記錄編號	6331
狀態	NC094FJU00105030
助教查核	
索書號	
學校名稱	輔仁大學
系所名稱	生命科學系
舊系所名稱	
學號	492546056
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論文名稱(中)	納豆醱酵分離菌株之鑑定與其蛋白質分解酵素之分析
論文名稱(英)	Identification of Bacterial Isolates from Natto Fermentative Broth and the Analyses of their Proteases
其他題名	
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校內全文開放日期	
校外全文	

開放日期	
全文不開放理由	
電子全文送交國圖.	
國圖全文開放日期.	
檔案說明	
電子全文	
學位類別	碩士
畢業學年度	94
出版年	
語文別	中文
關鍵字(中)	納豆 蛋白質分解酵素 醱酵基質
關鍵字(英)	Natto Protease
摘要(中)	納豆在日本是一種具有悠久歷史也是近年來最受矚目的傳統醱酵食品，是由枯草桿菌 (Bacillus subtilis natto) 將煮熟的大豆醱酵而來。本研究目的在探討納豆菌株之菌種鑑定及其蛋白質分解酵素 (pretease) 之分析。自西元 1987 年須見博士在納豆食品中發現能夠溶解血栓纖維的蛋白質分解酵素—納豆激? (nattokinase) 以來。從不同來源的 Bacillus 菌屬或其他微生物中，已發現許多菌株能夠產生功能類似的凝血纖維蛋白分解酵素。本研究從醱酵製程中篩選出高生長速率的菌株 FZ - 01、FZ - 02 與 FZ - 03，以菌種鑑定系統進行型態特徵，酸鹼度、生長溫度、耐鹽度等生理測試，碳源利用與酵素活性等生化測試及 16S rDNA 片段序列分析的遺

	傳鑑定，確認實驗菌株的分類地位；另外，本研究挑選菌株 FZ - 01，針對培養基組成，分別以脫脂奶粉、黃豆粉、小麥胚芽與酵母菌萃取物作為醱酵基質並收取發酵產物，探討不同醱酵基質對菌株產生蛋白質分解酵素的影響。結果顯示，在分類鑑定上 FZ - 01、FZ - 02、FZ - 03 與標準菌株 BCRC - 14718 在分類上皆屬於 Bacillus subtilis，且菌株 FZ - 01、FZ - 02 與 FZ - 03 有極為相近的親緣關係。在培養基組成不同的情況下，菌株 FZ - 01 所產生的蛋白質產物在 NATIVE - PAGE 的分子量、種類與表現量上皆有差異；從蛋白質分解酵素對血栓纖維蛋白與酪蛋白的蛋白質分解活性測試中可以發現活性表現的區域亦有所不同。從本研究結果可以推論單一菌株在不同培養條件下，可能產生不同種類的蛋白質分解酵素與血栓纖維分解酵素。
摘要 (英)	In Japan natto has a long history of being a premium traditional fermentive food, and been received much attention for health today. Natto is made of Bacillus subtilis natto in steamed soy bean, and was found containing with nattokinase, a fibrinolytic protease discovered by Dr. Sumi in 1987. According to many scientific publications, many similar proteases functioning in digesting fibrin were generated from a variety of bacillus strains and other microorganisms by using various substrates. Strains FZ-01, FZ-02 and FZ-03 were isolated from natto fermentation broth with high growth rates. The three strains were identified by analyzing following characterizations: pH, temperature, salt adaptation, carbon source and various enzyme activity. Furthermore, phylogenetic analysis was carried out by 16S rDNA fragment sequencing and showed that FZ-01, FZ-02 and FZ-03 are closely related and clustered with bacillus subtilis. In this study, strain FZ-01 was selected for protease production and analyses. Strain FZ-01was cultured with four different media: milk, soybean, wheat acrospire, and yeast extract-based media. Fermentative broths of strain FZ-01 were analyzed with protease using NATIVE-PAGE, which revealed that the produced proteins under four different media showed differences in molecular weight. The activities of these protease varied on fibrin- and casein-containing plates. From these results, we deducted that strain FZ-01 can produce different protease using different nitrogen sources.
論文目次	中文摘要 I 英文摘要 II 第一章 前言 1 1.1 納豆 1 1.2 菌種分類鑑定系統 2 1.3 Bacillus 的分類 4 1.4 納豆菌 5 1.5 蛋白質分解酵素 6 1.6 納豆激? 8 1.7 納豆激?之功能性 9 1.8 研究目的 11 第二章 材料與方法 12 2.1 菌株來源、培養與保存 12 2.2 本實驗使用之培養基 12 2.3 蛋白質分析實驗所使用之膠體配方、緩衝液與染劑 14 2.4 型態特徵與格蘭氏染色 17 2.5 基本生理特性-酸鹼度、生長溫度、鹽度的測試 17 2.5.1 酸鹼度生長範圍測試 17 2.5.2 溫度生長範圍測試 18 2.5.3 耐鹽度測試 18 2.5.4 生長曲線測定 18 2.6 基本生化特性-碳源利用與酵素活性測試 19 2.6.1 單一碳源利用能力 19 2.6.2 澱粉水解能力測試 19 2.6.3 Modified 酪蛋白 (Casein) 水解測試 19 2.6.4 白明膠 (Gelatin) 水解測試 20 2.6.5 解脂? (Lipase) 活性測試 20 2.6.6 API-ZYM 酵素活性檢測 20 2.7 菌種遺傳鑑定-16S rDNA 片段序列分析 20 2.7.1 細菌 genomic DNA 抽取 20 2.7.2 PCR 引子設計 21 2.7.3 PCR 反應 21

