# REFERENCES


Aketagawa, J., Miyata, T., Ohtsubo, S., Nakamura, T., Morita, T., Hayashida, H., Miyata, T., and Iwanaga, S. 1985. Primary structure of Limulus anticoagulant anti-lipopolysaccharide factor. J. Biol. Chem. 261 : 7357-7365.

Amersham Pharmacia biotech. 1999. Protein purification hand book. Sweden.

Amersham Pharmacia biotech. 1999. The recombinant Protein hand book. Sweden.

Anderson, I. 1993. The veterinary approach to marine prawns. In Brown (ed.), Aquaculture for veterinations: Fish husbandry and medicine, pp. 271-290. Amsterdam: Oxford Pergamon Press.

Aspán, A., Söderhäll, K. 1991. Purification of prophenaloxidase from crayfish blood cells, and its activation by an endogeneous serine proteinase. Insect Biochem. 21:363-373.

Aspán, A., Huang, T., Cerenius L., Söderhäll, K. 1995. cDNA cloning of prophenoloxidase from the freshwater crayfish *Pacifastacus lininusculus* and its activation. Proc. Natl. Acad. Sci. USA. 92 ; 939-43.

Bachère, E. 1999. Shrimp immunity and disease control. Aquaculture, inpress.

Bachère, E., Destoumieux, D., and Bulet, P. 2000. Penaeidins, antimicrobial peptides of shrimp: a comparison with other effectors of innate immunity. Aquaculture. 191 : 71-88.

Baily-Brook, J.H. and Moss, S.M. 1992. Penaeid taxonomy, biology and zoogeography. In A.W. Fast and L.J. Lester (eds.), Marine shrimp culture: Principles and practices, Amsterdam:Elsrvier Science Publishers.

Beschin, A., Bilej, M., Hanssens, F., Raymakers, J., Van Dyck, E., Revets, H., Brys, L., Gomez, J., De Baetselier, P., Timmermans, M. 1998. Identification and cloning of a glucan-and lipopolysaccharide-binding protein from *Eisenia foetida* earthworm involved in the activation of prophenoloxidase cascade. J. Biol. Chem. 273: 24948-24954.

Bode, W., and Huber, R. 1992. Natural protein proteinase inhibitors and their interaction with proteinase, Eur. J. Biochem. 204 : 433-451.

Bollag, D.M., Rozycki, M.D., and Edelstein, S.J. 1996. Protein methods. 2nd ed. New York: Wiley-Liss, Inc.

Borsing, L., Berger, E.G., Malissard, M. 1997. Expression and purification of His-tagged beta-1,4 galactosyltransferase in yeast and in COS cells. Biochem Biophys Res Commun 240:586–590

Boonyaratpalin, S., Supamattaya, K., Kasornchandra, J., Dierkkubsaracom, S.,Ekpanithanpong, U., Chantanachooklin, C. 1993. Non-occluded baculo-like virus, the causative agent of yellow head disease in the clack tiger shrimp (*Penaeus monodon*). Fish Pathol. 28:103-109.

Brock, J. A., and LeaMaster, B. 1992. A look at the principle bacterial, fungal and parasitic diseases of farmed shrimp. In : Wyban, J. (ed.). Proceeding of the special session on shrimp farming. World Aquaculture Society, Baton Rouge, La.

Browdy, C. 1998. Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. Aquaculture. 164:3-21.

Bulet, P., Kétru, C., Dimarcq, J.L., Hoffmann, D. 1999. Antimicrobial peptides in insects; structure and function. Dev. Comp. Immunol. 23:329-344.

Calera, J.A., Paris, S., Monod, M., Hamilton, A.J., Debeaupuis, J.P., Diaquin, M., Lopez-Medrano, R., Leal, F., Latge, J.P. 1997. Cloning and disruption of the antigenic catalase gene of *Aspergillus fumigatus*. Infect Immun. 65(11):4718-24.

Cerenius, L., Liang, Z., Duvic, B., Keyser, P., Hellman, U., Palva, E.T., Iwanaka, S. and Söderhäll, K. 1994. Structure and biological activity of a 1,3-beta-D-glucan-binding protein in crustacean blood. J. Biol. Chem. 269: 29462-29467.

Chantanachooklin, C., Boonyaratpalin, S., Kasonchandra, J., Direkbussarakom, S., Ekpanithanpong, U., Supamataya, K. 1993. Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by "yellow-head" disease. Diseases of Aquatic Organism. 17: 145-157.

Chen, B.P., Hai, T. 1994. Expression vectors for affinity purification and radiolabeling of proteins using *Escherichia coli* as host. Gene 139:73–75

Clare, J. J., Rayment, F. B., Ballantine, S. P., Sreekrishna, K., and Romanos, M. A. 1991a. High-level Expression of Tetanus Toxin Fragment c in *Pichia pastoris* Strains Containing Multiple Tandem Integrations of the Gene. Bio/Technology 9:455-460.

