

細菌 TKU008 所生產幾丁質？，帛J白？，妖瞻 峴w性

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摘要

本實驗研究目的為篩選能發酵蝦蟹殼幾丁質，具胞外蛋白？P幾丁質？‘艾牙鄙O之本土菌株，探討其較適培養條件及酵素之純化分離及定性。所得發酵上清液胞經硫酸銨沉澱、透析去除鹽類，DEAE-Sephadex及Sephacryl S-100管柱層析之分離步驟，純化出經由SDS-PAGE測得分子量分別為40 kDa與57 kDa之蛋白？」，帮X丁質？C細菌TKU008篩自台灣南部土壤，以蝦蟹殼粉(SCSP)當作主要碳源；此菌較適培養條件為1% SCSP、0.05% MgSO₄·7H₂O、0.1% K₂HPO₄於pH 7、100mL、30℃，150 rpm條件下，進行振盪培養4天後，可得最佳之蛋白？」，帮X丁質？“咁CTKU008以酪蛋白為基質所得到蛋白？搵ApH、最適反應溫度、pH穩定性及熱穩定性分別為pH 7, 50℃，pH 6 及 30℃；以懸浮態幾丁質為基質所得幾丁質？」，妊搵ApH、最適反應溫度、pH穩定性及熱穩定性分別為pH 6, 50℃，pH 7及 30~40℃。蛋白質？，妞“咁^收率為1%，純化倍數為2倍，比活性為0.36 U/mL，幾丁質？」，妞“咁^收率為50%，純化倍數為134倍，比活性為7.56 U/mL。由於蛋白質？”“咁被EDTA完全抑制，而可歸類為金屬型蛋白質；蛋白質？P幾丁質？”Cu²⁺、Mn²⁺抑制，蛋白質？P幾丁質？鴟韻肆？畯佢惇“尨祐 蟹筆酉w；其中蛋白？”b有機溶劑中儲存安定性又較幾丁質？”峩CTKU008蛋白質？巒T蛋白、彈性蛋白、人類白蛋白、血紅蛋白具有不同程度的水解能力。

關鍵詞：幾丁質；蛋白質？；幾丁質？；蝦蟹殼粉

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參考文獻

- 中文部份 1.王三郎。1996。水產資源利用學。高立圖書出版社。 2.王三郎。1999。海洋未利用生物資源之回收再利用-幾丁質及幾丁聚醣。生物資源 生物技術。1: 1-8。 3.王美人 , 游若荻。1995。篩選枯草桿菌之DNA轉型株用於蛋白? , 壞芝 HC 食品科學。22: 38-45。 4.王偉、秦汶。1989。脫乙醯甲殼素的超聲波降解。化學通報9: 44-52。 5.火田 野功太。1992。幾丁質、脫乙醯幾丁質與幾丁質水解? ' b 日本之研究發展現況。生物產業。3: 157-167。 6.徐世昌。2001。生物高分子-幾丁質與幾丁聚醣之介紹與應用。化工資訊月刊。15: 36-45。 7.莊榮輝 主編。2005。酵素化學實驗。第112-157頁。國立台灣大學。台北 , 台灣。 8.陳國誠。1989。微生物酵素工程學。藝軒圖書出版社。台北 , 台灣。 9.張珍田。2001。幾丁質? , 帚X丁聚醣? , 纽脕s。生命科學簡訊14: 3-7。 10.劉瓊淑, 幾丁質、幾丁聚醣及其相關酵素之特性與應用。1994。食品工業 , 第26-35頁。 11.蘇遠志 , 黃世佑。2002。微生物化學工程學。第372-374頁。華香園出版社。台北 , 台灣。 12.賴威安。2000。Bacillus sp. P-6蛋白?" 漸芟虫峯宅塙櫨R。第18-19、87頁。國立中興大學食品科學系碩士論文。台中 , 台灣。 13.蕭瑞昌。1997。利用水溶液性幾丁聚醣以薄膜超過濾法去除微量之金屬離子。私立元智工學院碩士論文。桃園。 英文部份 1.Alder-Nissen, J. 1986. Enzymic hydrolysis of food proteins. Elsevier applied science publ. 19-20. London and New york. 2.Allan, C. R. and Hadwiger, L. A. 1979. The fungicidal effect of chitsan on fungi of verying cell well composition. Exp. mycol.. 3: 285-298. 3.Akuzawa, R., Tottori, A., Tsukahara, K. and Okitani, A. 1997. Purification and characterization of a cysteine proteinase from *Lactococcus lactis* ssp. *lactis* IAM 1198 . Intl. Dairy J. 7: 429-434. 4.Amoozegar, M. A., Fatemi, A. Z., Hamid Reza Karbalaei-Heidari, H. R. and Razavi, M. R. 2006. Production of an extracellular alkaline metalloprotease from a newly isolated, moderately halophile, *Salinivibrio* sp. strain AF-2004. Microbiological Research. 5.Arora, N., Ahmad, T., Rajagopal, R. and Bhatnagar, R. K. 2003. A constitutively expressed 36 kDa exochitinase from *Bacillus thuringiensis* HD-1. Biochem. biophys. res. commun. 307: 620 – 625. 6.Austum, P. R. ,Brme, C. J., Castie, J.E. and Eikakis, J. P. 1981. “ Chitin ” . New Facets of research Science. 212: 749. 7.Beg, Q. K. and Gupta R., 2003. Purification and characterization of an oxidation-stable, thiol-dependent serine alkaline protease from *Bacillus mojavensis*. Enzyme microb. technol. 32: 294-304 8.Bersanetti, P. A., Park, H. Y., Bae, K. S., Son, K. H., Shin, D. H., Hirata, I. Y., Juliano, M. A., Carmona, A. K. and Juliano, L. 2005. Caracterization of arazyme,an exocellular matelloprotease isolated from *Serratia proteamaculans* culture medium. Enzyme microb. technol. 