

Effect of Environmental Factors on Keratinase Activity of Native Strain

任耕毅、吳建一

E-mail: 9607696@mail.dyu.edu.tw

ABSTRACT

The bacteria *Bacillus thuringiensis* Wu2 was isolated from poultry farm soil, it can degraded the feather completely in two weeks. The degraded feather was observed with Fourier transform-infrared (FTIR), the bonds between the function group of feather were discredited and decrease. Under the culture condition 37 °C and pH 7, the biomass were reached the maximum 1.135 g/L at 68 hour. The maximum activity was 3.50 kU/mL and the optimal pH and temperature of keratinase activity of *B. thuringiensis* Wu2 was pH 7 and 40 °C, respectively otherwise addition the nitrogen source of Peptone, Urea, NH₄Cl and NaNO₃ into the medium, it doesn't significance to the keratinase activity.

Keywords : *Bacillus* sp., Keratinase, Feed addition, Feather.

Table of Contents

目錄 封面內頁 頁次 簽名頁 授權書.....	iii	中文摘要.....	iv	英文摘要.....	x
要.....	v	誌謝.....	vi	目錄.....	vii
圖目錄.....	x	表目錄.....	xi	1. 前言.....	1
2. 文獻回顧.....	3	2.1 蛋白? (protease) 之簡介與工業上的應用.....	3	2.1.1 蛋白?之簡介.....	3
2.1.2 蛋白?在工業上的應用.....	3	2.2 角蛋白? (keratinase) 的介紹.....	4	2.2.1 角蛋白?之簡介.....	4
2.2.2 角蛋白?之特性.....	6	2.2.3 角蛋白?之生產菌株.....	6	2.3 角蛋白 (keratin) 之簡介.....	9
2.3.1 角蛋白的來源.....	9	2.3.2 角蛋白的結構.....	10	2.3.3 角蛋白的特性.....	14
2.4 角蛋白?生產菌株所生產之角蛋白?其應用與發展.....	14	2.4.1 飼料工業之應用.....	14	2.4.2 皮革工業.....	18
2.4.3 清潔劑工業.....	18	2.4.4 醫藥及美容用品.....	19	3. 材料與方法.....	20
3.1 藥品及儀器設備.....	20	3.1.1 藥品.....	20	3.1.2 儀器設備.....	21
3.2 菌株來源.....	22	3.3 培養基.....	23	3.4 培養方法.....	23
3.4.1 不同pH培養基之培養方法.....	23	3.4.2 不同溫度之培養方法.....	23	3.4.3 不同氮源培養基之培養方法.....	24
3.5 分析方法.....	24	3.5.1 角蛋白?活性之分析方法.....	24	3.5.2 蛋白質定量之分析方法(Folin-phenol法) ..	26
3.5.3 掃描式電子顯微鏡 (SEM) 檢測方法.....	27	3.5.4 氨基酸組成之分析.....	27	3.5.5 傅立葉轉換紅外線光譜分析.....	28
4. 結果與討論.....	29	4.1 角蛋白?生產菌株之篩選.....	29	4.2 不同培養溫度對酵素活性的影響.....	31
4.3 不同pH值的環境對角蛋白?活性的影響.....	32	4.4 不同氮源的環境對角蛋白?活性的影響.....	33	4.5 雞羽毛經 <i>B. thuringiensis</i> Wu2粗酵素作用後之 胺基酸組成與官能基變化.....	34
5. 結論.....	46	參考文獻.....	48		