Clare, J. J., Romanos, M. A., Rayment, F. B., Rowedder, J. E., Smith, M. A., Payne, M. M., Sreekrishna, K., and Henwood, C. A. 1991b. Production of Epidermal Growth Factor in Yeast: High-Level Secretion Using *Pichia pastoris* Strains Containing Multiple Gene Copies. Gene 105:205-212.

Chou, H.Y., Huang, C.Y., Wang, C.H., Chiang, H.C. and Lo, C.F. 1995. Pathologenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Dis. Aquat. Org. 23:16173.

Cowley, J.A., Dimmock, C.M., Wongteerasupaya, C., Boonsaeng, V., Panyin, S., Walker, P.J. 1999. Yellow head virus from Thailand and gill-assiciated virrus from Australia are closely related but distinct prawn viruses. Disease of Aquatic Organism. 36: 153-157.

Cregg, J. M., Barringer, K. J., and Hessler, A. Y. 1985. *Pichia pastoris* as a Host System for Transformations. Mol. Cell. Biol. *5*: 3376-3385.

Crosa, J.H., Hodges, L. L., and Schiewe, H. 1980. Curing of plasmids is correlated with an attenuation of virulence in the marine fish pathogen *Vibrio anguillarum*. Infect. Immun. 27:897-902.

Decker, H., and Tuczek, F. 2000. Tyrosine/catecholoxidase activity of hemocyanins: structural basis and molecular mechanism. TIBS. 25:392-397.

Despreaux, C. W., and Manning, R. F. 1993. The *dacA* Gene of *Bacillus stearothermophilus* Coding for D-Alanine carboxypeptidase: Cloning, Structure, and Expression in *Escherichia coli* and *Pichia pastoris*. Gene 131:35-41.

Destoumieux, D., Bulet, P., Loew, D., Van Dorsselaer, A. V., Rodriguez, J. and Bachère, E. 1997. Penaeidins: A new family of abtimicrobial peptides in the shrimp *Penaeus vannamei* (Decapoda). J. Biol. Chem. 272:28398-28406.

Destoumieux, D., Bulet, P., Strub, J.M., Dorsselaer, A. V., and Bachère, E. 1999. Recombinant expression and range of activity of penaeidins, antimicrobial peptides from penaeid shrimp. Eur. J. Biochem. 266:335-346.

Engstrom, Y. 1998. Insect immune gene regulation. In : Brey, P.T., Hultmark, D. (eds.). Molecular Mechanisms of Immune Responses In Insects. Chapman and Hall. pp. 211-244.

Faber, K.N., Harder, W., and Veenhuis, M. 1995. Review: Methylotropic Yeasts as Factories for the Production of Foreign Proteins. Yeast. 11:1331-1344.

Flegel, T.W. 1997. Special topic review: Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. World journal of microbiology & Biotechnology. 13:433-442.

Fryxell, K. B., O'Donoghue, K., Graeff, R. M., Lee, H. C., and Branton, W. D. 1995. Functional Expression of Soluble Forms of Human CD38 in *scherichia coli* and *Pichia pastoris*. Protein Expression and Purification 6:329-336.

Hall, M., van Heusden, M.C, Söderhäll, K. 1995. Identification of the major lipoproteins in crayfish hemolymph as proteins involved in immune recognition and clotting. Biochem. Biophys. Res. Commun. 216:939-946.

Hall, M., Wang R., Van Antewerpen, R., Söderhäll, K. 1999. The crayfish plasma clotting protein: a vitellogenin-related protein responsible for clot formation in crustacean blood. Proc. Nayl. Acad. Sci. U.S.A. 96:1965-1970.

Hasson, K.W., Lightner, D.V., Poulos, B.T., Redman, R.M., White, B.L., Brock, J.A., Bonami, J.R. 1995.Taura syndrome in *Penaeus vannamei*, demonstration of a viral etiology. Dis. Aquat. Org. 23:115-126.

Hoess, A., Watson, S., Siber, G.R., Liddington, R.. 1993. Crystal structyre of an endotoxin-neutralizing protein from the horseshoe crab. Limulus anti-LPS factor, at 1.5 Å A resolution. EMBO. J. 12:3351-3356.

Hoffman, J.A., Kafatos, F.C., Janeway, C.A., Jr., and Ezekowitz, R.A. 1999.Phylogenetic perspectives in innate immunity. Science. 284:1313-1318.

Hoffmann,J.A. and Reichart, J.M. 1997. Drosophila Immunity. Trends Cell Biol. 7:309-316.