37: 574 – 581 9.Benito, M. J., Rodr?櫻uez, M., N??羚z, F., Asensio, M. A., Berm?攔ez, M. E. and C?櫻doba, J. J. 2002. Purification and Characterization of an Extracellular Protease from *Penicillium chrysogenum* Pg222 Active against Meat Proteins. Appl. environ. microbiol.. 68: 3532 – 3536. 10.Blaiseau, P. L. and Lafay, J. F. 1992. Primary structure of a chitinase-encoding gene(chi 1)from the filamentous fungs *Aphanocladium album*: similarity to bacterial chitinase. Gene. 120: 243-248. 11.Bradford , M. 1976. A rapid and sensitine method for the determination of microgram quantities of protein unilizing the principles of protein-day binding. Anal. biochem. 72: 248-254. 12.Carroad, D. A. and Tom, R. A. 1978. Bioconversion of shellfish chitin waste : process conception and selection of microorganism. J. Food Sci. 43: 1158-1164. 13.Chen, T. E., Huang, D. J., Lin, Y. H. 2004. Isolation and characterization of a serine protease from the storage roots of sweet potato (*Ipomoea batatas* [L.] Lam). Plant Science. 166: 1019 – 1026. 14.Cosio, I. G. , Fisher, R. A. and Carroad , D. A. 1982. Bioconver -sion of shellfish chitin waste: waste pretreatment ,enzyme production, process design, and economic analysis. J . Food Sci. 47: 901-905. 15.Cowan, D., 1996. Industrial enzyme technology. Trends Biotechnol. 14: 177 – 178. 16.Elpidina, E.N., Tsybina, T.A., Dunaevsky, Y.E., Belozersky, M.A., Zhuzhikov, D.P. and Oppert. B. 2005. A chymotrypsin-like proteinase from the midgut of *Tenebrio molitor* larvae. Biochimie. 87: 771 – 779. 17.Fan, Z., Zhu, Q., Dai, J. 2001. Enzymatic treatment of wool. J. Dong Hua University (English edition). 18: 112 – 115. 18.Fich J, Pilet PE, Jolles P. 1992. What ' s new in chitinase research? Experientia. 48: 701-716. 19.Godfrey, T. 1986. Comparison of key characteristics of industrial enzyme by type and source. In: Industrial Enzymology. 466-557. (ed. T. Godfrey and J. Reichelt). Macmillan Publishers Ltd, New York. 20.Graham, D.E. and M.C. Phillips. 1979. Proteins at liquid interfaces. I. Kinetics of adsorption and surface denaturation. J. colloid interface sci. 70: 403-414. 21.Gunda, P. and Ralf, Malessa. 1998. Structural characterization of the pressure-denatured state and unfolding/refolding kinetics of staphylococcal nuclease by synchrotron small-angle x-ray scattering and fourier-transform infrared spectroscopy. J. Mol. Biol. 275: 389-402. 22.Gupta, A., Roy, I., Khare, S. K., Gupta, M. N. 2005. Purification and characterization of a solvent stable protease from *Pseudomonas aeruginosa* PseA. J.Chromatogr. A. 1069: 155 – 161. 23.Gupta, M. N. 1992. Enzyme function in organic solvents. Eur. J. Biochem. 203: 25 – 32. 24.Hackman, R. H. 1960. Studies on chitin. IV. The occurrence of complexes in which chitin and protein are covalently limked. Aust. J. Biol. Sci. 13: 568-579. 25.Hirano, S., Yamamoto, T., Hayashi, M., Nishida, T. and Inui, H. 1990. Chitinase activity in seeds coated with chitosan devates. Agric. Biol. Chem. 54: 2719-2720. 