2.7.4 16S rDNA 片段序列分析 22 2.8 醱酵培養與回收 23 2.8.1 醱酵前培養 23 2.8.2 醱酵實驗 23 2.8.3 醱酵液回收 23 2.9 醱酵產物分析 24 2.9.1 置備式電泳 24 2.9.2 膠體染色 24 2.9.3 血栓溶解活性測試 24 2.9.4 酪蛋白分解活性測試 25 第三章 結果 26 3.1 型態特徵與格蘭氏染色 26 3.2 基本生理特性-酸鹼度、生長溫度、鹽度的測試 26 3.2.1 酸鹼度生長範圍測試 26 3.2.2 溫度生長範圍測試 26 3.2.3 耐鹽度測試 26 3.2.4 生長曲線測試 27 3.3 基本生化特性-碳源利用與酵素活性測試 27 3.3.1 單一碳源的利用能力 27 3.3.2 澱粉水解能力測試 27 3.3.3 Modified 酪蛋白 (Casein) 水解測試 28 3.3.4 白明膠 (Gelatin) 水解測試 28 3.3.5 解脂? (Lipase) 活性測試 28 3.3.6 APIZYM 酵素活性檢測 28 3.4 菌種遺傳鑑定-16S rDNA 片段序列分析 28 3.5 醱酵培養與回收 29 3.6 醱酵產物分析 29 3.6.1 置備式電泳與膠體染色 29 3.6.2 血栓溶解活性測試 30 3.6.3 酪蛋白分解活性測試 30 第四章 討論 31 4.1 菌株 FZ-01、FZ-02 與 FZ-03 之菌種鑑定 31 4.2 不同醱酵基質對菌株 FZ-01 產生蛋白質產物的影響 32 4.3 不同醱酵基質對菌株 FZ-01 產生蛋白質分解酵素的影響 33 4.4 未來展望 34 參考文獻 36 圖表 44 附錄 附錄一、實驗菌株 FZ-01 之 16S rDNA 序列 72 附錄二、實驗菌株 FZ-02 之 16S rDNA 序列 73 附錄三、實驗菌株 FZ-03 之 16S rDNA 序列 74 附錄四、標準菌株 BCRC-14718 之 16S rDNA 75