Iwanaga, S. 1993. The limulus clotting reaction. Curr Opin. Immunol. 5:74-82.

Iwanaga, S., and Kawabata, S. 1998. Evolution and phylogeny of defense molecules associated with innate immunity in horseshore crab. Frontiers in Bioscience. 3:973-984.

Iwanaga, S., Kawabata, S., Miura, Y., Seki, N., Shigenaga, T., and Muta, T. 1994. Clotting cascade in the immune response of horseshoe crab. In. Hoffmann, J., Janeway, C.A., Jr., and Natori, S. 9ed.), Phylogenetic perspectives in immunity: the insect host defense, pp. 79-96. Austin: R.G. Landes Company.

Iwanaga, S., Kawabata, S. and Muta, T. 1998. New types of clotting fectors and defense molecules found in horseshoe crab haemolymph: Their structures and functions. J. Biochem. 123:1-15.

Iwanaga, S., Morita, T., Miyata, T., Nakamura, T., and Aketagawa, J. 1986. The hemolymph coagulation system in invertebrate animals. J. Protein Chem. 5 : 255-268.

Jaana, Toikkanen, Expression of proteins in yeast systems. VTT biotechnology. Available from: www.biocenter.helsinki.fi/biotechgs/media/toikkanen.pdf [2004, Apr 19]

Janeway, C.A. Jr. 1998. Approching the asymptole evoluation and revolution in immunology Colo. Spring Habour Symp. Quant. Bilo. 54: 1-13.

Janknecht, R., Nordheim, A. 1992. Affinity purification of histidinetagged proteins transiently produced in HeLa cells. Gene 121:321–324

Janknecht, R., de, Martynoff, G., Lou, J., Hipskind, R., Nordheim, A., Stunnenberg, G. 1991. Rapid and efficient purification of native histidine-tagged protein expressed by recombinant vaccina virus. Proc Natl Acad Sci USA 88:8972–8976

Jiang, H., Wang, Y., M.R. 1998. Pro-phenoloxidase activating proteinase from an insect, *Manduca sexta*: a bacteria-inducible protein similar to *Drosophila* easter. Pro.Natl. Acad. USA. 95: 12220-12225.

Jiravanichpaisal, P., Miyazaki, T., Limsuwan, C. 1994. Histopathology, biochemistry and pathogenicity of *Vibrio harveyi* infecting black tiger prawn *Penaeus monodon*. J. Aquat. Amin. Health. 6:27-35.

Johasson, M.W., and Söderhäll, K. 1989. A cell adhesion fector from crayfish haemocytes has degranulating activity towards crayfish granular cells. Insect Biochem. 19:183-190.

Karunasagar, I., Pai, R., Malathi, G. R., Karunasaga, I. 1994. Mass mortality of Penaeus monodon larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture. 128 : 203-209.

Kaslow, D.C., Shiloach, J. 1994. Production, purification and immunogenicity of a malarian transmission-blocking vaccine candidate: TBV25H expressed in yeast and purified using nickel-NTA agarose. Bio/Technology 12:494–499

Kawabata, S., Nagayama, R., Hirata, M., Shigenga, T., Agarwala, K.L., Saito, T., Cho, J., Nakajima, H., Takagi, T. and Iwanaga, S. 1996. Tachycitin a small granular component in horseshoe crab haemocytes, is an antimicrobial protein with chitin-binding activity. J Biochem (Tokyo). 120:1253-1260.

Kidd, I.M., and V.C., Emery, 1993. The use of baculovirus as expression vectors. App. Biochem. Biotech. 42:137-159

Kim, Y.S., Ryu, J.H., Han, S.J., Choi, K.H., Nam, K.B., Jang, I.H., Lemaitre, B., Brey, P.T., Lee, W.J. 2000. Gram-negative bacteria-binding protein, a pattern recognition receptor for lipopolysaccharide and β-1,3-glucan that mediates the signaling for the induction of innate immune genes in *Drosophila melanogaster* cell. J. Biol. Chem. 275:32721-32727.

Kroll, R.M., Hawkins, W.E.., Overstreet, R.M. 1991. Rickettsial and mollicute infection in hepatopancreatic cells of cultured Pacific white shrimp (*Penaeus vannamei*). J. Invertebr. Pathol. 57:3622-3660.

Kuusinen, A., Arvola, M., Oker-Blom, C., Kein_men, K. 1995. Purification of recombinant GluR-D glutamate receptor produced in Sf21 insect cells. Eur J Biochem 233:720–726

Lai-Fook, J. 1966. The repair of wounds in the integument of insects J. Insect Physiol. 12:195-226.