26.Imoto, T. and Yagishita, K. 1971. A simple activity measurement by lysozyme. Agric. Biol. Chem. 35: 1154 – 1156. 27.Jeuniax,C. 1964. Free chitin and masked chitin in invertebrate skeletal structures. Arch. Int. Physiol. Biochem. 72: 329-330. 28.Joo, H. E., Kumar, C. G., Park, G. C., Kim, K. T. and Paik, S. R. 2002. Optimization of the production of an extracellular alkaline protease from *Bacillus horikoshii*. Process Biochem. 38: 155-159. 29.Karadzic, I., Masui, A. and Fujiwara, N. 2004. Purification and Characterization of a Protease from *Pseudomonas aeruginosa* Grown in Cutting Oil. J. Biosci. Bioeng. 98: 145-152. 30.Kawasumi, T., Kiuchi, N., Futatsugi, Y., Ohba, K. & Yanagi, S. O. 1987. High yield preparation of *Lentinus edodes* (" Shiitake ") protoplasts with regeneration capacity and mating type stability. Agric. Biol. Chem. 51: 1649-1656. 31.Kelkar, H. S., Shankar, V. & Deshpande, M. V. 1990. Rapid isolation andregeneration of *Sclerotium rolfsii* protoplasts and their potential application for starch hydrolysis. Enzyme Microb. Technoogyl. 12: 510-514. 32.Khan, A., Williams, K., Molloy, M. P. and Nevalainen, H. 2003. Purification and characterization of a serine protease and chitinases from *Paecilomyces lilacinus* and detection of chitinase activity on 2D gels. Protein expr. purif. 32: 210 – 220. 33.Kim, H. K., Kim, G. T., Kim, D. K., Chio,W. A., Park, S. H., Jeong, Y. K. and Kong, I. S. 1997. Purification and Characterization of a Novel Fibrinolytic Enzyme from *Bacillus* sp. KA38

Originated from Fermented Fish. J. of Fermentation and bioengineering. 84: 307-312. 34.Koga, D., Sueshige, N., Orikono, K., Utsumi, T., Tanaka, S., Yamada, Y., and Ide, A. 1988. Efficiency of chitinolytic enzyme in the formation of trichoderma matsutake protoplasts. Agric. Biol. Chem., 52: 2091-2094. 35.Koga, D., Sueshige, N., Usumi, T. and Ide, A. 1989. Kinetics of chitinase from yam , Dioscorea oppsita thumb. Agric. Biol. Chem., 53: 3121-3127. 36.Knorr, D. 1984. Use of chitinous polymers in food. Food-A challenge for food research and development. Food Technol. 38: 85-97. 37.Kurtovic, I., S.N. Marshall, S.N. and Simpson, B. K. 2006. Isolation and characterization of a trypsin fraction from the pyloric ceca of chinook salmon (*Oncorhynchus tshawytscha*). Comp. biochem. physiol., Part B. 143: 432 – 440. 38.Kunz, C., Sellam, O. and Bertheau, Y. 1992. Purification and characterization of a chitinase from the hyperparasitic fungus *Aphanocladium album*. Physiol. mol. plant pathol. 40: 117- 131. 39.Laemmlli, U. K. 1970. Cleavage of structural proteins during the assembly of thehead of bacteriophage T4. Nature . 227: 680 – 685. 40.Lahl, W. J. and Braun, S. D. 1994. Enzymatic production of protein hydrolysates for food use. Food Technol. 48: 68-71. 41.Lee, J. H., Ahn, S.H., Lee, E. M., Young-Ok Kim, Y. O., Lee, S. J. and Kong, I. S. 2003. Characterization of the enzyme activity of an extracellular metalloprotease (VMC) from *Vibrio mimicus* and its C-terminal deletions. FEMS micro. biol. lett. 223: 293-300. 