S.A.)

Azocoll (Sigma,Mo. U.S.A.)

3-Aminopropionic acid (Sigma, Mo.,U.S.A.)

Beef extract (Gibco,U.S.A.)

Bis-acrylamide (LKB, Sweden)

Barium chloride (Baker's Analyzed, NY., U.S.A.)

Calcium chloride (Baker's Analyzed, NY.,U.S.A.)

Collagenase Type 1 A (Sigma, Mo., U.S.A.)

Coomasie brillant blue R 250 (Sigma,Mo.,U.S.A.)

Copper chloride (Baker's Analyzed, NY.,U.S.A.)

DEAE-cellulose (DE-52) (Whatman, U.S.A.)

Dipotassium phosphate (E.Merck, W.Germany)

Dialysis cellulose tubing ,WM cutoff 12,000-14,000
(Spectra/por, Spectrum,CA.U.S.A.)

Elastase Type 1 (Sigma,Mo. U.S.A.)

Elastin congo red (Sigma.Mo. U.S.A.)

Ethylenediaminetetraacetic acid (EDTA) (Sigma.Mo.,U.S.A.)

Glacial acetic acid (E.Merck.,W. Germany)

Glycine (Sigma, Mo.,U.S.A.)

Glycerol (E.Merck.,W.Germany)

Hydrochloric acid (E.Merck.,W.Germany)

p-Hydroxymercuribenzoate (Sigma.Mo.,U.S.A.)

Magnesium chloride (Baker's Analyzed,NY. U.S.A.)

Methanol (E.Merck.,W.Germany)

Mercury chloride (Baker's Analyzed.,NY.,U.S.A.)

Molecular weight markers for gel filtration (GF-1000)(Sigma.Ma.,U.S.A.)

Molecular weight markers for SDS-PAGE (low MW.) (Pharmacia,Sweden)

Potassium monophosphate (E.Merck.,W.Germany)

Phenylmethylsulfonylfluoride (Sigma.Mo.,U.S.A.)

1,10 phenanthroline monohydrate (Sigma.Mo.,U.S.A.)

Potassium chloride (E.Merck.,W.Germany)

Sodium dodecyl sulfate (Sigma.Mo.,U.S.A.)

Sodium hydroxide (E.Merck.,W.Germany)

Sephadex G-200 (Pharmacia,Sweden)

Riboflavin (Sigma.Mo.,U.S.A.)

N,N,N,N-tetramethylethylenediamine (TEMED) (LKB.,Sweden)

Trisma-base (Sigma.Mo.,U.S.A.)

Tris-HCl (Sigma.Mo.,U.S.A.)

Trichloreacetic acid (Sigma.Mo.,U.S.A.)

Trypsin (soy bean)inhibitor (Sigma.Mo., U.S.A.)

Zinc chloride (Baker's Analyzed, NY.,U.S.A.)

B. INSTRUMENTS

Fraction collector (2211 superRac,LKB, Bromma,Sweden)

Peristaltic pump (2232, microperpexs., LKB Bromma, Sweden)

Incubator (Type B-80, Memmert,W. Germany)

Shaker incubator (Lab-Line orbit environ-shaker,U.S.A.)

pH meter (Corning ,N.Y.,U.S.A.)

Ultrafiltration cell model 8050 (Amicon,Danver,U.S.A.)

Refrigerated superspeed centrifuge (Beckman J2-21,U.S.A.)

Magnetic stirrer (M21/1 Framo-Geratetechnik,W.Germany)

Spectophotometer (Beckman ACTA III UV-visible,U.S.A.)

Spectophotometer (HITACHI U-200, Japan)

APPENDIX II

REAGENTS AND PREPARATIONS

1. Media for preparation of protease

1.1 Glycerol beef extract broth (GB)

Beef extract (Gibco)	1 gm.
Glycerol	4 ml.
Phosphate buffer(0.005M) pH 7.0 to	100 ml.

1.2 Glycerol beef extract supplement with calcium chloride (GBC)

Beef extract (Gibco)	1 gm.
Glycerol	4 ml.
CaCl2 (0.05 M)	1 ml.
Phosphate buffer (0.005 M) pH 7.0 to	100 ml.