Laroche, Y., Storme, V., Meutter, J. D., Messens, J., and Lauwereys, M. 1994. High-Level Secretion and Very Efficient Isotopic Labeling of Tick Anticoagulant Peptide (TAP) Expressed in the Methylotrophic Yeast, *Pichia pastoris*. Bio/Technology 12:1119-1124.

Lavilla-Pitogo C.R., Baticafos M.C.L., Cruz-Lacierda, E.R., and de la Pena L.D. 1990. Occurrence of immunous bacterial disease of *Penaeus monodon* larvae in the Phillipines. Aquaculture. 91: 1-13.

Ledeboer, A.M., Edens, L., Maat, J., et al. 1985. Molecular cloning and characterization of a gene coding for methanol oxidase in *Hansenula polymorpha*. Nucl. Acids Res. 13:3063-3082.

Lee, S.Y., and Söderhäll, K. 2001. Early events in crustacean innate immunity. Fish&Shellfish Immunol. 12:421-437.

Lightner, D.V. 1983. Disease of culture penaeid shrimp. In : McVey J. (ed.) CRC. Handbook of Marineculture, vol. 1 Crustacean Aquaculture, pp. 289-320. Boca Raton: CRC Press.

Lightner, D.V. 1996. A Handbook of pathology and diagnostic procedures for disease of penaeid shrimp. World Aquaculture Soc. Baton Rouge, Section 3.11.

Lightner, D.V. 1998. Vibrio disease of penaeid shrimp. In:Sinderman, C.J., Lightner, D.V. (eds.). Disease Dianosis and Control in North American Marine Aquaculture. 2nd, pp. 42-47. Amsterdam: Elsevier.

Limsuwan, C. 1991. Handbook for cultivation of black tiger prawns. Bangkok: Tansetaki Co Ltd.

Liu, P.C., Lee, K.K., Chen, S.N. 1996. Pathogenicity of different isolates of *Vibrio harveyi* in tiger prawn, *Penaeus monodon*. Lett. Appl. Microbiol. 22:413-416.

Lo, C.F., Ho, C.H., Peng, S.E., and et al. 1996. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. Dis. Aquat. Org. 27:215-225.

Lotz, J.M. 1997. Special review: Viruses, biosecurity and specific pathogen free stocks in shrimp aquaculture. World journal of microbiology and Biotechnology. 13:405-413.

Mohney, L.L., and Lighter, D.V. 1994. An epizootic of vibriosis in Ecuadorian pond-reared *Penaeus vannamei* Boone (*Crustacea Decapoda*). J World Aquacult. Soc. 25.

Morita, T., Ohtsubo, S., Nakamura, T., Tanaka, S., Iwanaga, S., Ohashi, K., and Niwa, M. 1985. Isolation and biological activities of limulus anticoagulant (anti-LPS factor) which interacts with lipopolysaccharide(LPS). J. Biochem. 97:1611-1620.

Munford, R. S., C. L. Hall, J. M. Lipton, and J. M. Dietschy. 1982. Biological activity, lipoprotein-binding behavior, and in vivo disposition of extracted and native forms *Salmonella typhimurium* lipopolysaccharides. J. Clin. Invest. 70:877-888.

Muta, T., and Iwanaga, S. 1996. The role of hemolymph coagulation in inate immunity. Curr. Opin. Immunol. 8:41-47.

Muta, T., miyata, Y., Tokunaga, F., Nakamura, T., Toh, Y., Ikehara, Y. and Iwanaga, S. 1991. Limulus factor C an endotoxin-sensitive serine proteinase zymogen with a mosaic structure of complement-like, epidermal growth with factor-like and lectin-like domains. J. Biol. Chem. 266:6554-6561.

Nakamura, T., Furunaka, H., Miyata, T., Tokunaga, F., Muta, T., Iwanaga, S., Niwa, M., Takao, T., Shimonishi, Y. 1988. Tachyplesin a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*): isolation and chemical structure. J. Biol. Chem. 263:16709-16713.

Nathalie, MOREL, and Jean, MASSOULIÉ, 1997. Expression and processing of vertebrate acetylcholinesterase in the yeast *Pichia pastoris*. Biochem. J. 328:121–129

O'Reilly, D., L.K., Miller, and V.A. Luckow. 1992. Baculovirus expression vectors : A laboratory Manual. W.H. Freeman and company, New York, NY.

Queiroz, J.A., Tamaz, C.T., and Cabral, J.M.S. 2001. Hydrophobic interaction chromatography of proteins. J. biotechnol. 87:143-159

Paifer, E., Margolles, E., Cremata, J., Montesino, R., Herrera, L., and Delgado, J. M. 1994. Efficient Expression and Secretion of Recombinant Alpha Amylase in *Pichia pastoris* Using Two Different Signal Sequences. Yeast 10:1415-1419.