參考文獻

張文重 (1976) 蛋白質分解酵素(構造、功能、進化及應用)。國立編譯館。 王正仁，陳孟伶，林修平，陳啟祥 (1999) 水解酵素在工業上的利用。生物產業 10(1):1-11。 方繼等編譯。(1999) 現代食品微生物學。偉明圖書有限公司。 蘇遠志編著。(1999)應用微生物學。華香園出版社。 蘇遠志。(2003)。納豆菌代謝產物的開發與應用。生物產業 14(2)：117-30。 陳懋彥。(2002) 台灣地熱區嗜熱性細菌之研究。國立台灣大學植物學研究所博 士論文。 邱子玲。(2004) 自然環境及市售納豆產品中 Bacillus 分離株之特性分析及種類鑑定。天主教輔仁大學生命科學研究所碩士論文。 黃詩宜。(2005) 納豆菌發酵產物對人體血液生化因子之影響。天主教輔仁大學 生命科學研究所碩士論文。 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990. Basic local alignment search tool. J Mol Biol 215: 403-410. Ash, C., J. A. E. Farrow, S. Wallbanks, and M. D. Collins. 1991. Phylogenetic heterogeneity of the genus Bacillus revealed by comparative analtsis of small-subunit-ribosomal RNA sequences. Lett. Appl. Microbiol. 13:202-206. Atlas, R. M. & Bartha, R. 1998. Microbial Ecology : Fundamentals and Applications, 4th edn. Menlo Park Chang CT, Fan MH, Kuo FC, Sung HY. 2000. Potent fibrinolytic enzyme from a mutant of Bacillus subtilis IMR-NK-1. Journal Agricultural and Food Chemistry 48:3210-6. Daniels, L., Hanson, R. S. & Phillips, J. A. 1994. Chemical analysis. Methods for General and Molecular Bacteriology. pp. 655-682. Washington, D.C Felsenstein, J. 1989. PHYLIP -- Phylogeny Inference Package. Cladistics 5, 164-166. Ferreira, A. C., Nobre, M. F., Rainey, F. A., Silva, M. T., Wait, R., Burghardt, J.,Chung, A. P. & da Costa, M. S. 1997. Deinococcus geothermalis sp. nov. and Deinococcus murrayi sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. Int J Syst Bacteriol 47, 939-947. Forage, R. G., Harrison, D. E. F., Pitt, D. E. 1985. Effect of environment on microbial activity.

Comprehensive Biotechnology. 1, 253-279. . Fukutake, M., Takahahi, M., Ishida, K., Kawamura, H., Sugimura, T. and Wakabayashi, K. 1996. Quantification of genistein and genistin in soybeans and soybean products. Food and Chemical Toxicology 34:457-461. Gillis, M., Vandamme, P., De Vos, P., Swings, J. & Kersters, K. 2001. Polyphasic Taxonomy. Bergey’ s Manual of Systematic Bacteriology. 1: 43-48. H.Sumi, M. M. a. 1998. Effect of natto diet on blood pressure. Basic and Clinical Aspects of Japanese Traditional Food Natto II:1-3. Harwood, Colin R. 1989. Bacillus. Plenum Press, New York. Hosoi, T., A. Ametani, K. Kiuchi, and S. Kaminogawa. 2000. Improved growth and viability of lactobacilli in the presence of Bacillus subtilis (natto), catalase, or subtilisin. Can J Microbiol 46:892-7. Johnson, J. L. 1984. Nucleic acids in bacterial classification. Bergey’ s Manual of Systematic Bacteriology. 1:8-11. Johnson, J. L. 1994. Similarity analysis of DNAs. In Methods for General and Molecular Bacteriology, pp. 655-682. Edited by P. Gerhardt. Washington, D.C.: American Society for Microbiology. Jukes, T. H. & Cantor, C. R. 1969. Evolution of protein molecules. In Mammalian Protein Metabolism, pp. 21-132. Edited by M. H. N. New York: Academic Press. Kim S. H. and Choi N.S. 2000. Purification and characteration of subtilisin DJ-4 screened by Bacillus sp. Strain DJ-4 screened from Doen-Jang. Biosci. Biotechnol. Biochem. 64, 1722-1725. Kim W, Choi K, Kim Y, Park H, Choi J, Lee Y, OH H, Kwon I, Lee S. 1996. Purification and Characterization of a fibrinolytic enzyme producted form Bacillus sp. strain CK11-4 screened from Chungkook-Jang. Applied and Environmental Microbiology 62(7):2482-8. Ko J. H., YanJ. P., Zhua L., Qi Y. P., 2004. Identification of two novel fibrinolytic enzymes from Bacillus subtilis QK02. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 137(1):65-74. Krieg, N. R. 2001. Identification of Procaryotes. Bergey’ s Manual of Systematic Bacteriology. 1:33-38. Kroyer, J. and Chang, S. 1981. The promoter-proximl region of the Bacillus licheniformis penicillinase gene: Nucleotide sequence and predicted leader peptide sequence. Gene 15, 343-347. Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics Submitted. Li, Z., Kawamura, Y., Shida, O., Yamagata, S., Deguchi, T. and Ezaki, T. Bacillus okuhidensis sp. nov., isolated from the Okuhida spa area of Japan. Int. J. Syst. Evol. Microbiol. 52, 1205-1209. Ludwig, W. and Klenk, H. 2001. Overview: A phlogenetic backbone and taxonomic framework for procaryotic systematics. In Bergey’ s Manual of Systematic Bacteriology. 1: 43-48. Marteinsson, V. T., Hauksdottir, S., Hobel, C. F., Kristmannsdottir, H., Hreggvidsson, G. O. & Kristjansson, J. K. 2001. Phylogenetic diversity analysis of subterranean hot springs in Iceland. Appl Environ Microbiol 67, 4242-4248. Omura K., Hitosugi M., Zhu X., Ikeda M., MaedaH., and Tokudome S. 2005. A Newly Derived Protein From Bacillus subtilis natto With Both Antithrombotic and Fibrinolytic Effects. J Pharmacol Sci 99:247 – 251. Peng Y, Huang Q, Zhang RH, Zhang YZ. 2003. Purification and characterization of a fibrinolytic enzyme produced by Bacullus amyloliquefaciens DC-4 screened from douchi, a traditional Chinese soybean food. Comparative Biochemistry and Physiology Part B 134:45-52.