42.Leger, R.J. ST., Cooper, R.M. and Charnley, A. K. 1991. Characterization of Chitinase and Chitobiase Produced by the Entomopathogenic Fungus *Metarhizium anisopliae*. J. invertebr. pathol.. 58: 415-426. 43.Lilik, I. and David, A. M., 1996 .Leaching and characterization of *Rhizopus oligosporus* acid protease from solid-state formation, enzyme Microb Teachnol. 19: 175-177. 44.Margesin, R., Dieplinger, H., Hofmann,J., Sarg, B. and Lindner, H. 2005. A cold-active extracellular metalloprotease from *Pedobacter cryoconitis*—production and properties. Research in Microbiology. 156: 499 – 505. 45.Martinou, A., Koutsoulis, D. and Bouriotis, V. 2002. Expression, Purification, and Characterization of a Cobalt-Activated Chitin Deacetylase (Cda2p) from *Saccharomyces cerevisiae*. Protein expr. purif. 24: 111 – 116. 46.Matta, H. and Punj, V. 1998. Isolation and partial characterization of a thermostable extracellular protease of *Bacillus polymyxa* B-17. Int. j. food microbiol. 42: 139-145. 47.Melchers, L. S., Apotheker-de Groot, M., Ven der Knaap, J. A., Ponstein, A. S., Sela-Buurlage, M. B., Bol, J. F., Cornelissen, B. J. C. Vanden Elzen, P. J. M., Linthorst, H. J. M. 1994. Anew class of tobacco chitinases homologous to bacterial exo-chitinases displays antifungal activity. Plate J. 5: 469-480. 48.Minke, R. and Blackwell, J. 1978. The structure of -chitin. J. Mol. Biol. 120: 167-181. 49.Mozersky, S., Marmer, W., Dale, A.O. 2002. Vigorous proteolysis: Relining in the presence of an alkaline protease and baiting (Post -Liming) with an extremophile protease. JALCA. 97: 150-155. 50.Murao, S., Kawada, T., Itoh, H., Oyama, H. and Shin, T. 1992 Purification and characterization of a novel type of chitinase from *Vibrio alginolyticus* TK-22. Biosci. Biotech. Biochem. 56: 368-369. 51.Muzzarelli, R. A. A., G. and Rocchetti, R. 1978. Isolation of lysozyme on chitsan. Biotech. Bioeng. 20: 87-95. 52.Nanjo, F., Ishikawa, M., Katsumi, R. and Sakai, K. 1989. Purification, properties and transglycosylation reation of b-N-acetyl-hexosaminidase from *Nocardia orientalis*. Agric. Biol. Chem. 54: 899-906. 53.Nakamura T, Syukunobe Y, Sakurai T and Idota T. 1993 Enzymatic production of hypoallergenic peptides from casein. Milchwissenschaft. 48: 11-14. 54.Nduwimana, j., Guenet, L., Dorval, I., Blayau, M., Gall, J. L.and Treut, A. L. 1995. Proteases. Ann. Biol. Clin. 53: 251-264. 55.Ogino, H., Yasui, K., Shiotani , T., Ishihara, T. and Ishikawa, T. 1995. Organic solvent-tolerant bacterium which secretes an organic solvent-stable proteolytic enzyme. Appl Environ Microbiol. 61: 4258-4262. 56.Okazaki, K. and Tagawa, K. 1991. Purification and Properties of Chitinase from *Streptomyces cinereoruber*. J. of Fermentation and Bioengineering. 71: 237-241. 57.Ordentlich, A., Elad, Y. and Chet, I. 1988. The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phyto -pathol. 78: 84-87. 58.Preacott, M., Peek, K. and Daniel, R. M. 1995. Characterisation of a thermostable pepstatin-insenstivite acid protease from a *Bacillus* sp. Int. J. Biochem. Cell Biol. 27: 729-739. 