1.3 Beef extract broth (BB)

Beef extract	1 gm.
Phosphate buffer (0.005 M) pH 7.0 to	100 ml.

1.4 Glycerol beef extract broth supplement with 0.5 % glucose (GBG)

Beef extract (Gibco)	1 gm.
Glycerol	4 ml.
Glucose	0.5 gm.
Phosphate buffer (0.005 M) pH 7.0 to	100 ml.

All the medium was mixed well until dissolved completely and sterile by autoclaving at 121° C for 15 min.

2. Reagents for protease assay

2.1 Skim milk plate test

Solution 1	Skim milk	0.9 gm.
	Phosphate buffer pH 7.0	30 ml.
Solution 2	Noble agar	0.6 gm.
	Phosphate buffer pH 7.0	30 ml.

The mixture was heated until dissolved completely. Solution 1, 5 ml was mixed with solution 2, 5 ml ,then pour on petridisc which laid on the equilibrium level.

2.2 Azocasein test

2.2.1. 0.5 % azocasein

azocasein (Sigma)	0.5 gm.
Phosphate buffer pH 7.0	100 ml.

2.2.2. 0.5 % TCA

Trichloreacetic acid	5 gm.
D.W.	100 ml.

2.2.3 0.5 N NaOH

NaOH	2 gm.
D.W.	100 ml.

3. Reagents for Protein Estimation

3.1 Na_2CO_3, 2 % in NaOH, 0.1 M (solution A)

Ingradients per 200 ml

Sodium carbonate	4 gm.
0.1 M sodium hydroxide	200 ml.

3.2 $CuSO_4$ 5H2 O 0.5 % in $C_4H_4KNaO_6$ $4H_2O$, 1 % (solution B), Ingradients per 200 ml.

copper sulfate (1 %)	1	gm.
potassium sodium tartrate (2 %)	2	gm.
D.W. to	200	ml.

3.3 Solution C, Ingradients per 51 ml.

Solution A	50	ml.
Solution B	1	ml.

freshly prepared before used.

3.4 Solution D, Ingredients per 100 ml.

Folin Ciocalteus	50	ml.
D.W. to	100	ml.

Stored at 4° C in dark bottle.

4. Buffer solution

1. HCl-glycine 0.1 M pH 3.0

Glycine	7.5	gm.
adjust with 1 N HCl to pH 3.0		
D.W. to	100	ml.

2. HCl-glycine 0.1 M pH 4.0

Glycine	7.5	gm.
adjust with 1 N HCl to pH 4.0		
D.W. to	100	ml.

3. Acetic-acid-sodium acetate 0.1 M pH 5.0

Sodium acetate	0.14	gm.
1 M acetic acid	4.3	ml.
D.W. to	100	ml.

4. Phosphate buffer 0.1 M pH 6.0

0.5 M KH_2PO_4	17.5	ml.
0.5 M $K2HPO_4$	2.4	ml.
D.W. to	100	ml.

5. Phosphate buffer 0.1 M pH 7.0

0.5 M KH_2PO_4	7.8	ml
0.5 M KH_2PO_4	12.2	ml.
D.W. to	100	ml.

6. Tris-HCl buffer 0.1 M pH 8.0

Tris base	1.21	gm.
adjust with 1 N HCl to pH 8.0		
D.W. to	100	ml.

7. Tris-HCl buffer 0.1 M pH 9.0

Tris base	1.21	gm.
adjust with 1 N HCl to pH 9.0		
D.W. to	100	ml.

8. Sodium bicarbonate 0.2 M pH 10.0

$NaCO_3$	2.11	gm.
$NaHCO_3$	1.68	gm.
D.W. to	100	ml.

9. Phosphate buffer 0.01 M pH 7.0

Solution A

K_2HPO_4 (0.5 M)	68.04	gm.

Solution B

K_2HPO_4 (0.5 M)	87.09	gm.

Solution A ,7.7 ml, Solution 12.3 ml. and add D.W. to 100 ml.