REFERENCES

1. 王錦盟。1996。水解羽毛粉於肉雞飼糧中之應用。飼料營養雜誌。1:77-83。
2. 吳芝穎。2004。 *Bacillus licheniformis* THSC-1 角蛋白分解?之純化、定性與基因選殖。東海大學畜產學研究所碩士論文。台中，台灣。
3. 呂鋒洲，林仁混。1991。基礎酵素學。聯經出版社。台北，台灣。
4. 李京樺。2006。角蛋白?之生產菌篩選、純化及其特性。國立台灣海洋大學。食品科學研究所碩士論文。基隆，台灣。
5. 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。台北，台灣。
6. 台灣農業統計年報。2005。pp:122-125。
7. 張資奇。1999。蛋雞糞堆肥中篩選羽毛分解菌之研究。東海大學畜產學研究所碩士論文。台中，台灣。
8. 陳庭柔。2004。 *Bacillus licheniformis* CCRC 14353和 *Bacillus licheniformis* CCRC 115945之粗酵素對雞羽毛水解效果之評估。國立中興大學畜產研究所碩士論文。台中，台灣。
9. 楊建強、湯國營。2005。角蛋白?研究進展。生物技術通訊16 (2) :201-203。
10. 鄭永祥、詹德芳。1991。細菌性羽毛發酵產物羽毛溶成物作為飼料蛋白質的評估。飼料營養雜誌。10:10-17。
11. 鄭淑文。1998。 *Bacillus licheniformis* PWD-1 角蛋白?之生產、純化、特性及基因選殖研究。國立陽明大學生物化學研究所博士論文。台北，台灣。
12. 謝明國。2003。以 *Bacillus licheniformis* THSC-1 進行雞毛與豬毛分解效果之研究。東海大學畜產學研究所碩士論文。台中，台灣。
13. 謝魁鵬、魏耀揮。2000。最新生物化學實驗。藝軒圖書出版社。台北。p118-119。
14. 蘇睿綺。枯草菌屬角蛋白?之純化與性質研究。靜宜大學食品營養研究所碩士論文。台中，台灣。
15. 蘇遠志。1999。應用微生物學。華香園出版社。台北，台灣。
16. Atalo, K. and Gsahe, B. A. 1993. Protease production by a thermophilic bacillus sp. P-001A which degrades various kinds of fibrous proteins. Biotech. Lett. 15:1151-1156.
17. Baker, D. H., Blithenthal, R.

C., Boebel, K. P., Czamecki, G. L., Southern, L. L. and Willis, G. M. 1981. Protein-amino acid evaluation of steam-processed feather meal. *Poultry Sci.* 60:1865-1872.

18. Basma, G., Alya, S. and Moncef, N. 2003. Stability studies of protease from *Bacillus cereus* BG1. *Enzyme Microb. Tech.* 32:513-518.

19. Bernal, C., Cairo, J. and Coello, N. 2006. Purification and characterization of a novel exocellular keratinase from *Kocuria rosea*. *Enzyme Microb. Tech.* 38 (1-2) :49-54.

20. Bockle, B. and Muller, R. 1997. Reduction of disulfide bonds by *Streptomyces pactum* during growth on chicken feathers. *Appl. Environ. Microbiol.* 63 (2) :790-792.

21. Bockle, B., Galunsky, B. and Muller, R. 1995. Characterization of a keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530. *Appl. Environ. Microbiol.* 61 (10) :3705-3710.

22. Chellapandian, M. 1998. Preparation and characterization of alkaline protease immobilized on vermiculite. *Process Biochemistry.* 32 (2) :169-173.

23. Creighton, T. E. 1993. *Proteins: Structure and molecular properties*. 2 nd ed. New York, Freeman.

24. Farag, A. M. and Hassan, M. A. 2004. Purification, characterization and immobilization of a keratinase from *Aspergillus oryzae*. *Enzyme Microb. Tech.* 34 (2) :85-93.

25. Fraser, R. D. B. and Parry, D.A.D. 1996. The molecular structure of reptilian keratin. *Int. J. Biological Macromolecules.* 19:207-211.

26. Friedrich, A. B. and Antranikian, G. 1996. Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order Thermotogales. *Appl. Environ. Microbiol.* 62 (8) :2875-2882.

27. Friedrich, J. and Kern, S. 2003. Hydrolysis of native proteins by keratinolytic protease of *Doratomyces microsporus*. *J. Mol. Catal. B:Enzyme* 21 (1-2) :35-37.

28. Garrett, R. H. and Grisham, C. M. 2002. *Principles of biochemistry: with a human focus*. Fort Worth: Harcourt College Pub. pp.128-129.

29. Genckal, H. and Tari, C. 2006. Alkaline protease production from alkalophilic *Bacillus* sp. Isolated from natural habitats. *Enzyme Microb. Tech.* 39:703-710.

30. Gessesse, A., Hatti-Kaul, R., Gashe, B. A. and Mattiasson, B. 2003. Novel alkaline proteases from alkaliphilic bacteria grown on chicken feather. *Enzyme Microb. Tech.* 32 (5) :519-524.

31. Godfrey, T. and S. I. West. 1996. Introduction to industrial enzymology. In: Godfrey, T. and West, S. (ed.), *Industrial Enzymology*. 2nd ed. MacMillan Press LTD. pp. 609.

32. Goto A, and Kunioka M. 1992. Biosynthesis and hydrolysis of poly(γ -glutamic acid) from *Bacillus subtilis* IFO3335. *Biosci. Biotech. Biochem.* 56: 1031 – 1035.

33. Gradisar, H., Kern, S. and Friedrich, J. 2000. Keratinase of *Doratomyces microsporus*. *Appl. Microbiol. Biot.* 53 (2) :196-200.