Persson, M., Vey, A., and Söäderhll, K. 1987. Encapsulation of foreign particles in vitro by separated blood cells from cryfish, *Astacus leptodactylus*. Cell Tissue Res. 247:409-415.

Pizzutto, M., and Hirst, R.G. 1995. Classification of isolates of *Vibrio harveyi* virulent to *Penaeaus monodon* larvae by protein profile analysis and M13 DNA fingerprinting. Dis. Aquat. Org. 21:61-68.

Porath, J., Carlsson, J., Olsson, I., Belfrage, G. 1975. Metal chelate affinity chromatography, a new approach to protein fractionation. Nature 258:598–599

Rank, K.B., Mildner, A.M., Leone, J.W., Koeplinger, K.A., Chou, K.C., Tomasselli, A.G., Heinrikson, R.L., Sharma, S.K. 2001. [W206R]-procaspase 3: an inactivateable substrate for caspase 8. Protein Expr Purif 22:258–266

Relf, J.M., Chisholm, J.R.S., Kemp, G.D., Smith, V.J. 1999. Purification and characterization of a cysteine-rich 11.5 kDa antibacterial protein from the granular hemocytes of the shore crab, *Carcinus maenas*. Eur. J. Biochem. 264:350-357.

Ried, C., Wahl, C. Miethke, T., Wellnhofer, G., Landgraf, C., Scheider-Mergener, J., and Hoeses, A. 1996. High affinity endotoxin-binding and neutralizing peptides based on the crystal structure of recombinant Limulus anti LPS factor. J. Biol. chem. 271:28120-28127.

Rodriguez, M., Rubiera, R., Penichet, M., Montesinos, R., Cremata, J., Falcon, V., Sanchez, G., Bringas, R., Cordoves, C., Valdes, M., Lleonart, R., Herrera, L., and delaFuente, J. 1994. High Level Expression of the *B. microplus* Bm86 Antigen in the Yeast *Pichia pastoris* Forming Highly Immunogenic Particles for Cattle. J. Biotechnol. 33:135-146.

Romanos, M. A., Clare, J. J., Beesley, K. M., Rayment, F. B., Ballantine, S. P., Makoff, A. J., Dougan, G., Fairweather, N. F., and Charles, I. G. 1991. Recombinant *Bordetella pertussis* Pertactin p69 from the Yeast *Pichia pastoris* High Level Production and Immunological Properties. Vaccine 9:901-906.

Sambrook, J. Fritsch, E.F., Maniatis, T. 1989. Moleculaar cloning : A laboratory Manual. Clod Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

Satoh, D., Horii, A., Ochiai, M., and Ashida , M. 1999 Prophenoloxidase-activating enzyme of the silkworm, *Bombyx mori*: purification, characterization and cDNA cloning. J. Biol. chem. 274:7441-7453.

Schmidt, M., Tuominen, N., Johansson, T., Weiss, S.A., Keinamen, K., Oker-Blom, C. 1998. Baculovirus-mediated large-scale expression and purification of a polyhistidine-tagged Rubrella virus capsid protein. Protein Expr Purif 12:323–330

Shaw Warren, H., Maureen, L., Glennon, Norman, Wainwright, Stephen, J., Kirsch, Gilles, R., Riveau, Richard, I., Whyte, Warren, M., Zapol, and Thomas, J., Novitsky. 1992. Binding and Neutralization of Endotoxin by Limulus Antilipopolysaccharide Factor. Infection and Immunity June:2506-2513.

Söderhäll, K. 1999. Immediate immune reactions. Available:From http://www.jamfys.ebc.uu.se/dept/k_soderhall.html

Söderhäll, K., and Cerenius, L. 1992. Crustacean immunity. Annu.Rev. Fish. Dis. 2 : 3-23.

Söderhäll, K., and Cerenius, L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. Curr. Opin. Immunol. 10:23-28.

Söderhäll, K., and Cerenius, L., and Johansson, M. W. 1994. The prophenoloxidase activating system and its role in invertebrate defense. *Ann.* N. Y. Acad. Sci. 712:155-61.

Söderhäll, K., Smith, V.J., and Johansson, M.W. 1986. Exocytosis and uptake of bacteria by isolated haemocyte populations of two crustacean: evidance for cellular co-operation in the defence reactions of arthropods. Cell Tissue Res.245: 43-49.

Solis, N.B. 1988. Biology and ecology. In Taki, Y. Premavara, J.H., and Lobrera, J.(eds.), Biology and culture of *Penaeus monodon*, Aquaculture Department, Southeast Asia Fisheries Development Center.