5. Reagents for SDS-PAGE

5.1 Bis-acrylamide solution

Acrylamide	40	gm.
Bis-methyleneacrylamide	1.5	ml.
D.W. to	100	ml.

5.2 TEMED

TEMED	0.5	ml.
D.W. to	100	ml.

5.3 Buffer A (pH 8.8 ,store at 4° C)

Tris base	56.8	gm.
SDS	1.24	gm.
D.W. to	250	ml.

5.4 Buffer B (pH 6.8 ,store at 4° C)

Tris base	37.85	gm.
SDS	2.5	gm.
Adjust pH with HCl, D.W. to	250	ml.

5.5 0.5 % Ammonium persulfate

Ammonium persulfate	0.5	gm.
D.W. to	100	ml.

freshly prepared before used.

5.6 Electrode buffer running (stock solution)

Tris base	15.15	gm.
Glycine	72.0	gm.
SDS	5.0	gm.
D.W. to	100	ml.

Before used the electrode buffer is diluted 1:4.

5.7 Sample buffer

Tris-HCl	9.65	gm.
SDS	4.0	gm.
Glycerol	50.0	ml.
Bromphenol blue	2.0	mg.
D.W. to	100	ml.

5.8 Staining solution

Coomasie brillent blue R 250	0.75	gm.
Methanol	250	ml.
Glacial acetic acid	50	ml.
D.W. to	100	ml.

5.9 Destaining solution

Glacial acetic acid	100	ml.
Methanol	50	ml.
D.W. to	1000	ml.

6. Preparation of separating gel (10 %)

Bis-acrylamide	1.5	ml.
TEMED (0.5 %)	0.3	ml.
Buffer A	1.2	ml.
D.W.	1.5	ml.
0.5 % ammonium persulfate	1.5	ml.

7. Preparation of stacking gel (3 %)

Bis-acrylamide	1.0	ml.
TEMED (0.5 %)	1.0	ml.
Buffer B	1.0	ml.
D.W.	4.5	ml.
0.5 % ammonium persulfate	2.5	ml.

8. Polyacrylamide gel cationic system

(Reisfeld and Lewis and Williams)

Solution A	KOH 1 M	48 ml
	Gla. acetic acid	17.2 ml.
	TEMED	4 ml.
	D.W. to	100 ml.
Solution B	KOH 1 M	48 ml.
	Gla.acetic acid	2.9 ml.
	TEMED	0.45 ml.
	D.W. to	100 ml.
Solution C	Acrylamide	29.2 ml.
	Bis-acrylamide	0.8 ml.
	D.W. to	100 ml.
Solution D	Acrylamide	10 ml.
	Bis-acrylamide	2.5 ml.
	D.W. to	100 ml.
Solution E	Riboflavin	0.004 gm.
	D.W. to	100 ml.
Solution F	Ammonuim persulfate	0.28 gm.
	D.W. to	100 ml.

Electrode buffer (pH 4.5)

3-Aminopropionic acid	31.2 gm.
Gla. acetic acid	8.0 ml.
D.W. to	1000 ml.

Before use the electrode buffer is diluted 1:10

9. Preparation of lower gel 7.5 % (pH 4.3)

The above solution are mixed in following proportion:

A:C:H_2O:F = 1:1:1:4

Preparation of upper gel

Solution B:D:E:H2O = 1:2:1:4

For gel polymerization have to expose light about 1 hour.

10. Staining solution for polyacrylamide gels

1% Amido Black 10B in 7% acetic acid solution

Destaining solution : 7% acetic acid

11. Polyacrylamide gel electrophoresis (anionic system)

Solution A (pH 8.9)

1 N HCl	24.0 ml.
Tris base	18.3 ml.
TEMED	0.115 ml.
D.W. to	50 ml.

Solution B (pH 6.7)

1 N HCl	12 ml.
Tris base	1.495 ml.
TEMED	0.115 ml.
D.W. to	25 ml.

Solution C

Acrylamide	28.0 gm.
Bis-acrylamide	0.736 gm.
D.W. to	100 ml.