59.Rao, M., Tankasale, A., Ghatge, M., Desphande, V. 1998. Mole -cularand biotechnological aspects of microbial proteases. Micro -biol. Mol. Biol. Rev. 62: 597 – 634. 60.Robert A. C. 1994. Methods for protein analysis. A practical guide to laboratory protocols. Chapman andd Hall Publishing Co. New York. U.S.A. 61.Schokker, E. P., van Boekel, M. A. J. S. Production, purification and partial characterization of the extracellular proteinase from *Pseudomonas fluorescens* 22F. Int. Dairy J. 7:165 – 171. 62.Secades, P., Alvarez, B. and Guijarro, J. A. 2003. Purification and properties of a new psychrophilic metalloprotease(Fpp2) in the fish pathogen *Flavobacterium psychrophilum*. FEMS micro. biol. lett.. 226: 273-279. 63.Seong, C. N., Jo, J. S., Choi1, S. K., Kim, S. W., Kim, S. J., Lee, O. H., Han, J. M. and Yoo,J. C. 2004. Production, purification, and characterization of a novel thermostable serine protease from soil isolate, *Streptomyces tendae*. Biotechnol. lett. 26: 907 – 909. 64.Shahabuddin, M. and Kaslow, D. C. 1993. Chitinase: a Novel Target for Blocking Parasite Transmission? Parasitol. today. 9: 252-255. 65.Shapira, R., Ordentlich, A., Chet, I. and Oppenheim, A. B. 1989. Control of plant disease by chitinase expressed from cloned DNA in *Escherichia coli*. Phytopathol. 79: 1246-1249. 66.Sierecka, J. K. 1998. Purircation and partial characterization of a neutral protease from a virulent strain of *Bacillus cereus* . Int. J. Biochem.Cell Biol. 30: 579-595. 67.Singh, L. R., Devi, T. P. and Devi, S.K. 2004. Purification and characterization of a pineapple crown leaf thiol protease. Prep. biochem. biotechnol.. Feb. 34: 25-43. 68.Skujins, J. J., Potgieter, H. J. & Alexander, M. 1965. Dissolution of fungal cell walls by *Streptomyces* chitinase and ??1,3-glucanase. Arch. Biochem. Biophy. 111: 358-364. 69.Sormorin, O. and Nishi. 1979. Studies on chitin preparation of benzyl and benzyl chitins. Polym. J. 2: 391-498. 70.Souza, R.F., Gomes, R.C., Coelho, R.R.R., Alviano, C.S., Soares, R.M.A. 2003. Purification and characterization of an endochitinase produced by *Colletotrichum gloeosporioides*. FEMS micro. biol. lett. 222: 45-50. 71.Takaya, N., Yamazaki, D., Horiuchi, H., Ohta, A. and Takagi, M. 1998. Intracellular chitinase gene from *Rhizopus oligosporus*: molecular cloning and characterization. Microbiology. 144: 2647 – 2654. 72.Tanabe, T., Kawase, T., Watanabe, T., Uchida, Y. and Mitsutomi, M. 2000. Purification and Characterization of a 49-kDa Chitinase from *Streptomyces griseus* HUT 6037. J. of Bioscience and Bioengineering. 89: 27-32. 73.Taylor, M.M., Bailey. D.G. and Fearheller, S.H. 1987. A review of the use of enzymes in the tannery. J. Am Leather Chem. Assoc. 82: 153-165. 74.Tikhonov, V. E., Radigina, L. A., Yamskov, I. A., Gulyaeva, N. D., Ilyina, A. V., Anlsimova, M. V., Varlamov, V. P. and Tatarinova, N. Y. 1998. Affinity purification of major chitinases produced