Spán, K.M., and Lester, R.J.G. 1997. Special topic review: Viral diseases of penaeid shrimp with particular reference to four viruses recently found in shrimp from Queensland. World journal of microbiology and Biotechnology. 13:419-426.

Sreekrishna, K., Nelles, L., Potenz, R., Cruse, J., Mazzaferro, P., Fish, W., Fuke, M., Holden, K., Phelps, D., Wood, P., and Parker, K. 1989. High Level Expression, Purification, and Characterization of Recombinant Human Tumor Necrosis Factor Synthesized in the Methylotrophic Yeast *Pichia pastoris*. Biochemistry 28:4117-4125.

Sritunyalucksana, K., Cerenius, L., and Söderhäll, K. 1999. Molecular cloning and characterization of prophenoloxidase in the black tiger shrimp, *Penaeus monodon*. Dev. Comp. Immunol. 23:179-186.

Sunaryanto, A., and Mariam, A. 1986. Occurrence of a pathogenic bacteria causing luminescence in penaeid larvae in Indonesian hatcheries. Bull. Brackish Water Aqua. Devl. Cent. 8: 64-70.

Supungul, P., Klinbunga, S., Pichyangkura, R., Jitrapakdee, S., Hirono, I., Aoki, T., and Tassanakajon, A. 2002. Identification of immune-related genes in haemocytes of black tiger shrimp (*Penaeus monodon*). Mar. Biotechnol. 4: 487-494.

Terpe, K. 2003. Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. Mini-review. Appl. Microbiol. Biotechnol. 60:523–533

Tokunaga, F., Miyata, T., Nakamura, T., Morita, T., Kuma, K., Miyata, T., and wanaga, S. 1987. Lipopolysaccharide-sensitive serine-protease zymogen (factor C) of horseshoe crab produced during the activation show that it is a novel type of serine protease. Eur. J. Biochem. 167:405-416.

Tschopp, J. F., Sverlow, G., Kosson, R., Craig, W., and Grinna, L. 1987. High Level Secretion of Glycosylated Invertase in the Methylotrophic Yeast *Pichia pastoris*. Bio/Technology 5:1305-1308.

Wegner, G. H. 1990. Emerging Applications of the Methylotrophic Yeasts. FEMS Microbiology Reviews 87:279-284.

Weiss, H. M., Haase, W., Michel, H., and Reilander, H. 1995. Expression of Functional Mouse 5-HT5A Serotonin Receptor in the Methylotrophic Yeast *Pichia pastoris*: Pharmacological Characterization and Localization. FEBS 377:451-456.

Wongteerasupaya, C., Vickers, J.E., Sriurairatana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S., Tassanakajon.A., Withyachumnarnkul, B. and Flegal, T.W. 1995. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and cause high mortality in the black tiger prawn *Penaeus monodon*. Dis. Aquat. Org. 21: 69-77.

Yoshida, H., Kinoshita, K., Ashida, M. 1996. Purification of a peptidoglycan recognition protein from hemolymph of the silkworm, Bombyx mori. J. Biol. Chem. 271:13854-13860.

# APPENDICES

## APPENDIX A : Preparation for polyacrylamide gel electrophoresis

### 1. Stock reagents

**30 % Acrylamide, 0.8% bis-acrylamide, 100 ml**

| | | |
|---|---|---|
| Acrylamide | 29.2 | g |
| N,N′-methylene-bis-acrylamide | 0.8 | g |

Adjust volume to 100 ml with distilled water.

**1.5 M Tris-HCl pH 8.8**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 18.17 | g |

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

**2.0 M Tris-HCl pH 8.8**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 24.2 | g |

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

**0.5 M Tris-HCl pH 6.8**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 6.06 | g |

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

**1.0 M Tris-HCl pH 6.8**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 12.1 | g |

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

**Solution B (SDS PAGE)**

| | | |
|---|---|---|
| 2.0 M Tris-HCl pH 8.8 | 75 | ml |
| 10% SDS | 4 | ml |
| Distilled water | 21 | ml |

**Solution C (SDS PAGE)**

| | | |
|---|---|---|
| 1.0 M Tris-HCl pH 8.8 | 50 | ml |
| 10% SDS | 4 | ml |
| Distilled water | 46 | ml |

## 2. SDS-PAGE

### 15 % Seperating gel

| | | |
|---|---|---|
| 30 % Acrylamideml solution | 5.0 | ml |
| Solution B | 2.5 | ml |
| Distilled water | 2.5 | ml |
| 10% $(NH_4)_2S_2O_8$ | 50 | µl |
| TEMED | 10 | µl |

### 5.0 % Stacking gel

| | | |
|---|---|---|
| 30 % Acrylamideml solution | 0.67 | ml |
| Solution C | 1.0 | ml |
| Distilled water | 2.3 | ml |
| 10 % $(NH_4)_2S_2O_8$ | 30 | µl |
| TEMED | 5.0 | µl |

### 5X Sample buffer

| | | |
|---|---|---|
| 1 M Tris-HCl pH 6.8 | 0.6 | ml |
| 50% Glycerol | 5.0 | ml |
| 10% SDS | 2.0 | ml |
| 2-Mercaptoethanol | 0.5 | ml |
| 1 % Bromophenol blue | 1.0 | ml |
| Distilled water | 0.9 | ml |

One part of sample buffer was added to four parts of sample. The mixture was heated 5 min. in boiling water before loading to the gel.

### Electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 3.03 | g |
| Glycine | 14.40 | g |
| SDS | 1.0 | g |

Dissolve in distilled water to 1 litre. Do not adjust pH with acid or base (final pH should be 8.3).

# APPENDIX B : Preparation for Tricine-Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis

## 1. Stock reagents

**49.5 % Acrylamide, 3% bis-acrylamide, 100 ml**

| | | |
|---|---|---|
| Acrylamide | 48 | g |
| N,N′-methylene-bis-acrylamide | 1.5 | g |

Adjust volume to 100 ml with distilled water.

**Gel buffer, 100 ml**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 36.34 | g |
| SDS | 0.3 | g |

Adjust pH to 8.45 with 1 M HCl and adjust volume to 100 ml with distilled water.

## 2. Tricine-SDS-PAGE

**12 % Separating gel**

| | | |
|---|---|---|
| 49.5 % Acrylamide solution | 2.92 | ml |
| Gel buffer | 4 | ml |
| Distilled water | 3.88 | ml |
| 100% Glycerol | 1.2 | ml |
| 10% $(NH_4)_2S_2O_8$ | 70 | µl |
| TEMED | 15 | µl |

**4.0 % Stacking gel**

| | | |
|---|---|---|
| 49.5 % Acrylamideml solution | 0.25 | ml |
| Gel buffer | 0.775 | ml |
| Distilled water | 2 | ml |
| 10 % $(NH_4)_2S_2O_8$ | 30 | µl |
| TEMED | 5.0 | µl |

**2X Sample buffer**

| | | |
|---|---|---|
| Gel buffer | 3.0 | ml |
| 100% Glycerol | 2.4 | ml |
| SDS | 0.8 | g |
| 0.1 % Coomassie Blue G | 1.5 | ml |
| 0.1 % Phenol red | 0.5 | ml |

Adjust volume to 100 ml with distilled water.

One part of sample buffer was added to one parts of sample. The mixture was heated 5 min. in boiling water before loading to the gel.

**Electrophoresis buffer**

**Cathode buffer, 100 ml**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 1.21 | g |
| Tricine | 26.3 | g |
| SDS | 0.1 | g |

Dissolve in distilled water to 100 ml. Do not adjust pH with acid or base (final pH should be 8.3).

**Anode buffer, 100 ml**

| | | |
|---|---|---|
| Tris (hydroxymethyl)-aminomethane | 2.42 | g |

Adjust pH to 8.9 with 1 M HCl and adjust volume to 100 ml with distilled water.

## APPENDIX C : Preparation for silver staining

### 1. Stock reagents

**0.36% NaOH**

| | | |
|---|---|---|
| NaOH | 0.36 | g |

Adjust volume to 100 ml with distilled water.

**1% citric acid**

| | | |
|---|---|---|
| Citric acid | 1 | g |

Adjust volume to 100 ml with distilled water.

**50% methanol l0% acetic acid**

| | | |
|---|---|---|
| 100 % methanol | 50 | ml |
| Glacial acetic acid | 10 | ml |

Adjust volume to 100 ml with distilled water.

**1% acetic acid**

| | | |
|---|---|---|
| Glacial acetic acid | 1 | ml |

Adjust volume to 100 ml with distilled water.

### 2. Make fresh reagents

**Solution A**

| | | |
|---|---|---|
| Silver nitrate | 0.8 | g |

Adjust volume to 4 ml with distilled water.

**Solution B**

| | | |
|---|---|---|
| 0.36% NaOH | 21 | ml |
| 14.8 M (30%) ammonium hydroxide | l.4 | ml |

**Solution C**

Add Solution A to Solution B dropwise with constant vigorous stirring, allowing brown precipitate to clear. Adjust volume to 100 ml. Use within 15minutes.