Solution D

Acrylamide	5.0 gm.
Bis-acrylamide	1.25 gm.
D.W. to	100 ml.

Solution E

Riboflavin	2	mg./ 50 ml.

Solution F

Ammonium persulfate	5	gm /100 ml.

Preparation of lower gel: The above solution are mixed in the following proportion: $A:C:H_2O:F = 1:2:1:4$

Preparation of upper gel: $B:D:E:H_2O = 1:2:1:4$

For the gel polymerization have to expose light about 1 hr.

Sample buffer : 50 % glycerol, bromphenol blue 0.1 %

Electrophoresis buffer (pH 8.3)

Tris base	6.0 gm.
Glycine	28.8 gm.
D.W. to	1000 ml.

Before use the electrode buffer is diluted 1:10.

12. Reagents for immunoelectrophoresis

12.1 Sodium barbital buffer (0.05 M), pH 8.0

Barbital sodium	47.6	gm.
1 N HCl	69.0	ml.
10 % NaN_3	4.2	ml.
D.W. to	4200	ml.
adjust pH to 8.2		

12.3 Agar gel (1.5 %)

Special agar Noble	1.5	gm
Sodium barbital buffer.	100	ml.

The gel was heated untill dissolved and pipette 2 ml. onto a glass slide, then allowed to cool.

12.4 Dialyzing solution

NaCl	4	gm.
$N_2B_4O_7$	4	gm.
D.W. to	100	ml.

12.5 Destaining solution

Methanol	75	ml.
Glacial acetic acid	60	ml.
D.W.	30	ml.

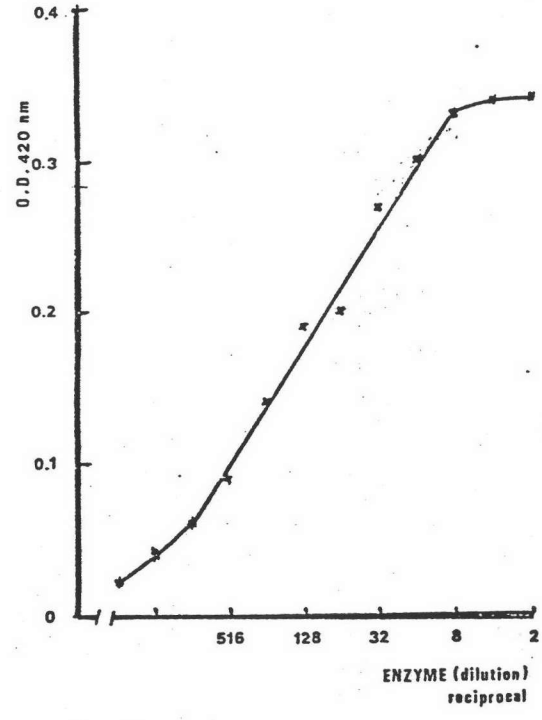

Fig. 18 Proteolytic activity against azocasein ; Plot O.D.420 nm. of azodye release versus reciprocal dilution of enzyme (*P. pseudomallei* crude filtrate).