34. Grazztoni, A., Pimentel, F. A., de Jong, E. V. and Brandelli, A. 2006. Nutritional improvement of feather protein by treatment with microbial keratinase. *Anim. Feed Sci. Tech.* 126 (1-2) :135-144.

35. Gupta, R., Beg, Q. K. and Lorenz, P. 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl. Microbiol. Biot.* 59:15-32.

36. Kim, J. M., Lim, W. J. and Suh, H. J. 2001. Feather-degrading *Bacillus* species from poultry waste. *Process Biochem.* 37 (3) :287-291.

37. Kim, J. S., Kluskens, L. D., de Vos, W. M., Huber, R. and van der Oost, J. 2004. Crystal structure of fervidolysin from *Fervidobacterium pennivorans*, a keratinolytic enzyme related to subtilisin. *J. Mol. Biol.* 335 (3) :787-797.

38. Kumar, C. G. and Takagi, H. 1999. Microbial alkaline protease: from a bioindustrial viewpoint. *Biotechnol. Adv.* 17 (7) :561-594.

39. Kunert, J. 1989. Biochemical mechanisms of keratin degradation by actinomycete *Streptomyces fradiae* and fungus *Microsporium gypseum*, a comparison. *J. Basic. Microbiol.* 29:597-604.

40. Latshaw, J. D., Musharf, N. and Return, R. 1994. Processing of feather to maximize its nutritional value for poultry. *Anim. Feed Sci. Technol.* 47:179-188.

41. Lehninger, A. L., Nelson, D. L. and Cox, M. M. 1993. The three-dimensional structure of proteins. In: *Principles of Biochemistry*. 2 nd ed. Worth Publishers. New York.

42. Lin, X., Kelemen, D. W., Miller, E. S. and Shin, J. C. H. 1995. Nucleotide sequence and expression of Ker A, the gene encoding a keratinolytic protease of *Bacillus licheniformis* PWD-1. *Appl. Environ. Microbiol.* 61:1469-1474.

43. Lin, X., Lee, C. G., Casale, E. S. and Shih, J. C. H. 1992. Purification and characterization of a keratinase from a feather-degrading *Bacillus licheniformis* strain. *Appl. Environ. Microbiol.* 58 (10) :3271-3275.

44. Macedo, A. J., da Silva, W. O. B., Gava, R., Driemeier, D., Henriques, J. A. P. and Termignoni, C. 2005. Novel keratinase from *Bacillus subtilis* S14 exhibiting remarkable dehairing capabilities. *Appl. Environ. Microbiol.* 71 (1) :594-596.

45. Malviya, H. K., Rajak, R. C. and Hasija, S. K. 1992. Synthesis and regulation of extracellular keratinase in three fungi isolated from the grounds of a gelatin factory, Jabalpur, India. *Mycopathologia.* 120:1-4.

46. Malviya, H. K., Rajak, R. C. and Hasija, S. K. 1993. In vitro degradation of hair keratin by *Graphium penicillodens*: evidences for sulfitolysis and peptidolysis. *Crypt. Bot.* 3:197-201.

47. McCullough, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. *Clin. Chim. Acta* 17:297 – 304.

48. Mehrotra, J., Mittal, A. and Rastogi, A. K. 1999. Antigenic definition of plasma membrane proteins of *Bacillus Calmette – Guerin*: predominant activation of human T cells by low-molecular-mass integral proteins. *Scand J. Immunol.* 50: 411 – 419.

49. Mehrotra, S., Pandey, P. K., Gaur, R. and Darmwal, N. S. 1999. The production of alkaline protease by a *Bacillus* species isolate. *Bioresource Tech.* 67:201-203.

50. Mitsui, S., Ichikawa, M., Oka, T., Sakai, M., Moriyama, Y., Sameshima, Y., Goto, M. and Furukawa, K. 2004. Molecular characterization of a keratinolytic enzyme from an alkaliphilic *Nocardia* sp. TOA-1. *Enzyme Microb. Tech.* 34 (5) :482-489.

51. Nduwimana, J., Guenet, L., Dorval, I., Blayau, M., Gall, J. L. and Treut, A. L. 1995. Protease. *Ann. Biol. Clin.* 53:251-264.

52. Ng, T. K., and W. R. Kenealy. 1986. Industrial applications of thermostable enzymes, pp. 197-215. In T. D. Brock (ed.) , *Thermophiles: general, molecular, and applied microbiology*. John Wiley & Sons, New York.