Plava, I. 1982. Molecular cloning of alpha-amylase gene from Bacillus amyloliquefaciens and its expression in Bacillus subtilis. Gene 19: 81-87. Priest, F.G. 1993. Systematics and ecology of Bacillus. In: Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physiology and Molecular Genetics (Hoch, J.A. and Losick, R., Eds.), pp. 3-16. American Society for Microbiology (ASM), Washington, DC. Roberto, M. L. Gabriella, C. Simon, M. C. and Martin, J. W. 2001. Bacillus subtilis spores competitively exclude Escherichia coli O78:K80 in poultry. Veterinary Microbiology 79: 133-142. Saito, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4, 406-425. Samy A, Aassar E. 1995. Production and properties of fibrinolytic enzyme in solid state cultures of Fusarium pallidoroseum. Biotechnology Letters 17(9):943-8. Simbert, R. M. and Krieg, N. R. 1994. Phenotypic characterization. In Methods for General and Molecular Bacteriology. pp. 607-654. Sneath, P. H. A. 2001. Numeriacl Taxonomy. In Bergey's Manual of Systematic Bacteriology, 1:39-42. Stackebrandt, E. and Goebel, B. M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rDNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Evol. Microbiol. 44:846-849. Steinmetz, K. A., Kushi, L. H., Bostick, R. M., Folsom, A. R., & Potter, J. D. 1994. Vegetables, fruit, and colon cancer in the Iowa women's health study. Am J Epidemiol 139: 1-15. Stephens, M. A., Ortlepp, S. A., Ollington, J. F. and McConnell, D. J. 1984. Nucleotide sequence of the 5' region of the Bacillus licheniformis alpha-amylase gene: Comparison with the B. amyloliquefaciens gene. J. Bacteriol. 158:369-372. Sumi H, Nakajima N, Yatagai C. 1995. A unique strong fibrinolytic enzyme (katsuwokinase) in skipjack ''Shiokara,'' a Japanese traditional fermented food. Compound Biochemistry Physiological 112B(3):543-7. Sumi, H., H. Hamada, H. Tsushima, H. Mihara, and H. Muraki. 1987. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. Experientia 43:1110-1. Suzuki Y., Kondo K., PhD, Ichise H, Tsukamoto Y, Urano T., and Umemura K. 2003. Dietary Supplementation With Fermented Soybeans Suppresses Intimal Thickening. Nutrition 19:261 – 264. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W.E.C. Moore, R. G. E. Murray, E. Stackerbrandt, M. P. Starr, and H. G. Truper. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int. J. System. Bacterial.37:463-464 Woese, C. R. & Fox, G. E. 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci USA 74, 5088-5090. Woese, C. R., Kandler, O. & Wheelis, M. L. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87, 4576-4579. Yamashita, T., E. Oda, J. C. Giddings, and J. Yamamoto. 2003. The effect of dietary bacillus natto productive protein on in vivo endogenous thrombolysis. Pathophysiol Haemost Thromb 33:138-43. Yang, M., Galizzi, A. and Henner, D. J. 1984. Cloning of the neutral protease gene of Bacillus subtilis and the use of the cloned gene to create an in vitro derived deletion mutation. J. Bacteriol. 160:15-

	21. Zheng Z. L., Zuo Z. Y., Liu Z. G., Tsai K. C., Liu A. F., and Zou G. L. 2005. Construction of a 3D model of nattokinase, a novel fibrinolytic enzyme from Bacillus natto A novel nucleophilic catalytic mechanism for nattokinase. J Mol Graph Model 23: 373 – 380.
論文頁數	86
附註	
全文點閱次數	
資料建置時間	
轉檔日期	
全文檔存取記錄	
異動記錄	M admin Y2008.M7.D3 23:18 61.59.161.35