by *Streptomyces kurssanovii*. Enzyme microb. technol. 22: 82-85. 75.Todd, E.W. 1949. Quantitative studies on the total plasmin and trypsin inhibitor of human blood serum. J. Exp. Med. 39: 295 – 308. 76.Toma, C., Ichinose, Y. and Masaaki Iwanag, M. 1999. Purification and characterization of an *Aeromonas caviae* metalloprotease that is related to the *Vibrio cholerae* hemagglutinin/protease. FEMS micro. biol. lett. 170: 237-242. 77.Tsutomu, T., Kasumi, A., Yasuyaki, T. and Venzo, S. 1991. Isolation and characterization of thermostable chitinase from *Bacillus licheniformis*. Biochim. Biophys. Acta. 1078: 404-411. 78.Uzui, T., Hayashi, Y., Nanjo, F., Sakai, K. and Ishido, Y. 1987. Transglycosylation reaction of chitinase purified from *Nocardio orientalis*. Biochim. Biophys. Acta. 9923: 302-309. 79.Venter, H., Osthoff, G., and Litthauer D. 1999, Purification and Characterization of a Metalloprotease from *Chryseobacterium indologenes* Ix9a and Determination of the Amino Acid Specificity with Electrospray Mass Spectrometry. Protein expr. purif.. 15: 282 – 295. 80.Vicente, J. I. D., Arriaga, D. D., Valle, P. D., Soler, J. and Eslava, A. P. 1996. Purification and Characterization of an Extracellular Aspartate Protease from *Phycomyces blakesleeanus*. Fungal genet. biol. 20: 115 – 124. 81.Venter, H., Osthoff, G. and Litthauer, D. 1999. Purification and Characterization of a Metalloprotease from *Chryseobacterium indologenes* Ix9a and Determination of the Amino Acid Specificity with Electrospray Mass Spectrometry. Protein expr. purif.. 15: 282 – 295. 82.Wang, S. L., Chen, Y. H., wang, C. L., Yen, Y. H. and chern, M. K. 2005. Purification and characterization of a serine protease extracellularly produced by *Aspergillus fumigatus* in a shrimp and crab shell powder medium. Enzyme Microl. Technol. 36: 660-665. 83.Wang, S. L. and Chio, S. H. 1998. Deproteinization of shrimp and crab shell with the protease of *Pseudomonas aeruginosa* K-187. Enzyme Microl. Technol. 22: 629-633. 84.Wang, S. L., Kao, T. Y., Wang, C. L., Yen, Y. H., Chern, M. K. and Chen, Y. H. 2006. A solvent stable metalloprotease produced by *Bacillus* sp. TKU004 and its application in the deproteinization of squid pen for -chitin preparation. Enzyme Microl. Technol. 85.Yasuda, M., Aoyama, M., Sakaguchi, M., Nakachi, K. and Kobamoto, N. 1999. Purification and characterization of a soybean-milk-coagulating enzyme from *Bacillus pumilus* TYO-67. Appl. microbiol. biotechnol. 51: 474 – 479. 86.You, M., Xuan, X., Tsuji, N., Kamio, T., Taylor, D., Suzuki, N. and Fujisaki, K. 2003. Identification and Molecular Characterization of a Chitinase from the Hard Tick *Haemaphysalis longicornis*. J. biol. chem. 278: 8556 – 8563 87.Yuli, P.E., Suhartono, M. T., Rukayadi, Y., Hwang, J. K. and Pyunb, Y. R. 2004. Characteristics of thermostable chitinase enzymes from the indonesian *Bacillus* sp.13.26. Enzyme Microl. Technol. 35: 147 – 153. 88.Zhang, Y., Bak, D.D., Heid, H. and Geider, K. 1999. Molecular characterization of a protease secreted by *Erwinia amylovora*. J. Mol. Biol. 289: 1239-1251.