53. Onifade, A. A., Al-Sane, N. A., Al-Musallam, A. A. and Al-Zarban, S. 1998. A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. *Bioresource Technol.* 66 (1) :1-11.

54. Papadopoulos, W. C. 1985. Processed chicken feather as feed stuff for poultry and swine. *Rev. Agricultural waste.* 14:275-290.

55. Podulka, S., Rohrbaugh, JR. R. and Bonney, R. (eds.) 2004. *Handbook of Bird Biology*. Cornell Lab. of Ornithology. Ithaca. New York.

56. Ramnani, P. and Gupta, R. 2004. Optimization of medium composition for keratinase production on feather by *Bacillus licheniformis* RG1 using statistical methods involving response surface methodology. *Biotechnol. Appl. Bioc.* 40:191-196.

57. Sangali, S. and Brandelli, A. 2000. Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. *J. App. Microbiol.* 89 (5) : 735-743.

58. Santos, R. M. D. B., Firmino, A. A. P., de Sa, C. M. and Felix, C. R. 1996. Keratinolytic activity of *Aspergillus fumigatus* Fresenius. *Curr Microbiol* 33 (6) :364-370.

59. Shih, J. C. H. 1993. Recent development in poultry waste

digestion and feather utilization. *Rev. Poultry Sci.* 72:1617-1620. 60. Shohe, Y., Yasutaka, M., Quamrul H., Sathuluri, R. R., Yuji, M., Kenji Y. and Ehchi, T. 2002. Characterization of a new keratin-degrading Bacterium isolated from deer fur. *J. Biosci. Bioeng.* 93 (6) :595-600. 61. Stilborn, H. L., Moran, E. T., Jr, Gous, R. M., and Harrison, M. D. 1997. Effect of age on feather amino acid content in two broiler strain crosses and sexes. *J. App. Poultry Res.* 6:205-209. 62. Takami, H., Akiba, T. and Horikoshi, K. 1989. Production of extremely thermostable alkaline protease from *Bacillus* sp. No. AH-101. *Appl. Microbiol. Biotechnol.* 30 (2) :120-124. 63. Takami, H., Akiba, T. and Horikoshi, K. 1990. Characterization of an alkaline protease from *Bacillus* sp. No. AH-101. *Appl. Microbiol. Biotechnol.* 33:519-523. 64. Takami, H., Nakamura, S., Aono, R. and Horikoshi, K. 1992. Degradation of human hair by a thermostable alkaline protease from alkalophilic *Bacillus* sp. No. AH-101. *Biosci. Biotech. Bioch.* 56 (10) :1667-1669. 65. Takami, H., Nogi, Y. and Horikoshi, K. 1999. Reidentification of the keratinase-producing facultatively alkaliphilic *Bacillus* sp. AH-101 as *Bacillus halodurans*. *Extremophiles* 3 (4) :293-296. 66. Tomarelli, R.M., Charney, J. and Harding, M.L. 1949. The use of azoalbumin as a substrate in the colorimetric determination of peptic and tryptic activity. *J. Lab. Clin. Med.* 34, 428 – 433. 67. Vignardet, C., Guillaume, Y. C., Michel, L., Friedrich, J. and Millet, J. 2001. Comparison of two hard keratinous substrates submitted to the action of a keratinase using an experimental design. *Int. J. Pharm.* 224 (1-2) :115-122. 68. Wang, X. 1997. Effect of processing systems on protein quality of feather meal and hair meals. *Poult. Sci.* 76:491 – 6. 69. Williams, C. M. and Papadopoulos, M. C. 1985. Processed chicken feathers as feed stuff for poultry and swine. *Rev. Agricultural waste.* 14:275-290. 70. Williams, C. M., and Shih, J. C. H. 1989. Enumeration of some microbial groups in thermophilic poultry waste digesters and enrichment of a feather-degrading culture. *J. Appl. Bacteriol.* 67 (1) :25-35. 71. Williams, C. M., Richter, C. S., Mackenzie, J. M., Jr. and Shih, J. C. H. 1990. Isolation, identification and characterization of a feather-degrading bacterium. *Appl. Environ. Microbiol.* 56 (6) :1509-1515. 72. Yamamura, S., Morita, Y., Hasan, Q., Yokoyama, K. and Tamiya, E. 2002. Keratin degradation: a cooperative action of two enzymes from *Stenotrophomonas* sp. *Biochemical and Biophysical Research Communications*, Vol. 294, pp.1138-1143. 73. Yu, R. J., Harmon, S. R. and Blank, F. 1968. Isolation and purification of an extracellular keratinase of *Trichophyton mentagrophytes*. *J. Bacteriol.* 96 (4) :1435-1436.