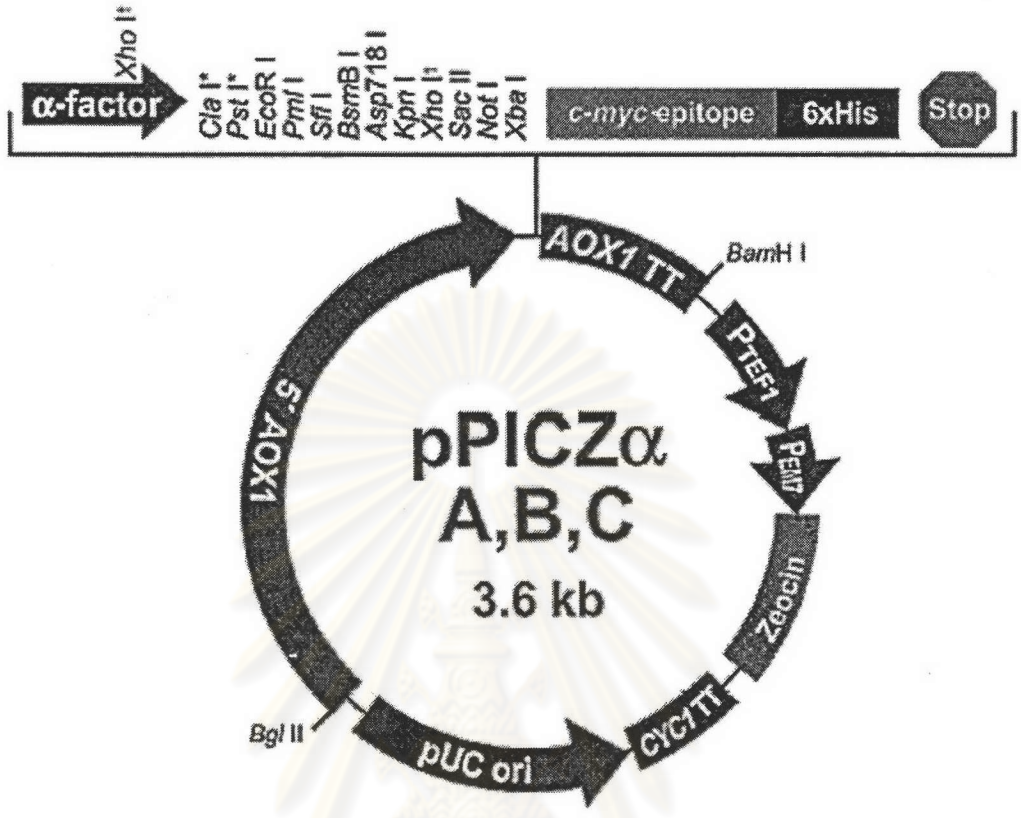
**Solution D**

Mix 0.5 ml 1%citric acid with 50 µl 38% formaldehyde, add water to 100 ml. Solution must be fresh.

**3. Silver Staining method**

1. Wearing gloves, pick up the gel and transfer it to a small container.Soak gel in 50% methanol l0% acetic acid for at least 1 hour with 2 -3 changes of methanol /acetic acid.
2. Rinse 30 minutes with water, with at least 3 changes.
3. Prepare Solutions A, B, then C.
4. Remove gel to a clean container and stain in Solution C for 15 minutes with gentle, constant agitation.
5. Rinse gel twice in deionized water, then soak 2 minutes with gentle agitation.
6. Prepare Solution D.
7. Remove gel to a clean container and develop by washing gel in Solution D. Bands should appear in less than 10 minutes or else change Solution D. If a pale yellow background appears, reaction should be stopped.
8. Stop development by rinsing in 1% acetic acid.
9. Wash gel in water for at least 1 hour with at least three changes of water.
10. Store gel in water or dry gel.

**APPENDIX D :** Transfer vector map and MCS sequence




[a]

```
                                                                    Xho I*
1136  ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA TCT CTC
      Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val Ser Leu

            Kex2 signal cleavage          EcoR I   Pml I        Sfi I          BsmB I Asp718 I
1187  GAG AAA AGA GAG GCT GAA GCT GAATTCAC GTGGCCCAG CCGGCCGTC TCGGATCGGT
      Glu Lys Arg Glu Ala Glu Ala
                        Ste13 signal cleavage
      Kpn I Xho I     Sac II Not I      Xba I      c-myc epitope
1244  ACCTCGAGCC GCGGCGGCC GCCAGCTTTC TA GAA CAA AAA CTC ATC TCA GAA GAG
                                        Glu Gln Lys Leu Ile Ser Glu Glu
```

[b]

[a] Map of pPICZαA

[b] Sequence around the multiple cloning sites

## Biography

Mr. Ekarin Achakulwisut was born on September 15, 1978 in Samutsakorn. He graduated with the degree of Bachelor of Science from the Department of Biochemistry, Chulalongkorn University in 1999. He has further studied in Master's degree of science (Biotechnology), Chulalongkorn University.