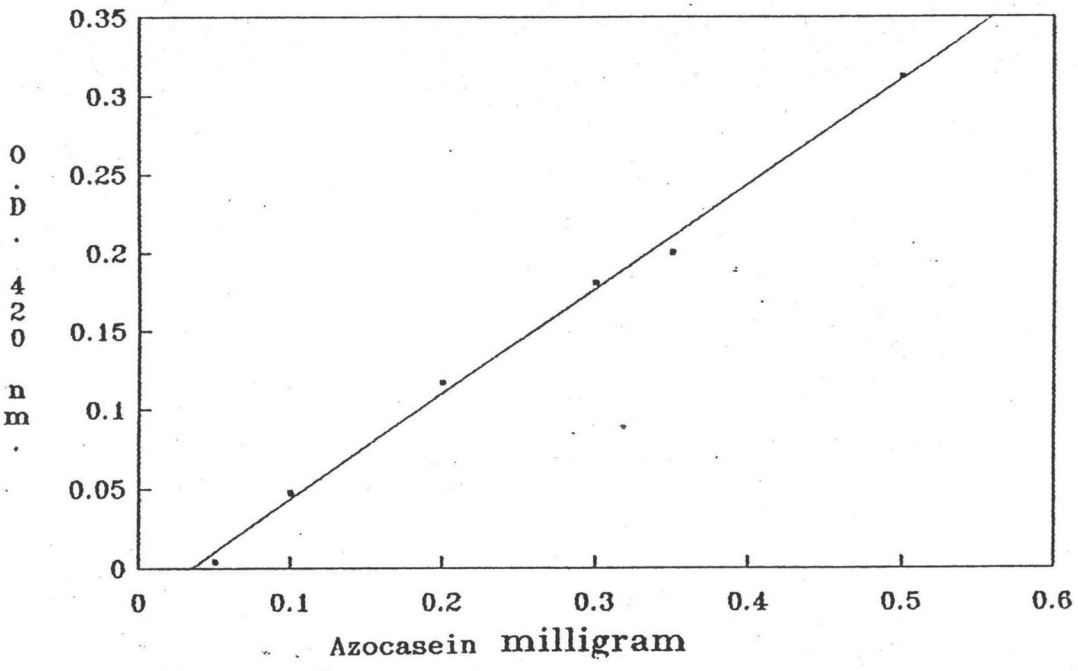

Fig. 19 Linear graph of azodye O.D. 420 nm against dilution of azocasein which was digested by trypsin 1 mg/ml.

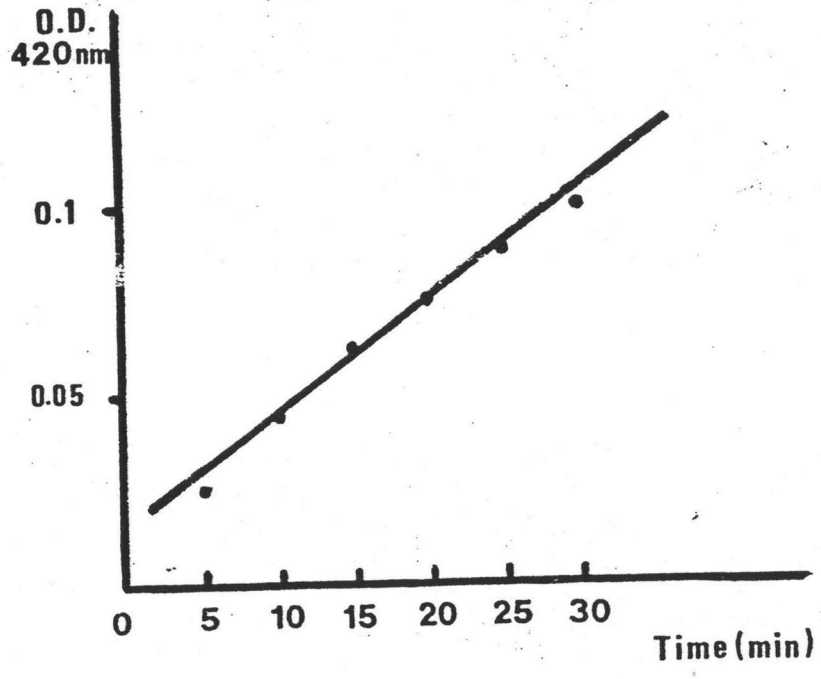

Fig. 20 Kinetic of trypsin 1 PU using azocasein as substrate,showed that 1 PU give 0.1 O.D. 420 nm ,30 min.

CURRICULUM VITAE

Miss Kanchalee Lertpocasombat was born on June 20, 1957, in Bangkok, Thailand. She graduated with the Bachelor of Science (Medical technology) degree from Chulalongkorn University in 1979. She obtained a diploma in Medical Microbiology from Institute for Medical reseach, Kuala lumpur, Malaysia and a certificate in Microbial Disease from the Research Institute for Microbial Diseases, Osaka University, Japan in 1980 and 1989 respectively. She has been working in Department of Microbiology; Bacteriology Unit,Faculty of Medicine, Chulalongkorn University since her graduation.